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COMPARISON ANALYSIS OF VOLATILES FROM THE LEAVES AND FLOWERS OF FOUR SAURURACEAE SPECIES

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Abstract

Saururaceae (lizard's tail family) comprises three genera and four species [*Saururus chinensis* (*Loureiro*) Baillon. (SC), *Gymnotheca chinensis* Decne. (GC), *Gymnotheca involucrata* Pei. (GI) and *Houttuynia cordata* Thunberg. (HC)] in eastern Asia, and they extend from the most primitive to the most evolutionary levels. The purpose of this study is to examine whether and to what extent the diversity of volatiles can support the accepted evolutionary scheme in Saururaceae for the four species. Volatiles from fresh leaves and flowers of Saururaceae species from different regions were analyzed comparatively by gas chromatography-mass spectrometry (GC-MS).The samples studied showed differences in the volatile profiles of leaves and flowers among the species. In the leaves and flowers, concentrations of all monoterpenes and oxides, all alcohols, all acids and all esters were highest in SC, lowest in HC, and the concentrations of these components for GC and GI were between those of SC and HC. Concentrations of all sesquiterpenes and oxides, all straight chain aliphatic hydrocarbons, all branched aliphatic hydrocarbons, all aldehydes, and all ketones were lowest in SC, highest in HC and the concentrations of these components for GC and GI were between those of SC and HC. The results in this study could support the accepted taxonomical scheme of four species in Saururaceae.

Key words: Saururaceae, *Gymnotheca chinensis, Gymnotheca involucrate, Saururus chinensis, Houttuynia cordata*.

Introduction

Some plants have been found to produce and emit volatile compounds that can be either defensive compounds emitted by plants in response to attack or injury or, alternatively, compounds that attract pollinators (Dötterl *et al*., 2006; Langenheim, 1994; Parker *et al*., 2012; Svensson *et al*., 2010). In most cases, flowers produce a wide variety of compounds that attract pollinators (Dobson, 1994; Knudsen *et al*., 1993), whereas leaves produce mainly lower molecular weight compounds that can attract the natural enemies of herbivores (Sørensen *et al*., 2003; Turlings *et al*., 1995).

Saururaceae species are distributed in eastern and southern Asia, and North America. The family comprises only 6 species. Two of them are distributed in North America. In eastern Asia, four species occur and are classified into three genera, determined by their morphological and physiological characteristics; while in China, *Houttuynia cordata* Thunb. (HC) is mainly distributed in the middle, southeastern and southwestern provinces and regions. In addition, *Saururus chinensis* (Loureiro) Baillon. (SC), *Gymnotheca chinensis* Decne. (GC), and *Gymnotheca involucrata* Pei. (GI) are mainly distributed in the southwest, including Guizhou, Sichuan, and Guangxi (Liang, 1995) provinces.

The purpose of this study is to examine whether and to what extent the diversity of volatiles can support the accepted evolutionary scheme in Saururaceae for the species SC, GC, GI, and HC. The profile of volatile compounds contained in the species' leaves and flowers will be used to investigate similarities among the species. The four species represent different evolutionary levels and have been placed in different genera. The most primitive species, SC, belongs to the genus *Saururus* L. (SL); GC and GI are more evolutionarily advanced than the SC and belongs to *Gymnotheca* Decne. (GD); HC is the most evolutionarily advanced among the four species and belongs to *Houttuynia* Thunb. (HT). According to morphological characteristics of insect and plant co-adaptation, the following features (leaves, color of stem top, and involucre color of basal inflorescence) (Tanaka, 1979) have the greatest importance for differentiation of the species into the genera: SC, "white" leaves of inflorescence in the stem top; GI, and HC, "white involucre" (Thien *et al*., 1994). All these features improve pollinators' co-adaptation. Another feature that determines the taxonomic allocation of the species into genus and family is growth of flower organs from Saururaceae species. Carpel and stamen number, shape thereof, and degree of fusion with other carpels and stamens have evolved in many different ways such that six stamens and four carpels of SL still separate for growth periods; the six stamens of GD, which keep some degree of fusion with other carpels in the initial stages, become a single ovary and separate with the style, while some of the stamens fuse with the ovary wall; and the stamens and carpels of HT were reduced to three stamens and three carpels, respectively (Liang, 1994).

In this paper, we compare volatile compounds from fresh leaves and flowers of SC, GC, GI, and HC from different China regions by gas chromatography-mass spectrometric (GC-MS) analysis of both solid phase microextraction (SPME) and adsorbent samples collected from headspace (HS). Indicated evolutionary and systematic characteristics of the volatile compounds were evaluated.

Material and Methods

Plant material: Samples of SC, GC, GI and HC, which were authenticated by Professor Chen Deyuan of Guiyang Chinese Medical College, were cultivated in conditions similar to the ones experienced by wild specimens at Liping, Luodian and Guiyang of Guizhou province in China in March 2009, respectively. Leaves and flowers of the species (Voucher specimens see Table 1) sealed, which were collected during May and June 2010, was kept on ice bags until return laboratory where the samples were rapidly required for analysis in order to avoid moisture and chemical changes.

Collections of volatiles by HS-SPME: The manual SPME holder was used with a 100 μm polydimethylsiloxane fibre assembly (Supelco, Bellefonte, USA). Before use, the fibre was conditioned as recommended by the manufacturer. The extraction experiments were carried out according to the literature method (Liang *et al*., 2005). Samples (0.5 g) were hermetically sealed in a 4 mL vial, the SPME fibre was then suspended in the HS and equilibrated for 60 min in a thermostatic bath, which was set at 50°C.

GC-MS conditions: The volatiles were analyzed on a Shimadzu GCMS-QP2010 gas chromatograph-mass spectrometer fitted with a DB-5ms capillary column (30 m, 0.25 mm *i.d*. and 0.25 μm). The injector temperature was 250°C. The column temperature program was 50°C for 3 min and was then increased at 8°C/min up to 240°C, where it was maintained for 15 min. The carrier gas was helium (0.78 mL/min) and the split ratio was 20:1. MS analyses were used with an ionization energy of 70 eV, a scan time of 0.5 s, and a mass range of 33-450 amu.

Identification of volatile compounds: Identification of volatiles was carried out by comparing the sample volatiles' peak relative retention times with those obtained for Alltech standards (Chyau *et al*., 1992), by comparing with two libraries (NIST 127 & NIST 147), and by comparing with references reported (Ahmad *et al*., 2006; Khan *et al*., 2013). This method is suitable for comparing the chemical composition of different organisms (Georgieva *et al*., 2005).

The precision of HS-SPME: The precision of HS-SPME was confirmed using six different working solutions prepared from leaves of sample S201106 under the optimum conditions. The precision was expressed as the relative standard deviation (R.S.D.s) of the peak areas.

Calculations and statistical analyses: Each sample was analyzed three times. The data obtained were entered into the Excel (2003) program (Microsoft Office) and reported as mean (n = 3). Each reported result is the average of three GC-MS analyses (different working solutions prepared independently from the same sample). The results were submitted to analysis of variance followed by R.S.D.s. Similarities among the species of Saururaceae were assessed by a tree clustering method based on percent disagreement (treating the presence or absence of each compound as a binary character); and single linkage. The cluster analysis was conducted using SPSS 13.0 (SPSS Inc., USA).

Table 1. Collection information of SC, GC, GI and HC.

No.	Plant material	Voucher specimen	Sources	Special remarks	Altitude	Acquisition time
1.	*S. chinensis*	S201101	Liping, Guizgou (N, 26°13'42.03"; E, 109°08'09.58")	In small frutex on the northern slope	617m	June 6, 2010
2.	*G. chinensis*	S201102	Liping, Guizgou (N, 26°13'42.03"; E, 109°08'09.58")	In small frutex on the northern slope	617m	June 6, 2010
3.	*G. involucrata*	S201103	Liping, Guizgou (N, 26°13'42.03"; E, 109°08'09.58")	In small frutex on the northern slope	617m	June 6, 2010
4.	*H. cordata*	S201104	Liping, Guizgou (N, 26°13'42.03"; E, 109°08'09.58")	In small frutex on the northern slope	617 m	June 6, 2010
5.	*S. chinensis*	S201105	Luodian, Guizgou (N, 25°26'20.75"; E, 106°46'08.38")	In grassland on the southern slope	385 m	May 26, 2010
6.	*G. chinensis*	S201106	Luodian, Guizgou (N, 25°26'20.75"; E, 106°46'08.38")	In grassland on the southern slope	385 m	May 26, 2010
7.	*G. involucrata*	S201107	Luodian, Guizgou (N, 25°26'20.75"; E, 106°46'08.38")	In grassland on the southern slope	385 m	May 26, 2010
8.	*H. cordata*	S201108	Luodian, Guizgou (N, 25°26'20.75"; E, 106°46'08.38")	In grassland on the southern slope	385 m	May 26, 2010
9.	*S. chinensis*	S201109	Guiyang, Guizgou (N, 26°35'30.40"; E, 106°43'10.55")	On the plain, drought stress	1099 m	June 17, 2010
10.	*G. chinensis*	S201110	Guiyang, Guizgou (N, 26°35'30.40"; E, 106°43'10.55")	On the plain, drought stress	1099 m	June 17, 2010
11.	*G. involucrata*	S201111	Guiyang, Guizgou (N, 26°35'30.40"; E, 106°43'10.55")	On the plain, drought stress	1099m	June 17, 2010
12.	*H. cordata*	S201112	Guiyang, Guizgou (N, 26°35'30.40"; E, 106°43'10.55")	On the plain, drought stress	1099 m	June17, 2010

Results

Fig. 1E showed additional chromatographic profiles of the volatiles from leaves of sample S201106 (six replicate analyses). The results of the precision were expressed as relative standard deviations (R.S.D.s) and are shown in Table 2. R.S.D.s values less than 5% suggest that the method had the good precision.

The GC-MS data showed differences in the profiles of compounds between leaves (Table 2, & Fig. 1A-D) and flowers (Table 3) and also among the four species from the three regions. Seventy-one compounds were identified. Table 2 shows the volatiles of leaves are monoterpenes and oxides (43.12-54.31%, SC, 23.23-27.23%, GC, 22.42-27.62%, GI, 2.38-3.88%, HC), sesquiterpenes and oxides (0.32-0.76%, SC, 2.88-4.66%, GC, 3.02-4.11%, GI, 8.63-14.66%, HC), straight chain aliphatic hydrocarbons (trace, SC, 3.37-4.38%, GC, 6.81-8.20%, GI, 10.02-14.29%, HC), branched aliphatic hydrocarbons (trace, SC, 1.54-2.20%, GC, 1.55-2.73%, GI, 4.47-6.26%, HC), alcohols (9.32-16.17%, SC, 7.71-10.08%, GC, 7.05-8.93%, GI, 1.65-2.89%, HC), aldehydes (0.25-0.45%, SC, 2.17-2.97%, GC, 2.67-3.87%, GI, 4.98-7.22%, HC), ketones (trace-0.51%, SC, 17.83-23.39%, GC, 21.87-25.17%, GI, 44.97-56.26%, HC), acids (9.38-13.31%, SC, 4.18-7.02%, GC, 4.13-5.98%, GI, 0.56-1.68%, HC) and esters (18.99-23.55%, SC, 12.46-17.74%, GC, 10.28-12.61%, GI, 0.53-1.90%, HC). Table 3 shows the volatiles of flowers are monoterpenes and oxides (44.66-59.68%, SC, 24.10-34.10%, GC, 15.68-22.48%, GI, 5.60-8.48%, HC), sesquiterpenes and oxides (1.06-1.64%, SC, 4.36-5.75%, GC, 5.02-7.54%, GI, 8.58-12.63%, HC), straight chain aliphatic hydrocarbons (trace, SC, 4.65-5.54%, GC, 5.24-7.78%, GI, 8.12-11.82%, HC), branched aliphatic hydrocarbons (trace, SC, 1.02-1.34%, GC, 1.07-2.35%, GI, 2.63-3.38%, HC), alcohols (7.17-9.39%, SC, 4.58-6.33%, GC, 3.23-5.45%, GI, 1.95-2.58%, HC), aldehydes (0.13-0.46%, SC, 2.13-2.96%, GC, 2.09-3.01%, GI, 3.85-5.43%, HC), ketones (1.20-1.56%, SC, 17.94-28.01%, GC, 24.23-35.45%, GI, 42.07-55.35%, HC), acids (7.34-11.24%, SC, 2.46-3.62%, GC, 2.01-4.00%, GI, 0.29-0.55%, HC) and esters (13.04-16.72%, SC, 9.98-14.03%, GC, 10.47-12.14%, GI, 1.93-2.99%, HC).

Fig. 2A & F-H show all contents of monoterpenes and oxides, alcohols, esters and acids were highest in the SC, lowest in the HC species, and their content in the species GC and GI was between SC and HC, respectively. Fig. 2B-E & I show all contents of sesquiterpenes and oxides, straight chain aliphatic hydrocarbons, branched aliphatic hydrocarbons and ketones were lowest in the SC, highest in the HC species, and their content in the species GC and GI was between SC and HC, respectively. Fig. 3A & F-H show all contents of monoterpenes and oxides, alcohols, esters and acids were highest in the SC, lowest in the HC species, and their content in the species GC and GI was between SC and HC, respectively. Fig. 3B-E & I show all contents of sesquiterpenes and oxides, straight chain aliphatic hydrocarbons, branched aliphatic hydrocarbons and ketones were lowest in the SC, highest in the HC species, and their content in the species GC and GI was between SC and HC, respectively. Fig. 4A-C show a dendrogram was generated. The four tested species from every region were divided into two main clusters. SC was in cluster A and GC, GI, and HC were in cluster B, which was then divided into two subgroups. HC was in the subgroup C and GC and GI were in subgroup D.

Discussion

Terpenoids and oxides, which are important allelochemicals, were found in all studied flower samples and in the volatiles from leaves of Saururaceae for the four species. The terpenoids and oxides and their contents exhibit significant diversity in different regions and different species, but at the same time, the terpenoids and oxides and their contents of a given species from different regions exhibit significant differences. *a*-Pinene, *β*-pinene, 3-carene, and linalool are important attractant pheromones for many insects (Borg-Karlson *et al*., 2003; Wu, 2010) and were found in all studied leaf and flower samples. Their content was highest in the SC, lowest in the HC species, and their content in the species GC and GI was between SC and HC (Figs. 2A & 3A). On the contrary, the content of all sesquiterpenes and oxides was highest in the HC, lowest in the SC, higher in the GC and GI between SC and HC (Figs. 2B & 3B). The characters of the terpenoid and oxide volatiles in the leaves and flowers of Saururaceae may be used as taxonomic characteristics of these species.

The volatiles from leaves and flowers of GC, GI and HC contained straight chain aliphatic hydrocarbons (C15-C26) along with low concentrations of branched aliphatic hydrocarbons, which were both absent in leaves and flowers from SC (Tables 2 & 3). The concentrations of all main straight chain aliphatic hydrocarbons (e.g. tridecane, tetradecane, pentadecane, eicosane, tricosane and pentacosane) in the volatiles of leaves and flowers from HC appeared, the highest concentrations of straight chain aliphatic hydrocarbons, while in the GD species (GC and GI), this group of hydrocarbons appeared in lower concentrations; the SC species did not contain straight chain aliphatic hydrocarbons (Fig. 2C & 3C). Branched hydrocarbons dominated the volatiles from leaves and flowers of HC. No hydrocarbons were found in the volatiles from leaves and flowers of SC, lower concentrations of branched hydrocarbons were found in GD species (Figs. 2D & 3D). Higher concentrations and diversity of branched aliphatic hydrocarbons were present in HC leaves and flowers. Branched hydrocarbons are involved in insect-plant relationships serving as attractants and pheromones of some insects. It is established that pentacosane is one of the components of the contact pheromone blend of some beetles (Matthew *et al*., 2003). It is present in almost all studied samples, except SC leaves and flowers. Branched aliphatic hydrocarbons are found almost entirely in the leaves and flowers of HC and GD. The presence of such higher hydrocarbons in the leaves and flowers of HC, low concentrations thereof in GC and GI, but not in SC, might be an indication of the most primitive and the less advanced evolutionary level of the SC species and GD species, respectively. According to the composition of the hydrocarbons in leaves and flowers, it could be assumed that the evolutionary level of GI is higher than GC which is being confirmed by flower morphology. GC and GI were the two species in the genus, and GI has kept white involucres under flowers, but this does not occur in GC. This involucre structure allows insects to easily reach nectar and pollen, but it determines less co-adaptive specialization of the flower (Tanaka, 1979). Some structures that increase the survival capacity of the species have formed in the Saururaceae plants.

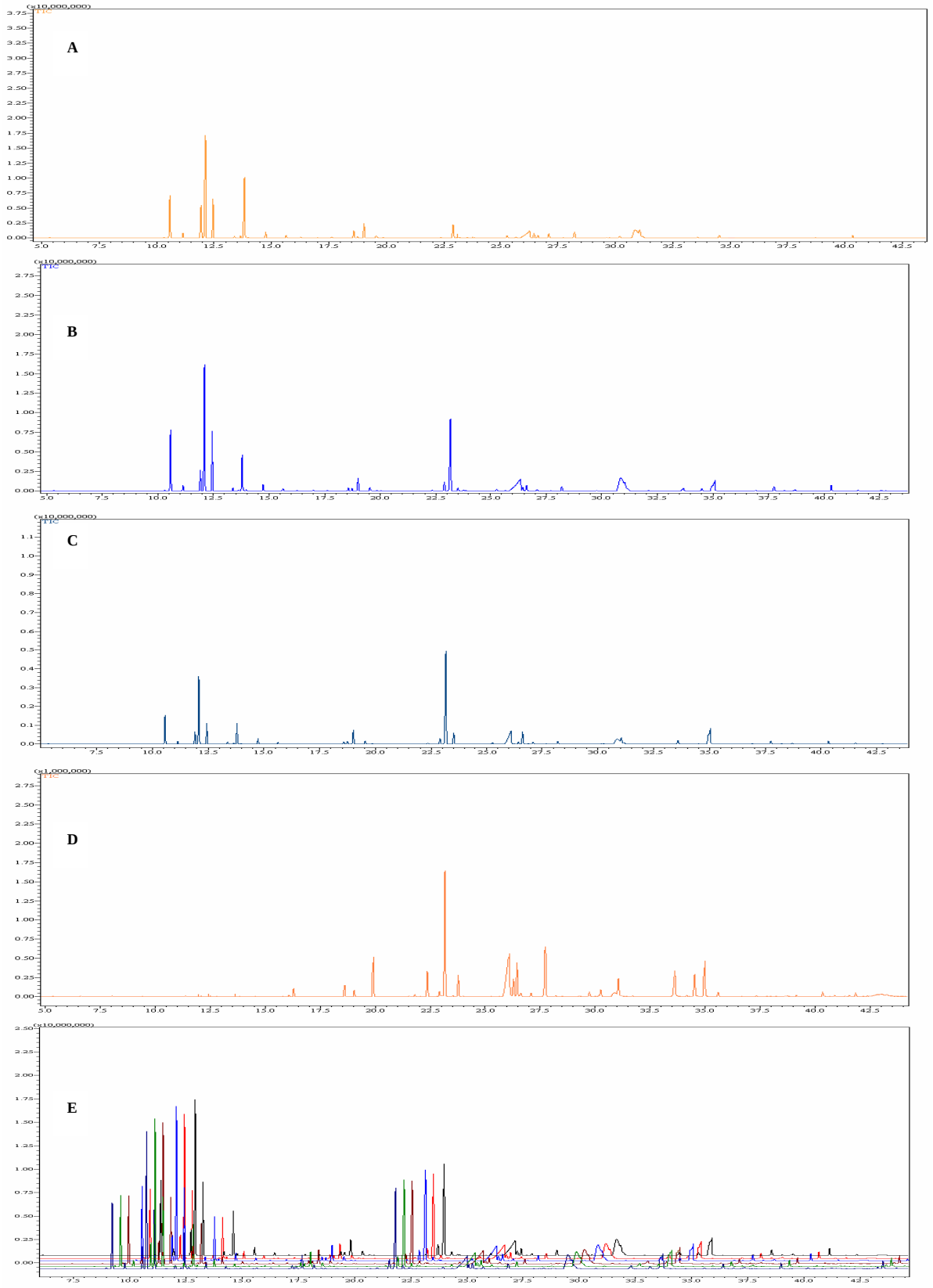

Fig. 1. The representative GC-MS total ion chromatogramic profiles of the volatile compositions in the leaves of SC (A), GC (B), GI (C) and HC (D) from Luodian (S201105, S201106, S201107 and S201108) by SPME; E, Additional chromatographic profiles of the volatile compositions of leaves sample from GC S201106 by SPME (six replicate analyses).

Table 2. Volatile compounds and their contents in leaves from the SC, GC, GI and HC, and R.S.D.s of precision analysis.

No.	Identified compounds [c, d, e]	Content of volatiles in the leaves from Saururaceae at different regions (%)												S201106 [a]R.S.D.s (%)
		SC			GC			GI			HC			
		S201101	S201105	S201109	S201102	S201106	S201110	S201103	S201107	S201111	S201104	S201108	S201112	
	Monoterpenes and oxides	**54.31**	**48.9**	**43.12**	**27.23**	**25.76**	**23.23**	**27.62**	**22.42**	**22.99**	**3.88**	**2.79**	**3.33**	
1.	3-Thujene	1.11	0.61	0.21			0.13		0.26	0.03				NC
2.	*α*-Pinene	10.33	11.81	12.79	1.02	2.65	3.27	0.89	1.55	1.65	0.28		0.48	3.2
3.	Camphene	0.25		0.78			0.43			1.33				NC
4.	Sabinene	1.10	1.01	1.56		0.91	0.34		0.81	0.24		0.12	0.50	NC
5.	*β*-Pinene	6.34	8.62	4.02	8.06	6.34	3.86	3.22	6.14	2.62	0.81	1.37	1.00	2.9
6.	*β*-Myrcene	12.35	11.36	9.29	9.53	11.29	9.23	16.45	10.13	9.19	0.29	0.41		2.1
7.	4-Carene	1.95			0.31			0.30			0.11			NC
8.	Limonene	3.36	2.19	1.34	5.21	2.12	1.11	3.16	0.15	0.87	1.10	0.23	1.00	3.3
9.	*β-trans*-Ocimene	0.45	0.23	0.12	0.21			0.26						NC
10.	*γ*-Terpinene	0.51	0.62	0.52		0.29	0.23		0.91	0.21		0.02		NC
11.	3-Carene	0.39	0.19	0.36	0.11	0.15	0.20	0.51	0.08	5.15	0.95	0.04	0.05	NC
12.	Linalool	15.65	11.4	10.59	2.01	1.23	2.95	2.37	2.06	0.85	0.34	0.60	0.30	3.9
13.	4-Terpineol	0.21	0.18	1.28			0.75			0.80				NC
14.	*p*-Menth-1-en-8-ol	0.31	0.68	0.26	0.77	0.78	0.73	0.46	0.33	0.05				NC
	sesquiterpenes and oxides	**0.32**	**0.52**	**0.76**	**2.88**	**3.66**	**4.66**	**3.02**	**4.41**	**3.55**	**8.63**	**10.86**	**14.66**	
15.	Caryophyllene			0.25	0.42	0.51	0.65	0.62	0.48	0.37	0.72	1.60	2.07	NC
16.	*α*-Guaiene	0.11				0.55	0.89		0.50	0.21	1.51	0.87	0.41	NC
17.	Caryophyllene-(11)		0.31	0.51	1.41	1.72	0.77	0.36	0.21	0.35	0.91	1.51	0.88	4.1
18.	*α*-Panasinsen		0.21			0.42	0.52	0.45	1.73	1.61	2.95	3.39	4.41	NC
19.	Caryophyllene oxide	0.21			1.05	0.46	1.83	1.59	1.49	1.01	2.54	3.49	6.89	NC
	straight chain aliphatic hydrocarbons				**4.38**	**3.37**	**4.34**	**6.81**	**7.55**	**8.20**	**14.29**	**10.02**	**11.09**	
20.	Pentadecane				0.13	0.12	0.21	0.22	0.19	0.47	0.82	0.60	0.47	NC
21.	Hexadecane					0.19	0.15		0.50	1.24	2.51	1.40	1.2	NC
22.	Heptadecane				0.20	0.37	0.24	0.66	0.30	0.41	1.91	1.51	0.81	NC
23.	Octadecane				0.38	0.53	0.57	0.85	0.53	0.56	1.87	0.39	0.21	NC
24.	Eicosane				0.25	0.13	0.14	1.39	2.19	0.35	0.54	0.49	1.04	NC
25.	Tricosane				1.39	1.55	0.21	0.22	1.08	3.55	0.22	0.62	0.61	NC
26.	Pentacosane				1.60	0.11	1.55	1.91	2.52	1.28	5.51	3.98	6.14	4.3
27.	Hexacosan				0.43	0.37	1.27	1.56	0.24	0.34	0.91	1.03	0.61	NC

Table 2. (Cont'd.).

		Content of volatiles in the leaves from Saururaceae at different regions (%)	S201106

No.	Identified compounds [c, d, e]	SC			GC			GI			HC			[a]R.S.D.s (%)
		S201101	S201105	S201109	S201102	S201106	S201110	S201103	S201107	S201111	S201104	S201108	S201112	
	branched aliphatic hydrocarbons	□	□	□	**1.54**	**2.11**	**2.20**	**2.00**	**1.55**	**2.73**	**5.56**	**4.47**	**6.26**	
28.	2,3,6-Trimethylheptane	□	□	□	0.33	0.65	0.68	0.22	0.18	0.31	0.22	0.60	0.37	NC
29.	Tetramethyloctane	□	□	□	□	0.39	0.15	□	0.10	0.25	0.51	0.62	0.42	NC
30.	2,2-Dimethyldecane	□	□	□	0.46	0.27	0.32	0.56	0.23	0.49	1.21	0.59	0.74	NC
31.	2,5,9-Trimethyldecane	□	□	□	0.12	0.22	0.55	0.40	0.60	0.27	1.65	0.52	2.01	NC
32.	4,6-Dimetylundecane	□	□	□	0.40	0.13	0.29	0.39	0.26	0.18	0.75	1.49	2.24	NC
33.	3-Methyl-5-propylnonane	□	□	□	0.23	0.45	0.21	0.43	0.18	1.23	1.22	0.65	0.48	NC
	Alcohols	**12.34**	**9.32**	**16.17**	**10.08**	**8.82**	**7.71**	**8.35**	**7.05**	**8.93**	**2.04**	**1.65**	**2.89**	
34.	3-Buten-2-ol	2.51	1.63	3.01	1.55	1.73	2.11	1.5	1.75	0.89	0.19	□	0.21	3.8
35.	2,3-Butanediol	3.12	2.35	6.41	2.12	1.37	1.88	1.41	0.9	3.01	0.36	0.46	0.87	3.6
36.	1-Nonanol	3.08	3.51	4.24	2.88	2.81	1.13	1.81	1.99	2.02	1.37	0.68	1.47	2.4
37.	Caprinic alcohol	3.63	1.83	2.51	3.53	2.91	2.59	3.63	2.41	3.01	0.12	0.51	0.34	2.9
	Aldehydes	**0.25**	**0.45**	**0.37**	**2.90**	**2.17**	**2.97**	**2.67**	**3.87**	**3.42**	**4.98**	**5.22**	**7.22**	
38.	2,3-Dihydroxy propanal	□	□	0.11	0.13	□	0.32	0.13	0.21	□	0.13	0.11	0.16	NC
39.	Benzaldehyde	□	□	0.12	□	□	0.22	0.53	□	□	0.62	0.35	0.41	NC
40.	Decanal	□	0.10	□	0.21	0.72	0.12	0.69	0.31	0.52	0.89	0.64	0.59	NC
41.	Nonanal	□	0.10	□	1.95	1.01	1.74	1.03	2.11	2.31	2.13	2.81	4.06	3.7
42.	Campholenal	0.15	0.25	0.14	□	□	0.36	□	□	□	0.62	0.45	0.31	NC
43.	*β*-Cyclocitral	0.10	□	□	0.61	0.44	0.21	0.29	1.24	0.59	0.59	0.86	1.69	NC
	Ketones	□	**0.34**	**0.51**	**19.83**	**17.83**	**23.39**	**24.25**	**25.17**	**21.87**	**48.74**	**56.26**	**44.97**	
44.	4'-methylacetophenone	□	0.21	□	2.47	0.89	2.74	1.51	1.07	0.88	2.42	1.37	1.88	NC
45.	8a-Methyloctahydro-4(1H)-azulenone	□	□	□	1.55	2.97	1.11	0.75	2.05	1.33	5.83	0.94	1.83	2.4
46.	2-Undecanone	□	□	□	10.19	9.61	14.93	19.18	16.42	13.4	33.18	49.42	39.4	2.1
47.	2-Dodecanone	□	0.13	0.18	3.16	2.58	1.40	1.25	3.61	2.64	0.85	0.51	0.24	NC
48.	2-Tridecanone	□	□	0.33	2.46	1.78	3.21	1.56	2.02	3.62	6.46	4.02	1.62	3.0

Table 2. (Cont'd.).

No.	Identified compounds [c, d, e]	Content of volatiles in the leaves from Saururaceae at different regions (%)												S201106 [a]R.S.D.s (%)
		SC			GC			GI			HC			
		S201101	S201105	S201109	S201102	S201106	S201110	S201103	S201107	S201111	S201104	S201108	S201112	
	Acids	**10.1**	**9.38**	**13.31**	**6.76**	**4.18**	**7.02**	**4.13**	**5.98**	**5.03**	**1.68**	**0.56**	**0.89**	
49.	Benzoic acid	3.31	3.61	2.04	1.31	□	□	□	□	□	□	□	□	NC
50.	Caprinic acid	2.10	1.81	1.59	1.24	0.40	0.41	1.06	1.09	2.01	0.36	0.11	□	NC
51.	Pentadecanoic acid, 2, 6, 10, 14-tetramethyl-	0.85	1.12	3.89	0.12	0.72	0.50	1.21	0.51	0.12	□	0.16	0.51	NC
52.	9,12-Octadecadienoic acid	0.99	0.54	0.91	1.26	□	1.04	0.24	1.86	0.61	0.17	□	0.15	NC
53.	10-Octadecenoic acid	1.84	0.83	1.88	0.71	2.15	0.52	0.26	2.52	2.14	□	0.12	□	4.0
54.	12-methyltetradecanoate	1.01	1.47	3.00	2.12	0.91	4.55	1.36	□	0.15	1.15	0.17	0.23	NC
	Esters	**18.99**	**23.55**	**19.84**	**12.46**	**17.74**	**14.37**	**11.44**	**10.28**	**12.61**	**1.90**	**0.57**	**0.53**	
55.	Vinyl methacrylate	1.31	0.21	2.27	1.53	2.27	0.58	1.41	1.22	0.91	0.11	□	□	3.5
56.	*trans*-Sabinenehydrate	3.59	6.82	2.38	□	0.24	0.15	0.54	0.24	0.21	□	□	0.19	NC
57.	Bornyl acetate	3.15	5.11	3.93	2.68	3.22	2.03	1.41	2.11	5.95	0.24	□	□	2.1
59.	*a*-Cyclogeraniol acetate	0.10	0.10	0.67	0.56	1.44	1.65	0.47	□	0.37	□	0.25	□	2.9
63.	(3-Isopropenyl-2-methylcyclopentyl) methyl acetate	1.26	0.16	2.25	1.87	2.1	2.94	1.25	2.09	□	□	□	0.13	1.8
64.	*p*-Menth-1-en-8-ol, acetate	4.11	5.98	1.21	1.33	0.41	□	□	0.31	0.31	□	□	□	NC
65.	Nerol acetate	1.39	0.19	1.26	2.19	2.23	4.15	3.01	1.12	3.12	□	□	□	3.4
66.	Geraniol acetate	2.56	4.16	4.52	1.04	2.77	0.54	1.11	1.47	0.40	1.55	0.32	0.21	NC
67.	Heptanoic acid, 3-methylbutyl ester	1.52	0.82	1.35	1.26	3.06	2.33	2.24	1.72	1.34	□	□	□	NC
	Others	**0.67**	**2.23**	**2.71**	**6.08**	**6.63**	**5.24**	**4.44**	**6.78**	**5.39**	**3.58**	**4.25**	**4.69**	
68.	Methyleugenol	□	□	□	2.95	1.05	1.54	1.27	2.4	2.12	1.12	2.47	1.38	4.2
69.	Isoeugenol	□	□	□	1.33	1.51	1.63	1.25	□	1.21	1.14	0.72	2.11	3.2
70.	Perillene	0.31	1.85	1.82	1.61	2.92	0.55	0.79	1.65	1.48	0.19	0.33	0.88	NC
71.	Propane, 2-ethoxy-	0.36	0.38	0.89	0.19	1.15	1.52	1.13	2.73	0.58	1.13	0.73	0.32	NC
	Unidentified	**3.02**	**5.31**	**3.21**	**5.86**	**7.73**	**4.87**	**5.27**	**4.94**	**5.28**	**4.72**	**3.35**	**3.47**	

[a] R.S.D. (%) = (S.D./mean) ×100. The analytes were the average of three GC-MS analyses and stable during the tested period; NC: not calculated; [b] '□': □ 0.05%; [c] by GC-MS; [d] literature (Kamal, *et al*., 2013; Liang *et al*., 2005; Lu *et al*., 2006; Qi *et al*., 2004; Xu *et al*., 2005); [e] KI: Kovats index (Chyau *et al*., 1992).

Table 3. Volatile compounds and their contents in flowers from the SC, GC, GI and HC.

No.	Identified compounds [c, d, e]	Content of volatiles in the flowers from Saururaceae at different regions (%)											
		SC			GC			GI			HC		
		S201101	S201105	S201109	S201102	S201106	S201110	S201103	S201107	S201111	S201104	S201108	S201112
	Monoterpenes and oxides	**59.68**	**44.66**	**56.08**	**34.10**	**32.59**	**24.10**	**17.38**	**22.48**	**15.68**	**7.23**	**5.60**	**8.48**
1.	3-Thujene	1.21	0.71	1.21	□	□	1.13	□	0.42	0.33	□	0.78	□
2.	*a*-Pinene	13.23	10.88	13.79	2.02	3.65	2.27	2.89	1.62	2.15	0.28	□	1.48
3.	Camphene	0.25	□	0.78	□	□	0.43	□	□	0.13	□	□	□
4.	Sabinene	1.10	1.01	0.56	0.45	2.91	1.34	□	1.87	1.24	0.51	0.12	0.50
5.	*β*-Pinene	5.91	7.62	4.02	5.26	6.44	3.86	3.22	5.14	3.62	1.81	1.37	1.52
6.	*β*-Myrcene	9.35	8.36	9.29	11.53	10.29	5.23	3.44	9.13	1.19	1.29	0.41	□
7.	4-Carene	1.25	0.89	□	5.31	0.38	0.59	1.36	□	□	0.11	□	1.10
8.	Limonene	3.36	2.19	1.34	3.21	2.12	1.12	1.16	0.15	0.87	1.10	0.23	0.53
9.	*β-trans*-Ocimene	1.45	0.93	3.12	0.21	0.54	□	0.26	□	0.19	□	□	□
10.	*γ*-Terpinene	1.51	0.62	2.52	0.42	2.29	1.23	0.71	0.91	0.21	□	0.20	□
11.	3-Carene	5.19	3.19	6.36	0.11	0.15	0.47	0.51	0.48	1.15	0.95	0.44	2.05
12.	Linalool	13.65	6.40	10.55	2.41	1.23	2.95	2.37	1.06	0.85	0.34	1.60	1.30
13.	4-Terpineol	0.91	1.18	1.28	□	0.81	1.75	□	0.37	2.70	0.84	□	□
14.	*p*-Menth-1-en-8-ol	1.31	0.68	1.26	3.17	1.78	1.73	1.46	1.33	1.05	□	0.63	□
	Sesquiterpenes and oxides	**1.06**	**1.64**	**1.20**	**4.98**	**5.75**	**4.36**	**5.02**	**6.35**	**7.54**	**12.63**	**9.66**	**8.58**
15.	Caryophyllene	□	0.81	0.18	0.82	1.51	1.45	2.72	1.48	1.47	2.72	1.60	2.47
16.	*a*-Guaiene	0.16	□	0.25	0.24	0.55	0.19	□	0.44	0.21	1.51	0.87	1.41
17.	Caryophyllene-(I1)	□	0.28	0.36	1.51	1.72	1.37	0.26	1.21	2.46	1.91	1.51	0.80
18.	*a*-Panasinsen	0.59	0.55	□	0.56	0.21	0.32	0.45	1.73	1.31	2.95	2.59	1.91
19.	Caryophyllene oxide	0.31	□	0.41	1.85	1.76	1.03	1.59	1.49	2.09	3.54	3.09	1.99
	Straight chain aliphatic hydrocarbons	□	□	□	**5.54**	**4.65**	**5.09**	**5.74**	**5.24**	**7.78**	**8.12**	**11.82**	**9.63**
20.	Pentadecane	□	□	□	□	□	0.21	0.22	□	0.47	0.85	0.79	0.52
21.	Hexadecane	□	□	□	□	0.19	□	□	0.60	1.24	1.51	1.34	1.7
22.	Heptadecane	□	□	□	□	0.37	0.24	0.41	0.56	0.41	0.91	1.58	0.81
23.	Octadecane	□	□	□	1.49	0.53	0.57	0.85	1.53	1.56	0.87	0.59	0.61
24.	Eicosane	□	□	□	0.35	0.13	1.14	1.59	0.11	0.35	0.51	0.89	1.26

Table 3. (Cont'd.).

No.	Identified compounds [c, d, e]	Content of volatiles in the flowers from Saururaceae at different regions (%)											
		SC			GC			GI			HC		
		S201101	S201105	S201109	S201102	S201106	S201110	S201103	S201107	S201111	S201104	S201108	S201112
25.	Tricosane	□	□	□	□	0.55	0.21	□	1.08	1.45	0.28	1.62	0.91
26.	Pentacosane	□	□	□	3.19	2.51	1.57	1.41	1.02	1.28	1.28	2.98	2.11
27.	Hexacosan	□	□	□	0.51	0.37	1.15	1.26	0.34	1.02	1.91	2.03	1.71
	Branched aliphatic hydrocarbons	□	□	□	**1.02**	**1.29**	**1.34**	**1.07**	**2.35**	**1.58**	**2.63**	**3.24**	**3.38**
28.	2,3,6-Trimethylheptane	□	□	□	0.51	□	0.58	□	0.13	0.25	0.28	0.41	□
29.	Tetramethyloctane	□	□	□	□	□	0.25	□	0.19	□	□	0.64	0.48
30.	2,2-Dimethyldecane	□	□	□	□	0.16	0.12	0.39	0.43	0.39	0.21	0.19	0.64
31.	2,5,9-Trimethyldecane	□	□	□	0.18	0.27	□	0.15	1.03	0.23	1.04	0.56	1.11
32.	4,6-Dimetylundecane	□	□	□	□	0.32	0.39	□	0.46	0.18	0.85	0.79	0.54
33.	3-Methyl-5-propylnonane	□	□	□	0.33	0.54	□	0.53	0.11	0.53	0.25	0.65	0.61
	Alcohols	**8.44**	**9.39**	**7.16**	**5.05**	**6.33**	**4.58**	**4.40**	**5.45**	**3.23**	**2.10**	**1.95**	**2.58**
34.	3-Buten-2-ol	2.51	0.63	1.01	0.61	1.93	1.01	0.41	1.05	0.69	0.21	□	□
35.	2,3-Butanediol	2.12	2.45	1.51	2.02	2.37	1.68	1.15	0.92	1.01	□	0.56	0.80
36.	1-Nonanol	2.08	4.51	3.13	1.89	1.12	0.81	2.31	1.99	1.02	1.57	0.68	1.47
37.	Caprinic alcohol	1.73	1.80	1.51	0.53	0.91	1.08	0.53	1.49	0.51	0.32	0.71	0.31
	Aldehydes	**0.13**	**0.46**	**0.34**	**2.63**	**2.13**	**2.96**	**2.09**	**2.88**	**3.01**	**4.61**	**3.85**	**5.43**
38.	2,3-Dihydroxy propanal	□	□	0.21	0.18	□	0.82	0.23	0.18	□	0.13	0.16	0.37
39.	Benzaldehyde	□	□	0.13	□	□	0.32	0.33	□	□	0.62	0.13	0.29
40.	Decanal	□	□	□	0.81	0.58	0.32	0.20	0.36	0.51	0.89	0.74	0.89
41.	Nonanal	□	0.15	□	0.95	1.01	1.14	1.08	2.00	1.81	1.94	2.41	2.86
42.	Campholenal	□	0.31	□	□	□	0.36	□	□	□	0.52	0.41	0.31
43.	*β*-Cyclocitral	0.13	□	□	0.69	0.54	□	0.25	0.34	0.69	0.51	□	0.71
	Ketones	**1.20**	**1.33**	**1.56**	**23.16**	**17.94**	**28.01**	**35.49**	**24.23**	**30.37**	**47.97**	**55.35**	**42.07**
44.	4'-methylacetophenone	□	0.70	□	2.47	0.81	1.74	1.41	1.07	0.80	2.11	0.47	1.09
45.	8a-Methyloctahydro-4(1H)-azulenone	□	□	□	1.95	2.92	2.11	7.75	3.01	4.31	5.89	1.94	1.83
46.	2-Undecanone	□	□	□	14.12	12.43	20.57	25.18	16.42	20.4	36.68	49.37	37.4

Table 3. (Cont'd.).

No.	Identified compounds [c, d, e]	Content of volatiles in the flowers from Saururaceae at different regions (%)											
		SC			GC			GI			HC		
		S201101	S201105	S201109	S201102	S201106	S201110	S201103	S201107	S201111	S201104	S201108	S201112
47.	2-Dodecanone	0.89	0.63	1.18	3.16	□	2.40	1.15	2.61	1.64	1.85	2.05	0.34
48.	2-Tridecanone	0.31	□	0.38	1.46	1.78	1.19		1.12	3.22	1.44	1.52	1.41
	Acids	**11.24**	**9.75**	**7.34**	**3.42**	**2.46**	**3.62**	**2.01**	**4.00**	**3.03**	**0.55**	**0.29**	**0.38**
49.	Benzoic acid	4.10	3.61	1.84	□	□	□	□	□	□	□	□	□
50.	Caprinic acid	2.01	0.65	0.91	□	□	□	□	□	□	□	□	□
51.	Pentadecanoic acid, 2,6,10,14-tetramethyl-	0.80	1.12	0.89	0.12	□	0.18	0.51	□	0.12	□	□	□
52.	9,12-Octadecadienoic acid	1.49	0.53	0.41	1.46	□	2.04	0.78	0.87	1.51	0.24	□	0.15
53.	10-Octadecenoic acid	2.44	2.63	1.88	0.71	1.55	0.59	0.16	2.12	1.24	□	0.12	□
54.	12-methyltetradecanoate	0.40	1.21	1.41	1.13	0.91	0.81	0.56	1.01	0.16	0.31	0.17	0.23
	Esters	**13.04**	**15.57**	**16.72**	**12.54**	**14.03**	**9.98**	**10.47**	**12.14**	**11.02**	**2.63**	**1.93**	**2.99**
55.	Vinyl methacrylate	0.31	0.18	0.22	1.63	0.77	0.68	0.41	□	□	□	□	□
56.	*trans*-Sabinenehydrate	1.39	2.82	1.38	□	0.28	0.15	0.58	0.24	□	□	□	□
57.	Bornyl acetate	2.31	5.11	3.93	1.68	1.81	1.03	1.51	1.81	1.95	□	□	□
59.	*a*-Cyclogeraniol acetate	0.14	0.12	0.67	0.56	1.51	1.15	0.42	□	0.32	□	□	□
63.	(3-Isopropenyl-2-methylcyclopentyl) methyl acetate	1.26	0.16	1.28	1.67	3.10	0.99	1.53	2.12	□	□	□	□
64.	*p*-Menth-1-en-8-ol, acetate	2.11	3.98	2.21	1.51	0.49	□	□	0.68	1.53	□	□	□
65.	Nerol acetate	1.39	0.49	1.26	2.19	3.25	2.15	1.71	2.12	2.13	□	0.51	1.31
66.	Geraniol acetate	3.56	1.16	4.52	1.04	0.58	3.44	2.62	1.45	2.95	1.51	1.42	1.56
67.	Heptanoic acid, 3-methylbutyl ester	0.57	1.55	1.25	2.26	2.24	0.39	1.69	3.72	2.14	1.12	□	0.12
	Others	**1.80**	**8.45**	**2.91**	**1.93**	**4.15**	**8.28**	**7.21**	**7.81**	**6.25**	**4.70**	**2.73**	**8.37**
68.	Methyleugenol	□	3.40	□	0.25	0.56	0.98	1.02	1.86	1.21	2.12	0.91	2.19
69.	Isoeugenol	□	2.30	□	□	1.51	2.13	1.2	3.45	3.02	1.14	0.72	1.98
70.	Perillene	0.15	2.34	2.22	0.41	0.92	3.89	2.78	1.69	1.48	0.19	0.31	2.88
71.	Propane, 2-ethoxy-	1.65	0.41	0.69	1.27	1.16	1.28	2.21	0.81	0.54	1.25	0.79	1.32
	Unidentified	**3.41**	**8.75**	**6.69**	**5.63**	**8.68**	**7.68**	**9.12**	**7.07**	**10.51**	**6.83**	**3.58**	**8.11**

Note: The analytes were the average of three GC-MS analyses and stable during the tested period; [b] '□ ': □ 0.05%; [c] by GC-MS; [d] literature (Kamal *et al*., 2013; Liang *et al*., 2005; Lu *et al*., 2006; Qi *et al*., 2004; Xu *et al*., 2005); [e] KI: Kovats index (Chyau *et al*., 1992)

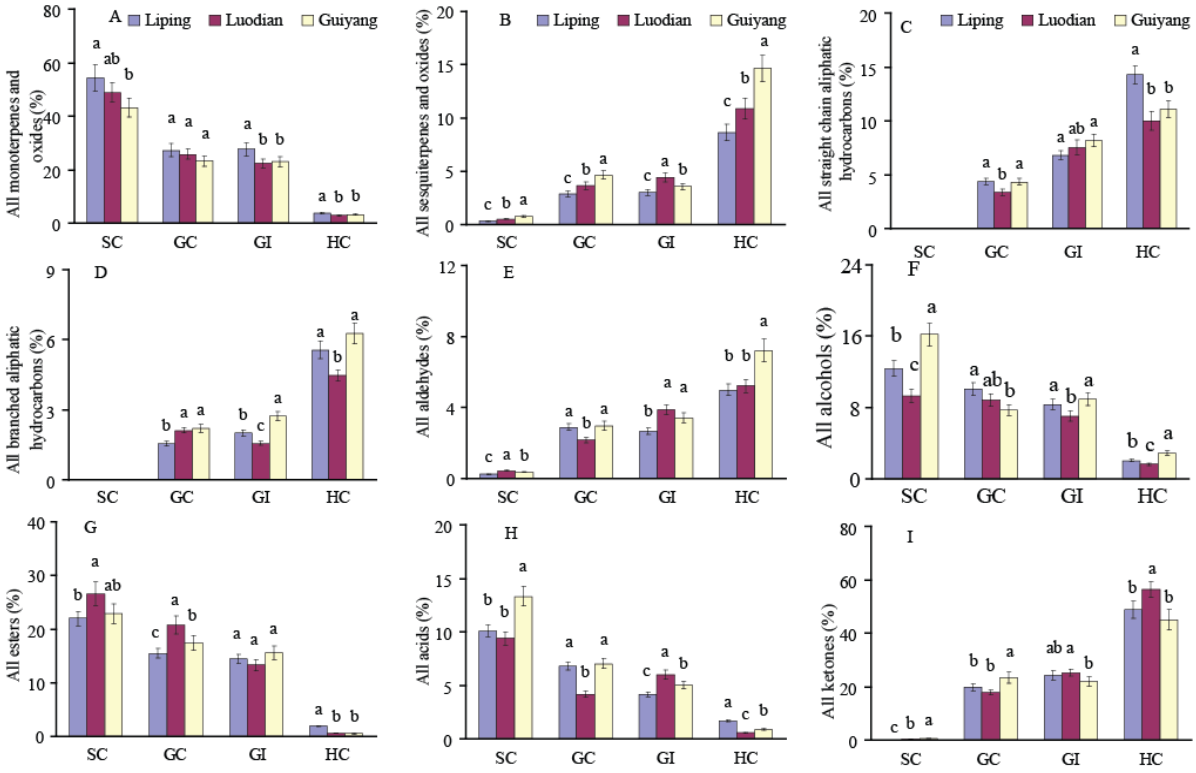

Fig. 2. Comparison of relative concentrations of all monoterpenes and oxides (A), all sesquiterpenes and oxides (B), all straight chain aliphatic hydrocarbons (C), all branched aliphatic hydrocarbons (D), aldehydes (E), all alcohols (F), all ester (G), all acids (H) and all ketones (I) in the leaves from SC, GC, GI and HC at different regions, respectively.

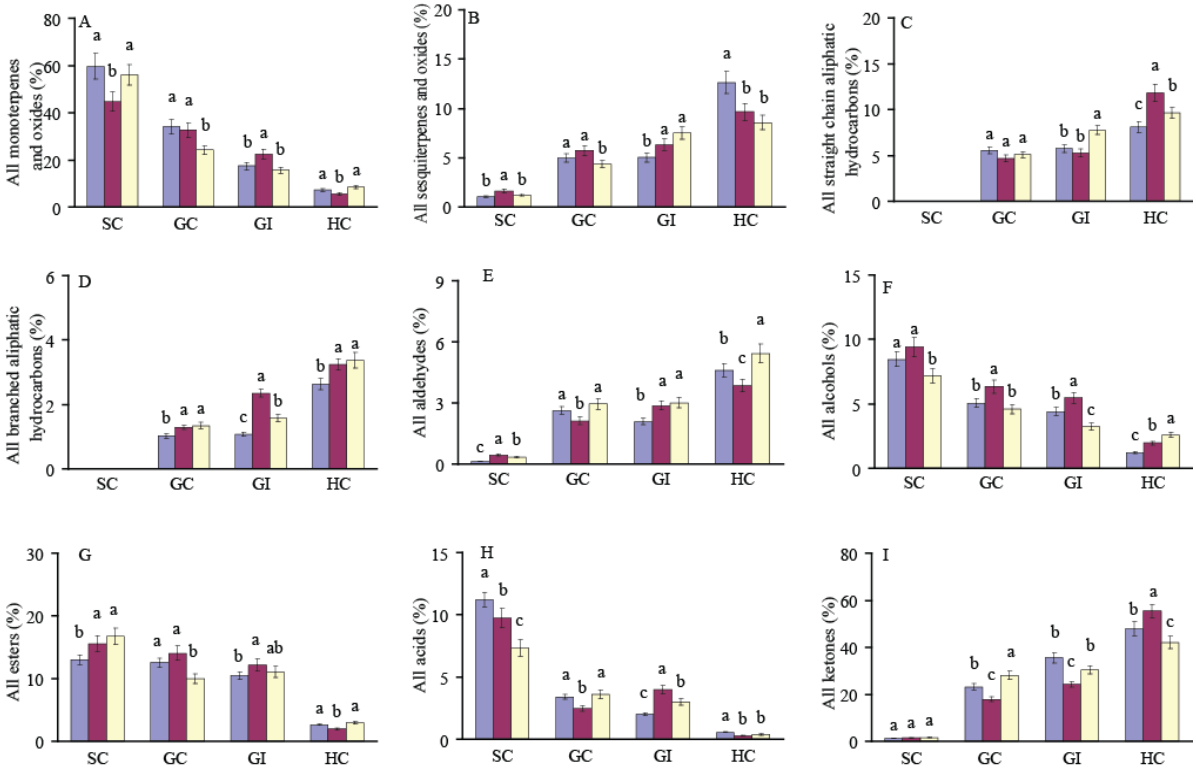

Fig. 3. Comparison of relative concentrations of all monoterpenes and oxides (A), all sesquiterpenes and oxides (B), all straight chain aliphatic hydrocarbons (C), all branched aliphatic hydrocarbons (D), aldehydes (E), all alcohols (F), all ester (G), all acids (H) and all ketones (I) in the flowers from SC, GC, GI and HC at different regions, respectively.

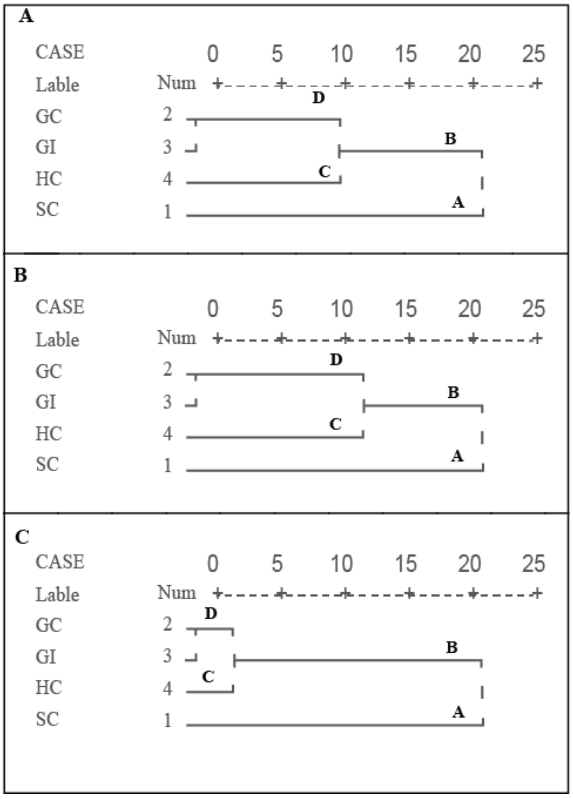

Fig. 4. Cluster analysis of the identified volatile compounds from the leaves and flowers of SC, GC, GI and HC from Liping (A), Luodian (B) and Guiyang (C), respectively. (Tree diagram for 4 cases single linkage percent disagreement).

One of those progressive modifications is the white leaves of inflorescence and white involucres. The evolutionary level of GC and HC with white involucres under flowers is higher than SC with white leaves of inflorescence on the stem top. Aldehydes (e.g., decanal, nonanal, 2,3-dihydroxy propanal, campholenal, *β*-cyclocitral and benzaldehyde), which are often important allelochemicals, were also identified in leaf and flower samples. It is established that decanal and nonanal, which is a constituent of volatiles excreted from leaves to attract wasps, attract some enemies of the Colorado beetle (Weissbecker *et al*., 2000) when caterpillars damage plant leaves (Mattiacci *et al*., 2001). Benzaldehyde is an attractant for some insects and a repellent to others depending on its concentration (Nuttley *et al*., 2001). Campholenal and *β*-cyclocitral possess antitermitic activity (Chang & Cheng 2002) and can act as a synergist to other allelochemicals (Deng *et al*., 2004). The content of all aldehydes were highest (HC), lower (GD) and lowest (SC), respectively (Fig. 2E & 3E) might reveal taxonomic characteristics. The alcohols (e.g., 3-buten-2-ol, 2,3-butanediol, 1-nonanol and caprinic alcohol) found in all leaf and flowers samples were often found in plants and algae and are components of the male pheromone of a beetle (Rochat *et al*., 2002). The content of all alcohols in the species might also reveal taxonomic characteristics (Fig. 2f & 3f). Esters (Fig. 2G & 3G), which were an important attractant pheromone, dominated in the SC and were less important in the GC and GI species. Acids (e.g., benzoic acid) possess antibacterial and antifungal activity and could have a defensive function (Terreaux *et al*., 1998). It was found that the content of all acids was highest in the SC, lowest in the HC and lower in the GD (Fig. 2H & 3H), which might also reveal systematic characteristics of Saururaceae species. Ketones (e.g., 4'-methylacetophenone, 8a-methyloctahydro-4 (1H)-azulenone, 2-undecanone, 2-dodecanone, and 2-tridecanone) were found only in GD species and HC, but not in SC. And the content of all ketones in the HC was higher than it in the GD (Fig. 2I & 3I). The results analyzed might also reveal taxonomic characteristics evolutionary significance. 2-Undecanone mainly dominated the volatiles from leaves of HC, and especially in flowers of HC. The concentrations of 2-undecanone, which were highest in the leaves (33.18-49.42%) and flowers (36.68-49.37%) of HC, were lower in the leaves (9.61-19.18%) and flowers (12.43-25.18%) of GD., but not in SL (Tables 2 & 3). There is some evidence that the highest concentrations of 2-undecanone in the leaves and flowers of HC, the lower concentrations thereof in the leaves and flowers of GD, and the complete absence thereof in SL may support for taxonomic characteristics of these species.

During the evolution of Saururaceae plants, some evolutionary structures have formed over time. Those more progressive modifications are the carpel and stamen; six stamens and four carpels of SL still maintain separation from initiation to maturity. The four carpels normally of GD**,** which keep some degree of fusion with other carpels in initiation, become a single ovary and separate from the style, while the underside of the stamens merge with the ovary wall. The stamens and carpels of HT were reduced to three stamens and three carpels, respectively (Liang, 1995). The carpel and stamen have evolved via fusion, reduction and multiplication; these changes were linked with concentration changes of all monoterpenes and oxides, all sesquiterpenes and oxides, all straight chain aliphatic hydrocarbons, all branched aliphatic hydrocarbons, all alcohols, aldehydes, all ketones, all acids and all esters in the leaves and flowers from SC, GC, GI, and HC from different regions (Fig. 4). It is suggested that these types of compounds may support taxonomic characteristics and evolutionary significance of these species.

The clustering process can be represented as a tree or dendrogram, where each step in the clustering process is illustrated by a joint in the tree (Li *et al*., 2007; Yu *et al*., 2007; Zou *et al*., 2005). In this study, the volatile composition of the samples was defined using characteristics in the analysis in order to analyze, differentiate and classify the four Saururaceae species from Liping, Luodian, Guiyang, respectively. A dendrogram was generated (Fig. 4A-C), which revealed the relationships between different species. The information from the cluster analysis confirms the accepted systematic scheme (Liang, 1995; Tucker *et al*., 1993). The highest similarity degree is between the species GC and GI, which are significantly distinguished from SC

and HC. There is a higher degree of similarity between the GD species and HC, which are significantly distinguished from SC. It can be concluded that GC and GI largely possess similar volatile profiles differing from that of SC and HC. The composition of SC and HC volatiles and some features of the volatile profiles of GC and GI are in agreement with their different evolutionary positions. The concentration and composition of every species' volatiles from different regions were different, but the result of clustering analysis was very similar. These results show that this pattern may reflect genetic and environmental factors.

Conclusions

Volatiles of plants may reflect genotype and environmental interactions affecting the expression and activity of volatiles with biosynthetic enzymes (Yang *et al*., 2011). The samples of Saururaceae species from different regions contained similar volatiles; however, the levels of specific chemical compounds differed. There is some evidence that the properties of the profiles and levels of the volatiles, which may be influenced by environmental locations and genotype and environmental interactions, can support taxonomic characteristics of these species, and be evaluated systematically. Further work is required to evaluate the relative contributions of genetic and environmental factors to the variability of volatiles of Saururaceae species.

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