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Composition and anti-microbial activity of essential oil of *Myristica fragrans* from Andaman Nicobar Island

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Abstract

The essential oil obtained by hydro-distillation of the fruits oil of *Myristica fragrans* Houtt (Nutmeg) collected from Andaman Nicobar Island the oil was analyzed by GC and GC-MS. Twenty eight compounds were identified, accounting for 92.9% of the contents. The major constituents were α-pinene (9.4%), Sabinene (41.7), β-pinene (7.3%), myrcene (2.5%), Limonene (3.7%), Terpine-4-ol (5.8 %), Safrole (1.4%) and Myristicin (2.7 %). This is the first report of the essential oil constituents of *M. fragrans* Houtt from Andaman Nicobar Island. The micro-biological activity of the isolated essential oil was investigated as well. It was found that the essential oil shows micro-biological activity except with *Pseudomonas aeruginosa* and *Candida albicans*.

Key-Words: *Myristica fragrans*, Essential oil, Anti-microbial activity Andaman Nicobar Island

Introduction

M. fragrans Houtt. (Fam. Myristicaceae) is a native of Moluccas, indigenous to India, Indonesia and Sri Lanka now cultivated in many tropical countries of both hemispheres. In India, it is grown in Madras state (Nilgiris, Coimbatore, Salem, Ramanathapuram, Tirunelveli, Kanyakumari and Madurai districts). Few trees are found in various localities in Kerala, Assam and other States. It is used as a remedy for stomach ache, rheumatism and vomiting in pregnancy[1-4]

Earlier studies reported the presence of major components Sabinene, myristin, safrole, elemicin ,α-pinene, β-pinene, sabinene[5-9] The presence of two compounds, myristicin and elemicin, is related to the hallucinogenic action of nutmeg while safrole has been suspected to be carcinogenic[10-12]. Myristicin is toxic effects against the pest[13]. Whereas, sabinene imparts sweetness to the products[14].

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Material and Methods

Plant material

The fruits of *M. fragrans* were collected in May 2006 from Andaman Nicobar Island. The plant was identified by Enthanobotany Section, National Botanical Research Institute, Lucknow, India. A voucher specimen (LMG-84365) was deposited at the Institute herbarium.

Isolation of volatile components

The plant material was chopped and subjected to hydro-distillation for 4 hours using Clevenger type apparatus. The oil obtained, in yield of 8.25% on dry weight basis. The oils were dried over anhydrous sodium sulphate. After dried, the oil was submitted to gas chromatographic analysis.

Gas-Liquid Chromatography (GC)

GC was performed on a Thermo Fisher TRACE GC ULTRA, under the following conditions: carrier gas, helium; injector temperature, 220^{0}C and 225^{0}C, respectively using a fused silica TR 50MS capillary column (30m x 0.25 mm, film thickness 0.25µm). Oven temperature was programmed, 5min. at 70^{0}C and rising 120^{0}C at 2^{0}C/min. and finally 240 ^{0}C at 3^{0}C /min.

GasChromatography-Mass Spectrometry (GC-MS)

GC-MS was recorded on a Thermo Fisher TRACE GC ULTRA coupled with DSQ II Mass Spectrometer instrument using a TR 50MS column (60m x 0.25mm ID, film thickness 0.25 µm,70^{0}C (2 min) and then heated at a rate of 3^{0}C/ min to 250^{0}C. The carrier gas was helium at a flow rate of 1 mL min–1, split ratio

1:50; ionization energy 70 eV, and acquisition mass range *m/z* 50–550.

Anti-microbial activity

The anti-microbial activity of the essential oil of *M. fragrans* was tested in vitro using the method of diffusion on disc[15] with the following microorganisms:

Botrytis cinerea	+
Staphylococcus aureus 3476	+
Penicillium frequentance	+
Pseudomonas aeruginosa	-
Sarcina lutea 6589	+
Aspergillus niger	+
Candida albicans	-
Bacillus subtilis 763	+

It was found that the essential oil of *M. fragrans* shows micro-biological activity with tested microorganisms, except with *Pseudomonas aeruginosa* and *Candida albicans*.

Identification of the components

The identity of the components was assigned by comparing their GC retention time with those of literature values, as well as with components of other essential oils which had been identified earlier, and confirmed by comparison of the fragmentation patterns of the mass spectra with those reported in literature[16-19] and the library established by us.

Results and Conclusion

In continuous our research work on the screening of aromatic flora for new sources of essential oils for perfumery and biological active compounds, the nutmeg of *M. fragrans* yielded oil (8.25% on a dry weight basis) on hydrodistillation. The oil was examined by GC and GC-MS. Twenty eight compounds were identified representing 92.9% of the oil shown in Table -1. The major constituents of the oil were α-pinene (9.4%), Sabinene (41.7%), β-pinene (7.3%), myrcene (2.7%), Limonene (3.7%), Terpine-4-ol (5.8%), Safrole (1.4%) and Myristicin (2.7%). The Major compositions of Indian *M. fragrans* nutmeg oil are different from the other fruits of different locations of India, Brazil, Indonesia and Kerala in south Indian[5-10].The fruits from Andaman Nicobar Island are found with high sabinene and low concentration of myristicin and elemicin. From the above facts it is evident that the quality of the essential oil from Andman Nicobar Island is competitive with the high sabinene and low concentration of myristicin contents than that produced in other parts of the country. The essential oil also showed the antimicrobial activity.

Based on the above study, it may be summarized that the low levels of myristicin, elemicin and safole coupled with high sabinene content in the essential oil of Andman Nicobar Island can be considered for future exploitation for food and pharmaceutical applications. The micro-biological activity of the essential oil of *M. fragrans* was tested using the method of diffusion on disc. As expected, the isolated essential oil shows microbiological activity with the tested microorganisms could be a useful starting point for the investigation of the course of biosynthesis of some components of the essential oil.

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Table 1. Composition of the essential oil of *Myristica fragrans* Houtt. (Nutmeg)

S/No.	No. of Compound	RI	GC Area (%)
1.	α-Thujene	930	2.8
2.	α- Pinene	938	9.4
3.	Camphene	953	0.5
4.	Sabinene	977	41.7
5.	β - Pinene	980	7.3
6.	Myrcene	990	2.5
7.	Phellanadrene	1006	1.5
8.	3-Carene	1012	0.6
9.	Terpinene	1018	1.6
10.	p-Cymene	1025	1.2
11.	Limonene	1033	3.7
12.	γ-Terpinene	1062	2.9
13.	Terpinolene	1089	1.3
14.	Linalool	1098	0.6
15.	cis-p-Menth-2-en-1-ol	1123	0.5
16.	β-Terpineole	1145	0.5
17.	Terpine-4-ol	1183	5.8
18.	α-Terpineol	1195	0.9
19.	Safrole	1287	1.4
20.	α-Cubebene	1352	0.1
21.	α-Terpinyl acetate	1354	0.1
22.	Citronellyl acetate	1357	0.3
23.	Eugenol	1360	0.5
24.	Geranyl acetate	1385	0.1
25.	Methyl eugenol	1415	1.5
26.	Myristicin	1521	2.7
27.	Vanillin acetate	1525	0.1
28.	Elemicin	1555	0.8
	Total		**92.9**

Elution order on TR 50 MS capillary column.