

Determination of Agarwood volatile compounds from selected *Aquilaria* species plantation extracted by Headspace-Solid Phase Microextraction (HS-SPME) method

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Abstract. A collection of inoculated heartwood of three *Aquilaria* species namely *A. crassna*, *A. sinensis* and *A. subintegra* were investigated for their volatile compounds. The volatile compounds were extracted using automated Headspace-Solid Phase Microextraction (HS-SPME) with fibre coating of 50/30 μm divinyl benzene/carboxen/poly-dimethylsiloxane (DVB/CAR/PDMS) and determined by gas chromatography/mass spectrometry (GCMS). The absence of proper scientific method in forming the agarwood has resulted in variations in agarwood quality and inconsistent grades in the market. Thus there is a need to quantify the amount of major volatile compounds found in the agarwood so that the quality can be scientifically verified. In this study, the commonly known volatile compounds present in agarwood sample were found but with varying amounts. At least three important compounds were obtained i.e. β -agarofuran, α -eudesmol and agarospirol. Based on the presence of these main volatile compounds, the study suggests that extraction by HS-SPME also useful instead of distillation process. The ability to identify key components in a short time can facilitate and shorten the analysis time. It further helps in developing the quality of agarwood industry standards.

1. Introduction

The *Aquilaria* tree content resinous heartwood commonly known as agarwood is a genus belonging taxonomically to the Thymelaeaceae. The agarwood tree produces resin as a defense mechanism against infection or injury. The resin is valued in many cultures for its distinctive fragrance, and is used as a principal component in incense and perfumes as well as in traditional medicine [1]. Agarwood chips produced significant aroma when it is heated or burned and they can be obtained from natural resources and nowadays it can be artificially induced [2]. However there are doubts about the quality of agarwood chips produced from the chemically induced tree. Usually, natural formed agarwood produce more resin and the price is higher than induced agarwood [2]. In addition, other uses of this product are constrained by limited supply and very expensive. One of the main reasons for the relative rarity and high cost of agarwood is the depletion of the wild resource [3]. This genus consists about 17 species, growing on particular sites of tropical Asia [3]. Among those species, five



are found in Malaysia, i.e. *Aquilaria malaccensis*, *A. sinensis*, *A. hirta*, *A. subintegra* and *A. crassna* [4].

Plantation for *Aquilaria* trees in combination with artificial agarwood-inducing methods serves as a way to supply agarwood and conserve of wild *Aquilaria* stock. Agarwood cultivation was increasing especially after several research studies showed that cultivated agarwood could provide feasible benefit [4]. Additionally, the existing agarwood-producing methods require a long time for agarwood formation, meanwhile, the resin produced in a short time in this inducing technique is deemed to be of inferior quality [5].

Studies on the chemistry of agarwood have reported presence of sesquiterpenes, chromone derivatives, sesquiterpene furanoids, tetradecanoic acid and pentadecanoic acid [6-10]. The sesquiterpenes and phenylethyl chromone derivatives are the main compounds in agarwood [11] and [12]. The sesquiterpene hydrocarbons contribute to the fragrant of wood aroma [13]. Based on the knowledge from the agarwood oil, studies had been carried out on the total volatile compounds from the agarwood chips using Headspace-SPME and by GC-MS [8, 14]. Chemical compounds were identified by matching their retention indices with literature values [9] and further verified by comparing with the Wiley and Adams library search data in GC/MS database. In this study, the chemical profiles of agarwood from different species of *Aquilaria* were examined using GCMS. The result is reported, compared and discussed in this paper.

2. Materials and methodology

Inoculated heartwood samples of three *Aquilaria* species namely *A. crassna*, *A. sinensis* and *A. subintegra* were investigated for its volatile compounds. *A. crassna* and *A. sinensis* samples were provided by RAL Plantation Sdn. Bhd, Kuala Kangsar, Perak meanwhile *A. subintegra* was obtained from Biobenua Teknologi Sdn. Bhd, Kuala Linggi, Melaka. The trees age were about 5 years old and all trees have been inoculated for 6 to 12 months.

2.1 Methods

2.1.1 Sample preparation. Samples are collected by cutting down the selected tree after being purchased from the planters. Infected wood known as agarwood removed from the white wood then the carving process was executed. In order to get agarwood, the dark wood will be separated from the white wood using sharp knives, a broken glass, or hooked knives and dried under the shade [15]. Under constant moisture content in room temperature, the samples were finely ground using basic analytical mill and ready to run the chemical analysis.

2.1.2 HS-SPME procedure. The agarwood volatile compounds were extracted using automated Headspace-Solid Phase Microextraction (HS-SPME) with fibre coating of 50/30 μm divinyl benzene/carboxen/poly-dimethylsiloxane (DVB/CAR/PDMS). Three replicates of 2 grams of each samples were prepared into 20 ml screw-top clear vial and tightly capped. The sample vials was introduced in an agitator for 10 min at incubation temperature of 80 °C. The extraction time was fixed at 10 min to trap volatile compounds. After sampling, the fibre was immediately injected into the GCMS injection port kept at 60 °C with a hold time of 10 min.

2.1.3 GCMS Analysis. The chemical constituents were determined by using gas chromatography/mass spectrometry (GCMS). Analysis was carry out using Agilent Technologies 7890A/5975C Series MSD with HP-5MS column (30 m x 0.25 mm ID x 0.25 μm film thickness). The programmed was set, initially at 60 °C for 10 min, increase to 180 °C for 1 min at 3 °C/min. Helium was used as carrier gas at a flow rate 1.0 ml/min and the ion source temperature was programmed at 280 °C. The chemical constituents were identified by mass spectral library (HPCH2205.L, Wiley7Nist05.L, and NIST05a.L). The results of the peak areas were expressed as peak counts.

3. Results and discussion

The volatile compounds identified in agarwood of three *Aquilaria* species namely *A. crassna*, *A. sinensis* and *A. subintegra* were shown in Table 1. Overall, a total of 20 compounds were identified in all of the woodchips of which 13 compounds from *A. crassna*, 17 compounds from *A. sinensis* and 17 compounds from *A. subintegra*. There are several common volatile compounds within these three species namely 4-phenyl-2-butanone, β -agarofuran, dihydroagarofuran, α -bulnesene, α -agarofuran, aromadendrene, 10-*epi*- γ -eudesmol, agarospirol, valencene, valerianol and α -eudesmol. Some of these compounds were major compounds in one species but not in other species. However, 9 similar compounds has been appeared in all study species which were 4-phenyl-2-butanone, α -guaiene, α -humulene, β -agarofuran, α -selinene, dihydroagarofuran, α -bulnesene, α -agarofuran and agarospirol exists either as major or minor compounds.

Table 1. Chemical compounds of *A. crassna*, *A. sinensis* and *A. subintegra* extracted by HS-SPME

No	Chemical compounds	Area (%)		
		<i>A. crassna</i>	<i>A. sinensis</i>	<i>A. subintegra</i>
1	4-phenyl-2-butanone	2.76	1.77	4.35
2	β -elemene	-	1.04	0.37
3	α -guaiene	0.97	3.43	0.59
4	α -humulene	0.59	3.23	1.51
5	β -agarofuran	8.55	2.13	1.9
6	α -selinene	0.88	5.44	1.88
7	ar-curcumene	-	6.07	5.39
8	dihydroagarofuran	8.41	5.44	3.96
9	α -bulnesene	6.17	5.36	1.26
10	α -agarofuran	4.06	4.91	1.7
11	Nerolidol	3.16	-	-
12	Aromadendrene	-	12.88	2.38
13	10- <i>epi</i> - γ -eudesmol	3.89	-	2.78
14	Agarospirol	14.85	2.48	2.92
15	Valencene	7.41	-	-
16	Valerianol	14.29	-	-
17	α -eudesmol	-	15.84	15.41
18	Santolol acetate	-	2.7	6.43
19	Cyclocolorinone	-	7.81	-
20	Aristolone	-	4.57	4.77

It is observed that temperature programmed at 80 °C, the fibre extracted most of the common major compounds in agarwood sample. Dihydroagarofuran was captured as one of major compounds in all three species. Agarospirol (14.85%) was the most abundance compounds for *A. crassna*, meanwhile α -eudesmol were the highest compounds in *A. sinensis* and *A. subintegra* with (15.84%) and (15.41%) respectively.

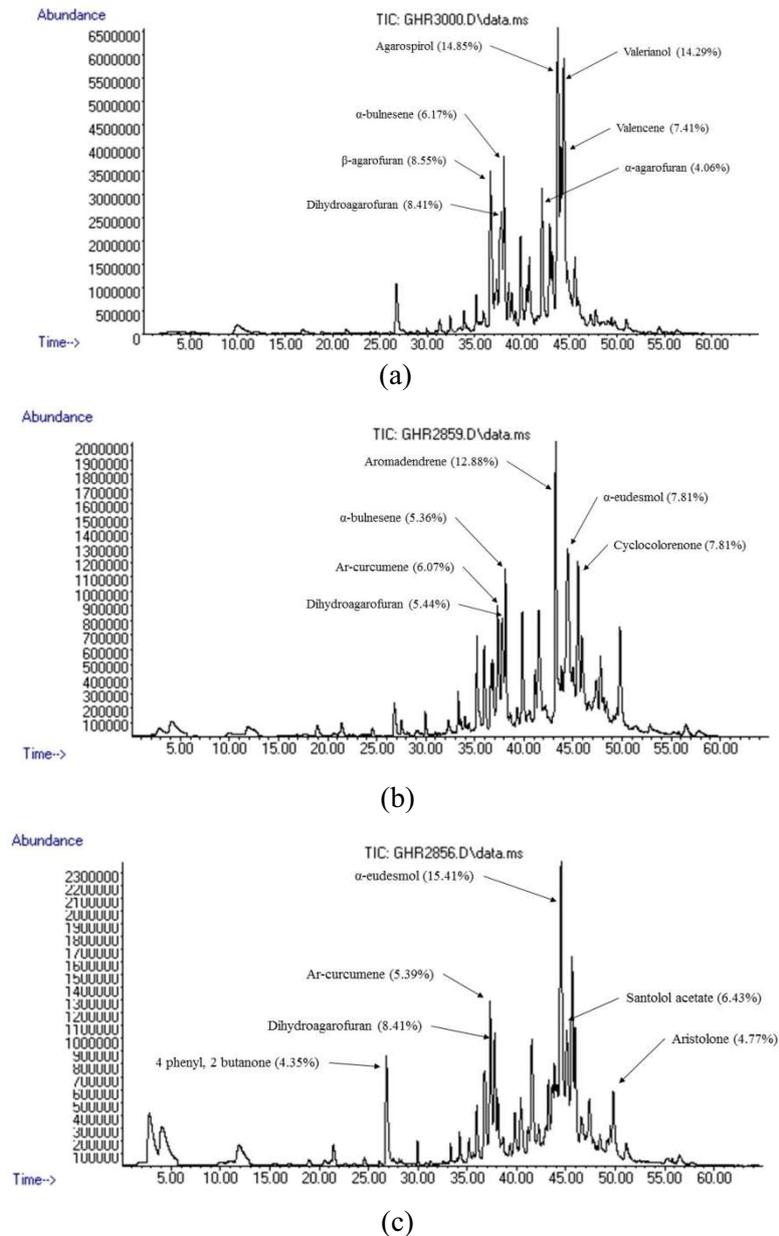


Figure 1. GCMS chromatogram profiles for each observed agarwood where (a) GCMS chromatogram of *A. crassna*, (b) GCMS chromatogram of *A. sinensis* and (c) GCMS chromatogram of *A. subintegra*.

All major volatile compounds presence in this study as chromatogram profiles shows in (Figure 1). These presented volatile compounds from wood chips were similarly found in agarwood essential oils. (Table 2) shows the studied from other researchers indicate that there are some similarities and variations in the volatile compounds of several agarwood oils, such as 3-Phenyl-2-butanone, β -agarofuran, α -agarofuran, agarospirol and 10-*epi*- γ -eudesmol were some of the volatile compounds found occurring in all the oils studied [16].

Table 2. Some chemical compounds detected in some Malaysian agarwood oils.

Compounds	RI	Selangor (%)	Kelantan (%)	Pahang (%)	Terengganu (%)
3-phenyl-2-butanone	1249	1.50	5.77	7.80	0.79
α -guaiene	1448	-	0.67	-	-
β -agarofuran	1477	1.69	1.98	0.69	0.50
α -agarofuran	1553	4.83	2.96	1.48	1.57
Nor-ketoagarofuran	1557	2.09	-	-	-
10- <i>epi</i> - γ -eudesmol	1618	11.54	9.03	8.10	3.32
Agarospinol	1631	14.86	5.49	7.11	18.86
β -eudesmol	1649	-	-	-	5.74
Jinkoh-eremol	1650	10.62	7.70	6.31	-
kusunol	1659	18.94	-	-	-
Jinkohol II	1751	4.71	-	-	-

Source: [16]

4. Conclusion

The analysis of the agarwood by GCMS has been extracted using HS-SPME on three *Aquilaria* species namely *A. crassna*, *A. sinensis* and *A. subintegra* successfully obtained all the major volatile compounds in these samples. All those volatile compounds obtained from these *Aquilaria spp.* mostly found by others research studies on the chemistry of agarwood oils. They have reported presence group of aromatic terpene compounds including monoterpenes, sesquiterpenes and diterpenes [16-18]. Currently GC and GC/MS profiles are being used essential oils extracted from distillation process to assist the detection and authentication of agarwood. By using HS-SPME, the time of determination process of volatile compounds can be reduced in order to generate the GCMS profiles in agarwood without involved any distillation methods. Generating GC/MS profiles of agarwood samples of various species and from different sources will be helpful towards developing suitable chemical marker for grading agarwood. The presence of these major volatile compounds can be used as a guideline in assessing the consistent quality of agarwood obtained from plantations.

Acknowledgements

This work was done under RMK10 project (Classification of karas and ramin based on a combination of technology, chemometric analysis chemistry, electronic nose and image analysis characterization) in Natural Products Division, Forest Research Institute of Malaysia (FRIM). The authors would like to thank all Product Development Programme (PPH) research group for supporting this research. Special thanks to the Institute Tropical Forestry and Forest Products (INTROP) University Putra Malaysia for support the participating into WOBIC 2017 Conference.

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