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Chemical composition and antimicrobial activity of essential oil from Brazilian plants *Acanthospermum australe*, *Calea fruticosa* and *Mikania glauca*

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The essential oils of leaves of *Acanthospermum australe*, *Calea fruticosa* and *Mikania glauca* (Asteraceae) from southeastern Brazil were obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Oxygenated sesquiterpenes were predominant in *C. fruticosa* (47.8%) whereas sesquiterpenes hydrocarbons constituents predominated in *A. australe* (85.1%) and *M. glauca* (63.3%) oils. Caryophyllene oxide, α-cadinol and selin-11-en-4-α-ol were the most abundant components in *C. fruticosa*. Germacrene D, (*E*)-caryophyllene and bicyclogermacrene were the major components observed in the essential oil obtained from the leaves of *A. australe* and *M. glauca*. The antimicrobial capacity of the oils was tested. The results showed that the oils have antimicrobial activity against Gram-negative bacteria and *Candida glabrata,* with minimal inhibitory concentration (MIC) values between 50 and 1000 µg/ml.

Key words: Asteraceae, essential oil, gas chromatography-mass spectrometry (GC-MS), antimicrobial activity, Brazilian flora.

INTRODUCTION

Essential oils are complex natural mixtures of volatile secondary metabolites, which sometimes can be isolated from different parts of plants. Most of them are used as flavours in the food and beverage industry, as well as in perfumery, and they are also recognized as having several therapeutic applications. They demonstrate pharmacological effects, such as anti-inflammatory, antioxidant, cytotoxic, and they are biocides against a broad range of organisms, such as bacteria, fungi, viruses, protozoa, as well as insects and plants

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(Vagionas et al., 2007). The main constituents of essential oils, for example monoterpenes and sesquiterpenes and phenylpropanoids including carbohydrates, alcohols, ethers, aldehydes and ketones, are responsible for the fragrant and biological properties of aromatic and medicinal plants (Astani et al., 2010).

Asteraceae is the largest family of angiosperms with 1600 to 1700 genera widely distributed and 24,000 species (Funk et al., 2009). *Acanthospermum*, *Calea* and *Mikania* comprise around 6, 110 and 430 species, respectively (Bremer, 1994).

Acanthospermum australe (Loefl.) Kuntze is an annual shrub widely distributed in South America. In Brazil, where it is popularly known as “carrapichinho” or “carrapicho-de-carneiro” it grows vigorously in agricultural fields, pasture and fallow soil. Its aerial parts are used in folk medicine as a tonic, diaphoretic, eupeptic, vermifuge, antidiarrheal, antimalarial, antigonorrheal, febrifuge and antianemic (Lorenzi and Matos, 2002). Previous phytochemical investigations of *A. australe* have led to the isolation of germacranolides, melampolides, diterpene lactones and 6-methoxyflavonoids (Bohlmann et al., 1979, 1981a; Matsunaga et al., 1996). *Calea fruticosa* (Gardner) Urbatsch, Zlotsky and Pruski is a synonym of *Calea morii* H. Rob (Urbatsch et al., 1986). The plant is not used in folk medicine, although other species of the same genus are used for stomach disease (Martinez et al., 1987; Steinbeck et al., 1997; Kato et al., 1994). Chemical studies carried out on *Calea* species have revealed the occurrence of a variety of compounds including sesquiterpene lactones (Ober et al., 1985), *p*-hydroxyacetophenone derivatives (Bohlmann et al., 1981b), thymol derivatives (Metwally and King, 1985), benzofurans (Bohlmann et al., 1982), chromenes (Steinbeck et al., 1997) and others.

Mikania glauca Mart. ex Baker is native and endemic in Brazil and its geographic distribution includes the South-eastern, particularly the State of Minas Gerais (Ritter et al., 2012). The plant is not used in folk medicine, although other species of the same genus known as “guaco” are used to treat fever, rheumatism, flu, asthmatic bronchitis, cough and hoarseness (Oliveira et al., 1984; Vilegas et al., 1997; Soares e Silva et al., 2012). Studies of the chemical composition of species of genus *Mikania* demonstrated the presence of sesquiterpene lactones and diterpenes, mainly of the *ent*-kaurene type (Herz, 1998).

In the present study, the chemical composition of the essential oils of three Asteraceae species from Brazil was investigated. The study included hydrodistillation of the leaves and gas chromatographic/mass spectrometric analysis of the essential oils from leaves of *A. australe*, *C. fruticosa* and *M. glauca*, and also an evaluation of the essential oils against a panel of microorganisms strains. There are no reports in the literature concerning the chemical composition of essential oil from *C. fruticosa*. To our knowledge, no study has shown the antimicrobial activity of the essential oils of *A. australe*, *C. fruticosa* and *M. glauca*.

MATERIALS AND METHODS

Plant

Samples of leaves from three Brazilian Asteraceae species were collected in the city of Ouro Preto, State of Minas Gerais (March to May, 2012). Voucher specimens have been deposited at the Herbarium José Badini, Universidade Federal de Ouro Preto-UFOP, voucher No. OUPR 25895 for *A. australe*, OUPR 26290 for *C. fruticosa* and OUPR 26457 for *M. glauca*.

Extraction of the essential oils

Fresh leaves were steam distilled using a modified clevenger apparatus for 4 h and the essential oils obtained were stored in sealed amber ampules at 4°C until chromatographic analysis could be performed. Oil yields were determined (w/w) based on the fresh plant material.

Gas chromatography-mass spectrometry (GC-MS) analysis

Analyses were performed on a Shimadzu QP-2010 gas chromatograph interfaced to a mass spectrometer (GC-MS). The following conditions were used: ZB-5MS column Phenomenex Zebron (30 m × 0.25 mm × 0.25 µm); helium (99.999%) carrier gas at a constant flow of 1.1 ml/min; 1 µl injection volume; injector split ratio of 1:40; injector temperature 240°C; electron impact mode at 70 eV; ion-source temperature 280°C. The oven temperature was programmed from 100°C (isothermal for 5 min), with an increase of 10°C/min to 250°C (isothermal for 5 min), and 10°C/min to 280°C (isothermal for 15 min).

Identification of constituents of essential oils

Individual identification of the constituents was accomplished by comparison of their GC retention indices determined with reference to a homologous series of normal C_9-C_{25} alkanes and comparison of the fragmentation patterns in the mass spectra with those from the software database (Wiley 7 lib and Nist 08 lib). The Kovats index was calculated for each constituent as previously described (Van den Dool, 1963) and the data were compared to the literature (Adams, 2009). The oil compositions are presented in Table 1.

Antimicrobial activity

The antimicrobial properties of the essential oils were examined using the broth microdilution method (96-well microtiter plates) as previously described by Salvador et al. (2002), to give a concentration between 12 and 5000 µg/ml. The minimal inhibitory concentration (MIC) was calculated as the lowest concentration showing complete inhibition of microbial growth. In these tests, chloramphenicol and ketoconazole were used as experimental positive controls for bacteria and fungi strains, respectively, while the solution of dimethyl sulphoxide (DMSO)-sterile distilled water (5:95, v/v) served as the negative control. Each sensitivity test was performed in duplicate for each microorganism evaluated and repeated 3 times. The strains of microorganisms utilized are shown in Table 2.

Table 1. Relative abundance of the constituents in the essential oils from the leaves of three species of Asteraceae from Brazil.

RI (lit.)[a]	RI [b]	Constituent	Relative peak area (%)		
			AA	CF	MG
932	939	α-Pinene	-	0.15	0.11
975	975	Sabinene	0.02	-	0.09
979	977	β-Pinene	-	-	0.38
990	988	Myrcene	0.12	-	2.55
1002	1007	α-Phellandrene	0.04	-	-
1024	1023	*p*-Cymene	-	0.12	-
1029	1028	Limonene	0.51	1.18	3.93
1037	1033	(*Z*)-β-Ocimene	-	-	0.15
1039	1033	Lavender lactone	-	0.50	-
1050	1044	(*E*)-β-Ocimene	0.18	-	1.01
1052	1048	*cis*-Arbusculone	-	0.16	-
1070	1067	*trans*-Arbusculone	-	0.23	-
1072	1069	*cis*-Linalool oxide	-	0.20	-
1086	1085	*trans*-Linalool oxide	-	0.15	-
1088	1085	Terpinolene	0.30	-	0.09
1096	1100	Linalool	0.05	1.28	0.06
1177	1180	Terpinen-4-ol	0.06	0.18	0.71
1182	1187	*p*-Cymen-8-ol	-	0.42	-
1188	1194	α-Terpineol	-	-	0.29
1216	1217	*trans*-Carveol	-	0.17	-
1243	1242	Carvone	-	0.20	-
1295	1297	Perilla alcohol	-	-	0.07
1338	1333	δ-Elemene	0.14	0.12	0.08
1351	1345	α-Cubebene	-	-	0.29
1371	1366	Cyclosativene	-	0.53	-
1376	1373	α-Copaene	0.12	1.36	0.61
1388	1381	β-Bourbonene	-	0.24	0.11
1388	1386	β-Cubebene	0.39	0.66	1.47
1390	1387	β-Elemene	0.16	0.79	0.88
1419	1417	(*E*)-Caryophyllene	9.92	0.25	8.58
1432	1427	β-Copaene	0.15	0.57	0.16
1439	1432	α-Guaiene	-	-	0.47
1450	1446	*cis*-Muurola-3,5-diene	-	-	0.52
1454	1452	α-Humulene	3.25	0.15	1.41
1460	1457	allo-Aromadendrene	0.19	0.27	0.07
1472	1472	Dauca-5,8-diene	-	1.49	-
1476	1470	*trans*-Cadina-3(6),4-diene	-	-	0.82
1479	1473	γ-Muurolene	0.53	-	0.23
1481	1481	Germacrene D	58.65	-	25.90
1484	1483	α-Amorphene	0.01	-	-
1490	1486	β-Selinene	0.16	1.47	-
1493	1489	*trans*-Muurola-4(14),5-diene	-	-	2.16
1494	1492	epi-Cubebol	-	1.14	-
1495	1489	γ-Amorphene	0.04	-	-
1500	1494	Bicyclogermacrene	7.80	-	11.77
1500	1496	α-Muurolene	0.90	1.14	0.92
1509	1499	α-Bulnesene	-	-	0.34
1509	1504	Germacrene A	0.34	-	0.23

Table 1. Contd.

1513	1510	γ-Cadinene	0.24	0.67	-
1515	1512	Cubebol	0.17	1.02	3.19
1522	1519	*trans*-Calamenene	-	0.45	0.79
1523	1516	δ-Cadinene	1.61	-	4.27
1529	1520	Zonarene	-	-	0.92
1534	1529	*trans*-Cadina-1,4-diene	0.04	-	0.13
1538	1533	α-Cadinene	0.18	-	-
1548	1545	Hedycaryol	-	-	0.91
1561	1555	Germacrene B	0.32	-	0.17
1563	1560	(*E*)-Nerolidol	3.40	1.54	-
1578	1574	Spathulenol	**-**	2.38	3.53
1583	1578	Caryophyllene oxide	0.27	15.01	1.41
1587	1584	Gleenol	**-**	0.31	-
1590	1587	Globulol	0.29	0.32	1.15
1592	1590	Viridiflorol	0.25	-	-
1595	1593	Cubeban-11-ol	0.03	-	-
1600	1593	Guaiol	-	-	0.21
1600	1603	Rosiflorol	0.09	-	-
1602	1599	Ledol	-	-	0.17
1608	1606	Humulene epoxide II	0.05	3.36	-
1619	1612	1,10-di-epi-Cubenol	0.04	-	-
1619	1618	Junenol	0.44	1.14	-
1628	1624	1-epi-Cubenol	0.26	3.71	1.48
1640	1638	Epi-α-Cadinol	0.61	-	-
1642	1641	Epi-α-Muurolol	1.18	-	2.27
1646	1643	α-Muurolol	0.43	1.38	0.56
1650	1651	β-Eudesmol	-	**-**	2.83
1653	1654	Pogostol	-	**-**	1.62
1654	1652	α-Cadinol	2.45	7.88	**-**
1659	1655	Selin-11-en-4-α-ol	-	7.32	-
1661	1660	*cis*-Calamenen-10-ol	-	0.88	0.08
1677	1669	Mustakona	-	-	0.32
1686	1681	Germacra-4(15),5,10(14)-trien-1-α-ol	0.13	-	0.54
1689	1689	Shyobunol	-	-	0.17
1736	1739	Eremophilone	-	0.44	-
1741	1731	Mint sulfide	-	-	0.33
2010	2007	13-epi-Manool oxide	-		0.09
		Monoterpenes	-	-	-
		Hydrocarbons	1.17	1.45	8.31
		Oxygenated	0.11	2.60	1.13
		Monoterpenes total	1.28	4.05	9.44
		Sesquiterpenes	-	-	-
		Hydrocarbons	85.14	10.16	63.30
		Oxygenated	10.09	47.83	20.44
		Sesquiterpenes total	95.23	57.99	83.74
		Others	-	0.89	0.42
		Total identified	96.51	62.93	93.60

[a]RI = Retention Indices. See Adams, 2009. [b]RI = Retention Indices on ZB-5MS column (relative to *n*-alkanes). AA = *Acanthospermum australe* (Loefl.) Kuntz; CF = *Calea fruticosa* (Gardner) Urbatsch, Zlotsky & Pruski; MG = *Mikania glauca* Mart. ex Baker. In bold: most representative components (>5%).

Table 2. Antimicrobial activity of the essential oils from the leaves of three species of Asteraceae from Brazil.

Microorganism	MIC[a] (µg/ml)			
	AA	CF	MG	Controls[b]
Proteus vulgaris (Pv)[c]	-	100	1000	50
Escherichia coli (ATCC 10538)[d]	-	-	-	50
Staphylococcus epidermidis (ATCC 12228)[d]	-	-	1000	50
Staphylococcus aureus (ATCC 14458)[d]	-	-	-	25
Candida glabrata (ATCC 30070)[c]	100	-	500	12
Candida tropicalis (CT)[c]	-	-	-	12
Candida albicans (ATCC 10231)[d]	-	-	-	12
Candida dubliniensis (ATCC 778157)[d]	50	-	-	12

[a]MIC: minimum inhibitory concentration in µg/mL; [b]Positive controls: chloramphenicol for bacterial strains and ketoconazole for yeast strains; [c]standard strain; [d]field strain; -: no inhibition of microbial development. AA = *Acanthospermum australe* (Loefl.) Kuntz; CF = *Calea fruticosa* (Gardner) Urbatsch, Zlotsky & Pruski; MG = *Mikania glauca* Mart. ex Baker.

RESULTS AND DISCUSSION

Extraction of the essential oils

Of all the plants analyzed in relation to their essential oil extraction, *M. glauca* presented the highest yield (0.227%). The yield was lower than presented by Guimarães et al. (2012), 0.65% for the same species. The yield of the essential oil of *A. australe* (0.030%) was also lower than that presented in the literature (0.13% of fresh mass) by Morais et al. (1997). *C. fruticosa* presented a yield of 0.041%.

Chemical composition

The compositions of the essential oils obtained by hydrodistillation from the leaves of *A. australe*, *C. fruticosa* and *M. glauca* analyzed by GC-MS are listed in Table 1. The percentage and retention indices of components are given. For the three species studied, 62.9 to 96.5% of all chemical constituents were identified. Sixteen compounds were common to these three species: limonene, terpinen-4-ol, δ-elemene, α-copaene, β-cubebene, β-elemene, (*E*)-caryophyllene, β-copaene, α-humulene, allo-aromadendrene, α-muurolene, cubebol, caryophyllene oxide, globulol, 1-epi-cubenol and α-muurolol.

Forty five compounds were identified in the essential oil of *A. australe*, accounting for 96.5% of the total oil. The oil from fresh leaves of *A. australe* was characterized by a high amount of sesquiterpenes hydrocarbons (85.1%), followed by oxygenated sesquiterpenes (10.1%). The major components were germacrene-D, (*E*)-caryophyllene and bicyclogermacrene which constitute, respectively 58.7, 9.9 and 7.8% of the total oil composition. In another investigation on chemical composition of essential oil from the leaves of *A. australe* collected in the Southern part of the Amazon Forest in Brazil, *E*-caryophyllene (16.0%), β-elemene (14.4%), γ-cadinene (13.0%), germacrene A (10.1%) and δ-cadinene (5.5%) were the major components in this oil, however it did not contain germacrene D (Morais et al., 1997). This difference probably occurred as a result of various factors that can affect the composition of the essential oils, such as genetic factors, growing location, the regional climate and the time of day at which it is collected (Burt, 2004).

The *C. fruticosa* essential oil was characterized by 43 constituents, representing 62.9% of the total oil composition. The essential oil is dominated by the presence of oxygenated sesquiterpenes constituting 47.8%, followed by sesquiterpenes hydrocarbons (10.2%) and the most abundant components were caryophyllene oxide (15.0%), α-cadinol (7.9%) and sellin-11-en-4-α-ol (7.3%). Other chemical constituents in lower quantities in the oil were ketones (0.4%) and lactone (0.5%). Among the species of the genus, only *C. pinnatifida*, *C. clematidea* and *C. serrata* were previously studied for the essential oil content. The major compounds found in the essential oil from aerial parts of *C. pinnatifida* were (*E*)-caryophyllene (15.2%), α-cadinene (8.2%) and α-coprene (4.9%) (Kato et al., 1994). The essential oil of the leaves from *C. clematidea* showed a high content of a natural epoxy terpenoid named clemateol (70.5%), with minor amounts of others compounds (Flach et al., 2002). Ribeiro et al. (2011) characterized the essential oil of *C. serrata* and encountered higher concentrations of precocene II (29.6%) and germacrene D (26.4%). The essential oils of these four species, including *C. fruticosa* are quite different, having in common only the sesquiterpene (*E*)-caryophyllene, indicating a considerable chemodiversity in the essential oils of *Calea* species. There are no data about the chemical volatile composition of specie *C. fruticosa* in the literature, which made difficult the discussion of the results.

The total number of chemical constituents identified in essential oils was 55 for *M. glauca*, representing 93.6%

of the total oil content. In essential oil, sesquiterpenes hydrocarbons were the main class of constituents (63.3%), followed by oxygenated sesquiterpenes (20.4%), and the most abundant components were germacrene D (25.9%), bicyclogermacrene (11.8%) and (*E*)-caryophyllene (8.6%). Previous report on essential oil composition of *M. glauca* is not completely in agreement with the present study. Guimarães et al. (2012) demonstrated that monoterpene hydrocarbons were the most abundant components in the essential oil of fresh leaves of *M. glauca*. The principal constituents were α-pinene, β-pinene, myrcene, (*E*)-caryophyllene and bicyclogermacrene. The predominance of terpene hydrocarbons has been reported for the essential oils of several species of *Mikania*. Germacrene-D and β-caryophyllene sesquiterpenes are commonly encountered as the principal constituents of the essential oils from several species of the genus.

Antimicrobial activity

The oils were tested against two Gram-positive and two Gram-negative bacterial strains and four yeast strains (Table 2). The results show that essential oil of *M. glauca* inhibited both Gram-negative (*Proteus vulgaris*, field strain) and Gram-positive (*Staphylococcus epidermidis* ATCC 12228, standard strain) bacteria and inhibited one yeast *Candida glabrata*. The antibacterial activity could have resulted from the presence of caryophyllene oxide, α-pinene, α-terpineol and linalool compounds that are known to possess antibacterial activity. Although present in low concentrations, these compounds could have imparted a significant effect on the antibacterial activity of the oil (Sivasothy et al., 2011; Magiatis et al., 1999).

The *C. fruticosa* essential oil exhibited antimicrobial activity only against Gram-negative bacteria *P. vulgaris* and no antimicrobial activity against yeast strains, suggesting its selectivity. The essential oil *C. fruticosa* showed a minimum inhibitory concentration (MIC) value greater than the essential oil of *M. glauca* to the same bacteria. This profile could be attributed to the high concentration in the oil of caryophyllene oxide (15.0%) with known antimicrobial activity (Vagionas et al., 2007). The antibacterial activity of the oil is suspected also to be associated with α-pinene and linalool, via a synergistic effect (Sivasothy et al., 2011). The essential oil of another plant of the same genus, *C. clematidea*, showed a moderate antifungal activity against multiple dermatophytes (Flach et al., 2002).

The results showed that the essential oil from *A. australe* was the only one that inhibited two of the four yeasts evaluated with MIC between 50 and 100 µg/ml. The most susceptible yeast was *C. glabrata* whose growth was inhibited by the essential oils of *A. australe* and *M. glauca* at concentrations of 100 and 500 µg/ml, respectively. The antimicrobial activities of the essential oils of *A. australe*, *C. fruticosa* and *M. glauca* confirm that the Asteraceae species are source of biologically active compounds. Further investigations are necessary to confirm the potential of these essential oils as bioactive agents useful for *in vivo* applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Adams RP (2009). Identification of essential oil components by gas chromatography/mass spectrometry. Allured Books, Carol Stream, IL.

Astani A, Reichling J, Schnitzler P (2010). Comparative study on the antiviral activity of selected monoterpenes derived from essential oils. Phytother. Res. 24(5):673-679.

Bohlmann F, Jakupovic J, Dhar AK, King RM, Robinson H (1981a). Two sesquiterpene and three diterpene lactones from *Acanthospermum australe*. Phytochemistry 20(5):1081-1083.

Bohlmann F, Zdero C, King RM, Robinson H (1981b). Heliangolides, and nerolidol and *p*-hydroxyacetophenone derivatives from *Calea* species. Phytochemistry 20(7):1643-1647.

Bohlmann F, Jakupovic J, Zdero C, King RM, Robinson H (1979). Neue melampolide und *cis*,*cis*-germacranolide aus vertretern der subtribus melampodiinae. Phytochemistry 18(4):625-630.

Bohlmann F, Marthur R, Jakupovic J, Gupta, RK, King RM, Robinson H (1982). Furanoheliangolides and other compounds from *Calea hymenolepis*. Phytochemistry 21(8):2045-2048.

Bremer K (1994). Asteraceae: Cladistics and Classification. Timber Press, Inc., Portland.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. Int. J. Food Microbiol. 94(3):223-253.

Flach A, Gregel B, Simionatto E, Silva UF, Zanatta N, Morel AF, Linares CEB, Alves SH (2002). Chemical analysis and antifungal activity of the essential oil of *Calea clematidea*. Planta Med. 68(9):836-838.

Funk VA, Susanna A, Stuessy T, Bayer RJ (2009). Systematics, evolution, and biogeography of the Compositae. IAPT, Vienna.

Guimarães LGL, Cardoso MG, Silva LF, Gomes MS, Andrade MA, Souza JA, Miranda CASF, Andrade J, Machado SMF, Figueiredo AC, Barroso JG, Mansanares ME, Nelson, DL (2012). Chemical analyses of the essential oils from leaves of *Mikania glauca* Mart. ex Baker. J. Essent. Oil Res. 24(6):599-604.

Herz W (1998). Terpenoid chemistry of *Mikania* species J. Indian Chem. Soc. 75(10-12):559-564.

Kato ETM, Akisue MK, Matos FJA, Craveiro AA, Alencar JM (1994). Constituents of *Calea pinnatifida*. Fitoterapia 65(4):377-377.

Lorenzi H, Matos FJA (2002). Plantas medicinais no Brasil: nativas e exóticas. Instituto Pantarum, São Paulo.

Magiatis P, Melliou E, Skaltsounis AL, Chonou IB, Mitaku, S (1999). Chemical composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var. chia. Planta Med. 65(8):749-752.

Martinez M, Esquivel B, Ortega A (1987). Two caleines from *Calea zacatechichi*. Phytochemistry 26(7):2104-2106.
Matsunaga K, Saitoh M, Ohizumi Y (1996). Acanthostral, a novel antineoplastic *cis*-*cis*-*cis*-germacranolide from *Acanthospermum australe*. Tetrahed. Lett. 37(9):1455-1456.
Metwally MA, King RM (1985). A thymol derivative from *Calea pilosa*. Indian J. Chem. B 24(9):982-982.
Morais SM, Machado MIL, Machado SMF, Facundo VA, Militão JSLT, Ribeiro AA (1997). Essential Oil of *Acanthospermum australe* DC. J. Essent. Oil Res. 9(5):601-602.
Ober AG, Urbatsch LE, Fischer NH (1985). Germacranolides, calbertolides A, B, and C, from *Calea berteriana*. Phytochemistry 24(8):1743-1745.
Oliveira F, Alvarenga M A, Akisue G, Akisue MK (1984). Isolamento e identificação de componentes químicos de *Mikania glomerata* Sprengel de *Mikania laevigata* Schultz Bip. ex Baker. Rev. Farm. Bioquím. Univ. São Paulo 20(2):169-183.
Ribeiro VLS, Santos JC, Martins JR, Schripsema J, Siqueira IR, von Poser GL, Apel MA (2011). Acaricidal properties of the essential oil and precocene II obtained from *Calea serrata* (Asteraceae) on the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). Vet. Parasitol. 179(1-3):195-198.
Ritter MR, Liro RM, Roque N, Nakajima (2012). *Mikania in* Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro, Rio de Janeiro.
Salvador MJ, Ferreira EO, Pral EM, Alfieri SC, Albuquerque S, Ito IY, Dias DA (2002). Bioactivity of crude extracts and some constituents of *Blutaparon portulacoides* (Amaranthaceae). Phytomedicine 9(6):566-571.
Sivasothy Y, Chong WK, Hamid A, Eldeen IM, Sulaiman SF, Awang K (2011). Essential oils of *Zingiber officinale* var. *rubrum Theilade* and their antibacterial activities. Food Chem. 124(2):514-517.
Soares e Silva L, Silva SL, Brumano L, Stringheta PC, Pinto MAO, Dias LOM, Muller CSM, Scio E, Fabri RL, Castro HC, Amaral MPH (2012). Preparation of dry extract of *Mikania glomerata* Sprengel (guaco) and determination of its coumarin levels by spectrophotometry and HPLC-UV. Molecules 17(9):10344-10354.
Steinbeck C, Spitzer V, Starosta M, Poser G (1997). Identification of two chromenes from *Calea serrata* by semiautomatic structure elucidation. J. Nat. Prod. 60(6):627-628.
Urbatsch LE, Zlotsky A, Pruski JF (1986). Revision of *Calea* sect. Lemmatium (Asteraceae: Heliantheae) from Brazil. Syst. Bot. 11(4):501-514.
Vagionas K, Ngassapa O, Runyoro D, Graikou K, Gortzi O, Chinou I (2007). Chemical analysis of edible aromatic plants growing in Tanzania. Food Chem. 105(4):1711-1717.
Van den Dool H, Kratz PD (1963). A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. J. Chromatogr. 11:463-471.
Vilegas JHY, Marchi E, Lanças FM (1997). Determination of coumarin and kaurenoic acid in *Mikania glomerata* ("guaco") leaves by high resolution gas chromatography. Phytochem. Anal. 8(2):74-77.