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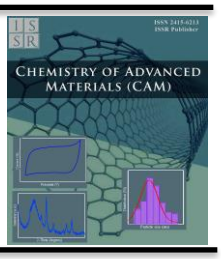

Original paper

Gas Chromatography Analysis of Artificially Inoculated Agarwood Compounds Related to High Quality Agarwood from Malaysia Plantation

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Abstract

The aim of this study is to determine the quality of artificially inoculated agarwood from *Aquilaria malaccensis* trees obtained from a plantation in East Malaysia using gas chromatography (GC). The agarwood quality was measured by existence and amount of aromatic and terpene compounds especially those which were recorded in high-quality agarwood such as α-guaiene, β-selinene, aromadendrene and agarospirol. For quality determination purpose of artificially inoculated agarwood, samples were collected from selected plantation area. Solid Phase Micro Extraction (SPME) method was performed to collect the volatile compounds released by wood sample. There were five commercial inoculants (Ino A, B, C, D and E) that had been used for inoculation of *A. malaccensis* in agarwood plantation. GC analysis revealed the presence of important compounds related to high-quality agarwood such as 4-phenyl-2-butanone, β-selinene, α-bulnesene, and agarospirol in agarwood sample produced from artificial inoculation using Ino A inoculant. Most of these compounds were mostly absent in the agarwood samples that have been inoculated with other inoculants (Ino B, C, D and E). Several important compounds related to high-quality agarwood were detected. The results obtained in this research clearly indicated a promising potential of artificially inoculated agarwood as an alternative source of high-quality agarwood.

1. Introduction

Agarwood, the highly valuable resinous woods derived from the diseased or wounded wood of *Aquilaria* plants, has been widely used as medicines and incenses. In Malaysia, agarwood is mainly produced by *Aquilaria malaccensis*. This plant is classified under *Thymelaeceae* botanical family and has been grouped as a non-timber wood which can grow vertically up to 30 m height. The bark of *A. malaccensis* appears to be soft and light color with greyish patches. Other than Peninsular Malaysia, A. *malaccensis* is found in India, Myanmar, Sumatra, Peninsular Malaysia, Singapore, Borneo and Philippines [1, 2].

Production of agarwood is a result of a plant defense mechanism where any physical, biological or chemical injury to the plant will initiate production of resin [3]. Resin will continue to accumulate in wood tissue as injury prolongs with pathogenic infection. Resin deposition may transform healthy wood into denser and darker wood that is known as agarwood [4]. Agarwood has been depleting from natural habitat due to over-logging and illegal logging [5]. Hence, all species of *Aquilaria* including *A. malaccensis* has been listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora [6].

Over a decade ago, for the protection of wild *Aquilaria* resources and their sustainable use, government and personnel make an attempt at cultivating *Aquilaria* sp. especially *A. malaccensis* in the plantation. In spites of the conservation effort, human-made artificial agarwood from plantation seems to create speculation of its doubted quality compared to natural agarwood. Formulation of agarwood inoculant and the invention of inoculating methods has been continuously studied in order to produce high quality of artificial agarwood which competent with natural agarwood quality. High-quality agarwood is enriched with aromatic and terpene compounds such as α-guaiene, β-selinene, aromadendrene, α-bulnesene and agarospirol. In case of artificial agarwood was proven to replace natural agarwood based on scientific analysis, the wrong speculation on artificial agarwood quality may be silenced and artificial agarwood would be accepted for international trade. It means a continuous supply of agarwood without interrupting with natural agarwood tree conservation, uprising economic benefit of agarwood trade and expanding agarwood-based aromatherapeutic and medical application.

Up to now the best inoculant formulation of agarwood in *Aquilaria* is remaining unknown but some commercial agarwood inoculants may work better than the other. The present study was, therefore, undertaken to study the qualitative differences in the agarwood obtained from artificially inoculated agarwood produced by different commercial inoculants in Malaysia plantation using gas chromatography.

2. Materials and Methods

2.1 Agarwood samples collection

Both agarwood and healthy wood sample were obtained from Agarwood Plantation in Merchang, Terengganu Malaysia. Five commercial inoculants (Ino A, B, C, D, and E) which have been tested for inducing artificial agarwood production from *A. malaccensis* in Merchang, Terengganu, were used. Different brands of these inoculants were obtained from different suppliers from the local markets. The exact composition of each inoculant was unknown but all inoculants were assumed to be organic as biological indicated through their sweet and sour odor. The healthy tree

wood was taken from a healthy tree that has never been wounded or inoculated before as agarwood control. The wounded agarwood tree used in this study was the agarwood from a hole made on *A. malaccensis* tree without an inoculant as inoculant control. Four months after the inoculation process, the artificially-inoculated agarwood was harvested and checked for its quality. The wood samples were air-dried and cut into small pieces.

2.2 Solid-phase micro extraction

Agarwood chemical analysis was performed using Solid Phase Micro Extraction (SPME) method to capture the volatile compound released by the wood sample. Resinous part of agarwood and healthy wood was cut into small pieces and put into 4 ml vial. Each of the wood samples was then, heated at 50 °C and exposed to SPME divinylbenzene-carboxen polydimethylsiloxane (DVB-CAR-PDMS) fiber for 30 minutes before being injected into the GC instrument.

2.3 Gas chromatography analysis

All the collected samples were analyzed using Gas Chromatography Flame Ionization Detector (GC-FID). Front inlet turns into a splitless mode with a heater of 200 °C, the pressure at 12.537 psi and septum purge flow at 3 ml /min. The oven was programmed at an initial temperature of 60 ºC which eventually increased to 250° C with an increment rate of 3 °C/min and the latter temperature was held for 5 minutes. Carrier gas flow in the oven was set at 1 ml/ min. Front detector heater was set at 250°C with hydrogen gas flow at 35 ml/ min and air flow at 350 ml/min with the flame on during the analysis.

2.4 Kovats index calculation

GC-FID results were based on retention indices or Kovats Index was calculated using a linear hydrocarbon C_8 to C_{20}. The formula of Kovats Index Calculation was as follow:-

$$I=100\times[n+(N-n)tr,a-tr,ntr,N-tr,n]$$

Where, I is the Kováts index, n is the number of carbon atoms in the alkane with the lower retention time, N is the number of carbon atoms in the alkane with the higher retention time, a is the analyte, tr is retention time, so tr, a is the retention time of the analyte.

3. Results and Discussion

There were five commercial inoculants (Ino A, B, C, D and E) that had been tested for artificial inoculation. Quality of four months agarwood inoculated with Ino A, B, C, D and E were evaluated to screen for the best inoculant producing high-quality agarwood based on chemical composition using SPME-GC-FID. The approach of SPME-GC-FID analysis is suggested to give better detection of initial quantitative sample composition [7]. The effect of inoculants toward agarwood production was compared to healthy wood and wounded wood.

Table 1 summarized all the chemical compositions of agarwood produced by inoculated *A. malaccensis* with commercial inoculants of Ino A, B, C, D and E. Based on the table, artificial agarwood using Ino A inoculant produced a higher percentage of important compounds, which usually found in high-quality agarwood, that are 4-phenyl-2-butanone, β-selinene, α-bulnesene, and agarospirol [8, 9]. In fact, comparison of GC-FID chromatogram of agarwood from Ino A, Ino E and healthy wood has obviously shown the difference (Figure 1). Sesquiterpene groups were more existed in agarwood Ino A with higher amount compared to Ino E and healthy wood. Most of these compound is absent in the agarwood samples with other inoculants (Ino B, C, D and E) and also in control samples of healthy and wounded wood.

Generally, 4-phenyl-2-butanone had been found in juvenile, matured agarwood and even in agarwood oil from *A. malaccensis* [10-12]. However, it was never been recorded in healthy agarwood tree. This data is contradicted to our finding where 4-phenyl-2-butanone compound, which also known as benzyl acetone, was found to be present in the healthy wood sample as shown as in Table 1. Thus, it is suggested that this compound may have functions differently rather than being specifically an indicator of agarwood production. 4-phenyl-2-butanone may play role in plant interaction with each other means for survival, attract pollinators, predators to the pathogen and to help affected tree nearby [13-15].

In comparison to artificial agarwood sample inoculated with Ino A, samples from Ino B, C and D shows only the present of 1 compound either benzaldehyde (Ino B and D) or 4-phenyl-2-butanone (Ino C). However, for sample inoculated with Ino E, none of the compounds related to agarwood was detected. Compared to agarwood samples inoculated with Ino B and Ino D (contained 2.62 and 1.53 area percentage of benzaldehyde, respectively), agarwood sample from Ino A contains no benzaldehyde component. Benzaldehyde was commonly found in high-quality agarwood [8, 9]. However, absent of benzaldehyde in agarwood was also recorded by Ishihara *et al*., [16], Nor Azah *et al*., [17] and Cui *et al*., [18]. According to Ismail *et al*., [9] chemical composition of agarwood smoke data, duration of SPME exposure may cause bias of benzaldehyde detection by gas chromatography. Benzaldehyde from high-quality agarwood was able to be detected between 15 and 60 minutes SPME fiber exposure but absent in 30 minutes exposure. Therefore, absent of benzaldehyde in other inoculated and wounded agarwood may be caused by exposure bias or literally absent of benzaldehyde. From the data provided by SPME-GC-FID analysis (Table 1), the best inoculant for agarwood inoculation was Ino A. Therefore, agarwood samples from Ino A inoculation were used for the next analysis.

Table 1. Chemical composition using SPME-GC-FID analysis of agarwood inoculated with commercial inoculant Ino A, B, C, D and E together with wounded and healthy wood samples as a control.

Compounds	**Ref KI**	**Area (%)**						
		Ino A	**Ino B**	**Ino C**	**Ino D**	**Ino E**	**Wounded wood**	**Healthy wood**
Benzaldehyde	935	-	2.62	-	1.53	-	-	-
4-phenyl-2-butanone	1210	2.4	-	1.58	-	-	3.57	6.53
β-selinene	1486	0.72	-	-	-	-	-	-
α-bulnesene	1503	2.55	-	-	-	-	-	-
Agarospirol	1631	15.65	-	-	-	-	-	-

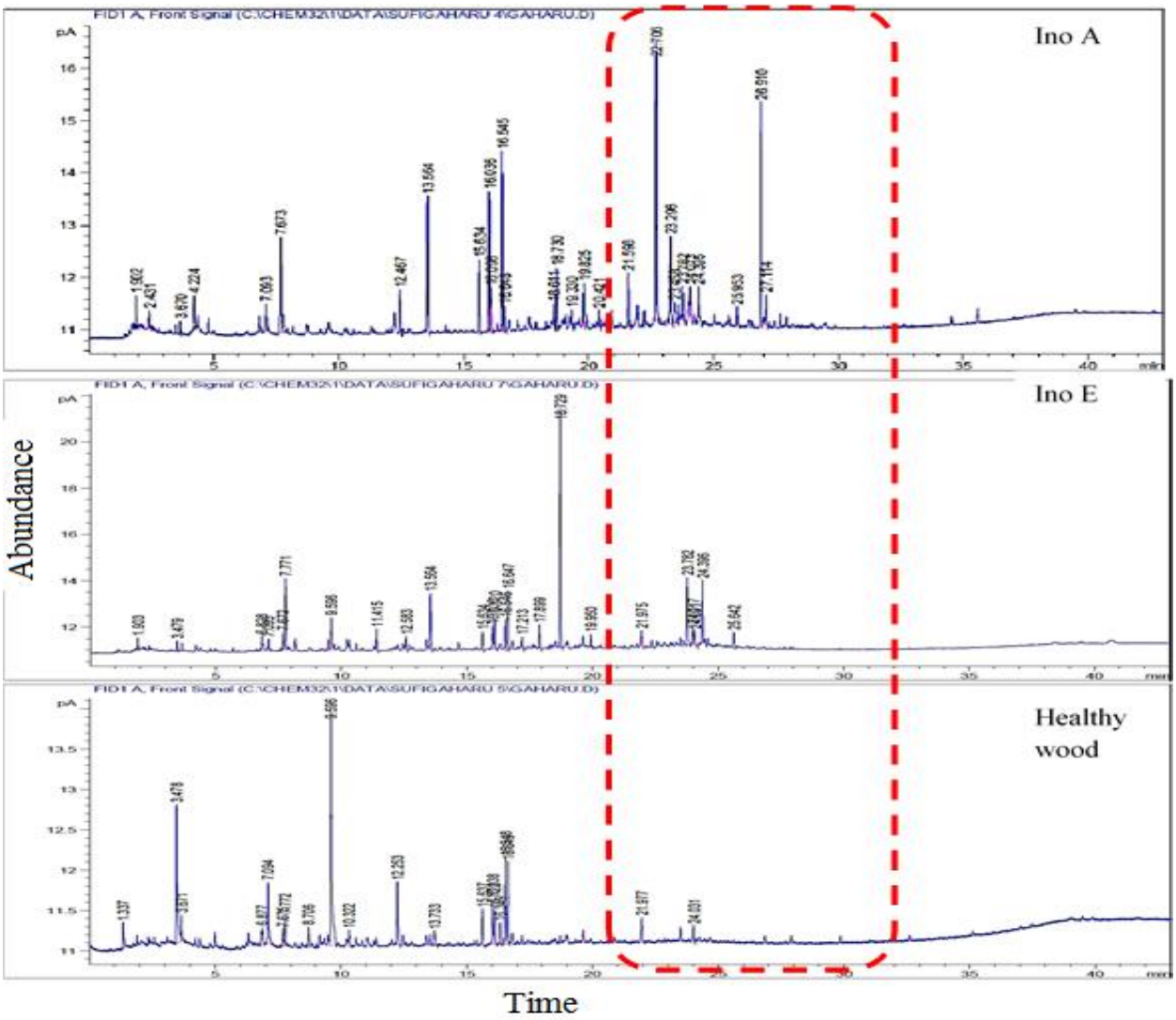

Figure 1. Comparison wood analysis chromatogram of agarwood from Ino A (above), Ino E (middle) and healthy wood (bottom). Sesquiterpene group peak was detected within the area in the red box. Ino A was proven to induce agarwood formation with more sesquiterpene groups contents and amount compared to Ino E and healthy wood.

4. Conclusions

From the current study, we demonstrated the presence of several important compounds related to high-quality agarwood such as 4-phenyl-2-butanone, β-selinene, α-bulnesene, and agarospirol in inoculated agarwood using Ino A and absent in other agarwood and control samples. It was found that agarwood originating from different inoculated samples share some common compounds but still, have several different compounds. Based on the results, further investigation should be carried out, which may contribute to the agarwood product development.

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