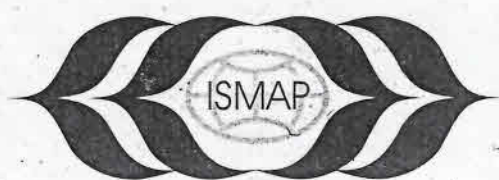


**III International Symposium Breeding  
Research on Medicinal and  
Aromatic Plants  
&  
II Latin American Symposium on the  
Production of Medicinal and Aromatic Plants  
and Condiments**



July, 5-8, 2004  
Campinas - SP - BRAZIL

**Program  
&  
Abstracts**



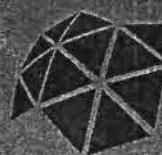
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## ORGANIZING



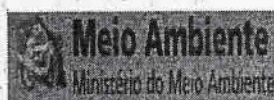
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## **Preface**

*On behalf of the organizing committee for ISMAP-2004, and, we could even say, on behalf of the Medicinal Plants themselves, we have the satisfaction to see such expressive attention from several groups in the world converged to focus on the raw material of phytomedicines.*

*The richness of the Latin American flora in terms of its wide range of potential medicinal species and the traditional knowledge associated to them, demands actions for its benefit to reach a greater number of people within several social realities. These actions go through integrated studies aiming to guaranty the quality and the quantity of MAPs, while assuming good practices of production.*

*These two events, presented now as one in Brazil, concern Agrotechnology and Plant Breeding of MAPs, building the basis for our major objective: **Use and Conservation**.*

*Every article sent to us and all interest for ISMAP-2004 had our most careful welcome, as it proves that participants are committed to this great resource of Nature.*

*To them all, we wish that this event may bring a small contribution to the noble work which they are developing.*

## **Prefácio**

*Em nome da comissão organizadora do ISMAP-2004, e, poderíamos até dizer, em nome das Plantas Medicinais, temos a satisfação em ver convergida a atenção de grupos do mundo todo para o foco da matéria prima dos fitomedicamentos.*

*A riqueza da flora Latina Americana quanto à diversidade de espécies com potencial medicinal e o conhecimento tradicional a ela associado demandam ações para que os benefícios dessas espécies atinjam maior número de pessoas nas diversas realidades sociais. Estas ações passam por estudos integrados visando garantir a qualidade e a quantidade das plantas medicinais, dentro de critérios de boas práticas de produção.*

*Estes dois eventos, apresentados agora conjuntamente no Brasil, tratam da Agrotecnologia e do Melhoramento Genético das MAPs formando a base para seu objetivo maior: **Uso e Conservação**.*

*Todos os trabalhos enviados e o interesse pelo ISMAP-2004 tiveram a nossa melhor acolhida e demonstram que os participantes estão compromissados com esta riqueza da Natureza.*

*A todos eles desejamos que este evento possa fortalecê-los ainda mais na nobre tarefa que realizam.*

Pedro Melillo de Magalhães, Dr.sc  
President of the III International Symposium  
Breeding Research on Medicinal and Aromatic  
Plants

Lin Chau Ming, Dr. sc.  
President of the II Latin American Symposium  
on the Production of Medicinal and Aromatic  
Plants and Condiments

**III International Symposium: Breeding Research on Medicinal and Aromatic Plants &  
II Latin American Symposium on the production of MAPs & Condiments  
BRAZIL, 5-8 July 2004**

**Monday, 5/07/2004**

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9:00-10:30 Registration and Material Distribution

10:00-10:30 Musical Presentation

10:30-11:00 Opening Session

11:00-12:30 Master lecture:

**L01** - Projects on Medicinal and Aromatic Plants in Brazil      Dr. Benjamin **Gilbert** (FIOcruz-Brazil)

12:30-14:00 Lunch

14:00-16:00 Round Table: **Agrotechnology and Quality Control**

Chairman: Peter **Griffie** (FAO-Italy)

14:00-14:40 **L02** - Research, discovery and application of Natural  
Products for Health and Medicine

Jim **Simon** (Rutgers-EUA)

14:40-15:20 **L03** - Cultivation and post harvest process for Aromatic  
Plants in Argentina

Ana **Curioni** (Argentina)

15:20-16:00 **L04** - Alternative methods for plant protection against  
diseases

Marcelo **Morandi** (EMBRAPA-Brazil)

16:00-16:30 Coffee

16:15-16:30 Musical presentation

16:30-17:30 Conference:

**L05** - Adaptation of medicinal and aromatic plants to  
contemporary requirements by breeding: aims,  
methods, and trends

Dr. Friedrich **Pank** (BAZ-Germany)

17:30-19:00 Poster section

- Biodiv. & Gen. Res. conservation
- Economic, Eth. & Legal aspects
- Biotechnology

**Welcome cocktail (Brazilian dishes) – Musical presentation**

**Tuesday, 6/07/2004**

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8:45-9:00 Laboral Gymnastics

9:00-11:00 Round table: **Breeding, Conservation and  
Biotechnology of MAP**

Chairwoman:

M. Beatriz **Perecin** (IAC Brazil)

9:00 - 9:40 **L06** - Medicinal and Aromatic Plants from Brazil: The  
Challenge of Genetic Resources Conservation

Roberto F. **Vieira** (Embrapa-Brazil)

9:40-10:20 **L07** - Regulation of essential oil biosynthesis and its  
implications in medicinal and aromatic plants  
breeding

Cícero **Dechamps** (UFPR-Brazil)

10:20-11:00 **L08** - *Stevia rebaudiana*: a genetic and genomic  
resource for breeding and secondary metabolism

Jim **Brandle** (AgriFood-Canada)

11:00 – 11:20 Coffee

11:20 – 12:10 Conference

**L 09** - Functional genomics and biotechnology to  
study aroma formation in aromatic plants

Efraim **Lewinsohn**

(Ministry of Agriculture-Israel)

12:30 – 14:00 Lunch

13:30 – 14:00 Capoeira presentation



14:00-16:30 **Oral presentations:** 10 lectures of selected posters -15'

- O1 (ISMAP-0047)** Up to date results in developing poppy (*Papaver somniferum* L.) cultivars of diverse chemical character  
J. Bernáth and É. Németh (Hungary)
- O2 (ISMAP-0339)** Evaluation of morphological and agricultural characteristics and their phenotypic correlations in genotypes of a *Pfaffia glomerata* (Spreng.) Pedersen population.  
Ilio Montanari Jr.; M.B. Perecin (Brazil)
- O3 (ISMAP-0365)** Polymorphisms of *cis*-Sabinene Hydrate and *cis*-Sabinene Hydrate Acetate in a Species Hybrid between Marjoram and Oregano  
Johannes Novak, Chlodwig Franz (Austria)
- O4 (ISMAP-0143)** A Mobile Distillation Unit for Essential Oils in Brazil  
Luiz Grossman (Brazil)
- O5 (ISMAP-0110)** Determination of drying and desorption isotherms parameters in carqueja (*Baccharis genistelloides* L.).  
Silva, E., Park, K.J., Magalhães, P. M., Pozitano, M. (Brazil)
- O6 (ISMAP-0046)** Development, biomass production and quercetin-3-sulphate contents in plants of *Cuphea carthagenensis* (Jacq.) MacBride, submitted to different substrates, irrigation systems and leaf pruning intensities.  
Dezanet, P. B., Krepsky, S. M., Mathioni, M. R., Farias, R. O., Nodari (Brazil)
- O7 (ISMAP-0195)** Biomass production and semi-quantitative determination of 4-nerolidylcatechol during cultivation of *Pothomorphe peltata* (Piperaceae).  
Pena, E.A.; Chaves, F. C. M.; Pohlit, A. M., Pinto, A.C.S. (Brazil)
- O8 (ISMAP-0080)** Chemical variability in essential oil of *Hyptis marrubioides* Epl.  
C.B.A. \*Oliveira, M.R. Santos, J.F. Sales, F.G. Silva, S.C. Santos, P.H. Ferri, J.R. Paula, J.C. Seraphin, V.E. Nascimento, and J.E.B.P. Pinto (Brazil)
- O9 (ISMAP-0140)** Composition of the essential oils of *Artemisia incana* (L.) Druce, *Helichrysum ocephalum* Boiss. and *Centaurea ispahanica* Boiss. three Asteraceae herbs growing wild in Iran  
A. Rustaiyan, A. Firouznia, M.T. Akbari, S. Masoudi and M. Bigdeli (Iran)
- O10 (ISMAP-0136)** Antimicrobial testings and chiral phase GC analyses of essential oils and related key aroma compounds  
Leopold Jirovetz, Gerhard Buchbauer, Erich Schmidt, Zapriana Denkova, Albena Stoyanova and Ivan Murgov (Austria/Germany/Bulgaria)

16:30-17:00 Coffee

17:00-18:00 Conference:

**L10** - The raw material for Pharmaceutical Industry

Luiz **Pianowski** (Aché-Brazil)

18:00-19:30 **Poster session**

- **Plant Breeding**
- **Agrotechnology: Prod./Post Harvest**

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**Confraternization diner (Barbecue) – Musical presentation**

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**Wednesday, 7/07/2004**

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8:00 –11:00 Field journey to CPQBA Agricultural Station  
&

P. Melillo **de Magalhães** (CPQBA-Brazil)

11:00-17:00 Botanical journey through the Atlantic Rain Forest\*

Botanicals of IB-UNICAMP (Brazil)

\* Scientific botanical walk at *Serra do Japi*. We will be visiting MAP from the Atlantic Rain Forest, one of the most endangered forest in the world. Bring your camera, adequate shoes, etc..

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**Thursday, 8/07/2004**

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8:45-9:00 Laboral Gymnastics

9:00-11:00 Round Table: **Ethnobotany and Legal Aspects of MAP**

Chairwoman: Elaine **Elisabetsky**

(UFRGS-Brazil)

9:00 - 9:40 **L11** - Aspects of Traditional Medicine: Ethnobotany and  
Malaria

Merlin **Willcox** (RITAM-UK)

9:40-10:20 **L12** - Legal markets and traditional medicine knowledge  
in Brazil: Implications of language, laws and  
market ideology

Charles Roland **Clement** (INPA-Brazil)

10:20-11:00 **L13** - Ethnobotany and benefit sharing in the context  
of technological innovation

Elaine **Elisabetsky** (UFRGS-Brazil)

11:00 – 11:20 *Coffee*

11:20 – 12:30 Conference:

**L14** - Ecocrop and EcoPort, and their relationship to  
medicinal plants

Peter **Griffie** (FAO-Italy)

12:30 – 14:00 *Lunch*

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14:00-16:30 **Oral presentations:** 10 lectures of selected posters 15'

- O11 (ISMAP-0316)** Production of aerial and underground biomass of *Jacaranda decurrens* Cham. - Bignoniaceae, in different harvest times. C.P.V. Guerreiro, L.C. Ming, and J.A. Marchese (Brazil)
- O12 (ISMAP-0377)** The Use of Geographic Information Systems as a Decision Making Tool for the Domestication and Conservation of Medicinal Species H. G. Momm, G. L. Easson, D. Ferreira, R. M. Moraes (USA)
- O13 (ISMAP-0284)** Distribution of proazulenes and productivity in *Achillea millefolium* s. l. spontaneous populations J. Radusiene, O. Gudaityte (Lithuania)
- O14 (ISMAP-0050)** Phytochemical value of Hungarian *Achillea* populations E. Nemeth, B. Vadady, E. Hethelyi, K. Demeter (Hungary)
- O15 (ISMAP-0103)** Multivariate analysis in fifty cultivars / landraces of *Piper nigrum* L. occurring in Kerala, India P. J. Mathew, P.M. Mathew and Vijayaraghava Kumar (India)
- O16 (ISMAP-0041)** Comparative study of the essential oil from leaves and flower stems of *Espeletia schultzii* at different blooming stages and at three different altitudes from the Venezuelan Andes. Ibañez, J., Usubillaga, A (Venezuela)
- O17 (ISMAP-0248)** Medicinal plants used by population served by "Programa de Saúde da Família", Governador Valadares, MG, Brazil B. G. Brasileiro; D. S. Matos; V. R. Pizziolo; Rezende, A.M.G. (Brazil)
- O18 (ISMAP-0265)** Ethnobotanical research at saúpe – ba: increasing value to traditional knowledge and contributing for regional sustainability Nina C.B. Silva; Martha Rocha; Rita M. Neri; Mara Zélia de Almeida. (Brazil)
- O19 (ISMAP-0129)** Influence of the different agricultural crops upon the antioxidant capacity of the broccoli and kale extracts using the DPPH\* free radical method K.A. Arbos, L. Borges, A.M. Weffort-Santos, C.A.M. Santos. (Brazil)
- O20 (ISMAP-0169)** Brazilian commercial exchange of spices Osvaldo Arizio y Ana Curioni (Argentina)

16:30-17:00 Coffee

16:40-17:00 Closing Musical Presentation

17:00-18:00 Conference:

**L15** *BIOProspectTA* – São Paulo State bioprospecting network associated with the BIOTA/FAPESP Program

Carlos Joly (UNICAMP-Brazil)

18:00 – 19:30 Poster Session

- Ethnobotany
- Chemistry & Quality Control
- Biological Activities

19:30 Closing session/ The next ISMAP.

Organisation Committee

### Notice !

**You can take part in Sanrisil social program by donating your backpack to the poor children in the Northern of Brazil, at the end of this Event.**



## Field Trip

### Atlantic Forest - "Serra do Japi"

"The so called "Atlantic Forest" of Brazil is a forest formation that originally stretched from thousand kilometers along the Atlantic coast, from the most Northeastern to the most Southern States of the country. The forest even extends inland to Paraguay and Northeastern Argentina. During the centuries of land occupation, most of its original vegetation was devastated. In spite of that, it still holds a large number of species, which makes it one of the biodiversity hotspots of the planet. The area is exceptionally diverse, with high numbers of rare and endemic species.

Today only about 7% (approximately 92,000 km<sup>2</sup>) of its original extent (approximately 1.2 million km<sup>2</sup>) still exists, and much of this acreage is not contiguous. Still, it currently makes up 13% of Brazil's territory and remains the third largest major vegetation formation in the country (after Amazonian and the Cerrado). Even in its shrunken state, the Atlantic Forest remains a place of great biodiversity. More than 6,000 of the estimated 20,000 plant species are thought to be endemic, 72 of 620 birds, 60 of 2000 reptiles, and 253 of 280 amphibians, as are 160 of 261 mammals (by comparison, the Amazon has 353 mammal species in five times the area of the Atlantic Forest).

There are only a few remaining areas of Atlantic Forest under protection, and "Serra do Japi" is one of them. It represents an important portion of the inland forest remnants of the São Paulo State.

Its predominant forest formation is called "semideciduous", which means that is not an "evergreen" forest, but it holds a number of plant species that drops at least part of its leaves in the winter season due to temperature and water restrictions. In Serra do Japi reserve, we can also see that it shows semi-arid species that are remains of the vegetation that predominated in the region millions of years before the present. Therefore, even Cactaceae species can be found on some spots as an evidence of past climate changes. Besides that, there are some altitude plants composing a true vegetal mosaic.

The botanical journey will consist of a visit to one of the two field tracks that are maintained at Serra do Japi reservation. The journey will last only a half day period and the field tracks are of two levels of difficulty in terms of walking distance and roughness. More details will be given during the event.



# Conferences



## **L01 - Projects on medicinal and aromatic plants in Brazil.**

Benjamin Gilbert, Far-Manguinhos – FIOCRUZ, Oswaldo Cruz Foundation, Rio de Janeiro.

In considering medicinal plant developments in Brazil one must distinguish industrial from small scale local production, finished pharmacy shelf products from state, municipal and privately run health care facilities, and phytomedicines directed at discomfort and superficial infirmities from those destined to the treatment of life-threatening disease.

Basically medicinal plants may be exotic adapted species mostly from the northern hemisphere. When these, as is usually the case, are approved for use in North American and European countries they can be registered in Brazil with relative facility. They therefore are the preferred items of the medicinal plant pharmaceutical industry. Native species, even if of long traditional use and generally regarded as efficacious, normally need to go through toxicological and clinical trials whose cost exceeds the expected rentability of the products in commerce. Manufacturers have therefore mostly limited registration to a few products that were successful market items before the present legislation came into force. Only a very few native species are thus available to the public as ethical medicines. Side by side with the industrialised phytomedicines we have the dispensing pharmacies. These do not have to register their products, which are made up for sale against doctor's prescription, sometimes only after the prescription has been handed in, and in no case may be stocked for more than about two weeks, that is, they are not made to have a shelf life and are for immediate use. Legislation here is designed to assure hygienic production facilities and care in quality control which has to be made by a certified external laboratory.

A third area of activity concerns the "green pharmacies", which are medicinal plant gardens associated to a laboratory that rarely conforms strictly with the legislation for dispensing pharmacies. Such green pharmacies serve lower income groups in towns and often are the only wide spectrum source of medicines for the rural population. Often local government runs or supports such initiatives and, in some states, like Ceará, the system is state recognised. In Goiânia, Goiás, a State hospital has been set up with an adjacent medicinal plant garden. These services are clearly supplemented by individual domestic plantations of medicinal plants for home use and some States, such as Amapá, distribute plants to the public for this purpose. Legislation has not yet been adapted to these forms of phytotherapy.

All of the previous categories deal mainly with common non-lethal diseases and conditions. However, traditional medicine as well as much scientific work has shown that life threatening infirmities like cancer, malaria, leishmaniasis and tuberculosis may also be susceptible to treatment by medicinal plants. Future developments should see these plants brought into use providing, as they did in some cases for the Indians and rural 'caboclos' in the past, alleviation and sometimes radical cure to patients in remote locations where modern medicine is not available.

Finally one should not lose sight of the bulk production of medicinal plants or their extracts or isolated substances for world commerce. These products do not have to pass through the same legislative barriers as the phytomedicines because they will not reach the public as such, but only after manipulation at a pharmaceutical factory. It is this final manufacturer who must obtain the license for his formulation. What is most important here is the care that must be taken not to collect the source plant to the verge of extinction and that is where agricultural advances and large scale extraction plant technology have revolutionised the trade.

Aromatic plants form a quite distinct legislative area although technically there is much in common with medicinal plant production. One of the common aspects is the high preference of the market for exotic plant oils and flavours. Although Brazil is said to possess a fifth of the world's flora, only a few native plants figure in international commerce and these entered centuries ago when Portugal and Spain were among the world's chief sources and brought their South American and Caribbean colonial plants to Europe. Work by several public institutions now justifies an effort to introduce new native aromatic plants into world commerce.



**L02 - Research, discovery and application of Natural Products for health and medicine.**

J.E. Simon, M. Wang, R. Juliani, H. Moharram and M. Rafi.

New Use Agriculture and Natural Plant Products Program, Rutgers University, New Brunswick, USA

This presentation will discuss the challenges and opportunities in integrating the use of plants as foods and spices with that of using them as a vehicle to improve human health. A model integrating both concepts resulting in the discovery of new bioactive compounds, and new application of known bioactive compounds will be illustrated. Using a scientific-driven and market-driven model, we will explore several case studies that facilitated the commercial introduction of new medicinal and aromatic plants from local use into regional and international commerce with a particular focus on botanical standardization and quality control. The successful introduction of botanicals, teas, culinary herbs, spices, or medicinals can each be enhanced with a multidisciplinary team of strategic partners that provide a complimentary set of skills and expertise that champion the new product and provide the needed technical, scientific and marketing expertise.



### L03 - Cultivation and post harvest process for aromatic and medicinal plants in Argentina.

Curioni, Ana Ofelia. Universidad Nacional de Luján:  
E-mail: aroma@infovia.com.ar/mora@mail.unlu.edu.ar

The international trade of aromatic products (spices, medicinal and seasoning herbs and essential oils) has increased since the 70s, at a rate that duplicates or triplicates the world population growth. At present, the international trade of spices is valued about 650.000ton per year, which means a value close to 2.500 million current dollars. That value depends on pepper's price, which is the spices that dominates the world market. Present valuations show the world trade of spices about 300.000 ton per year, which means about 1500 million dollars; during last decades this trade shows an increasing trend in value and in volume, with an average growth rate that overcomes the growth rate of the world population. In spite of the fact that Argentine presents optimal agronomical, edaphic and weather conditions to produce "spices seeds" and "seasoning herbs" of a temperate climate, only two spices have production areas and yields to allow the self-supply and exports, chamomile (Argentina is the main world producer) and coriander. The remainder spices production does not supply the domestic market. The objective of this work is to describe technological processes of cultivation, harvest and post-harvest of the main spices, seasoning and medicinal herbs produced in Argentina. These produced spices could be sorted out as extensive and intensive ones. The former present large and profitable production areas over 30-35 ha and the machinery used is the same used to crop the traditional crops, the economic yield is grain, some crops have annual cycle (coriander, anise, cumin, milk thistle) or perennial cycle as fennel (Table 1).

Table 1: Extensive crops: date of sowing and harvest, distribution spatial and yield.

Especie	Date of sowing	Date of harvest	Density (kg ha <sup>-1</sup> )	Distance of line (cm)	Yield (kg ha <sup>-1</sup> )
Coriander	15/5 al 30/7	25/11 al 20/12	15 y 25	15/17,5/20/35	1500-2200
Fennel	1/3 al 15/5	25/12 al 20/1	5 a 10	52/70	700-900 (1er año) 1000-1300 (2do año)
Anise	15/5 al 30/7	1/12 al 20/12	5 a 10	15/35 o 70	700 a 1000
Cumin	15/5 al 30/7	1/12 al 20/12	5 a 10	15/35 o 70	700 a 1000
M. thistle	1/3 al 15/5	20/11 al 15/12	8 a 12	52/70	600 a 1000

Intensive crops are capital and labor intensive and can be annual or perennial ones. Perennial spices as oregano, thyme, tarragon, mint, etc. are herbaceous and lavender, rosemary, cedron, etc. are woody plants; all of them are propagated vegetatively by cutting, seedling and rhizome. Among intensive annual spices chamomile, basil and parsley are propagated by seed, the sowing is made with the same machinery used for extensive crops, however harvest and post harvest are similar to intensive spices. Leaves and/or flowers represent economic yield of these intensive crops, its culture needs specialized equipment to do the harvest and post harvest treatment (dryer, thresher, classifier). Drying process produces physical and chemical changes such as: losses of volatile products (essential oils), rehydration capacity, discoloration, etc., these changes are according to drying conditions (speed, temperature, relative humidity, presence or lack of solar radiation, etc.) and to the characteristics of the products to be dried. There are different drying techniques such as natural, artificial and mix ones. The former could be sorted out as follows: outdoors with solar radiation, solar dryers or in the shade. Artificial techniques could be static or continuous, and "tower type" (source of heat is placed in a low side) or "type tunnel" where source of heat is placed at the end or laterally. The threshed, cleaned and classified product is pack up into polypropylene bags, or thick paper bags with polyethylene lining, or into boxes and bundles both covered with polypropylene. To be considered a high quality vegetal seasoning, means that exist a completely match between the spices named in the label and physical, chemical, microbiological, visual, olfactory and gustatory characteristics. The quality of the product is guaranteed with the quality of the process. Finding out critic control points is possible to avoid unknown material and polluting material and having appropriate records it is possible to build an agronomical routine in order to outlined the manage of the commercial raw material.



#### L04 - Alternative methods for plant protection against diseases.

Marcelo A. B. Morandi  
Researcher on Plant Pathology  
Embrapa Environment

The welfare of plants is of particular interest to those most directly concerned with the growth of plants and the manufacture and distribution of plant products. Most importantly, however, the welfare of plants should be of concern to every one of us as growers of plants for food or pleasure, as individuals concerned with the beauty and safety of our natural environment and, particularly, as consumers of plants and of the endless series of products derived from plants.

The growth and yield of plants depend on the availability of nutrients and water in the soil where they grow and on the maintenance within certain ranges of such environmental factors as light, temperature, and moisture. Plant growth and yield depend also on protecting the plants from parasites. Plant pathogens, unfavorable weather, weeds, and insects pests are the most common causes of reduction or destruction of plant growth and production.

A plant is healthy or normal when it can carry out its physiological functions to the best of its genetic potential. Whenever plants are disturbed by pathogens or by certain environmental conditions and one or more of these functions are interfered with beyond a certain deviation from the normal, then the plants become diseased.

A plant disease results from the interaction of a host and a pathogen as influenced by a favorable environment – the disease triangle. This figure illustrates one of the paradigms in plant pathology; that is, the existence of a disease caused by a biotic agent absolutely requires the interaction of a susceptible host, a virulent pathogen, and an environment favorable for disease development. Conversely, plant disease is prevented upon elimination of any one of these three causal components. The general principles of plant disease control (exclusion, eradication, protection, immunization, therapy, regulation and escape) are essentially based on the knowledge of how a disease occurs and change over time and space, i.e. the epidemiological approach.

The principle of **exclusion** includes all practices that prevent the entrance of a pathogen in an area not yet infested; the **eradication** includes the elimination of a pathogen from a previous infested area; the **protection** principle consists on the deposition of a protecting barrier (usually chemical) covering the susceptible surface of the plant before the arriving of the pathogen inoculum; the **immunization** principle is based on the selection/development of plants resistant or immune to the pathogen; the **therapy** aim at the stop of the progress of a already established infection; the **regulation** principle comprise the practices that modify the environmental condition and turn it unfavorable for the disease development; and, the **escape** principle can be defined as the prevention of the occurrence of a disease by the selection of season or area of planting when or where the pathogen is inefficient, rare or absent.

Although these principles are known since a long time, the plant protection in modern agriculture is largely dependent on the use of pesticides. This approach is extremely attractive, since it is simple, can be programmed (calendar, e.g.) and do not require profound knowledge of the basic process of the agroecosystem and the pathosystem for its application. In the other hand, the intensive use of pesticides in the agriculture is not sustainable over a long time and carries several environmental and public health concerns. On this context, the searching for environmental friendly, healthy and economically acceptable strategies and methods for reduce or eliminate the pesticide dependency on plant protection became a challenger task.

In a strict sense, "alternative methods" for plant disease control, in counterpoint of the use of synthetic pesticides, include the use of plant extracts, aqueous extracts of organic matter, products resulting from the fermentation of organic materials ("biofertilizer"), salts and diluted acids (e.g. bicarbonates, acetic acid), organic substances (e.g. milk) and biological control agents. However, in order to achieve the above stated "epidemiological approach", this concept should be enlarged to include other practices such as physical and cultural methods that contribute to the maintenance of the plant health.

Several alternative methods of diseases control feasible to be used in medicinal and aromatic plants cultivation are available and will be discussed.



### **Control of damping-off and soilborne pathogens**

Damping-off and root rot diseases occurs worldwide and affect seeds, seedlings, and roots of almost every cultivated plant. Losses vary considerably with soil moisture, temperature and other factors. In many instances, poor germination of seeds or poor emergence of seedlings is the result of damping-off infections in the pre-emergence stage. Older plants infect with root rot pathogens may have their development retarded considerably, and their yields may be reduced drastically. Several fungal pathogens are involved in damping-off and root rot diseases, including *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani*, *Fusarium* spp., and others.

The alternative methods for control of these diseases include:

#### Soil and substrate disinfestation by solar energy

##### **Soil solarization**

The method consists in covering the humid soil with a transparent plastic film, in pre-planting, over a period of intense solar radiation (variable for each region) for at least 30 days. The control of the pathogens propagules, weeds seeds and nematodes are resulting of several mechanisms, including physical stress and microbiological activation in soil.

##### **Solar collector**

A solar collector for substrate disinfestation was developed by the Embrapa Environment and the "Instituto Agronômico de Campinas". The equipment substitutes satisfactorily the methyl bromide use for substrate disinfestation. The solar collector is simple, easy to use and cheap. Complete information on how to construct and use the solar collector can be find at [http://www.cnpma.embrapa.br/download/circular\\_4.pdf](http://www.cnpma.embrapa.br/download/circular_4.pdf).

#### Fermented organic materials ("biofertilizer", composte, aqueous extracts)

The aerobic or anaerobic fermentation of organic matter (such as cattle manure, cereals bran, etc.) produce a rich biofertilizer, that can be used for nutritional and/or disease control purposes. Besides the inorganic nutrients, the biofertilizer contains a huge microbial community (especially bacteria and actinomycetes) that produces antibiotics, hormones and other antifungal compounds. The biofertilizer can be added to the soil or substrate by irrigation at pre- or post-planting stages. Considering the possibility of the presence of fecal coliforms in the biofertilizer made with animal excrements, it should not be used in post-planting of direct consumption vegetables.

#### Biological control agents

Fungal antagonists, especially those of the genera *Trichoderma* and *Gliocladium*, can be used to reduce the soilborne pathogens population. These antagonists control the pathogens by competition for space and nutrients, direct parasitism and antibiosis. Better results are achieved when the use of the antagonists is associated with other methods, for example, soil disinfestation followed by recolonization by the antagonists.

### **Control of the powdery mildews (*Oidium*) diseases**

Powdery mildews are probably the most common, conspicuous, widespread, and easily recognizable plant diseases. They affect all kinds of plants and are characterized by the appearance of spots or patches of a white to grayish, powdery, mildewy growth on young plant tissues, or of entire leaves and other organs being completely covered by the white powdery mildew. The symptoms are most commonly observed on the upper side of the leaves. The powdery mildews seldom kill their hosts but utilize their nutrients, reduce photosynthesis, increase respiration and transpiration, impair growth, and reduce yields, sometimes by as much as 20 to 40 percent.

The alternative methods for control of these diseases include:

#### Crude milk

The crude milk sprayed weekly at 10 to 20 % (milk:water, v/v) can control efficiently the powdery mildew on several crops. This mode of action include the formation of a biological film on the leaf surface and the inhibitory effects of the salts present in the milk. Complete information on how to use crude milk to control oidium diseases can be find at [http://www.cnpma.embrapa.br/download/comunicado\\_14.pdf](http://www.cnpma.embrapa.br/download/comunicado_14.pdf)

#### Salts

As stated above, salts have inhibitory effects on the powdery mildew development. Thus, the pulverization of diluted salts, such as sodium bicarbonate, monossodical glutamate and others can exert control of the disease. Caution should be taken with the possibility of phytotoxicity of some salts to more sensitive plants.



#### "Biofertilizers"

The liquid phase of the biofertilizer diluted to 10 to 20% (biofertilizer:water, v/v) and sprayed weekly is efficient in control the powdery mildew. Caution should be taken when using biofertilizer on direct consumption vegetables parts, to avoid biological contaminations.

#### **Control of general foliar pathogens**

The foliar spots and blights caused mostly by fungi affect numerous hosts and are quite diverse. The diseases cycles and controls of these diseases are quite similar, however. Nevertheless, considerable variability may exist among diseases on different hosts or when the diseases develop under different environmental conditions. Most foliar fungal pathogens reproduce by means of conidia (asexual spore), and, in some instances, by ascospores (sexual spore). On the infected areas, numerous spores are produced that spread to other plants by wind, wind-blown rain, water, and insects and cause more infections. In most cases these fungi overwinter and reproduce in fallen leaves or other plant debris. For some diseases, use of disease-free seed or removal and destruction of contaminated debris or both may be the most important control method.

The alternative methods for control of these diseases include:

##### Cultural practices and regulation of irrigation

Most of the foliar pathogens require high relative humidity and free water to cause infection. Thus, the irrigation, especially in greenhouse seedlings should be regulated to avoid excess of humidity for long periods of time. A nutritional balance, avoiding excess of nitrogen is another important measure to not predispose the plants to pathogen attack. As several pathogens can survive and multiply on plant debris, the sanitation practices (removal of dead tissues and plants, disinfestation of used pots and benches, etc.) should be taken regularly.

##### Biological control agents

Some biological control agents (including bacteria and fungus) are reported to be effective on the control of specific foliar diseases, especially in nursery and greenhouse cultivations. Unfortunately, there are only few commercial products available. Among them, includes products based on *Trichoderma* spp. and *Clonostachys rosea* for control of necrotrophic pathogens (such as *Botrytis cinerea*); *Ampelomyces quisqualis* for control of oidium diseases; *Bacillus subtilis* for control of some biotrophic pathogens (such as rusts and mildews).

##### "Biofertilizers"

In the same way as for the oidium diseases, the liquid phase of the biofertilizer diluted to 10 to 20% (biofertilizer:water, v/v) can be used to control general foliar pathogens. The efficacy of the treatment, however, varies with the pathosystem.

##### Plant extracts

Plant extracts are reported to be effective against some plant pathogens. Among them, there are several medicinal and aromatic plants extracts traditionally used by organic farmers. Several receipts can be found at the Web sites of organic agriculture.

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**L05 - Adaptation of medicinal and aromatic plants to contemporary requirements by breeding: aims, methods, and trends.**

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Breeding gives the opportunity to adapt crops to the requirements of seed companies, farmers, the processing industry, consumers and to general claims of the society. Cultivars complying with the required characteristics are an indispensable prerequisite for the production of high quality products in a sustainable and profitable way. Breeding aims at the change of the average trait expression of a genotype towards the desired level and at the reduction of its modification depending on environmental factors. These statements are valid for main agricultural crops in general but also in particular for medicinal and aromatic plants (MAP).

Breeders are confronted with some peculiarities of MAP: only a few results of breeding research - e.g. on genetics and on breeding methods - are available. MAP comprise a particularly great number of species, there are often different breeding aims in one and the same species depending on the field of usage, the costs of the analysis of important constituents are particularly high, only limited capacities for breeding research and breeding are reserved for these minor crops, refinancing of the breeders' expenses is insufficient due to the small saleable seed amount. On the other hand, there is also an advantage: the breeder can exploit high natural variability because breeding of MAP is at the beginning only. In view of these peculiarities and to ensure an adequate cost-value ratio, breeders have to plan very carefully which crops, breeding aims and breeding methods are to be chosen.

MAP breeding puts emphasis mainly on the following aims: high quality by ensuring the required composition and content of quality determining constituents, absence of harmful substances, and homogeneity of the herbal crude drug; sustainability by selection of appropriate genotypes for domestication from species endangered by depletion, low need of agrochemicals by breeding cultivars with resistance to pests and diseases and low nutritional requirements; profitability of the production by high yield, adaption to the climate of the cultivation region, technological suitability for the production processes in agriculture and in the processing industry, and good natural suitability for the protection of plant breeders' rights.

Adequate breeding methods must be chosen with respect to the biological peculiarities of a species. The evaluation of large collections of accessions of a species with subsequent selection of the desired genotypes is the main breeding method yet due to the prevailing high natural variability of these minor crops. New variability is being generated primarily by crossing and mutagenesis. The development of hybrid varieties and the exploitation of apomixis is at the beginning only. Biotechnological methods can be used only if adequate financial funds are available. Nevertheless, the clonal production of high performance elite plants in vitro and the use of molecular markers for the determination of the degree of relationship are being used increasingly. Some research teams are working to identify genes responsible for interesting characteristics and to establish gene transfer methods. But, unfortunately the European customers representing the biggest share of the world herb market disapprove genetically modified plants. Great progress has been achieved in the field of special trait assessment methods for breeding, e.g. the use of the near infrared spectroscopy that allows the non destructive analysis of important constituents of great sample numbers in a short time.

The rising number of registered MAP cultivars is clear evidence for the increasing activities in MAP breeding at present. The MAP branch has recognised that breeding of high performance cultivars is one of the most important measures to improve quality, sustainability, and profitability.



### **L06 - Medicinal and Aromatic Plants from Brazil: The Challenge of Genetic Resources Conservation.**

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Approximately two thirds of the biological diversity of the world is found in tropical zones, mainly in developing countries. Brazil is considered the country with the greatest biodiversity on the planet, with nearly 55,000 native species distributed over six major biomes: Amazon, Cerrado, Caatinga, Atlantic rainforest, Pantanal and the subtropical forest.

The National Center for Genetic Resources and Biotechnology - Cenargen, in collaboration with other research centers of Embrapa (Brazilian Agricultural Research Corporation), and with several universities, has been developing efforts to establish germplasm banks for medicinal and aromatic species. Several germplasm collections of medicinal and aromatic plants have been established in Brazil, and, around 80 species have been defined as priority for a germplasm conservation program. The main challenge now is to develop strategies for each of this species conservation, cultivation and sustainable management.

However, considering the Brazil biodiversity, very few germplasm collections have been established yet. Most consist of a large number of species, with few accessions within species. The concept of a germplasm bank with many species and accessions, including their different varieties, chemotypes and close related species is greatly needed. Few collections have started to treat their medicinal and aromatic plants as a germplasm bank. Rather the collections are more similar to the inclusion of accessions as used in botanical gardens. These collections are starting to more extensively gather additional plants to enrich their genetic variability of major species. A few species have been recognized as a priority for germplasm conservation, among them we can highlight Espinheira Santa (*Maytenus ilicifolia*), Ginseng brasileiro (*Pfaffia glomerata*), and Fava d'Anta (*Dimorphandra mollis*). It is an enormous task to elaborate a program for genetic resource conservation of medicinal and aromatic plants in Brazil, which require a multi-institutional and multi-disciplinary collaboration. This effort will have an important role in the future, providing genetic material for chemical characterization, breeding of new crops, improving our understanding of secondary metabolism, and in preserving an important part of our cultural and national heritage pathways.



**L07 - Regulation of essential oil biosynthesis and its implications in medicinal and aromatic plants breeding.**

Cicero Deschamps

Interest on studies of the regulation of essential oil biosynthesis has increased in the late years because of the economic importance of these compounds. Most of the information was initially obtained from investigations on monoterpenes accumulation in developing leaves of peppermint (*Mentha x piperita*). More recent research has been focused on basil (*Ocimum basilicum* L.) EMX-1 chemotype that has the volatile phenylpropane methylchavicol as the main constituent of the essential oil. Methylchavicol production has potential application as an antioxidant as well as a potential inhibitor of human carcinoma cell proliferation. Developmental studies showed that methylchavicol accumulation decreases overtime as leaves mature and it was significantly correlated to the activity of chavicol *O*-methyltransferase (CVOMT) and eugenol *O*-methyltransferase (EOMT), the enzymes that catalyze the final step in its biosynthesis. The CVOMT and EOMT transcript accumulation also follows the same pattern as its respective enzyme activity, indicating that methylchavicol biosynthesis is regulated at the transcriptional level. Furthermore, the CVOMT transcript levels are higher in peltate glands isolated from the youngest leaves compared to mature leaves. This provided for the first time evidence that CVOMT transcript level is developmentally regulated in basil glandular trichomes, and is not only a result of the trichomes density. Results from new investigations have also shown that several genes in the phenylpropanoid pathway are specifically expressed in the basil peltate glands and additional work has been focused on the isolation of specific promoters of glandular trichomes. It will assist in the evaluation of the roles of specific enzymes and regulatory factors involved in essential oil biosynthesis. The manipulation of phenylpropanoid pathway will be also possible, resulting in modified composition of essential oils. To implement this work, a transformation system has been developed for both *O. basilicum* and *O. citriodorum*.



**L08 - *Stevia rebaudiana*: a genetic and genomic resource for breeding and secondary metabolism.**

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*Stevia rebaudiana* Bertonii is a perennial herb native to Paraguay whose leaves accumulate high concentrations (up to 25 %) of a mixture of eight different glycosides derived from the tetracyclic diterpene steviol. In addition to the impressive ability to accumulate these compounds to such high concentrations their glycosylation pattern makes them taste intensely sweet. In fact, rebaudioside A, the second most abundant steviol glycoside in stevia leaves, is up to 300 times sweeter than sugar. Steviol is also structurally related to gibberellic acid. *Stevia* is able to separate primary from secondary metabolism, and GA and steviol use the same enzymes for cyclization and oxidation that lead from GGDP to kaurenoic acid. All of these factors combine to make *Stevia* an important model for the study of diterpene metabolism. We have taken a genetic approach and used it to understand how steviol glycosides are synthesized as well as to develop stevia cultivars with improved flavor. Our early work was focused on the establishment of breeding populations and the demonstration of genetic variability useful for cultivar development. To that end we found that half-sibs selected from a single land race cultivar had significant heritable genetic variation for that leaf yield, leaf:stem ratio and stevioside concentration. Furthermore, adapted lines were also present and we concluded that cultivar development for Southern Ontario was possible. We then developed a significant breeding effort in the mid-90's with a focus of leaf yield, glycoside yield and steviol glycoside profiles. The work resulted in the identification of three selections with novel glycoside compositions, one was composed of 93% stevioside (166-13), the second was 93% rebaudioside C (1306) and the third was 67% rebaudioside A (751). These were all single plant selections and, because of self-incompatibility, could only be reproduced vegetatively. At that point we focused on a single glycoside, rebaudioside A, the most sweet and least bitter of the steviol glycosides and the one that was most commercially desirable. We found that the presence of rebaudioside A was controlled by a single dominant gene, but after a certain base level concentration was quantitatively inherited. With the goal of creating a seed based cultivar and understanding the inheritance of rebaudioside A concentration we used recurrent selection to develop a synthetic cultivar, AC Blackbird, where rebaudioside A concentration was 83 % of the total. It was the first high rebaudioside A *Stevia* cultivar that could be reproduced with seed.

In a further effort to dissect steviol glycoside biosynthesis we applied the tools of molecular genetic and genomics. We first examined the two terpene cyclases involved in the synthesis of kaurene and found how *Stevia* may be able to separate the synthesis of steviol from that of gibberellic acid. Following that work we developed a library of expressed sequence tags (ESTs) and are using them to increase our understanding of steviol glycoside biosynthesis. 5548 ESTs from a *S. rebaudiana* leaf cDNA library were sequenced and candidate genes identified for 70% of the known steps in the steviol glycoside pathway. We used that EST resource to study of a diverse range of glucosylation reactions that occur with a single aglycone substrate. An electronic probe was constructed from a highly conserved region (PSPG box) from plant glycosyltransferases (UGT) along with key word searches to query the database and identify candidate UGT genes resident in our collection. Forty-three ESTs belonging to 17 clusters were found using this procedure. Full length cDNAs from 12 GTs were isolated and cloned into expression vectors and used to produce recombinant enzyme in *E. coli*. To screen candidates for *in vitro* glucosyltransferase activity an enzyme assay was conducted using quercetin, kampferol, steviol, steviolmonoside, steviolbioside and stevioside as acceptors, and <sup>14</sup>C-UDP-glucose as the donor. Thin layer chromatography was used to separate the products. Four of the recombinant enzymes produced labeled products that co-migrated with authentic standards. This was the first description of the steviol glycoside UGT genes.



## **L09 - Functional genomics and biotechnology to study aroma formation in aromatic plants.**

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Modern genomic and biotechnological methodologies have been applied to study the formation of aroma volatiles, insighting into the complexity of many of the biosynthetic pathways and their physiological and molecular regulation. The biotechnological potential in the utilization of the genes involved in fragrance formation either to produce expensive fragrances in transgenic microorganisms or plants, or to modify existing aromas and essential oil compositions of aromatic plants are beginning to be harnessed, and this has resulted in aromatic plants with altered essential oil compositions. Moreover, aromatic plants have been utilized as sources of genes coding for key enzymes in the formation of aroma chemicals. Many of such genes have been isolated using genomic methodologies, based on exploiting similarities between related genes at the DNA levels and their expression patterns, coupled to bioinformatic assignments to the putative roles of these genes in the formation of aroma chemicals. The final confirmation of the identity and function of a putative gene has been performed by its functional expression in heterologous or homologous systems utilizing gene silencing or overexpression methodologies. Although functional expression is often limiting in the characterization of many of the novel genes isolated, a few examples of such experimental successes will be reviewed. We have initiated genomic projects aimed at isolating and characterizing key genes involved in the formation of mono- and sesquiterpenes, as well as volatile ethers and esters that are prominent in the fragrance of roses, and components of the essential oil of sweet and lemon basil. These genes have been used through genetic engineering in attempts to improve the often lacking aroma characteristics of fruits, vegetables and flowers.



## **L10 - The raw material for Pharmaceutical Industry.**

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Regarding raw materials for phytomedicines use, Brazil is going through a moment which is unique. It owns the biggest biodiversity in the world as well as a market in expansion, which has been increasing well above average of the medicines in general. However, what we usually find in the market are phytomedicines procured with imported raw materials.

We also have in Brazil an empirical market in which the use of medicinal plants, as an alternative for health treatments, is quite common. What we need, and we are walking towards it, is to accomplish studies that show the safety and effectiveness of hundreds of potential species. The Universities and the pharmaceutical Industries have been walking side by side, in order to create a new cycle of economy, "The Cycle of Biodiversity".

The industry demands from its suppliers, raw materials with an uncontested quality standard. For that, the material suppliers look for an international standard to answer the internal and external demand.

The area of Botany and Agronomy is, basically, the responsible for the quality of these products, which should undergo a chemical standardization, by using active markers or the ones strictly close to the fraction which carries the active compound (s).

The supply of raw materials is, usually, garbled by a conjunction of facts such as: lack of organization in offering, unawareness of the demand, inappropriate cultivation, predatory collection, poor technology within the agriculture communities in producing and drying insumes, inadequate storage, among others.

The pharmaceutical industry searches for new alternatives for its line of products. However, there are some precedents so that it can be able to use this material, such as: quality in all the stages of the productive chain, botanical identification, plantation by using good agronomy techniques and/or exploitation within the criteria of supported development, drying and storage observing the good practices and the production of standardized extracts.

The suppliers, supported by research and development, will have to opt for standardization with differentiated markers. For instance, a specific chemical compound instead of groups of compounds, such as: total phenolics, total tanins, among others. Another important point in the choice of raw materials is its stability as well as its solvents in use and its methods of extraction.

Nowadays, the search for raw materials which had, throughout its chain, utilized organic techniques, is growing up. The plantation and the extraction with organic solvents are more and more common and tend to be consolidated in the pharmaceutical industry.

Hence, there is both the need for new products and the abundance of our biodiversity. To find a way in which these two realities walk together, is a task for the researchers of the various areas involved in the phytomedicines development.



## **L11 - Aspects of Traditional Medicine: Ethnobotany and Malaria.**

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### **Introduction**

Malaria causes at least 300 million episodes of illness every year, and kills over one million people. In certain parts of Africa, malaria mortality has increased 2-3 fold since the late 1980s, in tandem with the spread of chloroquine resistance. Today, *Plasmodium falciparum* causes more deaths than any other infectious agent in young African children; in Eastern and Southern Africa, it is now responsible for almost 40% of these deaths (WHO, 2003).

Malaria is not just a disease of poor countries; it is a disease of the poorest people in poor countries, which often strikes at the hardest times. Of the currently available antimalarials, only three cost US \$1 per treatment or less (White, 2003), which is the threshold for affordability set by WHO, and all of these are fast becoming useless because of drug resistance. Although other drugs exist, they are not as yet widely affordable or available. In these conditions, it is not surprising that under 20% of febrile episodes and deaths due to malaria come to the attention of any formal health system (Bremar, 2001). Many of these patients – up to 75% in some areas - use traditional herbal medicines to treat malaria (Willcox et al, 2004).

Western-trained doctors often dismiss traditional medicines as useless, and educate patients against their use. However, there is very little evidence to prove or disprove their safety and efficacy.

### **Objectives**

1. To present a review of ethnobotanical studies concerning antimalarial plants
2. To summarise proposed guidelines for studies on traditional herbal antimalarials
3. To present case studies of potentially useful antimalarial plants

### **Methodology**

1. An attempt was made to identify as many primary ethnobotanical studies as possible from malaria-endemic countries. MEDLINE, EMBASE, CAB, and SOCIOFILE databases were searched systematically using the terms "traditional medicine" and "malaria", and references to more studies were identified in these papers. Selected journals were searched by hand. Researchers and experts were also contacted to supply copies of unpublished work. A database is being constructed of all remedies and plant species reportedly used for the treatment and prophylaxis of malaria, fevers (including febrile convulsions) and splenomegaly. For each remedy, data recorded included the plant species and part(s) used, method of preparation, route of administration, dose, frequency, and duration, contraindications, and traditional use(s). Conservation information was found by consulting the 2002 IUCN Red Data book online (<http://www.redlist.org>).

Each species was assigned an "IVmal" (Importance Value for the treatment of malaria) according to how widely its use was reported. This scoring system was first developed for use at a local level by Leaman (1995), with values 1-4. We have extended this scoring system to apply at an international level by adding values 5-8 (see table 1).

2. An international network of researchers working on traditional herbal medicines for malaria (RITAM) was formed in 1999, and now has over 200 members from over 30 countries. Working groups were formed, and international meetings organised, to draft and refine guidelines for studies on traditional antimalarial plants.

3. Systematic literature reviews have been conducted on a small number of antimalarial plants with high importance values. Some of these findings will be summarised.

### **Results**

1. 94 original ethnobotanical publications have been included to date, concerning 33 tropical countries. Overall, 1277 species from 160 families have been reported for the treatment of malaria and/or fevers. Of these, five were endangered and 13 vulnerable. This data set is still incomplete, as there are many more studies which have yet to be included in the database.



Table 1: Number of plant species according to "IVmal", identified so far.

IVmal	No species	Definition
?	849	Insufficient data
1	95	reported once in a single ethnobotanical survey
2	30	reported twice in one community
3	6	reported at least three times in one community
4	42	reported in more than one community
5	91	reported in more than one survey, in the same country
6	106	reported in more than one country, in the same continent
7	47	reported in two continents
8	11	reported in three continents

2. Guidelines have been produced for the following types of study:

- Ethnobotanical studies
- Preclinical efficacy studies
- Safety studies
- Clinical case reports, cohort studies, and randomised controlled trials

These will be summarised in the presentation.

3. Systematic reviews have been prepared on the following traditional antimalarials, and some of the data will be summarised.

- Cinchona spp
- Artemisia annua
- Azadirachta indica
- Dichroa febrifuga
- Cryptolepis sanguinolenta
- Strychnos myrtoides
- "Malarial"
- "Ayush 64"

### Conclusions

Over 1200 species of plants are used traditionally for the treatment of malaria and fevers. Some of these are used independently in different tropical areas. There is some evidence from laboratory studies for their efficacy, but good quality clinical studies are needed before their use can be recommended. Meanwhile, conservation of endangered and vulnerable species is a priority.

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## **L12 - Legal markets and traditional medicinal knowledge in Brazil: Implications of language, laws and market ideology.**

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Any discussion of legal issues about traditional knowledge, especially the very visible medicinal plant component, must start with the Convention on Biological Diversity (CBD 1992), which attempted to stimulate conservation, sustainable use and equitable sharing of benefits derived from biodiversity and associated traditional knowledge in two ways of interest to us here. In the Preamble, the CBD affirms that States have sovereign rights over the biological resources within their frontiers. Before the CBD's ratification, these were considered common property and, in the case of genetic resources, a common heritage of humankind. The CBD essentially "enclosed" this common property and transformed it into national property, with the expectation that nations would assume the responsibility to conserve, use and share this resource, thus avoiding the "tragedy of the commons". This action only makes sense, however, within the dominant political-economic ideology of developed nations, while in the periphery of the developed world, where the majority of the remaining biodiversity, especially genetic resources and traditional knowledge, is located, this sense is less evident because the mechanisms to guarantee its function are absent or rudimentary. When mechanisms are absent, benefits tend to flow to the local and national elites, rather than being shared equitably.

In Article 2, the CBD defines "*Biological resources*" as including "genetic resources, organisms or parts thereof, populations, or any other biotic component of ecosystems with actual or potential use or value for humanity." It further defines "*Genetic resources*" as "genetic material of actual or potential value", where "*Genetic material*" is "any material of plant, animal, microbial or other origin containing functional units of heredity." It is curious that genetic resources have value but no use! These definitions are quite different from those previously used by the Food and Agriculture Organization and other institutions. The term "resource" implies both use and value, which makes up for the lack of use of genetic resources as defined. However, the term "potential" is much more problematic. Potential means to "have the capacity for existence *but not yet existing*." The use of this word essentially transforms all biodiversity into biological resources and then immediately into genetic resources, with the expectation that they would be conserved because of the use or value inherent in the word "resource", but neither use nor value exists for the majority, since the majority only has potential. This was an attempt to use law to create market value, hence guaranteeing use and conservation, at least in the minds of the authors of the CBD. When combined with the enclosure, followed by privatization, inherent in the Preamble, it is valid to doubt whether there will be equitable sharing of benefits.

Curiously, the CBD does not define traditional knowledge, although recognizing their great importance. Brazil's MP 2186 (see below) corrected this failing: "Associated traditional knowledge" is "individual or collective information or practice of the indigenous community or local community, with real or potential value, associated with genetic heritage". This definition does not explain where this traditional knowledge comes from, which is an integral part of deciding who should share any benefits derived from it, and also includes the problematic word "potential". One interesting definition of traditional knowledge is that it is "the intersection of two dimensions: presuppositions or unquestioned cultural truths; and the practices and experiences of living in a specific environment". While the 2<sup>nd</sup> dimension is easily defined geographically, the 1<sup>st</sup> is extremely problematic, since unquestioned cultural truths tend to have important time depths and wide geographical distributions. If we are to privatize traditional knowledge, even while attempting to share benefits, how widely must we spread the benefit net to capture all those who accept these unquestioned cultural truths? Unfortunately, neither the CBD nor the Brazilian MP 2186 offers assistance in answering this question.

In the Preamble, the CBD acknowledges "that substantial investments are required to conserve biological diversity", but says nothing about the substantial investments necessary to transform "potential" into use or value, certainly expecting that nations, businesses and entrepreneurs would take up this task. Again, this expectation might make sense in a developed nation, but in the periphery it is not clear that it makes sense and, in fact, experience has shown that investments are fewer than expected. A further problem is that an equitable sharing of benefits is rather unlikely when a resource has been privatized. This has always been especially true of the pharmaceutical industry, a major



market force and R&D practitioner, and a perceived threat to sovereignty over genetic resources and traditional knowledge in the periphery. This perception appears to be due to confusion between "potential" and real, and how "potential" can be transformed into use or value in a market.

The CBD appears to expect that the power of the market in our modern corporate capitalist economic system (or ideology?) will act to conserve, sustainably use and share benefits derived from biodiversity. Given 20<sup>th</sup> century experiences, this is a rather fantastic expectation. Biodiversity is important because of its variability in genes, species and ecosystems, but markets are not interested in variability, they demand uniformity, although they will accept numerous uniform products. The Rio+10 conference in Johannesburg, South Africa in 2002 showed the fragility of the CBD's reasoning and highlighted the continued loss of genetic resources, traditional knowledge and biodiversity in general. Nonetheless, the CBD's international legal framework was and is used to develop national legal frameworks in the periphery, many of which continue to enclose and open the way to privatization of these resources. In Brazil, the effort to internalize the CBD was initially led by Senator Marina Silva, of Acre, now Minister of the Environment, and was publically discussed for nearly 5 years. Unfortunately, the current legal framework was cobbled together (without the Senator's collaboration) in response to a multinational company's plans to obtain access and invest in Brazil's biodiversity, hence threatening to privatize a part of Brazil's sovereign genetic resources.

The Provisional Act 2186 (MP 2186-16, 2001; originally MP 2052, 2000) was written in response to a proposed contract between Novartis (now part of Syngenta) and BioAmazônia (a quasi-public NGO affiliated with the Ministry of the Environment) that was contested as being incompatible with the equitable-sharing-of-benefits philosophy of both the CBD and Senator Marina Silva's proposal for the Brazilian law (PL 305, 1995). The word "potential", combined with the power of Novartis' R&D investment capacity, created the perception that great wealth was being accessed for a pittance (US\$3 million for 3000 samples of microorganisms of unknown value or use). As a result, the MP 2186 focused on controlling access through a set of bureaucratic hurdles that only large companies, like Novartis, could handle. What an ironic result, although this is in line with expectations about enclosure and privatization in the periphery.

The MP 2186 created, and Decree 3945 (2001) defined and institutionalized, the Genetic Heritage Management Council (CGEN) that would regulate the hurdles, authorize access and over-see benefit sharing derived from Brazilian genetic resources and associated traditional knowledge. As initially defined, the Council was top-heavy with Ministerial representatives with little or no prior knowledge of the Council's subject matter. A few public R&D institutions were given seats also, but did not have enough weight to counterbalance that of the Ministries. Representatives of subsistence farmers and fishermen, indigenous groups, rubber tappers and other traditional peoples who actually use and conserve genetic resources and have associated traditional knowledge were not included in the Council until Senator Marina Silva became Minister (January 2003). The original composition of the Council, however, was in line with expectations about enclosure and privatization in the periphery. Additionally, "over-seeing benefit sharing" meant that benefits will flow to a federal government fund, which will then disperse benefits. In the periphery, bureaucratic costs of such a fund are sure to exceed benefits in the foreseeable future.

Federal and state governments, their agencies and institutions tend to be the major sources of investment in R&D in the periphery, especially with genetic resources and traditional knowledge. In Brazil, a small but growing number of companies are starting to invest, mostly based on information generated by public institutions. However, the hurdles created by the MP 2186 were too high and too numerous for these public R&D institutions to manage, even with the creation of institutional licenses to practice R&D with genetic resources and traditional knowledge, so public R&D is reputed to have slowed since the publication of the MP 2186, although there is no hard data to substantiate this appearance.

One of the many hurdles is "prior informed consent", since this requires scouting expeditions by a team of social and natural scientists, where the social scientists decode the natural scientists' plans for the local community to then decide about access to both genetic resources and traditional knowledge. Darrell Posey wrote that the negotiation of an agreed upon meaning for words and concepts between members of different groups within a society or between members of different societies (e.g., indigenous, traditional and modern) can be a long complex process. Honestly obtaining prior informed consent is thus a very difficult process. Scouting expeditions of this type are also incompatible with public funding of R&D in the periphery. They are compatible, however, with major private investment, opening the way for further privatization of national resources.



In response to constant pressure from the R&D community in Brazil, the government recently published Decree 4946 (2003). Although still based on the MP 2186, this decree was designed to lower the hurdles for Brazilian R&D institutions to access genetic resources and traditional knowledge. Whether CGEN and Ibama (Brazil's national environmental protection agency) will regulate this Decree expeditiously and uniformly across the country remains to be seen.

Many of the public R&D institutions claim that they want access to genetic resources and traditional knowledge for purely scientific reasons. Unfortunately, pure science related to resources is extremely rare in the modern world, so it is appropriate to question how the pure scientists will guarantee Brazilian sovereignty over these resources at the same time that they guarantee future equitable sharing of benefits for those who offer their traditional knowledge. After all, this is the same R&D community that is notoriously lax at protecting its own intellectual property rights. In a global scientific society, those who don't protect their rights lose them, either to the public domain or to competitors in the central economies. Either way, loss of rights over genetic resources and traditional knowledge forecloses the option of equitable sharing of benefits in the periphery.

On top of this, Brazilian investments in the rational use of its genetic resources and traditional knowledge are insufficient to take advantage of the numerous obvious opportunities available, especially the traditionally used medicinal plants. Fifteen thousand research groups have been identified by the Brazilian National Research Council (CNPq), of which 41% work with some aspect of biodiversity and its associated traditional knowledge. However, only 3.9% of these 15,000 work in Amazonia, the main focus of expectations about sustainably using biodiversity and sharing its benefits. The number that work directly on traditional medicinal knowledge is unknown, but knowledgeable sources suggest that it is much smaller than the number working in Amazonia. With this investment priority, biodiversity will continue to have "potential", but use and value will continue to "not yet exist".

Unless we clearly understand the implications of the words and concepts we use, laws will not be able to save biodiversity nor assure the equitable sharing of any benefits we hope to derive from it. As a consequence, the medicinal and aromatic plants that are the focus of this meeting will continue to be privatized for the benefit of the new owners of the intellectual property rights that flow from them.



### **L13 - Ethnobotany and benefit sharing in the context of technological innovation.**

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Intellectual capital is the critical resource in the current global economy. The capability of generate, have access to and rapidly organize the new knowledge, converting it into quality processes and products in the market is, today, the most important competitive advantage for industrial development. Brazil not only houses a monumental biodiversity, but also a diversity of ethnicities with different traditions and cultural heritage. Ethnicities are characterized by specific cultures, and cultures are prominently influent in systems of agriculture, medicine, hygiene, beauty and well being. Considering that various categories of economic activities (industry, commerce and service) incorporate biodiversity components in its business, not only biodiversity but also sociodiversity is of interest for technological innovation. Traditional communities live in close contact with the environment, make daily use of specific species for specific purposes, with specific collecting, processing and modes of use. The species are natural, but the products associated with these species are a product of human knowledge. The Brazilian sociodiversity should be looked upon as an advantage in global economy given that it stores a vast knowledge base that can be developed from the R&D perspective. Bioprospecting activities are no longer limited to the pharmaceutical industry, and now includes industrial activities as diverse as biological control, bioremediation, construction engineering, environmental monitoring, mining and industrial materials, where a variety of species coming from an array of habitats provide a service, a product or a model or inspiration for a new product. Even if historically dominated by companies based on the first world, Table 1 illustrates such activities in developing countries. Interestingly, in every case traditional knowledge is valued, and issues of sustainability (certification) and benefit sharing are contemplated. Despite its recognized richness in biodiversity and a well established scientific community, the use on biodiversity by Brazilian R&D is nearly incipient: according to Lucia R. Fernandes (National Institute of Technology, RJ) among the 1.119 plant species object of patents in the major international patent offices (USAPO, JAPO, EPO and WIPO), only 0,48% of the associated 418 companies are Brazilian. That is in contrast, for example, with the 19 patents required by Shisedo on Brazilian species only ! This narrow utilization of our own resources is still more striking if one compares the success rate of drug research programs collecting at random or based on traditional knowledge, as shown at Table 2. With anticancer candidates, the percentage of active genus/species cited in ethnomedical literature is consistently twice as much as for at random screening (Spuijt e Perdue, 1976). Regarding antivirals, plant collections associated with traditional use resulted in 5 times more hits in comparison with at random samples (Vlietinck, e Van Den Berghe, 1991). The data from Shaman Pharmaceuticals, using ethnopharmacology as the central axis of its DD program, corroborate the advantage observed in academic studies within the industrial context (Carlson et al, 1997; Oubré et al, 1997).

New laws that govern research with biodiversity and bioprospecting are articulated in three primary sources: international treaties (eg, Biodiversity Convention), national laws (in Brazil, Lei de Acesso a Recursos Genéticos e Conhecimentos Tradicionais) and self-regulation ruled by professional associations (ethics and conduct codes). In all these spheres the legitimacy of benefit sharing among players is recognized, in general with provisions for immediate, medium and long term benefit sharing. Benefit sharing is not necessarily monetary, and aims to adequate the community to a long term commercial relationship, if of mutual interest. This presentation discusses the value of traditional knowledge in R&D programs and the diverse forms of benefit sharing. The promotion of bioprospecting, economic development and conservation of biodiversity requires the development of new and creative legal frames and economic initiatives that value the sociobiodiversity in its many dimensions



Tabela 1. Examples of industrial activities associated with biodiversity.

Company's name	Country	Year*	Number of Products	Number of plant species	Sales 2002 (US\$ 1M)	Ethnomedical leads	Benefit Sharing Policy	Plant Certification#
Axxon Biopharm Inc.	USA/Nigeria	1999	12	10	Not available	✓	✓	✓
Centroflora	Brazil	1998 <sup>a</sup>	450	21 <sup>b</sup>	21.5	✓	✓	✓
Flora Medicinal	Brazil	1912	45 <sup>c</sup>	69	1.1	✓	✓	✓
Phytonova Ltd.	South Africa	1999	04	03	0.2	✓	✓	✓

\* Year when company became operational, or <sup>a</sup> when the "biodiversity" line started.

# Certification refers to either the accepted NTFP certification of sustainably collected or the companies individual strategies to ensure and stimulate that the species acquired by the company are properly planted.

<sup>a</sup>The company was founded in 1957; biodiversity police implemented in 1998; <sup>b</sup>top selling species (80% of sales).

<sup>c</sup>30 herbal drugs, 8 food supplements, 7 phytocosmetics

Tabela 2. Hit rates in ethnomedical and at random collections in diverse drug development programs.

Institution	Country	Program	Ethnopharmacology oriented Plant Collection	Hits %	At random Plant Collection	Hits %
NCI	USA	Anticancer	Arrow poison	52.2		10.4
			Fish poison	38.6		10.4
			Nematodes	29.3		10.4
NCI	USA	Antiviral		15.0		1.6
Antwerp Univ	Bélgica	Antiviral		25.0		5.0
Shaman	USA	Antiviral	Citomegalovirus	8.2	Herpes	0.013
			Influenza	1.6		
Shaman	USA	Diabetes		39.0* 57.0**		
UFMG	Brasil	Malaria		18.0		0.07
Walter Reed	USA	Malaria		70.0		

\*in vitro; \*\* in vivo



#### **L14 - Ecocrop and EcoPort, and their relationship to medicinal plants.**

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Ecocrop - the FAO crop environmental requirements database can be applied to four principal tasks. It can: identify plants for a specified environment, growth habit, defined use e.g. medicinal or aromatic and identify plants by a defined description. It is available on CD-ROM and on the Internet at URL: <http://ecocrop.fao.org/>.

Very little basic information is supplied on the species in Ecocrop. However, once a plant is chosen - a direct link exists to EcoPort. (<http://www.ecoport.org>).

FAO formed a consortium with the University of Florida (UF) and the National Museum of Natural History of the Smithsonian Institution (SI) in the USA, to build EcoPort: a service with ecology as cause and uses the pooled information power and institutional perspectives and mandates of FAO, UF and SI as a foundation to exploit and deliver the benefits of the Internet. At present 180 sponsors and over 500 editors contribute to on-line information.

EcoPort went public on 1 January 2000 and in June 2004, 370,000 entity records were established, including 88,000 plants. There are over 553,000 references, 120 slide shows, 55,000 glossary terms, 37,000 pictures, 440 methodologies, 80 taxonomic keys, 1560 Interactive tables, etc.

We now have records on over 500 plants which (also) have medicinal properties plus descriptions, cultivation, constituents, pharmacology etc and pictures. This PowerPoint presentation shows the power of the 'knowledge sponge'.

Each contributor receives a username and password that enables us to write information into the shared database, much as a group of authors write chapters for a book, except that the 'book' we are writing is a public database on the Internet. This process uses methods and tools invented at FAO which allow editors (not only webmasters) to write Hypertext directly.

Data quality is maintained by the same process of peer review that has kept scientific publishing going ever since it started by automatic email notification to supervisors. Each contributor's shared information is displayed under a banner and logo that reflects ownership and responsibility, and we clearly demonstrated that sharing and generosity does not threaten identity.

- As we all put sharing ahead of copyright and many other territorial aspects that unnecessarily increase the transaction costs associated with using data, our pooled knowledge grew very rapidly.

Many of these medicinal plant records need editors. Please contact [peter.griffiee@fao.org](mailto:peter.griffiee@fao.org) if interested.  
**You are invited to join the EcoPort community.**



**L15 - BIOprospecTA - São Paulo State bioprospecting network associated with the BIOTA/FAPESP Programme.**

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Undoubtedly, biological diversity possesses a priceless intrinsic value. Each specie is the unique result of the evolutionary process, as is the relationship among species and of these with the environment. Biological diversity also possesses value for the services that it renders (for instance, maintenance of the hydrological cycle, pollinator's populations, etc.) and for its scientific, educational, cultural, recreational and aesthetic meaning.

But it is important to keep in mind that biological diversity has also an extremely high economic value. The growing world market of biotech products is estimated in approximately US\$ 400 billion. In spite of occupying the top of the list of biodiversity rich countries, with approximately 20% of all species of the Earth (7.000 species of vertebrates, 55.000 species of higher plants, 2.000.000 of species of invertebrate, and an unknown amount of species of microorganisms), the Brazilian share in this market is still very low, something around US\$ 500 million.

The economic potential of the sustainable use of the Brazilian biodiversity is incalculable! How many times have we heard this statement? However, it seems that this "potential" never becomes effective. WHY?

In spite of highly reliable, the information available on our biological patrimony is generally fragmented, disperse, of difficult access and, consequently, underused. Besides, as a consequence of the lack of an updated cartographic base, the location of the points of collection of basic information, as the inventories of species, is usually inaccurate.

The great challenge is to systematise the collection, the storage and the integration of this information, using tools such as remote sensing, geoprocessing and bioinformatics.

Created officially in March, 1999, the Research Program on Conservation and Sustainable Use of the Biodiversity of the State of São Paulo, named BIOTA/FAPESP – The Virtual Biodiversity Institute ([www.biota.org.br](http://www.biota.org.br)) is the result of a prior period of three years of articulation of the Scientific Community. Since the program has been officially established, the scientific community has been working in an articulated manner on studies concerning the characterisation, conservation and sustainable use of biodiversity of the State of São Paulo, with the support of the Scientific Director of FAPESP (José Fernando Perez). The Program is based on the premises preconized by the Convention on Biological Diversity (CDB).

After five years, there are 50 large research projects being developed within the framework of the BIOTA/FAPESP Program. All major public Universities (USP, UNICAMP, UNESP, UFSCar), some private universities (PUC, UNAERP, UNISANTOS) research Institutes (such as the Instituto de Botânica, Instituto Florestal, Instituto Geológico, INPE), EMBRAPA Centers, and NGOs (such as Instituto Socioambiental, Fundação SOS Mata Atlântica, Conservation International and CRIA) are taking part of the Program. Considering only researchers linked with these institutions within the State of São Paulo, there are approximately 400 with at least a PhD level, plus 300 graduate students. There are another 80 collaborators from other Brazilian states and approximately 50 collaborators from abroad.

The common objective of the BIOTA/FAPESP research projects is to study the biodiversity (using the broadest definition of biodiversity as stated in the CDB) of the state of São Paulo seeking: a) to understand the processes that generate and maintain biodiversity, as well as those that can result in its deleterious reduction; b) to standardise sampling, gathering and making information relevant to biodiversity conservation and sustainable use available to decision makers; c) to ensure fast and free public access to this information; d) to improve teaching standards on subjects related to conservation and sustainable use of biodiversity.

The research projects linked to the Program are being developed to increase the academic knowledge on the State of São Paulo's biodiversity and to simultaneously produce results that can be used to improve State policies on biodiversity conservation and sustainable use. For this purpose, it was necessary to associate projects aimed at improving biological knowledge with projects seeking the economic use of species.



All projects are linked through the Environmental Information System of the BIOTA/FAPESP ([www.biota.org.br/sia](http://www.biota.org.br/sia)), developed by the Reference Centre on Environmental Information (CRIA) in collaboration with the State University of Campinas (Unicamp). This system is integrated with an electronic map base (scale 1:50.000) of the State ([www.biota.org.br/sia/atlas](http://www.biota.org.br/sia/atlas)), which was digitised by the Forest Institute of São Paulo. All information is freely available on the Internet and is starting to be used by the State branches in charge of planning its economic development.

After establishing a standard protocol for the biological material collected within the frame of the BIOTA/FAPESP Programme, it was of paramount importance to recover the enormous amount of information deposited in biological collections (historic data) of the State of São Paulo. Focusing on this specific target, the Programme stimulated and is supporting the project "Distributed Information System for Biological Collections: Integrating Species Analyst and SinBiota (FAPESP)"

(<http://splink.cria.org.br/>). Twelve collections (3 herbaria, 2 acari, 3 fish, 1 algae and 3 microorganism collections) are engaged on the first phase of this project.

With historical data and standardised sampling associated to a map base, there are a number of tools that may be developed. At the moment we are working in collaboration with the University of Kansas on geographic distribution models using GARP (Genetic Algorithm for Rule-set Production).

An enormous task to be addressed concerns authoritative taxonomic information. This is fundamental for data input and for searching purposes. There are a number of international initiatives that are aiming at implementing a complete catalogue of valid names (including synonyms and common names) and the Biota/Fapesp information system intends to collaborate with these initiatives. Examples are GBIF, and the Catalogue of Names ([www.gbif.org](http://www.gbif.org)), Species 2000 ([www.sp2000.org](http://www.sp2000.org)) and ITIS, the Integrated Taxonomic Information System ([www.itis.usda.gov](http://www.itis.usda.gov)).

A pilot project for bioprospecting plant species in the State of São Paulo has been pioneered within the BIOTA/FAPESP program, successfully demonstrating the viability of this integrated approach. The aim is now to expand this initiative, widening the scale of organisms and bioassays involved, and integrating all research groups interested in this effort. This is the scope of the **Rede Biota de Bioprospecção e Bioensaios-BIOprospecTA** ([www.redebio.org.br](http://www.redebio.org.br))

In order to establish a competitive bioprospecting program in the State of São Paulo to screen thousands of samples, it will be necessary to adapt the local expertise to the new needs. It is true that this network will not be able to compete with developed countries in the search for new drugs, especially those related to "developed countries diseases". Nevertheless, we can use the large experience of our research groups to create similar strategies to solve regional problems, especially to combat orphan diseases like leishmaniasis, Chagas, malaria and other tropical diseases. In addition, the chemical diversity of species from tropical forests is still a useful source of new potential anticancer, antioxidant, antifungal, anti-inflammatory or antibiotic compounds. Thus, these targets must also be taken into consideration, due to the potential benefits that a new discovery in these areas could bring to the Brazilian economy.

A first call for proposals was made by BIOprospecTA in June/2003 and the network received 57 projects covering the following topics: a) Standardized collection of biological samples (plants, microorganisms, marine species, insects, etc...) and pre-processing of raw materials for the subsequent preparation of extracts; b) Creation of a bank of extracts and pure compounds from plants, microorganisms, marine organisms and other natural sources, with the required automation and data management facilities; c) Natural Products Chemistry: Preparation of standardized extracts, fractionation and purification; d) Characterization of promising extracts/compounds (NMR, Crystallography, LC/GC-MS, etc...); e) Screening of extracts with existing in-vitro and in-vivo bioassays; f) Development of new bio-assays, particularly those adequate for High-Throughput Screening using small sample volumes; g) Pharmacology and toxicology of promising bioactive extracts or compounds; h) Synthesis of bioactive natural products and their derivatives; i) Medicinal chemistry and drug design applied to the development of promising compounds; j) Development of a database structure for the data processing of the program. These projects are now being evaluated by FAPESP's Scientific Directory.

Last, but not least, the BIOTA/FAPESP Programme launched an electronic peer reviewed journal, BIOTA NEOTROPICA ([www.biotaneotropica.org.br](http://www.biotaneotropica.org.br)) to publish research results relevant to biodiversity characterisation, conservation and sustainable use in the Neotropical region, obtained within the framework of the BIOTA/FAPESP Program or not.



# Biodiversity and Genetic Resources Conservation



**ISMAP0311 - Brazilian Ginseng [*Pfaffia glomerata* (Spreng.) Pedersen] Germplasm  
Ex-situ Conservation.**

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Fáfia (*Pfaffia glomerata*) is a large, shrubby ground vine, which has a deep root system. It grows mainly in the borders of Paraná river, and predatory collection has greatly reduced its natural populations. *Pfaffia* is known as "brazilian ginseng", since it is widely used as an adaptogen for many ailments and to promote weakness, much like American and Asian ginseng (*Panax* spp.).

This action is attributed to the anabolic agent, beta-ecdysterone, as well as three novel ecdysteroid glycosides, which are found in high amounts in *Pfaffia* roots. The root of *Pfaffia* also contains about 11% saponins. These saponins include a group of novel chemicals called pfaffosides as well as pfaffic acids, glycosides, and nortriperpenes. These saponins have clinically demonstrated the ability to inhibit cultured tumor cell melanomas and help to regulate blood sugar levels.

In 2001, Embrapa Genetic Resources and Biotechnology, in collaboration with the Emater Paraná (Paraná State Rural Assistance Corporation), and São Paulo State University (Unesp), Botucatu, has launched a field expedition collecting 15 accessions, including more than 200 hundred individuals, all over the borders of the rivers Paraná, Ivaí, and Paranapanema, including areas from Mato Grosso do Sul, Paraná and São Paulo states.

This material has been deposited in both field and *in vitro* germplasm collection. Preliminary studies on seed germination has showed a germination percentage from 12 to 67% and 9,6 to 12,5% relative humidity. A protocol for *in vitro* conservation for *Pfaffia* accessions was established, and ten populations containing 80 individuals have been deposited in our *in vitro* collection. A field collection has been established at Embrapa Genetic Resources and Biotechnology, and morphological and chemical descriptors will be applied. A preliminary survey has showed a presence of pathogenic fungi and root-knot nematodes. *Septoria* spot was detected in leaves of *P. glomerata* and *Meloidogyne incognita*, *M. javanica* and *Meloidogyne* sp. has been detected in roots. Germplasm collection will be useful to found sources of pathogenic fungi and nematode resistance.

**Keywords:** *Pfaffia glomerata*, Genetic Resources, Conservation,



**ISMAP0043 - *Buddleja globosa* Hope: Variability in natural populations and cultivated progenies.**

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*Buddleja globosa* is a medicinal shrub native to Chile with the common name "Matico". Its leaves, principally used for wound and ulcer healing, are mostly harvested from the wild. In each of three wild populations located between latitudes 35 and 38°S twenty individuals were randomly chosen to study different morphological characters, such like plant height and width, stem diameter, leaf length, width, form, density, and angle, teeth in margin, internodes length, inflorescence number and diameter, number of flowers, peduncle length, presence of resins, stipules and lateral buds, abundance of pilosity, leaf colour and contents of tannins and flavonoids in the leaves. Progenies of the three wild populations and of three individuals found in home gardens were cultivated in a random design in Universidad de Talca (latitude 35°S) and evaluated at the same moment as the natural populations. In most quantitative characters, i.e. plant height and width, leaf length, density and angle, inflorescence number, diameter and peduncle length, significant differences between natural populations were found, whereas the individuals of the same population did not differ significantly among them. No significant differences among populations were found for leaf width and most qualitative characters, such like presence of resin, lateral buds, abundance of pilosity on the upper side of the leaves, form of the leaf margin and inflorescence. The characters presence of stipules and presence of leaves in the raceme differed between the natural populations, whereas the individuals of the same population showed no significant differences. In the cultivated progenies differences between plant origins were found for the following characters: plant height, stem diameter, leaf length, width, density, and angle, number of teeth in the margin of the leaves, internode length, presence of stipules and lateral buds, abundance of pilosity on the upper and lower side of the leaves, form of the leaf margin and leaf colour. Flowering characters could not be evaluated in the plantation. All quantitative characters suffered significant variation by different irrigation treatments, whereas the quantitative characters were not affected, with the exception of the presence of resin. The presence of stipules was found to be the only character determined genetically without being apparently affected by environmental factors. We observed that the most southern origin adapted only with difficulties to the cultivation conditions, whereas it showed a normal behaviour in its natural habitat. The characters of agronomic interest were only evaluated in the plantation. The wild origins generally showed less leaf yield than the progenies of home garden plants. No differences could be observed in flavonoid and tannin content in leaves of different plant origin, neither in the natural habitat nor in the cultivated progenies.

**Keywords:** Matico, morphology, flavonoids, tannins

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**ISMAP0220 - Chemical characterization of Mint (*Mentha* spp.) germplasm at Federal District, Brazil.**

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The main goal of this work was to evaluate and compare Mint genotypes (*Mentha* spp.) at the Brazil Federal District conditions. Twenty-five genotypes introduced from Purdue University, USA collection and one Brazilian genotype were analyzed based on the following characters: Leaf fresh and dry weight, volatile oil content and relative percentage of oil constituents. Voucher specimens of each genotype included in our experiments were collected, dried and stored at the Embrapa Genetic Resources and Biotechnology (CEN). Leaves were harvested, bulked, weighted and placed in a paper bag, and dried in a forced-air drier at 38°C for 3 days for oil analysis. The essential oil was extracted by hidrodestillation in a modified Clevenger apparatus, in a 2 L flask during one hour. The collected and stored oil was analyzed by gas chromatography using FID and gas chromatography connected to mass spectrometry, to characterize the chemical composition of each genotype. The parameters were evaluated statistically using SAS program by Tukey test, at level of 5% probability. The genotypes showed a different behavior for each characters evaluated. "Hillary's Sweet Lemon" (*Mentha* sp.) showed the highest values for fresh and dry weight, 23,7%, 24,1% and 15,5% higher when compared to the second genotype. The genotype "Japanese Field Mint" presented the highest content of essential oil (4,17%) and genotypes: "Chinese Mint", "Grapefruit Mint", "Persian Mint Field" and "Eau de Cologne" showed the highest production of essential oil per hectare, 75,0 L/ha, 67,1 L/ha, 53,6 L/ha e 50,5 L/ha, respectively. Twenty-six genotypes of *Mentha* had been chemical evaluated, being detected as main constituents: 1,8-cineole, carvone, limonene, linalool, linalyl acetate, menthol, menthone, menthyl acetate, and piperitenone oxide. The genotypes showed between 20 ("Bergamot" - *Mentha gracilis*) and 66 ("Green Curly Mint" - *Mentha piperita*) essential oil constituents. Some genotypes were found to have essential oils with a high content of a particular constituent, like piperitone oxide (74,4% in "Pineapple Mint" - *M. suaveolens*), carvone (72,5% in "Chinese Mint" - *M. canadensis*) and linalool (67,9% in "Ginger Mint" - *M. arvensis*).

**Keywords:** chemical characterization, genetic resources, essential oil, *Mentha* spp.



**ISMAP0145 - Comparative Anatomical Study of *Anemopaegma arvense* Mart. (Bignoniaceae), from different regions of savanna from São Paulo, S.P., Brazil.**

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*Anemopaegma arvense* Mart., popularly known in Brazil as "catuaba", is a shrub from the Bignoniaceae family, native to the region of "cerrado", a savanna - like formation from central and southeast regions of Brazil. Considering that it is a certified drug, described in the Brazilian Pharmacopoeia and used by the population as aphrodisiac, it is important to establish its taxonomic characteristics for complete morphological and anatomical identification.

The objective of the present work was to investigate the anatomy of 4 individuals of *Anemopaegma arvense*, collected in different regions of savanna, of the state of São Paulo, to characterize the species unequivocally. The material was taxonomically classified by Prof. Dr. Marcos de Assis, from the Botany Department of Unesp from Rio Claro.

Stems and leaves were fixed with a mixture of ethanol, acetic acid and phormaldeide and stored in ethanol 70%. Histological preparations were obtained manually, with transversal sections of the stem and transversal and paradermic sections of the leaf. The sections were submitted to discoloration in sodium hypochlorite, followed by staining with alcian blue and basic fuchsin. Semipermanent slides were obtained, with glycerin between the slides and the preparation. Microscope analysis was carried out on a Coleman binocular microscope, and photomicrographs were obtained with a Karl Zeiss Jena Microscope.

Analysis of the anatomical characteristics of leaf blades, petioles and stems confirmed that it was possible to identify and distinguish the 4 individuals studied, through the leaf morphology, quantity and morphology of sclerenchyma fibers below the cortex region, in the stem, presence and quantity of non-glandular trichomes, and aspect of sclerenchyma fibers surrounding the vascular region of the leaf blade.

The samples analysed were formerly submitted to chemical analysis with triterpenes and to molecular analysis with RAPD, showing little difference between the four accesses. For this motive, the anatomical differentiation, thus delivered, is especially significative.

This study will proceed, with the anatomical analysis of a larger sample of each access. The present work is part of a multidisciplinary research, from the Biota Project, involving the characterization of accesses of *Anemopaegma arvense*.

We wish to thank Faculdades "Oswaldo Cruz" for financing the Project "Farmácia Viva" and for the fellowship granted to the student Renata B. Rinaldi.

We also thank Dr. Ana Maria Pereira, from UNAERP, for technical and financial help.

**Keywords:** catuaba, Bignoniaceae, anatomical markers, savanna flora.



**ISMAP0041 - Comparative study of the essential oil from leaves and flower stems of *Espeletia schultzii* at different blooming stages and at three different altitudes from the Venezuelan Andes.**

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*Espeletia schultzii* (Espeletiinae, Asteraceae) is a widely distributed endemic polycarpic caulirossette, with densely hairy leaves that grows between 2600 and 4200 m of altitude in the Venezuelan Andes. The plant has strongly aromatic leaves. A previous phytochemical study reported the isolation of (-)-kaur-9(11)-16-dien-19-oic acid, 15- $\alpha$ -hydroxy-(-)-kaur-16-en-19-oic acid and 15- $\alpha$ -acetoxyl-kaur-16-en-19 oic acid. No studies on the composition of the essential oil have been reported so the scope of this work was to analyze the essential oil of this plant collected at three different "paramos" (Culata 2800 m, Osos 3500 m and Pico Aguila 4100 m) and at different blooming stages. The oils were obtained by hydrodistillation and the analyses were carried out on a Hewlett Packard GC-MS system fitted with a 30 m long HP-5MS capillary column. Temperature was programmed from 60 to 260°C at 4°C/min. Monoterpene hydrocarbons constituted the most abundant fraction (80-97%), meanwhile oxygenated monoterpenes and sesquiterpenes occurred in small proportions. The most abundant compounds were myrcene,  $\alpha$ -pinene and  $\alpha$ -phellandrene. It was found that the first two components were more abundant in the oils of samples from Culata and Osos ( $\alpha$ -pinene:18-25%, myrcene: 9-25%) than from Pico Aguila ( $\alpha$ -pinene:13-17%, myrcene: 3-8%). They were found in larger proportions in the flowering stalks than in the leaves. In contrast  $\alpha$ -phellandrene was more abundant in the oil of plants from Pico Aguila (39-54%) than from Culata and Osos (14-25% and 17-20%, respectively). Furthermore it was found in larger proportions in leaves than in flowering stalks.  $\alpha$ -Thujene, which was the fourth most abundant constituent at Culata and Osos was practically absent in the oil from plants collected at Pico Aguila. In general, no significant differences were obtained in oil composition between plants collected at different blooming stages, but there were evident differences between the oil from plants collected at different places. It was found that the essential oil from *E. schultzii* collected at Osos and Culata differs considerably from the oil of *E. schultzii* collected at Pico Aguila. This may imply that altitude could be a factor influencing the chemistry of this plant.

**Keywords:** Essential oil, *E. schultzii*, Asteraceae, monoterpenes  
**Sponsor:** University of Los Andes, CDCHT, FONACIT (Venezuela)

**Table 1.** Main monoterpenes present in the essential oil of *E. schultzii*

Compound	CULATA						OSOS						PICO AGUILA					
	1			2			1			2			1			2		
	a	b		a	b	a	a	b	a	B	a		a	b		a	b	a
?-thujene	11.9	17.7		12.7	17.3	15.2	7.6	11.1	11.3	15.1	8.8	-	-	-		0.7	-	
?-pinene	15.0	26.2		9.8	17.7	27.9	23.3	24.3	16.2	24.9	30.2	11.5	18.2	12.8	15.9	15.7		
sabinene	3.1	2.9		5.2	1.6	2.5	1.7	2.3	4.9	1.4	2.5	-	4.3	-		1.4	-	
?-pinene	4.4	8.1		3.1	5.2	9.2	6.9	7.7	5.0	7.8	9.2	2.1	5.4	3.0	4.4	3.8		
myrcene	25.2	9.0		24.5	9.4	16.4	15.6	8.7	24.0	6.7	15.3	7.9	3.5	4.9	2.7	8.4		
?-	16.5	20.3		19.0	24.7	14.1	18.6	20.4	17.8	16.9	16.7	49.7	38.6	54.4	41.1	46.9		
phellandrene																		
(Z)-?-ocimene	1.4	0.6		1.4	0.7	1.0	1.0	0.6	1.3	0.5	0.8	0.6	-	-	-	0.6		
(E)-?-ocimene	4.0	1.6		4.5	2.2	2.9	2.9	1.7	4.1	1.5	2.2	1.8	0.9	0.8	0.5	1.6		

1: preblooming, 2: blooming, 3: after blooming    a: leaves    b: flowering stalks



**ISMAP0164 - Conservation ex-situ of *Physalis angulata* and *Anadenanthera colubrina* species of the Brazilian semi-arid.**

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The Brazilian Northeast is the third larger region of Brazil, with 1.548.672 Km<sup>2</sup> and has about 60% (900,000 Km<sup>2</sup>) recovered by the Bioma Caatinga. Although it is rich in different vegetal formations, it is suffering with the impact human beings activities. The man has degraded the ecosystems and extracted its resources looking for immediate profits. The disordered exploration of the vegetal biodiversity in the Brazil's Northeast region will bring losses of valuable therapeutical substances and also important genes that codify enzymes of potencial use in the vegetal improvement and biosynthetic studies of new natural products. In this context, the medicinal species of plants *Physalis angulata* L. and *Anadenanthera colubrina* (Vell.) Brenan are not only being extracted for Medical purposes but to plant others species with new source of incomes to the northeastern. The *P. angulata* specie belongs to the Solanaceae family and it is popularly known as mullaca, camapú, bucho-de-rã or juá-de-capote. In the popular medicine, it has been used as anticoagulating, antileucemic, antimutagenic, antiinflammatory, antispasmodic, and its fruits is used as feeding. Besides, the angico *A. colubrina* specie of the Mimosaceae family is used as tanniniferous, lumber, melliferous, ornamental and medicinal treatment of respiratory illnesses, ulcers and diarrhea. Because of the great importance of these two species, it had been made physiological and agronomic studies, objectifying bigger scientific knowledge and making possible its sustainable uses and its conservation, since they are considered, under extinguishing threat by the book Flora da Bahia. The species were cultivated in different types of substrates (vegetal land and vegetal land + sand) and luminosity (50% and 80%) in a delineation entirely randomized (factorial arrangement type 2<sup>2</sup>). Through 90 days, the plants were evaluated about the growth and characteristics of the vegetative development. They were analyzed about the average height with the aid of a milimetric ruler from the base of the stem to the insertion of the last leaf and the number of leaves. The results founded can be observed in Table 1. The table one concludes that the factor luminosity of 80% intervems in the height of the plants of both studied species, pomoting bigger height. There was significantly interaction of the substrate x luminosity in the level of 1% in the heigh of the *Physalis angulata* plant. About the number of leaves there was interference of the substrate promoting larger number of leaves per plant in the *Physalis angulata* specie what wasn't observe in the specie of *Anadenanthera colubrina*.

**Keywords:** Conservation, Angico, *Physalis angulata*, growth.

**Support:** Northeast Bank of Brazil, CNPq and FAPESB

Table 1. Values of F of the effect of the substratum and luminosity in the height and leaf number of the species *Physalis angulata* and *Anadenanthera colubrina*. Feira de Santana 2004.

Causes of variation	of G.L.	<i>Anadenanthera colubrina</i>		<i>Physalis angulata</i>	
		Height	Leaf Number	Height	leaf Number
Substratum (S)	1	95.64 <sup>ns</sup>	.977 <sup>ns</sup>	13.69 <sup>ns</sup>	1191.9**
Luminosity (L)	1	613.44*	.372 <sup>ns</sup>	193.21**	195.3 <sup>ns</sup>
Interaction (S X L)	1	.332 <sup>ns</sup>	6.69 <sup>ns</sup>	76.56*	55.8 <sup>ns</sup>
Treatments	3	248.9	2.68	94.48 <sup>ns</sup>	481.0 <sup>ns</sup>
Residue	12	113.0195	6.2550	11.7713	46.0544
Total	15	361.52	8.9350	106.2513	527.0544
C.V.%		36.53	25.95	7.57	12.5

(ns)-Not significant one to the level of 5% of probability; \* significant 5%; \*\* significant 1% Support



**ISMAP0066 - Cytological studies of the endangered medicinal plant  
*Podophyllum hexandrum*.**

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*Podophyllum hexandrum* is a perennial herbaceous species known as Indian Podophyllum, or Himalayan May Apple. The underground rhizomes and roots are targeted for their yield of lignans, mainly podophyllotoxin. The latter is in considerable demand because of increased clinical use of its derivatives as anticancer drugs. Podophyllotoxin is still obtained from plant sources, since its chemical synthesis is not economic on a commercial scale. However, natural sources of this product are limited. Consequently, it is important to preserve and to increase wild *P. hexandrum* germplasm which has become endangered through over-exploitation and lack of organized cultivation. Previously, plant regeneration was achieved through organogenesis from cultured tissues. In the present work, chromosome number and morphology of *Podophyllum* were investigated. Root tips excised from a plant 6 months after regeneration from cultured tissues, and from seed-grown (control) plants, were pre-treated with a 2.0 mM aqueous solution of 8-hydroxyquinoline; others were untreated. Root tips were kept at  $20 \pm 4^\circ\text{C}$  for 2.5 h, fixed in ethanol-acetic acid (3:1, v:v) and stored at  $4^\circ\text{C}$  in the dark. Root tip squashes were prepared using both Feulgen and aceto-orcein stains and chromosome preparations were examined using phase contrast microscopy. The somatic chromosome number was diploid ( $2n = 2x = 12$ ) for both seed- and tissue culture-derived plants. The large chromosomes (each 9–17  $\mu\text{m}$  in length) stained prominently, with low cytoplasmic background. The mitotic index was high (8%) in untreated cells from all plants. In the regenerant, the nuclear organiser regions were located on two pairs of chromosomes as secondary constrictions. Some cells showed evidence of chromosome breakage, including the presence of a decondensed chromatin body at late anaphase, which failed to segregate with the rest of the chromatin. It is possible that the damage observed to the chromosomes in the regenerated plant is symptomatic of a susceptibility to gross chromosomal modification and may be one of the reasons why this medicinally important species is so difficult to propagate by tissue culture.

**Keywords:** *Podophyllum hexandrum*, cytological analysis, endangered species, medicinal plant

**Sponsor:** CGS was supported by RHAE/CNPq and FUNED.



**ISMAP0284 - Distribution of proazulenes and productivity in  
*Achillea millefolium* s. l. spontaneous populations.**

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*Millefolii herba* is one of the oldest and most important drug in Lithuania used both in folk and traditional medicine with spasmolytic and anti-inflammatory effects due to present of proazulenes as most important constituent in the essential oil. The raw material usually is gathered from spontaneous populations. It would be reasonable to develop methods predicting which populations accumulate high enough levels of proazulenes being high productive in raw material, as well as to identify the relation between morphological characters and the presence of proazulenes. The vegetation records and morphometric measurements were made at 128 growing sites. Braun-Blanquet scale of abundance was used in phytosociological description of vegetation. 1,662 plants were tested for present of proazulenes. The proazulenes was indicated in the inflorescences applying a colorimetric assay described by Stahl. SPSS/PC was used for basic statistics and one-way analysis of variance (ANOVA). *A. millefolium* grew in 29 types of communities representing different growing habitats. The resources of milfoils prevailed in ruderal habitats of *Dauco-Melilotion* and grasslands of *Arrhenatheretalia* communities. The testing of *A. millefolium* plants in presence of proazulenes revealed high variation (0–85%) in different communities. The proazulenes-containing plants have been identified in 38% of cases, whereas individuals with high content of proazulenes comprised 85% of all proazulenes-containing plants. The highest percentage of proazulenes-containing plants was found in woodland and scrubland habitats. In segetal and wasteland communities of *Agropyretalia repentis*, *Sisymbietalia*, *Digitario-Setarion*, *Aperion spicae-venti*, *Senecion fluvialis* were found only proazulenes free plants. The ruderal habitats are usually used for gathering of raw material, however most of them appeared to be low pharmaceutical quality. A hierarchical cluster analysis dendrogram provides evidence that populations characterized by higher productivity possessed higher amount of proazulenes containing plants. Results of ANOVA showed that the width of leaves, number of internodes, and mass of stem deviated significantly ( $p < 0.05$ ) between the proazulenes-containing and proazulenes free plants. The characterization of proazulenes distribution may suggest the rapidly identification of proazulenes-containing and high productive populations for pharmaceutical industry, their purposive selection and conservation of resources from over exploitation.

**Keywords:** *Achillea millefolium*, proazulenes, phytocenological dependence, analysis of variance



**ISMAP0305 - Essential Oil Chemical characterization of *Piper* L. (*Piperaceae*) at Federal District, Brazil.**

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The Brazilian Cerrado contains a rich flora of medicinal use. The intense collection from wild species and the expansion of the agricultural have been contributing to the loss of genetic variability of the populations of medicinal and aromatic species in Cerrado. The family Piperaceae, well established in tropical areas, contain several aromatic species used traditionally in the Brazilian traditional medicine, and is also as insecticide. Piperaceae has five genera and 1,400 species. *Piper* L. is the most representative genus with 700 species. In the Federal District area, sixteen *Piper* species has been described, with some species containing aroma due the presence of essential oils. The main goal of this work is to verify the presence of essential oil, to evaluate its oil content and to identify the main constituents of the *Piper* species from Federal District, Brazil. Besides, the variability of major chemical constituents of *Piper aduncum* essential oil collected from 4 different places has been evaluated.

Leaf samples of 10 *Piper* species were collected at Fazenda Sucupira, Embrapa Genetic Resources and Biotechnology. For evaluation of the *Piper aduncum* chemical diversity, 10 healthy, adult leaves samples were collected from 4 population. Voucher specimens were stored at Embrapa Genetic Resources and Biotechnology herbarium (CEN) and determined by specialist. Leaves were harvested, weighted, placed in a paper bag, and dried in a forced-air drier at 38°C for 3 days for oil analysis. The essential oil was extracted by hidrodestillation in a modified Clevenger apparatus, in a 2 L flask during one and a half hour. The collected and stored oil will be analyzed by gas chromatography using FID and gas chromatography connected on Mass Spectroscopy to characterize the chemical composition of each species and variations in *Piper aduncum* samples.

Preliminaries results indicate two promising species reporting the yield of essential oil: *Piper xylosteoides* Kunth and *P. aduncum*, with 2,40% and 1,00% of essential oil in relation to the dry weight of the samples, respectively (Table 1). *Piper umbelatum* L. has shown only traces of essential oil considering the amount of distilled leaves (109g).

**Keywords:** *Piper*, essential oils, Piperaceae, Distrito Federal.

**Table 1.** Leaf essential oil content of seven *Piper* species from Federal District, Brazil.

Specie	Fresh weight (g)	Dry weight (g)	Content (ml)	Yield %
<i>P. dilatatum</i> L.C.Rich.	297,66	56,24	0,2	0,36
<i>P. xylosteoides</i> Kunth.	123,53	24,73	0,60	2,40
<i>P. amalago</i> L.	373,99	89,81	0,50	0,56
<i>P. aduncum</i> L.	1145,60	152,00	1,50	1,00
<i>P. umbelatum</i> L.	712,36	109,34	tr	-
<i>P. tectorifolium</i> Kunth.	768,30	160,89	0,40	0,25
<i>P. ovatum</i> Vahl.	245,80	59,19	0,20	0,34



**ISMAP0274 - Essential oil of *Ocimum gratissimum* L. from Pantanal of Cáceres, Mato Grosso, Brazil.**

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*Ocimum gratissimum* L. is a herbaceous plant, Labiatae (Lamiaceae), common name in the Brazil is alfavaca-cravo, alfavaca - cheiro, manjerição - cheiroso, erva - real and basílico-grande. In folk medicine is used as stimulant, carminative, soporific, febrifuge and diuretic, used also to manufacture liqueurs and for cooking. Recent studies have confirmed the insecticidal action of essential oil. The variations in chemical composition are important between plant populations. This work has as objective to describe the main constituents in the essential oil of leaves of *Ocimum gratissimum* growing at Cáceres (MT). The leaves were naturally dried, for three days, and stored in paper sacks on protected place of light and humidity. The essential oil analysis was done in the Adolpho Ducke laboratory of the Museu Paraense Emilio Goeldi, Belém (PA). It used GC/MS gas - liquid chromatography with silica capillary column DB-5 (30 m x, 0,25mm film thickness), helium carrier gas with flow adjusted to a linear velocity of 32 cm/sec (measured at 100 °C) programmed at 60 - 240 °C, with 3 °C/min gradient. Individual constituents were identified by comparison of mass spectro and retention table of standard substance. The essential oil yield was 1,63%. The chromatographic analysis allowed identifying the eugenol (60,43%) and 1,8-cineole (33,13%) as main components of essential oil. The data presented here suggests that chemical varieties of *Ocimum gratissimum* that grow at Pantanal are of the eugenol type.

**Keywords:** medicinal plant, eugenol, essential oil composition, chemical varieties.

**Table 1-** Essential oil composition in leaves of *Ocimum gratissimum* growing at Cáceres, Mato Grosso, Brazil.

Chemical Composition	Percentage (%)
$\alpha$ -pinene	0.2
sabinene	0.1
$\beta$ -pinene	0.9
myrcene	0.1
1,8-cineole	33.1
(E)- $\beta$ -ocimene	(tr)
linalool	(tr)
$\alpha$ -terpineol	0.1
safrole	0.1
eugenol	60.4
$\beta$ -caryophyllene	1.6
$\alpha$ -humulene	0.1
germacrene D	0.2
$\beta$ -selinene	2.4
$\alpha$ -selinene	0.4
Caryophyllene oxide	0.1



**ISMAP0168 - Essential oils from Myrtaceae species of  
the Brazilian southeastern coast.**

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The botanic family Myrtaceae comprises about two hundred genera and more than a thousand species. They usually concentrate volatile compounds in the leaves, and because of that, represent a renewable source of essential oils. This work reports the chemical composition of essential oils from seven Myrtaceae species, collected in the Brazilian seashore of Carapebus, Atlantic Forest, North of the state of Rio de Janeiro. Voucher specimens were catalogued in the herbal collection of Far-Manguinhos, FioCruz. Fresh leaves of *Eugenia prasina* Berg (EP), *Eugenia sulcata* Spring ex-Mart (ES), *Eugenia ovalifolia* Berg (EO), *Eugenia rotundifolia* Casaretti (ER), *Neomitranthes obscura* (DC.) Legrand (NO), *Myrciaria floribunda* Berg (MF) and *Myrciaria* spp. (MS) collected in the period from 1997 to 2000, were submitted to hydrodistillation (Clevenger, 8h). The oils were analysed by gas chromatography coupled to mass spectroscopy (30m x 0.25mm x 0.25mm HP-5 MS column, EI 70 EV, Ti 70 °C, Tf 290 °C, ti 5 min, rate 3 °C/min; injections of 1 from 2mg/mL CH<sub>2</sub>Cl<sub>2</sub> solution). The species were identified by Dr. G. M. Barroso. Refractive indices were obtained in Abbé refractometer. Oils yields (% w/w; h<sub>D</sub>) were 0.50 (EP; 1.4770), 0.60 (ES; 1.4772), 0.10 (EO; 1.4894), 0.30 (ER; 1.4908), 0.10 (NO; 1.4950), 0.70 (MF; 1.4950) and 0.40 (MS; 1.4777). Excepting EP (60% of monoterpenes), sesquiterpenes predominate, being practically the only species in ES, EO, NO and MS, and reaching high contents (74-82%) in MF and ER. Excepting EO and NO, most sesquiterpene species are oxygenated (mainly alcohols). These latter counterbalance the hydrocarbon species in MF. The majority of sesquiterpenes are represented by the eudesmane and selinane skeleta. Both *Myrciaria* species (MF and MS) showed 25 and 68% of nerolidol in their constitutions, respectively; and ES presented a high content of farnesol (50%) and a bicyclic selinenone (25%), what could be important as sources of new aromas and flavors.

**Keywords:** Myrtaceae, essential oil, *Eugenia*, *Myrciaria*



**ISMAP0028 - Evaluation of genetic variations, some agronomic and cytogenetic characters in Black cumin landraces.**

Bazoobandi, Mohammad; Faravani, Mahdi

Khorasan Agric. Res. Centre

Twenty eight *Nigella sativa* biotypes were collected from different areas of Khorassan province in 2000. An experiment was laid out in Randomized Complete Block Design using the collected biotypes as treatments. Various morphological characteristics were registered during growing season. To investigate anatomic difference between biotypes sampling was done over number of vascular stomata, xylem and phloem diameter. Agronomic traits were also registered after harvesting. Significant differences were observed between biotypes in respect of plant height, test weight, seed yield, number of branches, capsule weight, number of stomata, number of vascular, xylem and phloem diameter. Differences between harvest index, number of seed per capsule and single plant weight were insignificant. Biological yield and harvest index showed the highest heredity and genetic efficiency, which may be used in breeding.

**Keywords:** Black Cumin, Biodiversity, Cytogenetic



**ISMAP0025 - Fusarium / Alternaria Diseases Resistance Selection Among Local  
Cumin (*Cuminum cyminum* L.) Land Races.**

Akhavan, M., Bazoobandi, M., Faravani, M.

Cumin (*C. cyminum* L.) is one of the most important medicinal plant playing a key role in economic affairs of Khorasan province (Iran). About 90 % of produced Cumin is exported. There has been a significant reduction in area under Cumin cultivation due to *Fusarium* & *Alternaria* diseases outbreak. In order to find *Alternaria* & *Fusarium* resistant land races, an investigation was conducted. During the course of this study (2000–2001) 84 land races from different part of Iran were collected, date and percentage of germination, flowering time, plant height, infection level and some other important traits were registered. Since *Fusarium* is a soil born as well as seed born fungus, therefore seeds were planted in sterilized soil bed, they had already been treated with a proper fungicide seedlings were artificially incubated with *Fusarium* for two months. After germination sixty four resistant races were selected in first year to be evaluated in second year. Latice design was adopted to analyze data. Results revealed that there are significant differences among races for characteristics under study. It is clear that the most proper way for control of there diseases is to find resistant biotypes through breeding methods yet to be worked out.

**Keywords:** *Fusarium*, cumin, *alternaria*



**ISMAP0061 - Genetic diversity among mentrasto accesses by botanic-agronomical, molecular and phytochemical characteristics.**

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*Ageratum conyzoides* is recommended as antirheumatic and control of arthrosis with the decrease of the pain and inflammation or improvement in articulation mobility. This study aimed to evaluate the genetic divergence among five mentrasto accesses by multivariate methods using botanic-agronomical, molecular (RAPD markers) and phytochemical characteristics (content and composition of the essential oil). The accesses were obtained in Mariana-MG (AMA), Piranga-MG (API), Visconde do Rio Branco (ARB) and Viçosa-MG (AVB and AVP). The essential oil was obtained by hydrodistillation and the identification of the oil components by GC and GC/MS. The characteristics of the aerial part of fresh biomass, dry biomass, height, flowers, leaf area, number of seed by inflorescence, number of inflorescence by plant and number of seed by plant were measured. The grouping analysis by the Tocher's method in the botanic-agronomical, molecular and phytochemical characteristics, two groups were formed. In analysis of the genetic divergence by molecular markers, 14 primers gave rise to 26 polymorphic bands. Fresh biomass and dry biomass were the characteristics botanic-agronomical that most contributed for genetic diversity. Eleven chemical compounds were identified: terpinene-4-ol; bornyl acetate; (*E*)-caryophyllene; coumarin; precocene I; g-murolene; d-cadinene;  $\alpha$ -murolene; caryophyllene oxide; precocene II; and 6-hydroxymethyl-7-methoxy-2,2-dimethyl-2H-cromene. The compounds precocene I and precocene II were the phytochemical characteristics that most contributed for genetic diversity. The ARB access presented the higher essential oil content, that is 0,70 % ( $P < 0,05$ ). The precocene I was the main constituent in the access API, and the precocene II was the main constituent in the accesses AMA, ARB, AVB and AVP. Significant correlation coefficients were verified between dry biomass and precocene I. No significant correlation coefficient was found between the botanic-agronomical, molecular and phytochemical characteristics on the basis of the accesses distances.

**Keywords:** Genetic diversity, RAPD marker, essential oil, *Ageratum conyzoides*.

**Sponsor:** CNPq



**ISMAP0346 - Genetic diversity and similarity of wild germplasm of Brazilian ginseng [*Pfaffia glomerata* (Spreng) Pedersen] from three Brazilian States.**

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Plant populations occurring in natural habitats are sources of genetic diversity for conservation and breeding. The amount and distribution of the genetic variability available in these populations need to be investigated for better use of these resources (1). This paper describes the genetic diversity indexes, distances and similarities of *Pfaffia glomerata* accessions, a species which is one of the Brazilian species recognized as the "Brazilian ginseng". The conservation, breeding and cultivation of the species is on high priority, since its unwise exploitation is threatening the wild stocks and there are interests both from retailers and exporters for an increase in its availability. Isozyme markers were assayed in an average of 22 individuals from each of the four heterogeneous populations collected in three States of Brazil, which were Parana State (population A1 from an island of Parana River and A2 from Ivaí City), Mato Grosso do Sul State (A3) and São Paulo State (A4). Thirteen alleles were revealed, originated from five isozymic loci. The average genetic diversity ( $H_e$ ) was between 0.213 and 0.378 and the observed heterozygosity ( $H_o$ ) was between 0.112 and 0.231. These data show that the genetic diversity is of high magnitude for all accessions (2). Furthermore, accessions A3 and A4 presented higher diversity values in comparison to the other ones, suggesting that they can be more advantageous for germplasm conservation and use in breeding programmes. The pairwise similarities and distances were estimated according to Nei (2). An UPGMA dendrogram was built, revealing that populations A1 and A2 clustered together. They are from the same geographical region, which makes it predictable that they are genetically closer than the other ones that come from the other States. In conclusion, it can be considered that all four *P. glomerata* accessions are of great value for conservation and breeding, but if one wants to maximize diversity with the minimum number of individuals, it may be acceptable to collect less individuals from populations A1 and A2 than from A3 and A4.

**Keywords:** plant breeding; population genetics; genetic resources; medicinal plant

**Table 1.** Diversity indexes ( $H_e$ : genetic diversity and  $H_o$ : observed heterozygosity) of five polymorphic loci in individuals from four germplasm accessions of *P. glomerata* (Brazilian ginseng).

Loci	N	Germplasm accessions							
		A1		A2		A3		A4	
		$H_o$	$H_e$	$H_o$	$H_e$	$H_o$	$H_e$	$H_o$	$H_e$
PGI	24	0.095	0.455	0.043	0.426	0.333	0.452	0.500	0.522
6PGdh	24	0.238	0.285	0.130	0.125	0.381	0.455	0.333	0.494
MR	24	0.143	0.140	0.167	0.159	0.200	0.287	0.067	0.186
G6Pdh	24	0.143	0.285	0.174	0.232	0.095	0.483	0.095	0.176
DIA	24	0.095	0.260	0.043	0.125	0.143	0.215	0.000	0.273
<b>Average</b>		<b>0.143</b>	<b>0.278</b>	<b>0.112</b>	<b>0.213</b>	<b>0.231</b>	<b>0.378</b>	<b>0.199</b>	<b>0.330</b>

**Table 2.** Genetic similarities (upper diagonal) and distances (lower diagonal) of four germplasm accessions of *Pfaffia glomerata*.

Acessions	A1	A2	A3	A4
A1	-	0.9952	0.9754	0.9624
A2	0.0048	-	0.9654	0.9416
A3	0.0249	0.0352	-	0.9466
A4	0.0383	0.0602	0.0549	-

(1) Yeh. F. C. **Population Genetics**. In: Young. A.; Boshier. D.; Boyle. T. Forest Conservation Genetics. CSIRO/CABI Publ.. 2000.

(2) Frankel. O.T.; Brown. A. H. D.; Burdon. J. J. **The conservation of plant biodiversity**. Cambridge Univ. Press. 1996.



**ISMAP0183 - Genetic relationships between commercial cultivars and Brazilian accessions of *Salvia officinalis* L. based on RAPD markers.**

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Common sage (*Salvia officinalis* L.) was introduced in Brazil by European immigrants in the last centuries and has been maintained by seeds or stem cutting. Today, the introduction of commercial cultivars from Europe and North America has increased due to the condimentary, medicinal and aromatic importance of this plant. In the last decades, molecular markers have been successfully used to assess genetic relationships and genotype identification with great impact on plant breeding programs, germplasm management, and biodiversity preservation. In this context, the purpose of the present study was to evaluate the genetic relationship between Brazilian accessions and commercial cultivars of *S. officinalis* L. Ten accessions obtained from traditional agriculturists of the Northeast region of Rio Grande do Sul, five commercial cultivars, and a representative of *S. sclarea* L. were evaluated by the electrophoretic comparison of the random amplified fragments generated by polymerase chain reaction using decameric primers (RAPD analysis). A total of eighteen random primers used generated 195 bands of which 59,3% were polymorphic within *S. officinalis*. Genetic similarity was estimated and cluster analysis was performed. Three distinct groups were identified. Group I was formed by three accessions and a Brazilian cultivar. Group II was subdivided in three subgroups, one formed by the four European commercial cultivars and an accession from Fagundes Varela, and the other two by three Brazilian accessions each. As expected, the representative of *S. sclarea* formed Group III. These data are indicative that some common sage plants introduced in South Brazil by European immigrants represent a genetic pool different from that of commercial cultivars. The low variability observed within commercial cultivars can be considered as an indicative that a restrict genetic pool is explored in breeding programs, and that Brazilian accessions can be a source of genetic variability.

**Keywords:** Common sage; DNA markers; genetic relationship; PCR



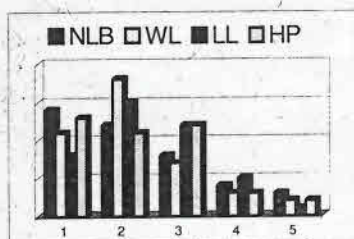
# ISMAP0113 - Genetic resources and improvement of *Piper aduncum* L. in the Amazonian.

José Maria D. Gaia<sup>1</sup>; Milton Guilherme of C. Mota<sup>1</sup>; Carmen Célia C. of Conceição<sup>1</sup>; Meirevalda do S. F. Redig<sup>1</sup>

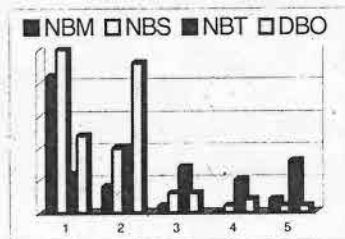
<sup>1</sup>Universidade Federal Rural of the Amazonian - FCAP, Av. Presidente Tancredo Neves s/n, 66077-530. C. P. 917, Belém-PA ([josegaia@interconnect.com.br](mailto:josegaia@interconnect.com.br)).

*Piper aduncum* L. is an aromatic species found abundantly in the Amazonian Brazilian. It possesses great potential for economical exploration in function of the proven usefulness of the essential oil in the agriculture and human health. Our objective was to collect germplasm in the area of natural occurrence to install a germplasm bank; to describe the phenotypic variability of the natural populations; to characterize the genetic diversity of the natural populations through markers of DNA (RAPD) and to evaluate the collected germplasm under the point of view of the production of essential oil to obtain variety for the production system. The following actions were developed: germplasm collect; germplasm bank installation; morphological, phytochemistry and molecular characterization; and germplasm evaluation. Were realized collect in the nine municipal district of de Brazilian Amazonian, included Amazonas e Pará States. After analysis of the figures 1, 2, 3, 4 and 5 it was observed that the been found species possesses a wide adaptation capacity to the environment conditions in the Amazonian, in different vegetation types, soil, climate and drainage. The collected material (seeds and branches) of 42 accessions were utilized to install a germplasm bank in the field, in the Federal University Rural of the Amazonian (UFRA)/BELÉM-PA-Brazil. It was concluded that exists enough phenotypic and genetic variability of superior genotypes. The figure 6 showed the good potential of oil production of this species.

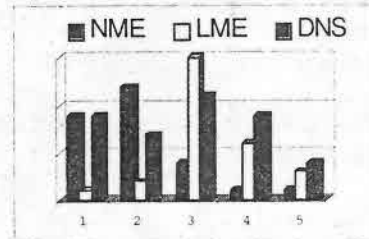
**Keywords:** germplasm, characterization, evaluation, essential oil.



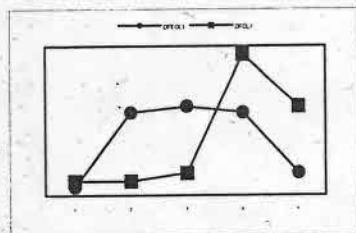
**Figure 1:** Distribution of frequencies for number of leaves for branch, width and length of the leaf and height of the plant. Belém-PA, 2001.



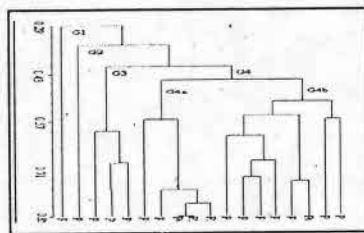
**Figure 2:** Distribution of frequencies for number of branches main, secondary and tertiary and diameter of the oldest branch. Belém-PA, 2001.



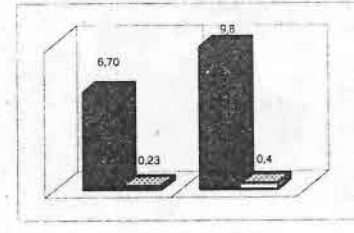
**Figure 3:** Distribution of frequencies for number and length medium of the ears and diameter medium of the nodal segment. Belém-PA, 2001.



**Figure 4:** Distribution of frequency of data of percentage of essential oil and dillapiole. Belém-PA, 2001.



**Figure 5:** Dendrogram of genetic similarity of four origins of *P. aduncum* L. Belém-PA, 2002.



**Figure 6:** Production of dry matter (black) and income of essential oil (white) in two cut times (kg/ha). Belém-PA, 2002.



**ISMAP0348 - Genetic resources conservation studies of *Lychnophora* species.**

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The *Lychnophora* genus have about 65 endemic species on Brazilian "campos rupestres", popularly used maybe it is medicinal properties. Due their large popular use and anthropic action in natural habitat, these plants can be extinct. This work relate the studies for genetic resources conservation of *Lychnophora* species. Three collecting expeditions were made at Delfinópolis, Carrancas and Alpinópolis, Minas Gerais state. It was collected plant cuttings, achenes (dry fruits) and exsiccates of *L. ericoides*, *L. pinaster* and *Lychnophora sp* (voucher MEP 001, MEP 002 and MEP 003 - UNICAMP Herbarium). Achenes of other species are given from UNICAMP Herbarium, for interchange of genetic material. All these genetic resources are forming the Active *Lychnophora* Genetic Bank of Botucatu (Table 1). The objective of the bank is to conserve *Lychnophora* genetic resources and promote propagation, crop production, and genetic researches. Some results of these experiments are shown here. The germination of achenes were evaluated on B.O.D. (10 hours dark and 14 hours light, temperature 20- 30° C), using several substrates. Best results were obtained on vermiculite, that presented more than 50% of germination, after densimetric selection, on *L. ericoides*, *L. pinaster*, *L. markgravii*, *L. martiana*, *L. uniflora* and *L. tomentosa*. Seed germination of *L. candelabrum*, *L. pohllii* and *L. passerina* was smaller than 5%. It was observed that over 80% of achenes were sterile to species studied, justifying the use of densimetric selection. Cuttings of *L. pinaster*, *L. ericoides* and *Lychnophora sp* were evaluated about root development at greenhouse (20- 30° C), irrigating 0.8L.hour<sup>-1</sup>. When the cuttings were treated with indol-butyric acid (IBA) at 300mg.g<sup>-1</sup> by 24 hours, the root development were over 75%, indicating their possible use on crop production studies. Presently the Germoplasm Bank has plants of 9 species of *Lychnophora*, and 7 accesses of *L. ericoides* growing at greenhouse. The results of this work show that it is possible to grow plants of this species at greenhouse and to promote studies about crop production of them. It is an important way for preservation of *Lychnophora* germoplasm variability and their future domestication.

**Keywords:** medicinal plant, germoplasm, seed germination, root development

**Sponsor:** CAPES

**Table 1.** Species of *Lychnophora* that are growing at greenhouse on Botucatu, SP

Specie	Local	Collector	Material	NGP*
<i>L. pinaster</i>	Carracas, MG	M.E.Paron	Achenes, Cuttings	124
<i>L. ericoides</i>	Delfinópolis, MG Paraiso	M.E.Paron	Achenes, Cuttings	20
<i>L. ericoides</i>	Delfinópolis, MG Santo	M.E.Paron	Achenes, Cuttings	20
<i>L. ericoides</i>	Alpinópolis, MG Pedreira	M.E.Paron	Achenes	14
<i>L. ericoides</i>	Delfinópolis, MG Palito	N.P. Lopes	Achenes	120
<i>L. ericoides</i>	Furnas, MG	N.P. Lopes	Achenes	33
<i>L. ericoides</i>	Grão Mongol, MG	N.P. Lopes	Achenes	12
<i>L. ericoides</i>	Delfinópolis, MG Babilônia	N.P. Lopes	Achenes	7
<i>Lychnophora sp</i>	Delfinópolis, MG Céu	M.E.Paron	Cuttings	12
<i>L. tomentosa</i>	Serra do Cipó, MG Juca	M.E. Mansanares	Achenes	56
<i>L. candelabrum</i>	Serra do Cipó, MG 1	M.E. Mansanares	Achenes	3
<i>L. martiana</i>	Será do Cipó, MG 2	M.E. Mansanares	Achenes	94
<i>L. markgravii</i>	Serra do Cabral, MG	M.E. Mansanares	Achenes	200
<i>L. uniflora</i>	Itabira, MG	M.E. Mansanares	Achenes	55
<i>L. pohllii</i>	Serra do Cipó, MG 3	M.E. Mansanares	Achenes	10
<i>L. passerina</i>	Serra do Cipó, MG Usina	M.E. Mansanares	Achenes	17

\* - number of growing plants at greenhouse



**ISMAP0322 - Genetic variability of *Jacaranda decurrens* checked by RAPD.**

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*Jacaranda decurrens* is an endemic medicinal plant native to scrub land regions of Brazil. The population has used indiscriminately root and leaf infusions of *J. decurrens* to heal wounds and to eliminate tumors, contributing for the genetic erosion of this species. Therefore, a genetic evaluation of the remainder resources is indispensable to establish conservation programs. The objective of this work was to determine the genetic diversity of *J. decurrens*, found in the state of São Paulo, using molecular markers. DNA of 93 plants randomly collected from 7 populations, approximately 14 individuals per population, in different localities of the state, were analyzed by PCR reactions using Applied Biosynthesis Taq polymerase and buffer. Genomic DNA (25 ng) was used for 25ml of reaction mixture. Amplifications were carried out in PTC-11 Programmable Thermal Controller MJ. Research. Inc. The amplification products were separated by electrophoresis in 1.5% agarose gels in 10 x TBE buffer, detected by ethidium bromide staining, developed under ultraviolet light and photographed using Polaroid 667 film. Reactions were carried out using 10 primers resulting in 78 polymorphic bands. The biometric method adopted was the PGMA. The mean diversity estimated inside the populations ( $H_{pop}$ ) was 0.086 and among the studied populations ( $H_{tot}$ ) was 0.144. Therefore, the proportion of the diversity inside populations obtained by the correlation  $H_{pop}/H_{tot}$ , was 0.6 whereas the diversity among populations [ $1-(H_{pop}/H_{tot})$ ] was 0.4. Analysis of the dendrogram constructed with the RAPD data, revealed that the greater genetic diversity of *J. decurrens* occurs inside the populations. Values of diversity index (index of Shannon) also indicated more heterogeneity among individuals of the same population. Although an intra-population variation (60%) was verified, it was not considered unexpected since it can be explained by the reproductive system of this species.

**Keywords:** Bignoniaceae, cerrado, medicinal plant



# ISMAP0378 - *In vitro* storage of *Echinacea tenneseensis*, an endangered North American medicinal species.

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Tissue culture protocols for *in vitro* propagation and storage were carried out as tools for developing an efficient conservation program on *Echinacea tenneseensis* (Beadle) Small, an endangered medicinal species. The response to additions of different osmotic agents into the media and incubation at low temperature were examined using shoot cultures of two clones. These clones were obtained from hypocotyls of germinated seeds donated by The North Central Regional Plant Introduction Station (NCRPIS) in Ames, PI 631250 accession. Each hypocotyl was the explant of one clone that has been chemically and morphologically characterized. For *in vitro* shoot multiplication and maintenance, explants were inoculated on Murashige and Skoog (MS) supplemented with 0.5 mg/l BA. Roots were induced in shoots transferred to MS media supplemented with 0.5 mg/l NAA. To develop *in vitro* protocol for germplasm storage of *E. tenneseensis*, shoots without roots were transferred to MS media supplemented with different concentrations of sorbitol and mannitol (20 g/l and 40 g/l each). These cultures were incubated at 25°C. Shoot survival and culture re-growth were examined after 2, 4, 6, and 8 months of storage and compared with cultures maintained at lower temperatures 5°C and 10°C for the same incubation time. Shoot growth was limited on MS media containing sorbitol or mannitol and at low temperature incubation, with the highest survival on media containing 2 % sorbitol and at 5°C (Fig 1). Vigorous re-growth was noticed only on shoots stored at low temperature 5°C. Shoots of clone 11 have resisted storage conditions better than clone 3. No abnormalities were noticed in our cultures during the 8- month period of this study (Fig 2).

**Keywords:** *Echinacea*, *in vitro*, micropropagation, germplasm.

**Sponsor:** The Office of Research and Sponsored Programs, The University of Mississippi

*In vitro* storage of *E. tenneseensis*

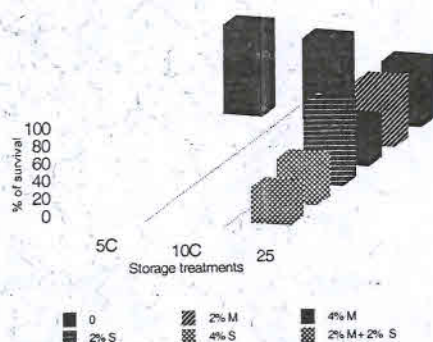


Fig. 1 demonstrates culture survival in storage

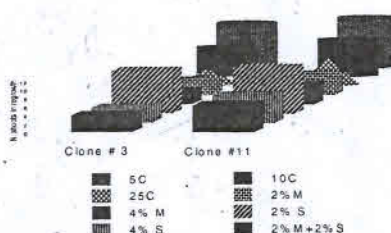


Fig. 2 shows shoot re-growth after storage



**ISMAP0313 - Initial approach to genetic diversity in the linalool rich species *Croton cajuçara* Benth. (sacaca).**

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In 1971, *Croton cajuçara* Benth. (sacaca) was identified as a source of linalool, which can be extracted from leaves and small branches, without having to harvest the entire plant. Linalool is commercially valuable, with 100 ml of 97% essence selling for US\$13 - 15. Purer forms, such as DL-linalool used for gas chromatography, may cost up to \$118/gr. Sacaca may be proposed as a potential crop for the Amazon Region, since it was originally collected at the edges of the forest, in transition zones, and may keep its properties under cultivation in similar areas. In view of its potential, a germoplasm collection was initiated at Embrapa Amazonia Ocidental in 1996. This collection is part of RENARGEN (Genetic Resources National Program) sponsored by Embrapa Genetic Resources & Biotechnology (Cenargen) and Ministry of Science & Technology (MCT) and holds 16 genotypes. The plants can be morphologically classified as red and white sacaca, according to the color of the leaves. The principal components of the essential oil from these plants have been previously demonstrated to be 5-hydroxi-calamenene and linalool for the red and white sacaca, respectively, and we suggested that they are chemotypes as well. The aim of this work was to search for genetic divergence between red and white genotypes based on RAPD patterns. DNA was obtained from leaves with DNEasy Extraction Kit (QIAGEN). PCRs were performed, in replica, as follows: DNA -10 ng; MgCl<sub>2</sub> - 2.5 mM; dNTPs - 400 mM; primer 10 mer - 375 nM; Taq DNA polymerase - 1.5 U; BSA 0.1% in 25 mL reactions, cycling 92 °C for 1 min; 35 x (92 °C for 1 min; 30 °C for 2 min e 72 °C for 2 min); 72 °C for 5 min; 4 °C forever. Ten random primers were used. *Manihot esculenta* Crantz. (cassava or manioc) was included in these analyses as a contrast of similarity between different species within the same botanical family (Euphorbiaceae). The software GENES (UFV) was used to estimate the genetic similarity indexes and for clusterization. Seventy one polymorphic fragments were analyzed and this number was reduced to 31 when fragments with reproducibility under 0.67 were eliminated from the analysis (it means that only data reproduced at least twice in three replicas were considered). Red and white sacaca plants had 80% similarity, cassava presented 45% similarity with white sacaca and 24% similarity with red sacaca.

**Keywords:** genetic diversity, chemotypes, RAPD, *Croton cajuçara*.



# ISMAP0081 - Intraspecific variability of *Origanum vulgare* L. naturally occurring in Poland.

Z. Węglarz, E. Osiedska, A. Gęszprych and J. Przybył

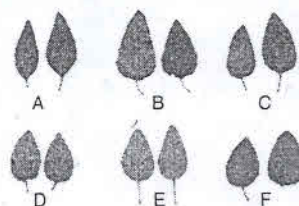
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Wild marjoram (*Origanum vulgare* L.) is a perennial growing wild in Poland on dry sunny slopes and forest margins. It grows in rather small clusters. The herb of wild marjoram is harvested from natural sites and used as a raw material for the pharmaceutical, cosmetic and food industry. Six wild marjoram populations originating from Central Poland were compared in *ex situ* studies. The morphological and developmental traits were described. The herb was harvested twice: at the full blooming stage and two months later – from the offshoots. The mass of herb per one plant was calculated. The content of essential oil, flavonoids, phenolic acids and tannins in the air-dry herb was determined according to Polish Pharmacopoeia VI. The qualitative analysis of essential oil and phenolic acids was carried out by GC method. For the investigated populations the most segregating morphological and developmental traits were: plant habit, number of lateral shoots, size of leaves, colour of flowers (white, pink and dark pink) and mass of herb (from 240 to 640 g · plant<sup>-1</sup>). The content of essential oil in the herb collected at the full blooming stage ranged from 0.35 to 0.55%. The populations differed in respect of the composition of essential oil. Its main constituents were: sabinene, cineol, 2-caryophyllene, caryophyllene oxide, terpinene-4-ol or 2-pinene. The content of flavonoids in the investigated raw materials ranged from 0.2 to 0.6%, phenolic acids – from 1.1 to 2.1%, and tannins – from 1.0 to 5.1%. Twenty one phenolic acids were identified. The populations differed in respect of the presence and percentage of particular phenolic acids. In all populations the dominant one appeared to be 2-hydroxycinnamic acid.

**Keywords:** wild marjoram, herb, essential oil, phenolic compounds

**Table 1.** The morphological characteristics of leaves

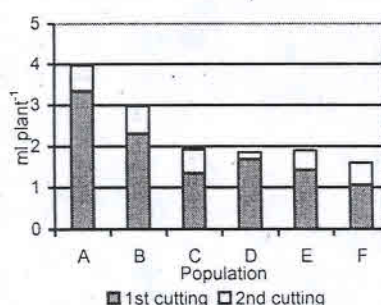
Population	Length of the leaf blade (mm)	Width of the leaf blade (mm)	Length of the petiole (mm)
A	33.0	14.5	7.0
B	35.0	19.0	10.0
C	34.5	18.5	11.0
D	23.5	15.0	5.0
E	29.5	18.5	12.0
F	29.5	20.0	11.0



**Figure 1.** The shape of leaves

**Table 2.** Main volatile compounds (% in essential oil)

Compound	Population					
	A	B	C	D	E	F
sabinene	5.46	10.77	15.45	17.98	35.52	10.90
β-pinene	1.27	6.34	2.37	0.72	1.12	18.03
linalool	15.60	-	0.57	1.86	1.18	0.35
γ-terpinene	9.24	3.26	1.41	-	0.96	2.24
terpinene-4-ol	18.48	2.79	2.29	5.14	5.64	0.59
p-cymene	1.03	-	7.50	0.26	6.78	8.44
cineol	0.75	10.98	13.03	10.02	12.40	3.08
thymol	0.21	0.43	0.69	2.81	1.72	7.35
β-caryophyllene	6.38	10.39	11.85	1.34	0.53	0.39
caryophyllene oxide	8.34	12.79	11.19	0.13	0.26	0.99



**Figure 2.** The yield of essential oil (ml · plant<sup>-1</sup>)



**ISMAP0282 - Mint (*Mentha* spp) Germplasm Collection in Brazil.**

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In the seventies Brazil was a large producer of mint essential oil (*Mentha arvensis*). Nowadays, Brazil became a large importer of this product, with the mint production been reduced to a few growers and backyards plants. However, there is now an improvement in the demand for Mint oil as source of menthol as well as for its use in phytotherapy.

As a first step towards this goal, Embrapa Genetic Resources and Biotechnology has collected and introduced a mint germplasm collection from several places. The main goal of this project is to evaluate mint germplasm in different Brazilian environmental conditions.

In 2002, twenty-seven mint germplasm accessions were introduced from Purdue University, USA. In 2003, forty-five mint accessions from northeast, southeast, and south Brazil were collected from Campinas Agronomic Institute (IAC), São Paulo, SP, CPQBA of Campinas University, Campinas, SP, Ceará Federal University (UFC), Fortaleza, CE, Brasília University (UnB), Brasília, DF, Paraná Agronomic Institute (IAPAR), Londrina, PR, West Paraná State University (Unioeste), Cascavel, PR. The total germplasm collection includes 11 species and 72 accessions, which are maintained at field and greenhouse conditions. Twenty seven accessions from USA have been maintained at *in vitro* conditions. The following species are included in this collection: *M. suaveolens* Ehrh. x *M. aquatica* L.; *Mentha* x *piperita* L.; *Mentha* x *villosa* Hudson; *Mentha aquatica* L.; *Mentha canadensis* L.; *Mentha* cf. x *gracilis* Sole; *Mentha* cf. *spicata* L.; *Mentha longifolia* L.; *Mentha spicata* L. e *Mentha suaveolens* Ehrh. Preliminary studies on cryopreservation of Mint have been initiated at Embrapa. The germplasm collection has been now multiplied and will be distributed to institutions of five Brazilian regions for essence oil evaluation. Oil samples of all accessions will be sent to natural products industries to be analyzed and market evaluated.

**Keywords:** germplasm, conservation, genetic resources



**ISMAP0345 - Morphological characterization of *Heteropterys aphrodisiaca* O. Mach.  
(Malpighiaceae) seeds and seedlings.**

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Studies dealing with the morphological characteristics of fruits, seeds and seedlings of species that are native to the cerrado region in Brazil, which are important for species identification, and for germination, regeneration, and dispersion studies, are still very scarce. Despite its proven medicinal potential, no morphological studies have been found for the species *Heteropterys aphrodisiaca*. The objective of this work was to characterize seed and seedling morphology in this species. The seeds used in the experiment were collected in the municipality of Chapada dos Guimarães, MT, in the year 2002; the external and internal characteristics of seeds and external characteristics of seedlings were observed, and structures were examined under a stereoscopic microscope. Seedling development was evaluated by means of four subsamples consisting of 25 seeds, incubated in a germination chamber at 30°C with a photoperiod of 8 hours. The seed is an indehiscent, exalbuminous samara, with a membranous-textured, crest-shaped, densely innervated wing; the seminiferous nucleus is basal, covered with abundant rusty-brown colored hairs; the hilum is ventral and the stigma is characteristically persistent. Germination began seven days after the experiment was installed, and extended through day 19, being of a hypogeal and cryptocotyledonary character, marked by the appearance of a structure that results from the prolongation of the cotyledons, referred to as the cotyledonary petiole.

**Keywords:** germination, morphology, medicinal plant, seed.

**Sponsor:** Biosintética Laboratory.



**ISMAP0381 - Multistrata and medicinal plants in Dii and Niza'a tribes.**

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Homegarden is one of the traditional agro forestry systems developed by the local population to improve their living standard while decreasing deforestation in natural ecosystems. The objective of the paper is to determine the role of this system in the conservation of the medicinal plants within the sudano - guinea savannah. The methodological approach was participative and reiterative. Ethnobotanical interviews were conducted in Dii and Niza'a tribes reputed for their culture. In each ethno - linguistic group, 150 farmers were questioned. Plant samples were either identified *in situ* or taken to the National Herbarium for species determination. In Dii seven villages were explored and nine for the Niza'a. More than 80 % of the respondents were familiar with medicinal plants in their production system. The average number of medicinal plants growing in the system varies from 10 (Niza'a) to 20 (Dii). These species are currently stored in strategic positions depending on the role played in the traditional medicine. The most frequent plants found in the two tribes are *Crinum* spp. (75,35%), *Cissus quadriangularis* (70,57%), *Dioscorea* spp (57,35%), *Piliostigma thonningii* (55,75%), *Upuntia* spp (23,36%). They are used to treat various illness and the protection of the concession and against devil spirits and the snake bite. In addition to these domesticated or protected wild plants in the production system, many other crops are used in local medicine. According to respondents malaria (37,5%), typhoid (37,5%) and amibiasis (37,5%) are the most frequent diseases of the area. The conservation of medicinal plants in the homegardens indicates a strong linkage between inhabitants and traditional medicine in the highlands of Adamawa. Homegardens representation and management by the local farmers show the key role of the endogenous knowledge on biodiversity conservation.

**Keywords:** Homegardens, Endogenous knowledge, Medicinal plants, Heath



**ISMAP0103 - Multivariate analysis in fifty cultivars / landraces of *Piper nigrum* L. occurring in Kerala, India.**

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*Piper nigrum* L., the source of 'black pepper' of commerce is considered to have originated in the Western Ghats of Indian peninsula. The Kerala State of India occupies a considerable portion of the Western Ghats and harbours a major share of the primary gene pool of the crop occurring both in wild and cultivated conditions. It is estimated that over 70 distinct cultivars of *Piper nigrum* are under cultivation in the State. But the genetic resources of the crop are getting lost as a result of gene erosion due to diverse factors, and the efforts in conserving and utilizing the resource are meager. A clear understanding of the genetic attributes of the cultivars and their interrelationships are advantageous for formulating strategies in conservation and utilization programmes of the resource. The present study deals with multivariate analysis based on 15 morphological characters in 50 cultivars / landraces of *P. nigrum* occurring in the State, using Mahalanobis D<sup>2</sup> statistics. On the basis of relative magnitude of D<sup>2</sup> values the genetic diversity between and among the cultivars were measured and grouped them in to 12 clusters (I to XII). The number of cultivars of the clusters ranged from 1 – 24. The D<sup>2</sup> values between the groups ranged from 20.74 to 177.61. The maximum intercluster distance was noted between the clusters VII and XII and the least between the clusters – I and IV. Intracluster distance ranged from 0.00 – 19.33. The least distance was observed in six clusters, which are single member clusters. The maximum intracluster distance was noted in cluster V. The results of the study are directly useful in selecting parent strains for hybridisation programmes aiming at exploiting hybrid vigour in the crop.

**Keywords:** *Piper nigrum* L., black pepper, genetic diversity, D<sup>2</sup> statistics



**ISMAP0361 - Phenotypical characteristics of *Maytenus ilicifolia* native populations, at Paraná State - Brazil.**

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*Maytenus ilicifolia* is a native medicinal tree from South Brazil. At Paraná State, native communities are rarely observed in Araucaria Forest, growing on the riparian forest and over rock lands. Although the medicinal importance of *M. ilicifolia* there are few systematized data about it, particularly on the environmental influence on morphological and phytochemical characteristics. At native populations there is a great diversity of foliar length, thickening, spine number and fruits color, length and form. These environmental links are very important to plant management and breeding. So, the objective of this work is to investigate possible correlations between the phenotypic characteristics of *Maytenus ilicifolia* growing in different environmental conditions. For this study it was select 16 trees as leaves and seeds sources, at Irati, Piraquara and Colombo localities, in Paraná State. The fruits were collected on January and February 2004, at random from in each tree source. The seeds number per fruit was evaluated. After processing the seeds were planted on plastic tubes with 75 cc<sup>3</sup> volume, two seeds per tube, and two kinds of substrate: 1 = 70% organic commercial substrate + 30% soil; 2 = 100% organic commercial substrate. After that, the tubes were placed under shady 50%. The germination evaluation was initiated a week after the seeding and it is being done weekly. The seeds germination is being very irregular among the 16 tree sources up to date. The seedling development will be evaluated until the definitive planting. The foliar samples were collected on the first April week, 2004. The foliar area (cm<sup>2</sup>) and mass (g) were measured in one year old leaves. For the 16 trees source was determinate the specific leaf weight (g/cm<sup>2</sup>) and the results change enough among the samples. Mainly the different trees foliar areas influenced this difference. This study will be complemented by seedling evaluations, chemical foliar and soil analysis. The results will be compiled at a data bank to *M. ilicifolia*.

**Keywords:** *Maytenus ilicifolia*, Phenotype, Specific leaf weight, Seed germination



### ISMAP0050 - Phytochemical value of Hungarian *Achillea* populations.

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Drugs of yarrow (*Achillea*) species (*herba, flos, Aetheroleum*) belongs to the items of most stable and significant market demand. Hungary is one of the greatest exporter (100-150 t/year) the drug is collected in wild populations. Official drugs originate from species of the section *Millefolium*, first of all from *A. collina*. However, their chemical and pharmaceutical value (mainly the content of essential oil and chamazulene) are extremely variable. Identification of species within the genus is often difficult because of its polymorphism and frequent interspecific hybridisation. Our intention was to clear up the degree and possible causes of variability concerning of productional and chemical characteristics of *A. collina* both at the level of populations and individuals.

The experiment was carried out in 2003 at the experimental station of our faculty in Budapest. Seeds of 19 population collected formerly at different Hungarian wild growing areas were sown in greenhouse and planted into open field at the end of April. During the vegetation period we evaluated the homogeneity of the stands measuring the plant height, the time of flowering, diameter of plant, overground biomass and flower mass. According to samples collected at full flowering we determined the essential oil content by steam distillation and the main components of the oil by GC with standards. For evaluation of the vegetative as well as the generative reproduction capacity, further measurements on the number of shoots, flowers, the ratio of seeds/ flowers and the germination power was determined.

A considerable variability among and inside the populations could be established concerning several morphological and productional characteristics. Two of the populations could not be identified as real *A. collina* species.

The height of flowering plants varied between 35 and 70 cm. According to the flowering time the populations could be grouped into three; each group opening at a week interval. The ratio of flowers in the *herba* mass was between 32-51% and the proportion of seeds in the dry flower mass reached 0,25-6,73%, in average 2,73%. Germination power differed also slightly.

The average content of essential oil in the *flos* samples reached 0,02-0,71% as means of populations, with considerable individual plant differences: marginal values 0,01- 1,77% d.w. Chamazulene proved to be the main component of nine populations, while it were other constituents registered as main component in four cases (mainly sesquiterpenes). In the remaining populations chamazulene together with another terpenoid (e.g. borneole) represented the major components. In the best individuals chamazulene accumulated as high as 80-90% of the oil.

Homogeneity inside the populations reached variable levels. No correlation in morphological and chemical homogeneity of the stands could be established. In correlation with our former (unpublished) results in *A. pannonica* and *A. ochroleuca*, the findings show, that the degree of homogeneity of the populations may be in connection with the form of natural multiplication.

Our results assure information for the cultivation of the species, developing the cultivation method and realisation of successful quality assurance in the production. As practical result, we have a collection of valuable raw for breeding of a high value cultivar.

**Keywords:** *Achillea*, essential oil, chamazulene, breeding, variability, chemism

**Sponsors:** Hungarian Ministry of Education (Project Nr. OM-00178/2001)



**ISMAP0287 - Phytochemicals in five tree species used as medicinal plants by local populations in the south-central Paraná state.**

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Five tree species typical of the Mixed Ombrofilous Forest at the south-central Paraná state (Iratí County) used as medicinal plants by local communities were screened for phytochemicals using standard laboratory procedures. The species studied were: *Drimys brasiliensis* (cataia), *Tabebuia alba* (ipê-amarelo), *Persea major* (pau-andrade) and *Picrasma crenata* (pau-amargo). Samples of sun and shade leaves as well as barks of one individual of each species were collected and analysed for the presence of tannins, flavonoids, anthraquinones, cardio tonic glycosides, steroids and essential oils. Morphological and chemical characteristics of the soils and light intensity of the surrounding environment in which the trees were growing were also determined and measured. The environment is characterized by relatively shallow, acidic and low fertility soils with an average of only 0, 45% of light intensity reaching the ground although tree heights were between 6 and 8 meters. The original environment of the forest has been altered by the "faxinal" system of soil use adopted by the local communities for at least 50 years consisting of the removal of shrubs and under story by grazing of domestic animals such as pigs, cows and horses, hastening natural regeneration. The careless removal of barks for medicinal purposes aggravates the situation. In none of the samples were detected anthraquinones or cardio tonic glycosides. The presence of tannins and flavonoids were detected in leaves (mainly shaded leaves) of *Picrasma crenata*. Flavonoids were detected also in leaves and bark of *Persea major*, while steroids were present only in the leaves. Leaves and bark of *Tabebuia alba* contained flavonoids and steroids. *Drimys brasiliensis* was the species with higher number of phytochemicals: tannins in the leaves, flavonoids and steroids in leaves and bark and essential oils mainly in leaves (1,9%) but also in the bark (0,75%). Further studies are needed to identify the specific active principles responsible for the therapeutic effects observed, as well as for the conservation of the genetic resource considering environmental degradation.

**Keywords:** phytochemicals, native trees, medicinal plants, Mixed Ombrofilous forest



# ISMAP0204 - Pilocarpine and molecular diversity in Jaborandi.

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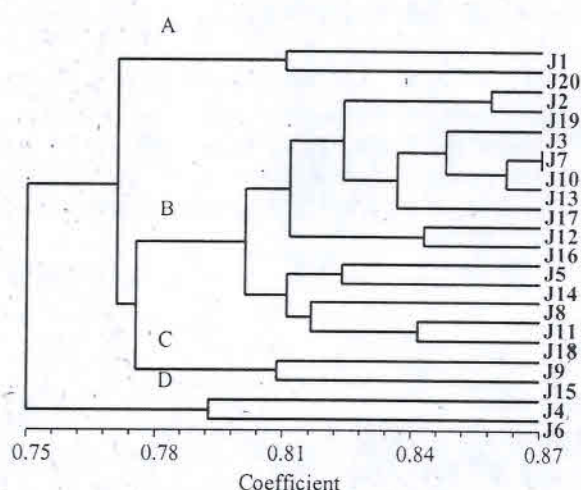
Pilocarpine is an imidazol alkaloid exclusively found in the genus *Pilocarpus* and *P. microphyllus* (Jaborandi) contains the highest content in the leaves. There is no report in the literature on the variability of pilocarpine in *Pilocarpus*. A population of 20 plants of *P. microphyllus* from the state of Maranhão was analysed for RAPD markers and pilocarpine content. According to the pilocarpine content it was possible to separate the plants in 4 groups (table 1). Among 33 primers tested, 18 showed variation in the amplification pattern. When this variation was compared with the pilocarpine data, it was observed that 9 primers could be used to differentiate the 20 genotypes regarding their amounts of pilocarpine. From the presence/absence of the polymorphic bands it was calculated the genetic similarity and the UPGMA analysis was used to obtain a dendrogram (Figure 1). It was possible to define four main heterotic groups (A, B, C and D). From the evolutionary point of view, the genetic structure observed might mean a response to the local environment from where the jaborandi seeds originated. However, it was not possible to establish a relationship between the genetic diversity revealed by RAPD markers and pilocarpine contents. Analysis of a larger number of individuals are necessary to confirm this conclusion.

**Keywords:** *Pilocarpus microphyllus*, RAPD, molecular markers, pilocarpine.

**Sponsor:** FAPESP

**Table 1.** Pilocarpine of the 20 tree genotypes of *P. microphyllus* grouped according to the alkaloid content.

Group	Genotype	Pilocarpine (ug/g dry weight)
1	J4	16.3
1	J17	19.8
1	J12	24.1
1	J3	24.1
1	J10	29.6
1	J13	39.4
2	J11	63.8
2	J8	67.5
2	J5	71.8
2	J16	74
2	J18	87.5
2	J20	85
2	J9	86.4
3	J14	129
3	J15	130.2
3	J19	145.1
3	J6	166.1
4	J2	215.7
4	J1	232.5
4	J7	235.9



**Figure 1.** Dendrogram (UPGMA) obtained from similarity matrix based on DICE coefficient estimated among 20 jaborandi genotypes based on 170 RAPD markers.



**ISMAP0150 - Plants diversity and traditional knowledge of Trans-Himalayas.**

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Holy Himalayas are known for its rich herbal resources since time immemorial in Indian subcontinent and Himalayan regions. Unlike other parts of Himalayas the trans-Himalayan regions has a barren landscape and lifeless appearance, but in its barrenness it possess a very rich biodiversity with more than one thousand rare and valuable plant species supporting large number of wild and domestic animals, birds and insects. The life and cultural diversity of inhabitants of these trans-Himalayan regions are largely influenced by these wild plants; basic commodities from food to shelter, medicine, fodder and plants for socio cultural needs were completely collected from wild plants sources.

The remoteness and inaccessibility had kept Trans-Himalayas regions in isolation from rest of the world during most part of the history. The people of these regions has to be a self-sustained society largely depending upon the surrounding wild plants for their basic needs like vegetables, medicines, fuel, fodder etc. This long association with plants and nature had enriched their knowledge about use of plants for various purposes. The rich and unique Amchi (Tibetan) medical system prevalent in these regions is specialized in medicinal use of Trans-Himalayan plants. Although from last five decades this scenario has been tremendously changed with better road networks, exportation of goods and advancement in agriculture etc. But there are many remote areas in Trans-Himalayas, who are still dependent on wild plants to fulfill their basic needs.

We have been conducting surveys of Trans-Himalayan regions of Ladakh and Lahoul-Spiti from last more than ten years. The plants of these regions come under alpine and high alpine zones and are dominated by annual and perennial herbs, followed by few stunted shrubs and bushes. Around 1000 plants species were recorded and ethno-botanical information of important plants were documented. A checklist book on medicinal and aromatic plants of Trans-Himalayan cold desert has been published based on author's extensive field and literary surveys. It contains information about 525 plants used in various Indian systems of medicines and folk traditions, commercially traded and endangered medicinal plants. This paper will highlight the rich plants diversity of these region and age old traditional knowledge of the indigenous people.

**Keywords:** Trans-Himalayas, Traditional medicine, Plants diversity, Traditional knowledge



**ISMAP0135 - Rose (*Rosa* spp.) germplasm resources of Turkey.**

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Turkey is one of the most important Rose germplasm centers. About 25% of all rose species are native to Turkey. Flowers of some rose species, such as *Rosa gallica* and *Rosa damascena*, have been used for rose oil and rose water production in Anatolia for a long time. Fruits (rose hip) of some other species have economic value and are also used for medicinal purposes. In this paper, *Rosa* species found in Turkey are listed, their distribution within the provinces is listed, as well as their characteristics and uses.

Keywords: Rose, Biodiversity, Turkey



**ISMAP0331 - Rosewood (*Aniba rosaeodora* Ducke): natural populations of an endangered species from the Amazon region are highly genetically diverse.**

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Rosewood (*Aniba rosaeodora* Ducke) is a very important species found in the Brazilian Amazon, especially in the Amazonas State, and it is the most explored source of linalool oil, used in perfumery and known as an ingredient for Channel Number 5 fragrance. In this reason, linalool is worthy. Decades of predatory and illegal exploitation of Rosewood trees have brought the species to the endangered degree. In order to evaluate the current levels of genetic diversity to four natural populations from Central and Médio Amazonas, RAPD molecular markers were used. Young leaves from 109 trees were collected in Manaus, Parintins, Silves and Maués, cities placed in Rosewood exploitation areas. DNA was extracted, quantified and then used for RAPD-PCR reactions with four arbitrary decamer primers. RAPD products were resolved in 1.5% agarose gels prepared in 1X TBE. The results were photo-documented and data were scored in a binary basis. The softwares POPGENE, NTSYS and TFGPA were used to calculate genetic diversity indexes for each of the sampled populations and among them. Fifty-one RAPD fragments were generated, from which 50 (98.04%) were polymorphic markers. The percentage of polymorphic *loci* ranged from 70.58 to 82.30%. Ht index (genetic diversity) was 0.387 and Hs (genetic diversity within populations) was found to be 0.310. Shannon Diversity Index ranged from 0.4258 to 0.4742. The group of plants located in Manaus was the most genetically diverse, in part because this population is included, since 1960, in a protected area, originally considered as highly biodiverse. It could be expected that diversity in an endangered species should be low. On the contrary, taken together the results demonstrated that, even submitted for decades to hard exploitation, these Rosewood populations have maintained high levels of diversity, whether the indexes are compared with those found for other tropical plant species. We can consider that the original genetic diversity levels for this species can no more be accessed and, in addition, that the exploitation process has eliminated essentially mature trees, keeping a great fraction of the genetic diversity preserved as plants in imature phases.

**Keywords:** *Aniba rosaeodora*, genetic diversity, linalool oil, Amazonia

**Sponsor:** FMNA/MMA.

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### ISMAP0058 - Seed Cryoconservation as up-to-date Tool for Diversity Preservation of Wild Ukrainian Flora.

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The flora of Ukraine contains a lot of plant species with limited distribution, many of which are under threat. The native mountain flora of Ukrainian Carpathian was found to consist of 2020 native species, of which more than 20% are endemic and relict. The biological characteristics of critical species are limited. Conservation of endangered native plants is an important priority for Ukrainian Botanic Gardens. The Botanic Garden-University of Lviv is one of the oldest in Ukraine. It was founded in 1823 as a part of the University with a scientific scope. The total area of the Botanic Garden is 18,5 ha. The Garden plays an important role in plant biodiversity conservation in Ukraine. Thematic collections are included collection of native flora that consists rare species. A programme for *ex-situ* conservation was established in order to save populations of relict, rare and threatened species. Some of these species have been introduced in Botanical Garden University of Lviv. Plant cryopreservation have been developed at the Lviv's University during last year. The work had been done with wild species from Ukrainian flora. This work will present research results of cryogenic conservation of native plant seed.

In this study seeds of *Silene vulgaris* (Moench.) Garske (from Caryophyllaceae family) which grows as endemic high mountain race in the Ukrainian Carpathian mountains, in eppendorf tubes were directly plunged into liquid nitrogen for 24 hours. The seeds were warmed in laboratory conditions and plated on agar medium. The biological and physical methods were held for seed characteristic after cryoconservation in liquid nitrogen. Seeds resumed growth within 3 days. The percentage of germination seeds was 44-46% in the control or in the experiments. The seedlings showed normal development. Preliminary results showed that ultra low temperature (-196°C) did not influence negatively on seed germination and growth.

**Keywords:** seeds, cryoconservation, germination, wild



**ISMAP0370 - Studies on distribution and growing behaviour of medicinal plant species in south-eastern part of India.**

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30 sites have been surveyed covering three forest divisions of Chhattisgarh forest, the south-eastern part of INDIA. Five sites have been identified as rich and potential sites for medicinal plant species. Commercially important species like *Abrus precatorius* L., *Aristolochia indica* L., *Curcuma caesia* L., *Hedychium coronarium* J.Koenig, *Operculina turpethum* L., *Rauwolfia serpentina* L. and *Zanthoxylum armatum* DC. have been recorded in negligible numbers at one or two sites. *Withania somnifera* L., *Gymnema sylvestre* Retz. and *Ruta graveolens* L. have not been recorded in the survey sites. 275 species have been collected and planted in the nursery to study the growing behaviour i.e germination through seed and vegetative plant parts, days to flowering and maturity. 47 species flowered within 100 days, 7 species within 200 days and 30 species flowered after 200 days of planting. Days to flowering of commercially important species i.e. *Andrographis peniculata* Burm.f. (100), *Spilanthes oleracea* DC. (100), *Plumbago zeylanica* L. (90), *Hedichium coronarium* J.Koenig. (180), *Gymnema sylvestre* Retz. (433), *Aristolochia indica* L. (401), *Cassia occidentalis* L. (55), *Curculigo orchoides* Gaertn. (60), *Costus speciosus* Koenig.(40), *Mucuna pruriens* (L.) DC (85), *Gloriosa superba* L. (90), *Abelmoschus moschatus* Medicus (80), *Cymbopogon flexuos* Roxb. (80), *Celastrus peniculatus* Willd. (700), *Operculina turpethum* L. (90), *Piper longum* L. (103), *Asparagus racemosus* Willd. (413), *Aborma augusta* L. (725) has been recorded. Seeds of important medicinal plant species were given various pre sowing seed treatments i.e. Hot water, Pacelling, Cow dung + Ash, Hydrochloric acid and Sulphuric acid. HCL (25 %) was found significantly effective in enhancing per cent seed germination of *Andrographis peniculata* Burm.f. (100.0), *Bixa orellana* L. (72.66) and *Crotalaria juncea* L. (58.33) whereas, hot water was effective for *Rauwolfia serpentina* L. *Clerodendroum seratum* L. (76.66), *Costus speciosus* Koenig. (100.0), and *Leea aspera* Edgew. (88.33).-Per cent seed germination was significantly higher in the seeds *Rauwolfia serpentina* L. treated with cow dung + ash (50.00) and pacelling (43.33). 125, 250 and 500 ppm concentrations of Indole-butyric acid (IBA) significantly enhance the per cent survival of cuttings in *Adhatoda vasica* Nees (100%), *Coleus barbatus* (Andr.) Benth. (88.23%) and *Vitex leucoxydon* L. (100.00).

**Keywords:** Medicinal plants, Distribution, Growing behaviour, Germination

**Sponsor:** IGAU



**ISMAP0347 - Substrate and temperature regime on *Heteropteris aphrodisiaca* O. Mach. (Malpighiaceae) seed germination.**

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The objective of this work was to study the germination of *Heteropteris aphrodisiaca* O. Mach. seeds, known as "nó-de-cachorro", on different substrates and under constant and alternate temperature regimes. The experiment was conducted with seeds from Santo Antônio do Leverger - MT, collected in September, 2003. Substrates tested included paper towels in the form of paper rolls, blotting paper with seeds upon the paper and between paper sheets, within sand, and within vermiculite, at temperatures of 30°C and 25-30°C, in a germination chamber under an eight-hour photoperiod. Results demonstrated that the best regime was constant temperature (30°C). The vermiculite and sand substrates provided the best germinative behavior, where the greatest percentage of normal seedlings and emergence velocity index results were observed.

**Keywords:** medicinal plant, seed, substrate, germination test.

**Sponsor:** Biosintética Laboratory.



**ISMAP0064 - Survey of occurrence and contents of linalol in sacaca (*Croton cajucara* Benth), in Region of northeast of Pará.**

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Sacaca (*Croton cajucara* Benth) is used in Brazilian Amazonia as phytoterapic product, by popular medicine and in pharmacist industry, by its effects as hypoglycemic and hypocolesterolenic, among others. It is alternative fountain of linalol, important fixative, in perfumery industry. This work aimed at to know the occurrence and potential of production of linalol in natural population of paraense northeast and to collect germoplasm of this species. The researches included: survey of information upon the area, expeditions for collecting, extraction and chemical of essential oil. Informations were collected about geographical distribution of this species, in paraense northeast and in other places of Amazonia. Also some informations were collected about climatic conditions of this area. Two transect were made in directions north-south and east-west, with collect of 30 plants. Data about places and these collected plants were made. In order to propagation of matrices and a work collection, the shoots are employed. The plants were scattered, with low density and different phases of growth or age, along of ways, brushwoods and yards. Also were observed variations of the leaves, in colour (green or brownish-green), size (length = 11,52 cm,  $\bar{A} = 2,34$ ; width = 3,88 cm,  $\bar{A} = 0,86$ ); pulverulence and contents of linalol. The contents of oil in leaves have had variation of 0,45% until 1,5% and linalol was from 1,64% until 34,20%, but that happened for epoch of material collected, because the larger contents of linalol were finded in plants collected in very dry period.

**Keywords:** Essential Oil, Genetics Resources, Amazonia Plant



**ISMAP0334 - The Brazilian Arnica (*Lychnophora ericoides* Less.)  
seed germination.**

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The vegetation of central Brazil, Brazilian savana, is distributed in the Central West, Southeast and Northeast regions, including eight states and the Federal District. The Brazilian savana occupies around 2 million km<sup>2</sup>, representing 23% of the Brazilian territory. The flora from this region is estimated at 7,000 plant species, which has been submitted to a strong anthropogenic action. The Brazilian savanna is rich in medicinal species consisting of leaves, developed roots ("xylopodios") and barks, which frequently accumulate pharmacologically active substances.

The Brazilian Arnica, or "Arnica de Goiás" (*Lychnophora ericoides* Less. - Asteraceae), is a shrub, up to 3m, growing in central Brazil region on rocky fields. The tincture of leaves of the Brazilian Arnica is used to treat hematomas, contusions, varicose vein and as an anti-inflammatory. The popular name "arnica" is probably given to these species in a reference to the same medicinal effects as the European arnica (*Arnica montana* L.).

The Brazilian Arnica seeds were collected from nine different locations in the Federal District area and Goiás, totaling ten populations and 107 plants. Fruits were collected in three different stages: a) stage E1: mature fruits without papus, with all flowers already pollinated; b) Stage E2: fruits showing papus but not all open or semi-open; and c) Stage E3: fruits with papus and totally open. For germination study, incubation temperature used was 25°C, using germination paper as substrate. Pre-treatments included chemical scarification with H<sub>2</sub>SO<sub>4</sub> for 15 and 20 minutes, exposure to temperatures below zero at - 20°C and - 196°C for 48 hours, with four replications, according to seed availability. Weekly counting of seed germination was done. Two hundred seeds from Fazenda Água Limpa (FAL), UnB, treated with Captan (2,4g/l) and Sodium hypochlorite 1% for 5 min, were used for *in vitro* germination test in 1/2 MS medium.

The highest germination rate (10%) was showed for seeds, at stage E3, exposed to H<sub>2</sub>SO<sub>4</sub> for 20 min. Seeds from FAL showed a 13.9% germination rate, with 9.2% humidity. Only 33% (66) of achenes showed presence of seeds (66 seeds), and from those 36 have germinated *in vitro*. Three different insects were observed damaging Brazilian Arnica fruits at field conditions, which can possibly explain the reduced germination rate.

**Keywords:** Brazilian Arnica, *Lychnophora*, seeds, Asteraceae

**Sponsors:** Fundo Nacional do Meio Ambiente (FNMA) and CNPq



# ISMAP0377 - The Use of Geographical Information Systems as a Decision Making Tool for the Domestication and Conservation of Medicinal Species.

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A Geographic Information System (GIS) is a computer system that has the tools to store, manage, analyze, query, and display data with spatial or locational attributes. provides tools for data and information management based on the features location or its attributes, by creating logical statements or expressions to access the features on the map and/or their records in the database. We are building attempting to create a database containing data information on the distribution of medicinal plant genotypes. Podophyllotoxin is the starting material for the semi-synthesis of the anticancer drugs etoposide, teniposide and etopophos. Since the total synthesis of podophyllotoxin is an expensive process, availability of the compound from natural renewable resources is an important issue for pharmaceutical companies that manufacture these drugs.

*Podophyllum peltatum* L. (American mayapple) colonies are source of podophyllotoxin. Colonies located in Mississippi have been collected, characterized chemically and morphologically (Fig 1). We created a GIS database to store and organize *P. peltatum* data that is easily updated and shared by different users in related fields. In this study, GIS has been used as a domestication tool to evaluate the eco-physical factors influencing drug yield.

The field data collection followed a standard procedure, which included geographical coordinates of each accession (determined using GPS equipment), sample of biomass (leaves randomly harvested of in each accession), date, location, and other information that were gathered in Table 1. Leaf samples were extracted and analyzed by HPLC. Chromatograms of each accession have been hyperlinked to the GIS database. Data are being gathered and organized in a geodatabase used as major input for in the GIS. Using the analytical tools of the GIS, biomass data was combined with By overlaying the lab data with geomorphologic data, to create thematic maps are being generated to identify chemical, physical, and microbiological elements of the environment that may contribute to yields of medicinal constituents, as shown in Figure 2.

Results showed that different *Podophyllum* lignan chemotypes were found in the same sites, indicating that the lignan production was mainly controlled by the genetic make-up. The database of genetic resources and drug yield linked to their spatial location supports the conservation and utilization of chemotypes for future studies.

**Keywords:** Mayapple, *Podophyllum*, Lignans, GIS

**Sponsor:** NCNPR and UMGC

**Table 1.** Field data organized into the GIS as table format

ACCESSION	FAMILY	COM-NAME	DATE	COLLEC-NAM	STATE	COUNTY	LONGITUDE	LATITUDE	PROJ	DATUM
LaMS-5	Berberidace	Mayapple	11/16/02	H. Momm	MS	Lafayette	89.5092000	34.3313900	MSTM	NAD83
UMMS-2	Berberidace	Mayapple	4/1/99	R. Moraes	MS	Univ. of Mis	89.5319900	34.3575500	MSTM	NAD83
NaMS-1	Berberidace	Mayapple	4/1/03	R. Moraes	MS	Natchez	W91 23.855	N 31 25.159	Lat/Long	WGS84
HsMS	Berberidace	Mayapple	4/1/03	R. Moraes	MS	Holly Spring	W89 26.268	N 34 49.203	Lat/Long	WGS84



**Figure 2.** Map of the accessions located in Lafayette County, MS overlaying the Soil Association Data



**Figure 1.** *P. peltatum* colony located at Lafayette County, MS



**ISMAP0089 - Variability of St. John's wort (*Hypericum perforatum* L.) Wild  
Accessions and Registered Cultivars.**

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St. John's wort (*Hypericum perforatum* L.) is medicinally used for the treatment of mild depression. High concentrations of the active compounds are found in late-stage flower buds and open flowers. Hypericin is used as the marker compound for medicinal activity. Since the early 1990ies the German pharmaceutical industry relies exclusively on cultivated raw material. Field cultivation requires high quality cultivars with low susceptibility to the fungus *Colletotrichum gloeosporioides*. The intensified cultivation of St. John's Wort was accompanied by intense breeding efforts. By now, 13 cultivars have been registered in Germany, characterized by diverse quality attributes.

In 2002 and 2003 we evaluated 10 popular cultivars for their yield, disease susceptibility, and active compound accumulation as well as the variability of 28 wild accessions from Chile and Germany. Results were compared to the Polish cultivar 'Topaz' (accession number 28).

Results of the evaluation of 10 cultivars of St. John's wort Germany in field experiments:

- The total yield of herbal drug of two years of cultivation (three cuttings) varied between 7,53 t/ha and 10,85 t/ha (mean 9,41 t/ha)
- In addition to agronomical characteristics, susceptibility to the fungus *Colletotrichum gloeosporioides* is a very important trait that also was evaluated. Until the end of the second year of cultivation 96,4% of the plants of the cultivar 'Gold' had died. The cultivar 'Hyperixtrakt' showed the lowest susceptibility to the fungus. Only 2,7% of the stems died (mean 21,2 %).
- Drug quality is determined by the concentration of active compounds. Highest hypericin contents (% photometrical measurement) were found in the plant material of the first harvest, varying between cultivars from 0,13% to 0,38% (cultivar 'Motiv').
- With increasing age of the flowers and the onset of fruiting, hypericin content was found to decrease whereas the hyperforin content increased.

Twenty-eight wild accessions of St. John's wort were infected with *C. gloeosporioides* spores supplied in open containers which were distributed within the culture on the field. We found that accessions containing high hyperforin concentrations were also highly sensitive to the infection. More accessions need to be analyzed to verify this correlation.

**Keywords:** *Hypericum perforatum* L., variability, hypericin, *Colletotrichum gloeosporioides*



# Plant Breeding



**ISMAP0091 - Adaptation of medicinal and aromatic plants to contemporary requirements by breeding: aims, methods, and trends.**

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Breeding gives the opportunity to adapt crops to the requirements of seed companies, farmers, the processing industry, consumers and to general claims of the society. Cultivars complying with the required characteristics are an indispensable prerequisite for the production of high quality products in a sustainable and profitable way. Breeding aims at the change of the average trait expression of a genotype towards the desired level and at the reduction of its modification depending on environmental factors. These statements are valid for main agricultural crops in general but also in particular for medicinal and aromatic plants (MAP).

Breeders are confronted with some peculiarities of MAP: only a few results of breeding research - e.g. on genetics and on breeding methods - are available. MAP comprise a particularly great number of species, there are often different breeding aims in one and the same species depending on the field of usage, the costs of the analysis of important constituents are particularly high, only limited capacities for breeding research and breeding are reserved for these minor crops, refinancing of the breeders' expenses is insufficient due to the small saleable seed amount. On the other hand, there is also an advantage: the breeder can exploit high natural variability because breeding of MAP is at the beginning only. In view of these peculiarities and to ensure an adequate cost-value ratio, breeders have to plan very carefully which crops, breeding aims and breeding methods are to be chosen.

MAP breeding puts emphasis mainly on the following aims: high quality by ensuring the required composition and content of quality determining constituents, absence of harmful substances, and homogeneity of the herbal crude drug; sustainability by selection of appropriate genotypes for domestication from species endangered by depletion, low need of agrochemicals by breeding cultivars with resistance to pests and diseases and low nutritional requirements; profitability of the production by high yield, adaption to the climate of the cultivation region, technological suitability for the production processes in agriculture and in the processing industry, and good natural suitability for the protection of plant breeders' rights.

Adequate breeding methods must be chosen with respect to the biological peculiarities of a species. The evaluation of large collections of accessions of a species with subsequent selection of the desired genotypes is the main breeding method yet due to the prevailing high natural variability of these minor crops. New variability is being generated primarily by crossing and mutagenesis. The development of hybrid varieties and the exploitation of apomixis is at the beginning only. Biotechnological methods can be used only if adequate financial funds are available. Nevertheless, the clonal production of high performance elite plants in vitro and the use of molecular markers for the determination of the degree of relationship are being used increasingly. Some research teams are working to identify genes responsible for interesting characteristics and to establish gene transfer methods. But, unfortunately the European customers representing the biggest share of the world herb market disapprove genetically modified plants. Great progress has been achieved in the field of special trait assessment methods for breeding, e.g. the use of the near infrared spectroscopy that allows the non destructive analysis of important constituents of great sample numbers in a short time.

The rising number of registered MAP cultivars is clear evidence for the increasing activities in MAP breeding at present. The MAP branch has recognised that breeding of high performance cultivars is one of the most important measures to improve quality, sustainability, and profitability.

**Keywords:** breeding, breeding research, medicinal and aromatic plants



**ISMAP0326 - Agronomic and chemical evaluation of a hybrid of the antimalarial species *Artemisia annua* L. for São Paulo state regions.**

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*Artemisia annua* L., one of nearly 400 species of this genera belonging to Asteraceae family, is listed in the Chinese pharmacopoeia as a remedy for various fevers including malaria, which is still one of the greatest causes of mortality in the word (in Brazil there are over 500,000 reported cases each year). *A. annua* contains the well-established antimalarial compound artemisinin and nowadays its cultivation has been encouraged in endemic countries, being successfully on some of them.

In order to evaluate the hybrid phenotypic development and production of artemisinin in São Paulo state, four experiments were carried out between October/2003 and February/2004. Seedlings were produced at CPQBA and plants were transplanted at the same time in Altinópolis, Campinas, Jales and São Carlos cities. Roughly 72 plants, under 1,00 x 0,60m spacing, were cultivated for 80 days and harvested and dried after this period. Biomass production (dry weight) was evaluated and artemisinin content was determinate by HPLC, using a mixture sample on each experimental area, not allowing mean contrast. The results are shown on table 1.

**Table 1-** Biomass (DW) and artemisinin evaluation on 72 plants of *A. annua* cultivated in different regions of São Paulo state.

Region	Biomass (g/plant)	Artemisinin (% w/w)	Artemisinin/plant (g)
Altinópolis	45.35	2.07	0.94
Campinas	113.33	1.86	2.11
Jales	65.39	2.39	1.56
São Carlos	127.45	1.49	1.90

Plants cultivated in São Carlos and Campinas showed better growth and development, resulting in higher biomass production/area. On the other hand, artemisinin concentration was higher in plants cultivated in Jales and Altinópolis. A negative correlation ( $r = -0,99$ ) was observed between biomass and artemisinin content, resulting in a compensation yield on artemisinin by plant.

**Keywords:** *A. annua*, artemisinin, biomass, HPLC



**ISMAP0328 - Agronomic and chemical preliminary evaluation of standardized genotype of *Phyllanthus amarus* for São Paulo state regions.**

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Toxicological and pharmacological studies of native species are currently being carried out with the objective of confirming their traditional medicinal use. *Phyllanthus amarus* Schum. & Thonn. (Euphorbiaceae) and other *Phyllanthus* spp. are well known on folk medicine for kidney stones. Different therapeutic indications have been scientifically evaluated, and prominent results have been observed on antiviral activity. As some scientific confirmation is obtained, an increase in the commercialization of such species, mainly by extraction from their natural environment, is observed. In order to reduce this practice it is necessary to develop production systems, including genotype evaluation. Aiming this objective, four experimental areas were carried out in São Paulo state region, between October/2003 and February/2004. Seedlings from a standardized material of *P. amarus* were produced at CPQBA and plants were transplanted at the same time and design in Altinópolis, Campinas, Jales and São Carlos cities. Roughly 200 plants, under 0,40 x 0,20m spacing, were cultivated for 80 days. After this period, plants were harvested; dried, and had biomass (dry weight), phyllanthine and hypophyllanthine contents determined by HPLC. The results are shown on table 1.

**Table 1-** Biomass (DW), phyllanthine and hypophyllanthine evaluation on 200 plants of *P. amarus* cultivated in different regions of São Paulo state.

Region	Biomass (g/plant)	Hipophyllanthine (% w/w)	Phyllanthine (% w/w)
Altinópolis	20.75	0.19	0.66
Campinas	20.62	0.13	0.52
Jales	16.97	0.24	1.00
São Carlos	18.80	0.16	0.60

All 4 evaluated areas represent a good potential for *P. amarus* production, as verified by biomass production and lignans contents. However, precocity was observed on *P. amarus* cultivated in Jales and these plants also showed higher levels of hipophyllanthine and phyllanthine. These results could be associated to growth under hotter and drier climates.

**Keywords:** *Phyllanthus amarus*, hipophyllanthine, phyllanthine, HPLC



**ISMAP0147 - Breeding strategy to increase oil yield and quality in Sweet Basil  
(*Ocimum basilicum* L.).**

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Tulsi (Sweet Basil), the Kalpabrikshs, is an aromatic herb that belongs to the genus *Ocimum*. It is distributed in the tropical and warm temperate regions of the world. Tulsi is considered to be a sacred plant in our country which has lots of uses in Ayurveds, Homeopathy, Naturopathy and even Aroma therapy. It is herbaceous, branched and annual plant of Indian origin (Bakhru, 1990) used all over the world. All parts of the plant have medicinal properties. The fresh leaves, inflorescence and roots are used either fresh or dried as powder or boiled as an infusion. The other important component is basil oil. The composition of essential oil varies in terms of quality and quantity among the different parts depending upon the habitat, package of practices and season of its collection. Chemically it contains eugenol, linalool, methyl chavicol and eugenol methyl ether.

Genetic variability, correlation coefficient and path analysis of oil yield and quality traits in Sweet basil (*Ocimum basilicum* L.) was carried out using 18 accessions at CCS Haryana Agricultural University, Hisar, India. The results indicated highly significant variation for all the characters studied. The highest genotypic and phenotypic coefficient of variation was observed for oil content, oil yield, linalool and methyl chavicol. High heritability coupled with genetic advance was observed for all the characters studied indicating the presence of additive gene effects for these characters. Herb yield per plant, herb yield per hectare and oil content exhibited positive and significant correlation with the oil yield. Accessions HOB-99-9, EC-338775, EC-338785, EC-338776, EC-338773, HOB-99-2, EC-291415 and HOB-99-13 were found to be high oil yielder and high methyl chavicol content whereas accessions EC-338788, EC-338772, EC-338794, EC-312204, HOB-99-6 and EC110264 were high oil yielder and high linalool content. Results showed a direct correlation between the appearance of linalool, methyl chavicol and leaf colour, stem or flower tissues represent the presence of oil content and quality.

**Keywords:** Correlation Coefficient, Heritability, Sweet basil, Variation



# **ISMAP0388 - Effect of competition on biomass and essential oil production of six *Aloysia citriodora* accessions.**

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Lemon verbena (*Aloysia citriodora*) is a perennial, arbustive and native species of South America. This species possess glandular trichomes located on the leaves that produce essential oil. Our objective was to evaluate the effect of competition on biomass and essential oil production of six *Aloysia citriodora* accessions. A field experiment was carried out at the Faculty of Agronomy of the University of Buenos Aires, Argentina. Accessions selected from two locations of Chile and four locations of Argentina were planted in a factorial design with five replications during spring summer 2002/3. The factors were accession (6 locations) and competition (with and without). Experimental units were harvested at full flowering. The biomass and essential oil concentration of leaves and inflorescences was determined. Leaves and inflorescences were hydro distilled for 1.30 hours using a Clevenger glass apparatus according to the European Pharmacopoeia method. Data was analysed with ANOVA. Not differences were detected for biomass and essential oil interaction (Table 1). Biomass and essential oil yield differed among accessions ( $P=0.02$ ,  $P=0.03$ ). "Experimental" accumulated less biomass than others accessions, while Mendoza yielded more essential oil concentration than Rancagua and Talca (Table 1). In competence, the biomass was significative different ( $P=0.0001$ ). In summary these results show that the behaviour among the different accessions are similar in competence. The differences in biomass and essential oil yield reflect genetic differences among accessions.

**Keywords:** *Aloysia*, biomass, essential oil yield.

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**Table 1:** Mean biomass and essential oil concentration of six accessions of *Aloysia citriodora*

Location	Means	
	Biomass (gr/plant)	Essential yield oil (%)
Accessions		
Mendoza	12.50	3.12
San Luis	11.72	3.00
Botanico	11.82	2.82
Experimental	9.40	2.98
Talca	11.39	2.76
Rancagua	10.81	2.70
P=	0.02	0.03
Intraespecific competition		
Without	16.02	3.20
With	5.30	2.80
P=	0.0001	0.27
Interaction Accession x Competition P=	0.83	0.75



**ISMAP0339 - Evaluation of morphological and agricultural characteristics and their phenotypic correlations in genotypes of a *Pfaffia glomerata* (Spreng.) Pedersen population.**

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*Pfaffia glomerata* (Spreng.) Pedersen, Amaranthaceae, occurs spontaneously over the whole Brazilian territory. Their roots are popularly used as tonic, anti-tumoral, aphrodisiac, nutritional complement, under other indications. Because of these uses, the species are drastically collected in the nature, imposing ecological risks to their natural populations. Cultivation is an alternative to ameliorate the ecological pressure that the species is suffering. The aim of this work was to characterize, following morphological and agricultural criteria, one cultivated population of *P. glomerata* and to look at associations among those criteria, in an attempt to find out characteristics that could help to identify promising plants in terms of roots productivity and other characteristics of interest in a breeding program. The evaluated germplasm belongs to a population of *P. glomerata* cultivated at CPQBA-UNICAMP, formed by 73 fourteen months old plants. The phenotypic characterization was made harvesting plants individually and measuring the characteristics shown in table 1. It was obtained the mean, standard deviation, the mean standard error, and the maximum and minimum values for each analyzed characteristic. It was calculated also the Pearson correlation for the characteristics considered of relevance to predict the individual cultivation potential (table 2).

**Table 1:** Basic statistics of the analyzed phenotypic characteristics of *P. glomerata*.

Variable	Number of plants	Mean	Standard deviation	Mean standard deviation error	Maximum values	Minimum values
Number of stems	73	13.11	9.95	1.17	44.00	1.00
Mean stem diameter (cm)	73	10.01	3.08	0.36	18.03	4.34
Plant high (cm)	73	1.71	0.61	0.07	3.40	0.35
Mass of fresh roots (g)	73	866.2	600.7	70.3	3100.0	120.0
Mass of dried roots (g)	73	329.5	232.6	27.2	1255.0	65.0
Aerial part mass (g)	73	1573	2754	322	17200	60
Number of roots per plant	73	14.15	5.81	0.68	20.00	1.00
Harvest index (%)	73	0.49	0.19	0.02	0.93	0.05

**Table 2:** Phenotypic correlations for the characteristics considered of relevance in a breeding program of the species *P. glomerata*.

Variables	Correlation coefficient of Pearson	Probability
Plant high x mass of the fresh aerial parts	0.536	< 0.001
Mean stem diameter x Mass of dried roots	0.371	0.001
Plant high x Mass of dried roots	0.469	< 0.001
Number of stems x Mass of dried roots	0.558	< 0.001
Number of stems x number of roots	0.427	< 0.001
Plant high x harvest index	-0.656	< 0.001
Aerial part mass x harvest index	-0.605	< 0.001
Number of stems x harvest index	-0.556	< 0.001

There was an expressive amplitude in the evaluated quantitative characteristics. The negative and positive correlations analyzed provide information of interest in the search for characteristics of agricultural importance for the species *P. glomerata*. The great phenotypic variation existing in this population suggests that it could be achieved expressive gains selecting the population.

**Keywords:** *Pfaffia glomerata*, phenotypic correlations, domestication, cultivation, selection



# ISMAP0338 - Four selection cycles in *Baccharis trimera* (Less.) D.C. aiming their commercial cultivation.

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*Baccharis trimera* (Less.) D.C., Asteraceae, popularly known as carqueja, is a dioic shrub, about 1 m high, perennial, branched from the ground level, with winged stems. It is of common occurrence in great part of South America. Brazil is considered its dispersion center. The species is rich in flavonoids such as apigenin, cirsimaritin, eupatorin, genkvanin, hispidulin and quercetin. Other chemical constituents found in the species are essential oils with carquejol, carquejilacetat and <sup>2</sup>-pinene as main constituents. In the folk medicine the aerial part of the plant is used against liver, stomach and intestine problems, besides cases of fever, influenza, common cold and rheumatism. The bitter constituents of the plant are also used in the drink industry. It is a not domesticated species and the wild collection is the main way to get the raw material to be used by the industry. To avoid the ecological problems caused by the wild collection and in the same time to improve the quality of the raw material and offer to the farmers a new agricultural option, we are conducting a domestication process of the species at CPQBA-UNICAMP. Three different populations, obtained from seeds from three different regions (Joaquim Egídio, Andradas and CPQBA), formed a unique population that was conducted through four generations selected following the massal method with gametic control. Each generation were selected in two steps: first at the greenhouse, where the seedlings were grown, and after that at the experimental field. At the greenhouse were selected plants that not lie down, with rapid germination and vigorous growth. At the field the plants were selected for not lie down plants, good vigour and regrowth after the harvest. The selection at the greenhouse were made 4 months after sowing, when the seedlings size were measured. The selected plants were than planted in the field where, after 12 months from planting, they were cut at 30 cm from the ground. Then, after three months, their regrowth capability was evaluated and those who do not present good regrowth were rooting out. The selected plants completed their reproductive cycle and the seeds were used to compose the next generation to be selected. The total time of each selection cycle was 24 months. The basic statistics, as well the progresses achieved in this process can be observed in table 1:

Table 1:

Population	Seedlings height mean (cm)	Seedlings height mean Standart Deviation	% of lie down plants (greenhouse)	% of plants selected (field)
Joaquim Egídio	19.165	4.461	15.25	5.83
Andradas	14.683	3.244	20.08	10.83
CPQBA	13.723	5.963	15.71	2.5
F <sub>1</sub>	16.08	8.645	17.84	26.08
F <sub>2</sub>	19.58	6.876	23.21	37.72
F <sub>3</sub>	26.97	4.653	10.71	57.9

The data shows that the selections made at the greenhouse improved the height of the seedlings trough the generations. This improved height could reflect the selection gains for faster growing plants, as well as an increasing number of plants without seed dormancy and therefore germinated and developed faster than those with dormancy. Both cases are very desirable for the *B. trimera* cultivation. However, the selection aiming erect plants did not seem to be efficient. The increase of selected plants in the field reflects the uniformity level obtained by the different generations, showing a clear progress in the direction of vigorous and good regrowth capability after harvest.

**Keywords:** *Baccharis trimera*, selection, domestication, cultivation



**ISMAP0364 - Influence of genotype and environment on secondary plant compounds of Chaste Tree (*Vitex agnus-castus* L.).**

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Chaste tree (*Vitex agnus-castus* L., Verbenaceae) is a shrub with its natural habitat ranging from the Mediterranean region to Central Asia. The fruits were formerly used as a substitute for pepper from Italy to Eastern Georgia, a use which is still reflected e.g. in some Italian synonyms of the plant's name ('albero del pepe', 'pepe falso'). Further application possibilities include ornamental purposes, as a fibre plant (also for basketry) and especially as a medicinal plant.

When it comes to medicinal plant uses, the dried fruits of *Vitex agnus-castus* are base raw materials for preparations against pre-menstrual disorders due to primary and secondary corpus luteum insufficiency, mastodynie and menopausal complaints.

Eight different accessions from the Mediterranean were transplanted on the Spanish Island of Mallorca and examined and sampled for three years to study the influence of genotype and environment on important traits of chaste tree.

Chemical traits analysed were the marker substances agnuside (an iridoid glycoside) and casticine (a flavonol glycoside) as well as diterpenes and the essential oil composition.

The content of agnuside was strongly depending on environmental influences and oscillated more between the experimental years than between the accessions. Casticine was in contrast to agnuside hardly influenced by the environment, while the accessions showed significant differences in its content. The reaction concerning the diterpenes was well balanced between agnuside and casticine. There were as well significant differences between the accessions as well as significant differences between the experimental years.

All three compounds were additionally characterised by a significant genotype x environment interaction.

The results clearly indicate the necessity to select for stability rather than to follow blindly selection to optimise yields of secondary compounds in one environment only.

**Keywords:** chaste tree, *Vitex agnus-castus*, genotypes, environmental influences



**ISMAP0165 - Phytochemical and genetic variability of *Casearia Sylvestris* Sw. from São Paulo State Atlantic Forest and Cerrado populations.**

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Two botanical varieties have been considered in *Casearia sylvestris* species: *C. sylvestris*, *lingua* variety with a shrubby characteristic, occurring in bushy savannah and *C. sylvestris*, *sylvestris* variety with an arboreous type in forest or shrubby vegetation. The limits of these varieties based on morphological parameters are very tenuous, making molecular and phytochemical markers very important to identify.

This work aimed to identify genetic and chemical differences between *lingua* and *sylvestris* varieties in six natural populations of *Casearia sylvestris* from Cerrado and the Atlantic Forest in São Paulo state, using RAPD (Random Amplification of Polymorphic DNA) molecular marker and chemical markers. Samples have been collected in order to analyze geographic location data (altitude, latitude and longitude). DNA extraction was performed using 100 mg of leaves through the CTAB (cetyltrimethylammonium bromide) procedure. Nine primers were used for the technique application. The dendrogram was obtained from the matrix of pairwise distances through the Jaccard's similarity index using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) grouping method. A Mantel test was used to analyze correlations between genetic and geographical distances, while the molecular variance analysis and the Shannon index were used to determine the intra- and interpopulation divergences. For the phytochemical study leaves of the species were submitted to extraction in methanol and rutin quantification was carried out using a high-performance liquid chromatographer.

Practically all species variability are represented by the intrapopulation variability and the grouping analysis showed higher similarity among populations of the same biome, revealing a genetic diversity between the two botanical varieties.

The majority of soils where collected "guaçatonga" is present is of high acidity and predominantly of medium texture. Nevertheless, most soil indicator values showed diversity among populations of savannah and forest.

There is a high variability in the rutin content among accessions within and among the study populations. The proposed comparisons among populations and the two study biomes in relation to the rutin content showed that the savannah populations have presented significant higher values. A positive correlation between altitude and rutin content has been clearly shown, i.e., higher altitudes are associated with higher rutin content and savannah biome.

In order to establish a germplasm bank of the species, it is necessary to collect a higher number of individuals within each population so that the existing variability in the species could be represented. On the other hand, for "in situ" conservation, the population maintenance with a great number of individuals has been necessary.

**Keywords:** *Casearia sylvestris*, Flacourtiaceae, RAPD, rutin.

**Sponsor:** FAPESP.



# ISMAP0365 - Polymorphisms of *cis*-Sabinene hydrate and *cis*-Sabinene hydrate acetate in a species hybrid between Marjoram and Oregano.

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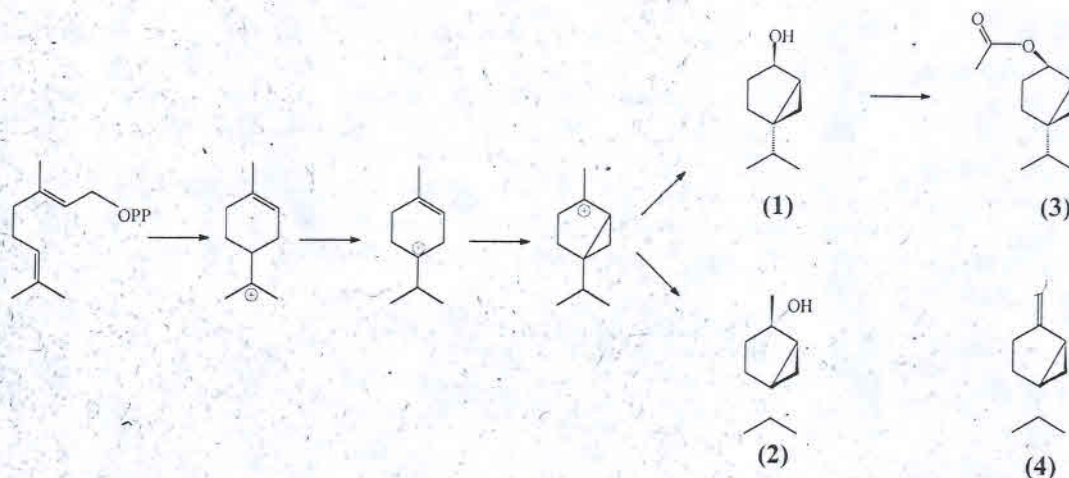
*Cis*-sabinene hydrate, a bicyclic monoterpene alcohol, and *cis*-sabinene hydrate acetate are character compounds of marjoram (*Origanum majorana* L. syn. *Majorana hortensis* Moench., Lamiaceae), responsible for the very distinct 'marjoramy' flavour of this aromatic plant. *Trans*-sabinene hydrate is present in marjoram too, but sensorially completely different ('mouldy'). Both, *cis*-sabinene hydrate and *trans*-sabinene hydrate are formed by one enzyme (sabinene hydrate synthase) and present in marjoram at always the same ratio<sup>1,2</sup>.

Within the genus *Origanum*, hybridisation occurs quite often, a fact that we used to establish a species hybrid between marjoram (*Origanum majorana*) and oregano (*Origanum vulgare*). In the segregating F<sub>2</sub>-generation, *cis*-sabinene hydrate followed a clear Mendelian segregation of 3:1 indicating that one gene is responsible for its formation that was not present in the oregano parent. *Cis*-sabinene hydrate acetate, too, followed this segregation of 3:1, but only in the presence of *cis*-sabinene hydrate. When *cis*-sabinene hydrate was absent, its acetate could not be formed, thus proving the necessity of *cis*-sabinene hydrate as direct source for the formation of the acetate. The formation of the acetate was very source specific, since *trans*-sabinene hydrate acetate was never identified.

**Keywords:** marjoram, oregano, *Origanum*, *cis*-sabinene hydrate

<sup>1</sup> Hallahan, T.W., R. Croteau, 1988. Monoterpene biosynthesis: demonstration of a geranyl pyrophosphate:sabinene hydrate cyclase in soluble enzyme preparations from sweet marjoram (*Majorana hortensis*). Arch Biochem Biophys 264: 618-631.

<sup>2</sup> Novak, J., Ch. Bitsch, J. Langbehn, F. Pank, M. Skoula, P. Gotsiou, Ch. Franz, 2000. Ratios of *cis*- and *trans*-sabinene hydrate in *Origanum majorana* L. and *Origanum microphyllum* (Benth.) Vogel. Biochem Syst Ecol 28: 697-704.



**Figure 1.** Biosynthetic "route" to the formation of *trans*-sabinene hydrate and *cis*-sabinene hydrate (Hallahan and Croteau, 1988) (modified and conversion of *cis*-sabinene hydrate to *cis*-sabinene hydrate acetate added by the author). (1)...*cis*-sabinene hydrate, (2)...*trans*-sabinene hydrate, (3)...*cis*-sabinene hydrate acetate, (4)...sabinene



**ISMAP0029 - Selection of superior lines of *Mucuna pruriens* for improved yield, quality traits and L-Dopa through breeding methods.**

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Seed of *Mucuna pruriens* is used as a natural source material for the treatment of Parkinson's disease. Among all plants, the legume family (particularly *Mucuna* species) received greatest attention for high L-Dopa (L-3, 4-Dihydroxyphenylalanine) content in the seeds. The seed is used as nerve tonic and aphrodisiac. Seed materials available from wild source in India were found to possess low quality and yield, variation in L-Dopa and showed itching trichome on pods which make it unsuitable for raising commercial cultivation. Therefore, the present study has been undertaken during 1996-2002 to develop superior lines associated with improved seed yield, seed quality, L-Dopa (in seeds) and with devoid/low intensity of itching trichomes on pods. 17 germplasm of *Mucuna pruriens* collected from wild growth have been characterized and used as parents in the breeding trials for developing hybrid progeny ( $F_1$ ) and back-cross generations.

Two selections for hybrid progeny have finally been identified from amongst 69 hybrids after detailed evaluation. Both these lines have with white colored seeds, medium-bold in size (seed) weighing 86-103 gm/100 seeds, yielding 491-532 gm (seed)/plant containing 5.5-5.6% of L-Dopa content. Finally, 3 promising selections through back-cross lines (BC) have been shortlisted from the 14 desirable lines initially shortlisted and evaluation for their seed associated characteristics. These 3 lines were found to have white colored seeds, medium-bold seed size (72-114 gm/100 seeds), medium to high seed yield (424-810 gm/plant) and > 4.5% L-Dopa content in seeds. Nutritional test conducted on these three lines showed that 56-58% carbohydrates, 25-27% protein and 5-6% fat. Minerals like calcium (204-353 mg/100 gm), phosphorous (254-256 mg/100 gm), iron (117-453 mg/kg), potassium (10-12 mg/kg) and sodium (167-261 mg/kg) have also been measured on these three lines. These 3 improved lines will be tested at multi-locations for bulk seed production and economics. The detailed yield and quality traits of these improved lines will be discussed.



**ISMAP0039 - Studies on evaluation and hybridization in *Coleus forskohlii* Briq.  
an endangered Medicinal Plant.**

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Eleven accessions of *Coleus forskohlii* Briq. collected from different parts of India were evaluated under replicated trial for morphological and yield characters. Studies showed wide variability among the collections, with accession 'K' as a superior and non-flowering type for tuber yield. Accession B, C and D were superior for harvest Index (HI). Intervarietal and backcross hybrids involving non-tuberous and tuberous types showed lack of crossability barrier and hybrids were inferior in tuber yielding character.

**Key Words:** *Coleus forskohlii*, forskolin, Hybridisation, tuberous roots



**ISMAP0083 - The biotechnological performance of soft-flesh hybrid fruit *Capsicum frutescens* L. x *Capsicum annuum* L.**

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Soft-flesh *Capsicum* fruits may be an interesting raw material for production of biologically active food, including nutraceuticals. They may be used for production of juices or pulps, and separation of edible parts of the fruit from inedible ballast, that is, the placenta with the seeds and the skin, consists in mechanical sieving of the pericarp. Thus, it is not necessary to apply high temperature treatment that adversely affects food quality. Active effect of the product, obtained from soft-flesh fruits, is in the first place determined by the content of capsaicinoids and antioxidant compounds. Creation of hybrids and selection of hybrid materials aimed at obtainment of genotypes combining the soft-flesh feature conditioned by the dominant *S* gene (*C. frutescens*) with high yield and good quality of crop fruits of *C. annuum* L. The research material was constituted by selected forms of  $F_2$  generation of interspecific hybrids *C. frutescens* L. x *C. annuum* L. Genotypes identified with symbols SF 32 and SF 33 were characterized by sweet flesh. The third one, SF 34 was distinguished by high content of capsaicinoids and hot taste of fruits. The yielding oscillated within a wide range. The highest yield of physiologically mature fruits were obtained from SF 34 genotype plants (Fig. 1). They were characterized by the lowest technological weight, that is, the weight of the fruit following removal of the pedicle along with the fleshy calyx sepal. Fruits prepared this way form a raw material that, when washed, may be subject to the process of separation of flesh from inedible parts. The average technological weight of fruits oscillated from 28 g with SF 34 to 104g with SF 32. Picking of small fruits is not labour-consuming because *S* gene conditioning the soft-flesh feature is of a pleiotropic nature, and the second feature it is responsible for is easy separation of the pericarp from the calyx. Fruits can be shaken off the plants. Apart from size, the average weight of fruits was determined by thickness of the pericarp walls reaching 6.31 mm with the heaviest fruit genotype. The latter feature had the greatest effect on biotechnological performance of fruits understood as the percentage share of the soft pericarp tissue obtained as a result of mechanical separation from the placenta with seeds and the skin of fruit in its weight. As indicated by the data of Figure 2 and mentioned above the highest biotechnological performance was characteristic of the fruit genotype with the highest average weight. The lowest biotechnological productivity was characteristic of fruits with the lowest technological weight. It means that along with decreasing weight of the fruit, the share of parts reducing its biotechnological performance relatively increases.

**Keywords:** capsaicinoids, technological weight, yield



**ISMAP0047 - Up to date results in developing poppy (*Papaver somniferum* L.) cultivars of diverse chemical character.**

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Since the isolation of morphine by *Sertürner* in 1805 more than 40 alkaloids have been isolated from the poppy (*Papaver somniferum*). The increasing demand for poppy alkaloids is the consequence of the widening of the medical application of morphine and its related compounds. However, the production of raw (either opium, or dried capsule is produced) has to be re-evaluated by countries interested in the production taking into consideration the UN Convention signed in 1988. This Convention forced the countries not only for controlling their cultivation methods and patrolling cultivation areas, but to build up a new strategy for making cultivars. Based on this strategy – applying different form of breeding (selfing, individual and population as well as accelerated selection under environmental pressure in phytotron, etc.) - new cultivars were developed and registered by us representing three different types of chemical character (Table 1.)

- Cultivars accumulating high morphine (18.0 – 20.0 mg/g), or special alkaloid content (narcotine, codeine, thebaine e.t.c.) for industrial extraction,
- Cultivars for seed and oil production, accumulating low level of alkaloids in the capsules (the amount of morphine is about 0.1 mg/g or less)
- Ornamental types with special flower or capsule form, accumulating restricted amount of alkaloids



**Figure 1.** Cultivar 'Ametiszt' selected for low alkaloid content in flowering stage

**Table 1.** Alkaloid patterns of cultivars

Name of the cultivar	Morphine (mg/g)	Codeine (mg/g)	Thebaine (mg/g)	Narcotine (mg/g)
a) 'Kék Gemona'	7.0 – 9.0	1.0 – 2.0	0.0 – 1.0	12.0 – 13.0
'Monaco'	7.0 – 10.0	4.0 – 6.0	2.0 – 3.0	-
'Tebona'	7.0 – 10.0	4.0 – 6.0	3.0 – 6.0	-
'New candidate'	18.0 – 22.0	2.0 – 3.0	4.0 – 8.0	-
b) 'Ametiszt'	0.1	-	-	-
c) 'Oriés Kék'	3-4	tr.	-	-

**Keywords:** selection, morphine, codeine, thebaine, narcotine

**Sponsors:** Hungarian Foundation OTKA T0 32393, OM 0005/2001



# **Agrotechnology (Production and Post Harvest)**



**ISMAP0134 - *Arrabidaea chica* verlot plants from stem cuttings in water.**

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As part of our research on the "chain of production" of economically important medicinal plants from the Amazon region, we have been studying different methods for the reproduction, standardization and industrialization of the species *A. chica*, or crajirú, as it is known popularly in Brazil. As this plant does not flower in this region, methods based on seeds are obviated. In this region, the local people grow crajirú from cuttings in small quantities, usually for personal consumption. The leaves are generally used in traditional medicine based on anti-inflammatory and astringency actions associated with this plant, in the treatment of intestinal cramps, bleeding during diarrheas, vaginal infections, anemia and leukemia. Our interest in large-scale production of *A. chica* is strategic, given its current and potencial exploration in cosmetics and phytotherapeutic agents. In this report, the objective was the production of young, viable plants from leafless (rootless) stem cuttings in water. We were induced to perform this systematic study after inicial trials showed fast root growth from stem cuttings in water. Stem diameter was varied systematically so as to establish parameters for stem selection in future larger-scale cultivation. All stem cuttings were 15 cm in length and had four distinct average diameters (T1 1.1, T2 0.5, T3 0.3, T4 0.2 cm), being prepared from stock plants located in the CPPN/INPA medicinal plant garden. For each stem size, 6 repetitions of 10 stems each were used. On July 20, 2003 (dry season), stems were placed in clear PET (polyethyleneterephthalate) soft drink container (top portion cut away) bottoms with 0.3 L capacity containing only common well water. The experiment was conducted in the greenhouse at CPPN under partial sunlight filtering conditions (sombrite) and daily irrigation. After ca. 90 days, the following variables were evaluated: % established plantlets, dry weight (g) of leaves and roots produced, no. and length (cm) of sprouted plant. The results were submitted to the Tukey Test (5 %). Among the most important results was the 95 % survival of stem cuttings with initial diameter of 1.1 cm (Table). Stems of smaller diameter presented lower survival rates, there being no statistical difference between the survival rates of T3 and T4. The largest growth of new shoots (18.6 cm / stem) was observed for T1, while T4 showed the least growth (2.6 cm), and T3 showed growth which did not differ statistically from T2 or T4. The largest (T1) and the smallest (T4) diameter stems differed significantly in the number of new shoots produced, however, stems of intermediate diameter did not produce statistically different numbers of shoots. Leaf production (based on dry mass) was highest in T1 stems which was statistically larger than for the other treatments. No significant difference was observed in the leaf mass of T3 and T4 stems. The dry weight of roots was not significantly different for T1-T3, however, T3 and T4 produced significantly different root masses, 1.6 and 0.7 g, respectively. Based on these results, it is concluded that the largest diameter stems (T1), which were obtained from the stem base of the stock plants, are overall the best material for the production of *A. chica* plantlets in water.

**Keywords:** Bignoniaceae, crajirú, stem cuttings.

**Sponsor:** CNPq, FINEP 1784/02.

**Table.** Averages of *Arrabidaea chica* plant growth parameters after 90 days in water, as a function of initial stem cutting diameter. INPA – Manaus/AM, 2004.

Treatment	Diameter (cm)	Survival (%)	Shoot length (cm)	Shoots / stem	Dry weight	
					Leaves	Roots
T1	1.1	95.0a	18.7a	2.3a	0.87a	1.75a
T2	0.5	78.3b	5.1b	2.2ab	0.39b	1.69a
T3	0.3	56.7c	3.8bc	2.0ab	0.22c	1.58a
T4	0.2	50.6c	2.6c	1.6b	0.14c	0.74b
SD		13.4	1.5	0.6	0.16	0.63
CV(%)		11.8	12.2	18.6	24.7	27.0



**ISMAP0264 - Effect of cold stress on surviving, development, yield and physical-chemical quality of chamomile from two origins.**

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The objective of the present trial was to evaluate the effects of low temperatures on the surviving of chamomile seedlings originated from two different places, local and imported, as the development, yield and final quality of vegetal raw materials. Forty seedlings of 7 to 10cm high from two locals were transferred to a germination chamber (BOD) during 24 hours in the following conditions: 10h with light and 15°C, 8h in the dark and 10°C, 3h in the dark and 5°C, and the last period of 3h in the dark and temperatures ranging from 0 (check), -2, -4, and -6°C. The local seedlings were more susceptible to the temperatures of -4 and -6°C. There were more flowers per plant (462% superior) and dry matter accumulation per plant (226% superior) in the plants originated from local seeds than the imported ones. The plants of chamomile began regrowth with a greater yield when submitted to temperatures of -6°C. The local and imported plants showed same concentration of quercetin.

**Keywords:** *Matricaria chamomilla*, medicinal plants, frost, resistance.

**Sponsor:** CAPES



# ISMAP0199 - Anatomy and effect of density and plant arrangement of plants on *Calendula officinalis* L. yield.

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Part of the thesis of Master in Agronomy of the first author presented to UFMS. <sup>1</sup>Awarders of productivity scholarship in research of CNPq.

*Calendula officinalis* L. is known by pot marigold, "malmequêr", "maravilha" and "malmequêr-dos-jardins". It has healing action, antiseptic and anti-inflammatory, among others. The experiment was carried out at Medicinal Plant Garden – HPM of the Federal University of Mato Grosso do Sul – UFMS, in Dourados-MS, from April to December, 2003. The objective was to describe anatomy and to evaluate calendula yield as a function of density and arrangement of plants. Four populations (60,000; 80,000; 100,000 and 120,000 plants ha<sup>-1</sup>) and two arrangements of plants (three and four rows per plot), which corresponded, respectively, to the following spaces between plants: 33.0; 24.7; 19.8 and 16.5 cm and 44.0; 33.0; 26.5 and 22.0 cm, besides spaces of 36.0 and 27.0 cm between rows, were studied. Treatments were arranged in a 4 x 2 factorial scheme, in a randomized block experimental design, with four replications. Anatomy of root, stem, leaf and disseminules was described qualitatively. Plant heights increase linearly in answer to dates of evaluation, from 11.22 cm to 41.53 cm since 15 until 105 days after transplant. Maximum yield of fresh biomass of aerial parts of plants was of 20,037 kg ha<sup>-1</sup>, under population of 91,037 plants ha<sup>-1</sup>. Dried biomass was not influenced by treatments and it was in average of 4,532.38 kg ha<sup>-1</sup>. Number of capitula decreased with the increase of rown in plot and it increased linearly with the increase of populations, which were 5.48 millions with 120,000 plants ha<sup>-1</sup>. Yields of fresh and dried mass of capitula were not influenced by treatments, which were, in average, of 4,565.88 and 834.50 kg ha<sup>-1</sup>, respectively (Quadro 1). Diameter and height of capitula decreased linearly as a function of harvest date (Figura 1).

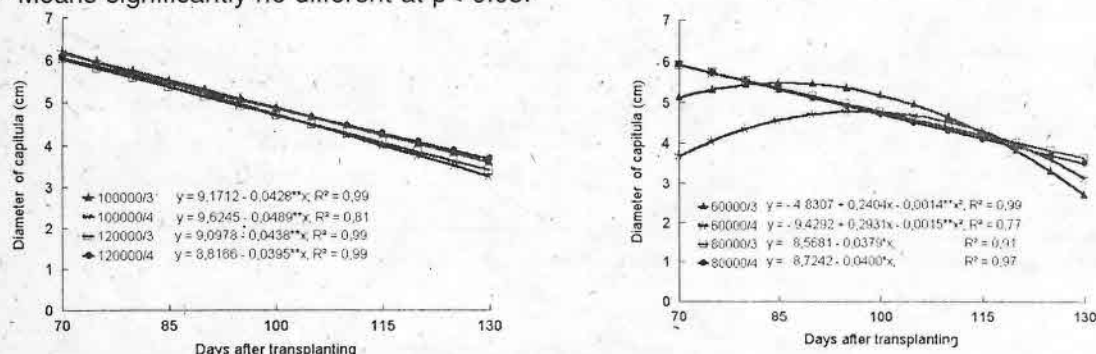
**Keywords:** Asteraceae, medicinal plant, population density, vegetal histology.

**Sponsor:** FUNDECT/CNPq.

**Table 1.** Fresh and dried biomass total (kg ha<sup>-1</sup>) e unit biomass (g capitula<sup>-1</sup>) of the capitulas of calendula plants, cultivated in four density and three or four rows per plot. Dourados, UFMS, 2003.

Density	Total fresh biomass	Total dried biomass	Unit biomass
60.000	4040,83 a <sup>1/</sup>	725,28 a	0,17 a
80.000	4344,26 a	835,42 a	0,17 a
100.000	4623,47 a	872,13 a	0,18 a
120.000	5254,95 a	905,18 a	0,16 a
<b>Rows</b>			
3	4860,90 a	862,59 a	0,17 a
4	4270,85 a	806,41 a	0,17 a
CV (%)	51,35	48,96	12,56

<sup>1/</sup> Means significantly no different at p< 0.05.





**ISMAP0112 - Biomass and essential oil variation of *Baccharis trimera* (Less) A. P. de Candolle submitted to different nitrogen fonts and concentrations.**

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*Baccharis trimera* (Less) A. P. de Candolle (Asteraceae) is a native specie from Uruguay, North of Argentina, Paraguay and Brazil. It is known in brazilian folk as carqueja and used to treat gastrointestinal and liver diseases, angina, poor blood circulation, diabetes and inflammatory process. The aim of this work was the evaluation of the influence of nitrogen font and concentration on the biomass, essential oil production and presence of other phytochemical compounds. The mineral and organic font of this element was used in the Experimental Station of Canguiri (UFPR), Brazil, with experimental design in random blocks with four repetitions and seven treatments. The distance within plants were 0,5m and 1m between parcels, evaluating a total of 224 plants. The treatments consisted of manure at 4, 8 and 16 grams of nitrogen/plant, urea was used as control in the same concentration. Fertilization was done in two times within interval of two months. Plants were collected nine months after transplant on field, harvesting aerial part upper 30 cm from the soil, then were conducted to the Laboratory of Ecophysiology (UFPR) for weighting green matter and dry matter 17 days after dried on shadow conditions. Essential oil was obtained by hidrodestillation, in a Clevenger apparatus during four hour using 100g of dry plant. The results for oil quantity and dry matter for plant and hectare did not show any statistical difference between treatments when means were compared with Tukey test (5%). The oil from each treatment is being conducted for qualitative analyses using GC-MS. Analysis of plant extracts showed the presence of glycosides antocianic, volatile and fixed acids, tannins, tannins hydrolisables and condensed (20%, aqueus extract); and amino groups, glycoside flavonics and esteroids and/or triterpenoids (20%, ethanolic extract). Which ones were determinate at the Phytochemistry Laboratory (UFPR).

**Keywords:** Medicinal plant, nitrogen fertilization, phytochemical analysis, productivity.

**Table 1.** Dry matter and oil yield per hectare and per plant.

Treatments	Yield / Hectare		Yield / Plant	
	Oil Liters	Dry matter Tons	Oil mL	Dry matter Grams
Control	56,27 <sup>a</sup>	3,97 <sup>a</sup>	2,12 <sup>a</sup>	149,58 <sup>a</sup>
4 gr N manure/plant	65,78 <sup>a</sup>	4,27 <sup>a</sup>	2,47 <sup>a</sup>	160,54 <sup>a</sup>
8 gr N manure/plant	78,74 <sup>a</sup>	4,73 <sup>a</sup>	2,68 <sup>a</sup>	178,08 <sup>a</sup>
16 gr N manure/plant	67,67 <sup>a</sup>	4,66 <sup>a</sup>	2,74 <sup>a</sup>	175,41 <sup>a</sup>
4 gr N Urea/plant	52,90 <sup>a</sup>	4,22 <sup>a</sup>	2,61 <sup>a</sup>	158,79 <sup>a</sup>
8 gr N Urea/plant	81,87 <sup>a</sup>	5,23 <sup>a</sup>	2,52 <sup>a</sup>	196,83 <sup>a</sup>
16 gr N Urea/plant	67,59 <sup>a</sup>	4,49 <sup>a</sup>	2,54 <sup>a</sup>	168,66 <sup>a</sup>

Means with the same letter in the column have not statistical difference by Tukey test to 5% of probability.



# ISMAP0170 - Biomass and leaf production of Sajonia Fennel (*Foeniculum vulgare* Mill. sweet var.) at initial stages during fall-winter sowing dates.

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Fennel is an aromatic, umbellifer and medicinal plant, which leaves and seeds, even the root have medicinal properties. It is used in pharmacological, food and cosmetic industry; its shoots are consumed as vegetable. The whole plant smells of anise scent. It is a temperate species, native of the Mediterranean region (South of Europe and North of Africa) and Asia Minor. It was introduced into North of Europe by Romans, and into South America by European settlers. It is cultivated in Germany, Southern Europe, South America, China and Japan. Its name comes from Latin, "foenum" that means hay straw due to leaf thinness. Three varieties are mentioned: sweet var. also called "Sajonia fennel", capillaceum var. or "wild fennel" and azoricum var. or "garden fennel". The objective of this work was to analyze phenological and phenometric aspects from sowing to the beginning of budding with the purpose to survey its behavior and adaptability to different environments. Macro plots were carried out in two different environments at Buenos Aires province: Luján (central north region) (Lu<sub>1</sub>) sowing date July 28, 2000 and (Lu<sub>2</sub>) March 27, 2003; Paso (west region) (Pa) sowing date July 2, 2000, Pehuajó (west region) (Pe<sub>1</sub>) sowing date September 13, 2000 and (Pe<sub>2</sub>) June 1, 2001. At vegetative stage (V<sub>4-6</sub>) and beginning of budding (Bfi) were recorded, the number of leaves and fresh and dry aerial biomass over 120 plants. At Bfi the biomass was split in dry leaf biomass and dry stem biomass. According to the length of the different stages, the leaf emerging rate, the growth rate per plant and the development rate were calculated. The number of leaves developed at V<sub>4-6</sub> stage was similar at the different locations and sowing dates (4,93 leaves per plant). At Bfi at Pe<sub>1</sub> the plants showed the number of leaves 26,6% higher than at Lu<sub>1</sub> due to higher temperatures during the crop cycle (Table 1). The biomass production per plant (table 2) was related with temperatures and rainfall. At Bfi the plants showed a low stem-leaf ratio (table 3). The possibility to cultivate Sajonia fennel at different locations and times during the year generates different soil and climate conditions which would influence its vegetative behaviour.

**Keywords:** *Foeniculum vulgare* sweet var., behavior, biomass, vegetative stage.

**Table 1:** The number of leaves and the leaf emerging rate in vegetative stage and beginning of budding stage.

	Pe <sub>1</sub>		Pa	Pe <sub>2</sub>	Lu <sub>2</sub>	Lu <sub>1</sub>	
	V <sub>4-6</sub>	Bfi	V <sub>4-6</sub>	V <sub>4-6</sub>	V <sub>4-6</sub>	V <sub>4-6</sub>	Bfi
Nh plants <sup>-1</sup>	5,3	10	5,2	4,53	4,6	5,0	7,9
Tah (Nhday <sup>-1</sup> )	0,061	0,088	0,071	0,061	0,12	0,05	0,06

**Table 2:** The fresh and dry aerial biomass production in vegetative stage.

	Pe <sub>1</sub> (g plant <sup>-1</sup> )	Pe <sub>2</sub> (g plant <sup>-1</sup> )	Lu <sub>1</sub> (g plant <sup>-1</sup> )	Lu <sub>2</sub> (g plant <sup>-1</sup> )	Pa (g plant <sup>-1</sup> )
Baf	14,99	2,38	4,62	1,99	7,67
Bas	1,764	0,22	0,58	0,26	0,95

**Table 3:** The dry aerial biomass production and stem-leaf ratio in beginning of budding stage.

	Pe (g plant <sup>-1</sup> )	Lu (g plant <sup>-1</sup> )
BSh	4,85	11,44
BSt	1,92	3,44
BSt/BS h	0,39	0,30



**ISMAP0054 - Biomass partitioning and cutting success as influenced by  
 Indolebutyric acid in softwood cuttings of Indian bdellium  
 [*Commiphora wightii* (Arnot.) Bhand.].**

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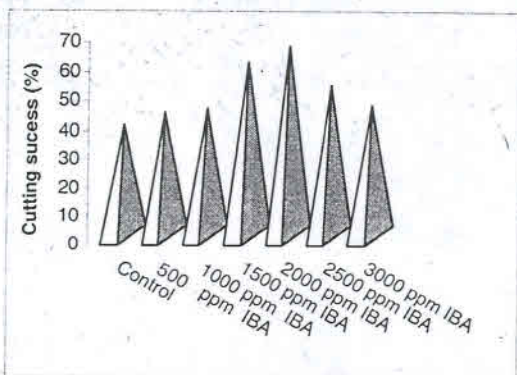
Indian bdellium (*Commiphora wightii*) is a large shrub of the family *Burseraceae* widely growing in arid and semi arid tracts of Rajasthan and Gujarat in India. The indiscriminate and faulty method of gum tapping led to destruction of the plants and consequently it is enlisted in category "A" of the endangered species. Its oleo-resin (gum) exudates from the deep incision(s) afflicted on the basal part of the stem, is used as a source of drug in Indian System of Medicine (ISM) since time immemorial to cure several ailments owing to its anti-inflammatory, antirheumatic, hypocholesteremic, hypolipidemic, and antifertility activities. For regeneration of softwood cuttings, the apical shoots (25 cm) were taken during first fortnight of June (premonsoon) and treated with various concentrations (0 – 3000 ppm) of Indolebutyric acid following quick dip method ( 5 seconds). The treated cuttings were planted in net shed having sandy loam textured soil (Fluventic Ustochrepts). The dry matter accumulation of leaves, twigs (newly emerged branches) and roots were recorded successively on 30, 60 and 90 days after planting. Medial concentrations of IBA (1500 or 2000 ppm) were found to be most effective in enhancing the cutting success and the dry matter accumulation (Table 1 and Figure 2. Softwood cuttings of Indian bdellium could be propagated successfully using IBA 1500 ppm.

**Keywords:** Indolebutyric acid, biomass partitioning, softwood cuttings, Indian bdellium

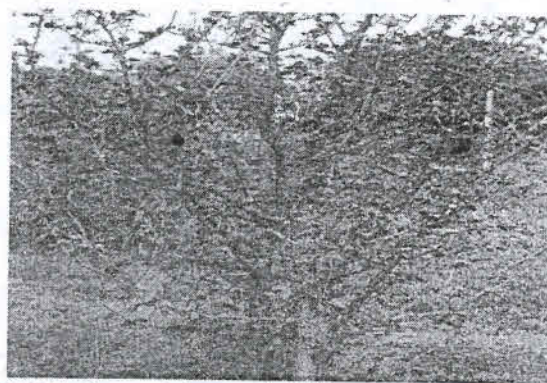
**Table 1** Influence of IBA on dry matter partitioning in softwood cuttings of Indian bdellium

Treatments	Leaf (g/cutting)			Twigs** (g/cutting)			Root (g/cutting)		
	30*	60*	90*	30*	60*	90*	30*	60*	90*
Control	0.03	0.25	0.84	0.35	0.42	0.48	0.07	0.08	0.10
500 ppm IBA	0.05	0.23	0.90	0.35	0.43	0.93	0.07	0.12	0.20
1000 ppm IBA	0.07	0.34	1.03	0.36	0.43	0.94	0.07	0.14	0.21
1500 ppm IBA	0.11	0.47	1.98	0.47	0.54	1.70	0.11	0.23	0.33
2000 ppm IBA	0.12	0.45	1.95	0.49	0.53	1.74	0.10	0.24	0.35
2500 ppm IBA	0.06	0.27	0.95	0.41	0.38	0.57	0.08	0.12	0.17
3000 ppm IBA	0.06	0.24	0.93	0.40	0.39	0.55	0.08	0.10	0.14
Mean	0.07	0.32	1.23	0.40	0.45	0.99	0.08	0.15	0.21
LSD (P=0.05)	0.03	0.09	0.28	0.05	0.11	0.30	0.01	0.05	0.06
SEm	0.01	0.05	0.13	0.02	0.05	0.14	0.005	0.02	0.03

\* Days after planting \*\* woody portion without leaves



**Figure 2** Response of IBA on cutting success



**Figure 1** *Commiphora wightii* image from National Research Centre for Medicinal and Aromatic Plants



**ISMAP0216 - Chamomile [*Chamomilla recutita* (L.) Raeuchert] crop production under organic manuring.**

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Chamomile is the most cultivated medicinal plant in the world and the largest production in Brazil concentrates on Mandirituba - Parana state. Its flower head inflorescence is rich in essential oil used for cosmetic and medicinal purposes. Agronomic studies developed in Mandirituba pointed out the lack of appropriate handling in the use of fertilizers and soil correctives. This represents the principal factor of productivity reduction of the chamomile cultivation. If the agricultural practices influence the production of the cultures, under the qualitative and quantitative aspects, then the cultivation will be able to be benefited by the rational use of organic manuring; specially in the composting form, where the nutrients become available slowly. The objective of this study was to evaluate the effect of different doses of organic compost (manure of cow milk pan and residue of corn silagem, composted for 90 days) in the mass production, plant height of essential oil yield of the chamomile, seeking to obtain a quality raw material. The experiment was carried out in the Municipal district of Mandirituba during 2003. The statistical design was in completely randomized blocs containing four repetitions and six treatments (0, 5, 10, 15, 20 and 25 t.ha<sup>-1</sup>). The analysis of variance of the data was performed and the averages compared by the Tukey's test at 5%. The dry matter production (kg.ha<sup>-1</sup>) increased according to the compost doses. No effect of the treatments was found for plant height and essential oil yield. The economic analysis indicated that the doses between 5 to 15 t.ha<sup>-1</sup> are the most appropriated.

**Keywords:** *Chamomilla recutita* (L.) Raeuchert; nutrition; composting.



**ISMAP0299 - Compounds of several parts of the fennel  
*Foeniculum vulgare* var. dulce.**

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The fennel (*Foeniculum vulgare* Mill.var. vulgare), it is an annual herbaceous plant, whose seeds are very used in the popular medicine. The fennel seeds produce a yellow-clear aromatic essential oil, used in the production of several beverages and cosmetics. Therefore, other structures of the species (stems, leaves, flowers bud, inflorescences) also produce essential oils with commercial interest. An experiment was carried in the farm of the Department of Plant Production of São Paulo State University - Campus of Botucatu-Brazil. The statistical design used was complete randomized block with 3 replications and 7 treatments. The treatments were the harvest time. The seedlings were sowed in summer of the 2001 (January) and harvested every 14 days, until the complete maturation, during one year. The results present high value of trans-anetole (74,95%) in winter in flowers buds, of Limonene (42,30%) in spring in stems/leaves and Fenchone (9,47%) in inflorescence phase in winter.

**Keywords:** fennel, phenological phases, seasons of the year, medicinal plants.

**Supported:** FAPESP: Nº 00/08372-4



**ISMAP0389 - Critical Fe concentration for better growth and productivity  
of Java Citronella.**

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Java citronella ( *Cymbopogon winterianus* Jowitt.) slips were raised in different concentrations of Fe from stress to toxicity levels in controlled glass-house conditions. Fe application stimulated both dry matter as well the fresh herb yield . Fe uptake 5.6 micro gram/ml was required for maximum response in dry matter production and oil concentration. Significant positive correlation was observed in between fresh herb yield and dry matter production with total monoterpene oil(s) and citronellal contents

**Keywords:** *Cymbopogon winterianus* Jowitt., Critical Fe concentration, essential monoterpene oil(s), Citronellal , Nutrient solution.



**ISMAP0024 - Cultivation and post-harvest technologies adopted in India for optimum productivity and economics of some important medicinal plants.**

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Cultivation and post-harvesting technological practices followed for optimum productivity and economics of ten important medicinal plants are discussed. Field trials conducted at the experimental farm of Zandu Foundation for Health Care, Ambach (Gujarat), India by modifying agricultural inputs and practices, and post-harvest operations for improved yield and economics recorded as ranging 3.2-5.5 metric tones dry leaf yield/hectare/6 coppicing (harvesting) in two years crop cycles with 1.0-2.4 % triterpenoid content in *Centella asiatica*, 1.0-1.5 metric tones seed yield/hectare/year with 3.9-4.6% L-Dopa content in *Mucuna pruriens*, 1.3-3.5 metric tones dry shoot yield/hectare/year with > 2.0% Andrographolide content in *Andrographis paniculata*, 1.3-2.0 metric tones dry root yield/hectare/year with > 0.2 % Plumbagin content in *Plumbago zeylanica*, 0.3-0.4 metric tones dry leaf yield/hectare/crop with > 2.5 % Phyllanthin content in *Phyllanthus amarus*, 1.8-5.2 metric tones dry tuber yield/hectare at 19 months in *Asparagus racemosus*, 0.6-1.5 metric tones of dry leaf yield/hectare/year with > 0.4 % Vasicine content in *Adhatoda zeylanica*, 2.0-3.0 metric tones of dry rhizome yield/hectare/year in *Acorus calamus*, 0.6-0.7 metric tones dry root yield/hectare/year in *Withnia somnifera* and 0.8-1.0 metric tones leaf yield/hectare/crop in *Cassia angustifolia* under the various field conditions. The production and post-harvesting technologies standardized and developed in the present field trials for various medicinal plants have been transferred to growers/farmers for large scale cultivation and arranged for bug-back through Indian leading user Industry to purchase their produce. The cultivation practices and post-harvest methods of all ten medicinal plants will be highlighted and discussed.

**Keyword :** Cultivation technology, medicinal plants, optimum productivity and economics



**ISMAP0110 - Determination of drying and dessorption isotherms parameters in carqueja (*Baccharis genistelloides* L.).**

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Carque (*Baccharis genistelloides* L.) has been used in official programmes of public health in the form of infusion, decoction or alcoholic tincture. The storage and drying process of the product must be studied so as to allow the use of dry vegetal raw-material, owing to its greater chemical stability. Therefore, the objective of this work is to describe dessorption isotherms in this species at three temperatures: 30, 45 e 60 °C, so as to check the influence of temperature on water activity and its respective drying curves. The experimental curves have been adjusted according to the following mathematical models: BET, BET LINEAR, GAB, HALSEY, OSWIN, PELEG, HENDERSON, LANGMUIR and CHUNG. The relative error between the experimental values and the estimated values have been calculated in each curve, so as to evaluate the equation that best adjusts to the experimental data. The model presenting the smaller relative error was GAB with 6,76 % at 60 °C, 7,95 % at 30 °C e 11,95, in the model BET LINEAR at 45 °C. The drying processes have been interpreted according to values of diffusivity, being  $1,31 \times 10^{-8}$ ,  $3,44 \times 10^{-8}$ ,  $11,2 \times 10^{-8}$ , respectively for temperatures at 30, 45 e 60 °C.

**Keywords:** water activity, post-harvest, medicinal herb, drying.

**Sponsor:** FAPESP/ CPQBA/UNICAMP



**ISMAP0360 - Development of *Elionurus latiflorus* nees as affected  
by number of tillers.**

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Usually known as "capim barba de bode" or Brazilian lemongrass, *Elionurus latiflorus* Nees (Poaceae) is a species native of South Brazil. It is almost being extinct because destruction of its natural occurrence areas by intensive agriculture.

It can be compared to *Cymbopogon citratus* (DC.) Stapf because its leaves contain essential oil rich in citral and became an alternative way for those who want to obtain essential oil with this compound. It has also a large genetic variability for several characters as weight and length, smell and colour of leaves. Few researches were made with this species in agronomical aspects and the aim of this work was to verify the influence of the number of tillers on development of the species, what brought answers to common questions of growers about development of a new species, such as mean further support to other researches about this plant. The experiment was carried out during the period of September 2003 to February 2004, at Department of Plant Production, Sector Horticulture, Agronomical Sciences College, in Botucatu, São Paulo, Brazil. The statistical design was completely randomized with three treatments and eight replications. The treatments were: T1: one tiller; T2: two tillers; T3: three tillers; and each parcel contained ten plants. The plants were put on sacks with soil, dung and carbonized rice straw in the proportion 3:1:1 and remained on nursery during 160 days. After this period the obtained data were: a) survival percentage: T1=73%; T2=89%; T3=90%. Statistical difference was observed between T1 and the other treatments. b) number of developed tillers: T1= 10,70; T2= 9,92; T3= 9,68. No statistical difference was found among the results. c) height: T1= 82,60cm; T2= 102,82cm; T3= 102,07cm. Statistical difference was observed between T1 and the other treatments. d) dry weight: T1= 2,17g; T2= 3,05g; T3= 2,92g. Statistical difference was observed between T1 and other treatments. As can be observed the number of tillers has influenced the development of the plant. The higher survival percentage, the higher height and the dry weight were obtained in plants with two or three tillers, resulting in a better development, so the number of tillers to initial propagation of the species must be two or higher.

**Keywords:** propagation, plant development, dry weight, tillers.



**ISMAP0271 - Development of guaco cuttings (*Mikania glomerata* Sprengel)  
produced in carbonized rice hulls and humus.**

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Considering the necessity of searching more efficacious technique in production of better quality cuttings of guaco (*Mikania glomerata* Spreng), a study was carried out, at Universidade Estadual de Londrina, Londrina-PR, to evaluate the guaco cuttings quality, in nursering condition, with different concentration of humus in carbonized rice hulls. The treatments were arranged in randomized blocks, with five doses (0%, 5%, 10%, 20% and 40% of humus) evaluated in five seedlings ages (60, 70, 80, 90 and 100 days). Each plot consisted of 30 seedlings. The cuttings were taken of only one matrix, to guarantee the genetic standard and came from different branches parts (15% apex, 70% half and 15% base) for each plot. From 60 days, it was evaluated the morphologic seedling parameters and some relation between them, that made possible the determination of seedling quality. The results showed there were no differences in the seedling quality and development between all tested dosages. The seedling quality improved with aging, seedlings between 90 and 100 days showed the best results.

**Keywords:** Medicinal plants, vegetative propagation, humus.



**ISMAP0046 - Development, biomass production and quercetin-3-sulphate contents in plants of *Cuphea carthagenensis* (Jacq.) MacBride, submitted to different substracts, irrigation systems and leaf pruning intensities.**

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*Cuphea carthagenensis* (Jacq.) MacBride Lythraceae (sete-sangrias) is a herbaceous plant that has been used as a medicinal plant. Previous study demonstrated the isolation of the flavonoid quercetin-3-sulphate from leaves of this plant. While the use of medicinal plants has been increased, there is lack of scientific and technical knowledge to support cropping on a commercial scale. Thus, the objective of this study was to evaluate the effects of different soil substracts, irrigation systems and leaf pruning intensity on the development, biomass production and quercetin-3-sulphate content in plants of *C. carthagenensis*. Analysis of variance (ANOVA) was performed and the differences between treatments were determined using Newman-Keuls' multiple range test ( $P < 0.05$ ). The effects of four distinct substracts (TUS - 50% commercial fertile turf + 50% sand, ATU - 70% argillaceous soil + 30% commercial fertile turf, ASA - 70% argillaceous soil + 30% sand and ATS - 50% argillaceous soil + 30% commercial fertile turf + 20% sand) and two irrigation systems (manual and automatic) were evaluated on the height of plant (HGT), number of leaves (NLE) and flowers (NFL), root length (ROL), total fresh weight and fresh weight of leaves (TFW and FWL), and total dry weight and dry weight of leaves (TDW and DWL). The TUS treatment was able to promote the highest performance of all analysed traits, except root length, when plants were grown with manual irrigation. Thus, *C. carthagenensis* plants demonstrated a better development in fertile and sandy soil, which has a good amount of organic matter, comparatively with other soil substracts. In addition, a leaf pruning experiment was designed with three treatments of pruning intensity (1/3, 2/3 and 3/3). Different leaf pruning intensities affected differently the evaluated traits. In DWL and NFL parameters the averages were superior by removing 1/3 or 2/3 of the leaves. However, statistically similar responses in NLE, TFW, TDW and FWL parameters were obtained in all treatments. These results suggest that the pruning practice can be done in this species as a strategy to increase biomass production. The quercetin-3-sulphate content analyses performed by HPLC demonstrated that there were not significant differences in the content of quercetin-3-sulphate among plants that were cultivated in different substracts (TUS, ATU, ASA and ATS) and in different irrigation systems (manual and automatic). In addition, the leaf pruning did not affect the production of that compound. Taking together these results, we can hypothesize that the different agronomic practices, including those ones that increase biomass, can be used without affecting the production of the quercetin-3-sulfate.

**Keywords:** Sete-sangrias, crop management, quercetin-3-sulphate, medicinal plant.

**Sponsor:** PADCT/CNPq



**ISMAP0253 - Effect of harvest time on the yield of the tannin and flavonoids of *Jacaranda macrantha* Cham.**

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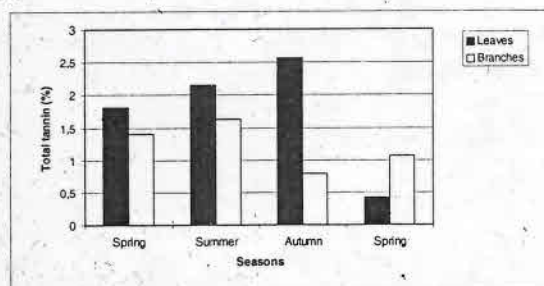
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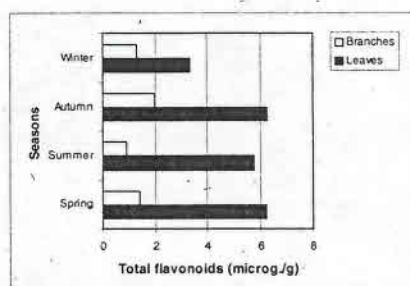
*Jacaranda macrantha* is a semideciduous tree of the family Bignoniaceae, native of Brazil. The leaves and roots of this plant have been used in folk medicinal as depurative, diuretic, anti-rheumatic, antitoxic and in the allergies treatment. This work had as objective to study the tannin and flavonoids production in function of the harvest time of the vegetable parts (leaves and branches), seeking the quality control in the management of natural populations of this species. The harvests were accomplished, monthly, in a secondary atlantic forest fragment, in Viçosa (MG). The tannin amount was determined by the Folin-Denis method and in the analysis of the data were considered the outline of subdivided fractions was considered the fractions were the time (seasons) and the sub fractions the vegetable parts, in the delineation in randomized blocks with 6 repetitions. The determination of the total flavonoids was accomplished HPLC-UV/DAD, adding the areas of all the identified peaks as flavonoids (254 and 337 nm) in the samples obtained in function of the harvest times, with 3 repetitions. The results showed that significant differences exist in the tannin production in function of the harvest time and of the vegetable parts (Figure 1). The flavonoids production was also larger in the leaves (Figure 2) and the cromatogramas of the extracts of leaves and branches showed there is chemical variability, qualitative and quantitative between the times and vegetable parts. A fall was verified in the production of phenolic compounds (tannins and flavonoids) in winter season, being the result in agreement with the used methodology, considering that the Folin-Denis method quantifies total phenols. In the harvest of *J. macrantha* it is just recommended the retreat of leaves, for the low production of active compounds and the low production of branches biomass. The harvest leaves, in agreement with the maxim tannin production and flavonoids, it should be accomplished in the summer season, period that precedes the foliated fall (autumn).

**Keywords:** harvest time, phenolic compounds, non-wood forest products, Atlantic Forest.

**Support:** CNPq



**Figure 1** – Tannin production of *Jacaranda macrantha* in function of vegetable parts and seasons, Viçosa (MG).



**Figure 2** – Flavonoids production of *Jacaranda macrantha* in function of vegetable parts and seasons, Viçosa (MG).



**ISMAP0373 - Effect of Harvest Time and Post-harvest Handling on the Content of Chemical Components in Ginkgo Leaves.**

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Ginkgo (*Ginkgo biloba* L.) leaves have been used for thousands of years in China as an astringent, a remedy for cancer, venereal disease, and on antitussive in asthma. Today, this ancient Chinese tree has been well accepted in the Western world for its memory improvement properties and to prevent or slow the progress of Alzheimer disease. The physiological benefits of ginkgo are generally attributed to its major constituents, ginkgolides, bilobalide, flavonoids, and ginkgolic acids. Ginkgo leaves are generally harvested in the fall before or shortly after leaf fall without consideration that chemical constituent levels may be affected by harvesting time. Information on the appropriate time to harvest leaves with maximum potency is almost nonexistent. In order to address this short coming, a study was conducted in order to provide an indication of when

Two each of male and female trees (> 30-yr-old) were randomly chosen for sampling from a city park in Kelowna, British Columbia. One hundred grams of leaves were hand picked once every two weeks from the same trees throughout the growing season starting from June 10, when the leaves were fully developed, until September 16. Harvested leaves were cleaned and stored in a freezer at -20 °C. In October, leaves were air dried in a forced air dryer at 30 °C for 48 hours, and freeze dried at -65 °C for 48 - 72 hours. Each dried leaf sample was ground in a Wiley Mill to pass a 1 mm screen and ginkgolides and bilobalide were extracted.

The contents of bilobalide and ginkgolides varies during the growing season in both male and female samples. Bilobalide contents ranged from 0.060 - 0.160 % and 0.004 - 0.037 % in freeze dried and 0.038 - 0.172 % and 0.020 - 0.048 % in air dried leaves of male and female trees, respectively. In ginkgolide A, the levels varied from 0.012 - 0.043 % and 0.003 - 0.008 % in freeze dried leaves, and 0.006 - 0.048 and 0.003 - 0.028 % in air dried leaves collected from male and female trees, respectively. The contents of ginkgolide B were small with little variation in either freeze or air dried leaves from male and female trees. On the average, no significant differences between freeze and air dried materials were observed for bilobalide and ginkgolides contents from both male and female trees. However, levels of both bilobalide and ginkgolide A were significantly higher in male than female trees after either freeze- or air-drying. The levels of bilobalide increased significantly starting from the first part of June and peaked in mid to late July in both freeze and air dried leaves of male and female trees and dropped significantly afterwards. In conclusion, The constituent levels varied during the growing season. On average, no significant differences of ginkgolides and bilobalide levels in freeze or air dried leaves. On the other hand, levels of both bilobalide and ginkgolide A were significantly higher in male than female trees after either freeze- or air-drying.

**Keywords:** Ginkgolide A, ginkgolide B, bilobalide, *Ginkgo biloba*



**ISMAP0372 - Effect of Nitrogen Application on Growth and Essential Oil Production of Peppermint ( *Mentha piperita* ).**

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Peppermint (*Mentha piperita* cv. Mitcham) plants were grown in Investigative Field of Ferdowsi University of Mashhad in IRAN (59°N latitude), and evaluated for their response to nitrogen rates. The form of applied nitrogen was urea. Plants were measured for total dry weight, final plant height, nodes number, internode length, total leaves number, and essential oil content in flowering stage. Rates of nitrogen applied were 0 (control), 75, 150, and 225 Kg ha<sup>-1</sup> nitrogen. Each treatment had three replications.

It was established that plant dry matter, plant height, internode length, leaves number, node number, and essential oil content were increased with increasing nitrogen rates (with the exception at 225 Kg ha<sup>-1</sup>N). With the increased fertilizer rates, the essential oil yield of the treated plants increased compared to the control. The highest and lowest essential oil content were shown in the plants that received 150 and 0 Kg ha<sup>-1</sup>N, respectively.

**Keywords:** *Mentha piperita*, Nitrogen Fertilizers, Essential Oil, Peppermint



**ISMAP0184 - Effect of stem size on vegetative reproduction of *Arrabidaea chica* Verlot.**

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The Amazon forests have a large number of medicinal plants, most of which have never been the subject of basic agronomic studies to establish viable methods for reproduction. *A. chica*, or crajirú as it is known popularly in the Amazon region, is grown by the local population in gardens, generally from stem cuttings. The leaves are generally used in traditional medicine based on anti-inflammatory and astringency actions associated with this plant, in the treatment of intestinal cramps, bleeding during diarrheas, vaginal infections, anemia and leukemia. Our interest in large-scale production of *A. chica* is strategic, given its current and potencial exploration in cosmetics and phytotherapeutic agents. In this report, the objective was the production of young, viable plants from leafless (rootless) stem cuttings. Stem diameter was varied systematically so as to establish parameters for stem selection in future larger-scale cultivation. All stem cuttings were 15 cm in length and had four distinct average diameters (T1 1.1, T2 0.6, T3 0.3, T4 0.2 cm), being prepared from stock plants located in the CPPN/INPA medicinal plant garden. For each stem size, 6 repetitions of 10 stems each were used. On July 20, 2003 (dry season), stems were placed in black, 1 kg capacity, polyethylene bags containing a composite substrate prepared in the following proportions: 3 sand, 2 argillaceous clay, 1 organic compost. The experiment was conducted in the greenhouse at CPPN under partial sunlight filtering conditions (sombrite) and daily irrigation. After ca. 90 days, the following variables were evaluated: % established plantlets, dry weight (g) of leaves and roots produced, no. and length (cm) of sprouted plant. The results were submitted to the Tukey Test (5 %). Among the most important results were 100 % survival of stems with initial diameters of 0.6 and 1.1 cm (Table). Stems of smaller diameter, had lower survival rates, there being no statistical difference between the survival rates of these thinner stems. The largest growth of new shoots (35, 2 cm / stem) was observed for the largest diameter stems and the lowest growth of shoots (13.1 cm / stem) was observed in the smallest diameter stems, however, the two intermediate treatments (T2 and T3) did not differ significantly in this parameter. Shoot growth in T2 and T3 did not differ significantly and T4 had the least growth of all the treatments (13.1 cm). Only the largest diameter stems differed from the other treatments in the number of new shoots produced, having an average of 3.3 shoots/stem. The leaf and root dry weights were larger and statistically significant for T1 (8.7 and 1.5 g, respectively), however, for T3 and T4, these variables were not statistically different. Based on these results, it is concluded that the largest diameter stems (T1), which were obtained from the stem base of the stock plants, are overall the best material for the production of *A. chica* plantlets.

**Keywords:** Bignoniaceae, crajirú, stem base cuttings.

**Sponsor:** CNPq, FINEP 1784/02.

**Table.** Averages of *Arrabidaea chica* plant growth parameters after 90 days, as a function of initial stem cutting diameter. INPA – Manaus/AM, 2004.

Treatment	Diameter (cm)	Survival (%)	Shoot length (cm)	Shoots / stem	Dry weight	
					Leaves	Roots
T1	1.1	100.0a	35.2a	3.3a	8.7a	1.5a
T2	0.6	100.0a	19.5b	2.5b	5.1b	1.0b
T3	0.3	73.3b	18.4b	2.3b	1.7c	0.2c
T4	0.2	68.3b	13.1c	2.0b	1.5c	0.1c
	SD	15.5	5.1	0.8	1.6	0.3
	CV(%)	10.5	14.7	20.3	23.6	26.9



**ISMAP0215 - Effect of cell size of tray on stakes of basil.**

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The objective of this work was to obtain information about rooting of the basil stakes (*Ocimum basilicum* L.) in different sizes and volumes of cells (72 and 128 cells per tray, the later with 60 and 90 cm height). The parameters studied were fresh and dry weight of root and upper ground parts, rooting index, and field surviving). In the production of basil transplants in plugs, it was used as substrate a commercial product, under conditions of protected environment, using floating system. The experimental design used was completely randomized blocks, with four replications. For the conditions of the experiment it was concluded that there was no differences among treatments for all studied parameters, or, basil stakes root in any size and volume cells.

**Keywords:** *Ocimum basilicum* L., rooting, transplants, trays.



**ISMAP0173 - Effects of light quality on seed germination of *Carapa guianensis* Aublet, popularly known as andiroba.**

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Roba-mahogany (andiroba) is well known for the quality of its timber and for the oil extracted from its seeds, used in the medical and cosmetics industries. The supply needs of the local and international markets have caused overexploitation of this important tree of Amazon Basin. In this work, we studied the necessary germination conditions of *Carapa guianensis* in order to support its production, conservation and use. For that, *Carapa guianensis* seeds, both with and without tegument, were inoculated in standard recipients, grown in constant volumes of a sterilized earth and vegetal humus mixture, and exposed to different light conditions: white, blue, green or red light, or kept in the dark for 80 days. For each experiment, sixty seeds underwent the treatment described above in a room with controlled and standardized irrigation, temperature, photoperiod and light intensity.

Our results showed that more seeds germinated in lesser time under dark conditions than those in other light conditions. In the dark treatment, 50% of all seeds had already germinated after 21 days, while only 28,58% of the seeds under green light, 26,66% of those under red light, 19,67% of those exposed to blue light and 16,66% of the seeds treated with white light had done so in the same period. Although the green light treatment caused more seeds to germinate faster, it was the red light treatment that showed the greatest frequency of germination, independent of time. Of the total seeds under red light, 56,66% germinated, while 53,32% of those exposed to white light and 50% of the seeds in both blue and green light treatments did so. Lack of tegument stimulated the frequency and increased the speed of germination in those seeds exposed to white light. The opposite was true for other treatments, where we observed that the presence of tegument stimulated frequency and increased speed.

**Keywords:** *Carapa guianensis*, Meliaceae, germination, light quality.

**Sponsor:** Estácio de Sá University.



**ISMAP0371 - Effects of Nitrogen Fertilizer on Growth , Seed Yield and Oil Seed  
Content of Naked Seed Pumpkin  
(*Cucurbita pepo* subsp. *pepo* convar. *pepo* var. *styriaca*).**

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Naked ( Styrian , Green Gold , Medicinal ) pumpkin (*Cucurbita pepo* convar. *pepo* var. *styriaca* ) is one of the valuable medicinal plants in pharmaceutical industries of developed countries. Nitrogen influenced the growth , development and productivity of the plants. In this study , nitrogen applied at four levels ( 0 , 50 , 100 and 150 kg ha<sup>-1</sup> ) with three replications in each treatment . Nitrogen fertilization were applied at three different stages of the plant growth : seed sowing time , fourth leaves and flowering stage . In order to study the effects of increasing nitrogen level on leaf total chlorophyll content , leaf dry matter , fruit and seed yield and oil seed content of the medicinal pumpkin were determined . The results indicated that nitrogen fertilization increased the amount of total leaves chlorophyll content in comparison to the control . The highest rate of chlorophyll content was obtained when nitrogen was applied at 150 kg/ha. There were significant differences between some treatments on fruit and seed yields , seed dry matter and oil seed content of the plants . The highest oil level was obtained at 100 and 150 kg ha<sup>-1</sup> of nitrogen fertilization.

**Keywords :** Nitrogen Fertilization , Medicinal Pumpkin , Seed Yield , Oil Seed Production



# ISMAP0288 - Enrootment of different species of thyme.

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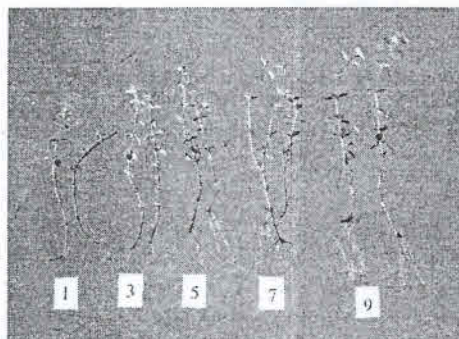
The thyme (Lamiaceae), an aromatic species whose main components of the essential oil are timol and carvacrol. Due to the great variability of species and possibilities of hybridization and mutation the propagation of seeds is not always appropriated because it results in not uniform stands. On the other hand the vegetative propagation permits to keep the characteristics of the mother plant. With the aim of checking the potencial of enrootment, stakes of accesses were assessed of the *Thymus vulgaris* L., *Thymus variegata* L. and *Thymus citriodorus* L. species, encountered in domestic cultivation in Rio Grande do Sul, Brazil. The stakes were carried out in multicelular trays in carbonized rice shell. The stakes were classified in 5 levels of enrootment (classes 1,3,5,7,9) (Table 01) (figure 01 and 02), being the two last classes (7 and 9) considered ready seedlings for transplantation. It was observed that on the enrootment of the stakes, each species had a different behaviour after 27 days of enrootment. The stake of the *T. vulgaris* species had the majority of stakes in classes 1, 3 and 5. The species *T. variegata*, in classes 1, 3 and 5 and the stakes of species *T. citriodorus* had an optimal enrootment, with the majority of the stakes in classes 7 and 9. Thus, for the production of seedlings in commercial scale, there will possibly be given a specific treatment, trying to optimize the qualities of each species.

**Keywords:** *Thymus* spp, Lamiaceae, vegetative propagation, stakes

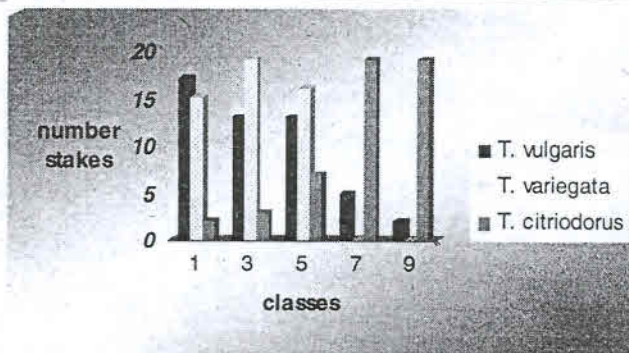
**Sponsor:** CAPES

**Table 01.**

Class	Description
1	Live stakes without any enrootment
3	Stakes with 1 to 4 roots, very tiny, up to 1,5 cm, developing 1 to 3 knots.
5	Stakes with 4 to 5 roots, well developed up to 4 knots with few secondary roots, up to 4 cm long.
7	Stakes with up to 4 enrooted knots with good development of primary roots up to 7 cm long and good development of secondary roots.
9	Stakes with 5 or more enrooted knots with optimal development of primary roots and lots of secondary well developed roots.



**Figure 01.** Types of thyme stakes classified according to the different grades of enrootment



**Figure 02.** Number of stakes contained in the different classes, showing the different grades of enrootment in the scale.



## ISMAP0200 - Essential oil yield and glandular trichomes density of mint species.

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Essential oils of *Mentha* species are valued commercially as additives for food products, cosmetics and pharmaceuticals. Monoterpenes are the major components of essential oil of the mint family and are presumed to function in the maintenance of the ecological fitness as the ecological defense against herbivores. The biosynthesis and accumulation of mint essential oils are regulated according to leaf development and environmental conditions. More recently, it has also been demonstrated that the site of essential oil production is specifically localized to the peltate glandular trichomes. This work had as major objectives to compare the essential oil yield and to investigate the glandular trichomes distribution among six leaf zones (abaxial apical, abaxial middle, abaxial basal, adaxial apical, adaxial middle and adaxial basal zones) of the mint genotypes Eau de Cologne<sup>®</sup> (*Mentha x piperita* L.), 'Lavander Mint' (*Mentha* cf. *aquatica* L.), and 'Banana Mint' (*Mentha arvensis* L.). Plants were propagated by stem cuttings at greenhouse conditions and transferred to the field (Experimental Station of Canguiri – UFPR, Brazil) previously fertilized with 20 kg N ha<sup>-1</sup>, 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 30 kg K<sub>2</sub>O ha<sup>-1</sup> following the results of soil analysis. After 30 days, a second N fertilization (30 kg ha<sup>-1</sup>) was performed. Completed expanded leaves from the sixth internodes were collected for trichome counting. Trichomes of the peltate type were counted using optical microscopy (Olympus CBA, 40 X). Essential oil was extracted from 100 g of fresh plant leaves by hydrodistillation using a Clevenger apparatus during six hours. The comparison between Mint genotypes showed that the highest trichome density was observed on abaxial middle and abaxial basal leaf zones of the Banana Mint genotype and adaxial basal of Eau de Cologne and Lavander. The only difference however on trichome distribution on Eau de Cologne genotype was found between adaxial apical and abaxial basal and on Lavander Mint between abaxial apical and adaxial basal. Other factors than trichome density seems to be more determinant on essential oil accumulation of these genotypes as higher yield was observed on *M. aquatica* genotypes Eau de Cologne (0,75 ml g fresh leaf<sup>-1</sup>) and Lavander Mint (0,68 ml g fresh leaf<sup>-1</sup>) compared to *M. arvensis* genotype Banana Mint (0,29 ml g fresh leaf<sup>-1</sup>)

**Keywords:** Mint, trichomes, essential oil.



**ISMAP0387 - First report on wilting of St. John's wort caused  
by fastidious bacteria.**

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Since 1998, in the Institute for Medicinal Plant Research »Dr. Josif Pančić« (Belgrade), *Hypericum perforatum* was put into cultivation, while cultivation of *Hypericum barbatum* started previous year. In the very start, in the period May-June 1998, decline of *H. perforatum* plants in the field has been recorded. At that time, from these plants several fungal species belonging to the genus *Fusarium* and *Colletotrichum* has been isolated and marked as possible cause of plant deterioration. However, it was not possible to explain symptoms in diseased plants with the presence of mentioned fungal species. Symptoms in diseased plants were as follows. In the period before blooming, leaves of plants have been changing colour, starting from the tops, to yellowish and then to red. At that phase further deterioration was progressing very fast through drying and foaling of leaves. Simultaneously, stem covered with red to dark vertical flakes became prematurely completely dry. In delayed infections in the lower half of the stem reddish colour appears, resulting in necrosis covering stem entirely. Before the end of vegetation drying and wilting of whole bushes occurred. Last years, disease appears in certain plots in epiphytic proportions. According to the level of damage of cultivated plants red wilting could be marked as highly destructive disease of St. John's wort. Appearance of disease of St. John's wort followed by symptoms described, has not yet been reported. Aetiology is not known. During the examination of possible causes of St. John's wort deterioration, among others, ultra thin cross sections of phloem tissue fragments of diseased plants were prepared and analysed by electron microscopy. By the use of this technique, followed by detailed analysis of appropriate micrographs, presence of rickettsia like organisms (RLO) has been recorded. This is the first foundation of such pathogens in the phloem tissue of St. John's wort. Related pathogenesis could lead to blockage of phloem tissue, as well as appearance of toxins, characteristic for fastidious bacteria.

**Keywords:** St. John's wort, red wilting, rickettsia like organisms, RLO



**ISMAP0321 - Growth of crajiru (*Arrabidaea chica* Verlot.) on different growing media.**

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The chemical and pharmacological properties of products extracted from medicinal plants native from the Brazilian Amazon region are being studied without taking into account agronomic development that could alleviate disorganized extractivist pressure on the ecosystem. One such species, crajiru (*Arrabidaea chica* Verlot) belonging to the botanical family Bignoniaceae, is currently extracted from the forest due to its anti-inflammatory and astringent properties, and is used by the regional population and small pharmaceutical companies for healing stomach and intestinal aches, diarrhea, anemia and leukemia. In spite of this, few agronomic studies have been conducted about this species, thus necessitating greater knowledge about its agronomic management techniques. The development of a production system for this species requires an appropriate growing medium for its propagation. Charcoal residues and pyroligenous acid are subproducts of the charcoal production process available in the region, and are considered as stimulants for the plants growing processes, especially when applied together. The objective of this research was to study the development of crajiru in different growing media in Manaus, Amazonas. The following growing media were used: Plantmax, charcoal + chicken manure, sand, sand + charcoal and soil + chicken manure. The pyroligenous extract (Biocarbo®) was applied in a 0.3% concentration four times, once per week. Semi-woody cuttings were taken from juvenile plants in the Sector of Medicinal and Aromatic Plants at Embrapa Amazonia Ocidental and cultivated in tubes. A completely randomized 5<sup>2</sup> factorial design was used corresponding to: five types of growing media, with and without application of pyroligenous acid. The experiment had three repetitions and each experimental plot was composed of nine tubes. The plants remained in the greenhouse, regularly watered, for 60 days. The evaluated variables were the number of survivors, height (cm) and number of new formed shoots, weight of the dry matter of the roots and aerial parts. The best growth rates were attained in charcoal or Plantmax. The plants showed a smaller number of shoots when growing in charcoal and soil when compared to sand, sand + charcoal and Plantmax. The highest plants showed a smaller number of shoots. The amounts of dry matter from aerial parts and roots were greater in charcoal and Plantmax growing mediums. Growing mediums sand + charcoal and sand showed the lowest values. In conclusion, from the evaluated parameters, it was observed that charcoal powder could substitute the Plantmax growing medium with similar results, since it shows the same advantages of easy access and low costs. Plants treated with pyroligenous extract tended to present greater height and greater dry matter values when growing in sand, Plantmax and soil. The opposite effect was observed in charcoal applications.

**Keywords:** charcoal, pyroligenous acid, growing media, Amazon.

**Sponsor:** Embrapa Amazônia Ocidental, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Biocarbo



**ISMAP0237 - Growth phase of potential harvest organs in *Pimpinella anisum* L.:  
Leaf polymorphism and dry matter partitioning.**

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The anise is known as medicine and spices since ancient times, it is named by Teofrasto, Dioscórides and Plinio who said that nightmares could be prevented by placing under the pillow anise seeds in order to smell them during the night. It is also said that anise has aphrodisiac properties. Between sowing and harvest the crops show a growth and development scheme codified by the genotype. Growth is set by increase of plant weight and how the dry matter is allocated to the different structures of the plant. (Andrade et al, 1996). The objective of this work is to describe the setting phase of potential harvest organs by recording the developed leaf number, total biomass production and split biomass production. Continued sowings were carried out at the University of Luján: sowing date March 4 and 24, June 28, and July 23, 2001; from the beginning of elongation (IE) up to the blooming of the main umbel (Upf) 15 plants per sowing date (FS) were random sampled and were recorded: leaf number (NH), fresh and dry matter weight, (PF, PS), total and split into stem biomass (PSt) and remains biomass (PSr). The leaf showing up rate (Tah), the development rate (TD), the growth rate per plant (TCp), the leaf and potential harvest organs growth rate (TCr) and the stem growth rate (TCt) were calculated with the data recorded and the length of the surveyed phase. The data were subjected to statistical descriptive analysis and regression analysis. The average NH at IE decreases as FS delays from 12 to 6 leaf per plant for the first and the last sowing date respectively. The leaf showing up rate (Tah) between IE and Upf was 0,76 leaf per day for the first FS and decreased to 0,48 leaf per day for the last FS. When the setting phase of potential harvest organs is over (Upf stage), plants show 58,8 leaf per plant for the first FS. From the first to the last FS was observed a decrease about 0,33 leaf per day due to every day of delay; related to the first sowing date the following decreases at NH were observed: 29%, 39% y 62% for 2nd, 3rd y 4th FS respectively. The development speed was similar for first and second sowing date (15,5 leaf per day), and increased 23% and 87% for 3rd and 4th FS, this fact could be explained due to higher temperatures during IE-Upf phase at the 4th FS. The regression analysis shows that NHT can explain PF and PS (NHT and PF,  $R^2$ : 0,7537; NHT and PS,  $R^2$ : 0,8947) and can explain in less way the partitioning because  $R^2$  calculated for NHT and PSr and NHT and PSt were 0,6444 y 0,6836 respectively. The fresh aerial biomass at IE was on average 3,89 gr. per plant for first and second sowing dates and decreased to 0,84 gr. per plant for 3rd and 4th sowing dates; at Upf the average weight was 16,9 gr. per plant for June sowing date and decreased to 7,49 gr. per plant for 3rd FS and 2,03 for the 4th. The dry aerial biomass represents between 15 and 17% of the fresh biomass; at IE the PS was highest for first and second FS related to 3rd and 4th (0,64 and 0,14 gr. per plant respectively; the difference between first and last sowing dates remains similar as at IE. The growth rate per plant between IE and Upf was  $4,1 \times 10^{-2}$ ;  $3,9 \times 10^{-2}$ ;  $3,3 \times 10^{-2}$  y  $1,2 \times 10^{-2}$  gr. per day for 1st, 2nd, 3rd and 4th sowing dates respectively. The PSr at IE for first and second FS was 0,55 gr. per plant, 3rd and 4th sowing dates represent 23% of the early ones. The stem begins its appearance since IE and when setting potential harvest organs is over its weight was 1,51 for the first and 0,32 gr. per plant for the last sowing date. At Upf for 4th sowing date The PSt per plant represents 21% of PSt for the first one. At IE and because it is starting the internodes elongation the Pft is insignificant in comparison to leaf biomass, hence PSh PSt ratio was 8,6 and increased 158% for the last two sowing dates. At Upf stage the total biomass is the result of leaf biomass plus the growing reproductive organs and PSr PSt ratio was 0,92 y 0,54 for first and last sowing dates respectively. The PF and PS regression analysis related to PSt and PSr show a moderated explanation of Pf and PS. The fresh and dry aerial biomass between IE and Upf decreases notably as sowing date delays. The development speed increases as sowing date delays. The growth rate per plant at setting potential harvest organs phase decreases as sowing date delays revealing the growth stem rate. PSr and PSt decrease as sowing date delays during IE-Upf phase, although the decrease of leaf and reproductive potential organs biomass is higher than stem decrease, so the sowing date delay does not affect the stem development as much as leaves and inflorescences.

**Keywords:** *Pimpinella anisum* L.; Leaf polymorphism; dry matter; partitioning.



**ISMAP0044 - Herbage and oil yield of Japanese mint (*Mentha arvensis*) as influenced by irrigation and nitrogen scheduling in Chhattisgarh region of India.**

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Japanese mint or *Mentha arvensis* has great potential in Chhattisgarh region of India. It can be cultivated commercially in the rice production area of the state, where the adequate irrigation facilities are available. *Mentha* is an important crop and has strong potential to become substitute for summer rice, as it is an economically more profitable crop, which could ensure net returns of Rs. 40,000 to 50,000 ha<sup>-1</sup>. Irrigation is a vital factor as *mentha* crop extends from mid winter through entire summer season, where its water requirement gradually increases with as increase in temperature. Besides irrigation, nitrogen management is another important factor for increasing the herbage and oil yields of mint. The optimum dose and proper splitting is essential for ensuring higher yields. Considering above facts an investigation has been carried out during winter season of 2001 and 2002 at Research Farm, Indira Gandhi Agricultural Farm, Raipur (CG) India. The soil of the experimental field was classified as *Vertisols* and texturally recognized as clay loam, containing 0.48% organic carbon, 198 kg available N, 19.0 kg available P and 306 kg available K. The three irrigation schedules- 1.1 IW/CPE, 7.8 IW/CPE and 4.6 IW/CPE and four nitrogen splitting – S<sub>10:30:30:30</sub>, S<sub>30:0:40:30</sub>, S<sub>40:0:30:30</sub> and S<sub>0:40:20:40</sub> were tested in split-plot design with four replications. The results reveal that I<sub>1.1 IW/CPE</sub> scheduling treatment proved to be the best one, because it increased the plant height, the number of branches and leaves, the dry matter accumulation and ultimately resulted in higher herbage and oil yields. It also recorded higher net returns and B: C ratio. The water stress conditions due to low frequency of irrigation (I<sub>4.6 IW/CPE</sub>) decreased the plant height, number of branches and leaves, which reduced dry matter production and resulted in lower herbage and oil yields. The oil content decreased with an increase in frequency of irrigation from I<sub>4.6 IW/CPE</sub> to I<sub>1.1 IW/CPE</sub>. Water use efficiency was higher in I<sub>4.6 IW/CPE</sub> scheduling, while it was lowest in I<sub>1.1 IW/CPE</sub> scheduling. Split application of N played an important role on herbage and oil yield in *mentha*. Higher yields were achieved when nitrogen applied in four splits S<sub>10:30:30:30</sub>. However, when N applied in splits S<sub>0:40:30:30</sub> also gave better yield.

**Keywords:** Japanese Mint, Irrigation, N splitting, Economics



**ISMAP0309 - Influence of Nitrogen Fertilizer Application in the content of colorant (curcuminoids) of *Curcuma longa* L. rhizomes.**

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The turmeric (*Curcuma longa* L.) oleoresin (crude extract) contains curcuminoids, which are used to impart flavor and color to a variety of food formulations. At middle of nineteenth century, organic dyes were developed, creating a large variety of coloring additives of economical importance, but lately the natural dyes or natural pigments are the ones that are revolutionizing the market. Curcuminoids have shown antimutagenic activities in different animals and cell cultures. The objective of this work was to characterize rhizomes of turmeric obtained with various type of nitrogen fertilizer, evaluating the curcuminoids amounts and the composition of rhizomes to maintain or to increase the yield of colorant. The species were cultivated in the Experimental Farm of the Agronomic Sciences College (Botucatu-SP). The fertilizers used were: urea - UR, nitrocalcium - NC, ammonium sulphate - AS and organic fertilization (cow manure) - OF, with dosis of 30, 45 and 60 kg/ha. Turmeric rhizomes were characterized by their content of sugar, protein, lipid, fiber, starch, ash, according to AOAC methods. Turmeric volatile oil was obtained by Hydrodistillation (HdE); turmeric oleoresins were obtained by Soxhlet (SoE) and supercritical fluid (SFE) extractions. The curcuminoids contents were analyzed by spectrophotometry. NC- 30, 45 kg/ha and OF- 45, 60 kg/ha produced the best results with respect to the biomass. The highest curcuminoids content was determined for the OF sample and was »2%. With respect to the reference sample, the OF sample had an increase of the total lipids (> 5%) for both tested levels, 45 e 60 kg/ha; nonetheless, with respect to the other compounds their contents were similar to the composition of the reference sample. The extract yield obtained by SFE for OF sample was 11.4 ± 0.2 %, at 300 bar, 30 °C and ethanol/isopropyl alcohol as cosolvent (10%). The best fertilizer to turmeric was organic fertilizer independent of the dosis, with higher curcuminoids content. The extract obtained by SFE had both fractions: the volatile oil and oleoresin.

**Keywords:** *Curcuma longa* L., curcuminoids, nitrogen fertilizers, supercritical fluid extraction

**Sponsors:** FAPESP 02/01608-6 (Ph.D) and 01/11134-0 (M.S.) assistantships of M.E.M. Braga.



**ISMAP0335 - Influence of NPK fertilization on *Cordia verbenaceae* DC.  
 biomass yield.**

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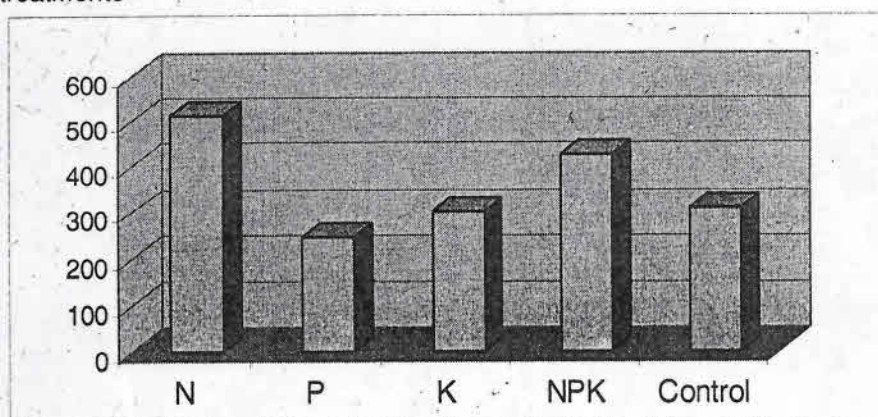
*C. verbenaceae*, *Boraginaceae*, is a perennial shrub species which occurs frequently in the Brazilian coast where it is used traditionally by local people with anti-inflammatory purposes. Due to its therapeutic properties, there is a general great interest in developing a large-scale system of production of this plant. In Brazil, the University of Campinas - UNICAMP through its Research Center (CPQBA) develops since 1991 a multi disciplinary study on *C. verbenaceae* involving: genetic breeding, large scale cultivation and post harvest processes, besides phytochemical and pharmacological studies. Considering that the drug is present in the essential oil from the leaves, we wanted to investigate the influence of NPK fertilization on the biomass yield of leaves. In a *C. verbenaceae* cultivated area at CPQBA, plants received in its first year 5 treatments within the following experimental design: parcels with 4 plants (spacing of 0.5 x 1.5m) in randomized blocks with 5 replications. The treatments were: 1) Nitrogen 100 kg/ha as Ammonia sulfate being applied 20% at the planting transfer to the field, and 20% after each 30 days (4 times); 2) Phosphorus 200 kg/ha as Simple super-phosphate applied once at the planting transfer to the field. 3) Potash, 90 kg/ha as Potash Chloride being 50% applied at transfer and 50% after 30 days; 4) NPK (100-200-90) kg/ha applied once at the transfer of the planting; and 5) The control. The evaluation was done after 6 months when the plants were harvested and dried at 40°C. The weight of dry leaves per plant is shown at table 1 and figure 1. In conclusion, we can say that on this kind and condition of soil, volcanic red soil and pH near to 6, we have no differences between the treatments, except for Nitrogen, which applied as Ammonia sulfate, 100 Kg/ha, sharing on 5 equal doses each 30 days, can promote such significant increase on the leaves yield. This treatment should be considered when intensive harvest has been done aiming at the oil extraction from the leaves.

**Table 1** - Biomass (g of dry leaves/plant) of *C. verbenaceae* influenced by fertilization treatments

Rep.\ Treat.	N	P	K	NPK	Control
I	460	130	200	490	420
II	420	350	240	360	240
III	520	130	400	560	290
IV	570	260	440	340	220
V	590	360	230	380	380
<b>Means</b>	<b>512 a</b>	<b>246 b</b>	<b>302 b</b>	<b>426 ab</b>	<b>310 b</b>

Means followed by different letters present significant differences at level of 1% (T test)

**Figure 1** - Biomass means (g dry leaves/plant) of *C. verbenaceae* influenced by fertilization treatments



**Keywords:** *Cordia verbenaceae*; Fertilization; Essential oil



**ISMAP0242 - Influence of temperature and day length on development rate of anise (*Pimpinella anisum* L.). Temperature sums and Base temperatures.**

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The anise (*Pimpinella anisum* L.) is a temperate climate species and presents economic value for Argentina. An important decrease of the cultivation area was observed at the traditional zone of production (northwest zone of our country) due to economic and social conditions, in consequence it is necessary to import grain from Egypt or Turkey in order to supply the domestic market. Therefore, it is thought about introducing the anise culture to the agronomic rotations at the pampeana zone as a new alternative for small and medium farmers. In order to achieve this goal it is necessary to improve the knowledge about its environmental requirements for growth and development. The objective of this work is to determine base temperature and temperature sums (degree-days) values required to complete anise phenological phases, and the influence of day length over development rate (DR).

The considered sub-periods were: Sowing-Emergence (S-E), Emergence-Umbel bud (E-Ub) and Umbel bud-Early Flowering (Ub-Ef). Phenological data were obtained from continued sowing dates field experiments carried out at University of Luján at Buenos Aires province during 2000 and 2001, and from experimental macro plots carried out by farmers at Pehuajó, Bolívar, Daireaux (Buenos Aires province) and Villa Mercedes (San Luis province) during 1998-99-00 growing seasons. Daily maximum and minimum air temperature and photoperiod value were used as meteorological data.

The duration of the following sub-periods was recorded for each experiment: Sowing-Emergence (S-E), Emergence-Umbel bud (E-Ub) and Umbel bud-Early Flowering (Bf-Ef). Temperature Sums

(ST) were calculated as follows: 
$$ST = \sum_{i=1}^n (T_m - T_b)$$

where ST is the temperature sums above base temperature required to complete the sub-period,  $T_m$  is daily mean air temperature,  $T_b$  is the base temperature at which development stops and  $n$  is the number of days of temperature observations used in the summation. The calculation of  $T_m$  was performed by averaging the daily maximum and minimum air temperatures.

The following methods were used to calculate base temperature: a) Least variation coefficient and b) regression coefficient and x-intercept.

The relationship between the development rate (DR), average air temperature ( $T_m$ ) and average photoperiod (N: sunshine duration in hours) were calculated by regression analysis for Emergence-Umbel bud (E-Ub) and Umbel bud-Early Flowering (Ub-Ef) sub-periods.

The duration of each sub-period decreases as average air temperature increases, although it was not observed a significant functional relationship for Umbel bud-Early Flowering sub-period.

Both methods used to calculate  $T_b$  showed to be accurate to estimate the thermal requirements for Sowing-Emergence sub-period. However, the least variation coefficient resulted to be better for Emergence-Umbel bud sub-period.

During Sowing-Emergence sub-period the temperature sums is 323 Degree-days, above  $T_b = 0^\circ\text{C}$  whereas Emergence-Umbel bud sub-period requires 804 Degree-days, above  $T_b = 4^\circ\text{C}$ .

The relationship of temperature and photoperiod to the development rate during Emergence-Umbel bud sub-period, is described as follows:

$$DR_{(E-Ub)} = 0,69 \times T_m + 1,83 \times N - 18,4.$$

The coefficient of determination of this relationship ( $r^2 = 0,90$ ), is higher than the one obtained when average air temperature and average photoperiod are related separately to development rate.

The development rate resulted to be independent of temperature and day length at Umbel bud-Early Flowering sub-period. These results about thermal and photoperiodical requirements of anise crop, allow to analyze the possibility to wide the production area and to carry out technological practices at the proper time of anise cycle.

**Keywords:** anise; phenological phases; degree-days; photoperiod.



**ISMÄP0222 - Occurrence of fungi on seeds of *Melissa officinalis* L. during fast aging test.**

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Phytoterapeutical and economic importances of medicinal plants has been adding grow expansion of many species. Right quality of seeds and proceeding to obtain better percentage of germination have great importance on domestic crop and commerce. Known as "true lemmon grass", *Melissa officinalis* L. (Lamiaceae) has been introduced in Brazil more than a century for flavoring and medicinal uses. Dried leaves are used on tea and condiment. The essential oil is largely used on pharmaceutical industry because it has activities as antioxidative, antiviral, antifungal, antibacterial, and sedative. Propagation of this specie can be done by section of clumps, cutting peaces or seeds. The last one can be change its germination in function of growing conditions. This trial had the purpose to evaluate sanitary quality of "true lemmon grass" seeds, submitted of different number of hours on test of fast aging. The trial was done since June to July of 2003, at laboratory of seeds analysis at FALM – Bandeirantes City – PR. Seeds were bought at local commerce and, for fast aging test, exposed on temperatures of 42°C during periods of: zero (control), 12h (T1), 24h (T2), 36h (T3), 48h (T4), 60h (T5), 72h (T6) and 84h (T7). After this it were submitted to germination test on dishes as "gerbox", on filter paper, moisted with destiled water, mantained in stove with permanent temperature of 30°C (day) and 20°C (night). It were done evaluations as Brasil (1992). Experimental design was randomized blocks, with 08 treatments and 04 replications using 50 seeds on each. Fungi were identified using estereocopic microscope. As results, is important to say that it has no germination on treatments and it had occurrence of following fungi (Tab. 1): *Penicillium* sp., *Cladosporium* sp., *Aspergillus* sp., *Fusarium* sp., *Helminthosporium* sp., *Nigrospora* sp., *Curvularia* sp. and *Alternaria* sp.. Genus with higher occurrence was *Penicillium* sp., found at all treatments, except T2, that had no fungi occurrence. At control it had higher diversity: *Penicillium* sp. (31%), *Fusarium* sp. (21%) and *Cladosporium* sp. (12%). Higher rate of affected seeds was on T7, showing 94% of contamination. It was conclude that fast aging test had effects on quantity and diversity of fungi growing on seeds of "true lemmon grass".

**Keywords:** *Melissa officinalis*, seeds quality, phytosanity.

**Tab. 1.** Percentage of obtained fungi during fast aging tast on true lemmon grass seeds in Bandeirantes City. – PR.

Fungi	Treatments							
	T0	T1	T2	T3	T4	T5	T6	T7
<i>Penicillium</i> sp	31	16,5	-	56,5	54	79	53,3	94
<i>Aspergillus</i> sp	-	1	-	-	1,5	-	-	-
<i>Cladosporium</i> sp	12	3	-	-	2	-	-	-
<i>Helminthosporium</i> sp	0,5	-	-	-	-	-	-	-
<i>Fusarium</i> sp	21	4	-	-	1	2	0,5	-
<i>Curvularia</i> sp	0,5	-	-	-	-	-	-	-
<i>Nigrospora</i> sp	5,5	1	-	-	2	-	-	-
<i>Alternaria</i> sp	1	-	-	-	-	-	-	-



## ISMAP0202 - Phosphorus and chicken manure on development and yield of *Tropaeolum majus* L.

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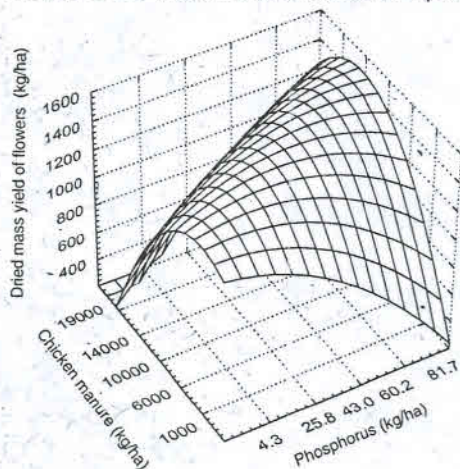
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Part of the thesis of Master in Agronomy of the first author presented to UFMS.

<sup>1</sup>Awarders of productivity scholarship in research of CNPq.

*Tropaeolum majus* L. (nasturtium) has large use as medicinal plant by its effects as anti-spasmodic, anti-scorbutic, antiseptic, stimulant of capillary tube, expectorant, disinfectant of urinary tract, digestive and dermatologic. Besides, it is considered mellific, natural coloring, ornamental and vegetable rich in mineral salts and C vitamine (Font Quer, 1993; Castellani, 1997). The objective of this work was to evaluate development and yield of nasturtium biomass as a function of phosphorus (P) and semi-decomposed chicken manure (C). Work was carried out at Medicinal Plant Garden – HPM of the Federal University of Mato Grosso do Sul – UFMS, in Dourados – MS, from April to August, 2003. Phosphorus – triple superphosphate (4.3; 25.8; 43.0; 60.2; 81.7 kg ha<sup>-1</sup>) and chicken manure (1,000; 6,000; 10,000; 14,000; 19,000 kg ha<sup>-1</sup>) were studied. Treatments were established by PLAN PLUEBA III experimental matrix, which resulted in the following combinations of P (kg ha<sup>-1</sup>) and C (kg ha<sup>-1</sup>): 4.3 and 6,000; 25.8 and 1,000; 25.8 and 6,000; 25.8 and 14,000; 43.0 and 10,000; 60.2 and 6,000; 60.2 and 14,000; 60.2 and 19,000 and 81.7 and 14,000. Used experimental design was randomized blocks, with nine treatments and four replications. Leaf area increased linearly with C, which maximum value was 11,350.77 cm<sup>2</sup>, that corresponded to those plants cultivated under 4.3 and 19,000 kg ha<sup>-1</sup> of P and C, respectively. The highest height of plants was of 46.38 cm at 94 days, which corresponded to the use of 25.8 and 14,000 kg ha<sup>-1</sup> of P and C, respectively. The highest fresh mass of aerial parts of plants were obtained with the use of the highest doses of C, while of dried mass were not influenced significantly by P neither C doses. The highest fresh (14,541.05 kg ha<sup>-1</sup>) and dried mass of flowers (1,445.28 kg ha<sup>-1</sup>) (Figure 1) were obtained under intermediary doses of P and C. Number of flowers was in average of 15.50 millions ha<sup>-1</sup>. The highest yields of fresh (2,739.38 kg ha<sup>-1</sup>) and dried fruits (619.41 kg ha<sup>-1</sup>) were obtained under the highest doses of phosphorus. In order to obtain great yield of flowers it is better the use of P and C under dose of 75 and 10,800 kg ha<sup>-1</sup>, respectively, for nasturtium.

**Keywords:** Nasturtium, medicinal plant, mineral nutrition, organic residue



**Figure 1.** Dried mass yield of nasturtium flowers as a function of phosphorus and chicken manure doses. Dourados, UFMS, 2004.

$$w = 1388,99 - 1,40679P + 0,02831C - 0,09190 P^2 - 0,000004 C^2 + 0,001142 PC; R^2 = 0,81$$

\* e \*\* significant 5 and 1% of probability. C.V. = 16,06 %

**Sponsor:** FUNDECT/CNPq.

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FONT QUER, P.V. **Plantas medicinales.** El dioscorides renovado. 7. ed. Barcelona: Labor, 1993. 421p.



**ISMAP0131 - Physiological quality and essential oil content of coriander seeds with different deterioration levels.**

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Dry seeds of coriander have essential oils that are very enjoyable for both aroma and odor. These essential oils are used in the food industry to manufacture several products. The objective of this study was to investigate the association of seed deterioration with coriander seed physiological quality and essential oil content. Coriander seeds cv. Verdao were artificially aged by placing seeds at 41°C and 100% humidity for 0, 48 and 96 hours, thus generating three seed lots varying in deterioration. Seeds were analyzed for germination test (20-30°C for 7 days), vigor (accelerated aging test, using saturated salt solution at 41°C/ 72 hours), and essential oil content. Distillation was accomplished with a vapor stream system using a modification of a Clevenger equipment to extract the oil content. The hydrolate obtained was partitioned with dicloromethane in a separation funnel. The organic fractions were dried in anhydrous magnesium sulphate and placed in a rotary evaporator (Büchi R-114 type), under reduced pressure. Seed aging affected physiological seed quality (germination and vigor). The maximum essential oil yield obtained was 0.7%. Seed deterioration did not affected essential oil yield. Possibly, the small increasing of oil content observed in aged seeds was due to the transfer of oil from the seed internal layers to the external ones, making oil extraction easier.

**Keywords:** *Coriandrum sativum* L., germination, vigor, aging



# ISMAP0231 - Postharvest sensitivity of sweet basil (*Ocimum basilicum* L.) to chilling injury.

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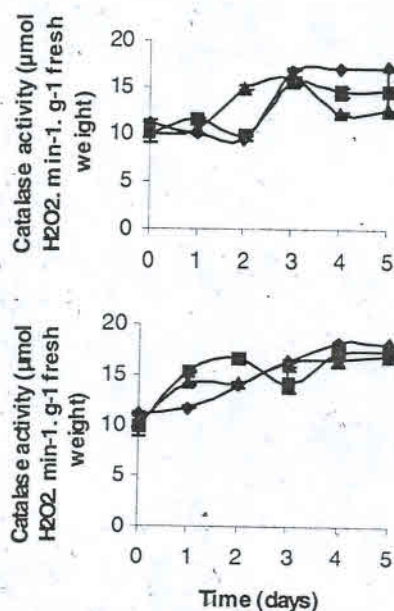
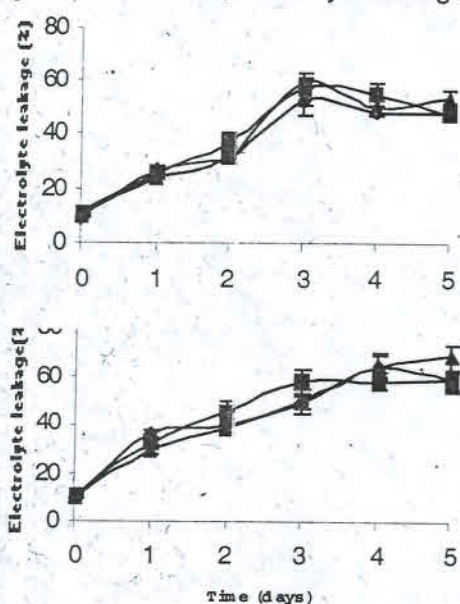
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Sweet basil has been used as culinary condiment, popular medicinal plant and as raw material for the pharmaceutical industry. Despite of sweet basil importance as fresh product, there is no information in the literature regarding the sensitivity of cultivars to chilling injury. The present work had the goal to investigate such differences among the cultivars Folha Larga, Semi-roxo and Branco of sweet basil leaves stored at 5 °C. Terminal stems of each cultivar were harvested, selected, packed in perforated boxes of cellulose acetate or unpacked and stored at 5 °C for five days. The leakage of electrolytes increased in all cultivars during storage. In those leaves packed in cellulose acetate boxes the increase in leakage was 48% for 'Folha Larga', 47% for 'Semi-roxo' and 54% for 'Branco', while in the control leaves the leakage was increased by 59%, 58% and 68%, respectively. Starch gels revealed that a single isoform of catalase, in both, chilling injured and not injured leaves in all cultivars. The catalase activity increased by 53%, 46% and 63% in the leaves of boxed 'Folha Larga', 'Semi-roxo' and 'Branco', respectively. In the control leaves, the increase in catalase activity was 61%, 74% and 63%, respectively. The increase in the leakage of electrolytes and catalase activity were coincident with the development of chilling injury symptoms (discoloration and browning). The changes in electrolyte leakage were not related to the sensitivity of the cultivar to chilling injury. The cultivar 'Folha larga' was the most resistant in developing discoloration and browning symptoms throughout storage at 5 °C.

**Keywords:** cultivars, electrolyte leakage, catalase.



**Figure 1-** Electrolyte leakage from cultivars Folha Larga(f&), Semi-roxo(%) and Branco(2%), packed(A) or unpacked(B)

**Figure 2 -** Catalase activity from cultivars Folha Larga(f&), Semi-roxo(%) and Branco(2%), packed(A) or unpacked(B)



**ISMAP0258 - Potential use of *Plectranthus barbatus* to control  
phytopathogenic fungi.**

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The metabolism of secondary plants compounds proves to be important for the plants which synthesize them. One of the functions of these substances is to provide protection from the attack of pathogenic organisms. This work aims at studying the effects of different plant extract on a phytopathogenic fungi, *Glomerella cingulata*, that causes post harvest disease on fruits. One gram of ethanolic extract from different plants ( *Costus pisonis*, *Achilla millefolium* and *Plectranthus barbatus* ) was diluted in five milliliters of organic solvent DMSO. The extract of *P. barbatus* was obtained from different plant parts, such as: leaf, stem and others. After resting in 5°C temperature, the plant extract which was dissolved in DMSO was diluted in Erlenmeyer of 50ml of medium PDA to obtain the concentrations of 400, 800, 1200, 1600 and 2000 ppm of extract in the PDA. After adding the extract, the PDA was flowed in Petri plate. The plates were put to sterilization under ultraviolet light for eight hours. Then, 0,70 cm diameters disks with *G. cingulata* were placed in the center of every plate. The fungi radial growth was evaluated in intervals that varied from 48 to 192 hours. Each treatment had 5 replications. Three extracts reduced the fungi radial growth significantly, with reduction values of 16, 20, and 37%. The *P. barbatus* extract was the one which presented the best results, reducing the fungi growth to 90%. There is a future possibility that this plant can be used in fruit post harvest disease control.

**Keywords:** Ethanolic extract, fungicide, plant disease



**ISMAP0337 - Production of *Cordia verbenaceae* D.C. according to their  
plantation density.**

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*Cordia verbenaceae* D.C., Boraginaceae, is a perennial aromatic shrub widely distributed on the South-eastern Brazilian coast and their leaves are used in folk medicine as anti-ulcer, anti-inflammatory, analgesic and cicatrizant drug. The chemical composition of their leaves shows that the species contains sesquiterpene compounds. Recent pharmacological investigations prove that its essential oil, rich in  $\alpha$ -humulen, posses a potent anti-inflammatory and analgesic effect. Due of this properties, an increasing interest of the pharmaceutical industry in the species can be noted. This fact led us to develop cultivation methods for the species. The aim of this work was to investigate the effect of the spacing on their leaf production. Seedlings grew in greenhouses were planted in the CPQBA experimental field in two densities: 1,0 x 2,5 m (treatment A, with 30 plants/parcel) and 0,5 x 2,5 (treatment B, with 60 plants/parcel). The experimental design was in randomized blocks, with 7 replications. The variances of the data analyzed by the Hartley test shows that they are homocedastic ( $H = 2,31^{**}$ ). The analysis of variance (table 1) shows that there is a significant difference in the leaf production between the two densities adopted ( $F = 27,14^{**}$ ): treatment A produces 55,9 kg/parcel (fresh matter) and treatment B produces 73,1 kg/parcel. The variation coefficient of this experiment was 9,57%. According to this work we can conclude that the treatment B is more efficient than treatment A to produce *Cordia verbenacea* leaves in our experimental field conditions.

**Table 1:** analysis of variance of the experiment.

Variation causes	d.f.	S.S	S.M.	F
Treatments	1	1035.44	1035.44	27.14**
Blocks	6	721.95	120.325	
Rest	6	228.83	38.138	
Total	13	1986.22		

**Keywords:** *Cordia verbenaceae*, plantation, cultivation, production



**ISMAP0316 - Production of aerial and underground biomass of *Jacaranda decurrens* cham. - Bignoniaceae, in different harvest times.**

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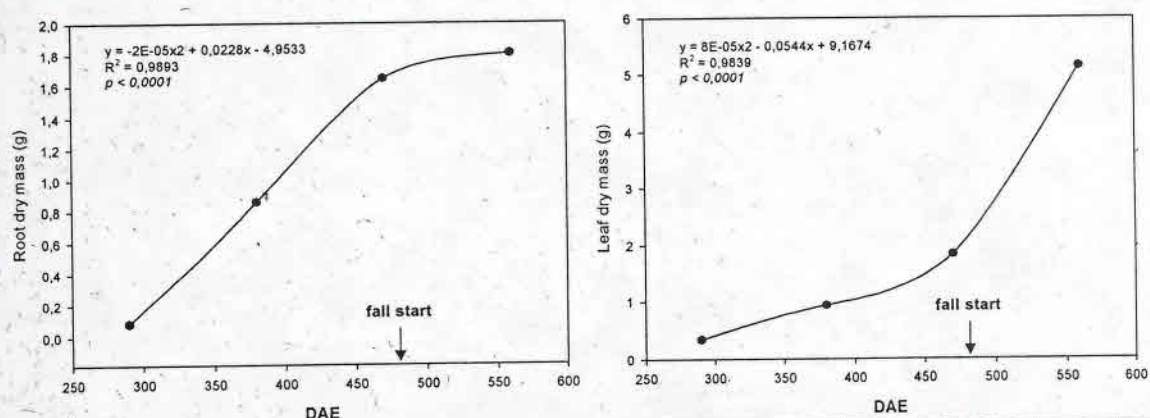
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*Jacaranda decurrens*, known popularly as carobinha, is a medicinal plant of the Brazilian Savannah, exposed to the genetic erosion provoked by the indiscriminate collection, and e/ou by the frequent deforestations in areas of natural occurrence. The *ursolic acid*ursólico, present in its roots, has antiinflammatory activity, it inhibits HIV-1 protease and it also presents antitumoral activity. The objective of the project was to study the production of biomass in the underground and aerial part of *J. decurrens*, determining the best harvest time. The work was accomplished in the Horticulture Division of the São Paulo State University, Botucatu-SP. Department of Vegetable Production - Setor Horticultura FCA/UNESP-Botucatu. THE The experimentproject was composed by 4 treatments (harvest times): 290, 380, 470 and 560 days after the emergency (DAE). The production of dry mass of the aerial and underground parts was evaluated. The used statistical delineamento was blocks entirely casualizados. After having harvest, the material was dried in greenhouse at 40°C until stabilization of the weight. The variance analysis showed the existence of significant differences among the treatments for root and leaf dry mass ( $p < 0.0001$ ). From 470 DAE, the root dry mass increased strongly, and the best production of the root dry mass was at 560 DAE, while the increment of the aerial part was smaller in the same period. In that period, that coincides with the fall start (short days), the flow of having assimilated was redirected from aerial part to roots, while the period from 290 to 470 days, among the beginning of spring and final of summer, the plants prioritized the allocation of biomass of the aerial part in detriment of the roots. In face of the results, the best time of the harvest is in the end of the autumn, when occurs the best biomass accumulation in the root, organ of commercial interest.

**Keywords:** medicinal plants, ursolic acid, harvest time, crop production

**Sponsor:** FAPESP



**Figure 1.** Leaf and root dry massBiomass evaporates of leaf and of raíz in function of different harvest times (02/09/02 - 290 DAE; 02/12/02 - 380 DAE; 02/03/03 - 470 DAE; 02/06/03 - 560 DAE) in *Jacaranda decurrens*. UNESP/BOTUCATU-SP, 2002/2003.



**ISMAP0304 - Production of biomass in *Pothomorphe umbellata* (L.) Miq. submitted to different photosynthetically active radiation.**

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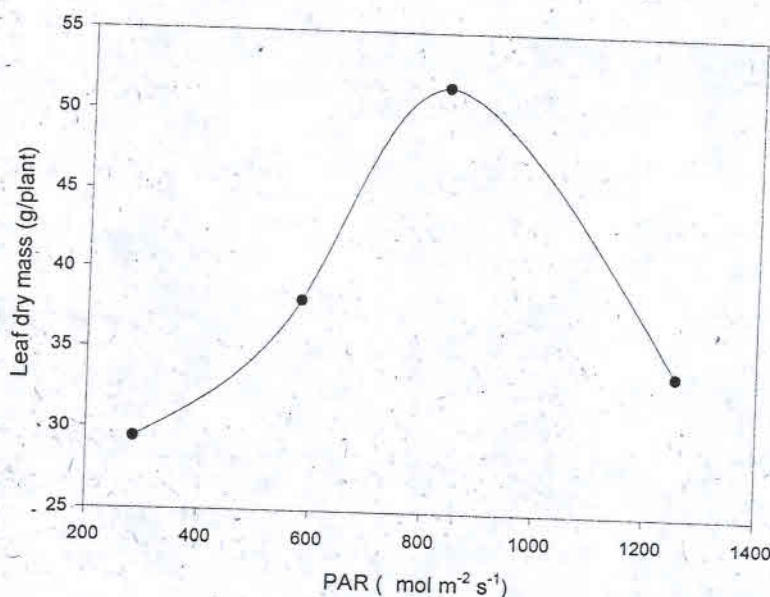
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*Pothomorphe umbellata* is a medicinal plant with a wide ethnotherapeutic uses. It a native species and grows in the borders of Brazilian Atlantic Forest, and brushwood (Guimarães *et al.*, 1978). Is a sciophyte species growing better under diffuse light and humid environment (Silva, 1998). The objective of this work was to evaluate the influence of the differents photosynthetically active radiation intensities on the biomass of *P. umbellata*. The work was accomplished at the Horticulture Division of the São Paulo State University, Botucatu-SP (22°51'S, 48°26'W, 786m). The treatments consisted of plants submitted to four intensities of photosynthetically active radiation (PAR): 1254,3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; 835,7  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; 580,6  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; and 284,8  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The production of dry mass of the leaves was evaluated. The used statistical delineamento was blocks entirely casualizados. After having harvest, the material was dried in greenhouse at 40°C until stabilization of the weight. The highest production of leaf dry mass was obtained in the PAR tratment 835,7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of leaves, confirming that *P. umbellata* is a shade plant (sciophyte). These data check with Marchese *et al.* (2003) that found higher photosynthetic rate in experiment with *P. umbellata* under a PAR of 835,7  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The plants cultivated in full sun presented clorosis and necrosis symptoms, due to degradation of the chromopigments. Similar symptoms are told by Silva (1998). The cultivation of *P. umbellata* is recommended in consortium or under canopy.

**Keywords:** leaf biomass, pariparoba, luminous intensities, medicinal plant



**Figure 1.** Production of leaf dry mass in *Pothomorphe umbellata* under different intensities of radiation photosynthetically active (PAR). Botucatu-SP, MAY/2003.



**ISMAP0302 - Productivity, Yield and Composition of the Essential Oil in seeds of fennel (*Foeniculum vulgare* var. *dulce*) in the season of the year.**

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The fennel (*Foeniculum vulgare* var. *dulce*), also called of “erva-doce” it is a well-known plant from the antiquity, because of its aroma, and is cultivated in many countries about the Mediterranean. The plant has adapted very well in the south states of Brazil. Four experiments were carried according to seedlings times, January/2001, May/2001, August/2001 and October/2001 to evaluate the productivity, yield and compounds of essential oil in seeds of fennel. The seeds were sowed in trays of poliestirene of 128 cells with organic substratum, cattle manure and charred straw of rice (3:1:1) and transplanted with 60 days of development. The treatments were established with base in seven harvesting, being the first carry out when the portions reached 70% of flowering after seed and the others in intervals of 14 days. The fruits were classified in the categories: green (green intense), mature (strong yellow) and dry (straw) for the determinations of the masses, the fruits, after classification were conditioned in heavy paper sacks, labeled, dry in temperature of 40 °C. The extraction of essential oil were done by hydrodistillation during three hours. The best result of productivity was in the summer with green seeds, with 490,62 g/36 plants and about to the yield of essential oil the best result was also in the summer with green seeds (3,08%). The main compounds in green seeds were: trans-anetole (73.81%), fenchone (15.08%) and limonene (5.26%); in mature seeds were respectively: 77.67; 15.14 and 2.74% and in dry seeds were, respectively 78,25; 13,98 and 3.67.

**Key words:** fennel, seeds, content of essential oil, productivity.  
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**ISMAP0366 - Relationships between plant density, biomass and essential oil production in seven *Aloysia citriodora* accessions from Argentina y Chile.**

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Lemon verbena (*Aloysia citriodora*) is a perennial, arbustive and native species of South America. This species have glandular trichomes on its leaves that produce essential oil. Genotype is a major determinant of how plants acquire and utilize resource and leads to large differences in biomass and essential oil production. Variations in the environment also influence the biomass and essential oil production. Biotic and abiotic stresses can increase essential oil percentage. Our objective was to evaluate the effect of density (competence intraspecific) on biomass and essential oil production of seven *Aloysia citriodora* accessions. A field experiment was carried out at the Faculty of Agronomy of the University of Buenos Aires, Argentina. Accessions selected from two locations of Chile and four locations of Argentina were planted in a factorial design with five replications during spring summer 2002/3. The factors were accession (7 locations) and density (1 and 5 plants per pot). Experimental units were harvested at full flowering. The biomass and essential oil yield of leaves and flowers was determined. Leaves and flowers were hydrodistilled for 1.30 hours using a Clevenger glass apparatus according to the European Pharmacopoeia method. Data was analyzed with ANOVA. No differences were detected for flowers plus leaves, stem biomass, and essential oil (expressed as % and ml) interaction (Table 1). Stem biomass and percentage essential oil differed among accessions ( $P=0.0004$  and  $P=0.03$ , respectively). The accessions from Salta showed the highest percentage of essential oil, but the lowest leaves and flowers biomass. In all accessions essential oil percentage remain similar in both density, but essential oil yield (ml per pot) were greater in high plant density, because of the increment in leaves and flowers biomass. In summary these results show the relationships between the plant density, biomass and essential oil production, and that the behavior among the different accessions are similar in high plant density.

**Table 1:** Mean biomass and essential oil expressed as % and ml, of seven accessions of *Aloysia citriodora*

Accessions	Leaves plus flowers g/pot	Stem biomass g/pot	Essential oil (%, w/v)	Essential oil ml
Mendoza	23.55	8.61	3.12	0.63
San Luis	21.86	12.34	3.00	0.56
Botanico	22.21	11.8	2.82	0.53
Experimental	15.97	9.3	2.98	0.36
Talca	20.10	8.1	2.76	0.45
Rancagua	19.42	6.49	2.70	0.44
Salta	8.71	12.55	4.48	0.30
P=	0.31	<b>0.0004</b>	<b>0.03</b>	0.70
Density				
Low	16.02	8.80	3.20	0.41
High	26.10	10.60	2.80	0.56
P=	<b>0.0003</b>	<b>0.0009</b>	0.27	<b>0.0003</b>
Interaction Accession x Density P=	0.96	0.57	0.75	0.14

**Keywords:** lemon verbena, density, yield.

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**ISMAP0166 - Respiration and ethylene production in sweet basil stored under chilling injury conditions.**

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In this experiment it was evaluated the behavior of respiration and ethylene production by the leaves of three cultivars of sweet basil stored under chilling injury conditions. Terminal stems of cultivar Folha Larga, Semi-roxo and Branco were harvested boxed or not in perforated cellulose acetate and stored at 5 °C for five days. Daily stems were removed from the cold storage and placed at 25 °C to determine the CO<sub>2</sub> and ethylene evolution by gas chromatography. The respiration and ethylene production experienced dramatic changes throughout the storage at 5 °C. In the stems boxed in cellulose acetate boxes, the respiration increased until the fourth day of storage, with enhancing the CO<sub>2</sub> production by 62.6% for 'Folha Larga', 48% for 'Semi-roxo' and 82.6% for 'Branco'. For the control treatment the respiration increased by 23.2%, 21.9% and 50.9%, respectively. The highest ethylene production for the boxed stems were observed between the second and third day of storage, with evolutions of 52.3 nL kg<sup>-1</sup> h<sup>-1</sup> for 'Folha Larga', 127.3 nL kg<sup>-1</sup> h<sup>-1</sup> for 'Semi-roxo' and 159.6 nL kg<sup>-1</sup> h<sup>-1</sup> for the 'Branco'. In the control treatment the maximum ethylene production were 26.6, 95.7 and 65.8 nL kg<sup>-1</sup> h<sup>-1</sup>, respectively. The highest rate of respiration was observed in the cultivar Branco, while the cultivar Folha Larga had the lowest production of ethylene throughout the storage. Both, respiration and ethylene production were stimulated with the development of the initial symptoms of chilling injury in the leaves.

**Keywords:** cultivars, respiration and ethylene



**ISMAP0111 - Row spacing effect on *Baccharis trimera* yield.**

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*Baccharis trimera* is a medicinal plant native from South America, known as carqueja. The objective of the present study was to evaluate the row spacing effect on *B. trimera* yield. The experiment was carried out in Mandirituba (PR) - Brazil, on field with acid soil (base saturation of 46.84%). Planting was done on November, 2001, with male plants propagated by stem cutting. A randomized block experimental design with three replications (20 plants each) was used. The spacing treatments were the following: 40x40cm, 40x50cm and 40x60cm. After one year from planting date (November 18<sup>th</sup>, 2002) the experiment was evaluated measuring height, fresh and dry mass per plant, weight loss after drying and fresh and dry mass yield. The plants were cut at 30cm of the ground. The height (50.7cm), fresh mass (174.1g) and dry mass (64.9g) of *B. trimera* at 40x60 spacing were higher than 40x40cm. No significant differences were observed for weight loss and yield in the row spacings. The average fresh yield was 7068.6kg.ha<sup>-1</sup> and dry yield was 2671.1 kg.ha<sup>-1</sup>. The 40x60cm was the best spacing as it showed better yield and easier walking between the plant rows.

**Keywords:** medicinal plant, spacing, *Baccharis*.

**Table 1.** Yield of *Baccharis trimera* cultivated on three row spacing at one year after planting. Mandirituba (PR) – Brazil.

Spacing	Height (cm)	Fresh mass per plant (g)	Dry mass per plant (g)	Fresh mass yield (kg.ha <sup>-1</sup> )	Dry mass yield (kg.ha <sup>-1</sup> )	Weight loss (%)
40x60cm	50.7a	174.1a	64.9a	7254.2a	2704.2a	62.7a
40x50cm	49.6ab	142.4ab	54.4ab	7118.3a	2721.7a	61.7a
40x40cm	47.7b	09.3b	41.4b	6833.3a	2587.5a	62,2a
CV (%)	1.7	11.1	12.2	10.3	11.7	2.5

Average in the same column followed by the same letter did not differ by Tukey test (5%).



# ISMAP0283 - Seedling growth of Mamacadela (*Brosimum gaudichaudii* Trec.) on six different substrates.

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The Brazilian Cerrado is rich in medicinal species which present morphological characteristics consisting of developed roots ("xylopodios") and barks, which frequently accumulate pharmacologically active substances.

Mamacadela (*Brosimum gaudichaudii*) is a tree or a shrub largely used in traditional medicine in the Cerrado region. The infusion of its roots is used as a blood depurative and to treat skin problems, itching, vitiligo, and bronchitis. The alcoholic extract of roots is used to externally treat vitiligo. Two furano-coumarins have been isolated (bergapteno and psoraleno) from roots of Mamacadela. Bergapteno is a photosensitive substance, which associated with the vitamins B1, B6 and A is used in the treatment of vitiligo, in form of topic solutions or cream (pomade), now being market as Viticromin®. The bergapteno promotes the re-pigmentation of the affected tissues.

Due to its commercial interest, Mamacadela has been intensive collected from the wild, having a strong anthropogenic exploitation pressure. Studies on propagation of this species is extremely necessary, since no horticulture technology is available so far.

The main goal of this paper is to evaluate the growth and development of Mamacadela on six substrates, using plastic bags with the following specifications: 20x30 cm (width x height), 4 L volume capacity and 0,02 mm espessure, with drainage. Seeds were grown at Embrapa Cerrados nursery at Planaltina, DF, Brazil, from September, 2002 to September, 2003. Five substrate types were prepared from a basic mixture (MB) of subsoil from Red Latosol with clay texture plus sandy thick soil from river in a proportion of 1:1, as follow: S1 – MB + 10% of cattle manure; S2 – MB + 20% of cattle manure; S3 – MB + 10% of cattle manure + 3 g/L of osmocote 6 months releasing; containing N (15%), P<sub>2</sub>O<sub>5</sub> (10%), K<sub>2</sub>O (10%), Ca (3,5%), Mg (1,5%), S (3%), B (0,02%), Cu (0,05%), Fe (0,5%), Mn (0,1%), Zn (0,05%) and Mo (0,004%); S4 – MB + 3g/L of osmocote; S5 - MB + 6g/L of osmocote. The sixth substrate type was composed using only thick river sand + 6g/L of osmocote.

The experimental design was completely randomized with six treatments and five replications with three plants per plot. Substrate S6 showed the least values for all variables evaluated, due to the high lixiviation capacity of the sandy texture. Substrates S3 and S5 (Figure 1) produced seedlings with higher dry above ground biomass and a tendency of higher weight of dry roots. Both treatments did not differ from others in height and stem diameter (Table 1). In the nursery phase, mamacadela has showed a higher investment in root growth than in the aerial part, which appear to be an adaptation related to the Cerrado dry season.

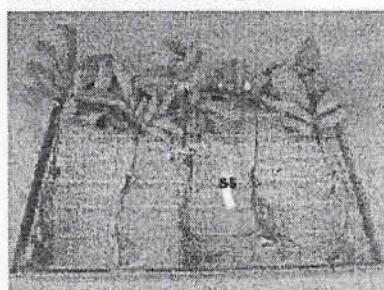
**Keywords:** Savannah, cerrado, seedling production, fertilization.

**Table 1.** Height, stem diameter, roots (RDW) and aerial part dry weight (APDW), from seedlings growing on six different substrates, after twelve months.

Substrates	Height (cm)	Diameter (mm)	APDW (g)	RDW (g)
S1	18,9 a	3,3 ab	3,1 bcd	7,2 ab
S2	18,3 a	3,6 a	2,9 cd	7,4 ab
S3	18,5 a	3,2 ab	5,2 ab	8,7 a
S4	18,2 a	3,6 a	4,0 bc	8,0 ab
S5	21,3 a	3,6 a	6,2 a	9,3 a
S6	12,1 b	2,7 b	1,6 d	4,6 b
CV (%)	13,5	13,6	29,0	23,4

Averages followed by the same letters do not differ statistically at 5% probability using Tukey test.

**Figure 1.** Seedlings of Mamacadela showing results from substrates S3 and S5.





## ISMAP0156 - Some experiences in indoor and outdoor production of Yellow gentian seedlings in Serbia.

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During the last decades, due to the overexploitation of Yellow gentian (*Gentiana lutea* L.) from its natural stands, a lot of efforts have been made to develop appropriate cultivation techniques, as a best way to exceed obvious threat to its survival. Following this road, the problems encountered with Yellow gentian seed dormancy and seedlings production may be considered as of utmost importance.

In few-years experiments several models of pre-sowing seed treatments, in order to induce seed germination, were tested, including the models that are appropriate for, recently very popular, organic way of Yellow gentian production. The best results (60% germination) were obtained in treatments with GA<sub>3</sub>. Very good results (>40% germination) are also achieved in non-hormonal treatments, with cooling of wet seeds in refrigerator at 2-4°C, in duration of 70 days. Also, the influence of climatic conditions during the fertilization and seed ripening period showed to be of great importance for seed quality, and will, consequently, influence seed germination and entire seedlings production.

In the case of production of seedlings in greenhouse conditions, the seed should be sown in the second half of February, germination and emerging of seedlings follows in 15-20 days, while 30-35<sup>th</sup> day the plantlets already have the first pair of permanent leaves. At beginning of May, the plantlets already have developed 3-4 pairs of permanent leaves and may be transplanted in the field, in mountainous regions. However, these plantlets are very delicate and require proper soil preparation and perfect weed control.

Out of tested methods of Yellow gentian seedlings production outdoors, in mountainous region, the autumn sowing of seeds without pre-sowing treatment showed to be the best choice. In this case, the seeds are left to hibernate in the open beds and seedlings emerged following year, in April. They were left in open beds during the vegetation period. In September they have developed rosette of 5-8 cm in diameter, with 6-8 pairs of permanent leaves. Such plantlets showed to be much stronger in comparison to those produced in greenhouse and were ready to be transplanted into the field weather in autumn or in spring of the following year.

**Keywords:** Yellow gentian, seed dormancy



**ISMAP0201 - Stomata density, physiological leaf parameters and essential oil yield of *Mentha x Piperita*, *M. cf. aquatica* and *M. arvensis*.**

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The biosynthesis and metabolism of monoterpenes in aromatic species are strongly influenced by environmental factors, including water, temperature and photosynthetic photon flux density, and the level of response varies according to the genetic material. Results obtained with *Mentha* species are not conclusive and present a wide range of responses. Investigations correlating plant gas exchange and essential oil yield represent an important way to understand the plant-environmental interaction. Because the level of gas exchange represent a result of stomata density and transpiration ratio, we determine in this work the stomata density in six zones of the leaf epidermises (abaxial apical, abaxial middle, abaxial basal, adaxial apical, adaxial middle and adaxial basal zones), transpiration ratio ( $\mu\text{g cm}^{-2} \text{s}^{-1}$ ) and stomata diffusive resistance ( $\text{s cm}^{-1}$ ) of the mint genotypes Eau de Cologne' (*Mentha x piperita* L.), 'Lavander Mint' (*Mentha cf. aquatica* L.), and 'Banana Mint' (*Mentha arvensis* L.). Mint plants were grown at field conditions (Experimental Station of Canguiri – UFPR, Brazil). The soil was previously fertilized with 20 kg N ha<sup>-1</sup>, 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 30 kg K<sub>2</sub>O ha<sup>-1</sup> following the results of soil analysis. After 30 days, a second N fertilization (30 kg ha<sup>-1</sup>) was applied. Environmental conditions and plant gas exchange measurements were performed on expanded leaves from the sixth internode using the equipment Porometer LI-1600C at three times (10:00, 13:00 and 16:00 hs) during 10 days. The average temperature, relative humidity and radiation for the period were 25.1 °C, 62.8 % and 504.4  $\mu\text{E s}^{-1}\text{m}^{-2}$ , respectively. At the end of the period, those leaves were collected to determine the stomata density by optical microscopy (Olympus CBA, 40 X). Essential oil was extracted from samples of fresh leaves (100 g each) by hydrodistillation using a Clevenger apparatus during six hours. The results showed that stomata density is concentrated on the abaxial epidermises of the three genotypes. The highest density was found on Banana Mint leaves (50,02 stomata.mm<sup>-2</sup>), followed by Lavander Mint (39,81 stomata.mm<sup>-2</sup>) and Eau de Cologne (34 stomata.mm<sup>-2</sup>). Differences on stomata density showed to be specific for some leaf epidermis zones. Leaves of the Banana Mint genotype presented higher number of stomata compared to the other genotypes on all abaxial zones (apical, middle and basal). Comparing the leaf zones of Banana Mint, we observed higher density on abaxial middle than on abaxial apical zone. The distribution of stomata showed to be uniform on leaf zones of the Lavander and Eau de Cologne. Transpiration ratio was also significantly higher on Banana Mint genotype (9,2 mg.cm<sup>-1</sup>.s<sup>-1</sup>), with intermediate values for Eau de Cologne (8,6 mg.cm<sup>-1</sup>.s<sup>-1</sup>) and lowers for Lavander Mint (7,7 mg.cm<sup>-1</sup>.s<sup>-1</sup>). No difference was found for stomata diffusive resistance (Lavander Mint, 1,5 s. cm<sup>-1</sup>; Eau de Cologne, 1,2 s. cm<sup>-1</sup>; Banana Mint, 1,1 s. cm<sup>-1</sup>) and leaf temperature (Eau de Cologne, 25,9°C; Lavander Mint, 25,7°C; Banana Mint, 24,8°C). The essential oil yield was superior on Mint genotypes Eau de Cologne (0,75 ml g fresh leaf<sup>-1</sup>) and Lavander Mint (0,68 ml g fresh leaf<sup>-1</sup>) compared to Banana Mint (0,29 ml g fresh leaf<sup>-1</sup>).

**Key words:** Mint; stomata, transpiration, essential oil



**ISMAP0281 - Storage effects on the germinative behaviour of *Chenopodium ambrosioides* L. seeds.**

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*Chenopodium ambrosioides* L., known in Brazil as *Erva de Santa Maria*, is a medicinal herb propagated by seeds and original from Tropical America. It is considered to be very significant in the drugs industry. The objective of this work was to evaluate the germinative behaviour of seeds stored in refrigerator for 0, 3, 6 e 12 months. The seeds have been sown in gerboxes, having *germiteste* paper as substrate; they have then been put in germination chamber (BOD) at an alternate temperature 20-30°C and a photoperiod of 8h light/ 16h dark. The assessment took place on the 7<sup>th</sup> and 15<sup>th</sup> days after sowing. It was used an experimental delineation entirely casualized with 4 repetitions (50 seeds/ repetition). The variables total germination and first counting of the germination test were determined, and the averages have been compared through the Tukey test to 5% of probability. The new seeds did not germinate, which means that just-harvested seeds are dormant. Those seeds stored for 3, 6 12 months did not present statistical difference in the percentage of germination: 35%, 32% e 36,5% respectively .

**Keywords:** *Chenopodium ambrosioides* L., seeds, storage

**Sponsors:** UENF/FAPESP/UNICAMP



# ISMAP0252 - Sustainable harvest of medicinal and aromatic plants from secondary tropical forest, Minas Gerais, Brazil.

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The non-wood forest products (NWFP) they are used thoroughly as foods, fibers, medicinal plants, cosmetics, etc. The growing population and the emergent demands of these resources for the pharmaceutical and cosmetic industry are stimulating the commercial harvest in native forest areas. This work had as objective to study the interrelation of the largest production of active compounds and better times of execution of the harvest activities, seeking quality of the raw material, smaller operational cost and smaller environmental impact in the sustainable management of medicinal and aromatic plants. The harvests were accomplished, monthly, in a secondary atlantic forest fragment, which is representative of the forest areas of the phytogeography zone, located in the "Zona da Mata Mineira". In the analysis of the data the outline of subdivided fractions was considered. The fractions were the time (seasons) and the sub fractions the vegetable parts, in the delineation in randomized blocks with 6 repetitions. *Anadenanthera macrocarpa* analysis was done in experimental delineation of the randomized blocks, with 4 treatments and 6 repetitions. The production of essential oils was monitored in *Casearia sylvestris*, *Ocotea odorifera*, *Siparuna guianensis* and *Trichilia catigua*, of phenolic compounds in *A. macrocarpa*, *Jacaranda macrantha* and *Luehea grandiflora*, and of friedelin in *Maytenus aquifolia* and *Maytenus robusta*. The amount of essential oils, phenolic compounds and friedelin was determined with the use of apparatus of adapted Clevenger, Folin-Denis method and chromatography respectively. The results concerning the time harvest of vegetable parts are shown in Table 1. The production of active compounds reached the following values: *C. sylvestris* - 1,12% leaf; *O. odorifera* 0,86% leaf - 0,83% branch - 1,37% bark; *S. guianensis*: 0,24% leaf - 0,09% branch; *T. catigua*: 0,21% leaf - 0,17% branch; *A. macrocarpa*: 37% bark; *J. macrantha*: 2,15% leaf; *L. grandiflora*: 3,76% branch - 19,72% bark; *M. aquifolia*: 22,94 mg /100 g leaves - 0,37 mg /100 g branches; *M. robusta*: 113,76 mg /100 g leaves - 58,14 mg/100 g branches. Seeking to minimize the impact on the export of nutrients, the vegetables parts picked and not marketed or with low amounts of active compounds, should be left at the place of occurrence. As for the relationship cost-benefit, it was verified that in the rainy season, the penetration in the forest and the operation of the activities related to the management become more difficult. On the other hand, during this time, it was observed that in the monitored individuals, a fast replacement of the removed biomass, caused by the vegetable growth takes place. For the system to allow the continuous acquisition of the products, future enrichment plantings with the species of interest is required. Studies referred to the management techniques and innovation in the administration of PFNM are also necessary.

**Keywords:** non-wood forest products, harvest time, management sustainable, Atlantic Forest  
**Support:** CNPq

**Table 1** – Harvest time of medicinal and aromatic plant material on yield of compounds atives of species from Atlantic Forest, Minas Gerais, Brazil.

Studied species	Harvest Time			
	Spring	Summer	Autumn	Winter
<i>C. sylvestris</i>	-	-	Leaves	leaves
<i>O. odorifera</i>	bark	leaf; branch	-	-
<i>S. guianensis</i>	-	-	leaves; stem	-
<i>T. catigua</i>	-	-	-	leaf; branch
<i>A. macrocarpa</i>	-	-	bark	-
<i>J. macrantha</i>	-	leaves	-	-
<i>L. grandiflora</i>	-	-	-	leaf; branch
<i>M. aquifolia</i>	leaf; branch	-	-	-
<i>M. robusta</i>	-	-	-	leaf; branch



**ISMAP0027 - The effect of different plant density and plant rates on yield on Saffron (*Crocus. Sativus*) and Black Zira (*Bunium persicum*) in Intercropping system.**

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Khorasan Agricultural Research Center

The effect of different plant densities and rates on saffron and black zira intercropping was studied in 2000 at Toragh Agricultural Research station in Mashhad. The experimental design was a randomized complete block in factorial arrangement with 4 replications and 15 treatments including 3 levels of plant populations (16.7, 8:3 and 4.17 plant per m<sup>2</sup>) (0, 33:67, 50:50, 67:33, 100) and 5 levels of two crops ratio in an intercropped arrangement based on replacement series techniques. The method of bivariate analysis was the good method of analysis and it made analysis simultaneously two values obtained from one plot related to a specific treatment.

Evaluation of Land Equivalent Ratio (LER) by bivariate method showed that the yield of all intercropped treatments, LER was higher than 1. Decreasing density from 33 to 67 plants per m<sup>2</sup> decreased the yield of black zira. But the yield of saffron wasn't significantly changed.

Maximum LER obtained with 33:67 saffron to black zira.

**Keywords:** Bivariate Analysis, black zira, Saffron Replacement series and LER.



**ISMAP0327 - Use of *Mentha piperita* to Multiply Arbuscular Mycorrhizal Fungi.**

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The objective of this work was to evaluate *Mentha piperita* Hudsson as a multiplying plant for three species of arbuscular mycorrhizal fungi (AMF) (*Glomus clarum* Nicol. & Schenck, *Glomus etunicatum* Becker & Gerd. e *Acaulospora scrobiculata* Trappe). Young stem cuttings of *Mentha piperita* were placed in sterilized substrate consisting of coco-pit and carbonized rice husk (1:1, v:v) in containers of different volumes (plastic trays with alveolis of 40 ml and 100 ml, respectively) containing 10 g of inoculum of each species of AMF. Twelve stem cuttings were grown in each plot, with four replicates and eight treatments in a subdivided design. Two weeks after rooting, the stem cuttings were transplanted to 5 liter polyethylene bags. Ninety days after the transplant, there was a colonization of 91,5%, 94% and 98% for the species *A. scrobiculata*, *G. clarum* and *G. etunicatum*, respectively. The three species of AMF showed better vegetative development of the aerial part, in terms of number of leaves, leaf area and fresh and dry matter of the aerial part. The most efficient species, *G. etunicatum*, presented 37% and 33% dry matter gain over not inoculated plants when grown on large and small alveolis, respectively. We concluded that *Mentha piperita* can be used to multiply the three AMF species tested in the present work; however, if the objective is to attain higher dry matter of the aerial part, inoculation with *G. etunicatum* and use of alveoli of higher volume is recommended.

**Keywords:** Aromatic plants, Lamiaceae, Mycorrhizae



# Biotechnology



**ISMAP0069 - 16S rRNA sequencing of Endophytic bacteria from micropropagated *Echinacea* plants.**

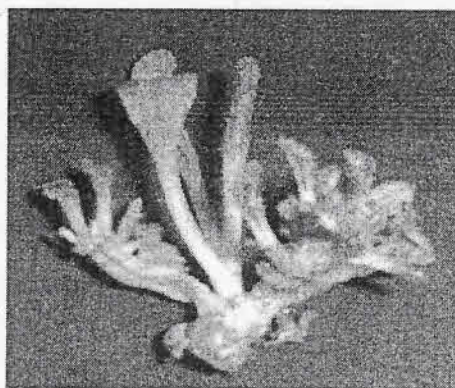
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Preventing or avoiding microbial contamination of plant tissue cultures is critical to successful micropropagation. Bacterial contaminants are often difficult to detect even after prolonged sterilization because they remain mostly within plant tissues as endophytes. The term endophyte refers to interior colonization of plants by microorganisms that do not have pathogenic effects on their host. The goal of this study was to develop a good detection method for bacterial contaminants of *Echinacea* plantlets (fig.1) and to isolate, identify and characterize the bacteria. Bacterial isolates from contaminated *E. angustifolia*, *E. pallida*, *E. purpurea* and *E. tennesseensis* shoot cultures were characterized. This study represents evidence that aseptically micropropagated *Echinacea* plantlets contained populations of endophytic bacteria which persisted in latent form. Analysis based on 16S rRNA sequencing provides a rapid and reliable approach. Sequencing of the 16S rRNA genes of isolated endophytes revealed a broad spectrum of bacteria. Our results indicated that endophytes associated with *Echinacea* plantlets are representatives of several genera, *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Ralstonia* and *Stenotrophomonas*. The significance of these bacteria in biology of host-bacterium interactions is unclear. Further experiments are in progress to determine the role of these bacteria in host-bacterium interactions.

**Key words:** *Echinacea*, endophytes, seed borne bacteria



**Fig 1.** *In vitro* micropropagated *Echinacea*



**ISMAP0182 - Actions of growth regulators on *Baccharis trimera* L. *in vitro* cultured.**

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"Carqueja" is known by several botanical names, including *Baccharis genistelloides*, Pers; *B. triptera*, Martius and *B. trimera* Less. It is found throughout the Amazon rainforest in Peru, Brazil and Colombia, as well as in tropical Argentina, Paraguay and Uruguay. Carqueja is one of the more widely known and used medicinal plants in Brazil and other parts of South America. In Brazil it is popularly employed as a natural herb to liver aid and to digestive aid. Phytochemically, carqueja is a rich source of flavonoids. The extracts contains up to 20% of flavonoids including quercetin, luteolin, nepetin, apigenin and hispidulin. Micropropagation yields plants that are genetically identical with the donor plants, and has been achieved through rapid proliferation of shoot-tips and axillary buds in culture. This work presents data about the development of *Baccharis trimera* plants, in function of plant growth regulators added to culture medium. Nodal segments obtained from *in vitro* germinated seeds on basal Murashige & Skoog medium (MS) were subcultured on MS without growth regulators (MS0) or added with different growth regulators (n=31 for each treatment): kinetin (KIN); 6 - Benzylaminopurine (BA) and Indolacetic Acid (IAA). The cultures were maintained at  $25\pm 1^\circ\text{C}$ , with illumination of  $23.0 \mu\text{moles.m}^{-2}.\text{s}^{-1}$  and a 16-hour photoperiod, pH = 5.8. Calli were formed in axillary shoot explants cultured in MS with KIN and BAP, after 60 days. Shoots developed in MS when transferred to KIN, after 60 days, elongated to 1.3 cm, 3.9 new nodal segments were formed with 6% of rooting formation. The use of BA resulted in 0.98-cm shoot elongation, production of 3 new nodal segment without rooting induction. The maximum elongation was obtained with MS0 and MS + IAA, that presented 2.8 and 3.1 cm and 92 and 97% of rooting, respectively, which were considered statistically similar. Nodal segments formation was achieved with MS0 and MS + IAA, wherein after 60 days 8 and 7.3 new buds were developed, respectively. The results were submitted to ANOVA statistic analysis. Taking into account that the main objective of this work is to produce selected monoclonal plants to be used in phytomedicin industries, it is important that the method presents a large number of buds per explant. In this work, the best treatment used was MS0, because it produced the best multiplication rate (1:25).

**Keywords:** *Baccharis trimera*, carqueja, medicinal plant, growth regulators

**Sponsor:** CNPq



**ISMAP0077 - Analysis of rutin in leaves and flowerings buds of fava d'anta  
(*Dimorphandra mollis*).**

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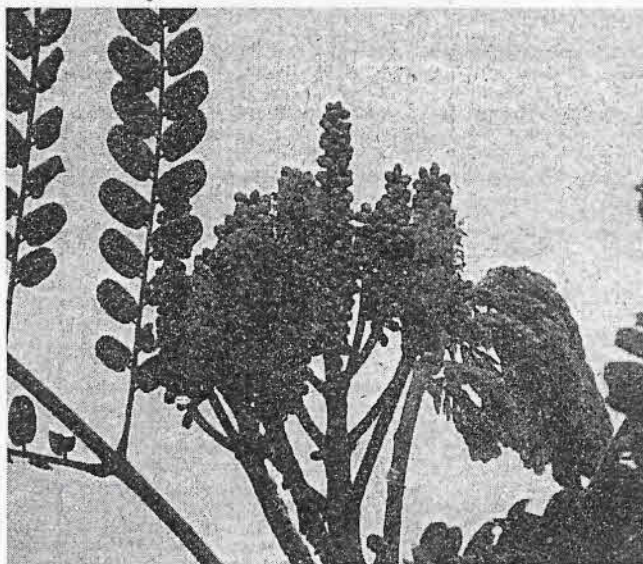
Fava d'anta is native to vast areas in Brazil and produces the flavonoid rutin. Rutin has the ability to increase the strength of the capillaries (blood vessels) and to regulate their permeability. The objective of this work was to determine the rutin contents in leaves and flowering buds of *Dimorphandra mollis* (Figure 1). The plant samples used were collected in Montes Claros, North of Minas Gerais State. The plant materials were dried at room temperature and powdered using a mill. The plant materials (10 mg) were next extracted at room temperature with one aliquot of 10 mL of methanol. Quantitative analysis was performed using a rutin calibration curve with ultra-violet spectrophotometer in the wavelength of 285 nm. The results obtained in this study compared well with the results recorded from other studies. The flowering buds contained a higher rutin content (10,39%) than the leaves (8,2%) (Table 1).

**Keywords:** Fava d'anta, rutin, leaves, flowering buds, quantitative analysis

**Sponsor:** Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG

Plant part	Rutin content % (w/w)
Leaves	8,2%
Flowering buds	10,4%

**Table 1:** Rutin content obtained in leaves and flowering buds.



**Figure 1:** Fava d'anta. Leaves and flowering bud.



**ISMAP0022 - Bangladeshi plant extracts on gene expression profiling:  
from Macro to Microarray Technology.**

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**Introduction:** DNA hybridization arrays (macroarrays and microarrays) are very useful tools for the analysis of gene expression profiles in human pathologies. In addition, macro- and microarrays can be used in pharmacogenomic and toxicogenomic experiments, aimed at extensive analyses of the effects of therapeutic drugs on overall gene expression of target cells. Despite the fact that extracts from medicinal plants have been described to retain interesting biological activity, including anti-inflammatory, antitumor and antimicrobial effects, few data are present in the literature on gene expression profile studies.

**Experimental:** Here, we review results on the effects of anticancer plant extracts from *Moringa oleifera* on the gene expression profile of a human tumor cell line, the K562 cell line, originally isolated from a patient with chronic myelogenous leukemia (CML) in blast crisis, very useful for the identification of antitumor compounds.

**Results:** The data obtained using macroarrays were compared with those obtained using reverse-transcription polymerase-chain reaction (RT-PCR). Effects of *Moringa oleifera* extracts were compared to those of *Embllica officinalis*. The results obtained suggest that a general strategy for the development of specific therapeutic approaches could be proposed starting from gene expression studies employing macro- or microarray. Treatment of target cells with plant extracts will allow to identify genes which are down- or up-regulated. For these genes, the molecular analysis of the promoter regions and coding sequences could allow to design decoy ODN, antisense DNA or RNA, peptides and monoclonal antibodies expected to mimic the biological effects of the employed plant extracts.

**Conclusion:** Using these macroarrays and microarrays techniques we have identified several potential lead molecules for the treatments of several types of cancer.

**Keywords:** macroarrays, microarrays, *Moringa oleifera*, *Embllica officinalis*, antitumor.



**ISMAP0195 - Biomass production and semi-quantitative determination of 4-nerolidylcatechol during cultivation of *Pothomorphe peltata* (Piperaceae).**

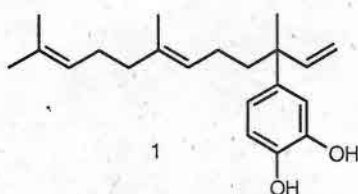
Pinto, A.C.S.<sup>1</sup>; Pena, E.A.<sup>2</sup>; Chaves, F. C. M.<sup>3</sup>; Pohlit, A. M.<sup>4</sup>

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*Pothomorphe peltata* is known by the common names caapeba, pariparoba, caapeba-do-Norte among other names and is a small shrub or treelet measuring 1 to 2 m in height and is distributed in the Americas. It has many uses in traditional medicine, such as the treatment of malaria using roots and the leaves are reportedly ingestible as a foodstuff. In previous work, a phytochemical study yielded 4-nerolidylcatechol (**1**) which was shown to be present in the root, leaf and fruit of adult plants using normal phase TLC and development with FeCl<sub>3</sub> (intense blue color). This compound was also shown to be lethal *in vitro* towards brine shrimp larvae (*Artemisia franciscana*), as well as larvae of the hemorrhagic dengue fever vector *Aedes aegypti*. Furthermore, in a preliminary trial, this compound presented *in vitro* inhibition of the malaria parasite, *Plasmodium falciparum* (Pinto, A.C.S. Master's Thesis, UFAM, Manaus, Brazil, 2002). In the present study the aim is to evaluate the leaf, root and stem masses, the proportion of leaf/stems (m/m) and also to quantify the 4-nerolidylcatechol content in different parts of the plant, as a function of harvest time. Seeds were obtained from the fruit of one year old parent plants present in the garden of INPA's Natural Products Division (CPPN) using a simple wetting / washing technique. Substrate was prepared using local soil and chicken manure (3:1, m/m) in black polyethylene bags. Seeds were placed directly onto the surface of the substrate in each bag during Manaus's late dry season (date: 9/9/03). Germination occurred after 30 days and on 2/11/03 plants were thinned, leaving the single most vigorous plant in each bag. The plants were irrigated in the greenhouse until their definitive plantation in the Experimental Area at EMBRAPA, in Manaus, Amazonas State (22/12/03, beginning of the rainy season). The experimental design was in four randomized blocks, using a spacing of 1.0 m x 1.0 m. Plots of 6 plants in four replicates were analyzed. The first harvest was on 11/2/04 and the average dry masses of leaf, stem and roots were 3.84, 1.14 and 1.56 g / plant, respectively. The proportion of leaf to stem was 3.6. The treatments will be harvested at 60 day intervals for a total of about 20 months. The semi-quantitative determination of **1** in the different parts of the cultivated plants is underway and involves exhaustive solvent extraction under nitrogen followed by total evaporation. Analysis of extracts versus external standard at different dilutions, first semi-quantitatively using thin-layer chromatography, then quantitatively using high performance liquid chromatography (HPLC) will be performed at each interval to establish the existence of any seasonal and developmental variation in the concentration of **1**.

**Keywords:** biomass, cultivation, 4-nerolidylcatechol, *Pothomorphe peltata*.

**Sponsors:** FAPEAM, Bioamazonia





**ISMAP0056 - Callus Culture in Worm wood (*Artemisia absinthium*.L) from Leaf.**

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*Artemisia absinthium*.L (Wormwood), Asteraceae, is one of the medicinal and aromatic plants in Iran. It is traditionally used because of its pharmaceutic and aromatic properties. It is showed that the culture of callus tissue is an important technique that can be preliminary to the regeneration of whole plants. Because of the potential genetic variability associated with this system, regenerated plants may assume importance for genetic improvement and selection strategies when evaluted for somaclonal variation. For study of callus culture, explants (5x5mm) from leaf of *Artemisia absinthium* were cultured on MS basal medium (agar added at 8 gL<sup>-1</sup> and sucrose 30 gL<sup>-1</sup>) supplemeted with different concentrations of BA, Kn , NAA , IAA and 2,4-D in combination or singly. The pH of the medium was adjusted to 5.8. All the callus culture were incubated at 25°C under a 16h light( 35µmolm<sup>-2</sup> s<sup>-1</sup> ) and 8h dark cycle. The fresh weight of callus was measured after 25 days. However, cultures grew slowly following the second subculture and the majority turned brown and died within the next month. Results showed that supplementing the medium with 1 mgL<sup>-1</sup> NAA in combination with 1 mgL<sup>-1</sup> Kn , and 0.2 mgL<sup>-1</sup> BA in combination with 0.5 mgL<sup>-1</sup> NAA enhanced the growth rate of callus cultures.

**Keywords:** Wormwood , Callus

**Sponsor:** Bu Ali Sina University



# ISMAP0259 - Carotenoids determination of *Pothomorphe umbellata* L. "caapeba do sul".

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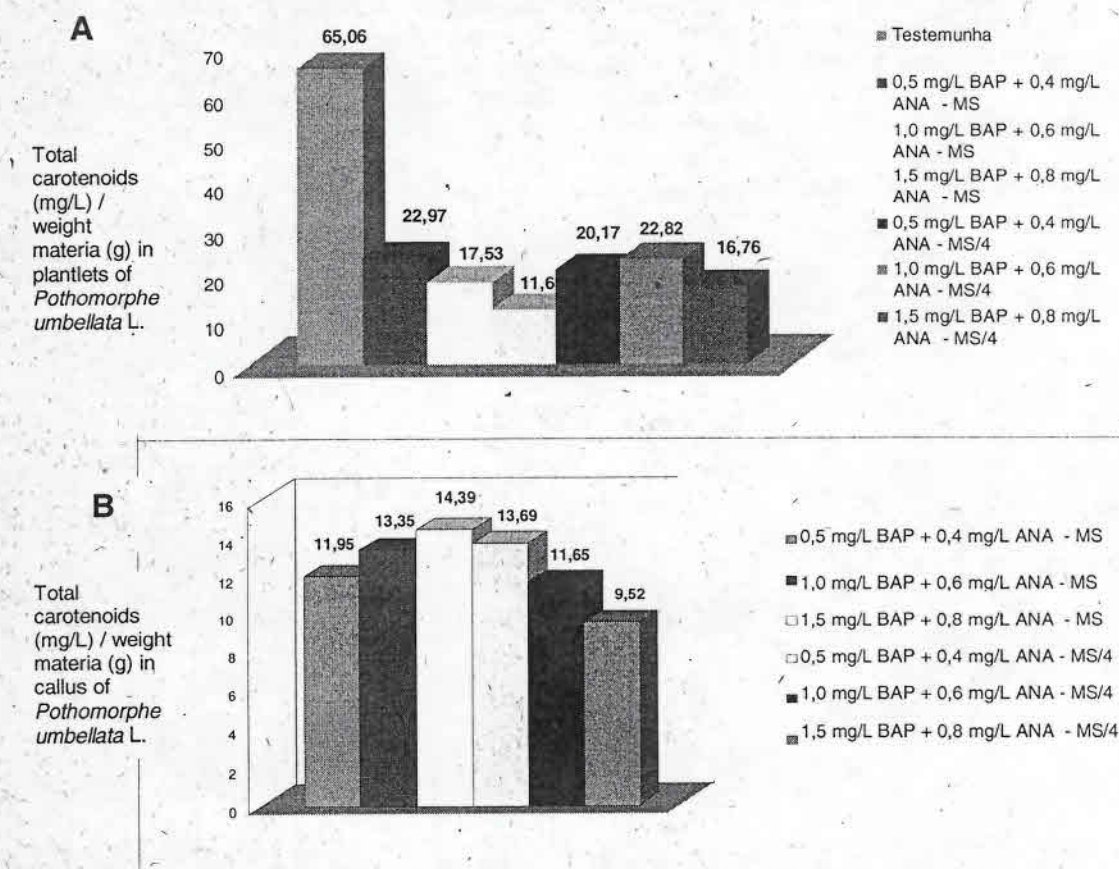
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Carotenoids are a class of more important than 600 natural pigments that are present in fruits and vegetables. Antioxidants properties were determined in *Pothomorphe umbellata* L. The scope of this work was to determined a protocol of micropropagation including the induction of callus formation. Besides, it was compared the carotenoids quantity between *Pothomorphe umbellata* callus and plantlets. The vegetal material (seed) was obtained from Adranópolis - PR. *Pothomorphe umbellata* germinated seeds were inoculated in different concentrations of BAP (0,5 mg.L<sup>-1</sup>; 1,0 mg.L<sup>-1</sup>; 1,5 mg.L<sup>-1</sup>) and NAA (0,4 mg.L<sup>-1</sup>; 0,6 mg.L<sup>-1</sup>; 0,6 mg.L<sup>-1</sup>) respectively, in order to stimulate callus induction and increase quantity of carotenoids. After 60 days, callus which contained shoots were inoculated in plantlets differentiation medium (MS and MS/4, GA<sub>3</sub> 0,1 mg.L<sup>-1</sup>, BAP 0,5 mg.L<sup>-1</sup>) during 40 days and transferred to plantlets growth medium. Callus collected after 60 days and plantlets were collected after 140 days. There were frozen in liquid nitrogen and maintained in freezer -80°C to be used in further carotenoids test. The best treatment for callus production and shoots elongation was NAA 0.6 mg.L<sup>-1</sup> in association with BAP 1.0 mg.L<sup>-1</sup>. The higher carotenoids concentration was in plantlets without growth regulators, compared with callus (Figure 1 a,b).

**Keywords:** carotenoids, callus, micropropagation, plant tissue culture.

**Figure 1.** Total carotenoids (mg/L) / weight materia (g) in plantlets (a) and callus (b) of *Pothomorphe umbellata* L.





**ISMAP0227 - *In vitro* development of *Malva sylvestris* L.**

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*Malva sylvestris* L. (Malvaceae) is popularly known as “malva grande” (big mallow), “malva rosa” (pink mallow) and “malva selvagem” (wild mallow). It has been used in cases of chronicle bronchitis, intestinal constipation, inflammatory diseases of mucous membrane and colitis. The plant is presented like an emollient, diuretic, laxative, anti-septic, expectorant and bacterial. Like extern use, it is employed in skin's affections, contusions and haemorrhoids. In its chemical composition there are essential oils, anthocyanins, mucilage, tannin and vitamins (A, B, C). The chemicals constituents responsible for biological actives variated depending how plant is cultured, harvested and transported; thus *in vitro* micropropagation is appropriated to obtain homogeneous plant quality which will be furnish for phytomedicine producers. The objective of this work was to determine the effects of different growth regulators (kinetin, indoleacetic acid and 2,4-dichlorophenoxyacetic) on organogenesis of *M. sylvestris* *in vitro* cultures. Nodal segments were isolated from plants cultured in MS0 medium (MS medium without growth regulators), supplemented with 30 g/L of sucrose, solidified with agar (8 %), pH 5.8 and cultured in some concentrations of kinetin (KIN), indoleacetic acid (IAA) and 2,4-dichlorophenoxyacetic (2,4-D). The experiments were realized with n = 33 per treatment and the results were analyzed by ANOVA ( $p \leq 0.05$ ). The cultures were maintained in culture room at  $25 \pm 1^\circ\text{C}$  with 16-h photoperiod under irradiance of  $23.0 \mu\text{moles.m}^{-2}\text{s}^{-1}$ . Growth analysis were realized, during 45 days, appraising number and length of shoots. The different growth regulators employed ( $1.8 \mu\text{M}$  KIN;  $1.8 \mu\text{M}$  IAA and  $1.8 \mu\text{M}$  2,4-D) didn't induce roots in cultures of *M. sylvestris*. In relation to shoots length, the all media tested didn't induce significant difference in 45 days of culture, where the media MS0, MS +  $1.8 \mu\text{M}$  IAA and MS +  $1.8 \mu\text{M}$  KIN induced average height of 1.35 cm, while the medium MS +  $1.8 \mu\text{M}$  2,4-D induced average of 0.91cm. The cultures with MS0, IAA, KIN e 2,4-D didn't induce statistics difference in development of shoot number, which had average of 1.34; 1.48; 1.84 and 1.13, respectively. The results relative to callus development, in 45 days of culture showed that the medium supplemented with  $1.8 \mu\text{M}$  2,4-D induced greater callus than others media with rate of 50 %.

**Keywords:** medicinal plants, tissue culture, malvaceae and growth regulators

**Sponsor:** CNPq and PBV



**ISMAP0363 - Effect of inoculation of the different strains of *Agrobacterium rhizogenes* on phenotypes and terpenes production of *Artemisia annua* L. transformed roots.**

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Transformed roots were obtained after the inoculation of nodal segments of *Artemisia annua* L. with two strains of *Agrobacterium rhizogenes*: one agropine strain (15834) and one manopine strain (8196). The confirmation of the transgenic nature of the roots was obtained by the "Dot Blot" technique. The transformed and normal roots were established and cultivated *in vitro* with success on hormone-free liquid Murashige & Skoog (1962) medium. The established hairy root lines displayed three morphological phenotypes. Most of the cultures resulting of strain 15834 inoculation showed the characteristic traits of hairy roots. (lines FI, C1, C3, C5, C6, C7, C8, C10, C11, C12, C15). except C14 line that showed callus-like aspect. The cultures resulting of strain 8196 inoculation (C1\*, C2\*, C3\* and C4\*) were thin without branching. The growth rate of the transformed root lines was always higher than that of untransformed roots, showing that the genetic changes caused by the *A. rhizogenes* transformation conditioned a higher biomass formation. The roots extracts were chemically analysed by CG/MS for evaluation of relevant terpenes, such as: artemisinin, artemisinic acid, as well as two other compounds with strong antiulcerogenic activity: dihydro-epideoxi-artenuin B and deoxiartemisinin. The analysis of results revealed that none of the afore mentioned terpenes were present in the none of the different transformed root phenotypes extracts. The chromatograms of the extracts taken from normal roots cultivated in MS liquid medium deprived of phyto regulators revealed the presence of the antiulcerogenic compound dihydro-epideoxi-artenuin B.

**Keywords:** Hairy root cultures, agropine and manopine *Agrobacterium rhizogenes* strain, Dihydro-epideoxi-artenuin B, antiulcerogenic and antiproliferative activities.

**Sponsor:** CNPq



**ISMAP0076 - Effects of harvest time on the rutin content in fava d'anta leaves  
(*Dimorphandra mollis*).**

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H.S., Durães<sup>1</sup>, D. A., Oliveira.<sup>1</sup>

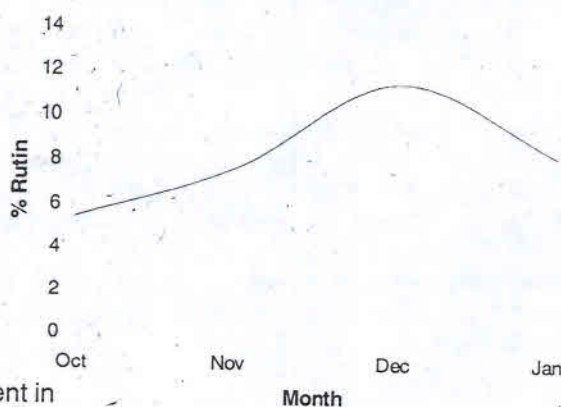
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MG, 39401-089, Brasil.

Rutin ( $C_{26}H_{26}O_{16}$ ) is a flavonoid that has the ability to increase the strength of the capillaries (blood vessels) and to regulate their permeability. This substance is found in great concentration in *Dimorphandra mollis*, a native plant species from the Brazilian cerrado. The objective of this work was to determine the content of rutin in fava d'anta leaves harvested at different times and with different ages (Figure 1A). The samples were collected in Montes Claros, North of Minas Gerais state. Leaves were collected from October 2003 to January 2004. Leaves were dried at room temperature and three leaf repetitions (10 mg) were powdered in a mill and then the extraction of rutin was carried out with one aliquot of 10 mL of methanol. The extracts were submitted to absorbance in the spectrophotometer in the wavelength of 285 nm. The concentration of rutin increased from October to December 2003 and decreased in January 2004 (Figure 1B).

**Keywords:** Fava d'anta, rutin, leaves, quantitative analysis

**Sponsor:** Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG



**Figure 1:** A) Fava d'anta leaf and B) Rutin content in fava d'anta leaves harvested at different times.



**ISMAP0142 - Effects of pH and major nutrients on *in vitro* growth of a Brazilian plant: *Baccharis tridentata* Vahl. (Asteraceae).**

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*B. tridentata* Vahl is an aromatic and medicinal plant that grows wild in small cavities in rocky surfaces of the Itatiaia Plateau, at nearly 2400 m elevation. The aim of this study was to evaluate the effects of initial pH variation, different nitrogen sources and lower concentrations of the main macroelements, on *B. tridentata in vitro* growth. Shoot tips of a previously established *in vitro* clone were inoculated in liquid MS (Murashige & Skoog) basal medium, on a filter paper bridge. MS basal medium was modified in relation to pH to give a gradient of initial pH values ranging from 4,0 to 6,5 at 0,5 pH unit intervals, besides pH 5,8 used as a control. The effect of nitrogen source was investigated through follow treatments: (T1) complete MS medium with mixed source ( $\text{NO}_3^- + \text{NH}_4^+$ ), (T2)  $\text{NO}_3^-$  and (T3)  $\text{NH}_4^+$ , as the only N source. These treatments were done with full, half and quarter strength MS medium. Lower concentrations of each macroelement (N, P, K, Ca and Mg) were tested using MS standard medium concentration diluted to  $\frac{1}{2}$  and  $\frac{1}{4}$ . Results showed that, independently of the initial pH values, after 31 days, the growth of the clone resulted in acidification of the media (final pH= 3,6). Moreover, there was growth of the clone in these acid media, for another 31 days of *in vitro* cultivation, without any symptoms of nutritional deficiency or toxicity. The mixed source of N significantly favoured, growth of the clone and root formation. Decrease of Mg concentration to  $\frac{1}{2}$  and  $\frac{1}{4}$ , did not affect shoot growth and increased the root/shoot dry matter ratio, in comparison to the standard concentration of MS medium. This results point out the importance of physiological studies in native plants to reach a better understanding of mechanisms of adaptation, as well as for the preservation of these plants. Moreover, lowering the concentration of main macroelements, showed that native plants have specific requirements and concentrations of standard culture medium can be unnecessary.

**Keywords:** *Baccharis*, brazilian plant, nitrogen source, acidification

**Sponsor:** CAPES



**ISMAP0197 - Evaluation of naphthodiantrones' Level in *Hypericum perforatum* L. plants growing *in vitro*.**

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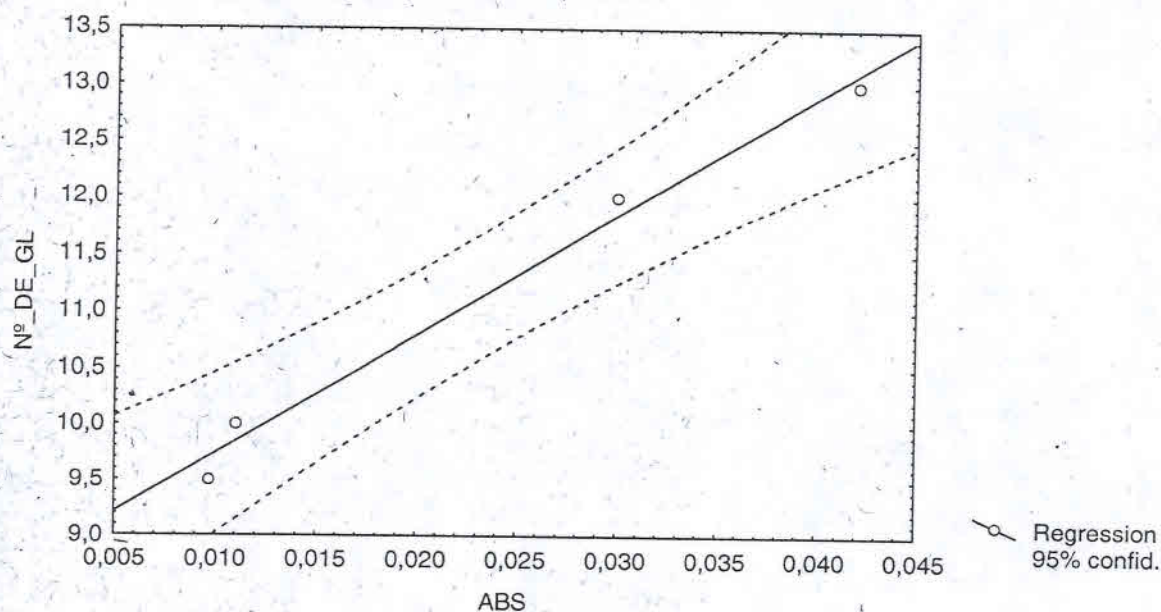
*Hypericum perforatum* L., Clusiaceae, also known as St. John's Wort, is a perennial herbaceous plant native from Europe. *H. perforatum* is a medicinal plant that produces a wide spectrum of secondary quite a few of which are of pharmaceutical interest, as anti depressive (mild to moderate depression), antiviral, antiretroviral and anti-inflammatory. The objective of this work was to establish the development *in vitro* culture and evaluation of naphthodiantrones' level in *Hypericum perforatum* L. plants cultivated *in vitro*. For shoots regeneration was utilized organogenic calli from leaves, cultivated on basal medium of Murashige & Skoog (1962) with 7.5 g/L Agar (MS) add 2.69  $\mu$ M NAA and 0.88  $\mu$ M BA. For micropropagation was utilized the growth regulators: 2.28  $\mu$ M IAA; 2.2  $\mu$ M BA; 2.28  $\mu$ M IAA and 2.2  $\mu$ M BA, for nodal segment in nine weeks of culture. The number of dark glands that containing hypericin, localized on border of leaves, was count by microscopy. The hypericin's analysis was performed by spectrophotometry (DAC, 1986). Figure 1 shows the correlation observed between number of dark glands and naphthodiantrones' level on plants growing *in vitro* on different growth regulators. The most adequate medium for shoot induction and elongation, root development and hypericin's production was MS add IAA.

**Keywords:** *Hypericum perforatum*, glands, regeneration, hypericin.

ABS vs. Nº\_DE\_GL (Casewise MD deletion)

$$\text{Nº\_DE\_GL} = 8,6874 + 105,18 \cdot \text{ABS}$$

Correlation:  $r = ,99369$



**Figure 1:** Correlation between number of dark glands and naphthodiantrones' level (% DW) on plants growing *in vitro* on different growth regulators



**ISMAP0362 - Evaluation of relevant terpenes presence in normal and transformed root cultures of *Artemisia annua* L.**

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Plants of the *Artemisia* genera are a rich source of sesquiterpenes, which have received great attention from researchers due to their biological and chemical diversity. The continued investigation of the presence of secondary compounds is highly recommended and necessary for the exploration of this rich source of new chemotherapeutic and agricultural agents. The plant targeted in this study was the CPQBA 2/39 x PL5 hybrid of the *A. annua* species, obtained through the genetic improvement program of MEDIPLANT (Centre de Recherches sur les Plantes Médicinales et Aromatiques, Suíça) and CPQBA. Methodologies were developed for the establishment and cultivation of roots, both normal and transformed with two strains of *Agrobacterium rhizogenes* (15834 and 8196). The confirmation of the transgenic nature of the roots was obtained by the "Dot Blot" technique. The plant material extracts were analyzed by CG/MS according to the analytical methodology incorporating high sensitivity terpene detection developed by CPQBA/UNICAMP researchers. The transformed and normal roots established and cultivated in different culture media under different photoperiodic conditions were chemically analysed for evaluation of relevant terpenes, such as: artemisinin, artemisinic acid, as well as two other compounds with strong antiulcerogenic activity: dihydro-epideoxi-artenuin B and deoxiartemisinin. The analysis of results revealed that none of the afore mentioned terpenes were present in the callus extracts or transformed roots. The chromatograms of the extracts taken from normal roots cultivated in MS liquid medium deprived of phyto regulators revealed the presence of the antiulcerogenic compound dihydro-epideoxi-artenuin B and another as yet unidentified compound. The characteristics of the mass fractioning pattern observed on CG/MS suggest that this unidentified compound is also a terpene, however confirmation of this hypothesis requires further studies. Photoperiod during cultivation influenced the production of both these terpenes, with the production of the unidentified compound being inversely proportional to the production of dihydro-epideoxi-artenuin B. Under continuous darkness dihydro-epideoxi-artenuin B was intensely produced and the unidentified compound present in small proportions, whereas the inverse occurred in a 16 hour photoperiod. The quantification of dihydro-epideoxi-artenuin B by CG/FID revealed an approximately fivefold increase in the production of this compound by normal roots cultivated under continuous darkness compared to roots cultivated in the presence of 16 hour of light. The presence of auxin in the MS medium maintained under 16 hour of light resulted in substantial production of the unidentified compound by normal roots, while the production of dihydro-epideoxi-artenuin B was not detected in these roots by the methods employed.

**Keywords:** Roots *in vitro* cultures, sesquiterpenes, antiulcerogenic and antiproliferative activities, *Agrobacterium rhizogenes*.

**Sponsor:** CNPq



**ISMAP0153 - Extraction and quantitative analysis of rutin in fava d'anta leaves  
(*Dimorphandra mollis*) from different origins.**

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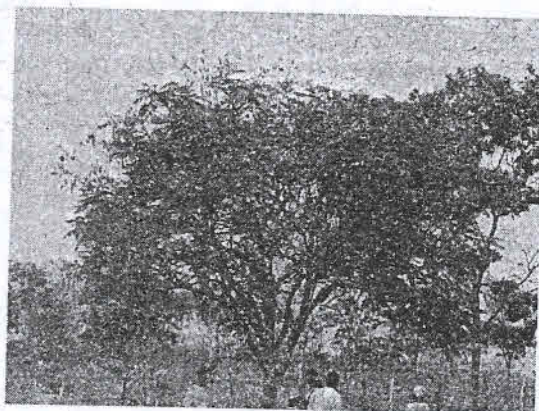
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Rutin is a bioflavonoid extracted from fruits of the Fava d'Anta tree. Fava d'anta is native to vast areas in Brazil. Rutin has the ability to increase the strength of the capillaries (blood vessels) and to regulate their permeability. It is essential for proper absorption and use of vitamin C; It is beneficial to hypertension; helps hemorrhages and ruptures in the capillaries and connective tissues and builds a protective barrier against infections. The objective of the work was to extract and to determine the content of rutin in fava d'anta leaves proceeding from different origins. Fava d'anta leaves were collected in the cities of Jequitai, Mirabela, Patis, Januária, and Montes Claros, North of Minas Gerais State (Figure 1). Three repetitions of fava d'anta leaves were powdered and the extraction of rutin was carried out with one aliquot of 10 mL of methanol. The extracts were submitted to absorbance reading in the spectrophotometer in the wavelength of 285 nm. A rutin calibration curve developed before was used and the following results in w/w of rutin in fava d'anta leaves were obtained: Januária 4.1%, Jequitai 9.4%, Montes Claros 2.9%, Mirabela 4.7% and Patis 5.2%. The discrepancy found in the different places might be explained by the different ages of the leaves. Research is being carried out with the purpose of determining the period when the production of rutin in leaves and fruits of fava d'anta is higher.

**Keywords:** Fava d'anta, rutin, leaves, origin

**Sponsor:** Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG



**Figure 1:** Fava d'anta tree studied in Mirabela, North of Minas Gerais State



**ISMAP0392 - *In vitro* conservation of *Podophyllum peltatum* using synthetic seed technology and osmotic agents.**

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Germplasm conservation of *Podophyllum peltatum* L. was attempted by encapsulating shoot buds using synthetic seed technology and storing buds, shoots and plantlets on medium containing mannitol and sorbitol. Shoot buds excised from *in vitro* plantlets were encapsulated in calcium alginate beads stored on different substrates at 5°, 10° and 25° C for 12 months. In evaluating the effects of storage conditions on the shoot cultures, samples were taken after 4, 8 and 12 months of storage for re-growth evaluation. Encapsulated buds survived better on substrate containing water solidified with 1 % agar under 10° C storage condition. Maintenance of encapsulated buds at 25°C was poor since germination was not controlled in such temperature, thus during re-growth these buds produced low percent of plantlets that re-grew. For storing shoot cultures under minimal growth, Murashige and Skoog (MS) media was supplemented with sorbitol and mannitol (20 g/l and 40 g/l). Inclusion of 20 g/l sorbitol into conservation media increased culture survival however higher concentrations affected their re-growth. Addition of mannitol or sorbitol to the media was less effective in reducing growth than encapsulated shoot buds.

**Keywords:** Mayapple, germplasm, tissue culture, lignans



# ISMAP0261 - In vitro germination of *Pothomorphe umbellata* L.

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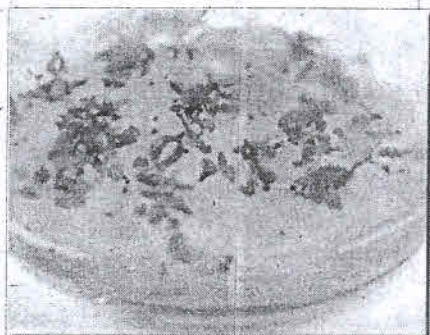
In Brazil, *P. umbellata* L. is called "caapeba do sul", "pariparoba do sul" and "malvarisco". (Figure 1). This **plant** is known by its medicinal properties like anti-inflammatory, painkiller, anti-fungi, etc. The present research aimed at establishing a protocol of sterilization and germination of seeds. The plant material was obtained from Adrianópolis-PR. Seeds were immersed in water for 12 hours and decontaminated with alcohol 70% v/v (30 seconds) and sodium hypochloride (Q-Boa) 20% v/v (2% active chlorine) for 5, 10, 15 and 30 minutes followed by inoculation in MS62 medium. Finally, the seeds were submitted to 16 h/d, light continuous and darkness. The seeds immersed in water for 12 hours didn't present difference in the germination, compared with the seeds germinated without immersion. The best treatments of sterilization were 10 and 15 minutes, with 32,5% and 30% plantlets production, respectively (Table 1). The plantlets that were germinated in 16 h/d photoperiod (43,20%) presented better development compared with light continuous and darkness (Figure 2).

**Keywords:** germination, contamination, photoperiod, plant tissue culture.

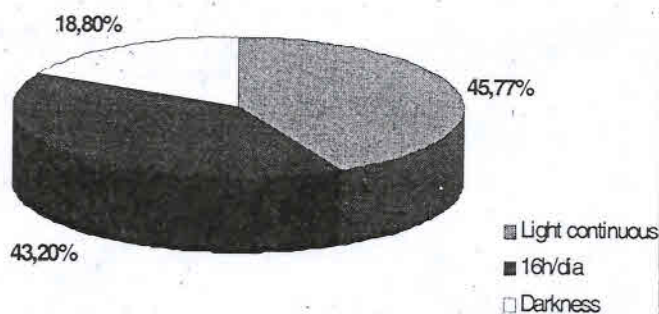
**Table 1.** Contamination, germination and plantlets production of *Pothomorphe umbellata* L. submitted to different times of immersion of sodium hypochloride.

TRATAMENT (Immersion time in sodium hypochloride)	CONTAMINATION (5 days)	SEED GERMINATION (7 days)	PLANTLETS PRODUCTION (30 days)
5 minutes	67%	40	17,55%
10 minutes	----	45	32,5%
15 minutes	----	45	30%
30 minutes	----	42,5	25%

**Figure 1.** *Pothomorphe umbellata* L. plantlets submitted to 16 h/d after days



**Figure 2.** Percent of seeds germination *Pothomorphe umbellata* L. submitted to different photoperiods





# ISMAP0002 - In vitro propagation of *Cordia verbenaceae* L. (Boraginaceae).

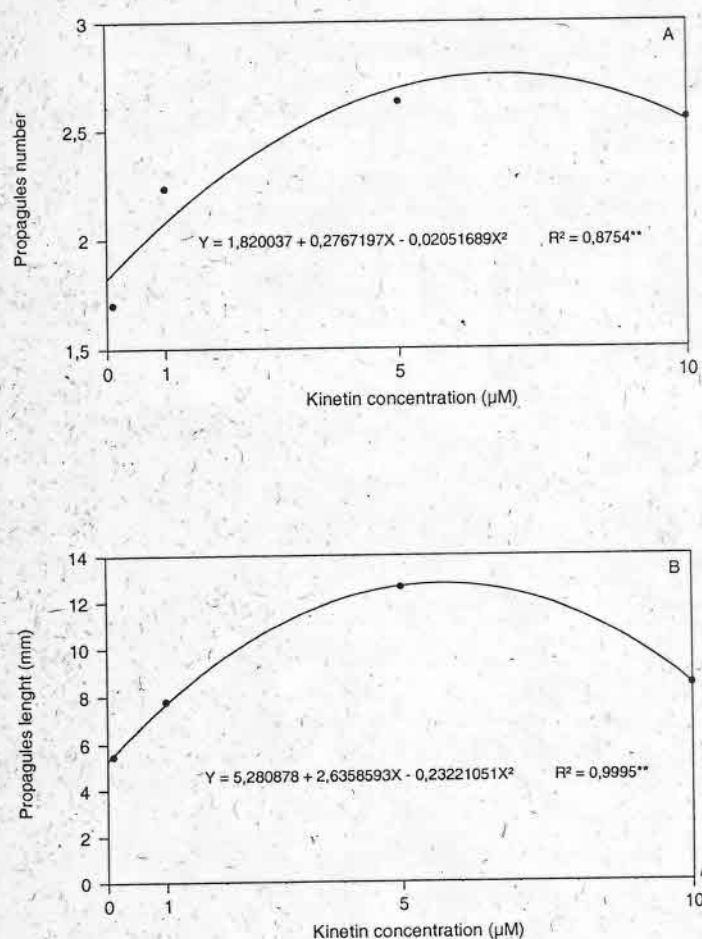
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Clonally propagating by tissue culture is highly desirable to regenerate sufficient populations of plants with similar characteristics, decreasing or eliminate the possibility of anomaly what occurring with others methods. There are no previous reports of micropropagation of *Cordia verbenaceae*. In this study, in vitro techniques are applied multiply this important Brazilian medicinal plant. Shoot tip and nodal explants were cultured on Murashige and Skoog solid (0,6%) medium (MS) supplemented with 0.1, 1.0, 5.0 and 10.0 µM kinetin and 0.01µM naphthalene acetic acid (NAA). Shoot tips yielded more propagules than nodal explants. The number and length of propagules increased unclear linearly with in kinetin levels at 5 µM, decrease with more concentration. The treatment containing shoot tip explants cultured in 5 µM kinetin and 0.01µM NAA yielded 2.7 propagules per explant. Propagules rooted on MS medium without growth regulators. Finally, 90-95% of the rooted plants transferred to potting medium survived.

**Keywords:** *Cordia verbenaceae*, growth regulators, micropropagation, shoot tip.



**Fig. 1-** Effect of kinetin on propagules number (A) and length (B) of *Cordia verbenaceae*



**ISMAP0225 - Initiation and growth characteristics of callus cultures of *Ginkgo biloba* L.**

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*Ginkgo biloba* L. is a native of China and the only living representative of a once large group of plants from the late Mesozoic period that flourished for centuries in temple gardens of China and Japan. It is important as an ornamental tree as well as a source of pharmaceutical products, including ginkgolides, diterpene compounds that have been used to treat asthma, heart disease, stroke and Alzheimer's patients. The tree may require up to 20 yr to reach maturity, and ginkgolides are found in the root bark (1%) and in leaves (0,2%). Thus, mass production of ginkgo trees as a source of plant material for harvesting ginkgolides is of commercial interest. As concerns this plant, little work has been done on *in vitro* cultures. The objective of this study was to establish an *in vitro* culture protocol for callus induction for *Ginkgo biloba* L. Leaves are collected from trees growing at Ijuí - RS- Brasil in January of 2003, washed with water and commercial liquid detergent and superficially disinfested by being soaked in 70% sodium hypochlorite plus two drops Tween 20/100 ml for 20 min and rinsed twice in sterile distilled water. Two different explant sizes are tested: 0,1 and 1,0 cm<sup>2</sup>. Explants were placed in Petri dishes containing 20 ml Murashige and Skoog minimal organics medium plus 1 mg/l Benlate. Various levels and combinations of naphthalene acetic acid (NAA) 0,1 to 1,0 mg/l and benzyladenine (BA) 0,5 to 1,0 mg/l were added to the medium. Cultures were incubated at 25 ± 2°C, in the dark and under a 16 h photoperiod. Callus induction was recorded after 2 weeks as a function of hormone exposure. Media tests had 10 replications for each explant and the experiment was repeated. The greatest amount of callus as measured by fresh weight was observed with 1,0 mg/l NAA and 1,0 mg/l BA in the dark, and the mediums containing 0,1 mg/l NAA did not produce any response. Our results established a protocol for the induction of callus in *Ginkgo biloba* L., and now we are working in order to the establishment of cell suspensions to the future extraction of medicinal ginkgolides.

**Financial Support:** UNICRUZ / FAPERGS.



**ISMAP0079 - Isolation and culture of protoplasts from the medicinal plant  
*Centella asiatica*.**

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*Centella asiatica* (L.) is a medicinal plant which is used to treat several ailments, including diarrhoea and eye infections, and to give partial relief from leprosy. The application of protoplast technologies to medicinal plant species has been relatively poorly exploited. However, such somatic cell approaches may be employed for genetic modification of chemical composition or other characteristics, such as disease resistance. Additionally, the culture of protoplasts is one of the most effective ways of isolating lines of single cell origin which can be selected for their high secondary metabolite synthesis. To date, there are no reports of the isolation, culture and plant regeneration from protoplasts of *C. asiatica*. In the present investigation, protoplasts were isolated from cell suspensions initiated from leaf laminae and petioles using an enzyme mixture consisting of 1.5% (w/v) Cellulase R10, 1.0% (w/v) Macerozyme R10 and 0.5% (w/v) Driselase in CPW salts solution with 13% (w/v) mannitol as osmotic stabilizer. Digestion was carried overnight for 16 hours on a rotary shaker (20-30 rpm) in the dark at  $25 \pm 2^\circ\text{C}$ . Yields and viabilities of isolated protoplasts were  $6.5 \times 10^4 \pm 0.8 \text{ g}^{-1}$  fresh weight and  $24.4 \pm 6.8\%$  for protoplasts from lamina-derived cell suspensions and  $7.9 \times 10^5 \pm 1.5 \text{ g}^{-1}$  fresh weight and  $79.3 \pm 13.4\%$  for protoplasts from petiole-derived cell suspensions. Protoplasts from lamina explant-derived cell suspensions were cultured at plating densities of  $0.25 \times 10^5 - 2.0 \times 10^5$  protoplasts  $\text{ml}^{-1}$  in half-strength B5 based medium (Gamborg *et al.*, 1968), containing  $0.1 \text{ mg l}^{-1}$  2,4-dichlorophenoxyacetic acid (2,4-D) and  $0.3 \text{ mg l}^{-1}$  zeatin, dispensed as semi-solid agarose droplets (each approx. 70  $\mu\text{l}$  in volume) in 5.5 cm diameter Petri dishes (10 droplets per dish). First mitotic divisions of protoplast-derived cells were observed after 4 d of culture at an optimum plating density of  $0.5 \times 10^5$  protoplasts  $\text{ml}^{-1}$ , giving an initial plating efficiency at this time of  $12.7 \pm 0.6\%$ . After 42 d of culture, protoplast-derived cell colonies were creamy-white in colour and each approx. 1 mm in diameter, with a final plating efficiency of  $0.6 \pm 0.2\%$ . Cell colonies transferred to semi-solid proliferation medium containing 2,4-D ( $4.0 \text{ mg l}^{-1}$ ) and zeatin ( $0.2 \text{ mg l}^{-1}$ ) were creamy-yellow in appearance, whereas colonies cultured on medium devoid of these growth regulators became light green and compact. In the case of protoplasts from petiole-derived cell suspensions, culture in Murashige and Skoog (1962)-based medium supplemented with  $2.0 \text{ mg l}^{-1}$  alpha-naphthaleneacetic acid and  $0.5 \text{ mg l}^{-1}$  6-benzylaminopurine resulted in an initial plating efficiency of  $19.3 \pm 4.2\%$  at an optimum plating density of  $1.0 \times 10^5$  protoplasts  $\text{ml}^{-1}$ . However, mitotic division was not sustained after this stage. Plant regeneration studies are on-going from protoplasts isolated from lamina-derived cell suspensions.

**Keywords:** *Centella asiatica*, cell suspensions, medicinal plant, protoplasts

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**ISMAP0187 - Micropropagation of *Cunila incisa* Benth., a potential source of 1,8-cineole.**

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*Cunila incisa* Benth. (family Lamiaceae, subfamily Nepetoideae, tribe Mentheae) is one of the twelve South-American species of the genus *Cunila*. The essential oil of this aromatic perennial scrub is characterized by high concentration of 1,8-cineole. Leaves and flowers infusions are used in popular medicine for the treatment of chronic cough and respiratory diseases. *C. incisa* can be propagated by seeds and cuttings, but the application of these conventional propagation methods are limited by poor seed viability, low germination rate and scanty rooting of stem cuttings. In this context, we report a protocol for the micropropagation and rooting of this species. Axillary buds of adult selected plants of *Cunila incisa* were used to evaluate the effect of growth regulators and culture media on micropropagation. The highest propagation rate was obtained using Murashigue and Skoog (MS) medium supplemented with 4.4  $\mu$ M of benzyladenine (BA). High concentrations of BA resulted in hyperhydricity. Auxine/cytokinin combinations were not benefic to the micropropagation process. Rooting was achieved on MS medium without growth regulators. Indolbutyric acid addition to rooting media was detrimental for both proliferation and rooting. Micropropagated plants transferred to the field showed normal morphology, and were more vigorous than stem rooting derived plants.

**Keywords:** *Cunila incisa*, in vitro culture, growth regulators.



**ISMAP0226 - Micropropagation of *Lantana camara* L. under different experimental conditions.**

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*Lantana camara* L., a member of the Verbenaceae family, is native to tropical and subtropical America; however, this species is cultivated world-wide as an ornamental plant. Although some taxa of the widely variable *L. camara* are toxic to ruminants, the plant has been used in folk medicine, in many parts of the world, to treat fever, influenza, asthma, bronchitis and a variety of others disorders. The scope of this work was to evaluate the effect of IAA (indole-3-acetic acid), BA (benzylaminopurine) and TDZ (thidiazuron) on *in vitro* shoot number, shoot size and rooting development of *Lantana camara* L. To examine the response of nodal segments to growth regulators, plantlets cultivated on MS medium (Murashige & Skoog, 1962) solidified with 0.8% agar, containing 3% sucrose were subcultured to MS medium supplemented with IAA at 0.044, 0.44 and 4.4  $\mu$ M; BA at 0.044, 0.44 and 4.4  $\mu$ M; TDZ at 0.044, 0.44 and 4.4  $\mu$ M. In addition, a control without growth regulators was included (MS medium). The cultures were incubated at  $25 \pm 2^\circ\text{C}$  under a 16/8 h light/dark photoperiod (daylight fluorescent lamps,  $23 \mu\text{mol m}^{-2}\text{s}^{-1}$ ). During 8 weeks, with 20 days intervals, observations were recorded on the number of shoots per explant, shoot height (cm), number of nodal segments and percent root formation. There were 30 explants per treatment and each experiment was repeated twice. Data were subjected to analysis of variance (ANOVA) and the means compared by the Tukey's test at 5% significance. The greatest shoot number and nodal segments were obtained with 4.4  $\mu$ M BA while the highest shoots were obtained with 0.44  $\mu$ M IAA. *L. camara* rooted on all treatments, although 0.044, 0.44  $\mu$ M IAA and 0.044  $\mu$ M BA resulted on the highest root percentage (93.7%). In conclusion, micropropagation of *Lantana camara* can be promoted by culturing nodal segments on shoot multiplication medium with 4.4 BA and then transferring the resultant plantlets to a elongation and rooting medium containing 0.44  $\mu$ M IAA.

**Keywords:** *Lantana camara*, growth regulators, in vitro culture

**Sponsor:** PBV/UFRJ



**ISMAP0141 - Micropropagation of homogeneous plants of the Brazilian species  
*Baccharis tridentata* Vahl. (Asteraceae).**

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*B. tridentata* Vahl is an aromatic, medicinal plant, which essential oil is constituted mainly of nerolidol and spathulenol. The aim of this study was to establish a protocol for micropropagation of this species. Shoot tips of a single greenhouse grown plant were used as explants. They were washed in running water for 3 hours, sterilized with NaOCl (1%) and inoculated in Murashige & Skoog (MS) basal medium, containing activated charcoal (1% p/v). No growth regulators were added to the medium. During the five initial days after inoculation, explants were maintained in darkness to overcome oxidation, and were cultivated at 25-27°C under 16 hour photoperiod. Half strength MS (1/2MS) and half strength B5 media were also tested. Full strength MS medium resulted in taller plants with greater values of shoot and root dry matter. Rooting was induced in the same medium, without addition of growth regulators. Acclimatization gave 80% plant survival. This protocol constitutes an efficient method that could potentially produce one thousand (1000) homogeneous plants from a single matrix plant in a relatively short time (six months).

**Keywords:** *Baccharis*, clonal propagation, shoot tips, *in vitro* culture.

**Sponsor:** CAPES



**ISMAP0102 - Molecular studies on the multigene AOX as a functional marker candidate for postharvest ripening of "graviola" (*Annona muricata* L.).**

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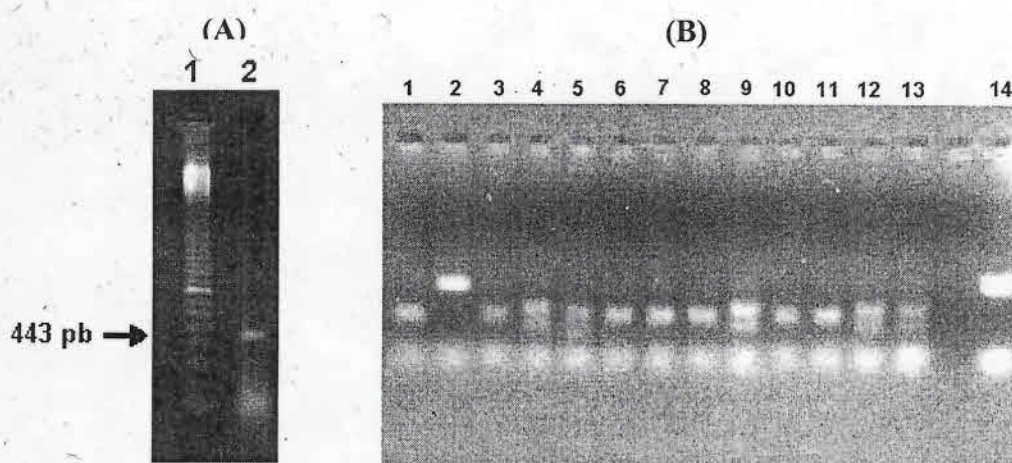
<sup>3</sup> Universidade Nacional de Rosario, Argentina.

<sup>4</sup> University of Hamburg, Alemanha.

*Annona muricata* L. (bras. "graviola") is a tropical tree, which is highly appreciated especially because of its delicious fruits. In Brazil, the fruits are mainly used in the northern and northeastern part of the country as a valuable part of nutrition. *Annona muricata* L. is also traditionally used as a medicinal plant in various tropical countries due to different properties attributed to the different parts of the plant. Fruit and fruit juice are taken in medicinal care for parasites, worms and diarrhea, as well as to cool fevers and as a lactagogue (Taylor, 2002). The rapid ripening of soursop fruit and consequent intense softening, make this fruit of difficult postharvest handling. This fact generate a problem to introduce the fruit to the market (Mosca, 1996). A better knowledge of key components in fruit development is crucial to enable initiation of new strategies in breeding as well as technology development to minimize postharvest loss. Climacteric fruits are defined by a respiratory burst during ripening, which is related to enhanced activity of the non-phosphorylating mitochondrial alternative pathway. The objective of the study is to analyse the role of the gene(s) of alternative oxidase during postharvest of commercially used graviola fruits. Genome DNA from soursop leaves was obtained through nucleus isolation and CTAB method. PCR primers, based on conserved regions from exon 3 of AOX from different plant species were used to amplify fragments from soursop DNA. A fragment of 443 bp obtained by PCR was cloned in the pGEM<sup>R</sup> T-easy vector. Individual colonies were analyzed by restriction fragments polymorphism using *Alu* I enzyme. Three different restriction patterns were identified: AC (assymetric cut), SC (symetric cut) and NC (non cut). The expression of AOX was evaluated by immunoblotting analysis. The results reveal the presence of a multigenic family of the alternative oxidase gene (AOX) in the soursop fruit. Differential expression profiles during postharvest ripening suggest a significant role for the multigene AOX family during postharvest behaviour of graviola.

**Keywords:** *Annona muricata*, postharvest ripening, alternative oxidase

**Sponsors:** FUNCAP/CNPq/EMBRAPA/DAAD



**Figure 1:** (A) PCR analysis: Marker (line 1); amplified Aox fragment (line 2). (B) Restriction fragment length polymorphism (RFLP) of cloned AOX fragments to select and isolate multigene AOX gene sequences: AC clones (lines 4, 5, 9, 12, 13); SC clones (lines 1, 3, 6, 7, 8, 10, 11); NC clones (lines 2, 14).



**ISMAP0198 - Exogenous Polyamines in the micro propagation and subterranean organ formation in *Colocasia esculenta* SCHOTT (L.).**

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The objective of this research project was to check the hypothesis of exogenous polyamines promoting an increase in the multiplication rate in *Colocasia esculenta* (inhamé) *in vitro* and its action in the subterranean organ formation with a higher protein rate in the subterranean organs. Axillary gems were used, with a length of about 0,5 cm developed from rhizomes treated with fungicide and submitted to different commercial NaClO<sub>4</sub> concentrations. The explants were washed 3 times, in distilled water and autoclaved and after that they were inoculated in test tubes containing 15 mL of MS (Murashige & Skoog) medium. The tubes were kept in a cultivation room with its light and temperature controlled. After the plants were got, four treatments were established, using exogenous polyamines (putrescine and spermidine) during the rhizome formation stage. The gems aseptis in commercial NaClO<sub>4</sub> at 50% during 10 minutes, showed itself to be much more efficient in order to prevent the fungi / bacteria from coming on the plant. The multiplication rate analysis (number of leaves and roots, height, tubes containing fungi, bacteria and/or oxidation, formation of calluses and subterranean organ formation), shows that the Putrescine application induced the formation of more roots and leaves in quantity and height.

**Keywords:** micro propagation, formation, disinfection, polyamines

**Sponsor:** CNPq



**ISMAP0278 - Production of betulinic acid and phenolic compounds in seedlings of  
*H. brasiliense* under stress conditions, *in vitro*.**

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Extracts from *Hypericum brasiliense*, an herb growing in the Southern and Southeastern part of Brazil, contain quercetin, rutin, betulinic acid and 1,5-dihydroxyxanthone. Recent reports showed that 1,5-dihydroxyxanthone inhibited the enzymes MAO A and B. Quercetin and rutin are flavonols widely distributed in plants and it has been shown that they play a role against heart diseases, virus infection, prostate cancer and protection against oxidative damages. Several pharmacological reports have shown that betulinic acid is a potent and selective replication inhibitor of the HIV type I and it has been used in pre-clinical studies for the treatment and prevention of malignant melanomas, by inducing apoptosis in p53 cells. The aim of the present work was to study the biosynthesis of these substances in *H. brasiliense*. Seedlings (12cm) growing in MS liquid medium for 20 days were submitted to the following treatments: jasmonic acid, NaCl, PEG, BION, 3xN and 3xB (1N/B = concentration of MS), salicylic acid e brassinosteroid. Three replicates were made for each treatment and samples were collected after 5 and 10 days from beginning of the treatments. The shoots were extracted with dichloromethane (betulinic acid) or methanol (rutin, quercetin and xanthone). Jasmonic acid induced an increase of betulinic acid with jasmonic acid/10 days. However, for all other treatments there was a reduction of this terpenoid when compared with control seedlings kept at normal MS. In the treatments 3xB and salicylic acid quercetin increased after 5 days treatment, decreasing to very low concentrations after 10 days. On the other hand quercetin increased with brassinosteroid and BION only after 10 days treatment. Salicylic acid and PEG also induced an increase of rutin after 5 days but there was a marked decrease of this flavonoid in the osmotic stress after 10 days. The 1,5-dihydroxyxanthone content was increased by salt and osmotic stress. To our knowledge this is the first study on the production of bioactive substances of *H. brasiliense in vitro*. Our results show that the production of betulinic acid is related with optimal growth conditions while the phenolic substances varied according to the stress conditions which may affect growth. Therefore, plants growing at the ambient may also respond to environmental conditions, what may affect the concentration of bioactive substances.

**Keywords:** Quercetin, Rutin, 1,5-dihydroxyxanthone, stress.  
Work granted by FAPESP.



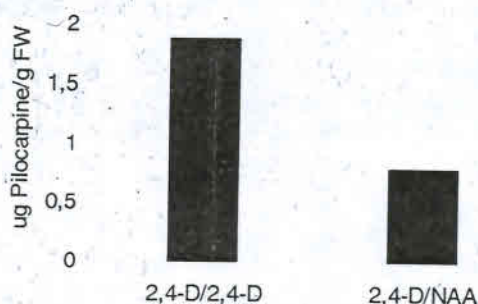
# ISMAP0205 - Production of pilocarpine *in vitro*.

I.N. Abreu, P. Mazzafera.

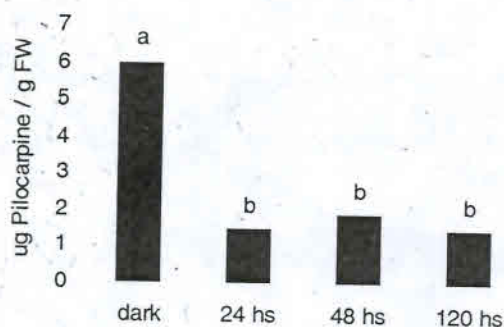
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*P. microphyllus* (Jaborandi) is a species found in the North and Northeast of Brazil, and it contains in the leaves the imidazole alkaloid pilocarpine. Pilocarpine has been extracted from leaves collected by local population from plants growing in the forest. The extractivism of Jaborandi led IBAMA to include this species in the list of endangered Brazilian species. It is not of our knowledge any work concerning *in vitro* production of pilocarpine by plant cells. Therefore, the aim of this work was to establish protocols for the production of this alkaloid in calli and cell suspension cultures from *P. microphyllus*. Hypocotyl, epicotyl, cotyledon and leaves from seedlings obtained from seeds germinated *in vitro*, and leaves, stem, petiole and petiolule from plants growing at greenhouse were used as explants to initiate the cultures. MS and Schenk & Hildebrandt media were used to test different combinations of 2,4-D and NAA. Friable calli were obtained from stem, petiole and petiolule using MS medium containing 5,77  $\mu$ M 2,4-D, in the dark. When used to produce cell cultures these calli formed cell aggregates instead of cell clusters. HPLC analysis showed that pilocarpine production was highest with 2,4-D in calli maintained in the dark (fig. 1). The alkaloid content was decreased when calli were transferred from darkness to light (fig. 2). Cultured cells released pilocarpine into the medium (fig. 3). The release of pilocarpine in the medium is an interesting characteristic to explore the production of this alkaloid using bioreactors.

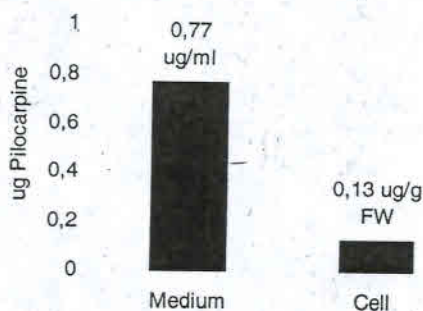
**Keywords:** Jaborandi, *Pilocarpus microphyllus*, pilocarpine, cell suspension  
Work granted by FAPESP



**Fig. 1:** Pilocarpine (ug/g Fresh Weight) in callus of *P. microphyllus* transferred to 2,4-D or NAA, in the dark, after 30 days.



**Fig. 2:** Pilocarpine (ug/g Fresh Weight) in callus of *P. microphyllus* induced in the dark and transferred to light during 24, 48 e 120 hs.



**Fig. 3:** Pilocarpine in cells (ug/g Fresh Weight) of *P. microphyllus* and in culture medium (ug/ml), after 15 days of culture, in the dark.



**ISMAP0009 - Recurrent production of plants of Wood Apple  
[*Feronia limonia* (L.) Swingle]  
from in vitro cultured hypocotyl and internodal explants.**

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The hypocotyl and internodal segments from *in vitro* grown seedlings of wood apple (*Feronia limonia* (L.) Swingle) were cultivated on Murashige and Skoog's (1962, MS) medium supplemented with N<sup>6</sup>-benzyladenine (BA) or adenine (ADE) or kinetin (KN) at 0.5 to 5  $\mu$ M. The optimum response was recorded on the medium containing 2  $\mu$ M BA. An average of 12.48 and 8.37 shoots were developed from hypocotyl and internodal explants, respectively, after eight weeks of culture. The shoots were excised, and the residual explants were transferred to fresh medium where again they developed shoots. Up to three such passages resulted in the production of shoots from repeatedly subcultured explants and an average of 24 - 36 shoots per explant was obtained. The *in vitro* developed shoots produced roots when transferred to half strength MS medium supplemented with 1  $\mu$ M 1-naphthaleneacetic acid (NAA). The developed plantlets were successfully transferred to mixture of soil, sand and coco-peat (1:1:1) and hardened in controlled environment. Hardened plants were transplanted to soil in greenhouse. Thus, a protocol has been developed to raise plants of wood apple at any time of the year.

**Keywords:** adventitious shoots, medicinal plant, micropropagation, organogenesis.



**ISMAP0172 - Tissue culture of *Cyrtopodium cardiochilum* Lindl. (Orchidaceae).**

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In Brazil, orchids of the *Cyrtopodium* genus are known popularly for the anti-inflammatory and cicatrization properties of its glucomannan-rich extract. *Cyrtopodium* species' survival is threatened due to both overexploitation and its difficult dissemination in nature, which is made worse by the continuous destruction of its natural habitat. *In vitro* propagation techniques reduce genetic diversity when used to introduce a single clone, and its possible mutants, into the environment. However, this procedure is preferable to the extinction of the species. In this work, we establish a fast and efficient protocol for the development of monoclonal shoots of *Cyrtopodium cardiochilum* for reintroduction in nature and production of genetically standardized phytotherapies, in accordance with the RDC no. 48 guideline. For that, protocorms induced in an agar-solidified hormone-free Murashige & Skoog basic medium, supplemented with sucrose and vitamins, were subgrown in the same type of medium with or without (control media) various concentrations of 2,4-D, TDZ, kinetin and AIA. The explants were kept in a photoperiod, lighting and temperature-controlled environment.

The protocorm dissemination was measured by a weekly evaluation of fresh weight. The most efficient media for protocorm-like body (PLB) induction were those that contained solely TDZ or TDZ associated with 2,4-D. We observed fresh weight increases of up to 50 times, when compared with the control media (hormone-free) PLBs. The TDZ-containing media also showed the largest number of differentiated plants/initial explants: 5,7. The increase in the plants' differentiation rate was directly related to the increase in cytokinin concentration. Root formation occurred in the AIA-containing media, although no PLB proliferation was observed. The media containing solely 2,4-D did not stimulate either PLB proliferation or root and plant differentiation.

**Keywords:** *Cyrtopodium cardiochilum*, Orchidaceae, tissue culture, protocorms.

**Sponsor:** Estácio de Sá University.



# Chemistry and Quality Control



**ISMAP0190 - Analysis of antioxidant activity of epicuticular wax of  
*Baccharis uncinella*.**

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*Baccharis uncinella* is a member of Asteraceae family. The genus *Baccharis* consists of more than 400 species, many of them already chemically studied. In the literature, there are reports of studies about flavonoids and terpenes isolation, but very few studies about antioxidant activity.

The antioxidant activity of epicuticular wax extract of *Baccharis uncinella* was evaluated considering the reactivity of the plant material with DPPH and its action against lipid peroxidation, using rat liver microsomes as lipid membrane model.

The plant material was collected in Urubici (SC) and dried at 50 °C. The epicuticular wax was obtained by dipping entire dried leaves in acetone for one minute followed by a concentration stage. DPPH assay was carried out with a medium containing an ethanolic solution of 200 µM of the free radical and monitored at 515 nm. The decrease in DPPH absorption was calculated through the comparison with control group (100% DPPH). The lipid peroxidation was induced by FeCl<sub>3</sub>/ascorbic acid system and monitored spectrophotometrically by the TBARS (thiobarbituric reactive substances) method, at 535 nm. The microsome membranes were obtained by differential centrifugation.

The extract of epicuticular wax yielded was 10%. The DPPH test provided information about radical scavenging effect of the stable DPPH free radical; the IC<sub>50</sub> obtained was 280 ± XX µg/mL much less than that obtained with quercetin, a positive control (4,7 ± XX mg/mL). The peroxidation test provided information about the microsome protection against oxidation and the IC<sub>50</sub> obtained was 148 ± XX µg/mL, also much less than the positive control (42,3 ± XX mg/mL).

These results showed for the first time that the extract of epicuticular wax of *B. uncinella* possesses very active compounds with significant antioxidant activity. Further studies have been carried out to isolate them.

**Keywords:** epicuticular wax, antioxidant activity, *Baccharis*

**Sponsors:** CNPq and FUNCITEC



**ISMAP0228 - Analysis of epicuticular wax of *Baccharis uncinella*:  
isolation ursolic acid.**

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The genus *Baccharis* consists of more than 400 species, many of them already chemically studied. In literature, there are reports of studies about flavonoids and terpenes isolation in the genus *Baccharis*. Particularly, there are only one study with volatile oils isolation from *B. uncinella*.

Isolation and identification of triterpenes from the epicuticular wax of *B. uncinella*.

The vegetal material was collected in Urubici (SC) region and dried at 40 °C for 36 hrs. The dried leaves (320g) were immersed in acetone for one minute to remove the wax layer. The extract was filtered and concentrated to dryness under reduced pressure to determine total surface wax. The residue was dissolved in methanol : water (25:75), which resulted in the precipitated that was separated. The precipitated was acetylated and purified by chromatography on silica gel using dichloromethane. The substance isolated was analysed by TLC using a mixture dichloromethane:methanol (97:3) and comparison with an authentic sample. The substance isolated also was analysed through spectroscopical analysis (NMR and IR).

The yield of surface wax extracted from leaves was 10% (32g) and the yield of precipitated was 2g. The chromatographic procedure resulted in the isolation of one pure substance. This substance showed chromatography conduct identical the authentic sample of ursolic acid. By spectroscopical analysis IR verified the signals: 2940, 1730, 1690, 1460, 1330 and 1250 cm<sup>-1</sup>. The most important signals in the spectrum NMR are: signals of 32 atoms C; signal in  $\delta_c$  137,9 e  $\delta_c$  125,7 that indicating the presence of the double bond, what is feature of the structure of triterpene with nucleus ursane (in triterpene with nucleus olean this signal occurs in  $\delta_c$  143 – 145 and  $\delta_c$  121-122) (;  $\delta_H$  5,3 (t) confirm the presence of double bond; and  $\delta_H$  2,2 (d - 1H) that indicating H-18.

The results of comparison of substance BU1 with an authentic sample and spectroscopic analysis showed that pure substance is the ursolic acid.

**Keywords:** ursolic acid, epicuticular wax, *Baccharis*



**ISMAP0060 - Anatomy and histochemistry of *Siegesbeckia orientalis*:  
vegetative part.**

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*Siegesbeckia orientalis* L. (Asteraceae) is an annual herb used in the traditional Chinese medicine in anti-allergic treatments, rheumatic arthritis, among others. In Brazil it is frequently found in the Southeast area, could be considered weed in some cultures. The anatomy of the vegetative organs of *S. orientalis* in primary structure was described, with emphasis on characterization and histochemistry of its secretory structures. Leaves, stems and roots were fixed in FAA<sub>50</sub> and ferrous sulphate and stored in 70 % ethanol. Cross and longitudinal sections were submitted to toluidine blue, pH 4.0, ruthenium red, PAS reaction, ponceau Xylidine reactive, Sudan black B and Dragendorff reactive. Anatomically, *S. orientalis* vegetative organs are similar to the characters described for the Asteraceae. The root is triarch and the stem, eustele. Ducts are absent in the root and present in the stem and leaf. These structures are of low diameter, being delimited by 4 to 5 epithelial cells. In the stems, the ducts were present in the cortex, next to the endoderm and in the pith. In the leaves, they were associated to the vascular bundles, facing both the xylem and the phloem. The leaves are dorsiventral and amphistomatic. Three types of secretory structures were observed: ducts, hydathodes and glandular trichomes. The histochemical tests applied showed the presence of phenolic and alkaloid compounds in the ducts, while the presence of lipid and phenolic compounds was found in the trichomes. Such results indicate the complex secretion produced by the structures in this species.

**Keywords** – weed, medicinal plant, hythiem, shrimp-claw plant, Heliantheae.

**Sponsor:** FAPEMIG



**ISMAP0325 - Antiedematogenic and antiproliferative activity of crude extract and fractions obtained from *Plathymenia reticulata* benth. (Leguminosae).**

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The species *Plathymenia reticulata*, known popularly as "vinhático" is used in folk medicine for treatment of several inflammatory processes. The scope of this work was to evaluate the antiedematogenic and antiproliferative activity of crude hidroalcoholic extract (CHE) as well as the basic (BF), neutral (NF) and acid (AF) fractions from CHE. The crude extract and fractions were evaluated on paw edema model induced by carrageenan (1%). The crude hidroalcoholic extract (1000 mg/Kg) inhibited the inflammation process by 42, 37, and 41% after 3, 4, and 5 hours of carrageenan subplantar administration, respectively. Under the same conditions, only fractions FB and FN (500 mg/Kg) showed significant antiedematogenic activity after 4 and 5 hours. Indomethacin (10 mg/kg) produced 46, 60 and 50% inhibition after 3, 4 and 5 hours, respectively. Antiproliferative activity was tested employing an *in vitro* assay using human cancer cell lines. The results were detected with Sulforhodamine B assay (SRB). The crude extract and fractions (FB and FN) gave cytostatic inhibition from 25 µg dose and cytotoxic inhibited at 250µg dose with UACC-62 (melanoma) OVCAR (ovarian) and NCI460 (lung) cell lines. Indomethacin gave cytostatic inhibition from 25 µg dose and cytotoxic inhibited at 250µg dose with NCI-460 (lung), PCO<sub>3</sub> (prostate), UACC-62 (melanoma) and 786-0 (renal) cell lines. These results may suggest that the pharmacological mechanism of crude extracts and fractions of *P. reticulata* is similar to that observed for the non-steroidal antiinflammatory drugs.

**Keywords:** *Plathymenia reticulata*, , antiinflammatory activity, antiproliferative activity, medicinal plants

**Sponsor:** FAPESP; CPQBA/Unicamp



## ISMAP0136 - Antimicrobial testings and chiral phase GC analyses of essential oils and linalool.

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**Introduction:** Various essential oils and aroma compounds possess antimicrobial activities and are used since ancient times e.g. as preservatives in foods and cosmetic products. For the testing of these effects especially agar diffusion and agar dilution methods are preferred. Although many single aroma components have been tested for their antimicrobial potential against different bacteria, yeasts and fungi, only few data of enantiomers are available in literature [1]. Therefore, some essential oils and their key aroma compounds were analyzed by means of chiral phase GC (GC-FID and GC-MS) to get informations about the chirality of these target components. These data were compared with those of antimicrobial testings of the samples to find a connection between enantiomeric and biological properties.

**Methodology:** Agar diffusion and agar dilution methods were used for the antimicrobial testings, chiral phase (cyclodextrin) gas chromatography (GC-FID and GC-MS) for separation of aromatic enantiomers and olfactive techniques (e.g. olfactive evaluations and GC-olfactometry) for aroma control.

**Results and discussion:** The monoterpene alcohol linalool in (-), (+) and racemic form, eighteen linalool-rich essential oils (different lavender/lavandin, coriander and basil samples from various origin) and the phenolic compound eugenol (used as reference) were tested by agar diffusion/dilution methods for the antimicrobial activities against Gram-(+)-bacteria *Staphylococcus aureus*, Gram-(-)-bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as well as the yeast *Candida albicans*. (-)-Linalool (main enantiomer in lavender/lavandin oils) was most effective against *Klebsiella pneumoniae* and *Candida albicans*, (+)-linalool (main enantiomer in e.g. coriander oils) against *Escherichia coli* and racemic linalool against *Candida albicans*. In contrast to this result, most of the used lavender and lavandin samples were also very active against *Staphylococcus aureus* (beside expected high activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* – see above).

By means of chiral phase GC the enantiomeric purity of linalool samples as references and as key aroma compound in basil, coriander, lavandin and lavender oils was analyzed and the reported high percentage of the correct enantiomer in each oil (e.g. (-)-linalool in lavandin/lavender and (+)-linalool in coriander samples) ascertained.

**Conclusion:** Some of the antimicrobial data of tested reference samples and essential oils are in agreement with those obtained by chiral phase GC. On the other hand, a significant different antibacterial activity of lavandin and lavender oils in correlation to linalool samples was found. This result seems to be an additional confirmation for the hypothesis that an antimicrobial activity of terpenoids is more depending on the hydrogen-bonding parameters and lesser on the enantiomeric quality [1,2]. In addition, synergistic and antagonistic effects of further constituents may play an important role for antimicrobial effects of the tested essential oil.

**Keywords:** Linalool, linalool-rich essential oils, antimicrobial testings, chiral phase GC.

### Literature:

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[2] Griffin, S.G., Wyllie, S.G., Markham, J.L., Leach, D.N., *Flavour Fragr.J.* 14, 322-332 (1999).



**ISMAP0320 - Antiproliferative activity of crude extract and fractions obtained from *Aspidosperma tomentosum* Mart.**

Kohn, Luciana Konecny<sup>1</sup>; Pizão, Paulo Eduardo<sup>2</sup>; Foglio, Mary Ann<sup>1</sup>; Antônio, Márcia Aparecida<sup>1</sup>; Amaral, Maria do Carmo E.<sup>3</sup>; Bittrich, Volker<sup>3</sup>; Carvalho, João Ernesto<sup>1</sup>.

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This study evaluated the antiproliferative activity of *Aspidosperma tomentosum* Mart ("peroba do campo") dichloromethane and ethanol extracts, a typical tree of the Brazilian *cerrado*. *In vitro* assay using five human cells line: K562 (leukemia), MCF7 (breast), NCIADR (breast expressing the multidrug resistance phenotype), NCI460 (lung) and UACC62 (melanoma) were employed. The results were detected with Sulforhodamine B assay (SRB). The crude extracts, showed a concentration dependent inhibition on MCF7, UACC62, NCIADR and NCI460. The dichloromethane extract (CHD) had better cytotoxic (46%) activity for MCF7 at 125 mg/mL than the ethanol extract (CHE). Therefore the CHD was submitted to column chromatography on silicagel providing fractions denominated unpolar fraction (UP), terpenic medium polar fraction (TF), alkaloid medium polar fraction (AF) and polar fraction (PF). The best results were observed on MCF7 and NCI460 strains with TF fraction, whereas PF fraction was unspecific, inhibited all the cells lines giving cytotoxicity at 125 mg/mL. Fractions AF and UF did not present a significant inhibition in the tested concentrations. This information suggests that the active principle(s), which is (are) responsible for the antiproliferative activity is (are) found in fraction TF and PF demonstrating a concentration dependent inhibition.

**Keywords:** *Aspidosperma tomentosum*, cancer



**ISMAP0383 - Aroma compounds of essential oils of two varieties of the spice plant  
*Ocimum canum* Sims from Cameroon.**

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*Ocimum canum* Sims (Lamiaceae) is a well known spice in Cameroon Africa and Asia. locally name "cotimajo, or Werere", the aerial plant part is used to flavour fish soup. The essential oils of flowers, and leaves of two chemo types of *O. canum* from Cameroon were analysed by GC/FID and GC/MS. The oils of type I were characterized by a high percentage of oxygenated monoterpenes (71.7% - 91.9%), represented especially by linalool (44.92%) and geraniol (39.18%). The oil from chemotype-II contain lower oxygenated monoterpenes and higher sesquiterpene derivatives (47.75% - 71.84%), the main components respectively in the oils of leaves and the flowers are, limonene (41.52% and 5.69%) 1,8-cineole (10.13% and 18.49%),  $\delta$ -cadinene (4.03% and 18.01%) and  $\alpha$ -terpineol (6.89% and 6.4%).

**Keywords:** *Ocimum canum*, essential oil, linalool geraniol



**ISMAP0343 - Caulinar morpho-anatomical characters of *Himatanthus sucuuba*.**

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*Himatanthus sucuuba* (Spruce) Woodson, Apocynaceae, popularly known as sucuuba and janaguba, is a latescent woody species from Amazonian region. The stem is upright and the bark is rugous, and it has been confirmed its anti-inflammatory and analgesic activity as well as low toxicity. Aiming to establish anatomical characters for the medicinal plant identification and drug quality control, the stem in secondary growth has been analysed. The botanical material was collected at the Instituto Nacional de Pesquisas Amazônicas (INPA) and caulinar fragments were fixed in FAA, free-hand sectioned and stained according to usual microtechniques. Histochemical essays were applied for starch, lignin, lipophilic and phenolic substances and for confirming crystal chemical nature. In secondary growth, the stem transection is circular and it is found periderm as the dermal system, being the first phellogen formed in the subepidermal layers. The cortex shows numerous branched and non-articulated laticifers, idioblasts containing many prisms and few druses of calcium oxalate, and parenchymatic cells with amiloplasts. A non-continuous sclerenchymatic sheath consisting of non-lignified fibres encircles the vascular system, which is bicollateral (Figure 1 and 2). The pith cells are similar to the cortex ones.

**Keywords:** *Himatanthus sucuuba*, quality control, stem, laticifers

**Sponsor:** CAPES

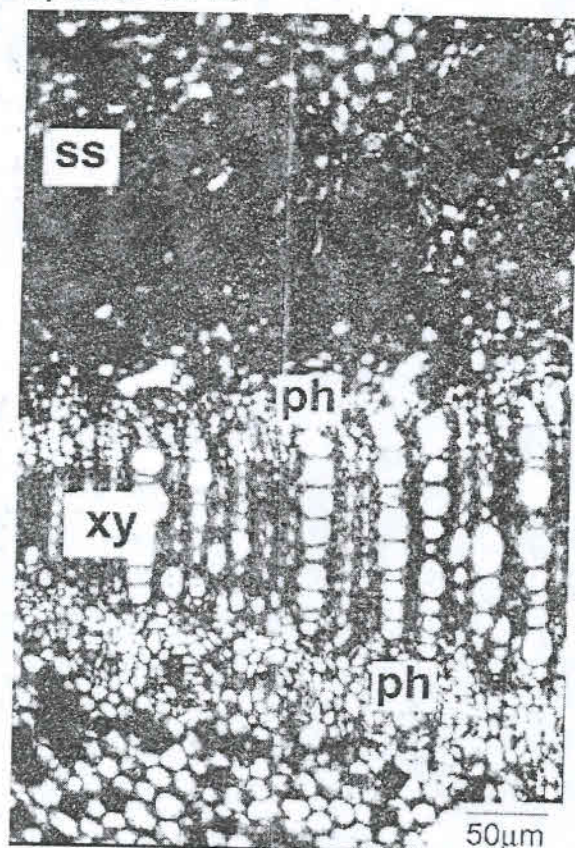


Figure 1 – Stem in transection, showing sclerenchymatic sheath (ss), phloem (ph) and xylem (xy)

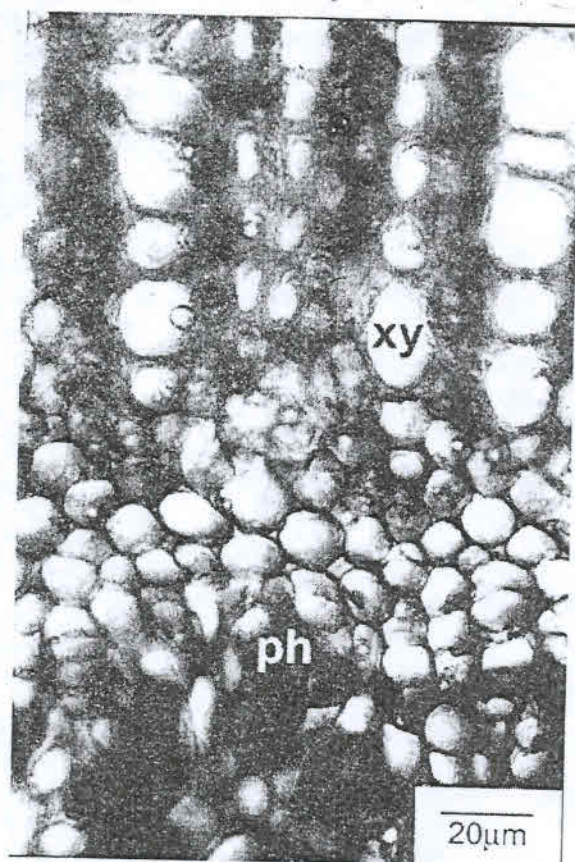


Figure 2 – Detail of the vascular system, indicating the xylem (xy) and the internal phloem (ph)



**ISMAP0155 - Characterization of major anti-fungal compound from the bark of  
*Ailanthus excelsa*.**

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*Ailanthus excelsa* Roxb (Simarubiaceae) known as Maharukh in Hindi is a fast growing tree distributed in the Central and Southern India. The bark is bitter, refrigerant, astringent and antihelmintic. It has anti-spasmodic and expectorant properties and is used for asthma, bronchitis, dysentery and cure of skin diseases. Previously four quassinoids separation from the root bark have shown substantial anti-tumour and cytotoxic activities against the lymphocytic leukemia. The compounds of this class will continue to be of interest. Besides the quassinoids, the bark also contains alliantic acid, 2, 6 – dimethoxy benzoquinone,  $\beta$  - sitosterol, malantin triacontane and hexatriqcontane. The crude alcoholic extract of *A. excelsa* show anti-fungal activity. Separation and purification of compounds from *A. excelsa* have been done. Antifungal activity was determined by minimum inhibition concentration with agar serial dilution test. Spectral analysis show major anti-fungal compound as excelsin.



**ISMAP0104 - Chemical composition of essential oil from *Ocimum sanctum* L. leaves grown in Londrina, Parana, South Brazil.**

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Holy Basil (*Ocimum sanctum* L., Fam. Lamiaceae); native to the Indian subcontinent is used in folk medicine to relief digestive problems. The leaves of this shrub have been traditionally used in religious rituals by the Hindu, and by Australian Aborigines to treat dysentery, fevers, and sickness; European settlers ingested a tea, made from the leaves, to treat septic sores on hands. The essential oils have been used to kill or repel insects, as well as to flavor foods and oral products, in fragrances. Although being tropical specie, *O. sanctum* has successfully been grown in the colder areas of southern Brazil. This specie has produced around 5 ton/ha of fresh leaves with a very rich aroma. Its leaves are high in several essential oil constituents such as methyl-chavicol. The main objective of this work was to evaluate the essential oil yield and composition of this genotype of Holy basil when grown in southern Brazil. In August 2003, 100g of fresh leaves from *O. sanctum* were collected from plants cultivated at medicinal plant garden of IAPAR in Londrina, Parana, South Brazil. The freshly harvested leaves were then subjected to oil extraction and analysis to determine oil yield and aromatic volatile composition. Leaves were steam distilled to extract the essential oil. Physiology laboratory of IAPAR and the chemical characterization of the essential oils was performed at the New Use Agriculture and Natural Plant Products Program at Rutgers University, USA. Volatile oils (10 µl) were dissolved in 1 ml of hexane, after dehydration over sodium sulfate, and analyzed using a gas chromatograph (GC) coupled to a mass spectrometer (MS) (Agilent GC System 6890 Series, Mass Selective Detector, Agilent 5973 Network, FID detector). Samples of 0.5ml were prepared in duplication and were injected with an auto sampler (Agilent 7683 Series), the Inlet temperature was 220°C, in a HP5-MS (30m, 0.25 mm ID, 0.25mm Film) column, temperature program, 60°C 1 min, 4°C/min, 200°C 15 min. Helium constant flow was set at 1 ml/min. Individual identifications were made by matching their spectra with those from mass spectral libraries (Wiley 275.L). From 100 g of fresh leaves was obtained 2.0 ml of essential oil, representing 2 % of oil yield. The main essential oil compounds were found to be Methyl chavicol (95.35 %), Germacrene-D (1.46 %), and Bicyclogermacrene (2.03%). The results showed that *O. sanctum* grown in Londrina presented a similar oil yield and oil composition to other producer regions and could be a promising source of Methyl chavicol.

**Keywords:** Methyl-chavicol, oil yield, alfavaca anisada.



**ISMAP0224 - Chemical study of the apolar constituents of *Cladocolea micrantha* Kuijt ("erva-de-passarinho"), an ethnomedicinal mistletoe of the Amazon Region.**

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**Introduction.** The Loranthaceae family comprises about 70 genera and 950 species, which are mainly distributed in tropical regions. The ethnomedical use of these species includes their antimicrobial, antihypertensive, relief of stomach ache, anti-inflammatory and anticancer properties. *Cladocolea micrantha* Kuijt, known as "erva-de-passarinho" is an endemic Amazonian Loranthaceae species, which grows from Venezuela to Brazil and has been popularly used as an anti-tumoral agent and to heal wounds. **Object.** The hexane and chloroform extracts, which showed "in vitro" activity against five neoplastic cell lines: SP2/0 (mouse plasmacytoma), J774 (mouse monocytic cell line), BW (thymoma), P3653 (plasmacytoma), MK2 (monkey kidney cell), were chemically evaluated. **Experimental.** The plant was collected in the Campus of the University of Amazonas (UA), Manaus (UA Herbarium No. 6213). Air dried and powdered plant were extracted by hot maceration with ethanol:water 7:3. The combined extracts were evaporated and redissolved in distilled water, and partitioned by successive solvent extraction, using n-hexane, chloroform and ethyl acetate and the extracts evaporated to dryness. The more active extracts, hexane and chloroform, were evaluated by thin layer chromatography (TLC), silica gel normal and reverse (C18) phases and GC-MS, <sup>1</sup>H and <sup>13</sup>C-RMN. **Results.** Chemical tests, TLC and spectrometric analysis showed the presence of terpenes and the absence of alkaloids and flavonoids. The triterpene lupeol, present in all fractions, was the main component in these extracts. Chromatographic assays also permitted identification of the triterpenes  $\alpha$ -amyrin,  $\beta$ -amyrin, and their acetylated derivatives;  $\beta$ -sitosterol, cycloartenol and a euphane triterpene, butyrospermol. **Conclusions.** The evaluation of hexane and chloroform active extracts of *C. micrantha* showed the major presence of triterpenes. These finding suggest that the anti-neoplastic activity could be due to the combination of these substances. This is the first chemical study of the genus *Cladocolea* and is relevant to the medicinal uses of *C. micrantha* by the Amazonian people.

**Keywords:** Loranthaceae, mistletoe, *Cladocolea micrantha*, triterpenes



ISMAP0080 - Chemical variability in essential oil of *Hyptis marrubioides* Epl.<sup>a</sup>

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*Hyptis* species (Labiatae) are quite aromatic and are frequently reported in the treatment of gastrointestinal infections, cramps, and pain, as well as in skin infections. The chemical components and biological activities of a few species have been examined, although the Brazilian Cerrado is characterised by explosive evolution of *Hyptis*, as a major area of morphological variation. In the present work, the composition and chemical variability of essential oils of *H. marrubioides* were examined by GC/MS. For that purpose, two sampling sites (Lavras and Tiradentes cities, Minas Gerais State, Brazil) were chosen and essential oils from stems and leaves (fresh or dried, and whole or sliced) were obtained by hydrodistillation with average yield of 0.09±0.08% (wt/wt). In total, 24 compounds were identified, with oxygenated sesquiterpenes as main constituents (Table 1). Identification of the oil components were made based on the retention indexes and by comparison of mass spectra with the NIST libraries and literature. Volatile components were submitted to multivariate analysis (754 data) using SAPD.N (CISIA, France) package. Principal Component (PCA) and Cluster Analysis were applied to the study of the similarity of individuals on the basis of constituent distribution. From PCA the whole data set could be projected in the space defined by the 2 first PCs retaining 80% of the total variance. First PC separates the dried leaves from fresh stems and leaves, and the Second PC the sampling sites (Lavras from Tiradentes). Hence, three clusters were found: I (fresh or dried stems and fresh leaves from Lavras site), characterised by a high percentage of 7 (16.7±1.6%) ( $P<0.003$ ), 8 (5.7±0.8%) ( $P<0.002$ ) and 9 (5.3±0.7%) ( $P<0.002$ ); II (dried leaves and stems from Tiradentes site) those having 3 (4.7±2.1%) ( $P<0.02$ ) and 5 (16.2±2.6%) ( $P<0.001$ ) as principal constituents, and cluster III (dried leaves from Lavras site) containing a high content of 1 (10.1±0.1%) ( $P<0.02$ ) and 2 (17.3±1.3%) ( $P<0.01$ ). The whole or sliced plant materials resulted in similar chemical composition. On the other hand, there is correlation between essential oil composition and geographic variation. Sesquiterpenes 3 and 5 were mainly produced at lower latitudes and longitudes (Tiradentes), whereas 7 and 9 at higher ones (Lavras).

**Keywords:** Chemical composition, volatile components, multivariate analysis  
**Sponsor:** PADCT, CNPq and FUNAPE/UFG

**Table 1.** Percentage of major components of *H. marrubioides* essential oils.

Component	RI <sup>a</sup>	Mean	SD <sup>b</sup>	Min.	Max.
1 $\alpha$ -copaene	1380	4.3	4.3	0.1	10.5
2 (E)-caryophyllene	1423	8.3	5.9	1.3	18.7
3 Germacrene D	1485	2.7	2.5	0.0	7.5
4 $\gamma$ -cadinene	1526	4.6	0.9	3.4	6.8
5 <i>epi</i> -longipinanol	1562	7.4	6.9	0.1	20.1
6 globulol	1586	10.1	3.1	5.8	15.8
7 caryophylla-4(14),8(15)-dien-5- $\beta$ -ol	1642	13.2	3.6	7.9	18.9
8 cubenol	1650	4.0	1.7	1.8	7.2
9 himachalol	1654	3.1	2.2	0.1	6.6
10 guaia-3,10(14)-dien-11-ol	1675	4.5	1.9	2.1	8.2
11 eudesma-4(15),7-dien-1- $\beta$ -ol	1691	11.5	2.5	7.4	15.3

<sup>a</sup>Retention Index. <sup>b</sup>Standard deviation



**ISMAP0369 - Comparison between different coffee microcapsules by GC-MS qualitative profile.**

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Microencapsulation is the process whereby particles of a compound are formed and covered with a wall providing protection. The most common covering materials are hydrocolloids, gums, dextrans and modified starches and the most used methods are spray drying, extrusion, coacervation and molecular inclusion.

The aim of this study is compare coffee microcapsules using three different wall material monitoring them by GC/MS qualitative profile.

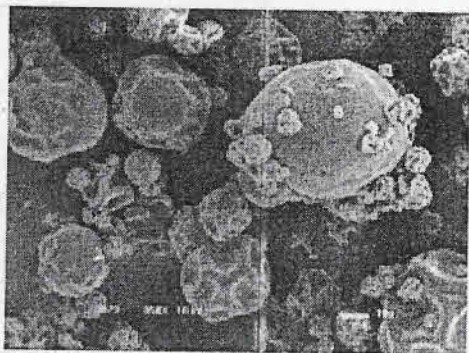
A suspension containing crioconcentrated coffee extract and the wall material was prepared in water, homogenized and passed through in a mini spray dryer LabPlant model SD-04 (Leeds,UK) using 180 °C of entrance temperature, 90 °C of exit temperature, 10 mL/min of liquid flow rate, 10 L/min of air pressure and 1 mm of nozzle diameter.

The coffee microcapsules was obtained from different wall materials, maltodextrin, acacia gum and cashew tree gum, using wall material concentration of 30% and 30% of crioconcentrated coffee extract of total solids in suspension. The microcapsules was extracted using microwave-assisted extraction (4g, 8min, 500W) in triplicates. The morphology of the materials was analysed by scanning electron microscopy to verify the structural characteristics of the microcapsules. The capsules produced with acacia gum were smaller ( $d_{50\%}=42,8 \mu$ ) in the range of 3-450  $\mu$  (Figure1).

The aroma of three coffee microcapsules samples from spray drying technique were studied by GC/MS qualitative profile analysis. The identities of compounds were confirmed by comparison of their mass spectral with those of library spectra.

It has been demonstrated that the three wall materials have a similar GC/MS qualitative profile.

**Keywords:** *aroma, coffee, microencapsulation, spray drying.*



**Figure 1.** Illustration of microcapsules from acacia gum



**ISMAP0140 - Composition of the essential oils of *Artemisia incana* (L.) Druce.  
*Helichrysum ocephalum* Boiss. and *Centaurea ispahanica* Boiss. three  
Asteraceae herbs growing wild in Iran.**

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The genus *Artemisia* (Asteraceae tribe Anthemideae) belongs to the useful aromatic and medicinal plants comprising about 300 species found in the northern hemisphere. Thirty-four of this genus are found in Iran, among which two are endemic: *A. melanolepis* Boiss. and *A. kermanensis* Podl. Chemical composition of some *Artemisia* species has shown them to contain monoterpenes, sesquiterpene lactones, flavonoids and other constituents. The genus *Helichrysum*, consists of a few hundred species widespread throughout the world. Nineteen species of this genus are found in Iran, among which eight are endemic. Seventy-four species of the *Centaurea* species have shown the presence of flavonoids, sesquiterpene lactones specially guaianolides and germacranolide type sesquiterpene lactones. A literature survey revealed that the oils of *Artemisia incana*, *Helichrysum ocephalum* and *Centaurea ispahanica* have not been previously studied, so we decided to examine these oils. Water -distilled essential oils from the aerial parts of *Artemisia incana* (L.) Druce., *Helichrysum ocephalum* Boiss. and from the flower of *Centaurea ispahanica* Boiss. were analyzed by GC and GC/MS. Nineteen components of the oil of *A. incana* were characterized, representing 90.6% of the total components detected. The major constituents were identified as 1,8-cineole (23.3%), chrysanthenone (21.3%) and davanone (19.3%). The oil of *H. ocephalum* was characterized by higher amount of 2-caryophyllene (13.5%), menthone (10.8%), dodecane (9.1%) and menthol (8.9%) among the eighteen components comprising 95.6% of the total oil detected. Benzyl benzoate (26.5%), hexadecanoic acid (17.1%), benzyl salicylate (16.6%) and caryophyllene oxide (12.8%) were the main components among the thirteen constituents characterized in the flower oil of *C. ispahanica* representing 91.8% of the total components detected.

**Keywords:** *Artemisia incana*, *Helichrysum ocephalum*, *Centaurea ispahanica*; essential oils, GC/MS.



**ISMAP0203 - Development of methyleugenol and estragole concentrations during the ontogenesis of basil cultivars and wild provenances in the field and in the greenhouse.**

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In 2001 the Council of Europe published two opinions, which refer to the carcinogenic potential of methyleugenol and estragole. Dependent on the individual chemotype both substances may appear as main component in the essential oil of basil. The knowledge received to date suggests a very large variability in the occurrence of these two natural substances [1]. A comparison of cultivars and provenances in the field and in the greenhouse should therefore supply reliable statements regarding the biosynthetic potential concerning both components, in order to lower the toxic risk by selection of the varieties and suitable cultivation procedures.

The examinations of cultivars showed corresponding results in 2002 and 2003. The commercially available linalool types possess a very different methyleugenol base level. For these types a reduction of the methyleugenol concentration in the leaves takes place in all cases during the ontogenesis. But the red-leaf cultivars „Opal“ and „Osmin“ always contain higher contents than the green varieties. A comparable tendency cannot be determined for estragole in the typical estragole cultivars „Mittelgroßblättriges Grünes“ and „NUFAR“.

The evaluation of wild provenances from a gene bank shows that some forms are methyleugenol-free already in the young plant stage. But these accessions exhibit completely nontypical ingredient profiles for using as spices. Other forms however always contain methyleugenol and a reduction cannot be recognized during the cultivation period. Obviously the reduction of methyleugenol during ontogenesis is not specific for the entire genus *Ocimum*.

Generally, during ontogenesis basil plants cultivated under greenhouse and field conditions show a similar trend regarding the methyleugenol content. At the same time it becomes visible that there exists a strong dependence on the individual cultivar as well as on the harvest time. Beyond that it becomes clear that red leaf varieties present higher amounts of methyleugenol than green leaf basil plants. In the gene bank collection certain nontypical spice basil forms were found, which did not contain any methyleugenol or in which methyleugenol did not show any change in the level during ontogenesis.

**Keywords:** *Ocimum*, methyleugenol, estragole, ontogenesis

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**ISMAP0033 - Domestication of *Vitex madiensis* Oliv. : vegetative propagation by cuttings and essential oil chemical composition.**

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Domestication of tree species which provide non timber forest products is an important need in agroforestry development. *Vitex madiensis* is a natural shrub valorised by indigenous population for food and medicinal purposes. The genus *vitex* is known as an important source of essential oil. Therefore, the present work was designed to investigate the composition of the essential oil and to identify the rootstock height which can improve the rooting ability of the species. The essential oil of the leaves and flowers were analysed by GC/FID, GC/MS. The juvenile cuttings harvested from mature rootstocks felt at 0, 25, 50, 100, 150, 200 and 250 cm above the ground level were grown in polypropagators using a RCBD with three replicates. The essential oil of the leaves was characterized by a greater content of monoterpene hydrocarbons (27.9%) and sesquiterpene hydrocarbons (32.7%) while the flowers exhibited a higher content of oxygenated sesquiterpenes (48.1%). The main components of oil in leaves were  $\beta$ -myrcene (20.9%),  $\beta$ -caryophyllene (15.6%), linalool (7.7%),  $\alpha$ -copaene and caryophyllene oxide (7.8%). The oil extracted from flowers revealed mainly caryophyllene oxide (33.1%), linalool (6.9%),  $\alpha$ -copaene (5.8%) and calamenene-10- $\alpha$ -ol (5.3%). The rooting potential of the species was significantly ( $p < 0.001$ ) influenced by the height of the rootstock. This effect was more pronounced at 0 cm height with 45.83 % of the rooted cuttings. The height of 0 cm significantly enhanced the number of roots per cutting (8.72) as well as the length of roots (12.73 cm). These results suggest that it is possible to develop clones of genetically superior trees of *V. madiensis* for use in agroforestry as potential source of essential oil.

**Keywords:** *Vitex madiensis*, Essential oil, Rooting ability, Elite trees



**ISMAP0262 - DPPH scavenger profile of some Piperaceae from Vale do Itajaí.**

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Increased consumption of fruits and vegetables has been associated with protection against various diseases, including cancers and cardio-and cerebral-vascular diseases. This association is often attributed to the antioxidants in the fruits and vegetables such as vitamin C; vitamin E, carotenoids, lycopenes and flavonoids that prevent free radical damage. Thus, in order to identify antioxidants in plant extracts, some *Piper* genus species were assessed for potential capacity to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. The *Piper* genus (Piperaceae) is represented for 700 species distributed in both hemispheres. Some investigations have already confirmed anti-oxidative properties for *Piper betle* and *Piper fulvescens*. The object of the present study was to measure the antioxidant properties of five *Piper* species from the Atlantic forest in the Itajaí Valley in Santa Catarina State, by using DPPH radical-scavenging assay. *P. Lindbergii*, *P. cernuum*, *P. caldense* and *P. gaudichaudianum* were collected at Parque São Francisco, in Blumenau city. *P. methysticum* was obtained commercially. The free radical-scavenging activity of methanolic extracts (400, 200, 100, 50, 25, 12.5 ppm) was measured adding 2 mL of a MeOH solution of DPPH 0.004 %. The decrease in absorbance at 517 nm was measured after 30 min. The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (EC<sub>50</sub>). The EC<sub>50</sub> value for each extract was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentrations. The total flavonoid contents of these species were determined by AlCl<sub>3</sub> spectrophotometric method. Standard concentrations of quercetin (0.0026, 0.0065, 0.0091, 0.0130 and 0.0195 mg/mL) and methanol extracts of species (0.4 g/ 50 mL) was added to 2% AlCl<sub>3</sub> solution, and absorbance at 410 nm was determined after 30 min. The total flavonoid contents are expressed as quercetin. The methanol extracts of leaves of *P. cernuum* and *P. gaudichaudianum* were the most active among all extracts tested, presenting an EC<sub>50</sub> approximately 17 and 9 times greater than EC<sub>50</sub> of ascorbic acid, and 28 and 15 times greater than quercetin, respectively. For *P. caldense*, *P. lindbergii* and *P. methysticum* the EC<sub>50</sub> value is above 400 ppm. Total phenolic concentration, expressed as quercetin equivalents showed correlation with the scavenging activity on DPPH test, being highest in *P. gaudichaudianum* and lowest in *P. caldense*.

**Keywords:** Piper, flavonoids, antioxidants, DPPH

**Sponsor:** CNPq, FURB.

**Table 1.** The EC<sub>50</sub> values and the content of flavonoids of the different *Piper* species.

Specie/ compound	IC <sub>50</sub> , µg/mL	Flavonoid content, %
<i>P. cernuum</i>	148.0	0.166
<i>P. caldense</i>	380.0	0.005
<i>P. lindbergii</i>	> 400.0	0.031
<i>P. gaudichaudianum</i>	80.0	0.197
<i>P. methysticum</i>	> 400.0	-
Quercetin	5.15	-
Ascorbic acid	8.4	-
Galic acid	2.6	-



**ISMAP0245 - Effect of an accidental herbivore attack on concentration of  
dihydrocucurbitacin B and cucurbitacin B  
in root of *Wilbrandia ebracteata* Cogn (Cucurbitaceae).**

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**Introduction:** *Wilbrandia ebracteata*'s roots (Cucurbitaceae), used in folk medicine to treatment of rheumatic disease, are rich in cucurbitacins. The anti-inflammatory activity of the dichloromethanic fraction has been previous demonstrated as well a quantitative assay by HPLC has been developed. Dihydrocucurbitacin B (DHCB) is the most abundant cucurbitacin while cucurbitacin B (CB) is a minor component of the dichloromethanic fraction. It was also previous demonstred the high toxicity of the CB in relation to that of the DHCB. There are no previous studies on environmental factors that influence the concentration of cucurbitacins on *W. ebracteata*'s root. But some secondary metabolites can have a defensive action against parasites and herbivores. These organisms can influence on secondary metabolites concentration. **Objectives:** Monitoring the concentration of DHCB and CB in root of *W. ebracteata*. **Methodology:** A root's part of the same especimen of *W. ebracteata* was collected in Florianópolis, Santa Catarina state, Brasil, in three different years: 2002 (January), 2003 (May) and 2004 (February). The plant material was dried at 35-40°C and fragmented (<1mm). Each sample was analyzed in triplicate by HPLC method for simultaneous quantitative assay of DHCB and CB in the roots. **Results:** The results were presented at table 1. It was observed that the concentration of CB, a cytotoxic compound, was higher in 2003. As the concentration of DHCB was reduced, the ratio between DHCB and CB was very small at this year. The fact can be explained by herbivore attack to the root. At 2004 the concentration of DHCB returns to original level and the concentration of CB was reduced. This result presents an example of great variability on the concentration of a toxic secondary metabolite on a medicinal plant and the fundamental importance of the quality control, determination of toxicity and validation of pharmacological activity. **Conclusion:** The results suggest that herbivore attack could increase the concentration of CB.

**Table 1:** Concentration of and cucurbitacin B (CB) and dihydrocucurbitacin B (DHCB) in the root of *Wilbrandia ebracteata* collected on three years.

Year	CB (mg/100g)	DHCB (mg/100g)	DHCB/CB
2002	3.81 ± 0.06	60.2 ± 1.18	15.8
2003	35.8 ± 2.89	39.8 ± 4.6	1.11
2004	13.0 ± 0.12	61.5 ± 3.2	4.73

**Keywords:** *Wilbrandia ebracteata*, quantitative analysis, cucurbitacin, environment influence

**Sponsor:** CNPq



**ISMAP0186 - Essential oil changes in capsules of carqueja (*Baccharis genistelloides* (Lam.) Pers.).**

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*Baccharis genistelloides* (Lam.) Pers (carqueja), Asteraceae, used medicinal plant popularly in the hepatic and gastrointestinal disease treatments. The essential oil changes in capsules (PEC) and the rude powder (PBC) of dry powder of 'carqueja' was evaluated during the period of 320 days of storage. For such task, the powder was encapsulated in colorless gelatinous capsules, number zero, stored in white bottles, while the rude powder was maintained in bottles amber. At about 50 g of powder PEC and PBC were submitted to distillation process by vapor hydro distillation for 4 hours, at regular intervals of 80 days at 80, 160, 240, 320 days of storage, with four repetitions. The essential oil values had been calculated and the data were submitted to analyses of variance and regression analysis. The models chosen based were based on the significance of the regression coefficients, using the t-test at 1% of probability and the coefficient of determination ( $r^2 = \text{SQ Regression/SQ time}$ ). The results from the essential oil analysis had content higher than 0.5% in both PBC and PEC. The essential oil yield, in the first sampling, reached the following significant values of 2.3% for PEC and 2.8% for PBC. The results after 80 days, in the second sampling, showed 2.4% for PEC and 2.9% for PBC. After 160, 240 and 320 days the content of essential oil were 2.4, 2.5 and 2.1% for PEC, respectively. While for PBC the values were 2.9, 3.2 and 2.5%, respectively. The regression analysis for PEC gave  $w = 2225$  and for PBC  $w = 2,44929 + 0,0048303^{**}EP - 0,0000145089 EP^2$  and  $r^2 = 0.5243$ . We conclude that during storage no significant losses were present and the essential oil contents were higher than the mentioned by Brazilian Pharmacopoeia IV (2003). The analysis of the data allow to conclude that the essential oil content in PEC did not change by the length of time in storage, by contrast the PBC had a maximum content of 2.85% at 166 days.

**Keywords:** carqueja, essential oil, quality control, *Baccharis*



**ISMAP0138 - Essential Oil from *Lippia lacunosa* and  
*Lippia rotundifolia* (Verbenaceae).**

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The genus *Lippia* (Verbenaceae) comprises *circa* 200 species occurring mainly in Central and South America and also in some areas of Tropical Africa. Some species, like *L. alba* and *L. graveolens* are largely used in folk medicine and culinary. As part of our continuing study on Brazilian species of the genus *Lippia*, the species *L. lacunosa* Mart. & Schau. and *L. rotundifolia* Cham. were selected for investigation. To the best of our knowledge, no previous studies concerning these species were published. Both form a complex of very difficult taxonomic delimitation, and were considered as synonyms in many Herbarium samples. However, pollen analysis showed relevant differences. Further taxonomic studies lead to the classification of these plants as two separate species. In this way, the chemical study might contribute as an extra tool to reinforce this delimitation. The objective of this work was to investigate the chemical composition of their essential oils. Aerial parts (leaves and flowers) of *L. lacunosa* and *L. rotundifolia* (cultivated, from original clones brought from Diamantina, MG, Brazil) were collected in the campus of Federal University of Juiz de Fora, Juiz de Fora, Brazil, in October 2003. The essential oils were obtained by hydrodistillation in a Clevenger-type apparatus and yielded 0.4% (v/w) for *L. lacunosa* and 0.6% (v/w) for *L. rotundifolia*. Analyses were performed in a HP 5890 gas chromatograph equipped with a FID detector and a HP5 capillary column (30m X 0.25mm X 0.25µm), using H<sub>2</sub> (1.0mL/min) as carrier gas. The oven temperature was programmed from 60 to 240°C at 3°C/min. Pure oil (0.03µL) were injected in split mode (100:1). Mass spectra were recorded using a GC/MS Agilent 5973N system, operating in electron impact mode at 70eV, using the same column and chromatographic conditions, but helium as the carrier gas. The constituents of the oils were identified by comparing their mass spectra with those in a spectral database (Wiley 6<sup>th</sup> ed) and by their retention indices. Major components identified in the oil of *L. lacunosa* were myrcenone (53.1%), myrcene (21.1%) and E-ocimenone (11.7%). In the oil of *L. rotundifolia* were found limonene (28.3%), trans-thujone (15.7%) and sabinene (12.5%). The differences observed in the chemical composition of the oils can be used as an aid in the botanical classification of these species.

**Keywords:** *Lippia lacunosa*, *Lippia rotundifolia*, essential oil, Verbenaceae.



**ISMAP0105 - Essential oil from Alecrim pimenta (*Lippia sidoides* Cham.) leaves grown in Parana, South Brazil.**

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Alecrim-pimenta (*Lippia sidoides* Cham. Fam. Verbenaceae) is a wild aromatic shrub from northeastern Brazil. Its leaves are rich in several essential oils, including thymol and carvacrol. In previous studies, the essential oil of this species has exhibited bactericidal, bacteriostatic, fungicidal and fungistatic activities. While being tropical specie, *L. sidoides*, has grown well in southern Brazil despite the low temperature common during the winter. The objective of this study was to evaluate the essential oil accumulation and composition from leaves of *L. sidoides*, grown in Londrina, Parana, southern Brazil. In August, 2003, 100g of fresh leaves were manually harvested from plants growing in the Medicinal Plants Collection of IAPAR. Essential oils were extracted by steam distillation at the Physiology Laboratory of IAPAR and the chemical characterization of the oils was conducted at the New Use Agriculture and Natural Plant Products Program, Rutgers University, USA. The volatile oils (10 µl) were dissolved in 1 ml of hexane and then dried over sodium sulfate and then analyzed by a gas chromatograph (GC) coupled to a mass spectrometer (MS) (Agilent GC System 6890 Series, Mass Selective Detector, Agilent 5973 Network, FID detector). Samples were injected with an auto sampler (Agilent 7683 Series), the Inlet temperature was 220°C, in a HP5- MS (30m, 0.25 mm ID, 0.25mm Film) column, temperature program, 60°C 1 min, 4°C/min, 200°C 15 min. Helium constant flow was set at 1 ml/min. Individual identifications were made by matching their spectra with those from mass spectral libraries (Wiley 275.L). Results indicated that the essential oil yield was 1 ml (1%) and the main aromatic volatile constituents were in sequence: Carvacrol (37.85 %), Paracymene 4-isopropyltoluene (24.57 %), Gamma-terpinene (16.14 %), and Thymol (3.70 %). The results showed that despite the appearance of a vigorous growth, the specie does not appear to accumulate as much essential oil as it may in its native region as the oil yield was less than half than reported from the same specie when collected in northeastern Brazil.

**Keywords:** thymol, carvacrol, environment adaptation.



### ISMAP0055 - Essential Oil Composition of In vitro-Grown, Greenhouse-Grown and Field-Grown Plants of *Artemisia absinthium*.L from Iran.

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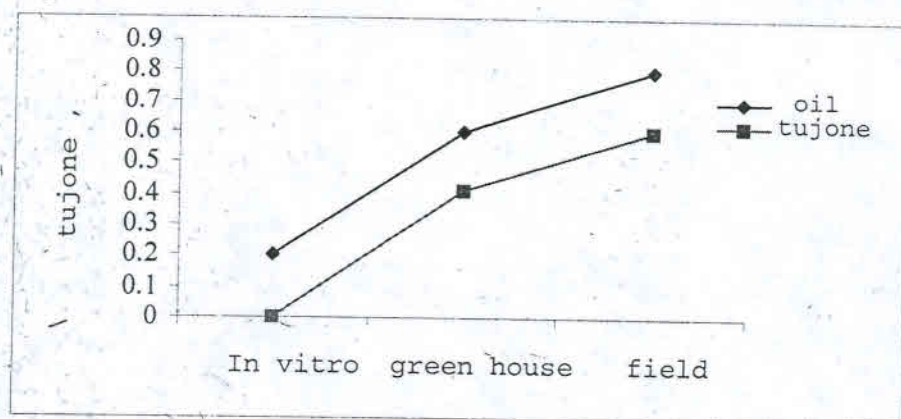
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*Artemisia absinthium*.L (Wormwood), is one of the medicinal and aromatic plants in Iran. It is a perennial shrub traditionally used because of anti helminthic, insecticidal, stomachic, tonic-bitter and aromatic compounds. The essential oil obtained from this plant is used as flavoring agent in some foods and beverages too. In order to study of qualitative and quantitative difference of essential oil composition in different conditions of growth; seeds were planted in greenhouse and field. For In vitro propagation were used MS medium and 0.5 mg/l NAA+ 0.2 mg/l BAP. Essential oil was extracted from leaves by Clevenger - water circular distillation. The essential oils were obtained from In vitro-grown, greenhouse-grown and field-grown plants in yields of 0.24%, 0.63% and 0.81% (w/w) respectively based on dry weights. GC-MS was used for identification of essential oil components obtained from In vitro greenhouse and field grown plants. The components of essential oils were analyzed, identified and determined type and percentage for each of components by retention time and kowats index. Results show that difference between essential oil composition in In vitro conditions and other conditions was very much.  $\pm$ - tujone was the main compound in the essential oil obtained from field and greenhouse grown plants. The percentage of  $\pm$ - tujone was 60% in field and 41% in greenhouse plant but it was not produced in In vitro condition. Citronellil isovalerat, Trans-nerolidol, Terpinil isobutyrate, and Borneol were main components of essential oil of In vitro plants. Cumazulen was produced in In vitro plants.

**Keywords:** essential oil, in vitro,  $\pm$ - tujone, Cumazulen

**Sponsor:** Bu Ali-Sina University



**Figure 1.** Essential oil and Tujone percentage changes in different conditions.



**ISMAP0356 - Essential oils composition of leaves, inflorescences and seeds of  
*Mikania laevigata* Schultz Bip and *Mikania glomerata* Sprengel.**

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*Mikania laevigata* Sch. Bip. ex Baker and *M. glomerata* Spreng., both known popularly as "guaco", are species very similar morphologically, causing great confusion in their identification. Individuals of both species are cultivated under the same conditions at CPQBA in Paulínia. The leaves of some species are used in folk medicine to treat respiratory tract diseases. Frequently encountered secondary metabolites are coumarin, diterpenes of the kauranes type and sesquiterpene lactones. The purpose of this work is to describe the comparative study the essential oil components obtained from leaves, inflorescences and seeds of *Mikania laevigata* and *Mikania glomerata*. The plants studied were grown of cultivate to full sun in the Experimental Field of CPQBA. The essential oils were obtained from 100 g fresh leaves, inflorescences and seeds by hydrodistillation using a Clevenger-type apparatus for 3 h. All extractions were realized in duplicate and GC/MS analyses were performed on a Hewlett Packard 5980/5971 gas chromatograph/mass detector equipped with HP-5 column (25m X 0,2mm X 0,33 $\mu$ ). The identification of the chemical constituents was based on comparisons of their retention indices and mass spectra with those obtained from authentic samples and NIST-98, Wiley libraries' spectra as well as with literature data. The oil content of fresh leaves, inflorescences and seeds of *M. laevigata* were 0,34, 0,08 and 0,22 %, respectively. To *M. glomerata* this values were 0,30, 0,04 and 0,07 %. Regarding the composition of the oils, significant differences were found in their monoterpenes percentage (14.12–23.30% *M. laevigata*, 0.80-3.43% *M. glomerata*). For the oils of *M. laevigata*, the bM. *glomerata*. The sesquiterpenoid fraction was higher for leaves, inflorescences and seeds oils for *M. glomerata*. The main sesquiterpene of leaves and inflorescences oils for *M. laevigata* and *M. glomerata* was germacrene D, while for seed oils was spathulenol. The coumarin presence was just detected in the essential oil of leaves of *M. laevigata* (5.37%).

**Keywords :** *Mikania laevigata*, *Mikania glomerata*, *guaco*, *essential oil composition*.

**Acknowledgment:** FAPESP for financial support and Agrotecnological Division of CPQBA/UNICAMP for supply plant material.



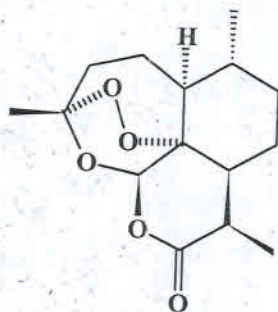
**ISMAP0214 - Evaluation of *Artemisia annua* L. clean up methods for Artemisinin Quantification by HPLC.**

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Artemisinin (1), an endoperoxide sesquiterpene lactone isolated from *Artemisia annua* L. (Asteraceae), has received considerable attention due to its antimalarial activity. The limiting step in sample preparation is result of large amounts of chlorophyll present. In this work we describe several sample preparation methods using solid-phase extraction (SPE) and compare them with conventional liquid-liquid extraction method. One gram of *Artemisia annua* L. was extracted with three portions of 5 mL methanol for 2 minutes with mixer at room temperature. After filtration the sample was submitted to clean-up procedures and was analyzed by high performance liquid chromatographic using a refractive index detector with Zorbax SB- CN column (150mm x4.6mm x 5µm), eluted with methanol: water (60:40v/v), flow-rate 1.0 mL/min, sample injection 20µL and detector and column temperature were 35 °C. The aim of this work was to define the optimum conditions for a clean-up procedure of plant extract. Silica, C<sub>18</sub>, C<sub>18</sub> with active coal were evaluated as solid supports for SPE and liquid-liquid extraction. The best result was obtained with silica. The crude extract was filtered on a column containing 3 g of silica that was preconditioned with 20 mL hexane. The filtrate was discarded and the artemisinin retained in the solid-phase were eluted with 50 mL the hexane 100%, 50 mL the hexane: ethyl acetate 20% and ethyl acetate 100%. Artemisinin eluted in the second fraction, was evaporated to dryness, dissolved in 5 mL methanol, and filtered before being subjected to HPLC analysis. Table 1 show that yields recoveries with standard silica. The low recoveries obtained with adsorptive materials, such as C<sub>18</sub>, C<sub>18</sub> with active coal and liquid-liquid extraction, are a result of bad separations efficiency among artemisinin and chlorophyll.

**Keywords:** *Artemisia annua*, artemisinin, clean up, High Performance Liquid Chromatography  
**Sponsor:** FAPESP



(1) Artemisinin

**Table 1** - artemisinin standard % Recovery by SPE with silica

Concentration	% Recovery
150 µg/ml	98,23 %
350 µg/ml	96,88 %
550 µg/ml	94,68 %



**ISMAP0250 - Fractionation of Carbohydrates from the Bark  
of *Ocotea puberula* (Reich.) Nees.**

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Medicinal plants are popular used, although people do not concern which compound is responsible for the biological activity. This work has as objective extraction of the main carbohydrates in the mucilage of the bark of *Ocotea puberula* (Reich.) Nees (Lauraceae), a brazilian native tree frequently used for wounds treatment. The dried and crushed bark was submitted to the extraction with  $\text{CHCl}_3$ :MeOH (2:1, v/v) to give extract  $E_1$  and residue  $R_1$  (83% yield\*).  $R_1$  was extracted with MeOH:H<sub>2</sub>O (4:1, v/v) resulting in fraction  $E_2$  and residue  $R_2$  (64% yield\*).  $R_2$  was extracted with water at 25°C resulting in extract  $E_3$  and residue  $R_3$  (60% yield\*). The extract  $E_3$  was precipitated with ethanol (3:1, v/v), resulting an insoluble ( $E_{3p}$ ) and supernatant fractions ( $E_{3s}$ ). The residue  $R_3$  was submitted to aqueous extraction at 70°C giving rise to extract  $E_4$  and residue  $R_4$  (55% yield\*).  $E_4$  was precipitated with ethanol (3:1, v/v) to give  $E_{4p}$  and  $E_{4s}$ . The residue  $R_4$  was extracted with 2% aqueous NaOH (w/v) to give extract  $E_5$  that was precipitated with ethanol providing  $E_{5p}$ ,  $E_{5s}$  and residue  $R_5$  (21% yield\*). The isolated fractions were analysed by gas-chromatography (GC) as alditol acetates. The results were  $E_2$ : Rha (31%), Ara (2%), Xyl (2%), Man (8%) and Glc (57%);  $E_{3s}$  Rha (57%), Ara (6%) and Glc (36%);  $E_{3p}$  Rha (3%), Fuc (1%), Ara (47%), Xyl (44%) and Glc (4%);  $E_{4s}$  Rha (35%), Fuc (9%), Ara (5%), Man (2%) and Glc (48%);  $E_{4p}$  Rha (5%), Ara (37%), Xyl (35%) and Glc (23%);  $E_{5s}$  Rha (10%), Fuc (3%), Ara (69%), Xyl (10%), and Glc (8%);  $E_{5p}$  Rha (2%), Ara (38%), Xyl (24%), Man (2%) and Glc (34%). The low carbohydrate compounds extracted with MeOH:H<sub>2</sub>O showed rhamnose and glucose as main monosaccharides. Polysaccharides on  $E_{3p}$  showed arabinose and xylose, but fractions  $E_{4p}$  and  $E_{5p}$  showed also glucose on its composition. This fractionation would be important to determinate the biological activity.

\*Based on the bark.

**Keywords:** *Ocotea puberula*, carbohydrates, fractionation, mucilage.



**ISMAP0330 - Hydrolysis of turmeric and ginger starches using subcritical water and CO<sub>2</sub>: the effect of the SFE pre-treatment.**

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Supercritical fluid extracts from turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinale roscoe*) have coloring and flavor properties; they also exhibit antioxidant and anticancer activities. These rhizomes contain approximately 25 and 30% of starch, respectively. Starch is not soluble in supercritical CO<sub>2</sub>, therefore, the starch matrix remains intact after the supercritical extraction process. The supercritical fluid extraction (SFE) conditions reported, in the literature, are pressures from 250 to 300 bar and temperatures from 25 to 35 °C. The SFE step can act as a pre-treatment to the hydrolysis because the pressure acts over the starch structure loosening it. In this work, the hydrolysis was made using a fixed bed extractor (reactor) of 5mL; the reactor vessel was filled with solid matrix at a pre-selected humidity level. The hydrolysis was done using subcritical water and CO<sub>2</sub> at 150 bar, 200 °C and a reaction time of 11 minutes. The reaction was monitored through the amount of starch hydrolyzed; process yields were calculated using the amount of reducing and total reducing sugars formed. The effect of the supercritical extraction step was evaluated by comparing the hydrolysis products obtained from *in natura* rhizomes with that obtained from ginger and turmeric bagasses or the SFE residues. Higher degree of hydrolysis (96.9%) and higher total sugars yield (26.9%) were established for the *in natura* turmeric. The SFE process did not alter significantly the hydrolysis rates in the tested conditions.

**Keywords:** turmeric, ginger, subcritical hydrolysis, supercritical process

**Sponsor:** FAPESP 99/12868-6 (Ph.D) and 1999/01962-1.



**ISMAP0034 - Hypoglycaemic and antioxidant activity of *Brassica oleracea* var. *gongylodes* in streptozotocin induced diabetic rats.**

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A study to investigate the effect of petroleum ether extract of *Brassica oleracea* var. *gongylodes* on blood glucose levels and on activities of antioxidant enzymes in streptozotocin induced diabetic rats was conducted. Diabetes was induced in rats by single injection of streptozotocin (STZ 65mg/kg, i.p.). After 60 days of treatment with *Brassica oleracea* extract (15.12 mg/kg, p.o.) glucose level was measured in blood and activities of antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH Pxase) were measured in erythrocytes. Concentration of glutathione (GSH) and lipid peroxidation product viz. malondialdehyde (MDA) were also measured. Petroleum ether extract of *Brassica oleracea* var. *gongylodes* significantly ( $P < 0.0001$ ) lowered the STZ induced hyperglycemia. It also produced a significant ( $P < 0.0001$ ) decrease in peroxidation product viz. MDA. The activity of antioxidant enzymes such as SOD, CAT and GSH Pxase were found to be increased in the erythrocytes of diabetic animals treated with the *Brassica oleracea* extract. Section from *Brassica oleracea* extract treated diabetic rats showed good number of regenerating tiny islets whereas the diabetic control showed minute islets.

**Keywords:** *Brassica oleracea*, streptozotocin diabetes, antioxidants.

**Table.1** Effect of Knol-Khol (Petroleum ether extract) on concentration of MDA, GSH, blood glucose and activities of antioxidant enzymes in streptozotocin induced diabetic rats

Group	MDA (nmol/100ml)	GSH (mg/100ml)	Blood glucose (mg/100 ml)	SOD (IU/gm of Haemoglobin)	CAT (IU/gm of Haemoglobin)	GSH-Pxase (IU/mg of Haemoglobin)
I	11.566 ± 0.33	63.8 ± 0.84	82.83 ± 1.19	681.16 ± 4.83	8.96 ± 0.27	31.30 ± 0.67
II	171.83 ± 0.48 <sup>#</sup>	41.16 ± 0.47 <sup>#</sup>	400.33 ± 2.67 <sup>#</sup>	539.33 ± 10.75 <sup>#</sup>	4.52 ± 0.18 <sup>#</sup>	17.68 ± 0.52 <sup>#</sup>
III	134.5 ± 0.34 <sup>*</sup>	57.0 ± 0.74	151.16 ± 7.78 <sup>*</sup>	639.33 ± 1.04 <sup>*</sup>	7.96 ± 0.04 <sup>*</sup>	27.36 ± 0.91 <sup>*</sup>
IV	130.3 ± 0.5 <sup>*</sup>	60.16 ± 0.84 <sup>*</sup>	112.5 ± 2.53 <sup>*</sup>	664.16 ± 0.9 <sup>*</sup>	8.12 ± 0.02 <sup>*</sup>	29.65 ± 0.20 <sup>*</sup>

Group - I : Non - diabetic rats.

Group - II: Streptozotocin induced diabetic control rats.

Group - III: Extract treated rats. Group - IV: Insulin treated rats.

Each value is the mean ± SEM of 6 rats.

<sup>#</sup>  $P < 0.001$  as compared to group-I, <sup>\*</sup>  $P < 0.001$  as compared to group-II (ANOVA followed by Dunnet's 't' test).



**ISMAP0351 - *In vitro* antimicrobial activity of *Copaiba Balsam* using ELISA microplates.**

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Copaiba oils, also described as copaiba balsams or oleoresins, are produced by trees belonging to the genus *Copaifera*, largely distributed in northern South America, mainly in the Amazonas rain forest. *Copaiba Balsam* is widely used in popular medicine for the treatment of cancer, ulcer, syphilis, bronchitis, diarrhoea and as bactericidal, anti-helminthic, analgesic, anti-inflammatory, antiseptic, gastro-protective, antitumor and trypanomicidal agent. However there are more than 20 species of *Copaifera* in Brazil, occurring significant differences of chemical composition among them. Thus, analysis and chemical standardization of the oleoresins of different species of *Copaifera* is essential to permit validation and quality control. The objective of the project are phytochemical, pharmacological and toxicological studies of oils, fractions and isolated compounds from different species of *Copaifera*. Present communication describes the antimicrobial tests realized with 11 oleoresins from different trees collected in Amazonas and Pará States. The activity was tested against Gram-positive (*Staphylococcus aureus* CCT 2740, *Bacillus subtilis* CCT 2576) and Gram-negative (*Escherichia coli* CCT 0547, *Pseudomonas aeruginosa* ATCC 13388) bacteria. Bacterial cultures was developed in Nutrient Agar, and inocula were prepared by dilution overnight cultures with the Muller-Hinton liquid medium ( $10^4$  u.v./ml). The *in vitro* evaluation of antimicrobial activity was performed with ELISA microplates. Results were expressed as minimal inhibitory concentration (MIC) and are showed in Table 1. Cloranphenicol was used as positive control. Results revealed inactivity of all the samples against Gram-negative bacteria at the level of 1000  $\mu$ g/ml. However, oils 2, 5 and 10 shown significant activity against *B. subtilis* and *S. aureus*. Most oils showed different levels of inhibition, confirming the importance of the standardization, once *Copaiba Balsam* is freely used in folk medicine.

**Keywords:** copaiba oil, *Copaifera* sp, Leguminosae, antimicrobial activity

**Sponsor:** CNPq

**Table 1.** Minimal inhibitory concentration (MIC,  $\mu$ g/ml) of eleven Copaiba oils.

Copaiba oils	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
1	-	550	-	-
2	5	5	-	-
3	350	150	-	-
4	300	150	-	-
5	5	5	-	-
6	450	125	-	-
7	550	225	-	-
8	550	250	-	-
9	550	-	-	-
10	5	5	-	-
11	-	-	-	-
Control <sup>a</sup>	15,6	7,8	1.000	15,6

<sup>a</sup> Cloranphenicol



# ISMAP0163 - Influence of agronomic management on flavonoid content in the leaves of cultivated *Phyllanthus niruri* L.

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*Phyllanthus niruri* L. (Euphorbiaceae) is a little shrub, widely distributed in tropical and subtropical countries. In the Brazilian folk medicine the whole plant is used as a tea in the management of genitourinary disorders. Although many classes of constituents, such as flavonoids, alkaloids, terpenes, lignans, and coumarins have been isolated, the compound – or class – related to this therapeutic application remains so far unknown. Many research results indicated the polyphenolic compounds - mainly flavonoids - as one of the biological active groups for this plant. Therefore flavonoids were chosen as marker substances in the leaves, where they are mostly found, in order to evaluate the influence of agronomic parameters such as planting periods and harvesting conditions. The cultivation was managed under the same agronomic practices at the CBPQA agronomic station in Campinas/SP. Three planting periods were carried out: April; May and October, 2002. The aerial parts were firstly reaped after complete plant development (harvest 1), and a second harvest was taken after the development of new aerial parts growing from the first harvest remaining (harvest 2) (Table 1). The leaves were manually separated and dried in an air oven (40 °C) until a moisture content of 6 to 8 % was reached, and ground in a knife mill (outlet sieve = 1 mm). Total flavonoid content, expressed as quercetin (% w/w) was determined spectrophotometrically (420 nm), after selective extraction, acid hydrolysis and complexation with AlCl<sub>3</sub>, following a previously validated method based on the German Pharmacopoeia. The results (Table 1) were analyzed by ANOVA and Tukey multi-comparison test (p = 0.05). The statistical analysis demonstrated that there are significant differences among all samples. The highest flavonoid content was measured in the leaves, which grew over the summer, and the least one was observed in those that grew over the winter. Enhanced flavonoids concentrations were also related to the second harvesting regardless of its growing season. Probably, the flavonoid increment that usually occurred after second sprouting was induced by the climatic conditions, specially in the summer. This behavior may be in accordance to the flavonoids function in plants, since the protection against solar radiation is more evident during the summer. Besides the flavonoid accumulation in the leaves, as related in previous studies, these results suggest that even with less favorable climatic conditions, in winter, the pruning seems to cause a strong influence in the flavonoid production.

**Keywords:** Flavonoids, *Phyllanthus niruri*, leaves, cultivation

**Sponsor:** CNPq

**Table 1.** Total Flavonoid content (g %, w/w, mean ± standard error) in the dry powdered leaves of *P. niruri*

Planting period	Harvest 1	Harvest 2
A (April 2 <sup>nd</sup> , 2002)	1.01 <sup>d</sup> ± 0.005 (June 13 <sup>th</sup> , 2002) fall	1.23 <sup>c</sup> ± 0.021 (September 9 <sup>th</sup> , 2002) winter
B (May 10 <sup>th</sup> , 2002)	0.96 <sup>e</sup> ± 0.019 (September 3 <sup>rd</sup> , 2002) fall/winter*	1.31 <sup>b</sup> ± 0.040 (December 5 <sup>th</sup> , 2002) winter/spring*
C (October 24 <sup>th</sup> , 2002)	1.26 <sup>c</sup> ± 0.009 (January 9 <sup>th</sup> , 2003) spring*/summer	1.53 <sup>a</sup> ± 0.059 (March 24 <sup>th</sup> , 2003) summer

\*Predominant growing season. Means followed by the same letter do not differ according to the Tukey multi-comparison test (p = 0.05).



# ISMAP0121 - Influence of edaphic factors on tannin levels of *Stryphnodendron* spp.

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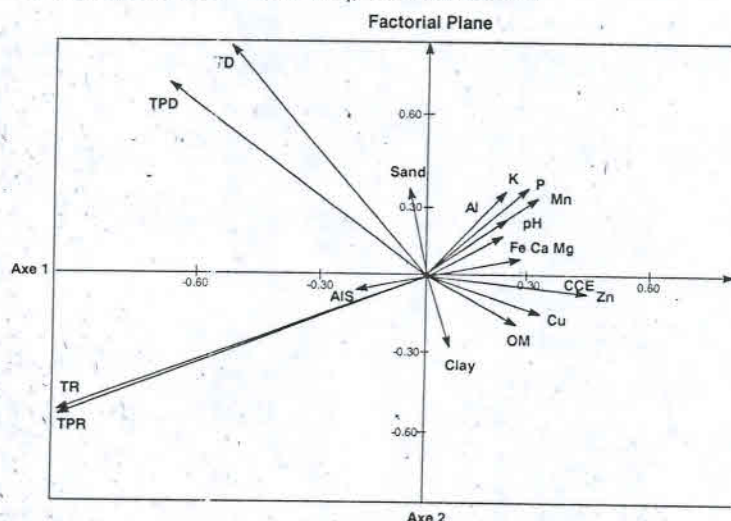
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*Stryphnodendron adstringens* (Martius) Coville and *S. polyphyllum* M. (Mimosaceae), both known as Barbatimão, are medicinal plants used, as an aqueous extract of the bark, for the treatment of gynaecological problems, wounds, ulcer and diarrhoea. Pharmacological activities of these plants possibly are related with the high levels of condensed tannins present in their barks. In order to correlate the variability in the levels of tannins with edaphic factors, barks were collected at four sites in the cerrado area, Goiás State, in the dry (July, 2001) and rainy (February, 2002) seasons. Five individuals of *S. adstringens* and *S. polyphyllum* were sampled in each of the municipalities of Rio Verde and Bela Vista; in Silvânia five individuals of *S. adstringens* were collected, and five individuals of *S. polyphyllum* in Abadia de Goiás, summing up 30 individuals. The collected material comprised one sample of the bark by specimen and date, and a soil sample at two depths (0-20 and 20-40 cm) in the dry season. The freeze-drier acetone:water (7:3) extracts were analysed by colorimetric assays to quantify Total phenolics (FeCl<sub>3</sub>) and Protein precipitation (with BSA). Chemical and physical soil analysis followed the standard methods. Results obtained from the assays together with soil analysis of each local were used in the statistical analysis performed by the program SPAD.N. The majority of the data, in the principal components analysis (PCA), could be represented in two main axes (Figure 1), which contained 90,17% of total variance (x = 53.5 and y = 36.7 %). In the first axis, which is related with chemical characteristics of the soil, aluminium saturation (AIS) and cationic capacity exchange (CCE) appeared on the opposite sides, confirming the inverse proportionality of them. In the second axis, which is related with texture characteristics of the soil and phenolics levels in the barks, samples from the rainy season are separated from the dry one, forming two distinct groups. Total phenols and tannins (dry season) are directly correlated with sand, while samples from the rainy season are positively correlated with aluminium saturation. All other chemical characteristics of the soil are negatively correlated with total phenols and tannins from both seasons. There is no separation between the species, despite *S. polyphyllum* accumulated higher levels of phenols in the dry and rainy seasons. The statistical analysis suggested that edaphic factors can affect strongly the production of tannins by both species. As shown, sandy soils with low fertility and high aluminium saturation promoted higher levels of total phenols and tannins. These results are in accordance with the growth-differentiation balance hypothesis which postulates that when resources (i.e. nutrients) are limited, growth is retarded and surplus carbon is diverted to phenolic compounds biosynthesis.

**Keywords:** *Stryphnodendron*, tannins, phenols, edaphic factors.

**Sponsor:** PADCT III, CNPq, FUNAPE/UFG



**Figure 1** – Principal Component Analysis (PCA) between edaphic factors and phenols levels.

TPD – total phenols dry season  
TD – tannins dry season  
TPR – total phenols rainy season  
TR – tannins rainy season  
OM – organic matter  
AIS – Aluminium saturation



**ISMAP0312 - Intensity of inhibition and bacterial inactivation of 32 different vegetal condiments on standardized species of bacteria.**

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It was determined Intensity of Activity of Inhibition (IINIB) and the Intensity of Activity of Inactivation (IINAB) of hidro-alcoholic extracts of 32 plants with ethnograph indicative to spices in the metropolitan region of Porto Alegre/RS/BR, submitted to fracionated destillation in route-vapor, with posterior rehidratation, , over standardized steins of bacteria like *Escherichia coli* (ATCC 11229), *Staphylococcus aureus* (ATCC 25923), *Salmonella enteritidis* (ATCC 11076) and *Enterococcus faecalis* (ATCC 19433). They were collected stems, leves, fruits or bulbs preferential in period of budding. Extracts of 12 plants had presented inhibition capacity and or selective inactivation on the standardized species of bacteria. The plants that better had been distinguished were sage (*Salvia officinalis* L.), poró garlic (*Allium porrum* L.), nirá garlic (*Allium tuberosum* L.) and types of peppers like, "Garden" (*Capsicum annuum* L.), "malagueta" (*Capsicum frutescens* L.), "calabreza" (pool of *Capsicum* sp.) e young-woman-finger (*Capsicum baccatum* L.). *S. aureus* demonstrated the biggest resitence, while that *S. enteritidis* was the most sensible agent.

**Keywords:** antibacterial activity, vegetal condiments, bacterial inhibition, spices.

**Sponsor:** CNPq



**ISMAP0384 - Investigation on medicinal aromatic plants from Cameroon: GC/MS analysis of essential oils of *Ocimum suave* Willd.**

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*Ocimum suave* Willd. (Lamiaceae) is a plant with insecticide activities found in Africa and Asia. The essential oils of flowers, leaves and stems of *O. suave* from Cameroon were analysed by GC, GC/MS and olfactometry. The olfactory characteristics of the oil are: animal, unpleasant fish notes, fatty-train-oil-like (whale), green-fatty sides notes and weak repellent odour. Using GC/FID and GC/MS the oils were characterized by a high percentage of oxygenated aromatic compounds (26.2% - 91.6%), represented especially by elemicine (9.8% - 38.5%), eugenol (1% - 33.1%) and cis-methyl eugenol (6.8% - 19.3%). The oil from dried leaves and dried flowers and dried stems contain a higher percentage of sesquiterpene derivatives (46.9%, 57.3% and 78.2% resp.), the main sesquiterpenes are  $\beta$ -caryophyllene,  $\beta$ -bisabolene, humulene oxide I and humulene oxide II.

**Keywords:** *Ocimum suave*, essential oil, elemicine, eugenol



**ISMAP0007 - Investigation the effect of harvesting time and drying methods and packaging in jojob in Iran.**

Mokhtarian, A.; Azarpazhooh, E.

In order to determination the best harvesting time , drying ,packaging and preservation of jujub fruit, on experiment was done in city of Birgand for 3 years . The experimental design was on the basses of two Factors factorial in frome of completely randomized design with 3 replicates, Factor A was harvesting time (100 days after full bloom , 120 days after full bloom) and factor B was drying methods (drying on tree , drying among the ash ,industrial drying). Quality parameters in cluding moisture percent, ash,contamination and panel tests were done on samples.

The results of first phase showed that the best harvesting time was 120 days after full bloom, that fruits had the more weight, width, Length, TSS and TA. The best drying methods was drying in ash that the fruits had better texture color, taste and overall acceptance. In the second phase the selected fruits of the first phase (harvested 120 days after full bloom dried in ash) Packaged with 3 methods (Cartoon , low density polyethylene , celophon),and preserved in (25 , 10 , 4) degrades centigrade for 1 years.

The factors including weight changes , Contamination to insicts , moisture, test panels (Texture , color , taste , overall acceptance) were tested every 6 months . The results showed that low dnsity polyethylen were the best packaging material and the samples preserved in this condition had the best quality after 1 years.

**Keywords:** Jujuba, Harvesting time, Drying, Packaging , Storage



**ISMAP0341 - Leaf anatomy of *Himatanthus sukuuba* (Apocynaceae).**

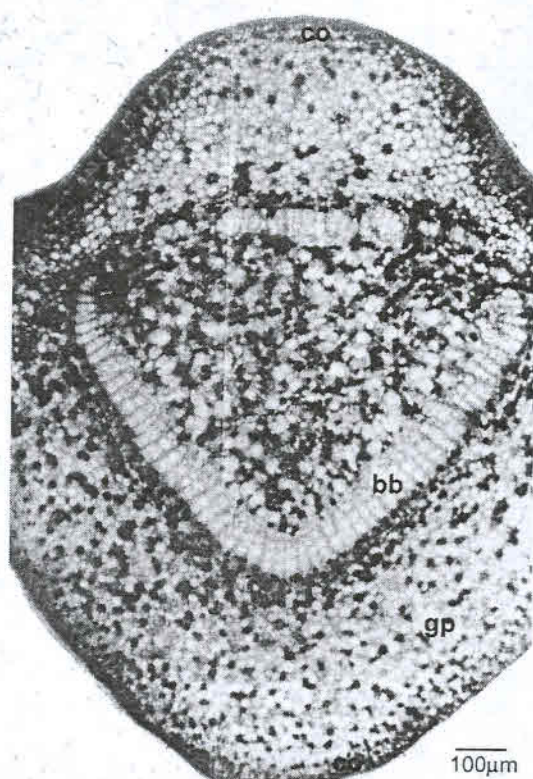
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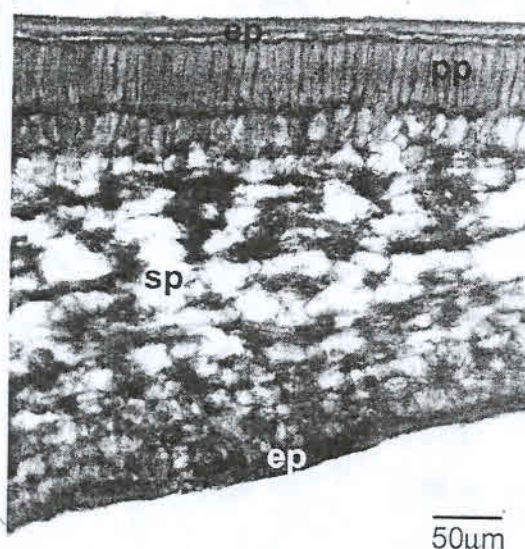
*Himatanthus sukuuba* (Spruce) Woodson, Apocynaceae, is a species employed in the traditional medicine for treating skin problems, constipation and gastric irritation. For this purpose, the population uses the latex from the stem and leaves. This study was carried out to analyse the leaf anatomical characters, in order to contribute to the medicinal plant identification and drug quality control. Adult leaves were collected at the Instituto Nacional de Pesquisas Amazônicas (INPA), Manaus-AM, in 2003 July. They were fixed in FAA, free-hand sectioned and stained either with toluidine blue or basic fuchsin and astra blue. Histochemical reactions were accomplished with hydrochloric phloroglucin (for lignin), iodine-iodide (for starch), Sudan III (for lipophilic substances), ferric chloride (for phenolic compounds) and sulphuric acid (for calcium oxalate). The epidermis is uniseriate, the anticlinal cell wall is undulate and a striated and thick cuticle is observed. The leaf is hypostomatic, showing anomocytic stomata. The mesophyll is dorsiventral and the minor vascular bundles are encircled with a parenchymatic sheath (Figure 2). The midrib has biconvex transection and it occurs a subepidermal layer and annular collenchyma, as well as a bicollateral bundle embedded in the ground parenchyma (Figure 1). The petiole has plain-convex contour with two lateral wings, bicollateral bundles and two amphicribral ones. Many branched laticifers, amyloplasts and idioblasts containing phenolic compounds and calcium oxalate crystals are found in the midrib and petiole.

**Keywords:** *Himatanthus sukuuba*, quality control, leaf, laticifers

**Sponsor:** CAPES



**Figure 1** – Midrib transection, revealing collenchyma (co), bicollateral bundles (bb) embedded in the ground parenchyma (gd)



**Figure 2** – Blade transection showing uniseriate epidermis (ep), palisade parenchyma (pp) and spongy parenchyma (sp)



**ISMAP0363 - Microwave-assisted extraction of coffee microcapsules.**

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Volatiles in solid foods can be frequently extracted by steam distillation in the Likens/Nickerson apparatus (LNA) -Figure 1. An aqueous slurry of the sample is put in a flask attached to the left side of the apparatus, while a flask connected to the other side comprise the organic solvent. Both sides are heated separately; condensed in the cooled section, and subsequently separated in the "U" section, resulting in a continuous extraction during 1, 2 or sometimes 3 h. This apparatus doesn't allow simultaneous extraction.

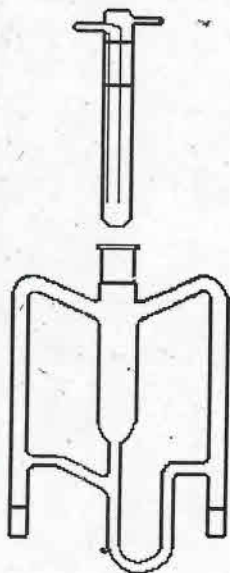
Microwave ovens technology has proven to be useful in extraction and have been used in laboratories over the past twenty years. The microwave-assisted extraction (MAE) improved dramatically the speed extraction for chemical analysis but has the disadvantage of being yet an expensive equipment. Closed-vessel microwave extraction allows the extraction with solvents to be faster, that is in shorter extraction times (10-30 min).

The most suitable method should be chosen to be fast with avoiding any losses of compounds. The aim of this study is compare two techniques for volatile extraction of coffee microcapsules by GC/MS qualitative profile. The microcapsules were produced by spray drying with concentrated coffee extract and maltodextrin.

The optimal extraction involved time consuming and sample scale for LNA and also electric power for MAE. The LNA involved sample (2 and 4 g) and time consuming (1 and 3h). The MAE involved sample (10, 250, 500 and 4000 mg), time consuming (4 and 8 min) and electric power (250 and 500W).

A fast and simultaneous method (12 samples) for extraction of coffee microcapsules is proposed and established using MAE system with 4 g and 500 W during 8 min. The GC/MS profiles of both systems were similar.

**Keywords:** *aroma, coffee, extraction, microwave.*



**Figure 1-** Illustration of Likens/Nickerson apparatus



**ISMAP0070 - Optimization of a protocol for extraction and quantitative analysis of rutin in fava d'anta (*Dimorphandra mollis*).**

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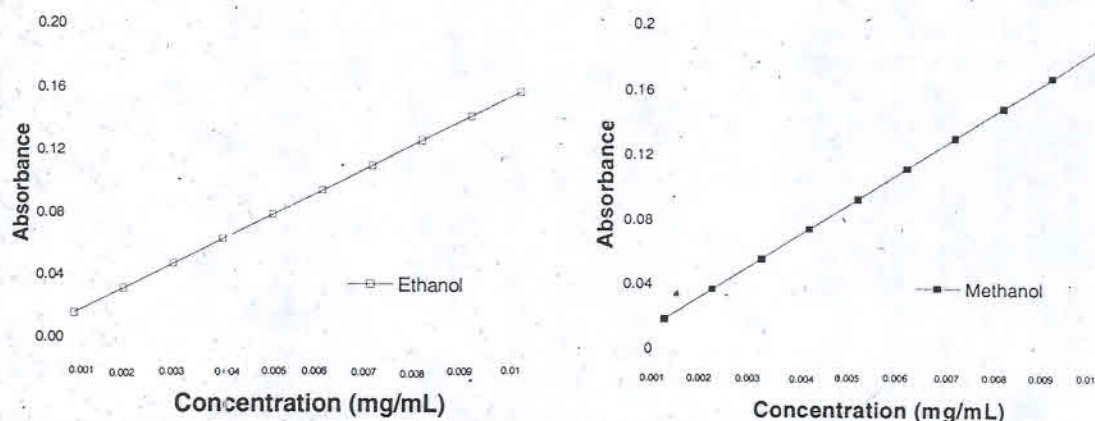
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Fava d'anta (*Dimorphandra mollis*) is a plant that produces a bioflavonoid known as rutin. Rutin is used in the pharmaceutical and cosmetics industry. The northern region of Minas Gerais State produces about 23% of the rutin produced in Brazil. The objective of this work was to establish a protocol for the extraction and quantitative analysis of rutin in fava d'anta. Dried plant material (10 mg) was extracted at room temperature with one aliquot of 10 mL of methanol and 10 mL of ethanol. Quantitative analysis of rutin was achieved by using an ultra-violet spectrophotometer to compare the absorbances obtained through suitable dilutions of a rutin standard in methanol and ethanol extracts. A known concentration of the rutin standard in methanol and ethanol ( $5 \times 10^{-3}$  mg/mL) was scanned from a wavelength of 250 to 315 nm. Selected peaks were 285 and 280 nm for methanol and ethanol, respectively. A rutin calibration curve was plotted using absorbance values obtained for the rutin standard in methanol and ethanol concentrations in the range of 0 mg/mL and 0,001 mg/mL. The regression equations obtained were Absorbance = 15,7576(concentration) + 0,0027 for methanol and Absorbance = 18,1550(concentration) + 0,0062 for ethanol. Methanol presented greater efficiency in the extraction of rutin. Methanol extracted 12,5% and ethanol extracted 2,34% of rutin in fava d'anta fruits.

**Keywords:** Fava d'anta, rutin, extraction, quantitative analysis

**Sponsor:** Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG



**Figure 1:** Rutin calibration curve obtained for the rutin standard in A) Ethanol and B) Methanol concentrations.



**ISMAP0185 - Organoleptic analyses in capsules of carqueja (*Baccharis genistelloides* (Lam.) Pers.).**

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Color, flavor, odor and texture are standards of most importance to be established quality properties medical dry plant. It was verified through organoleptic analyses (color, flavor and odor) of *Baccharis genistelloides* (Lam.) Pers (carqueja) the possibility of contamination for other vegetal species or for parts of other plants. Regarding the pureness of the sample, in Brazil alone there are more than 400 species of 'carquejas', increasing the chances for impurity. With the goal to evaluate such variations, a work was established where dry powder was put in capsules (PEC) and with rude powder (PBC) as control treatment. The samples were stored for 320 days allowed by the Brazilian Pharmacopoeia IV (2003). For encapsulated samples, 1500 g of dry powder were distributed in gelatinous colorless capsules, number zero and stored inside of white bottles, while rude powder (PBC) was stored in amber bottles. At regular intervals of 80 days it was carried out organoleptic and botanical assays for determination of color, flavor, odor, texture, composition, authenticity and pureness recommended by the Brazilian Pharmacopoeia IV (2003). Testing the behavior of capsules in the water, sulfuric acid, alcohol, hydroxyl bases and other common organic solvents had not been detected the presence of mucilage gums and other contaminants, but there was the presence of saponins, essential oils and resins. By treating the samples with 5% sodium hydroxide it was disclosed the presence of intense yellow color, while the treatment with 66% sulfuric acid the a chestnut the color colored was evident and without bubbling, demonstrating to be a genuine material without contaminants. Treatment with ferric chloride both PEC and PBC had disclosed black-bluish, which is indicative coloration of the bark tannin presence. The flavor of the PEC and rude powder PBC were intensely bitter, when compared with the brucine solution, and very less sweet than sucrose. The odor test revealed no sweet smell in any sample. The characteristic odor of PEC and PBC were formed by some strong floral grades, with light sugars tone and discrete woody fragrance, but citric characters or astringents had not been observed. The analyzed samples evidenced 99.9% authenticity, constituting, therefore, legitimate samples of *Baccharis genistelloides* (Lam.) Pers. No contaminants have been identified in none of the analysis at 80, 160, 240 and 320 days, including strange fragments with dubious nature. The pureness of the fresh source material was analyzed at initial stage 24 hours after harvest, which allowed to visual the presence of one Lepidopteran larvae, however no presence of dirt or contaminants were observed.

**Keywords:** organoleptic analyses, carqueja, *Baccharis*, contaminants



# ISMAP0219 - Phytochemical comparison by HPLC and GC/MS between two species of genus *Himatanthus*.

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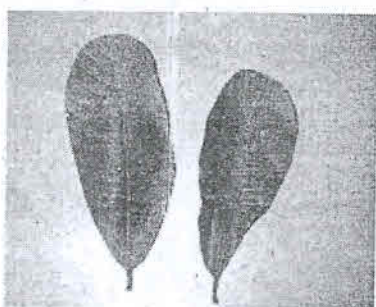
*Himatanthus sucuuba* (Spruce) Woodson and *H. obovatus* are plants of the Apocynaceae family. The first one is popularly known in the northern region of Brazil as sucuuba, sucuba or janaguba while the second one is known as tiborna. These plants are commonly used in Brazilian and Peruvian folk medicine for the treatment of gastritis, haemorrhoids, anaemia, arthritis, worms and cancer. This work has the objective to promote a phytochemical comparison between *H. sucuuba* and *H. obovatus* by HPLC and GC/MS. The powdered barks and leaves were macerated in ethanol and the extracts were evaporated to dryness, suspended in methanol/H<sub>2</sub>O (8:2) and partitioned with hexane, dichloromethane and ethyl acetate. The crude extracts and the hexane fractions were analyzed and compared by HPLC/UV and GC/MS, respectively. In the crude extract of *Himatanthus sucuuba* the same major compound, plumieride, was found with amount of 43.5% at leaves and 77.5% at barks. *Himatanthus obovatus* showed plumieride as the major compound to the barks (44.8%) a flavonoid as the major compound to the leaves. The hexane fraction of *H. sucuuba* and *H. obovatus* present some compounds previously related as fungicide, they are:  $\alpha$ -amyrin,  $\beta$ -amyrin, lupeol cinnamates and lupeol acetate. The hexane fractions of leaves and barks of *H. sucuuba* showed lupeol acetate as the major compound at amount of 40.5% to the barks and 36.6% to the leaves. Lupeol acetate was the major compound of leaves (34.2%) and barks (36.0%) of *H. obovatus*. The lupeol cinnamate was found only in leaves and barks of *H. sucuuba*, 32.7% and 36.3%, respectively. These results showed the great similarity between both species and justify in part, the use of them to the same medicinal purpose.

**Keywords:** *Himatanthus*, HPLC, iridoid, flavonoid.

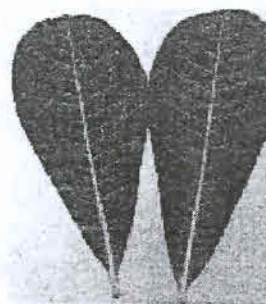
**Sponsor:** CNPq.

**Table 1.** Comparative table between *H. sucuuba* e *H. obovatus* (hexane extracts):

	Leaves	Barks
<i>Himatanthus sucuuba</i>	Lupeol acetate (36.6%) Lupeol cinnamate (32.7%)	Lupeol acetate (40.5%) Lupeol cinnamate (36.3%)
<i>Himatanthus obovatus</i>	Lupeol acetate (34.2%)	Lupeol acetate (36.0%)



**Figure 1.** *Himatanthus sucuuba*-Leaves



**Figure 2.** *Himatanthus obovatus*-Leaves



**ISMAP0223 - Phytochemical screening of *Achillea millefolium* harvested at Araraquara – SP.**

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*Achillea millefolium* (Asteraceae) is a native plant from Europe, North America, South Australia and North Asia, but perfectly adapted for Brazil. *A. millefolium*, 30 – 90 cm higher with abundant leaves and rose or white flowers is popularly known by “mil-folhas”, “novalgina”, “mil-em-rama”, “erva-de-carpinteiro”, “milefólio”, “erva-de-cortaduras”, “erva-dos-carreiros”, “aquiléia”, “macelão” and “yarrow”. Its popular use includes antiinflammatory and astringent actions, which are proportionate for the presence of the azulene, one of the main components of the crude essential oil. Phytochemistry studies carried out with this species describes the presence of tannins, amino acids, sugars, mucilage, resins, coumarins, cyanogenic heteroside, betains, chlorogenic acid, vitamin C, salicylic and caffeic acids, fatty acids, phytosterol, sesquiterpenic lactones and the presence of flavonoids in its leaves like apigenin, luteolin, artemetin and rutin. The objective of this work was to search the presence of secondary metabolites in leaves of *A. millefolium* cultivated at the “Horto de Plantas Medicinais e Tóxicas da Faculdade de Ciências Farmacêuticas de Araraquara-UNESP, São Paulo”. To the characterization of flavonoids, tannins, alkaloids, saponins, glycosides (antraquinonic and cardiac) and antraquinones were used chemical reactions specific of precipitation and development of colour. In *A. millefolium* leaves the specific characterization reactions for alkaloids, antraquinonic and cardiac glycosides and antraquinones presented negative results. The reactions of Taubock (yellow-green fluorescence), ferric chloride (green-brown colour) and aluminium chloride (yellow-green colour) presented positive results to flavonoids (flavonols). The positive results to condensed and hydrolyzable tannins were demonstrated by precipitation in the reactions of gelatin and lead acetate, as well as the development of green-brown colour by iron salts. The formation of abundant and persistent foam showed the presence of saponins in leaves of *A. millefolium*. Our results of phytochemistry-screening indicated the presence of flavonoids, tannins and saponins in *A. millefolium* leaves.

**Keywords:** *Achillea millefolium*, tannins, flavonoids, saponins

**Support:** FAPESP; PADC – FCF – UNESP



**ISMAP0358 - Production of standardized *Cordia verbenacea* D.C. essential oil.**

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*Cordia verbenacea* known popularly as "erva baleeira" occurs in Brazil from the Amazonian region to Rio Grande do Sul State. Pharmacological studies demonstrated a potent anti-inflammatory activity of the essential oil of leaves of *C. verbenacea*. The aim of this work was to produce a standardized *C. verbenacea* essential oil and to reach a quality control protocol for the use in pharmaceutical industry. Erva baleeira has been cultivated in large scale in Experimental Field of CPQBA/UNICAMP. The essential oil was obtained using fresh aerial parts of plant by vapor distillation in steel reactor with 450 kg capacity. The yield of extractions was 0.12% (fresh base). Some analytical methods used were based in European Pharmacopoeia fourth edition but tests such identification and quantification of active constituents were defined for us. The assays defined in essential oil analysis for pharmaceutical product (topical use) were: density (0.870 to 0.900 g mL<sup>-1</sup>), acid value (no more than 0.5 determined on 10 g dissolved in 50 mL of ethanol 96% v/v and ether), water (absent), foreign esters (absent), solubility in alcohol (it is soluble in 5.0 – 8.0 volumes of ethanol 90% v/v), fatty oils and resinified essential oils (absent) and description of colour and odour. Complementary tests for identification using GC-MS and assay of  $\alpha$ -humulene by GC-FID (2.3 to 2.9% w/w) were also included after validation protocol. This compound is responsible for the anti-inflammatory activity of this essential oil.

**Keywords:** *Cordia verbenacea*, essential oil, anti-inflammatory



**ISMAP0119 - Production, transformation and essential oils composition of flowers leaves and stems of lemon verbena [*Aloysia triphylla* (L'Herit.) Britton] grown in Portugal.**

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*Aloysia triphylla* (L'Herit.) Britton (Fig.1), has been experimentally grown in four different sites of the Northern region of Portugal to be transformed and commercialised as lemon verbena tea with guaranty certificate. The drug assay usually includes quantification of the essential oil and TLC analysis to show the presence of citral. However, for a guaranty certificate much more compounds may be included for detection of alterations of the respective characteristics. In our view, the percentages of the ten major compounds of the essential oils can be considered a reasonable compromise as the main marker to be used in the respective certificate of origin. In this work lemon verbena plants were propagated by cuttings which after root induction in rooting desk were transferred to soil, planted at distance of 0,7 cm in rows separated 1,10 m each other. The leaves biomass, harvested in September and dried in a solar dryer (place of Covide), showed humidity percentages of 20%, 7,8% and 5,5% at the end of 96, 120, and 144 h, respectively, and gave an essential oil yield of 2,18%. However, the essential oil yields from leaves ranged from 1,00 to 2,18 % of the dried biomass depending of the site, harvest date and, drying method used. More than sixty compounds were identified, majority distributed by monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes (Table 1).

**Keywords:** lemon balm, *Aloysia triphylla*, essential oils

**Sponsor:** INIAP/IFADAP - Programme AGRO / 8 / 8.1 / Project HERBAROM, Ref. 338.



**Figure 1.** *Aloysia triphylla*

**Table 1.** Compound groups of the essential oils from lemon balm

Compound group	Flowers	Leaves	Stems
Monoterpene hydrocarbons	30%	22%	5%
Oxygenated monoterpenes	57%	58%	69%
-oxides	6%	4%	1%
-alcohols	2%	2%	3%
-aldehydes	48%	53%	64%
-esters	1%	1%	1%
Sesquiterpene hydrocarbons	7%	7%	16%
Oxygenated sesquiterpenes	3%	5%	3%



**ISMAP0120 - Production and essential oils composition of lavender [*Lavandula angustifolia* Miller] grown in Northern of Portugal.**

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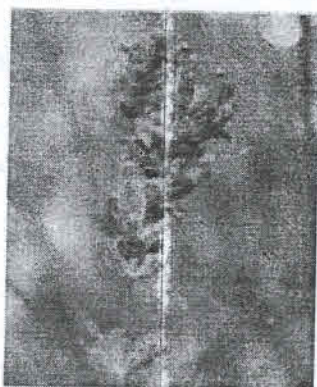
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Lavender [*Lavandula angustifolia* Miller] (Fig. 1) is a shrub medicinal plant (1) growing in the low mountains (800-1300 m) of the Mediterranean basin. To fulfil the requirements of the French Pharmacopeia the essential oil of *Lavandula angustifolia* flowers must contain 25-38% linalool, 25-45% linalyl acetate, 0.1-0.5% limonene, 0.3-0.5% camphor, and 0.3-1% aher. The flowers, harvested in July, from plants grown in the localities of Covide and Mesio were dried in a solar and in a warm air dryer, respectively. After hydrodistillation in a Clevenger type apparatus, the dried flowers from Covide and Mesio gave essential oil yields of 10.1% and 8.4%, respectively. The essential oils composition was determined by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC-MS), in the conditions reported before (2). More than seventy compounds were identified. The composition profile of the essential oil from the aerial part of lavender, determined through periodical sampling in two experimental fields, changed drastically over the year in what concerns the levels of some of its most significant constituents, namely linalyl acetate. The great variation in composition of the essential oils of this species has already been reported (1). However, the major constituents present in essential oil of flowers from lavender plants grown in Covide and Mesio kept basically the same (Table 1). Obviously that the characteristics of the essential oils here reported do not correspond to parameters defined in the French Pharmacopeia. Given such differences we consider that the commercialization of these type of products would be accompanied with the respective certificate of origin.

**Keywords:** lavender, *Lavandula angustifolia*, essential oils

**Sponsor:** INIAP/IFADAP - Programme AGRO / 8 / 8.1 / Project HERBAROM, Ref. 338.



**Table 1.** Main essential oil constituents of the lavender flowers

Compounds	Covide	Mesio
linalool	47%	48%
(Z)-?-ocimene	2%	2%
borneol	6%	6%
1,8-cineole	7%	5%
linalyl acetate	4%	8%
camphor	13%	10%
terpinen-4-ol	5%	4%
(Z)-?-farnesene	1%	1%
bornyl acetate	1%	2%
germacrene D	0,2%	0.2%
limonene	0.2%	0.1%

**Figure 1.** *Lavandula angustifolia*

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**ISMAP0323 - *Pterodon pubescens* (Leguminosae) active compound:  
antiinflammatory activity, isolation and identification.**

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Based on the popular use of *Pterodon pubescens* (Leguminosae), usually known as "sucupira", markedly in the treatment of several inflammatory processes, we decided to investigate the antiedematogenic activity of its fruit's crude oil and to identify the active (s) compound (s) responsible for this pharmacological activity. The chosen models were the paw edema assay induced by carrageenin (also applied to adrenalectomized animals), histamine and bradykinin, as well as ear croton oil edema. The isolation of the active compound, from the crude oil, was monitored by carrageenin paw edema animal model, from which three compounds were isolated. The maximum inhibitory effect for the crude oil observed on carrageenin paw edema was 65% (300mg/kg) ( $P<0,01$ ) and 25% (200mg/kg) ( $P<0,05$ ). This activity was also observed in adrenalectomized rats. After 30 minutes of paw edema induction by histamine and bradykinin, there was a significant inhibition of 77% and 46% ( $P<0,01$ ), respectively. Our results indicate Compound 3 (vouacapan diterpenoid) as the responsible for the most remarkable anti-inflammatory activity in the paw edema model.

**Keywords:** *Pterodon pubescens*, vouacapan, antiinflammatory, diterpenoids

**Sponsor:** CNPq and FAPESP



**ISMAP0042 - Quality Control and Phytochemical Investigation  
of *Terminalia chebula*.**

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In the present study an attempt is made carry out quality control and phytochemical investigation of *Terminalia chebula*. Initially physicochemical investigation of the plant is carried out so as to study the organoleptic characters, fluorescent analysis, extractive values, ash values etc. In addition to above investigation, the different extracts from solvents like petroleum ether, benzene, chloroform, alcohol, water of the plant were obtained, these extracts further screened for the presence of active principles like alkaloids, glycosides by adopting standard procedures. In response to above investigation, different values are obtained for physicochemical investigation and in case of later part of the study, where extracts are studied. Active principles like steroids in petroleum ether, benzene and chloroform, triterpenoids in all the extracts except aqueous extract, glycosides in aqueous extract, carbohydrates and tannins. Alcohol and aqueous extracts and proteins in aqueous extract of plant were investigated.

**Keywords:** Physico chemical, quality control, *Terminalia chebula*, triterpenoids.

**Table 1:** Qualitative chemical investigation of crude extracts of *Terminalia chebula*

Tests	<i>Terminalia chebula</i>				
Extracts	Pet-ether	Benzene	Chloroform	Alcoholic	Aqueous
<b>1 Tests for Steroids</b>					
a) Salkowski test	+	+	+	-	-
b) Liebermann-Burchard Test	+	+	+	-	-
c) Sulphur test	+	+	+	-	-
<b>2 Tests for Triterpenoids</b>					
a) Salkowski test	+	+	+	+	-
b) Liebermann-Burchard test	+	+	+	+	-
c) Tschugajew test	+	+	+	+	-
d) Briekorn and Brinar test	+	+	+	+	-
<b>3 Tests for Glycosides</b>					
a) Baljet test	-	-	-	-	+
b) Keller-Killani test	-	-	-	-	+
c) Raymond test	-	-	-	-	+
<b>4 Tests for Carbohydrates</b>					
a) Molisch's test	-	-	-	+	+
b) Barfoed's test	-	-	-	+	+
c) Benedicts test	-	-	-	+	+
<b>5 Tests for Tannins</b>					
a) Ferric chlorid test	-	-	-	+	+
b) Gelatin test	-	-	-	+	+
<b>6 Tests for Proteins</b>					
a) Millon's test	-	-	-	-	+
b) Xanthoproteic test	-	-	-	-	+
c) Biuret test	-	-	-	-	+

"+" Present. "-" Absent.



**ISMAP0349 - Quality control of annato (*Bixa orellana* L.): optimization of the routine analysis of bixin content in the seeds.**

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Annato (*Bixa orellana* L.), or "urucu" and "urucum" in Brazil, is a plant species that has been used by indigenous peoples in South America for body paintings and other traditional uses. Frequently, it has also been considered a medicinal plant (1), but presently its main use is for its colouring material that are used in the food industry that, due to restrictions on artificial colourings, is turning its interest to the natural sources. The dyeing substance of annato is mainly a carotenoid called bixin that is produced in the outer part of the seeds. The substance is extracted to be concentrated and further used in many products such as in dairy products, pasta and as a condiment. The content of bixin surrounding the seeds is genetically and environmentally determined and the extraction industry only has interest in seed material that presents at least 2.5% bixin (2). For that reason, the quality control of bixin content of annato seeds is very important for the producers and industry. The current methods used for analysis are not the subject of a consensus (3,4), specially the one-based in organic solvents are troublesome when applied to a great amount of samples. In this paper it is investigated a more reliable and easy way to perform the analysis of bixin content in annato seeds, based in the traditional KOH method. Three experiments were performed. The first one dealt with the sample size; it was used samples of 2, 8, 14, 20 and 26g of seeds, with five replicates. The second experiment was designed as a 4 x 3 factorial, with four replicates, where the temperatures of 50, 65, 80 or 95 °C were applied through a hot bath during the period of 5, 10 or 15 min. The third experiment was also a factorial with four replicates where the initial volumes of 60, 80 and 100 ml and the dilution volumes of 100, 150 or 200 ml were used. The analysis of variance for sample sizes, duration of bath and initial volumes were not significant, but temperature and dilution volumes were significant to determine the reliability of the analysis. The best conditions found in the set of experiments showed that a sample of 14g of seeds, with a temperature of 80 °C, during the period of 10 min, with an initial volume of 80 ml and a dilution volume of 200 ml should be used in routine analysis according to these findings.

**Keywords:** annato breeding; genetic resources; natural dye; natural colouring

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**ISMAP0382 - Quality evaluation of herbal drinks using selected herbs.**

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Medicinal plants and herbs contain substances known to modern and ancient civilization for their healing properties. Herbal teas can be prepared by simplest technique using selected herbs. An attempt was made to select suitable herbs for standardization and preparation of herbal drinks mix. The study also aims at analysis of chemical constituents, microbial counts, storage life and organoleptic characteristics. The selected herbs were rose (*Rosa damascena*), lotus (*Nelumbo nucifera*), avaram (*Cassia auriculata*) shoe flower (*Hibiscus rosa-sinensis*) and cutch tree bark (*Acacia catechu*). Herbs after cleaning, shade dried and finely powdered. The herbal powder at different concentrations 1%, 3% and 5% were used for preparation of Herbal tea. Herbal powders was also incorporated with tea dust in different ratio viz., 1:1, 2:1 and 1:2. The herbal powders were packed in high-density polyethylene packages, metallized polyester polypropylene laminated pouches and in pet jars and stored at room temperature to study the storage life. The moisture content, pH and total sugars of all the five herbal powders were decreased during six months storage. The crude fibre content of rose powder, lotus powder, avaram powder, shoe flower and cutch tree bark powder were found to be 9.64, 11.35, 10.24, 13.29 and 8.44 g per hundred grams respectively. Organoleptic characteristics have revealed higher scores when herbals powders were packed in metallised polyethylene laminated packs.



**ISMAP0260 - Seasonal evaluation of TLC profile of *Zollernia ilicifolia*.**

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*Maytenus ilicifolia* Martius ex Reissek (Celastraceae) is known in Brazil as “espinheira-santa”. Their leaves have been used as anti-ulcer and to other gastric perturbances, and have proven activity on gastric ulcers in laboratory animals and humans. Adulterations in this specie are very common and quality control is required, both for crude drugs and phytomedicines, in order to assure the safety and efficacy of *Maytenus* preparations. The most common adulterants of “espinheira-santa” are *Zollernia ilicifolia* (Brongniart) Vogel (Fabaceae) and *Sorocea bonplandii* (Baillon) Burger, Lanjouw & Boer (Moraceae). In previous studies, we identified chemical markers by TLC for *Zollernia ilicifolia* and *Sorocea bonplandii*. In present study, we investigated the seasonal profile of the main chemical marker, detected in *Zollernia ilicifolia*, ZI-2. This compound was characterized as a triterpene. *Zollernia ilicifolia* leaves were monthly collected (from march/2000 to march/2001), crushed and macerated in dichloromethane at room temperature for 6 days. The extracts were filtrated and concentrated under low pressure and dried extracts were dissolved in CH<sub>2</sub>Cl<sub>2</sub> in accordance to the relation 1:1 (dried plant weight/ solvent volume), where 1 mL of extractive solution corresponds to 1 g dried plant. Chromatographic analysis of dichloromethane extracts of samples of *Z. ilicifolia* was performed on 20 x 10 cm silica gel 60 GF<sub>254</sub> TLC plates (Merck). Aliquots (10 µL) of each sample and of the marker ZI-2 were applied to the plates. Separation was performed using light petroleum: ethyl acetate (85:15) as mobile phase. After development, the plates were dried and derivatized with a thymol-sulphuric solution. The TLC plates were digitalized in both color and black and white scanning process and the concentrations of spots corresponding to ZI - 2 were determined by the software *Scion Image* (NIH). The sample with the highest concentration was considered equal to 100 %, and concentration in the other samples was expressed as percentual values. All monthly samples of *Z. ilicifolia* presented ZI-2, the proposed TLC marker for this specie. The relative concentrations of ZI-2 from march/2000 to march/2001 were, respectively: 82.62 %; 57.0 %; 100.00%; 60.90%; 63.74%; 72.95%; 75.65%; 55.57%; 61.16%; 50.78%; 96.67%; 60.52%; 76.4%. The highest concentrations of ZI 2 were detected in samples collected in May (100 %) and January (96,67%). The lowest concentration was found in December and it was nearly the half of the highest concentration. The presence of high concentrations of ZI-2 in all samples allows the application of this substance as chemical marker in the quality control of ‘espinheira-santa’ products in order to detect adulterations with *Z. ilicifolia*.

**Keywords:** Espinheira-santa, *Maytenus ilicifolia*, *Zollernia ilicifolia*

**Sponsor:** Capes



# ISMAP0151 - Seasonal variability in essential oil of *Baccharis trimera*.

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*Baccharis trimera* (Less.) DC. (Asteraceae) is a widespread South American plant known as carqueja. This plant is used to treat stomach pain and renal diseases, and is also known to have an antiinflammatory and antihepatotoxic effects. In the present work, the diurnal and seasonal chemical variability of *B. trimera* essential oil were examined by GC/MS. For that purpose, a natural population (Lavras city, Minas Gerais State, Brazil) and a cultivated sampling were chosen and essential oils from aerial parts were obtained by hydrodistillation. In total, 25 compounds were identified, with sesquiterpene hydrocarbons (mean value, 71.5±8.9%) as main constituents (Table 1). Principal Component (PCA) and Cluster Analysis were applied to the study of the similarity of individuals on the basis of constituent distribution using SPAD.N (CISIA) package. From PCA the whole data set could be projected in the space defined by the 2 first PCs retaining 72% of the total variance (Fig. 1). First PC separates the natural and cultivated sampling in March-May months, which were characterised by the highest level of oxygenated sesquiterpenes (35.0±7.2%), and the Second PC separates the natural from cultivated populations in June-December period, with sesquiterpene hydrocarbons reach higher levels in natural sampling (82.2±1.1%). Hence, three clusters were found: I (□; natural and cultivated sampling from March-May), characterised by a high percentage of spathulenol (mean value, 8.6±2.6%) ( $P<0.001$ ) and globulol (8.6±1.3%) ( $P<0.0001$ ); II (■; cultivated sampling from June-December) containing a high content of ledol (12.6±1.6%) ( $P<0.001$ ), and cluster III (●; natural sampling from June-December) those having germacrene D (25.5±2.8%) ( $P<0.004$ ), bicyclogermacrene (20.9±2.1%) ( $P<0.005$ ), and (E)-caryophyllene (17.3±1.7%) ( $P<0.012$ ) as principal constituents. The diurnal variation (6, 12 and 18h) resulted in similar chemical composition. The observed seasonal variation has a possible ecological role, which are probably caused by a combination of irradiance, climate, nutrients and water availability in soil.

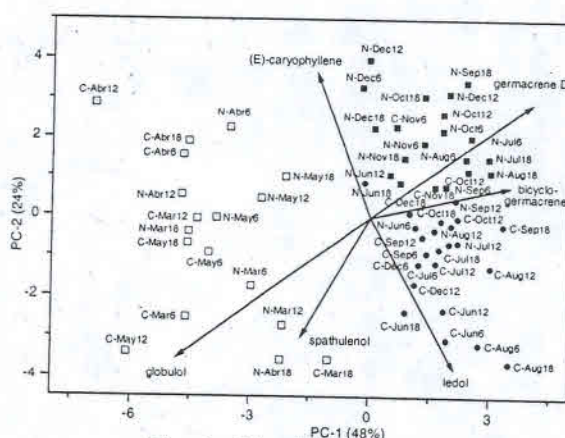
**Keywords:** *Baccharis trimera*, essential oil, multivariate analysis

**Sponsor:** PADCT, CNPq and FUNAPE/UFG

**Table 1.** Major components (%) of *B. trimera*

Compound	Mean±SD <sup>a</sup>	Min.	Max.
(E)-caryophyllene	15.9±2.5	0.2	22.6
α-guaiene	2.5±0.8	10.3	4.9
γ-muurolene	1.7±0.8	1.1	3.9
germacrene D	19.7±7.3	0.7	30.3
bicyclogermacrene	17.9±4.0	3.0	24.9
δ-cadinene	5.8±1.7	0.0	9.3
ledol	8.9±4.2	3.6	18.2
spathulenol	5.6±3.0	0.2	13.6
globulol	5.9±2.4	1.9	15.6
viridiflorol	1.9±1.0	3.1	4.2
guaiol	2.4±1.1	0.5	4.4

<sup>a</sup>Standard deviation



**Fig. 1.** Principal Component Analysis



**ISMAP0359 - Tropanic alkaloids in leaves of *Brugmansia suaveolens* (WILLD.)  
SWEET (SOLANACEAE).**

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Of the numerous plant species producing tropane alkaloids (TAs), solanaceous genera *Atropa*, *Datura*, *Scopolia* and more recently the genus *Brugmansia*, are the most important. Considerable effort is presently being invested in the development of methods for the production of these tropane alkaloids (specifically atropine and scopolamine), the two most useful ones from the medicinal point of view. Many reports have been published on the screening of plants possessing good biosynthetic capabilities for tropane alkaloid production. In this work, we describe a simple method of extraction and determination of TAs present in leaves of *B. suaveolens*. The TAs were isolated by acid-base extraction and the alkaloidal content analysed by GC-MS in a Hewlett Packard 5988 equipped with a capilar column SA-5 (30 m x 0,25 mm x 0,25  $\mu$ , Sigma-Aldrich) directly coupled to a selective mass detector Hewlett Packard 5970. The retention index (RI's) of the TAs were calculated according to van den Dool & Kratz (1963) and the fragmentation features of TAs were compared with those reported by Ethier & Neville (1986). 10 TAs were identified in the extracts (Table 1) and they are present mostly as free bases. The leaves of *B. suaveolens* contain scopolamine, atropine, hyoscyamine and norscopolamine as major TAs.

**Table 1.** TAs identified in leaves of *Brugmansia suaveolens*.

	Alkaloids	RI's	M+
1	3,6 dihydroxytropane	1365	157
2	3-hydroxy-6-methyl-butenoyl-oxytropane	1785	239
3	apopatropine	2032	271
4	norhyoscyamine	2165	275
5	aposcopolamina	2131	285
6	hyoscyamine	2170	289
7	norscopolamine	2277	289
8	atropine	2281	289
9	scopolamine	2288	303
10	6-hydroxyhyoscyamine	2355	305

RI's - retention index

M+ - molecular ion

Van den Dool H, Kratz PD (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr 11:463-471  
Ethier JC, Neville GA (1986) Quadrupole EI and CI mass spectra of some principal tropane alkaloids. Dan J Spectr 31:81-88

**Keywords:** *Brugmansia suaveolens*, tropanic alkaloids, Solanaceae



# **Economic, Ethical, and Legal Aspects**



# ISMAP0169 - Brazilian commercial exchange of spices.

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Brazil is the main regional market and argentine's commercial partner; because of this the analysis of this market is essential in order to detect business opportunities for our country.

Last quinquennial averages, percentage of participation per spice, commercial balance, and interchange terms, were calculated based on data about imports and exports of spices from the Development Industry and Commerce Ministry, during the highest level of opening by the period 1990-2002. The average annual rate of growth for the last decade was calculated by linear regression method.

It is concluded that Brazil has raised their imports and exports of spices at a significative rate (5.9% y 6.1% respectively in terms of volume). It is observed an excellent complement with our country, due to Brazil produces and exports tropical climate spices, whereas Argentina produces and exports temperate climate spices. (Table 1)

The brazilian exports of spices, their commercial balance and interchange terms are highly influenced by changes of the pepper's price, which is a main exporting product. Important commercial opportunities for Argentina are detected related to cumin, anise, coriander and fennel. (Graphic 1).

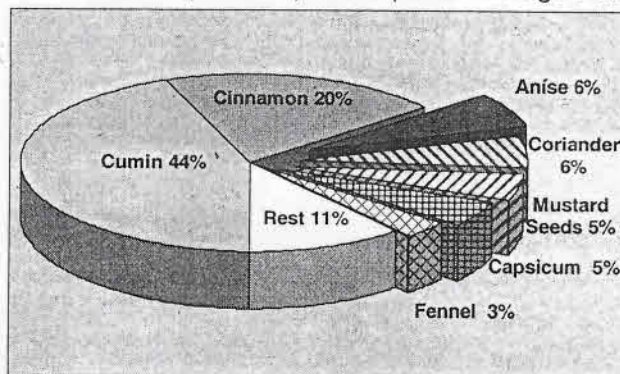
**Keywords:** spices, Brazil, exchange, opportunities.

**Table 1:** Brazilian commercial exchange of spices 1990-2002

Year	Imports		Exports		Commercial Balance
	u\$s CIF	Vol. kg	u\$s FOB	Vol. kg	u\$s
1990	7,409,851	5,546,653	50,688,387	37,401,354	43,278,536
1991	8,064,681	6,095,015	56,312,718	54,891,989	48,248,037
1992	9,024,096	5,547,674	32,151,313	34,145,322	23,127,217
1993	10,752,258	6,394,741	35,344,097	34,576,334	24,591,839
1994	10,868,721	8,454,252	49,525,228	30,437,558	38,656,507
1995	12,648,749	8,852,852	58,694,787	28,510,739	46,046,038
1996	14,198,812	9,702,840	61,116,809	29,642,315	46,917,997
1997	14,850,099	9,504,748	65,623,159	20,230,310	50,773,060
1998	18,610,545	11,803,723	86,099,678	25,859,974	67,489,133
1999	13,906,434	10,257,226	103,403,201	32,494,395	89,496,767
2000	14,594,321	9,303,029	83,943,919	32,341,599	69,349,598
2001	18,220,197	10,078,728	93,212,779	55,782,333	74,992,582
2002	17,526,671	15,457,589	103,417,974	57,612,323	85,881,303
Average 93/97	12,663,728	8,581,887	54,060,816	28,679,451	41,397,088
Average 98/02	16,571,634	11,380,059	94,013,510	40,818,125	77,441,877
Rate 93/02	5.4	5.9	10.7	6.1	

Alice Data Base. Development Industry and Commerce Ministry

**Graphic 1:** Brazilian most important spices imports. Average volume 1998/2002.



Alice Data Base. Development Industry and Commerce Ministry



### ISMAP0194 - Culture of medicine plants.

#### L.C.Alcântara

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The Dr. Cândido Ferreira Health Service Center, located in the district of Sousas, in the city of Campinas, in the State of São Paulo, 93 Km from the Capital City of São Paulo. It is considered a model in terms of mental health treatment in Brazil, having been recognized by the World Health Organization as early as 1993. The projects major objective is to build services to allow for the release of patients from mental institutions and their consequent reinstatement in social activities. Also to give the right of the mentally ill patient to participate in society. The Dr. Cândido Ferreira Health Service Center assists approximately 990 people per month. The Cornélia Vlieg Association, supported by the Cândido Ferreira Hospital, is a non-profit society that is acknowledged as an organization of Municipal and State public interest. The Cornélia Association was established in 1993, with headquarters in Campinas, São Paulo. The association was founded by patients, their family members and by the mental service workers who, at that time, proposed to de-hospitalize their patients. Since then, the association has been active in the psychological and social rehabilitation of mentally ill persons, seeking to unite health, social and cultural work. The Cornélia Association started workshop centers in 1990, there are now 12 workshops, all participants are granted a scholarship, which guarantees them the freedom to live alone. These grants are funded from the results of the production and sale of their products. One of the workshops is agricultural, due to the Hospital having a large area for cultivation, about 12 hectares. This workshop now develops a project for the production and sale of medicinal plants, in partnership with the City Hall of Campinas. The project target is to supply dried herbs for the public pharmacies, such as Espinheira Santa *Maytenus ilicifolia* (Schrader) Planch. *Malva Malva sylvestris* L., Guaco *Mikania glomerata* Spreng., Maracujá *Passiflora* spp., Quebra-Pedra *Phyllanthus niruri* L. and Carqueja *Baccharis trimera* (Lees.) DC. As a result of this project the number of participants increased and guaranteed their scholarships. Two Hectares of Espinheira Santa has been planted and in a short time other medicinal herbs will occupy the Hospital fields. This will go a long way to forming of qualified professionals and establishing a qualified workforce.

**Keywords:** Medicine Plants, Social Works, Pharmacy.



**ISMAP0139 - Participatory approach for sustainability of *Swertia chirayita*:  
sustaining rural livelihoods and poverty reduction in eastern Nepal.**

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The Ayurvedic medicinal system has considered the Himalayan herbs as prized ingredients of its medicine since inception during the Vedic time. It is generally agreed that more than seventy percent of our population is benefiting from such herbal medicines. It has been estimated that 605 of these drug resources are obtained from the Himalayan region. *Swertia chirayita* locally is known as Chirayito has intimately been associated with human being since ancient time in Nepal. Cultivation of Chirayito through the herbal users cooperative group is in practice in some parts of eastern Nepal as income generating program. Adaptable agro-technologies and other means of verification have been continuing as scientific intervention. The study is an attempt to provide necessary information on participatory approach for utilization pattern, cultivation trend, marketing and developmental implications of Chirayito that includes its role towards poverty reduction in Nepal.

Discussion was also made on collections-permit system and attitude of grower and government and non-government organization. Seed processing, storage and supply system has mentioned as additional income sources. Socioeconomic profile of traders and perception about the difficulty faced by the growers in selling Chirayito is mentioned with reference to farm gate price and volume of supply. Financial aspects of commercial cultivation of Chirayito have analyzed in the basis of information provided by the growers and results obtained from experimental trial plots.

**Keywords:** Integrated research, cultivation, utilization pattern and sustainability



**ISMAP0062 - Projects on medicinal and aromatic plants in Brazil.**

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In considering medicinal plant developments in Brazil one must distinguish industrial from small scale local production, finished pharmacy shelf products from state, municipal and privately run health care facilities, and phytomedicines directed at discomfort and superficial infirmities from those destined to the treatment of life-threatening disease.

Basically medicinal plants may be exotic adapted species mostly from the northern hemisphere. When these, as is usually the case, are approved for use in North American and European countries they can be registered in Brazil with relative facility. They therefore are the preferred items of the medicinal plant pharmaceutical industry. Native species, even if of long traditional use and generally regarded as efficacious normally need to go through toxicological and clinical trials whose cost exceeds the expected rentability of the products in commerce. Manufacturers have therefore mostly limited registration to a few products that were successful market items before the present legislation came into force. Only a very few native species are thus available to the public as ethical medicines. Side by side with the industrialised phytomedicines we have the dispensing pharmacies. These do not have to register their products, which are made up for sale against doctor's prescription, sometimes only after the prescription has been handed in, and in no case may be stocked for more than about two weeks, that is, they are not made to have a shelf life and are for immediate use. Legislation here is designed to assure hygienic production facilities and care in quality control which has to be made by a certified external laboratory.

A third area of activity concerns the "green pharmacies", which are medicinal plant gardens associated to a laboratory that rarely conforms strictly with the legislation for dispensing pharmacies. Such green pharmacies serve lower income groups in towns and often are the only wide spectrum source of medicines for the rural population. Often local government runs or supports such initiatives and, in some states, like Ceará, the system is state recognised. In Goiânia, Goiás, a State hospital has been set up with an adjacent medicinal plant garden. These services are clearly supplemented by individual domestic plantations of medicinal plants for home use and some States, such as Amapá, distribute plants to the public for this purpose. Legislation has not yet been adapted to these forms of phytotherapy.

All of the previous categories deal mainly with common non-lethal diseases and conditions. However, traditional medicine as well as much scientific work has shown that life threatening infirmities like cancer, malaria, leishmaniasis and tuberculosis may also be susceptible to treatment by medicinal plants. Future developments should see these plants brought into use providing, as they did in some cases for the Indians and rural 'caboclos' in the past, alleviation and sometimes radical cure to patients in remote locations where modern medicine is not available.

Finally one should not lose sight of the bulk production of medicinal plants or their extracts or isolated substances for world commerce. These products do not have to pass through the same legislative barriers as the phytomedicines because they will not reach the public as such, but only after manipulation at a pharmaceutical factory. It is this final manufacturer who must obtain the license for his formulation. What is most important here is the care that must be taken not to collect the source plant to the verge of extinction and that is where agricultural advances and large scale extraction plant technology have revolutionised the trade.

Aromatic plants form a quite distinct legislative area although technically there is much in common with medicinal plant production. One of the common aspects is the high preference of the market for exotic plant oils and flavours. Although Brazil is said to possess a fifth of the world's flora, only a few native plants figure in international commerce and these entered centuries ago when Portugal and Spain were among the world's chief sources and brought their South American and Caribbean colonial plants to Europe. Work by several public institutions now justifies an effort to introduce new native aromatic plants into world commerce.



**ISMAP0297 - Some questions on the Brazilian sanitary legislation related to medicinal plants.**

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In the last two decades, Brazil has been a scenario for several debates concerning to the legality of medicinal plants' use, resulting in regulations supported by federal laws. Regardless to the progress attained, improvement of these regulations must be pursued through continuous debates and proposals. The aim of this work is to present a general discussion of the Brazilian sanitary legislation followed by a more specific ones concerning to its regulation. A historical background with a broad view of the present situation, including success achieved and mistakes, is given as well as several analyses of the Act Nr. 6360/76 (drugs' sanitary vigilance), Nr. 519/98 and RDC Nr. 23/00 (both related to teas as food), and RDC Nr. 17/00 and Nr. 48/04 (phytotherapeutic drugs' registration). Also, the implications of the Act Nr. 6437/77 (federal sanitary legislation transgression), and Nr. 9695/98 (crimes related to therapeutic and medicinal drugs' abuse and the Penal Code) are discussed. The most relevant aspect of this complex legislation found was the possibility of removing poor quality phytotherapeutic products from the market. Among the imperfections of the legislation, as lack of clear definitions, inadequate demands and incoherence between the characteristic of the country facing the applied legal policy that favors the work with exotic plants, even part of the Government does not fulfill some rules. It is also discussed in this paper the lack of rules and policies to strengthen the activities with native plants and the consequent disencouragement of popular phytotherapy practice. The later faces directly against the WHO orientation, which suggests that practices of traditional medicine should be encouraged, broadening the popular access to medicinal plant-related drugs.

**Keywords :** phytotherapeutic products; laws, resolutions; phytotherapy.



# Ethnobotany



**ISMAP0263 - *Abarema cochliacarpus* ethnopharmacology  
and phytochemistry study.**

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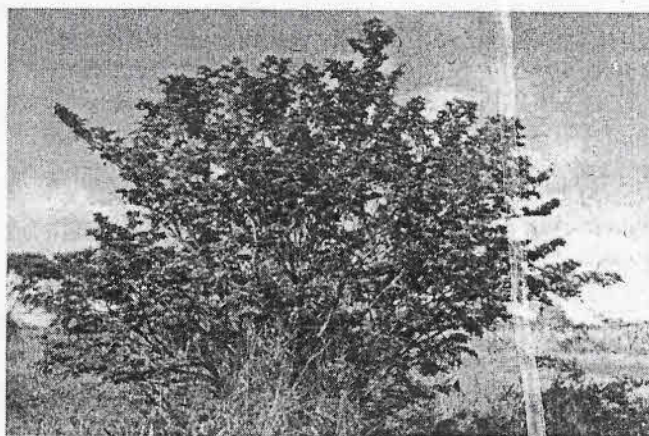
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For a long time mankind has relied on nature for the cure of its ailments. From that knowledge, acquired and disseminated for many generations, new modern drugs have emerged. Ethnopharmacology research has been developing rationality for new plant drug search, minimizing costs incurred and time spent. Based on this, a research of medicinal plants was developed with Barra II community members. Barra II is situated near Morro do Chapéu, North of “Chapada Diamantina” - Bahia. 131 plants were identified, almost at genus level, almost all used for medicinal purposes. *Abarema cochliacarpus* (B.A. Gomes) Barneby & J.W Grimes (Mimosaceae) was the most cited specie. Popularly known as “Barbatimão”, that specie is used for antiinflammatory and healing purposes, as tea or bath, prepared from stem bark. Several species from Leguminosae family are known with the same popular name but scientific literature doesn't register any research about the specie that occurs at Barra II. The goal of this work is the phytochemistry investigation of *A. cochliacarpus* beginning from ethnopharmacological directions. 2267 g from bark stem were collected. For the extraction procedure, 294 g of dried and powdered bark were used for continue extraction with CH<sub>2</sub>Cl<sub>2</sub>, CHCl<sub>3</sub>, CH<sub>3</sub>OH, 264 g were used for cold aqueous extract preparation and 128 g for hot aqueous extract preparation. Extracts analyses, by TLC with specific revelation reagents, pointed the presence of triterpene/saponins and phenolic compounds, possibly tannins and/or flavonoids and/or catequins. Alkaloids presence was detected in methanolic extracts, using Dragendorff reaction. The high methanol soluble substances level, around 12.3 %, indicates the polar nature of metabolites in bark stem. These results indicate possible toxicity of that plant for oral administration, information already passed to community. Further more, microbiological, antiinflammatory and analgesic activity tests are being carried out. That work shows the importance of ethnopharmacology as a strategy for medicinal plant investigation and the needs of combining information acquired from communities with phytochemistry studies.

**Sponsor:** CAPES, UFBA

**Keywords:** Ethnobotany, phytochemistry, medicinal plants



**Figure 1:** *Abarema cochliacarpus* tree – Barbatimão



**ISMAP0229 - Antimicrobial screening of species used in folk medicine in  
Governador Valadares, Minas Gerais, Brazil.**

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Since 1998, the BIOPLANTA research group from Universidade Vale do Rio Doce, UNIVALE, is carrying on a screening of species used as folk medicine for the people of Governador Valadares, Minas Gerais. Besides the rescue of the ethnobotanical knowledge, we have been search a possible activity of the collected species. The scope of this work was to investigate the antimicrobial activitie of medicinal plants. Aerial parts of each species were collected and oven dried at 40 °C. The powdered material was extracted by maceration using 95% ethanol as solvent. The obtained crude extracts were submitted to antimicrobial assays, performed by CBM determination, in triplicate, using *Staphylococcus aureus* (ATCC 25985) and *Escherichia coli* (ATCC 25922). From the 8 tested crude extracts, 4 species presented activity against *S. aureus*, and only *Pyrostegia venusta* (Bignoniaceae) presented activity against both *S. aureus* and *E. coli* (S > 5,0mg/mL and S > 10,0mg/mL, respectively), (Table 1).

**Table 1:** Antimicrobial activity of species growing in Governador Valadares, MG, Brazil

Species (Family)	Used part	Popular uses	<i>S. aureus</i>	<i>E. coli</i>
<i>Syzygium jambolanum</i> (Myrtaceae)	Leaves	anti-inflammatory, analgesic, diabetes and hypertension.	Resistent	Resistent
<i>Costus pisonis</i> (Costaceae)	Stern	Anti-hepatotoxic, renal diseases	S > 10mg/ml	Resistent
<i>Merremia</i> sp (Convolvulaceae)	Aerial parts	No ethnobotanic indication	Resistent	Resistent
<i>Pyrostegia venusta</i> (Bignoniaceae)	Aerial parts	Anti-stomach pain, analgesic.	S > 5mg/ml.	S > 10mg/ml.
<i>Argemone mexicana</i> (Papaveraceae)	Aerial parts	Stimulant	S > 5mg/ml	Resistent
<i>Gomphrema celosioides</i> (Amaranthaceae)	Aerial parts	No ethnobotanic indication	S > 5mg/ml	Resistent
<i>Jatropha multifera</i> (Euphorbiaceae)	Leaves	Cicatrizo	Resistent	Resistent
<i>Ipomoea glandifolia</i> (Convolvulaceae)	Aerial parts	No ethnobotanic indication	Resistent	Resistent

**Keywords:** Antimicrobial, *Staphylococcus aureus*, *Escherichia coli*, *Pyrostegia venusta*  
**Sponsor:** Fundação Percival Farfuar / Universidade Vale do Rio Doce



**ISMAP0211 - Botanical systematical aspects of medicinal flora in Lithuania.**

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The use of medicinal and aromatic plants is widespread and has old traditions in Lithuania. The ethnobotanical knowledge on plant usage was assembled from all known sourcebook on medicinal plant. The medicinal flora of Lithuania represented 465 species belonged to 91 *Angiosperm* families. The seven families included 9 species of fungus, lichen, algae, and moss. The spontaneous species comprised 62%, introduced – 28%, and alien – 10% of all species used in ethnopharmacology. The official medicine classified 189 species as medicinal plants for usage in galenical preparations. 127 species of this list are found in spontaneous populations or agrocenosis, or cultivated in home gardens. In order to determine the ethnomedical importance of the plant families', regression analysis method previously used by Dan Moerman (1991) was applied for *Angiosperm* species. The families were ranked according to their decreasing residuals, which exposed relative importance of medicinal plant usage. The residual is the difference between the number of medicinal species predicted by regression analysis and the true number of medicinal species. According to the residuals of the regression analysis ( $y=0.1528x + 1.0242$ ) of medicinal flora, the top six families of usage were: *Laminaceae*, *Apiaceae*, *Ranunculaceae*, *Asteraceae*, *Solanaceae*, *Polygonaceae*. The lowest level of usage in the regression analysis exposed *Poaceae*, *Cyperaceae*, *Polygalaceae*, *Caryophyllaceae*, and *Chenopodiaceae*. Based on the data of userreports, gastrointestinal disorders and respiratory ailments form the largest groups of illnesses treated by medicinal plant. Out of 94 species of the *Laminaceae*, 36 species are medicinal used in treatment of gastrointestinal ailments. The organoleptical characteristics of the species in *Laminaceae* are very relevant in usage. Most of them are introduced and grown in home gardens for medicinal purposes or used as culinary herbs. The *Poaceae* and the *Cyperaceae* are the most abundant families, however they are not important in medicinal usage. We compare the relatedness of the Lithuanian medicinal flora with one of the South American ethnobotanical medicinal floras of Veracruz (Mexico) using the Pearson correlation coefficient of the residuals. The medicinal flora of Lithuania shows a correlation of 0.482 with the flora of Veracruz. The correlation is high compare with geographical differences of both countries. The results demonstrated the process of incorporation of the different flora in usage.

**Keywords:** ethnobotany, medicinal flora, regression analysis



**ISMAP0265 - Ethnobotanical research at Sauípe – BA: increasing value to traditional knowledge and contributing for regional sustentability.**

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Sauípe is situated in the northern coast of Bahia, at Costa dos Coqueiros region. The community produces handcrafts with straw from different regional palm trees and uses plants for many purposes such as aromatic, food and condimental applications. The ethnobotanical research carried out within an ongoing “Sustained Development Program / Family Agricultural Project” contributed to the establishment of development directives, promoting the rational use of some plant species and increasing value of products obtained from that. So, the goal of this work was the creation of a pharmacobotanical garden and community members capacity building for products generation from material cultivated at this garden. Workshops about cultivation, collecting and drying plant material, preparation of disinfection products, candles, jelly, compotes, cosmetics and condiments were performed at Pharmacy Faculty/ UFBA and at Community Production School of Sauípe. At the garden (Figure 1), species are cultivated using organic techniques including capim-santo (*C. citratus*), capim-citronela (*C. nardus*), different pepper species (*Capsicum* sp.), manjeriço (*O. basilicum*), quioio (*O. gratissimum*), coentro de rigor (*Erigium* sp.) and orelha de maroto (*P. amboinicus*). During the workshops, massage oils, shampoo and soaps (Figure 2), dry condiments, olive oils with aromatic herbs, aromatic pillows, disinfection's products, bath salts and candles were prepared. The pharmacobotanical garden has been providing plant material for handcrafts production that is being commercialized at a Community Production School and at “Casa do Artesão” shop, situated in Sauípe hotel complex. These actions contributed for local traditional knowledge rescue and increased community income.

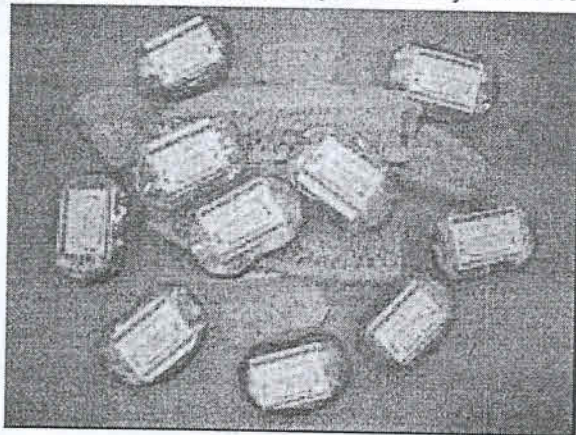
**Keywords:** ethnobotany, sustained development; pharmacobotany, organic cultivation

**Sponsor:** SEBRAE; Instituto Souza Cruz.

**Figure 1:** Pharmacobotanical garden at Sauípe



**Figure 2:** Soaps produced by community members





**ISMAP0059 - Ethnopharmacological study of plants used topically by the community of Macabú (RJ) and its essential oil chemistry evaluation.**

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**INTRODUCTION:** Macabú is a small town in the Rio de Janeiro's mountain area that shows great natural resources around the characteristic Atlantic Forest. Rural people, mainly Italians and Germans descendants, compose the Macabú's community. An ethnopharmacological approach was run in this community to know the species more used in dermatological treatments, in order to correlate the popular use with the essential oil composition achieved from each cited plant. **METHODOLOGY:** Structured interviews were done with the community residents using a previously elaborated questionnaire about dermatological pathologies. The questionnaire have approached questions about the use of medicinal plants, their frequency of use, mode of prepare and therapeutics indications. When possible, the indicated plants were collected to botanical identification and the essential oil extraction by hydrodistillation using Clevenger-type apparatus and investigated by GC/MS. Bibliographic researches of the identified species were evaluated searching scientific comprovation for the pharmacological activities cited and in the main patent offices (American, European union and Brazilian) related to cosmetic-like products based on those medicinal plants. **RESULTS AND DISCUSSION:** The Macabú population cited nine species for topical treatment, however, only five of them could be identified botanically and/or had the essential oil composition analyzed. **CONCLUSIONS:** All of the essential oil analyzed has shown the presence of a-bisabolol or its derivate, b-bisabolene, that is very used in topical products. We can conclude that the efficacy of those plants in dermatological diseases can, probably, be due to the presence of this compound or its derivate.

Popular name	Botanical name	Therapeutic indications	Mode of preparation	Essential oil composition
Erva de bicho	<i>Polygonum punctatum</i> E.	Skin allergies, itch and hemorrhoids	Bath with leaves or root tea (fresh or dry)	$\beta$ -bisabolene
Mostarda	<i>Brassica campestris</i> L.	Skin inflammations and allergies	Triturate the fresh leaves in fresh water and take a bath	<i>Trans</i> -cariophyllene $\alpha$ -bisabolol
Transsage	<i>Plantago major</i> L.	Skin inflammations, skin stones and mouth injuries	Bath with the whole fresh plant tea or only the fresh leaves tea	<i>Trans</i> -cariophyllene $\alpha$ -bisabolol
Hortelã-preta	<i>Mentha</i> sp.	Skin inflammations	Bath with the fresh leaves tea	4-terpineol $\alpha$ -copaene piperitenone oxide $\alpha$ -bisabolol
Aroeira	<i>Schinus terebinthifolius</i> R.	Skin allergies	Bath with the fresh leaves tea	Not analyzed

**Keywords:** Essential oils, Topical Use, Medicinal Plants, Macabú.

**Financial Support:** CAPES, FAPERJ, PRONEX.



**ISMAP0065 - Indigenous phytotherapeutical leads from the tribals of orissa, India for asthma and respiratory disorders.**

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Plants and their constituents have become chief ingredients of a number of pharmaceutical preparations used for a variety of diseases and more than 75 % of the leads for the discovery of new drugs have come from ethnophytotherapy. Of the various ailments, asthma is attracting the global attention as 15-20 % of world population suffer from this malady unabated. Asthma is a chronic inflammatory disorder of the airways of bronchi and bronchioles characterized by i). breathlessness or shortness of breath, ii). Wheezing, iii). chest tightness and iv). coughing. During asthma attack, 3 phenomena take place to narrow down the airway – a) the muscle wall of the airway contracts (squeezes) reducing the size of the lumen, b) increases mucus secretion that further blocks the airway and c) the inner lining of airway becomes inflamed and swells, adding to further narrowing of the airway. The resulting narrowing of the airways in the lungs makes breathing difficult and the inflammation of the airways make them super-sensitive to irritants. There is a high prevalence of usage of complementary medicine for asthma. The historical importance of herbals for asthma is indisputable as four of the five classes of drugs currently used to treat asthma – namely B2 agonists, anticholinergics, methylxanthines and cromones – have origins in herbal treatments going back at least 5000 years. Orissa state is one of the tribal rich states of India with 62 different tribes. Nearly 25 % of the tribal population belonging to 18 tribes can be termed as most primitive as they still eke out their living as 'food gatherers' in secluded hilly terrain away from civilization. In the absence of any modern medical facility in their remote areas, they still depend on plants for their various ailments. In the present study, data is generated by critically studying the herbs used and methodology adopted by the tribal healers and interacting with the patients in different tribal communities on the aspects of bronchodilation, reduction of bronchial mucosa oedema, lessening of airway secretions etc. after administration. In all, 36 tribal healers and 225 patients were interviewed periodically by visiting the tribal hamlets. Tribal preparations and prescriptions vary from single plant to polyherbal combinations. Kolha tribe of Dhenkanal use *Toddalia asiatica* (leaf - nitidine, magnoflorine) with 40% recovery where as Santhals, Ho, Munda and Bhumij of Sundargarh, Mayurbhanj and Sambalpur districts employ *Adhatoda vasica* (leaf) and the success varies between 40-45%. The 'Juang tribe' prescriptions are rated high for curative value (85%) and invariably contain four plants viz *Toddalia asiatica* (leaf - nitidine, magnoflorine) + *Adhatoda vasica* (leaf – vasicine, vasicinone) + *Terminalia chebula* (fruit - tannins) + *Solanum xanthocarpum* (berry - solasodin). The 'Bonda' tribe of Malkangiri use *Boswellia serrata* (gum - boswellic acids) + *Tylophora indica* (leaf - tylophorine, tylophorinine) + *Terminalia belerica* (fruit - tannins) and the success rate is 60%. Savara (Lanja subsect) and Kondha tribes of Ganjam district employ *Toddalia asiatica* (leaf - nitidine, magnoflorine), *Tephrosia purpurea* (root - Rutine) and *Solanum (S. trilobatum or S. xanthocarpum, berries - solasodin)* with 55% results. The effect of the SBR (Strengthening Body Resistance) was taken care by supplementing with 2 spoonfuls i.e. about 30 gm twice a day of *Withania somnifera* (root), *Curculigo orchioides* (root-tubers), *Asparagus recemosus* (root-tubers) and *Chlorophytum tuberosum* (root-tubers) either alone or in combination and this depends upon the availability of these plants in their neighbourhood. Poly-herbal approach appears to be the best for effective tackling of asthma and other respiratory disorders because of their synergic effect.

**Keywords:** Ethnotherapeutic leads, asthma, Orissa tribes, india



**ISMAP0248 - Medicinal plants used by population served by "Programa de Saúde da Família", Governador Valadares, MG, Brazil.**

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The cumulated traditional knowledge from human groups about the environment and natural sources are very important to quantify and compare this cultural heritage with another similar groups. This fact is true for every cultural and historical files of a people, and botanical and medicinal knowledge of this people or which this people had been assimilated from others are included. About medicinal plants, in Brazil until few years ago, before natural medicines became "fashion life way", usually the use of them are related to poor or non-urban communities. In addition, only ancients had been care about preserve this knowledge. About urban population, few are known concerning the use of medicinal plants. The "Programa de Saúde da Família (PSF)" is a federal program that attends the population in several cities in Brazil. The scope of this work was verify the population profile and to compile a list of medicinal plants used, including scientific and vernacular name, therapeutic use and plant part used. This work was carried out by interviewing local people to get information about the personal profile and about medicinal use of plants. We carried on 2455 interviews and the results showed that 36,0% of the interviewed use medicinal plants frequently, mainly the people with age above 50 years old and with instruction up to Fundamental degree. When asked about why they use medicinal plants, 67,0% said they follow the family tradition. 232 plants was indicated for treatment of several diseases and the most voluntaried cited plants were *Cymbopogon citratus* (Poaceae), *Coleus barbatus* (Laminaceae), *Gossypium sp* (Malvaceae), *Mentha sp* (Laminaceae), *Mentha pulegium* (Laminaceae), *Plantago Major* (Plantaginaceae), *Solidago microglossa* (Asteraceae), *Coreopsis grandiflora* (Asteraceae), *Citrus aurantium* (Rutaceae) and *Rosmarinus officinalis* (Laminaceae).

**Keywords:** Medicinal plants, ethnobotany,

**Sponsor:** Fundação Percival Farquhar / Universidade Vale do Rio Doce



# ISMAP0315 - Medicinal plants used by a traditional community in cerrado areas of Mato Grosso state (Brazil).

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Located in central Brazil, cerrado vegetation constitutes an important ecosystem, for both economic and ecological reasons. The present work registers the ethnobotanical uses for a traditional community in an areas covered by cerrado. The people (5) were interviewed about the ethno biological aspect being regarded old age (old aged) and time of residence (olden) in the community; a semi structured open form was used. The community Sao Joao (S 15°46' 567", W51°46'302") is pertaining at municipal district of the Porto Esperidião-MT, constituted by habitants that came from several regions, in and out of the State. This society exists for about 90 years in there and it conserves traditional habitants using plants as medicaments. The species identified by specialist from botanic laboratory of UNEMAT. Were registered 19 species distributes at 14 family. Among the indicated plants detaches 5 species and ethno botanical uses: Araçá (*Psidium guineense* Sw.) as astringent, Aroeira (*Myracrodruon urundeuva* (Engl.) Fr All.) To antiseptic use, Carapiá (*Dorstenia brasiliensis* Lam.) to breath infection, Fedegoso (*Senna occidentalis* (L.) Link.) to diuretic treatment and Mamica cadela (*Brosimum gaudichaudii* Trec.) to purify the blood. The people studied had a great Knowledge about the native vegetation, being this information important for to guarantee the conservation this species and consequently the maintenance of the culture habits this population.

**Keywords:** Ethnobotany, Medicinal plants, Cerrado, Brazil.

**Table 1:** Medicinal plants more cited for community São João – Mato Grosso – Brazil.

Common name	Science name	Family	Indication	Medical Parts
Araçá	<i>Psidium guineense</i> Sw.	Myrtaceae	Astringent	Bark
Aroeira	<i>Myracrodruon urundeuva</i> (Engl.) Fr All.	Anacardiaceae	Antiseptic	Bark
Carapiá	<i>Dorstenia brasiliensis</i> Lam	Moraceae	Expectorant	Root
Fedegoso	<i>Senna occidentalis</i> (L.) Link	Leguminosae	Menstrual disorders	Seed
Mamica Cadela	<i>Brosimum gaudichaudii</i> Trec.	Moraceae	Depurative	Root



**ISMAP0390 Medicinal plants used in  
the Nova Russia, Brazilian Atlantic Rain Forest.**

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Since the beginning of our civilization, human have been used the medicinal plants to protect their health and to cure theirs infirmity. During years, this knowledge was transmitted form generation to generation, constructing true natural pharmacies constituted of plants found in natural habitats and exotic plants. This knowledge is preserved in any traditional communities, as the community Nova Russia, located in Blumenau city, Santa Catarina state, Brazil, is located in the neighborhoods of the Natural Municipal Park "Nascentes do Garcia", a region occupied by the Atlantic Rain Forest. The objective of this work was to know the medicinal plants used in this community and to realize ethnobotany research. Forty-eight interviews were made with the use of a questionnaire containing 17 questions. Besides that medicinal plants were collected and identified. Thus 110 species of plants were identified, belonging to 49 families and 98 genera: 44,6% of species grow spontaneously, 52,7% are cultivated and 2,7% are purchased. The most frequent used species were: mint or peppertree (*Mentha* sp), "oregano" (*Origanum vulgare* L.), common parsley (*Petroselinum crispum* (Mill) A.W.Hil), "boldo" (*Plectranthus barbatus* Andrews and *Plectranthus neochilus* Schlechter), lemon balm (*Melissa officinalis* L.), rose mallow (*Malva sylvestris* L.), lemon gras (*Cymbopogon citratus* (DC) Stapf.), garlic (*Allium sativum* L.), green onion (*Allium fistulosum* L.), "alecrim" (*Rosmarinus officinalis* L.), and aniseed (*Foeniculum vulgare* Mill.). The most representative families sampled were Asteraceae (14 species), Lamiaceae (11), Liliaceae (6), Myrtaceae (6), Rosaceae (5), Rutaceae (4) e Solanaceae (4). The habitants obtain these plants in the yards (71%), friends and relatives house (21%), forest (6%) or purchased (2%). The medicinal plants are widely use by this community, this data can be used as instrument for programs of preservation of the Atlantic Rain Forest. It was also verified that the habitants used cultivated plants in the yards whose often that those collected directly in the forest. Despite of the big diversity observed in Atlantic Rain Forest, the plants more ofter are not native, demonstrating the necessity to continue works to systematize this knowledge and return to community, throughout a Environmental Educational Program, searching to valorize folk knowledge.

**Keywords:** Medicinal plants, Atlantic Rain forest, Ethnobotany, Diversity.



**ISMAP0206 - Participatory research of species from Cinchona genera, other antimalarial and biocidal species in 7 Indigenous communities of the Manu Biosphere Reserve.**

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**Introduction**

The present research work identifies species of Cinchona genera, other antimalarial and biocidal species of medicinal use by indigenous people of Manu Biosphere Reserve. The area of study comprises province of Manu and Kosñipata, with participation of 07 indigenous communities of the region. The research study was developed between years 2001-2002

This study research exemplifies a model to elaborate a proposal for sustainable management of biodiversity and natural resources, integrating the indigenous and scientific knowledge.

**General Objective**

- To implement a research plan for the identification of new species of flora that contributes to the cure of malaria with participation of indigenous communities of Manu Biosphere Reserve

**Specific Objectives**

- To evaluate the status of natural populations of Cinchona genera, other antimalarial and biocidal species.
- To train indigenous communities in techniques or methods to evaluate flora medicinal species
- To sensitize indigenous communities on the importance of medicinal resources conservation

**Methodology**

The research study comprised the identification of flora species, their medicinal use by indigenous communities through interviews and ethnobotanical questionnaires to the local communities; inventory of medicinal species, abundance and tree diameters, associated vegetation; the proper botanical identification; the evaluation and establishment of monitoring plots of identified species; determination of current biologic status.

**Outcomes:**

- Natural populations of *Cinchona pubescens* Vahl, known as "cascarilla roja", identified in the study area.
- 07 species of flora used as antimalarials for the indigenous population and 04 species used as repellent o biocidal, identified
- Ecosystems where these species grow and present state of natural populations, evaluated
- Sistematization of results reached, shared within indigenous communities
- Indigenous communities motivated and organized for the conservation and management of natural resources and aware on value of traditional knowledge and culture
- Other organizations motivated to support indigenous communities in conservation and sustainable management of natural resources efforts

**Conclusions**

- Species from botanical families like, Rubiaceae, Arecaceae, Solanaceae, Fabaceae are some of the major findings of the research
- At present Indigenous communities are still using these identified species for malaria treatment and control of vectors
- Scientific knowledge can help to find the way to get new natural drugs against malaria, based in traditional knowledge of indigenous people.
- Based in the research performed, indigenous communities have formulated a plan for conservation and sustainable management of Cinchona, other antimalarial and biocidal species in the Biosphere Reserve

**Keywords:** antimalarial and biocidal species, indigenous knowledge, ethnobotany, biodiversity and genetic resources conservation

**Sponsor:** PROMANU - European Union Community



**ISMAP0350 - A Phytotherapy Program for public health in Brazil - Campinas, SP.**

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In Campinas, São Paulo (Brazil), the city hall's Phytotherapy Program has been going on for 13 years. It started in 1991 in a health unit in Joaquim Egídio, a rural area, which disposes of a garden containing medicinal plants. In 1993, the first phytotherapies were obtained at CPQBA-UNICAMP and used with good results. Professionals learned Phytotherapy and the medicines were prescribed by physicians. We have now 12 standardized phytomedicines: Aloe vera, Arnica montana, Calendula officinalis and Hamamelis virginiana, as lotion and gel for external treatments; Chamomilla recutita, Maytenus ilicifolia, Passiflora alata and Malva sylvestris (topic) as tea; Ginkgo biloba and Hypericum perforatum medicines (dry extracts); and the syrup of Mykania laevigata. The phytomedicines were standardized and manipulated according to Pharmacopoeia (a manual of application and use is available). The access to the phytomedicines is also available to homecare and to family health programs in Campinas. Phytotherapy was legalized in Campinas, Brazil, under the law 13/01 of 2001.

Methodology: the clinical characteristics of the pathologies were described in the clinical cards. The evaluation consisted in quantifying the cases of CURE (referred by the patient or from clinical evidences), SUSPENSION and ABANDONMENT. The results obtained presented approximately 60 to 70% of cure.

Therefore Phytotherapy was incorporated in the therapeutic arsenal of health units. It started to be a part of routine in health services, providing satisfaction from the good results it obtained. We believe it is an effective therapeutic option besides being economically viable for public health.

**Keywords:** Phytotherapy; Public Health; social program.



**ISMAP0344 - The use of Phytomedicines by Public Health Services in Brazil (for external treatments).**

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The use of phytomedicines are taking place at 4 public health centres in Campinas, Brazil (A, B, C and D), prescribed by physicians and supervised by trained nurses. The access to these phytotherapies is also available to homecare and family health programs in Campinas.

The following phytomedicines for external use treatments have been in use: Gel and cream of *Aloe vera* at 25% for secondary skin lesions from burns; *Calendula officinalis* at 5% for skin lesions in general (wounds infected or not-infected, eczema, milk crust, dermatitis atopica, fissures of mamma, ulcers and acne); *Arnica montana* at 3% for bruises, muscular pain, joint pain, local inflammations, bruises; and *Hamamelis virginiana* at 5% for varicose ulcers, wounds (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> degrees), phlebitis, hemorrhoids, dermatitis, and exudative wounds. Through medical reports, the clinical characteristics of the lesions were described at the beginning and throughout the treatment: causing agent, location, extension and depth; presence of bubbles, erythema or necrosis, secretion and edema. The evaluation consisted in quantifying the cases of CURE (referred by the patient or from clinical evidences), SUSPENSION and ABANDONMENT.

In unit A *Calendula* and *Aloe* were applied on 30 patients for one year, starting on May/1998; in unit B *Hamamelis*, *Calendula*, *Arnica* and *Aloe* were used on a total number of 50 patients along 2 years; unit C carried out a evaluation from July/1998 to December/1999 with 72 cases of *Calendula* and *Arnica*; and in unit D 100 patients from January/99 to January/2000 who made use of *Arnica*, *Aloe*, and *Calendula* were evaluated.

The results obtained were the following: in unit (A) *Calendula*: among 19 patients, 79% presented cure, 16% abandon, 5% suspension; *Aloe vera*: among 11 patients, 73% presented cure and 27% abandon. In unit (B), from 50 patients, 77% presented cure. In unit (C), from 62 cases of *Calendula*: 76% presented cure, 21% abandon and 3% suspension of the use; and from 10 cases of *Arnica*: 80% presented cure and 20% abandon. In unit (D), 20 cases of *Arnica*: 85% presented cure and 15% abandon; from 25 cases of *Aloe vera*: 84% presented cure and 16% abandon; and from 55 cases of *Calendula*: 85,5% presented cure, 11% abandon and 3,5% suspension.

It was observed that there was a significant improvement in several of the treated cases, which evidenced the potential therapeutic use of phytotherapy, incorporated in the therapeutic arsenal of the units. Therefore, Phytotherapy started to be a part of the routine in some services, providing satisfaction from its positive results. We believe it is an effective therapeutic option besides being economically viable for public health.

*Obs:1- The phytomedicines listed are standardized and manipulated according to Pharmacopoeia.*



**ISMAP0379 - Therapeutic/toxic categorization of medicinal plants used in Maquiné  
– Rio Grande do Sul, Brazil.**

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In Rio Grande do Sul, the southernmost state of Brazil, the use of medicinal plants is especially common in rural areas, where the primary health care is particularly inadequate. A remarkable grass root health movement known as "Farmácias Caseiras Comunitárias" (FCC, "Community home pharmacies"), is supported by the Rural Female Workers Association as well by NGOs associated with the Catholic Church. Currently the FCCs are present in 23 municipalities of Rio Grande do Sul. All FCCs follow the "Manual das Bruxinhas de Deus" ("God's witches manual") for preparing the remedies, which include 55 tinctures, 25 elixirs and 8 ointments. In order to support the utilization of medicinal plant at FCCs, this work aimed to categorize the used medicinal plants concerning its therapeutic/toxic properties, in the lines of the Caribbean TRAMIL Project. Species were botanically identified, and a thorough review was made with appropriate scientific literature (Nápralert, WHO monographs, etc). Based upon chemical and pharmacological data available plants were classified as follows: A - recommended, B - need more studies, C - toxic. The results show that 25.4% of the species can be categorized as A, 47.5% as B, 22% as A/B and 8.5% as C. The C category was given to systemic use of *Aristolochia triangularis* Cham., *Chenopodium ambrosioides* L., *Luehea divaricata* Mart., as well as for the topic use of *Sambucus australis* Cham. & Schldl. and *Symphytum officinale* L.. The results were organized in a folder for sharing the results with the community. Meetings were realized with FCC leaders, the NGO ANAMA (working with sustainable development), and the university results were discussed. The possibility of substituting the banned tinctures internally used is being discussed. This experience is useful for rationalizing the use of medicinal plants in established systems of traditional medicine in primary health care.

**Keywords:** Medicinal plants, Etnopharmacology, therapeutic/toxic categorization

**Financial support:** CNPq



# Biological Activities



**ISMAP0307 - Antiedematogenic and antinociceptive activities of *Bauhinia forficata*.**

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Several species of the *Bauhinia* genus have been investigated phytochemically and pharmacologically. *B. manca*, *B. candicans*, *B. uruguaensis*, *B. purpurea*, *B. forficata* and *B. splend* were recorded to have medicinal values for containing lactones, flavonoids, terpenoids, steroids, tannins and quinones. The objective of the present study was to evaluate the antiedematogenic, antinociceptive activities as well as ulcerogenic effects of crude aqueous extract of *B. forficata* callus and aerial parts. *B. forficata* crude extracts (5, 20 and 50 mg/Kg) were administered orally on male Wistar rats (200g), one hour before the experiments. The antiedematogenic activity was evaluated on carrageenin-induced paw edema in rats (0,1 mL of solution in 1,0 %), measured by Plethysmometer (Ugo Basile), antinociceptive activity was evaluated using Hargreaves test (Ugo Basile). For the ulcerogenic effects evaluation, crude extracts were administered orally to rats 24 h and an extra dose 1 h before the test. The ulcerogenic effects were determined by direct observation of the gastric mucous, no antiedematogenic effects were observed after administration of the *B. forficata* callus extracts. However, the administration of extract (5 mg/Kg) of *B. forficata* aerial parts (AP) showed significant antiedematogenic activity 2h after the administration reducing the edema volume (AP =  $1,95 \pm 0,1$ ) when compared with saline control (sal =  $2,26 \pm 0,13$ ;  $p < 0,05$ ), 20 mg/Kg (AP =  $1,81 \pm 0,04$ ; sal =  $2,26 \pm 0,13$ ;  $p < 0,01$ ) and 50 mg/Kg (PA =  $1,84 \pm 0,05$ ; sal =  $2,26 \pm 0,12$ ;  $p < 0,05$ ). The administration of the extracts of *B. forficata* callus and aerial parts did not induce ulcerogenic effects in the different doses tested when compared with control (indomethacin-induced ulcer). The use of the AP extracts 20 mg (1h) exhibited antinociceptive effect when compared with control (AP =  $12,15 \pm 2,1$ ; sal =  $6,75 \pm 0,39$ ;  $p < 0,05$ ), though it was not observed with the other doses and periods evaluated. The administration of different doses of crude extract of aerial parts of *B. forficata* showed significant antiedematogenic activity in the course of 4 hours. The administration of *B. forficata* extracts seems did not induce aggressive effects to the gastrointestinal tract.

**Keywords:** *Bauhinia forficata*, antinociceptive, antiedematogenic, activities.

**Sponsors:** Capes, Unaerp.



**ISMAP0189 - Antilisterial activity of essential oils isolated from *Thymbra capitata* and *Origanum* spp.**

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**Aims:** *Listeria monocytogenes* is a foodborne pathogen and the use of essential oils isolated from aromatic plants can constitute an alternative method to the use of antibiotic and other chemicals agents in the control of the pathogen. **Methods:** The oils were isolated from fresh material by hydrodistillation, for 4 hours, using a Clevenger-type apparatus and analysed by gas chromatography and gas chromatography coupled to mass spectrometry. The antilisterial activity of essential oils *Thymbra capitata* and *Origanum* spp were tested against 41 strains of *L. monocytogenes* isolated from food and other sources using the disc agar diffusion technique. Chloramphenicol and water were used as positive and negative control, respectively. **Results:** The oils of *Thymbra capitata* were mainly constituted by one component, carvacrol (78.6%), while in the oils of *Origanum* spp three components constituted 69.2% of the oils. These components were thymol (32.6%),  $\gamma$ -terpinene (25.9%) and p-cymene (10.7%). Both essential oils demonstrated antilisterial activity. However the essential oil of *Thymbra capitata* had a significantly higher activity ( $P < 0.05$ ) against *L. monocytogenes* strains. The activity of *Origanum* spp essential oil was similar ( $P > 0.05$ ) to chloramphenicol. No significant differences ( $P > 0.05$ ) in *L. monocytogenes* susceptibilities to the essential oils tested were registered. **Conclusions:** The use of *Thymbra capitata* and *Origanum* spp essential oils can constitute a powerful tool in the control of *L. monocytogenes* in food and other industries.

**Keywords:** *Listeria monocytogenes*, essential oils



**ISMAP0051 - Antimicrobial activities of fractions obtained from the stem bark of  
*Buchholzia coriacea*.**

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The crude methanol extract of the stem bark of *Buchholzia coriacea* exhibited intrinsic in vitro antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and a *klebsiella spp*, a yeast, *Candida albicans* and a mould, *Aspegillus flavus*. This study reports the in vitro antimicrobial properties of four fractions obtained by partitioning the crude methanol extract based on polarity. Fractions were tested at concentrations of 50mg/ml and 1000mg/ml respectively. The degree of susceptibility of the microorganisms to the fractions varied. The ethyl acetate fraction showed the highest antibacterial activity at 1000mg/ml while the chloroform fraction displayed the highest antifungal activity at 1000mg/ml. Results were comparable to the activity of ampicillin and tioconazole. Thin layer chromatographic analysis of these fractions indicated the presence of cyanogenetic glycosides except in the hexane fraction. The plant may be a potent lead for antimicrobial drug discovery.

**Keywords:** *Buchholzia coriacea*, antibacterial, antifungal, Chromatography.



**ISMAP0221 - Antimicrobial properties of *Arctium lappa* extracts.**

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Infectious diseases still remain one important cause of death in the world and the spreading of multidrug resistant strains of microorganisms has added a dramatic characteristic in this situation. The researches are searching for novel sources of molecules that present this capacity. This study is part of the search of antimicrobial agents from plant extracts, by *in vitro* determination of the minimum inhibitory concentration (MIC). The antimicrobial activity of hydroalcoholic extract of *Arctium lappa* (Bard Tint); root (ART) and leaves hydroalcoholic extracts (AFT), were assayed to *M. tuberculosis* H37Rv (ATCC 27294), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228) and *Candida albicans* (ATCC 64548). The antimicrobial activity was done in microdilution plates inoculating plant extracts and strains to be tested. The antimycobacterial activity was assayed using microdilution method with Alamar blue oxidation-reduction dye. The bacterial strains in concentrations of  $1 \times 10^5$  cells/mL was incubated for 24 hours at 37°C in Brain Heart Infusion medium or for yeast assay in RPMI 1640 medium according NCCLS method, standard M27-A. The antimycobacterial activity obtained to *M. tuberculosis* H37Rv for Bard Tint, ART and AFT extracts were 2000, 4000 and 2000 µg/mL respectively. The MICs for Bard Tint were the same to the strains *S. aureus* and *S. epidermidis* with values of 250 µg/mL. In front of these strains, AFT and ART extracts exhibited MICs of 125 and 62,5 µg/mL, respectively. The extract Bard Tint when tested to *C. albicans* showed MIC of 250 µg/mL. Our results showed that *A. lappa* extracts presents antimicrobial activity that was more relevant to bacterial strains and *C. albicans*. The extracts tested presented lower antimycobacterial activity. These results indicated that further studies should identify fractions with antimicrobial activity.

**Keywords:** *Arctium lappa*, antimicrobial activity, *Mycobacterium tuberculosis*, *Candida albicans*  
**Support:** UNAERP



**ISMAP0254 - Antimycobacterial activity of extracts of *Curcuma longa* obtained via supercritical fluid extraction.**

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There has been an increase of *Mycobacterium tuberculosis* strains that are resistant to the current anti-TB agents, mainly through acquired resistance by therapeutic failure. This fact has underscored the need of a quick development of antimycobacterial drugs that are more effective than those currently in use. Screening of plant extracts for natural antimycobacterial is one way to find new therapies. The substances extracted from turmeric (*Curcuma longa* Linneu) have been used for hundred years to impart colour to foods, medicinal preparations, and related products. The aim of this study was determine the antimycobacterial activity of turmeric using the tetrazolium microplate assay (TEMA). A total of 8 plant extracts were evaluated. The extracts were obtained by supercritical fluid extraction using a mixture of ethanol and isopropyl alcohol as cosolvent. The extraction temperature was 30 °C and pressures of 200 and 300 bar were used. The Minimal Inhibitory Concentration (MIC) was 32µg/mL and were comparable to those obtained with the Microplate Alamar Blue Assay (MABA); one extract had a MIC of 64 µg/mL. The turmeric extract is an oleoresin formed by a light (volatile oil) fraction and a heavy fraction of a yellowish-brown color. The compounds responsible for the yellow color are the curcumin (1,7 bis (4-hydroxy-3-methoxyfenil)-1,6-heptadiene-3,5-diona) and two curcuminoids: demethoxy-curcumin (DMC) and bis-demethoxy-curcumin (BDMC). The light fraction contained as the main compounds: ar-turmerone, (Z)-γ-atlantone, (E)-γ-atlantone. The MIC results indicated significant antimycobacterial activity of the turmeric extracts and suggest that they may serve as sources for compounds with therapeutic potential, but further studies should identify the active compounds.

**Keywords:** Antimycobacterial activity, *Curcuma longa*, MTT, supercritical fluid extraction.

**Sponsor:** FAPESP



**ISMAP0255 - Antimycobacterial activity of *Byrsonima* spp. leaves extracts.**

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Tuberculosis still remains an important public health problem worldwide, accounting for 8 million new cases per year. The tuberculosis agent *Mycobacterium tuberculosis* kills almost 3 millions of people per year around the world. Despite the improvements of the chemotherapeutic, tuberculosis is severely affected by the development of multi-drug resistant *M. tuberculosis* strains. Therefore, the search for new drugs with antimycobacterial activities from natural or synthetic compounds is strongly recommended, especially in developing countries. The search for active compounds from the huge Brazilian flora is a central point of our researches. *Byrsonima* species (Malpighiaceae) are used in Brazilian folk medicine for the treatment of diseases related mainly to gastric disorders, diarrhea and infections. We investigated the antimycobacterial activity of chloroformic and methanolic extracts of *B. crassa* leaves. The analytical method used was the Microplate Alamar Blue assay (MABA) that determines the Minimal Inhibitory Concentration (MIC) of extract that kills mycobacteria in vitro. The MIC of methanolic extract of *B. crassa* was 1000 µg/mL and the chloroformic extracts was 62,5 µg/mL. Phytochemical analyses of apolar extracts allowed to identify lupeol, α-amirine, β-amirine and their acetates, as well as α-amirenone. Our results suggest that the terpenoids from *Byrsonima crassa* leaves can be the responsible compound to the observed antimycobacterial activities. The next step will be evaluate the activities of an enriched terpenoid fraction against *M. tuberculosis*.

**Keywords:** Antimycobacterial activity, *Byrsonima* spp., Alamar Blue, *Mycobacterium tuberculosis*  
**Sponsor:** FAPESP



**ISMAP0296 - Antiophidic properties of the Vegetal Extract of *Mikania glomerata* and Partially Isolated Fractions.**

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Nowadays several plants with possible antiophidic activities have been studied for new natural compounds which may complement the current serotherapy. The *Mikania glomerata*, species which is commonly known as guaco, is commonly used in the treatment of several pathologies and also against the ophidic poisoning. This study seeks to characterize and to isolate the substances of the different vegetal extracts of *M. glomerata* (leaves, roots and stems) responsible for the antiophidic effect against snakes of the gender *Bothrops* and *Crotalus*. The tested enzymatic and pharmacological assays were: anti-phospholipasic activity in plates with gel which is made up of agar, egg yolk and human blood; anti-clotting on the human plasma thus preventing the formation of clots through the venom, Anti-hemorrhagic in camundongos by means of intradermal administration of the venom in the dorsal region; anti-edema activity in the camundongo's paw, as well as interaction studies using electrophoresis in polyacrilamide gel with desnaturating agents (SDS-PAGE). In all assays the venom was pre-incubated with the plant extracts for 30 minutes at 37°C. The vegetal material was collected from the Collection of Medicinal Plants of UNAERP. The extracts were initially prepared from fresh material by using hot water and were triturated in a blender. 24 hours later such material was filtered and lyophilized. For the preparation of the dry material extracts, such were collected on the same day and submitted to drying process in a circulating hot-air heater, then they were ground and submitted to extraction, according to previous proceeding. All extracts were submitted to successive partition with hexane and EtOAc. The roots aqueous extracts were also submitted to purification in chromatographic column by using C-18, as stationary phase, and initial elution with water followed by methanol. The results demonstrate that the fresh extracts of the leaf, root and stem inhibited 100% of the phospholipasic activity of the *Crotalus d. terrificus* venom; however they did not present inhibiting activity of the *Bothrops jararacussu* venom. Among the dry extracts, only the root presented inhibiting activity against the *C. d. terrificus* venom. In the anti-clotting assay the dried-root extract inhibited 100% of the *B. jararacussu* venom and the dried-stem extract also inhibited 100% of the *B. neuwiedi* venom and 50% of the *B. moojeni* venom, while the fresh-stem extract inhibited 100% of both. The dried-root extract were also proven capable to inhibit between 40% and 50% of the hemorrhagic activity of both the *B. moojeni* and *B. alternatus* venom respectively; however the fresh-root and stem extracts inhibited 100% of the *B. jararacussu* and *B. moojeni* venom. The tested extracts were poorly efficient in inhibiting the edema induced by *C.d. terrificus* and *B. jararacussu* venom. As for the purified fractions of the dried extracts, only the fractions of the aqueous root extracts purified in column of reversed phase C-18 as well as the aqueous stage obtained from the root aqueous extract partition presented anti-phospholipasic activity of 50% on the *C. durissus terrificus* venom. Therefore the extract of *M. glomerata* is a rich source of active compounds with antiophidic properties, and in the future it may help in alternative treatment to snakebite poison.

**Keywords:** Antiophidic plants, *Mikania glomerata*, snakes venom, pharmacological bioassays  
**Financial support:** FAPESP and UNAERP.



**ISMAP0137 - Antioxidant capacity of *Caryocar brasiliense* Cambess leaves and internal mesocarp extracts.**

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Free radicals are formed throughout cellular metabolism. As fairly unstable and highly reactive substances, they are able to cause oxidation and sometimes-irreversible damage to several human cells, compromising their function. Cumulative evidences are connecting oxidative stress caused by free radical generation with several degenerative disorders, ranging from premature aging, neutrophil-mediated inflammatory processes, neuronal diseases through cancer. However, although produced at high levels, most of the generated free radicals can be inactivated by endogeneous and/or exogenous antioxidants. The Brazilian plant *Caryocar brasiliense* Cambess, known by the popular name *pequizeiro*, is a typical tree that grows abundantly in Brazil central area, producing *pequi*, its exotic fruit, which is fairly consumed by the local population. Its high level of nutritional content, particularly vitamin A, proteins, and lipids has already been reported. The aim of this work was to investigate whether extracts prepared from leaves and fruit of *pequizeiro* could have antioxidant potential. To achieve this, we have prepared extracts from its leaves and internal mesocarp and assayed them using the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH<sup>•</sup>) and phosphomolybdenum complex chemical methods, in which increasing concentrations of the extracts (10<sup>-4</sup> – 10 mg/ml) were tested. The antioxidant activities of rutin and ascorbic acid, two compounds largely known by their strong antioxidant effects, were also investigated under similar conditions and have been used as controls. The capacity of the *pequizeiro* extracts in reducing in 50% the initial DPPH<sup>•</sup> concentration, expressed as Efficient Concentration (EC<sub>50</sub>), was 0.054 and 0.459 mg/ml, respectively, for the leaves and mesocarp, while 0.056 and 0.053 mg/ml were the values obtained for ascorbic acid and rutin. In the phosphomolybdenum assay, the results obtained for the leaves, mesocarp, ascorbic acid, and rutin were 0.43, 0.11, 1.0, and 0.32; respectively. These results suggest that this plant has, although variable depending on the part evaluated and the methodology used, an unexpected antioxidant potential, which should be more explored to define the compounds responsible for this activity.

**Keywords:** *Caryocar brasiliense*, Caryocaraceae, antioxidant activity.



**ISMAP0128 - Antioxidant capacity of edible cruciferous from different agricultural crops employing the phosphomolybdenum method.**

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The Cruciferae family has many species important for the regular human diet as they provide important antioxidant components such as vitamins, minerals, fibers, flavonoids, carotenoids amongst other phytochemicals. As they are routinely present as part of the Brazilian folk meals such, and protection against several diseases has been attributed to the various antioxidants phytonutrients contained in these vegetables, it is important to evaluate their antioxidant potential. Thus, in the present work, the antioxidant capability of the aqueous extracts prepared from white cabbage (*Brassica oleracea* var. *capitata* L.), kale (*Brassica oleracea* L. var. *acephala* D.C.), cauliflower (*Brassica oleracea* L. var. *botrytis* L. subv. *cauliflora*), broccoli (*Brassica oleracea* L. var. *botrytis* L. subv. *cymosa*), *Nasturtium officinalis* (water-cress) and radish (*Raphanus sativus radiculat* L.), obtained from conventional, hydroponic, and organic agricultural procedures whereas possible, was investigated *in vitro* by the chemical method of phosphomolybdenum complex, which is based on the reduction of Mo<sup>+6</sup> to Mo<sup>+5</sup> by antioxidants present in the sample with subsequent formation of a green phosphate-Mo<sup>+5</sup> complex at acidic pH. The antioxidant activity of ascorbic acid (200mg/ml), assayed under similar conditions, was normalized at 1.00 and was used for comparing the results. The most significant antioxidant capacity found was for the broccoli inflorescences extract obtained from organic crops, which was 7.13±0.24 fold higher than that of the control, followed by the organic white cabbage (6.06±0.02), organic kale (5.49±0.07), organic cauliflower (5.47±0.07), conventional cauliflower stems (5.36±0.10), conventional broccoli stems and leaves (5.3±0.08), conventional cauliflower (5.16±0.07), organic radish (4.94±0.13), hydroponic kale (4.51±0.17), conventional broccoli inflorescences (4.2±0.06), conventional kale (3.87±0.05), and hydroponic water-cress (2.34±0.06) extracts. These results have shown that (1) all Cruciferae extracts evaluated presented significant antioxidant capacity when compared to the control; (2) the antioxidant capacity of the various Cruciferae species assayed varied according to the agricultural technique by which the vegetables are grown (organic > hydroponic > conventional), and also (3) to the parts of the vegetables tested within the same crop type (conventional broccoli stems and leaves > conventional broccoli inflorescences). Although the antioxidant potential of the most Cruciferae vegetables assayed here has been already reported, to our acknowledge, this is the first report describing such activity for water-cress and radish. Although the antioxidant results herein presented and discussed must be interpreted with caution as a list of factors such as soil composition, climate or culture technology can influence the vegetables' quality, they favor the beneficial effects of consuming Cruciferae vegetables, particularly those obtained from organic culture, including their advantage of having less fertilizer and toxic residues associated with high levels of solid nutrients.

**Keywords:** Cruciferae, antioxidant, phosphomolybdenum complex, agricultural type



**ISMAP0303 - Aqueous extract of *Bauhinia forficata* aerial parts inhibits the enzymatic and pharmacologic activities of serpents' poisons.**

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The medicinal plants and their derived consisted for a long time the base of therapeutics. It is believed that 80 % of world population uses the plants as first therapeutic resource like antiophidian activities of plants that have been studied to complement the current serumtherapy. *Bauhinia forficata*, belongs to a well-known popularly leguminous family like cow paw and used in the Brazilian popular medicine as diuretic agent, hypoglycemic, tonic, purifier, to elephantiasis combat, and in glucosic reduction. This study seeks to demonstrate the possible antiophidian effect of aqueous extract of *Bauhinia forficata* aerial parts, through several assays: Anti- Phosfolipasica Activities, Anti-coagulable, Anti-Hemorrhagic, Anti-edema and Interaction of the aqueous extract of *Bauhinia forficata* with poisons of *B. jararacussu* and *C. d. terrificus*, incubation for 30 minutes, 37°C and after poliacrylamide gel eletrophoresis in presence of denaturing agents (Biondo, A.M. Pereira, S. Marcussi, P.S. Pereira, S.C. França, A.M. Soares, Biochimie 85 (2003) 1017-1025). The results show that aqueous extract of *Bauhinia forficata* aerial parts (50ug/ul) didn't inhibit the phosfolipasica activity of *Crotalus durissus terrificus* poisons (10ug/ul), *Bothrops alternatus* (10ug/ul), *B. jararacussu* (5ug/ul), *B. moojeni* (2ug/ul) and *B. Neuwiedi* (2ug/ul). The coagulable activity on human plasma induced by *B. jararacussu* poison (2ug/ul)\* with time of 1:20 minutes, in relation of 1/10 the extracts had inhibited in average .6 minutes, while that in relation of 1/50 it inhibited with average time of 8 minutes and was effectively inhibited by extract of *Bauhinia* when incubated for one hour in relation of 1/100, 1/200, 1/300, 1/400, but it didn't inhibit the *B. moojeni* poison. The extract didn't demonstrate effectiveness in the inhibition of hemorrhagic activity of *B. Neuwiedi* (10ug/ul), *B. Moojeni* (10ug/ul), *Bothrops alternatus* (10ug/ul). For the assay anti-edema measured in time of 30, 60 and 80 minutes with application of the extract of *Bauhinia* (200ug/ul) only *C. d. terrificus* poison (10ug/ul) was inhibited. The interaction of aqueous extract with *B. jararacussu* and *C. d. terrificus* poisons demonstrated that there was not any degradation of poisons proteins.

**Keywords:** Antiophidian Plants, *Bauhinia forficata*, serpents' poisons, pharmacologic bioassays.

**Sponsor:** FAPESP and UNAERP.



**ISMAP0174 - Biological activity of 4-nerolidylcatechol isolated from *Pothomorphe peltata* (Piperaceae).**

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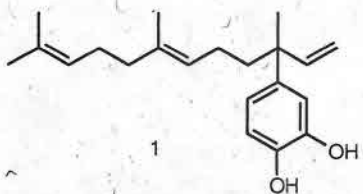
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*Pothomorphe peltata* (L.) Miquel (Piperaceae) which is known in Brazil by the popular names caapeba, pariparoba, caapeba-do-Norte, among other names, is a small bush or treelet 1 to 2 m in height and is distributed in the Americas. It is used in traditional medicine in the treatment of malária, inflammation, ulcers, hepatitis and leishmaniasis in different locations in the Amazon Region. In what follows, we describe the results obtained from the screening of extracts of this plant for biological activity during a phytochemical study on the roots of *P. peltata*. The phytochemical work was bioguided using lethality to larvae of *Artemia franciscana* and also by the characteristic phenol reaction on thin-layer chromatography (TLC) plates developed with FeCl<sub>3</sub> (blue spot). 4-nerolidylcatechol (**1**) was obtained from the ethanol root extract and identified using spectroscopic methods and comparison with literature data. This substance was shown to be present in leaf and fruit extracts by comparative TLC. Compound **1** presented activity *in vitro* against *A. franciscana* larvae (LC<sub>50</sub> 8 ± 1 µg / mL), as well as larvicidal activity *in vitro* to the hemorrhagic dengue fever vector *A. aegypti* (LC<sub>50</sub> 26 ± 3 µg / mL) and inhibited *in vitro* growth of the human malária parasite *Plasmodium falciparum* (LC<sub>50</sub> 9 ± 4 µg / mL) (Pinto, 2002). An earlier study showed that this substance presents cytotoxicity to KB cells (Mongelli *et al.*, 1999), however, cytotoxic activity in other tumor cell lines *in vitro* has not been shown previously. We therefore tested compound **1** in human leukemia (CEM and HL-60), human breast tumor (MCF-7), human colon tumor (HCT-8) and murine melanoma cell lines provided by the Mercy Children's Hospital (EUA). The cells were cultivated in RPMI 1640 medium supplemented with 10 % bovine fetal serum and 1 % antibiotics in an incubator at 37 °C and atmosphere containing 5% CO<sub>2</sub>. The samples were diluted in DMSO at a stock concentration of 5 mg / mL and tested in triplicate. For analysis of cytotoxicity, the MTT method was used (3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2-H-bromide tetrazolium salt]. The IC<sub>50</sub> and standard deviation was calculated from the non-linear regression using the program *GraphPad Prism* and compound **1** was shown to be highly active in general in all of the tumor cell lines, having the following IC<sub>50</sub> values: CEM 6.78 ± 0.06, HL-60 5.06 ± 0.53, MCF-7 7.43 ± 0.28, HCT-8 5.40 ± 0.26 and murine melanoma 6.07 ± 0.10.

**Keywords:** 4-nerolidylcatechol, tumor cell lines, larvicidal activity, *Plasmodium falciparum*

**Sponsor:** FAPEAM



Mongelli, E.; Romano, A.; Desmarchelier, C.; Coussio, J.; Ciccía, A, G. Cytotoxic 4-nerolidylcatechol from *Pothomorphe peltata* Inhibits Topoisomerase I Activity. *Planta Medica*, v. 65, n.4, p. 376-78, may. 1999.

Pinto, A.C.S. *Estudo Fitoquímico e Biológico de Pothomorphe peltata* (L.) Miquel (Piperaceae). 2002. Dissertação (Mestrado) - Universidade Federal do Amazonas, UFAM, Manaus, 156p., 2002.



# ISMAP0257 - Comparative effect of a phytotherapeutic product as cicatrizing.

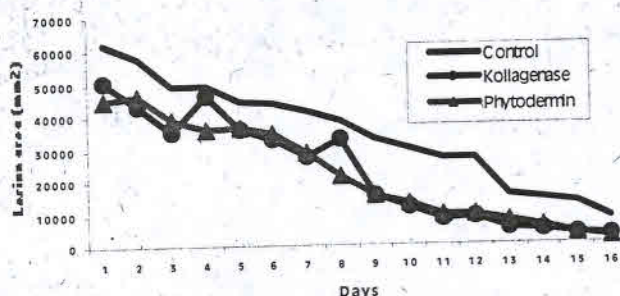
M. B. Silva<sup>1</sup>, C. Alberto-Silva<sup>2</sup>, L. C. C. Malaquias<sup>2</sup>, M. M. Sarandy<sup>1</sup>, F. R. Antunes<sup>2</sup> and M. C. M. Freire<sup>2</sup>

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The use of the phytotherapy is very old, and now it is expanding again all over the world. The new phytotherapeutic products needs to have its effects assured with base in the scientific experimentation, as well as toxicological studies, what justifies the use of biological experiments, with the test of several models. In this work the results obtained with the cicatrization utilizing a product obtained of a *Brassica* sp. extract (Phytodermin<sup>®</sup>) were compared to a standard industrialized product (Kollagenase<sup>®</sup>), and to treatment without control. Each treatment had three replications. The tests were accomplished in nine animals (*Cavia cobaya*), weighing around 500 to 600 grams. The hair of the lateral right of the animals were removed mechanically. Soon after it was made a surgical incision of approximately 2,0 cm<sup>2</sup> of diameter. The animals received a local anesthesia with 0,5 mL of lidocain 2% + fenilferin 1:1000. The animals were treated with the ointments, applied twice a day until the end of the cicatrization process. The cicatrization was accompanied being measured the length and the width of the wounds daily. The cicatrization of the wounds happened about twenty days after the begin of the experiment. In the control treatment (only used of the wash of the wound with water) was observed a delay of cicatrization process. To the data of cicatrization evolution, it was possible to adjust one curve in each treatment, with aid of the lineal model. It was compared the values of the rates estimated in each treatment by the Tukey (p=0,05) test. The cicatrization rates obtained with use of Phytodermin<sup>®</sup> and with Kollagenase<sup>®</sup> didn't differ to each other. Both rates were larger than the rate observed in the controls treatment.

**Keywords:** phytotherapy, *Cavia cobaya*, plant extract  
**Sponsor:** FAPEMIG



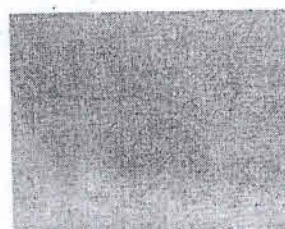
**Figure 1.** Evolution of cicatrization process in wounds treated with different products

**Figure 2.** Statistical comparison between cicatrization rates observed on different treatments

Treatment	Cicatrization rate (mm <sup>2</sup> /day)
Kollagenase <sup>®</sup>	6866,67 a
Phytodermin <sup>®</sup>	6461,33 a
Control	3456,67 b



before the treatment



after the treatment



**ISMAP0298 - Effects of essential oils from medicinal plants used in Brazilian folk medicine against EPEC and ETEC *Escherichia coli*.**

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Diarrhea caused by *Escherichia coli* infection is a emergent problem in both developing and developed world. Enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) are the most important among the different known serotypes in terms of total diarrhea episodes on a global scale. The diarrhea is often treated with antibacterial drugs, but this treatment is generally ineffective, due in part to the presence of drug resistant strain. In the present study, essential oils from 34 native and exotic medicinal plants traditionally used in Brazil were screened for antimicrobial activity against EPEC and ETEC *E. coli* serotypes. The oils were obtained from 40g fresh plants by hydrodistillation using Clevenger system during 3h, dried over anhydrous sodium sulfate, preserved in sealed flasks and stored at 4-6°C until analysis. The *E. coli* inoculum was prepared in nutrient broth with D.O. (optical density) similar to 0.5 of Mc Farland scale and diluted suitably. Minimal inhibitory concentration (MIC) from oils was determined by microdilution method according to Ellof (1998). The main results obtained from the bioassays are presented in Table 1. The results showed that among the 34 tested species, essential oils from 11 plants presented mainly moderate activity against the *E. coli* serotypes tested (MIC between 0,50-1,00 mg/mL). *Cymbopogon martini* and *Cyperus articulatus* were able to inhibit the strain ETEC TR441/4 at MIC between 0,25-0,50 mg/mL. Regarding to standard used in the tests, the MIC for chloranphenicol was 0,004 mg.mL<sup>-1</sup>. All remaining plants and extracts investigated presented MIC above 2.0 mg.mL<sup>-1</sup>. In conclusion, the results of the present study indicate that the essential oils obtained from 11 out of 34 plants commonly used in Brazilian folk medicine had anti-*E. coli* activity.

**Keywords:** *Escherichia coli*, essential oils, minimal inhibitory concentration  
**Sponsor:** FAPESP (03/02668-7)

**Table 1.** Antimicrobial activity from medicinal plants against EPEC and ETEC *E. coli*.

Medicinal plants	EPEC O551-1	EPEC O119	ETEC O63	ETEC TR441/4	ETEC 6/81-H5J
<i>Achyrocline satureoides</i>	M	M	M	M	M
<i>Artemisia annua</i>	M	M	M	M	M
<i>A. camphorata</i>	M	M	M	M	M
<i>Aloysia triphylla</i>	M	M	M	M	M
<i>Baccharis trimera</i>	M	M	M	M	M
<i>Cymbopogon martinii</i>	M	M	M	S	M
<i>C. winterianus</i>	M	M	M	M	M
<i>Cyperus articulatus</i>	M	M	M	S	M
<i>Mikania laevigata</i>	M	M	M	M	M
<i>Ocimum gratissimum</i>	M	M	M	M	M
<i>Origanum vulgare</i>	M	M	M	M	M

S = Strong activity – MIC between 0,25-0,50 mg/mL

M = Moderate activity – MIC between 0,50-1,00 mg/mL



**ISMAP0088 - Hepatoprotective activity of *Punica granatum* (Linn) leaves in rats.**

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The effect of various extracts like petroleum-ether (40-60° C), chloroform, methanol and water was studied on carbontetrachloride induced liver injury in rats. Hepatotoxicity was induced by administering carbontetrachloride (2 ml/kg body wt.) and olive oil (2 ml/kg body wt.) orally for 2 days. The administration of various extracts (200 mg/kg) orally was given for 4 days and along with CCl<sub>4</sub> for 2 days. Petroleum ether, chloroform and aqueous extracts produced significant ( $p < 0.01$ ) lowering of serum AST, ALT along with histopathological recovery of liver. The results were comparable to Liv-52 (5 ml/kg orally). The study shows that there was reduction in liver damage by treatment with petroleum ether, chloroform and aqueous extracts.

**Keywords:** Hepatotoxicity, serum AST, serum ALT, Liv-52.



**ISMAP0129 - Influence of the different agricultural crops upon the antioxidant capacity of the broccoli and kale extracts using the DPPH free radical method.**

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Vegetables play an important role in human diet and increased consumption of vegetables containing high levels of phytochemicals has been recommended for preventing chronic diseases related to oxidative stress in the human body. Cruciferous vegetables contain high levels of vitamins and phytochemicals that can act as antioxidants, compounds that may protect against several degenerative diseases. The aim of this work was to evaluate the antioxidant potential of ethanolic extracts from the most common edible portions of broccoli (*Brassica oleracea* var. *botrytis*) and kale (*Brassica oleracea* var. *acephala*) extracts, obtained from organic and conventional crops. Increasing concentrations of the extracts (0,01 – 10 mg/ml) were analyzed using the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH<sup>•</sup>), which has an absorption band at 518 nm that disappears upon reduction by antioxidant compounds present in the sample. The results, which have been expressed as Efficient Concentration (EC<sub>50</sub>), defined as the amount of antioxidant necessary to decrease the initial DPPH<sup>•</sup> concentration by 50%, showed that the extract of organic broccoli has the highest DPPH scavenging activity with an EC<sub>50</sub> of 0,53 mg/ml, followed by the organic kale (EC<sub>50</sub> 0,60 mg/ml), the conventional broccoli (EC<sub>50</sub> 0,62 mg/ml) and the conventional kale (EC<sub>50</sub> 0,67 mg/ml) extracts. Moreover, at the concentration of 10 mg/ml, all extracts assayed were capable of reducing more than 90% of the DPPH<sup>•</sup> present. The results herein presented have revealed, for the first time, that different types of growing Cruciferous vegetables could influence their antioxidant potential evaluated by the traditional DPPH chemical method. They also may contribute to stimulate the consume of edible plants with antioxidant power recommended regularly as they may collaborate with the free radical scavenging activity necessary to prevent the development of several diseases related to oxidative stress.

**Keywords:** Cruciferae, agricultural crop, free radicals, DPPH.



**ISMAP0317 - *Maytenus ilicifolia* Mart.: 90-day toxicological studies in rats**

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*Maytenus ilicifolia* Mart. (Celastraceae), native plant from the Tropical Atlantic Forest (Mata Atlântica, Brazil) popularly known as 'espinheira santa', is traditionally used as an efficient antiulcerogenic agent. The toxicological effects of this species were evaluated after a 90-day oral treatment in male and female rats. Experimental groups consisted of saline (received 10mL/kg/day of 0.9% saline solution), sham (no treatment) and three oral doses (100, 300 and 1000 mg/kg/day). During the experimental period, body weight and food consumption of all animals were monitored and registered. Nontoxicity was evidenced by the body weight gain especially observed in females. On the 90<sup>th</sup> day, the rats were submitted to hippocampal screening and later anesthetized for peripheral blood collection. Complete hematological studies were carried out as well as biochemical, enzymatic, lipidic and electrolytic analysis of the serum. In a sequence, the animals were euthanized and samples of organs were weighed and sent to histopathological analysis. In the view of all data obtained, the main aspect was the decrease in the female reproductive tract weight (ovaries and uteri). These findings are compatible with reports about another popular use of this species as abortifacient and contraceptive. The probable pharmacological effect is in the hypothalamus-pituitary-gonad path, already observed as a reduction in the embryonic implantation and viability in mice (Montanari et al; *Contraception* 65 (2002) 171-175).

**Sponsors:** CNPq, Fundação Herbarium de Saúde e Pesquisa.



**ISMAP0249 - Pharmacological study of *Syagrus oleracea* (Martius) Beccari and *Mauritia vinifera* Martius fruit extracts.**

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Palmae species are very interesting by the chemical and pharmacological point of view. However, the oil from *Mauritia vinifera*'s fruit has being used in the treatment of burn without any previous pharmacological studies. The fruits from *Syagrus oleracea* haven't being studied either. The target of this work was to evaluate the anti-inflammatory and analgesic activities of the ethanolic and hexanic extracts of fruits from those two Palmae species. The extracts were tested for the analgesic activity by three methodologies: writhing, tail flick and hot plate. They have being tested too for anti-inflammatory activity by formaline and ear oedema induced by croton oil methodologies. Each test group consisted of five Swiss 44 male mice that received 0.1ml of an extract solution, with doses of 100 or 400mg/kg, by *p.o.* The assays evaluated the ethanolic extracts from epicarp/mesocarp of *S. oleracea*, epicarp/mesocarp of *M. vinifera* and mesocarp/endocarp of *M. vinifera*; the hexanic extract from endosperm of *S. oleracea*; the hexanic fractions from epicarp/mesocarp of *S. oleracea* and epicarp/mesocarp of *M. vinifera*. With the doses used, only the hexanic extracts from epicarp/mesocarp and from endosperm of *S. oleracea* were significantly active. These results suggest that the compounds responsible for the analgesic and anti-inflammatory activities of *S. oleracea* are concentrated in the lipophilic extracts. For *M. vinifera*, in a general way, the mesocarp/endocarp extract had the best results for analgesic and anti-inflammatory activities, suggesting that compounds of the endocarp from the fruits are those responsible for activities (see table 1).

Extracts	Writhing (number of writhings)	Tail flick (time of reaction) (seconds)	Hot plate (time of reaction) (seconds)	Formaline (time of reaction) (seconds)	Ear oedema (difference of weight between the ears) (mg)
Hexanic fraction from epic./mesoc. of <i>S. oleracea</i> (100mg/kg)	43.75 ± 0.74	6.98 ± 0.04	16.93 ± 0.18	91.51 ± 0.89	2.90 ± 0.03
Hexanic extract from endosperm of <i>S. oleracea</i> (100mg/kg)	-----	7.00 ± 0.50	18.75 ± 0.53	78.03 ± 0.79	2.30 ± 0.24
Ethanolic extract epic./mesoc. of <i>M. vinifera</i> (400mg/kg)	22.68 ± 1.20	-----	-----	-----	-----
Ethanolic extract mesoc./endoc. of <i>M. vinifera</i> (400mg/kg)	17.00 ± 0.82	6.64 ± 0.31	19.40 ± 0.35	95.54 ± 0.87	2.16 ± 0.09
Positive control	24.71 ± 0.52	11.03 ± 0.24	29.35 ± 0.38	71.32 ± 0.78	0.35 ± 0.05
Negative control	54.14 ± 1.16	3.30 ± 0.06	12.64 ± 0.51	116.50 ± 1.14	3.21 ± 0.06

**Table 1:** Results of pharmacological tests of the active extracts from *S. oleracea* and *M. vinifera* (mean ± standard deviation, n = 5). P < 0.05 (Student T test).

**Keywords:** *Syagrus oleracea*, *Mauritia vinifera*, pharmacological studies

**Financial Support:** CAPES, FUJB, FAPERJ, CNPq, PRONEX



**ISMAP0234 - Study on the cicatrisation activity of carbohydrates of the bark of  
*Persea major* Kopp (Lauraceae).**

**L. W. P. CASTRO, I. H. BINI, A. T. B. GUIMARÃES, L.T. MARANHO, C. M. RIBAS DE OLIVEIRA**

Although the popular use of mucilages of native species in health treatment is constant, further studies are necessary in order to confirm their activity. The object of the present study is to characterize the main carbohydrates in the mucilage of the bark of *Persea major* (locally known as "pau-andrade") and the analysis of the cicatrisation activity of various fractions, *in vivo* using 3 cm incisions in 6 rats per group. Powdered bark was defatted with  $\text{CHCl}_3$ :MeOH (2:1, v/v) resulting in Fraction FCM (4.3% yield\*), and the residue was submitted to the extraction with MeOH:H<sub>2</sub>O (4:1, v/v), to give Fraction FMW (10.3% yield\*). Insoluble material was then extracted with water at 25°C, and treatment with excess ethanol (x 3. v/v) gave rise to precipitated Fraction FCW (6.15% yield). The residue of cold water extraction was treated with hot water obtaining Fraction FHW (4.8 % yield)-after treatment with 3 volumes of ethanol. To evaluate the cicatrisation activity in the rats, they were accompanied day by day with largest and length measures. The monosaccharide composition of the fractions was analyzed by GC: FCM - Rha 23.5%, Xyl 15.8%, Man 11.6%, Glc 49.1%; FMW - Rha 39.2%, Man 2.3%, Glc 58.0%; FCW - Rha 0.2%, Ara 65.6%, Xyl 34.3%. Using the t-student parametric test for dependent samples, the groups FCM, FCW, FHW were analyzed and showed a medium cicatrisation period ( $\pm$  SD) of  $14 \pm 1$ ,  $16 \pm 2$ , and  $17 \pm 4$  days, respectively, while the positive (PC) and negative controls (NC), had averages of  $17 \pm 2$  and  $19 \pm 2$  days respectively. Fraction FCM showed a significant difference in comparison to PC ( $p = 0.00$ ,  $t = -7.11$ ) and NC ( $p = 0.00$ ,  $t = -8.99$ ). Fraction FHW gave results close to those of FCM, but with a difference between the averages of PC and FCW ( $p = 0.009$  and  $t = -2.67$ ), and NC x FCW ( $p = 0.0$  and  $t = -5.59$ ). Fraction FCW showed close to the PC ( $p = 0.34$  and  $t = -0.96$ ) and clearly different to NC ( $p = -5.76$  and  $t = 0.00$ ). The group treated with FPCW had a behavior similar to PC Kamillosan®. In spite of fractions FCM and FHW having similarities when compared to the PC and NC controls, the process of cicatrisation completion of the FCM group was on the average 5 days faster than the group treated with FPHW, showing that FCM had the highest activity.

**Keywords:** Cicatrisation; carbohydrate; mucilage; *Persea major* bark.

\*yield based on the bark



**ISMAP0085 - Toxicological study of the flowers of Brazilian *Calendula officinalis* L.**

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The men have used medicinal plants for a long time. The lack of appropriate information of the toxicity of the plants can produce serious risks to the health. The *Calendula (C.) officinalis* is known to have medicinal activities, as: antiinflammatory, diuretic, hypoglicemiant, antibacteriam and stimulant of immune response, among others. It is prescribe in many therapeutic formulations. This work aimed to evaluate the toxicity of the powdered flowers of *C. officinalis*, planted in Brazil, to establish initial information of its safe use.

Obtaining the extract: The *C. officinalis* ethanolic extract (CEE) was obtained by cold maceration of 200g of powdered flowers in 1 liter of 96% ethanol alcohol for 72 hours. It was concentrated in a rotoevaporator under reduced pressure and temperature. Test of toxicity: The animals were individually acclimatized in metabolic cages. Groups of ten were daily observed and treated (0.1, 0.3 and 1.0g/Kg of CEE or 5mL/Kg of distilled water, orally) for twenty-eight days. In the 29<sup>th</sup> day the animals were sacrificed through heart puncture. The blood collection was made for blood tests and evaluation of the biochemical profile.

Were expressed as percentage of the control group. The treatment with 0.1g/Kg of CEE significantly increased the urinary volume to  $128.6 \pm 5.0\%$  (control =  $11.9 \pm 0.6\text{mL}$ ). The treatment with 1g/Kg of CEE significantly decreased the number of lymphocytes to  $82.6 \pm 5.2\%$  (control =  $80.4 \pm 2.3\%$ ) and significantly increased the number of total neutrophils to  $190.2 \pm 24.5\%$  (control =  $16.3 \pm 1.9\%$ ). The treatment with 0.1g/Kg and 0.3g/Kg of CEE significantly decreased the glycemia to  $78.3 \pm 4.7\%$  and  $71.7 \pm 5.4\%$  respectively in relation to control ( $72.2 \pm 4.7\text{mg}\%$ ). The treatment with 0.1g/kg and 0.3g/Kg of CEE significantly increased the creatinine rate to  $900 \pm 100\%$  and  $700 \pm 200\%$  respectively, in relation to control ( $0.1 \pm 0.1\text{mg/dL}$ ). The treatment with 0.1g/kg of CEE significantly decreased the total proteins rate to  $88.5 \pm 1.6\%$  (control =  $6.1 \pm 0.2\text{g}\%$ ). The treatment with 0.1g/Kg of EEC significantly decreased the albumins rate to  $91.7 \pm 2.8\%$  (control =  $3.6 \pm 0.1\text{g}\%$ ).

CEE orally administered shown a little of nephrotoxicity evidenced by the creatinine increase that can restrict the orally use of *C. officinalis* until new studies of toxicological effects were accomplished. CEE shown diuretic action that can justify its related hypotension activity. The hypoglycemiant effect of CEE could be a starting point for studies that objectives elucidate the action mechanism and the active principles so that the plant would be used in diabetics in a safe and appropriate way. Although in agreement with literature (Amirghofran *et al*, 2000), this work was not establish in safe way the CEE immunological effect.

**Keywords:** *Calendula officinalis* - toxicity, safe use, Brazil.

**Financial support:** FUNAPE/UFG- Goiânia, Brazil.



**ISMAP0277 - Toxicity acute of *Diploptropis ferruginea* Benth.**

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The medicinal plants have been constituting in an of the important therapeutic alternatives used in the search of some diseases, especially those that are related with the Central Nervous System (CNS). The *Diploptropis* genus, of the Fabaceae family, comprises about 23 species, including *Diploptropis ferruginea* Benth, and little is known about its chemistry. There are only two papers in the literature describing the isolation of quinolizidine alkaloids from *Diploptropis martiusii* and flavonoids, steroids and triterpenes from *Diploptropis purpurea*. *Diploptropis ferruginea* a tree found in Northeastern Brazil commonly know as "sucupira-preta", and it used as folk medicine to treat arthritis. The tree is widely distributed in the States of Bahia and Rio Grande do Norte, Brazil. In the present paper, we report results of the first toxicological study of the species *Diploptropis ferruginea*. The objective of this work went evaluate the toxicity acute induced by the administration acute of the extracts of the peel of the stem bark of *Diploptropis ferruginea*. Mice swiss was used, of both sexes, weighing among 20-40g, submitted to the administration of doses, progressive up to 5g/Kg orally and 2g/Kg for road intraperitoneal, the mice was observed by a period of four uninterrupted hours and accompanied for seven days, where the behaviors were observed, evolution weight, consumption of water and ration of the treated animals with extracts and of the group it controls negotiated with saline. After seven days the animals were sacrificed and the organs submitted to exam pathological anatomy. The treated mice with extract for both roads exhibited alterations behavior as activity excess, aggressiveness, tremors, irritability and excessive movements, what demonstrates that *Diploptropis ferruginea* Benth presents a compatible profile with stimulating drugs of CNS, while the treated animals with saline didn't present any alteration behavior. Significant alterations were not observed in the consumption of water, ration and us organ examined. With base in these results tests behavior they will be accomplished using other methodologies for confirmation of the stimulating effects of *Diploptropis ferruginea* on CNS, besides study of chronic toxicity in rodents and dogs

Keywords: Toxicity acute, *Diploptropis*, *Diploptropis ferruginea*



**ISMAP0308 - Triperpene isolated from *Gaylussacia brasiliensis* (spreng.) Meisn. (ericaceae): the correlationship between its antiproliferative activity and cox inhibition.**

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*Gaylussacia brasiliensis* (Spreng.) Meisn. (Ericaceae) is used in Brazilian folk medicine in the treatment of several inflammatory processes. *G. brasiliensis* crude dichlorometanic extrate (CHD), its active compound and indomethacin were evaluated on sulforhodamine B antiproliferative assay in a four-level concentration (0,25 to 250 µg/mL), for 48 h, showing a concentration dependent inhibition on every cell line. Based on experimental evidence that inhibition of COX 2 causes tumor regression, the scope of this work was to evaluate the correlationship between CHD and Compound 1 antiproliferative active and COX inhibition. The CHD *in vivo* anti-inflammatory activity was analyzed through the antiedematogenic model induced by carrageenin in rats. CHD (1000 mg/kg, po) inhibited paw edema and its maximum inhibitory effect (44%) was obtained after 5h of the carrageenin injection. Indomethacin (10 mg/kg, po) started to reduce the paw edema on the second hour after the edema induction, with a maximum inhibitory effect (64%) obtained after 4h of the injection. The systemic effect of CHD (500 mg/kg, po) over the synthesis of PGE<sub>2</sub> in the gastric mucosa of rats, with or without previous indomethacin (5 mg/kg, sc) treatment, was measured. CHD inhibited 50% of its PGE<sub>2</sub> synthesis, which was potentiated (82%) by indomethacin previous administration. To evaluate the same pharmacological activity of Compound 1 (250mg/mL), the amount of PGE<sub>2</sub> in human cancer cell lines (HT-29 - colon) was measured, for 48 h. Both Compound 1 and indomethacin reduced the synthesis of PGE<sub>2</sub> *in vitro*, at 64 and 57%, respectively. Overall, experimental data suggest that the Compound 1 antitumor mechanism is related to COX inhibition. Nowadays, the investigation involving other mechanisms of action of the Compound 1 are being carried out and include: DNA damage (common pharmacological effect of several anti-neoplastic drugs), interference with cell division, as well as antiangiogenic and proapoptotic effects.



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**Kalyankar, R.N.** - ISMAP0088 (A08-16)  
**Kaplan, M.A.C.** - ISMAP0249 (A08-19)  
**Khan, I.** - ISMAP0378 (A01-22)  
**Khan, M.T.H.** - ISMAP0022 (A04-6)  
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- Kokate, C.K. - ISMAP0042 (A05-46)  
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Krepesky, P.B. - ISMAP0046 (A03-16);  
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Krishnamurthy, R. - ISMAP0024 (A03-12);  
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Kumar, A. - ISMAP0147 (A02-6)  
Kumar, D. - ISMAP0054 (A03-8)  
Kumar, V. - ISMAP0103 (A01-28)  
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Leite, C.Q.F. - ISMAP0254 (A08-7);  
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Leme, E.E. - ISMAP0298 (A08-15)  
Leopold, J. - ISMAP0383 (A05-9);  
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Li, T.S.C. - ISMAP0373 (A03-18)  
Li, X.C. - ISMAP0069 (A04-3)  
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Lima, G.P.P. - ISMAP0198 (A04-26);  
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Lima, J. - ISMAP0104 (A05-12); ISMAP0105  
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Lima, M.G.S. - ISMAP0102 (A04-25)  
Lima, S.S. - ISMAP0226 (A04-23);  
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Lino, P.L. - ISMAP0215 (A03-21)  
Lira, M.P.S. - ISMAP0313 (A01-23)  
Lorscheiter, Rafael - ISMAP0327 (A03-51)  
Lotufo, L.V.C. - ISMAP0174 (A08-13)  
Lourenço, M.V. - ISMAP0307 (A08-3)  
Lowe, K.C. - ISMAP0079 (A04-21)  
Luz, S.C.S. - ISMAP0190 (A05-3)  
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Maass, B. - ISMAP0043 (A01-4)  
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Madan, V.K. - ISMAP0147 (A02-6)  
Magalhães, P.M. - ISMAP0110 (A03-13);  
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Maia, G.A. - ISMAP0102 (A04-25)  
Maia, J.G. - ISMAP0274 (A01-12)  
Maia, N.B. - ISMAP0349 (A05-47)  
Maiorano, V.A. - ISMAP0296 (A08-9);  
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Maranho, L.T. - ISMAP0234 (A08-20);  
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Marchese, J.A. - ISMAP0299 (A03-10);  
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Marcussi, S. - ISMAP0296 (A08-9);  
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Marques, D.A. - ISMAP0362 (A04-15);  
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Marques, M.O.M. - ISMAP0299 (A03-10);  
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Marques, V.B. - ISMAP0252 (A03-49);  
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Martins, A.L.M. - ISMAP0349 (A05-47)  
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Mathioni, S.M. - ISMAP0046 (A03-16)  
Matiucci, C. - ISMAP0253 (A03-17)  
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Mattana, R.S. - ISMAP0304 (A03-40)  
Mattos, C.A.M. - ISMAP0287 (A01-31)  
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Mazzafera, P. - ISMAP0204 (A01-32);  
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Melo, J.M. - ISMAP0264 (A03-4)  
Melo, L.Q. - ISMAP0334 (A01-40)  
Mendes, M.A. - ISMAP0311 (A01-3)  
Mendes, R.A. - ISMAP0282 (A01-25);  
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- Menut, C.** - ISMAP0033 (A05-18)  
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**Montanari Junior, I.** - ISMAP0335 (A03-31);  
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**Moraes, R.M.** - ISMAP0069 (A04-3);  
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**Moreira, R.R.D.** - ISMAP0223 (A05-41)  
**Moreschi, S.R.M.** - ISMAP0330 (A05-28)  
**Morgante, P.G.** - ISMAP0362 (A04-15);  
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**Nakashima, T.** - ISMAP0112 (A03-6)  
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**Nascimento, W.M.** - ISMAP0131 (A03-35)  
**Nemeth, E.** - ISMAP0047 (A02-16);  
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**Nery, P.S.** - ISMAP0070 (A05-38); ISMAP0076  
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**Ngassoum, M. B.** - ISMAP0033 (A05-18)  
**Ngassoum, M.B.** - ISMAP0383 (A05-9);  
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**Nitta, V.** - ISMAP0287 (A01-31)  
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**Noghteh, A.C.** - ISMAP0371 (A03-23);  
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**Novak, J.** - ISMAP0364 (A02-10); ISMAP0365  
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**Oliveira, C.M.R.** - ISMAP0234 (A08-20);  
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**Oliveira, C.Z.** - ISMAP0296 (A08-9);  
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**Oliveira, D.A.** - ISMAP0070 (A05-38);  
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**Oliveira, E.A.** - ISMAP0229 (A07-4)  
**Oliveira, G.M.C.** - ISMAP0250 (A05-27)  
**Oliveira, H.R.C.** - ISMAP0119 (A05-43);  
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**Oliveira, J.A.** - ISMAP0166 (A03-43)  
**Oliveira, J.D.** - ISMAP0198 (A04-26)  
**Oliveira, J.M.D.** - ISMAP0198 (A04-26)  
**Oliveira, L.O.** - ISMAP0061 (A01-16)  
**Oliveira, O.R.** - ISMAP0200 (A03-25);  
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**Oliveira, P.M.C.** - ISMAP0070 (A05-38)  
**Oliveira, R.G.** - ISMAP0315 (A07-10);  
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**Oliveria, J.A.** - ISMAP0231 (A03-36)  
**Orellano, E.G.** - ISMAP0102 (A04-25)  
**Ortiz, M.S.** - ISMAP0206 (A07-12)  
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**Otoch, M.L.O.** - ISMAP0102 (A04-25)  
**Pacheco, T.A.R.C.** - ISMAP0351 (A05-30)  
**Pal, M.** - ISMAP0155 (A05-11)  
**Palsuledesai, M.R.** - ISMAP0029 (A02-13)  
**Pandey, A.** - ISMAP0155 (A05-11)  
**Pank, F.** - ISMAP0091 (A02-3)  
**Parente, L.M.L.** - ISMAP0085 (A08-21)  
**Park, K.J.** - ISMAP0110 (A03-13); ISMAP0281  
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**Pasa, T.B.C.** - ISMAP0190 (A05-3)  
**Pathak, J.M.** - ISMAP0024 (A03-12);  
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**Patil M.B.** - ISMAP0088 (A08-16)  
**Patil, M.B.** - ISMAP0034 (A05-29);  
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**Paula Júnior, W.** - ISMAP0137 (A08-10)  
**Paula, J.R.** - ISMAP0080 (A05-14);  
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- Pavlovi, S.** - ISMAP0387 (A03-26)  
**Peixoto, P.H.P.** - ISMAP0138 (A05-22)  
**Pellegrino, A.P.** - ISMAP0225 (A04-20)  
**Pena, E.A.** - ISMAP0195 (A04-7)  
**Perecin, M.B.** - ISMAP0338 (A02-9);  
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**Pereira, A.M.** - ISMAP0145 (A01-6)  
**Pereira, A.M.S.** - ISMAP0165 (A02-11);  
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**Pereira, A.V.** - ISMAP0283 (A03-45)  
**Pereira, E.B.C.** - ISMAP0283 (A03-45)  
**Pereira, F.M.** - ISMAP0254 (A08-7)  
**Pereira, J.O.** - ISMAP0322 (A01-21)  
**Pereira, P.S.** - ISMAP0165 (A02-11);  
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**Pereira, R.S.** - ISMAP0131 (A03-35)  
**Perotti, J.C.** - ISMAP0225 (A04-20)  
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**Pessoa, C.O.** - ISMAP0174 (A08-13)  
**Petrere, V.G.** - ISMAP0225 (A04-20)  
**Petrovick, P.R.** - ISMAP0163 (A05-31)  
**Pianowski, L.F.** - ISMAP0358 (A05-42)  
**Pietro, R.C.L.R.** - ISMAP0221 (A08-6);  
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**Pilla, C.M.P.** - ISMAP0221 (A08-6)  
**Pimentel, E.C.** - ISMAP0344 (A07-14);  
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**Pinto, A.C.S.** - ISMAP0174 (A08-13);  
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**Pinto, J.E.B.P.** - ISMAP0002 (A04-19);  
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**Piri, K.** - ISMAP0055 (A05-24); ISMAP0056  
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**Pizziolo, V.R.** - ISMAP0229 (A07-4);  
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**Pizzolatti, M.G.** - ISMAP0262 (A05-19)  
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**Pohlit, A.M.** - ISMAP0134 (A03-3);  
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**Power, J.B.** - ISMAP0066 (A01-9);  
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**Pozitano, M.** - ISMAP0110 (A03-13)  
**PrzybyB, J.** - ISMAP0081 (A01-24)  
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**Queiroz, L.** - ISMAP0282 (A01-25)  
**Quisen, R.C.** - ISMAP0331 (A01-35)  
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**Radusiene, J.** - ISMAP0211 (A07-5);  
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**Rangel, V.L.B.I.** - ISMAP0223 (A05-41)  
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**Rau, C.** - ISMAP0341 (A05-36); ISMAP0343  
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**Razmilic, I.** - ISMAP0043 (A01-4)  
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**Rehder, V.L.G.** - ISMAP0298 (A08-15);  
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**Reis, R.A.** - ISMAP0321 (A03-27)  
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**Rocha, E.A.** - ISMAP0076 (A04-12);  
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**Rocha, G.R.** - ISMAP0274 (A01-12)  
**Rocha, G.S.** - ISMAP0315 (A07-10)  
**Rocha, J.N.** - ISMAP0264 (A03-4)  
**Rocha, M.** - ISMAP0265 (A07-6)  
**Rocha, S.A.** - ISMAP0198 (A04-26)  
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**Rodrigues, M.V.N.** - ISMAP0356 (A05-25);  
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**Rodrigues, R.A.F.** - ISMAP0323 (A05-45);  
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**Sakiyama, N.S.** - ISMAP0061 (A01-16)  
**Sales, J.F.** - ISMAP0080 (A05-14);  
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**Salimena, F.R.G.** - ISMAP0138 (A05-22)  
**Salomão, A.N.** - ISMAP0311 (A01-3);  
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**Sama, W.** - ISMAP0051 (A08-5)  
**Sampaio, P.T.B.** - ISMAP0331 (A01-35)  
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**Santos, A.S.** - ISMAP0358 (A05-42)  
**Santos, C.A.M.** - ISMAP0128 (A08-11);  
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**Santos, I.R.I.** - ISMAP0282 (A01-25);  
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**Santos, M.R.** - ISMAP0080 (A05-14);  
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**Santos, R.P.** - ISMAP0331 (A01-35)  
**Santos, S.C.** - ISMAP0080 (A05-14);  
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**Sartoratto, A.** - ISMAP0298 (A08-15);  
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**Sato, A.** - ISMAP0182 (A04-4); ISMAP0197  
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**Siani, A.C.** - ISMAP0168 (A01-13);  
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**Silva, C.A.** - ISMAP0258 (A03-37)  
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