Tropical Journal of Pharmaceutical Research September 2018; 17 (9): 1839-1845
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i9.23

Original Research Article

Chemical components of volatile oil from *Cinnamomum jensenianum* Hand Mazz leaf in Yongzhou, and its antibacterial and antioxidant properties

Shao Jinhua[1,2], Zhang Yufei[3], Zhu Zhiyong[1,2], Chen Xiaoming[1,2], He Fulin[1,2]*
[1]Hunan Key Laboratory of Plant Resources Utilization, Hunan University of Science and Engineering, [2]Hunan Provincial Engineering Research Center for Ginkgo Biloba, Yongzhou, [3]Mudanjiang Medical University, Mudanjiang, Heilongjiang 157011, China

For correspondence:* ***Email:* *ra1216@163.com*

Sent for review: 11 June 2018 *Revised accepted: 26 August 2018*

Abstract

Purpose: *To study the chemical components, and in vitro antibacterial and antioxidant properties of volatile oil extracted from Yongzhou Cinnamomum jensenianum Hand. Mazz leaves.*
Methods: *The extraction process of volatile oil in the leaves of Yongzhou Cinnamomum jensenianum Hand. Mazz was optimized with respect to volatile oil yield, and the oil was subjected to gas chromatography-mass spectrometry (GC-MS) analysis. In vitro antimicrobial activities against bacteria and fungi were evaluated by the filter paper method, while in vitro antioxidant potential was determined by assaying its ability to scavenge DPPH radicals.*
Results: *The optimized extraction conditions for volatile oil from the leaves of Yongzhou Cinnamomum jensenianum Hand. Mazz were 3.5 h extraction time, solid-liquid ratio of 1:10, and soaking time of 4 h, which resulted in volatile oil yield of 2.4 ± 0.19 %. A total of 37 components were isolated and identified, accounting for 99.19 % of the volatile oil. The in vitro antimicrobial activities against bacteria and fungi were significant, with minimum inhibitory concentration (MIC) between 10 and 20 uL. At a volatile oil concentration of 0.09 mg/mL, the DPPH scavenging ability of the oil was stronger than that of BHT.*
Conclusion: *There is a high level of extractability of volatile oil from the leaves of Yongzhou Cinnamomum jensenianum Hand. Mazz. The main component is linalool (94.45 %), and it has good antibacterial and antioxidant properties.*

Keywords: *Yongzhou Cinnamomum jensenianum Hand. Mazz, Volatile oil, Antibacterial, Antioxidant*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Cinnamomum jensenianum Hand. Mazz is a member of the genus *Polygonaceae* and is distributed in Hubei, Hunan, Guizhou, Sichuan and Jiangxi Province [1-3]. Its branches and fruits contain aromatic oils that can be used as industrial raw materials. At present, only few studies have been carried out on the chemical components of *Cinnamomum jensenianum* Hand. Mazz, and there are only two studies on the analysis of the volatile components of the leaves of Yongzhou *Cinnamomum jensenianum* Hand. Mazz and their antimicrobial activities.

There are significant differences in the anatomical characteristics, tissue structure, and material content of *Cinnamomum jensenianum* grown in different regions [4-6]. Therefore, it is necessary to study the extraction process and the *in vitro* biological activities of Yongzhou *Cinnamomum jensenianum* Hand. Mazz [7-9].

In this study, volatile oil was extracted from *Cinnamomum jensenianum* by stream distillation, and analyzed by GC-MS. The results were compared with previous data through database search for qualitative and quantitative analyses. The oil was also assessed for antioxidant property using DPPH* scavenging assay, in addition to determination of its *in vitro* antibacterial activity.

EXPERIMENTAL

Yongzhou *Cinnamomum jensenianum* Hand. Mazz was purchased from Hunan Health-Guard Bio-tech Inc. (product number = 151109). The product was shade-dried to a constant weight, and then ground, sifted, sealed in a plastic bag, and stored in the refrigerator at 4 °C

Reagents

Sodium sulfate (Na_2SO_4) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China); ethyl ether was purchased from Shenzhen Jianyuan Chemical Co., Ltd. (Shenzhen, China); *Bacillus subtilis* ATCC6633, *Staphylococcus aureus* ATCC06538 and *Escherichia coli* CMCC44103 were provided by Chengdu Institute of Biology, Chinese Academy of Sciences, Culture Collection Center. *Penicillium notatum* and *Aspergillus niger* were provided by the microbiology laboratory of Hunan University of Science and Engineering.

Extraction of volatile oil

The plant product (100 g) was soaked in distilled water in a 2,000-mL round-bottomed flask for a period of time, and extracted with a volatile oil extractor. The oily liquid in the volatile oil extractor was dehydrated over anhydrous Na_2SO_4.

Estimation of volatile oil yield

The yield of volatile oil was calculated according to Eq 1.

$$C = \{(m_1 - m_2)/m_3\} \ldots\ldots\ldots (1)$$

where C (in %) is the yield of volatile oil; m_1 (in g) is weight of the bottle and oil; m_2 (in g) is the weight of bottle; m_3 (in g) is the weight of dried powder of product.

Optimization of extraction conditions of volatile oil

Influence of duration of extraction on volatile oil yield

Five (5) parts (100 g each) of dry powder were placed separately in 2000-mL round bottom flasks, and 1200 mL of distilled water was added. The mixture was allowed to soak for 8 h, and then extracted with a volatile oil extractor for different periods i.e. 2, 3, 4, 5, and 6 h. In each case, the oily liquid in the volatile oil extractor was collected and dried over anhydrous sodium sulfate. The yield of volatile oil was calculated to determine the optimum extraction time.

Influence of powder: liquid ratio on yield of volatile oil

Five (5) parts (100 g each) of dry powder were put separately in 2000-mL round bottom flasks, and 600, 800, 1000, 1200, and 1400 mL of distilled water were added to different flasks. The mixtures were allowed to soak for 8 h, and then extracted with a volatile oil extractor for 4 h.

The oily liquid in the volatile oil extractor was collected and dried over anhydrous sodium sulfate. The yield of volatile oil was calculated to determine the optimum solid-liquid ratio.

Impact of soaking duration on volatile oil yield

Five parts (100 g each) of dry powder were mixed separately with1200-mL portions of distilled in 2000-mL round bottom flasks for different periods of time, ranging from 2 to 10 h. They were then extracted with a volatile oil extractor for 4 h.

The oily liquid in the volatile oil extractor was collected and dried as usual over anhydrous sodium sulfate, and the oil yield was calculated to determine the optimum soaking time.

Orthogonal experiment

In order to determine the optimum extraction conditions for the volatile oil, the extraction time (A), liquid-solid ratio (B) and soaking time (C) were evaluated, and L9 (3^3) was used for orthogonal studies on the basis of outcomes of the single factor extraction studies above. The design is shown in Table 1.

Table 1: Design used for orthogonal test

No.	A (h)	B (g/mL)	C (h)
1	3	10:1	6
2	4	12:1	8
3	5	14:1	10

Conditions for GC-MS analysis

The volatile oil was subjected to GC–MS analysis in a Perkin Elmer TurboMass GC mass spectrometer at a temperature of 270 ℃ and 5-min desorption. An Agilent HP-1 column of dimensions 30 m × 0.25 mm, and stationary phase thickness of 0.25 µm (J & W Scientific) was used, with helium as carrier gas at the rate of 1 mL/min. The injection volume and split ratio were 1 µL and 20:1, respectively, and the pre-column pressure was 8.04 psi (55.44 k Pa). The column temperature was maintained for 3 min at 60 ^{o}C, raised at 10 ^{o}C per min to 280 ℃, and kept constant for 10 min at 280 ^{o}C. The mass detector used functioned at 70 eV within 30 – 600 m/z in ionization mode, with an interface temperature of 230 ℃. The MS database was NIST05a. 5L.

Determination of antibacterial activity of volatile oil

Disc diffusion procedure was used to determine the diameter of the zone of inhibition of the volatile oil. The minimum inhibitory concentration (MIC) was estimated as outlined previously [10].

Evaluation of DPPH radical scavenging capacity of volatile oil

Equal volumes of 0.03, 0.06, 0.09, 0.12, 0.15, and 0.18 mg/mL *Cinnamomum jensenianum* Hand. Mazz volatile oil in different test tubes were incubated with 2 mL of 0.1 mmol / L DPPH solution for 30 min in the dark. The absorbance (A_i) of each tube was measured at 518 nm [11]. A mixture of equal volumes of ethanol solution of volatile oil, and anhydrous ethanol was similarly treated, but without DPPH•, and the absorbance (A_j) was measured at 518 nm. In another tube, equal volumes of ethanol and DPPH• solution were also treated similarly, and the absorbance (A_0) was determined. A tube containing BHT in place of volatile oil served as control, and the DPPH• scavenging capacity (W) was estimated using the equation 2.

$$W = \{1-[(A_i-A_j)/A_0]\} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where W (in %) is the DPPH radical scavenging capacity; A_i is the absorbance of volatile oil solution and DPPH; A_j is the absorbance of oil solution and anhydrous ethanol; and A_0 is the absorbance of the control.

Statistical analysis

The results are presented as mean ± standard deviation (SD). They were analyzed for statistically significant differences using Duncan multiple comparison (SSR method). All analyses were done with Microsoft Office 2007, Design Expert 8. 0.5b and SPSS. Values of $p < 0.05$ were taken as indicative of significant differences between treatments groups.

RESULTS

Single-factor optimization

Influence of duration of soaking on volatile oil yield

Figure 1 shows the effect of soaking time on the yield of volatile oil. The yield of volatile oil increased with increase in the duration of soaking, and attained a maximum value at a soaking time of 8 h. Beyond 8 h, the yield of volatile oil was almost constant.

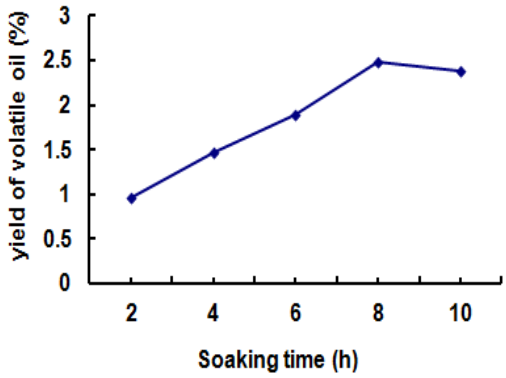

Figure 1: Effect of soaking time on the yield of volatile oil. Different letters indicate significant differences between groups

Effect of extraction time on the yield of volatile oil

The yield of volatile oil increased as extraction time was increased, and reached maximum when the duration of extraction was 4 h (Figure 2). However, the yield stabilized beyond 4 h and became almost constant, indicating that the volatile oil was already completely extracted.

Effect of powder:liquid ratio on the yield of volatile oil

The effect of powder : liquid ratio on the yield of volatile oil is shown in Figure 3. The yield of

volatile oil increased with increase in the amount of distilled water used. The yield of volatile oil reached maximum at powder : liquid ratio of 1:12. However, with further increase in distilled water, the yield of volatile oil showed a downward trend.

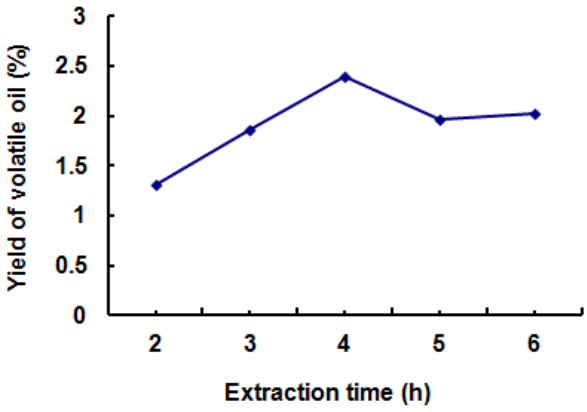

Figure 2: Effect of extraction time on the yield of volatile oil. Different letters indicate significant differences between groups

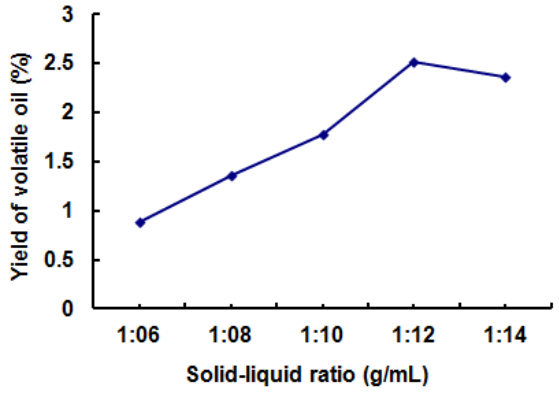

Figure 3: Influence of solid (powder): liquid ratio on oil yield. Different letters indicate significant differences between groups

Orthogonal optimization

From Table 2, the order of factors that affect the yield of volatile oils was: A > B > C, and the optimum process parameters were A_3 B_2 C_3, i.e. extraction time of 5 h, solid-liquid ratio of 1:12, and soaking duration of 10 h.

GC-MS results

Thirty-seven compounds were isolated and identified, accounting for 99.19 % of the volatile oil. The main chemical component was linalool (1,6-octadien-3-ol) which accounted for 94.45 %). The other components were present in trace amounts (Table 3).

Antibacterial activity

As shown in Table 4, the volatile oil exerted the strongest antibacterial ability against *Aspergillus niger* while the effect on *Penicillium, B. subtilis, S. aureus*, and *E. coli* was milder. The antibacterial activity against fungi was stronger than that against bacteria.

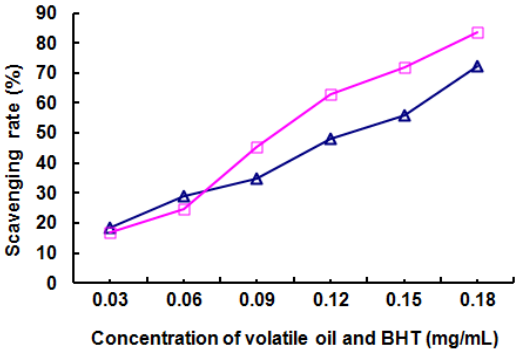

Figure 4: DPPH• scavenging capacity of volatile oil, relative to that of BHT. Different letters indicate significant differences between the two. □ = Scavenging by volatile oil; △= Scavenging by BHT

Table 2: Outcome of orthogonal experiment

Test number	A （h）	B （mL/g）	C (h)	Yield of volatile oil (%)
1	1	1	1	0.6
2	1	2	2	1.6
3	1	3	3	1.5
4	2	1	2	1.8
5	2	2	3	2.3
6	2	3	1	2.0
7	3	1	3	2.1
8	3	2	1	2.0
9	3	3	2	2.2
k_1	1.233	1.500	1.533	
k_2	2.033	1.967	1.867	
k_3	2.100	1.900	1.967	
R	0.867	0.467	0.434	

Table 3: Chemical components of volatile oil from Yongzhou *Cinnamomum jensenianum*

S/no.	Retention time/min	Chemical components	Molecular Formula	Molecular weight	Relative Content (%)
1	5.395	Alpha-pinene	$C_{10}H_{16}$	136.2	0.071
2	6.694	Alpha-phellandrene	$C_{10}H_{16}$	136.3	0.02
3	7.062	1-Methyl-4-(1-methylethyl) benzene	$C_{10}H_{14}$	134.1	0.059
4	7.135	D-Limonene	$C_{10}H_{16}$	136.2	0.056
5	7.467	4-Hydroxybenzaldehyde	$C_7H_6O_2$	122.03	0.054
6	7.933	Alpha-methyl-alpha-[4-methyl-3-pentenyl]oxiranemethanol	$C_{10}H_{18}O_2$	170.13	0.628
7	8.216	2-Furanmethanol,5-ethenyltetrahydro-alpha, alpha,5-trimethyl-, cis-	$C_{10}H_{18}O_2$	170.13	0.772
8	8.537	1,6-Octadien-3-ol	$C_{10}H_{18}O$	154.13	94.449
9	9.612	2H-Pyran-3-ol,6-ethenyltetrahydro-2,2,6-trimethyl-	$C_{10}H_{18}O_2$	170.13	0.059
10	10.005	Estragole	$C_{10}H_{12}O$	148.08	0.055
11	10.802	1,6-Octadien-3-ol,3,7-dimethyl-,3-(2-aminobenzoate)	$C_{17}H_{23}NO_2$	273.17	0.052
12	11.062	2,6-Octadienal, 3,7-dimethyl-	$C_{10}H_{16}O$	152.12	0.025
13	11.147	Cinnamaldehyde, (E)-	C_9H_8O	132.05	0.069
14	11.304	1,7,7-TRIMETHYLBICYCLO [2.2.1] HEPT-2-YL PHENYLACETATE	$C_{12}H_4O_3$	196.14	0.183
15	12.325	Eugenol	$C_{10}H_{12}O_2$	164.08	0.029
16	13.195	*(1R,4Z,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene*	$C_{15}H_{24}$	204.18	0.289
17	13.491	Benzofuran	C_8H_6O	118.04	0.12
18	13.636	alpha-Caryophyllene	$C_{15}H_{24}$	204.18	0.256
19	14.464	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	$C_{15}H_{24}$	204.18	0.271
20	14.736	alpha-Calacorene	$C_{15}H_{20}$	200.15	0.024
21	14.881	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	$C_{15}H_{26}0$	222.19	0.526
22	15.183	1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,	$C_{15}H_{24}O$	220.18	0.261
23	15.267	Caryophyllene oxide	$C_{15}H_{24}O$	220.18	0.638
24	15.376	Guaiol	$C_{15}H_{26}0$	222.19	0.077
25	15.57	3-Cyclohexen-1-carboxaldehyde	$C_9H_{14}O$	138.10	0.11
26	15.878	1-Methyl-4-((E)-1,5,9-trimethyl-8-methylene-dec-4-enyl)-2,3-dioxa-bicyclo[2.2.2]oct-5-ene	$C_{15}H_{24}$	204.18	0.156

Table 4: Inhibition zone diameter and MIC of volatile oil from Yongzhou *Cinnamomum jensenianum*

Volume of volatile oil (µL)	Diameter of growth (mm)				
	Escherichia coli	***Bacillus subtilis***	***Staphylococcus aureus***	***Penicillium***	***Aspergillus niger***
5	12±0.23a	15±0.27a	18±0.34a	24±0.31a	36±0.32a
10	21±0.3b	29±0.22b	32±0.23b	35±0.28b	46±0.25b
15	34±0.2c	41±0.31c	47±0.39c	54±0.33c	-
20	56±0.3d	69±0.28d	-	-	-
25	-	-	-	-	-

"-" indicates no cell growth; each value is mean of 3 replicates ± SD. Different letters depict significant differences at $p = 0.05$

The minimum inhibitory concentration (MIC) of volatile oil was determined for each strain tested. The MIC value for *Aspergillus niger* was 10 uL; while the MIC for *Penicillium* and *Staphylococcus aureus* was 15 uL. For *Escherichia coli* and *Bacillus subtilis,* the MIC was 20 uL.

DPPH• scavenging potential

The DPPH• scavenging ability gradually increased with increase in volatile oil and BHT concentrations. When the concentration of volatile oil reached 0.09 mg/mL, the volatile oil scavenged DPPH• more efficiently than BHT.

DISCUSSION

The essential oil produced by the secondary metabolism of *Cinnamomum jensenianum* Hand. Mazz is the main active component of the plant. Studies have demonstrated variabilities in characteristics, tissue structure, and material

contents of the same plant resources grown in different regions [12-13]. This necessitated the study of Yongzhou *Cinnamomum jensenianum* Hand. Mazz. In this study, steam distillation method was used to extract volatile oil from the plant. Optimization of the extraction process showed that extraction duration of 5 h, solid-liquid ratio of 1:12, and soaking duration of 10 h resulted in volatile oil yield of 2.4 ± 0.19 %. This yield is higher than what was obtained in previous studies.

The major component of the volatile oil was linalool, which accounted for 94.45 %. Linalool is widely used in flavors and fragrances, health care, synthetic vitamin A, synthetic anticancer drugs, deodorants, dental applications, insect repellents and insecticides. The annual demand of linalool for the formulation of various flavors worldwide is as high as 10,000 tons [13-15]. The present study has demonstrated that the volatile oil has good antibacterial and antifungal properties. The MICs were in the range 10 - 20 uL, which are better than MICs reported in previous studies. At a concentration 0.09 mg / mL, the DPPH scavenging capacity of the oil was stronger than that of BHT, and its antioxidant activity was also better. The antioxidant and antibacterial properties of the oil may be due to linalool, which is consistent with the report of Deepak [16-20]. Future studies will be focused on the composition, antibacterial activities and antioxidant activities of volatile oils of different origins, so as to provide theoretical support for research and comprehensive development of the volatile oils from this plant.

CONCLUSION

The content of volatile oil in Yongzhou *Cinnamomum jensenianum* Hand. Mazz is relatively high. The method of steam distillation is a simple and efficient method for obtaining volatile oil from this species. The volatile oil of *Cinnamomum jensenianum* Hand. Mazz has good antibacterial and antioxidant activities.

DECLARATIONS

Acknowledgement

We greatly acknowledge the financial supports from the Hunan Provincial Natural Science Fund (2018JJ3194).

Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. He Fulin conceived and designed the study. Shao Jinhua, Zhang Yufei, Zhu Zhiyong, Chen Xiaoming, He Fulin collected and analysed the data, while Shao Jinhua wrote the manuscript.

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