Haya: The Saudi Journal of Life Sciences (SJLS)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2415-623X (Print)
ISSN 2415-6221 (Online)

Chemical Composition and Antimicrobial Activity of the Essential Oils from *Aquillaria malaccensis* in Bangladesh

Md. Najmol Hoque[1], Md. Fuad Mondal[2], Mohammad Mehedi Hasan Khan[1*]
[1]Department of Biochemistry and Chemistry, Sylhet Agricultural University, Bangladesh
[2]Department of Entomology, Sylhet Agricultural University, Bangladesh

Original Research Article

***Corresponding author**
Mohammad Mehedi Hasan Khan

Article History
Received: 03.10.2018
Accepted: 11.10.2018
Published: 30.10.2018

DOI:
10.21276/haya.2018.3.10.2

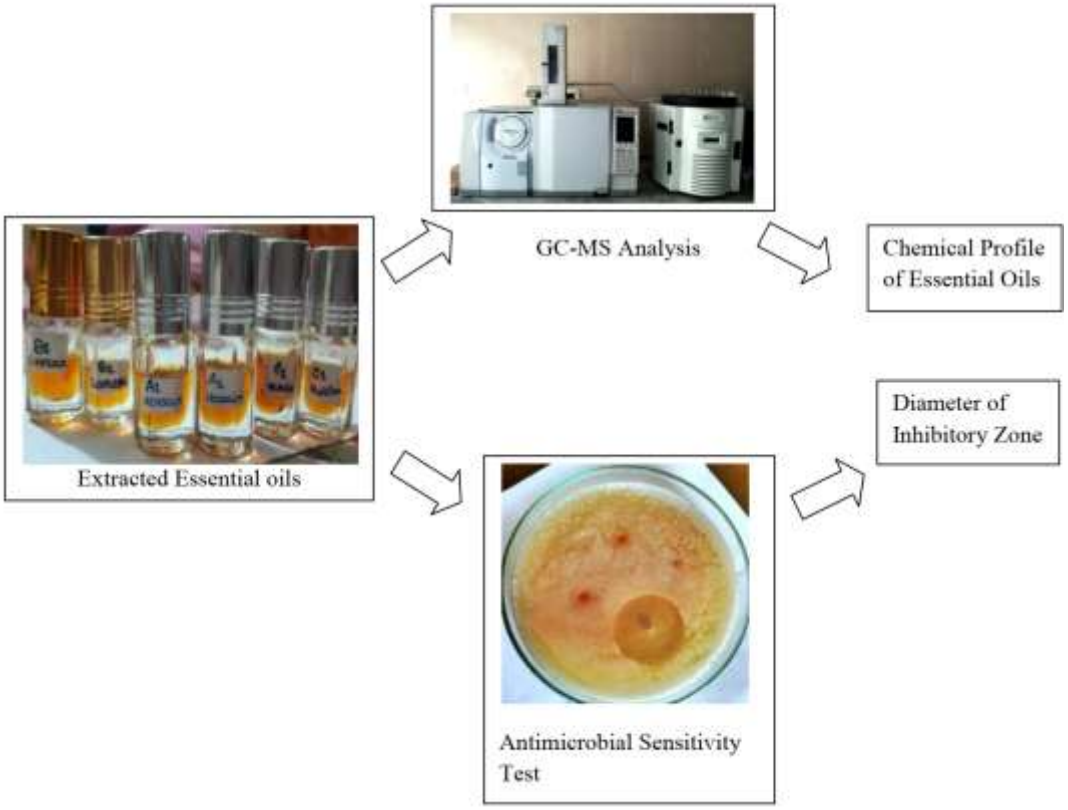
Abstract: Agarwood oil is a highly priced type of oil due to its unique aroma. Agarwood oil is widely used in perfume, medicine and cosmetic industries due to its unique properties and high medicinal value. The oil is generally extracted from the fragrant resins that form in the heartwood of agarwood tree. These unique properties of agar oils are contributed by the presence of certain chemical compounds. The market demand for agarwood oil is very high. Agarwood oil is traded based on grade, corresponding to expensive and cheap price. Currently, the grading of agar oil depends on its physical appearance such as color, odor and essential oil components, like present of sesquiterpenes. The present research analyzed essential oils from *Aquilaria malaccensis* obtained from Moulvibazar, Bangladesh based on GC-MS data and antimicrobial properties. GC-MS analysis found Sesquiterpenes, alcoholics, fatty acids and other chemical groups in the exportable agar oils. Farm-1 contained 94.95%, Fram-2 contained 95.37% and Farm-3 contained 93.90% sesquiterpenes which is the most important properties for stronger odors and high quality. All the agar oils contained about 75% 5-Azulenemethanol-1,2,3,4,5,6,7,8-octahydryo.alpha.alpha.3.8-tetramethyl-3,7-Cyclodecad iene-1- ethanol.alpha.alpha.4,8–tetramethyl-[s-(Z,Z)]. They also contained other sesquiterpenes like Ledene oxide- II; Aromandendrene; Bicyclo[4.4.0]dec-5-ene,1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-1H-3 a,7-methanolazulene, deca hydro-1,4,9,9-tetramethyl-Diethyl Phthalate; Isoaromadendrene epoxide. Other groups like alkane ~2%, alcoholics ~1% and fatty acid were found above 2%. The essential oils from all the farms except farm-2 have shown non-significant sensibility in antimicrobial test against *E. coli, S. aureus, Salmonella,Vibrio.*

Keywords: Agarwood, Chemical profile, Antimicrobial activity, Bangladesh.

INTRODUCTION

Agar tree belongs to Thymelaeaceae family was cultivated from 3000 years ago in the Middle East, China and Japan [1] and which is endemic to the Indomalesia region [2]. Indian sub-continent was considered as main source of agar tree for many centuries but now it is cultivated throughout the world especially in Bangladesh, India, Myanmar, Bhutan, Papua New Guinea, Thailand, Laos and Vietnam [3]. In Bangladesh, this tree is cultivated mostly in the greater Sylhet region and some parts of Chittagong and Chittagong hill tracts [2]. About 21 familiar *Aquilaria* species recorded, of which 13 are apparently fragrant resin producers [2]. Theoretically agarwood can be produced from all members [4]. Agarwood is the resinous heartwood of agar tree, comparatively light and pale colored, produce in response to natural infection (natural pathological infection such as insects, bacteria) or artificial induction [5, 40] (mechanical injury by human being or outer factors, wounding) that is very dense, dark when the wood matured and the tree produces a dark aromatic resin [6, 7]. The resin contains different plant secondary metabolites like aromatic terpenes. Agarwood are produced from *A. malaccensis, A. agallocha and A. secundaria*; *A. crassna* and *A. sinensis* are under *Aquilaria* genus [8]. In Bangladesh, *Aquillaria malaccensis* and *A. sinensis* are grown in a huge area in Sylhet and Chittagong divisions. Agar oils (essential oils) are a very highly valuable product of agar tree [9]. This is tremendously demanded in several countries which is further processed into perfumes, incenses and decorative displays and used as a raw material in traditional and modern medicines [2]. These fragrant oils have been mostly used as perfumery ingredients and as flavor and aroma enhancers [10]. Agar wood products have many different uses. Many religious groups honor it as a meditation incense to calm the mind and spriit, use in traditional Chinese unanai ayuravedic and have medicinal remedy for nervous disorders, stimulants, carminatives and sedatives [11, 12], anti-inflammatory property and treatment for lung and stomach tumors [13]. Oil is also sold in Vietnam for internal use. Agarwoods are marketed as the world's most expensive resin.

International agarwood market has experienced fluctuation of agarwood price. In 2013, global trade in agarwood chips and powder was about 4.7 million kg. Highest price of agarwood for first-grade agarwood, is the most expensive natural raw materials in the world. Based on presence of resin, agarwood chips are sold from $30 to $10,000 per kilogram [14]; high quality woods are usually sold for up to $30,000 US per kilogram [15, 16]. The global market for agar oil and other agarwood related products was estimated in the year of 2013 in the range of $6 to 8 billion [17]. Agar oil industrial buyers are expected to exceed it up to $36 billion in 2017 [18]; By 1970s, agar products were sold up to $42.5 per kg and then raised straightly $1250 and $2500 per kg in 2000 and 2005 respectively [16]. The demand of agarwood oils are very much high in the Middle East, where this oil symbolizes the society ranking, wealthiness and hospitality. It is an important ingredient in the perfumery industry and to produce aroma in wedding ceremonies or banquets. The agarwood oil is traded worldwide and major importers of agarwood essential oils are Saudi Arabia, United Arab Emirates, Bahrain, Malaysia, Singapore, China, Taiwan and Japan [18]. India, Bhutan, China, Indonesia, Malaysia, Myanmar, Thailand, Vietnam are cultivating, producing and exporting more amount of agarwood products than Bangladesh [19]. Bangladeshi are unknown about what chemical constituents their extracted oils bear, what are the variations of their products from the other growers and why they should demand more price of their product than the others. Focusing on their demand and opportunities on this field in the development of our country, the present research work had been taken to analyze the chemical composition of exportable agarwood essential oils from Moulvibazar, Sylhet, Bangladesh using GC-MS technique, to develop proper grading system so that its market value can be increased and to analyze the antimicrobial quality present in the exportable oils from Moulvibazar, Sylhet, Bangladesh.



Pictorial abstract showing the process

MATERIALS AND METHODS

Sample collection

Agarwood oil samples were collected from three different upazila of Moulvibazar (24° 29' 19.97" N latitude and 91° 46' 14.70" E longitude), Sylhet, Bangladesh during 2016 by the relevant three factories worker of the selected region. And these upazilas are Kamalgonj (24° 22' 0.12" N latitude, 91° 52' 0.12" E longitude), Rajnagar (24°56' 02" N latitude, 91°87' 09"E longitude), Baralekha (24° 70' 83" N latitude, 92°20' E longitude). These collected oils were further treated with different methods.

Extraction of essential oil

Essential oils were extracted from healthy and artificially screws injected plants using same local extraction techniques by three factories. All these three types of plant materials were collected from Moulvibazar, Sylhet, Bangladesh crashed and dried and then grinded individually. The grinded materials were

soaked in distilled water up to 14 days and filtered them separately.

Fig-1: Hydro-distillation method of agar oil extraction

The filtrate water mixtures were placed with Clevenger-type apparatus individually for the isolation of oils by hydrodistillation. After several hours essential oils were collected separately and then these exportable oils were then stored in sealed container prior to analysis [20].

GC-MS analysis

The three types of essential oil from three different factories of *Aquilaria* trees were analyzed by GC-MS (USA) method on Shimadzu GC-2010 Plus gas chromatograph in Institute of National Analytical Research and Service (INARS), Bangladesh Council of Scientific and Industrial Research (BCSIR). GC-MS analysis was performed with a Varian 450 gas chromatograph (USA) equipped with a VF-5MS capillary column (30 m × 0.25 mm i.d., flim thickness 0.25 µm) and a quadruple mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV).

Fig-2: Shimadzu GC-2010 Plus gas chromatograph GC-MS

The carrier gas was helium, at a flow rate of 1.03 mL/min. The injections were performed in split less mode at 35 °C. 1 µL essential oil solution in hexane (HPLC grade) was injected. The operating parameters were the temperature program of 35 °C for 1 min, ramp of 3 °C/min, subsequent increase to 2.0 °C with an 80 °C/min heating ramp, and keeping at 80 °C for 1 min. 10^0 C with an 120^0 C heating ramp, and keeping at 120 °C for 1 min. 20 °C with an 300 °C/min heating ramp, and keeping at 300 °C for 1 min. The scan range was 20–500 amu under full scan. 1 µL C8-C40 n-alkanes was injected separately and ran in the same program as the essential oils.

Identification of the compounds

Compound identification was carried out by comparing the NIST (*National Institute of Standards and Technology*) library data of the peaks with those reported in literature, mass spectra of the peaks with literature data. Percentage composition was computed from GC peak areas on with DB-5 ms column without applying correction factors.

Antimicrobial activity

Test microorganisms

Four clinical bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio* and *Salmonella* were used as test organisms in the screening. The microbial strains were obtained from the Department of Microbiology and Immunology, Sylhet Agricultural University, Sylhet.

Media preparation

LB media was prepared for the isolation of pure bacterial culture from stock culture. 6.5 g of nutrient agar was dissolved with 50 ml of distilled water

and then the solution was heated, sterilized by autoclave at 15 lbs pressure and 121^0 c temperature. In this LB media sub-cultures of four clinical bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio* and *Salmonella* were done. For antibiotic sensitivity test Mueller Hinton Agar media was used. About 11.4 g of powdered Mueller Hinton Agar was dissolved with 300 ml of distilled water, after that the solution was heated, sterilized by autoclave at 15 lbs pressure and 121^0 C temperature and then was cooled at 45-50^0 c on Mueller Hinton Agar Plate. Dilution of gentamicin was done from 100 mg/ml to 10 mg/ml (1 ml gentamicin + 9 ml PBS) and from this to 1 mg/ml (1 ml gentamicin + 9 ml PBS) or 1 µg/µl.

Determination of diameters of inhibition zone

Simple susceptibility screening test through agar well diffusion method was used [21]. The inocula of the bacterial strains were adjusted to 0.5 McFarland standard turbidity (approximately 10^8 CFU/mL) [22, 23]. One hundred micro liter of a suspension containing approximately 10^8 CFU/mL of each microorganism was spread on nutrient agar (NA). 6 mm diameter wells were cut from the agar using a sterile cork-borer, and 50 µL of the oil solution in a concentration of 50 mg/mL (dissolved in DMSO) were delivered into the wells. Negative controls were prepared using DMSO. Gentamicin (50 µg/well) was used as the positive reference standards. The plates were incubated for 18–24 h at 37 °C. The antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

RESULTS

Chemical constituents of exported Agarwood oil

Agarwood essential oils were extracted from different farms under Moulvibazar, using same extraction method (Hydro-distillation method). To ensure accurate results, chemical analysis of agar oils was done through GC-MS that confirm grading of the agarwood oils. The oils contained a complex mixture of secondary metabolites consisting of mainly oxygenated monoterpene and sesquiterpene hydrocarbons.

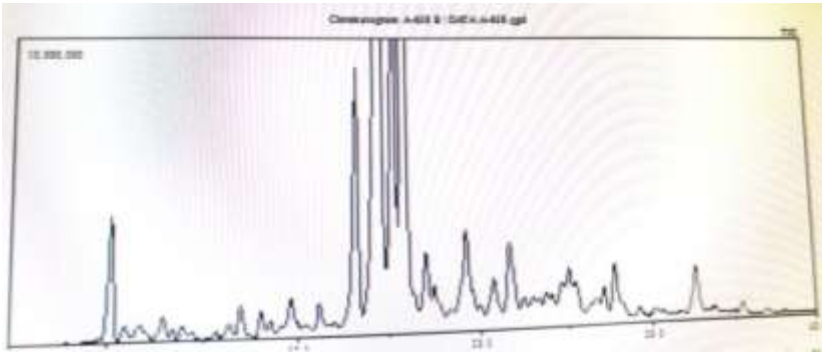


Fig-3: GC chromatograms of the farm-1 agarwood essential oils

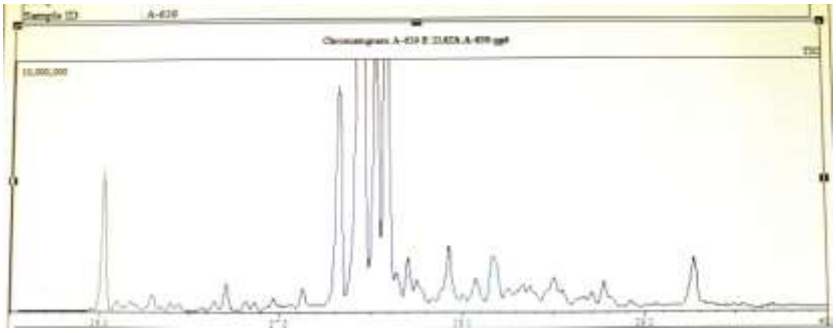


Fig-4: GC chromatograms of the farm-2 agarwood essential oils

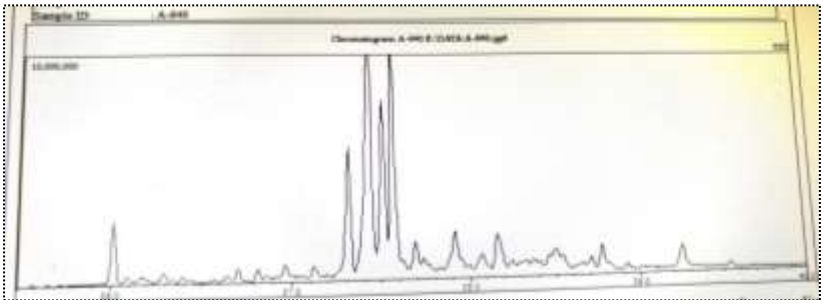


Fig-5: GC chromatograms of the farm-3 agarwood essential oils

Total sixteen essential compounds were identified from the three samples (Table-1 and Figure-3, 4, 5) of different farms under Moulvibazar, Sylhet, Bangladesh was found from GC-MS peak. The peaks obtained by this method were shown in fig 3, 4 and 5 respectively represent farm-1, farm-2 and farm-3. Thirteen essential compounds were identified from farm-1, fourteen from farm-2 and sixteen from farm-3. The major compounds detected from the oil samples were 2-butanone, 4-phenyl; Cyclohexanemethanol,4-ethenyl–alpha.alpha.4–trimethyl–3-(l-methylethenyl)-[1R-(1.alpha.,3.alpha.4.b); N–benzyl-oxycarbonyl–l–serine,methylester; 6,7–dimethyl–1,2,3,5,8, 8a–Hexahydronaphthalene; Ledene oxide – II ; Aromandendrene; 2H –3,9a–Mehtano-1-benzoxepin, octahydro–2, 2, 5a,9–tetramethyl-[3R-(3,alpha, 5a,alpha. 9.alpha, 9a.alpha)]. gamma.elemene; Tricyclo [5.2.2. 0(1,6)undecen-3-ol, 2-methylene-6,8,8-trimethyl- Aromandendrene; Bicyclo [4.4.0]dec-5–ene,1,5–dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-1H-3a,7-methanol azulene, decahydro-1,4,9,9-tetramethyl-Diethyl Phthalate ;5-Azulenemethanol,1,2,3,4,5,6,7,8–octahydro–alpha. alpha. . 3,8–tetramethyl–3,7–Cyclodeca- diene-1-methanol.alpha..alpha.4, 8–tetramethyl-[s-(Z,Z)]; Tricyclo [4.4.0.0 (2,7)dec-8-ene–3-methanol. alpha. alpha. 6,8–tetra methyl-stereoisomer; Isoaromadendrene epoxide; 3-Tetra decyn-1-ol; 4-Isopropenyl-4 ,7-dimethyl-1-oxaspiro[2.5]octane; 2-Octylcyclopropene–1 -heptanol; n-Hexadecanoic acid. Among these essential oils carbohydrate and alkane group consist of 1.719% from farm-1, 0.895% from farm-2 and 1.921% from farm-3.Sesquiterpenes and aromatics group were found94.95%, 95.37% and 93.895% respectively from farm-1, farm-2 and farm-3. On the other hand alcoholic group occupied 1.876%, 1.358% and 1.59% for farm-1, farm-2 and farm-3 respectively and fatty acid group occupied 2.011%, 2.239%, and 1.796% for farm-1, farm-2 and farm-3 respectively. There were many chemical compounds existing in agarwood oils and these compounds contribute to the characteristic of the agarwood oils odor and color. These essential oils compounds from different farms were shown in table-1.

Table-1: Constituent of Exportable Agarwood Essential Oils from Three Different Farms under Moulvibazar, Bangladesh

SL. No.	Chemical Group	Essential oils	% Conc. of essential oils		
			farm-1	farm-2	farm-3
1	Alkane	2-butanone,4-phenyl	1.70	0.90	1.80
2		N-benzyloxycarbonyl-l-serine, methyl ester	-	-	0.13
3	Sesquiterpenes and aromatics	6,7-dimethyl-1,2,3,5,8,8a-Hexahydronaphthalene	-	-	0.002
4		Ledene oxide- II	4.60	14.07	4.78
5		Aromandendrene	2.88	1.68	2.32
6		2H-3,9a-Mehtano-1-benzoxepin,octahydro-2,2,5a,9-tetramethyl-,[3R-(3,alpha,5a,alpha.,9.alpha,9a.alpha.)].gamma.elemene	0.30	0.47	0.37
7		Tricyclo[5.2.2.0(1,6) undecen-3-ol,2-methylene-6,8,8-trimethyl-Aromandendrene	0.43	0.17	0.74
8		4-Isopropenyl-4,7-dimethyl-1-oxaspiro[2.5]octane	0.90	0.60	0.32
9		Bicyclo[4.4.0]dec-5-ene,1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-1H-3a,7-methanol azulene,decahydro-1,4,9,9-tetramethyl-Diethyl Phthalate	4.34	1.31	3.43
10		5-Azulenemethanol,1,2,3,4,5,6,7,8-octahydro-alpha.,.alpha.,3,8-tetramethyl-3,7-Cyclodecadiene-1-methanol,.alpha.,.alpha.4,8-tetramethyl-,[s-(Z,Z)]	77.28	74.15	8.61
11		Tricyclo[4.4.0.0(2,7)dec-8-ene-3-methanol,.alpha.,.alpha.,6,8-tetramethyl-, stereoisomer	2.60	2.0	2.30
12		Isoaromadendrene epoxide	1.10	0.95	1.07
13		Cyclohexanemethanol,4-ethenyl-alpha.,.alpha.,4-trimethyl-3-(l-methylethenyl)-[1R(1.alpha.,3.alpha.,4.b)	-	-	0.42
14	Alcoholics	Tetradecyn-1-ol	0.94	0.78	0.80
15		2-Octylcyclopropene-1-heptanol	0.92	0.65	0.81
16	Fatty acid	n-Hexadecanoic acid	2.01	2.27	1.80
	Total		100	100	100

Antimicrobial activity test

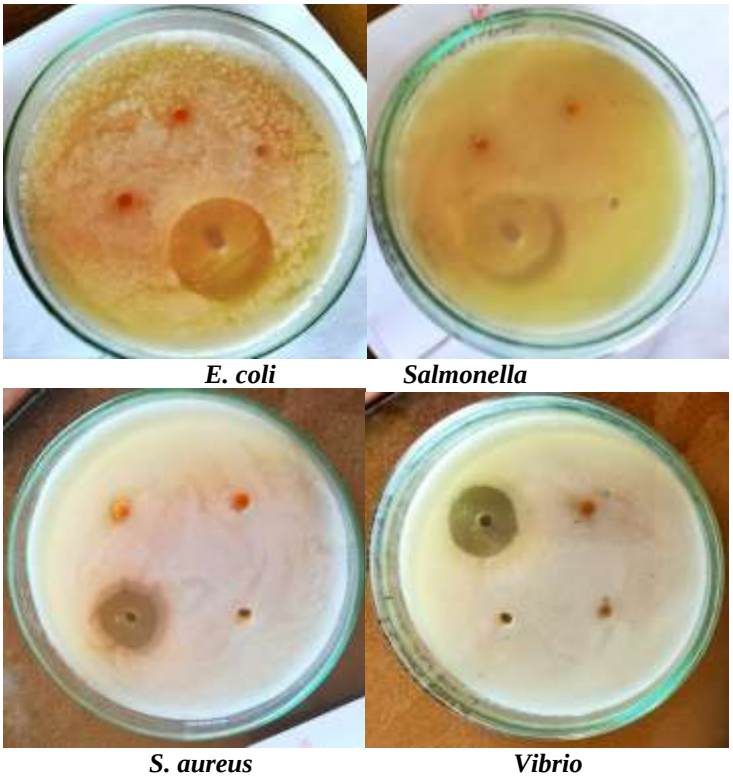
Agarwood essential oil samples from Moulvibazar district of Bangladesh were taken into antimicrobial activity test against four different bacterial strains (*E. coli, S. aureus, Salmonella,Vibrio).* All these bacterial strains are responsible for specific

disease in human being. Simple susceptibility screening test through agar well diffusion method was used to test antimicrobial activity of the exportable agarwood oils against these specific bacterial strains**.** The antimicrobial activity was determined from the diameter of inhihibitory zone. The antimicrobial activities of the three agarwood essential oils from three different farms were shown in Table-2.

Table-2: Screening Results for Antimicrobial Activity of the Exportable Essential Oils

Farms	Bacterial species			
	E.coli	*S. aureus*	*Salmonella*	Vibrio
Farm-1	NS	NS	NS	NS
	NS	NS	NS	NS
	NS	NS	NS	NS
Farm-2	LS	LS	LS	LS
	LS	LS	LS	LS
	LS	LS	LS	LS
Farm-3	NS	NS	NS	NS
	NS	NS	NS	NS
	NS	NS	NS	NS
Gentamycin	+++	+++	+++	+++
PBS	0	0	0	0

**NS indicate non-significant sensitivity; +++ indicates High Sensitivity; PBS means Phosphate Buffer Solution



E. coli ***Salmonella***

S. aureus ***Vibrio***

Fig-6: Screening results for antimicrobial activity of the exportable essential oils

DISCUSSION

Chemical constituents of exported Agarwood oil

The farms under study are situated in three different locations within the same district (Moulvibazar) using same extraction method were shown nearly similar in sesquiterpenes compositions. Farm-1 contains 94.95%, Farm-2 contains 95.37% and Farm-3 contains 93.895%. These results mean that all the three farms export agar oils which contain similar stronger odors that showed more value than the oils exported from China [24] and some other countries. Sesquiterpenes is one kind of terpenes which have attracted significant interest as it can play significant roles in biological systems and responsible for agarwood's nice scent [25]. The structure of this sesquiterpenes is the 15-carbon skeletons which make up the backbone of all sesquiterpenes. Sesquiterpenes are thought to be less volatile than other terpenes, have a greater potential for stereochemical diversity and have stronger odors. They are antiinflammatory [26], strong antioxidant [27], antitumor activity [28] and have bactericidal properties [29]. Sesquiterpenes can be

monocyclic, bicyclic or tricyclic and are a very diverse group [30]. Among the sixteen essential oils compound, ten are found sesquiterpenes and aromatics compound. Aromadendrene is one of the most important sesquiterpene derivatives. It was presumed that its compound is one of the distinguisher compounds (chemical marker) of agarwood [31]. The increasing aromadendrene chemical content indicated increasing agarwood level [31]. It was found a marked difference of sesquiterpenes compound in the agarwood from Bangladesh 94-95%, China 80-89% [24], Malayasia 80-85% [21].

The agarwood oils contained ledene oxide-(II) and 3-Tetradecyn-1-ol. According to several researches, these have enormous potential to strongly inhibit microbial pathogens [32]. The active antimicrobial compounds of essential oils are terpenes, which are phenolic in nature and seem reasonable that their antimicrobial or anti-fungal mode of action might be related to that of other compounds. The activity of ledene oxide-(II); Tricyclo [4.4.0.0(2,7) dec-8-ene-3-methanol,.alpha..alpha..6,8-tetrethyl-stereoisomer can significantly inhibit the growth of all the phytopathogens present in the oils .

Alcoholic compound such as 3-Tetradecyn-1-oland 2-Octylcyclopropene-1-heptanol are present in many essential oils such as perfumes and soaps and are responsible for tertiary odors. Alcohols are known to possess bactericidal rather than bacteriostatic activity against vegetative cells [33]. We have found nearly similar alcoholic essential in concentration from the three different farms. 3-Tetradecyn-1-ol was found 0.94%, 0.78%, 0.8% and 0.92%, 0.65%, 0.81% 2-Octylcyclopropene-1-heptanol respectively from Farm-1, Farm-2 and Farm-3.

Common occurrence of 2-butanone, 4-phenyl compound was commonly identified in all the three sample farms which responsible for a waxy herbal odor type with medium odor strength [34]. This essential oil was observed in all agarwood oil (*A. malaccensis)* obtained from different sources in Moulvibazar, Bangladesh. This compound has gave slight abundance in concentration which show more or less similar concentration and peak in case of farm-1 (1.70 %) and farm-3 (1.80 %) but slightly lower in case of farm-2 (0.90 %).

Cyclohexanemethanol,4-ethenyl-alpha.alpha.,4-trimethyl-3-(l-methylethenyl)-[1R(1.alpha.3 .alpha.4.b) is an essential compounds which was absent in case farm-1 and farm-2 where 0.42% in case farm-3. It is a naturally occurring monoterpene alcohol. It is also used as a solvent. It is an important constituent of pine oil disinfectants. Further, it is used as a fragrance in perfumes, fat denaturant for soap production and synthetic flavoring agent [35].

N-hexadecanoic acid is fatty acid showed more or less similar concentration in GC-MS accounted for 2.01 %, 2.27 % and 1.80 % respectively of the total essential oil from farm-1, farm-2 and farm-3, which explained its smell and state that potentially useful in the medicines and perfumery purposes. This result in comparisons with the other country was shown much lower in performance where Chinese agarwood contained 5-6% [24], 1.26% from naturally healthy agar and 7.06% from artificially screw injected wood[36].

The grading system is based on the color, weight and odor [31]. These oils were looked light brown to deep brown in color and stronger in odor, which indicated their stronger physical quality and higher in grade. The grading of the agarwood oils to the high and low based on its quality is done using manually such as human trained grader. It is performed based on the agarwood oils physical properties such as human experience and perception, the oil colour, odor, long lasting aroma and chemical profiles of the oil which is must to overcome the problem facing by manual techniques i.e. human nose cannot tolerate with the many oils at the same time. So to ensure accurate results which can be obtained by chemical analysis of agar oils that confirm grading of the agarwood oil [31]. Forest Research Institute Malaysia (FRIM) has carried out classification of agarwood oils quality based on its wood physical properties, long lasting aroma when burnt, color, resin content, high fixative properties and consumer perception [38]. Based on the chemical composition and agar oil color, it can be concluded that exportable agarwood oils from Bangladesh can be classified into high quality agarwood and must deserve high market value.

Antimicrobial activity test

The results indicated that agar oils had some activity against almost all the tested bacteria. All the essential oils showed non-significant sensitivity against *E. coli, S. aureus, Salmonella, Vibrio.* The essential oils of farm-1 and farm-3 had lower antimicrobial activities than that of farm-2. This is probably because of the higher Ledene oxide II essential oils present in farm-2 than farm-1 and farm-3 as Ledene oxide II has higher antimicrobial properties [32, 39]. Ledene oxide II is one kind of sesquiterpenes usually possess antimicrobial activity. If these active components are isolated and purified, their antimicrobial activities could become stronger [24]. The negative-control experiments, including the antimicrobial test for PBS indicated no microbial contamination, 50 µg/well PBS, the maximum concentration used for dissolving essential oil was shown no inhibition on the microbial growth and Gentamycin was used as a positive control was shown higher sensitivity which indicated highest microbial contamination in the essential oils and media, as shown by the data in Table-2. This is one of the premier reports concerning the antimicrobial activities

to the four bacterial strains of Bangladeshi agarwood oil from *A. malaccensis species.*

CONCLUSION

The chemical compositions of the agarwood essential oils were determined from different farms under Moulvibazar, Sylhet, Bangladesh originated from *A. malacensis.* The grading of the agarwood oils is performed based on the physical properties such as human experience and perception, the oil colour, odor, long lasting aroma and chemical profiles of the oil which overcome the problem facing by manual techniques i.e. human nose cannot tolerate with the many oils at the same time. So accurate results which be obtained by chemical analysis of agar oils that confirm grading of the agarwood oil. This is one of the premier reports concerning the antimicrobial activities to the four bacterial strains of Bangladeshi agarwood oil from *A. malaccensis species.* The essential oils of farm-1 and farm-3 had lower antimicrobial activities than that of farm-2. Information on exportable essential oils from Moulvibazar is very important for getting desirable market price of the products in the international market, in making grade of the products by the export promotion bureau of Bangladesh. These results indicated that agarwood oils exported from Bangladesh have higher sesquiterpenes which mainly responsible for its unique aroma. So, it can be concluded that exportable agarwood oils from Bangladesh can be classified into high quality agarwood and should deserve high market value.

ACKNOWLEDGEMENT

This work was financed by the National Science and Technology Ministry, Government of Bangladesh, Dhaka, Bangladesh. Special thanks to Mr. Ataur Rahman, Mohammad M. A. Laskar and M. Hossain Ahmed for the supply of raw oils of *Aquillaria malaccensis* wood.

Conflict of interest

The authors have declared that there is no conflict of interest.

REFERENCES

1. Le, C. K. (2003, November). History and ecology of agarwood in Vietnam. In *Proceedings of the 1st International Agarwood Conference*. The Tropical Rainforest Project.
2. Mohamed, R., & Lee, S. Y. (2016). Keeping up appearances: Agarwood grades and quality. In *Agarwood* (pp. 149-167). Springer, Singapore.
3. CITES. (2004). Amendments to appendices I and II of CITES. In Proceedings of Thirteenth Meeting of the Conference of the Parties, Bangkok, Thailand, on 2nd October.
4. Ng, L. T. (1997). A review on agar (gaharu) producing Aquilaria species. *J. Tropic. Forest Prod.*, *2*, 272-285.
5. Akter, S., Islam, M. T., Zulkefeli, M., & Khan, S. I. (2013). Agarwood production-a multidisciplinary field to be explored in Bangladesh. *International Journal of Pharmaceutical and Life Sciences*, *2*(1), 22-32.
6. Blanchette, R. A. (2006). The genus Gyrinops is closely related to Aquilaria and in the past all species were considered to belong to Aquilaria. Cultivated Agarwood Training programs and Research in Papua New Guinea. *Forest Pathology and Wood Microbiology Research Laboratory, Department of Plant Pathology, University of Minnesota*.
7. Chowdhury, M., Rahman, A., Hussain, M. D., & Kabir, E. (2017). The economic benefit of agarwood production through aeration method into the Aquilaria malaccensis tree in Bangladesh. *Bangladesh Journal of Agricultural Research*, *42*(1), 191-196.
8. Broad, S. (1995). Agarwood harvesting in Vietnam. *TrAFFIC Bulletin*, *15*(2), 96.
9. Ba, X. D. (2010). A Vietnamese technique of agarwood inducement.
10. NorAzah, M. A., Majid, J. A., Mailina, J., Said, A. A., Husni, S. S., Hasnida, H. N., ... & Chang, Y. S. (2009). Profiles of selected supreme Agarwood oils from Malaysia. *Herbal Globalisation: A New Paradigm for Malaysian Herbal Industry (MAPS 2008)*, 393-398.
11. Abas, M. A., Zubir, N. S. A., Ismail, N., Yassin, I. M., Ali, N. A. M., Rahiman, M. H. F., ... & Taib, M. N. (2017). Agarwood oil quality classifier using machine learning. *Journal of Fundamental and Applied Sciences*, *9*(4S), 62-76.
12. Chakrabarty, K., Kumar, A., & Menon, V. (1994). Trade in agar tree. TRAFFIC India and WWF-India, New Delhi. 51.
13. K Yadav, D., Mudgal, V., Agrawal, J., K Maurya, A., U Bawankule, D., S Chanotiya, C., ... & T Thul, S. (2013). Molecular docking and ADME studies of natural compounds of Agarwood oil for topical anti-inflammatory activity. *Current computer-aided drug design*, *9*(3), 360-370.
14. Babatunde, O. J. (2015). Oud: Arabia's traditional scent. Available at: http://www.masterpiece-ng.com/ 2015/09/01/oud-arabias traditional-scent on 10th December 2017.
15. Joynal, M. A. (2014). Development of SMEs: replicating top foreign models. Available SSEN:http://dx.doi.org/10.2139/ssrn.2504480.
16. Abdin, M. (2014). The agar wood industry: yet to utilize in Bangladesh.
17. Akter, S., Islam, M. T., Zulkefeli, M., & Khan, S. I. (2013). Agarwood production-a multidisciplinary field to be explored in Bangladesh. *International Journal of Pharmaceutical and Life Sciences*, *2*(1), 22-32.
18. Tripathi, H., Suresh, R., Kumar, S., & Khan, F. (2016). International Trade in Essential Oils and Resinoids: A Case Study of Past 18 Years. *Journal*

of Medicinal and Aromatic Plant Sciences, *38*(1-4), 1-28.

19. Kassan, I. (2014). Menjanahasildamarkaras. Seminar GaharuKebangsaan 1–4.
20. Liu, X., Xu, D., Yang, Z., & Zhang, N. (2017). Chemical Composition of Essential Oils from the Heartwood of Pterocarpus macrocarpus by Different Extraction Methods in Southern China. *Journal of Essential Oil Bearing Plants*, *20*(1), 110-115.
21. Yusoff, N. M., Tajuddin, S. N., Hisyam, A., & Omar, N. M. (2015). Agarwood Essential Oil: Study on Optimum Parameter and Chemical Compounds of Hydrodistillation Extraction. *Journal of Applied Science and Agriculture*, 1-5.
22. Cao, L., Si, J. Y., Liu, Y., Sun, H., Jin, W., Li, Z., ... & Le Pan, R. (2009). Essential oil composition, antimicrobial and antioxidant properties of Mosla chinensis Maxim. *Food Chemistry*, *115*(3), 801-805.
23. Sokmen, A., Gulluce, M., Akpulat, H. A., Daferera, D., Tepe, B., Polissiou, M., ... & Sahin, F. (2004). The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic Thymus spathulifolius. *Food control*, *15*(8), 627-634.
24. Chen, H., Yang, Y., Xue, J., Wei, J., Zhang, Z., & Chen, H. (2011). Comparison of compositions and antimicrobial activities of essential oils from chemically stimulated agarwood, wild agarwood and healthy Aquilaria sinensis (Lour.) Gilg trees. *Molecules*, *16*(6), 4884-4896.
25. Pasaribu, G., Waluyo, T. K., & Pari, G. (2013). Analysis of chemical compound in some of agarwood quality by gas chromatography mass spectrometry. *Journal Forest Product Research*, 31(3) 181–185.
26. Jeena, K. O. T. T. A. R. A. P. A. T., Liju, V. B., & Kuttan, R. A. M. A. D. A. S. A. N. (2013). Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. *Indian J Physiol Pharmacol*, *57*(1), 51-62.
27. Ornano, L., Venditti, A., Ballero, M., Sanna, C., Quassinti, L., Bramucci, M., ... & Bianco, A. (2013). Chemopreventive and Antioxidant Activity of the Chamazulene-Rich Essential Oil Obtained from Artemisia arborescens L. Growing on the Isle of La Maddalena, Sardinia, Italy. *Chemistry & biodiversity*, *10*(8), 1464-1474.
28. Park, D., Hadžić, T., Yin, P., Rusch, J., Abruzzi, K., Rosbash, M., ... & Taghert, P. H. (2011). Molecular organization of Drosophila neuroendocrine cells by Dimmed. *Current Biology*, *21*(18), 1515-1524.
29. Ishnava, K. B., Chauhan, J. B., & Barad, M. B. (2013). Anticariogenic and phytochemical evaluation of Eucalyptus globules Labill. *Saudi journal of biological sciences*, *20*(1), 69-74.
30. Başer, K. H. C., & Demirci, B. (2007). Studies on Betula essential oils. *Arkivoc*, *7*, 335-348.
31. Pasaribu, G., Waluyo, T. K., & Pari, G. (2015). Analysis of chemical compounds distinguisher for agarwood qualities. *Indonesian Journal of Forestry Research*, *2*(1), 1-7.
32. Çakir, T., Kirdar, B., & Ülgen, K. Ö. (2004). Metabolic pathway analysis of yeast strengthens the bridge between transcriptomics and metabolic networks. *Biotechnology and Bioengineering*, *86*(3), 251-260.
33. Khan, R. A. G., Khan, F. A., & Khan, M. A. (2011). Impact of training and development on organizational performance. *Global Journal of Management and Business Research*, *11*(7).
34. Hashim, Y. Z. H. Y., Ismail, N. I., & Abbas, P. (2014). Analysis of chemical compounds of agarwood oil from different species by gas chromatography mass spectrometry (GCMS). *IIUM Engineering Journal*, *15*(1).
35. Zhang, Z., Zhang, X., Yang, Y., Wei, J. H., Meng, H., Gao, Z. H., & Xu, Y. H. (2014). Hydrogen peroxide induces vessel occlusions and stimulates sesquiterpenes accumulation in stems of Aquilaria sinensis. *Plant growth regulation*, *72*(1), 81-87.
36. Bhuiyan, M. N. I., Begum, J., & Bhuiyan, M. N. H. (2009). Analysis of essential oil of eaglewood tree (Aquilaria agallocha Roxb.) by gas chromatography mass spectrometry. *Bangladesh J Pharmacol*, *4*, 24-28.
37. Ismail, N., Ali, N. A. M., Jamil, M., Rahiman, M. H. F., Tajuddin, S. N., & Taib, M. N. (2014). A review study of agarwood oil and its quality analysis. *Jurnal Teknologi (Sci Eng)*, *68*(1), 37-42.
38. NorAzah, M. A., Majid, J. A., Mailina, J., Said, A. A., Husni, S. S., Hasnida, H. N., ... & Chang, Y. S. (2009). Profiles of selected supreme Agarwood oils from Malaysia. *Herbal Globalisation: A New Paradigm for Malaysian Herbal Industry (MAPS 2008)*, 393-398.
39. Jamal, M. A. H. M., Rahman, M. S., Hossain, M. B., Sharma, S. P., Chung, H. J., Kim, H. J., & Hong, S. T. (2017). Antibacterial Properties and Chemical Composition of Essential Oil from Aegle marmelos (L.) Corr. Leaves Growing in Bangladesh. *Journal of Essential Oil Bearing Plants*, *20*(1), 155-174.
40. http://www.ouddh.com/?cid=2065877 on 20th December 2017.