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Evaluation of a Method of Separating Agarwood Absolute from *Aquilaria crassna* Pulp by Soxhlet and Soaking for Fixative Substance in Blending Fragrance

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Authors' contributions

This work was carried out in collaboration among all authors. Author LHH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LMXT and NQT managed the analyses of the study, managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Agarwood resin is a precious material that has been widely used in industry and life. The aim of this study is the evaluation of a method of separating *Aquilaria crassna* wood pulp with ethanol by Soxhlet and solvent immersion method for fixative substance in blending fragrance. Agarwood pulp of *Aquilaria crassna* tree is grown in Binh Thuan province, Vietnam. Our research team has extracted the Agarwood absolute F.1 and F.2 from *Aquilaria crassna* by Soxhlet extraction and soaking method. The fragrance is diluted 10 times by odorless solvent diethyl phthalate (DEP), then use paper and olfactory of the nose to evaluate the aroma. The yield of absolute Agarwood extracted by the method of Soxhlet is 5.56% weight. The yield of Agarwood absolute extracted by the method of soaking is 4.58% weight. Agarwood absolute F.1 is a fixative substance, which is

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capable of storing fragrance better than Agarwood absolute F.2. The scent of fragrance Fr.F.2 using Agarwood absolute F.2 is more like a natural scent than fragrance Fr.F.1 with fixative F.1. Separating Agarwood absolute F.1 from *Aquilaria crassna* pulp by Soxhlet provided yield and fixative capabilities higher than separating Agarwood absolute F.2 from *Aquilaria crassna* pulp by soaking. The scent of fragrance Fr.F.2 using Agarwood absolute F.2 is more like a natural scent than fragrance Fr.F.1 with Agarwood absolute F.1.

Keywords: Agarwood absolute; extraction; fragrance; natural fixative; soxhlet.

1. INTRODUCTION

Agarwood, known as chenxiang in Chinese and called aloeswood, eaglewood, jinkoh, agalloch, gaharu, or kanankoh in different regions, is a highly valuable fragrant wood [1]. Many species of Agarwood are found worldwide in Indonesia, Malaysia, China, India, Philippines, Vietnam, Laos, Cambodia, Thailand, Singapore, and Papua New Guinea [2]. Some species produce incense after being attacked by force [3,4], insect [5], or bacterial, fungal infection [6,7].

Agarwood resin is a precious material that has been widely used in industry and life. Aromatic substance from Agarwood resin is a valuable material, it is used in traditional medicine preparations [8] and a material for perfume and cosmetics [9]. Many studies have been carried out to analyze the quality of them [10,11,12]. Recently, the phytochemical investigations have achieved fruitful results, and more than 300 compounds have been isolated, including numerous new compounds that might be the characteristic constituents with physiological action [13,14,15].

The chemical compounds of Agarwood resin show a complex mixture of monoterpenes, sesquiterpenes, and its chromones derivatives [16,17,18,19]. Sesquiterpenes have been reported to be the main active components that play important roles in giving the aroma and pleasant odor of Agarwood [20]. Aromatic resins, polymer compounds found in Agarwood are good ingredients for fixative substance.

Agarwood oil has been extracted using various techniques, equipment and solvents. Solvent extraction and supercritical liquid extraction are commonly used for Agarwood pulp [21]. The method of extraction of Agarwood is usually dependent on the purpose of the extract. There are many types of solvents commonly used in the extraction of Agarwood essential oils namely methanol, ethanol ... Each solvent produces different extracts in terms of quantity and quality of the final product.

Solvent extraction has certain advantages and disadvantages, so the extraction method depends on the intended use of the final product such as Agarwood oil, Agarwood extract. With the purpose of taking the end product for blending fragrance, scientists often choose the extraction method with ethanol solvent.

There are four species (i.e., *Aquilaria crassna, Aquilaria baillonnii, Aquilaria banaensis, Aquilaria rugosa*) of Agarwood recorded natural distribution in Vietnam. *Aquilaria crassna* is a kind of big timber tree that contains a high volume of Agarwood oil and has been growing popularly in Vietnam [22,23,24]. Currently, research on Agarwood resin as a fixative substance for fragrance has not been focused on in Vietnam and around the world. Therefore, research to separate Agarwood resin for fixative purposes is very important and necessary.

The aim of this study is the evaluation of a method of separating *Aquilaria crassna* wood pulp with ethanol by Soxhlet and solvent immersion method for fixative substance in blending fragrance.

This study is valuable in exploiting available resources for making natural fixative to replace traditional fixative substance synthesized from chemicals to protect human health and friendly to the environment.

2. MATERIALS AND METHODS

2.1 Materials

Agarwood pulp of *Aquilaria crassna* tree is grown in Binh Thuan province, Vietnam. The material used in the experiment for the natural fragrance was taken from project VIE86033 and applied according to Indian Standards for aromatherapy. In our experiments, we used the volatile solvent is a solvent of alcohol 96% and an odorless solvent diethyl phthalate (DEP).

2.2. Extraction Methods

2.2.1 Extraction method by Soxhlet

Soxhlet apparatus kits use for extraction by an automatic continuous extraction method with high extraction efficiency, requiring less time and solvents compared to other methods.

Agarwood contains aromatic resins that are cut and ground into fine flour. Add 50g of Agarwood pulp to soxhlet, 500ml of ethanol 96% into a 1000ml round bottom flask. Heating and opening cooling water for condensed - evaporated ethanol passes through the powdered wood pellets back to the flask. Continue heating up to 4 hours, each time increase $3^{O}C$, the aromatic ethanol liquor is black-brown. Then add 5 g Na_2SO_4 to adsorb water in the solution, use glass chopsticks to stir for 2 minutes, then settle for 2 hours and filter. Setting vacuum evaporator at 0.4 atm and a temperature of $70^{O}C$, temperature of cooling water is $16^{O}C$. The process ends when the evaporation is completed, we have Agarwood absolute F.1.

2.2.2 Separation by the soaking method

Soak and extract in a volatile solvent at room temperature $25\text{-}30^{0}C$. This method is based on the principle of using an appropriate solvent to dissolve the constituents that bring scent into a solvent. After extraction, we distillate to isolate solvent at low pressure to obtain the absolute resin. In this study, we used alcohol 96% to dissolve Agarwood resin.

We put in a 1000ml Erlenmeyer flask with 100g of Agarwood pulp and 500ml ethanol 96%. Every day, we shake the wood and ethanol a few times. The ethanol solvents have a black- brown color due to the resin dissolved in the ethanol. After 15 days, the solvent is removed in other Erlenmeyer flasks. Then add 300ml of ethanol 96% and continue to soak, shake as above. After 10 days, the solvent is removed into Erlenmeyer flasks. We continue to add to each flasks 200ml of ethanol 96% and continue soaking, shake on. After 5 days, the new resin grade is removed with normal patterns for the Erlenmeyer flasks. Vacuum filter is set at 0.6 atm pressure. 840ml of solvent is recovered, 160ml of solvent is the loss because it is left in wood pulp and evaporates in the process. Let 10g of Na_2SO_4 absorb water, use glass chopsticks to stir for 2 minutes, then settle for 2 hours and filtrate the solution through a filter. Setting a rotary vacuum evaporator at 0.4 atm and temperature of $70^{O}C$, a temperature of cooling water $16^{O}C$. The process ends when the evaporation is completed, we have Agarwood absolute F.2.

2.2.3 Method of assessment of product quality

Although many modern types of equipment such as gas chromatography/mass spectrometric (GC/MS), electronic nose... [13,14,15] but the Perfumer still has to evaluate the quality of fragrance with the human sense. The olfactory nerves of humans can recognize pleasant or unpleasant odor to humans that modern machines cannot do. The Perfumer can know the odor of fragrance is suitable for a product as cosmetic, soap, detergent. The machinery can analyze the chemical composition of the aroma but cannot recognize the aroma that the human like or not. The fragrance is diluted 10 times by odorless solvent diethyl phthalate (DEP), then use paper and olfactory of the nose to evaluate the aroma. The method of assessment of product quality in this study is using the olfactory to assess the odor of samples over time according to the evaluation of Jiang John et al. [16], to record odor quality and odor retention time for comparative evaluation. Based on this scent classification, we construct similar levels (Table 1.).

3. RESULTS AND DISCUSSION

3.1 Evaluation of Time and Yield Extraction

Properties of Agarwood absolute F.1 and F.2 are in Table 2. Separated Agarwood absolute F.1 by the method Soxhlet from the wood pulp of *Aquilaria crassna* to the Agarwood absolute is a short time of 8 hours. Separated Agarwood absolute F.2 by the method of soaking from the wood pulp of *Aquilaria crassna* to the Agarwood absolute is a long time of 31 days.

The yield of Agarwood absolute F.1 extracted by the method Soxhlet is 5.56%. The yield of Agarwood absolute F.2 extracted by the method of soaking is 4.58%. The yield of Agarwood absolute F.1 extracted by the method Soxhlet is higher than the yield of Agarwood absolute F.2 extracted by the soaking method because of the effect of the temperature of the solvent ethanol. The temperature of the ethanol solvent in the Soxhlet method is about $78\text{-}88^{0}C$ which is higher than that of the ethanol solvent of the soaking method at room temperature. At a temperature of 78-88^{0}C, the aromatic constituents of Agarwood pulp easily enter the solvent than at room temperature 25-30^{0}C, so the yield of Agarwood absolute F.1 extracted by the method Soxhlet is higher.

3.2 Evaluation of the Odor of the Agarwood Absolute F.1 and F.2

The Agarwood absolute F.1 and Agarwood absolute F.2 have a warm, sweet, woody odor, typical of Agarwood. The odor of Agarwood absolute F.2 is extracted by soaking, which odor in a more natural and harmonious woody odor than Agarwood absolute F.1. The Agarwood absolute F.2 is extracted by an ethanol solvent at room temperature of 25-30^{0}C, so there is little chemical change in the extraction process, so the odorous substances from Agarwood pulp are not changed and remain the natural fragrance. The Agarwood absolute F.1 is extracted by ethanol solvent by the method Soxhlet at a temperature of about 78-88^{0}C, so there is a chemical change in the extraction process by polymerization at the separation temperature, so some the substances from Agarwood pulp is slightly modified and does not retain its natural odor.

3.3 Evaluation of Fixative Capabilities of the Agarwood Absolute F.1 and F.2

We created fragrance Fr.F.1 and fragrance Fr.F.2 with similar structure and differences in fixative note. Agarwood absolute F.1, F.2. The structure of fragrances includes top group, body group, fixative group (Table 3).

The odor of Fragrance Fr.F.1 and Fr.F.2 are floral with natural rose odor. The main constituents that make up the rose smell are Geraniol, Geranyl acetate, Geranyl butyrate, Geranyl formate, Citronellol, Citronellyl acetate, Citronellyl butyrate, Citronellyl formate, Nerol, Neryl acetate, Neryl butyrate, Palmarosa oil, Phenyl ethyl alcohol, Phenyl ethyl formate. These are the aromatic substances of natural origin that make up the natural rose scent of Fragrance Fr.F.1 and Fr.F.2. A little difference between Fragrance Fr.F.1 and Fr.F.2 is that Fragrance Fr.F.2 has a more interesting, more attractive, and natural fragrance than Fragrance Fr.F.1. Fixative Agarwood absolute F.2 makes Fragrance Fr.F.2 more attractive than Fragrance Fr.F.1. Agarwood absolute F.2 is extracted from *Aquilaria crassna* pulp by soaking at room temperature 25-30^{0}C, so its natural aroma is more attractive than Agarwood absolute F.21, extracted by Soxhlet extraction.

From the experiment, it shows that the method of separating Agarwood absolute from Agarwood pulp by soaking to create a fixation is more suitable than the Soxhlet method in blending fragrance.

Table 4. and Fig.1. show that: Fixative capabilities of fragrance Fr.F.1 and Fr.F.2 are the same at the first 47 hours. Then the fragrance Fr.F.2 reduced its odor faster than Fr.F.1 and after 95 hours Fr.F.2 had completely disappeared. Fragrance Fr.F.1 retains better odor, after 105 hours completely lose its odor. The fixative capabilities of Agarwood absolute F.1 are better than the fixative capabilities of Agarwood absolute F.2. It is possible that the extraction by Soxhlet method is at a temperature of 78-88^{0}C, so the polymeric substances contained in Agarwood pulp are separated more than the separation by the soaking method at room temperature. Fixative capabilities of polymeric substances are good, so the fixative capabilities of Agarwood absolute F.1 are better than Agarwood absolute F. 2.

Agarwood absolute is extracted from Agarwood pulp by Soxhlet method at a temperature of 78-88^{0}C has the advantage of a short separation time, high yield compared to the separation by the soaking method at room temperature, but the odor of Agarwood absolute separation by the

Table 1. Descriptive statistics on the ability to retain odor by smell

Rating level	Classifying odor rating	Description of the smell
0	No odor	The smell did not recognize
1	Very weak (odor threshold)	The smell was initially very difficult to receive
2	Weak	The odor was mild
3	Medium (normal)	Smell initially decreased significantly
4	Strong	The initial odor is slightly reduced
5	Very strong	The original aroma of the sample

Table 2. Properties of the Agarwood absolute F.1 and F.2

N^0	Properties of the extract	Agarwood absolute	
		F.1	**F.2**
1	Separation method	By soxhlet	By soaking
2	Yield% weight	5.56	4.58
3	Appearance	Dark colored resin	Dark colored resin
4	Density	0.988	0.986
5	Odor	Warm, sweet, woody odor, typical of agarwood.	Warm, sweet, woody odor, typical of agarwood. More natural and harmonious woody odor

Table 3. The composition of aromatic groups

N^o	Material	Fragrances % weight		Note
		Fr.F.1	**Fr.F.2**	
1	Cinnamaldehyde	1	1	Top note
2	Citronellal	1	1	
3	Eugenol	1	1	
4	Isoeugenol	2	2	
5	Geraniol	6	6	Body note
6	Geranyl acetate	4	4	
7	Geranyl butyrate	3	3	
8	Geranyl formate	3	3	
9	Citronellol	6	6	
10	Citronellyl acetate	4	4	
11	Citronellyl butyrate	3	3	
8	Citronellyl formate	3	3	
9	Hydroxy citronellal	3	3	
10	Nerol	6	6	
11	Neryl acetate	4	4	
12	Neryl butyrate	3	3	
13	Palmarosa oil	2	2	
14	Phenyl ethyl alcohol	2	2	
15	Phenyl ethyl formate	2	2	
16	Linalool	2	2	

N^{o}	Material	Fragrances % weight		Note
		Fr.F.1	Fr.F.2	
17	Linalyl acetate	2	2	
18	Linalyl cinnamate	2	2	
19	Heliotropin	2	2	
20	Citral diethyl acetal	1	1	
21	Ionone	3	3	
22	Methyl ionone	3	3	
23	Methylheptenone	1	1	
24	Tecpineol	1	1	
25	Tecpinyl acetate	1	1	
26	Ginger oil	1	1	
27	Ylang Ylang oil	2	2	
28	Jasmine oil	2	2	
29	Extract laurel flower	2	2	
30	Orange oil	1	1	
31	Grapefruit oil	1	1	
32	Lemon oil	1	1	
33	Mandarin oil	1	1	
34	Poumu oil	1	1	
35	Sandalwood oil	1	1	
36	Cedar wood oil	1	1	
37	Vetiver oil	1	1	
38	Agarwood absolute F.1	8		End note Fixative group
39	Agarwood absolute F.2		8	
	Total	100	100	

Table 4. Review and evaluate the fixative capabilities of fragrance Fr.F.1 and Fr.F.2

Nº	Time (hours)	Fragrance	
		Fr.F.1	Fr.F..2
1	0	5	5
2	3	5	5
3	11	4	4
4	23	4	4
5	35	3	3
6	47	3	3
7	59	3	2
8	71	2	1
9	83	2	1
10	95	1	0
11	107	0	0

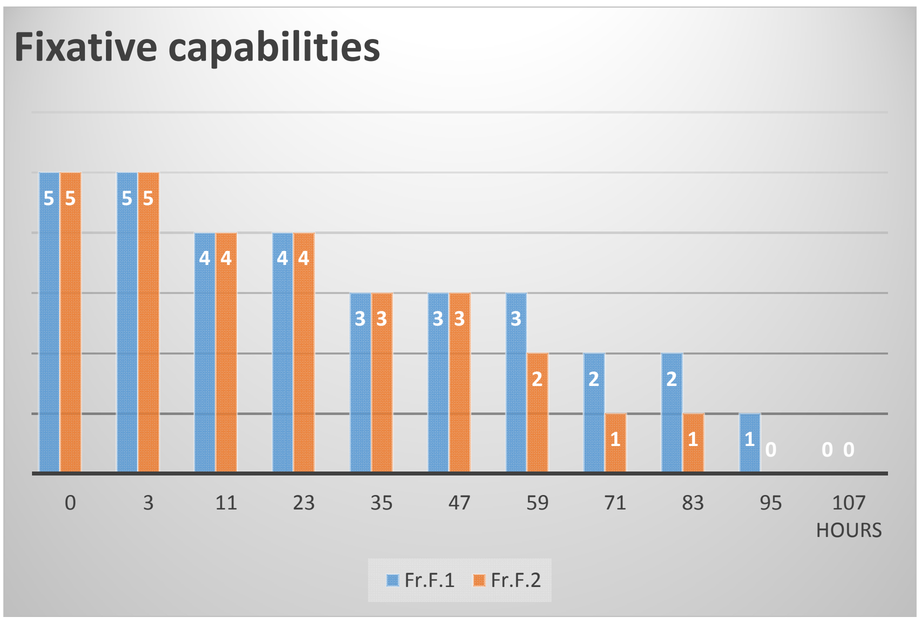

Fig.1. Fixative capabilities of fragrance Fr.F.1 and Fr.F.2

soaking method at room temperature has more natural and harmonious woody odor. In the field of blending fragrance, Perfumers pay more attention to odor, so the method of separating Agarwood absolute from Agarwood pulp by soaking to create a fixation is more suitable than the Soxhlet method in blending fragrance.

4. CONCLUSION

Our research team has extracted the Agarwood absolute F.1 and F.2 from Aquilaria crassna by Soxhlet extraction and soaking method. Separating Agarwood absolute F.1 by the method Soxhlet from the wood pulp of *Aquilaria crassna* is a short time of 8 hours while separating Agarwood absolute F.2 by the method of soaking is a long time of 31 days. Separating Agarwood absolute F.1 from *Aquilaria crassna* pulp by Soxhlet has a higher yield than separating Agarwood absolute F.2 by the soaking method.

Fragrance Fr.F.2 has a more interesting, more attractive, and natural fragrance than Fragrance Fr.F.1. Agarwood absolute F.2 is extracted from *Aquilaria crassna* pulp by soaking, so its natural aroma is more attractive than Agarwood absolute F.1 extracted by Soxhlet extraction.

The method of separating Agarwood absolute from Agarwood pulp by soaking for fixative substance is more suitable than the Soxhlet method in blending fragrance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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