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Chemical analyses of the essential oils from leaves of *Mikania glauca* Mart. ex Baker

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The essential oils obtained by hydrodistillation of two samples of fresh *Mikania glauca* leaves collected in different periods were analyzed by gas chromatography (GC) and by GC–mass spectrometry (GC–MS). Twenty compounds, representing 99% of the total oil composition, were identified and quantified in sample 1, while forty-five compounds, also representing 99% of the total oil composition, were identified in sample 2. The essential oils from the fresh leaves of both samples were rich in the terpenes. In sample 1 and 2, respectively, α-pinene (27% and 26%), β-pinene (22% and 36%), myrcene (24% and 18%), β-caryophyllene (9% and 6%) and bicyclogermacrene (8% and 4%) were the principal constituents. This demonstrated the predominance of monoterpene hydrocarbons in the essential oil of *M. glauca* and the influence of the collection period on the concentrations of the components.

Keywords: *Mikania glauca*; Asteraceae; β-pinene; α-pinene; myrcene

Introduction

The plants of the Asteraceae family constitute a systematic and numerous group belonging to the Angiosperms, and involve approximately 1100 genera and 25,000 species. They are plants of varied aspect, including small plants (98%), such as herbs and bushes, in the majority. They are mainly found in South America in all types of habitat (1). They are very common in the open formations of Brazil, mainly in the *cerrado*, where the family is well represented by approximately 250 genera and 2000 species (2).

The *Mikania* genus, which includes around 450 species, belongs to this family. Its species are mainly distributed in neotropical regions, there being around 150 species in the Andes countries and 171 species in Brazil, where they are mainly found in the states of Minas Gerais, Rio de Janeiro and São Paulo (3, 4). Despite the fact that the *Mikania* genus presents a large number of species, there are few studies of plants belonging to this genus in Brazil; only the species *Mikania glomerata* Spreng. and *Mikania laevigata* SCH. Bip. *ex* Baker have been studied. These species are known as *Guaco*, and they are widely used in Brazil in the formulation of syrups for the treatment of the respiratory system. The use of the *M. glomerata* species has been official since the first edition of the Brazilian Pharmacopoeia in 1929, whereas the *M. laevigata* species had its monograph included in 2005 (4, 5).

Mikania glauca Mart. *ex* Baker is a herbaceous climber found in the state of Minas Gerais in gallery forests and in rupestrian fields (6). There are no accounts of phytochemical studies with this species. However, it is found in the list of the species threatened by extinction among the flora of Minas Gerais State (7).

The essential oils extracted from this genus of plants are generally rich in terpenoid compounds, which have made them the object of several studies since many terpenes present biological properties that are of interest to the pharmaceutical and cosmetics industries. Thus, the objective of this work was to identify and quantify the chemical constituents of the essential oil extracted from the leaves of *M. glauca* Mart. *ex* Baker.

Experimental

Plant material

Two samples of leaves of *M. glauca* (Asteraceae) were collected in different periods in the municipality of Itumirim, Minas Gerais, Brazil. The first sample was collected in October 2007, and the second in June

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2011. The species was identified by Doctor Mariana Esteves Mansanares of the Federal University of Lavras (UFLA), and a specimen was registered at the ESAL Herbarium, located in the Biology Department of UFLA, under the registration number 233.

The essential oils from both the samples were extracted by hydrodistillation of the fresh leaves (100 g) from *M. glauca* during 4 hours using a Clevenger-type apparatus, according to the method of the European Pharmacopoeia (8). The essential oils were stored at −20°C in the dark prior to analysis. Parallel to the extractions, the moisture test was performed according to the official method of the AOCS, followed by alterations (9). The yield of the essential oil was calculated and expressed as the weight of the oil divided by the weight of the leaves on a moisture-free basis (% w/w). The extraction was performed in triplicate.

Analysis of the essential oils

The qualitative analysis of the essential oils from the two samples was performed by gas chromatography with a mass spectrometer detector (GC/MS). The analysis of the first sample (October 2007) was performed utilizing a Shimadzu model G-17A chromatograph equipped with a P5050A mass-selective detector and a DB-5MS capillary column (5% phenylmethylsiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm; Folsom, CA, USA). The oven temperature was programmed from 50°C for 2 minutes with an increase of 4°C/minute to 200°C, followed by an increase of 10°C/minute to 300°C, where the temperature was maintained for 10 minutes. The carrier gas was helium (1 mL/minute); the split ratio was 1:83, and 0.5 μL of a 1% solution of the oil in dichloromethane was injected. The configuration of the mass spectrometer was as follows: energy of impact, 70 eV; scan range, 40–550 u; temperature of the ion source, 280°C; decomposition interval, 0.50. A series of linear hydrocarbon (C_9H_{20}–$C_{26}H_{54}$) standards was injected under the same conditions to determine the linear retention indices (10). The spectra obtained were compared with the data bank of the Wiley 229 library, and the retention index calculated for each constituent was compared with tabulated values (11). Whenever possible, co-injection with an authentic sample was performed.

The qualitative analysis of the second sample (June 2011) were performed on a Perkin–Elmer Autosystem XL chromatograph coupled to a Perkin–Elmer Turbomass mass spectrometer. The device was operated under the following conditions: DB-1 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific Inc., Rancho Cordova, CA, USA); the oven temperature was programmed from 45° to 175°C at 3°C/minute, followed by heating at 15°C/minute up to 300°C, and then held isothermally for 10 minutes. The carrier gas was helium (linear velocity of 30 cm/second); the split ratio was 1:40, and 0.2 μL of the sample (1% solution in pentane) was injected. The configuration of the mass spectrometer was: energy of impact, 70 eV; ionization current, 60 μA; scan range, 40–300 u; scan time, 1 second. Utilizing the same conditions as those of the sample, a series of linear hydrocarbon standards was injected to determine the retention indices. The spectra obtained were compared with the resident data bank, and the retention indices calculated for each constituent were compared with tabulated values (12, 13). Whenever possible, co-injection with an authentic sample was performed.

The quantitative analyses were accomplished by internal normalization (expressed in relative percentage of the peak areas corresponding to each constituent, assuming the same detector response factor for all the constituents), calculated as mean values for two injections of each oil without the use of correction factors. The analyses of the first sample were performed using a Shimadzu CG–17A gas chromatograph equipped with a flame ionization detector (FID) and a DB5 fused silica capillary column (5% phenylmethylsiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm; Folsom, CA, USA). The oven temperature was maintained at 60°C for 2 minutes, followed by an increase of 4°C/minute to 200°C, and then an increase at 10°C/minute to 300°C, where the temperature was maintained for 10 minutes; the temperature of the injector was 250°C; the temperature of the FID, 280°C. The carrier gas was nitrogen (2.2 mL/minute); the split ratio was 1:20, and the injected volume of the oil dissolved in hexane (1:100 v/v) was 1 μL.

The second sample was analyzed on a Perkin–Elmer Autosystem XL gas chromatograph equipped with an FID (Perkin–Elmer, Shelton, Connecticut, USA) and a fused silica capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm; J & W Scientific Inc.). The oven temperature was programmed from 45 to 175°C, at 3°C/minute, followed by an increase at 15°C/minute to 300°C, where it was held isothermally for 10 minutes; the temperature of the injector was 280°C; the temperature of the FID, 300°C. The carrier gas was hydrogen with a linear velocity of 30 cm/second; the split ratio was 1:50, and the injected volume of the sample was 0.2 μL (1:100 v/v in pentane).

Results and discussion

The mean essential oil content of the fresh *M. glauca* leaves was 0.65% (w/w). The chemical constituents of the essential oil from *M. glauca* leaves, the calculated and tabulated retention indices, and their concentrations expressed in percentage are found in Table 1. An example of this species gas-chromatography profile is given in Figure 1.

Table 1. Chemical constituents of the essential oil from fresh *Mikania glauca* leaves.

Components	RI[a] DB-5	RI[11] DB-5	RI[b] DB-1	RI[13] DB-1	*Mikanua glauca* Sample 1	*Mikanua glauca* Sample 2
Tricyclene			921	924		t
A-Thujene			924	928		t
A-Pinene	924	932	930	937	27.1	26.2
Camphene	942	946	938	952	t	t
Sabinene	968	969	958	974	1.8	0.9
B-Pinene	973	974	963	977	21.9	36.0
Dehydro-1,8-cineole			973			t
β-Myrcene	987	988	975	984	23.8	17.6
α-Phellandrene			995	1010		t
α-Terpinene			1002	1015		t
p-Cymene			1003	1018		t
β-Phellandrene	1029	1025	1005	1021	1.7	0.1
Limonene			1009	1022		4.3
cis-β-Ocimene	1035	1032	1017	1028	1.2	t
trans-β-Ocimene	1046	1044	1027	1036	t	0.4
γ-Terpinene			1035	1047		t
trans-Sabinene hydrate			1037	1054		t
Terpinolene			1064	1077		t
trans-Pinocarveol			1106	1111		t
cis-Verbenol			1110	1124		t
Pinocarvone			1121	1139		t
Terpinen-4-ol	1181	1174	1148	1160	t	0.1
α-Terpineol			1159	1168		t
Myrtenol			1168	1178		t
δ-Elemene			1332	1344		0.1
α-Cubebene			1345	1347		t
α-Copaene	1357	1374	1375	1374	t	t
β-Cubebene			1385	1384		t
β-Elemene	1389	1389	1388	1386	0.4	0.1
β-Caryophyllene	1419	1417	1414	1412	8.5	6.1
α-Guaiene	1434	1437			t	
α-Humulene	1455	1452	1447	1449	0.9	0.4
allo-Aromadendrene			1456	1461		0.1
trans-Cadina-1(6),4-diene			1469			t
Germacrene-D	1483	1484	1474	1481	1.0	0.9
Bicyclogermacrene	1496	1500	1487	1500	8.4	4.1
Germacrene A	1506	1508			0.5	
γ-Cadinene	1514	1513	1500	1511	0.3	0.4
δ-Cadinene	1517	1522	1505	1519	0.7	0.2
Elemol			1530	1541		0.1
Spathulenol			1551	1552		0.3
β-Caryophyllene oxide	1582	1582	1561	1578	0.5	0.1
Viridiflorol			1569	1582		t
Guaiol			1575	1588		t
Ledol			1580	1600		t
α-Muurolol			1618	1622		t
β-Eudesmol			1620	1639		t
% Identification					98.7	98.5
Grouped components						
Monoterpene hydrocarbons					77.5	85.5
Oxygen-containing monoterpenes					t	0.1
Sesquiterpene hydrocarbons					20.7	12.4
Oxygen-containing sesquiterpenes					0.5	0.5

Notes: RI[a], calculated retention index relative to C_9–C_{26} *n*-alkanes on the DB-5 column; RI[11], literature retention indices on DB-5 (5% diphenyl, 95% dimethylsiloxane); RI[b], calculated retention index relative to C_9–C_{17} *n*-alkanes on the DB-1 column; RI[13], literature retention indices on DB-1 column (100% dimethylpolysiloxane); t, trace (<0.05%).

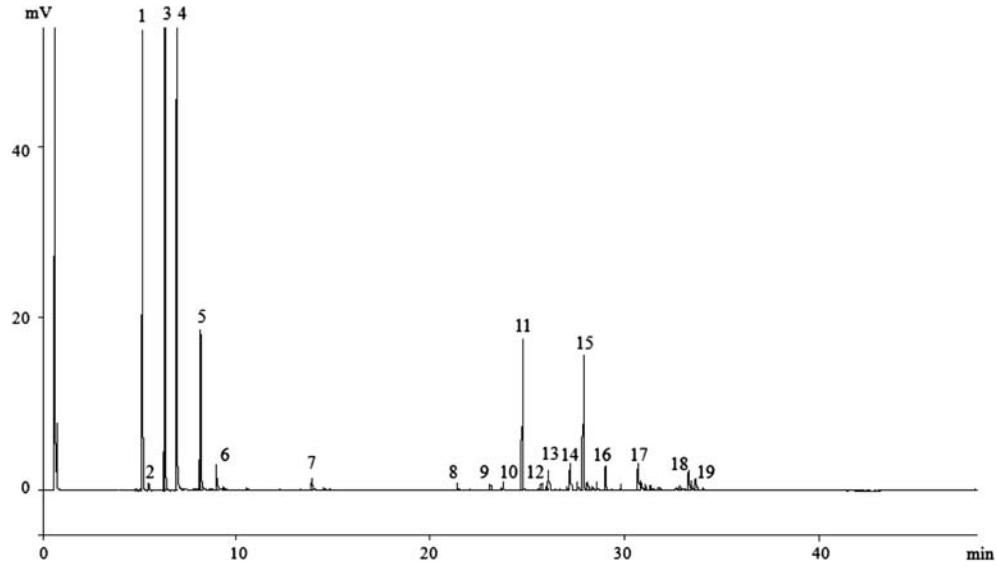

Figure 1. Gas chromatography profile of the essential oil isolated from the leaves of *Mikania glauca*, collected in June 2011 (sample 2). **1**: α-pinene; **2**: sabinene; **3**: β-pinene; **4**: β-myrcene; **5**: limonene; **6**: *trans*-β-ocimene; **7**: terpinen-4-ol; **8**: δ-elemene; **9**: α-copaene; **10**: β-elemene; **11**: β-caryophyllene; **12**: α-humulene; **13**: *allo*-aromadendrene; **14**: germacrene-D; **15**: bicyclogermacrene; **16**: γ-cadinene; **17**: δ-cadinene; **18**: spathulenol; **19**: β-caryophyllene oxide.

Twenty compounds, representing 99% of sample 1, and 45 compounds, representing 99% of the total oil from sample 2, were identified.

Monoterpene hydrocarbons were the predominant components, in both samples, contributing with 78% and 86% of the compositions of samples 1 and 2, respectively, of the essential oil of *M. glauca*. The compounds present in sample 1 were mainly represented by α-pinene (27%), β-pinene (22%) and myrcene (24%). These compounds were also the main constituents of sample 2 (Figure 1), although the concentration of β-pinene (36%) was higher, and the concentrations of α-pinene (26%) and myrcene (18%) were slightly lower. Essential oil samples 1 and 2 contained 21% and 13%, respectively, of sesquiterpene hydrocarbons, their concentrations being lower in sample 2 than in sample 1. β-Caryophyllene (9%) and bicyclogermacrene (8%) were the main constituents belonging to this class observed in samples 1 and 2, but their concentrations were lower in sample 2, where the concentrations of β-caryophyllene and bicyclogermacrene were 6% and 4%, respectively.

The predominance of terpene hydrocarbons has been reported for the essential oils of several species of *Mikania*. The essential oil of *Mikania cordata* (Burm. f.) B.L. Robison was rich in α-pinene (20%) and in germacrene-D (20%) (14). In another study, β-caryophyllene (12 and 13%) and germacrene-D (22 and 60%) were observed to be the principal constituents in the essential oil of the same species (15). The presence of several chemotypes, characterized predominantly by monoterpenes such as α-pinene, β-pinene, limonene and β-phellandrene and by oxygenated compounds such as α-terpineol, geranial, viridiflorol, γ-eudesmol and phytol, was observed in plants belonging to the same species and collected in nine different locations of the Côte d'Ivoire (16).

The germacrene-D and β-caryophyllene sesquiterpenes are commonly encountered as the principal constituents of the essential oils from several species of *Mikania*, such as that from *M. glomerata* Spreng, with contents of 41% and 15%, respectively (17). They are also found in the essential oils from *Mikania burchellii* (18), *Mikania micrantha* (18), *Mikania paranensis* (18), *Mikania involucrata* (19) and *Mikania laevigata* (20). These compounds were also found in the essential oils obtained from the inflorescence and seeds of *M. laevigata* and *M. glomerata*. The principal constituents in the essential oil from the inflorescence of *M. glomerata* were germacrene-D (25%) and β-caryophyllene (16%), while germacrene-D (19%), followed by myrcene (18%), were the predominant constituents in the essential oil from the inflorescence of *M. laevigata* (20).

Studies of the chemical composition of the essential oils from three species of *Mikania* found in the Chapada Diamantina, Bahia, Brazil, demonstrated the presence of 46 terpenes. In the essential oil from *Mikania hookeriana*, the principal constituents were α-pinene (23%) and germacrene-D (11%). The principal constituents in the essential oil from *Mikania hagei* were β-selinene (46%) and limonene (31%). β-Pinene (26%) and α-pinene (17%) were the principal compounds found in the oil from *Mikania jeffreyi* (91%). However, the monoterpenes α- and β-pinene, limonene and

β-thujene and the sesquiterpenes β-caryophyllene and germacrene-D were encountered in all of the essential oils from these species (21).

Through the use of bioassays, β-caryophyllene was observed to significantly inhibit the germination and growth rates of *Brassica grassland* cuttings and the oil raddish (22) in the concentration of 3 mg/L. This observation showed that this sesquiterpene can function as an allelochemical that can influence the growth of neighbouring plants. Therefore, it can have an important role in allelopathy among several species of plants.

The decrease in the sesquiterpene hydrocarbon contents in sample 2 is mainly the result of the increase in the concentration of β-pinene and the presence of limonene (4%) in this same sample. Although the principal constituents of the two samples were the same, there were a larger number of constituents in sample 2. These variations can be explained by environmental factors such as seasonal variations, rainfall, temperature and the vegetative cycle, among others. These environmental conditions can influence enzymatic activities in certain plant species and, consequently, interfere in the biosynthesis of their metabolites (23–25). Thus, it is important to emphasize that, although both samples were collected at the same location, they were collected at different times of the year.

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