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Research Article

Chemical Constituents of Essential Oils of *Cymbopogon distans* from Two Producing Areas during Different Storage Periods

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Abstract

The essential oils of *Cymbopogon distans* from Gansu and Sichuan were obtained by dynamic headspace method and analyzed by gas chromatography and mass spectrometry for different storage periods (1 month, 3 months, 6 months, and 12 months). The types of chemical constituents of essential oil from Gansu *C. distans* with different storage periods are alkanes, esters, amides, and ketones. In addition to the above four, compounds of Sichuan *C. distans* essential oil also include aldehydes, benzenes, and carboxylic acids. Alkanes of the essential oil collected from the two regions increased with storage time. After 12 months of storage, amides was the largest in the essential oil of Gansu, while that of Sichuan was ketones. The essential oils of Gansu and Sichuan *C. distans* were identified 24 and 27 compounds, respectively. Among these compounds, hexadecane and heneicosane are the major compounds inessential oil of Gansu and Sichuan *C. distans*, respectively. Dibutyl phthalate, hexacosane, and heneicosane were produced in the two places in the same period, which can be used to identify the chemical fingerprint of C. distans. Six differential metabolites (nonadecane, heptadecane, octadecane, dibutyl phthalate, 2-methyl-hexacosane, and heneicosane) can be used as a basis for judging the origin of *C. distans*.

Keywords: *Cymbopogon distans*; essential oil; chemical constituents; producing areas; storage periods

Introduction

Cymbopogon distans is a perennial herb of genus *Cymbopogon* in Gramineae, widely distributed in Gansu, Sichuan, Shaanxi, Tibet, and other provinces of China [1]. Plants of the genus *Cymbopogon* (*C. martini*, *C. flexuosus*, *C. khasianus*, and *C. distans*) have been developed as commercial production of essential oils [2-4]. *C. distans* has a strong fragrance and great commercial value. Both the stems and leaves of *C. distans* can be used to extract aromatic oil [5]. The major usages of *C. distans* essential oil are perfumery, medicine, insect repellant, and other related industrial products [6]. And this oil can also be used to treat asthma and coughs [7]. *C. distans* essential oil displayed antifungal [8], antiviral, antibacterial, and insecticidal activities. For example, essential oil of *C. distans* exhibited strong repellency against *Liposcelis bostrychophila* Badonnel and *Tribolium castaneum* Herbst [9].

Various chemical constituents have been found in essential oil of *C. distans*, such aspiperitone, geraniol, neral, citronellal, geranyl acetate, geranial, limonene, and so on [10-11]. Geraniol and citronellol showed stronger resistance against red flour beetle than DEET [12]. In addition, p-menth-2-en-1-ol and piperitol showed significant antifungal and antibacterial activities against *Candida kefyr*, *Bacillus subtilis*, and *Salmonella typhimurium* [13].

However, the chemical constituents of *C. distans* essential oil from Gansu and Sichuan of China have not been studied. Climatic conditions are different between the two places. Gansu is located in north China with dry and little rain throughout the year. Sichuan is located in the zone of subtropical monsoon climate with plentiful rainfall. Therefore, in this paper, the essential oils from the two places with different storage periods were collected by dynamic headspace method, and its chemical composition was analyzed by GC-MS.

Materials and Methods

Preparation of essential oils

Gansu (GS) and Sichuan (SC) *Cymbopogon distans* were sealed with sample bags and collected with an atmospheric sampling instrument for 3 hours with airflow rates of 200 mL/min. The adsorption materials were eluted with n-hexane (chromatographic pure grade). The essential oils (EOs) of *C. distans* were storage at -20°C, and then analyzed and identified by GC/MS.

GC-MS analysis

GC-MS analysis was used an Agilent gas chromatograph. The chromatography was performed on a VF-5ms (30m×250μm×0.25μm) capillary column. Programmed temperature: the initial temperature was 50°C for 3 minutes, 5°C/min to 210°C for 3 min, and then 15°C/min to 280°C for 3 min; Injector temperature was 250°C; The carrier gas was helium; The flow rate was 1.0 mL/min. The shunt ratio was 30:1; the injection volume was 500μL. Mass spectrometry conditions: ion source temperature (230°C), quadrupole temperature (150°C), interface temperature (280°C). The ionization mode is electron bombardment ion source. Electron energy was at 70 eV. Mass number (m/z) scanning range from 30 to 600. Solvent delay was 3 min. RI values were obtained by comparing with mass spectra of n-alkanes under the identical conditions. The composition of the compounds was identified by comparing the RI with the National Institute of Standards and Technology (NIST) chemistry online book and published literature [14]. The relative percentages of compounds were calculated by the peak area normalization method without taking into account the response factors of individual compounds.

Results and discussion

Chemical constituents of Eos obtained from GSC. distans at different storage periods

The chemical constituents of Eos from GS*C. Distans* are shown in Table 1. A total of 24 compounds were identified in the essential oils with four different storage periods (A, B, C, and D in the figures and tables represent 1 month, 3 months, 6 months, and 12 months, respectively). These identified compounds were alkanes, esters, amides, and ketones. During A storage period, alkanes, esters, and amides accounted for 78.6%, 7.7%, and 13.7% of the total compounds, respectively (Figure 1). The compound types in the B period were the same as those in the A period, accounting for 98.7%, 1.0%, and 0.3%, respectively. The main compounds in the Eos at C storage period were alkanes (98.7%) and esters (1.3%). Amides (61.7%), alkanes (21.4%), esters (9.1%), and ketones (7.8%) are obtained from the EOs of *C. distans* during the D storage period.

Alkanes increased from 78.6% to 98.7% and then gradually decreased to 21.4%with increase of storage time. The content of esters (7.7% to 1.0%) and amides (13.7% to 0.3%) decreased first and then increased (9.1% and 61.7%). Ketones (7.8%) were only discovered in the last storage period.

S. No	*Compound*	*RI^exp^*	*RI^lit^*	*Peak area%*			
				A	*B*	*C*	*D*
1	Heptadecane	1711	1700	1.2	7.2	-	-
2	Dibutyl phthalate	2037	2037	1.5	0.3	1.2	0.06
3	Hexacosane	2606	2600	6.4	46.4	59.4	-
4	Octadecane	1810	1800	-	4.0	0.1	0.04
5	Hexadecanoic acid ethyl ester	1978	1959	0.6	0.3	-	0.03
6	Octadecanoic acid ethyl ester	2177	2196	1.5	0.2	-	0.05
7	(Z)-9-Octadecenamide	2228	2401	2.1	0.3	-	1.0
8	Heneicosane	2109	2100	8.9	11.5	8.4	-
9	2-methyl-Octacosane	2840	2862	3.8	-	3.9	-
10	2-methyl-Hexacosane	2641	2641	2.1	-	-	-
11	2-methyl-Eicosane	2045	2064	0.1	-	-	-
12	(Z)-13-Docosenamide	2625	2625	4.1	-	-	-
13	Tetratetracontane	4395	4395	5.5	11.7	15.1	-
14	Nonacosane	2904	2900	3.3	-	-	-
15	Tetradecane	1413	1400	2.5	-	-	-
16	Pentadecane	1512	1500	0.1	-	-	0.02
17	Nonadecane	1910	1900	1.6	-	-	-
18	Octacosane	2804	2800	0.2	-	1.3	-
19	Tritriacontane	3301	3300	0.2	-	-	-
20	3-methyl-Nonacosane	2939	2974	0.1	-	-	-
21	Pentacosane	2506	2500	-	-	0.2	-
22	Decane	1015	1000	-	-	-	0.1
23	Indane	1047	1041	-	-	-	0.2
24	Acetophenone	1029	1059	-	-	-	0.1
*RI^{exp}: Experimental Retention Index; RI^{lit}: Literature Retention Index							

Table 1: Chemical constituents of EOs of GS *C. distans*.

The EOs composition of GS *C. distans* changed with storage period. During A storage period, 20 compounds were obtained, and the five compounds with the highest content are (z)-13-docosenamide, (z)-9-octadecenamide, 2-methyl-eicosane, 2-methyl-hexacosane, and 2-methyl-octacosane.During B storage period, 9compounds were identified, and the content of hexacosane, tetratetracontane, heneicosane, heptadecane, and octadecane is higher than other periods.Hexacosane, tetratetracontane, heneicosane, 2-methyl-octacosane, and octacosane were major compoundsatCstorage period. At D storage period, nine compounds were collected, and the content of (z)-9-octadecenamide, indane, acetophenone, dibutyl phthalate, and decane were ranked from first to fifth, respectively. In addition, only dibutyl phthalate was found in all four storage phases.

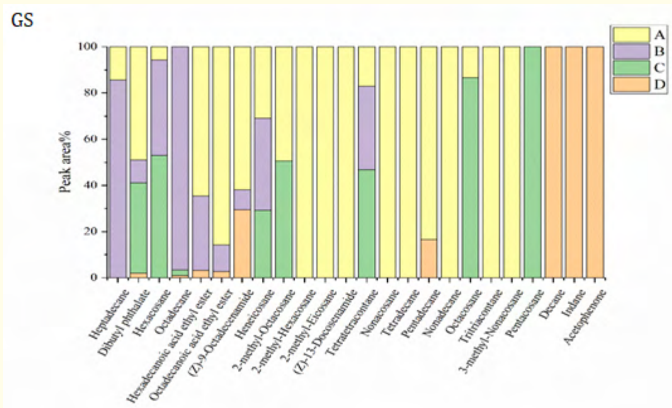

Figure 1: Chemical constituents of EOs of GS *C. distans*.

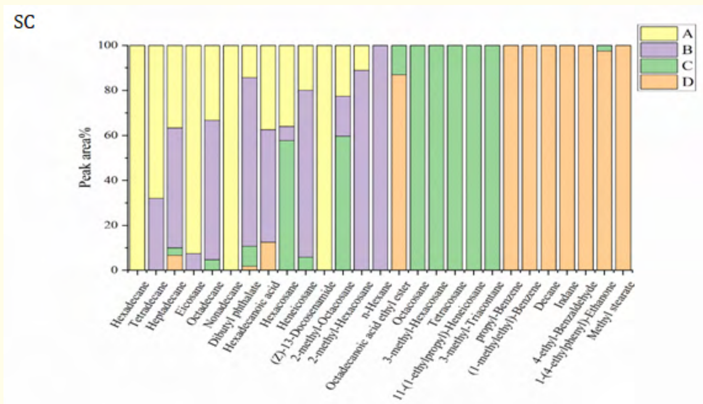

Figure 2: Chemical constituents of EOs of SC *C. distans*.

Chemical constituents of Eos obtained from SCC. Distans at different storage periods

The chemical constituents of EOs of SC *C. distans* is shown in Table 2. Twenty-seven compounds were identified in EOs of SC*C. distans*, which were aldehydes, benzenes, ketones, esters, amdies, alkanes, andcarboxylic acids.The chemical constituents of EOs from SC*C. Distans* were similar to that of GS. In the first three storage periods, alkanes are the most abundant in the EOs, accounting for 95.2%, 94.2%, and 99.1%, respectively (Figure 1). At D storage period, ketones (69.5%) was the largest content inessential oil, followed by benzenes (9.6%), esters (8.3%), alkanes (6.8%), aldehydes (4.5%), and carboxylic acids (1.3%).

With the storage time increase, alkanes increased from 95.2% to 99.1% and then gradually decreased to 6.8%; esters increased from 1.2% to 5.3%, then to 0.7%, and finally gradually to 8.3%. Amides accounted for 3.2% in the first storage periods. Carboxylic acids and ketones increased from 0.4% to 1.3% and 0.1% to 69.5%, respectively. Aldehydes (4.5%) and benzenes (9.6%) were only found in the D period.

S. No	Compound	RI^{exp}	RI^{lit}	Peak area%			
				A	B	C	D
1	Hexadecane	1612	1600	0.3	-	-	-
2	Tetradecane	1413	1400	1.7	0.8	-	-
3	Heptadecane	1711	1700	1.1	1.6	0.1	0.2
4	Eicosane	2009	2000	4.9	0.4	-	-
5	Octadecane	1810	1800	0.7	1.3	0.1	-
6	Nonadecane	1910	1900	0.4	-	-	-
7	Dibutyl phthalate	2037	2037	0.8	4.2	0.5	0.1
8	Hexadecanoic acid	1978	1959	0.3	0.4	-	0.1
9	Hexacosane	2606	2600	33.8	5.9	54.2	-
10	Heneicosane	2109	2100	15.0	55.8	4.4	-
11	(Z)-13-Docosenamide	2625	2625	2.0	-	-	-
12	2-methyl-Octacosane	2840	2840	1.4	1.1	3.7	-
13	2-methyl-Hexacosane	2641	2641	0.5	4.0	-	-
14	n-Hexane	618	623	-	3.5	-	-
15	Octadecanoic acid ethyl ester	2177	2196	-	-	0.03	0.2
16	Octacosane	2804	2800	-	-	1.4	-
17	3-methyl-Hexacosane	2641	2641	-	-	0.3	-
18	Tetracosane	2047	2400	-	-	13.4	-
19	11-(1-ethylpropyl)-Heneicosane	2477	2477	-	-	0.5	-
20	3-methyl-Triacontane	3039	3060	-	-	0.5	-
21	propyl-Benzene	992	962	-	-	-	0.2
22	(1-methylethyl)-Benzene	928	929	-	-	-	0.3
23	Decane	1015	1000	-	-	-	0.1
24	Indane	1047	1041	-	-	-	0.2
25	4-ethyl-Benzaldehyde	1195	1182	-	-	-	0.3
26	1-(4-ethylphenyl)-Ethanone	1242	1279	-	-	0.1	3.9
27	Methyl stearate	2077	2124	-	-	-	0.2
*RI^{exp}: Experimental Retention Index; RI^{lit}: Literature Retention Index							

Table 2: Chemical constituents of EOs derived from *C. distans* in SC.

During the four storage periods, 13, 11, 13, and 11 compounds were identified, respectively. In the first three periods, hexacosane and heneicosane are the major compounds in the EOs. In addition to these two compounds, periods A (eicosane, (z)-13-docosenamide, and tetradecane), B (dibutyl phthalate, 2-methyl-hexacosane, and n-hexane), and C (tetracosane, 2-methyl-octacosane, and octacosane) also include other high content compounds. During D storage period, 1-(4-ethylphenyl)-ethanone, (1-methylethyl)-benzene, propyl-benzene, 4-ethyl-benzaldehyde, and octadecanoic acid ethyl ester are main compounds. Heptadecane and dibutyl phthalate were found in EOs of SC*C. distans* at four storage stages.

The same metabolites in EOs of GS and SC C. distans at different periods

The metabolites produced by GS*C. distans* were 22 compounds, which were identical with those from SC, mainly including alkanes, amdies, and esters (Figure 2). During A storage period, nine same metabolites were obtained in the EOs of GS and SC*C. distans*, which were heptadecane, dibutyl phthalate, 2-methyl-octacosane, 2-methyl-hexacosane, (z)-13-docosenamide, tetradecane, hexacosane, heneicosane, and nonadecane. Among these identified chemical constituents, the content of the latter three compounds is higher than other compounds. During B storage period, the same metabolites from the two places were heptadecane, dibutyl phthalate, hexacosane, octadecane, and heneicosane. There were five same metabolites (dibutyl phthalate, hexacosane, octadecane, heneicosane, and 2-methyl-octacosane) at C storage period. During D storage period, the same compounds were dibutyl phthalate, decane, and indane. The same chemical constituents of EOs produced by *C. distans* from GS and SC in the same storage period can be used as a chemical fingerprint to identify whether the plant is *C. distans*.

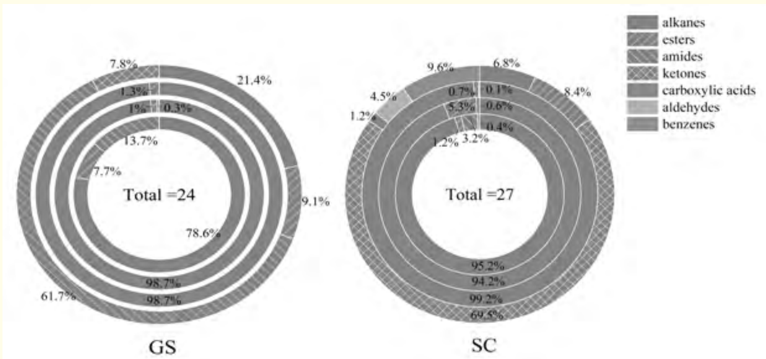

Figure 3: The proportion of compound types in *C. distans* at different storage stages in GS and SC (The rings are, from the inside to the outside, in order of period A, B, C, and D).

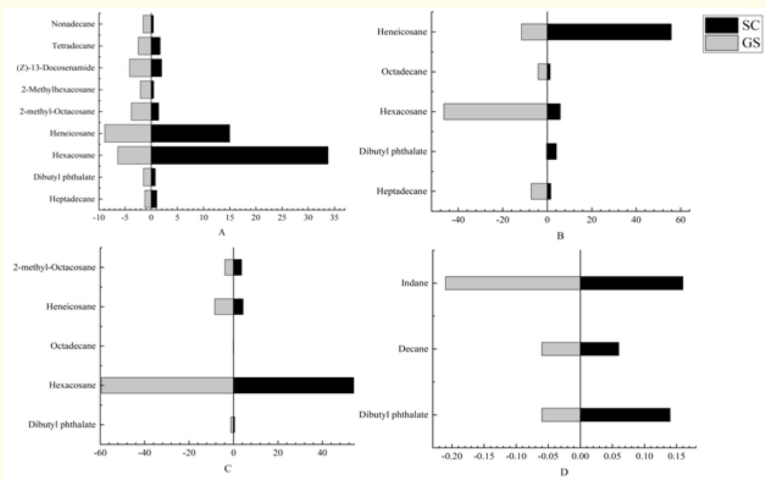

Figure 4: Comparison of the contents of the same compounds in *C. distans* from GS and SC in different periods.

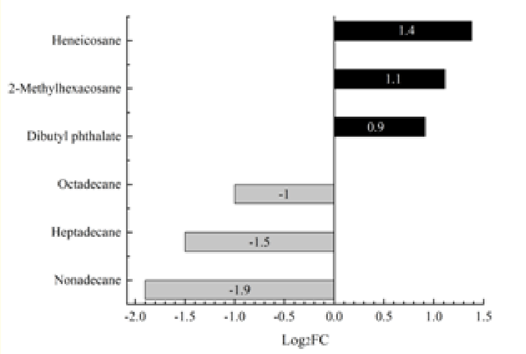

Figure 5: Comparison of differential metabolites of *C. distans* from GS and SC in different periods.

The differential metabolites in EOs of GS and SC C. distans at different periods

OPLS-DA model Variable Importance in Projection (VIP) was used to screen differential metabolites combined with Fold Change. Metabolites with Fold Change≥1.5, Fold Change≤0.8, and VIP≥1 were recognized as differential metabolites. The horizontal coordinate is the log 2 FC of differential metabolites, and the vertical coordinate is the differential metabolite. Greyre presents up-regulated differential metabolites, and black represents down-regulated differential metabolites. There are six differential metabolites, namely nonadecane, heptadecane, octadecane, dibutyl phthalate, 2-methyl-hexacosane, and heneicosane (figure 3). The first three differential metabolites were up-regulated, while the last three were down-regulated. These results showed that the chemical components of EOs were different due to producing areas.

In this study, alkanes accounted for the largest proportion of *C. distans* EOs under different climatic conditions in Sichuan and Gansu province. Nerol (36.72%) was the main compound in EO of *C. distans* grown in Hubei province of China [15]. In Liu's study, the major compound was piperitone (30%-40%), followed by geraniol (10%) [16]. Ma also reported that piperitone (20.02%) was the main compound in *C. distans* EOs, but the content of δ-4-carene (15.65%) ranked second [17]. The above research results are inconsistent with the research results of this paper. One of the reasons may be the different producing areas (geographical environment) of *C. distans* resulting in different chemical constituents of EOs; another reason is that the compounds of EOs obtained by different collection methods are also different. This paper adopts dynamic headspace method, and the above research is mainly water distillation method.

Conclusions

The chemical constituents of the EOs produced by *C. distans* from one producing area at different storage periods are different, and the compounds of the EOs from different producing areas in the same period are also different. The same metabolites (dibutyl phthalate, hexacosane, and heneicosane) obtained from different places can be used as the chemical finger print of *C. distans*. In addition, nonadecane, heptadecane, octadecane, dibutyl phthalate, 2-methyl-hexacosane, and heneicosane can be used as marker compounds to determine the origin of plants.

Acknowledgments

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