Indian Journal of Natural Products and Resources
Vol. 5(1), March 2014, pp. 44-47

Analysis of different grades of Agarwood (*Aquilaria malaccensis* Lamk.) oil through GC-MS

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Received 1 February 2013; Accepted 26 June 2013

The study was carried to find out differences in the composition of Agarwood oil obtained from highly infected (Grade 1), moderately infected (Grade 2), less infected (Grade 3) and healthy wood (Grade 4) by using gas chromatography mass spectrometry analysis (GC-MS). Highly infected wood oil (G1) contains aromadendrene2 (24.76%), valencene2 (17.53%), + calarene (9.63%), 1(5), 6-Guaiadiene (8.76%), etc. Moderately infected wood oil (G2) contains Tau-cadinol (16.90%), valencene2 (1.73%), aromadendrene2 (1.73%), etc. Less infected wood oil (G3) contains 1-Methyl-1-caprolactone (39.10%), 7(Hydroxymethyl)-2-methoxy-xanthone (32.06%), aromadendrene2 (1.58%), valencene2 (1.58%), etc. Healthy wood oil (G4) contains 3-Carbethoxy-3-butenyl Crotonate (29.12%), Methyl 5, 5-dimethoxy-4, 4-dimethyl-3-hydroxy-2- vinylpentanoate (24.68%), etc. Since the the presence of aromadendrene and valencene plays an important role in grading of Agarwood oil, quantity of these two compounds in the above mentioned four grades of wood are in the following order G1>G2>G3 and absent in G4 grade wood oil.

Keywords: *Aquilaria malaccensis*, Agarwood, Aromatic oil, Aromadendrene, Valencene.

IPC code; Int. cl. (2013.01)−A61K 36/00.

Introduction

Agar a valuable aromatic oleoresin deposit found in the stem of *Aquilaria malaccensis* Lamk. (syn. *Aquilaria agallocha* Roxb., Family-Thymelaecae). It is a fast-growing tree which can be found growing from the foothills of the Himalayas to the rain forests of Papua New Guinea[6]. It is one of the 15 tree species in the Indo-Malaysian genus *Aquilaria*. It is a large evergreen tree, growing over 15-40 m tall and 0.6-2.5 m in diam and has white flowers[3, 10]. *A. malaccensis* and other species in the genus *Aquilaria* sometimes produce resin-impregnated heartwood. There are many names for this resinous wood, depending on the oleoresin content agar, agarwood, aloeswood, eaglewood, *gaharu* and *kalamabak*. This wood has high demand for medicine, incense and perfumes across Asia, Middle East and Europe[1]. First-grade agarwood is one of the most expensive natural raw materials in the world, with prices in consumer countries ranging from a few dollars per kg for low quality to more than US$30,000 per kg for top quality wood.

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The Agarwood in general has three major uses, viz. medicine, sedative, perfume and incense. Smaller quantities are used for carving purposes[9]. Agarwood has been a traditional medicinal source for Ayurvedic, Tibetan and East Asian Medicine. It is used for the treatment of pleurisy. Three new sesquiterpenic furanoids of the selinane group from agarwood oil, obtained from the fungus infected plant and their structures and an absolute configuration was determined by degradative studies and physical measurements[8]. Degradative and physical measurements supported by an unambiguous synthesis of the derived ketone have led to the assignment of a novel spiroskeleton to agarospirol, a sesquiterpene alcohol isolated from the essential oil of infected agarwood[14, 15]. *Aspergilus niger* is a dominant fungi which infects stem of Aquilaria. Other pathogenic fungus that found in the agarwood are *Fusarium*, *Penicillium*, and *Trichoderma* sp. Vesicular-arbuscular myccorhizal association in the tree species and changes in amino acid composition due to pathogenesis were also studied[11-13].

This investigation shows a marked difference in the oil compositions among the treatments with regards to their quality. So far the qualitative study of the different grade oils of eaglewood

not available. Hence, the present investigation was undertaken to study the qualitative differences in the four grades of oils obtained from infected and non-infected eaglewood.

Materials and Methods

Plant material

The wood samples of *A. malaccensis* were collected from Hojai, Assam during April 2012 and it was brought to Forest College and Research Institute, Mettupalayam, Tamil Nadu.

Extraction of essential oil

The oil was extracted from three different infected and non-infected wood samples. The oil was graded according to the intensity of the infection in the wood samples. The Grade G1 was assigned to the oil extracted from the highest infected wood followed by G2 (moderately infected), G3 (less infected) and G4 which was extracted from the healthy wood sample. All the four types of wood samples were crashed, dried and ground individually. The ground materials were soaked in distilled water for a time period of 14 days and filtered separately. The filtrate water mixtures were placed with Clevenger-type apparatus individually for isolation of oils by hydro distillation[4]. After 72 h oil was collected separately and dried over anhydrous sodium sulphate. The oils were then stored in sealed container under refrigeration prior to analysis.

GC-MS analysis

The four grades of essential oil in different types of woods from *A. malaccensis* were analyzed by GC-MS Thermo GC - Trace Ultra Ver: 5.0, Thermo MS DSQ II; DB 5 – MS, Capillary standard non - polar column (30 Mts, ID: 0.25 mm, FILM: 0.25 µm), column temperature / oven temp 80° C raised to 260° C AT 5° C /min; carrier gas, He, flow: 1.0 ML/Min. Injection volume 1 micro litre.

Identification of the compounds

Compound identification was done by comparing the NIST 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.

Results and Discussion

Seventeen (17) compounds in the highly infected (G1) agarwood oil were identified followed by sixteen (16) compounds in the moderately infected (G2), nine (9) compounds in the less infected (G3) and six (6) compounds in the healthy (G4) agarwood oil. Significant difference among the oils obtained from different categories of infected and non-infected wood samples for their quality was observed.

Highly infected wood oil (G1) contains aromadendrene2 (24.76 %), valencene2 (17.53 %), + calarene (9.63%), 1(5), 6-Guaiadiene (8.76%), Spathulenol (7.45 %), Peroxygibberol (5.90 %). Moderately infected plant wood oil (G2) contains Tau-cadinol (16.90%), Ethyl (E)-2-acetyl-2-methyl-4-phenylbut-3-enoate (12.10 %), 1-Butyl-4-pentylbenzene (11.01 %), valencene2 (1.73 %), aromadendrene2 (1.73 %). Less infected plant wood oil (G3) contains 1-Methyl-1-caprolactone (39.10 %), 7-(Hydroxymethyl)-2-methoxy-xanthone (32.06 %), 2, 3, 4, 12-Tetrahydrofuro [2, 3-b] naphtho [2, 3-b] pyrrolo-5,10-quinone (9.09 %), aromadendrene2 (1.58 %), valencene2 (1.58 %). Healthy plant wood oil (G4) contains 3-Carbethoxy-3-butenyl Crotonate (29.12 %), 3-Methyl-3-buten-1-yl 4-(4-methyl-4-pnten-1-yl) benzene-sulfonate (29.12%), Methyl 5, 5-dimethoxy-4, 4-dimethyl-3-hydroxy-2- vinylpentanoate (24.68 %) (Table 1).

The oils obtained from different categories of infected plants showed almost unique distribution of the components. Major volatiles detected in this study were consistent with those of previously published studies in which volatile components were isolated by various organic solvent extractions[5,7].

But some of the compounds like aromadendrene and valencene were commonly found in the different categories of infected wood oils. The G1 (Highly infected) contains aromadendrene 2 (24.76 %) and valencene2 (17.53 %), G2 (Moderately infected) contains valencene2 (1.73 %) and aromadendrene 2 (1.73 %), G3 (Less infected) contains aromadendrene2 (1.58 %) and valencene 2 (1.58 %). But these two compounds were totally absent in the wood oil from healthy plants (G4) (Plate 1) (Table 1). The compounds found in *A. malaccensis* were also found in the species Thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.) as major compounds[2, 16 & 17].

Table 1—Constituents of different grades of Agarwood oil

Highly infected Agarwood (G1)			Moderately infected Agarwood (G2)			Less infected Agarwood (G3)			Healthy Agarwood (G4)		
RT (min)	Components	%	RT (min)	Components	%	RT (min)	Components	%	RT (min)	Components	%
30.86	Aromadendrene 2	24.76	3.05	Methyl-î-caprolactone	39.10	3.05	Methyl-î-caprolactone	39.10	39.09	3-Carbethoxy-3-butenyl Crotonate	29.12
30.86	Valencene 2	17.53	38.96	7-(Hydroxymethyl)-2-methoxy-xanthone	32.06	38.96	7-(Hydroxymethyl)-2-methoxy-xanthone	32.06	39.09	3-Methyl-3-buten-1-yl 4-(4-methyl-4-pnten-1-yl) benzenesulfonate	29.12
30.47	Calarene	9.63	35.43	2,3,4,12-Tetrahydrofuro[2,3-b]naphtho[2,3-b]pyrrolo-5,10-quinone	9.09	35.43	2,3,4,12-Tetrahydrofuro[2,3-b] naphtho[2,3-b]pyrrolo-5,10-quinone	9.09	3.05	Methyl 5,5-dimethoxy-4, 4-dimethyl-3-hydroxy-2-vinylpentanoate	24.68
30.25	1(5),6-Guaiadiene	8.76	38.10	1-Deutero-2-allyloctanol	7.26	38.10	1-Deutero-2-allyloctanol	7.26	36.24	1-Butyl-2-(1-hexynyl)-3, 4-dimethylbenzene	7.34
25.10	Spathulenol	7.45	42.54	(Z)-9-Docosene-1,22-diol	3.57	42.54	(Z)-9-Docosene-1, 22-diol	3.57	35.56	(Z)-6-methyl-1-trimethylsilyloxy-1-heptene	6.27
27.29	Peroxygibberol	5.90	40.67	11-Hexadecen-1-ol	3.40	40.67	11-Hexadecen-1-ol	3.40	32.57	Ethyl 1-Methyl-4-phenylpyrrole-3-carboxylate	3.47
31.14	Eremophilone	3.51	30.33	2,3,5-Trimethylenehexane	2.36	30.33	2,3,5-Trimethylenehexane	2.36			
			25.81	Aromadendrene 2	1.58	25.81	Aromadendrene 2	1.58			
31.14	Quindoline	3.51	25.81	Valencene 2	1.58	25.81	Valencene 2	1.58			
31.64	trans-1,2,3,4,4a,5,6,7-Octahydro-à,à,4a-trimethyl-2-naphthalenemethanol	3.50									
29.91	Octahydronaphthalene	3.46									
32.78	Aristol-9-en-12á-ol	3.32									
33.19	Lepidozenol	1.62									
28.58	(7R,10R)-carota-1, 4-dien-14-ol	1.43									
28.58	Santalal	1.43									
32.31	Benzene, (1-Pentylheptyl)	1.43									
24.48	à-Helmiscapene	1.38									
33.39	1-Ethyldecylbenzene	1.38									

RT - Retention Time, % - Percentage

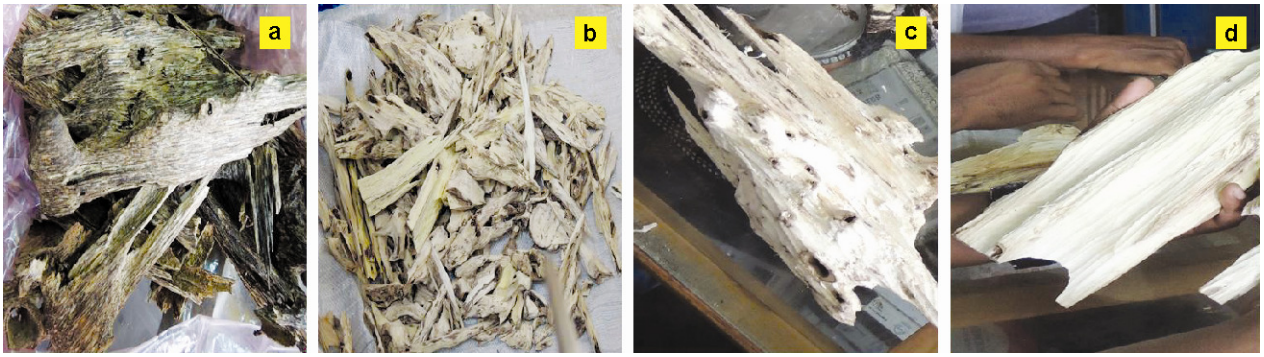

Plate 1− Different grades of Agarwood collected for GCMS analysis: a. Highly infected wood (G1); b. Moderately infected wood (G 2); c. Less infected wood (G3); d. Non-infected wood (G4)

Conclusion

Analysis of the different grades of *Aquilaria malaccensis* (Agarwood) essential oil through Gas Chromatography Mass Spectrometry (GC-MS) indicated that the presence of aromadendrene and valencene plays an important role in grading of Agarwood oils. Further studies can be elaborated to find out the role of above mentioned compounds in the development of aroma and quality of the Agarwood oils as well as role of the fungus infecting these trees.

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