PROFILING OF COMPOUNDS IN HYDROSOL EXTRACT OF *AQUILARIA* (AGARWOOD) SPECIES USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GCMS)

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ABSTRACT: Most research involving agarwood mainly focused on extracting the agarwood oil from the infiltrated resin, the most sought non-timber forest product from *Aquilaria* species with high demand and prices. This led to other parts of this valuable plant species being considered as waste. This includes the agarwood hydrosol (by-product of water distillation). Hydrosol has been reported to retain certain aromatics of its primary essential oil. Therefore, the aim of the present study is to identify the composition of the agarwood hydrosol extracts from *Aquilaria* species by using gas chromatography-mass spectrometry (GCMS). Six agarwood hydrosol samples from different batches of production were used in this study and designated as S1 until S6. The hydrosol extracts were prepared in three steps; isolation of dissolved essential oil, filtration of the hydrosol extract and finally the evaporation of solvent (hexane). The resulting extracts were subjected to GCMS analysis. A total of 46, 48, 40, 53, 58 and 62 compounds were detected in hydrosol extract samples of S1, S2, S3, S4, S5 and S6, respectively. Accordingly, the oxygenated and phenolic compounds were found to be present in all hydrosol extract samples. The detected phenolic compounds include phenol, 2,5-bis (1,1-dimethylethyl)-,.phenol, 2,4-bis (1,1-dimethylethyl)- and 9-octadecenoic acid (Z)-, methyl ester. These compounds are believed to possess various potential biological activities including antioxidant activities. Sample S1 and S2 consist of sesquiterpene compounds such as (-)-aristolene, naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-,(4aR-trans), azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]- and alloaromadendrene where the later had the highest number of oxygenated and antioxidant compounds. In conclusion, results from this study suggests that agarwood hydrosol possesses myriad of compounds that warrant further investigation based on their potential medicinal benefits. This in turn could help realize the waste to wealth paradigm as well as promote the material as sustainable source for development of halal therapeutics.

KEY WORDS: *Agarwood, Aquilaria, Compounds, Gas chromatography-mass spectrometry, and Hydrosol.*

1. INTRODUCTION

The bioactive compounds extracted from higher plants represent a reservoir of effective non-phytotoxic therapeutic agents which are more systemic and easily biodegradable [1-3]. Higher plants including *Aquilaria* species continue to play a dominant role as a source of bioactive compounds with promising pharmacological activities such as anticancer, anti-inflammatory, antioxidant, antimicrobial, antidiabetic and antiallergic [4-8].

Aquilaria species is very well-known for its aromatic resin-containing heartwood which is termed as agarwood. It has been utilized for various types of application including as folk medicine, perfume and incense [6, 9-10]. Technically, the term 'agarwood' refers to the resin-containing heartwood which naturally forms in response to fungal infection or through feedback mechanism of wounding [6, 11]. Agarwood is called by various names based on cultures around the world such as 'gaharu' (Malay), 'oud' (Arab), 'agar' (India), 'jin-koh' (Japan) and 'chenxiang' (Chinese) [6, 12]. There are thirty one species of *Aquilaria* which can be found worldwide including in Malaysia, Indonesia, Thailand, China and India [10], with nine species reported to potentially produce agarwood with *A. malaccensis*, *A. agallocha*, and *A. secundaria* being the primary producers [6].

For many years, most research involving *Aquilaria* species mainly focused on extracting the agarwood oil from the infiltrated resin, the most sought non-timber forest product from *Aquilaria* species that has high demand and prices. This led to other parts of this valuable plant species such as leaf and bark being considered as waste. Further, in agarwood oil distillation; hydrosol which is the by-product of the process is also considered as waste.

Hydrosol has been reported to retain certain aromatics of its primary essential oil. Recently, chemical profile of agarwood hydrosol and its anticancer effects has been reported. The author revealed that the potential anti-tumor compounds identified in agarwood hydrosol of *A. malaccensis* such as 16- hentriacontanone, benzaldehyde and 1-tricosene may justify the anti-attachment and cytotoxic effects on Calu-3 lung cancer cells [13]. Another work reported that agarwood hydrosol was found to possess anti-attachment and cytotoxic effects on MCF-7 breast cancer cells *in vitro* [14]. However, the author did not perform any profiling of compounds in the hydrosol sample which could assist to elucidate its anticancer effects. Despite its potential biological activities, studies on profiling of compounds in agarwood hydrosol is still scarce. In commercial setting of agarwood oil distillation, the raw material (agarwood chips) used were a mixture of species originating from various locations. Other parameters include various extraction conditions. This results in differences in the yield and other characteristics of oil and its hydrosol.

Therefore, the present study was conducted to identify the composition of the agarwood hydrosol extracts from *Aquilaria* obtained from different batches of production; by using gas chromatography-mass spectrometry (GCMS). The findings from this study have unraveled the presence of various compounds in the agarwood hydrosol extract that could be potentially used as source of halal therapeutics while also realizing the waste to wealth paradigm.

2. METHODOLOGY

2.1. Raw materials and chemicals

Six samples of agarwood hyrdosols were obtained from Agargreen Sdn. Bhd. in Seri Kembangan, Selangor, Malaysia. The hydrosols are by-product of different batches of commercial process of agarwood oil distillation of which the raw material (agarwood chips)

were a mixture of species. Hexane used was from Systerm Chemicals (Malaysia) and anhydrous sodium sulphate (Na_2SO_4) was from Merck (U.S).

2.2 Preparation of hydrosol extracts

The procedure for preparation of *Aquilaria* hydrosol extracts involved three steps; isolation of dissolved essential oil, filtration of the hydrosol extract and finally the evaporation of solvent (hexane). These steps were based on Verma et al. (2012) [15] with some modification as specified in the following sections.

2.2.1 Isolation of dissolved essential oil

The hydrosol samples were filtered prior to isolation of dissolved essential oil. The extraction was carried out with the ratio of 10:1 of hydrosol sample to hexane at room temperature. Two beakers were labelled with 'oil' and 'waste' separately for each hydrosol sample. For each sample, 100 mL of agarwood hydrosol was filtered and 10 mL of hexane was added into the filtered hydrosol and the mixture was shaken vigorously for 30 minutes and transferred into the separatory funnel by using filter funnel. The funnel was placed in a retort stand and the mixture was allowed to settle and well-separated. The 'waste' labelled beaker was placed under the separatory funnel. The stopper was removed, and the bottom layer was drained in the beaker. The upper layer was drained into the beaker with 'oil' label as shown in Fig. 1.

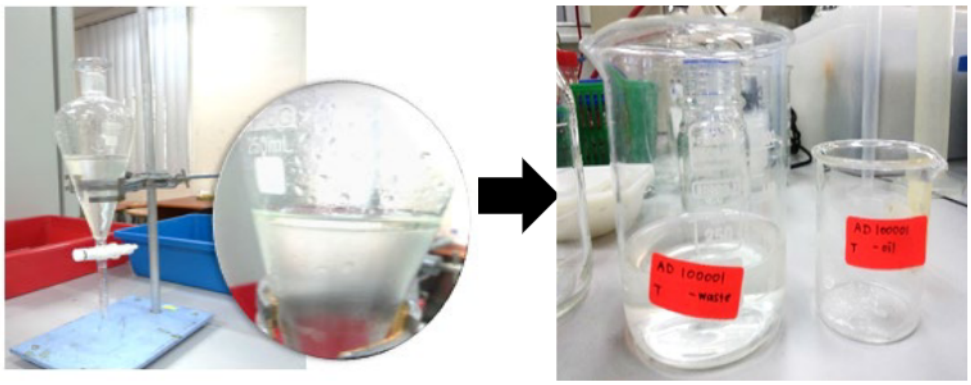

Fig. 1. Isolation of the dissolved essential oil from *Aquilaria* hydrosol extract.

2.2.2 Filtration of the hydrosol extract

A teaspoon of dehydration agent; anhydrous sodium sulphate (Na_2SO_4) was added into the 'oil' labeled beaker and the mixture was stirred well. The filter paper was folded and 2 to 3 drops of hexane were dropped onto the filter paper for better filtration and refold. The folded filter paper was put into the new beaker with label of 'new oil'. The mixture (hydrosol extract) was filtered. The filtration process was carried out at room temperature.

2.2.3 Evaporation

The solvent from the filtered hydrosol extract was evaporated using rotary evaporator (Buchi, Switzerland) under low pressure with the temperature below 25 °C. Solvent was well-evaporated once the pressure reached at 272 millibar (mbar). The volume of oil produced from this procedure was recorded and the yield was calculated as per Eq. (1). The hydrosol extract was placed in a cool dark place for further analysis. All steps were repeated for each hydrosol sample.

$$Yield\ of\ dissolved\ oil\ (\%) = Volume\ of\ oil\ recovered/100\ mL\ x\ 100 \quad (1)$$

2.3 Gas chromatography-mass spectrometry (GCMS)

The procedure for GCMS analysis of *Aquilaria* hydrosol sample extract involved sample preparation and the gas chromatography-mass spectrometry. The methods were based on Hashim et al. (2014) and described in more detail in the following sections [16].

2.3.1 Sample preparation

About 10 µL of the hydrosol extract was added into a new 1 mL microcentrifuge tube. 990 µL of hexane was added and mixed into the tube. The mixture was stirred well. The vial was labelled according to the sample of the hydrosol extracts. Septa was attached with the cap and 250 µL insert was inserted into the vial. 250 µL of the mixture was added into the prepared vial and capped. All steps were repeated for each hydrosol extract sample and 250 µL of hexane solvent was prepared as blank sample and labelled as depicted in Fig. 2.

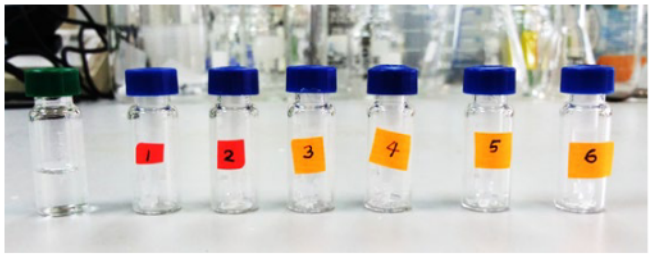

Fig. 2. Blank and hydrosol extract samples (S1-S6).

2.3.2. GCMS analysis

The volatile constituents of agarwood hydrosol extract samples were analyzed by using gas chromatography system; Agilent 7890A (Agilent Technologies) coupled with Agilent 5975C quadrupole mass spectrometer and autosampler. Hewlett Packard HP-5MS ultra inert silica capillary column (30 m x 0.25 mm; 0.25 µm) was used. The analytical conditions for GCMS are listed in Table 1. The detected peaks from the total ion chromatography (TIC) and mass chromatograms were identified based on National Institute of Standards and Technology (NIST) 2008 mass spectral library.

Table 1. Parameters for GCMS analysis

Program	**Conditions**				
Oven Program		Rate °C/min	Value °C	Hold Time (min)	Run Time (min)
	Initial		80	2	2
	Ramp	10	250	10	29
Carrier gas	Helium				
Gas flow	2 mL/min				
Split ratio	1:50				
Injection Volume	1 µL				
Mode	Split				
Interface temperature	250 °C				
Electron impact (emission current)	70 eV				
Scan range	500 amu				

3. RESULTS AND DISCUSSION

Hydrosol samples were commercially obtained from six different batches of agarwood oil distillation. The yield and chemical profile of agarwood hydrosol extract from the six samples are discussed further in the next section.

3.1 Yield of agarwood hydrosol extract

The volume of oil produced from the preparation of hydrosol extract samples were recorded in percentage as listed in Table 2. The percentage of oil yields (%) were compared. Sample S5 had the highest percentage oil yield of 39.47 % followed by sample S3 (21.05 %), S6 (15.79 %), S4 (13.16 %), S2 (6.58 %), and S1 (3.95 %) respectively. The differences in the yield obtained may be due to the variance in the hydrosol samples tested in terms of parameters such as species, plant (raw material) characteristics, extraction conditions and quality of oil.

Table 2. The percentage yield of agarwood hydrosol extract obtained from six *Aquilaria* species

Sample	Oil Yield Percentage (% *v/v*)
S1	3.95
S2	6.58
S3	21.05
S4	13.16
S5	39.47
S6	15.79

3.2 Chemical profile of agarwood hydrosol extract

The chemical profile of six hydrosol samples were analyzed using Agilent GCMS. GCMS analysis revealed that sample S1 had the highest total number of compounds (142) while sample S3 has the lowest number of compounds (101). Sample S4, S5 and S6 constituted of 128, 125 and 127 compounds, respectively. Meanwhile, among the six hydrosol samples, sample S6 had the highest number of compounds with mass spectral matching of more than 80% (Qual > 80) with the registered compounds in NIST mass spectral library within the retention times (RT) of 6.93 to 21.71 minutes. Table 3 summarizes the composition of agarwood hydrosol extract of sample S1 to sample S6 while Fig. 3 and 4 depict the integrated total ion chromatogram (TIC) and exemplary chromatograms of each samples, respectively.

Table 3. Total number of compounds in each agarwood hydrosol extract sample

Sample	Total compound number				
	Overall	Qual >80*	Oxygenated	Phenolic	Sesquiterpene
S1	142	46	9	1	1
S2	134	48	12	2	3
S3	101	40	5	1	0
S4	128	53	4	1	0
S5	125	58	4	1	0
S6	127	62	6	1	0

*Confidence measure and matching of compounds with NIST mass spectral libary. This is listed under the 'Qual' tab in GCMS result. The closer to 100 the value is the greater the confidence.

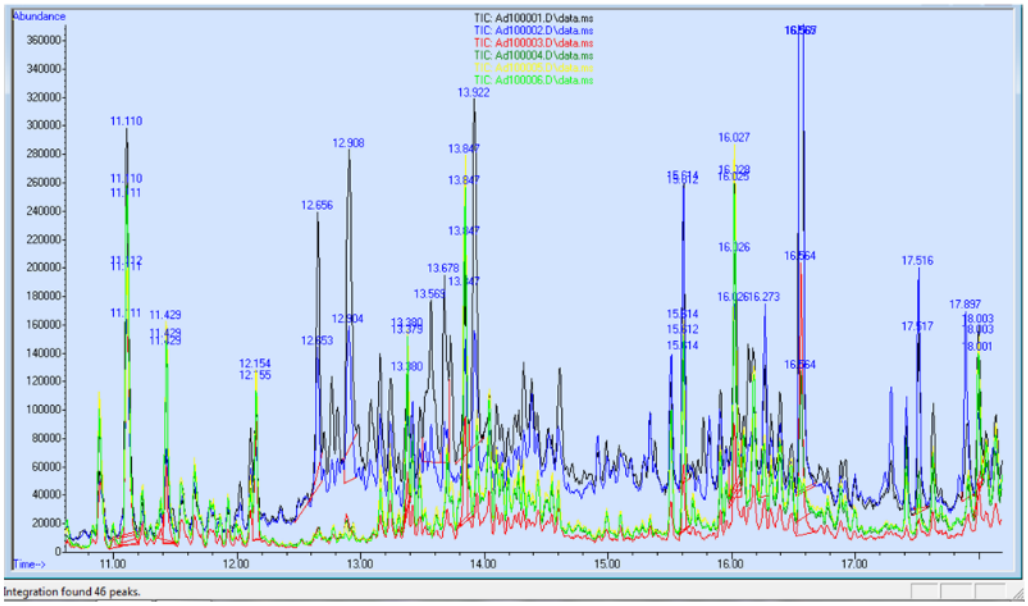

Fig. 3. Integrated TIC of blank and six samples of agarwood hydrosol extract.

Subsequently, based on GCMS analysis, oxygenated and phenolic compounds were detected in all samples while sesquiterpene compound were only present in S1 and S2. In terms of phenolic compound, S1 and S2 had similar compound, which was phenol, 2,5-bis (1,1-dimethylethyl)-, $C_{14}H_{22}O$. Meanwhile, for S3, S4, S5 and S6 had identical phenolic compound, which was phenol, 2,4-bis (1,1-dimethylethyl)-, $C_{14}H_{22}O$. The phenolic compound was selected based on the presence of OH group and benzene ring, which may be related to the antioxidant properties of the samples. Hence, the presence of 9-octadecenoic acid (Z)-, methyl ester, $C_{19}H_{36}O_2$ present in S2 showed that the sample may have the antioxidant and anticancer properties as per reported by previous researchers [17, 18, 19]. More specifically, Abdullah and Moosa (2010) [20] reported that agarwood hydrosol contain phenolic content, that is correlated with its antioxidant properties.

According to Stewart (2015), the compound which consists of $C_{15}H_{24,}$ or three isoprene units can be expressed as sesquiterpene. Sesquiterpene compound is unsaturated and hence has the ability as free radical scavengers [21]. Based on the results, S1 and S2 had two and four sesquiterpene compounds respectively. S1 had (-)-aristolene and naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-. Concurrently, S2 comprised of naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-,[2R-(2.alpha.,4a.alpha.,8a.beta.)]-, azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]- and alloaromadendrene. However, sesquiterpene compound was absent in hydrosol extract S3, S4, S5 and S6. Guaiane, a type of sesquiterpene has been reported to be present in agarwood hydrosol extract [22].

The compounds revealed in this study have been shown to have biological properties in previous work. For instance, Tyagia and Argawak (2017) reported that the oxygenated compounds such as phenol, 2,4-bis(1,1-dimethylethyl)- and 10-Octadecenoic acid, methyl ester had the biological activities of antibacterial, antifungal and antioxidant [23]. Moreover, phenol, 2,4-bis(1,1-dimethylethyl)- and 9-Octadecenoic acid (Z)-, methyl ester showed anticancer property [24, 25, 26] while hexadecanoic acid, methyl ester has anti-inflammatory effect [27, 28].]. To this end, these compounds worth further investigation in

the effort to fully use the otherwise underutilize agarwood hydrosol towards development of therapeutics. Nevertheless, based on findings of this work, a more controlled raw material and processes at agarwood distillation stage is required to enable collection of agarwood hydrosol having uniform characteristics and similar quality for effective product development.

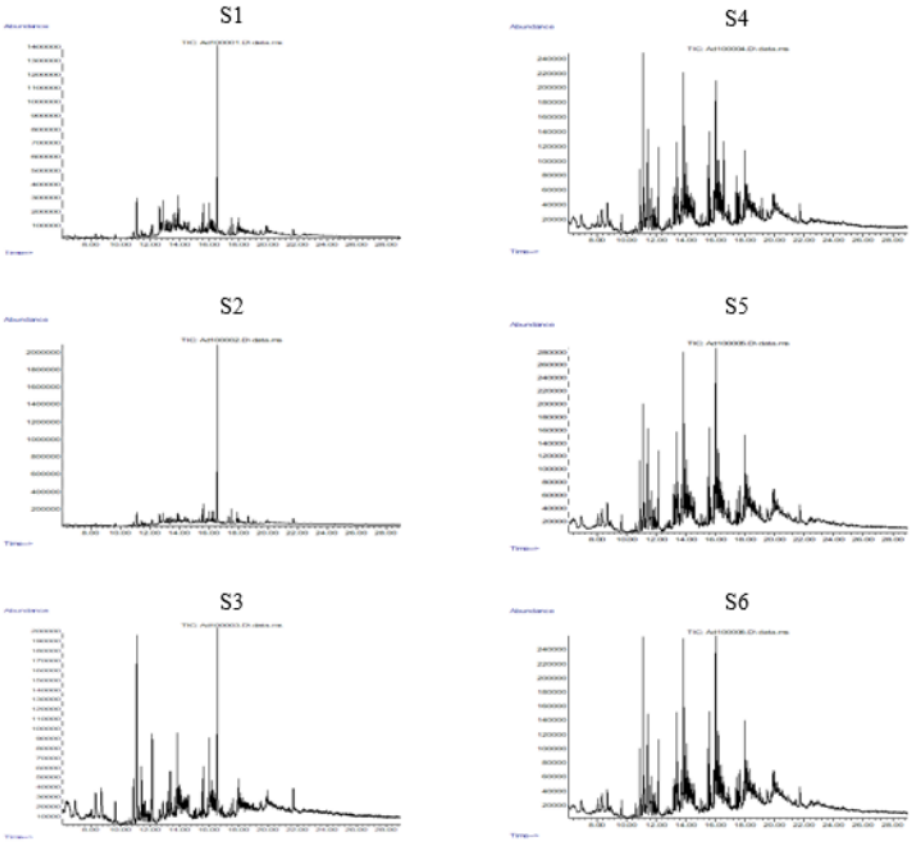

Fig. 4. Exemplary chromatograms of agarwood hydrosol samples obtained using GCMS.

4. CONCLUSION

In conclusion, profiling of compounds in agarwood hydrosol extracts revealed the presence of some potential bioactive particularly oxygenated compounds, phenolics and sesquiterpenes which suggest that the agarwood hydrosol extracts can be further explored for its biological activities such as antioxidant, anticancer and others. This could help realize the waste to wealth paradigm as well as promote the material as sustainable source for development of halal therapeutics.

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