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Comparison of volatile and polyphenolic compounds in Brazilian green propolis and its botanical origin *Baccharis dracunculifolia*

Mário Roberto MARÓSTICA JUNIOR¹, Andreas DAUGSCH¹, Cleber Silveira MORAES¹, Carmen Lucia QUEIROGA², Gláucia Maria PASTORE¹, Yong Kun PARK¹*²

1 Introduction

Propolis (also referred to as bee's glue) possesses a broad spectrum of biological activities, such as anti-hepatotoxic, antitumor, antiviral, antioxidant, antimicrobial and anti-inflammatory properties, and is therefore used as a constituent of health foods and functional foods (BURDOCK, 1998). Propolis is in general composed of 50% resin and balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other substances, such as aliphatic acids, esters, aromatic acids, fatty acids, carbohydrates, aldehydes, amino acids, ketones, chalcones, dihydrochalcones, terpenoids, vitamins (B1, B2, B6, C and E) and minerals (aluminum, antimony, calcium, cesium, copper, iron, lanthanum, manganese, mercury, nickel, silver, vanadium and zinc) (ALMEIDA; MENEZES, 2002).

1 Introduction

Propolis is collected from the leaf buds of numerous tree species (alder, birch, palm, pine, poplar and willow). Other plant exudates and secretions, such as substances secreted by plants to seal wounds, lipophylic substances on leaves, mucilages, gums, resins, latices, etc. are also used (BANKOVA; CASTRO; MARCUCCI, 2000). Brazilian Green Propolis is collected from the tender sprouts of *Baccharis dracunculifolia*, which is commonly found in the States of Minas Gerais, São Paulo, Rio de Janeiro and Paraná (ALENCAR et al., 2005; PARK; ALENCAR; AGUIAR, 2002; PARK; IKEGAKI; ALENCAR, 2000). In Brazil, twelve distinct groups of propolis have been classified according to their botanical origin and biological properties. Brazilian Green Propolis is classified as Group 12, which is the most commercialized and largely used in foods and beverages to improve health and prevent diseases (PARK et al., 2005).

Propolis is in general composed of 50% resin and balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other substances, such as aliphatic acids, esters, aromatic acids, fatty acids, carbohydrates, aldehydes, amino acids, ketones, chalcones, dihydrochalcones, terpenoids, vitamins (B1, B2, B6, C and E) and minerals (aluminum, antimony, calcium, cesium, copper, iron, lanthanum, manganese, mercury, nickel, silver, vanadium and zinc) (ALMEIDA; MENEZES, 2002).

Abstract

Ethanolic extracts and essential oils from Green Propolis from southeastern Brazil and leaf buds from its botanical origin *Baccharis dracunculifolia* were analyzed by Reversed Phase High Performance Liquid Chromatography (RP-HPLC), Reversed Phase High Performance Thin Layer Chromatography (RP-HPTLC) and Gas Chromatography – Mass Spectrometry (GC-MS). The essential oils were obtained by hydro-distillation. Both ethanolic extracts and essential oils showed similar chromatographic profiles. Fifteen flavonoids were identified by RP-HPLC and RP-HPTLC analyses in both samples. Twenty-three volatile compounds were identified by GC-MS analyses. Seventeen were present in both essential oils. The major flavonoid compound in both extracts was artepillin C. The major volatile compound in both essential oils was nerolidol. The major compounds identified in this work could be used as chemical markers in order to classify and identify botanical origins of propolis.

Keywords: essential oil composition; *Baccharis dracunculifolia*; propolis; “Alecrim do campo”; flavonoids.

Resumo

Extratos etanólicos e óleos essenciais de própolis verde do sudeste brasileiro e gemas de sua origem botânica (*Baccharis dracunculifolia*) foram analisados por CLAE-FR (Cromatografia Líquida de Alta Eficiência em Fase Reversa), CCDAE (Cromatografia em Camada Delgada de Alta Eficiência) e CG-EM (Cromatografia Gasosa acoplada a Espectrometria de Massas). Os óleos essenciais foram obtidos por hidrodestilação. Extratos etanólicos e óleos essenciais de *Baccharis dracunculifolia* e de própolis mostraram perfis cromatográficos similares entre si. Treze flavonoides foram identificados por CLAE-FR e CCDAE em ambas as amostras. Vinte e três compostos voláteis foram identificados por CG-EM, sendo dezessete deles presentes em ambos os óleos essenciais. Artepillin C foi o flavonóide encontrado em maiores concentrações em ambas as amostras, enquanto nerolidol foi o volátil majoritário em ambos os óleos essenciais. Os compostos majoritários identificados neste trabalho podem ser utilizados como marcadores químicos para classificar de forma prática e identificar origens botânicas de própolis.

Palavras-chave: composição de óleos essenciais; *Baccharis dracunculifolia*; própolis; Alecrim do campo; flavonoides.
The health benefits of propolis are related to its content of flavonoids and other phenolic compounds (BANKOVA; CASTRO; MARCUCCI, 2000). As volatile components, various mono- and sesquiterpenes are found (BANKOVA et al., 1994, FERRACINI et al., 1995).

2 Materials and methods

2.1 Propolis and its botanical origin

Brazilian Green Propolis, produced by africanized Apis mellifera, and B. dracunculifolia leaf buds were collected in southeastern Brazil and immediately analyzed.

2.2 Distillation

The essential oil of B. dracunculifolia leaves was extracted by hydrodistillation and collected in a bottom with methylene chloride. After 1h of distillation, the oil was separated in a funnel. The aqueous portion was extracted twice with methylene chloride. The organic phases were dried with anhydrous sodium sulfate, filtered and dried under vacuum. The same procedure was performed with the propolis.

2.3 GC-MS analyses

Were carried out in a GC 6890N Agilent gas chromatograph with a 5975 mass spectrometry detector equipped with a HP5MS column (30 m × 0.25 mm × 0.25 µm). Column temperature was programmed as follows (QUEIROGA; FUKAI; MARSAIOLI, 1990): 55 to 120 °C at 20 °C/min, 120 to 150 °C at 1.5 °C/min, 150 to 250 °C at 20 °C/min, 250 °C (10 minutes). Carrier gas was helium (1 mL/min). Injector and detector temperatures were 220 and 250 °C, respectively. The identification of the compounds was based on retention indices relative to C9-C20 n-alkane series (ADAMS, 1995), by computer search using the NIST107 library, and by comparison with the spectra data in the literature.

2.4 Preparation of propolis and B. dracunculifolia leaf bud ethanolic extracts

The ethanolic extracts were prepared as previously described (PARK et al., 2005). Two grams of propolis and 25 mL of 80% (v/v) ethanol were used for extraction at 70 °C for 30 minutes. After extraction, the mixture was centrifuged at 10,000 x g and the resulting supernatant was evaporated to dryness at 40 °C and vacuum. The leaf buds were removed with a knife without breaking them into pieces, and two grams of the samples were rinsed immediately with 50 mL of 80% ethanol, at 70 °C, for 1 hour, to remove superficial resins and then centrifuged. The supernatant was evaporated to dryness at 40 °C and vacuum. The dried propolis and Baccharis dracunculifolia leaf bud resin extracts were dissolved in methanol and used for RP-HPLC and RP-HPTLC analyses.

2.5 Reversed-phase high-performance liquid chromatography (RP-HPLC)

The analyses of the chemical compounds in propolis and leaf bud extracts were performed by RP-HPLC, with a liquid chromatograph equipped with a YCM Pack ODS-A column and photodiode array detector (SPD-M10-A, Shimadzu Co., Kyoto, Japan). The mobile phase was acetic acid:methanol:water (5:75:60 by vol). The flow rate was 1 mL/min. For quantification, a DAD trace of 254 nm was used. The identification of the flavonoids was carried out through retention times and co-chromatography with authentic standard substances (Extrasynthese Co., Genay Cedex, France).

2.6 Reversed-phase high-performance thin layer chromatography (RP-HPTLC)

Precoated RP-18 F Sub silica gel plates (Merck Co., Darmstadt, Germany) were used. Six microliters of the ethanolic extracts were applied, and an ascending chromatographic run with a mobile phase of ethanol:water (55:45 vol/vol) was performed during 1.5 hour. The detection of flavonoids was carried out by UV radiation at 366 nm (PARK et al., 2004).

3 Results and discussion

As shown in Figures 1 and 2, ethanolic extracts of G12 propolis and its botanical origin B. dracunculifolia show a similar chromatographic profile. The thirteen flavonoids identified in...
G12 propolis were also present in the ethanolic extracts of *B. dracunculifolia* leaf buds, although in different relative amounts (Table 1). The major compound in both extracts was artepillin C. The relative amounts of flavonoids in the ethanolic extracts of *B. dracunculifolia* leaf buds are higher than in the extracts of G12 propolis. This is due to the fact that propolis contains about 30% wax, 10% essential and aromatic oils, 5% pollen and 5% other substances, and only about 50% resins of *B. dracunculifolia*. In addition to the comparison of the flavonoid profile, we also compared the volatile composition of G12 propolis and its botanical origin.

The GC analyses of *B. dracunculifolia* and propolis essential oils revealed a great similarity between them, as shown in Figures 3 and 4. Twenty-three compounds were identified. Seventeen were present in both oils. The relative amount of each compound was different between the oils. Several components were present in trace amounts in both samples, such as beta-pinene, benzeneethanol, 4-terpineol and alpha-humulene. The components present in concentrations higher than 1% in both oils were: alpha-pinene, 1-phenyl-ethanone, linalool, trans-caryophyllene, delta-cadinene, nerolidol, spathulenol and globulol. The components contribute to more than 40% of the total oil for both samples, as shown in Table 2. The compound present in the highest concentration in both of the oils was nerolidol: 14.82% in *B. dracunculifolia* essential oil and 6.64% in Green Propolis oil. Excepting epi-alpha-cadinol, all the major compounds (>1%) present in the highest concentration in both of the oils was alpha-pinene, 1-phenyl-ethanone, linalool, trans-caryophyllene, delta-cadinene, nerolidol, spathulenol and globulol. The components contribute to more than 40% of the total oil for both samples, as shown in Table 2. The compound present in the highest concentration in both of the oils was nerolidol: 14.82% in *B. dracunculifolia* essential oil and 6.64% in Green Propolis oil. Excepting epi-alpha-cadinol, all the major compounds (>1%) present in *B. dracunculifolia* were also present in the propolis oil.

According to a previous study (FERRACINI et al., 1995), alpha-pinene, beta-pinene, limonene, alpha-terpineol, trans-caryophyllene, aromadendrene, alpha-humulene, delta-cadinene, nerolidol, spathulenol and globulol were identified in *B. dracunculifolia* essential oil (female and male plant). Our results confirm the presence of these 11 compounds in *B. dracunculifolia*, as well as in G12 propolis. Additionally, we identified benzeneacetaldehyde, 1-phenyl-ethanone, linalool, benzeneethanol, benzeneacetonitrile and 4-terpineol in both essential oils. 1-Phenyl-ethanone and linalool were the major compounds in both essential oils. The remaining compounds were found only in minor quantities. Myrcene, benzenepranoic acid methyl ester and ethyl 3-phenylpropionate were found in G12 propolis essential oil, but were not present in *B. dracunculifolia* oil. This might have been caused by the action of africanized *Apis mellifera*, adding other materials during the production of propolis. Gamma-muurolene, cis-beta-guaiene and epi-alpha-cadinol (torreyol) were found in *B. dracunculifolia* but not in G12 propolis.

### 4 Conclusion

We reported the identification of 11 major volatile compounds and 13 flavonoids in G12 propolis and *B. dracunculifolia*. The major compounds identified in this work could be used as

![Figure 3. Chromatographic profile of *Baccharis dracunculifolia* essential oil obtained by GC-MS.](image)

![Figure 4. Chromatographic profile of the volatile portion of G12 propolis essential oil obtained by GC-MS.](image)

### Table 1. Flavonoid composition of G12 propolis and *Baccharis dracunculifolia*.

<table>
<thead>
<tr>
<th>#</th>
<th>Name</th>
<th><em>Baccharis dracunculifolia</em></th>
<th><em>G12 propolis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention time (minutes)</td>
<td>Area (mAU)</td>
<td>Content (mg g⁻¹ dry extract)</td>
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<tr>
<td>1</td>
<td>Coumaric acid</td>
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<td>2</td>
<td>Rutin</td>
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<td>Pinobanksin</td>
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<tr>
<td>4</td>
<td>Quercetin</td>
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<td>5</td>
<td>Kaempferol</td>
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<td>6</td>
<td>Apigenin</td>
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<td>7</td>
<td>Pinocembrin</td>
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<td>8</td>
<td>Pinobanksin - 3-acetate</td>
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<td>9</td>
<td>Chrysir</td>
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<td>Galangin</td>
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<td>Kaempferide</td>
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<td>13</td>
<td>Artepillin C</td>
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</table>
chemical markers in order to classify and identify botanical origins of propolis. Further research in this area could focus on the evaluation of the biological activity of the compounds described in this investigation.

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References


