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Chemical Composition and Antibacterial Activity of the Essential Oils of *Blainvillea rhomboidea* (Asteraceae)

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The essential oils of *Blainvellea rhomboidea* (Asteraceae) were obtained by hydrodistillation and analyzed by GC/MS and GC/FID. Initially, the essential oil from the aerial parts was investigated. From the 18 identified components, 5-indanol (14.5%) followed by *p*-cymen-8-ol (10.1%), β-caryophyllene (9.6%), caryophyllene oxide (9.6%), limonene (8.6%), terpinolene (7.8%), and spathulenol (7.7%) were the major constituents. The oil was tested against seven bacterial strains and the results showed significant antimicrobial activity. As a consequence, the essential oils from leaves and from flowers were analyzed separately. The major constituents of the leaf oil were terpinolene (21.2%), β-caryophyllene (19.2%), spathulenol (9.1%), caryophyllene oxide (7.4%), and bicyclogermacrene (7.1%), while the oil of the flowers contained terpinolene (28.1%), 5-indanol (16.3%), *p*-cymen-8-ol (15.3%) and limonene (14.7%) as prevalent compounds. The oils were tested against the same bacterial strains and the flower oil was the more active. These results indicated that the components of the essential oil from flowers seem to be responsible for the activity.

Keywords: *Blainvillea rhomboidea,* Asteraceae, essential oil, antibacterial activity.

The Asteraceae family is a well-known source of aromatic and medicinal plants rich in bioactive compounds. Due to their ethnopharmacological and economical importance, an increasing number of plants belonging to this family have been investigated from the chemical and biological viewpoints [1-6]. The *Blainvillea* genus (tribe Heliantheae, subtribe Ecliptinae) is represented by ten species of pantropical dispersion [7]. In accordance with a literature survey, five entries (*B. rhomboidea*, *B. latifolia*, *B. gayana*, *B. acmella*, and *B. dichotoma*) have been cited as chemically investigated. The same survey revealed *Blainvillea* plants as a rich source of sesquiterpene lactones of different skeletal types, such as melampolides, germacranolides, guaianolides, and heliangolides [7-12]. As part of the interest on the essential oils from plants of the Asteraceae family, the chemical composition and antimicrobial activity of the essential oils of *Blainvillea rhomboidea* Cass is reported. *B. rhomboidea* is an aggressive, difficult to control weed that infest pastures and several important agronomical cultures, including sugar cane crops [13,14]. In spite of several chemical studies, no report on the volatiles constituents of *Blainvillea* ssp. has been found.

The results of the qualitative and quantitative analysis of the oils from *B. romboidea* enabled the identification of thirty-one volatile constituents for the three sample oils, which are listed in Table 1, including their respective retention indices and relative percentages. Except for the presence of a phenylpropanoid, the oils were exclusively characterized by terpenoids, including mono- and sesquiterpenes. Initially, the essential oil from the aerial parts of *B. rhomboidea* at the flowering stage was analyzed. The phenylpropanoid 5-indanol

Table 1: The chemical composition of the essential oils of *Blainvillea rhomboidea*.

Compounds[a]	RI[b]	Aerial parts	Leaves	Flowers
α-Pinene	939	1.2	2.1	1.1
Sabinene	973	-	-	1.9
β-Pinene	979	-	0.9	-
Myrcene	980	0.9	0.5	2.7
Limonene	1011	8.6	3.0	14.7
β-Ocimene	1030	-	2.3	1.4
Terpinolene	1068	7.8	21.2	28.1
p-1,3,8-Menthatriene	1119	-	-	0.5
Terpinen-4-ol	1164	-	-	0.5
p-Cymen-8-ol	1168	10.1	0.9	15.3
5-Indanol	1350	14.5	-	16.3
α-Copaene	1372	2.4	3.2	-
β-Elemene	1390	-	0.8	-
β-Caryophyllene	1419	9.6	19.2	3.8
Aromadendrene	1437	-	0.4	-
α-Humulene	1452	0.9	1.1	-
allo-Aromadendrene	1459	-	0.9	-
Germacrene D	1482	-	3.9	-
epi-Cubebol	1495	1.4	-	-
Bicyclogermacrene	1498	-	7.1	-
α-Muurolene	1503	-	0.5	-
γ-Cadinene	1515	-	0.6	-
Cubebol	1516	1.2	-	-
δ-Cadinene	1526	1.2	1.8	-
E-Nerolidol	1570	1.5	1.1	-
Spathulenol	1581	7.7	9.1	1.1
Caryophyllene oxide	1584	9.6	7.4	1.2
1,10-di-*epi*-Cubenol	1628	1.6	2.1	-
β-Caryophylla-4(14),8(15)-dien-5-ol	1635	-	0.8	-
epi-α-Muurolol	1642	3.3	3.6	-
α-Cadinol	1654	1.6	1.5	-
Total		85.1	96.0	88.6

[a]Compounds are listed in order of elution on a DB-5 column;
[b]Retention indices;
[c]percentages calculated from GC-FID.

was the main constituent, but others components, such as *p*-cymen-8-ol (10.1%), β-caryophyllene (9.6%), caryophyllene oxide (9.6%), limonene (8.6%), terpinolene (7.8%), and spathulenol (7.7%) were also detected in significant amounts. Table 2 shows the *in vitro* antimicrobial activities of the *B. rhomboidea* oils. The inhibition zones caused by standard antibiotics are shown for comparison.

As can be seen, the results of this screening showed that the oil from the aerial parts was moderately active. For this reason, the oils from the leaves and flowers were individually screened in order to identify which of these plant parts was responsible for the observed antimicrobial activity. As expected, the oils showed significant qualitative and quantitative differences. Comparison of their chemical composition showed that the oil from the leaves contained a larger percentage of sesquiterpenes (63.6%), whereas the oil from the flowers had higher concentration of monoterpenes (66.2%). Moreover, the oil from flowers was marked by the presence of 5-indanol (16.3%), which was absent in the leaf oil. Twenty-four constituents were identified in the essential oil of the leaves representing 96.0% of the total oil composition. In the monoterpene fraction, terpinolene (21.2%) appeared as the prevalent compound, while β-caryophyllene (19.2%), caryophyllene oxide (9.6%), spathulenol (7.7%), and bicyclogermacrene (7.1%) were the major identified sesquiterpenes. In the oil from the flowers, thirteen constituents were detected accounting for 88.6% of the oil composition. The most prominent components were terpinolene (28.1%), 5-indanol (16.3%), as above mentioned, *p*-cymen-8-ol (15.3%), and limonene (14.7%).

Table 2: Antibacterial activity of the essential oils of *Blainvillea rhomboidea*.

	Inhibition zone diameter (mm)			
Microorganisms tested	Aerial parts	Leaves	Flowers	Tobramicin[b]
Chromobacterium violaceum	21.5	15.0	21.3	22.0
Enterobacter aerogenes	13.3	a	15.0	17.0
Klebsiella pneumoniae	14.6	a	15.6	18.0
Pseudomonas aeruginosa	14.6	a	14.6	34.0
Salmonella choleraeasuis	14.0	a	13.3	22.0
Bacillus subtilis	20.3	13.0	18.6	31.0
Staphylococcus aureus	17.6	11.3	16.0	25.0

[a] Absence of inhibition at 60 µg/disk
[b] Tobramicin disk (10 µg)

As can be seen in Table 2, the oil from the flowers exhibited activity against all tested organisms, while the leaf oil showed effect against only three.

According to the chemical profiles of the oils, and considering the results of the biological screening, it is possible to speculate that the monoterpene terpinolene, which was the main compound of both oils, is not a bioactive component. This indicates that other components, such as limonene, *p*-cymen-8-ol, and 5-indanol could be the responsible for the antibacterial activity. Indeed, limonene the major compound of citrus oils has shown both antibacterial and antifungal activities [15]. *p*-Cymene has been related to antimicrobial activities [16], but no mention of any activity for its derivative *p*-cymen-8-ol was found. Although, no specific activity has been found for 5-indanol it is well known from literature that several essential oils rich in phenol like compounds do show activity [17,18]. However, the synergistic action of the oil constituents can not be disregarded.

Experimental

Plant material: The aerial parts of *B. rhomboidea* used for this study were collected during their full flowering period from a population found on the campus of the Universidade Federal do Ceará, in July 2004. In the subsequent year (July 2005), leaves and flowers were harvested from the same site. After identification by Dr. Edson P. Nunes a voucher specimen (No 33.879) was deposited at the Herbário Prisco Bezerra (EAC) of the Departamento de Biologia, Universidade Federal do Ceará.

Extraction of the essential oils: Portions of the fresh aerial parts (450 g), leaves (300 g), and flowers (260 g) of *B. rhomboidea* were subjected to hydrodistillation for 2 hours in a Clevenger-type apparatus to produce yellowish oils in yields (w/w) of 0.2, 0.3 and 0.06%, respectively. The isolated essential oils were dried over anhydrous sodium sulfate, filtered and stored under refrigeration in sealed vials before analysis.

GC-FID analysis: The quantitative analysis was performed on a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector using a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Hydrogen was used as carrier gas at a flow rate of 1 mL/min and 30 psi inlet pressure; split ratio 1:30; the column temperature was programmed from 35ºC to 180ºC at a rate of 4ºC/min, then heated at a rate of 17ºC/min to 280ºC and held isothermal for 10 min; both injector temperature and detector temperature were 250ºC.

GC-MS analysis: The GC-MS analysis was carried out on a Hewlett-Packard Model 5971 GC/MS using a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness); carrier gas helium, flow rate 1 mL/min and with split mode. The injector temperature and detector temperature were 250ºC and 200ºC, respectively. The column temperature was programmed from 35°C to 180ºC at 4ºC/min and then 180°C to 250ºC at 10ºC/min. Mass spectra were recorded at an ion voltage of 70 eV over a scan range of 30 – 450 atomic mass units.

Compounds identification: All components of the oils were identified by comparison of their mass spectrum with those stored in the spectrometer data base using the Wiley L-built library and two other computer library MS searches using GC retention indices as a preselection [19,20], and were further confirmed by comparison of the fragmentation pattern of each mass spectrum with those reported in the literature [21,22].

Tested microorganisms: A panel of eight bacterial strains obtained from the American Type Culture Collection (ATCC), were used in this work. They included the Gram-negative bacteria: *Chromobacterium violaceum* (ATCC 12472), *Enterobacter aerogenesis* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 25319), and *Salmonella choleraeasuis* (ATCC10708), and the Gram-positive bacteria: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538P), and *Staphylococcus aureus* (ATCC 25923). The bacterial strains were maintained on Nutrient agar (Difco Laboratories, Detroit, MI) slants.

Screening for antibacterial activity: Inhibition of bacterial growth by the oils was determined as described by Bauer and co-workers [23]. Briefly, bacterial cultures were cultivated in Müller Hinton broth (Difco Laboratories, Detroit, MI) for 18 hours. Sterile swabs were immersed in the microbial suspensions adjusted to McFarland scale 0.5 [10^8 colony-forming units (CFU)/mL] and confirmed by spectrophotometrical reading at 600 nm and evenly applied to Petri dishes containing Müller Hinton agar (Difco Laboratories, Detroit, MI). For the assay, the oil samples were diluted in chloroform (40 mg/mL) and immediately applied (15 μL) into sterile Whatman AA filter paper disks (6 mm in diameter) reaching a final concentration of 60 μg/disk. After 10 minutes, for the chloroform

evaporation, the disks were placed on the agar plates. Tobramicin disks (10 μg; Cecon, São Paulo, SP, Brazil) were used as positive control, and chloroform (15 μL) was used as a negative control. The plates were incubated overnight at 35ºC and then examined for zones of growth inhibition around each disk. The inhibition zone was measured in millimeters and the assay was carried out in triplicate for each oil.

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