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HS-SPME-GC-MS ANALYSIS OF VOLATILE AND SEMI-VOLATILE COMPOUNDS FROM DRIED LEAVES OF *Mikania glomerata* Sprengel

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This paper reports on the identification of volatile and semi-volatile compounds and a comparison of the chromatographic profiles obtained by Headspace Solid-Phase Microextraction/Gas Chromatography with Mass Spectrometry detection (HS-SPME-GC-MS) of dried leaves of *Mikania glomerata* Sprengel (Asteraceae), also known as 'guaco.' Three different types of commercial SPME fibers were tested: polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB) and polyacrylate (PA). Fifty-nine compounds were fully identified by HS-SPME-HRGC-MS, including coumarin, a marker for the quality control of guaco-based phytomedicines; most of the other identified compounds were mono- and sesquiterpenes. PA fibers performed better in the analysis of coumarin, while PDMS-DVB proved to be the best choice for a general and non-selective analysis of volatile and semi-volatile guaco-based compounds. The SPME method is faster and requires a smaller sample than conventional hydrodistillation of essential oils, providing a general overview of the volatile and semi-volatile compounds of *M. glomerata.*

Keywords: *Mikania glomerata* Sprengel (Asteraceae); HS-SPME-HRGC-MS; coumarin; monoterpenes; sesquiterpenes.

INTRODUCTION

Mikania glomerata Sprengel (Asteraceae) is one of the *Mikania* species known in Brazil by the popular name "guaco," whose leaves serve as raw material for commercial phytopharmaceuticals (extracts and syrups) used as expectorants and bronchodilators. A review of the situation of herbal medicines registered in Brazil revealed that *M. glomerata* is the native plant species with the largest number of registered phytomedicines (14 products in March 2008).[1] In 2012, Brazil's Ministry of Health included *M. glomerata* in the official list of drugs used by the country's National Health Service (SUS). This official list includes only herbal medicines that have proven effectiveness and are manufactured and registered with Brazil's National Health Surveillance Agency (ANVISA - Agência Nacional de Vigilância Sanitária).[2]

Data obtained from a comparative study of the anatomy and molecular biology of *M. glomerata* and *M. laevigata* Schultz Bip. ex Baker (another *Mikania* species also popularly known in Brazil as "guaco") suggest that these two species could be unified in terms of nomenclature, due to their similarity.[3] Phytochemical, biological and pharmacological investigations of "guaco" were reviewed, and the main compounds reported are coumarin and terpenoids (monoterpenes, sesquiterpenes and diterpenes), among others.[4] The GC-MS analysis of the essential oil (EO) obtained by the hydrodistillation of fresh *M. glomerata* leaves led to the identification of oxygenated sesquiterpenes (72.5% of the EO), sesquiterpene hydrocarbons (22.6%), monoterpene hydrocarbons (1.7%) and oxygenated monoterpenes (0.3%); and another report of an analysis of EO from *M. glomerata* leaves, flowers and seeds also indicated that sesquiterpenes are the major compounds (81.5% of the EO from fresh leaves).[5]

GC techniques have been employed for the quantitative analysis of coumarin and kaurenoic acid from *M. glomerata* leaves.[6] However, the quantification of coumarin by HPLC-UV has been suggested as a procedure for the quality control of guaco phytomedicines based on hydroalcoholic extracts,[7] and this analytical strategy has been applied in the development of guaco phytomedicines.[8] Pharmacological studies of the antiallergic activity of ethanol extracts of *M. glomerata* leaves confirm the use of coumarin as one of the markers of this species.[9] Moreover, coumarin as well as *o*-coumaric acid have been quantified by HPLC-UV in a comparative study of *M. glomerata* and *M. laevigata*, and these two compounds have been identified as partly responsible for the therapeutic activity of both guaco species.[10]

The first step in the production of standardized phytomedicines is to ensure the quality of herbal material used as raw material. Moreover, there is an increasing demand for the development of versatile and faster analytical methods suitable for routine quality control analysis on the scale required by the phytopharmaceutical industry. In this context, headspace solid-phase microextraction (HS-SPME) has proved to be a successful technique for studying the composition of the volatile fraction of a plant. A review by Bicchi and co-workers summarizes the evolution of vapor phase sampling of the volatile fraction of vegetable matrices, including HS-SPME applications. In their review, the authors also point out the confusion between the definitions of headspace (HS) and EO in the literature, although they recognize that HS and EO composition may sometimes be similar, in the case of plant matrices. Another review by Bicchi and co-workers, covering the field of aromatic and medicinal plants, discusses the advantages and limitations of HS-SPME as an alternative or a complement to EO analysis, and these authors also discussed further examples of the advantages and limitations of HS-SPME in the analysis of the EO of medicinal plants EO.[11]

A recent review of Jeleńa and co-workers, about analysis of food flavor volatile compounds, shows the leading position of SPME among the most popular microextraction methods of volatile compounds important for food quality, including vegetable foods such as fruits and vegetables, spices and herbs, among others.[12]

Despite the evolution and widespread acceptation of HS-sampling in the analysis of plant material, most of the official methods for plant drug analysis still do not consider SPME sampling for the quality control of medicinal plants. Therefore, within the scope of the proposition of faster analytical methods that are alternatives to the conventional procedures for the analysis of Brazilian phytomedicines, this study compares the HS-SPME-HRGC-MS analysis of volatile and semi-volatile compounds from dried *Mikania glomerata* leaves, using three different commercial fibers, and suggests HS-SPME as an alternative analytical approach that can be used as a complement

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in the quality control of *M. glomerata* phytomedicines, particularly in the analysis of herbal raw material.

EXPERIMENTAL

Plant material

Mikania glomerata leaves (Voucher UNESP BOTU 19813) were obtained from specimens grown in Ribeirão Preto, SP, Brazil. Plants were harvested in the morning, and the leaves were immediately separated from the other aerial parts and dried in the shade at room temperature (average temperature of 22 to 30 °C) until they reached a constant weight. The leaves were separated and samples of 10 g each were stored separately in hermetically sealed plastic bags. These bags were opened immediately prior to HS-SPME sampling.

Table 1. Relative (%) chemical composition of the volatile and semi-volatile compounds from *Mikania glomerata* dried leaves found by HS-SPME-HRGC-MS using different SPME fibers. Peak numbering as in Figure 1

Peak	Compound	PA (%)	DVB/PDMS (%)	PDMS (%)
Monoterpene hydrocarbons		**2.52**	**6.26**	**8.33**
10	α-thujene	n	-	-
11	**α-pinene**	1.75	3.80	5.97
12	thuja-2,4(10)-diene	0.10	0.33	0.10
15	**sabinene**	0.57*	1.17*	1.70*
16	**β-pinene***			
20	β-myrcene	0.10	0.75	0.46
23	p-cymene	-	0.14	0.07
24	**limonene**	-	0.07	0.03
Oxygenated monoterpenes		**4.89**	**5.91**	**2.80**
29	acetophenone	-	-	-
34	perillene	-	0.10	0.13
35	α-canpholenal	0.14	0.22	0.13
36	*trans*-pinocarveol	0.79	0.70	0.38
38	*trans*-verbenol	1.17	0.85	0.47
41	pinocarvone	0.62	0.87	0.42
44	p-cymen-8-ol	-	0.17	-
45	*cis*-3-hexenyl butyrate	-	-	-
47	myrtenal*	0.91*	1.14*	0.51*
48	myrtenol			
50	carvomenthone	-	-	-
51	verbenone	1.01	1.53	0.72
52	*trans*-carveol	0.25	0.33	0.04
Other oxygenated compounds				
68	**Coumarin**	**28.37**	**18.11**	**7.27**
Sesquiterpene hydrocarbons		**8.18**	**16.44**	**16.75**
57	**δ-elemene**	0.17	0.33	0.41
58	α-cubebene	0.38	1.45	1.25
60	**α-ylangene**	-	0.15	0.11
61	α-copaene	0.42	1.36	1.26
A	3,4 dihydrocoumarin*⁺	2.92*	4.13**	3.52**
62	β-bourbonene**⁺			
63	β-cubebene	0.28	0.47	0.70
64	β-elemene	0.25	0.59	0.52
65	**β-caryophyllene**	0.89	1.98	2.43
66	unknown (MW 204; fragmentation similar to cubebene)	0.25	0.60	0.57
70	**humulene**	0.58	1.13	0.94
71	allo-aromadendrene	0.11	0.28	0.30
72	**germacrene-D**	1.18	1.72	2.99
73	**bicyclogermacrene**	0.38	0.56	0.85
74	α-muurolene	-	0.31	0.25
77	**γ-cadinene**	0.16	0.54	0.36
78	**δ-cadinene**	0.21	0.72	0.29
79	**α-calacorene**	-	0.12	-
Oxygenated sesquiterpenes		**44.38**	**38.47**	**51.39**
81	unknown (MW 206; possibly a sesquiterpene ketone)	1.60	1.86	2.09
84	1,5-epoxy salvial-4(14)-ene	0.34	0.50	0.63
86	**spathulenol** (co-elution)*	20.79	20.41*	17.33
87	**caryophyllene oxide** (co-elution)	8.02	(co-elution)	10.88
89	salvial-4(14)-en-1-one	0.52	0.56	0.68
91	humulene-epoxide II	1.99	0.29	2.13
94	nor-copaanone	0.27	0.25	0.35
95	unknown (MW 220, possibly a sesquiterpene alcohol)	1.57	1.53	1.87
96	caryophylle-4(14), 8(13)-dien-5β-ol	1.15	2.80	2.93
100	unknown (MW 222, possibly a sesquiterpene alcohol)	1.64	1.25	1.68
102	unknown (MW 206, possibly a sesquiterpene ketone)	2.67*	6.14*	6.31*
B	caryophylla -3,8(13)-dien-5β-ol*			
103	unknown (MW 220, possibly a sesquiterpene alcohol)	1.58	1.30	1.64
104	unknown (MW 220, possibly a sesquiterpene alcohol)	0.37	0.27	0.41
105	unknown (MW 236, possibly a carbonilic compound)	0.27	0.28	0.44
107	γ-1-cadinal	-	-	-
110	14-oxi-α-muurolene	0.34	0.26	0.55
113	10-oxo-isodauc-3-en-15-ol	1.26	0.77	1.47
Diterpene hydrocarbons		**0.48**	**0.38**	**0.72**
116	beyerene	0.48	0.38	0.72
120	kaurene	-	-	-
125	atiserene	-	-	-
Oxygenated diterpenes		**0.37**	**0.18**	**1.76**
114	hexahydrofarnesyl acetone	0.14	0.10	0.22
119	epi-13-manoyl oxide	-	-	-
123	beyeren-18-al	-	-	-
124	beyeren-18-ol	-	-	-
126	kauran-16-ol	0.23*	0.08*	1.54*
127	beyeren-19-ol *			
128	unknown (MW 316, fragmentation similar to a beierene methyl ester)	-	-	-
	TOTAL (%)	**89.19**	**85.75**	**89.02**

PA: polyacrilate; **DVB**: divinylbenzene; **PDMS**: polydimethylsiloxane.
names in bold: compounds previously reported in *M. glomerata* EO.[5]
(-) compound with peak area smaller than the integration threshold parameters.
(n) not detected or not identified.
(* and **) most abundant compound of two co-eluting peaks.
(⁺) 3,4-dihydrocoumarin (peak **A**) and β-bourbonene were quantified together.
peak **A:** tentative identification.
peak **B:** tentative identification.

HS-SPME sampling

The SPME device and the fused silica fibers were purchased from Supelco (Bellafont, PA, USA). The following fibers were employed: 100 mm poly(dimethylsiloxane) (PDMS), 65 mm poly(dimethylsiloxane)-divinylbenzene (PDMS-DVB), 65 mm polyacrylate. All fibers were conditioned prior to use as recommended by manufacturer. The analytical conditions described for HS-SPME sampling were chosen after preliminary assays using different amounts of dried plant material, different extraction and desorption conditions (time, temperature, equilibrium time). Dried *M. glomerata* leaves samples were hand-cut with a scissor and powdered using a domestic blender and grounded, and only particles smaller than 50 mesh were utilized for the HS-SPME step. A sample of plant material (60 mg) was sealed in 2 mL vials and stored at -18 °C for 2 h at least) before HS-SPME sampling. The SPME device was inserted into the sealed vial containing the plant sample and the fiber was exposed to the headspace (equilibration time 15 min and temperature 50 °C; sampling time 120 min and temperature 50 °C). After sampling, the SPME device was immediately inserted into the GC injector and the fiber was thermally desorbed for 15 min at 230 °C.

Gas chromatography – mass spectrometry analysis

Analysis were carried out on an Agilent GC-6890 Plus system coupled to a 5973 MSD (Little Falls, DE, USA). For HS-SPME-HRGC-MS analysis, the injector was operated in the splitless mode at 230 °C during 5 min and thereafter split (split ratio 1:10), and the chromatographic conditions were: column, HP-5 MS (25 m × 0.25 mm i.d., 0.3 mm film thickness; Agilent); temperature programme, from 40 °C (held for 3 min) to 125 °C (0 min) at 4 °C min^{-1}, then at 1.5 °C min^{-1} to 140 °C (4 min) and finally at 5 °C min^{-1} to 250 °C (5 min); carrier gas was He at flow rate of 1.0 mL min^{-1} in constant flow mode. Interface temperature was 280 °C and the MS were obtained in the electron impact mode at 70 eV, scan mode, range *m/z* 35-350 at a rate of 2.36 scans s^{-1}. Data were acquired in a PC system using software ChemStation G1701CA (Agilent). Compounds were identified by comparison of their linear retention indices, relative to C_8–C_{25} n-alkanes, and their MS with those of authentic samples or components of reference essential oils.

RESULTS AND DISCUSSION

Most of the compounds extracted by HS-SPME are mono- and sesquiterpenes, and 59 compounds were fully identified by HS-SPME-HRGC-MS (Table 1). Several peaks were not fully identified, but fragmentation data also furnished strong evidence about their partial structural information, which is also included in Table 1. Previous reports of the analysis of EO from *M. glomerata* are described in the literature,[6] but the direct comparison of EO and HS-SPME is not strictly correct, since the fundamental principles of the two techniques differ completely, which influences the quantitative and qualitative chemical composition of EO and headspace.[11] Taking these considerations into account, however, HS-SPME-HRGC-MS analysis led to the identification of several volatile compounds from *M. glomerata* not previously reported.[5]

A comparison of the chromatographic profiles obtained by HS-SPME-HRGC-MS (Figure 1) indicates that the main difference between the three HS-SPME profiles was the extraction efficiency of different fibers under the same conditions, since the number of extracted compounds is almost the same. Considering the sum of the areas of the detected peaks in TIC (ΣA) as a criterion to measure extraction efficiency, PDMS-DVB fiber showed the highest efficiency ($\Sigma A_{PDMS\text{-}DVB} = 6.10 \times 10^9$, s.d. ±5.2%), i.e., almost twice that of PA ($\Sigma A_{PA} = 3.65 \times 10^9$, s.d. ±4.9%) and PDMS ($\Sigma A_{PDMS} = 3.31 \times 10^9$, s.d. ±3.5%) fibers. Coumarin was extracted with greater efficiency by PA fiber (28.37% of ΣA_{PA}, compared to 18.11% of $\Sigma A_{PDMS\text{-}DVB}$ and 7.27% of ΣA_{PDMS}).

HS-SPME-HRGC-MS showed a good performance, considering the result achieved in the extraction of coumarin (a chemical marker of *M. glomerata*). Moreover, the SPME procedure offers the advantages of requiring smaller samples and shorter time of analysis than the HRGC-FID and HPLC-UV methods previously reported for *M. glomerata*.[6] HS-SPME-HRGC-MS is also faster than the conventional hydrodistillation procedure for EO analysis (around 4 hours only for hydrodistillation step), and the latter procedure has the additional disadvantages of requiring hundreds of grams of plant material and of potentially generating artifacts due to the

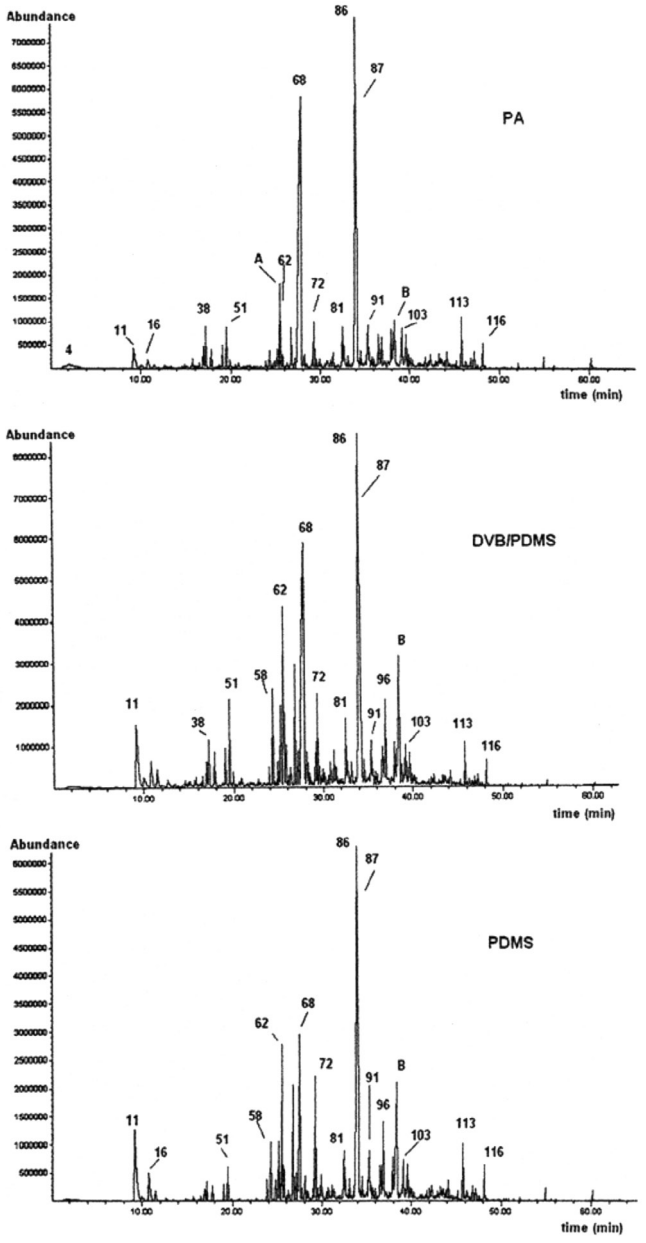

Figure 1. *TIC-HRGC-MS profiles of volatile and semi-volatile compounds found by HS-SPME from Mikania glomerata dried leaves, using different SPME fibers. For identification of the peaks, see Table 1.* ***PA****: polyacrilate;* ***DVB/PDMS****: divinylbenzene / polydimethylsiloxane;* ***PDMS****: polydimethylsiloxane*

long exposure of plant material to water steam. Hence, the SPME method may be a valuable tool for screening and characterizing the chemical composition of volatile and semi-volatile compounds of *M. glomerata* in studies requiring a large number of plant samples and/or when a limited amount of plant material is available for several samplings, such as dynamic plant biological processes (including metabolomic studies), micropropagation or cloning for cultivation purposes, etc. In addition, it is easier to automate the HS-SPME approach using online systems that combine sample preparation and GC analysis.

CONCLUSIONS

The chemical analysis of any phytomedicine must be standardized, and this perforce includes the volatile fraction. Considering that quality assurance in routine processes such as health surveillance, or during the industrial production of phytomedicines, requires reproducible and fast methods, and also in view of the increasing interest in "environmentally friendly" techniques such as SPME to replace some procedures adopted in Pharmacopoeias that are difficult to adapt to modern and automated analytical methods, the results presented here suggest HS-SPME-HGRC-MS as a potential analytical tool for the analysis of volatile and semi-volatile compounds of *M. glomerata*.

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