



**Abstract Book**  
of the  
**First International Conference**  
**on Api-Microbiology**  
online 28 and 29 May 2022

## Final Program

Time Zone: **UTC/GMT + 3 hours**  
(Summer time in Bucharest, Romania)

Online (ZOOM), free of charge

Main organizers: [Prof. Narimane Segueni](#) (Algeria) and [Dr. Stefan Stângaciu](#) (Romania)

**Saturday, May 28<sup>th</sup>. 2022**

<b>Api-Pharmacopeia and Microbiology</b>	
09:00-9:10	Online connexion of participants
09:10-09:30	Opening speeches
<b>Session 1 on Propolis</b> Moderators: Narimane Segueni (Algeria) and Badiaa Lyoussi (Morocco)	
9:30-10:00	❖ <b><i>Influence of the Used Solvent during Extraction on the Antibiofilm Properties and Chemical Composition of Propolis</i></b> - <a href="#">Narimane Segueni</a> , Amina Daikh, Nazime Mercan Dogan, Sevki Arslan, Dogukan Mutlu, Ibrahim Kivrak, Salah Akkal & Salah Rhouati (Algeria, Turkey)
10:00-10:30	❖ <b><i>Propolis – an old and new medicine with antimicrobial, antibiofilm and antipathogenic activity</i></b> - Veronica Lazar (Romania)
10:30-11:00	❖ <b><i>Impact of Biohybrid Magnetite Nanoparticles and Moroccan Propolis on Adherence of Methicillin Resistant Strains of Staphylococcus aureus: Application fields</i></b> - Badiaa Lyoussi (Morocco)
11:00-11:30	❖ <b><i>In vitro Effect of Ethanolic Extract of Propolis on the Growth of Enterococcus faecalis</i></b> - Ardo Sabir (Indonesia)
11:30-12:00	❖ <b><i>Summary of antimicrobial activity studies on Turkish Propolis</i></b> - Sevgi Kolayli (Turkey)
<b>Session 2 on Bee pollen and Bee bread</b>	
12:00-12:30	❖ <b><i>Antimicrobial and Antioxidant Activities of Natural and Fermented Bee Pollen</i></b> - <a href="#">Adriana Cristina URCAN</a> , Adriana Dalila CRISTE, Otilia BOBIS, Daniel Severus DEZMIREAN (Romania)
12:30-13:30 Lunch break	

<p align="center"><b>Session 3 on Honey</b> Moderator: Narimane Segueni (Algeria)</p>	
13:30-14:00	❖ <b>Evaluation of the Antidiabetic and Healing Honey from southern Algeria “in vivo” study</b> - <u>Noureddine Djebli</u> and Rabia Eladaouia Taleb (Algeria)
<p align="center"><b>Session 4 on Propolis</b> Moderators: Narimane Segueni (Algeria) and Sibel Silici (Turkey)</p>	
14:00-14:30	❖ <b>Antifungal Activity of Olive Oil and Ethanol Extract of Propolis</b> - Sibel Silici (Turkey)
14:30-15:00	❖ <b>Phytochemicals and antibacterial properties of honeybee propolis from selected regions in Kenya</b> - <u>Timothy M. Kegode</u> & H. Michael G. Lattorff (Kenya)
15:00-15:30	❖ <b>Mechanisms of action of green and red Brazilian propolis against pathogene bacteria. A short review</b> - Niraldo Paulino
15:30-16:00	❖ <b>Seasonal studies of the Greenish-brown propolis from Alagoas, Brazil: preliminary results</b> - <u>Ticiano Gomes do Nascimento</u> , Emanoel Guilhermino da Silva Junior, João Victor Lessa de Oliveira, Matheus Vinicius Guimarães de Melo, Monique Almeida Vila Nova, Lucas Rafael de Oliveira Silva, Nataly Christine Soares Gama, Kathylen V. Ferreira dos Santos, Arthur L. Tavares Ferreira Borges, Fernanda Geny Calheiros Silva, Salvana Priscylla Manso Costa (Brazil)
16:00-16:30 Coffee break	
16:30-17:00	❖ <b>Propolis effects on people living with HIV under retroviral therapy</b> - José Maurício Sforcin (Brazil)
17:00-17:30	❖ <b>Phytochemical Composition and bioactivity of Propolis from Arid and Semi-arid Regions of the Sonoran Desert in North-Western Mexico</b> - <u>Efrain Alday</u> , Dora Valencia, Adriana Garibay, Claudia Virués Colorado, Carlos Velazquez (Mexico)
17:30-18:00	❖ <b>Cycloartanes: Major Beehive Protective agents in Southern Nigerian Propolis</b> - S. I. Iyen, J.V. Anyam, J.O Igoli and T.A. Tor-Anyiin (Nigeria)
<p align="center"><b>Session 5 on Royal jelly and Apilarnil</b></p>	
18:00-18:30	<b>Comparison of antifungal activity of Royal jelly and Apilarnil</b> – Sibel Silici (Turkey)
<p align="center"><b>Session 6 on Bee Venom</b> Moderators: Dr Stefan Stângaciu and Pr Maria Cristina Marcucci</p>	

18:30-19:00	❖ <b><i>Antibacterial and anti-biofilm effect of bee venom from the East-West region of Algeria against multi-resistant Gram positive bacteria of hospital origin</i></b> – <u>Aissaoui Hadjira, Hassaine Hafida, Gaouar Sara, Bellifa Samia</u> <b>TECHNICAL problem !</b>
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**Session 8 on Several bee products**

19:00-19:30	❖ <b><i>Antimicrobial activity of Brazilian bee products</i></b> - Maria Cristina Marcucci (Brazil-Italy)
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19:30-20:00 Dinner

**20:00-21:00 Api-Expo**

**Free Discussions 21:00-22:00**

**Sunday, May 29<sup>th</sup>.**

**Clinical use of bee products against pathogenic micro-organisms**

09:15-09:30	Online connexion of participants
<b>Session 1</b> <b>Moderators: Stefan Stângaciu (Romania) and Mamdouh Abdulrhman (Egypt)</b>	
09:30-10:00	❖ <i>Efficacy of poplar propolis enriched in procyanidins and green tea catechins against pandemic H1N1 influenza A strain</i> – Marco Biagi (Italy)(pre-recorded video)
10:00-10:40	❖ <i>Clinical use of honey dressings in the management of infected wounds</i> - Andrei Zbucea (Romania)
10:40-11:30	❖ <i>Use of honey for children under the age of 1 year.</i> ❖ <i>Use of honey and propolis in the treatment of pneumonia</i> - Mamdouh Abdulrhman (Egypt)
11:30 – 12:00 Coffee break	
12:00-12:30	❖ <i>Use of beehive air, as anti-microbial agent, in the practice</i> – Tiago Guardia (Canada/Brazil), Stefan Stângaciu (Romania)
12:30-13:00	<b><i>Antimicrobial Effect of Propolis on MRSA and MSSA Isolated from Subclinical Bovine Mastitis. A Pilot Project - Nataša Rajić Savić, Nemanja Zdravković, Stanislav Savić, Aleksandar Furdek (Serbia)</i></b>
13:00-13:30	<b>Round Table</b>
13:30-14:30 Lunch break	

<b>Session 2</b> <b>Moderator: Stefan Stângaciu (Romania)</b>	
14:30-15:00	❖ <b><i>Use of bee venom against infectious diseases. A short review</i></b> – Stefan Stângaciu (Romania)
15:00-15:30	❖ <i>Rules and Principles on the Clinical use of beehive products against Infectious diseases</i> – Dr Stefan Stângaciu (Romania)

15:30-16:00	<b>Prevention and treatment of vaginal candidiasis with Api-Phyto-Aromatherapy</b> - Elbens Azevedo (Canada/Brazil)
16.00-16.05	<b>Microbiological Evaluation of bee bread (perga)</b> - <u>Sibel Silici</u> (Turkey)
16.05-16.10	<b>Evaluation of the therapeutic potential of some apicultural products with essential oils for cutaneous wounds in cats and dogs</b> - <u>Octavia TAMAS-KRUMPE</u> , Cornelia DOȘTEȚAN ABĂLARU, Ramona GROSU, Cecilia Gabriella DANCIU, Cristina TODORAN and Laurenț OGNEAN (Romania).
16.10-16.15	<b>Correlative research regarding the total polyphenolic content, antioxidant, and antibacterial activity of three types of Romanian honey</b> - Octavia Tamas-Krumpe, Otilia Bobiș, Rodica Mărgăoan, Flore Chirilă, Călin Lațiu, Laurenț Ognean (Romania).
16.20-16.25	<b>Composition influenced in vitro antibacterial activity of honeys' on bee associated and non-bee associated (environmental) bacteria</b> - <u>Solomon I. Chogo</u> , John M. Onyari, Geoffrey O. Bosire, H. Michael G. Lattorff (Kenya)
16.25-16.30	<b>Isolation and characterization of alpha and beta amyryns from propolis obtained from Benue state</b> - <u>S. S. Ipav</u> J. O. Igoli, T. A. Tor-Anyiin and J. V. Anyam (Nigeria)
16:30-17.00	<b>Open debate</b> on the best recommendations of the « International Api-Microbiology Group » on the use of bee products against pathogenic micro-organisms.
17:00	<b>Final Round Table with Group Photos</b>

**Nota bene:**

The organizers will offer Certificates of participation to all speakers and moderators

The conference will be streamed online, live, in Facebook and will be also recorded.

After the conference, we will offer to the participants our electronic book of abstracts and technical guidelines on the use of beehive products in Microbiology and Clinical practice.

The **link** to our ZOOM conference room is:

<https://us02web.zoom.us/j/7677675492>

Password: 1234

## Influence of the Used Solvent during Extraction on the Antibiofilm Properties and Chemical Composition of Propolis

Narimane Segueni<sup>1,2</sup>, Amina Daikh<sup>1</sup>, Nazime Mercan Dogan<sup>3</sup>, Sevki Arslan<sup>3</sup>,  
Dogukan Mutlu<sup>3</sup>, Ibrahim Kivrak<sup>4</sup>, Salah Akkal<sup>5</sup> & Salah Rhouati<sup>1</sup>

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### Abstract:

Antimicrobial agents are one of the strategies for inhibition of biofilm formation. However, most antimicrobials are not often effective in controlling of biofilm formation. Therefore, finding of new materials that have biofilm inhibitory effects is so important. In this regard, we aimed to determine the antibiofilm effect of five Algerian propolis extracts obtained by extraction in solvents of varying polarity. Propolis extracts were tested for their ability to inhibit biofilm formation and to reduce preformed biofilm of eight bacterial strains including reference strains of *Staphylococcus aureus* (*S. aureus* ATCC29213 and *S. aureus* ATCC33862), three methicillin-resistant *S. aureus* (M10-1, M18-3, and M20-1), *Enterococcus faecalis* ATCC19433, *Micrococcus luteus* NRRL-B1013, and *Yersinia enterocolitica* RSKK1501. Chemical investigation was performed using ultra-performance liquid chromatography with electrospray ionization coupled to tandem mass spectrometry (UPLC-ESI-MS/MS). All tested extracts exhibited the highest eradicating capability for *S. aureus* reference strains and methicillin-resistant strains, especially MRSA18-3 and MRSA20-1. The reduction of biofilm formation was found to be significantly affected by the used solvent for maceration, the tested bacterial strains, and the origin of tested propolis. In addition, biofilm reduction of propolis seemed to be dose-dependent. In addition, twenty-six phenolic compounds were detected. Difference between the amounts of detected compounds was found to be significant. Caffeic and ferulic acids were the main compounds in the tested extracts. These results suggest that those compounds might be responsible for the observed antibiofilm and cytotoxic activities of propolis extracts.

**Keywords:** Algerian propolis, antibiofilm activity, chemical composition, solvent

## **Propolis – an Old and New Medicine with Antimicrobial, Antibiofilm and Antipathogenic Activity**

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### **Abstract**

There are actual data showing a high level of multidrug resistance and indicating that we are approaching to the post-antibiotic era. There is a crisis of antibiotics and the research in these field is trying to identify new anti-infectious drugs, without selection pressure and potential to induce resistance. Apart from this genetic, inherited antibio-resistance, the medical field is facing with another form of resistance named phenotypical, behavioural resistance, or tolerance of adherent and biofilm embedded cells to all kind of antimicrobials. The interest in biofilm associated infections and the ways to efficiently treat them is motivated by their increased proportion, around 80% of all infections. The high cellular density of a biofilm and the consequent accumulation of signal or quorum sensing (QS) molecules is contributing to a better adaptation of the metabolic activity of bacterial/microbial cells, including their tolerance to antimicrobials and synthesis of virulence factors, activating their genes expression at a critical point of cell density and in accordance with different steps of the infectious process. In this manner, the pathogens can inactivate the host unspecific and specific immune defence mechanisms, which ensures their survival and host colonization. As the intercellular communication by QS mechanisms and signal molecules is well documented, a new antibiofilm and antipathogenic strategy is based on QS inhibitors (QSIs). After the discovery of such molecules at a red algal species, a lot of microbial and vegetal species were searched for the presence of QSIs, being identified a lot of species which are QSIs producers. It was shown that even honeybees can protect them against their own pathogens producing some antimicrobials which are valuable natural products used also by humans since ancient times. These molecules, including QSIs, have double origin: from the bees' organism and from the visited plants and, by consequence, their chemical composition varies according to plant source and geographical area. From all kinds of hive products, propolis is one of the most valuable bee products, due to its rich content in nutrients and active substances with a lot of prophylactic and therapeutic effects, which must now be well studied and scientifically documented. Among these potential therapeutic effects, it will be discussed the antimicrobial, antibiofilm and antipathogenic effects of propolis, by its content in QSIs targeting the QS circuits, which is considered a new and intelligent anti-infectious strategy (alternative or complementary to antibiotics), efficient and without capacity to induce antibio-resistance.

**Keywords:** Propolis, Biofilms, Biofilm associated infections, Quorum sensing inhibitors



# **Impact of Biohybrid Magnetite Nanoparticles and Moroccan Propolis on Adherence of Methicillin Resistant Strains of *Staphylococcus aureus* : Application fields**

Pr Badiaa Lyoussi

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The biologically synthesized nanomaterials are an important aspect in biotechnology as well as in the field of nanomedicine. The capacity of nano-antimicrobial agents to penetrate bacteria and biofilm can make them as potential agents for controlling infectious diseases. Magnetite nanoparticles (MNPs) have been evaluated for inhibiting microbial growth and biofilm formation. In this study the effect of the nanocomposite Moroccan propolis extract / MNPs acting against methicillin resistant strains of *Staphylococcus aureus* (MRSA) was evaluated. MNPs were obtained through the co-precipitation method. The fabricated nanostructure was characterized by X-ray Diffraction (DRX), Transmission Electron Microscopy (TEM), and Fourier Transform-Infrared Spectroscopy (FTIR). TEM of MNPs provided a particle average size of 15 nm, FTIR spectral analysis enabled a fast way of identification of MNPs, attesting the occurrence of the different combinations. The use of MNPs loaded with propolis and the antibiotic chloramphenicol at Minimum Inhibitory Concentration (MIC) value inhibited the bacterial growth of MSSA (methicillin susceptible strain of *S. aureus*) and MRSA strains. After the treatment with MNPs-OA-P-CLO nanocomposite (MNPs with oleic acid, propolis and chloramphenicol), the disruption of the bacterial cell was observed by TEM. The combination of propolis and chloramphenicol in free form at MIC value largely impaired both MSSA and MRSA strains as, after 2 h of treatment, no viable cells of MRSA 2 and MRSA 16 were recovered. Hence, the results elucidated a new antibacterial nanocomposite synthesis, for possible applications as prospective nano-antibacterial agents or drug carriers.

The importance of nanoparticles as drug delivery systems with optimized physicochemical and biological properties consists in the fact that they are taken up by the cells more easily than larger molecules, so they can be successfully used as delivery tools for currently available bioactive compounds. Overall, in this study, we report the synthesis of a 15 nm nanostructure, which when combined with propolis extract and the antibiotic CLO, exhibited antibacterial activity against different *S. aureus* strains, including MRSA. The action of the propolis nanocomposite targeted the bacterial cell wall evidencing their disruption and protrusions formation. The obtained results demonstrated that the proposed strategy prove to have an important benefit due to its antibacterial properties on demand for numerous medical applications.

## ***In vitro* Effect of Ethanolic Extract of Propolis Toward the Growth of *Enterococcus faecalis***

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### **Abstract:**

Bacteria has a pivotal role to the occurred of dental pulp and periradicular disease. *Enterococcus faecalis* (*E faecalis*) was known as major bacteria that caused the failure of endodontic treatment. Propolis is a natural resinous product by honeybee from different plant sources. It has been used as a traditional antibacterial and anti-inflammatory medicine for many centuries. The purpose of this *in vitro* study was to know the most effective concentrations of Ethanolic Extract of Propolis (EEP) solution to inhibit the growth of *E. faecalis* bacteria. Propolis was collected from honeycombs in South Sulawesi, Indonesia. Propolis was subjected to maceration process using 95% ethanol solution for 5 days and evaporating to get EEP, which was then diluted in DMSO to 1%; 2%; 3%; 4% and 5% concentrations. *Aquadest* and 5.25% NaOCl solutions were also used as negative and positive control solution. *E. faecalis* were grown in medium nutrient broth and incubated with EEP solutions for 24 and 48 hours, at temperature 37°C. Antibacterial activity was reflected by the diameter of the inhibition zones which occurred around the stainless steel cylinder. Data was statistically analyzed using ANOVA followed by LSD test with significance level of 5%. The results of this study showed that after incubated for 24 and 48 hours, all EEP concentrations and 5.25% NaOCl significantly ( $P < 0.05$ ) inhibit the growth of *E. faecalis*. In conclusion, 1% EEP solution was the most effective concentration to inhibit the growth of *E. faecalis* bacteria after 24 and 48 hours incubated at temperature 37°C.

**Keywords:** Propolis, *Enterococcus faecalis*, antibacterial, *in vitro*, dental pulp.

## Summary of Antimicrobial Activity Studies on Turkish Propolis

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### **Abstract:**

My first encounter with bee products was with biological activity study including antimicrobial of three Turkish honeys. The article published in Food Chemistry in 2007, and the study was the highest referenced study with 732 citations. The next antimicrobial activity study was published in the International Journal of Food Properties in 2013 with 78 references. In this study, ten different Turkish raw propolis extract were used and their antimicrobial activities were determined by zone radius and MIC values by agar well diffusion method against six bacteria and two fungi. Next study in 2016, it was determined that Turkish propolis is very effective on *Helicobacter pylori* bacteria and inhibited its urease enzyme. In the next study, it was determined that propolis played a role as a stomach protector in experimental animals with experimental ulcers. In our study in 2021, it was determined that propolis was effective in the inhibition of Covid19 as an antiviral. In 2021, we had another study showing that propolis chewing gums are important for oral hygiene. Based on this study, *Staphylococcus aureus*, which is important for oral and dental health, we showed that Turkish propolis is effective against *Enterococcus faecalis*, *Streptococcus mutans*, *Lactobacillus acidophilus* and *Lactobacillus casei*. Our recent studies, we determined that ethanolic propolis samples have showed anti-quorum sensing, anti-biofilm and anti-swarming activity against some antibiotic resistant bacteria such as *Chromo bacterium violaceum*, *Chromo bacterium violaceum* and *Pseudomonas aeruginosa* PO1. As a result, it is possible to say that ethanolic Turkish propolis rich in caffeic acid, CAPE, chrysin, pinocembrin and hesperidin have high antimicrobial and antifungal potentials.

**Key words:** Propolis, antimicrobial, anti-quorum sensing, anti-biofilm and anti-swarming activities.

## **Antimicrobial and Antioxidant Activities of Natural and Fermented Bee Pollen**

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### **Abstract**

Bee pollen is a natural honeybee product promoted as a valuable source of nutritional substances, rich in biologically active substances, which gives this product antioxidant and antimicrobial properties. Bee pollen became more and more appreciated by consumers and it is used for therapeutic purposes. The natural fermentative process of transforming pollen into bee bread is accompanied by complex biochemical reactions as a result of the activity of microorganisms, especially lactic acid bacteria. The presence of lactic acid together with the low pH helps to preserve the product for a longer period. This study was performed to highlight the antibacterial activity of bee pollen and fermented bee pollen extracts, in various concentrations, against gram-positive and gram-negative bacterial strains, such as: *Escherichia coli*, *Salmonella enteritis*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*. Antimicrobial activity of samples was determined using broth dilution assays and disc diffusion methods. Antioxidant activity was measured by spectrophotometric methods. The results show that all pollen samples tested had significant antimicrobial activity, but the fermented pollen samples had a higher efficiency on Gram-negative bacteria. Minimum inhibitory concentration (MIC) range between <3.10 to 25.00 μL/g. Also, in the case of fermented pollen samples the antioxidant activity was higher. These biological activities can be explained by the high content in polyphenols, flavonoids, and carotenoids from bee pollen, but also by the synergism of these compounds. All this shows that fermented pollen can be used as a food supplement bringing an important supply of biological substances to the human body.

## **Evaluation of the Antidiabetic and Healing Honey from southern Algeria. “In vivo” study**

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### **Abstract**

Honey have long been used in traditional medicine, but their health benefits have been explained in recent decades, when the scientific world became concerned with tests and thus explained their physicochemical characteristics that show their qualities and their origins. The present study aims to demonstrate whether honey an ant diabetic, and wound healing effect in rats. Analysis of phenolic compounds was determined by HPLC and in vitro antioxidant activity was determined by two methods free radical scavenging (DPPH) and iron reduction (FRAP). Antidiabetic activity was evaluated in Wistar rats made diabetic by injection of streptozotocin (STZ) for 2 months. The wound healing capacity of honey was measured at the level of wounds in diabetic and non-diabetic rats. The analysis of the results of the physico-chemical parameters showed that the Algerian Sidr honey meets international standards. This honey is made up of 37.27% of fructose, 29.23% of glucose and 6.39% of sucrose and a total absence of maltose. Rich in polyphenols and flavonoids ( $47.35 \pm 3.35$  mg GAE / 100 g and  $1.20 \pm 0.20$  mg QE / 100 g, respectively). This richness in polyphenols has given Sidr honey significant antioxidant activity. Resveratrol, chrysin, protocatechoic acid, caffeic acid phenyl ester (CAPE), and rutin were the major phenolic compounds detected by HPLC. Oral administration of this honey at doses of 250, 500 and 1000 mg / kg in our experimental model of diabetes has shown a hypoglycemic effect and leads to appropriate changes in lipid, hepatic and even pancreatic histology profiles. This honey has significant wound healing activity in diabetic and non-diabetic rats. To conclude that the honey studied have antioxidant and anti-diabetic activities.

**Keywords:** honey, antioxidant, anti-diabetic, wound healing, HPLC.

## Antifungal Activity Of Olive Oil And Ethanol Extract Of Propolis

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### Abstract:

Propolis is a bee product that has been known for many years, but its popularity has increased in recent years. One of its beneficial biological activities is its antimicrobial property. Crude propolis is extracted using different solvents before use. The aim of this study is to determine the antifungal activity of propolis extracted using different solvents (water, ethanol and olive oil) by ultrasound assisted extraction method. A total number of 81 strains which isolated from various clinical samples in the study. These strains are *Candida albicans*, *C. parapsilosis*, *C. tropicalis*, *Saprochaete capitata*, *C. inconspicua*, *Candida glabrata*, and *C. krusei*. In vitro antifungal susceptibility testing was performed according to CLSI. Fluzonazole (FLU) served as positive control. The geometric mean MIC (GMMIC) of ethanol, olive oil and water extracts of propolis for *C.albicans* and *C. tropicalis* were 2.3-4.2 µg/ml, 55.8-64.9 µg/ml, 236.5-277.5 µg/ml. As a result, the MIC value of the ethanol extract of propolis against the tested microorganisms was lower than the water and olive oil extract, while the olive oil extract of propolis showed higher antifungal activity than the ethanol extract against the *Saprochaete capitata* strains. Olive oil extract, one of the non-ethanolic extracts of propolis, showed antifungal activity, showing that it can be used as an alternative to ethanol extract.

**Key words:** propolis, ethanol, olive oil, *Candida*, antifungal

## **Phytochemicals and Antibacterial Properties of Honeybee propolis from selected Regions in Kenya**

Timothy M. Kegode<sup>1</sup>, H. Michael G. Lattorff<sup>1</sup>

[www.icipe.org](http://www.icipe.org)

### **Abstract**

The demand for natural products to be used as antimicrobial agents is on the rise. This is being precipitated by microorganisms developing resistance against the conventional antimicrobial agents. This will lead to the development of antimicrobial resistant (AMR) strains. Bee products are among the natural products that exhibit antimicrobial activity. Propolis is of interest due to its plant derived composition and hence being rich in phytochemicals. Despite this, there is little information on the chemical composition of African propolis and its bio-functional properties. Hence in our study, we quantified the bioactive compounds, and determined the anti-microbial properties of honeybee propolis from various regions in Kenya, against Gram-positive and Gram-negative bacteria. Analysis using Principal Component Analysis (PCA) showed that the sampling region had a remarkable impact on the propolis's composition and its antibacterial properties. Furthermore, analysis of propolis by Gas Chromatography-Mass Spectrometry (GC-MS) indicated the presence of different compounds with varying antibacterial activities. These compounds included triterpenoids alpha- and beta-amyrin, oleanen-3-yl-acetate, urs-12-en-24-oic acid, and lanosta-8, 24-dien-3-one, which have been reported to have antimicrobial activities. The propolis samples collected from hotter climatic conditions contained relatively higher concentration of total phytochemicals, correlating with the anti-microbial activities as compared to those from relatively cold climatic conditions. Of great interest is that all propolis samples exhibited anti-microbial activities. Key findings of this study demonstrate the occurrence of relatively higher phytochemical content in Kenya's propolis, with antimicrobial properties. Hence, this potential of propolis could be harnessed for disease control.

**Key words:** Antimicrobial, Gram-positive, Gram-negative.

## Seasonal Studies of the Greenish-brown Propolis from Alagoas, Brazil: Preliminary Results

Ticiano Gomes do Nascimento<sup>1</sup>, Emanuel Guilhermino da Silva Junior<sup>1</sup>, João Victor Lessa de Oliveira<sup>1</sup>, Matheus Vinicius Guimarães de Melo<sup>1</sup>, Monique Almeida Vila Nova<sup>1</sup>, Lucas Rafael de Oliveira Silva<sup>1</sup>, Nataly Christine Soares Gama<sup>1</sup>, Kathylen V. Ferreira dos Santos<sup>1</sup>, Arthur L. Tavares Ferreira Borges<sup>1</sup>, Fernanda Geny Calheiros Silva<sup>1</sup>, Salvana Priscylla Manso Costa<sup>1</sup>.

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### Abstract:

The greenish-brown propolis from the União dos Palmares-Alagoas-Brazil region has been showing chemical characteristics of transition areas between an Agroforestry System comprising the agricultural production of tropical fruits near an area of wild forest in the Quilombos region in Alagoas. This research aimed to evaluate the variation of total flavonoid concentration and its influence on antioxidant, antibacterial and antifungal activities over a 12-month seasonal cycle. The samples were collected in apiaries in the region of União dos Palmares and Branquinha, Alagoas, Brazil. A comparative analysis of the results of antimicrobial activity assays (bacteria and fungi), antioxidant activity by the DPPH method, total flavonoids and HPLC-DAD allowed to identify months in which there was a lower concentration of flavonoid compounds (November 2020 and March and June 2021) with antioxidant activity and the percentage of total flavonoids decreasing similarly, while in the months of higher concentration of these compounds (December 2020 and January, April and May 2021), which presented better microbial activity and quantification of flavonoids. This study demonstrated the sensitivity of the methods in detecting variations in chemical composition in samples from the different months and apiaries studied. Some apiaries show characteristics of greenish-brown propolis from areas of wild forest and other apiaries of brown propolis from areas of agroforestry plantations.

**Keywords:** greenish-brown propolis, Total flavonoids content, Microbial activity, antioxidant activity, seasonal variation.



## **Propolis effects on people living with HIV under retroviral therapy**

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### **Abstract:**

Despite the benefits of the antiretroviral therapy (ART), its prolonged use and earlier initiation indicate the need of interventions to reduce its harmful effects, especially regarding the oxidative stress, which contributes to accelerate the inflammaging, a commonly phenomenon observed in people living with HIV/aids (PLWHA). Propolis is a bee product exhibiting several properties such as antioxidant and anti-inflammatory. This work aimed at evaluating whether the daily use of propolis could affect the metabolic/biochemical, nutritional and inflammatory/immune profile of PLWHA. A double-blind study enrolled 40 virologically suppressed PLWHA: 20 under intake of 500 mg/day of propolis and 20 placebo. Blood samples and medical records were collected at two periods of time, before and after three months of intervention. There were no changes in the food pattern during the intervention nor side effects or complaints, showing that the daily intake of propolis for 3 months is safe for asymptomatic PLVHA on ART. The viral load remained undetectable, showing no propolis interference in the therapeutic treatment. T CD4<sup>+</sup>/CD8<sup>+</sup> cell count remained elevated. Propolis increased magnesium concentration, lymphocyte proliferation and led to an anti-inflammatory profile. Such findings were not associated to sociodemographic and therapeutic features, nor to changes in food habits.

**Keywords:** propolis, apitherapy, HIV, antiretroviral therapy, immunomodulation.

## Phytochemical Composition and bioactivity of Propolis from Arid and Semi-arid Regions of the Sonoran Desert in North-Western Mexico

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### Abstract:

Propolis is a bioactive chemical matrix produced by honey bees (*Apis mellifera*) from resinous plant material. The pharmacological potential and phytochemical composition of propolis samples collected from arid (Caborca) and semi-arid zones (Ures and Pueblo de Álamos) of the Sonoran Desert in North-western Mexico, have been studied. At present, chromatographic and spectroscopic methods have revealed more than 30 compounds in propolis from Ures, Pueblo de Álamos and Caborca, samples mainly composed by phenolic acids, flavonoids and their ester derivatives, including chemical markers of poplar-type propolis, such as pinocembrin, chrysin, pinobanksin-3-O-acetate and galangin. The resinous material from *Populus fremontii* has been authenticated as the main plant origin for Sonoran propolis (SP). Several *in vitro* biological activities have been reported for SP and some of its constituents, including antioxidant, antibacterial, antiparasitic, antiproliferative activity in cancer cells and immunomodulatory effects. In addition, the chemical composition and pharmacological properties of SP have shown to be significantly influenced by a seasonal effect. In conclusion, SP represents a bioactive matrix with potential clinical applications. Further studies focused on the molecular mechanisms underlying the pharmacological potential of SP are needed.

**Keywords:** propolis, arid and semi-arid regions, flavonoids, poplar type propolis

## **Cycloartanes: As major Beehive Protective Agents in Southern Nigerian Propolis**

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### **Abstract:**

Cycloartane-type triterpenes: Cycloeucalenol and 24-methylene cycloartanol were isolated from all five (5) southern Nigerian propolis samples. The known compounds were identified, and structures elucidated by NMR experiments. The cycloartane triterpenes were major constituents of all the propolis samples under study. This may be attributed to the plants/trees sources foraged by bees in the region for propolis production. The antimicrobial assay of the Awka propolis crude extracts showed good antibacterial activities (between 23-28mm diameter zone of inhibition) and antifungal activities (25-34mm diameter zone of inhibition). The MIC and MBC/MFC values were between 0.625mg/ml and 2.5mg/ml respectively. This showed the extracts were highly bactericidal and fungicidal at low concentrations hence maybe of pharmaceutical relevance for drug/ supplements for various ailments.

**Keywords:** Propolis, Cycloartane-triterpene, cycloeucalenol, 24-methylene cycloartanol.

## Comparison antifungal activity of royal jelly and apilarnil

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### **Abstract:**

Royal jelly and apilarnil are bee products used for their beneficial biological activities in apitherapy. Since Apilarnil is a newly recognized product, there are more studies on the antimicrobial activity of royal jelly. The aim of this study was to examine the antifungal activity of royal jelly and apilarnil against 84 clinical yeast isolates. The isolated yeast strains were identified as *Candida albicans* (n: 13), *C. parapsilosis* (n: 13), *C.tropicalis* (n: 13), *Saprochaete capitata* (n: 13), *C. inconspicua* (n: 13), *Candida glabrata* (n: 10), *C.krusei* (n:9) by conventional methods and DNA sequence analysis. *In vitro* antifungal susceptibility testing was performed according to the recommendations proposed by the Clinical and Laboratory Standards Institute. The antifungal activity of lyophilized apilarnil against *C. albicans* was approximately 8 times higher than that of royal jelly.

**Key words:** royal jelly, apilarnil, yeast, antifungal activity

## **Antibacterial and anti-biofilm effect of bee venom from the East-West region of Algeria against multiresistant Gram positive bacteria of hospital origin**

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### **Abstract :**

The rapid increase in drug resistance and the failure of available antibiotics to treat biofilm-associated infections is a major health concern. There is thus an urgent need to develop naturally bioactive compounds. This study aims to identify the most common MDRBs found on medical devices and responsible for biofilm formation to meet the urgent need to develop new antibacterial substances to control infection and eradicate biofilm. An evaluation of the *in vitro* activities of the apitoxin produced by the bee *Apis mellifera* from Tlemcen-Algeria using the electric shock method on the growth of the bacteria *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis* clinical isolates. The antibacterial activity of bee venom by the determination of the minimum inhibitory concentration and (CMIB) for planktonic and sessile cells, performed by the broth microdilution method. The results revealed that the amount of bee venom collected for a week was 236 mg/colony of dry venom and the MIC values obtained for the clinical isolates varied from 23.43 µg/ ml à 11.71 µg/ mL and the CMIBs was higher than and equal to the MICs. These results indicate that bee venom inhibits the growth and survival of multiresistant strains. Therefore, we suggest that bee venom can be used as a natural antibiofilm antimicrobial agent. This survey aims to promote the use of natural products, in particular bee products (venom) in Algeria, to find alternative solutions to reduce and inhibit the adhesion of microorganisms to medical devices and consequently the eradication of biofilms and infections related to medical implants.

**Keywords:** Bee venom, *Apis mellifera*, Biofilm, Clinical isolates, Multiresistant, Antibacterial, Antibiofilm.

## **Clinical use of Honey Dressings in the management of Infected Wounds**

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### **Abstract:**

Honey has been used since ancient times to treat wounds, especially infected ones. Modern scientific studies and research have revealed its complex properties and effects on wound healing: antibacterial, anti-inflammatory, anti-edematous and anti-exudative, antioxidant; free radical control; ensuring moist local environment through the viscosity of honey and drawing fluids by osmosis; wound debridement; increasing the rate of healing and nourishing action in the wound, indirectly through osmotic flow of lymph and also directly through the intake of easily metabolized nutrients from honey. In particular, laboratory experiments and clinical trials have shown the broad-spectrum antibacterial action of honey due to its acidity, osmotic effect, hydrogen peroxide content, nutritional and antioxidant content, immune boosting and other non-peroxide compounds. Resistance to the application of honey in wound therapy, due to its lack of standardization and its sticky and fluid nature, can now be overcome by the production and marketing of honey-based medicinal products, licensed and approved for local wound treatment. There are currently several brands (L-Mesitran, Activon, HoneySoft, Manuka Health, Medihoney, MelMax, MelDra, etc.) and a wide range of sterilized products containing honey and registered as medical devices available on the market for the topical treatment of wounds. This work also presents the favorable results obtained in a lot of patients with infected wounds, who were treated in the Plastic Surgery Department of the District Emergency Hospital of Ploiești, Romania. Medical grade honey dressings exerted a beneficial effect on the healing of burns and other types of wounds, with gradual remission of the infection, sometimes in combination with surgical treatment, being applied in these cases before or after surgery. Further clinical trials are needed to establish the best indications, methods, and forms of administration for honey-based dressings.

**Keywords:** medicinal honey, wound infection, wound healing

## **Anti-microbial Properties of the Beehive air, a Short Review**

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### **Abstract:**

Beehive air therapy has been used to promote wellbeing and treat different diseases of the respiratory system, improve respiratory capacity, regulate the immune system and even to help people suffering from depression. Each one of the products elaborated by the bees has an incredibly rich chemical composition, and the environment inside the hive is rich and chemically complex. If we consider all the products of the bees, there are thousands of different molecules inside the hive, and the volatile compounds also combine one to another thereby creating new compounds in the air. A few studies on chemical composition of beehive's air have already identified more than 50 volatile compounds present in the products of the beehive (e.g., honey, beeswax, propolis, etc.), and indicated that the pharmacological properties of the beehive's air compounds are closely related to their anti-inflammatory, anti-asthmatic, and antimicrobial actions. Regarding its antimicrobial properties, the air of the beehive has been shown to be effective when tested against different types of bacteria that can affect the respiratory system, indicating a promising future for the beehive air therapy. This presentation aims to provide a short review on the antimicrobial properties of the volatile components of the air of the beehive.

**Keywords:** beehive air therapy, antimicrobial properties, scientific validation.

## **Prevention and treatment of vaginal candidiasis with Api-Phyto-Aromatherapy Elbens Azevedo<sup>1</sup>**

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### **Abstract:**

*Candida* is an opportunistic organism and can promote fungal infections when there is an imbalance of the microbiota of the female external genitalia. Pre-existing chronic diseases, such as diabetes, the use of antibiotics, hormonal contraception, and other drugs as well as inappropriate eating habits and clothing can favor the installation of fungal infection. Increased resistance to available antifungals has become a concern. The products of the hive, mainly propolis and beeswax associated with essential oils, extracted from aromatic plants, are well-known antimicrobial agents, characterized by a wide spectrum of activities, including antifungal properties. We formulate a vaginal cream composed of Propolis (botanical origin *Populus* species) 1%, 50µ/ml; Beeswax 100g; Coconut oil 25g; essential oils (*Melaleuca alternifolia* 49.8 mg; *Mentha spicata* 49.8 mg and *Rosmarinus officinalis* 49.8 mg). Still, the intention is not only to do symptomatic treatment but a holistic apitherapeutic approach considering the woman as a single indivisible being. Hopefully, we can contribute to women's health concerning that very common and annoying disease.



## Microbiological Evaluation of Perga

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### Abstract:

Bee bread has rich nutrient content and probiotic properties and is widely used in traditional medicine. We aimed to investigate whether there is difference between fresh and stored perga in terms of microbiological reproduction, and susceptibility of isolated strains. The 34 strains were isolated in taken fresh perga samples. No microorganism was growth in stored perga samples. The isolated strains were identified by conventional and MALDI-TOF MS methods. The 34 strains were identified as *Aspergillus spp.* (n:12), *Rhizopus oryzae* (n:6), *Mucor circinelloides* (n:1), *Bipolaris* (n:2), *Trichoderma* (n:3), *Paecilomyces variotii* (n:1), *Penicillium chrysogenum* (n:1), *Kodamaea ohmeri* (n:1), *Bacillus altitudinis/pumilus* (n:3), *Bacillus licheniformis* (n:1), *B. megaterium* (n:1), *Micrococcus luteus* (n:1) and *Serratia marcescens* (n:1). MICs values of itraconazole, voriconazole, anidulafungin and caspofungin for *Mucor* and *Rhizopus* strains had high ( $\geq 32$   $\mu\text{g/ml}$ ) except amphotericin B, posaconazole. MICs values of drugs for *Aspergillus* strains (n:9/12) were low (1  $\mu\text{g/ml}$ ). *Trichoderma* strains had low MIC values ( $\leq 0.50$   $\mu\text{g/ml}$ ) for other drugs except itraconazole. *P. chrysogenum* was found to have low MIC value ( $\leq 0.25$   $\mu\text{g/ml}$ ) for POS, AND, CS. *Bipolaris*, *P. variotii* and *K. ohmeri* as had low MIC values to all antifungals.

**Key words:** Bee bread, bacteria, fresh or stored, molds, susceptibility, yeast.

## **Composition Influenced *in vitro* Antibacterial Activity of Honey's on Bee Associated and non-bee Associated (environmental) Bacteria**

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### **Abstract:**

Honey has proven antibacterial activity against a range of bacteria. Honey's antibacterial activity depends on its phytochemical composition. Honey is increasing in popularity as alternative medicine because of its antibacterial effects. It is claimed that bacteria might not develop resistance to honey as a range of phytochemical might confer the antibacterial effect. However, bacteria might adapt to their environmental niches to enhance survivability. This work investigated six honeys' composition and their antibacterial effect against four honeybee-adapted bacteria (HAB) and two honeybee-non-adapted (HNB) (environmental) bacteria testing whether HAB might have adapted to honey and are suppressed less than HNB. Additionally, the bacterial candidates were grouped into Gram-positive and Gram-negative bacteria and their antibacterial response to the different honey investigated. Spectrophotometric and colorimetric methods were used to quantify the honeys' physico-chemical parameters, phytochemicals, and antioxidant activity. In addition, gas chromatography-mass spectrometry (GC-MS) was used to analyse honey volatiles and dichloromethane (DCM) extract chemical profiles. The zone of inhibition assay was used to assess antibacterial activity. The honey had variable composition with high bioactive phytochemical content. They also possessed varied physicochemical parameters. The HAB experienced slightly reduced growth inhibition by the honey compared to the HNB. The position of the bacteria within the honeybees' gut corresponds to the level of resistance. Bacteria residing in the crop and frequently exposed to honey's phytochemicals have a stronger resistance to honey, while those originating from the midgut show a higher suppression. Some honey samples showed enhanced and bacteria-specific inhibition, while none of the honey was superior in suppressing all bacteria. Bacteria can develop resistance against honey upon frequent exposure, which needs to be considered when promoting honey as alternative medicine.

# ISOLATION AND CHARACTERISATION OF ALPHA AND BETA AMYRINS FROM PROPOLIS OBTAINED FROM BENUE STATE, NIGERIA

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## ABSTRACT

Chemical composition of propolis has been reported to be phyto-geographical in nature. The aim of this study was to isolate and characterize chemical compounds from hexane and ethyl acetate extract of propolis obtained from Gboko, Benue State, Nigeria. To isolate compounds, extract was subjected to column chromatography by gradient elution using two solvent mixtures - hexane: ethyl acetate and ethyl acetate: methanol. The structure of the isolated compound was established using <sup>1</sup>H-NMR and further verification of data on the compound by comparison with literature reports.  $\alpha$ -amyrin,  $\beta$ -amyrin,  $\alpha$ -amyrin acetate and  $\beta$ -amyrin acetate, known pharmacologically active pentacyclic triterpenoids, were isolated from the hexane and ethyl acetate extract of this propolis.

**Keywords:** propolis, column chromatography, ethyl acetate, <sup>1</sup>H-NMR,  $\alpha$ -amyrin,  $\beta$ -amyrin,  $\alpha$ -amyrin acetate and  $\beta$ -amyrin acetate.

*N.B. For any questions regarding this document feel free to contact*

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