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MARÓSTICA JUNIOR, Mário Roberto; DAUGSCH, Andreas; Silveira MORAES, Cleber; QUEIROGA, Carmen Lucia; PASTORE, Gláucia Maria; PARK, Yong Kun
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Comparison of volatile and polyphenolic compounds in Brazilian green propolis and its botanical origin *Baccharis dracunculifolia*

Comparação de volatilidade e compostos polifenólicos na própolis verde brasileira e sua origem botânica Baccharis dracunculifolia

Mário Roberto MARÓSTICA JUNIOR[1], *Andreas DAUGSCH*[1], *Cleber Silveira MORAES*[1], *Carmen Lucia QUEIROGA*[2], *Gláucia Maria PASTORE*[1], *Yong Kun PARK*[1]*

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. Thirteen 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 hidro destilação. Extratos etanólicos e óleos essenciais de *Baccharis dracunculifolia* e de própolis mostraram perfis cromatográficos similares entre si. Treze flavonóides 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; flavonóides.*

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 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, lattices, 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).

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[1] *Departamento de Ciências de Alimentos, Faculdade de Engenharia de Alimentos – FEA, Universidade Estadual de Campinas – UNICAMP, Barão Geraldo, CP 6177, CEP 13081-970, Campinas - SP, Brasil, E-mail: ykpark@fea.unicamp.br*
[2] *Centro Pluridisciplinar de Pesquisas Químicas e Biológicas – CPQBA, Universidade Estadual de Campinas – UNICAMP, Barão Geraldo, CP 617, CEP 13083-970, Campinas - SP, Brasil*
**A quem a correspondência deve ser enviada*

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 x 0.25 mm x 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 $F_{254}S$ 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

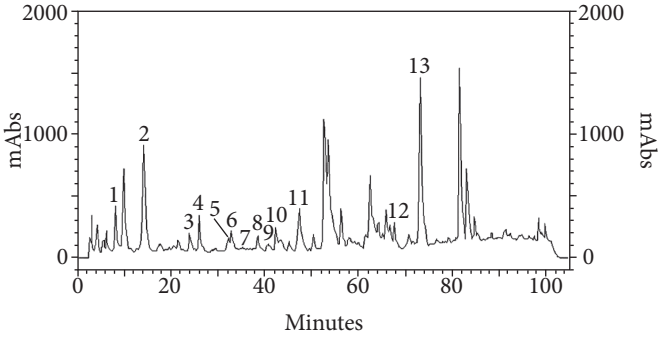

Figure 1. RP-HPLC of G12 propolis ethanolic extracts: 1 = Coumaric acid; 2 = Rutin; 3 = Pinobanksin; 4 = Quercetin; 5 = Kaempferol; 6 =Apigenin; 7 = Pinocembrin; 8 = Pinobanksin-3-acetate; 9 = Chrysin; 10 = Galangin; 11 = Kaempferide; 12 = Tectochrysin; and 13 = Artepillin C.

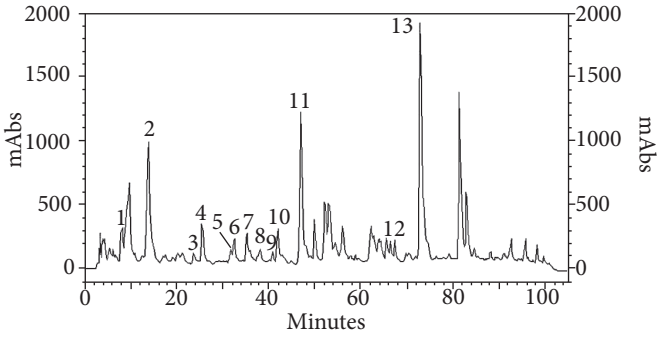

Figure 2. RP-HPLC of *Baccharis dracunculifolia* ethanolic extracts: 1 = Coumaric acid; 2 = Rutin; 3 = Pinobanksin; 4 = Quercetin; 5 = Kaempferol; 6 = Apigenin; 7 = Pinocembrin; 8 = Pinobanksin-3-acetate; 9 = Chrysin; 10 = Galangin; 11 = Kaempferide; 12 = Tectochrysin; and 13 = Artepillin C.

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 *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 benzeneacethaldehyde, 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, benzenepropanoic acid methyl ester and ethyl 3-phenylpropionate were found in G12 propolis essential oil, but were not present in *B. dracunfulifolia* 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

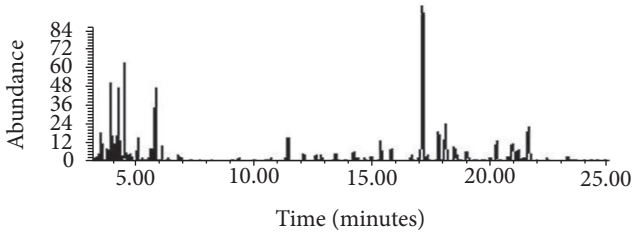

Figure 3. Chromatographic profile of *Baccharis dracunculifolia* essential oil obtained by GC-MS.

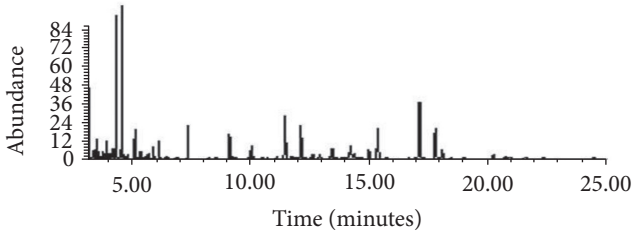

Figure 4. Chromatographic profile of the volatile portion of G12 propolis essential oil obtained by GC-MS.

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

#	Name	*Baccharis dracunculifolia*			G12 propolis		
		Retention time (minutes)	Area (mAU)	Content ($mg.g^{-1}$ dry extract)	Retention time (minutes)	Area (mAU)	Content ($mg.g^{-1}$ propolis)
1	Coumaric acid	8.165	9332459	2.49	8.324	8744338	2.33
2	Rutin	13.972	67294468	24.49	14.320	47908775	17.44
3	Pinobanksin	23.672	7210896	7.19	24.034	8470280	8.45
4	Quercetin	25.556	15028197	3.78	25.978	11944283	3.00
5	Kaempferol	31.926	6219622	0.91	32.397	6444702	0.94
6	Apigenin	32.638	13752477	2.51	33.072	13525546	2.47
7	Pinocembrin	35.329	9704040	27.07	36.417	2802988	7.82
8	Pinobanksin-3-acetate	38.170	9771622	6.59	38.614	11535066	7.78
9	Chrysin	40.915	6093583	1.13	41.277	3498328	0.65
10	Galangin	41.987	12318031	2.30	42.480	9369716	1.75
11	Kaempferide	46.974	49555936	7.11	47.413	25225045	3.62
12	Tectochrysin	67.394	13244539	2.93	67.725	14505198	3.21
13	Artepillin C	72.899	92100059	56.09	73.183	59455291	36.21

Table 2. Volatile composition of G12 propolis and *Baccharis dracunculifolia* essential oils.

Constituents	MW	R.I.	R.I. lit.	*B. dracunculifolia*		G12 propolis	
				t_r (minutes)	Area (%)	*tr* (minutes)	Area (%)
Alpha-pinene	136	930	932	3.20	1.56	3.20	1.59
Beta-pinene	136	983	980	3.53	0.64	3.53	0.63
Myrcene	136	990	991	-	-	3.57	0.14
Limonene	136	1036	1031	3.93	2.17	3.93	0.72
Benzeneacethaldehyde	120	1047	-	4.06	0.97	4.05	0.30
Ethanone, 1-phenyl-	122	1071	-	4.30	1.92	4.30	4.85
Linalool	154	1102	1098	4.57	2.82	4.57	4.99
Benzeneethanol	122	1115	-	4.79	0.18	4.79	0.24
Benzeneacetonitrile	117	1135	-	5.10	0.75	5.10	1.19
4-terpineol	154	1170	1177	5.68	0.43	5.68	0.23
Alpha-terpineol	154	1181	1189	5.85	2.55	5.86	0.51
Unknown	120	1198	-	6.13	0.65	-	-
Unknown	154	1242	-	6.84	0.28	-	-
Benzenepropanoic acid, methyl ester	164	1274	-	-	-	7.35	1.94
Ethyl 3-phenylpropionate	178	1349	1350	-	-	9.11	1.72
Unknown	204	1382	-	-	-	10.02	0.97
Trans-caryophyllene	204	1414	1418	11.44	1.76	11.45	4.05
Aromadendrene	204	1442	1439	12.11	0.48	12.11	3.13
Alpha-humulene	204	1457	1454	12.63	0.40	12.64	0.48
Gamma-muurolene	204	1478	1477	13.45	0.77	-	-
Cis-beta-guaiene	204	1488	1490	14.22	0.77	-	-
Delta-cadinene	204	1524	1524	15.36	1.70	15.36	3.37
Nerolidol	222	1564	1564	17.13	14.82	17.12	6.64
Spathulenol	220	1577	1576	17.80	2.85	17.80	3.57
Globulol	222	1583	1583	18.06	4.21	18.08	1.25
Epi-alpha-cadinol (torreyol)	222	1640	1640	20.93	1.66	-	-

MW = Molecular Weight; R.I. = Retention Index relative to n-alkanes; R.I. lit. = Retention Index as found in literature (ADAMS, 1995); and t_r = retention time, in minutes.

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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