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ORIGINAL PAPER

Rapid Determination of Volatile Compounds in *Gymnotheca involucrata* Pei. by MAE–HS-SPME Followed by GC–MS

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Abstract Microwave-assisted extraction (MAE) and headspace solid-phase microextraction (HS-SPME) followed by gas chromatography–mass spectrometry (GC–MS) was developed for the rapid determination of the volatile chemical compositions of *Gymnotheca involucrata* Pei. The experimental parameters of the MAE–HS-SPME were optimized by orthogonal experimental design. The results indicated that the optimal experimental parameters for determination of the volatile constituents of *Gymnotheca involucrata* Pei. were: sample weight 2.0 g, microwave power of 400 W and irradiation time of 3.0 min. Employing these conditions, 106 volatile constituents in the dry stems and 103 volatile constituents in the dry leaves of *Gymnotheca involucrata* Pei. were separated and identified, amounting to 93.58 and 98.53% of the total peak areas, respectively. The major components found in the oil extracted from the stems and leaves were nerolidol (16.57, 17.50%), sabinene (3.35, 3.65%), *cis, cis, cis*-1,1,4,8-tetramethyl-4,7,10-cycloundecatriene (4.64, 5.03%), α-copaene-8-ol (6.01, 6.33%) and phytol (2.92, 3.03%), respectively. Relative standard deviation (RSD) values of <9.0% showed that the MAE–HS-SPME method followed by GC–MS has good precision. The experimental results demonstrated that this solvent-free method is simple, time-efficient, and is a potentially suitable analytical tool for the determination of volatile compounds from vegetative and other materials.

Keywords *Gymnotheca involucrata* Pei. · Volatile composition · Gas chromatography-mass spectrometry · Headspace solid-phase microextraction · Microwave-assisted extraction

Introduction

The glabrous Bai zheer (*Gymnotheca involucrata* Pei., is a very rare type of Chinese Miao medicinal herb (CMMH) [1]. Flowering from May to July, it has white flowers in spikes, growing in size to about 30–70 cm long. It is a member of the Saururaceae family, which is comprised of four genera and six species native to Eastern Asia and North America [2–4], including *Saururus chinensis* (*Loureiro*) *Baillon.*, *Houttuynia cordata* Thunberg., *Gymnotheca involucrata* Pei. and *Gymnotheca chinensis* Decaisne. *Gymnotheca* is endemic to China, *Gymnotheca involucrata* Pei., one type of *Gymnotheca*, is widely distributed throughout southwest and south China, especially in the Guizhou and Sichuan provinces. Because of their high levels of pharmacological activity, low toxicity, and rare complications, CMMH have been used clinically for several 1,000 years for the therapeutic treatment of many diseases. *Gymnotheca involucrata* Pei., a typical Chinese Miao folk medicinal herb, is used extensively to treat urinary tract infections, nephritis, edema, jaundice, leucorrhea, external boil and carbuncle swelling and skin eczema.

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It is also used to eliminate beriberi in Miao populations in rural zones of Guizhou, China [1].

In recent years, studies have focused largely on Saururaceae Linn and *Houttuynia* Thunb. have reported that a variety of active compounds have been separated from Saururaceae *Houttuynia* and Saururaceae [5–10], including lignans, alkaloids and flavonoid essential oils [5, 11–19]. The main chemical component of the essential oil of Saururaceae is sesquiterpene, while the main constituent of the essential oil of *Houttuynia* is monoterpene [5, 11, 16]. However, the essential oil components of *Gymnotheca involucrata* Pei. had not received much attention until this study. Therefore, microwave-assisted extraction (MAE) [17–23] followed by headspace solid-phase microextraction (HS-SPME) [24–29] and GC–MS was developed for the rapid determination of volatile chemical compositions in the dry stems and leaves of *Gymnotheca involucrata* Pei. The experimental parameters were determined by orthogonal array design [30], and the precision of the method was also investigated.

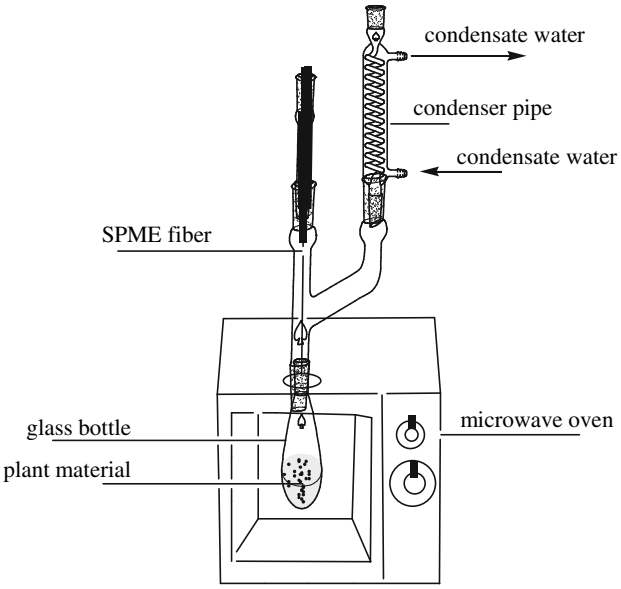

Fig. 1 The MA-HS-SPME apparatus

Experimental

Vegetative Material, SPME Fibers and Microwave Oven Sources

Dry stems and leaves of *Gymnotheca involucrata* Pei. were collected from Duyun in Guizhou province, China. They were identified by Associate Professor Zhiyou Guo (Department of Life Sciences, Qiannan Normal University for Nationalities, city of Duyun in Guizhou, China). After being ground to a fine powder, the *G. involucrata* Pei. samples were extracted using MAE–HS-SPME. The 65 μm poly-dimethylsiloxane/divinylbenzene (PDMS/DVB) fiber coating materials were purchased from Supelco (Bellefonte, USA). The microwave oven, with a maximum power of 700 W, was purchased from Qingdao Haier Microwave Products Co., Ltd. (Qingdao, China).

MAE–HS-SPME Procedure

The MAE–HS-SPME apparatus used in this study was custom built and is illustrated in Fig. 1. Two-gram samples of the plant material were ground to a fine powder and introduced into a 25-mL glass bottle. In order to absorb enough microwave energy, 2.00 g of water was used to moisten the plant material samples. The bottles containing the plant material samples were microwaved at 200, 400 and 700 W for 1–5 min, respectively, and a reflux condenser with a continuous flow of chilled water was used to continually condense the vapors. Throughout the heating process, the volatile compounds were extracted from *Gymnotheca involucrata* Pei. by the fiber coating. All the volatile compounds absorbed on the SPME fiber were desorbed at the GC injector (250 °C for 3 min), and then analyzed by GC–MS.

Gas Chromatography

GC analysis was performed on an Agilent (GC 6890 N) gas chromatograph fitted with a FID and an HP-5 MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm; stationary phase: diphenyl–95% dimethyl siloxane copolymer; Agilent). Nitrogen was used as the carrier gas at a flow rate of 1.00 mL min^{-1}. The temperature program was as follows: 50 °C for 1 min; then increased to 120 °C at a rate of 3 °C min^{-1}, then to 210 °C at 4 °C min^{-1}, at which temperature the column was maintained for 10 min.; injection port temperature was maintained at 260 °C; detector temperature at 250 °C; split-less mode. The percentage composition of the constituents of the oil was determined by area normalization (Table 4). The major constituents, viz. sabinene, nerolidol and phytol were quantified and the data provided in Table 3.

Gas Chromatography–Mass Spectrometry

The oil was analyzed using a Agilent GC-6890 N gas chromatograph equipped with 5973 mass spectrometer and HP-5 MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm; stationary phase: diphenyl–95% dimethyl siloxane copolymer; Agilent). Helium was used as the carrier gas at a flow rate of 1.00 mL min^{-1}. The injection port was maintained at 250 °C; the detector temperature and ion source temperature was 230 °C; quadrupole temperature was 150 °C; oven temperature was programmed as described above. Split-less

mode, and ionization energy 70 eV with 2.0 scans/s and mass range *m/z* 33–450. The Kovats indices (KI) [18–21, 23–28] were calculated relative to C_8–C_{25} *n*-alkanes. The constituents of the oil were identified by comparison of Kovats indices with those reported in the literature [22–29], by matching the mass spectral data with those stored in NIST147 and Wiley275 libraries, wherever possible, by co-injection with authentic standards.

The Precision of MAE–HS-SPME

The precision of the MAE–HS-SPME was studied by six replicate analyses of the volatile compounds in *Gymnotheca involucrata* Pei. under the optimum conditions. The precision was expressed by relative standard deviation (RSD, %). The peak areas of the volatile compounds in the plant material obtained by replicate analyses were used for the calculation of their RSD values.

Results and Discussion

Optimization of the MAE–HS-SPME Parameters

The optimization of the experimental conditions represents a critical step in the development of an MAE–HS-SPME method because various parameters will potentially affect the extraction process. In fact, the sample weight, the microwave power and irradiation time are generally considered to be the most important factors. The optimization of the method can be carried out step-by-step or by using an experimental design. Table 1 shows the results of MAE–HS-SPME extractions of *G. involucrata* Pei. carried out under different conditions according to the Taguchi experimental design (All experimental designs and statistical data were analyzed using software DPS v7.05 and SPSS15.0) [30]. The selected factors were examined using a three-level orthogonal array design with an L_9 (3^4) matrix as shown in Table 1. The peak area sum of *G. involucrata* Pei. obtained under orthogonal conditions are also shown in Table 1. The sums of the peak areas were 2.70×10^8–9.67×10^8.

Table 1 The results of orthogonal tests L_9 (3^4)

No.	Micro-wave power (W)	Irradiation time (min)	Samples weight (g)	Sum of peak area $\times 10^8$
1	200	1.00	1.00	2.70
2	200	3.00	1.50	4.80
3	200	5.00	2.00	5.53
4	400	1.00	1.50	6.95
5	400	3.00	2.00	9.67
6	400	5.00	1.00	6.12
7	700	1.00	2.00	5.46
8	700	3.00	1.00	3.81
9	700	5.00	1.50	3.24
k_1	4.34	5.04	4.21	
k_2	7.58	6.09	5.00	
k_3	4.17	4.96	6.89	
R	3.41	1.13	2.68	

Table 2 The results of variance analysis

Error sources	SS	*f*	*S*	*F*	*P*
Micro-wave power	22.13	2	11.07	187.33	<0.01
Irradiation time	2.40	2	1.20	20.30	<0.05
Samples weight	11.36	2	5.68	96.11	<0.05
Error	0.06				

$F_{0.01}(2,2) = 99.00$, $F_{0.05}(2,2) = 19.00$

The value of peak area sums obtained at different sample weights, microwave powers and irradiation times are shown in Table 1, and the optimal conditions for extraction efficiency as determined by the sum of peak areas were determined to be: microwave power of 400 W, irradiation time of 3 min, and a sample weight of 2.0 g. The results of variance analysis showed that there are three significant factors and Table 2 indicates that the three factors having the most significant impact on volatile compounds extracted in *Gymnotheca involucrata* Pei., are microwave power, followed by sample weight and irradiation time. Therefore, we should pay special attention to the control of microwave power in the experiment.

Precision of MAE–HS-SPME

To obtain the precision of the method, six replicate analyses of the volatile compounds in *Gymnotheca involucrata* Pei. were performed by MAE–HS-SPME at the optimum conditions. The RSD values were calculated by the peak areas obtained by replicate analyses. As shown in Table 4, the calculated RSD values <9% showed that MAE–HS-SPME followed by GC–MS had an acceptable level of precision.

Quantitation of Sabinene, Nerolidol and Phytol

Known concentrations (20–60 μg/ml) of sabinene, nerolidol and phytol in hexane were used for preparing calibration curves. The calibration curves were linear over the concentrations tested, for the regression equations $Y = 2.1365X - 0.614$, $Y = 1.5287X - 0.723$ and $Y = 1.451X - 0.146$ for sabinene, nerolidol and phytol, respectively. The contents of the sabinene, nerolidol and phytol in the oils were determined by external standard in the sample with those found for the standards in Table 3.

Table 3 Contents of the three compounds in *Gymnotheca involucrata* Pei.

Compound	Content (μg/ml)	
	Stem	Leaf
Sabinene	1.85	1.99
Nerolidol	11.31	11.92
Phytol	2.11	2.17

Analysis of the Volatile Compounds in *Gymnotheca involucrata* Pei.

Under the optimal MAE–HS-SPME conditions, the volatile compounds in *Gymnotheca involucrata* Pei. were extracted and concentrated by MAE–HS-SPME, then were analyzed by GC–MS (see Figs. 2, 3). Results of the GC/MS analysis of the oils are shown in Table 4, where the constituents are listed in the order of their elution from the HP-5MS column. 106 volatile constituents and 103 volatile constituents in the dry stems and leaves, respectively of *Gymnotheca involucrata* Pei. were identified by mass spectra library and Kovats indices, representing 93.58 and 98.53% of the total chromatographic area, respectively. They were all identified for the first time; the stem oil was characterized by a large amount of nerolidol (16.57%), sabinene (3.35%), *β*-bourbonene (2.36%), *cis, cis, cis*-1,1,4,8-tetramethyl-4,7,10-cycloundecatriene (4.64%), (-)-humulene epoxide II (3.43%), α-copaene-8-ol (6.01%), 6,10,14-trimethyl-2-pentadecanone (3.12%) and phytol (2.92%). The major constituents of the leaf oil were also nerolidol (17.50%), sabinene (3.65%), *cis, cis, cis*-1,1,4,8-tetramethyl-4,7,10-cycloundecatriene (5.03%), (-)-humulene epoxide II (3.57%), α-copaene-8-ol (6.33%), 6,10,14-trimethyl-2-pentadecanone (3.02%) and phytol (3.03%). In addition,

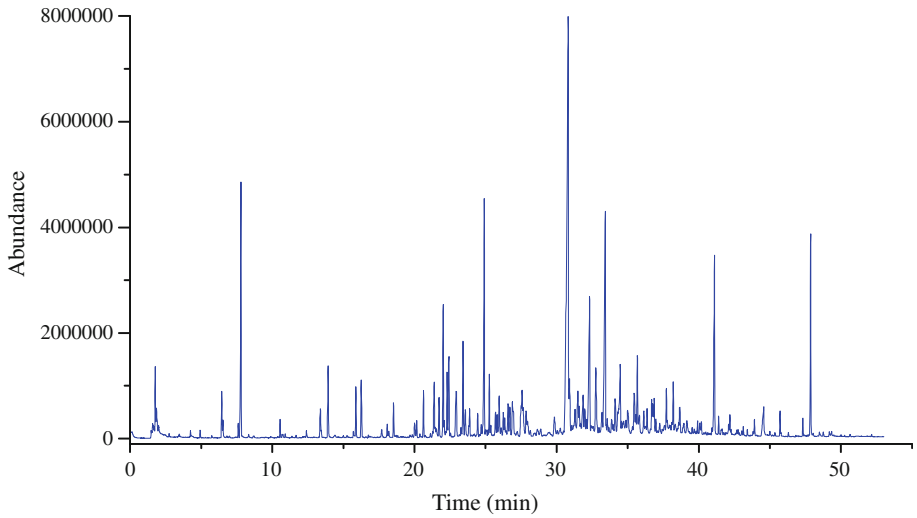

Fig. 2 The total ion current chromatogram of essential oils from stems of *Gymnotheca involucrata* Pei.

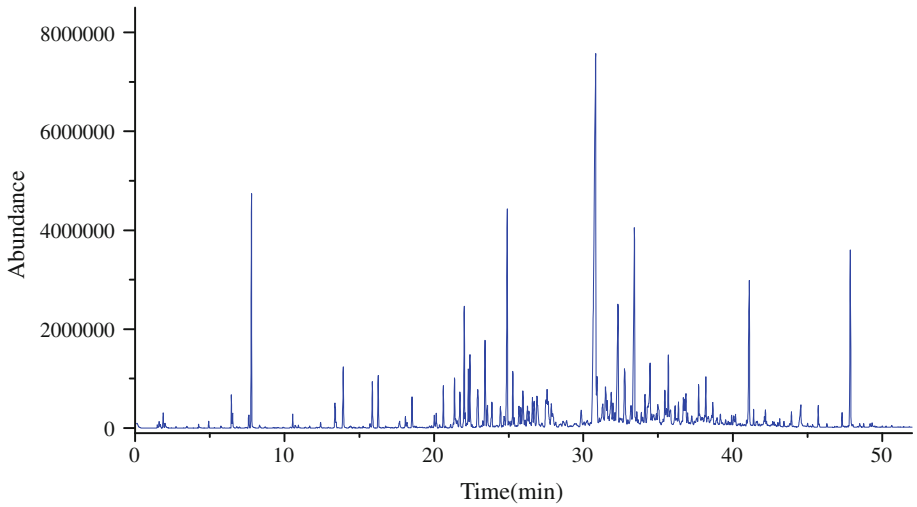

Fig. 3 The total ion current chromatogram of essential oils from leaves of *Gymnotheca involucrata* Pei.

Table 4 Composition of *G. involucrata* Pei. essential oils

RT (min)	Compound	*KI*	Relative content (%)		RSD (%)	Method of identification
			Stem	Leaf		
1.77	Acetic acid	605	0.77	–	6.8	KI, MS
1.87	2-Methyl-butanal,	613	0.65	0.26	3.3	KI, MS
2.00	2-Ethyl-furan	623	0.26	–	2.1	KI, MS
4.92	Cyclofenchene	927	0.10	0.10	3.9	KI, MS
6.44	6-Methyl-5-hepten-2-one	982	0.56	0.45	2.6	KI, MS
6.53	Myrcene	986	0.27	0.23	4.2	KI, MS
7.61	*p*-Cymene	1,018	0.19	0.20	7.7	KI, MS
7.79	Sabinene	1,023	3.35	3.65	6.2	KI, MS, CI
10.55	L-Linalool	1,096	0.24	0.20	2.6	KI, MS
12.40	1-Methyl-2-methylene-cyclopentane	1,141	0.11	0.12	3.5	KI, MS
13.38	Phellandral	1,164	0.09	–	4.5	KI, MS
13.44	Dehydrocineole	1,168	–	0.26	7.2	KI, MS
13.92	Cryptone	1,177	1.09	1.10	8.3	KI, MS
15.71	Nerol	1,219	0.13	–	2.9	KI, MS
15.89	Thymol methyl ether	1,223	0.76	0.81	5.3	KI, MS
16.27	Cuminic aldehyde	1,232	0.86	0.93	3.8	KI, MS
17.69	*p*-Menth-1-en-7-al	1,265	0.20	0.18	6.1	KI, MS
18.09	1-Phenyl-1-butanol	1,275	0.24	0.17	4.7	KI, MS
18.19	Anethole	1,277	0.11	–	5.4	KI, MS
18.53	4-(1-Methylethyl)-benzenemethanol	1,285	0.50	0.57	7.5	KI, MS
20.02	α-Terpinene	1,320	0.25	0.26	3.9	KI, MS
20.16	δ-Elemene	1,324	0.28	0.28	4.6	KI, MS
20.64	α-Cubebene	1,335	0.69	0.75	3.3	KI, MS
21.39	Cyclosativene	1,353	1.00	0.96	1.4	KI, MS
21.45	α-Ylangene	1,354	0.14	0.14	3.4	KI, MS
21.73	α-Copaene	1,361	0.67	0.70	6.7	KI, MS
22.02	β-Bourbonene	1,369	2.36	2.55	3.2	KI, MS
22.30	β-Cubebene	1,375	1.02	1.09	4.8	KI, MS
22.42	β-Elemene	1,377	1.22	1.31	8.7	KI, MS
22.95	Methyl-cyclohexane	1,390	1.08	1.03	6.5	KI, MS
23.26	Tetradecane	1,400	0.17	0.13	5.3	KI, MS, CI
23.43	*trans*-Caryophyllene	1,402	1.60	1.71	4.5	KI, MS
23.89	β-Cubebene	1,412	0.59	0.63	5.4	KI, MS
24.45	Aromadendrene VI	1,426	0.39	0.46	7.4	KI, MS
24.71	α-Elemene	1,431	0.25	0.26	6.5	KI, MS
24.91	*cis, cis, cis*-1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene	1,436	4.64	5.03	5.8	KI, MS
25.01	Alloaromadendrene	1,438	0.11	–	6.6	KI, MS
25.17	Bicyclosesquiphellandrene	1,442	0.11	–	4.5	KI, MS
25.28	*trans*-β-Farnesene	1,445	0.96	1.01	7.1	KI, MS
25.37	Germacrene-d	1,447	0.19	0.19	8.2	KI, MS
25.70	(+)-δ-Selinene	1,455	0.52	0.52	4.5	KI, MS
25.81	α-Amorphene	1,457	0.39	0.41	6.3	KI, MS
25.96	Germacrene-d	1,461	0.87	0.92	5.2	KI, MS
26.11	β-Ionone	1,464	0.18	0.23	8.7	KI, MS
26.25	β-Selinene	1,467	0.45	0.47	3.8	KI, MS
26.36	2-Isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	1,470	0.39	0.40	2.9	KI, MS

Table 4 continued

RT (min)	Compound	*KI*	Relative content (%)		RSD (%)	Method of identification
			Stem	Leaf		
26.59	α-Selinene	1,475	0.68	0.72	5.7	KI, MS
26.71	*β*-Cubebene	1,478	0.58	0.61	7.2	KI, MS
26.9	α-Muurolene	1,483	1.17	1.21	8.1	KI, MS
27.51	α-Amorphene	1,497	0.57	0.61	6.1	KI, MS
27.56	Pentadecane	1,500	0.99	0.93	3.8	KI, MS, CI
27.68	*δ*-Cadinene	1,502	1.31	1.29	6.6	KI, MS
27.96	1s, *cis*-Calamenene	1,506	0.45	0.37	2.2	KI, MS
28.64	Solanesol	1,519	0.29	0.28	4.5	KI, MS
28.88	Calacorene	1,524	0.23	0.23	6.3	KI, MS
29.85	[3S-(3.α.,3a.α.,6.α.,8a. α.)]-4,5,6,7,8,8a-HexaHydro-3,7,7-trimethyl-8-methylene-3*H*-3a,6-methanoazulene	1,543	–	0.57	2.2	KI, MS
30.26	(7*S*,10*S*)-2,6,10-Trimethyl-7,10-epoxy-2,11-dodecadien-6-ol	1,551	0.29	0.27	6.7	KI, MS
30.82	Nerolidol	1,562	16.57	17.50	7.8	KI, MS, CI
30.92	(-)-Caryophyllene oxide	1,564	1.19	1.23	6.9	KI, MS
31.29	Hydroxy-6-cytosine	1,571	–	1.01	5.7	KI, MS
31.49	Salvial-4 (14)-en-1-one	1,575	1.04	1.07	5.2	KI, MS
31.58	6,6-Dimethyl-3-methylene-bicyclo[3.1.1]heptane,	1,577	–	0.66	4.3	KI, MS
31.87	4,8,8-Trimethylspiro[2.6]non-4,6-diene	1,582	1.23	1.23	8.5	KI, MS
31.99	Guaiol	1,584	0.58	0.60	8.8	KI, MS
32.11	α-Cedrol	1,587	0.38	0.39	6.6	KI, MS
32.32	(-)-Humulene epoxide II	1,591	3.43	3.57	8.2	KI, MS
32.77	Hexadecane	1,600	1.75	1.77	7.5	KI, MS, CI
33.19	*cis*-Asarone	1,610	0.71	0.74	7.3	KI, MS
33.42	α-Copaene-8-ol	1,616	6.01	6.33	8.2	KI, MS
33.57	7-Methyl-3,4,5,6,7,8-hexahydronaphthalen-1(2H)-one,	1,620	0.29	0.30	6.7	KI, MS
33.88	2-Isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-en	1,628	0.31	0.32	4.8	KI, MS
33.98	3-Oxo-α-ionol	1,630	–	0.24	5.9	KI, MS
34.13	1,4,6,7,8,9-Hexahydro-2-methoxy-3-methyl-6,6-diisopropyl-naphthalene	1,634	–	0.79	7.7	KI, MS
34.3	Alloaromadendrene	1,638	0.86	0.44	6.3	KI, MS
34.47	(1S-*cis*)-1,2,3,4-Tetrahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	1,643	1.63	2.18	7.4	KI, MS
34.58	1-Butyl-bicyclo[3.2.0]hept-2-ene-6-one	1,645	0.24	0.25	4.1	KI, MS
34.99	(+)-Spathulenol	1,656	0.87	0.90	7.8	KI, MS
35.33	2-Methyl-hexadecane	1,665	0.21	0.21	3.6	KI, MS
35.47	Dehydroaromadendrene	1,668	0.86	0.89	7.2	KI, MS
35.69	2-Hexyl-1-decen-3-yne	1,674	1.41	1.51	7.5	KI, MS
35.83	1-Pentadecene	1,677	0.589	0.58	6.3	KI, MS
36.13	4-(3-Hydroxybutyl)-3,5,5-trimethyl-2-cyclohexen-1-one	1,685	0.48	0.49	3.2	KI, MS
36.36	(3*E*,5*E*,8Z)-3,7,11-Trimethyl-1,3,5,8,10-dodecapentanene	1,691	0.50	0.53	6.7	KI, MS
36.69	Heptadecane	1,700	0.57	0.54	5.8	KI, MS, CI
36.77	2,6,10,14-Tetramethyl-pentadecane	1,701	0.54	0.56	6.1	KI, MS

Table 4 continued

RT (min)	Compound	*KI*	Relative content (%)		RSD (%)	Method of identification
			Stem	Leaf		
36.86	2-(1-Cyclohexen-1-yl)-3-hydroxy-5,5-dimethyl-2-cyclohexen-1-one	1,704	0.68	0.71	7.3	KI, MS
36.98	Citronellol acetate	1,708	0.29	0.29	6.4	KI, MS
37.26	Dibenzothiophene	1,717	0.26	0.26	8.2	KI, MS
37.59	Oplopenone	1,727	0.22	0.22	2.7	KI, MS
37.72	[*R*-(*R**,*R**)]-6-(1,5-Dimethyl-4-hexenyl)-3-methyl-2-cyclohexen-1-one	1,731	0.75	0.80	4.8	KI, MS
38.11	2-Ethyl-1-methyl-1-butenyl-cyclohexane	1,743	0.17	0.18	6.9	KI, MS
38.20	Anthracene	1,746	0.89	0.99	8.5	KI, MS
38.35	Vulgarol B	1,750	0.32	0.34	7.2	KI, MS
38.59	(-)-Spathulenol	1,758	0.18	0.18	4.5	KI, MS
38.66	Calamenene	1,760	0.47	0.48	8.4	KI, MS
38.95	Tetradecanoic acid	1,769	0.26	0.23	3.7	KI, MS
39.17	4-Hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one	1,776	0.24	0.24	5.5	KI, MS
39.53	*β*-Sesquicyclocitral	1,787	0.11	–	2.7	KI, MS
39.93	Octadecane	1,800	0.19	0.18	6.7	KI, MS, CI
40.06	2,6,10,14-Tetramethyl-hexadecane	1,803	0.18	0.18	8.5	KI, MS
40.19	7-Butyryl-4,6-dihydroxy-3-methylbenzo[b]furan	1,808	0.19	0.20	5.1	KI, MS
40.93	Neophytadiene	1,834	0.10	0.10	6.3	KI, MS
41.10	6,10,14-Trimethyl-2-pentadecanone	1,840	3.12	3.02	7.6	KI, MS
41.39	Isobutyl phthalate	1,850	0.24	0.25	2.5	KI, MS
42.11	2-Methyl-2-butenoic acid	1,875	0.11	0.12	6.4	KI, MS
42.19	1-Hexadecene	1,877	0.21	0.20	5.9	KI, MS
43.14	1-Propyl-1*H*-1,2,4-triazol-5-amine	1,912	–	0.13	4.2	KI, MS
43.42	Hexadecanoic acid, methyl ester	1,922	0.08	0.08	8.6	KI, MS
43.92	1,2-Benzenedicarboxylic acid, dibutyl ester	1,942	0.26	0.27	7.9	KI, MS
44.56	Hexadecanoic acid	1,966	0.91	0.79	8.8	KI, MS, CI
45.72	(*E*,*E*,*E*)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	2,012	0.26	0.26	6.3	KI, MS
47.32	(*E*)-5-Octadecene	2,079	0.26	0.24	4.4	KI, MS
47.87	Phytol	2,102	2.92	3.03	5.7	KI, MS, CI
	Alkanes, cycloalkanes and alkenes, cycloolefines (ACAC)		15.96	16.98	7.5	
	Fatty acids and esters (FAE)		2.91	2.02	8.2	
	Alcohols and carbonyl compounds (ACC)		13.24	12.73	5.7	
	Terpenes compounds (TC)		28.26	28.87	7.3	
	Terpenes oxygen derivatives compounds (TODC)		32.91	34.79	6.1	
	Other compounds (OC)		0.30	3.13	8.7	
	Total identified (%)		93.58	98.53	7.6	

RT retention time, *KI* Kovats indices obtained using series of *n*-alkanes on HP-5MS, *MS* mass spectrum, *CI* co-injection with authentic standards

the contents of some constituents were almost identical; *β*-elemene (1.22, 1.31%), *trans*-caryophyllene (1.60, 1.71%), *δ*-cadinene (1.31, 1.29%) and (-)-caryophyllene oxide (1.19, 1.23%) were detected in both the stem and leaf oils of *G. involucrata* Pei., respectively.

A comparison of the chemical constituents in this study revealed that the composition of the stem oil of *Gymnotheca involucrata* Pei. was not distinctly different from that of leaf oil. The stem and leaf oil were dominated by terpenes and terpene oxygen derivatives, accounting for

61.17 and 63.66%, respectively, followed by alkanes/cycloalkanes and alkenes/cycloolefins, up to 15.96 and 16.98%.

Nerolidol (3,7,11-trimethyl-1,6,10-dodecantriene-3-alcohol), a sesquiterpene present in the essential oils of several plants [31]., is approved by the US Food and Drug Administration as a food flavoring agent. Nerolidol exhibits antineoplastic activity [32], and has been tested as a skin penetration enhancer for the transdermal delivery of therapeutic drugs [33, 34]. Lopes et al. [35] had reported the activity of nerolidol against the malaria parasite. Therefore, it is believed that *G. involucrata* Pei. might potentially be used as a medicinal raw material.

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