


REVIEW

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# Stingless bee propolis: composition, biological activities and its applications in the food industry

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## Abstract

The breeding of stingless bees, known as meliponiculture, can be found throughout the world, and is closely linked to the history of the first human communities in the Americas. This activity provides products of industrial interest (food, cosmetics, pharmaceuticals), and among them propolis stands out. This matrix is a resinous material but being difficult to classify and conceptualize due to its variability according to the environment and producing species. The propolis produced by these bees is a source of compounds with nutritional and functional potential, but the main interest is focused on the phenolic compounds. This potential makes propolis a product that can be used in the prospection of new molecules with antimicrobial and antioxidant activity. Thus, the scientific literature describes propolis activity (generally extracts) against bacteria and fungi, but with a higher microbicidal activity against gram-positive bacteria. The high antioxidant activity of this bee product is a consequence of the presence of several phenolic compounds. The use of propolis from SBP (Stingless bee propolis) is still quite limited, generally restricted to its antioxidant potential, but studies with application in other sectors can benefit this productive sector, as with the propolis produced by the bee *Apis mellifera*. This work presents and discusses the composition of stingless bee propolis and its consequent biological activities, as well as its potential applications in the food industry.

**Keywords** Antioxidant, Antimicrobial, Functional food, Meliponiculture, Phenolics

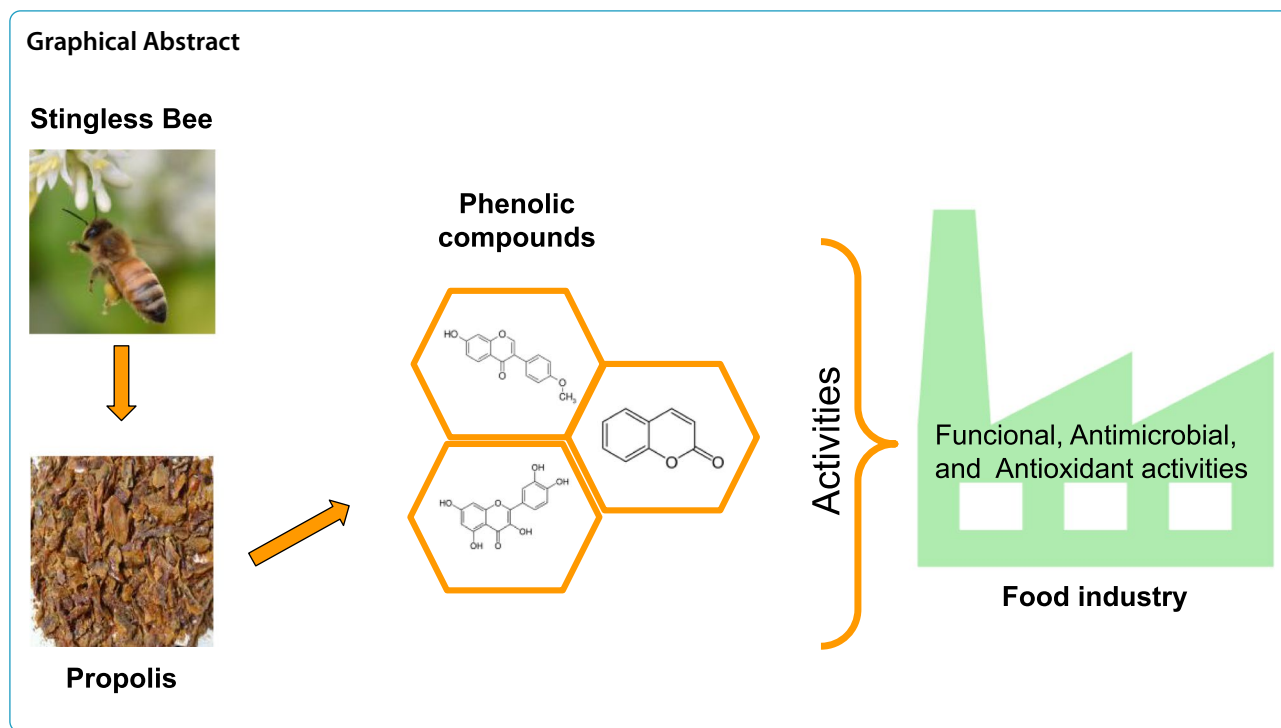
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### Introduction

Stingless bees are so called because of their atrophied sting, but they are also known as indigenous bees. They are mainly found in the tropical and subtropical areas of the globe. The breeding of these insects is known as meliponiculture. Stingless bees play an important role since they are the main pollinators of agricultural crops and natural environments (Barbiéri & Franco 2020). In addition to the influence of the pollination process in food safety and production of vegetables, bees have a portfolio of products that have been explored for a long time, such as honey, pollen, wax and propolis. These products have been used both for food and for traditional therapeutic purposes for several centuries (Maroof & Gan 2022; Quezada-Euán 2018).

The word “propolis” comes from the junction of the Greek words “pro”, which means defense, and “polis”, city (Zulhendri et al. 2021). The name highlights its function, since it is a mixture of plant resins, parts of plant tissues, bee’s saliva and wax, with the aim of protecting the hive against invaders, being used to seal openings and to embalm dead individuals (Zulhendri et al. 2021; Dezmirean et al. 2020; Shanahan & Spivak 2021).

The interest in stingless bee propolis has grown in recent years as a way to value and preserve native bees, and to investigate compounds with biological functionality. The typical composition presented in works on propolis is 50% resins and vegetable balm, 30% beeswax,

5% pollen, and 10% essential and aromatic oils (Anjum et al. 2019). However, this composition is not definitive and can vary considerably according to the locality, and the existing international parameter is resumed to the acceptance of propolis with more than 25% wax (Hogendoorn et al. 2013). The profile of chemical compounds of propolis is heavily studied, mainly with regard to its richness of phenolic compounds, due to the wide therapeutic application of these compounds (Popova et al. 2019). Furthermore, a line of investigation that has gained more and more space is the use of propolis as a technological component in foods, especially in regard to preservation against oxidizing agents and spoilage and pathogenic microorganisms (Santos et al. 2019; Thamnopoulos et al. 2018; Vasilaki et al. 2019). Recently, with the development and improvement in technology for medicines, there has been an emerging interest in the study of propolis and its diverse constituents (Zullkiflee, Taha, & Usman 2022).

In this work, it has been addressed: characteristics of stingless bees, description of Meliponiculture, Propolis and its functional characteristics, biological activity, compounds (mainly phenolic) responsible for antimicrobial, and antioxidant activity.

The information presented in this work was selected from scientific articles related to propolis, stingless bee propolis, and food science, with information organized in tables (functional and nutritional activity, chemical composition, antimicrobial, antioxidant), and text.

The databases used were PubMed, Scientific Electronic Library Online (Scielo), SCOPUS and Web of Science, using keywords alone or in combination and referring to the topics stingless bee, propolis, antimicrobial, antioxidant, chemical profile, and food. The review included 40 works that specifically deal with the propolis of stingless bees.

Given the possibilities of use as a functional food and the presence of molecules with technological effects, the objective of this work was to understand the application of propolis in the area of Food Science.

### Meliponiculture

The term “meliponiculture” was introduced in 1953 by one of the pioneers in the research on stingless bees in Brazil, Paulo Nogueira Neto, and is defined as the rational breeding of stingless bees. However, the practice of breeding these insects has been carried out since before the colonization process of the Americas (Barbiéri & Franco 2020). Nowadays, this activity is seen as an income opportunity for small producers, since the value for stingless bee honey can reach \$100/kg in the international market, while for the sting bee (*Apis mellifera*), honey costs \$20–40/kg (Se et al. 2018; Shadan et al. 2018).

It is important to emphasize that not all stingless bees are suitable for the practice of breeding and obtaining products, so the definition can be restricted only to stingless bees that produce and store a greater amount of honey (Abduh et al. 2020). The Meliponini tribe is composed of more than 500 species divided into 61 genera, which are distributed in tropical areas of the globe (Popova et al. 2019; De Menezes Pedro 2014); only in Brazil, 244 species are found, of which 87 are endemic and divided into 29 genera (Hrncir et al. 2016). These bees make meliponiculture viable because they are easy to maintain without the need to use special equipment for their handling, since they are docile bees, in addition to being an activity that can be developed both in rural and urban areas (Braghini et al. 2021). In Latin America, countries like Brazil, Mexico and Costa Rica maintain a long tradition of stingless bee breeding. This activity can also be found in the African and Asian continents, in addition to Oceania, more specifically in Australia (Cor-topassi-Laurino et al. 2006).

In Mesoamerica, meliponiculture is believed to have started in the Yucatan Peninsula and northern Guatemala, where the activity progressed along with the people who inhabited this region, where honey was used as food and as part of rituals (Quezada-Euán et al. 2018). In Mayan society, meliponiculture may have been an activity considered to be highly specialized, and honey and

wax played a fundamental role in trade, but were also offered as gifts and tribute payments (Paris et al. 2018). Nowadays, Mexico plays an important role in research on bee products, being one of the main countries to produce research on this specific topic (Popova et al. 2019).

Meliponiculture has also developed in aboriginal tribes from Australia, but the activity, despite being conducted for a long period, remains small and lacks research, which is important considering the process of pollination of these bees in agricultural crops (Quezada-Euán et al. 2018). Research with native species of stingless bees has already been started in Australia with the purpose of evaluating contamination carried by these insects that can harm the environment, the bees themselves and humans (Halcroft et al. 2013). The global trend of valuing stingless bee products in tropical countries is repeated in Australia, promoting the interest of breeders in obtaining greater economic income than products originated from Africanized bees (bees originated from the crossing of the European bee with the African bee) (Zhou et al. 2018).

In the African continent, meliponiculture is currently practiced mainly in tropical Africa, but the greatest diversity of stingless bees is found in the equatorial part of the continent (Salatino et al. 2019). However, meliponiculture lacks historical information in this part of the world, and its practice is still quite reduced when compared to traditional beekeeping, being that 50 species of stingless bees have already been identified in Africa (Vit et al. 2012). Thus, this activity is still quite artisanal in Africa, where some communities use logs and clay pots as breeding sites, but there is an interest by some African countries in the development of meliponiculture (Chidi & Odo 2017).

In Asia, particularly in Malaysia, the stingless bee, which is known locally as *lebah kelulut* in Malaysia, is an important species that is well adapted for tropical countries and has emerged as an alternative source of honey. More than 38 stingless bee species have been identified in Malaysia but only four species are commercially cultivated: *Geniotrigona thoracica*, *Heterotrigona itama*, *Lepidotrigona terminata* and *Tetragonula leviceps* (Mustafa et al., 2018).

### Propolis

Propolis is a product made by bees from the mixture of parts of plant tissues, bee wax and saliva, and plant resins, which has the function of protecting the hive both physically and microbiologically (Zulhendri et al. 2021; Dezmirean et al. 2020; Shanahan & Spivak 2021). It has a composition of 50% resins and vegetable balm, 30% beeswax, 5% pollen, and 10% essential and aromatic oils (Anjum et al. 2019), but this composition varies greatly

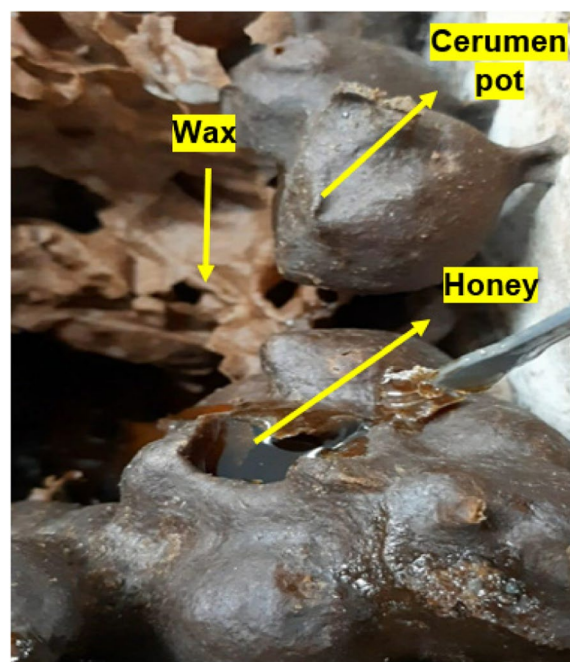
in relation to the producing species, place of origin and time of the year in which it was collected. For example, it is possible to question the frequently cited content of 50% resin and balm in propolis (Hogendoorn et al. 2013), since there are few studies where the quantification of balms and resins was performed, there are reports of values greater than 70%, contrary to the 50% widely publicized (Papotti et al. 2012). There are also resins that are deposited on the floor or walls of the bee nest and which, for some producers, are considered as a “pre-propolis”, being also known as resin deposit or viscous propolis deposit (Shanahan & Spivak 2021).

There is another product known as geopropolis, which basically is propolis mixed with soil, and which only a few species of stingless bees can produce (*Melipona scutellaris*, *Melipona marginata*, *Melipona quadrifasciata*), but it also plays a role in nest defense and structuring and there are works that have inadequately treated propolis and geopropolis as a single product (Popova et al. 2019). A problem with its study is that the addition of soil confers physical and chemical characteristics quite different from the pure propolis, such as a greater rigidity, as well as peculiar chemical profiles, such as the composition of minerals, directly related to their presence in the soil of a specific region (da Cruz Ferreira et al. 2020; Ferreira et al. 2019).

In addition to geopropolis, another product that causes divergence in the literature is cerumen, as the term is mistakenly used as propolis in some works, since cerumen is resin mixed with beeswax (Popova et al. 2019). As it can be seen in Fig. 1, the chemical composition of cerumen, which forms the honey storage pot, directly interferes with the chemical characteristics of this product, due to the transfer of antimicrobial and antioxidant biocompounds. This fact increases the divergence of the data found in the literature, because, many times, the type of sample analyzed is not well explained, whether it is propolis or geopropolis (Souza et al. 2021).

In an attempt to classify propolis (Park et al. 2000; Kasote et al. 2022), they have been categorized into 12 groups, where the parameters used were the physicochemical characteristics and the region of collection, but the work itself admits the non-inclusion of some propolis due to particularities of vegetation and frequency of appearance. After a few years, one more group was added to the other 12, the red propolis, that is present in mangroves in Northeast Brazil, whose botanical origin is the plant *Dalbergia ecastophyl* (Alencar et al. 2007; Dausch et al. 2008). No studies were found relating the propolis produced by stingless bees to any of these groups.

The knowledge of the botanical origin of propolis is extremely important due to the strong correlation of the



**Fig. 1** Structure of a stingless bee nest

chemical profile of the product with that of the botanic source used as a source (Dausch et al. 2008). For stingless bees, broader studies are still lacking, and the plant sources used by them and that are most described are *Mouriri* (Melastomataceae), *Drymonia* (Gesneriaceae) and *Stigmaphyllon* (Malpighiaceae) (Buchmann 1987). This situation points to an important gap to be elucidated in the knowledge related to stingless bees.

The resin is one of the main elements within the hive, as it is involved in its construction and maintenance, defense against invaders and microorganisms, being a source of biocompounds for the various products made by indigenous bees (Souza et al. 2021; Leonhardt & Blüthgen 2009). An interesting fact to note is that the amount of resin produced by a plant is not decisive for the bee to forage it (Lavinias et al. 2019), and not every tree attracts these insects. Regarding the attraction made by plants to bees (colors, volatile compounds) terpenoids are the most important volatile constituents in propolis, and together with phenolics, they constitute the main biocompounds found in the product (Lavinias et al. 2019).

#### **Propolis as a functional food**

A food is classified as functional when its properties are beyond nutritional ones, generating physiological and/or metabolic effects that are beneficial to health in general, without the need for medical supervision for its consumption (Stringheta et al. 2007). In Brazil, for example, the National Health Surveillance Agency



(ANVISA), a Brazilian regulatory agency, does not recognize the term “functional food”, but rather, “food with claims of functional properties”, being characterized by the fact where the food acts on the growth, development and maintenance of the organism in a proven way. This second definition makes more sense, including for the stingless bee’s propolis, as this propolis can present various biocompounds with anti-inflammatory, immunomodulatory, antimicrobial, anticancer and antioxidant activity (Sanches et al. 2017; Zullkiflee, Taha, Abdullah, et al. 2022).

Propolis is undoubtedly a great source of molecules with different biological properties, being used in traditional medicine of different peoples, and there are possibilities for new discoveries, with the search for phenolic compounds being the most promising. For the propolis of the *Trigona minor* bee, for example, 15 cycloartane triterpenoids have already been isolated, 5 of which have not been previously described in any propolis (Nguyen et al. 2017). A new alkylphenol with anticancer properties was identified in the same propolis (Nguyen et al. 2018). In another study, two new compounds derived from dihydrochromene and xanthone were identified in the propolis of the bee *Lysotrigona furva* (Oanh et al. 2021). In the propolis of the *Tetragonula biroi* bee, present in Indonesia, three new compounds with antioxidant activity were identified (Miyata et al. 2019).

These findings provide a basis for further research, but it is already known that, in addition to phenolic compounds, it is possible to find alcohols, amino acids, fatty acids, minerals, vitamins, and a variety of other nutritional elements (Anjum et al. 2019). In a preliminary study of a type of African propolis, more than 50 constituents were identified, including sugar and fatty acid derivatives (Popova et al. 2021). Fructose,

glucose, cholesterol, retinol (derived from vitamin A) and tocopherol (from vitamin E) are also examples of propolis constituents (Campos et al. 2015). There are reports showing the presence of tocopherol, in addition to phytosteroids and triterpenes, in the propolis of the bee *Plebeia droryana* (Bonamigo, Campos, Oliveira, et al. 2017). The propolis of *Heterotrigona itama*, when analyzed, showed that there were 43% of lipids and to a lesser extent proteins, carbohydrates and fibers, in addition to essential minerals for the activities of the human organism, such as calcium, magnesium, iron and zinc (Abdullah et al. 2019).

Another biological function related to stingless bee propolis is a prominent anti-inflammatory activity (Campos et al. 2015; Brodkiewicz et al. 2018; Sabir & Sumidarti 2017), and also the ability to regulate cardiovascular disorders (Massaro et al. 2013). Table 1 summarizes the different activities attributed to propolis of different stingless bee species from various geographical origins.

It is difficult to trace a relationship between the constituents of propolis (quantitatively or qualitatively) and its benefits, due to the synergistic relationships between the molecules (Santos et al. 2019). However, considering the different scientific sources, it is certain that propolis can exert not only nutritional functions, but also other important functions (healing, anti-inflammatory, etc.) for humans and animals (Bankova et al. 2016; Santos et al. 2020).

**Phenolic compounds in propolis**

Phenolic compounds are part of secondary metabolites of plants, which are produced when the plant undergoes stress or injury, and it is possible to find them in exudates such as those produced by *Dalbergia ecastophyllum*, which gives rise to red propolis, and by

**Table 1** Different biological activities described for stingless bee propolis. These activities are correlated with the bee species and its geographical origin

Producing species	Geographical origin	Activity	Reference
<i>Tetragonula carbonaria</i>	Australia	Cardiac regulator	(Massaro et al. 2013)
<i>Tetragonisca fiebrigi</i>	Brazil	Anti-inflammatory Nutritional	(Campos et al. 2015)
<i>Plebeia droryana</i>	Brazil	Nutritional	(Bonamigo, Campos, Oliveira, et al. 2017)
<i>Trigona minor</i>	Vietnam	Antitumor	(Nguyen et al. 2017; Bankova et al. 2016)
<i>Trigona</i> sp.	Indonesia	Anti-inflammatory	(Sabir & Sumidarti 2017) <sup>a</sup>
<i>Scaptotrigona jujuyensis</i> ; <i>Tetragonisca fiebrigi</i>	Argentina	Anti-inflammatory	(Brodkiewicz et al. 2018) <sup>a</sup>
<i>Tetragonula aff. Biroi</i>	Indonesia	Antioxidant (xanthin oxidase)	(Miyata et al. 2019)
<i>Heterotrigona itama</i>	Brunei	Nutritional	(Abdullah et al. 2019)
<i>Lisotrigona furva</i>	Vietnam	Antitumor	(Oanh et al. 2021)
<i>Meliponula ferruginea</i>	Tanzania	Nutritional	(Popova et al. 2021)

<sup>a</sup> In vivo and in vitro experiments, without <sup>a</sup> only in vitro

*Baccharis dracunculifolia*, which gives rise to green propolis (Daugusch et al. 2008; Abdullah et al. 2019, Moise and Bobis, 2020). It makes sense then that these plant exudates have compounds that exert, for example, antimicrobial activity, since the attack of a microorganism must be fought by the plant; therefore, the effect may be similar on human and animal pathogens (Santos et al. 2020). In addition, phenolic compounds constitute chemical markers for propolis, such as isoflavones (formononetin, biochanin A) and chalcones (isoliquiritigenin), that relate red propolis to its botanical origin (Daugusch et al. 2008).

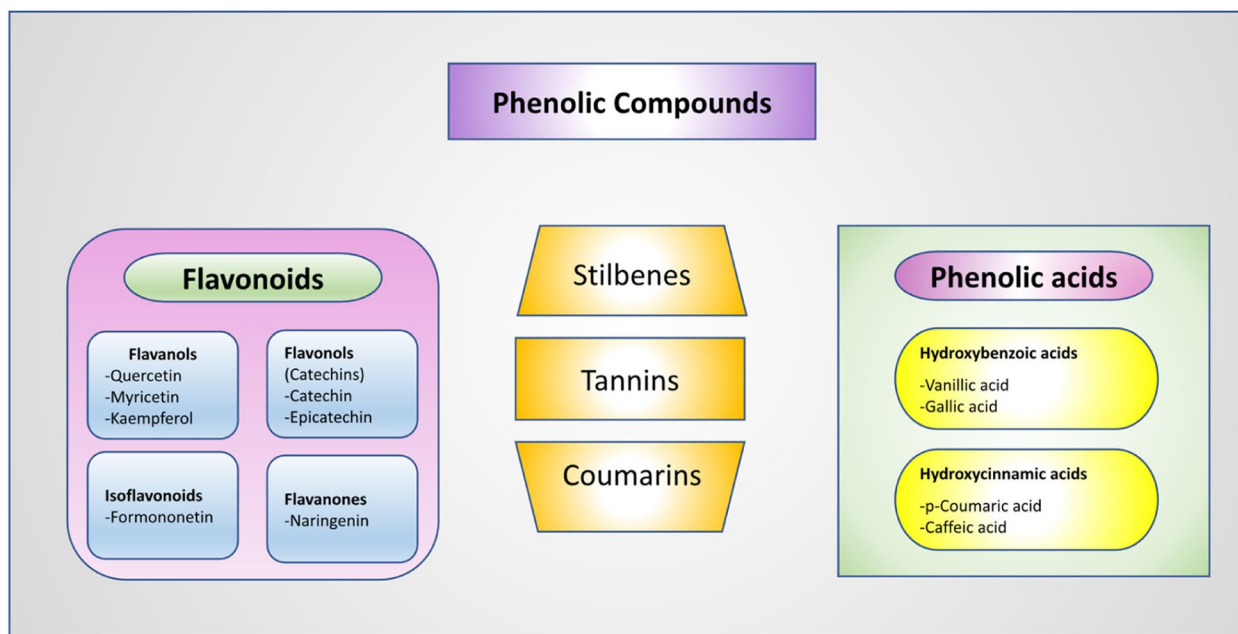
As can be seen in Fig. 2, phenolics are divided into classes and subclasses, being defined as compounds with one or more aromatic rings linked to one or more hydroxyl groups (Liu 2004). There are several examples of these classes and subclasses that are already described in scientific works on propolis from stingless bees, as can be seen in Fig. 2. The main phenolic compounds described in this type of propolis are phenolic acids (Bonamigo, Campos, Alfredo, et al. 2017; Campos et al. 2014), catechins (Araújo, dos Santos Júnior, et al. 2016; Hochheim et al. 2019), flavonols (Torres et al. 2018), stilbenes (Torres et al. 2018), and tannins (Hasan & Kuswandi 2011), among other constituents.

Compounds based on natural and synthetic coumarins have attracted the interest of drug design scientists due to the various pharmacological and biological properties observed, including: anti-inflammatory, antibacterial, antiviral, cyclooxygenase inhibition, antioxidant, antithrombotic, lipoxygenase inhibition, xanthine oxidase inhibition,

anti-Alzheimer’s disease (AD) as well as antitumor effects (Al-Warhi et al. 2020).

In addition, other phenolic compounds present in propolis, such as caffeic acid, cinnamic acid, rutin and apigenin, may also be responsible for different functions. The propolis produced by *M. q. anthidioides*, has the potential to be used in the prevention or treatment of diseases related to oxidative stress and mutagenesis confirmed in in vitro tests, as well as the propolis produced by *P. droryana* and *A. mellifera* contains important compounds capable of minimizing the action of oxidizing substances in organism and reducing the viability of erythroleukemia cells (Bonamigo, Campos, Oliveira, et al. 2017; Campos et al. 2018). However, a problem with natural products is related to the amount that must be consumed and the wide variety of compounds present, which makes it difficult to know which compounds are acting on the given target, and the exact amount of each one that must be consumed in order to exert its biological activity.

It is known that the chemical composition of propolis is not significantly altered by bees of the same species (Bankova & Al 2000). Therefore, its chemical profile is entirely dependent on the vegetables that the bee forages. So, considering this finding, it becomes impossible to establish a universal extraction method for all types of propolis, given the particularities of the product (Bankova 2005). Regarding the way to extract compounds from propolis, it is known that organic solvents are the most used extractors, or the mixture of these solvents with



**Fig. 2** Classes, subclasses and some phenolic compounds found in propolis from stingless bees

water (Amorim et al. 2008). A problem in the literature is that some authors use their own propolis extraction methodology. This fact makes it difficult to compare the results, as the extraction method may not be the most efficient for specific molecules, and interfere with the final objective of the work. There are many ways to extract compounds from propolis: maceration, soxhlet, ultrasound, microwave, supercritical extraction, among others. The most traditional and used one is maceration; however, in terms of cost at the laboratory level, time and sustainability, since less energy and resources are used, ultrasound extraction is the most viable (Bankova et al. 2021; Silva-Beltrán et al. 2021).

#### **Coumarins in stingless bee propolis**

Coumarins are compounds already reported in studies for propolis from stingless bees, however, in a restricted way (Araújo et al. 2010; Ibrahim et al. 2016; Mat Nafi et al. 2019). These compounds do not fit the classical definition of a phenolic compound, as there is no hydroxyl in their structure and there is an oxygen heteroatom attached to the aromatic ring (Dos Anjos et al. 2011). However, the basic structure of a coumarin is present in the so-called hydroxylated derivatives, for example scopoletin, which have the presence of the hydroxyl, which makes these derivatives part of the phenolic compounds. Therefore, coumaric compounds can be classified as phenolics, but also as a separate class.

The presence of this compound may be associated with the ability of propolis to promote antimicrobial, anti-inflammatory, antioxidant, anticancer action, among others (de Mendonça et al. 2020; Pereira et al. 2021; Franchin et al. 2016). But more research should be done in order to understand the action pathways and synergy with other compounds for a better understanding of the activity of coumarins present in propolis.

#### **Antimicrobial activity of stingless bee propolis**

Many scientific studies have focused on the antimicrobial potential of extracts from different propolis. In this way, the effect of inhibiting the growth or inducing the death of microorganisms of interest to human and veterinary health, and even industrial health is sought in propolis. As can be seen in Table 2, for bacteria, the cited studies have used *Staphylococcus aureus*, present in 12 of the 13 studies, as a Gram-positive model, and for fungi, *Candida albicans*, present in 5 of the 13 studies. *Staphylococcus aureus* is a gram-positive, anaerobic bacterium present in the mucosa of animals and humans, environments, food and water, which produces symptoms due to the production of enterotoxins, being considered as one of the main foodborne pathogens (Rubab et al. 2018). Stainless steel is one of the

main materials used in the food industry, so research with *Candida albicans* is necessary, since it is one of the main fungi resistant to antifungal agents that is capable of forming biofilms in this type of material and promoting food contamination (Tomičić & Raspor 2017).

Some of the microorganisms mentioned in Table 2 are not part of the scope of food science. However, there are others of great importance with regard to infections, such as *Enterococcus faecalis*, *Escherichia coli*, and *Aspergillus niger*. *A. niger* is a filamentous fungus of importance to the food industry due to its ability to produce enzymes, as a spoilage agent in cereals and grapes, and as a producer of mycotoxins (Gil-Serna et al. 2019). Propolis is an emerging antibacterial option against the bacterium *E. coli*, as this microorganism has shown resistance against classical antibiotics, including strains isolated from food (Pormohammad et al. 2019). The bacterium *E. faecalis* is an important pathogen present in water. In this way, the treatment of these waters by future technologies using propolis can be a cheap and sustainable possibility, as treatment with chemical products is currently preferred (Ersoy et al. 2019).

The activity of propolis on microorganisms depends on multiple variables. It is even possible that there is no efficacy against *E. coli*, a Gram-negative bacterium; however, there was a significant effect against Gram-positive *S. aureus* (Carneiro et al. 2016). On the other hand, it is possible to verify the effect of propolis on *S. aureus*, in addition to other bacteria tested, whether gram-positive or gram-negative (Zullkiflee, Taha, Abdullah, et al. 2022). Other studies corroborate the difficulty of controlling *E. coli* (Campos et al. 2014; Velikova et al. 2000), and this difficulty possibly extends to other Gram-negative bacteria (Sanpa et al. 2015). But for the control of *S. aureus*, the results have been promising, regardless of the species that produced the propolis (Massaro et al. 2013; Campos et al. 2014; Umthong et al., 2009; Yusop et al. 2019). The difference in the efficiency of propolis on the control of Gram-positive and Gram-negative bacteria may be due to the difference in the structure of the cell wall, where the Gram-negative ones are more complex, preventing the action of compounds with antimicrobial effect. It is important to note that both studies tested propolis from bees of the genus *Tetragonisca* spp.

Regarding another bacterium of importance in food safety, *E. faecalis*, studies have shown that there is antibacterial activity, although less significant than what was found for *S. aureus* (Campos et al. 2015; Torres et al. 2018). It is possible to verify a remarkable antimicrobial activity of an Italian propolis, from an unidentified species, against *Listeria monocytogenes* isolated from milk and cheese (Pedonese et al. 2019). Other works also report an antimicrobial activity of propolis from the stingless bees

**Table 2** Antimicrobial activity of stingless bee propolis. For each propolis, it is referred its producing species, its geographic origin, and the microorganisms on which each extract had an antimicrobial effect

Producing species	Geographic origin	Target microorganism	Reference
<i>Melipona quadrifasciata anthidioides</i>	Brazil	<i>Staphylococcus aureus</i> ; <i>Escherichia coli</i>	(Velikova et al. 2000)
<i>Trigona laeviceps</i>	Thailand	<i>Staphylococcus aureus</i> ; <i>Escherichia coli</i> ; <i>Candida albicans</i> ; <i>Aspergillus niger</i> .	(Umthong et al. 2009)
<i>Frieseomelitta varia</i>	Brazil	<i>Aeromonas hydrophila</i> ; <i>Pseudomonas aeruginosa</i> ; <i>Bacillus subtilis</i> ; <i>Staphylococcus aureus</i> .	(Campos et al. 2011)
<i>Tetragonula carbonaria</i>	Australia	<i>Staphylococcus aureus</i> ; <i>Pseudomonas aeruginosa</i>	(Massaro et al. 2014)
<i>Melipona orbigny</i>	Brazil	<i>Escherichia coli</i> ; <i>Staphylococcus aureus</i> ; <i>Candida albicans</i> .	(Campos et al. 2014)
<i>Tetragonisca fiebrigi</i>	Brazil	<i>Staphylococcus aureus</i> ; <i>Staphylococcus epidermidis</i> ; <i>Enterococcus faecalis</i> ; <i>Klebsiella pneumonia</i> ; <i>Pseudomonas aeruginosa</i> ; <i>Proteus mirabilis</i> ; <i>Candida glabrata</i> ; <i>Candida albicans</i> .	(Campos et al. 2015)
<i>Tetragonula laeviceps</i> ; <i>Tetrigona melanoleuca</i>	Thailand	<i>Listeria monocytogenes</i> ; <i>Micrococcus luteus</i> ; <i>Pseudomonas aeruginosa</i> ; <i>Staphylococcus epidermidis</i> ; <i>Staphylococcus aureus</i> ; <i>Streptococcus pyogenes</i> ; <i>Serratia marcescens</i> ; <i>Salmonella typhimurium</i> ; <i>Bacillus cereus</i> ; <i>Escherichia coli</i> .	(Sanpa et al. 2015)
<i>Tetragonisca angustula</i>	Brazil	<i>Staphylococcus aureus</i> ; <i>Streptococcus mutans</i> ; <i>Escherichia coli</i> .	(Carneiro et al. 2016)
<i>Trigona thoracica</i>	Malaysia	<i>Candida albicans</i> ; <i>Cryptococcus neoformans</i> .	(Shehu et al. 2016)
<i>Melipona quadrifasciata</i> ; <i>Tetragonisca angustula</i>	Brazil	<i>Staphylococcus aureus</i> ; <i>Enterococcus faecalis</i> ; <i>Escherichia coli</i> ; <i>Klebsiella pneumonia</i>	(Torres et al. 2018)
<i>Frieseomelitta longipes</i> ; <i>Apis mellifera</i>	Brazil	<i>Bacillus cereus</i> ; <i>Staphylococcus aureus</i> ; <i>Pseudomonas aeruginosa</i> ; <i>Escherichia coli</i> ; <i>Candida albicans</i> ; <i>Candida tropicalis</i>	(De Souza et al. 2018)
<i>Heterotrigona itama</i>	Brunei	<i>Staphylococcus aureus</i> ; <i>Bacillus subtilis</i> ; <i>Escherichia coli</i> ; <i>Pseudomonas aeruginosa</i>	(Abdullah et al., 2019)
not described	India	<i>Staphylococcus aureus</i> ; <i>Bacillus subtilis</i> ; <i>Escherichia coli</i> ; <i>Pseudomonas aeruginosa</i> ; <i>Candida albicans</i>	(Kasote et al. 2019)
<i>Trigona itama</i>	Malaysia	<i>Staphylococcus aureus</i> ; <i>Escherichia coli</i>	(Yusop et al. 2019)
<i>Apis mellifera scutellata</i> ; <i>Scaptotrigona bipunctata</i> ; <i>Melipona quadrifasciata quadrifasciata</i> ; <i>Plebeia remota</i>	Brazil	<i>Escherichia coli</i> ; <i>Klebsiella pneumonia</i> ; <i>Pseudomonas aeruginosa</i> .	(Surek et al. 2021)

*Tetragonula laeviceps* and *Tetrigona melanoleuca* against the bacterium *L. monocytogenes* (Sanpa et al. 2015).

Regarding the antifungal activity of propolis, studies are very scarce and focused on yeasts of the genus *Candida* spp., especially *Candida albicans* (Umthong et al. 2009). Significant inhibition of two yeast species has already been found by the propolis of the bee *Tetragonisca fiebrigi* (Campos et al. 2015). Other studies corroborated these results, using propolis from stingless bees, such as *Frieseomelitta longipes*, *Melipona orbigny* and *Trigona thoracica* (Campos et al. 2014; Shehu et al. 2016; De Souza et al. 2018).

#### Antioxidant activity of stingless bee propolis

The antioxidant capacity of natural products is one of its most important activities, due to its value against diseases and aging, and also for the generation of new technologies, specifically in the field of food science. It is important to know that free radicals are free fragments

of highly reactive molecules, accidentally or even normally produced by cells, which can generate oxidative damage to the genetic material or to the cell membrane (Piantadosi 2020). Reactive oxygen species are the main radicals to be inhibited, with hydrogen peroxide being the most important of them. Against free radicals, a full physiological and homeostatic balance is important, but it is also possible to increase this activity through diets and treatments containing antioxidant components (Kieliszek et al. 2018).

The works cited in Table 3 do not specify which molecule acts as an antioxidant, because when considering the diversity of compounds present in propolis, it is difficult to determine which molecule has a greater influence on this activity. However, it is possible to verify the existence of vitamin E (tocopherol) in propolis, an important antioxidant that is present mainly in the lipid phase of some foods (Campos et al. 2015; Bonamigo, Campos, Oliveira, et al. 2017; Bonamigo, Campos, Alfredo,



**Table 3** Antioxidant activity of stingless bee propolis

Specie	Geographic origin	Method	AA	Reference
<i>Melipona orbignyi</i>	Brazil	DPPH	IC <sub>50</sub> 40%	(Campos et al. 2014)
		OHI	125 µg/mL	
		LPI	125 µg/mL	
<i>Tetragonisca fiebrigi</i>	Brazil	ABTS	IC <sub>50</sub> 119.6 µg/mL	(Campos et al. 2015)
		OHI	50–125 µg/mL;	
		LPI	50–125 µg/mL	
<i>Melipona scutellaris; Melipona fasciculata; Apis mellifera</i>	Brazil	DPPH	IC <sub>50</sub> 29–845 µg/mL	(Araújo, Bosco, & Sforcin, 2016)
<i>Tetragonisca angustula</i>	Brazil	DPPH	IC <sub>50</sub> 293–734 µg/mL	(Carneiro et al. 2016)
<i>Scaptotrigona depilis; Melipona quadrifasciata anthidioides</i>	Brazil	DPPH	IC <sub>50</sub> 60.91 µg/mL	(Bonamigo, Campos, Alfredo, et al. 2017)
		ABTS	IC <sub>50</sub> 13.4–80 µg/mL;	
		OHI	125 µg/mL;	
<i>Melipona quadrifasciata; Tetragonisca angustula</i>	Brazil	RP	657.5 mg/100 g	(dos Santos, Hochheim, et al. 2017)
		DPPH	IC <sub>50</sub> > 1000 µg/mL	
		LPI	75 µg/mL	
<i>Melipona quadrifasciata anthidioides; Tetragona clavipes; Scaptotrigona spp.</i>	Brazil	DPPH	IC <sub>50</sub> 8.4–512 µg/mL	(Pazin et al. 2017)
		SOD	21 µg/mL	
<i>Plebeia droryana; Apis mellifera</i>	Brazil	DPPH	IC <sub>50</sub> 49.8–182.4 µg/mL	(Bonamigo, Campos, Oliveira, et al. 2017)
<i>Frieseomelitta longipes; Apis mellifera</i>	Brazil	DPPH	IC <sub>50</sub> 3.74–8.81 µg/mL;	(De Souza et al. 2018)
<i>Melipona quadrifasciata quadrifasciata; Tetragonisca angustula</i>	Brazil	DPPH	IC <sub>50</sub> 241.8–2433 µg/mL	(Torres et al. 2018)
<i>Heterotrigona itama</i>	Brunei	DPPH	IC <sub>50</sub> : 76.5–1905 mg/L	(Abdullah et al. 2019)
<i>Melipona quadrifasciata</i>	Brazil	DPPH	IC <sub>50</sub> 151–1000 µg/mL;	(Hochheim et al. 2019)
		RP	16.9–127.83 mg /100 g;	
		LPI	10%–38,48%.	
<i>Geniotrigona thoracica; Heterotrigona itama; Tetrigona binghami</i>	Brunei	DPPH	IC <sub>50</sub> 76.5–1975 mg/L	(Abdullah et al. 2020)
<i>Tetrigona apicalis</i>	Malaysia	DPPH	37–92,70%	(Zainal et al. 2021)

For each propolis, it is described its producing species, geographic origin, and activity

DPPH 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, OHI Oxidative hemolysis inhibition, LPI Lipidic peroxidation inhibition, RP Reduction potential, ABTS Diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) assay, SOD Superoxide dislocation, IC<sub>50</sub> Concentration able to inhibit 50% oxidization

et al. 2017). The presence of this vitamin is of great technological relevance, as it can contribute to the stability of oils and meats due to its high antioxidant activity. In addition to tocopherol, the presence of phenolic compounds is seen as the main justification for the antioxidant activity, and the extraction method influences the efficiency of obtaining these constituents, and polar solvents, mainly ethyl acetate, have been highlighted in this obtaining process (Hochheim et al. 2019; Pazin et al. 2017; Zainal et al. 2021).

As can be seen in Table 3, the most used test to verify the antioxidant activity corresponds to the use of the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical, and the antioxidant power of propolis is clearly variable, although the assays were conducted with propolis from the same stingless bee species, showing the importance of geographic origin (Bonamigo, Campos,

Alfredo, et al. 2017; Pazin et al. 2017; Dos Santos, David, & David 2017). Of the 13 studies cited, 7 tested *Melipona* propolis, which is the most studied genus. Phenolic compounds, such as benzoic acid, p-coumaric acid, caffeic acid, cinnamic acid, among others, are identified as important for antioxidant activity (Bonamigo, Campos, Oliveira, et al., 2017; Bonamigo, Campos, Alfredo, et al. 2017). These compounds exert antioxidant activity by inactivating free radicals due to the hydroxyl group normally present in the molecules of these phenolic compounds; this activity can preserve cells, and consequently prevent diseases associated with the oxidant effect of these free radicals, such as cardiac problems, cancer and diabetes (Campos et al. 2014; De Souza et al. 2018). However, it is possible that the amount of wax in propolis and the time (season/month) of collection may influence the

antioxidant activity of propolis (Carneiro et al. 2016; Araújo, Bosco, & Sforcin 2016).

#### **Propolis applications in the food**

In the same way as vegetable oils can inhibit oxidizing agents, thus preventing the unwanted appearance of flavors and odors, and consequently the loss of food safety (Del Ré & Jorge 2012), propolis is studied as an antioxidant component mainly for meats, but this type of conservation also covers other foods, such as vegetables, fruits and juices (Pobiega et al. 2019). In food science, the research on stingless bee propolis is focused on its antioxidant potential associated with encapsulation or microencapsulation, thus preserving the compounds and their bioactive activities. The propolis of the Tubuna bee, when encapsulated in alginate, in order to obtain an antioxidant color, was successful in the encapsulation process and high efficiency in the tests of antioxidant activity (Dalponte Dallabona et al. 2020). Another microencapsulation technique that can be used is spray-drying, thus maintaining a high content of total phenolics and the antioxidant capacity of propolis (Pratami et al. 2019). Microencapsulation is an alternative to retain the strong aroma of propolis (which could be negative in food) and thus take advantage of its preservative power (antimicrobial, antioxidant)) (Maroof et al. 2022).

However, the application of propolis is still far below the possibilities of its use, and it becomes even more restricted when considering the propolis of stingless bees. Still, new fields are emerging or expanding for propolis, such as its use in improving livestock productivity, food preservation, and incorporation into materials (Bankova et al. 2016). In addition to the aforementioned fact of the incorporation of nutrients and functional aspects that prevent diseases, propolis is able to promote physical, chemical and sensory aspects to food in a safe and non-toxic way (Pobiega et al. 2019).

#### **Conclusions**

Stingless bee propolis is a product with many aspects to be investigated, through a rational and sustainable exploitation by producers and researchers. Even considering its widely studied chemical profile, it is still needed to establish common research parameters, so that the data can be properly compared, and the concept of propolis begins to be unified, with no further doubts and misunderstandings regarding the material to be analyzed. Considering the scientific literature, the importance given to phenolic compounds is remarkable, since these compounds exert preventive activities against diseases, but also against microorganisms and free radicals, which makes propolis a source for the isolation of bioactive compounds. In

the same way, its use as a functional food is important, considering that propolis has nutritional constituents and compounds that are not restricted to phenolics. Technologically, the use of propolis from stingless bees is very restricted to the encapsulation technique, but it is already known that this product has potential as a substitute for chemical additives, increasing the shelf life of meats, oils and vegetables, in addition to a wide horizon of its use as a sanitizer in the food industry.

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#### **Code availability**

Not applicable.

#### **Authors' contributions**

VMR, JPA, RDP, COS, MAUG performed the bibliographic revision. VMR, JPA and COS wrote the manuscript. RDP and MAUG critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

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#### **Availability of data and materials**

Not applicable.

#### **Declarations**

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#### **Competing interests**

The authors declare no conflicts of interest.

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