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Chemical Compositions of Essential Oil of Agarwood (*Aquilaria crassna*) Harvested in Phu Quoc Island, Vietnam

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Abstract. The importance of natural products derived from agarwood species is increasingly emphasized in producing perfumery or cosmetic products made from agarwood species. On the other hand, due to the predominant amount of aromatic compounds in essential oils, frankincense essential oil is increasingly popular with high economic value. Therefore, in this study, we focused on the extraction process of essential oils and evaluated the chemical composition of the essential oils by the GC-MS method. Differences in the composition of essential oils between natural and artificial agarwood may be due to species, habitat conditions, climate, geographical conditions, and extraction methods in different regions. Specifically, volatile components in essential oils were identified as neopetasone (14.43%); dihydroagarofuran-15-al (9.20%); jinkho-eremol (5.02%); valerianol (4.94%); β-agarofuran (8.02%); dihydrokaranone (3.25%), valenca-1(10), 8-dien-11-ol (5.95%) and selina-4,11-dien-14-ol (2.73%), contribute to determining the main aroma in agarwood essential oil.

Introduction

Currently, with consumers tending to use natural compounds, the world's demand for essential oils is increasing, especially those used in the aromatherapy and cosmetic industry [1–9]. Vietnam is in the tropical monsoon region, with high temperatures, rainfall, and relative humidity. Therefore, creating a rich and diverse flora, especially plants for essential oils; in which agarwood essential oil is often known as fragrant oil, the high-grade fragrance is an important product popular. Agarwood belongs to the family Thymelaeaceae; there are 19 species of Aquilaria found in the Asian region [10]. As a species of tree endemic to the hot and humid equatorial climate, it is commonly found in Southeast Asia and New Guinea islands in Oceania. In Vietnam, agarwood is distributed in provinces such as Ha Giang and Phu Quoc. A number of studies have confirmed that agarwood is a wood containing many aromatic resins produced from the trunk of the *Aquilaria crassna* tree. When the tree is injured, the oil in the tree gathers to resist the infection from the outside, the oil that remains gradually denatures and becomes agar, depending on the time of formation and the degree of infection that will give out large and small bass blocks and different shapes [11]. However, only about 7-10% of trees in natural forests contain agarwood [10]. Besides, if there is human intervention in creating the wound of the tree, it is artificial agarwood. On the other hand, chemical studies on agarwood wood have

shown that aromatic compounds characterize the main chemical constituents of essential oils, such as sesquiterpenes, sesquiterpene paranoids, chromone derivatives, pentadecanoic acid, and tetradecanoic acid [12–15].

Some extraction methods for essential agarwood oil may include hydrogen distillation, steam distillation, carbon dioxide extraction, solvent extraction, florasol/phytol extraction, etc. [12,16,17]. Each extraction method has its advantages and disadvantages. However, hydrodistillation is a popular process used in essential oil extraction because of its environmental friendliness, low operating costs, and the ability almost completely to extract the essential oil contained in the material. The objective of this study was to extract Agarwood essential oil by hydrodistillation method and chemical compound analysis based on Gas Chromatography - Mass Spectrometry (GC-MS). The recovery efficiency of essential oils was calculated and compared with previously published parameters.

Experimental Methods

Plant material. Raw materials used in this study were collected from agarwood trees on Phu Quoc Island, Kien Giang province, Vietnam. Professor Le Cong Kiet (University of Natural Sciences, Vietnam National University, Ho Chi Minh City) botanically identified *Aquilaria crassna* Pierre ex. Lecomte belongs to the family Thymelaeaceae. Agarwood is transported to the laboratory, where the specimens are kept. Agarwood was taken from a 15-year-old tree that has been symbiotic with a specific fungus (a product licensed by Lam Vien Co., Ltd.). After transplanting mushrooms, the tree will be harvested after 3 years; agarwood is obtained after the manual process of removing the unaffected woody parts. To preserve and avoid damage, agarwood samples were air-dried, ground into powder, and stored in paper bags at room temperature.

The essential oil extraction process. The process of extracting essential agarwood oil is done through two stages: First, a mixture of 500 g of agarwood samples and water is stored in a glass jar and kept for 3 weeks. In the next stage, the above mixture was distilled in a Clevenger-type apparatus for 72 hours until the first drop of oil appeared, heated to 120 °C. After the distillation is finished, a mixture of essential oils and water is obtained; the water is removed and anhydrous with Na_2SO_4, and the essential oils will be stored in dark bottles for analysis.

The GC-MS analysis of essential oils. Agarwood essential oil was analyzed GC-MS on an Agilent Technologies HP7890A GC instrument, combined with an Agilent Technologies HP5975C mass spectrometry detector and a DB-XLB column (60 mm 0.25 mm, film thickness 0.25 μm, Agilent Technologies). The elution times of components are expressed by the retention time index (RI) and are related to the retention times of a series of n-alkanes with the same GC program. Based on peak area GC (MSD response) calculates relative amounts of individual components without correction.

Results and Discussion

The essential oil obtained from agarwood powder after soaking in beams combined with the hydrodistillation method gave a 0.13% yield. Agarwood essential oil is a dark yellow liquid with a characteristic woody odor. The hydrodistillation process depends on the diffusion of oil droplets from the wood mass and their loss in the condensate. Therefore, the soaking process increases the efficiency of recovering the essential oil content. When soaking, due to the difference in water content inside and outside the wood cells, water penetrates the wood cell membrane and swells the wood pulp, but it depends on the size and shape of the wood pulp. When the cell membrane expands wide enough, it is easier for essential oils to penetrate the cell membrane and go to the outside environment. However, if the soaking time is too long, it will decompose some compounds in the essential oil, and the essential oil is lost during the soaking process, and the essential oil's performance will be degraded. Some published documents show that recovery efficiency depends on the extraction process or the input material. Huaiqiong Chen et al. (2011) conducted hydrodistillation extraction with three different raw materials and obtained the following results: agarwood is stimulated by chemical method (S1-0.042%), wild agarwood (S2-0.32%), and healthy plants (S3-0.0128%) as a control [18]. Patcharee Pripdeevech et al. (2011) showed that the extraction efficiency of essential oil

was up to 0.8% when using the solid-phase microextraction method (SPME) for *Aquilaria crassna* sample (Rayong - Thailand) [19].

Table 1. The chemical composition of Vietnamese agarwood essential oil

Chemical name	Molecular Weight	Molecular Formular	CAS Number	This study Vietnam
Benzylacetone	148.20	$C_{10}H_{12}O$	2550-26-7	1.53
2,6-Di-tert-butyl-4-methylphenol	220.30	$C_{15}H_{24}O$	128-37-0	0.35
4,5-di-epi-Aristolochene	204.35	$C_{15}H_{24}$	26620-71-3	0.31
β-Agarofuran	206.32	$C_{14}H_{22}O$	6040-08-0	8.02
β-Dihydroagarofuran	222.37	$C_{15}H_{26}O$	5956-09-2	0.40
Anisylacetone	178.23	$C_{11}H_{14}O_2$	104-20-1	0.21
Agarofuran <a->	220.36	$C_{15}H_{24}O$	5956-12-7	0.49
nor-Ketoagarofuran	222.33	$C_{14}H_{22}O_2$	5986-25-4	0.36
Epoxybulnesene	204.35	$C_{15}H_{24}$	36911-11-0	1.27
Eudesmol <10-epi-g->	222.37	$C_{15}H_{26}O$	15051-81-7	2.44
Agarospirol	222.37	$C_{15}H_{26}O$	23811-08-7	5.64
Hinesol	222.37	$C_{15}H_{26}O$	23811-08-7	0.54
Jinkho-eremol	220.35	$C_{15}H_{26}O$	86747-08-2	5.02
Valerianol	222.37	$C_{15}H_{26}O$	20489-45-6	4.94
Eudesmol <a->	222.37	$C_{15}H_{26}O$	473-16-5	2.17
Valenca-1(10),8-dien-11-ol	220.35	$C_{15}H_{24}O$	168099-20-5	5.95
Dehydrojinkho-eremol	220.35	$C_{15}H_{24}O$	150034-02-9	1.65
4,15-Epoxy-dihydroagarofuran	222.37	$C_{15}H_{26}O$	15052-76-3	1.72
Cadina-1(10),4-dien-8a-ol	220.35	$C_{15}H_{24}O$	147853-18-7	0.95
Dihydroagarofuran-15-al	222.37	$C_{15}H_{26}O$	20053-66-1	9.20
Selina-3,11-dien-9-ol	220.35	$C_{15}H_{24}O$	133593-96-1	1.97
Neopetasone	218.33	$C_{15}H_{22}O$	13902-42-6	14.43
Selina-4,11-dien-14-al	218.34	$C_{15}H_{22}O$	150034-05-2	2.73
Dihydrokaranone	218.34	$C_{15}H_{22}O$	19598-45-9	3.25
Nootkatone	218.34	$C_{15}H_{22}O$	91416-23-8	0.61
oxo-agarospirol	236.35	$C_{15}H_{24}O_2$	93133-69-8	2.59
Total				78.74

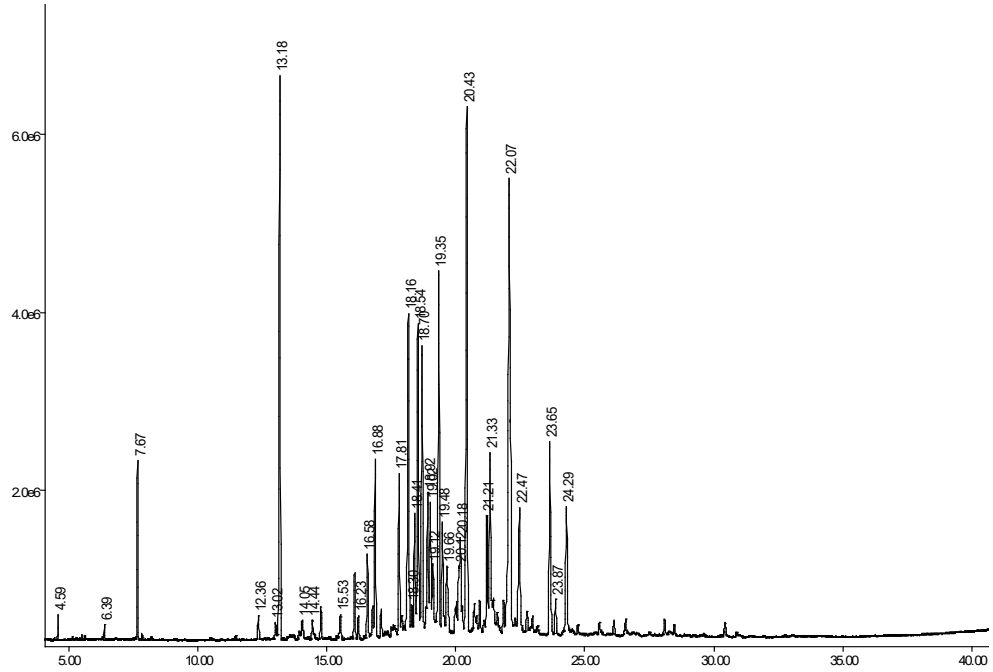

Fig. 1. Chromatogram of volatile compounds present in Vietnamese agarwood essential oil (absis?)

The volatile components of Frankincense essential oil were subjected to GC-MS analysis; specifically, the qualitative and quantitative results of the components are shown in Table 1. At the same time, the elution times of the compounds are characterized by the retention time (RI) value as the chromatographic configuration shown in (Figure 1). Most essential oils are made up of complex compounds such as sesquiterpene hydrocarbons, monoterpene hydrocarbons, and oxygenated sesquiterpenes. Through the recorded results, 27 different retention time values corresponding to 28 components were identified in the essential oil. Some components dominate in essential oils such as β-agarofuran (8.02%); agarospirol (5.64%); valeriano (4.94%); valenca-1(10),8-dien-11-ol (5.95%); dihydroagarofuran-15-al (9.20%); neopetasone (14.43%) and dihydrokaranone (3.25%), eluted in order of increasing retention time. Next is the component Benzylacetone (1.53%); Epoxybulnesene (1.27%); eudesmol <10-epi-g-> (2.44%); eudesmol <a-> (2.17%); dehydroJinkho-eremol (1.65%); 4,15-epoxy-dihydroagarofuran (1.72%); selina-3,11-dien-9-ol (1.97%); selina-4,11-dien-14-al (2.73%) and oxo-agarospirol (2.59%), and some components were detected at low concentrations (<1%). Similarly, Nor Atikah Mat Yusoff et al. (2015) study surveyed the optimal conditions for extracting agarwood essential oil. The highest essential oil yield (0.78%) was obtained when a combination of hydrodistillation and agitator was involved in the extraction process.

At the same time, the research team analyzed the chemical compounds in essential agarwood oil and identified some key compounds agarospirol, 4-phenyl 2-butanone, α-elemol, α-muurolene and selina-3,11-dien-9-ol [20]. In another publication, Saiful Nizam Tajuddin et al. (2010) compared the chemical composition of agarwood essential oil extracted directly by hydrodistillation method and commercial essential oils. The differences were evident in the content of the major compounds present in specific essential oils, such as the laboratory essential oil α-guaiene (5.8%), jinkho-eremol (6.5%), and 4-phenyl-2-butanone (32.1%).) and commercial essential oils are eudesmol (3.2%), caryophellene oxide (8.6%) and α-guaiene (10.3%) [21]. Depending on geographical location, climate, and origin of raw materials or variations, ecological types exist within agarwood tree species; essential agarwood oil will have different qualities in terms of quality compounds as well as recovery efficiency. Typical in the study of Patcharee Pripdeevech et al. (2011) used the same species of *Aquilaria crassna* from Thailand for the experiment, but the main compounds in the essential oil were β-agarofuran, isoamyl dodecanoate, dehydroJinkho-eremol, kusunol and 9,11-eremophiladien-8-one. The research team also showed that the main aroma activity of essential oils is characterized by compounds of terpenes, of which β-agarofuran based on high aroma concentration and specificity, should be considered the most important aroma contribution [19]. In addition, in 2019, the Dinh Thi

Thu Thuy et al. research group conducted a survey and evaluated essential agarwood oil from three different raw materials Khanh Hoa, Bac Giang, and Phu Quoc Island (Kien Giang province). Recovery efficiency was 0.27%, 0.32%, respectively, and 0.25% (w/w) based on the hydrodistillation method. Also, the volatile compounds were determined dihydrokaranone (2.63–3.59%), agarospirol (2.98–3.42%), β-agarofuran (3.04–6.18%), and neopetasone (7.47–8.29%) were mainly responsible for the quality of all essential oil samples. It can be seen that in the same extraction process or analysis method, different input materials also lead to different yields and qualities of essential oils [22].

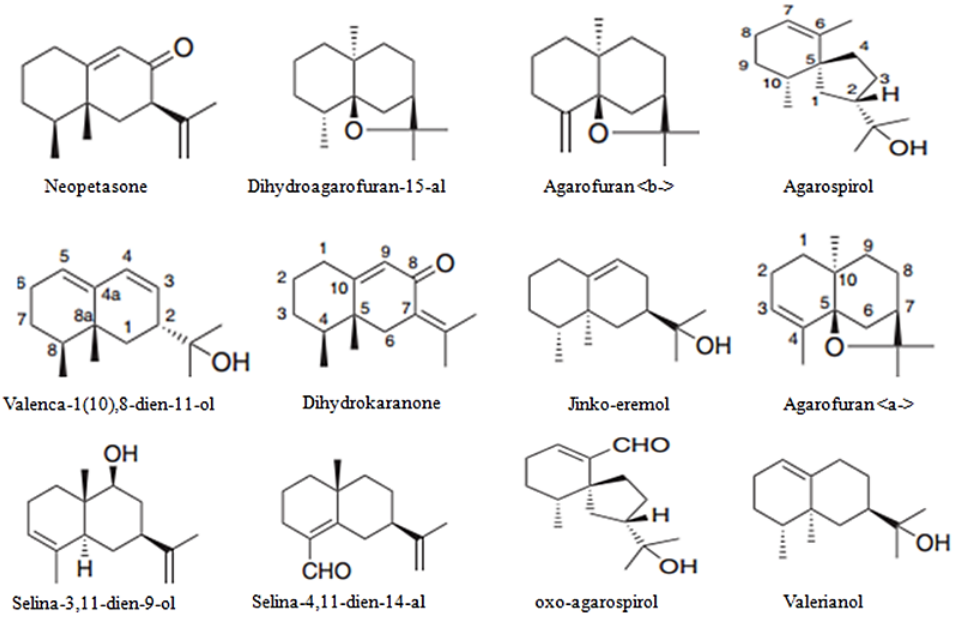

Fig. 2. Structure of important sesquiterpene compounds in essential agarwood oil in Phu Quoc island of Vietnam

Antibacterial and anti-inflammatory activities have been found in the literature as two prominent properties of essential agarwood oil. On the other hand, Penpun Wetwayaklung et al. (2009) detected the characteristic compounds in agarwood essential oil such as selina-4,11-dien-14-al, γ-selinene, and selina-4,11-dien-14-al and tested the activities. Inhibitory antibacterial activity against some strains of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [11]. Specifically, Chen et al. found that essential oils extracted from artificial and natural agarwood have significant inhibitory effects on *Bacillus subtilis* and *Staphylococcus aureus* [23]. Similarly, *in vitro* testing of essential agarwood oil against *Staphylococcus epidermidis* also revealed a strong disrupting effect of the oil on cell walls, contributing to cell biofilm inhibition [24]. Regarding the anti-inflammatory effects, it was found that Jinkho-eremol and agarospirol were the main compounds responsible for the potent inhibitory activity of agarwood essential oil. Saad Sabbar Dahham et al. (2015) discovered that essential agarwood oil strongly inhibits the metastasis and proliferation of pancreatic cancer cells (MIA PaCa-2) through an *in vitro* model. Inhibitory effect of cells at (10 µg/ml) and MIA PaCa-2 cytotoxic activity with IC_{50} (11 ± 2.18 µg/ml) on agarwood essential oil [25]. In addition, the inhibitory properties of cancer cells from the essential oil extract from the frankincense tree were further reported by Saad Sabbar Dahham et al. (2016) conducted an in vivo model of rat subcutaneous tumors, demonstrating that essential oils are safe with an LD_{50} above 2000 mg/kg [26]. The research team has opened up a potential avenue for preclinical use in animals against pancreatic and colorectal cancer based on the chemotherapeutic effects of the essential oil extract [25,26].

Summary

The recovery efficiency of agarwood essential oil was 0.13% through the experimental results of extraction by the soaking process combined with the hydrodistillation method. The GC-MS method was used to identify and quantify the chemical components present in essential oils, with some of the

main components including agarospirol, β-agarofuran, jinkho-eremol, valeriano, etc. Research on the extraction and identification of highly active compounds will contribute to opening up many reliable and valuable applications of frankincense oil. Therefore, the plants that give precious essential oils, which are highly applicable in many fields of production and life, have been studied, exploited, and processed by scientists to improve their use-value.

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