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Original Article

GAS CHROMATOGRAPHY- MASS SPECTROMETRIC ANALYSIS OF THE ESSENTIAL OIL OF EAGLEWOOD (*AQUILARIA AGALLOCHA* ROXB)

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ABSTRACT

Objective: The investigation was carried out to study the phytochemical constituents of agar oil of *Aquilaria agallocha* grown in Assam.

Methods: Fungal infected agar laden wooden chips grown in Assam were soaked in water for 6 to 7 days and grounded into smaller pieces and hydro distilled to obtain the oil. 2 µl of the oil sample diluted in methanol was used for GC/MS analysis to study the chemical constituents.

Results: GC-MS analysis of the oil has shown the presence of at least 35 different compounds, out of which 17 compounds were identified. Three furanoids viz. 3 methyl-2-(2 methyl-2-butenyl)-furan; 2-isobuteyl-3 methyl furan and 3-methyl-2-(2-oxopropyl)furan were identified as the main aromatic components of the oil. Besides these some acids, ketones alcohol and aldehydes were also identified and reported here.

Conclusion: Variation in the quality of the oil has been observed depending upon geographical region on which the trees are grown, age of the plant and extent of disease lesions formed in the wood, which was evident by studying the phytochemical constituents of the oil.

Keywords: GCMS, Eaglewood, Essential oil.

INTRODUCTION

Aquilaria agallocha Roxb., is an evergreen tree of medium size, which belongs to the family Thymeliaceae. In India it is confined to the hills of the North -eastern states and in Assam, it is mainly distributed it the upper Assam Districts of the Brahmaputra valley and certain areas of the Barak valley. It is also distributed in several Southern and Southeast Asian countries like Bhutan, Bangladesh, Thailand, Burma, Malaysia, Indonesia and Vietnam.

The tree is of great commercial and economical importance due to several products derived from it, the most important of all being the aromatic oil –the agar oil that accumulates in its infected wood. Eaglewood or agarwood oil is an excellent perfume retainer and is highly prized by the European perfumers. It finds wide application in high grade perfumes as fixatives for mixing with scents. Agar laden wood chips are also widely marketed. The essential oil could be obtained through hydro-distillation of the fungal infected wood.

Agar oil, which is produced as a result of fungal infection, is one of the highly prized essential oil in the middle-east countries. It is believed that only the diseased and mature agar trees yield best grade of agar oil. Even the non-infected young trees of more than six years old yield low quality essential oil with high content of fatty acids, which also finds application in perfumery and cosmetics products in India. The oil also has several medicinal values. It has been used as stimulant cardiac tonic, carminatives and beneficial for skin diseases. It is used as anti-asthmatic, aphrodisiac, astringent and effective against diarrhea, dysentery, gout, rheumatism and paralysis. It also acts as a preventive against microbes, fleas and lice. The wood is used for making fumigators, pastilles and agarbatties [1]. The secret of agar formation in the wood is still not clear. Some believe that the oil is the secondary metabolite of the plant, while others believe that it to be a fungal metabolite or a product induced as a result of infection by fungus.

Chemically agar oil is rich in sesquiterpene hydrocarbons. Many workers have isolated and characterized several chemical compounds of the oil [2,3,4]. It has been seen that the chemical components of the oil vary depending upon the region on which the trees are grown, age of the plant and extent of disease lesions formed in the wood. Therefore there is lot of variations in the quality of agar oil originating from different countries as well as oil extracted from different agar plant materials. Considering the importance of the oil in perfumery and medicine and also very limited work done on the nature of the components of the oil, the present study was considered necessary.

MATERIALS AND METHODS

Collection of plant material

Fungal infected agar laden wooden chips were collected from Hozai a small township in middle Assam which is considered a hub for agar oil cottage industry.

Extraction of the oil

The oil rich chips were soaked in water for 6 to 7 days and grounded into smaller pieces. These pieces were hydro distilled in a Clevenger type distillation plant. The oil so obtained was dried in an oven at 40°- 45° C for 2-3 hours to remove traces of moisture present in the oil.

GC/MS analysis

The oil so obtained was analyzed by GC/MS to study the presence of different components, by using Fisons MD 800 GCMS model. The oil was diluted in HPLC grade methanol and 2 µl of the solution was injected for the analysis.

Operating conditions of the GC/MS

The operating condition of the GC/MS are as follow. Oven Temperature was programmed at 100 º C for 1 minute to 200 ºC @ 10 º C/min, held for 5 minutes, then increased to 250 º C at the rate of 15ºC/ minutes and held for 8 minutes. Temperature maintenance were as follow- Injection temperature was 200 ºC, interface temperature 250 °C and source temperature 200 ºC. Carrier gas used was Helium with flow Rate of 1 ml / minute and split ratio 1: 25. The Column used in the system was fused silica capillary column cross linked with 5% Phenyl-Methyl Silicone, (25 mt × 0.20 mm internal diameter × 0.33 µm film thickness, HP-ultra 2) and ionization source was Electron impact ionization(EI) at 70 ev.

Identification of the compounds

Identification of the compounds were done by studying the peaks obtained in the total ion current chromatogram, separately from the mass fragmentation pattern and then comparing the same with the standard library present in the data station.

RESULTS

GC/MS analysis of the chemical constituents present in the essential oil obtained by hydro- distillation of fungal infected agarwood showed the presence of nearly thirty-five different compounds, out of which only 17 compounds could be identified. The main group of compounds was found to be the furanoid monoterpenoids, methyl esters of higher fatty acids, fatty acids, ketones, aldehydes and alcohols. The list of compounds identified were Cyclopentadecanol; Decanoic acid; 4-(4-methoxyphenyl)-2-butanone; Pentadecanoic acid; 3-mehtyl-2- (2-methyl-2-butenyl)-furan; Pentadecanoic acid-15 Bromo; 2-isobutyl-3methyl furan; Oleic acid; 3-methyl-2-(2-oxopropyl)furan; Tetradecanoic acid; Hexadecanoic acid,14 methyl, methyl ester; Heptatriactontadien-2-one; 4-hydroxy-3,5-dimethoxybenzyldehyde; 4 phenyl-2-butatone; (S)-4a-methyl-2-(1-methylethyl)-3,4,9,5,6,7-hexahydro-naphthalene; 4-hydroxy-3,5-dimethoxybenzyldehyde. (Table 1). The furano-monoterpenoids, comprised to be the major aromatic compounds of the oil. Among the acids viz. decanoic acid, pentadecanoic acid, oleic acid and tetradecanoic acid, oleic acid was found to be very prominent. Compounds with alcoholic and aldehyde group were also found to be present in the oil.

DISCUSSION

GC/MS studies of the constituents of the essential oil have been reported in several plant species. Skaltsa *et al* [5], reported the presence of monoterpenes and sesquiterpene hydrocarbons in the essential oil obtained by steam distillation from several *Xeranthemum* species which was analyzed by GC/MS. Other reports involving GC/MS studies of the phytochemical of essential oil has been reported in *Achillea tenuifolia* Lam [6], *Calamintha nepeta* [7], *Salvia atropatana* Bunge [8], *Dittrichia graveolents* L. [9] *Valeriana wallichii* DC. [10], *Pinus sylvestris* L. [11] and *Eucalyptus spp*. [12]. Studies on the constituents of the agar oil have been done by several workers in the past. A GC/MS study of the components from three different varieties of agar oil from Bangladesh was reported by Bhuyan *et al* [13]. Masakazu *et al* [14], working with agar oil sample from Vietnam, reported the identification of three new sesquiterpenes i.e. (-)- guaia-1(10),11-dien-15-al; (-)- selina-3,11-dien-9-one and (+)- selina-3,11-dien-9-ol by GC and GC/MS analysis of the wood extracted with acetone and diethyl ether. Some studies of the oil regarding compound isolation and its structural elucidation by IR and absolute configuration determined by degradative studies and physical measurements have been reported by Maheswari *et al* [15].

They have isolated three furanoid sesquiterpenes- α-agarofuran., ß-agarofuran and dihydroagarofuran of the selinoic group in small quantities. Besides these, three more furanoids of the selinane group consisting of Nor-ketoagarofuran, 4-Hydroxydihydroagarofuran and 3,4-Dihydroxydihydroagarofuran have also been isolated and characterized by Maheswari *et al* [4].

Study by Maheswari *et al* has considerable corroboration with our studies with GC/MS, where we have reported several compound with furano-monoterpenoids group. Cyclopentadecanol, an alcholic constituent was identified in our oil sample whereas investigation of the alcoholic constituents by NMR and spectroscopic studies by Verma *et al* [16] has led to the isolation of a monoetenoid bicyclic sesquiterpene alcohol as a major component which was named as agarospirol. Similarly solvent extracted essential oil of the fungal infected plant led to the identification of a monoethynoid eudalenic primary alcohol named agarol, which has been isolated in pure form by batch strip fractionation and column chromatography [17].

It appears that the agar oil samples obtained from different regions vary considerably in terms of their constituents class of compounds, although there broad functional group in conjugation with other functional groups were found to be same. Hence this may results in distinct variation in fragrance characteristics of different oil samples obtained from different geographical locations.

Table 1: Identified compounds from the agar oil extracted from fungal infected agarwood by GC/MS

S. No.	Identified Compounds	Retention Time
1	Cyclopentadecanol	7.421
2	Decanoic acid	8.839
3	4-(4-methoxyphenyl)-2-butanone	8.914
4	Pentadecanoic acid	9.139
5	3-mehtyl-2- (2-methyl-2-butenyl)-furan	10.190
6	Pentadecanoic acid-15 Bromo	10.715
7	2-isobutyl-3methyl furan	10.940
8	Oleic acid	11.107
9	3-methyl-2-(2-oxopropyl)furan	11.307
10	Tetradecanoic acid	11.723
11	Hexadecanoic acid,14 methyl, methyl ester	13.675
12	Heptatriactontadien-2-one	13.501
13	4-hydroxy-3,5-dimethoxybenzyldehyde	13.767
14	4 phenyl-2-butatone	11.857
15	(S)-4a-methyl-2-(1-methylethyl)-3,4,9,5,6,7 -hexahydro-naphthalene	12.808
16	4-hydroxy-3,5-dimethoxybenzyldehyde	15.360
17	Bis (2-ethylhexyl) phythalate	15.410

CONCLUSION

Agar oil is considered as a liquid gold because of its high demand in the international market. It has been seen that the chemical components of the oil vary depending upon the region on which the trees are grown, age of the plant and extent of disease lesions formed in the wood.

It has been concluded that some variation in the quality of the oil from North east India is seen compared to the oil obtained from the trees grown in other geographical locations of the world.

CONFLICT OF INTERESTS

Declared None

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