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CONSTITUENTS OF THE ESSENTIAL OIL FROM *AQUILARIA AGALLOCHA* ROXB BY GC-MS AND ITS CYTOTOXIC ACTIVITIES

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ABSTRACT

Aquilaria agallocha (Thymelaeaceae) is an important medicinal plant.[1] It is also used for the production of essential oils which is widely used in various pharmaceutical and cosmetic industries .[2] Some of the constituents are also used for food processing and many other purposes. The essential oil was isolated from *Aquilaria agallocha* by steam distillation. Brine Shrimp bioassay result of this oil showed cytotoxic activity (LD_{50} 0.683 mg/mL after 24 hours and LD_{50} 0.232 mg/mL after 48 hours). From the plant eighteen (18) compounds have been isolated.[3-7] The composition of hexane soluble part of this oil was analyzed by GC-MS. Thirty eight (38) different compounds were identified among which n-Hexadecanoic acid (17.34%), 1(11)-Spirovetiven-11-ol (10.42%), Guai-1(5)-en-11-ol (8.98%), (-)-Aristolene (6,52%), Tetradecanoic Acid (5.43%) and 4-Eudesmen-11-ol (5.0%) are the major constituents.

KEY WORDS: *Aquilaria agallocha; LD50; GC-MS; Essential Oils; Brine Shrimp Bioassay.*

1. INTRODUCTION

The production and use of essential oils widely used in various pharmaceutical, cosmetic industries and some of the constituents are used for food processing and many other purposes. The revolution in the science of essential oils began at the end of the 18th century with the work of Lavoisier (1743-1794) which led to new and illuminating studies by the scientists to explore the various facets of science of essential oils.

Aquilaria malaccenis Lamk. which is also known as *A. agallocha* Roxb. belongs to the family Thymelaeaceae. Its common name is agar wood, eagle wood tree, aloe wood etc. It is a moderate-sized evergreen large tree, is widely distributed in India, China, Thailand, and Malaysia.[10] In Bangladesh, it grows in the forests of Sylhet. On the basis of traditional information it is an important medicinal plant. It is used for rheumatism.[1,10] for abdominal disorders, as an incense, stimulant, tonic, carminative, stomachic, laxative, diuretic and aphrodisiac.[9,10,11] It is also useful in leucoderma and other skin diseases, hiccup, bronchitis, asthma, chronic diarrheoa and diseases of the ear.[12] Powder wood is used as a perfume. From bioassay results it was found that *A. agallocha* is mild cardiotoic.[13] The plant is infected by fungus. Crud agar oil has a brown color that freezes under low temperature. A review of the chemical literature revealed that 18 oily compounds have been isolated from *A. agallocha*.[14-20]

Present study was designed to determine the constitutes and its composition of n-Hexane soluble part of oil obtained by water extraction followed by distillation using Gas Chromatography coupled with Mass Spectrophotometer. Study was extended to determined cytotoxic study Brine Shrimp Lethality Assay (BSLA) for LD50 which is an inexpensive bioassay technique used for determine the efficacy of chemical components present in the plant extracts. A poster was presented on this work at Asian Symposium on Medicinal Plants, Spices and Other Natural Products (ASOMPS X).[8]

2. MATERIALS AND METHODS

2.1 Plant Materials

The black portion of *A. agallocha* Roxb. was collected from Sylhet, Bangladesh and was identified by Prof. Salar Khan, Bangladesh National Herbarium (BNH). A voucher specimen has been deposited in the BNH.

2.2 Isolation of the compounds

One kilogram fresh black portion of the plant was kept under water for 20 days then it was crushed in grinder. The crushed materials were again kept under water for ten days. Then the brown color essential oil was extracted from it by steam distillation for 36 hours. The amount of the oil was 11.5 gm.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

n-Hexane was added to the oil sample extracted by distillation and was sonicated for an hour. Finally it was kept at room for 30 minute to settle down. The n-Hexane part was taken for the GC-MS analysis to identify the constituents and its composition.

The oil compositions of *A. agallocha* oil were analyzed by GC/MS. GC-17A Model (Shimadzu) with fused silica capillary column DB-1 (J&W), 0.25 µm film thickness was used. Helium was the carrier gas with flow rate 5 ml/min and 90 kPA pressure. The GC was programmed from 80-240^{0}C at 3^{0}C / min with MS operating at 70eV and ion source temperature of 240^{0}C. The GCMS-QP 5050A mass spectrometer equipped with NIST 107 Library (Shimadzu Corporation) was used for the comparison with published spectra. Percentages of important constituents were quantitatively determined on the basis of respective area of the eluted peaks and identified on mass matching with the mass of NIST 107 Library of Shimadzu Corporation. Minimum count for the peak integration is 10x10^6. The chemical compositions of the constituents of *A. agallocha* n-Hexane soluble oils are summarized in Table 1 (major constituents) & Table 2 (minor constituents).

2.4 Screening of Cytotoxic Activity

2.4.1 Instrumentation

Rectangular glass vessel, Measuring cylinder, Table salt, Spatula, Brine shrimp eggs (5 gram), Air pump, Pasteur pipette, Table Light, Micro pipette, Test tubes, Magnifying glass, n-Hexane soluble part of plant oil.

2.4.2 Brine Shrimp Lethality Bioassay

Shrimp was hatched in a rectangular glass vessel filled with five liter salted water. Air was passed from bottom of the jar and few gram egg was added and kept for 24 hours under table lamp. n-Hexane part was taken for study. Different concentration (5mg/mL, 2.5mg/mL, 0.5mg/mL and 0.1mg/mL) were exposed to ten fully grown nauplii and survivors counted after 24 hours and 48 hours. Three replications were used for each this concentration. The mortality endpoint was considered when forward motion is absent during 30 sec of observation. Mortality Rate Brine Shrimp assay, Log concentrations and probit values when exposed to n-Hexane soluble oil after 24 and 48 h of *Aquilaria agallocha are* presented in Table 3.

The death percentage was calculated as per (Equation: 1)

Percentage of Death (%): (Total naupii – Alive naupii) × 100%/Total naupii ------- Equation: 1

Lethal dose (LD_{50}) for each time points were determined using statistical analysis.

RESULTS AND DISCUSSION

Upon analysis of n-Hexane soluble part of water distillated extract of *A. agallocha.* by GC-MS resulted thirty eight (38) compounds. Among them there were six major constitutes (5.00% - 17.34%) and these are listed in Table 1. The other thirty two constituents are present in the range 0.65% to 4.8% and those are presented in Table 2. Some of these compounds were identified by different scientists but most of them are new from this plant extracts.

Table: 1 Major constitutes of n-Hexane soluble oil extracts of *A. agallocha.*

S/N	Name of the Compound	Retention Time (Min)	Area %
1	n-Hexadecanoic acid	43.68	17.34
2	1(11)-Spirovetiven-11-ol	31	10.42
3	Guai-1(5)-en-11-ol	31.63	8.98
4	(-)-Aristolene	31.29	6.52
5	Tetradecanoic Acid	36.16	5.43
6	4-Eudesmen-11-ol	30.38	5.00

Table: 2 Minor constitutes of n-Hexane soluble oil extracts of *A. agallocha.*

S/N	Name of the Compound	Retention Time (Min)	Area %
1	n- Heptanoic acid	8.68	0.65%
2	2-Butanone	14.08	0.83
3	2-methylene -6,8,8-trimethyl-tricyclo[5,2,22,0(1,6)]undecan-3-ol	24.57	2.98
4	Lenden alcohol	27.57	2.98
5	Diethyl Phthalate	28.16	2.79
6	3,7-Dimethyl-6-octen-1-ol	28.35	0.65
7	Dodecanoic acid	28.65	0.87
8	3-Ethyl-4,4-dimethyl-2-(2-methylpropenyl)cyclohex-2-enone	29.53	0.67
10	1(11)-Spirovetiven-11-ol	31.00	10.42
13	3-Eudesmen-11-ol	31.81	0.92
14	Tricyclo[4.4.0.2,7]dec-8-ene-3-methanol	32.14	2.72
15	4,11-Eudesmadien-2-ol	32.37	1.25
16	1-Heptatriacotanol	32.73	2.63

17	3,11-Eudesmadien-2-ol	32.96	1.00
18	Aristolene epoxide	33.19	1.02
19	4,11(13)-Eudesmadien-12-ol	34.05	1.18
20	11,11-Dimethyl-bicyclo[6.3.0.0(1,8)]undeca-1(8),9-diene	34.35	0.75
21	Myristic acid	34.47	0.78
22	Hexadecahydropyrene	34.60	1.13
23	2-methylene-6,8,8-trimethyl-tricyclo[5.2.2.0(1),60]undecan-3-ol	34.70	0.67
24	ɣ-Gurjunenepoxide-(2)	34.84	0.68
25	2,5-diprop-2-enyl-decahydroquinoline	35.25	1.40
26	5(1H)-Azulenone	35.43	1.07
28	2-Methyl-4-phenyl-2,3-pentadenoic acid	36.50	0.68
29	4,11-Eudesmadien-12-hydroxy-2-one	36.73	1.19
30	1-(*p*-Toluidino)-1-deoxy-β-D-idopyranose	38.13	0.76
31	Eicosanoic acid	38.75	4.79
32	Pentadecanoic acid	39.79	4.50
33	9-Hexadecenoic acid	42.18	2.26
35	Ethylhexadecanoate	43.94	0.65
36	1,3-Di-palmitin	45.88	0.65
37	12-Methyl-E,E-2,13-octadecadien-1-ol	48.58	1.39
38	2-Hydroxy-cyclopentadecanone	48.65	0.78

The brine shrimp lethality assay (BSLA) is a simple and less expensive bioassay used for testing the cytotoxic efficacy of chemical isolated from plant extracts. With this study it was found that the extent of lethality was directly proportional to the concentration of the extract. The LC50 (median lethal concentration) values were calculated by using the regression line obtained by plotting the concentration against the death percentage on a probit scale.

Studies indicated that LD50 of n-Hexane soluble compounds after 24h is 0.684 mg/mL and LD50 of the same compounds set after 48h is 0.232 mg/mL. Statistical Analysis of Mortality Rate Brine Shrimp assay after 24h and 48h are presented in Table 4 and Table 5 respectively. Moreover, fit plot and normal probability plot of 24h and 48h are presented in Figure 1 & 2 and Figure 3 & 4 respectively.

Table: 3 Mortality Rate Brine Shrimp assay, Log concentrations and probit values when exposed to n-Hexane soluble oil after 24 and 48 h of *Aquilaria agallocha.*

Amount Dose (mg/ml)	Log10 Conc	No of Shrimps in each test	After 24 hours						After 48 hours					
			No Alive	No Dead	% Mortality	% Corrected	Probit	LD50 (mg/ml)	No Alive	No Dead	% Mortality	% Corrected	Profit	LD50 (mg/ml)
5	0.6990	10	0	10	100	97.5	6.96	0.684	0	10	100	97.5	6.96	0.232
	0.6990	10	0	10	100	97.5	6.96		0	10	100	97.5	6.96	
	0.6990	10	0	10	100	97.5	6.96		0	10	100	97.5	6.96	
2.5	0.3979	10	0	8	80	80	5.84		0	10	100	97.5	6.96	
	0.3979	10	0	9	90	97.5	6.28		0	10	100	97.5	6.96	
	0.3979	10	0	8	80	80	5.84		0	10	100	97.5	6.96	
0.5	0.3010	10	7	3	30	30	4.48		1	9	90	90	6.28	
	-.3010	10	7	3	30	30	4.48		2	8	80	80	5.84	
	-.3010	10	7	3	30	30	4.48		3	7	70	70	5.52	
0.1	-.0000	10	10	1	10	10	3.72		8	2	20	20	4.16	
	-.0000	10	9	1	10	10	3.72		8	2	20	20	4.16	
	-.0000	10	10	0	0	2.5	3.03		8	2	20	20	4.16	

Table: 4 Statistical Analysis of Mortality Rate Brine Shrimp assay after 24 hours.

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	20.98654	20.98654	226.5018	3.38629E-08
Residual	10	0.926551	0.092655		
Total	11	21.91309			
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*
Intercept	5.331868	0.088135	60.49643	3.7E-14	5.135490013
X Variable 1	2.01256	0.133725	15.04998	3.39E-08	1.714601669

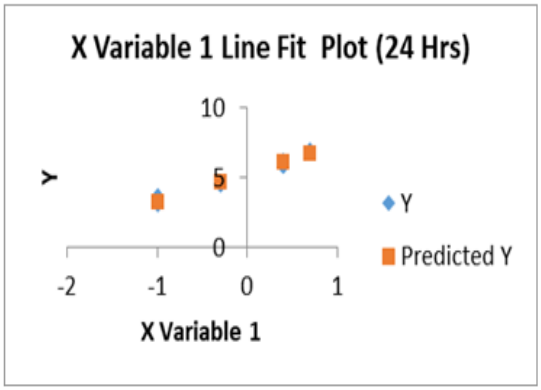

Figure: 1 Fit Plot Analysis 24h Data.

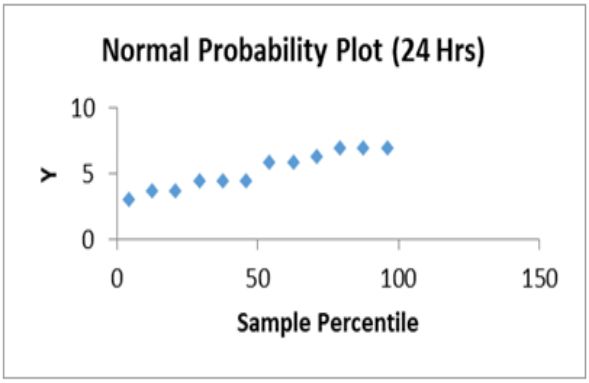

Figure: 2 Normal Probability Plot of 24h Data.

Table: 5 Statistical Analysis of Mortality Rate Brine Shrimp assay after 48 hours.

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	14.88261	14.88261	130.8947	4.57E-07
Residual	10	1.136991	0.113699		
Total	11	16.0196			
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*
Intercept	6.076486	0.097632	62.23848	2.79E-14	5.858947
X Variable 1	1.694798	0.148135	11.44092	4.57E-07	1.364733

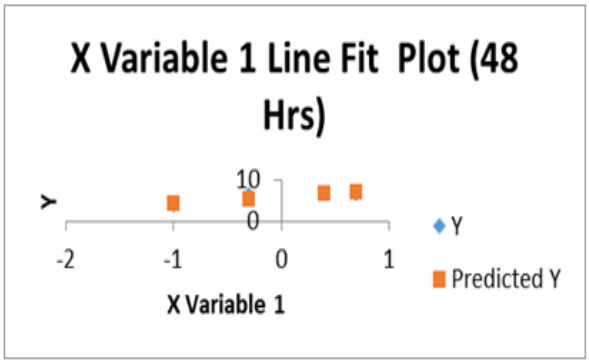

Figure: 3 Fit Plot Analysis of 48h Data

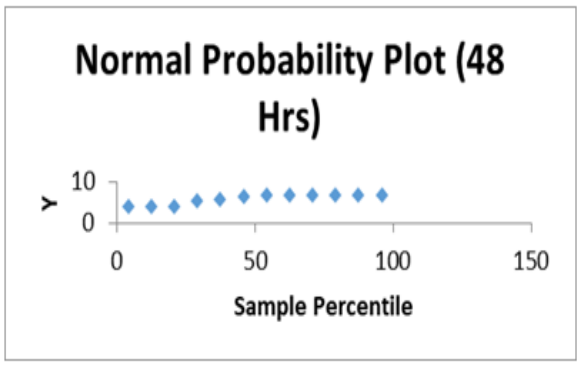

Figure: 4 Normal Probability Plot of 48h Data

CONCLUSION

Thirty eight (38) different compounds were isolated and identified by GC-MS from *Aquilaria agallocha* by steam distillation and among which n-Hexadecanoic acid (17.34%), 1(11)-Spirovetiven-11-ol (10.42%), Guai-1(5)-en-11-ol (8.98%), (-)-Aristolene (6,52%), Tetradecanoic Acid (5.43%) and 4-Eudesmen-11-ol (5.0%) are the major constituents. Most of these compounds are new identification from this plant. Brine Shrimp bioassay result of this isolated compounds showed cytotoxic activity (LD_{50} 0.683 mg/mL after 24 hours and LD_{50} 0.232 mg/mL after 48 hours), no such cytotoxic activities were reported for *Aquilaria agallocha* until completion of this studies.

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REFERENCES

1. Ghani, A, Medicinal plants of Bangladesh, Asiatic Society of Bangladesh, 1998; 85.
2. Opdyke, DLJ, Food Cosmet. Toxicol, 1975; 13: 93.
3. Ishihara, M, et.al., Phytochemistry, 1993; 33: 1147.
4. Ishihara, M, et.al., Phytochemistry, 1991; 30: 3343.
5. Ishihara, M, et.al., Phytochemistry, 1991; 30: 563.
6. Thomas, AF, Tet. Lett., 1976.
7. Jain, TC, et.al., Tet. Lett., 1969; 9: 13.
8. Abm Mahfuz Ul Alam, Md. Zia ul Abedin, Mohammed Mosihuzzaman, Nilufar Nahar, Mozaffar Hussain, Md. Fazal, Constituents of the essential oil of Aquilaria agalocha Roxb, Asian Symposium on Medicinal Plants, Spices and Other Natural Products (ASOMPS X), 2000; 7: 191. https://www.researchgate.net/, DOI: 10.13141/RG.2.2.16576.21129
9. Chakrabarty, K., A. Kumar and V. Menon Trade in Agarwood. In: Barden, A., A.A. Noorainie, T. Mulliken, and M. Song Heart of the matter: Agarwood use and trade and CITES implementation

for Aquilaria malaccensis. TRAFFIC International, 2000.
10. Kirtikar, K.R. and Basu, B.D., Indian Medicinal Plants, Bishen Sing and Mahendra Pal Sing, Dehra Dun, India, 1984; 2: 2171.
11. Ponglux, D, Wongseripipatana, S, Phadungcharoen, T., Ruangrungsri, N. and Likhitwitayawuid, K., Medicinal Plants, The First Princess Chulabhorn Science Congress, 1987; 33.
12. Dictionary of Natural Products on CD-ROM, Chapman & Hall Chemical Database, 1998.
13. Meyer, B.N, Ferrigni, N. R., Putnam, J.E., Jacobsen, L.B., Nicholas, D.E., McLauglin, J.L., Planta Med, 1982; 45: 31-34.
14. Maheshwari ML, Jain TC, Bates RB, Bhattacharyya SC. Terpenoids-xli: structure and absolute configuration of α-agarofuran, agarofuran and dihydroagarofuran. Tetrahedron, 1963; 19(6): 1079-1090.
15. Maheshwari ML, Varma KR, Bhattacharyya SC. TerpenoidsXLVII: structure and absolute configuration of norketoagarofuran, 4-hydroxydihydroagarofuran, 3,4-dihydroxydihydroagarofuran and conversion of β-agarofuran to α-agarofuran. Tetrahedron, 1963; 19(10): 1519-1525.
16. Jain TC., Battacharrya SC. Structure, stereochemistry and absolute configuration of agarol, a new sesquiterpene alcohol from agarwood oil. Tetrahedron Letters, 1959; 1(9): 13-17.
17. Naf R, Velluz A, Brauchli & Thommen W. Agarwood oil (Aquilaria agallocha Roxb.). Its composition and eight new valencane, eremophilane, vetispiranederivatives. Flav. FragJ, 1995; 10: 147-152.
18. Naf R, Velluz A, Busset N, Gaudin JM. New Norsesquiterpenoids with 10epiEudesmane Skeleton from Agarwood (Aquilaria agallocha Roxb.) Flav & Frag J, 1992; 7(6): 295-298.
19. Naf R, Velluz A, Thommen W, Brauchli R. New Compounds Identified in Agarwood (Aquilaria agallocha Roxb.). Flavour & Fragrance J, 1993; 8(6): 307-313.
20. Nagashima T, Kawasaki I, Yoshida T, Nakanishi T, Yoneda K, Miura I.New Sesquiterpenoids from agarwood. Paper IXth International essential oil congress. Singapore, 1983; 12-16.