Isolation Process and Compound Identification of Agarwood Essential Oils from *Aquilaria crassna* Cultivated at Three Different Locations in Vietnam

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Abstract: Agarwood and agarwood essential oils are commodities with great commercial value. In Vietnam, the agarwood industry has been growing, with more than 10,000 ha of forest land reserved for the cultivation of *Aquilaria crassna*, an agarwood-producing tree. The aim of this study was to present a hydrodistillation process to recover agarwood essential oil and to compare chemical compositions of agarwood samples harvested from various locations in Vietnam. Three agarwood samples representing products from *A. crassna* trees cultivated in the provinces of Bac Giang and Khanh Hoa, and on the Phu Quoc island (Kien Giang province) of Vietnam were subjected to hydrodistillation, resulting in essential oil yields of 0.32%, 0.27%, and 0.25% (w/w), respectively. Using GC–MS analysis, a total of 44 volatile compounds were identified in the obtained oils. Most of the constituents were oxygenated sesquiterpenes and had been previously found in other agarwood oil samples. Notable compounds of other chemical classes were aromatics and fatty acids. The three oil samples showed a common volatile profile, which is characterized by the dominance of eremophilane, agarofurans, and eudesmane sesquiterpenes, while vetispirane and guaiane sesquiterpenes were found in smaller quantities. Desired compounds, such as neopetasane (7.47–8.29%), dihydrokaranone (2.63–3.59%), β-agarofuran (3.04–6.18%), and agarospirol (2.98–3.42%), were present in substantial quantities, suggesting that the essential oils could be commercialized as fragrant materials of high value.

Keywords: agarwood; *Aquilaria crassna*; essential oils; neopetasane; dihydrokaranone; β-agarofuran; agarospirol

1. Introduction

Agarwood, the fragrant resinous heartwood originating from Aquilaria and a number of other plant species of the Thymelaeaceae family, is a non-timber forest product highly prized for its values as aromatic material and herbal medicine [1,2]. Due to its value and uniqueness, the chemistry of agarwood has been extensively studied since the 1960s until today, which has been excellently summarized in recent reviews [3–5]. As reported by Kalra and Kaushik in 2017 [5], a total of more than 250 compounds, mainly sesquiterpenes, chromones, and aromatic compounds, were identified in various samples of agarwoods and agarwood oils. Most of the identified sesquiterpenes and chromones from agarwood products were either new or the first isolated compounds from nature [3].
In Vietnam, the use and trade of agarwood has a long history, which dates back to ancient times [1]. Before 2000 in Vietnam, all agarwoods of various quality grades were naturally collected. Among quality grades, Qinan, termed Kyara in Japanese grading, was the most valuable agarwood and the tag of natural origin of the product had further raised the value of natural agarwoods to exorbitant numbers. The agarwoods of lower qualities were used to extract agarwood oil at many small distilleries. Ultimately, most of the Vietnamese agarwood products were exported to Taiwan, Hongkong, and Singapore, where they were re-exported elsewhere [6].

Earlier studies proposed that the source plant of Vietnamese agarwood samples was *A. agallocha* Roxb., which was later recognized as an unresolved botanical name [7]. Subsequent investigations found that four *Aquilaria* spp. in Vietnam, namely, *A. crassna* Pierre ex. Lecomte, *A. baillonii* Pierre ex. Lecomte, *A. bauanensis* P. H. Hô., and *A. rugosa* K. Le-Cong & Kessler, were responsible for accumulation of most of natural agarwood reserve [6,8–11]. The determination of the source plants of agarwoods is very difficult because, besides the leaves, exact identification of *Aquilaria* spp. also requires flowers and fruits [11]. At present, *A. crassna* Pierre ex. Lecomte (also named Dô Trâm or Dô Bâu in Vietnamese), which is distributed throughout the country, is generally considered the main source plant for the production of Vietnamese commercial agarwoods [12–14].

In the late 1990s, as agarwood from the wild was nearly exhausted, attempts were made to cultivate *A. crassna* for the large-scale production of agarwoods. Such efforts were first expended spontaneously by agarwood collectors in collaboration with forest engineers at a small scale, and then by private entrepreneurs with bigger investments. The government also encouraged this development by supporting a number of scientific studies on the breeding and cultivation of the plants, as well as on agarwood-inducing techniques. Progress in these initiatives resulted in about 10,000 ha of *A. crassna* cultivation in Vietnam today, and has made production of agarwood a promising industry [13]. Currently, commercial agarwood essential oils are locally produced by a hydrodistillation technique that calls for the development of authenticity and adulteration tests for this valuable new product. However, comprehensive characterizations of this source of essential oil have been lacking since most of studies involving compositional determination of Vietnamese agarwoods were conducted using agarwood extracts, rather than essential oils. In view of this development, there is an urgent need for a reliable chemical evaluation of the end products, especially of the various agarwood oils obtained by hydrodistillation and other methods [15]. In this study, we extracted essential oils by hydrodistillation from agarwoods originating from *A. crassna* cultivated in three production areas in Vietnam, and determined their chemical composition using GC–MS techniques.

2. Materials and Methods

2.1. Materials

The agarwoods used in this study were provided by Mr. Nguyen Thoan in 2016, a forest engineer and director of Lam Vien Co. Ltd., Hanoi, Vietnam, a company specializing in agarwood trading and *Aquilaria crassna* cultivation. The cultivation of agarwood-producing plants was located in three different provinces in Vietnam, including Bac Giang, Khanh Hoa, and on the Kien Giang (Phu Quoc island). The agarwood-producing plant was botanically 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. The voucher specimens were stored in University of Science, Vietnam National University Ho Chi Minh City. To obtain the agarwood, 15-year-old *Aquilaria crassna* trees were first inoculated with a preparation containing proprietary fungi of Lam Vien Co. Ltd. After three years, the unaffected heartwoods of the tree were manually removed, leaving agarwood, which was subsequently subject to air drying and grinding into powder. The final product was kept in paper bags at room temperature before distillation.
2.2. Hydrodistillation of Essential Oils

Similar to the material pretreatment process, the extraction procedure described herein was devised to represent the actual process practiced at the site of production of agarwood essential oils. Specifically, 500 g of each agarwood sample was immersed in distilled water in a 2 L round-bottom glass flask for 3 weeks. Following that, the agarwood was subject to hydrodistillation for 72 h in an all-glass Clevenger-type distillation apparatus. The rate of distillation was adjusted so that the temperature of the obtained distillate was maintained at lower than 40 °C, ranging from 30 to 35 °C. This corresponds to the flow rate of 3–10 mL/min. The volatile oil recovered from the recuperating container was transferred into a clean and previously weighed sample bottle and refrigerated until analysis. The samples were labeled as Bac Giang, Khanh Hoa, and Phu Quoc for the essential oils obtained from the Bac Giang, Khanh Hoa, and Phu Quoc materials, respectively.

2.3. GC–MS Analysis of Essential Oils

GC–MS analysis of the essential oils was in accordance with a previous study [16] and was carried out on an Agilent Technologies HP7890A GC equipped with a mass spectrum detector (MSD) Agilent Technologies HP5975C and a DB-XLB column (60 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies). The injector and detector temperature were set at 250 and 280 °C, respectively. The column temperature progress initiated at 40 °C, followed by an increase to 140 °C at 20 °C/min and then to 270 °C at 4 °C/min. The carrier gas was helium at a flow rate of 1 mL/min. Samples were injected by splitting. The split ratio was 100:1. The volume injected was 1 µL of essential oils. The MSD conditions were as follows: ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range 35–450 amu under full scan. A homologous n-alkane series was used as the standard to calculate the retention time indices (RIs) of each component. The relative amounts of individual components were calculated based on the GC peak area (MSD response) without correction.

2.4. Identification of the Constituents

MassFinder 4.0 (Dr. Hochmuth, Hamburg, Germany) software connected to the HPCH1607, W09N08 libraries, and the NIST Chemistry WebBook (National Institute of Standards and Technology, Gaithersburg, MD, U.S.) was used to match mass spectra and retention indices. To confirm these results, further comparisons were made with the data of authentic compounds reported in the original literature.

3. Results and Discussion

The agarwood samples in this study were chosen to represent products originating from A. crassna cultivated in three geographically different areas of Vietnam, namely, in Bac Giang province, Khanh Hoa province, and on the Phu Quoc island (Kien Giang province), which are situated in North, Central, and South Vietnam, respectively. The yields of essential oils obtained after hydrodistillation were 0.32%, 0.27%, and 0.25% (w/w) for the Bac Giang (BG), Khanh Hoa (KH), and Phu Quoc (PQ) samples, respectively.

In an independent preliminary GC–MS study, we analyzed a representative sample of agarwood oil on 3 different capillary GC columns, namely DB-1, HP-5, and DB-XLB (results not shown here). The DB-XLB column was chosen in this comparative study because of its thermal stability and separation efficiencies. Under the GC–MS conditions described in the experimental section, the obtained gas chromatograms of the three oil samples were found to be very complex. Even with peaks below 0.1% being excluded, about 70 peaks were still registered for each sample.

The identification of the individual components of the oils was based on matching of their mass spectra and retention indices with those of authentic compounds available in GC–MS libraries or reported in the original literature. Inseparable pairs of compounds were found for peak number 6 (4-ethylphenol and 4-vinylanisol), 20 (7-epi-eremophila-1(10),8,11-triene and an unknown compound), and 40 (karanone and oxo-agarospirol). Peak number 25 could be attributed either to hinesol (identified
by MassFinder 4.0 or isoagarospirol [3]. In total, 44 compounds could be identified. All of them had been previously found in agarwood samples from different sources [3]. Detailed analysis results of GC–MS of the three oil samples are shown in Table 1. The chemical structures of important sesquiterpene constituents are presented in Figure 1.

**Table 1. Constituents of essential oils obtained from agarwood samples produced by *A. crassna* growing in the provinces Bac Giang (BG) and Khanh Hoa (KH), and on the island Phu Quoc (PQ) of Vietnam.**

| Peak No. | Compound a | RI 1 XLB | RI 1-2 HP-5 | RI 2-3 DB-1 | Peak Area (%) b |
|---------|------------|----------|-------------|-------------|----------------|
| 1       | Benzaldehyde| 1081     | 961         | 941         | 0.53           |
| 2       | Phenylethylacetaldehyde | 1082 | 1042 | 1012 | 0.52 |
| 3       | Salsicylaldehyde  | 1087 | 1045 | 1020 | 0.24 |
| 4       | Acetylene | 1107 | 1065 | 1039 | 0.28 |
| 5       | n-Nonane-1-ol | 1102 | 1169 | 1149 | 0.17 |
| 6       | 4-Ethylphenol and/or 4-vinyltos | 1192 | - | 1139 | 0.30 |
| 7       | n-Normorac acid | 1290 | 1271 | 1263 | - |
| 8       | Benzylacetone  | 1295 | - | 1207 | 0.10 |
| 9       | 4-Pentan-2-one | 1299 | - | - | 0.32 |
| 10      | 3-Aranaldehyde | 1314 | 1250 | 1218 | 0.30 |
| 11      | (1R,6S,9R)-6,10-Trinormethyl-11-oxatricyclo[7.2.1.03,6]-dodecane | 1441 | - | - | 0.21 |
| 12      | 4,8-di-Eudesmol | 1500 | - | 1470 | 0.18 |
| 13      | β-Agarofuran | 1507 | - | 1474 | 4.66 |
| 14      | n-Butanal | 1530 | - | 1494 | 0.24 |
| 15      | Di-β-agarofuran  | 1533 | - | 1480 | 0.70 |
| 16      | Anisylacetone | 1549 | - | - | 2.27 |
| 17      | β-Agarofuran  | 1582 | 1550 | 1535 | 0.53 |
| 18      | non-Keto-agarofuran | 1608 | - | 1554 | 2.92 |
| 19      | 1-Propanol | 1619 | - | 1572 | 2.77 |
| 20      | 7-epi-Eremophila-1(10),11-ol and unknown [11,220(20)] | 1629 | - | 1584 | 2.12 |
| 21      | (1R,2R,5S,9R)-6,10-Trinormethyl-11-oxatricyclo[7.2.1.03,6]-dodecane-2-ol, epimer 1 | 1637 | - | - | 1.21 |
| 22      | (1R,2R,5S,9R)-6,10-Trinormethyl-11-oxatricyclo[7.2.1.03,6]-dodecane-2-ol, epimer 2 | 1651 | - | - | 1.27 |
| 23      | 10-epi-Eudesmol | 1660 | 1624 | 1609 | 1.68 |
| 24      | Aganospirol | 1672 | 1648 | 1635 | 2.98 |
| 25      | Hexadecane | 1675 | 1642 | 1622 | 1.08 |
| 26      | Jinko-eremol | 1686 | - | 1643 | 3.50 |
| 27      | Valencene | 1693 | 1658 | 1647 | 3.91 |
| 28      | β-Eudesmol | 1699 | 1651 | 1641 | 2.96 |
| 29      | α-Eudesmol | 1704 | 1654 | 1643 | 2.74 |
| 30      | Valencene-1(10),8-dien-11-ol | 1723 | - | - | 0.07 |
| 31      | Dehydrojinko-eremol | 1730 | - | - | 0.37 |
| 32      | Epvinyl-agarofuran | 1742 | - | 1673 | 1.42 |
| 33      | Cedrane-1(10),4-dien-8-ol | 1748 | - | 1657 | 1.64 |
| 34      | (1R,2R,5S,9R)-6,10-Trinormethyl-11-oxatricyclo[7.2.1.03,6]-dodecane-2-carboxylic acid | 1753 | - | 1673 | 4.67 |
| 35      | (1R,2R,5S,9R)-6,10-Trinormethyl-11-oxatricyclo[7.2.1.03,6]-dodecane-2-carboxylic acid | 1753 | - | 1673 | 4.67 |
| 36      | Neopentane | 1814 | - | 1753 | 7.96 |
| 37      | Selina-4,11-dien-14-ol | 1825 | - | 1734 | 1.48 |
| 38      | Dihydrokaranone | 1870 | - | 1759 | 3.56 |
| 39      | Norkaranone | 1877 | 1807 | 1782 | 0.98 |
| 40      | Karanone and re-agarofuran | 1892 | - | 1812 | 1.20 |
| 41      | n-Hexadecanoic acid | 1995 | - | 1951 | 5.69 |
| 42      | Oleic acid | 2164 | - | - | 0.88 |
| 43      | Total of identified compounds | 8354 | 8430 | 7411 | 74.11 |
| 44      | Sesquiterpenes hydrocarbons | 2543 | 2434 | 2302 | 7.96 |
| 45      | Oxypregn-8-ene-3,14-diene-11,12-dihydrodiol | 5904 | 6894 | 6168 | 74.11 |

a Elution order on DB-XLB column. Compounds were named according to the nomenclature summarized by Naef (2011) [3]; b Experimental RI values on DB-XLB column; c RI values on HP-5 column according to Adams (2004) [16]; d RI values on DB-1 column; e RI values on DB-1 column according to Koening et al., (2018) [17]; f MS data of unknown compound with m/z of the base peak followed by the highest m/z value and its intensity in%; g Peak areas were calculated following the chromatogram obtained from the DB-XLB column.

In the Bac Giang oil, 41 components were identified, representing 83.54% of the total volatiles. Major constituents were benzylacetone (10.46%), neopentane (Peak no. 36, 7.96%), n-hexadecanoic acid (5.69%), β-agarofuran, (Peak no. 13, 4.86%), the dihydroagarofuran aldehyde (1S,25,6S,9R)-6, 10,10-trimethyl-11-oxatricyclo[7.2.1.03,6]-dodecane-2-carboxylic acid (Peak no. 34, 4.67%), valerenol (Peak no. 27, 3.91%), and dihydrokaranone (Peak no. 38, 3.59%).

Forty-one components were identified in Khanh Hoa oil, representing 74.11% of the total volatiles, with major constituents being neopentane (8.29%), valencia-1(10), 8-dien-11-ol (30, 6.05%), valerenol (5.37%), n-hexadecanoic acid (4.98%), the dihydroagarofuran aldehyde (1S,25,6S,9R)-6, 10,10-trimethyl-11-oxatricyclo[7.2.1.03,6]-dodecane-2-carboxylic acid (4.73%), β-eudesmol (Peak no. 28, 4.38%), jinko-eremol (Peak no. 26, 3.87%), and epoxy-β-agarofuran (Peak no. 32, 3.84%).
Figure 1. Structures of important sesquiterpene compounds identified in Bac Giang, Khanh Hoa, and Phu Quoc agarwood oils. The compounds were numbered in accordance with corresponding peaks.

In the Phu Quoc oil, 39 components were identified, representing 84.30% of the total volatiles, with major constituents being neopetasane (7.47%), \( n \)-hexadecanoic acid (6.43%), \( \beta \)-agarofuran (6.18%), valerianol (4.56%), jinko-eremol (4.51%), \( \beta \)-eudesmol (4.18%), dihydrokaranone (3.52%), agarospirol (Peak no. 24, 3.42%), and nor-keto-agarofuran (Peak no. 18, 3.31%).

There were 23, 26, and 33 unknown compounds, representing 16.46%, 15.70%, and 25.89% of the total volatiles of the Bac Giang, Khanh Hoa, and Phu Quoc oils, respectively. Among the three essential oil samples, the Bac Giang oil stood out by the abundance of benzylacetone (10.46%), the Khanh Hoa oil was characterized by the presence of valenca-1(10),8-dien-11-ol (6.05%), and the Phu Quoc oil was characterized by the higher number and larger quantity of unidentified compounds.

Identified volatile components in the three samples were grouped based on their structural skeletons for better characterization and comparison. The results are shown in Table 2. In this approach, agarofurans and \( \text{epi-}\gamma\)-eudesmanes were put in one group considering their close biosynthetic relationship [18]. As shown in Table 2, major groups found in these oils included the valencanes and eremophilanes, the agarofurans and \( \text{epi-}\gamma\)-eudesmanes, the selinanes and eudesmanes, the fatty acids and aliphatics, and the aromatic compounds. The remaining minor groups were the vetispiranes, the guaianes, and the cadinanes. In the eremophilane and valencane group,
the most abundant compounds were neopetasane (7.47–8.29%), valerianol (3.91–5.37%), jinko-eremol (3.50–4.51%), and dihydrokaranone (2.63–3.59%). In the agarofurans and \textit{epi-\gamma}-eudesmane group, the most abundant compounds were \textbeta-agarofuran (3.04–6.18%), (1S,2S,6S,9R)-6,10,10-trimethyl-11-oxatricyclo[7.2.1.0^1,6]dodecane-2-carbaldehyde (2.07–4.73%), epoxy-\textbeta-agarofuran (0.94–3.84%), and 10-\textit{epi-\gamma}-eudesmol (1.28–2.11%). In the selinane and eudesmane group, the most abundant compounds were \textbeta-eudesmol (2.96–4.38%), \alpha-eudesmol (2.27–3.15%), selina-3,11-dien-9-ol (35, 1.34–2.40%), and selina-4,11-dien-14-al (37, 0.33–2.34%). Important compounds in other groups were agarospirol, hinesol (25), epoxybulnesene (19), benzylacetone, and \textit{n}-hexadecanoic acid. Neopetasane, dihydrokaranone, \textbeta-agarofuran, and agarospirol are desirable aromatic compounds [19,20].

Table 2. Structural profiles of the constituents of essential oils obtained from Bac Giang (BG), Khanh Hoa (KH), and Phu Quoc (PQ) agarwood samples.

| No. | Compound Class                  | Number of Compounds | Total Area% |
|-----|---------------------------------|---------------------|-------------|
|     |                                 |                     | BG         | KH   | PQ   |
| 1   | Eremophilanes and valencanes    | 9                   | 23.39      | 32.03| 24.09|
| 2   | Agarofurans and \textit{epi-\gamma}-eudesmanes | 10 | 19.47 | 16.98| 20.02|
| 3   | Selinanes and eudesmanes       | 5                   | 9.05       | 11.79| 9.17 |
| 4   | Fatty acids and aliphatics      | 4                   | 6.74       | 8.52 | 7.56 |
| 5   | Aromatic compounds             | 9                   | 15.22      | 3.41 | 2.85 |
| 6   | Vetispiranes                   | 3                   | 5.26       | 5.68 | 5.38 |
| 7   | Guaiolanes                     | 1                   | 2.77       | 3.47 | 2.86 |
| 8   | Cadinanes                      | 1                   | 1.64       | 2.42 | 2.18 |

The compositional results in the present study share similarities to that of a Vietnamese agarwood sample reported previously. To be specific, Yoneda et al. (1984) performed a comparative GLC and GC/MS analysis of sesquiterpenes in the benzene extracts of two agarwood samples. One sample was from Vietnam (designated by the authors as type A agarwood) and the other was from Indonesia (designated by the authors as type B agarwood) [20]. While the Indonesian sample was different from the present results in terms of composition, the Vietnamese sample (source plant: \textit{A. agallocha Roxb.}) was characterized by the dominance of \textit{oxo}-agarospirol (5.8%), agarospirol (4.7%), jinko-eremol (4.0%), valerianol (2.9%), and dihydrokaranone (2.4%), all of which were also found in the present study. Furthermore, the identified compositions in this study were quite similar to that of oil obtained from Indian \textit{A. agallocha Roxb.} [21].

Despite aforementioned similarities, the profiles of volatile composition of all three oil samples were different from results of studies involving agarwood samples from various areas, including those from Vietnam. To be specific, notable compositional difference could be observed when comparing with essential oils obtained from Chinese agarwood from \textit{A. sinensis}, in which the major component was found to be the vetispirane sesquiterpene \textit{oxo}-agarospirol [22]. The three oil samples were also different from the oil obtained from Indonesian \textit{A. malaccensis}, which was characterized by the abundance of prezизzane sesquiterpenes jinkohol II and jinkohol [23].

Some important compounds that were previously identified are also missing in the present results. In a series of analysis of another Vietnamese agarwood sample, graded and designated in Japan as Kanankoh (Ryoku-yu), 14 new compounds, including 9 guaiane and 5 selinane sesquiterpenes, were isolated and structurally characterized [7,24–26]. Notable identified constituents were eremophilane sesquiterpenes dehydrojinko-eremol and neopetasane. In a further comparative study by GC–MS, the acetone extracts of four agarwood samples from Vietnam, including three Kanankoh samples (graded and designated in Japan as Ryoku-yu, Cha-yu, and Murasaki) and one Jinkoh (Bateikei) sample were investigated [18]. It was found that guaiane and selinane sesquiterpenes were major components of the three Kanankoh samples, while the Jinkoh sample was characterized by the abundance of \textit{oxo}-agarospirol (11.6%) and neopetasane (8.1%). A later study also isolated a new spirovetivane sesquiterpene, (4R,5R,7R)-1(10)-spirovetiven-11-ol-2-one, together with a known
2-(2-phenylethyl)-chromone from the ethyl ether extract of Kyara, the agarwood of highest grade from Vietnam [27]. This new sesquiterpene has been shown to exhibit significant inducing effect on brain-derived neurotrophic factor mRNA expression in vitro. In addition to the aforementioned compounds, a number of nonvolatile chromones were also isolated and characterized from Kyara [28] and Kanankoh [29] agarwood imported from Vietnam, as well as from agarwood originating from A. crassna cultivated in Vietnam [30].

In general, volatile composition of essential oils obtained from the present agarwood samples showed both similarities and differences in comparison with previous results. More importantly, the present results revealed the absence of several valuable guaiane and selinane sesquiterpenes existing in extracts from other Vietnamese agarwood samples (Kyara, Kanankoh, and Jinkoh), which were probably collected in nature.

4. Conclusions

Essential oils of three agarwood samples representing products from A. crassna cultivated in the provinces Bac Giang and Khanh Hoa, and on the Phu Quoc island (Kien Giang province) of Vietnam were obtained by hydrodistillation. The process afforded an essential oil yield of 0.32%, 0.27%, and 0.25% (w/w) for three agarwood samples, respectively. A total of 44 volatile compounds which were previously found in agarwood oils were identified in these oils by GC–MS analysis. Most of the constituents were oxygenated sesquiterpenes. Notable compounds of other chemical classes were aromatics and fatty acids. The three oil samples shared a similar composition profile which is characterized by the dominance of eremophilane, agarofuran, and eudesmane sesquiterpenes, while vetispirane and guaiane sesquiterpenes were found in smaller quantities. Desired compounds, such as neopetasane (7.47–8.29%), dihydrokaranone (2.63–3.59%), β-agarofuran (3.04–6.18%), and agarospirol (2.98–3.42%), were present in substantial quantities, suggesting a use for the oils as high value fragrant materials.

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References
1. Arlene, L.-S.; Tony, P. History of Use and Trade of Agarwood. *Econ. Bot.* 2018, 72, 1–23.
2. Liu, Y.; Wei, J.; Gao, Z.; Zhang, Z.; Lyu, J. A Review of Quality Assessment and Grading for Agarwood. *Chin. Herb. Med.* 2017, 9, 22–30. [CrossRef]
3. Naef, R. The volatile and semi-volatile constituents of agarwood, the infected heartwood of Aquilaria species: A review. *Flavour Fragr. J.* 2011, 26, 73–87. [CrossRef]
4. Chen, H.-Q.; Wei, J.-H.; Yang, J.-S.; Zhang, Z.; Yang, Y.; Gao, Z.-H.; Sui, C.; Gong, B. Chemical Constituents of Agarwood Originating from the Endemic Genus Aquilaria Plants. *Chem. Biodivers.* 2012, 9, 236–250. [CrossRef] [PubMed]
5. Kalra, R.; Kaushik, N. A review of chemistry, quality and analysis of infected agarwood tree (*Aquilaria* sp.). *Phytochem. Rev.* 2017, 16, 1045–1079. [CrossRef]
6. Broad, S. Agarwood Harvesting in Vietnam. *Traffic Bull.* 1995, 15, 96.
7. Ishihara, M.; Tsuneya, T.; Shiga, M.; Uneyama, K. Three sesquiterpenes from agarwood. *Phytochemistry* 1991, 30, 563–566. [CrossRef]
8. Ho, P.H. *An Illustrated Flora of Vietnam*, 2nd ed.; Young Publishing House: Hanoi, Vietnam, 1999; Volume II, pp. 36–37. (In Vietnamese)
9. La, D.M.; Luu, D.C.; Tran, M.H.; Nguyen, T.T.; Nguyen, T.P.T.; Tran, H.T.; Ninh, K.B. *Essential Oil Plant Resources in Vietnam*; Agriculture Publishing House: Hanoi, Vietnam, 2001; Volume I.

10. Kiet, L.C.; Kessler, P.J.A.; Eurlings, M.; Pierre, A.; Hô, A.P.H.H. A New Species of Aquilaria (Thymelaeaceae) from Vietnam during a survey on wild and cultivated aquilaria populations in the Central Highlands of Vietnam, one interesting Aquilaria was identified in the Sa Thay district, Kontum province. It is similar. *Blumea* 2005, 135–141. [CrossRef]

11. Ito, M.; Honda, G. Taxonomical identification of agarwood-producing species. *Nat. Med.* 2005, 59, 104.

12. Quan, L.T.; Qui, K.T.; Kouda, K.; Nguyen, N.T.; Maruyama, Y.; Saiki, I.; Kadota, S. A survey on agarwood in Vietnam. *J. Tradit. Med.* 2003, 20, 124–131.

13. Son, H.N. *Final Report of the Project “Assessment of the Current Situation and Sustainable Development of the Plant Do Tram Aquilaria spp.” of the Ministerium of Agriculture and Rural Development of the Socialist Republic of Vietnam, Implemental 2007-2010*; Non-Timber Forest Product Research Center, Vietnamese Academy of Forest Sciences: Hanoi, Vietnam, 2010. (In Vietnamese)

14. Nguyen, H.T.; Min, J.E.; Long, N.P.; Thanh, M.C.; Van Le, T.H.; Lee, J.; Park, J.H.; Kwon, S.W. Multi-platform metabolomics and a genetic approach support the authentication of agarwood produced by Aquilaria crassna and Aquilaria malaccensis. *J. Pharm. Biomed. Anal.* 2017, 142, 136–144. [CrossRef] [PubMed]

15. Thuy, D.T.T.; Tuyen, T.T.; Chien, N.Q.; Thuy, T.T.T.; Bich, H.T.; Toan, T.Q. Comparative Study on Volatile Compounds of Agarwood from Khanh Hoa Province Extracted by Different Methods. *VJST.* 2008, 56, 266–272. [CrossRef]

16. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*, 3rd ed.; Allured Publishing Corporation: Carol Stream, IL, USA, 2004.

17. König, W.A.; Joulain, D.; Hochmuth, D.H. Terpenoids Library—Terpenoids and Related Constituents of Essential Oils. Available online: https://massfinder.com/wiki/Terpenoids_Library (accessed on 30 December 2018).

18. Ishihara, M.; Tsuneya, T.; Uneyama, K. Components of the volatile concentrate of agarwood. *J. Essent. Oil Res.* 1993, 5, 283–289. [CrossRef]

19. Zviely, M.; Ming, L. Sesquiterpenoids—The Holy Fragrance Ingredients—Part 1. *Perfum. Flavorist* 2013, 38, 52.

20. Zviely, M.; Boix-Camps, A. Sesquiterpenoids—The Holy Fragrance Ingredients—Part 2. *Israel Chem. Eng.* 2015, 1, 27–36.

21. Näf, R.; Velluz, A.; Braunlich, R.; Thommen, W. Agarwood oil (Aquilaria agallocha Roxb.). Its composition and eight new valencane-, eremophilane- and vetispirane-derivatives. *Flavour Fragr. J.* 1995, 10, 147–152. [CrossRef]

22. Chen, H.; Yang, Y.; Xue, J.; Wei, J.; Zhang, Z.; Chen, H. Comparison of compositions and antimicrobial activities of essential oils from chemically stimulated agarwood, wild agarwood and healthy Aquilaria sinensis (Lour.) Gilg trees. *Molecules* 2011, 16, 4884–4896. [CrossRef] [PubMed]

23. Yoneda, K.; Yamagata, E.; Nakanish, T.; Nagashima, T.; Kawasaki, I.; Mori, H.; Miura, I. Sesquiterpenoids in two different kinds of agarwood. *Phytochemistry* 1984, 23, 2068–2069. [CrossRef]

24. Ishihara, M.; Tsuneya, T.; Uneyama, K. New sesquiterpene from agarwood. *Phytochemistry* 1991, 30, 3343–3347. [CrossRef]

25. Ishihara, M.; Masatsugu, Y.; Uneyama, K. Preparation of (-)-guaia-1(10),11-dien-15,2-olide and (-)-2α-hydroxyguaia-1(10),11-dien-15-oic acid, fragrant sesquiterpenes in agarwood (Aquilaria agallocha Roxb.). *Tetrahedron* 1992, 48, 10265–10276. [CrossRef]

26. Ishihara, M.; Tsuneya, T.; Uneyama, K. Fragrant sesquiterpenes from agarwood. *Phytochemistry* 1993, 33, 1147–1155. [CrossRef]

27. Ueda, J.; Imamura, L.; Tezuka, Y.; Tran, Q.L.; Tsuda, M.; Kadota, S. New sesquiterpene from Vietnamese agarwood and its induction effect on brain-derived neurotrophic factor mRNA expression in vitro. *Bioorganic Med. Chem.* 2006, 14, 3571–3574. [CrossRef] [PubMed]

28. Hashimoto, K.; Nakahara, S.; Inoue, T.; Sumida, Y.; Takahashi, M.; Masada, Y. A new chromone from agarwood and pyrolysis products of chromone derivatives. *Chem. Pharm. Bull. (Tokyo)* 2011, 33, 5088–5091. [CrossRef]
29. Nakanishi, T.; Inada, A.; Nishi, M.; Yamagata, E.; Yoneda, K. A New and a Known Derivatives of 2-(2-Phenylethyl) Chromone from a Kind of Agar Wood (“Kanankoh,” In Japanese) Originating from Aquilaria Agallocha. *J. Nat. Prod.* **1986**, *49*, 1106–1108. [CrossRef]

30. Yagura, T.; Shibayama, N.; Ito, M.; Kiuchi, F.; Honda, G. Three novel diepoxy tetrahydrochromones from agarwood artificially produced by intentional wounding. *Tetrahedron Lett.* **2005**, *46*, 4395–4398. [CrossRef]