Chemical Diversity of Propolis from Meliponinae: An Ancestral Treasure to be Preserved

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ABSTRACT

Propolis is a mixture made by bees consisting of plant resins and metabolites, salivary enzymes and wax. Some species of bees add soil to this mixture forming geopropolis. This material is used in the defense of the hive as a physical barrier and antimicrobial agent, ensuring the health of the colony. Propolis has been extensively studied and several chemical constituents have been identified, mainly flavonoids, terpenes and phenolics. With the emerging microbial resistance to antibiotics, the interest in the search for active compounds, mainly secondary metabolites of plants, has been increasing significantly. In this paper, we describe the characteristics of the main species of native stingless bees found in South America, especially in Brazil, the ancestral use of propolis produced by them, its chemical composition and its potential for the development of new therapeutic compounds, along with the challenges that the survival of bees face.

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I. INTRODUCTION

Since immemorial times, the therapeutic resources used by humanity came from nature, notably from plants, animals and minerals. The search for pain relief and cure of illnesses through the ingestion of herbs may have been one of the first ways of using natural products (VIEGAS JUNIOR; BOLZANI; BARREIRO, 2006). Plants produce a huge variety of compounds, commonly related to defense mechanisms, including antimicrobial activity. This great diversity of phytochemicals is due, in part, to the evolutionary need to struggle with microorganisms, insects, nematodes and other plants. This defense system effectively prevents infections caused by most plant pathogens (SIMÕES; BENNETT; ROSE, 2009).

Propolis is one of the natural products that has been used by mankind for various therapeutic purposes. It consists of a mixture of resin from plants collected by bees with the wax and saliva of these insects (LAVINAS et al., 2019). Some species of stingless bees mix this resinous material with the soil, forming the so-called geopropolis (LIBERIO et al., 2011). It has great importance for the health of the colony, as it is used in the construction of nests and to seal the hive, preserving the inside temperature and preventing the entry of unwanted visitors. Propolis also acts as a chemical defense against microorganisms, due to its antimicrobial properties (LAVINAS et al., 2019).

In its constitution it can be found about 50% vegetable resins, 30% beeswax, 10% essential oils, 5% pollen and 5% wood and earth waste (MENEZES; 2005). However, the yield of the volatile fraction has usually been reported in the order of 1% (BANKOVA; POPOVA; TRUSHEVA, 2014). The volatile compounds are responsible for the characteristic aroma of propolis and have significant biological activity. Several compounds were identified in their composition, mainly mono and sesquiterpenoids (BANKOVA; CASTRO; MARCUCCI, 2000). Studies have demonstrated the importance of these

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compounds against pathogenic microorganisms, such as Gram-positive and negative bacteria of medical importance (SIMIONATTO et al., 2012; BANKOVA; POPOVA; TRUSHEVA, 2014).

II. IMPORTANCE OF THE PROSPECTION FOR NEW MEDICINES AND THE ROLE OF PROPOLIS

Newman and Cragg (2020) highlighted, in an extensive review, the importance of natural products and structures derived from or related to natural products as a source for new molecules. In the period between January 1981 and September 2019, 126 antibacterial molecules were approved (disregarding prophylactic agents). Of the 126, about 48% (78) are derived from natural products or unaltered natural products. However, since the advent of antibiotics in the 1950s, virtually no antimicrobials have been developed from plant sources. The main natural sources of these agents were bacteria and fungi. With the emergence of resistance to antimicrobials, interest in the search for other sources, mainly secondary metabolites from plants, has increased significantly (SIMÕES; BENNETT; ROSE, 2009; ABREU; MCBAIN; SIMÕES, 2012).

To produce propolis, bees collect the plant resins by selecting the best compounds capable of protecting the nest and ensuring its survival. Considering this natural pre-selection based on the knowledge of these insects, the composition of propolis and its biological activities have aroused the interest of several researchers (BANKOVA et al., 1999a). Despite the great diversity of the bees native to Brazil, most of the studies investigating the composition and activities of propolis are carried out with samples produced by exotic bee species introduced in the country. Studies on its volatile fraction are even more scarce. Knowing that the chemical composition of propolis is quite variable and is related to the species of bee that produces it, seasonality and its geographical location, studies with propolis and geopropolis of native stingless bees provide potential unprecedented results and the discovery of compounds with antimicrobial activity (OLIVEIRA et al., 2009).

2.1 Bee species

Since ancient times, bees have aroused curiosity and human interest. In Greece, they were seen as priestly and chaste animals. The local currency had the image of a bee, symbolizing wealth. The Romans considered them as a representation of territorial defense (SFORCIN et al., 2017). In Christianity they were related to various qualities, above all hope, due to their tireless work, and resurrection, for disappearing in winter and resurfacing in spring (LEXIKON, 1997).

Bees have developed behavioral characteristics, such as the distinction of colors and aromas, that aid in the search for nectar and pollen: their main food sources. These characteristics also benefit the plants, because during foraging, incidentally, they transfer the pollen of the anther of one flower to the stigma of another, performing pollination (TRIPLEHORN; Johnson, 2005).

The order *Hymenoptera* is one of the most diverse groups of species in the class *Insecta*. *Hymenoptera* presents a wide variety of complex habits and behaviors, culminating in the social organization of wasps, ants and the most important pollinating agents: bees (TRIPLEHORN; Johnson, 2005). The bees belong to the superfamily *Apoidea*, which has several families, among them the *Apidae*, which has more advanced social habits and is divided into four subfamilies: the *Apinae* (bees of the genus *Apis*, with about 11 species), the *Meliponinae* (stingless bees, with hundreds of species), the *Bombinae* (bumblebees, with about 250 species) and the *Euglossinae* (orchid bee, with about 175 species) (NOGUEIRA-NETO, 1997).

The subfamily *Meliponinae* is believed to have been the oldest to branch off from less social ancestors and develop high social behavior. A fossil of a female stingless bee about 80 million years old has been found wrapped in amber. This bee is considered the oldest known species of social bee and has characteristics similar to living species (CRANE, 1999).

2.2 Meliponinae subfamily

The first written records about the meliponines arrived in Europe in the sixteenth century and were made by Spanish and German explorers in Central and South America. These reports and later archaeological studies, particularly in the Mesoamerican region, contributed to the discovery of important data on the traditional search for honey and on the management of meliponines, which was already well structured at least 300 BC. More advanced research occurred only in the nineteenth century, almost two centuries after the beginning of scientific research on bees (HRNCIR et al., 2016).

Meliponines are also known as stingless bees (SB) or indigenous bees because they are traditionally bred by indigenous populations (SILVEIRA et al., 2002). More than 600 species have been described and are found in South America, Central America, southern North America, Africa, Southeast Asia and Northern Oceania. In Brazil more than 200 species can be found, distributed in 29 genera, being *Plebeia, Trigona, Melipona, Scaptotrigona* and *Trigonisca* the ones with the largest number of known species (LAVINAS et al., 2019).

Brazil is home to the largest diversity of SBs in the world. Native species are distributed throughout the Brazilian territory, mainly in the Amazon region. The warm climate and the abundant flora in species that can provide nectar, pollen and resin are favorable conditions for the existence of these bees (VENTURIERI, 2008). They are especially sensitive to low temperatures and depend on the structure of their nests for the thermoregulation of the hive (KLEINERT et al., 2009).

The nests of the meliponines are considered the most elaborate among bees. Some species nest in exposed places, such as on branches, but more often the nests are found in pre-existing cavities in tree trunks or anthills and abandoned termite mounds. They are protected by one or several layers of a casing composed of wax and resin, which assists in maintaining the temperature. The colony is delimited by the bitumen, which consists of a mixture of wax, resin and clay (NOGUEIRA-NETO, 1997; MICHENER, 2000). The upper layer of bitumen is usually compact to avoid infiltration and the lower one is riddled, allowing the flow of water (VILLAS-BÔAS, 2012).

The entrance of the nest presents particular characteristics for each species and can be constituted of geopropolis, clay, cerumen or pure wax (VILLAS-BÔAS, 2018). Some species build quite narrow entrances, which are guarded by a single bee, others build larger entrances that allow the circulation of multiple guards (FONSECA et al., 2017). Camouflage and occlusion of the entrance are strategies that can also be undertaken to prevent invaders (SFORCIN et al., 2017).

Female bees have a well-developed ovipositor (egg-laying organ), which can be modified to form a stinger, which is used as a defense mechanism (TRIPLEHORN; Johnson, 2005). However, female meliponines have a stunted stinger and are therefore unable to use it, and so are known as stingless bees (SFORCIN et al., 2017).

They are considered more docile; however, they are not helpless bees. When they feel threatened by large invaders, such as men, they can curl up in their hair or fur, pinch with their sharp jaws, penetrate holes such as ears and nostrils, or release unpleasant odors. Some species produce formic acid in their mandibular glands, which when released, can cause serious burns (BALLIVIÁN et al., 2008; SFORCIN et al., 2017). The SBs with tamer behavior protect their nests by building them in places of difficult access, including inside anthills or near more defensive bee nests (OLIVEIRA et al., 2013).

These bees have eusocial behavior, that is, they live in well-organized communities, where the queen is responsible for reproductive work and the workers take care of the offspring, the provision of food and the construction and defense of the nests (FONSECA et al., 2017). Food storage is done in pots consisting of cerumen, where pollen and honey are stored separately (VILLAS-BÔAS, 2012).

SBs differ from bees of the genus *Apis* in several aspects. While *Apis* exhibit uniform behaviors and morphology, meliponines are quite diverse. Some species are very small, smaller than fruit flies and others have robust bodies (VELIKOVA et al., 2000; HRNCIR et al., 2016).

The size of the colony is also variable and can house tens to thousands of individuals. The nests may be arranged in clusters or combs. Foraging can be done by groups or solitary bees and is generally characterized by shorter flights, which implies the elaboration of propolis using the plant resources closest to the colony (VELIKOVA et al., 2000; HRNCIR et al., 2016).

The ecological relevance of these insects is undeniable. They act as pollinators of several native and cultivable plants, contributing to the conservation of different biomes and agricultural production, directly impacting the production of fruits and seeds and consequently the economy (BONSUCESSO et al., 2018). The honey produced by ASF is marketed in some regions of Brazil, for its appreciated flavor and medicinal properties. These bees also produce propolis, which has been the subject of studies around the world due to its pharmacological properties (LAVINAS et al., 2019).

2.3 Native bees: sociocultural importance and ethnoknowledge

Indigenous peoples have a close relationship with the meliponines. Before the introduction of the species *Apis mellifera* and the culture of sugar cane in America, the honey of the native bees was used by the indigenous as an indispensable source of energy for the great journeys in the food search. The popular names of many species such as Jataí, Tiúba, Jandaíra, Guarapu and Manduri are part of the sociolinguistic indigenous heritage (VILLAS-BÔAS, 2012).

Indigenous knowledge is closely related to nature. The legacy of these peoples has immeasurable value and has been passed down through the generations. In Mexico the creation of SBs and the use of its products date back to the pre-Columbian period. For the Maya people, one of the most relevant ancient civilizations in history, the SBs played an important role in religious ceremonies, feeding and treating diseases (AYALA et al., 2013).

In Brazil, the Kayapó people use elaborate techniques for the management of swarms, such as platforms that allow the reach of hives in tall trees. In this people, bee specialists are shamans, who possess extensive knowledge about the anatomy and behavior of various SB species. The people use honey and pollen in food, cerumen and resins in the waterproofing of canoes, in addition to being inspired by the social organization of these insects (BALLIVIÁN et al., 2008). For the Pankararé, indigenous people in northeastern Brazil, the creation of 'abeia mansa' (meek bee) is a recreational and medicinal activity. Honey is used in food as an energy source and is extracted in a non-predatory way, ensuring the maintenance of the tree where it was collected and the colony (FONSECA et al., 2017).

The knowledge about the SBs of the Guarani Mbyá people of the Morro da Saudade village, in the city of São Paulo, is transmitted orally through the generations. The teachings are followed and improved by the younger ones and some members of the community are seen as great connoisseurs of the subject. The products from bees are used in handicrafts, religious rituals and medicinal potions, as well as activities related to spiritual and contemplative life (RODRIGUES, 2005). Santos and Antonini (2008) conducted a study on the traditional knowledge about the SBs of the Enawene-Nawe people of the state of Mato Grosso. They were able to conclude that the people were able to identify several species of SBs not only by morphology but also by the ecological and social characteristics of these insects.

Meliponiculture has also been practiced for generations by other traditional communities such as 'quilombolas' (descendants of black slaves), 'ribeirinhos' (inhabitants of riverbanks), 'sertanejos' (inhabitants of the hinterland), 'caipiras' (countrymen) and 'caiçaras' (traditional fishing communities). The products from the SBs are used for subsistence family consumption and are a source of complementary income. These peoples contribute to the conservation of bees, through the management of species that are practically no longer found in natural habitat, due to the devastation of the native forest. The rational beekeeping of SBs can therefore be a sustainable strategy to promote biodiversity conservation (VILLAS-BÔAS, 2018).

2.4 Introduction of exotic bee species in Brazil

The introduction of the European bee species *Apis mellifera* in Brazil occurred around 1839, brought by the Portuguese to produce wax candles. In 1845 German immigrants brought more bees and settled in the south of the country. Other colonizers have also introduced European bees into different regions (ATHAYDE et al., 2016). In 1956, the biologist and geneticist Dr. Warwick Kerr, at the request of the Brazilian government, brought from Africa some queens of bees to perform crossing by artificial insemination, which would originate a species capable of producing more honey in the tropical climate (BALLIVIÁN et al., 2008; ATHAYDE et al., 2016).

The following year, swarms and their respective queens escaped accidentally and ended up crossing with the European species already inserted in the Brazilian territory, emerging hybrid populations, today called Africanized bees. This incident had an extensive environmental impact since these bees spread to almost the entire American continent (BALLIVIÁN et al., 2008; ATHAYDE et al., 2016).

2.5 Impact of anthropogenic actions on bees

The introduction of exotic species, accidentally or for economic purposes, can cause significant changes in natural environments, such as changes in habitats, hybridization and competition with native species. In addition, the intensive occupation of the environment by man impacts bee populations by eliminating food sources, destroying substrates necessary for the construction of nests and by pesticides (SILVEIRA et al., 2002).

Due to the intense bee die-off, several studies were conducted to evaluate the toxicity of agrochemicals, but most of them were carried out with the species *Apis mellifera*, endemic in several regions of the world. The study conducted by Dorigo et al. (2019) evaluated the effect of the insecticide Dimethoate on the SB species *Melipona scutellaris*, where the average lethal concentration (LC_{50}) was 320 times lower than for *Apis mellifera* larvae, demonstrating the sensitivity of this species to the pesticide and the need to conduct further studies using native bees.

The deforestation of forest areas for urban and agricultural use has also significantly reduced bee populations. The scarcity of resources, competition between species, predation by invaders and inbreeding due to population decrease, can make swarms captive to a small territorial space, or lead them to extinction (SILVEIRA et al., 2002).

Even if the mellipones adapt, Portal et al. (2023) verified that the major volatile compounds in the propolis of native bees in the Itajaí Valley region, in southern Brazil, are compounds found in Pinus and Eucalyptus, indicating that this is the most important plant source in the elaboration of propolis by those specimens. This 'reforestation' with exotic trees certainly contributes to the difficulty of discovering new molecules with biological activity and potential for the development of new drugs.

As bees contribute to the maintenance of forests through pollination, several species of *Meliponinae* depend on forest environments and are not found in anthropogenic environments, except near forests. This issue is an aggravating factor in Brazil, considering that some species of the genus *Melipona* are translocated to regions distant from their natural occurrence (SILVEIRA et al., 2002).

2.6 Propolis

Propolis is a mixture of substances used by bees in the defense of the hive. Worker bees collect resinous materials from shoots, exudates, and other parts of plants and pack them in their corbicles (part of the posterior tibia used to transport pollen, clay, and resin). These resins are biotransformed by bees with the addition of their salivary enzymes and wax (PRZYBYŁEK; KARPINSKI, 2019; BREYER; BREYER; CELLA, 2016). Physically, propolis has quite variable characteristics. They may have a hard and brittle consistency or be sticky and elastic. The coloration can also vary between cream, yellow, green, light brown or dark brown (SALATINO et al., 2005).

The name propolis is derived from the Greek words *pro*, in defense of, and *polis*, the city, e.g.: "in defense of the city or the hive." In fact, this material has great importance for the health of the colony. Bees use propolis in the sealing and repair of crevices to prevent the entry of invasive insects and maintain the internal temperature (MARCUCCI, 1996; SALATINO et al., 2005). In addition, it is used against microorganisms in the asepsis of the places where the laying of eggs is made. And if the bees cannot remove a dead invader from the hive, they use propolis to embalm it, which prevents decomposition and bacterial proliferation in the nest (MARCUCCI, 1996; SALATINO et al., 2005).

The plant materials used in the composition of propolis are produced by various botanical processes in different parts of the plants. The collection of these materials is a difficult activity to observe and often occurs high in the trees. Among the substances collected, lipophilic compounds from the leaves, mucilages and resins can be highlighted (BANKOVA; CASTRO; MARCUCCI, 2000).

2.7 Historical and popular use of propolis

Propolis has been used in traditional medicine since antiquity. The Egyptians used it to embalm the dead and thus prevent putrefaction. Its properties were also recognized in Greek and Roman medicine by Aristotle, Dioscorides, Pliny and Galen, who employed it in asepsis and wound treatment and as an oral disinfectant. Its use was perpetuated in the Middle Ages in Arab medicine and was recognized by New World civilizations (CASTALDO; CAPASSO, 2002).

In the seventeenth century, propolis was included in the London Pharmacopoeia. Between the seventeenth and twentieth centuries, its use became popular in Europe due to its antibacterial action. It is currently used in various pharmaceutical forms such as extracts, mouthwash, lozenges and formulations for topical use. Also, its employment in the food and cosmetic industries have been benefiting from the propolis properties (CASTALDO; CAPASSO, 2002).

In 1908 the first scientific work on propolis, its chemical properties and composition was indexed in Chemical Abstracts. The first patent appeared decades later in the same index of scientific literature, in 1968, where Romanian propolis was employed in the production of bath lotions. Since then, several studies on propolis have been published, possibly due to its panacea characteristic and its added value (PEREIRA; SEIXAS; NETO, 2002).

Currently, propolis is used for various therapeutic purposes as an antibacterial, antifungal, antiviral, anti-inflammatory agent and to increase the body's natural resistance to infections. Formulations for external use are employed in the treatment of dermatitis and wounds. It is also available in capsules of pure extracts or in combination with other natural products. Throat lozenges and *spray*, mouthwash and hydroalcoholic or glycolic extracts are widely used as a folk remedy (CASTALDO; CAPASSO, 2002).

2.8 Geoprópolis

Some SB species add soil to propolis, giving rise to the so-called geopropolis. Although it is not its main constituent, the presence of earth is a differential in the composition of this product. Its coloration is variable and is related to the plant origin, the soil used in its constitution and the species of bee that produces it. The chemical composition and biological activities of this bee product are still little known (BONSUCESSO et al., 2018).

Fritz Müller, a German naturalist who migrated to Santa Catarina in the mid-nineteenth century, in one of his letters to Charles Darwin, about the habit of various insects, mentioned his observations on the genus *Melipona* and the constitution of propolis. He observed that these bees not only used wax in the construction of the nest structures but added other materials such as resins and soil (MÜLLER, 1874).

III. CHEMICAL COMPOSITION OF GEOPROPOLIS FROM MELIPONINES

In 1998(a), Bankova et al. identified more than 50 compounds present in the Brazilian geopropolis. Among them, the geopropolis of Melipona *compressipes* and *Melipona quadrifasciata anthidioides* collected in the states of Piauí (Northeast) and Paraná (South) respectively. The extracts of these samples were submitted to chemical analysis through GC-MS and a complex chemical composition was observed. The samples showed significant amounts of lactic, phosphoric and long-chain fatty acids, such as stearic and palmitic acid.

Araújo et al. (2015) analyzed the chemical composition of *Melipona fasciculata* geopropolis, collected in Maranhão state (North). The main constituents found were carbohydrates and their derivatives (19.8%), triterpenes (15.9%), hexoses (11.9%), anacardic acid (8.3%), lupeol (7.3%) and alkylresorcinols (5.9 %). Disaccharides, glucuronic acid, salicylic acid and isomers, β-amyrin, inositol, and xylitol, among others, were also found.

In the work of Silva, Muniz and Nunomura (2013) samples of geopropolis of the species *Melipona interrupta* and *Melipona seminigra*, collected in municipalities of Amazonas (Nort), were evaluated for their composition, evidencing the significant presence of phenolic compounds.

Santos et al. (2017) evaluated the composition of the aqueous and hydroalcoholic extracts of *Melipona quadrifasciata*. Rutin, gallic acid, gallocatechin, epicatechin gallate and syringic acid were identified in the aqueous extract. In the hydroalcoholic extract, the main constituents found were quercetin, epigallocatechin, p-hydroxybenzoic acid, epigallocatechin gallate and coumaric acid.

Propolis contains volatile constituents to a lesser extent. This fraction, however, can provide relevant information about the antimicrobial activity and elucidate the classes of compounds present in its composition, contributing to the identification of its botanical origin (TORRES et. al., 2008). Volatile compounds are among the main secondary metabolites present in plants. They perform important functions that ensure survival and adaptation to the environment. Among these functions can be mentioned attraction of pollinators and seed dispersers, protection through repulsion or intoxication and antibacterial, fungicidal and insecticidal action (BAKKALI et al., 2008).

Volatile oils (VO), also called essential oils (EO) when obtained directly from plants, are a complex mixture of lipophilic substances, consisting mainly of terpenes, fatty acid derivatives, amino acid derivatives and phenylpropanoid compounds. In propolis, terpenes constitute a large part of the volatile compounds (RUFATTO et al., 2017).

Terpenes are classified according to the number of isoprene units (C_5H_8) present in their structure: monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{40}$), tetraterpenes ($C_{40}H_{64}$) and polyterpenes (DUBEY; BHALLA; LUTHRA, 2003). Monoterpenes make up a large part of the EO and can contribute more than 90% of its total composition. Sesquiterpenes are another important group found in the EOs. Terpenes can be biosynthesized by the classical mevalonate pathway in the cytosol or by the alternative deoxylulose pathway that occurs in plastids (CABALLERO; TRUGO; FINGLAS, 2003).

EOs can be obtained by distillation or pressing of plants or parts thereof. At room temperature, they are liquid and volatile, with a characteristic odor. The density of EOs is generally lower than that of water, but sassafras, cinnamon and clove oils may have higher densities (CABALLERO; TRUGO; FINGLAS, 2003). The composition of an VO can present a major constituent, facilitating chemical correlation with biological activity. However, small amounts of other substances present can act synergistically, contributing to a certain biological action (YUNES; CECHINEL FILHO, 2016). However, the composition of these oils is variable and is directly related to seasonality and the place of collection (OLIVEIRA et al., 2009).

IV. BIOLOGICAL ACTIVITIES OF GEOPROPOLIS COMPOUNDS

Some studies have highlighted the importance of investigating the biological potential of geopropolis, mainly the antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antimycoplasmic and antimutagenic properties of SBs geopropolis of the genus *Melipona*.

Dutra et al. (2014) evidenced the antioxidant potential of the hydroalcoholic extract of geopropolis of *M. fasciculata*. The action was correlated with the presence of phenolic compounds such as phenolic acids, gallotannins and ellagitannins. Batista et al. (2016) also suggested that the high concentration of phenolic acids, such as gallic and ellagic acid, was responsible for the antioxidant action of the samples tested.

In the work of Santos et al. (2017) the antibacterial activity of the hydroalcoholic extract of M. *orbignyi* geopropolis was evaluated against the Gram-positive bacterium *S. aureus* (sensitive and methicillin-resistant strains) and Gram-negative *E. coli* (sensitive and cephalosporin-resistant strains) and *P. aeruginosa* (strains sensitive and resistant to amphotericin B). The antibacterial action was attributed to the presence of phenolic compounds, which have some known mechanisms of action, among them the permeabilization of the microbial cytoplasmic membrane.

Valcanaia et al. (2022) investigated the antimicrobial potential of the volatile oil of *Melipona q. quadrifasciata* geopropolis against bacteria with and without cell walls. The antimicrobial action with the lowest minimum inhibitory concentration was against the bacterium without cell wall *Mycoplasma pneumoniae* 129 strain. The volatile oil was fractionated and its subfractions were tested, but these showed no improvement in antibacterial activity. This characteristic had already been observed by Kujumgiev, Tsvetkova and Serkedjieva (1999), who concluded that the isolated compounds from propolis did not present better results regarding the activities tested, suggesting that the synergy between the compounds favors biological actions.

In the literature, it is possible to find studies that confirmed the antimicrobial activity of the volatile constituents of propolis against several microorganisms. Against Gram-positive bacteria such as *S. aureus*, *Staphylococcus epidermidis*, *Micrococcus glutamicus*, *Bacillus subtilis*, *Bacillus cereus*, *Sarcina lutea*, *Streptococcus pyogenes*, *Streptococcus mutans, Streptococcus faecalis* and Gram-negative bacteria such as *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *P. aeruginosa* (BANKOVA; POPOVA; TRUSHEVA, 2014).

A study conducted in Greece investigated the chemical composition of volatile propolis compounds from different geographic regions. The predominant constituents were terpenoids, especially α-pinene. Other components have also been identified as α-eudesmol, δ-cadinene, α-muurolene, guaiol and trans-β-terpineol. The *in vitro* antimicrobial activity was evaluated against 6 species of bacteria, including *S. aureus*, *P. aeruginosa* and *E. coli*, confirming the antimicrobial potential of the samples tested (MELLIOU; STRATIS; CHINOU, 2007).

In the study by Simionatto et al. (2012), the volatile constituents of propolis samples collected in three municipalities of Rio Grande do Sul, α-pinene and β-pinene were presented as major constituents. The antimicrobial activity was evaluated by the agar diffusion method against *S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *E. coli* type strains. The antibacterial activity was classified as moderate and the presence of monoterpenes in the samples was attributed to this activity.

Fernandes et al. (2015) evaluated the antimicrobial potential of the volatile oil of *Apis mellifera* propolis collected in Mato Grosso do Sul, Brazil (West). The activity was evaluated by the broth microdilution method against strains of *P. aeruginosa, Klebsiella pneumoniae, Enterococcus faecalis* and *S. aureus*. The volatile oil and two of its isolated constituents - (E)-nerolidol and spathulenol - showed antimicrobial properties.

Oliveira et al. (2009) tested the antimicrobial activity of the volatile oil of *Apis mellifera propolis* collected in Rio de Janeiro. The microorganisms *Staphylococcus aureus, S. epidermides, Streptococcus pyogenes* and *E. coli* were susceptible to the sample tested. The chemical composition presented as major constituents β-caryophyllene (12.7%), acetophenone (12.3%) and β-farnesene (9.2%).

Portal et al. (2023) found that the *M. b. schencki* geopropolis VO with the best minimal inhibition concentration (MIC) was 424±0 µg mL^{-1} against all the mycoplasma strains evaluated. Fractionation of the VO resulted in a reduction of 50% of the MIC. However, its compounds' synergism seems to be essential to this activity. Antibiofilm assays demonstrated 15.25% eradication activity and 13.20% inhibition of biofilm formation after 24h for one subfraction at 2x its MIC as the best results found. This may be one of the essential mechanisms by which geopropolis VOs perform their antimicrobial activity.

V. BACTERIAL RESISTANCE TO ANTIBIOTICS AND THE NEED FOR NEW DRUGS

The increasing microbial drug resistance has been observed worldwide and with increasing mortality, prolonged hospital stays and rising costs, with other sectors of society being impacted beyond healthcare (WHO, 2015).

Between 2011 and 2014, the National Health Safety Network in the U.S. reported high levels of resistance to various antibiotics in Gram-positive bacteria including methicillin-resistant

Staphylococcus aureus, and Gram-negative bacteria such as third-generation cephalosporin-resistant *Escherichia coli* and carbapenem-resistant *Pseudomonas aeruginosa* (WHO; EMP; IAU, 2017).

Data from the European Centre for Disease Prevention and Control from 2015, when compared to US data, showed lower rates of resistance in Gram-positive bacteria and equally worrisome rates among Gram-negative bacteria (WHO; EMP; IAU, 2017).

The U.S. Centers for Disease Control and Prevention estimates that at least 23,000 deaths from resistant infections occur each year in the U.S. In Europe, in the year 2007 alone, 25,000 deaths were attributed to infections by resistant microorganisms (YU et al., 2016).

If this current trend continues, it is estimated that by 2050 there will be 10 million additional deaths due to antimicrobial resistance, surpassing other significant diseases such as cancer and diabetes. In addition, in the same year, microbial resistance is estimated to cumulatively cost $100 trillion worldwide (YU et al., 2016).

Natural products offer an obvious source for the research and development of new pharmacological treatments of infections. Moreover, it is more rational to use the millenary wisdom of bees, that learned to look for the best chemical compounds in nature to produce their propolis for the defense of their hives, in every environment, instead of testing individually each plant species for antimicrobial compounds.

VI. CONCLUSION

Propolis from native stingless bees have an enormous potential to be unveiled, both in its use in the form of herbal medicines and in the discovery of new molecules with potential for drug development. Many species of bees have not even had their propolis chemically characterized. However, it is necessary that their environment be preserved, and that the bees themselves be preserved, so that humankind can benefit from this ancestral treasury.

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