Journal of Spices and Aromatic Crops
Vol. 17 (1) : 10–15 (2008)

Indian Society for Spices

Chemical composition of leaf oils of *Myristica beddomeii* (King), *Myristica fragrans* (Houtt.) and *Myristica malabarica* (Lamk.)

T John Zachariah, N K Leela, K M Maya, J Rema, P A Mathew, T M Vipin & B Krishnamoorthy

Indian Institute of Spices Research
Post Box No. 1701, Marikunnu P.O., Calicut–673 012, Kerala, India
E-mail: john@spices.res.in

Received 4 January 2007; Revised 8 October 2007; Accepted 16 December 2007

Abstract

Essential oil constituents of leaves of three *Myristica* species namely, *Myristica beddomeii, M. fragrans* and *M. malabarica* were determined by gas chromatography and gas chromatography-mass spectrometry. *M. fragrans* was dominated by monoterpenes (91%), *M. beddomeii* contained mono- (48%) and sesquiterpenes(35%) whereas *M. malabarica* was dominated by sesquiterpenes (73%). The leaf oil of *M. beddomeii* was dominated by α-pinene (19.59%), t-caryophyllene (14.63%) and β-pinene (12.46%). The leaf oil of *M. fragrans* contained sabinene (19.07%), α-pinene (18.04%), 4-terpineol (11.83%), limonene (8.32%) and β-pinene (7.92%) as major compounds, while t-caryophyllene (20.15%), α-humulene (10.17%), nerolidol (9.25%) and δ-cadinene (6.72%) were predominant in the oil of *M. malabarica*. Linalool, α-terpineol, t-caryophyllene, β-elemene and γ-elemene were present in all the three species. This is the first report on the essential oil composition of *M. beddomeii* leaves.

Keywords: leaf essential oil, *Myristica beddomeii, M. fragrans, M. malabarica.*

Introduction

The genus *Myristica* (Myristicaceae), comprising of 72 species, is distributed from India and South East Asia to North Australia and the Pacific Islands. *M. fragrans* (Houtt.) is the commercially important species, which yields two distinct spice products, nutmeg and mace. The volatile oils of nutmeg and mace have been widely studied (Lawrence 1997, 2000, 2005; Mallavarapu & Ramesh 1998). The major constituents of oils of both nutmeg and mace are monoterpene hydrocarbons, together with smaller amounts of oxygenated monoterpenes and aromatic ethers (Purseglove *et al.* 1981). Among monoterpene hydrocarbons, pinene and sabinene dominate in the oil and the major aromatic ether constituent is myristicin. The aromatic ethers, myristicin, safrole and elemicin determine the flavour and medicinal properties of nutmeg to a great extent. *M. beddomeii* (King) and *M. malabarica* (Lamk.) are two species occurring in the evergreen forests of Western Ghats in India. The nut and mace of *M. malabarica* are known as Bombay nutmeg and Bombay mace respectively, and are used to adulterate *M. fragrans* products (Gamble 1967; Hooker 1973). Phytochemical studies have revealed the occurrence of several terpenes, flavones and diarylnonaoids in *M. malabarica* (Purushothaman *et al.* 1997; Talukdar *et al.* 2000; Sabulal *et al.* 2007). There is no report on the chemistry of *M. beddomeii,* till date. This prompted us to investigate the essential oil composition of *M. beddomeii* leaf which is reported here and is compared with that of *M. fragrans* and *M. malabarica*.

Materials and methods

Leaves of *M. beddomeii, M. fragrans* and *M. malabarica* (250 g each), were collected from Indian Institute of Spices Research, Experimental Farm, Peruvannamuzhi (Kerala). Fresh leaves were cut into small pieces and hydro distilled using a Clevenger trap to yield the essential oil (AOAC 1975). The separated oil was quantified and traces of moisture were removed using anhydrous sodium sulphate. The constituents of the leaf oil were analysed using gas chromatograph-flame ionization detector (GC-FID) and gas chromatograph-mass spectrometer (GC-MS).

Gas chromatography

GC-FID analysis of the leaf oil was conducted on a Perkin-Elmer Autosystem gas chromatograph equipped with FID, PE-Nelson 1022 GC plus integrator and SE-30 column. Oven temperature was programmed from 70°C to 210°C at the rate of 5°C/min. FID temperature and injection port temperature were maintained at 300°C.

Gas chromatography-mass spectrometry

GC-MS analysis of the oil was carried out using a Shimadzu GC-2010 gas chromatograph equipped with QP 2010 mass spectrometer. RTX-5 column (30 m x 0.25 mm, film thickness 0.25 ìm) was used. Helium was used as the carrier gas at a flow rate of 1.67 ml min^{-1}. The injection port was maintained at 250°C; the detector temperature was 220°C. Oven temperature was programmed as follows: at 60°C for 5 min, 60°C to 110°C @ 5°C/min, 110°C to 200°C @ 3°C/min and up to 220°C @ 5°C/min, at which the column was maintained for 5 min. The split ratio was 1:40 and ionization energy 70eV. The retention indices were calculated relative to C_8–C_{20} n-alkanes. The constituents of the oil were identified by comparison of retention indices with those reported in literature, by matching the mass spectral data with those stored in NIST and Wiley libraries and the published data, and wherever possible, by co-injection of authentic standards (Adams 1989; Gianni *et al*. 2005; Quijano *et al*. 2007; Rout *et al*. 2007; Tzakou *et al.* 2007).

Results and discussion

The leaves of *M. beddomeii, M. fragrans* and *M. malabarica* yielded 0.13%, 1.20% and 0.05% oil, respectively. The essential oil composition of the three oils is indicated in Table 1. In the essential oil of *M. beddomeii* leaf, 42 constituents representing ~88% of the oil were identified of which monoterpenes (~48%) predominated. α-Pinene (19.59%) and β-pinene (12.46%) accounted for ~32% of the oil (Table 1). The oil contained ~35% sesquiterpenes among which t-caryophyllene (14.63%) was the predominant component. The oil contained α-humulene (5.00%), β-myrcene (3.25%), limonene (3.28%), α-copaene (2.77%), t-α-bergamotene (2.27%) and caryophyllene oxide (3.84%) as minor components. Caryophyllene oxide was the only oxide present in the oil. The compounds cis-α-bergamotene, α-gurjunene, germacrene-D, β-bisabolene, caryophyllene oxide, (2E, 2E)-farnesol and benzyl benzoate were present only in the oil of *M. beddomeii. M. beddomeii* oil contained ~2.5% straight chain compounds. The oil contained higher level of hydrocarbons compared to oxygenated compounds. This is the first report on the essential oil composition of *M. beddomei* leaf oil.

In *M. fragrans* leaf oil, 41 constituents contributing to ~94% of the oil were identified of which, monoterpenes (~91%) predominated. The oil contained the following major compounds: α-pinene (18.04%), sabinene (19.07%), 4-terpineol (11.83%), limonene (8.32%) and β-pinene (7.92%), which contributed to ~66% of the oil. Minor compounds included β-myrcene, α-phellandrene, δ-3-carene, α-terpinene, α-terpinolene, α-terpineol, t-2-menthen-1-ol and myristicin. Madhavan *et al*. (1991) also reported similar composition of leaf oil of *M. fragrans*. Zachariah *et al*. (2000) have reported 3%–11% myristicin and 0.3%–7.0% elemicin in the leaf oil of *M. fragrans*. Steam-distilled leaf essential

Table 1. Leaf oil constituents of *Myristica* spp.

Compound	RRI	Lit. RI	*M. beddomeii* (%)	*M. fragrans* (%)	*M. malabarica* (%)	Method of identification
Hexanal*	801	-	0.32	-	-	MS
2-Hexenal*	845	-	0.47	0.11	0.66	MS
4-Hexenol*	848	-	-	-	0.44	MS
t-2-Hexen-1-ol*	849	-	-	0.02	-	MS
1-Hexanol	860	871	1.8	0.03	0.85	RI, MS
α-Thujene	927	930	-	0.61	-	RI, MS
α-Pinene	936	935	19.59	18.04	-	RI, MS, CI
Camphene	948	954	0.54	0.62	-	RI, MS
Sabinene	982	975	-	19.07	-	RI, MS, CI
β-Pinene	985	979	12.46	7.92	-	RI, MS
β-Myrcene	993	991	3.25	3.72	-	RI, MS, CI
α-Phellandrene	1008	1003	-	2.14	-	RI, MS
δ-3-Carene	1014	1008	-	3.54	-	RI, MS
α-Terpinene	1018	1017	0.16	3.61	-	RI, MS
p-Cymene	1026	1025	0.73	0.17	-	RI, MS
Limonene	1031	1029	3.28	8.32	-	RI, MS, CI
1,8-Cineol	1033	1032	0.42	-	-	RI, MS, CI
β-cis-Ocimene	1041	1037	0.26	0.03	-	RI, MS
β-t-Ocimene	1050	1050	0.49	0.22	-	RI, MS
α-Terpinene	1060	1060	0.35	-	-	RI, MS
cis-Sabinene hydrate	1070	1070	-	0.09	-	RI, MS
Terpinolene	1090	1089	0.49	4.13	-	RI, MS
Linalool	1101	1097	0.34	0.96	0.12	RI, MS, CI
cis-2-Menthen-1-ol	1125	1122	-	1.12	-	RI, MS
t-2-Menthen-1-ol	1143	1145	-	0.45	-	RI, MS
4-Terpineol	1185	1177	0.25	11.83	-	RI, MS, CI
α-Terpineol	1194	1189	1.35	1.93	0.25	RI, MS, CI
cis-Piperitol	1201	1196	-	0.16	-	RI, MS
t-Piperitol	1211	1208	-	0.27	-	RI, MS
β-Citronellol	1226	1226	0.16	-	-	RI, MS
t-Geraniol	1254	1249	-	-	0.16	RI, MS,CI
Bornyl acetate	1286	1267	-	0.23	-	RI, MS
Safrole	1289	1287	-	0.11	-	RI, MS
α-Cubebene	1350	1351	0.22	-	0.31	RI, MS
α-Terpenyl acetate	1351	1350	-	0.23	-	RI, MS,CI
α-Neryl acetate	1365	1362	0.43	0.03	-	RI, MS
α-Copaene	1378	1377	2.77	0.26	4.28	RI, MS

Continued next page

Table 1 continue

Compound	RRI	Lit. RI	*M. beddomeii* (%)	*M. fragrans* (%)	*M. malabarica* (%)	Method of identification
Damascenone	1389	1385	-	-	1.3	RI, MS
α-Elemene	1395	1391	1.28	0.01	0.98	RI, MS
α-Gurjunene	1403	1410	1.24	-	-	RI, MS
cis-α-Bergamotene	1421	1413	1.84	-	-	RI, MS
t-Caryophyllene	1428	1419	14.63	0.08	20.15	RI, MS, CI
α-Ionone*	1433	-	-	-	1.55	MS
t-α-Bergamotene	1440	1435	2.27	-	0.14	RI, MS
Aromadendrene	1441	1441	0.45	-	0.53	RI, MS
t-Isoeugenol	1457	-	-	0.59	-	MS
α-Humulene	1460	1455	5.00	-	10.17	RI, MS
Epibicyclosesquiphe-llandrene*	1476	-	-	-	0.32	MS
α-Muurolene	1482	1480	-	-	1.11	RI, MS
Germacrene-D	1489	1485	0.75	-	-	RI, MS
Bicyclogermacrene	1503	1500	1.08	0.13	1.63	RI, MS
α-Muurolene	1505	1500	-	-	1.27	RI, MS
(E,E)-α-Farnesene	1511	1506	-	0.28	-	RI, MS
β-Bisabolene	1512	1506	1.06	-	-	RI, MS
cis-Caryophyllene	1512	1510	-	-	1.04	RI, MS
α-Cadinene	1519	1514	-	-	0.86	RI, MS
Myristicin	1526	1519	-	1.55	-	RI, MS, CI
δ-Cadinene	1530	1523	-	-	6.72	RI, MS
4,10-Dimethyl-7-isopropyl bicyclo (4,4,0)-1,4-decadiene	1537	1535	-	-	0.41	RI, MS
α-Calacorene	1548	1546	-	-	0.42	RI, MS
Elemol	1553	1547	-	0.44	0.20	RI, MS
Nerolidol	1569	1563	0.87	-	9.25	RI, MS, CI
Spathulenol	1586	1578	0.48	-	0.71	RI, MS
Globulol	1591	1583	-	-	2.58	RI, MS
Caryophyllene oxide	1591	1583	3.84	-	-	RI, MS
Guaiol	1603	1595	-	0.43	-	RI, MS
α-Eudesmol	1637	1629	-	0.14	-	RI, MS
Agaruspirol*	1639	-	-	-	0.54	MS
α-Muurolol	1649	1646	-	-	1.92	RI, MS

Continued next page

Table 1 continue

Compound	RRI	Lit. RI	*M. beddomeii* (%)	*M. fragrans* (%)	*M. malabarica* (%)	Method of identification
γ-Cadinol	1654	1654	0.41	-	1.13	RI, MS
β-Eudesmol	1656	1649	0.22	-	0.28	RI, MS
α-Cadinol	1663	1654	-	-	2.31	RI, MS
Bulnesol*	1673	-	-	0.17	-	MS
α-Bisabolol	1700	1686	0.33	-	0.28	RI, MS
(2E, 2E)-Farnesol	1725	1725	0.44	-	-	RI, MS
Benzyl benzoate	1770	1760	0.71	-	-	RI, MS, CI
Hexahydrofarnesyl acetone*	1874	-	-	-	0.22	RI, MS
Phytol	1948	1949	-	-	0.66	RI, MS
Hexadecanoic acid	1978	2010	-	-	4.34	RI, MS
Kaurene*	-	-	-	0.19	-	MS
Neophytadiene*	-	-	0.37	-	3.50	MS
Oleic acid*	-	-	-	-	0.42	MS
Total			87.40	93.98	84.01	

RRI=Relative retention indices; Lit. RI= Literature values of retention indices ; MS=Mass spectrum, CI=Co-injection;
* Tentatively identified based on mass spectrum

oil from Indonesia contained 80% α-pinene and 10% myristicin (Varghese 2001).

In *M. malabarica* leaf oil, 39 constituents contributing to ~84% of the oil were identified. The oil was predominated by sesquiterpenes (~73%). The oil contained ~0.5% monoterpene alcohols, ~2% straight chain compounds and ~8.0% miscellaneous compounds also. The leaf oil contained t-caryophyllene (20.15%) and α-humulene (10.17%) as major components. Nerolidol (9.25%), δ-cadinene (6.72%), α-copaene (4.28%), β-cubebene (3.29%) and epiglobulol (2.58%) were minor constituents. Sabulal *et al*. (2007) reported that *M. malabarica* leaf oil from South Kerala was predominated by t-caryophyllene (27.3%), α-humulene (13.8%), α-copaene (11.5%) and δ-cadinene (5.4%). Their oil contained relatively higher level of α-copaene and lower level of nerolidol compared to that of ours. This difference could be due to the influence of location on the formation of secondary metabolites.

The three oils showed some differences in chemical composition. The monoterpene, sabinene was the predominant compound in *M. fragrans*, whereas, it was absent in *M. beddomeii* and *M. malabarica.* The leaf oils of *M. beddomeii* and *M. malabarica* contained higher level of t-caryophyllene compared to that of *M. fragrans* (0.08 %). *M. fragrans* leaf oil contained about 1.55% myristicin and 0.11% safrole, the hallucinogenic principles, whereas, these could not be detected in the oils of *M. malabarica* and *M. beddomeii*. Similarly, nerolidol, α-cubebene, t-α-bergamotene, α-humulene, β-eudesmol and α-bisabolol which were present in *M. malabarica* and *M. beddomeii* leaf oils were absent in the *M. fragrans* leaf oil. Zheng *et al*. (1992) reported the beneficial effects of the essential oil constituents namely, myristicin and t-caryophyllene. t-Caryophyllene is well known for its anti-inflammatory property and myristicin is a scavenger of cancer causing compounds.

Caryophyllene is anti-inflammatory in nature and nerolidol is an important component of perfume industry. Hence these species have very good prospects in naturopathy.

Acknowledgements

The authors thank the Director, Indian Institute of Spices Research, Calicut, for providing facilities.

References

Adams R P 1989 Identification of Essential Oils by Ion Trap Mass Spectroscopy. Academic Press, San Diego.

AOAC (Association of Official Analytical Chemists) 1975 Official Methods of Analysis. 12th Edn. Association of Official Analytical Chemists, Washington DC.

Gamble J S 1967 Flora of the Presidency of Madras. Vol. II. Botanical Survey of India, Calcutta.

Gianni S, Silvia M, Mariavittoria M, Martina S, Stefano M, Matteo, Renato B 2005 Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry 91: 621–632.

Hooker J D 1973 Flora of British India. Periodical Experts, New Delhi.

Lawrence B M 1997 Progress in essential oils Perfum. Flav. 22 (4): 57–74.

Lawrence B M 2000 Progress in essential oils Perfum. Flav. 25 (5): 52–71.

Lawrence B M 2005 Progress in essential oils Perfum. Flav. 30 (5): 52–66

Madhavan L, Gopalakrishnan M & Narayanan C S 1991 Chemical composition of nutmeg leaf and flower oils. Indian Perf. 35: 161–163.

Mallavarapu G R & Ramesh S 1998 Composition of essential oils of nutmeg and mace. J. Med. Arom. Pl. Sci. 20: 746–748.

Purseglove J W, Brown E G, Green C L & Robbin S R L 1981 Spices Vol. I. (pp. 174–228), Longman Inc., London.

Purushothaman K K, Sarada A & Connolly J D 1997 Malabaricones A-D, novel diarylnonanoids from *Myristica malabarica* Lam. (Myristicaceae). J. Chem. Soc. Perkin (I) 5: 587–588.

Quijano C E, Salamanca G & Pino J A 2007 Aromavolatile constituents of Colombian varieties of mango (*Mangifera indica* L.). Flav. Fragr. J. 22: 401–406

Rout P K, Rao Y R, Sree A & Naik S N 2007 Composition of essential oil, concrete, absolute, wax and head space volatiles of *Murraya paniculata* (Linn.) Jack flowers. Flav. Fragr. J. 22: 352–357.

Sabulal B, Kurup R, Sumitha B & George V 2007 Chemical composition of the leaf oils of *Myristica malabaricum* Lamk and *Gymnocranthera canarica* (King) Warb. J. Essen. Oil Res. 19: 323–325.

Talukdar A C, Jain N, De S & Krishnamurty H G 2000 An isoflavone from *Myristica malabarica.* Phytochemistry 53: 155–157.

Tzakou O, Bazos I & Yannitsaros A 2007 Volatile metabolites of *Pistacia atlantica* Desf. from Greece. Flav. Fragr. J. 22: 358–362.

Varghese J 2001 Nutmeg and Mace-IV. Essential oils of *Myristica fragrans* Houtt.-1. Spice India 14: 24.

Zachariah T J, Shajiprabha S P & Krishnamoorthy B 2000 Major chemical constituents of nut, mace and leaf of *Myristica fragrans* In: Ramana K V, Eapen S J, Babu K N, Krishnamurthy K S & Kumar A (Eds.) Spices and Aromatic Plants-Challenges and Opportunities in the New Century. Contributory Papers, Centennial Conference on Spices and Aromatic Plants, 20–23 September 2000, Calicut (pp. 297–300). Indian Society for Spices, Calicut.

Zheng G Q, Kenney P M & Lan L K T 1992 Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. J. Nat. Prod. 55: 999–1003.