Research Article

Antioxidant Activity of a Geopropolis from Northeast Brazil: Chemical Characterization and Likely Botanical Origin

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Geopropolis is a product containing wax, plant resin, and soil particles. It is elaborated by stingless bees of tribe Meliponini. Methanolextracts of sample of geopropolis produced by Scaptotrigona postica (“mandaguari”) in the state of Rio Grande do Norte (RN, northeast Brazil) were analyzed for the determination of standard parameters (total phenols, total flavonoids, and radical scavenging activity) and chemical characterization by HPLC-DAD-MS/MS analysis. The sample analyzed has high content of total phenols and flavonoids, as well as high antioxidant activity. The constituents characterized were mainly flavonols, such as quercetin methyl ethers, and methoxylchalcones. Such chemical profile is similar to the composition of a green propolis from the same area of RN, which is produced by Africanized Apis mellifera, using shoot apices of Mimosa tenuiflora, popularly known as “jurema-preta.” This finding provides evidence that “mandaguari” geopropolis and honeybee propolis have the same botanical origin in RN. The sharing of a plant resin source by phylogenetically distant bees (Apinae and Meliponinae) suggests that bee genetic factors play little role in the choice of plants for resin collection and that the availability of potential botanical sources plays a decisive role.

1. Introduction

Apidae and Meliponinae correspond, respectively, to a family and a subfamily of tropical bees. An important tribe in the group is Meliponini, whose members are known as stingless bees. There are over 300 species of Meliponini, distributed into several genera, including Melipona and Trigona. The former genus comprises about 50 exclusively neotropical species, while the former is a larger group, represented by over 130 pantropical species. They are valuable pollinators of either native or crop plants [1]. The hives of stingless bees are built as honeycombs for the larvae and as pots for honey and pollen keeping [2, 3].

Geopropolis is a product elaborated by stingless bees. It is similar to honeybee propolis, since both products contain plant resin and beeswax. They are distinct, however, because geopropolis contains variable content of soil material. As is the case with propolis, geopropolis is used to line the entrance of the hive and seal holes on the hive nest. Much have been published about chemistry of propolis, its botanical origin, and biological activity [4–6]. In comparison, considerably less has been done on geopropolis, in spite of the huge biological diversity of stingless bees [7, 8]. In Brazil, geopropolis has been used for the treatment of respiratory diseases and dermatoses [9]. Brazilian geopropolis revealed antioxidant, anticancer, anti-inflammatory, and antimicrobial activities [10], while Indonesian geopropolis has cytotoxic activity against several human cancer cell lines [11]. Most papers about chemistry of Brazilian geopropolis have dealt with Melipona species. Melipona fasciculata produces different types of geopropolis: carbohydrates, triterpenes, anacardic acid, alkylresorcinols, and sugar alcohols characterize a
geopropolis from the northeast Brazil [7], while phenolic acids, gallo- and ellagitannins, are constituents of a geo-
propolis from the lowlands of the state of Maranhão (western 

northeast Brazil) [2, 3]. *Melipona subnitida* from the state of 

Paraíba (northeast Brazil) produces geopropolis containing 

phenylpropanoids and flavonoids [12]. *Melipona scutellaris*
from the state of Bahia (southern northeast Brazil) produces 

distinct types of geopropolis, for example, a sample contain-

ing benzophenones as main constituents [13] and another 

one characterized by cinnamic acid esters and coumarins 
[8]. Flavonoid glycosides were detected in geopropolis of 

two Amazonian stingless bees, *Melipona interrupta* and 

*M. seminigra* [14], and phenolic compounds and terpenes 

were reported for a geopropolis of *M. orbignyi* from Mato 

Grosso do Sul (central-west Brazil) [15]. Much less has been 

published about geopropolis of other species of stingless 

bees. A geopropolis of *Trigona spinipes* contains triterpenes 

and magniferolic acid, among other constituents [9]. A 

geopropolis of *Scaptotrigona postica* from Maranhão has a 

peculiar chemical profile: in addition to caffeoyl-quinic acids, 

it contains flavone-C-glycosides (chiefly vicenin-2) and a 

pyrrolizidine alkaloid derived from retronecine [16].

Studies about botanical origin of geopropolis are scarce. 

Evidence has been raised that fruits of *Corymbia torelliana* 

(Myrtaceae) provide resin for the production of geopropolis 

by the Australian *Tetragonula carbonaria* (Meliponini), due 

to a great similarity in the methylated flavanone profiles of 

both materials [17].

To our knowledge, nothing has been published about the 

chemistry of geopropolis of *Scaptotrigona* from the Brazilian 

Semiariad, a region designated as the “drought polygon.” It 

has been shown recently that a dark green honeybee propolis 

from Rio Grande do Norte (RN) is produced with shoot 
apices of *Mimosa tenuiflora* (Wild.) Poir. (Leguminosae, 

Mimosoideae), a plant locally known as “jurema-preta” [18]. 

It is a hardy and abundant species in the caatinga biome, 
a typical dry forest of Brazilian northeast, characterized by 

spiny small trees and shrubs, cacti, and euphorbs. Most 

caatinga plants are devoid of leaves over the long dry season, 

but “jurema-preta” remains green all year long. In the same 

area, a geopropolis with dark green color is produced by bees 

of *Scaptotrigona postica* Latreille, 1807, popularly known as 

“mandaguari.” It is a highly adaptable and active pollinator 

stingless bee widespread in several parts of Brazil. Many 

meliponaries produce “mandaguari” pollen and propolis, 

the latter being reputed as having quality superior to the 

green propolis from southeast Brazil. The aim of the present 

study was to determine standard parameters (total phenolic 

substances, total flavonoids, and antioxidant activity) of *S.* 

*postica* geopropolis, characterize its constituents, and test the 

hypothesis that “jurema-preta” might be its resin source.

2. Material and Methods

2.1. Material. A geopropolis sample of *S. postica* was collected in 

January 2014 in the meliponary at UFERSA (Rural Fed-

eral University of the Semiariad), municipality of Mossoró, 

RN state (eastern northeast Brazil; 05°11′16″S 37°20′38″W). 

Nearly 3 g of material was collected from three boxes, by 

scraping the product accumulated between the hive and its 

cover. The geopropolis collected is dark green and somewhat 

sticky, with resinous smell and moldable texture. The sample 

was cleaned of impurities, placed inside plastic bags, and kept 
in freezer.

2.2. Preparation of Ethanol Extracts. The geopropolis sample 

was powdered using liquid nitrogen, mortar, and pestle. 

Powdered material (1 g) was treated with 150 mL of ethanol 
in Soxhlet for 6 h. The extract was filtered and kept overnight 
in a dark flask in freezer at −20°C. The cold extract was filtered 
again to eliminate wax excess.

2.3. Standard Chemical Parameters. An aliquot of 20 mL of 

the wax free extract (WFE) was evaporated to dryness under 
nitrogen flow and weighed for determination of total solids. 

Triplicates of 5 mL of WFE were used for determination of 

the contents of total phenolic substances and total flavonoids, 

the former by the method of Folin–Ciocalteu and the latter by 

the method of the aluminum chloride. The procedures were 
described elsewhere [19]. Reference compounds used were p-

coumaric (total phenols) and quercetin (total flavonoids).

2.4. Antioxidant Activity. Triplicates of 5 mL of WFE were 

used for analysis of DPPH (2,2-diphenyl-1-picrylhydrazyl) 

free radical scavenging activity, according to Righi and 

collaborators [21], with modifications. Detailed procedure is 
available in a recent publication [18].

2.5. HPLC/DAD and HPLC-DAD-ESI-MS/MS Analyses. 

Aliquots of 1 mL of WFE were evaporated to dryness under 
nitrogen flow and the residue was dissolved in HPLC grade 

methanol to obtain solutions at 10 mg mL−1. The solutions 

were purified through 0.45 μm filters. Aliquots (10 μL) were 

analyzed by HPLC-DAD with a HPLC HP 1260 chromato-

gram (Agilent Technologies), using a Zorbax 5B-RP-18 

column. Analysis was also carried out by HPLC-DAD-ESI-

MS/MS with a DADSPD-MIOAVP Shimadzu chromatograph 

equipped with degasser, two LC-20AC pumps, CTO-20A 

column oven, SIL 20AC autoinjector and SPD-20A, and a 

reverse phase column Phenomenex Gemini C-18, protected 

by a guard column. The chromatograph was coupled to an 
esquire 2000 Plus Bruker Daltonics spectrometer, equipped 

with a quadrupole ion trap mass analyzer. Details of the 

analysis procedures, such as gas flows, oven temperatures, 

solvent gradients, and databases for chemical characteriza-

tion of constituents, are available in a recent publication [18].

Constituents’ characterization was based on UV-DAD and 

MS data in the negative and positive ionization modes and 

comparison with previously published data [22–25].

3. Results and Discussion

3.1. Standardization and Antioxidant Activity. The contents of 
soluble solids, total phenolic compounds, and total flavonoids 

are shown in Table 1, in addition to the minimum acceptable 

values given by the Technical Regulation of Propolis Identity 

and Quality (TRPIQ) (standards for comparison of physical
Table 1: Chemical parameters (g kg⁻¹) and DPPH radical scavenging activity (μg mL⁻¹) of a sample of geopropolis of *Scaptotrigona postica* from Mossoró (state of Rio Grande do Norte, northeast Brazil), compared with mean values of “jurema-preta” propolis [18] and limit values of the Technical Regulation of Propolis Identity and Quality (TRPIQ) [20].

| Sample                | Soluble solids | Total phenols | Total flavonoids | EC₅₀       |
|-----------------------|----------------|---------------|------------------|------------|
| Geopropolis           | 550            | 111.5 ± 5.4   | 98.5 ± 8.6       | 74.1 ± 0.5 |
| “Jurema-preta” propolis | 537            | 140           | 105              | 65         |
| TRPIQ                 | Minimum 350    | Minimum 50    | Minimum 5        | —          |
| Quercetin             | —              | —             | —                | 7.4 ± 0.1  |

and chemical characteristics of propolis produced in Brazil) [20]. All parameters analyzed comply with TRPIQ standards. As apparent in the table, they are similar to values reported for RN green propolis derived from “jurema-preta” [18] and considerably higher than the corresponding parameters of the internationally marketed green propolis from southeast Brazil [19]. The EC₅₀ antioxidant activity of the geopropolis sample is also shown in Table 1. As expected from the similarity between RN green geopropolis and “jurema-preta” propolis, the DPPH radical scavenging capacity of both bee products is also similar. The antioxidant activity of propolis has been attributed to its phenolic profile [26].

3.2. Chemical Characterization and Comparison with “Jurema-Preta” Propolis. Table 2 summarizes UV-DAD and MS data in the negative and positive ionization modes. Flavonoids were characterized by UV/vis bands at 345–360 nm (band I) and 255–280 nm (band II); chalcones were characterized by bands at 340–370 nm (band I) and 255–280 nm (band II); chalcones were characterized by bands at 340–370 nm (band I) and 255–280 nm (band II); chalcones were characterized by fragments generated by Retro-Diels-Alder reaction [23–25]. Details about characterization of the compounds based on UV/vis, MS⁺, MS⁻, and sodium adducts ([M + Na]⁺) are available in the paper by Ferreira and collaborators [18].

Only phenolic compounds, virtually only flavonoids, were characterized as constituents of the analyzed extracts of geopropolis (Table 2). This observation is consistent with the exceptionally high flavonoid content shown in Table 1. Biological properties of propolis are often attributed to their flavonoid constituents. Compounds 1, 4, and 11 are simple flavonol glycosides. Compounds 2, 5, 7, and 9 are flavon methyl ethers, compounds 3, 6, 8, and 13–15 are chalcones, compound 11 is a flavanone, and compounds 12 and 16 are flavones. Thus, the main constituents of the analyzed *S. postica* geopropolis are flavonoids and chalcones. Figure 1 depicts the HPLC-DAD chromatogram of the geopropolis ethanol extract. So far the origin of resin of propolis containing chalcones has been attributed to Leguminosae species, for example, *Dalbergia ecastophyllum*, the plant source of Brazilian red propolis [21, 27, 28]. The main plant sources of chalcones are species of Asteraceae, Leguminosae, and Moraceae [29]. The present report of chalcones as major constituents of both geopropolis of *S. postica* and green propolis of *Apis mellifera* from RN is coherent with the hypothesis that “jurema-preta” is one of their relevant botanical sources [18]. Data of Table 2 evidences the high similarity between chemical compositions of both products. Among the 16 constituents detected in the ethanol extract of the geopropolis, 10 were also detected as constituents of the green propolis from the same area (Table 2). “Jurema-preta” is abundant in the caatinga vegetation and its pollen is an important nutritional resource for the local bees.

3.3. Comparison with Other Geopropolis. The chemical profile seen in Table 2 is distinct from the composition reported for other Brazilian geopropolis. To our knowledge, no chalcones have so far been detected in geopropolis. A geopropolis of *Plebeia aff. flavocincta* from RN has been reported to contain high contents of phenols and flavonoids, but no detailed analysis was carried out for characterization of individual constituents [30]. The geopropolis analyzed in the present work is much distinct from a geopropolis produced by *Scaptotrigona postica* from Maranhão [16]. It is worth observing that stingless bee colonies of the same species may produce geopropolis much distinct from one another.
Table 2: Distribution of constituents of ethanol extracts of geopropolis of Scaptotrigona postica (Meliponini) from the meliponary at the University of Mossoró (state of Rio Grande do Norte, northeast Brazil), as characterized by HPLC-DAD-ESI-MS/MS analysis. Data are also given about presence/absence of the constituents in propolis of Apis mellifera from the same area, derived from shoot apices of “jurema-preta” (Mimosa tenuiflora, Leguminosae), according to published data [18].

| Compound number | Retention time | UV (nm) | MS⁻ (m/z, %) | MS⁺ (m/z, %) | Proposed characterization | References | Presence/absence in “jurema” propolis |
|-----------------|----------------|---------|--------------|--------------|--------------------------|-----------|-------------------------------------|
| 1               | 35.2           | 260, 365| 301 [M - H]⁻ | 303 [M + H]⁺ | Quercetin                | Comparison with standard          | +                     |
| 2               | 38.2           | 260, 365| 315 [M - H]⁻ | 339 [M + Na]⁺, 317 [M + H]⁺, 302 (100) | Quercetin methyl ether          | [19, 21] | +                                   |
| 3               | 39.6           | 270, 365| 315 [M - H]⁻ | 339 [M + Na]⁺, 317 [M + H]⁺, 197 (100) | Trihydroxy-dimethoxy chalcone   | [17–19, 22] | +                                   |
| 4               | 41.0           | 260, 360| 285 [M - H]⁻ | 287 [M + H]⁺ | Kaempferol               | Comparison with standard          | –                     |
| 5               | 42.4           | 260, 360| 299 [M + H]⁺ | 323 [M + Na]⁺, 301 [M + H]⁺, 286 (100) | Kaempferol methyl ether         | [19, 21] | +                                   |
| 6               | 43.0           | 250, 270sh, 365 | 329 [M - H]⁻ | 353 [M + Na]⁺, 331 [M + H]⁺, 316 (100) | Dihydroxy-trimethoxy chalcone   | [19, 21] | +                                   |
| 7               | 44.4           | 255, 355| 313 [M - H]⁻ | 337 [M + Na]⁺, 315 [M + H]⁺, 300 (60), 282 (100) | Kaempferol dimethyl ether       | [19, 21] | +                                   |
| 8               | 45.1           | 370     | 315 [M - H]⁻ | 317 [M + H]⁻ | Uncharacterized flavonol | —                           | —                     |
| 9               | 45.6           | 255, 355| 329 [M - H]⁻ | 353 [M + Na]⁺, 331 [M + H]⁺ | Quercetin dimethyl ether       | [19, 21] | +                                   |
| 10              | 47.1           | 255, 355| 327 [M - H]⁻ | 329 [M + H]⁺ | Uncharacterized flavonol | —                           | —                     |
| 11              | 48.3           | 260, 360| 271 [M - H]⁻ | 273 [M + H]⁺ | Trihydroxy flavonol      | [19, 21] | –                                   |
| 12              | 50.2           | 270, 340| 329 [M - H]⁻ | 331 [M + H]⁺ | Trihydroxy-dimethoxy flavone | [19, 21] | –                                   |
| 13              | 52.2           | 370     | 269 [M - H]⁻ | 271 [M + H]⁺, 161 (100), 137 (70) | Methoxy-dihydroxy chalcone     | [17–19, 22] | +                                   |
| 14              | 56.6           | 370     | 299 [M - H]⁻ | 301 [M + H]⁺, 167 (100) | Dimethoxy-dihydroxy chalcone   | [17–19, 22] | +                                   |
| 15              | 58.5           | 370     | 313 [M - H]⁻ | 315 [M + H]⁺ | Hydroxy-trimethoxy chalcone | [17–19, 22] | –                                   |
| 16              | 59.9           | 270, 340| 343 [M - H]⁻ | 367 [M + Na]⁺, 345 [M + H]⁺ | Dihydroxy-trimethoxy flavone   | [17–19, 22] | +                                   |
3.4. Perspectives. Favorable prospects regarding biological activities are suggested by the high content of flavonols and chalcones and the expressive antioxidant activity of the RN “mandaguari” geopropolis. In addition to many other biological activities, flavonols such as quercetin and isorhamnetin derivatives exhibit coronary heart disease prevention, as well as antioxidant, hepatoprotective, anti-inflammatory, and anticancer effects [32, 33]. Chalcones are flavonoids recognized as possessing many activities, such as antioxidant, anti-inflammatory, antibacterial, antifungal, cytotoxic, antitumoral, and chemopreventive activities [29, 34–36].

4. Conclusion

Most propolis in the commerce derives from honeybees. Some stingless bees, however, may produce propolis in amounts commercially feasible, as is the case of “jataí” (Tetragonisca angustula), from the northeast of Brazil. Bees of S. postica (“mandaguari”) reveal promising prospects for becoming a relevant source of commercial geopropolis. The already reputed high quality of its geopropolis is consistent with results of the present paper, such as the high antioxidant activity and the exceptional predominance of flavonoids, including a high content of chalcones.

Taking into account the economic and social difficulties afflicting the population of northeast Brazil, including the population of RN, the implementation of actions stimulating the production of honeybee green propolis and “mandaguari” geopropolis, both products derived from “jurema-preta,” might afford benefits for the local economy, mainly during the harsher dry seasons.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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