Compositional Profiling of Agarwood Oil (*Aquilaria beccariana*) from Malaysia using GC-MS and GC-FID for Enhanced Analytical Characterization

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ABSTRACT

Agarwood oil is one of the most beneficial and sought-after oils, which is significant. It's considered an expensive natural raw world resource due to its quality and high demand. It is very beneficial and valuable and used worldwide, especially in fragrances, incense, perfumes, ceremonies, medicines, and symbols of wealth. The presence of various chemical compounds contributes to the unique aroma and reflects the quality of essentials and automatically affects the price of the oil. Nowadays, the quality of agarwood oil is classified based on the chemical constituents of the oil. This strategy can improve the exactness of the evaluating framework, hence illuminating the issue expressed by the conventional method which is based on color and odor. This existing method has a limitation which is the result varies for each panel and is based on individual experiences. The existing study mentioned that the main chemical constituents of agarwood oil consist of sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, and chromone derivatives. This study uses GC-MS coupled with GC-FID analysis to identify the chemical compounds of agarwood oil from *Aquilaria beccariana* species.The result showed that this type of agarwood oil is categorized as C grade with sesquiterpene and sesquiterpenoid content of 21.94% (ABS1) and 21.90% (ABS2). Two adulterants namely benzyl benzoate (10.61% and bis (2-ethylhexyl) phthalate (10.09% - 12.31%) were found to indicate that the oil is impure. The study's findings play a crucial role in emphasizing the compounds that contribute to agarwood oil's significance as a prized and versatile natural resource, underscored by its diverse applications and unique chemical composition. The future direction entails refining chemical-based classification methods for precise quality assessment and fostering sustainable practices to ensure its continued value and impurity.

Keywords: Agarwood oil; Chemical compound; GC-MS analysis; Identification; *Aquilaria beccariana*

1. Introduction

Aquilaria trees produce resin-infused wood known for its fragrance, referred to as Agarwood [1]. Agarwood is recognized by various names globally, often associated with countries or religions. In Malaysia, it is commonly termed "Gaharu," while in China, it's referred to as "Chexiang." Additionally, other appellations include "Kalambak," "Kanankoh," "Agalloch," "Jinkoh," "Eaglewood," and "Aloeswood," as noted in [2-4]. Notably, agarwood holds immense significance as a non-timber forest resource due to its high demand. It plays a vital role in traditional medicine, contributes to fragrant product production, and serves as a key element in religious and cultural ceremonies and festivals across various nations and faiths [5-6].

Agarwood oil has garnered global and Malaysian recognition for its manifold and valuable applications, notably in fragrances, incenses, perfumes, ceremonies, medicines, and as symbols of affluence [7-8]. The distinctive aroma of agarwood is underpinned by a medley of chemical compounds, serving as a testament to the essential oil's quality [9]. In the 20th century, a paradigm shift emerged in grading agarwood oil, pivoting towards chemical attributes. This approach stands as a preeminent strategy for assessing oil quality, heralding the potential to refine the precision of evaluations and ameliorate challenges posed by established methods [10-11]. At the heart of agarwood's allure lie sesquiterpenes, the principal active components constituting almost 20% of detected chemicals, bestowing upon it its captivating fragrance

[12]. Collaborative efforts among academia, industry partners, and key players like AJMAL have endeavored to pinpoint pivotal marker compounds responsible for agarwood's unique bouquet. Research outcomes advocate for the classification of agarwood, or *Aquilaria*, based on their chemical makeup, predominantly focusing on sesquiterpenes and sesquiterpenoids, known to be the prime contributors to the robust woody aroma [9-11]. In light of this, an imperative method arises to discern the pivotal chemical entities that underlie the diverse qualities of agarwood oils.

However, an internationally recognized standard for agarwood oil remains absent to this day [13]. Diverse countries employ varying methods to grade agarwood oil [14-15], with the existing grading heavily reliant on consumer perception and preferences, thus fostering adulteration within the agarwood trade [13-15]. The market value of agarwood oil hinges upon its inherent quality, with higher-quality variants commanding elevated prices [15-16]. As previously mentioned, the prevailing method for evaluating agarwood oil quality involves human sensory panels, a practice riddled with inefficiencies, fatigue, and time consumption [17-18]. Consequently, doubts can arise, and the grading process differs among exporting nations [13,19]. The absence of a standardized classification method perpetuates the potential for manipulations and fraudulent practices, where subpar oil may be sold at premium prices.

A comprehensive study conducted by the CITES team (2010) regarding the UAE's agarwood market pinpointed critical quality assessment criteria, encompassing factors such as country of origin, fragrance longevity, aroma, and oil color, thickness, and density [20]. Since 2007, a collaborative effort between Universiti Malaysia Pahang (UMP), Universiti Teknologi MARA (UiTM), industrial partners, and key players like AJMAL has been dedicated to identifying pivotal marker compounds shaping agarwood's distinctive aroma [19, 21]. In light of these endeavors, it is proposed that agarwood classification aligns with its chemical composition, with sesquiterpenes and sesquiterpenoids standing out as primary contributors [21-23]. Extensive research underscores the prominence of these compound groups in imparting the robust woody scent to agarwood [21-23].

2. Experimental

2.1 Materials and Characterization Methods

Two samples from the same species which is *Aquilaria beccariana* namely ABS1 and ABS2 were used in this study. These two samples are from Malaysia. The volume for each sample is 5ml. The study uses an agarwood chip, obtained from a natural forest located in Rompin, Pahang, and authenticated by Saiful Nizam Tajuddin (BARCE, UMPSA). The extraction was done by using the hydrodistillation (HD) method. Several parameters of each extraction were applied to obtain the best and optimum condition for agarwood oil extract. Before extraction, the ground agarwood chip was soaked in water for several days sequentially to break down the parenchymatous and oil glands. The samples were diluted in dichloromethane (DCM) with an analytical grade for gas chromatography-mass spectrometer (GC-MS) and gas chromatography system (GC-FID) determination [21].

GC-MS (MS) is identified by comparison of the mass spectrum generated from sample analysis with a library of the National Institute of Standards and Technology (NIST) with acceptance similarity ≥80%. GC-FID (FID) identification by linear retention indices, determined relative to the retention times on DBI column of homologous series of C [22]. Kovats retention indices are determined by comparing the retention times of sample peaks with the retention times of a series of n-alkanes under the same chromatographic conditions. In this experiment, the DB-1ms column is used and the retention times are determined relative to this column. Furthermore, compound identification was solely based on retention times, simplifying the analysis approach and avoiding additional complexity introduced by Kovats retention indices. Mass spectral libraries (HPCH2205.L, Wiley7Nist05.L, and NIST05a.L) were used to identify the chemical components. Peak counts were used to expressing the findings of the peak areas [21].

The GC-MS and GC-FID are outlined as follows: for the GC-MS system, the model is Agilent 7890B GC System is coupled with an Agilent 5977A MSD. The inlet temperature is set at 250 °C, and the Agilent DB-1ms column with dimensions 30 m × 250 µm × 0.25 µm is employed. The flow rate is maintained at 1.0 mL/min with helium as the carrier gas. The oven program initiates at 80 °C, followed by an increase of 3 °C/min until reaching 250 °C, where it is held for 3 minutes. Additionally, the auxiliary heater is set at 260°C, the MS source at 230°C and the MS quad at 150°C. Ionization is achieved using Electron Impact (EI) mode with an energy of 70 electron volts (eV). On the other hand, the GC-FID system utilizes an Agilent 7890B GC System. The parameters for this system include an inlet temperature of 250 °C, the same Agilent DB-1ms column, and a flow rate of 1.0 mL/min with helium as the carrier gas. The oven program mirrors that of the GC-MS system, starting at 80 °C, increasing by 3 °C/min until 250 °C, and maintaining this temperature for 3 minutes. Unlike the GC-MS system, the GC-FID system does not employ an auxiliary heater. Instead, the FID detector operates at a temperature of 250 °C.

3. Results and Discussion

Figure 1 presents the chromatogram analysis of ABS1, revealing the presence of four noteworthy peaks: peak no. 30 (benzyl benzoate), peak no. 34 (selina-3,11-dien-14-oic acid), peak no. 37 (guaia-1(10),11-dien-15-oic acid), and peak no. 41 (2-hydroxyguaia-1(10),1 -dien-15-oic acid). Furthermore, additional compounds are also observable, with comprehensive information provided in Table 1.

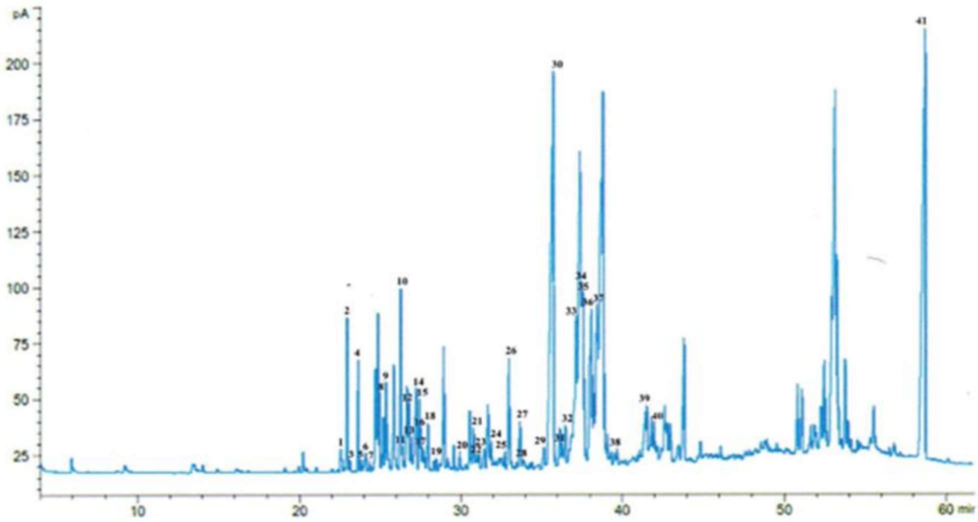

Figure 1. Chromatogram of the analysis by gas chromatography (GC) for agarwood essential oil for sample ABS1

Refer to Table 1, a total of 41 compounds for ABS1 are tabulated based on their retention times (minutes), ranging from 22.576 to 58.726. Notably, bis(2-ethylhexyl) phthalate exhibited the highest peak area (%), registering at 12.31%. Subsequently, benzyl benzoate followed with a peak area of 10.61%. Among these compounds, ß-gurjunene exhibited the lowest peak area (%) at 0.11%.

Table 1. Chemical Compounds for ABS1

No.	Ret. time, tr (min)	Peak area (%)	Compounds	Ident. Mode
1	22.576	0.14	n-Decanoic acid	FID,MS
2	22.970	1.53	B-Patchoulene	FID,MS
3	23.116	0.11	ß-Elemene	FID,MS
4	23.654	1.14	Cyperene	FID,MS
5	23.830	0.13	-Gurjunene	FID
6	24.099	0.31	Isocaryophyllene	FID
7	24.339	0.10	ß-Gurjunene	FID,MS
8	24.983	0.73	a-Guaiene	FID,MS
9	25.373	1.38	Aromadendrene	FID
10	26.272	1.98	allo-Aromadendrene	FID,MS
11	26.387	0.22	Y-Gurjunene	FID
12	26.770	0.66	B-Selinene	FID,MS
13	26.956	0.31	Valencene	FID,MS
14	27.256	1.25	Dihyro-B-Agarofuran	FID,MS
15	27.462	0.74	8-Guaiene	FID,MS
16	27.563	0.37	Y-Cadinene	FID
17	27.670	0.17	cis-Calamenene	FID
18	27.981	0.55	8-Cadinene	FID,MS
19	28.494	0.13	-Calacorene	FID
20	29.948	0.18	Y-Vetivenene	FID

21	30.801	0.39	Caryophyllene oxide	FID
22	31.133	0.13	Tetradecanal	FID
23	31.496	0.34	10-epi-y-Eudesmol	FID,MS
24	31.919	0.26	Y-Eudesmol	FID,MS
25	32.747	0.16	Jinkoh-eremol	FID
26	33.008	1.33	Kusunol	FID
27	33.658	0.56	Dehydrojinkoh-eremol	FID
28	33.818	0.16	Cyperotundone	FID,MS
29	35.160	0.31	Selina-3,11-dien-9-ol	FID
30	35.740	10.61	Benzyl benzoate	FID,MS
31	36.29	0.28	9,11-Eremophiladien-8-one	FID
32	36.491	0.55	Guaia-1(10),11-dien-9-one	FID
33	37.178	1.03	Nootkatone	FID
34	37.522	1.61	Selina-3,11-dien-14-oic acid	FID
35	37.601	1.44	Sinenofuranol	FID
36	38.099	1.60	Hexadecanal	FID
37	38.445	2.47	Guaia-1(10),11-dien-15-oic acid	FID
38	39.679	0.15	Pentadecanoic acid	FID,MS
39	41.538	0.51	Methyl palmitate	FID
40	41.984	0.50	2-hydroxyguaia-1(10),1 dien-15-oic acid	FID
41	58.726	12.31	Bis(2-ethylhexyl) phthalate	MS

Figure 2 illustrates the chromatogram analysis of ABS2, revealing four prominent peaks corresponding to peak no. 30 (benzyl benzoate), peak no. 37 (hexadecanal), peak no. 38 (guaia-1(10),11-dien-15-oic acid), and peak no. 42 (bis(2-ethylhexyl) phthalate). Additionally, other compounds are also evident, and further information is available in Table 2.

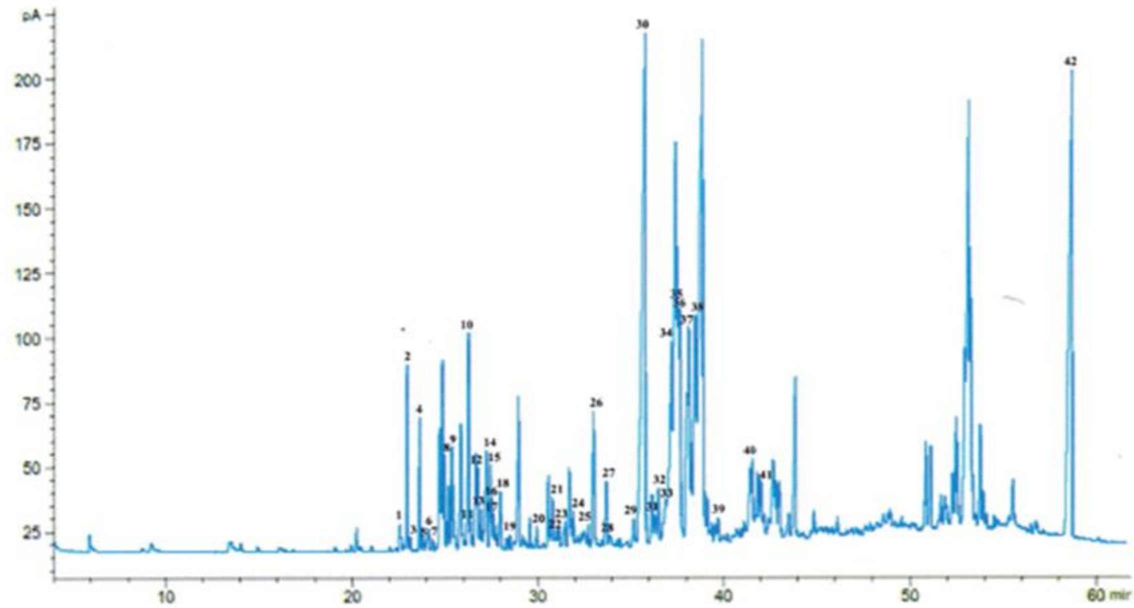

Figure 2. Chromatogram of the analysis by gas chromatography (GC) for agarwood essential oil for sample ABS2

Table 2. Chemical Compounds for ABS2

No.	Ret. time, tr (min)	Peak area (%)	Compounds	Ident. Mode
1	22.581	0.31	n-Decanoic acid	FID,MS
2	22.977	1.43	B-Patchoulene	FID,MS
3	23.121	0.10	ß-Elemene	FID,MS
4	23.661	1.07	Cyperene	FID,MS
5	23.837	0.12	-Gurjunene	FID
6	24.103	0.29	Isocaryophyllene	FID
7	24.344	0.09	B-Gurjunene	FID,MS
8	24.990	0.68	a-Guaiene	FID,MS
9	25.380	1.28	Aromadendrene	FID
10	26.280	1.80	allo-Aromadendrene	FID,MS
11	26.393	0.18	Y-Gurjunene	FID
12	26.779	0.65	B-Selinene	FID,MS
13	26.967	0.35	Valencene	FID,MS
14	27.265	1.24	Dihyro-B-Agarofuran	FID,MS
15	27.470	0.68	8-Guaiene	FID,MS
16	27.572	0.34	Y-Cadinene	FID
17	27.678	0.16	cis-Calamenene	FID
18	27.988	0.51	8-Cadinene	FID,MS
19	28.502	0.12	-Calacorene	FID
20	29.957	0.17	y-Vetivenene	FID
21	30.808	0.38	Caryophyllene oxide	FID
22	31.146	0.12	Tetradecanal	FID
23	31.506	0.32	10-epi-y-Eudesmol	FID,MS
24	31.928	0.25	Y-Eudesmol	FID,MS
25	32.758	0.16	Jinkoh-eremol	FID
26	33.022	1.45	Kusunol	FID
27	33.676	0.59	Dehydrojinkoh-eremol	FID
28	33.829	0.15	Cyperotundone	FID,MS
29	35.173	0.39	Selina-3,11-dien-9-ol	FID
30	35.789	11.37	Benzyl benzoate	FID,MS
31	36.306	0.30	9,11-Eremophiladien-8-one	FID
32	36.514	0.58	Guaia-1(10),11-dien-9-one	FID
33	36.891	0.15	Dehydrofukinone	FID,MS
34	37.213	0.99	Nootkatone	FID
35	37.553	1.58	Selina-3,11-dien-14-oic acid	FID
36	37.642	1.66	Sinenofuranol	FID
37	38.135	1.69	Hexadecanal	FID
38	38.481	2.68	Guaia-1(10),11-dien-15-oic acid	FID
39	39.709	0.25	Pentadecanoic acid	FID,MS
40	41.564	0.72	Methyl palmitate	FID
41	42.015	0.67	2-hydroxyguaia-1(10),1 -dien-15-oic acid	FID
42	58.712	10.09	Bis(2-ethylhexyl) phthalate	MS

Both Table 1 and Table 2 collectively unveil that agarwood oil is predominantly composed of a mixture of compounds. These compounds have been categorized into four distinct groups: carboxylic acid, other compounds, sesquiterpene, and sesquiterpenoid. Table 3 presents the chemical composition of the agarwood oils identified through GC-FID and GC-MS analysis. Regarding the sesquiterpene compounds, allo-aromadendrene exhibited the most prominent peak area, constituting 1.98% in ABS1 and 1.80% in ABS2. Among the sesquiterpenoid compounds, guaia-1(10),11-dien-15-oic acid exhibited values of 2.47% in ABS1 and 2.68% in ABS2. Notably, all the aforementioned compounds hold significant presence within the agarwood oil samples under study.

Table 3. Chemical composition of agarwood oils identified through GC-FID and GC-MS analysis

Compounds	Molecular formula	Area, %		Ident. mode
		ABS1	ABS2	
Carboxylic acid				
n-Decanoic acid	$C_{10}H_{20}O_2$	0.14	0.31	FID,MS
Pentadecanoic acid	$C_{15}H_{30}O_2$	0.15	0.25	FID,MS
Other compounds				
Tetradecanal	$C_{14}H_{28}O$	0.13	0.12	FID
Benzyl benzoate	$C_{14}H_{12}O_2$	10.61	11.37	FID,MS
Sinenofuranol	$C_{10}H_{12}O$	1.44	1.66	FID
Hexadecanal	$C_{16}H_{32}O$	1.60	1.69	FID
Methyl palmitate	$C_{17}H_{34}O_2$	0.51	0.72	FID
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	12.31	10.09	MS
Sesquiterpene				
B-Patchoulene	$C_{15}H_{24}$	1.53	1.43	FID,MS
B-Elemene	$C_{15}H_{24}$	0.11	0.10	FID,MS
Cyperene	$C_{15}H_{24}$	1.14	1.07	FID,MS
a-Gurjunene	$C_{15}H_{24}$	0.13	0.12	FID
Isocaryophyllene	$C_{15}H_{24}$	0.31	0.29	FID
ß-Gurjunene	$C_{15}H_{24}$	0.10	0.09	FID,MS
a-Guaiene	$C_{15}H_{24}$	0.73	0.68	FID,MS
Aromadendrene	$C_{15}H_{24}$	1.38	1.28	FID
allo-Aromadendrene	$C_{15}H_{24}$	1.98	1.80	FID,MS
Y-Gurjunene	$C_{15}H_{24}$	0.22	0.18	FID
B-Selinene	$C_{15}H_{24}$	0.66	0.65	FID,MS
Valencene	$C_{15}H_{24}$	0.31	0.35	FID,MS
8-Guaiene	$C_{15}H_{24}$	0.74	0.68	FID,MS
Y-Cadinene	$C_{15}H_{24}$	0.37	0.34	FID
cis-Calamenene	$C_{15}H_{22}$	0.17	0.16	FID
8-Cadinene	$C_{15}H_{24}$	0.55	0.51	FID,MS
a-Calacorene	$C_{15}H_{20}$	0.13	0.12	FID
Y-Vetivenene	$C_{15}H_{22}$	0.18	0.17	FID
Sesquiterpenoid				
Dihyro-B-agarofuran	$C_{15}H_{24}0$	1.25	1.24	FID,MS
Caryophyllene oxide	$C_{15}H_{24}0$	0.39	0.38	FID
10-epi-y-Eudesmol	$C_{15}H_{26}0$	0.34	0.32	FID,MS
Y-Eudesmol	$C_{15}H_{26}0$	0.26	0.25	FID,MS
Jinkoh-eremol	$C_{15}H_{26}0$	0.16	0.16	FID
Kusunol	$C_{15}H_{26}0$	1.33	1.45	FID
Dehydrojinkoh-eremol	$C_{15}H_{24}0$	0.56	0.59	FID

Cyperotundone	$C_{15}H_{22}0$	0.16	0.15	FID,MS
Selina-3,11-dien-9-ol	$C_{15}H_{24}0$	0.31	0.39	FID
,11-Eremophiladien-8-one	$C_{15}H_{22}0$	0.28	0.30	FID
Guaia-1(10),11-dien-9-one	$C_{15}H_{22}0$	0.55	0.58	FID
Dehydrofukinone	$C_{15}H_{22}0$		0.15	FID,MS
Nootkatone	$C_{15}H_{22}0$	1.03	0.99	FID
Selina-3,11-dien-14-oic acid	$C_{15}H_{22}0_2$	1.61	1.58	FID
Guaia-1(10),11-dien-15-oic acid	$C_{15}H_{22}0_2$	2.47	2.68	FID
2-hydroxyguaia-1(10),11- dien-15-oic acid	$C_{15}H_{22}0_3$	0.50	0.67	FID
Total of carboxylic acid		0.29	0.56	
Total of other compounds		26.60	25.65	
Total of sesquiterpene		10.74	10.02	
Total of sesquiterpenoid		11.20	11.88	

The analysis of agarwood oil samples, with 21.94% (ABS1) and 21.90% (ABS2) sesquiterpene and sesquiterpenoid content determined through GC analysis, places both oils within the C grade category [21]. Furthermore, adhering to the stringent Ajmal standard, these oils meet the criteria for C grade, exhibiting sesquiterpenoid content of 11.20% (ABS1) and 11.88% (ABS2). Notably, both oils contain notable levels of benzyl benzoate (ranging from 10.61% to 11.37%) and bis(2-ethylhexyl) phthalate (ranging from 10.09% to 12.31%). The presence of these compounds, often used as fixatives and fragrance carriers in perfumes and personal care products, suggests impurity and potential adulteration, likely aimed at extending the duration of aroma. Furthermore, benzyl benzoate was reported used as a carrier and fixative agent in perfumes [24]. Meanwhile, bis (2-ethylhexyl) phthalate (di-2-ethylhexy phthalate, diethylhexyl phthalate, DEHP; dioctyl phthalate, DOP) was reportedly used as a fragrance carrier and personal care products [25]. Figure 3 visually illustrates the sesquiterpene and sesquiterpenoid constituents (Green) contributing to the robust woody scent of agarwood essential oil, alongside adulterants (Red) intentionally introduced into the oils.

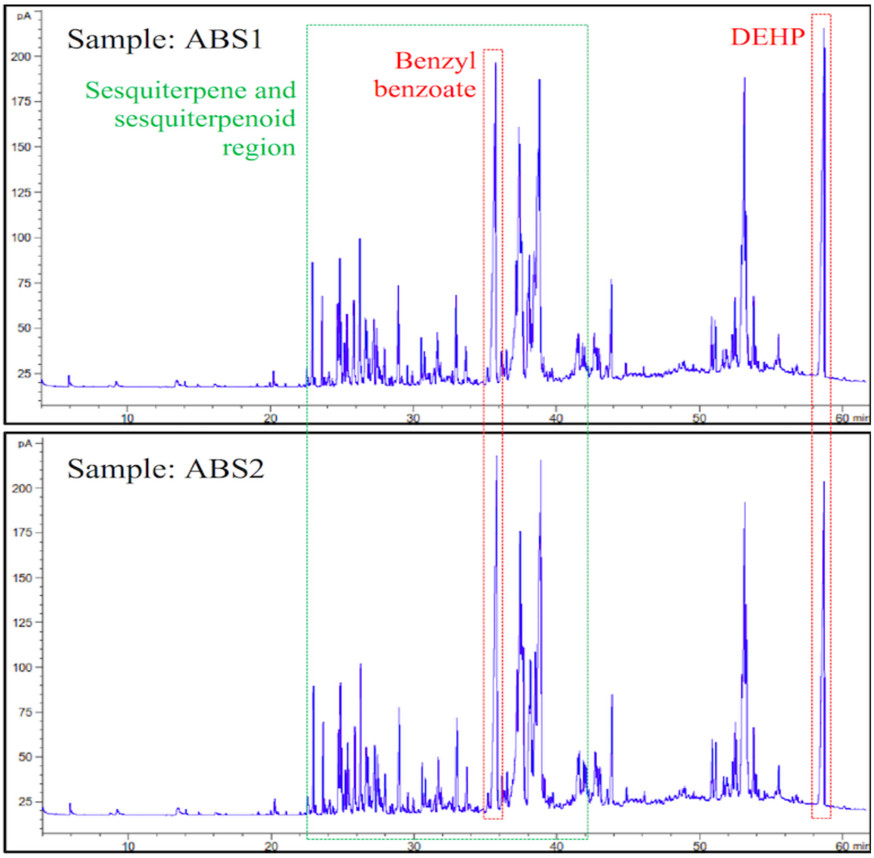

Figure 3. Chromatogram of GC for agarwood oils (ABS1 and ABS2)

4. Conclusions

In conclusion, the chemical composition in agarwood essential oil was successfully identified using GC-MS coupled with GC-FID analysis. Both agarwood oil samples are low quality according to the recommended grading system and reached the C grade with sesquiterpene and sesquiterpenoid content of 21.94% (ABS1) and 21.90% (ABS2). Two adulterants namely benzyl benzoate (10.61% and bis (2-ethylhexyl) phthalate (10.09% - 12.31%) were found to indicate that the oil is impure.

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