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Artigo

Physicochemical characteristics of the essential oils of *Baccharis dracunculifolia* and *Baccharis uncinella* D.C. (Asteraceae)

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RESUMO: "Características físico-químicas dos óleos essenciais de *Baccharis dracunculifolia* e *Baccharis uncinella* D.C. (Asteraceae)". Os óleos essenciais de *Baccharis dracunculifolia* e *Baccharis uncinella*, obtidos por hidrodestilação, foram caracterizados físico e quimicamente em relação ao seu rendimento, sua densidade relativa (d_{20}^{20}), seu índice de refração, sua solubilidade em etanol e sua composição química por meio de cromatografia gasosa acoplada à espectrometria de massas (CG/EM). Os resultados mostraram um rendimento de 1,5 e 1,65 mL/100g^{-1}, uma densidade relativa (d_{20}^{20}) de 0,9151 e 0,9147, um índice de refração de 1,4593 e 1,4602, um poder rotatório de +1,99 e +2,18, a sua solubilidade em etanol 70% foi 3,0, em etanol 80% foi 1,0 e em etanol 96,5% também foi de 1,0, para o óleo de *B. dracunculifolia* e *B. uncinella*, respectivamente. Os resultados mostraram que os óleos avaliados são semelhantes, apresentando 26 compostos, com destaque para β-pineno, ε-nerolidol, limoneno e espatulenol.

Unitermos: *Baccharis dracunculifolia, Baccharis uncinella*, óleos essenciais, cromatografia.

ABSTRACT: The essential oils of *Baccharis dracunculifolia* and *Baccharis uncinella,* which were obtained by hydrodistillation, were physically and chemically characterized for their yield, relative density (d_{20}^{20}), refraction index, solubility in ethanol and chemical composition through gas chromatography coupled to mass spectrophotometry (GC/MS). The results showed a yield of 1.5 and 1.65 mL/100g^{-1}, relative density (d_{20}^{20}) of 0.9151 and 0.9147, refraction index of 1.4593 and 1.4602, rotatory power of +1.99 and +2.18. Solubility in 70% ethanol was 3.0, in 80% ethanol was 1.0 and in 96.5% ethanol was 1.0, for the oils of *B. dracunculifolia* and *B. uncinella* respectively. The evaluations in gas chromatography coupled to mass spectrophotometry showed that the oils studied are similar, presenting 26 constituents among which β-pinene, ε-nerolidol, limonene and spathulenol are highlighted.

Keywords: *Baccharis dracunculifolia, Baccharis uncinella*, essential oils, chromatography.

INTRODUCTION

The essential oils are the odoriferous principles found in several parts of the plant and perform the function of adapting the plant organism to the environment. The names given to these oils are due to their physicochemical features. As they evaporate when exposed to air under room temperature, their major characteristic, they are termed volatile oils; as they bear intense and pleasant scent, making them true essences, essential oils; and as they are soluble in apolar organic solvents, such as ether, they can be named ethereal oils (Robbers et al., 1997; Simões et al., 2000).

Most of the essential oils consist in a mixture of hydrocarbons (terpenes, sesquiterpenes, among others) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, phenolic ethers, and others). Chemically, these compounds derive from terpenoids, stemming from mevalonic acid, or from phenilpropanoids, from chichimic acid (Guenther, 1977; Simões et al., 2000).

The physical characteristics of the volatile oils are common, despite presenting different chemical constitutions. They are generally insoluble, or very little soluble, in water, but solubilize in alcohol, ether and many organic solvents. They exhibit unique odors, high refraction index and are optically active (Robbers et al., 1997; Tyler et al., 1988; Cowan, 1999).

According to the family and the species they belong to, the volatile elements can be concentrated in specific anatomic organs. The essential oils are located in special secretory structures, such as cavities,

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schizogenous or lysigenous channels (pockets), oily channels, glandular hairs, differentiated parenchymatous cells. They can be stored in flowers, leaves, trunk bark, wood, roots, rootlets, fruits and seeds, and can vary in their composition according to the location in a single species (Queiroga et al., 1990; Janssen et al., 1987; Coutinho et al., 2006).

In addition to the variation according the organ of location, other factors can interfere with the composition of the essential oil of a single plant species, such as: time of collection; weather and soil conditions; geographic location; vegetative cycle of the species; and the process of obtaining (Moreira et al., 2003; Robbers et al.,1997; Mejdoub & Katsiotis, 1998; Wirthensohn & Sedgley, 1998; Simões et al., 2000; Siani et al., 2000; Franco et al., 2005; Tavares et al., 2005; Oliveira et al., 2005).

Biologically, these essential oils perform the function of adaptation of the plant to the environment, acting in the defense against the attack of predators, attraction of pollination agents, protection against water loss and temperature increase and as inhibitors of germination (Feresin et al., 2001, Jassim & Naji, 2003).

Economically, they are employed in food, cosmetic and cleaning products industries, as well as in alternative medicine due to their many therapeutic properties. However, despite their many beneficial characteristics, toxic effects of these substances cannot be discarded, which could lead from a simple skin reaction to convulsive and psychotropic effects (Grassmann et al. 2000).

The Asteraceae family is the most numerous systematic group within the Angiosperms, encompassing about 1100 genera and 25000 species. These are plants of extremely varied aspect, including especially small herbs or bushes and rarely trees (Queiroga et al., 1990). About 98% of the genera are composed of small-sized plants, and are found in all kinds of habitats, but mainly in the mountainous tropical regions of South America (Navarro et al., 1996; Willians et al., 1998). Among them, there is the genus *Baccharis*, represented by more than 500 species distributed mainly in Brazil, Argentina, Colombia, Chile and Mexico (Queiroga et al., 1990). The high concentration of species in Brazil and the Andes indicates that these areas are the putative centers of origin of this genus. In Brazil there are 120 species of *Baccharis* described, most of them at the Southeast region. It is estimated in 100 the number of species in Argentina, 28 in Mexico and about 40 in Colombia, constituting one of the most important plant groups in this country, of which 38% are endemic (Loayza et al. 1993; 1995; Ferracini et al., 1995).

The species of *Baccharis* are generally bushes such as "carqueja", "vassoura" or "vassourinha", and measure on average 0.5 to 4.0 m tall. They present high socio-economical value, with wide dispersion in the States of Santa Catarina, Paraná, São Paulo and Rio Grande do Sul, among other regions of the country, where large numbers of them are used in folk medicine for the control or treatment of several diseases (Nunes et al., 2003; Agra et al., 2007; Oliveira et al., 2007). They are consumed mainly as teas with indications for illnesses of the stomach and liver, anemia, inflammations, diabetes, prostate diseases, and are also described as a medicine for disintoxication (Weyerstahl et al., 1996). For instance, in Brazil and Argentina, *B. crispa* and *B. notosergila* are used to heal wounds and fight inflammations. Other well-recognized species in alternative medicine are *B. trimera* and *B. articulata*. *B. genistelloides* is a medicinal herb frequently used in Brazil for a variety of diseases, such as digestive and liver disorders, malaria, ulcers, diabetes, anemia, diarrhea, urinary inflammations, tonsillitis, parasitic infections, Hansen's disease, and others (Verdi et al., 2005).

Their phytochemistry is highlighted due to the occurrence of flavonoids, diterpenes and triterpenes, being observed a greater amount of flavones, flavonols and labdane and clerodane diterpenes (Barroso, 1976; Queiroga et al., 1990; Fullas et al., 1994; Ferracini et al., 1995; Borella & Fontoura, 2002; Verdi et al., 2005; Borella et al., 2006; Mendes et al., 2006).

Approximately 120 species of *Baccharis* were chemically assessed and among them, about 30 were subjected to studies of biological activity. In general, the most evidenced compounds are the flavonoids, clerodanes and labdanes, although the presence of kauranes, triterpenes, germacrene, coumaric acid, trichotecenes, sesquiterpenes and phenylpropanoids has been frequently observed. In the studies on biological activity the allelopathic, antimicrobial, cytotoxic and anti-inflammatory effects are stressed. *B. megapotamica*, *B. incarum*, *B. trimera*, *B. trinervis*, *B. salicifolia*, *B. crispa, B. coridifolia*, *B. dracunculifolia*, *B. uncinella, B. grisebachii* and *B. tricuneata* are among the most researched species for chemical composition and/or biological activity (Loayza, et al. 1993 and 1995; Budel et al., 2004; Ferronatto et al., 2006 and 2007, Marchesan et al., 2006; Ferrante et al., 2007).

Considering that the genus *Baccharis* has a very large diversity and is important in many ecosystems, the purpose of this work was to evaluate the physicochemical characteristics of the essential oils produced by *B. dracunculifolia* and *B. uncinella* D.C. Asteraceae.

MATERIAL AND METHODS

The essential oil was obtained through direct hydrodistillation by vapor drag of the dry leaves using a Clevenger device. The species under study are *B. dracunculifolia* D.C. and *B. uncinella* D.C. (Asteraceae), collected in Southwestern Paraná from

February to May, 2006. The material was collected in the field, transported to the laboratory, selected and standardized, so as to obtain leaves with the same pattern and age. This material was weighted and the humidity determined by the difference of initial and final weight. Drying was carried out at 60 °C until constant weight, then the material was stored in room with dehumidifier. The process of extraction was carried out during three hours. The dry samples of this material are stored at the Botany Laboratory of the Universidade Paranaense - UNIPAR - Francisco Beltrão Campus, under numbers 28-A and -B.

The yield of the essential oil was given in volume/mass %, that is, volume (mL) of essential oil per mass (g) of plant material (Farmacopéia Brasileira, 1988; Fabrowski, 2002).

The relative density (d_{20}^{20}) was determined according to the Farmacopéia Brasileira (1988), which refers to the ratio of mass of the liquid sample and the mass of water, both at 20 °C. Pycnometers were used according the amount of essential oil available. For samples obtained with less than 1 mL on distillation, a previously calibrated capillary tube was used, repeating the same procedure used for the pycnometer. During the tests the samples were kept at a temperature of 20 °C.

The refraction index was determined in a refractometer using sodium light of wavelength of 589.3 nm (D ray), which was adjusted with distilled water (refraction index of 1.3330), the samples being kept at 20 °C.

The solubility of the essential oil was carried out in 70%, 80%, 90% and 96.5% ethanol, according to the Pharmacopoeia Helvetica (1993). This solubility consists in the volume of ethanol needed to solubilize one volume of the essential oil (v/v).

The identification of the chemical components of the samples of the essential oil was made through gas chromatography coupled to mass spectrometry (GC/MS). The constituents of the oils were identified through comparison of their mass spectra to those of the Wiley library (GC/MS) and those described by Adams (1995), was well as through comparison of the Kovats' retention index with literature data (Adams, 1995).

The GC analyses were carried out in a Hewlett Packard 6890 Series Chromatograph, equipped with a HP-Chemstation data processor, using a HP-Innowax column (30 m x 320 μM i.d.) and 0.50 μM film thickness (Hewlett Packard, Palo Alto, USA), temperature of the column 40 °C (8 min) to 180 °C at 3 °C/min, 180-230 °C at 20 °C/min, 230°C (20 min); injector temperature 250 °C; split ratio 1:50, flame ionization detector with temperature of 250°C; drag gas H2 (34Kpa), injected volume 1 μL diluted in hexane (1:10).

The analyses in GC/MS were carried out in gas chromatograph coupled to a Hewlett Packard 6890/MSD5973 mass spectrometer equipped with HP Chemstation software and Wiley 275 spectrum library (MacLafferty et al., 1997). It was used a HP-Innowax column (30 m x 250 μM) and 0.50 μM film thickness (Hewlett Packard, Palo Alto, USA). The temperature program was the same used in GC: 280 °C interface; split ratio 1:100; drag gas He (56 Kpa); flow ratio 1.0 mL/min; ionization energy 70 eV; injected volume 0.6 μL diluted in hexane (1:10).

The data from essential oil yield, relative density, refraction index and optic rotation were subjected to analysis of variance and the means were separated by Tukey's test.

RESULTS AND DISCUSSION

Determination of the yield of the essential oil

Table 1 indicates the yield of the essential oils of *B. dracunculifolia* and *B. uncinella.*

Through this methodology it was observed that most of the essential oil is extracted at the beginning of the distillation, keeping the levels of yield satisfactory during the first hour of extraction, and decreasing considerably along the process.

Fabrowski (2002), when evaluating the yield of the essential oil of *Eucalyptus smithii*, observed the same behavior, although Mejdoub & Katsiotis (1998) indicated three hours as the ideal time interval for distillation of the essential oil of *Eucalyptus citriodora*.

The process of hydrodistillation, which lasted three hours, yielded 1.54 mL.100 g^{-1} for *B. dracunculifolia* and 1.65 mL.100 g^{-1} for *B. uncinella*.

Comparing the results of this investigation with those of Souza et al. (2007), we noticed that the values are similar, because in three hours of extraction of volatile oils of *B. dracunculifolia,* he obtained 1,72 mL.100 g^{-1}, very similar to this work. Also, in the first hour Souza et al. (2007) obtained 50% of the oil, coinciding with the extraction times used in this work.

Physicochemical analyses of the oils

The relative density, refraction index and optic rotation are presented in Table 2. It is observed that the essential oil of *B. dracunculifolia* is a little denser than that of *B. uncinella*, showing relative density of 0.9151 ± 0.0021, while that of *B. uncinella* was 0.9147 ± 0.0006.

The refraction index revealed that there is not a significant variation between the two oils assessed, yet a higher value was obtained for the oil of *B. uncinella* (index of 1.4602 ± 0.0015) compared to the oil of *B. dracunculifolia* (index of 1.4593 ± 0.0005).

As for the analysis of the rotatory power, the results obtained with the samples tested indicated that these are dextrorotatory (deviate the plane of polarized light to the right), the sample of *B. uncinella* having a larger angle of deviation than the oil of *B. dracunculifolia*, showing values of +2.18 ± 0.10 and

+1.99 ± 0.06, respectively.

As for the solubility in ethanol it can be seen in Table 3 that the oils display the same behavior, 3 mL of 70% ethanol being necessary to solubilize 1.0 mL of the oils, while at the ethanol concentrations of 80%, 90% and 96.5% 1 mL was needed.

Considering that published scientific works on the physicochemical characteristics of these oils are inexistent, it is difficult to make any further discussion concerning the results obtained. In general, the essential oils rich in oxygenated constituents are more soluble in diluted alcohol than the essential oils rich in terpenes (Fabrowski, 2002). As for the results obtained in this work, we cannot state that the solubility is related to the oil composition, because the percentage of terpenic compounds is similar to the percentage of oxygenated compounds.

Gas chromatography coupled to mass spectrophotometry

The chemical composition of the essential oils of *B. dracunculifolia* and *B. uncinella* D.C. (Asteraceae) is presented in Table 4.

After the assessments it was possible to identify 93.43% of the components of the oil of *B. dracunculifolia* and 91.34% of those of *B. uncinella*; 48.42% and 37.86% of these are monoterpenes and 45.01% and 53.48% are sesquiterpenes in the oils of *B. dracunculifolia* and *B. uncinella*, respectively. This identification was possible using the spectrum library of Wiley and bibliographic references such as Adams (1995), Frizzo et al., (2005), Ferracini et al., (1995), Loayza et al., (1993), Loayza et al., (1995), Queiroga et al. (1990).

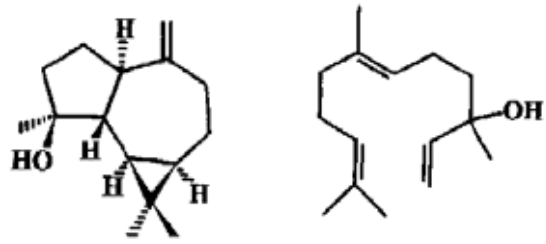
From these data we could verify that the constituents of the essential oils of *B. dracunculifolia* and *B. uncinella* are basically monoterpenes e sesquiterpenes. The chemical constitution of the oils is very similar, differing only in minor constituents. As for the major constituents, it is observed the occurrence of the monoterpenes β-pinene and limonene in both species and of the sesquiterpenes ε-nerolidol and spathulenol (Figure 1a and 1b), known for their commercial value.

The ε-nerolidol is of great interest for the perfume industry because it is a natural fixative, fundamental compound of perfume composition. As for spathulenol, it has anti-carcinogenic activity, as reported in the literature by Fullas et al. (1994). Essential oils having low concentrations of spathulenol and ε-nerolidol are considered of lower quality and are not accepted by many pharmacopeias (Wagner & Bladt, 1995).

ε-Nerolidol was first detected in the oil of *B. dracunculifolia* by Motl & Trka (1983), making up 14% of the whole components of the oil. In this same work, Motl & Trka, (1983) also detected spathulenol, although spathulenol was first detected in the essential oil of *Eucalyptus spathulata* var. *grandiflora* by Bowier and Jefferies in 1963 apud Motl & Trka (1983). In its pure state, ε-nerolidol is a colorless oil with a tender but persistent floral scent (Wagner & Bladt, 1995).

The results obtained are similar to those obtained by Frizzo et al. (2005) when assessed the composition of essential oils of *B. dracunculifolia*, obtained by vapor drag and supercritical extraction.

Other similar results were obtained during the investigation of 120 species of the genus *Baccharis*, where the most evidenced chemical compounds are the flavonoids and the terpenoids, such as monoterpenes,



(a) **(b)**

Figure 1. (**a**) Chemical structure of Spathulenol
(**b**) Chemical structure of Nerolidol

Table 1. Yield of the essential oil of *B. dracunculifolia* and *B. uncinella*.

Samples	Yield of the essential oil (mL/100 g)	Processing time
B. dracunculifolia	1.54a*	3 hours
B. uncinella	1.65a*	3 hours

* means followed by the same letter do not differ at 5% probability by Tukey's test.

Table 2. Relative density, refraction index and rotatory power of the essential oils of *B. dracunculifolia* and *B. uncinella* D.C. (Asteraceae).

Samples	Relative density (d_{20}^{20})	Refraction index	Rotatory power
B. dracunculifolia	0.9151 ± 0.0021a*	1.4593 ± 0.0005a*	+1.99 ± 0.06a*
B. uncinella	0.9147 ± 0.0006a*	1.4602 ± 0.0015a*	+2.18 ± 0.10a*

* Means followed by the same letter do not differ at 5% probability by Tukey's test.

Table 3. Solubility of the essential oils of *B. dracunculifolia* and *B. uncinella* D.C. (Asteraceae) in ethanol.

Samples	70% ethanol	80% ethanol	90% ethanol	96.5% ethanol
B. dracunculifolia	3.0	1.0	1.0	1.0
B. uncinella	3.0	1.0	1.0	1.0

Solubility: volume of ethanol to solubilize 1 volume of essential oil (v/v).

Table 4. Percent composition of the essential oils produced by *Baccharis dracunculifolia* and *Baccharis uncinella* D.C. (Asteraceae).

Compound		TR*	*B. dracunculifolia* (%)	*B. uncinella* (%)
Monoterpenes			48.42	37.86
α-Pinene	1	4.32	5.33	6.45
α-Tujene	2	4.98	2.42	1.42
β-Pinene	3	7.63	27.45	18.76
β-Mircene	4	11.83	1.93	2.78
Limonene	5	12.78	10.67	6.70
(E)- β-Cimene	6	13.32	0.62	1.25
p-Cimene	7	17.11	-	0.50
Sesquiterpenes			45.01	53.48
β-caryofilene	8	30.49	0.75	1.76
Aromadendrene	9	31.20	0.08	-
α-Humulene	10	33.21	0.24	1.12
Germacrene D	11	34.82	0.67	1.34
γ-Murolene	12	35.74	0.17	-
Bicyclogermacrene	13	35.86	1.67	2.33
α-Cadinene	14	36.34	1.35	2.98
δ-Cadinene	15	36.77	0.87	-
Cis-Calamenene	16	38.45	0.95	1.05
ε-Nerolidol	17	42.46	14.02	12.96
Caryofilene oxide	18	43.88	5.90	8.22
Globulol	19	47.02	3.08	4.22
Viridiflorol	20	47.23	3.14	3.05
α-Guaiene	21	49.72	1.21	1.87
Spathulenol	22	51.01	9.54	10.54
T-Cadinol	23	52.34	-	1.68
α-Murolol	24	52.56	-	1.32
T-Murolol	25	54.15	1.56	-
α-Bisabolol	26	56.54	-	2.02
Total			93.43%	91.34%

* TR Time of retention of the compound.

sesquiterpenes, diterpenes and triterpenes (Moreira et al, 2003; Davies, 2004; Verdi et al., 2005).

In studies carried out by Boldt (1989) with 12 species of *Baccharis*, it was possible to see that the terpenoids occur as components of leaves and branches of species of this genus, and can be ε-nerolidol, spathulenol, δ-cadinene e β-caryophilene found in *Baccharis dracunculifolia*. Other species showing terpenoids in their compositions are *Baccharis salicifolia* with α-phelandrene, germacrene-D, bicyclogermacrene e δ-cadinene, and *Baccharis latifolia* with α-tujene, α-pinene, limonene, germacrene-D and ledol as major terpenoids (Queiroga et al., 1990; Loaysa et al., 1995).

In assessments carried out by Agostini et al. (2005) on the chemical composition of the oils obtained from *B. articulata, B. semiserrata, B. milleflora, B. uncinella, B. cognata* and *B. oxyodonta*, the oils had quali-quantitative variations on their chemical composition, related to the concentration of ·α-pinene, β-pinene, limonene, spathulenol and ε-nerolidol. The major components were β-pinene, reaching 58.43% in *B. articulata*, followed by ε-nerolidol with 25.5% in *B. uncinella* and spathulenol with 23.4% in *B. milleflora*. Both evaluations also had variations in the yield. It is probable that these differences were due to both the stage of development of the plant and the general environmental and geographic conditions, once the samples were collected in different locations of Rio Grande do Sul.

We should also take into consideration that the genus *Baccharis* produced many secondary metabolites; about 15% of the species of *Baccharis* have been analyzed from the phytochemical point of view, but few of them have complete studies (Boldt, 1989).

CONCLUSION

After these studies using the methodology described, we can conclude that the best yield was of *B. uncinella*, with 1.65 mL, followed by *B. dracunculifolia* with 1.50 mL.100 g^{-1}; the relative density (d_{20}^{20}) of *B. dracunculifolia* was 0.9151 and of *B. uncinella* was 0.9147. The refraction index was 1.4593 for *B. dracunculifolia* and 1.4602 for *B. uncinella*. The rotatory power was +1.99 for *B. dracunculifolia* and +2.18 for *B. uncinella,* while the solubility in 70% ethanol was 3.0, in 80% ethanol was 1.0 and in 96.5% ethanol was also 1.0 for both oils analyzed. The evaluations using gas chromatography coupled to mass spectrophotometry showed that these oils are similar, showing 26

compounds, of which β-pinene, ε-nerolidol, limonene and spathulenol are highlighted.

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