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COMPARATIVE STUDY ON VOLATILE COMPOUNDS OF AGARWOOD FROM KHANH HOA PROVINCE EXTRACTED BY DIFFERENT METHODS

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ABSTRACT

Agarwood from *Aquilaria crassna* cultivated in Khanh Hoa province was extracted by 5 methods, including hydrodistillation, enzyme-assited, microwave-assisted hydrodistillation, extraction with solvent and supercritical carbon dioxide. The volatile compounds of the products were analyzed by GC-MS. Almost all samples contain some common characteristic components, such as benzylacetone, 10-epi-γ-eudesmol, agarospirol, valerianol, β-eudesmol, α-eudesmol, neopetasane, and dihydrokaranone. However, there were obvious differences in yields, composition, and number of identified components. The application of the more advanced methods for the extraction of agarwood resulted in saving of time and energy, and some improvement of product yield. These methods also lead to unconventional products with characteristic chemical compositions, what requires further investigations.

Keywords: agarwood, *Aquilaria crassna*, essential oil, hydrodistillation, extraction.

1. INTRODUCTION

Agarwood (also known as Aloeswood, Eaglewood and Gaharu, among many common names) is the highly valuable resinous and fragrant heartwood of the *Aquilaria*, a genus of the Thymelaeaceae family which has damage on trunks or branches of the trees. Agarwood and its essential oil can be used to produce incenses for religious ceremonies, perfumes and medicines. Nineteen species are found in the areas from India eastward throughout the Southeast Asia, south China. In Vietnam, four species, *A. crassna* Pierre ex. Lecomte, *A. banaensae* Phamh., *A. baillonii* Pierre ex. Lecomte, and *A. rugosa* K.Le-Cong & Kessler, were found distributed in the provinces Quang Binh, Quang Tri, Thua Thien Hue, Quang Nam, Da Nang, Quang Ngai, Binh Dinh, Ninh Thuan, Binh Thuan, Tay Nguyen, An Giang, Kien Giang and on the island Phu Quoc. However, *A. crassna* Pierre ex. Lecomte is the main source of agarwood cultivated in Vietnam [1-5]. Studies showed that characteristic components of essential oil of agarwood are sesquiterpenoids, chromones, and volatile aromatic compounds [6-8]. Many methods were

developed to extract essential oil from Agarwood [9]. In this report, we examined and compared the chemical composition of the essential oils and oleoresins obtained from a Vietnamese agarwood (*Aquilaria crassna*, cultivated in Khanh Hoa province) by different extraction methods.

2. MATERIALS AND METHODS

2.1. Materials

The agarwoods using as raw material for this study were supplied by Mr. Nguyen Thoan, forest engineer and director of the Lam Vien Co. Ltd, Hanoi, Vietnam. The agarwood producing plants were cultivated in Khanh Hoa province and were identified as *Aquilaria crassna* Pierre ex. Lecomte (family Thymelaeaceae) by Professor Le Cong Kiet, Faculty Biology and Biotechnology, University of Science, Vietnam National University Ho Chi Minh City, where the voucher specimens are deposited. The pleasant smelling, resinous woods were obtained from 15 years old trees which were artificially inoculated with a preparation containing specific fungi (a product supplied by Lam Vien Co. Ltd). Three years after inoculation, the trees were harvested; the unaffected parts of the heartwoods were removed by hand to yield the agarwoods. The agarwood samples were air dried, ground to a powder, packed in paper bags and kept at room temperature before extraction.

2.2. Extraction methods

2.2.1. Hydrodistillation

500 g of agarwood sample were soaked in a 2 L round-bottom glass bottle with distilled water. After 3 weeks, hydrodistillation was carried out in an all-glass Clevenger-type distillation apparatus for 72 h. The volatile oil was collected from the receiver arm of the apparatus and placed into a clean and previously weighed sample bottle and refrigerated until analysis. The sample was labelled E1.

2.2.2. Enzyme-assisted hydrodistillation

500 g of agarwood sample were soaked in a 2 L round-bottom glass bottle with distilled water for 24 hours. Then a mixture of the enzymes Laccase 1.0 ml/g and Htec-2 1.5% was added. After 10 hours incubation at 40 ^{o}C, hydrodistillation was carried out in an all-glass Clevenger-type distillation apparatus for 72 hours. The volatile oil was collected and treated as described above and was labelled E2.

2.2.3. Microwave-assisted hydrodistillation

500 g of agarwood sample were soaked in a 2 L round-bottom glass bottle with distilled water. After 3 weeks, hydrodistillation was carried out for 6 hours in an all-glass Clevenger-type distillation apparatus using microwave oven at 650 W. The volatile oil was collected and treated as described above and was labelled E3.

2.2.4. Solvent extraction using chloroform

50 g of agarwood sample were extracted with hot chloroform (50 ^{o}C for 5 hours). The obtained solution was filtered and concentrated under reduced pressure to yield a dark brown, viscous residue. The residue was refrigerated until analysis. The sample was specified as "agarwood oleoresin by chloroform extraction" and labelled E4.

2.2.5. Extraction using supercritical CO_2

50 g of agarwood sample were extracted with supercritical CO_2 (sCO_2). The obtained solution in ethanol was filtered and concentrated under reduced pressure to yield a dark brown viscous residue. The residue was refrigerated until analysis. The sample was specified as "agarwood oleoresin by supercritical CO_2 extraction" and labelled E5.

2.3. Analysis of essential oils

Gas chromatography (GC) analyses of the essential oils were carried out on an Agilent Technologies HP7890A GC equipped with a mass spectrum detector (MSD) Agilent Technologies HP5975C and a DB-XLB column. The dimensions of the column are 60 m x 0.25 mm x 0.25 µm. The injector was set at 250 ^{o}C. The temperature program was 40^{o}C ramp of 20 ^{o}C/min up to 140 ^{o}C, subsequent increase to 270 ^{o}C with a 4 ^{o}C/min heating ramp. The carrier gas was Helium at a flow rate of 1 $mL.min^{-1}$. The split ratio was 100:1 and 1 µL of essential oils was injected into the GC. The MSD conditions were: full scan modes under electron impact ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range 35-450 amu under full scan.

Retention time indices RI of each component was determined relative to the retention times of a homologous *n*-alkane series with the same GC program. The relative amounts of individual components were calculated based on the GC peak area (MSD response) without correction.

2.4. Identification of constituents

The identification of the constituents was performed by MassFinder 4.0 using their mass spectra and retention indices (RI). Further identification was performed by comparing their RI and their mass spectrum with those from HPCH1607, W09N08 libraries and NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/) database.

3. RESULTS AND DISCUSSION

3.1. Extraction products and yields

Fragrant volatiles containing products, including essential oils and oleoresins, were obtained from agarwood by 5 different extraction methods. The product type and yield of each extraction method are shown on Table 1. Hydrodistillation has low yield and high energy consumption (soaked in water for 3 weeks and distilled for 72 hours). Adding enzymes mixture during soaking (1 - 2 days) before hydrodistillation improved the yield of essential oil. Using microwave oven in distillation has low yield but save more energy than hydrodistillation (6 hours vs. 72 hours). Extraction with chloroform has the lowest yield but take only 5 hours for all steps as compared to conventional method (up to 4 weeks). The sCO_2 extraction has very high yield and do not need soaking.

Table 1. Product types and yields of agarwood extraction by 5 different methods.

Sample	Extraction method	Type of product	Yield (%, w/w)
E1	Hydrodistillation (HD)	Essential oil	0.25
E2	Enzyme-asisted HD	Essential oil	0.32
E3	Microwave-assisted HD	Essential oil	0.20
E4	Solvent extraction with chloroform	Oleoresin	0.15
E5	Supercritical CO2-extraction	Oleoresin	0.27

3.2. Volatile components of the extraction products

Samples E1 - E5 representing products from the 5 extraction methods were analyzed by GC-MSD. Totally, 57 peaks on the gas chromatogram with RI ranging from 1000 to 2600 were studied. The results are shown in Table 2. A great number of other minor peaks visible in the gas chromatograms could not yet be studied.

Table 2. Chemical composition (Area %) of the extraction products.

No.	RI	Compound	E1	E2	E3	E4	E5
1	1001	Benzaldehyde	0.13	0.28			0.23
2	1192	4-Ethylphenol		0.18			
3	1211	Decan-2-one				1.39	
4	1285	Undecan-2-one				0.41	
5	1288	Benzylacetone	2.88	1.07	1.09	0.91	0.62
6	1507	Selina-4(15),7,11-triene		0.28		0.27	
7	1509	10-Epi-γ-selinene		0.16		0.18	
8	1511	α-Guaiene			0.59	0.50	
9	1519	ß-Agarofuran	7.19	3.21		13.20	
10	1537	α-Bulnesene		0.39	0.22	0.47	
11	1550	ß -Dihydroagarofuran	0.34			0.58	
12	1562	Anisylacetone					0.42
13	1599	α-Agarofuran		0.36		0.54	
14	1628	Epoxybulnesene	1.49	2.61	1.79	3.21	
15	1641	2,14-Epoxy-vetispir-6-ene			2.91		
16	1644	Unknown	2.73			1.33	
17	1676	10-Epi-γ-eudesmol	2.31	1.16	0.94	2.19	0.59
18	1682	γ-Eudesmol		1.49		2.66	
19	1685	Agarospirol	5.58	0.97	4.55	4.72	2.33
20	1688	Guaiol		0.40		0.72	2.06
21	1690	Hinesol		0.45	0.85	1.34	
22	1694	Unknown	2.08	1.97	1.31	2.19	
23	1699	Jinko-eremol	5.00	2.08	5.53	5.19	

24	1704	Valerianol	4.93	2.67	5.38	5.88	2.10
25	1713	β-Eudesmol	4.08	1.43	2.77	5.48	1.10
26	1716	α-Eudesmol	2.09	2.44	2.93	4.71	1.02
27	1719	Unknown	1.77		1.84	1.12	1.30
28	1727	Valenca-1(10),8-dien-11-ol	5.06	4.97	6.22	1.40	
29	1733	Unknown	2.11		1.48		
30	1739	Dehydrojinko-eremol	1.37	1.05	1.36	0.89	
31	1746	Eudesma-3,11-dien-8-one		0.47		0.81	
32	1750	Unknown		1.32			
33	1757	Vetispira-2(11),6(14)-dien-7-ol + unknowns	2.03				
34	1759	Cadina-1(10),4-dien-8a-ol		2.10	1.55	0.57	
35	1762	Unknown	2.31				
36	1766	Vetispira-2(11),6-dien-14-al + selina-3,11-dien-9-one	6.90	1.43	4.79		1.52
37	1774	Unknown		5.05		1.27	
38	1777	γ-Costol	2.77				
39	1794	Selina-3,11-dien-9-ol	2.43	2.00	2.67	0.81	
40	1800	Selina-3,11-dien-14-al	2.25	1.51			
41	1821	Unknown		1.91			
42	1828	Neopetasane	8.44	1.62	7.58		2.88
43	1832	Unknown	2.09	2.90	2.26		1.10
44	1842	Selina-4,11-dien-14-al + unknowns	2.84	3.19	1.59	1.44	
45	1872	n-pentadecanoic acid		0.61			
46	1885	Unknown					1.32
47	1891	Dihydrokaranone	2.82	1.79	2.57	5.15	1.46
48	1897	Nootkatone		0.58			1.18
49	1915	Karanone + unknowns	2.11		6.51		7.84
50	1974	n-hexadecanoic acid	0.93	16.06		3.94	
51	1978	Unknown				2.14	1.36
52	2072	Dihydrocolumellarin				1.41	
53	2100	1,5-diphenyl-pentan-3-one				1.08	
54	2158	Oleic acid		4.00			
55	2475	2-(2-phenylethyl)chromone			1.18		5.07
56	2566	Diisooctyl phthalate				1.38	
57	-	6-Methoxy-2-(2-phenylethyl)chromone					7.28
		Total	**89.06**	**76.16**	**72.46**	**81.48**	**42.78**

In the essential oil E1, obtained by simple hydrodistillation, Table 2 listed 30 components representing 89.06 % of the total volatiles. The major identified compounds were neopetasane (8.44 %), ß-agarofuran (7.19 %), agarospirol (5.58 %), valenca-1(10),8-dien-11-ol (5.06 %), jinko-eremol (5.00 %), valerianol (4.93 %), β-eudesmol (4.08 %), benzylacetone (2.88 %), dihydrokaranone (2.82 %), selina-3,11-dien-9-ol (2.43 %), 10-epi-γ-eudesmol (2.31 %), and

selina-3,11-dien-14-al (2.25 %). Neopetasane, ß-agarofuran, agarospirol, dihydrokaranone, and selina-3,11-dien-14-al are very valuable fragrant materials.

In the essential oil E2, obtained by enzyme-assisted hydrodistillation, Table 2 listed 37 components representing 76.16 % of the total volatiles. The major identified compounds were n-hexadecanoic acid (16.06 %), valenca-1(10),8-dien-11-ol (4.97 %), oleic acid (4 %), ß-agarofuran (3.21 %), valerianol (2.67 %), epoxybulnesene (2.61 %), α-eudesmol (2.44 %), cadina-1(10),4-dien-8a-ol (2.1 %), jinko-eremol (2.08 %), selina-3,11-dien-9-ol (2.00 %), dihydrokaranone (1.79 %), neopetasane (1.62 %), and selina-3,11-dien-14-al (1.51 %). Using enzyme mixture to assist the extraction process shortened the distillation time and lead to higher oil yield, but the oil has high percentage of fatty acids and fatty acid derivatives which decrease the quality of the oil.

In the essential oil E3, obtained by microwave-assisted hydrodistillation, only 26 components representing 72.46 % of the total volatiles were found in Table 2. The major identified compounds were neopetasane (7.58 %), valenca-1(10),8-dien-11-ol (6.22 %), jinko-eremol(5.53 %), valerianol (5.38 %), agarospirol (4.55 %), α-eudesmol (2.93 %), 2,14-epoxy-vetispir-6-ene (2.91 %), β-eudesmol (2.77 %), selina-3,11-dien-9-ol (2.67 %), dihydrokaranone (2.57 %), epoxybulnesene (1.79 %), cadina-1(10),4-dien-8a-ol (1.55 %), dehydrojinko-eremol (1.36 %), 2-(2-phenylethyl)chromone (1.18 %), and benzylacetone (1.09 %).

In the oleoresin E4, obtained by extraction with chloroform, Table 2 listed 37 components representing 81.48 % of the total volatiles. The major identified compounds were ß-agarofuran (13.20 %), valerianol (5.88 %), β-eudesmol (5.48 %), jinko-eremol (5.19 %), dihydrokaranone (5.15 %), agarospirol (4.72 %), α-eudesmol (4.71 %), n-hexadecanoic acid (3.94 %), epoxybulnesene (3.21 %), γ-eudesmol (2.66 %), and 10-epi-γ-eudesmol (2.19 %). Compared with E1, the extraction products E3 and E4 contained more desired volatile components.

In the oleoresin E5, obtained by supercritical carbon dioxide extraction, Table 2 listed 21 components representing 42.78 % of the total volatiles. The major identified compounds were 6-methoxy-2-(2-phenylethyl)chromone (7.28 %), 2-(2-phenylethyl)chromone (5.07 %), neopetasane (2.88 %), agarospirol (2.33 %), valerianol (2.10 %), guaiol (2.06 %), dihydrokaranone (1.46 %), nootkatone (1.18 %), β-eudesmol (1.10 %), α-eudesmol (1.02 %), benzylacetone (0.62 %), 10-epi-γ-eudesmol (0.59 %), and anisylacetone (0.42 %). The high boiling components contained in E5 are important ingredients of agarwood smoke.

4. CONCLUSIONS

Our preliminary study showed that the application of more advanced methods for the extraction of agarwood using enzymes, microwave oven, organic solvent, and supercritical carbon dioxide resulted in saving of time, energy consumption, and some improvement of product yield. These methods also lead to unconventional products with characteristic chemical compositions, what requires further investigations.

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