Research Article

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Geopropolis: taxonomic dependence and compositional drawbacks

Abstract

The contents of total ashes and ethanol soluble substances were determined for propolis samples from species of two Meliponini genera: *Melipona* and *Scaptotrigona*. All *Melipona* samples corresponded to geopropolis (propolis containing aggregated soil material), with ash contents above 40%. Only one species of *Scaptotrigona* was shown to produce geopropolis, but with ash content (12%) much lower than *Melipona* samples. In *Melipona* a high negative correlation was observed between ash and ethanol solubles. Not only aggregated soil may contribute to lower the content of ethanol solubles. It is hypothesized that wax content may have a similar influence.

Keywords: apidae, stingless bees, *melipona, scaptotrigona*, propolis, phenolic substances

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Introduction

Propolis is a resinous apiculture product used commercially in industries of food and hygiene products, as well as in traditional and popular medicine. Nearly all marketed types of propolis derive from honey bees (*Apis mellifera*). While bee laborers produce honey from nectar collected from a wide diversity of plant species, they elaborate propolis by mixing resin from a relatively low diversity of plant species, which they mix with beeswax.[1,2] Propolis has complex chemical composition. Most propolis types contain predominantly phenolic compounds, a class of secondary metabolites known to exert a wide diversity of biological activities.[3,4] Among members of the family Apidae, *Apis mellifera* is not the only propolis producer species. Many species of stingless bees (Meliponini) also produce propolis, often with composition similar with honey bee propolis.[5] Meliponine propolis has been shown to exert a diversity of biological properties,[6] including antitumor activity.[7] There is a high diversity of meliponines in tropical habitats, with the number of species in the New World reaching nearly 400. They are relevant pollinators of native plants and crops.[7] Main meliponine genera in Brazilian biomes are *Melipona, Scaptotrigona and Tetragonisca*. Comparing with honey bees, the productivity of meliponines is considerably low. For this reason, meliponine propolis is seldom commercialized. However, in recent years there has been a growing interest aiming to produce meliponine honey and propolis. In several parts of Brazil, meliponiculture has been practiced by leisure or desire to contribute with native bee conservation.[8] Similar practices have been noticed in Australia.[9]

Some meliponines produce propolis containing soil material mixed with resin and wax, a reason why this type of propolis is called geopropolis.[8] It is not clear the extent among meliponines of the behavior of aggregating soil to propolis. In some papers, it is implied that geopropolis production is generalized among stingless bees.[10] Other papers deal with results about geopropolis, but also of propolis of meliponines.[11] So far, it is not known if geopropolis is characteristic of species or genera of meliponines, or, alternatively, if the same species may produce propolis and geopropolis. A proper procedure to verify if a meliponine product is propolis or geopropolis is to determine its content of ashes. Ash contents similar with the corresponding parameter of honey bee propolis indicate absence of aggregated soil, hence the material is not geopropolis. The maximum admitted content of ashes in honey bee propolis is 5%.[12] Ash contents of several samples of Brazilian green propolis were found to be lower than 4%.[13] Ash contents considerably higher than 5% are characteristic of geopropolis. It seems logical that the higher the content of aggregated soil in geopropolis, the lower the content of biologically active substances, such as phenolic substances, since these constituents are soluble in alcohol. The aim of the present work is to obtain a first evaluation of the extent of production of propolis and geopropolis by Brazilian native meliponines. Two main genera of tropical meliponines were selected for this purpose: *Melipona* and *Scaptotrigona*. It is aimed also to determine the content of ethanol solubles in all products and verify possible correlations between the two parameters.

Material and methods

Propolis/geopropolis samples

Sixteen samples of propolis or geopropolis were analyzed. They were produced by species of two genera: *Melipona and Scaptotrigona*. The samples were produced in meliponaries from the Brazilian states Pará (northern Brazil), Maranhão and Rio Grande do Norte (northeast), and Minas Gerais and São Paulo (southeast) (Table 1).

Total ethanol solubles

The procedure by Matsuda[13,14] was used, with modifications. Crude propolis was extracted with ethanol for 5 h in Soxhlet. The extract was maintained at -20 °C overnight and filtered through Whatman filter paper n. 1 and evaporated to dryness in flasks previously weighed.

Ashes contents

The samples of crude propolis or geopropolis (3g) were placed in previously weighed crucibles and incinerated over Bunsen burner until final carbonization. The crucibles were transferred to a muffle furnace and maintained at 650 °C for 5 h. After cooling in desiccator at room temperature, the crucibles were weighed.

Statistical analysis

All analyses were performed in triplicates. Means and standard errors were determined. The results were distributed in a scatter plot using Cartesian coordinates. Equation slopes and correlation coefficients (R^2) were calculated separately for points relative Melipona and Scaptotrigona.

Results and discussion

Highly distinct means were obtained regarding the contents of ashes, comparing the samples of *Melipona* and *Scaptotrigona.* Values regarding materials from *Scaptotrigona* were in the range 1%-12%, while values from *Melipona* varied from 43% to 81%. However, the range of variation concerning total ethanol solubles was not much different comparing the samples from both genera: *Melipona* – 1%-46%; *Scaptotrigona* – 12%-38%. The standard errors corresponding to ash contents were negligible. However, high variation was noted concerning values of ethanol total solubles, even regarding samples of the same species and meliponary (Figure 1). A scatter plot combining the two series of parameters of *Melipona and Scaptotrigona* (Figure 1) enabled the attainment of regression slopes. The regression equations and correlation coefficients for slopes of the two genera were very distinct: $y = 98 – 1.2x$ and $R^2 = 0.96$ for *Melipona,* and $y = 8 + 2.7x$ and $R^2 = 0.64$ for *Scaptotrigona.* The results regarding *Melipona* indicate that all samples analyzed correspond to geopropolis because their ash contents exceeded by far the upper limit acceptable for honey bee propolis (5%; Brasil 2001). On the other hand, among the samples of *Scaptotrigona* only *S. tubiba* (**St;** meliponary from Guarujá; Table 1), with ash content of 12% (Figure 1) is a geopropolis. All other samples of *Scaptotrigona* **(Sp, Sb$_1$, Sb$_2$, Sx$_1$** and **Sx$_2$**) contained low amounts of ashes (1% or 2%, Figure 1). Another way to distinguish between propolis and geopropolis is the color of the ashes inside the crucible: ashes derived from calcination of propolis have grey color, while ashes of geopropolis have brown-yellowish color, due to the presence of clay iron salts. Samples from *Melipona* provided brown-yellowish ashes, while most samples from *Scaptotrigona* provided grey ashes (Figure 2 A and B, respectively). The results obtained in the present work indicate that most species of *Melipona and Scaptotrigona* differ in their behavior regarding the production of propolis: the former aggregate soil material or mud to propolis, hence they are geopropolis producers; most species of *Scaptotrigona,* on the contrary, produce propolis devoid of soil material. Even the unique example in the present work of a *Scaptotrigona* geopropolis producer (*S. tubiba*) is distinct from all species of *Melipona*, if we consider the ashes content obtained: 12% for *S. tubiba* and 43%-81% for *Melipona* species (Figure 1).

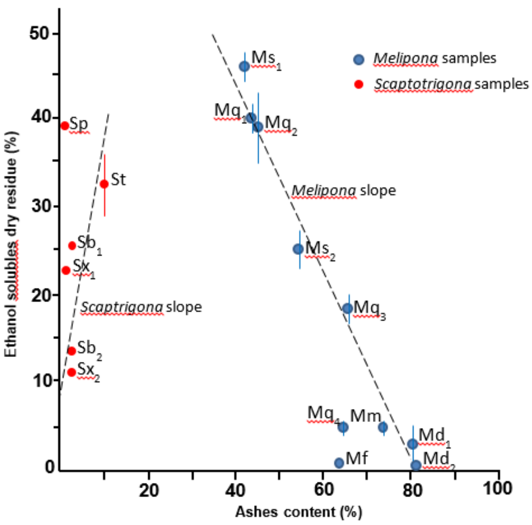

Figure 1 Relationship between contents of total ashes and total ethanol solubles of propolis and geopropolis "from species of *Melipona* and *Scaptotrigona* (Meliponini, Apidae)".

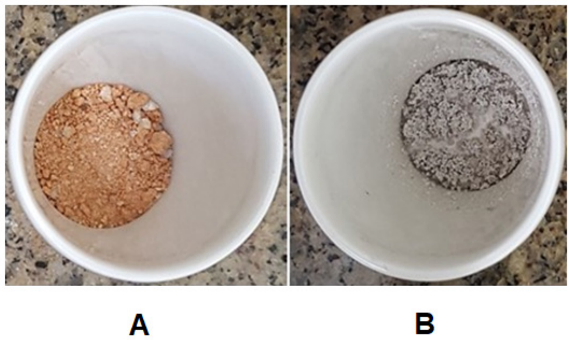

Figure 2 Ashes obtained from calcination of a geopropolis from a species of *Melipona* (A) and a species of *Scaptotrigona* (B; Meliponini, Apidae).

Because the contents of ashes and ethanol solubles in *Melipona* are highly correlated ($R^2 = 0.96$; Figure 1), geopropolis with higher contents of soil material contain lower amounts of biologically active substances. As seen in Figure 1, for several samples with ash contents higher than 60% (**Md$_1$, Md$_2$, Mf, Mm, Mq$_3$ and Mq$_4$**) the contents of ethanol solubles lie in the range of 2%-6%. The lowest officially admitted content of ethanol solubles in propolis of *Apis mellifera* is 35% (Brasil 2001). In the present work, only four samples complied with this requisite: **Mq$_1$, Mq$_2$, Ms$_1$ and Sp** (Figure 1). High intraspecific contents of both total ashes and total ethanol solubles were observed for samples of *Melipona*. For example, the contents of ashes and ethanol solubles were, respectively, 43% and 40% for **Mq$_1$**, but 18% and 63% for **Mq$_3$**. Such difference may be attributed to geographic factors, which are known to influence considerably propolis composition.[15] Regarding *Scaptotrigona*, there is virtually no correlation ($R^2 = 0.64$) between the contents of total ashes and ethanol solubles. Although practically no variation was observed in the contents of ashes of samples **Sb$_1$,Sb$_2$, Sp, Sx$_1$** and **Sx$_2$**, their content of ethanol solubles varied from 11% (**Sx$_2$**) to 38% (**Sp;** Figure 1). Therefore, other major propolis constituents exert influence, reducing the content of ethanol solubles. A major propolis constituent that may be pointed out in this regard is the wax content. It is suggestive that **Sb$_1$** and **Sx$_1$**, both from a meliponary in the municipality of Guarujá (Table 1) had higher contents of ethanol solubles than **Sb$_2$** and **Sx$_2$**, both samples derived from a meliponary in the municipality of Jacuí (Table 1; Figure 1). Availability of plant sources of resin, which is affected by several factors, including geography[16] may account for the observed results.

Table 1 Species of Meliponini which produced propolis and geopropolis analyzed in the present work, respective codes mentioned in the text, and localities (municipalities and states) of the corresponding meliponaries

Species	Codes	Localities (municipalities/ states)
Melipona flavolineata (Friese, 1900)	**Mf**	Castanhal; Pará
M. marginata (Lepeletier, 1836)	**Mm**	Guarujá; São Paulo
M. mondury (Smith, 1863)	**Md$_1$**	Cotia; São Paulo
M. mondury	**Md$_1$**	Cotia; São Paulo
M. quadrifasciata (Lepeletier, 1836)	**Mq$_1$**	Cotia; São Paulo
M. quadrifasciata	**Mq$_2$**	Cotia; São Paulo
M. quadrifasciata	**Mq$_3$**	São Paulo; São Paulo
M. quadrifasciata	**Mq$_4$**	São Paulo; São Paulo
M. subnitida (Ducke, 1910)	**Ms$_1$**	Mossoró; Rio Grande do Norte

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Table Continued...

Species	Codes	Localities (municipalities/ states)
M. subnitida	**Ms_2**	Mossoró; Rio Grande do Norte
Scaptotrigona bipunctata (Lepeletier, 1836)	**Sb_1**	Guarujá; São Paulo
S. bipunctata	**Sb_2**	Jacuí; Minas Gerais
S. postica (Latreille, 1804)	**Sp**	Barra do Corda; Maranhão
S. tubiba (Smith, 1863)	**St**	Guarujá; São Paulo
S. xanthotricha (Moure, 1950)	**Sx_1**	Guarujá; São Paulo
S. xanthotricha	**Sx_2**	Jacuí; Minas Gerais

Conclusions

The results indicate that the habit of aggregating soil to propolis is taxonomically dependent. Probably most species of *Melipona* are geopropolis producers, while most *Scaptotrigona* species produce propolis. A high content of ashes implies that the corresponding geopropolis is a poor vehicle of biologically active substances. However, propolis from meliponine taxa devoid of soil material may also present reduced flavonoids and other phenolic substances, possibly due to high wax content. A proper quality evaluation of meliponine propolis and geopropolis requires the quantitative determination of classes of several chemical constituents.

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Conflicts of interest

None.

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