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Analysis of the essential oils of *Alpiniae Officinarum* Hance in different extraction methods

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Abstract. It was developed for the analysis of the essential oils of *Alpiniae Officinarum* Hance extracted by steam distillation (SD), ultrasonic assisted solvent extraction (UAE) and supercritical fluid extraction (SFE) via gas chromatography mass spectrometry (GC-MS) combined with retention index (RI) method. There were multiple volatile components of the oils extracted by the three above-mention methods respectively identified; meanwhile, each one was quantified by area normalization method. The results indicated that the content of 1,8-Cineole, the index constituent, by SD was similar as SFE, and higher than UAE. Although UAE was less time consuming and consumed less energy, the oil quality was poorer due to the use of organic solvents was hard to degrade. In addition, some constituents could be obtained by SFE but could not by SD. In conclusion, essential oil of different extraction methods from the same batch of materials had been proved broadly similarly, however, there were some differences in composition and component ratio. Therefore, development and utilization of different extraction methods must be selected according to the functional requirements of products.

1. Introduction

The rhizome of *Alpinia Officinarum* Hance (galangal), belongs to the *Zingiberaceae* family [5]. At present, the main producing area of the plant is located in Xuwen, Guangdong [17] .Galangal has been used as a traditional Chinese medicine for warming stomach for dispelling cold and relieving pain and digestion [13] . Therefore, the quality of galangal oil is directly affected the sensorial quality of fresh and processed products [9,10] . Steam distillation (SD) is a kind of traditional method of essential oil extraction, which was used in a majority of galangal oils extraction in past researches [12,14] . However, the method required long heating time and high temperature to gain the heat sensitive and easily hydrolyzed components. Accordingly, using different extraction methods are conducive to obtain the galangal oil of the actual demand.

Due to the components of essential oils are too complex to be identified by standard components individually. Meanwhile, the performance of an instrument and the separation effect of a column are different from one another, which could seriously affect the determination of components. Therefore, it is found that there is still widespread uncertainty in the determination of the components of the corresponding peaks with the matching degree of mass spectra. Kovats retention index (RI), the most widely used and accepted as a qualitative analysis method, which is an important parameter for the qualitative analysis of chromatography. As long as the chromatographic column is of the same property, the conditions are similar, and the RI calculated by different components in different instruments is usually constant. Consequently, the complex components of essential oils can be identified more accurately through the matching degree of the mass spectrum and RI [7,16] .

2. Materials and methods

2.1. Materials and chemical

Galangal was provided by the agricultural products processing institute of the Chinese Academy of Tropical Agricultural Sciences (Zhanjiang, China). Eucalyptol std. (1,8-Cineole) , *n*-alkanes std.(C7~C30) , methanol and *n*-hexane (both chromatographic grade) were all acquired from Sigma-Aldrich Chemical Co. (USA). Water was Milli-Q (Millipore, USA). Other reagents were all of analysis grade.

2.2. Extraction of essential oil

2.2.1 Steam distillation (SD). Initially, galangal plants were cleaned, dried, and crushed. Subsequently, the essential oil was gained by hydrodistillation in accordance with the method in Pharmacopoeia of the People's Republic of China [4].

2.2.2 Ultrasonic assisted solvent extraction (UAE). Firstly, the galangal powder was dealt with like above. Secondly, the powder with equal weight of ethyl ether were extracted by ultrasonic (Shenzhen haoshun ultrasonic instrument Co., Ltd., China), 360 W, 45℃，2 times, each time 20 min. Thirdly, The two filtrate were merged and placed in the rotary evaporator to gain concentration of 2 mL (Heidolph, German).

2.2.3. Supercritical fluid extraction (SFE). The powder dealt with like above was put into a bag, which was put in the reaction kettle. Then, the powder was extracted by supercritical CO_2 in 35℃ and 8 MPa, 3 times, each time 30 min.

After drying of the above three oils on anhydrous sodium sulphate (Na_2SO_4), the corresponding oils were respectively measured in a yield. And then the essential oils were kept at 4°C until further analyses.

2.3. Essential oils analysis

The identification and semi-quantification of three essential oils were performed on a GCMS-QP2010Plus instrument equipped with a quadrupole mass analyzer (Shimadzu, Japan). A fused silica capillary Rtx-5 ms (Restek, USA) column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used for the separation. The GC injection and MS interface temperatures were both maintained at 250 °C. Electron ionization (EI) was used as the ion source; and the electron impact energy was 70 eV as well as the ion source temperature was 230 °C. The following temperature program was used with a 2.5 min solvent delay. Initially, the temperature began at 60°C (held for 6 min) and then increased to 160℃ (held for 5 min) at a rate of 10°C/min, and then gradually increased to 240 °C (held for 10 min) at a rate of 20 °C/min using split injection mode. The constant flow rate of the carrier gas (Helium) was 1mL/min at a split ratio of 30:1. The EI mass spectra were set to scan from 40 to 450 atomic mass units (amu).

2.4. Compounds identification

The essential compounds were identified on the basis of a comparison of their retention index (RI) relative to *n*-alkanes (C_7~C_{30}), standard substance, as well as published data and EI mass spectra from the literature. Compounds were further compared and authenticated their MS data to the National Institute of Standards and Technology mass spectral library (NIST14) [2] and Wiley Registry of Mass Spectral Data, 9th Edition (Wiley9).

3. Results and discussion

3.1. Essential oil yield

Variation in essential oil yield among different extraction methods was observed (Table 1). Eucalyptol(1,8-Cineole),which has the pharmacological activity like insecticidal, antibacterial, antipyretic, dispelling wind and dispelling dampness, auxiliary drug penetration strong, is considered as the index active compound of the galangal oil [11] . In Pharmacopoeia of the People's Republic of China [3] , eucalyptolwas considered as the main compound to quantitative.

Table 1 The essential oil yield of galangal extracted by different methods (X±sd, n=3)

Methods	SD	UAE	SFE
Dry weight（g）	500.3±0.2	499.8±0.3	500.1±0.1
Essential oil yield（mL）	7.50±0.05	5.01±0.10	16.30±0.05
1,8-Cineole （mL）	1.69±0.12	1.04±0.03	1.72±0.2
Extraction ratio (mL/kg)	15.09	11.14	32.6

Although there was a huge difference among essential oil extraction ratio of different extraction methods, according to the results of GC-MS, essential oil yield and extraction ratio of three methods was not a positive proportion. This may be due to the concentration of essential oil in the UAE method by rotary evaporator, so that some essential oil was steamed out with ethyl ether simultaneously. In addition, the SFE method can be used to obtain a lot of low-polarity constituents, including certain heat sensitive and easily hydrolyzed compositions, so that the content of essential oil is higher than traditional SD method.

3.2. Composition of essential oil

The components of essential oils were identified by comparing their relative retention times, mass spectra similarity, retention index and comparison with standards. The results of analyses were listed in Table 2, in which the compounds were shown in the order of their elution time on the column.

Chemical compositions of galangal oils in different extraction methods were led to the identification of 52 constituents, respectively accounting for 98.67%, 88.76% and 98.36%. As shown in Table 2, 1,8-Cineole (22.59%, 36.82%, 10.55%) was the main compound of galangal oil extracted by different methods [8] . As the Table 1 shown, due to the different essential oil yield, the content of 1,8-Cineole extracted by the SD method was equivalent to the SFE method. The content of 1,8-Cineole gained by the UAE method was the lowest.

Table 2 Essential oil constituents of galangal oils from three different extraction methods

No.	**Constituents[1]**	**Ident[2]**	**Retention Times[3]**	**RI(Lab)[4]**	**RI[5]**	**Relative amount (%)**		
						SD	**UAE**	**SFE**
1	*α*-Thujene	a,b	6.109	924	925	0.72	-[6]	-
2	*α*-Pinene	a,b	6.431	931	933	5.92	2.21	0.54
3	Camphene	a,b	7.080	947	949	6.76	2.25	0.47
4	*β*-Pinene	a,b	8.055	976	978	7.12	5.93	1.25
5	*β*-Myrcene	a,b	8.341	990	991	3.69	1.66	0.39
6	*α*-Terpinene	a,b	8.934	1016	1018	2.98	0.8	0.65
7	*p*-Cymene	a,b	9.138	1024	1025	1.14	1.27	0.27
8	1-Limomenen	a,b	9.253	1028	1030	2.82	1.7	0.39
9	1,8-Cineole	a,b,c	9.369	1033	1032	22.59	36.82	10.55
10	*γ*-Terpinene	a,b,c	10.193	1065	1064	3.32	6.22	0.22
11	*α*-Terpinolene	a,b,c	10.295	1069	1063	0.32	2.42	0.16
12	Linalool	a,b	11.049	1099	1100	0.6	0.64	0.64
13	D-Fenchyl alcohol	a,b	11.354	1116	1115	0.71	0.16	0.21
14	Camphor	a,b	11.915	1147	1149	3.07	0.98	0.94
15	Terpinen-4-ol	a,b,c	12.564	1183	1180	2.42	0.76	1.1
16	*α*-Terpineol	a,b	12.828	1197	1195	4.89	2.21	4.27
17	Fenchyl acetate	a,b	13.349	1226	1228	1.05	0.24	0.45
18	2-Hydroxycineole	a,b	13.397	1230	1230	-	0.66	8.12

Continued to Table 2

19	Isobutyl benzoate	a,b	15.115	1334	1331	0.62	-	0.24
20	*α*-Ylangene	a,b	15.759	1382	1377	0.73	0.35	1.51
21	*α*-Copaene	a,b	15.934	1386	1382	0.35	0.57	0.84
22	*β*-Elemene	a,b	16.124	1400	1399	-	-	0.48
23	Phenethyl isobutyrate	a,b	16.139	1402	1402	0.6	-	0.88
24	*α*-Santalene	a,b	16.57	1427	1425	0.58	0.64	0.92
25	1,7-Diphenyl-(3,4-dihydroxyphenyl)-4-ene-3-heptanone	a,b	16.617	1430	1428	-	-	1.44
26	*β*-Caryophyllene	a,b	16.642	1431	1434	3.62	1.75	3.66
27	*trans*-Bergamotene	a,b	16.806	1441	1439	3.22	1.21	4.31
28	*α*-Guaiene	a,b	16.889	1446	1445	0.38	0.27	1.18
29	Alloaromadendrene	a,b	17.096	1458	1458	0.24	0.46	0.96
30	*α*-Caryophyllene	a,b	17.213	1465	1464	1.45	0.67	2.35
31	Germacrene D	a,b	17.426	1478	1480	0.66	0.58	0.86
32	Valencene	a,b	17.563	1486	1484	0.36	0.21	1.88
33	Cycloisolongifolene	a,b	17.802	1500	1502	-	-	1.05
34	*β*-Selinene	a,b	17.815	1501	1503	0.78	0.45	1.96
35	*α*-Farnesene	a,b	18.008	1509	1507	2.94	2.66	8.53
36	*β*-Bisabolene	a,b	18.099	1513	1511	0.51	0.61	1.15
37	*β*-Panasinsene	a,b	18.161	1516	1513	-	0.59	1.04
38	*γ*-Cadinene	a,b	18.328	1524	1524	4.12	5.11	7.34
39	*δ*-Cadinene	a,b	18.476	1530	1528	0.62	1.64	3.54
40	Selina-3,7(11)-diene	a,b	18.81	1552	1551	0.39	0.51	1.82
41	Germacrene B	a,b	19.345	1569	1562	1.16	0.32	3.67
42	Nerolidol	a,b	19.417	1570	1571	0.33	0.24	0.51
43	Caryophyllene oxide	a,b	19.932	1599	1595	2.81	-	0.73
44	Viridiflorol	a,b	20.007	1613	1609	0.75	0.28	0.88
45	*β*-Eudesmol	a,b	20.37	1651	1649	-	1.32	3.34
46	Cubenol	a,b	20.874	1632	1636	0.76	0.32	0.98
47	*α*-Muurolol	a,b	21.262	1650	1651	-	0.55	1.8
48	*α*-Cadinol	a,b	21.518	1661	1659	-	0.52	5.16
49	*α*-Bisabolol	a,b	22.339	1695	1695	-	-	0.38
50	*α-trans*-Bergamotol	a,b	22.551	1714	1708	0.25	-	0.55
51	*α*-Sinensal	a,b	22.642	1725	1731	0.32	-	0.78
52	5-Hydroxy-1,7-diphenyl-3-heptanone	a,b	22.828	1809	1805	-	-	1.02
	Total (%)					98.67	88.76	98.36

[1] Compounds by order of elution within each category.
[2]Method of identification: a: mass spectra; b: retention index, c: comparison with standard.
[3] Retention time in order with respect to the chromatogram.
[4] Retention Index on Rtx-5ms column in the laboratory.
[5] Retention Index on HP-5ms column [2].
[6] (-) Not detected.

It can be seen from Tables 1 and 2, the composition of essential oils of *Alpinia Officinarum* Hance were affected using different extraction methods. Compared with the SD method, the extraction temperature of the UAE method and the SFE method was no higher than 45℃ and the extraction system was without water. Therefore, it is possible to gain the heat sensitive and easily hydrolyzed components in the two methods, such as 2-Hydroxycineole, *β*-Panasinsene, *α*-Muurolol and α-Cadinol. 2-Hydroxycineole, is a hydroxyl functionalized 1,8-Cineole, which is similar with molecule structure of

1,8-Cineole. Hence, the odor and activity of the two is similar with each other. Due to the hydroxyl group, antimicrobial activity and aroma of 2-Hydroxycineole is better than 1,8-Cineole; meanwhile, the compound is soluble easily in water and decomposed rapidly at high temperature [15]. Accordingly, the compound could not be detected in the essential oil obtained by SD method.

The extractant of SFE method was supercritical fluid, so could be gained several unique compound, such as *β*-Elemene, 1,7-Diphenyl-(3,4-dihydroxyphenyl)-4-ene-3-heptanone, Cycloisolongifolene, *α*-Bisabolol, 5-Hydroxy-1,7-diphenyl-3-heptanone. 1,7-Diphenyl-(3,4-dihydroxyphenyl)-4-ene-3-heptanone and 5-Hydroxy-1,7-diphenyl-3-heptanone both belong to diphenylheptane compounds, which are mainly found in the Zingiberaceae plants. These compounds have been discovered a variety of biological and pharmacological activities, such as antioxidant, anti-inflammatory, antiemetic, antitumor and so on [1]. The diphenylheptane compounds are a class of low polar compounds with low volatility and thermal instability, which could not be obtained by SD method [6] .

4. Conclusion

The essential oils extracted by three different extractions were analyzed by GC-MS. It was found that most of volatile components were similar. The essential oil has the function of the auxiliary medicine transdermal absorption, which is one of the indexes to measure the drug quality and clinical curative effect. It could be seen that 1,8-Cineole, the main compound, obtained by the SD method was equivalent to the SFE method, and gained lowest by the UAE method. And 2-Hydroxycineole and diphenylheptane compounds were only extracted by the SFE method. Therefore, the SFE method can be used in some production for special needs. The volatile components of *Alpinia Officinarum* Hance from different extraction methods were various. It should be chosen suitable method according to the actual needs of development and utilization of specific functions.

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