Antimicrobial natural products: apitherapy

D. Mello Bobáný and R. R. Cabral Martins

1Course of Veterinary Medicine, Center for Health Sciences, University Center Serra dos Órgãos, Campus Quinta do Paraíso, Estrada da Prata S/N, Prata, CEP 25.976-340, Teresópolis, Rio de Janeiro, Brasil.

The current world trend in the search of alternative therapies in the treatment of common diseases comes from interest to minimize side effects normally present in conventional therapies and the need of resources accessible to the population. Why are alternative therapies economically viable, socially just and environmentally correct, based on clean and sustainable technologies, are particularly important in societies where there is social inequality deep, due to the high cost of scientific medicine, examinations sophisticated, complex surgical interventions and the modern equipment of diagnosis. Honey has been used by man for over 2000 years, in the most varied forms of application and with different objectives inherent in their use. However, in more recent years, there has been a revival of interest in use of honey as a therapeutic agent with antimicrobial active ingredients. Although little known, the honey bee sting (ASF) has a similar therapeutic action to the honey of *Apis* and has been used in popular therapies.

Keywords Complementary Therapies; Apitherapy; Antibiosis.

1. Introduction

Bees belong to the class Insecta, order Hymenoptera and Apidae's family. There are about 20 thousand species of bees described and distributed in nine families throughout the world. However, less than 5% of these species are social and belong to the families Halactidae, Anthophoridae and Apidae. The family Apidae is divided into four subfamilies: Bombinae, Euglossinae, Meliponinae and Apinae. The sub-family Meliponinae is composed of stingless bees (SB) and divided into two tribes: Meliponini and Trigonini. Already Euglossinae and Bombinae correspond to solitary bees, and in the Apinae's sub-family are found bees that have stingers, genus *Apis*, primarily responsible for the production of honey in the world.

The bees give us honey, propolis, pollen, beeswax, royal jelly and poison, in addition to being the most important insect pollinators of the planet.

In addition to countless nutritional properties, the honey bees of the genus *Apis* has medicinal properties fairly known, with studies in humans in the treatment of some diseases since ancient medicine. Their action as topical antibacterial and healing has already been proven, being an alternative to low financial cost.

The honey of stingless bees (SB) presents different composition of the honey of *Apis mellifera*, differentiated in its consistency, aroma, color and flavor, and lately has been studied, together with the propolis, for their antimicrobial properties.

However, the study of medicinal properties both of *Apis* and SB, in application in veterinary medicine is very recent, requiring greater attention by researchers, which may contribute to the minimization of undesirable effects normally attributed to conventional therapies.

2. Definition and characteristics of main product of bees with antimicrobial action

2.1. Honey and its characteristics

Honey is known by man since prehistoric times. Always was used as food as well as for its medicinal properties. Currently the man learned to protect swarms, install them in rational hives and handle them with purpose both to reap the products produced by bees, as well as to study them and get to know better their therapeutic activities, particularly its antimicrobial action.

Honey is a viscous substance, aromatic and sweet produced by bees from the nectar of flowers.

The average composition of honey summarizes at three components:

- Sugars (monosaccharides such as glucose and fructose and disaccharides including maltose and sucrose);
- Water (which in honey has strong interaction with the sugar molecules, leaving fewer water molecules available for microorganisms) and;
- Various elements that depend on the specific characteristics of different floral sources (enzymes, proteins and amino acids, organic acids, minerals, vitamins, flavorings, hidroximetilfufural, among others).

Research has shown that many pathogenic bacteria and some fungi are susceptible to the antimicrobial activity of honey.
2.2. Honey and antimicrobial properties

2.2.1. Antibacterial action

Several factors contribute to the antimicrobial activity of honey as the high osmotic pressure (bactericidal and bacteriostatic actions), low water activity, low pH, low protein content, high carbon nitrogen ratio, high content of reducing sugars, low dissolved oxygen content.

The antimicrobial property may vary according to the floral source of the honey. Analysis shows that monofloral honey has antimicrobial potential higher than the honey mixed.

Studies "in vitro" have observed that the honey from *Melipona subnitidae* is effective against antibiotic-resistant bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The honey bee is a supersaturated solution (strong osmotic activity) between pH 3.2 and 4.5 (inhibits the growth of many microorganisms) that contains hydrogen peroxide produced by glucose oxidase (the most important antibacterial component of honey). The honey of *Melipona subnitidae* over infected skin of rats stimulated the immune response and reduced infection and healing time. [1]

Researchers noticed some important facts, such as the observation of high antimicrobial activity of honey obtained from *Nannotrigona testacea* against Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus spp.*), also the observation of the sensitivity of Gram-positive (*Staphylococcus spp. coagulase-negative Streptococcus pyogenes*). [2]

Our studies "in vitro" with honey *Tetragonisca angustula* aimed to analyze the effectiveness of honey on the microorganisms found in inflammations of the auditory meatus of dogs. In preliminary microbial identifications was seen in secretion of the ear a mixed infection comprising *Staphylococcus* sp., *Bacillus* sp. and yeast.

It is noteworthy that, in this experiment, there was no microbial growth in the honey sample seeded in Tryptone Soya Broth (TSB) and the result of the microbiology of the material from Petri dishes of the antibiogram, other microorganisms were not identified beyond of preliminarily found, confirming the safety of the product.

When compared to conventional antibiogram, the honey of *Tetragonisca angustula* showed antimicrobial activity less than the CFL (cephalexin - 40 mm), higher than OFX (ofloxacin - 15 mm), PEN (penicillin - 10 mm) and ENO (enrofloxacin - 10 mm) and very close to AMP (ampicillin - 21 mm) and CIP (ciprofloxacin - 20 mm), demonstrating the excellent therapeutic potential of the honey of this species on the microorganisms tested (Fig. 1).

![Fig. 1](image)

Honey of *Apis mellifera* has a proven antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

2.2.2. Action against fungi and yeasts

Our studies "in vivo" showed that in ear secretions of dogs (Canis familiaris) and cats (Felis catus), which lies a mixed microfauna of bacteria often associated with yeast species Malassezia pachydermatis, the topical use of honey "in natura" of *Tetragonisca angustula*, had antimicrobial activity in external otitis of these animals, being effective in controlling Malassezia spp. as well as the bacteria *Staphylococcus spp.*

The fact that the pH of honey of *Tetragonisca angustula* be acid (around 3.27) by itself qualifies it as an inhibitor of growth both of *Malassezia spp* (Fig. 2) as *Staphylococcus spp.*, which has its pH optimum between 5 and 7.
Another work developed by our research team demonstrated, "in vitro", the antifungal activity of honey bees of Tetragonisca angustula against Sporothrix schenckii, a fungus responsible for sporotrichosis, important to be a zoonosis. In the present study, the material was collected from skin lesions in cats.

2.2.3. Antiviral action

Researchs with antiviral action of honey bees are not yet available in the literature and studies are needed to check this possibility.

2.3. Propolis and its characteristics

We called propolis the resinous substance with complex composition collected by bees of various plant’s parts such as buds, buds, bark and resinous exudates. It has this name, which derives from the Greek (pro means "defense" and polis "city") since it is used by bees to defend the hive.

Propolis is used to repair cracks or damage to the hive (thermal insulation and enemies) in the preparation of sites for aseptic posture of Queen Bee and mummification of invasive insects, since to propolis is attributed still an antimicrobial action. Their properties vary according to their botanical origin. It has also highlighted as anti-inflammatory, healing, anti-ulcer and anti-interference, as well as functional food.

Its chemical composition is complex and varied, being closely related to the ecology of the flora of each country visited by bees and the period of resin's collection, featuring a range of pharmacological activities of propolis best in most tropical regions of the planet and lower in the temperate.

The identification of components of propolis has been performed by techniques of high performance liquid chromatography, capillary gas chromatography and mass spectrometry, which demonstrated the great complexity and diversity of these components.

Some components are present in all samples of propolis, while others derived from propolis occur only in particular species of plants.

Propolis has in its basic composition approximately 50% of vegetable resins, 30% beeswax, 10% essential oils, 5% pollen, 5% waste wood and soil.

The average chemical composition of propolis include flavonoids (such as galangin, quercetin, pinocembrin, kaempferol, apigenin, hesperetin, rutin, luteolin, genistein, daidzein and anthocyanidin), aromatic acid and esters (for example palmitic acid, oleic acid, stearic acid, malic acid, succinic acid, benzoic acid, trans-4-coumaric acid, furluc acid, caffeic acid and 3,4-dimethoxycinnamic), aldehydes and ketones, terpenoids and phenylpropanoids (such as caffeic acid and chlorogenic acids), steroids, amino acids, polysaccharides, hydrocarbons, fatty acids, vitamins B1, B2, B6, C, E and PP, and various other compounds in small quantities. There is also in its constitution inorganic elements such as sodium, potassium, magnesium, barium, strontium, cadmium, lead, copper, manganese, iron, calcium, vanadium, silicon, aluminum, nickel, zinc, chromium, titanium, silver, molybdenum and cobalt.

Of all these groups of compounds, the flavonoid and phenolic acids certainly has been calling the attention of researchers, since it are granted the antibacterial, antiviral and antioxidant properties.

The tropical propolis samples, especially the Brazilian's propolis, are shown significant differences in their chemical compositions relative to propolis from temperate zone. For this reason, the Brazilian propolis becomes the subject of great interest from scientists.

A study of propolis samples from Tetragonisca angustula and Apis mellifera from different regions of Brazil showed that propolis from Apis mellifera presents wide variation in the composition of the samples determined by their geographical origin. However, the samples of propolis of Tetragonisca angustula showed the same profile on all regions analyzed, indicating that these bees are very selective about the plant used as source resins.
2.4. Propolis and antimicrobial properties

2.4.1. Antibacterial action

The antibacterial properties of propolis are attributed mainly to flavonona pinocembrine, flavonol galagina and phenethyl ester of caffeic acid, with a mechanism of action that is probably based on the inhibition of bacterial RNA polymerase. Other flavonoids, caffeic acid, benzoic acid and cinnamic acid must act on the membrane or cell wall of the microorganisms, causing structural and functional damage.

Studies with propolis of Apis mellifera demonstrate effectiveness against gram-positive bacteria (Staphylococcus aureus and Enterococcus sp) and gram negative (Escherichia coli, Pseudomonas aeruginosa) being greater action against Gram positive than Gram negative that is limited.

Still cannot say clearly the reason of lower biological activity of propolis against Gram-negative bacteria, however, it is suspected that this increased resistance is due to the fact that these bacteria possess a cell wall chemically more complex and higher lipid content, that somehow hinders the action of the active components of propolis.

Some bacteria have tried to analyze the antimicrobial activity of propolis of Apis mellifera.

- Escherichia coli,
- Escherichia cloacae,
- Staphylococcus aureus,
- Staphylococcus epidermidis,
- Bacillus subtilis,
- Bacillus cereus,
- Klebsiella pneumoniae,
- Listeria monocytogenes,
- Pseudomonas aeruginosa,
- Salmonella typhimurium,
- Salmonella enteritides.

Research shows the inhibition of gastric ulcers by intake of propolis, possibly related to anti-Helicobacter activity.

The propolis of stingless native bees (SB), such as Tetragonisca angustula, Nannotrigona testaceicornis, Scaptotrigona sp. and Melipona sp. showed that are highly effective against Staphylococcus aureus and Escherichia coli. [3]

Our studies using the geopropolis of Tetragonisca angustula in different concentrations, in cultures using milk from positive animals test with California Mastitis Test (CMT), the extract of geopropolis to 80%, formulated using the methodology described by Park et al. [4] demonstrated to be effective against pathogenic bacteria like Staphylococcus sp. coagulase-negative, one of the most common bacteria responsible for subclinical mastitis cows. (Fig. 3 e 4).

Fig. 3 Microbial growth of Staphylococcus sp. present in mastitic cows milk indicated by turbidity in TSB culture medium.

Fig. 4 Comparison of antibiogram:
(A) clear appearance (B) microbial culture turbid.
Authors found antimicrobial properties of propolis against experimental rat caries caused by Streptococcus mutans and Streptococcus cricetus related to elements in their composition, analyzed by chromatography and classified as cinnamic acid, ethyl ester cinnamic acid and caffeic acid.

In human researchs conducted in "in vivo" and "ex vivo", it can be concluded that propolis extracts at different concentrations have the same antimicrobial efficacy, and compared to the efficacy of manufactured products, the 11% propolis extract appears to be the most suitable, due to their antimicrobial efficacy associated with the lowest concentration. [5]

2.4.2. Action against fungi and yeasts

Propolis, according to some researchers, has antifungal activity even in very small quantities, but its activity is proportional to the concentration of propolis, particularly when used alcoholic extracts.

Propolis has also shown excellent activities fungistatic and fungicidal in tests "in vivo" against yeasts identified as causes of onychomycosis.

Some fungi and yeasts that are sensitive to propolis:

- Actinomyces sp,
- Candida albicans,
- Candida tropicalis,
- Candida parapsilosis.

2.4.3. Antiviral action

Antiviral drugs intervene in some stage of virus replication and depend of the metabolism on the virus / cell for its action. There is no report that the antiviral are able to effectively eliminate the virus from the infected body, simply because its metabolism is fully associated with the host cell.

One of the goals of antiretroviral therapy is, selectively, to inhibit induced enzymes encoded or enzymes by the virus by using a drug that does not affect specifically the enzymes normally involved in biochemical processes similar to the host cell.

To be effective and have few side effects, antiviral drugs have to be more toxic to the virus than to the cell.

An antiviral substance must meet features like:

- Provide broad spectrum of antiviral activity and be effective in a large group of viruses;
- Have enough power for complete inhibition of viral replication;
- Have favorable pharmacokinetic properties to react only in the target organ,
- Provide minimal toxicity to the host cell,
- Do not suppress the normal development of active immunity of the individual,
- Not be susceptible to the development of viral resistance variants.

There are not many reports on the antiviral activity of propolis but a virucidal action of propolis on herpes simplex virus (HSV) and vesicular stomatitis virus (VSV) has been observed. [6]

Applications of propolis in the dermatological area have demonstrated activity on simplex herpes and genital herpes. [7]

In a study conducted in Ukraine was compared the efficacy of propolis ointment Canadian with acyclovir and placebo in the treatment of patients with recurrent genital herpes type 2. The preparation of propolis containing flavonoids proved to be more effective than the other two in wound healing and reduction of local symptoms. [6]

Studies "in vitro" suggests that propolis has potent antiviral activity against variants X4 and R5 HIV-1, affecting replication of influenza virus (influenza type A and B) of vaccinia virus and avian herpes. Similar activity was observed with CD4 + lymphocytes operating, at least in part, as an inhibitor of viral entry. [9]

In our research we evaluated "in vitro" the antiviral activity of propolis bees of Apis mellifera and Tetragonisca angustula against viral strain identified as bovine herpesvirus (BHV). The resins of propolis samples were diluted in dimethyl sulfoxide (DMSO) at a dose of 100% 10mg/ml. The bovine kidney cells (MDBK) were cultured in EAGLE HANKS medium containing 5% inactivated fetal bovine serum. Cell viability was determined by the activity test by the method mitochondrial reduction of MTT (3 - (dimethylthiazol-2-yl) 2,5 diphenyltetrazolium bromide).

In this experiment, all concentrations of propolis Tetragonisca angustula proved to have greater activity in inhibiting the production of viral particles than Apis mellifera propolis although both have shown an anti-BHV.

In this experiment, the analysis of cytotoxicity, we found that propolis of Apis mellifera is less toxic than Tetragonisca angustula and compared with acyclovir (ACV), propolis has a much lower cytotoxicity.

With regard to the concentration of compound able to inhibit by 50% the production of viral particles, we observed a dose-dependent behavior, characterized by an increase in viral inhibition with increasing the concentration of propolis.

For the Selectivity Index (SI), the propolis Tetragonisca angustula presents a greater degree of safety "in vitro".
2.4.4. Antiprotozoal action

Although Castro and Higashi have demonstrated antiprotista action of propolis on mice infected with Trypanosoma cruzi, studies to deepen this knowledge are scarce yet. [10]

Acknowledgements

Would like to thank the Veterinary practitioners graduates by UNIFESO, Adonis Moreira de Oliveira, Bruno Soares Palmieri, Carlos Bastos Augusto Constancio and Cintia Raye Maciel, who during their studies collaborated with this research team in the development of work in the line of apitherapy.

References

[9] FARNESI AP. Efeitos da própolis de abelhas africanizadas e meliponíneos em microorganismos. 2007. 89f. Dissertação (mestrado) - Faculdade de Medicina de Ribeirão Preto (USP), Ribeirão Preto.