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Research Article

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Ultrasound-Assisted Extraction and GC-MS Analysis of Zanthoxylum Oil from Zanthoxylum

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ABSTRACT

The volatile oil of zanthoxylum was extracted by ultrasound-assisted extraction method and its chemical composition was analyzed by GC-MS. Using an orthogonal array design, we determined the optimal extraction conditions to be 20 min, 60 kHz and 1:30 (g/mL) for extraction time, ultrasound frequency and solid-to-solvent ratio, respectively. The maximum yield of volatile oil under the optimized conditions was 8.61%. A total of 33 compounds were detected in the volatile oil, of which 28 were identified for the fi rst time, and its chemical composition was analyzed by GC-MS. accounting for 96.96% of the total volatile compounds. The major compounds identified were linoleic acid (27.43%), oleic acid (15.77%), limonene (14.25%), linalool (8.36%), linolcnic acid (6.85%), β-ocimene (3.78%), 1-(2-hydroxy-methyl-pyrrol-1-yl) ketone (2.74%), linaly acetate (2.72%), and β-myrcene (2.52%) etc.

Keywords: Zanthoxylum Oil, Gas Chromatography-mass Spectrometry (GC-MS), Ultrasound-Assisted Extraction.

INTRODUCTION

Ultrasonic assisted extraction is a new separation technology, it has a short extraction time, high efficiency, low energy consumption, the characteristics of this technology was widely used natural spices, plant enzymes, the extraction of active ingredients of Chinese herbal medicine, etc. [1-3] The mechanism of ultrasonic assisted extraction is the use of mechanical effect, mechanism of cavitation effect and heat effect, By increasing medium molecular movement speed, increase the penetration of the medium to the active components of extraction of biological [4, 5]. In the experiment, we chosed the ultrasonic assisted extraction of zanthoxylum oil extraction method, and analysesed the prickly ash oil, using gas chromatography-mass spectrum usage, the purpose for the sake of zanthoxylum oil edible value and medicinal value of development and utilization to provide certain scientific basis.

EXPERIMENTAL SECTION

Material, reagents and equipments

Material: Commercially available. After destemmed, air drying, crushed and sieving,set aside.Other are all analytical reagent.

Main instrument: DH-101 Electro- thermostatic blast oven; 601 Thermostatic water-circulator bath; TRACE GC UltraT Gas chromatography -ITQ1100 mass spectrometry; KQ100VDB Ultrasonica extractor; FA1104N Electronic analytical balance.

Experimental process

Take 1g pricklyash peel Powder (m_{powder}) in a bottle, join the petroleum ether soaking, then carries on the ultrasonic extraction, using ultrasonic extraction. In order to prevent the volatile oil damage due to high temperature in the ultrasonic extraction apparatus with ice, keep the temperature of water at 0□, filtering after ultrasonic extraction, put the filtrate into dry small beaker, its quality for m_1. In the fume hood the filtrate after natural volatilization, weighed for m_2.

Process flow diagram: Zanthoxylum particles→sorting→drying→weighing→leaching→ ultrasonic extraction→filter→volatiling→zanthoxylum oil.

Zanthoxylum oil yield =$[(m_2-m_1/m_{powder})] \times 100$ %.

GC-MS conditions

GC-MS conditions: HP-5 Quartz capillary column, 30 m×0.25mm×0.25μm; carrier gas: helium; temperature of the injection port: 250□; temperature of the sample port: 280□; column temperature: maintain 70 □ for 1 min, and thenincrease according to 2□/min to 150 □, again thenincrease according to 5 □/min to 250 □ lasts for 5 min; sample quantity: 1 μL; electron energy: 70 ev; ion source temperature: 200□.

Calculate the relative mass fraction of each component with the area normalization method, composition of relative mass fraction is more than 0.01% points for identification. On total ion flow chart of each chromatographic peak corresponding to mass spectrogram, reference to NIST2005 edition spectral library retrieval, artificial spectrogram analysis. According to the mass spectrum of each peak lobes, reference literatures and manual, compare the base peak, mass ratio and relative abundance, confirm each peak. According to the retention time and other reliable standard of mass spectrometry, for the final appraisal.

RESULTS AND DISCUSSION

Select extractants

With methanol, ethanol and petroleum ether as extraction solvent, solid-to-solvent ratio 1:20, ultrasonic assisted extraction time and temperature was fixed, study the effect of these three solvents on extraction rate of zanthoxylum oil, choose the best solvent.

Table 1 Effect on extraction rate of zanthoxylum oil

Items	1	2	3
Extraction solvent	Methanol	Anhydrous ethanol	Petroleum ether
Temperature（℃）	60	60	60
Ultrasonic extraction time（min）	20	20	20
Ultrasonic frequency（KHz）	40	40	40
Solid-to-solvent ratio（g/mL）	1:30	1:30	1:30
Extraction ratio（%）	7.62	5.36	8.07

The experimental results show that: under the same condition, the highest extraction yield of extraction solvent to zanthoxylum oil was petroleum ether, and easy to operate, so the petroleum ether was selected as extraction solvent in this experiment.

Single factor experiment

With petroleum ether as extraction agent, the influence of three factors on the zanthoxylum oil yield was respectively investigated: the particle size (30, 40, 50, 60, 80 mesh), ultrasonic extraction time (10, 15, 20, 25, 30 min) and solid-to-solvent ratio (1:10, 1:20, 1:30, 1:40, 1:50 (g/mL)).

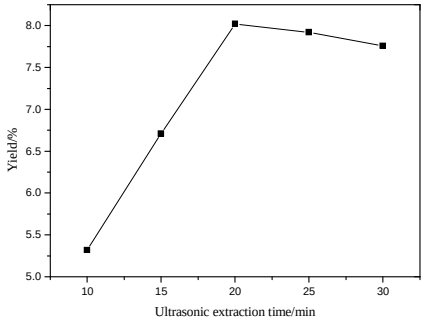

Fig. 1 Influence of ultrasonic extraction time on oil rate

Under the grain size 40 mesh, solid-to-solvent ratio 1:30 conditions, the influence of ultrasonic extraction time on zanthoxylum oil yield is shown in figure 1. The figure 1 shows that zanthoxylum oil yield achieved the maximum when ultrasonic extracted for 20 min, increase the extraction time, the oil rate gradually decreased. The reason was some essential oil and water emulsified, dispersed in water was difficult to separate, so the ultrasonic extraction for 20 min is advisable.

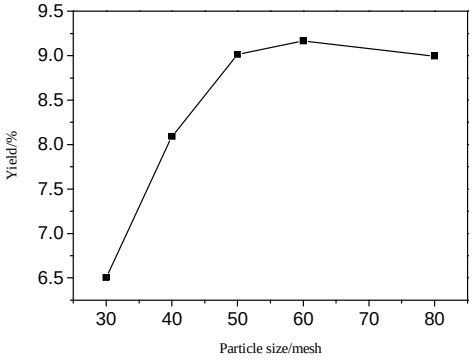

Fig. 2 Influence of particle size on oil rate

Ultrasonic extracted for 20 min, other conditions as above, the influence of particle size on zanthoxylum oil yield as shown in figure 2. The figure 2 shows that small particles prickly ash increases the contact area of extraction agent, and helpful to improve the extraction rate, but when the particle size greater than 60 mesh, oil extraction rate began to fall, the reason is the raw material grinding particle size has a dual effect on zanthoxylum oil yield efficiency. On the one hand, the mass transfer area of small particles increases, reduces the mass transfer distance and mass transfer resistance, is conducive to extraction, on the other hand, particles is too small, will be compacted under high pressure, thus increase the packing density of the material, as to make the zanthoxylum oil droplets is difficult to through the material layer into the solvent, eventually lead to lower yield efficiency. Comprehensive consideration, the 60 mesh chinese prickly ash particles is the best.

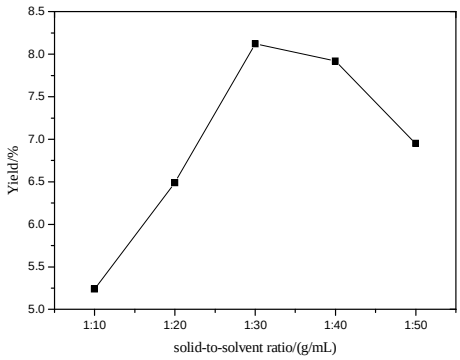

Fig.3 The influence of material liquid ratio on oil rate

Under the grain size 60 mesh, other conditions as above, the influence of material liquid ratio on oil rate as shown in figure 3. The figure 3 shows that when the material liquid is small, when the material liquid ratio is small, oil rate increases with the increase of material liquid ratio increasing, when solid-to-solvent ratio 1:30 the maximum yield efficiency is got, and then decreases with the increase of material liquid ratio. This is due to excessive solvent in the process of exsolution flavor material loss bigger, and material liquid ratio is too large, the subsequent processing costs will increase. So the solid-to-solvent ratio is suitable for 1:30.

Zanthoxylum oil extraction process optimization

On the basis of single factor experiment, the process conditions was optimized by orthogonal experiment. A: ultrasonic extraction time(min); B: particle size(mesh); C : solid-to-solvent ratio (g/mL).

Table 2 Factors level of orthogonal array design ($L_9(3^4)$)

Level / Factor	A	B	C
1	10	50	1:20
2	15	60	1:30
3	20	80	1:40

Table 3 Orthogonal array design and results for the optimization of extraction conditions

NO	A	B	C	Extraction ratio(%)
1	10	50	1:20	5.913
2	10	60	1:30	6.770
3	10	80	1:40	6.531
4	15	50	1:30	7.530
5	15	60	1:40	7.061
6	15	80	1:20	6.346
7	20	50	1:40	7.872
8	20	60	1:20	8.066
9	20	80	1:30	8.597
Ⅰ	6.397	7.105	6.908	
Ⅱ	7.112	7.299	7.766	
Ⅲ	8.172	7.152	7.155	
R	1.775	0.194	0.858	

The figure 5 shows that ultrasonic extraction time has the greatest influence on the extraction ratio, material liquid ratio the second, particle number is minimal. Extract optimum technological conditions were determined for A3B2C2, that is ultrasonic extraction time 20min, particle size 60 mesh, solid-to-solvent ratio 1:30 (g/mL). Repeat test three times in this conditions, the average oil at a rate of 8.607%.

Results of GC-MS usage analysis

Table 4 Chemical composition and relative content of zanthoxylum oil

NO	RT/min	Composition	Molecular formula	Relative content	Molecular weight
1	3.41	α-pinene	$C_{10}H_{16}$	2.51	136
2	4.33	β-phellandrene	$C_{10}H_{16}$	0.23	136
3	4.37	limonene	$C_{10}H_{16}$	14.25	136
4	4.47	β-ocimene	$C_{10}H_{16}$	3.78	136
5	4.74	β-myrcene	$C_{10}H_{16}$	2.59	136
6	6.27	α-ocimene	$C_{10}H_{16}$	1.63	136
7	6.39	linalool	$C_{10}H_{18}O$	8.36	154
8	6.80	terpinyl acetate	$C_{12}H_{20}O_2$	0.49	196
9	7.11	linaly acetate	$C_{12}H_{20}O_2$	2.72	196
10	7.73	ganyl acetate	$C_{12}H_{20}O_2$	0.37	196
11	9.17	nary acetate	$C_{12}H_{20}O_2$	0.15	196
12	10.23	Neroli	$C_{10}H_{18}O$	0.84	154
13	10.47	germacreneD	$C_{15}H_{24}$	0.52	204
14	11.23	β-caryophllene	$C_{15}H_{24}$	0.13	204
15	11.50	cetylic acid	$C_{16}H_{32}O_2$	1.86	256
16	14.43	caryophyllene oxide	$C_{15}H_{24}O$	0.17	220
17	14.65	linoleic acid	$C_{18}H_{32}O_2$	27.43	278
18	14.92	linolcnic acid	$C_{18}H_{30}O_2$	6.85	278
19	15.21	oleic acid	$C_{18}H_{34}O_2$	15.77	282
20	15.67	1-(2-hydroxy-methyl- pyrrol-1-yl) ketone	$C_7H_{13}NO_2$	2.74	144
21	16.97	5,8,11-seventeen carbon leukotriene -1-ol	$C_{17}H_{30}O$	0.18	250
22	17.49	Tributyl phosphate	$C_{12}H_{27}O_4P$	0.21	266
23	19.20	submersion	$C_{21}H_{27}NO_3$	0.85	342
24	22.12	erucic acid	$C_{22}H_{42}O_2$	0.32	338
25	24.16	camp sterol	$C_{28}H_{48}O$	0.15	400
26	26.46	β-sitoserol	$C_{29}H_{50}O$	0.09	414
27	27.96	2,4,6,8 - twelve carbon four en-1-carboxyl ate	$C_{14}H_{20}O_2$	1.41	220
28	30.38	α-tocopherol	$C_{29}H_{50}O_2$	0.36	430

A total of 33 compounds were detected in the volatile oil, of which 28 were identified for the fi rst time, and its chemical composition was analyzed by GC-MS. accounting for 96.96% of the total volatile compounds. The major compounds

identified were linoleic acid (27.43%), oleic acid (15.77%), limonene (14.25%), linalool (8.36%), linolcnic acid (6.85%), β-ocimene (3.78%), 1-(2- hydroxy-methyl-pyrrol-1-yl) ketone (2.74%), linaly acetate (2.72%), and β-myrcene (2.52%). Linalool, limonene is the main flavoring substances. GermacreneD content is high, at the same time also contains very rich in unsaturated fatty acid in zanthoxylum oil[7-9]. Oleic acid, linolenic acid, linoleic acid and other unsaturated fatty acid content is high in zanthoxylum oil, it is rich in nutritional value, followed by terpenoids, content of at least 30%, besides, limonene has antitumor, cough expectorant, dissolve gallstones, and other functions, and linalool is composed, antiviral effect[10], etc. therefore, zanthoxylum oil has very high medicinal value, is a collection of nutrition, delicious and health preservation in the integration of cooking oil.

CONCLUSION

Based on the single factor test the optimum conditions of ultrasonic assisted extraction of volatile oil from zanthoxylum were determined with orthogonal experiments. The optimum condition as follows: extracting 20min at 0□, ultrasonic frequency is 60KHz, and the rapeseed flowers/ethyl ether ratio(g:mL) 1:30, The yield achieved to 8.61%. The chemical components were qualified and quantified by gas chromatography-mass spectrometry (GC-MS). This method is low energy consumption, low cost, high efficiency, extract components completely, a new way of textracting similar natural is provided.

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