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Research Article

Microwave-assisted Extraction and GC-MS Analysis of *Zanthoxylum* Oil from *Zanthoxylum bungeanum*

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Abstract: The conditions for microwave-assisted extracting oil from *Zanthorylum bungeanum* seeds were studied and its chemical composition was analyzed by GC-MS. The effects of extract medium, microwave power, time and ratio of liquid to solid on extraction yield of *Zanthorvlure bungeanum* oil were investigated, using an orthogonal array design. The experimental results showed that using petroleum ether as extract medium, microwave power 350 w, solid-to-solvent ratio 1:5 (g/mL), 60 sec, can obtained 7.85% of *Zanthorylum bungeanum* oil. Thirty-three substances in *Zanthorylum bungeanum* oil were authenticated by GC-MS, of which 33 were identified for the first time and its chemical composition was analyzed. Accounting for 97.82% of the total volatile compounds.

Keywords: Analyses, Gas Chromatography-Mass Spectrometry (GC-MS), microwave-assisted extraction, *Zanthorylum bungeanum* oil

INTRODUCTION

Microwave-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., (Fu *et al*., 2006; Borges *et al*., 2004; Zhao *et al*., 2004). Microwave radiation induced to promote technology has high selectivity, short operation time, consumption, small volume, high rate of effective ingredients, does not produce noise, suitable for hot unstable substances, etc., (Zhao *et al*., 2005; Zhou *et al*., 2001). In the experiment, we chosed the microwave assisted extraction of *Zanthoxylum* oil extraction method and analyses 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.

MATERIALS AND METHODS

Materials: 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; TOPEX Microwave extraction plant; FA1104N Electronic analytical balance.

Experimental process: The Take 1 g pricklyash peel Powder (m_{powder}) in a bottle, join the petroleum ether soaking, then carries on the microwave extraction, using microwave extraction. Set the microwave power, temperature, time and solid-liquid ratio, reflux extraction. Extraction end, take out the flask, filtration, the filtrate after depressurized boiled water, put in the dryer to constant weight, weighing, calculating rate, the quality of dry small beaker 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 (Wang *et al*., 2014):

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

GC-MS conditions: HP-25 Quartz capillary column, 30m×250×0.25μm; carrier gas: helium; temperature of the injection port: 250°C; temperature of the sample port: 280°C; column temperature: maintain 70°C for 1 min and thenincrease according to 2°C/min to 150°C, again thenincrease according to 5°C/min to 250°C lasts for 5 min; sample quantity: 1 μL; electron energy: 70 ev; ion source temperature: 200°C.

The 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

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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 (Wang *et al*., 2014).

RESULTS AND DISCUSSION

Select extractions: With methanol, ethanol and petroleum ether as extraction solvent, ratio of liquid to solid at 3:1, microwave assisted extraction time and microwave power was fixed, study the effect of these three solvents on extraction rate of *Zanthoxylum* oil, choose the best solvent (Table 1).

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: microwave power (300, 350, 400, 450 and 500 w, respectively), particle size (30, 40, 50, 60 and 80 mesh, respectively), microwave extraction time (40, 50, 60, 70 and 80 sec, respectively) and solid-to-solvent ratio (1:3, 1:4, 1:5, 1:6, 1:7 (g/mL), respectively).

Influence of microwave extraction time on oil rate: With ether as extraction agent, extraction time was 60 sec and solid-liquid ratio was 1:5, the influence of different microwave power on the *Zanthoxylum* oil yield as shown in Fig. 1.

Figure 1 shows that the extraction rate was different under different microwave power. High microwave power is damage to essential oils, under high power, the extraction material of temperature rises in an instant, part of the essential oil losses on account of oxidation, results in the decrease of extraction yield. Under the condition of low power, reaction temperature does not rise too fast, resulting in higher extraction rate. But the microwave power is too low and reaches the purpose of heating. By the experiment that when microwave power 350 w, best extraction yield.

Influence of microwave extraction time on oil rate: Under the grain size 40 mesh, solid-to-solvent ratio 1:5 conditions, microwave power 350 w, the influence of microwave extraction time on *Zanthoxylum* oil yield is shown in Fig. 2. Figure 2 shows that *Zanthoxylum* oil yield achieved the maximum when microwave extracted for 60 sec, 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 60 sec is advisable.

Table 1: Effect on extraction rate of *Zanthoxylum* oil

Items	1	2	3
Extraction solvent	Methanol	Anhydrous ethanol	Petroleum ether
Extraction time (sec)	30	30	30
Microwave power (w)	300	300	300
Ratio of liquid to solid (mL/g)	3:1	3:1	3:1
Extraction ratio (%)	6.62	4.36	7.07

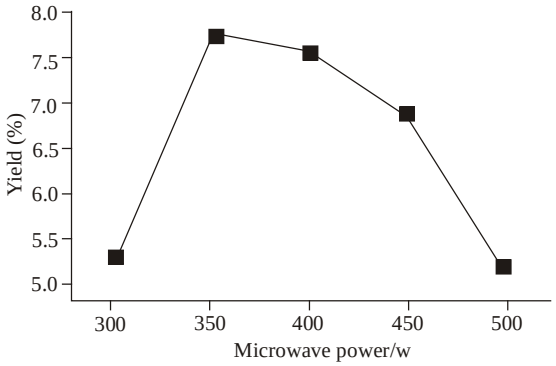

Fig. 1: Influence of microwave power on oil yield

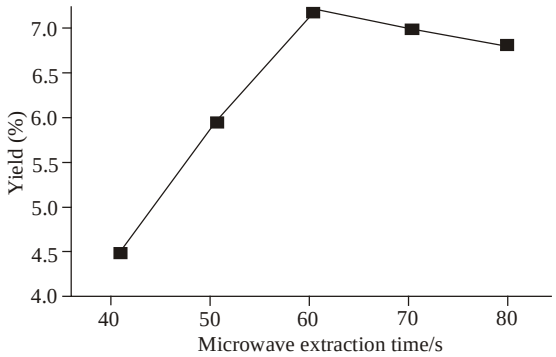

Fig. 2: Influence of microwave extraction time on oil rate

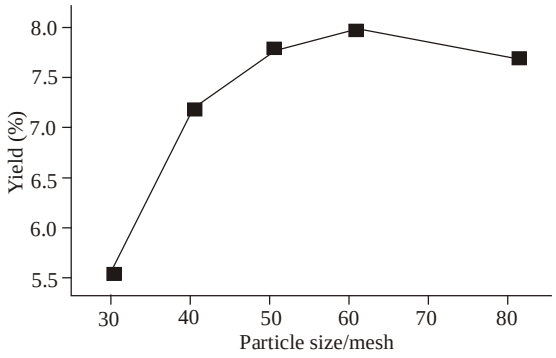

Fig. 3: Influence of particle size on oil rate

Influence of particle size on oil rate: Microwave extracted for 60 sec, other conditions as above, the influence of particle size on *Zanthoxylum* oil yield as shown in Fig. 3. Figure 3 shows that small particles prickly ash increases the contact area of extraction

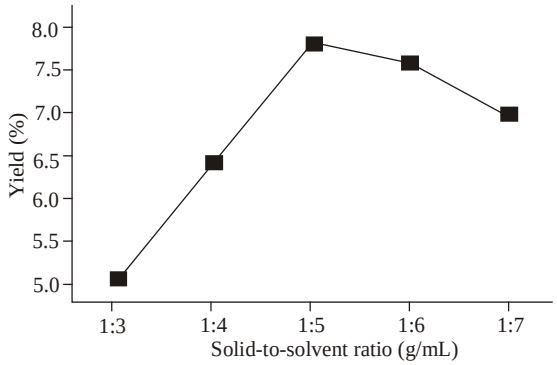

Fig. 4: The influence of material liquid ratio on oil rate

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.

Table 2: Factors level of orthogonal array design (L9 (34))

Level/factor	A	B	C
1	40	50	1:4
2	50	60	1:5
3	60	80	1:6

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

No.	A	B	C	Ction ratio (%)
1	40	50	1:4	5.41
2	40	60	1:5	6.27
3	40	80	1:6	6.03
4	50	50	1:5	7.03
5	50	60	1:6	6.56
6	50	80	1:4	5.85
7	60	50	1:6	7.27
8	60	60	1:4	7.57
9	60	80	1:5	8.10
I	5.903	6.570	6.277	
II	6.480	6.800	7.133	
III	7.647	6.660	6.620	
R	1.744	0.230	0.856	

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 Fig. 4. Figure 4 shows that when the material liquid is small, when the material liquid ratio is small, oil rate

Table 4: Chemical composition and relative content of *Zanthoxylum* oil

No.	Composition	Molecular formula	Relative content	Molecular weight
1	1, 3-dihydroxyacetone	$C_3H_6O_3$	0.67	90
2	2, 4-heptadine	$C_7H_{10}O$	6.31	110
3	2-heptene	$C_7H_{12}O$	0.23	112
4	Heptanal	$C_7H_{14}O$	0.21	114
5	Heptoic acid	$C_{10}H_{14}O_2$	1.25	130
6	α-pinene	$C_{10}H_{16}$	2.51	136
7	β-phellandrene	$C_{10}H_{16}$	0.23	136
8	Limonene	$C_{10}H_{16}$	14.15	136
9	β-ocimene	$C_{10}H_{16}$	3.57	136
10	β-myrcene	$C_{10}H_{16}$	2.52	136
11	α-ocimene	$C_{10}H_{16}$	1.63	136
12	Linalool	$C_{10}H_{18}O$	8.16	154
13	Terpinyl acetate	$C_{12}H_{20}O_2$	0.49	196
14	Linaly acetate	$C_{12}H_{20}O_2$	2.62	196
15	Ganyl acetate	$C_{12}H_{20}O_2$	0.37	196
16	Nary acetate	$C_{12}H_{20}O_2$	0.15	196
17	Neroli	$C_{10}H_{18}O$	0.84	154
18	GermacreneD	$C_{15}H_{24}$	0.52	204
19	β-caryophllene	$C_{15}H_{24}$	0.13	204
20	Cetylic acid	$C_{16}H_{32}O_2$	1.86	256
21	Caryophyllene oxide	$C_{15}H_{24}O$	0.17	220
22	Linoleic acid	$C_{18}H_{32}O_2$	27.32	278
23	Linolcnic acid	$C_{18}H_{30}O_2$	6.62	278
24	Oleic acid	$C_{18}H_{34}O_2$	15.57	282
25	1-(2-hydroxy-methyl- pyrrol-1-yl) ketone	$C_7H_{13}NO_2$	2.64	144
26	5, 8, 11-seventeen carbon leukotriene-1-ol	$C_{17}H_{30}O$	0.18	250
27	Tributyl phosphate	$C_{12}H_{27}O_4P$	0.21	266
28	Submersion	$C_{21}H_{27}NO_3$	0.85	342
29	Erucic acid	$C_{22}H_{42}O_2$	0.32	338
30	Camp sterol	$C_{28}H_{48}O$	0.15	400
31	β-sitoserol	$C_{29}H_{50}O$	0.09	414
32	2, 4, 6, 8 - twelve carbon four en-1-carboxylate	$C_{14}H_{20}O_2$	1.41	220
33	α-tocopherol	$C_{29}H_{50}O_2$	0.36	430

increases with the increase of material liquid ratio increasing, when solid-to-solvent ratio 1:5 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 1:5.

***Zanthoxylum* oil extraction process optimization:** On the basis of single factor experiment, with ethyl ether as extraction agent, the microwave power 350 w, the process conditions was optimized by orthogonal experiment. A: microwave extraction time (sec), B: particle size (mesh), C: solid-to-solvent ratio (g/mL) (Table 2 and 3).

The Table 3 shows that microwave 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, which is microwave extraction time 60 sec, particle size 60 meshes and solid-to-solvent ratio 1:5 (g/mL). Repeat test three times in this conditions, the average oil at a rate of 7.85%.

Results of GC-MS usage analysis: A total of 33 compounds were detected in the volatile oil and its chemical composition was analyzed by GC-MS. accounting for 97.82% of the total volatile compounds. The major compounds identified were linoleic acid (27.32%), oleic acid (15.57%), limonene (14.15%), linalool (8.16%), linolcnic acid (6.62%), β-ocimene (3.57%), 1- (2-hydroxy-methyl-pyrrol-1-yl) ketone (2.64%), linaly acetate (2.62%) and β-myrcene (2.52%). Linalool, limonene is the main flavoring substances (Thomas, 1998). Germacrene D content is high, at the same time also contains very rich in unsaturated fatty acid in *Zanthoxylum* oil (Bülow and Konig 2000; Magnusson *et al*., 1997; Gomes-Carneiro *et al*., 1998). 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, etc., (Zhou *et al*., 2002). Therefore, *Zanthoxylum* oil has very high medicinal value, is a collection of nutrition, delicious and health preservation in the integration of cooking oil (Table 4).

CONCLUSION

Based on the single factor test the optimum conditions of microwave assisted extraction of volatile oil from *Zanthoxylum* were determined with orthogonal experiments. The optimum condition as follows: extracting 60 sec, microwave power 350 w and the rapeseed flowers/ethyl ether ratio (g:mL) 1:5, The yield achieved to 7.85%. The chemical components were qualified and quantified by Gas Chromatography-Mass Spectrometry (GC-MS). This method is low energy consumption, low cost, high efficiency, extracts components completely, a new way of textracting similar natural is provided.

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