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Propolis of Stingless Bees: a Promising Source of Biologically Active Compounds

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ABSTRACT
The information published about chemical composition and biological activity of propolis from stingless bees (Meliponinae) of different genera is reviewed. The available data on the biological action of individual constituents is presented. The plant sources of this propolis are also considered. The perspectives of research and therapeutic use of Meliponinae propolis are discussed.
KEYWORDS: Propolis, Meliponini, Antimicrobial activity

INTRODUCTION
The tribe Meliponini belongs to the group of corbiculate bees (subfamily Apinae) and encompasses all the bees know as "stingless bees", found throughout the tropical and subtropical areas of the world (1). They are the major visitors and native pollinators of flowering plants in the tropics, comprising a large group of small to medium sized bees (from 2 mm up to 1.5 cm) with a level of social organization comparable to that of honeybee *Apis mellifera* (2). As they are resistant to the diseases and parasites of honeybees, the propagation of their colonies contributes to the preservation of biodiversity, however, deforestation has lead to decreased density of these eusocial bees (3). Stingless bees have been kept in the Americas for centuries, long before the arrival of Columbus and common honeybees. In the Yucatan peninsula, Mayan people developed meliponiculture to a level similar to that of management of honeybees during Medieval times in Europe (4).Nowadays, meliponiculture is gaining popularity in Brazil (5). As with *Apis mellifera*, people keep stingless bees for honey, which is prized as a medicine and is more expensive than honeybee honey. Stingless bees also collect plant resin and store it in large deposits within their nests. These deposits are much easier to harvest compared to honeybee propolis and they can be used in a similar way as honeybee propolis: in Brazil ,stingless bees' propolis is used as a traditional remedy for healing wounds, gastritis, etc., it has also demonstrated strong antibacterial properties (4, 6). Scientific research proving the medicinal properties of stingless bee propolis could result in marketing of this product as a natural and organic specialized item. However, it is necessary to characterize the chemical composition and biological activity of this product beforehand (4). Such studies have been started only in the last 10 – 15 years. In this article, we summarize for the first time all the available information concerning the chemistry and biological properties of propolis from stingless bees.

Chemical composition and plant origin of Meliponinae propolis - The first communication concerning chemical composition of stingless bee propolis was published in 1993 (7). Propolis from five indigenous Meliponinae species from Venezuela was analyzed by HPLC-MS and it was found that the main components were prenylated benzophenones originating from *Clusia* trees. Phenolic compounds were not detected in propolis from Paratrigona stingless bees. In this study, no individual compounds have been identified. Further studies appeared in the late 1990's. Most of them have been carried out by GC-MS of both volatiles and alcohol extracts and lead to the identification of numerous compounds of different structural types. These data are summarized in Table 1. The only individual compounds isolated from stingless bee propolis are diterpenic acids of kaurene type: kaurenoic acid **1**, *ent*-15β-(3-methylbutanoyloxy)-16-kauren-19-oic acid **2** and *ent*-15β-hydroxy-16-kauren-19-oic acid **3**, found in propolis from *Melipona quadrifasciata anthidioides* (8). Very often the studies have been performed in comparison with propolis from common honeybees, collected at the same locations. These data demonstrated that in most cases, the propolis sources used by *A. mellifera* and stingless bees did not coincide. Also, they revealed that there were several types of Meliponinae propolis, according to the prevailing type of compounds in alcohol extracts: diterpenic, triterpenic, and gallic acid type (9). Mixed-type propolis was also observed. In the diterpenic type, most compounds were tentatively identified as diterpenic acids. It is interesting to note that the prenylated p-coumaric acids, typical for green Brazilian propolis (from *Baccharis dracunculifolia*) have not been identified in any of the samples analyzed. It is noteworthy that in some studies (9, 10) the latter compounds were identified as main component in *A. mellifera* propolis from the same locations as stingless bee propolis. There was only one exception: Miorin et al. (10) found some typical components of green propolis in propolis from *T. angustula*, but their concentrations were much lower in the latter. There are cases, however, where striking similarity of common honeybee and Meliponinae propolis has been observed.

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Table 1. Literature data about chemical composition of Meliponinae propolis

Bee species	Origin	Compound type	Analytical method	Extract studied	Ref.
Friesomelitta silvestrii	Brazil, Goias	Monoterpenes	GC-MS	Solid samples	18
		Sesquiterpenes	GC-MS	Solid samples	18
Friesomelitta silvestrii languida	Brazil, Minas Gerais	Monoterpenes	GC-MS	Solid samples	18
		Sesquiterpenes	GC-MS	Solid samples	18
		Diterpenes	GC-MS	Solid samples	18
		Triterpenes	GC-MS	Solid samples	18
Friesomelitta varia	Brazil, Sao Paulo	Monoterpenes	GC-MS	Solid samples	18
		Sesquiterpenes	GC-MS	Solid samples	18
		Triterpenes	GC-MS	Solid samples	18
	Venezuela	Perenylated benzophenones	HPLC-MS	Methanol	7
***Lestrimellata* spp.**	Brazil, Parana	Diterpenes	GC-MS	70% ethanol	9
		Triterpenes	GC-MS	70% ethanol	9
Melipona beechei	Mexico, Yucatan	Monoterpenes	GC-MS	Volatiles	17
		Sesquiterpenes	GC-MS	Volatiles	17
Melipona compressipes	Brazil, Piaui	Sesquiterpenes	GC-MS	Essential oils	19
		Phenolic acids	GC-MS	70% ethanol	20
		Flavonoids	GC-MS	70% ethanol	20
		Sugars and sugar alcohols	GC-MS	70% ethanol	20
		Diterpenes	GC-MS	70% ethanol	20
		Triterpenes	GC-MS	70% ethanol	20
		Fatty acids	GC-MS	70% ethanol	20
	Venezuela	Prenylated benzophenones	HPLC-MS	Methanol	7
Melipona favora orlinge	Brazil, Mato Grosso do Sul	Diterpenes	GC-MS	70% ethanol	9
		Triterpenes	GC-MS	70% ethanol	9
Melipona favosa	Venezuela	Prenylated benzophenones	HPLC-MS	Methanol	7
Melipona marginata	Brazil, Pernambuco	Phenolic acids	GC-MS	70% ethanol	9
		Sugars	GC-MS	70% ethanol	9
Melipona quadrifasciata	Brazil, Parana	Phenolic acids	GC-MS	70% ethanol	9, 20
		Sugars and sugar alcohols	GC-MS	70% ethanol	9
		Monoterpenes	GC-MS	Essential oils	19
		Sesquiterpenes	GC-MS	Essential oils	19
		Diterpenes	GC-MS	70% ethanol	9, 20
		Diterpenic acids	Isolated, characterized by spectral methods	70% ethanol	8
		Triterpenes	GC-MS	70% ethanol	9
		Fatty acids	GC-MS	70% ethanol	20
	Brazil, Espiritu Santo	Phenolic acids	GC-MS	70% ethanol	20
		Diterpenes	GC-MS	70% ethanol	9
	Brazil, Sao Paulo A	Phenolic acids	GC-MS	70% ethanol	9
		Triterpenes	GC-MS	70% ethanol	9
		Sugars	GC-MS	70% ethanol	9
	Brazil, Sao Paulo B	Diterpenes	GC-MS	70% ethanol	9
		Phenolic acids	GC-MS	70% ethanol	9
		Triterpenes	GC-MS	70% ethanol	9
	Brazil, Minas Gerais	Phenolic acids	GC-MS	70% ethanol	9
		Triterpenes	GC-MS	70% ethanol	9
		Sugars	GC-MS	70% ethanol	9
	Brazil, Pernambuco	Phenolic acids	GC-MS	70% ethanol	9
		Sugars	GC-MS	70% ethanol	9
Melipona scutelaris	Brazil, Pernambuco	Phenolic acids	GC-MS	70% ethanol	9
		Sugars	GC-MS	70% ethanol	9
Nanotrigona testacularis	Brazil, Minas Gerais	Diterpenes	GC-MS	70% ethanol	9
		Triterpenes	GC-MS	70% ethanol	9
Plebeia remota	Brazil, Parana	Diterpenes	GC-MS	70% ethanol	9
		Triterpenes	GC-MS	70% ethanol	9
		Phenolic acids	GC-MS	70% ethanol	9
		Sugars	GC-MS	70% ethanol	9
***Plebeia* spp.**	Brazil, Parana	Diterpenes	GC-MS	70% ethanol	9
		Triterpenes	GC-MS	70% ethanol	9
		Phenolic acids	GC-MS	70% ethanol	9
		Sugars	GC-MS	70% ethanol	9

Plebeia droriana	Brazil, Minas Gerais	Triterpenes	GC-MS	70% ethanol	9
		Phenolic acids	GC-MS	70% ethanol	9
Scaptotrigina bipunctata	Brazil, Parana	Phenolic acids	GC-MS	70% ethanol	9
		Sugars	GC-MS	70% ethanol	9
		Diterpenes	GC-MS	70% ethanol	9
		Triterpenes	GC-MS	70% ethanol	9
Scaptotrigona depilis	Venezuela	Prenylated benzophenones	HPLC-MS	Methanol extarct	7
Tetragona clavipes	Brazil, Parana	Fatty acids	GC-MS	70% ethanol	20
		Phenolic acids	GC-MS	70% ethanol	9, 20
		Sugars and sugar alcohols	GC-MS	70% ethanol	9, 20
		Monoterpenes	GC-MS	Essential oils	19
		Sesquiterpenes	GC-MS	Essential oils	19
		Diterpenes	GC-MS	70% ethanol	9, 20
		Triterpenes	GC-MS	70% ethanol	9, 20
Tetragonicsa angustula	Brazil (Sao Paulo, Parana, Minas Gerais)	Triterpenes	GC-MS	70% ethanol Dichloromethane, acetone, methanol	9, 11
		Phenolic acids	GC-MS	70% ethanol Methanol	9, 11
		Sugars and sugar alcohols	GC-MS	70% ethanol Methanol	9, 11
	Sao Paulo	Amino acids	GC-MS	Methanol	11
		Hydroxy acids	GC-MS	Methanol	11
		Fatty acids	GC-MS	Acetone, methanol	11
	Parana, Minas Gerais	Prenylated derivayives of *p*-coumaric acid	HPLC-DAD	Ethanol	10

Tomas-Barberan et al. (7) (1993) have found identical compounds in propolis from honeybees and from four stingless bee species from Venezuela. Also, Pereira et al. (11) demonstrated that triterpenes comprised more than 35% of the total amount of the propolis samples of both *A mellifera* and *T. angustula* propolis of the same location, and the chemical profiles were almost identical. Two recent works deal with fingerprinting of propolis of native Brazilian stingless bees by electrospray ionization mass spectrometry (ESI-MS). In these studies, no chemical constituents have been identified but the correlation among propolis samples was studied via chemometric analysis based on their composition patterns (12, 13). This type of studies is very useful with respect to identification of the plant sources of propolis of different bee species and for revealing correlations between propolis composition and bee species. It was found (12) that *Tetragonisca angustula* propolis from different regions in Brazil had uniform composition pattern, and *Shinus terebentifolius* was suggested as one of the main plant sources of this propolis type. Further, Sawaya et al. analyzed propolis from 12 Brazilian Meliponinae species (13) and found that *S. terebentifolius* is an important source of propolis not only for *T. angustula* but also for other stingless bee species. Velikova et al. (9) found that *T. angustula* propolis was rich in triterpenic compounds, so the observed “triterpenic” type of stingless bee propolis could be connected to *S. terebentifolius*. Another important source turned out to be *Araucaria* (13). As *Araucaria* resins have been found to contain significant amounts of diterpeneic acids, this fact may account for the “diterpenic type” Meliponinae proipolis (9).

Although *Tetragonisca angustula* obviously has a preferred resin source, *Shinus terebentifolius,* this behavior seems not to be characteristic for all Meliponinae species. From *Melipona quadrifasciata,* Velikova et al. (9) analysed 7 propolis samples and these samples belonged to different chemical types: diterpenic (three samples), gallic acid (two samples) and mixed type (two samples). The results of Sawaya et al. (13) support these findings: one of their *M. quadrifasciata* samples had the typical ions for propolis derived from *S. terebentifoulius*, another one demonstrated diagnostic ions for propolis from *Araucria* resin, and two samples were of mixed origin. On the other hand, all three samples from Pernambuco state, Brazil, independently of the bee species, demonstrated absence of diterpenes and triterpenes. They contained phenolic acids (mainly gallic acid) and sugars only (9).

It is of significant importance that sometimes native bees seem to ignore a potential plant source used by *A. mellifera*: none of the characteristic ions of green *A. mellifera* propolis (*Baccharis dracunculifolia* propolis) have been observed in the fingerprints of any of the samples of stingless bees, confirming the observations of Velikova et al. (9) and indicating that *B. dracunculifolia* has not been used as a source of resin by any of the 12 species studied (13).

Biological activity

Meliponinae propolis has been tested mainly for its antibacterial and antimicotic activity. The data available are summarized in Table 2. In general, the antibacterial activity

Table 2. Literature data about antimicrobial activity of Meliponinae propolis

Bee species	Microorganisms	Reference
***Lestrimellata* spp.**	*Staphylococcus aureus*	9
	Candida albicans	9
Melipona favora orlinge	*Staphylococcus aureus*	9
	Esherichia coli	9
	Candida albicans	9
Melipona mandacaia	*Staphylococcus aureus*	14
	Enterococcus spp.	14
Melipona marginata	*Staphylococcus aureus*	9
	Candida albicans	9
Melipona quadrifasciata	*Staphylococcus aureus*	9
	Esherichia coli	9
	Candida albicans	9
Melipona scutellaris	*Staphylococcus aureus*	9, 14
	Candida albicans	9
	Enterococcus spp.	14
***Melipona* spp.**	*Staphylococcus aureus*	14
	Enterococcus spp.	14
	Esherichia coli	14
Nanotrigona testaceicornis	*Staphylococcus aureus*	9, 14
	Esherichia coli	9
	Candida albicans	9
***Partamona* spp.**	*Staphylococcus aureus*	14
	Enterococcus spp.	14
Plebeia droriana	*Staphylococcus aureus*	9
	Candida albicans	9
Plebeia remota	*Staphylococcus aureus*	9
	Esherichia coli	9
	Candida albicans	9
***Plebeia* spp.**	*Staphylococcus aureus*	9
	Candida albicans	9
Scaptotrigona bipunctata	*Staphylococcus aureus*	9
	Esherichia coli	9
	Candida albicans	9
***Scaptotrigona* spp.**	*Staphylococcus aureus*	14
	Enterococcus spp.	14
Tetragona clavipes	*Staphylococcus aureus*	9
	Esherichia coli	9
	Candida albicans	9
Tetragonisca angustula	*Staphylococcus aureus*	9, 10, 11, 14
	Esherichia coli	9
	Candida albicans	9
Trigona spinipes	*Staphylococcus aureus*	14

of stingless bee propolis was found similar to that of honeybee propolis. As expected, Meliponinae propolis was active against Gram-positive microorganisms and less active or inactive against Gram-negative ones. Individual samples of propolis from *Partamona* spp. (14), *Melipona* spp.(14), *Melipona quadrifasciata* (9), *Tetragonsisca angustula* (10) have demonstrated higher antibacterial activity than *A. mellifera* propolis. In *Melipona quadrifasciata* propolis, diterpenic acids have been identified as antibacterial components (8). A statistical study, however, has demonstrated that Brazilian Meliponinae propolis (from 12 different species) is of lower antibacterial activity against *S. aureus* than the Brazilian and European bee glue from honeybees (15). There is scarce data concerning other types of activities. The potential cytotoxicity of stingless bee propolis from several species has been studied (9). Samples from *Mellipona quadrifasciata* and *Nanotrigona testacularis* demonstrated very promising toxicity in this test, much better than honeybee propolis. It is interesting to note that all these propolis samples belonged to the diterpenic type (9). There is also one communication about antioxidant activity of Meliponinae propolis (from *Melipona quadrifasciata*, *Tetragonsica angustula* and *Nannortrigona* spp.) (16).

CONCLUSION

Obviously, the study of Meliponinae propolis is even more challenging than the study of honeybee propolis. Information on the chemical composition of propolis of these native bees, as well as the plants they visit as sources of resin, is of great importance, both for conservation purposes and for meliponiculture. Also, more research is needed to scientifically support the medicinal properties of stingless bee propolis. This could lead to increased prizes for Meliponinae propolis and help to revive meliponiculture, providing an additional source of income for farmers in rural communities.

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