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Chemical Constituents in *Baccharis dracunculifolia* as the Main Botanical Origin of Southeastern Brazilian Propolis

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Previously, it was reported that one group of propolis (Group 12) was identified in southeastern Brazil, and the botanical origin of the propolis was *Baccharis dracunculifolia* resinous exudates. It was also observed that honeybee (Africanized *Apis mellifera*) mainly visited the leaf buds or unexpanded leaves of *B. dracunculifolia* but rarely expanded leaves. *B. dracunculifolia* is dioecious with male and female inflorescences, and RPHPLC of the ethanolic extracts of the respective male and female bud resinous exudates showed the same profiles. RPHPLC profiles of propolis G12 leaf buds and unexpanded and expanded leaves of *B. dracunculifolia* showed similarity, but unexpanded leaves quantitatively decreased in chemical constituents as compared with leaf buds. In the case of expanded leaves, all chemical constituents were severely decreased or disappeared. Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) was also identified in both propolis and resinous exudates, and both ethanolic extracts contained the highest concentrations of this compound as compared with the rest of the chemical constituents.

KEYWORDS: Propolis G12; resinous exudate; flavonoids; africanized *Apis mellifera*; *Baccharis dracunculifolia*

INTRODUCTION

Propolis is the generic name for the resinous substances collected by honey bees from various plant resins and has been used as a folk medicine since ca. 300 BC (*1*). Recently, numerous biological properties have been reported, including cytotoxic (*2*), antiherpes (*3*), antitumor (*4*), free radical scavenging (*5*), antimicrobial (*6*), and anti-HIV activities (*7*). Because of the wide range of biological activities, propolis is now increasingly being used as a health food supplement (*8*, *9*).

Previously, we reported that Brazilian propolis had been classified into 12 groups based on physicochemical characteristics, five in southern Brazil, one in southeastern Brazil, and six in northeastern Brazil (*10*, *11*), and it was also reported that the main botanical origin of propolis group 3, group 6, and group 12 were resins of the poplar tree, *Hyptis divaricata*, and *Baccharis dracunculifolia*, respectively (*12*).

Of the 12 groups of propolis, group 12, which can be collected in southeastern and central western Brazil, has been extensively used in foods and beverages to improve health and prevent diseases. Therefore, the objective of this work was to investigate further and obtain detailed knowledge of the chemical constituents in *B. dracunculifolia*, which is the botanical origin of propolis group 12.

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MATERIALS AND METHODS

Propolis and Plant Resins. We were informed by a beekeeper in the southeastern region of Brazil that bees (Africanized *Apis mellifera*) mainly visited the leaf bud or the unexpanded leaves of *B. dracunculifolia* to collect resins. These three stages (bud, unexpanded, and expanded) of the growing leaves were collected separately, then ethanolic extracts of the resins were prepared immediately as described below. In the case of propolis, approximately 30 g of the propolis were collected from one beehive, which was put in the same area where the leaf buds from *B. dracunculifolia* were collected.

Preparation of Ethanolic Extracts of Propolis and Plant Resins. The ethanolic extracts of propolis and plant resins were prepared as described previously (*12*). The respective leaf buds, unexpanded, and expanded leaves, were removed with a knife without breaking them into pieces, and immediately 2 g of the respective samples were rinsed with 20 mL of 80% ethanol at 70 °C for 1 h to remove superficial resins and then centrifuged to separate the supernatant. In the case of propolis, 2 g of the propolis powder was mixed with 25 mL of 80% ethanol and shaken at 70 °C for 30 min. After extraction, the mixture was centrifuged to separate the supernatant, and the supernatant was used for analysis.

Reversed-Phase High-Performance Liquid Chromatography (RPHPLC). The analysis of flavonoids from bud exudates and propolis was performed by reversed-phase HPLC with a chromatographer equipped with YMC Pack ODS-A column and diode array detector (SPD-M10A, Shimadzu Co., Japan). The column was eluted by use of a linear gradient of solvent water (solvent A) and methanol (solvent B), starting with 30% of B (0−15 min), raising until 90% (15−95 min), and decreasing to 30% of B (95−105 min). The absorption spectra

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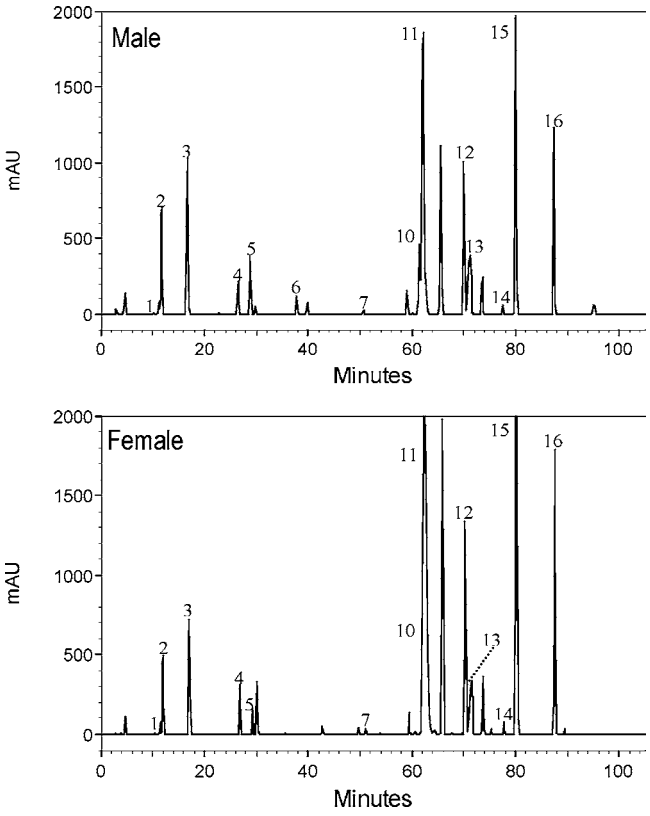

Figure 1. RPHPLC of ethanolic extracts of male and female *Baccharis dracunculifolia*.

resulting from diode-array detection were used to distinguish peaks. The chromatogram measured at 260 nm was used for qualification of flavonoid and other phenolic constituents. Identification of flavonoid and other phenolic constituents was carried out by direct HPLC comparison with authentic standards and was based on retention time, co-chromatography, and on the identity of absorption spectra. Furthermore, some identifications were also confirmed by GC-mass spectrometry, as described previously (*12*). The following authentic standards of flavonoids (Extrasynthese Co., France) were examined: quercetin, kaempferol, apigenin, pinocembrin, chrysin, acacetin, galangin, kaempferide, isosakuranetin, and sakuranetin. Additionally, pinobanksin, pinobanksin-3-acetate, and dimethylallyl caffeic acid (kindly provided by E. Wollenweber, Darmstadt, Germany), and *p*-coumaric acid (4-hydroxycinnamic acid, Extrasynthese Co.) and ferulic acid (Extrasynthese Co.) were used in this assay.

Method of NMR. The ^{1}H NMR spectra were obtained on a Varian Inova spectrometer at 500 MHz, using 5-mm tubes. The extracts were dried and dissolved in $CDCl_3$, and tetramethylsilane was used as the reference.

RESULTS AND DISCUSSION

Propolis Group 12 and Its Botanical Origin. As described in previous publications (*10*−*12*), one group of propolis (Group 12), was identified in southeastern Brazil, and the botanical origin of this propolis was the *B. dracunculifolia* resinous exudate. Currently the Brazilian Cerrado (Brazilian geographical name), which is intermediate between forest and pasture, is one of the richest areas in *Baccharis* sp., and the plants are a group of woody perennial shrubs, which are dioecious with male and female inflorescences appearing on separate plants (*13*). Of the various *Baccharis* species, *Baccharis dracunculifolia* is the dominant propolis source in southeastern Brazil (State of São Paulo and Minas Gerais cerrado area), where the majority of the commercialized propolis products are produced (*10*, *11*, *14*).

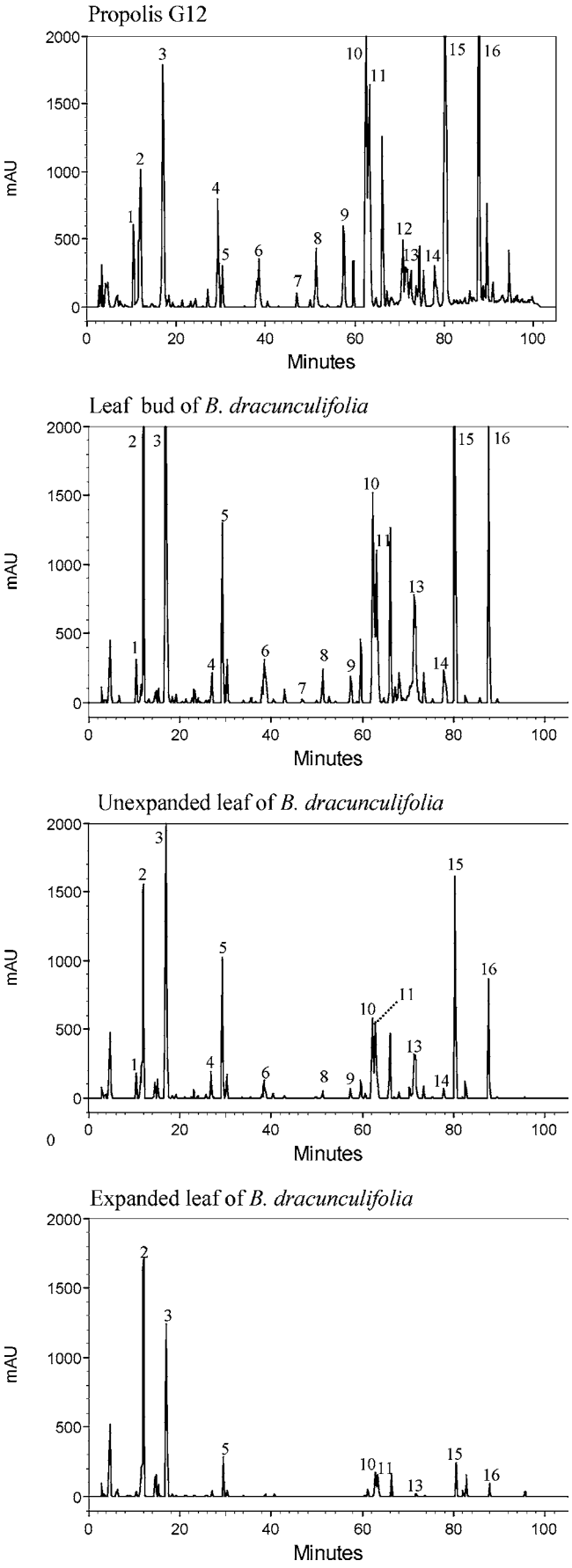

Figure 2. RPHPLC chromatograms of the ethanolic extracts of propolis G12 and *B. dracunculifolia* resin.

Table 1. Flavonoids and Other Chemical Constituents of Propolis and *B. dracunculifolia*, Determined by RPHPLC (mg/g)

peak no.	retn time (min)	compound	propolis[a] G12	leaf bud[a]	unexpanded leaf[a]	expanded leaf[a]
1	10.40	coumaric acid	10.67	4.23	2.68	–
2	12.08	ferulic acid	2.40	4.80	3.82	4.18
3	17.50	λ 245 nm[b]	+	+	+	+
4	28.20	cinnamic acid	2.62	0.68	0.60	–
5	30.10	pinobanksin	1.66	7.20	5.54	1.66
6	38.85	kaempferol	1.30	1.10	0.49	–
7	48.20	isosakuranetin	4.87	1.22	–	–
8	52.10	chrysin	1.86	1.05	0.23	–
9	57.65	acacetin	6.65	2.33	1.15	–
10	62.90	kaempferide	12.57	8.15	3.95	1.61
11	64.15	λ 244 nm[b]	+	+	+	+
12	71.15	λ 230 nm[b]	+	+	–	–
13	72.00	λ 245 nm[b]	+	+	+	+
14	78.15	λ 228, 246 nm[b]	+	+	+	–
15	80.20	artepillin C	38.58	40.54	13.75	1.68
16	88.15	λ 223, 276 nm[b]	+	+	+	+

[a] Quantity of constituents is mg/g of propolis, or leaf bud, or unexpanded leaf, or expanded leaf. Symbols: +, present, but not quantified; –, not detected. [b] Unidentified constituents represent only UV spectral absorption maxim.

Furthermore, this plant also produces vassoura oil, which is used in perfumery (*13*). RPHPLC of the ethanolic extracts of the respective male and female *B. dracunculifolia* leaf bud was performed, and the results showed the same profiles as those shown in **Figure 1**. It was also found that on rare occasions, other plants are used as propolis sources by the bees where the *B. dracunculifolia* is not present. These propolis samples were not included in this investigation because they did not show a significant presence of polyphenol compounds and biological activities.

Qualitative and Quantitative Comparisons of the Flavonoid and Other Phenolic Compound Profiles. Flavonoid and other phenolic compound profiles of the ethanolic extracts of propolis G12 and *B. dracunculifolia* resinous exudates were carried out by RPHPLC. Flavonoid and other phenolic compounds were quantified by HPLC on a C-18 reversed-phase column, and the absorption spectra resulting from diode-array detection were used to distinguish peaks. Identification of peak was carried out by HPLC comparison with authentic standards. The results were shown in **Figure 2** and **Table 1**. The HPLC profiles of propolis G12 are exactly the same as those of *B. dracunculifolia* leaf bud extracts, and it is apparent that, qualitatively, flavonoid and other phenolic compound profiles of leaf bud resemble those found in unexpanded and expanded leaves of *B. dracunculifolia*. However, in quantitative contrast, the chemical constituents of unexpanded leaves were decreased as compared with leaf bud. In case of expanded leaves, all chemical constituents were severely decreased, and coumaric acid, cinnamic acid, kaempferol, isosakuranetin, chrysin, and acacetin are not apparent.

Identification of Artepillin C. Artepillin C, 3,5-diprenyl-4-hydroxycinnamic acid, is a distinct antimicrobial compound and was isolated from Brazilian propolis by Aga et al. (*15*). We have examined the presence of artepillin C in the ethanolic extracts of all groups of Brazilian propolis and *B. dracunculifolia* resinous exudates by HPLC, and identification of peak was carried out by comparison with an authentic standard, which was donated by Hayashibara Biochemical Laboratories, Okayama, Japan. It was found that peak 15 of the HPLC chromatograms (**Figure 2**) of propolis G12 and *B. dracunculifolia* was identified, as artepillin C and **Table 1** indicated the highest concentrations as compared with other chemical constituents. The presence of artepillin C in the ethanolic extracts was also confirmed by ^{1}H NMR. The peaks observed in the spectrum of the authentic sample of artepillin C (δ 7,69 (1H, d, $J = 15.9$); δ 7.20(2H s); δ 6.29 (1H, dd, $J = 15.9$); δ 5.31(2H, t, $J = 7.2$); 2H δ 3.35(4H, d, $J = 7.2$); s δ 1.79 (6H, s); δ 1.78 (6H, s)) were all clearly observed in the spectra of the extracts of propolis and of *B. dracunculifolia*, except for the extract of the expanded leaf. As a final check, artepillin C was added to the solution of the propolis extract and an increase in the peaks attributed to artepellin C was observed.

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