SEGUNDO FORUM INTERNACIONAL

APIMEDICA & APIQUALITY 2008

PAPERS CIENTÍFICOS

STUDY OF BEE ROYAL JELLY IMMUNISTIMULATING CHARACTERISTICS IN "IN VITRO" EXPERIENCES

Presenter: Professor Dr Vasily Krylov

Other Authors: I.B. Ulitin, E.V. Krylova, S.S. Sokolsky

Organisation: Nizhni Novgorod State University, Russia

Email: kfg@bio.unn.ru

The immunomodulate activity of bee Roual Jelly (RJ) is its one of the most important mechanisms of therapeutic effects. However known RJ immunomodulate characteristics are based on apitherapy phenomena most often and do not open mechanisms, through which these characteristics reveal itself in the organism. We suppose that listed below experiments on insulated immunocompetent human blood cells will contribute a certain contribution to the study of these mechanisms.

In first series of experiences it was studied a lymphocytes viability on the system chosen immunocompetent human blood cells (lymphocytes) practically sound people, i.e. range optimum physiological and a RJ rapids toxic concentrations required for the following experiments on revealing its immunomodulate activity mechanisms. Viability valued, painting dead cells by 0.5 % solution of tripane blue. It was installed that possessing toxic action on the cells RJ minimum concentration has formed 750 mkg/ml. Not influencing upon a lymphocytes viability physiological concentration was within the range of 62.6 - 500 mkg/ml.

A T-lymphocytes rosette modeling ability defined a method of spontaneous formation with ram erythrocytes after their incubations with RJ different concentrations solutions. It was installed that T-lymphocytes most activity show at RJ concentrations 125 mkg/ml, that on 32 % more than in checking.

A lymphocyte incubated with RJ solution adhesive characteristic valued on degrees their adhesia on a synthetic filament. On immunocompetent blood cells adhesia influence of the RJ different concentrations studyes have shown that cells showed maximum ability to adhesia at incubations them with RJ in concentrations 125 mkg/ml. Under given concentrations of under investigation material the cells ability to adhesia increased on 22.2% in contrast with factors in the checking group (indicator panel. 3). Lymphocytes possessed the least ability to adhesia, which were added a RJ in 500 mkg/ml concentrations.

Circulating Immune Complexes (CIC) level valued [illegible] method, which on principle is

concluded in CIC precipitating by polyethyleneglycole. CIC maximum amount was observed in the blood serum at the RJ accompaniment in 400 mkg/ml concentrations. Concentrations of 250 mkg/ml and 1000 mkg/ml enlarged a CIC amount less significant.

In the under investigation substratum activation of complement system (CS) studied on ram sesibilisating erythrocytes haemolysis degrees. Studies have shown that maximum CS activity was observed when using a RJ in 500 mkg/ml (on 40% on the comparison with checking). with reducing an activity to scale down concentrations.

On the grounds of conduct experiments it is necessary to conclude that bee Royal Jelly possesses denominated immunomodulate action just on chosen immunocompetent blood cells in the broad interval of solutions. This action can be the basis of raising as specific, so and non-specific resistency of human organism when RJ using.

Improved Antitumor Effect By Combined Treatment Of Propolis And Chemotherapeutic Drugs

Presenter: Prof. Nada Orsolic

Other Authors: Vesna Benkovic, Ivan Basic

Organisation: Faculty of Science, University of Zagreb, Croatia

Email: norsolic@yahoo.com

Using a Swiss albino mouse model of Ehrlich ascites tumor (EAT), we investigated antitumor efficacy of two preparations of propolis (water and ethanolic extract) or quercetin, a compound of propolis, combined with chemotherapeutic drugs (Cisplatin and Doxorubicin). Egzamination was directed to suppression of tumor progression expressed as the percent increased life span. Propolis or quercetin were administrated ip to animals at doses of 50 mg kg-1 body weight for three consecutive days before tumor inoculation. Doxorubicin (20 mg kg-1) or cisplatin (10 mg kg-1) were given to mice two days after tumor cell inoculation. The results of log rank test between treated and control mice showed:WSDP, p=0.00291; wsdp + cis, p=0.00781; WSDP + DOX, p=0.4998; EEP, p=0.00902; EEP + CIS, p=0.27603; EEP + DOX, p=0.22014; QU, p=0.27454; QU + CIS, p=0.72713; QU + DOX, p= 0.02667; CIS, p=0.00350; DOX, p= 0.2593. Combined treatment with test compounds and Cisplatin resulted in pozitive outcome (p=0.00853; p=0.00767; p=0.02500) versus cisplatin alone, however combined treatment with doxorubicin was ineffective. Our findings indicate that WSDP, EEP, and quercetin significantly erhanced the antiproliferative and cytotoxic action of cisplatin and that propolis compounds may be usefully used for lowering the toxicity and other deteriorating activities chemotherapeutic drugs.

Summary Of A Comparison Of Two Types Of Pollen Supplement With Respect To Brood Area, Honey Production And Nosema

Presenter: Mr Peter Warhurst

Organisation: Biosecurity, QLD Australia

Email: diane.werner@dpi.qld.gov.au

An apiary of 36 hives was evaluated and divided into three groups of 12 containing hives of equivalent population or hive strength. Treatments were assigned randomly to the groups.

Group 1 was fed 400 g of Palmer's protein cakes (locally made)

Group 2 was fed 600 g of Feed Bee (formulated in Canada using a recipe developed by the University of Guelph researchers)

Group 3 control

All hives had access to 40 litres of sugar syrup fed twice a week in an open feeder. Measurements taken at three weekly intervals include brood area (visual assessment), number of frames covered with bees (visual assessment), honey production (honey supers weighed) and Nosema counts (laboratory analyses).

Discussion

Brood area was not correlated to the number of frames covered with bees or honey production. This was an unexpected result which could only be explained by the variable lifespan of the bees.

The number of frames covered with bees was highly correlated to the honey production which as was increased due to feeding supplements. The two supplements were not significantly different from each other and the Feed Bee was not significantly different from the control.

Costs

Assuming both types of cakes cost $6.50 per kg, each hive in the treatment consumed $31.20 of Palmer's protein cakes and $46.80 of Feed Bee. Over this twelve week period each hive consumed approximately 13 kg of sugar costing $13.00. No costing for labour or travelling is included.

PERFIL DE FLAVONOIDES E INDICES DE OXIDACION DE ALGUNOS PROPOLEOS COLOMBIANOS

Presenter: Dr Guillermo Salamanca Grosso

Organisation: Universidad del Tolima Tolima Colombia

Email: salamancagrosso@gmail.com

Los flavonoides son compuestos químicos de origen botánico con marcada actividad biológica y han sido usados como marcadores de la calidad del propóleo. En este trabajo se presenta la metodología necesaria en la determinación cuantitativa de flavonoides totales usando dos métodos calorimétricos complementarios, basados en la reactividad diferencial del tricloruro de aluminio y el la 2-4 dinitrofenilhidrazina, frente a flavonas, flavonoles y flavanonas. las determinaciones se hicieron mediante espectrofotometría Ultravioleta-Visible. Adicionalmente se evaluaron los índices de oxidación en un grupo de muestras de propóleos colombiano. Las determinaciones se realizaron sobre extractos alcohólicos usando etanol del 96% v/v (EEP). Las muestras fueron colectadas en los Departamento de Arauca y Boyacá, en las zonas de vida de bosque húmedo tropical (*bh-T*) y seco montano bajo (*bs-MB*). Los índices de oxidación se determinaron siguiendo el test del permanganato de potasio en medio acido. Las determinaciones para el contenido total de flavonoides vario entre 7.50±0.48 y 22.3 ± 5.41%; los valores observados en el test de tricloruro de aluminio (Flavonas y Flavanoles), estuvieron entre 0.52±0.03 y 3.26±0.26% entre tanto para 2,4D (Flavanonas) 9.90± 0.20 y 11.7±0.20% respectivamente. Los índices de oxidación observados en muestras con altos contenidos de flavonoides totales estuvieron por el orden de 2.0 a 10 segundos, demostrándose así la actividad antioxidante de las muestras analizadas y la calidad de los EEP de la zona estudiada. Se concluye que la calidad de los EEP está influenciada por el método de recolección de las muestras, su almacenamiento y manejo durante la preparación de los extractos.

Therapeutic effects of honeybee (Apis Mellifera L.) venom injection on bovine mastitis

Presenter: Dr Sangmi Han

Other Authors: Dr Kwanggill Lee Dr Joohong Yeo Dr Haeyong Kweon Mrs Soonok Woo Mr Haju Baek Dr Soontae Kim

Organisation: National Institute of Agricultural Science and Technology Kyonggi-do South Korea

Email: sangmih@rda.go.kr

The therapeutic effective way of honeybee venom collected using bee venom collector on bovine mastitis was investigated. Bovine mastitis is the most serious problem in the dairy industry in terms of economic losses to the dairy industry. Bovine mastitis can broadly be classified into contagious mastitis and environmental mastitis. *Staphylococcus auresus* and *Streptococcus agalactiae* are the most important contagious agent of bovine acute and chronic mastitis. Environmental mastitis is caused primarily by *Escherichia coli*, *Streptococcus dysgalactiae* and *Streptococcus uberis*. We was carried to evaluate the potential application of treating bovine intramammay infections by applying honey bee venom collected in Korea to inactivate the primarily bacterial mastitis pathogens in milk. Mastitis cows from four farms were selected in the Yang-pyeong areas. Chronic mastitis cows were injected with the various concentrations of honey-bee venom per day. There was significant difference in the reduction rates of SCC according to treatment concentration and method of bee venom. The milk somatic cell counts (SCC) were significantly lower on 3 days in all concentrations after bee venom treatment. The reduction rates of SCC for treatment of 3, 6, 12 and 24 honeybee venom were 20, 43, 63.3 and 65.8% respectively. Hone venom treatment ways divided by a syringeful and bovivet spenstift. The treatment ways with Bovivet Spenstift was higher the reduction rates of SC compared with s syringeful. Thirty two out of 53 quarters were cures were c by bovivet spenstift with 12 bee venom per day for 14 days. The cure rate bee venom for *Escherichia coli*, *Stapylococcus aureus*, Gram positive bacte and Gram negative bacteria were 33.3, 75, 75 and 43.8% respectively. The results suggested that bee venom treatment (by bovivet spenstift with 12) be effective for treatment of bovine mastitis.

Effect of protein hydrolysates of bee collected rape pollen on superoxide anion radical

Presenter: Mrs Liping sun

Other Authors: Ms Xiaoli Zhu Ms Jie Dong

Organisation: Bee Research Institute, China Academy of Agricultural Sciences China

Email: apis@vip.sina.com

Protein hydrolysates were prepared from bee collected rape pollen by enzymatic treatment using an alkaline protease in order to increase the water solubility and bioactivity of the pollen. Ultrafiltration was applied to separate protein hydrolysates into five fractions based on the molecular weights. The antioxidant activity of the proteins hydrolysates was evaluated by their abilities to scavenge superoxide anion radical produced by the action of xanthine oxidase on xanthine. Significant radical scavenging capacity was demonstrated by the fractions with molecular weight higher than 1000. Membrane filtration was found to be effective to concentrate functional ingredients.

Chemical composition and antimicrobial activities against five oral pathogens of Chinese propolis extracts

Presenter: Dr Zhiwu Zhang

Organisation: Bee Research Institute. China Academy of Agricultural Sciences Beijing China

Email: zhangzhiwu@yahoo.com

Ethanol extract of Chinese propolis was assayed for their abilities to inhibit oral pathogens and scavenge DPPH radicals. Propolis extracts could be effective at low concentrations in inhibiting *Actinomyces viscosus* and *Streptococcus mutans*, i.e. minimum inhibitory concentration of 50 µg/ml. Growth of *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum* was inhibited in higher propolis concentration. It was believe that 14% of flavonoid in the extract contributed to the stronger radical scavenging activities and antimicrobial activities of propolis. HPLC profiles indicated the major bioactive ingredients to be phenolic compounds such as flavonoids, phenolic acids and some unidentified phenolics.

The Brazilian Red Propolis: Botanical origin, chemical composition and physiological activities

Presenter: Dr Andreas Daugsch

Other Authors: Prof. Dr. Yong Kun Park

Organisation: UNICAMP SP Brazil

Email: andreas@daugsch.com

The Brazilian red propolis is found in the northeast of Brazil inside the mangrove swamps. Brazilian propolis was divided into 12 distinct groups according to its botanical origin and chemical composition. The red propolis was recently classified as group 13 with the botanical origin *Dalbergia ecastophyllum* (Leguminosae). The botanical origin was verified by observation and different chromatographic methods / histological exams. Propolis of group 13 is rich in flavonoids and other phenolic compounds with highly interesting physiological properties. The following flavonoids were identified in Brazilian red propolis from *Dalbergia ecastophyllum*: liquiritigenin, isoliquiritigenin, daidzein, dalbergin, formononetin and biochanin A. Isoliquritigenin and liquiritigenin inhibited the growth of prostate cancer *in vitro*. It also inhibited the enzyme xanthine oxidase. Xanthine oxidase inhibitors were suggested in the treatment of hepatitis and cerebral tumors, as these diseases are augmenting the levels of xanthine oxidase in the serum. Isoliquiritigenin and liquiritigenin demonstrated also an anti-allergic activity, which was dose-dependant. Formononetin and biochanin A are isoflavonoids with estrogen and anti-fungal activity. They are metabolized to daidzein and genistein, respectively, which are well-known isoflavonoid aglycons found e.g. in soy and are largely used to treat women with problems related to menopause. They showed activity against prostate cancer and breast cancer. Red propolis had shown analgesic effects *in vivo*, in concentrations of 25 – 40 mg dry extract/kg body mass. It has also shown high free radical scavenging and hepato-protective activity.

Antibacterial activity of Melipona honey from Yucatán

Presenter: Dr Elizabeth Ortiz-Vazquez

Other Authors: Francisco Caballero MSc Luis cuevas-Glory Dr Elizabeth Ortiz-Vázquez

Organisation: Insitituto Tecnólogico de Mérida Yucatán México

Email: eortiz@itmerida.mx

The melipona honey has been used since pre-hispanic time, by the Mayan civilization, such as a medicinal product. Yucatán has sixteen species of stingless bees that belong to the tribe Meliponini. The melipona honey has many properties, including antibacterial activity, this property is an important parameter for medicinal use, healing to wound and to avoid bacterial infection.

Cultured either *Staphylococcus aureus* or *E. coli* were added to warm nutrient agar, which was poured into plates, wells were punched in the set agar. Honey samples were tested at a concentration of 80%, 50% and 30% for antibacterial activity. Catalase solution was used for analyzing non-peroxide activity. It was measured the diameter of the area of inhibition around the wells. Minimum inhibitory concentration was determined using 0 to 10% of honey with nutrient Agar.

The melipona honey had inhibitory activity against *S. aureus* equivalent to that of 10% (w/v) phenol, as determined by an in vitro assay, the effect was greater against *E. coli*. Differences in the average of diameters of inhibition zones were found among honey samples of Melipona honey. Apis honey was not able to inhibit *S. aureus* growth as it has been shown with other researchers. However, it has been reported that *S. aureus* was not susceptible to that honey from Trigona. The minimum inhibitory concentration of Melipona honey was 5%, in Manuka honey has been reported 3%. We are concluding that Melipona honey has a potential as antimicrobial agent.

Royal Jelly Prevents Osteoporosis in Rats: Beneficial Effects in Ovariectomy Model and in Bone Tissue Culture Model

Presenter: Prof Saburo Hidaka

Other Authors: Lecturer Yoshizo Okamoto Ph.D Satoshi Uchiyama Master Kaori Ishiyama Ph.D Ken Hashimoto Prof Masayoshi Yamaguchi

Organisation: Fukuoka College of Health Sciences Fukuoka Japan

Email: sabrnrn@college.fdcnet.ac.jp

Royal jelly (RJ) has been used worldwide for many years as medical products, health foods, and cosmetics. Since RJ has some steroid hormone-type activities, we hypothesized that it may have beneficial effects on osteoporosis. Rats were divided into eight groups: sham-operated (Sham), ovariectomized (OVX), OVX given 0.5% (w/w) freeze-dried royal jelly (RJ), OVX given 2.0% (w/w) RJ, OVX given 0.5% (w/w) protease-treated RJ (pRJ), OVX given 2.0% (w/w) pRJ, OVX given 17-estradiol, and OVX given its vehicle, respectively. The administrations of 2.0% (w/w) RJ and 0.5-2.0% (w/w) pRJ to OVX rats recovered the tibial bone mineral density (BMD) by 85% or more. This result indicates that both RJ and pRJ are almost as effective as 17-estradiol in preventing the development of bone loss induced by ovariectomy in rats. In tissue culture models, both RJ and pRJ increased calcium contents in femoral-diaphyseal and -metaphyseal tissue cultures obtained from normal male rats. However, in a mouse marrow culture model, they neither inhibited the parathyroid hormone (PTH)-induced calcium loss nor affected the formation of osteoclast-like cells induced by PTH. Therefore, our results suggest that both RJ and pRJ may prevent osteoporosis by enhancing intestine calcium absorption, but not by directly antagonizing the action of PTH.

Botanic Origin and Chemical Composition of a Novel Brazilian propolis: Red Propolis

Presenter: Dr. Severino Alencar

Other Authors: Severino M. Alencar Masaharu Ikegaki Pedro L. Rosalen

Organisation: ESALQ/USP São Paulo Brazil

Email: alencar@esalq.usp.br

The Brazilian propolis is a resinous non-toxic substance, collected from buds or exudates of plants by *Apis mellifera* bees, which was classified in 12 types according to physicochemical properties and related with geographic localization. The chemical composition of propolis is quite complicated. More than 300 compounds such as polyphenols, phenolic aldehydes, sequiterpenes, coumarins, and steroids have been identified in propolis. A new propolis named Brazilian red propolis (BRP) because of its color, non-classified, has been found in Maceio City (Alagoas state, Northeastern Brazil) and has been speculated by the international market.This unique type of propolis is not found in any other Brazilian State, which can be explained by the local biodiversity characterized by mangrove around that city, not present in any other Brazilian ecosystem. The objective of this study was to identify botanic etiology and chemical composition of the new Brazilian red propolis by exploratory phytochemistry analyses of the microflora resin produced in the mangrove area. Thus, the novel Brazilian propolis and the plant secretion from 20 species, most frequently mentioned as probable botanical sources of this type of propolis, were investigated by RP-HPTLC. Based on phytochemical evidence provided by Absorbance of UV-visible spectrum, RP-HPLC and CG-MS, *Dalbergia ecastophyllum* (L.) Taub. was shown to be the main propolis source of red propolis in Alagoas state. Both the propolis and the plant resin showed high relative percentages of isoflavones 3-Hydroxy-8,9-dimethoxypterocarpan and medicarpin. This appears to be the first report on the occurrence in the leguminosae family as a botanical source of Brazilian propolis.

Anticancer Activity of A New Type of Propolis (G6) Provided From Bahia - Brazil

Presenter: Dr. Severino Alencar

Other Authors: Severino M Alencar João E. Carvalho

Organisation: ESALQ/USP São Paulo Brazil

Email: alencar@esalq.usp.br

Propolis has been used in folk medicine for centuries and is appreciated for its anti-microbial, antioxidative, anti-ulcer, hepatoprotective, anti-inflammatory and anticancer activities. More than 300 compounds, such as polyphenols, phenolic aldehydes, sequiterpenes, coumarins, and steroids have been identified in propolis samples. Thus, the scope of this work was to evaluate the antiproliferative activity of a new type of propolis (G6), collected in the State of Bahia, Brazil. The crude extract (CEG6) and a semi-pure fraction (F1G6) were evaluated on sulforhodamine B antiproliferative assay at different concentration levels against nine human tumor cell lines: leukemia (K-562), prostate (PC0-3), kidney (C786-0), ovary (OVCAR), melanoma (UACC-62), colon (HT-29), lung (NCI-460), breast (MCF-7), multi-drug resistant breast cells (NCI-ADR), and one normal cell, human skin fibroblast (HF). The crude extract (CEG6) displayed antiproliferative activity against cancer cells tested in a concentration-dependent way (IC_{50} 3.2-40). The semi-pure fraction (F1G6) displayed similar antiproliferative activity profile as CEG6 for most cancer cell lines, presented lower IC_{50} values (0.5-1.7), and showed selective activity against leukemia (K-562). Both EBG6 and F1G6 displayed a lower antiproliferative activity against normal cell (FH), IC_{50} (66 and 1000). Most chemotherapeutic agents present "antiproliferative" rather than "anticancer" activity; hence there is a growing need for anticancer drugs presenting less toxicity and more efficacy and selectivity. In this study we observed this characteristic when crude propolis (CEG6) and one of its semi-pure fraction (F1G6) were evaluated. CEG6 presented a significant anticancer activity, whereas F1G6 was more effective, selective and presented less toxicity.

How To Obtain Bees From The Hive For Use In Apitherapy

Presenter: Mrs Barbara Dalby

Organisation: Pebadale Apiairies Herts ENGLAND

Email: apither-queen.eng@virgin.net

The purpose of this presentation is to describe and demonstrate, graphically and verbally, some of the different methods of obtaining and maintaining bees from the hive for use in Bee Venom Therapy.

Bees need to be removed from the hive and maintained away from the hive environment to enable non-beekeeping live Bee Venom Therapy users to have a ready supply of bees for their use. Users need to be aware of any maintenance needed to keep bees alive for a period of time as it may not be practical to obtain bees on a daily basis.

Bees should be removed from the hive with minimum disturbance to the colony ensuring the queen stays in the main colony. There are various methods suggested for this by many people and the aim of this presentation is to show some of those and discuss any advantages and disadvantages of them.

Removal of bees from the hive is possible at all times of the year and in all weathers but care needs to be taken to ensure minimal disruption to the mother colony. With information from this presentation it may be easier to perform this procedure swiftly and safely.

Prevention and Treatment of Bee Venom related Adverse Reactions

Presenter: Dr. Stefan Stangaciu

Organisation: AC&TI Romania

Email: drstangaciu@apitherapy.com

The adverse reactions to bee products, including the anaphylactic shocks, as rare as they are, are causing though worldwide problems in the acceptance of beekeeping and Apitherapy.

If we want to prevent and treat the adverse reactions to a bee product, we must concentrate our efforts in order to find what is wrong with:

A) The bee product itself.

B) The client/customer, seen as a person with possible medical problems.

Among the problems that are related more often to the **bee product** itself are:

- Poor quality (inappropriate processing and storage);
- Poor or wrong description written on the commercial label, etc.

Among the problems that are related more often to the **customer** itself are:

- History of various intolerances and allergies to other foods and/or remedies;
- Weaknesses in the adrenal glands area;
- Weaknesses or malfunctioning in the immune, nervous and endocrine system of various causes, etc.

With enough patience and a good knowledge of the bee products and the functioning of the human body we can establish a practical general **set of rules and principles** that can easily **prevent** and **treat** most of the adverse reactions, even in remote areas like isolated apiaries.

Antimicrobial activity of honey and microflora in bee brood of stingless bee (Trigona laeviceps)

Presenter: Assist. Prof. Dr. Panuwan Chantawannakul

Organisation: Department of Biology, Faculty of Science, Chaing Mai University Chiang Mai Thailand

Email: panuwan@yahoo.com

Antimicrobial activities of honey and microflora in bee brood of stingless bee (*Trigona laeviceps*) were assessed against 10 bacterial species (i.e. *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Serratia marcescens*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Staphylococcus epidermedis*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*) and four fungal genus (i.e. *Mucor* sp., *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp.) by using agar well method. Results showed that three different sources of honey can inhibit all bacteria with most inhibitory effect on *Staphylococcus aureus*. However fungal inhibition was not found. Antibacterial activities of honey from each hive of stingless bee were statistically different. Microflora in bee brood of stingless bee (*Trigona laeviceps*) from 5 hives were studied by using standard plate count method. The amount of bacteria were 1.23 ± 0.35 -7.10 ± 2.05 x 10 CFU/g after 24 hr 2.17 ± 0.86 -7.97 ± 2.30x 10 CFU/g after 48 hr at 30 °C. Fifteen bacterial isolates were isolated and identified by biochemical tests. They were identified as *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus* spp.

Total antioxidant activity of hive products

No

Presenter: Prof. Patricia Vit

Other Authors: Prof Antonio Rodriguez Malaver Prof Patricia Vit

Organisation: Universidad de Los Andes Merida Venezuela

Email: vit@ula.ve

It has been reported that honey, pollen and propolis have antioxidant activity, however, little is known about the antioxidant activity of other hive products. Studies carried out by several authors have sheered light on the role that different honey components play as antioxidants. Although the antioxidant capacity of honey seems to be the result of combined action of all its components, it has been suggested that phenolic compounds (tocophenols, flavonoids and phenolic acids) represent essential elements that in most cases define the level of antioxidant activity of certain honey sample. Beside phenolic compounds honey contains a variety of natural compounds such as nitrogen compounds (alkaloids, chlorophyl derivatives, amino acids and amines), carotenoides and vitamin C which are widely known for their antioxidant activity. The study of antioxidant activity of honey has the attractive aspect that in the near future it could be used as a parameter for detecting fraud in the hive products. In this work the total antioxidant activity (TAA) of wax, honey, royal jelly, propolis tincture, propolis extract, and pollen in water, methanol or ethanol was studied. Water, ethanol and methanol exhibited TAA values near nil, as well as wax. The bee honey and royal jelly had a low TAA near 0.1 μM. Propolis tincture TAA was four times higher than wax values, and was similar in all the three extracts. However, the propolis extract had a high TAA, similar to alcoholic extracts of pollen. In contrast, water-extracted pollen had a TAA similar to propolis tincture, approx. one fourth times of the propolis extract and methanol or ethanol extracted pollen. In the same way, antioxidant substances of the bee pollen were extracted better with ethanol and methanol, for this reason, one could recommend an alcoholic preparation for the antioxidant activity of this product of the hive.

Effects Of Royal Jelly Of Different Forms On The Amount Of 5-HT On Rat In Vivo

Presenter: Prof Jihong Yan

Other Authors: Dr Xue Wang Dr Rong Zhang

Organisation: Apicultutal Research Institute of Chinese Academy of Agricultural Sciences P.R.China

Email: yanjihong@vip.sina.com

Introduction

This study chooses 5-HT as the analysis subject to investigate the mechanism on sleeping quality improvement by royal jelly in order to get the effects of royal jelly of different forms on the amount of 5-HT on rat in vivo.

Method

This study is based on the experimental method. The investigation was composed by three groups, i.e. digestive royal jelly group, royal jelly group and blank control.

Results

It is demonstrated in the experimental results that, the digested royal jelly can obviously improve the 5-HT levels in cerebral cortex, rapheal nuclei, hippocampal gyrus and serum in rats ($P<0.001$), and the values are obviously higher than those obtained from undigested royal jelly ($P<0.005$), so it is suggested that the small peptides from digested royal jelly can smoothly pass blood brain barrier and improve the level of 5-HT and it is helpful on the improvement of insomnia. Moreover, -aminobutyric acid (GABA) in undigested

HONEY ADDED WITH PROPOLIS: PHYSICOCHEMICAL QUALITY CONTROL

Presenter: Prof. Ligia Bicudo Almeida-Muradian

Organisation: University of Sao Paulo Sao Paulo Brazil

Email: ligiabi@usp.br

Propolis is a glue-like substance composed of plant resins, bee wax and pollen with antibacterial activity As the alcoholic extract has a strong taste and may provoke rejection, it is added in honey, so that it can be better accepted by consumers. In Brazil, there is a special regulation for honey (Instrução Normativa nº11/2000) and propolis (Instrução Normativa nº03/2001) separately, but not for their combination. Despite this lack of regulation, the product is sold with the authorization of the Ministry of the Agriculture. However, it is important to state the methods that can be used for the quality control of this product. The objective of this work is to verify if the same methods for the quality control of pure honey can be used for honey added with propolis. The methods used were based on the honey regulation methods: moisture, reducing sugars, apparent sucrose, ash, hydroxymethylfurfural, water insoluble solids, diastase activity and acidity. Honey samples with 2-5% of propolis were used. The results suggested that all the methods were suitable for all the samples except for diastase activity method which had to be adapted for honey with propolis samples.

Biological Properties of Australian Propolis

Presenter: Dr Colin Duke

Other Authors: Dr Van Tran Ms Seokkwan Tan Mr Abdallah Abu-Mellal

Organisation: University of Sydney New South Wales AUSTRALIA

Email: colind@pharm.usyd.edu.au

Introduction: Propolis is a complex resinous substance produced by worker honeybees from exudates and secretions of young shoots and buds collected from certain trees and shrubs. The chemical composition of propolis is variable depending on the plant source, geographic location and honeybee species. Propolis has been reported to exert biological activities such as anti-bacterial, anti-viral, anti-inflammatory, anti-cancer, antioxidant actions and also having benefit in lowering blood glucose and lipids. The purpose of this study is to determine the chemical constituents contributing to the biological properties of Australian propolis, and identify biologically active components that may be used to define medicinal propolis products of consistent quality or used as lead active substances for the development of pharmaceutical products.

Methods

Samples of propolis from various locations in Australia.

Fractionation of the propolis by chromatography and spectroscopic analysis of the fractions to detect and characterise components.

Biological properties of detected substances are evaluated from published literature or from in-house testing of pure isolated substances.

Results

Propolis constituents identified from:- Sydney; flavonoids (40%), caffeic acid ester derivatives (10%), cinnamyl cinnamate and derivatives (5%), "triterpenoid" substances (30%). Helensburgh NSW; flavonoids (40%), cinnamyl cinnamate (10%). Wolumla NSW; flavonoids, caffeic acid isopentenyl esters. Kangaroo Island; prenylated substances (70%), coumaric acid derivative (3%).

Discussion

Based on composition and biological testing evaluation useful but non-identical medicinal properties may be attributed to the propolis samples. The sample from Kangaroo Island is of particular interest as it appears to contain novel substances.

Phenylacetaldehyde in Honey - Residue or natural compound?

No

Presenter: Mrs Gudrun Beckh

Other Authors: Dr Cord Lüllmann Prof Dr Karl Speer

Organisation: Quality Services International GmbH Bremen Germany

Email: beckh@qsi-q3.de

In its report about chemical analyses of honey, Stiftung Warentest, in its April issue 2004, rejected some of the products tested due to an increased concentration of phenylacetaldehyde, although there is no legal maximum limit for it. Phenylacetaldehyde can be used as a bee repellent for a simplified harvest of honey, which means that the substance could be regarded as residue. But phenylacetaldehyde can also be generated in the honey itself from the amino aci phenylalanine, for example with the help of enzymes or in the Maillard Reaction.

In this research project the attempt was made to find a relationship between the content of phenylacetaldehyde and phenylalanine, respectively, under consideration of several parameters, e.g. temperature and light. The analysis of phenylalanine was carried out with an amino acid analyser. It was shown that the content of phenylalanine largely depends on the botanical origin of the honey. Concentrations from 5 ppm (Fir honey) up to more than 1000 ppm (Lavender honey) were found. These results correspond to literature. To determine the concentration of phenylacetaldehyde a Headspace-GC/MS method without special sample preparation was used. Honey samples with known concentrations spiked with phenylalanine, were analysed as well. The more phenylalanine the samples contained, the higher was the detected amount of phenylacetaldehyde, this same relationship occurred between the incubation temperature during Headspace analysis and the concentration of phenylacetaldehyde: the more the temperature was increased, the more phenylacetaldehyde was found. Due to the fact, that the Headspace-GC/MS method was not suitable another method has to be developed in which phenylacetaldehyde was extracted with TBME and the extract analysed by GC/MS.

In the next step, storage tests were carried out. Certain honey samples and also a honey-like sugar syrup (pure and spiked with phenylalanine) were analysed for their respective content of phenylalanine and phenylacetaldehyde. Then, the samples were divided and stored (A) in darkness at room temperature, (B) at increased temperature and (C) under UV lighting for a few weeks. The concentrations of phenylacetaldehyde in each sample were determined gradually. Under (A) the concentrations remained as at the beginning of the experiment,

Total antioxidant activity, polyphenols, total flavonoids and color of Czech Republic honey.

Presenter: Prof. Patricia Vit

Other Authors: Prof Antonio Rodríguez-Malaver Ing Michal Bednář Dr Dalibor Titěra Prof Patricia Vit

Organisation: Universidad de Los Andes Merida Venezuela

Email: vit@ula.ve

Honey is known as the product of hive that is obtained after the collection of nectar or honeydew, mixed with substances produced by bees, stored and matured in the honey comb. Beside 20% of water and 80% of sugar, honey contains organic acids, flavonoids, enzymes, pollen, etc. The bioactivity of honey has been used in apitherapy, however theoretical basis of its medical properties waits for experimental demonstrations. Floral origin and entomological origin cause variations in the active compounds of a product seemingly homogeneous, colored in a range from almost no color until dark brown. The antioxidant activity of this food was evaluated for its capacity to act as free radical scavenger. In this work, it was examined the total antioxidant activity (TAA), polyphenolic content, total flavonoids and color of Czech Republic floral and honeydew honeys sent to the Analytical Service of Apicolar Research Institute at Dol. Following methods based on ABTS cation radical, Folin-Ciocalteu's phenol reagent diluted 1:10 of honey with Batterfield's phosphate buffer 0.25 M KH2PO4, pH 7.2 adjusted with NaOH and measured absorbance at 593 nm. All samples showed antioxidant activity, and contained polyphenols, particularly flavonoids. Also, their color was variable. Some authors found a correlation between honey antioxidant activity and its polyphenolic content. This trend also was found in our preliminary study with fifty Czech honey samples.

Anti-allergic properties of Brazilian propolis and its components

Presenter: Ph.D Hiroko Tani

Other Authors: Ph.D Kiyoshi Okihara Ph.D Tomoki Tatefuji Ph.D Ken Hashimoto

Organisation: Yamada Apiculture Center, Inc Okayama Japan

Email: 0807@mail.yamada-bee.com

Propolis has been used in folk medicines in many regions of the world and has been reported to have various biological activities such as anti-inflammatory and anti-allergic properties. Cysteinyl leukotrienes (cys-LTs: LTC4, LTD4, and LTE4) are chronic allergic substances generated predominantly by mast cells and eosinophils, and induce airway smooth muscle contraction, microvascular leakage, and mucous hypersecretion. During the investigation of effectiveness of propolis, we found that the ethanol extract of Brazilian propolis inhibited release of cys-LTs from human peripheral blood leukocytes. We, therefore, searched for cys-LTs release inhibitors in the ethanol extract.

Brazilian propolis, from Minas Gerais, was extracted with ethanol and the ethanolic extract was filtrated and evaporated *in vacuo*. The residue was purified by chromatographic separations which were guided by the assay using rat peritoneal mast cells. The purification steps showed the existence of some active compounds and finally purifications by HPLC gave artepillin C as one of the active compound. Artepillin C strongly inhibited cys-LTs release from rat peritoneal mast cells, and the IC_{50} values of ethanol extract and artepillin C are 4.3 and 3.0 g/ml, respectively. A study of remaining compounds is now in progress.

Investigating the prebiotic potential of Australian Honeys

Presenter: A/Prof Patricia Conway

Other Authors: A/Prof Patricia Conway

Organisation: University of New South Wales NSW Australia

Email: P.Conway@unsw.edu.au

It has been shown in the US and New Zealand that honeys can promote the growth of beneficial bacteria, including Lactobacillus and Bifidobacterium. These bacteria have been shown to have positive effects on intestinal health and the immune system. Compounds that can promote the growth of these beneficial bacteria are referred to as prebiotics. Most prebiotics that are commercially available are sugars of plant origin. The aim of this study is to investigate the potential of Australian honeys to promote the growth of bacteria proven to have beneficial effects on health. Australian honeys are unique because they are produced from indigenous flora, specific for this country. It is therefore of interest to examine the prebiotic capacity of various floral species of honeys sourced from Australia. Each honey is being tested for its capacity to selectively support the growth of a range of beneficial bacteria. From the findings, statements can be made about the potential health benefits of the various species of Australian honeys. It can be concluded that evidence of the prebiotic benefits of the Australian honeys will give the food industry a valuable functional food ingredient. The term "functional food" is used to refer to a food ingredient that provides a health benefit beyond basic nutrition. This will give the honeybee industry a marketing claim that will add value to honey and in turn increase both sales and price of Australian honeys.

PHYSICOCHEMICAL EVALUATION OF BRAZILIAN HONEY FROM JATAÍ BEE (Tetragonisca.angustula)

Presenter: Prof. Ligia Bicudo Almeida-Muradian

Other Authors: Fernando Barion Ligia B. Almeida-Muradian

Organisation: University of Sao Paulo Sao Paulo Brazil

Email: ligiabi@usp.br

Honey is considered as a food that provides energy, being elaborated from the dehydration and transformation of the nectar of the flowers by the bees. For the human consumption, the honey needs to attend the minimum requirements of identity and quality demanded by their regulation. In Brazil beekeepers can be divided in two practical distinct ones: the traditional ones, which use *Apis mellifera* bees and the Meliponiculture which uses shingless bees as Jataí bee (*Tetragonisca angustula*). There is no identity and quality parameters or regulation for this type of honey. The results of the physicochemical analysis of Jataí honey from southeast region of Brazil (city of Lins - State of São Paulo), were: moisture (21.21 %), total acidity (37.05 meg/ kg), reducing sugars (67.71 %) apparent sucrose (0.86 %), HMF (0.09 mg/100g), diastase activity (DN= 16.6), insoluble solids (0.099 %), ashes (0.17 %), proteins (1.09%), lipids (0.07%) and carbohydrates (77.22%). This works aims to collaborate with the International Honey Commission (IHC), International Bee Research Association (IBRA), and Brazilian government to collaborate with the future regulation for stingless bee honey legislation.

Apitherapy For Systemic Lupus Erythematosus

Presenter: Dr. Cristina Aosan

Organisation: Nippon Apitherapy Society Romania

Email: draosan@gmail.com

C.V. – 30 years (woman)

Diagnosis: SYSTEMIC LUPUS ERYTHEMATOSUS. VASCULARY PURPURA

Debut: at 17 years old (vasculary skin eruption on both calfs; swelling and pains burning-like to the same level; fever; in time: appearing pains of the joints and of the muscles)

Evolution: progressively bad: at 29 years old: has pension off for sickness reasons

First consultation (June 30, 2004): the eruption: all the inferior limbs, including posteriors; extended to the forearms; is permanent and it appears even if she is sitting on a chair; she is practically obligated to sit almost al the time straight in the bed; lupus cells: present in the blood; taking prednisone 15 mg/day from 6 months, with no changing of the symptoms

Treatment with herbs and bee products (excepting bee venom): internally administration; all symptoms begins to diminished

- after 6 weeks: starting to diminished the doses of prednisone; the eruption appears only on the right calf, with a few elements of purpura

warehouses. Total carotenoids and-carotene were quantified and then stored for four months in packages (one of them protected from light) supplied by the producer. Carotenoids were quantified in spectrophotometer (=450nm) and - carotene was analyzed by open column chromatography. The average content of total carotenoids and-carotene ranged from 86.39 to 222.76µg/g and from 4.89 to 19.57µg/g, respectively. The pro-vitamin A value was between 0.41 and 1.63 µg retinol/g sample. Losses of total carotenoids and -carotene of 44 to 60% and 53 to 84%, respectively, were observed when exposed to light during storage and losses of 37 to 61% and of 53 to 56%, in that order, when stored in the dark. The difference between the two storage conditions was not significant ($p>0.05$).

Antioxidant Activity and Chemical Composition af a New Type of Brazilian Propolis

Presenter: Dr. Severino Alencar

Other Authors: Ivani A. M. Moreno Pedro L. Rosalen Masaharu Ikegaki Severino M. Alencar

Organisation: ESALQ/USP São Paulo Brazil

Email: alencar@esalq.usp.br

Propolis is a resinous substance collected by honeybees (Apis mellifera) from several parts of plants. It has been used in folk medicine for centuries and shows a variety of biological effects such as antiviral, antimicrobial, antioxidant, anti-inflammatory, anticancer, anaesthetic, and cytostatic activities. In vitro studies have shown that a new type of Brazilian propolis, known as red propolis, presents important biological activities. The objective of this study was to evaluate the chemical composition and antioxidant properties of this new type of Brazilian propolis, collected in the State of Alagoas. The ethanolic extract of propolis (EEP) was analyzed by CG/MS. This analysis made possible the identification of 20 compounds, among which the isoflavones homopterocarpin, medicarpin, and 4',7-dimethoxy-2'-isoflavonol showed to be present in highest amounts, through the use of CG/MS. Afterwards, EEP was fractionated by liquid-liquid extraction with hexane and chloroform, with yield of two fractions, FR-Hex and FR-Chlo, respectively. Fr-Hex and Fr-Chlo were refractionated by preparative RP-HPLC, originating a semi-pure fraction (SP-F) and a pure compound, identified as vestitol, respectively. Their antioxidant activity was determined by two assays: free radical scavenging activity on DPPH and inhibition of linoleic acid oxidation by -carotene bleaching. The results showed that vestitol presented higher activity against the oxidation of linoleic acid (39.5%) than SP-F (35.6%); SP-F showed a strong activity for the free radical scavenging (89.9%), while vestitol showed activity of 9.5%. Consequently, SP-F was selected to continue identification process due to its important antioxidant properties.

Apitherapy For A Group Of Patients With MS

Presenter: Dr. Cristina Aosan

Organisation: Nippon Apitherapy Society Romania

Email: draosan@gmail.com

QUESTION: CAN WE CURE MULTIPLE SCLEROSIS ?

1. Presentation of **the group of patients** (treated many years)
2. Presentation of **the diagnose criterions** (date of the **MRI** and date of starting Api – Phyto - Therapy.
3. The **length of the disease** from the first symptoms until the moment of starting the Api – Phyto – Therapy.
4. General presentation of the **symptoms** and associated health problems
5. **Treatments** made **before** the Api – Phyto – Therapy
6. **The Natural Therapy**:
   - Preparation part for the bee venom therapy
   - Apitherapy: detailed presentation of the treatment
   - Phyto - Therapy
   - Basic elements for diet, life style, psychotherapy
7. **Clinical results:**

   for one part of the patients all the symptoms have almost disappeared;

   for some patients the symptoms have an important diminishing, and some of them have disappeared

   MRI results (presenting MRI imagines for each of the patients)

   making control MRI after one year, and then year by year, we have observed the disappearing of some of the demyelization lesions and decrease of other lesions.

   this MRI evolution was continuously good for many years; as the clinically evolution, too
8. **Comments**

   MRI evolution for many years: it is possible the regeneration of the demyelization lesions

The protocol treatment is individual for each patient, depending all the health problems and the individual evolution in the time of the treatment

9. **ANSWER :** the actual results give us the **HOPE** that it is **POSSIBLE.** The time will give to us the complete answer...

Effects Of The Therapeutic Nutrition For Liver Diseases In Dogs

Presenter: Dr Adrian Siceanu

Other Authors: Dr Mateescu Cristina Dr Radoi Cecilia Dr Cauia Eliza Dr Radoi Ion

Organisation: Institute for Beekeeping Research& Development -Bucharest Romania ROMANIA

Email: beeswoborders@yahoo.com

Introduction

The ever increasing interest for the alternative and complementary medicine and especially for the veterinary api-phyto-therapy led to the development of several researches on the preparation of an api-therapeutic product (Hepro-vit) and the study on its therapeutic effects in some liver diseases in dogs.

Material and method

The researches on the preparation of Hepro-vit were carried out in the Institute for Beekeeping Research and Development while the preclinical and clinical studies were carried out at the Medical Clinic of The Faculty of Veterinary Medicine from University of Veterinary Medicines Bucharest. The studied api-therapeutic product, prepared from a mixture of bee products (bee pollen and propolis), a plant extract (common celandine tincture (*Chelidonium majus*), microelements and vitamins was administered alone or in various therapeutic schemes, according to the disease. The clinical tests were carried out on company animals (dogs) of various ages and races, showing diagnosed hepato-billiary diseases (liver insufficiency, acute hepatitis, liver cirrhosis, cholestasis). The clinical investigations were completed by echo-graphic and biochemical examinations (BIL, ALAT, GGT, PAL), and the values were registered before and after the treatment.

Results and discussions

14 days after the application of Hepro-vit, the best results were noted in dogs with light hepatic insufficiency where the analyses showed a normalization of the BIL values (0,3-0,5 mg/dl) and a decrease of transaminases (ALAT-33-51,5UI/l). These results were superior to those obtained following the classic medicinal treatment (BIL 0,5-0,6 mg/dl, TRANS, ALAT-60-67,8 UI/l).

In dogs with acute hepatitis, after the application of Hepro-vit, the decrease of BIl values ranged between 0,85 -1,1 mg/dl as compared to 1,9-2,2 mg/dl obtained after the medicinal treatment. A decrease of ALAT to values ranging between 33-68UI/l as compared to 88-109UI/l in medicinal treatment, were also noted.

Conclusions

The methods of nutritional therapy with Hepro-vit, applied in hepatic diseases, were at least as efficient as the standard treatment methods.

Comparative analysis of the different beesting methods

Presenter: Mr Aleksey Mazhov

Other Authors: Dr Rail Khusmatullin

Organisation: The Tentorium Apicompany Russia

Email: kate@tentorium.ru

The aim of the work is to estimate efficiency of the multiple beestings method by separated sting for the patients with diffuse lesion of musculoskeletal system.

Two groups of patients with low back pain and arthropathies of dystrophic genesis had a course of beestings.

The first group was ten patients with lesion of musculoskeletal system with allergic anamnesis, concomitant pathology as well as beesting symptomatic local reaction.

As a result the multiple beestings method by separated sting into acupuncture points and affected joints was chosen. The basal value of pain sensation was evaluated by visual analog scale (VAS). on average.

Sensation of pain in cervical spine was 4 points; in lumbar spine - 7.2 points; horacic spine – 5.3 points; in small joints - 6.83 points; in extremities joints – 6.2 points on average.

The second group was twenty people with extensive dystrophic lesion of musculoskeletal system. The method of classical beesting into acupuncture points and affected joints was chosen for treatment. The basal value of pain sensation according to VAS was 6.75 points in cervical spine; 6.2 – in lumbar spine; 5.88 – in small joints of extremities; 6.79 – in big joints.

The treatment for the patients of the both groups included 8 procedures on average. The total number of beestings was 36 and the total exposure of sting in body was 123 minutes for the second group during the procedures.

Positive result was obtained for the both groups.

Reduction of pain sensation was noticed by the patients of the first group in cervical spine by 90% of the basal value; in lumbar spine by 51.6%; in thoracic spine by 87.5%; in small joints of extremities by 65%; in big joints by 75.7%. The patients of the second group noticed pain reduction in cervical spine by 86.37% of the basal value; in lumbar spine by 83.8%; in small joints of extremities by 70%; in big joints by 70.9%.

Thus, the multiple method of beestings by separated sting for the patients with diffuse lesion of musculoskeletal system is as effective as the classical method

INTRODUCTION OF BEE PRODUCTS IN THE ORGANISM

Presenter: Professor Dr Vasily Krylov

Other Authors: S. Kopylova S Peretyagin O. Sandova V. Novikov

Organisation: Nizhni Novgorod State University Russia

Email: kfg@bio.unn.ru

The influation of water-alcohol suspension of royal jelly and propolis mixture of inhalation (preparation "Apinhalin") in the burn of human and animals. In connection with this we have made up the inhalative preparation Apinhalin which represents aerosol of water-alcohol mixture of propolis and royal jelly. Mixture consistend of propolis 1; etyl alcohol (96%) 12; royal jelly 2; deionized water 85% from weight, respectively (drug Apinhalin , patent RU 2174002 C1).

In the first serieses there are 15 patients with III degree A-B of a burn (30-40 % of body surface) at the age from 20 up to 40 years old were observed. In the third series isolated rat s trachea was placed into "Apinhalin". The analysis of blood composition, of lipid peroxidation activity, of erythrocytes, some ferments of plasma, the dynamic of spirometric parameters of patients was made. In experiments of isolated rat's trachea the influence of preparation on contract activity was researched. Polyorganic alteration bringing to the breakup of blood composition, level of aminotransferase balance, increase of lipid peroxidation rate appeared as a result of burn disease. Becides that, burn disease reduced of minute volume and respiratory volume, alveolar oxygen concentration decreased.

Antibacterial Effects of Australian, Nepalese, and Thai Propolis against the Pathogenic Bacteria of External Wounds

Presenter: Dr. RATNA THAPA

Organisation: Mae Fah Luang University Chiang Rai THAILAND

Email: rthapa@yahoo.com

Propolis is a resinous substance collected by honeybees: *Apis mellifera* from various plants. The medicinal properties of propolis, and its use as a therapeutic substance have been known since ancient times. The main purpose of this research was to evaluate antibiotic effects of three geographically different countries; Australia, Nepla, and Thaialnd.

A total of 6 species of pathogenic bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Enterococcus faecium*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, collected from the Department of Microbiology, Chiang Rai Hospital, Chiang Rai, Thailand, were used to test the antibacterial effects of propolis. Propolis samples collected from Kuyan Apiaries, Gin-gin, Perth, Western Australia; Kathmandu, Nepal, and Chiang Saen, Chiang Rai, Thailand, were extracted with 70 percent ethanol to prepare three different concentrations: 10, 20, and 30 percent (w/v), respectively. The antibacterial activities of three different concentrations of propolis were tested by the agar disk diffusion method. Pathogenic bacteria previously isolated from wounds were cultured (10^7 CFU/ml), and incubated at 37 C for 24 hr. Antibiotic disk filter papers were dipped in 10, 20, and 30 percent propolis for 15 minutes. The disks were then placed directly on the plates, and incubated at 37 C for 24 hr. Ethanol (70 percent), and distilled water were used as control solvents. The inhibitory zone was measured by a vernier caliper.

The results showed clear zones with 10 percent of Australian, Nepalese, and Thai propolis against *S. aureus*, *S. epidermidis*, *A. baumannii*, *E. faecium*, *K. pneumoniae*, and *P. aeruginosa* of 1.2, 4.8, 7.0, 4.3, 10.0, and 3.0; 2.3, 5.3, 6.0, 6.0, 10.0, and 5.0mm; and 2.6, 6.0, 5.0, 3.5, 6.0, and 2.3mm, respectively, for these country sources. The clear zones with 20 percent Australian, Nepalese, and Thai propolis against *S. aureus*, *S. epidermidis*, *A. baumannii*, *E. faecium*, *K. pneumoniae*, and *P. aeruginosa* were 2.0, 6.7, 7.2, 1.9, 11.9, and 5.5; 3.7, 3.4, 1.8, 4.5, 6.3, and 5.0; and 4.0, 4.7, 3.5, 6.0, 4.5, and 4.5mm, respectively, for these country sources. The clear zones with 30 percent Australian, Nepalese, and Thai propolis against *S. aureus*, *S. epidermidis*, *A. baumannii*, *E. faecium*, *K. pneumoniae*, and *P. aeruginosa* were 2.5, 3.0, 2.4, 2.4, 8.9, and 5.0; 6.1, 3.6, 4.2, 4.3, 3.4 and 5.1; and 4.2, 5.9, 3.1, 3.6, 4.3, and 3.6mm, respectively, for these country sources..

In conclusion, *K. pneumoniae* was found to be highly sensitive to all three types of propolis. Nevertheless, *S. aureus*, *S. agalactiae*, *S. epidermidis*, *A. baumannii*, *E. faecium* and *P. aeruginosa* also showed sensitivity to the propolis collected from three different geographical regions-- Australia, Nepal, and Thailand.

Refractometric Determination Of Water Content In Royal Jelly

Presenter: Dr Livia Persano Oddo

Other Authors: Lorenzo Lusco Dr Livia Persano Oddo

Organisation: Istituto Sperimentale per la Zoologia Agraria, Sezione di Apicoltura Italy

Email: livia.persano@apicoltura.org

The determination of water content is a significant part of royal jelly (RJ) quality control, and up to now different methods have been used for its determination: gravimetric (freeze drying and oven drying) and Karl Fischer.

Although accurate and precise these methods are time consuming or require specific equipment. The aim of this work is to verify if the simple refractive index measurement, already used for honey moisture determination, can provide also for activity that help the body to cope with oxidative stress. *In vitro* studies indicate that phenolic compounds, widely present in the vegetable kingdom, might play a key role in the protective effect of plant foods. Evidences suggest that honey may have antioxidant properties, due to its content in phenolics. In this research the *in vitro* antioxidant activity of 13 unifloral honey types produced in Italy (*Arbutus*, *Castanea*, *Citrus*, *Erica*, *Eucalyptus*, *Hedysarum*, *Helianthus*, *Robinia*, *Taraxacum*, *Thymus*, *Tilia*, *Trifolium* and Honeydew) and of royal jelly, have been assessed through the measurement of reducing power and chain breaking antioxidant activity, respectively with FRAP (Ferric Reducing Antioxidant Power) and TRAP (Total Radical Trapping Antioxidant Parameter) assays.

The FRAP values were quite different in the different matrices examined, ranging from about 9 to less than 1 mmol Fe^{++}/kg FW. The highest values were recorded for *Arbutus* honey (8.86±0.49), honeydew honey (6.56±0.15), royal jelly (4.94±0.49) and *Erica* honey (4.17±0.26).

At the TRAP test, only *Eucalyptus* and *Helianthus* honeys showed a marked antioxidant activity (8.62±1.18 and 5.37±0.85 mmol/kg FW, respectively).

Eucalyptus honey, displaying the highest *in vitro* antioxidant activity, was selected to perform an *in vivo* test. Ten healthy volunteers, non-smoking, normolipidaemic, taking no supplements or medication, were asked to eat, in a crossover design, 80 g of honey. Basal venous blood samples have been collected before and at different times after honey ingestion. The data resulting are under elaboration.

CHARACTERISTIC EVALUATION OF ANTIBIOTIC ACTIVITY OF VARIOUS HONEY SAMPLES FROM KARNATAKA.

Presenter: Mr BHARGAVA H R

Other Authors: Dr JYOTHI J V A

Organisation: BANGALORE UNIVERSITY KARNATAKA INDIA

Email: bhargavahr@rediffmail.com

Honey is a natural and sweet substance produced by honeybees from the nectar of flowers. Honey is composed of sugars, acids, proteins, vitamins, enzymes and mminor ingredients. Honey is a readily available source of glucose and fructose. Thus it plays an important role in food and medicine. Honey also acts as an antibiotic agent on few strains of bacteria. To analyse this function, different strains of bacteria were collected and cultured under the laboratory conditions. The effect of different concentrations of honey on these bacterial strains is tested. It is found that the honey samples showed antimicrobial activity on different bacterial strains. The minimum inhibitory zones of different strains of bacterial plates are recorded. The honey samples collected from Coorg district showed higher antimicrobial activity than the other regions of Karnataka