BIOCHEMICAL VARIABILITY IN NUTMEG
(*Myristica fragrans*)
AND RELATED TAXA

Thesis submitted to the University of Calicut in partial fulfillment of the requirement for the degree of Doctor of Philosophy (Biochemistry)

By

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CERTIFICATE

This is to certify that the thesis entitled "**Biochemical variability in nutmeg (*Myristica fragrans*) and related taxa**", submitted to the University of Calicut by Maya K M in partial fulfillment for the award of the degree of Doctor of Philosophy in Biochemistry, is an original research work carried out by her at Indian Institute of Spices Research, Calicut, under my guidance. No part of the work has formed the basis for the award of any other degree or diploma previously.

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DECLARATION

I, hereby, declare that the thesis entitled "**Biochemical variability in nutmeg (*Myristica fragrans*) and related taxa**", submitted by me for the award of the degree of Doctor of Philosophy of the University of Calicut, is an original research work carried out by me, at Indian Institute of Spices Research, Calicut, under the guidance of Dr T. John Zachariah. No part of the work has formed the basis for the award of any other degree or diploma previously.

K.M. Maya

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30.09.2005

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Introduction

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Myristica fragrans produces two spices, nutmeg and mace. It belongs to the *Myristicaceae* family with about 18 genera and 300 species. It is native to the Moluccas in the East Indian Archipelago. Indonesia and Grenada are the major producers of nutmeg. Nutmeg was introduced in India for quite a long time. It is seen mainly in Kerala, Tamil Nadu and Karnataka. Though it is dioecious, male and female flowers are sometimes seen on the same tree (Purseglove *et al.*, 1981).

1.1 FAMILY *MYRISTICACEAE*

The nutmeg family is composed of aromatic evergreen trees with their bark abounding in a viscid reddish juice. The leaves are simple, alternate, without stipules and penninerved. The flowers are small and dioecious. The perianth is valvately three to four lobed. The stamens are several and united at the base. The fruit is fleshy with the covering splitting into halves and exposing a nut-like bright red seed (Parkinson, 1972).

It includes evergreen trees that are often stellately tomentose. The leaves are alternate, entire and often pellucid-punctate. They have no stipules. The flowers are small, dioecious, regular and in axillary or lateral panicles, cymes, umbels or fascicles and rarely in racemes. The perianth is gamophyllous, inferior, with 2, 3 or 4, lobes valvate in bud. Three or more anthers are connate in a sessile or stipitate column, ring or disk. The anthers are two-celled, linear or ovate. The ovary is superior, free and single-celled. There is one basal ovule that is erect and anatropous. The stigma is capitate, discoid or lobed. The fruit is more or less fleshy, splitting usually into two, rarely four, valves (Gamble, 1967).

Under the family *Myristicaceae*, the following species are highly relevant for variability study.

1.1.1 *M. fragrans* Houtt

It is a native of the Eastern Moluccas. It is cultivated in the Malayan Peninsula, Penang and the Malay Islands. It is a lofty tree with slender branches. The leaves are elliptic-lanceolate, acuminate which are 76 mm to 127 mm (3 to 5 inches) long and 38 mm to 50 mm (1.5 to 2 inches) broad. They are coriaceous, sometimes oblanceolate and the tip is caudate with acute base. They are pale yellow brown, paler with red-brown nerves beneath. They have glabrous leaves that are 76 mm to 89 mm (3 to 3.5 inches) long. They are elliptic-oblong or lanceolate, acuminate, and glaucous beneath. The nerves are about 8 pairs and slender. The petiole is 6 mm to 12 mm (0.25 to 0.5 inch) long. The cymes have few flowers, at the most three to five in the male but fewer in the female. The male racemes are 25 mm to 50 mm (1 to 2 inches) long, and the flowers are 6 mm (0.25 inch) long, ellipsoid or urceolate and nodding. The flowers are bracteolate. The anthers are nine to twelve in number, connate in a cylindrical stipitate column. The fruit is ovoid, subglobose or pyriform and 38 mm to 50 mm (1.5 - 2 inches) long. The pericarp is yellow with the arillus red and laciniate (Gamble, 1967; Hooker, 1973).

1.1.2 *M. andamanica, Hook. F.*

It is a slender handsome tree with slender horizontal branches seen in the Andaman Islands. The branches are slender and quite glabrous. The leaves are 76 mm to 102 mm (3 to 4 inches) inches in diameter and pale brown when dry on both surfaces. The leaves are pale silvery or coppery beneath. The base is acute with spreading nerves. It has glabrous petioled leaves, which are membranous, elliptic-oblong and subacute. The nerves are twelve to fifteen pairs and very slender. Flowers are axillary and fasciculate. The male flowers are in sessile or peduncled clusters. The perianth is quite glabrous and smooth. The staminal column is oblong and obtuse. The fruit has the size and shape of a hen's egg. The pericarp is thick and brown with a blood-red seed. (Parkinson, 1972; Hooker, 1973).

1.1.3 *M. malabarica*, Lamk

It is seen in the evergreen forests of Western Ghats, up to 300 m. It is seen in the Konkan, Canara and North Malabar. It is a tree reaching 15 m. in height with a diameter of 0.5 m. It is nearly glabrous with slender flowering branches. The leaves are 100 mm to 200 mm x 38 mm to 100 mm and linear-oblong or elliptic-lanceolate. The nerves are very slender with 19 mm to 26 mm petiole. The male panicles are 25 mm to 38 mm long long, axillary and supra-axillary. The male flowers are in subcymose panicles and bracteolate. The anthers are ten to fifteen in number. The anthers are connate in a cylindrical shortly stipitate column. The ripe fruits are called Bombay nutmeg and Bombay mace that are used as adulterants of *Myristica fragrans*. They have no odour and taste. The aril is reddish yellow, irregularly lobed, laciniate and extending to the apex of the seed (Anonymous, 1962; Gamble 1967; Hooker, 1973).

1.1.4 *M. magnifica*, Beddome Fl. Sylv.

It is found in the swampy ground in evergreen forests of Western Ghats, Travancore and parts of Tinnevelly and Canara. It is an immense gregarious tree, 30 m high. The leaves are linear-oblong, acute or acuminate. They are densely stellately, tomentose beneath and glabrate with 20 to 26 pairs of nerves. The petiole is stout, 12 mm to 25 mm long and channelled. The flowers are ovoid in short cymes that are fascicles on thick woody peduncles. The male flowers are tomentose and densely crowded. The female flowers are slightly larger but less numerous than the male. The fruit is oblong; up to 100 mm long, densely tomentose with a much deeply laciniate orange-red arillus. The red arils from seeds are used for dyeing purpose. The seeds yield oil that is used for burning and making candles (Anonymous, 1962; Gamble 1967; Hooker, 1973).

1.1.5 *M. amygdalina*, Wall.

It is found in Tenasserim, Tavoy and Moulmein. It is a tall perfectly glabrous tree. The leaves are 38 mm to 50 mm in diameter, coriaceous and pale brown

on both surfaces with a 12 mm long petiole. They are 150 to 200 mm long, elliptic-lanceolate and acute at both ends. There are eight to twelve pairs of nerves. The male panicles arise from the axils of fallen leaves and are 75 mm to 125 mm long. They are branched from the base and quite glabrous. The flowers are loosely clustered and the pedicels are as long as the perianth and slender. The anthers are about eight and combined. The fruit is shortly peduncled and 38 mm long. The pericarp is rather thin and glabrous. The aril is yellow and lacerate at the tip only (Hooker, 1973).

1.1.6 *M. beddomeii*

It is seen in evergreen forests of Western Ghats up to 1,500 m. It is a large tree reaching 27 m in height with a diameter of about 0.8 m. The leaves are oblong or elliptic-lanceolate. They are glabrous beneath and usually glaucous and smooth above. The leaf nerves are transverse; nervules are conspicuous with leaves 125 mm to 250 mm long and 60 mm to 100 mm broad. The flowers are in cymes and are dioecious. The fruit is globose, 50 to 63 mm in diameter with a fleshy pericarp. The arillus is orange-red and laciniate with their ends separate. This tree has been used as a rootstock for the vegetative propagation of *M. fragrans* (Gamble 1967; Anonymous, 1962).

1.1.7 *M. prainii*

It is fairly frequent in semi-deciduous and evergreen forests. Here the leaves are 150 mm to 300 mm long, elliptic to oblong. The flowers are seen in branched panicles. The leaves are 150 mm to 300 mm long, 75 mm to 125 mm broad, elliptic-oblong to broadly elliptic, acute, and base broad and somewhat rounded. It has 15 to 18 pairs of lateral nerves. The flowers are small and the fruit is ovoid. It is 38 mm long with a thick pericarp with a red laciniated seed (Parkinson, 1972).

1.1.8 *Knema andamanica*

It is also seen in the evergreen forests of Western Ghats up to 600 m. It is a moderate-sized tree with oblong-lanceolate acuminate leaves upto 200 mm long, 50 mm to 75 mm broad, prominently and regularly nerved, glaucous and rusty pubescent beneath. The flowers are stellately pubescent. The fruit is ovoid, 38 mm long and the aril has a brilliant crimson color (Gamble, 1967).

1.2 USES OF NUTMEG AND MACE

The spices are used as condiments and in medicine. The dried nutmeg and mace are used as spices. The essential oil (also called volatile oil) and the oleoresin are the major products of interest from the spice. Nutmeg is a stimulant, carminative, astringent and aphrodisiac. It is used in tonics and electuaries; and forms a constituent of preparations for dysentery, stomachache, flatulence, nausea, vomiting, malaria, rheumatism, sciatica and early stages of leprosy. Higher doses have a narcotic effect. Delirium and epileptic convulsions are found to occur. Mace is also used similarly. It is chewed for masking foul breath. It also prevents dental caries.

In India, both the spices are used as drugs. Alcoholic extracts of nutmeg have antimicrobial activity. They have applications in bakery products, soups, preserves, sauces and diary products. The leaf essential oil has herbicidal properties and is used for preparing soaps and chewing gum. The rind of the fruit is used in pickles, jams and jellies (Anonymous, 1962; Purseglove *et al.*, 1981).

1.3 COMPOSITION OF NUTMEG AND MACE

Nutmeg has the following composition: 14.3% moisture, 7.5% protein, 36.4% ether extracts, 28.5% carbohydrates and 11.6% fibre. It has a mineral content of 1.7% with 0.12% calcium, 0.24% phosphorous and 4.6 mg% of iron. The essential oil has a range of 6 to 16% and a starch content of 14.6 to 24.2%, 2.25% pentosans, 1.5% furfural and 0.5% to 0.6% pectin.

Mace, on the other hand, has following composition: 15.9% moisture, 6.5% protein, 24.4% ether extract, 47.8% carbohydrate and 3.8% fibre. It has 1.6% of mineral content with 0.18% calcium, 0.10% phosphorous and 12.6 mg% of iron. It has 4 to15% of essential oil, 25% amylodextrin, reducing sugars, pectin and resins. The mace oil resembles nutmeg oil in odour, flavour and composition. Thus no distinction is made between them in trade (Anonymous, 1962).

1.4 ESSENTIAL OIL

The yield of essential oil in nutmeg has a range from 6 to 16% based on the origin and quality of the spice. Mace has 4 to15% of essential oil. The essential oil is colourless or pale yellow coloured with the characteristic spicy odour. The major constituents of oil are β-pinene, sabinene, dipentene, *p*-cymene, *d*-linalool, terpinen-4-ol, *dl*-α-terpineol, geraniol, safrole, eugenol, isoeugenol, an aldehyde with citral odour, myristicin (3-methoxy-4, 5-methylenedioxy-1-allylbenzene), myristic acid and esters of myristic acid and other fatty acids (Anonymous, 1962).

Essential oils are used for flavouring food products and liquors. It replaces ground nutmeg to avoid leaving particles in foods and beverages. Thus, it is used to flavour baked items, beverages, candies, meats and syrups. It is also used for scenting soaps, dental creams and perfumes. It is mildly counter-irritant and is used in ointments, hair-lotions and cosmetics. Nutmeg oil is mainly used in the pharmaceutical industry. It is used to treat illnesses ranging from the nervous system to the digestive system.

1.5 MYRISTICIN

Myristicin present in the oils of nutmeg and mace is believed to be responsible for the toxicity of nutmeg. Five to 15 gm of nutmeg causes symptoms similar to atropine poisoning – flushing of skin, tachycardia, absence of salivation, excitation of central nervous system etc.

Myristicin is also endowed with many beneficial properties. It has been proved to induce Glutathione-S-Transferase (GST) that protects the body from harmful peroxides and toxic carcinogens. It thus has potent chemopreventive properties (Ahmad 2001). It also has antioxidant properties (Hattori *et al.*, 1993). Myristicin is found to be toxic to insects and synergised the insecticidal activity of synthetic insecticides (Lichtenstein *et al.*, 1974). The insecticidal property of myristicin is believed to protect many plants from the insect attack (Stahl, 1981).

1.6 OLEORESIN

It consists of both volatile and non-volatile components. The volatile components include the essential oil constituents that are responsible for the flavouring and scent properties of the spice. The non-volatile components of the oleoresin include carotenoids, steroids, alkaloids, anthoyanins, glycosides etc. They are important for the taste, colour, texture and antioxidant properties of the product (Boelens, 2000).

1.7 FAT (BUTTER)

Nutmegs contain 25-40 % of fixed oil, otherwise called *oleum myristiceae expressum.* It is a highly aromatic, orange-yellow coloured fat with the consistency of fat at room temperature. It has the odour and taste of nutmeg. It is composed of mainly trimyristin with a high proportion of essential oil. It is an ester of glycerol and tetradecanoic acid (myristic acid).

The other major sources of trimyristin are coconut oil and palm kernel oil. (Purseglove *et al.*, 1981).

1.8 MEDICINAL PROPERTIES OF NUTMEG AND MACE

When the effects of dietary administration (3.9 mg/day for 17 months) of essential oil on the polyunsaturated fatty acid composition of the retina of aged rats were studied, its levels were increased (Recsan *et al.*, 1997). Thus, there is a possible relationship between the antioxidant properties and the prevention of age related macular degeneration.

Sait & Satyaputhra (1995) have worked out the effect of deterpenation on the medicinal quality of nutmeg oil. Deterpenation was carried out by alcoholic extraction or chromatographic separation. Alcoholic extraction gave complete removal of monoterpene hydrocarbons whereas chromatographic separation gave only partial removal. Deterpenation caused a downgrading of the medicinal quality of the oil. This was due to an increase in the myristicin concentration

Janssens *et al.*, 1990, have identified the most active constituent as inhibitors of platelet aggregation from nutmeg oil. It showed that, medicinally, nutmeg oil and nutmeg powder can be replaced by eugenol and isoeugenol.

Flavonoids from several plants including *Myristica fragrans* were found to have hypolipidaemic activity (Koshy & Vijayalakshmi, 2001).

The possible insulin function effects of several herbs, spices and medicinal plants were evaluated by Broadhurst *et al.* (2000). It was found that cinnamon was the most bioactive product followed by witch, hazel, green & black teas, allspice, bay leaves, nutmeg, clove, mushroom etc. This glucose-oxidation enhancing property was lost when treated with polyvinylpyrrolidone (PVP) treatment. This shows that the active compound may be phenolic in nature. This study shows the specific effects of plant extracts on insulin activity.

Two antimicrobial resorcinols isolated from mace showed strong antifungal and antibacterial activities that were reduced by structural modifications (Orabi *et al.*, 1991). The phenylpropanoids including myristicin, elemicin and *trans-*

isoelemicin also showed antifungal activity (Marston *et al.*, 1995). Nutmeg exhibited potent activity against *Bacillus subtilis, E.coli* and *Saccharomyces cerevisiae* (De *et al.,* 1999). The nematicidal activity of *Myristica fragrans* against *Meloidogyna incognita* was reported by Gotke *et al.* (1990). Their study revealed mace oil to be the most effective antimicrobial agent followed by nutmeg oil.

When the antioxidant property of many essential oils was studied, the oil from *Myristica fragrans* was among the most effective antioxidants in both the egg-yolk assay and in the chick-liver assay (Dorman *et al.,* 1995).

Dorman *et al.* (2000) studied the antioxidant property of nutmeg essential oil using an antioxidant assay and demonstrated an antioxidant capacity superior to the synthetic antioxidants (like Butylated Hydroxy Anisole, Butylated Hydroxy Toluene, etc). When the antioxidant property of nutmeg was studied, it was shown to prevent the oxidative bleaching of β-carotene.

There are only few reports available on the biochemical variability among the cultivated *M. fragrans* and the wild taxa. This study aimed at studying the variability in relation to primary and secondary metabolites.

1.9 OBJECTIVES

Objectives of the study are the following

1.9.1 Evaluation of Biochemical Variability

Here *M. fragrans* accessions are grouped into three based on the yield of nutmeg.

i) High yielding (above 1500 fruits per year)
ii) Medium yielding (between 1500 to 1000 fruits per year)
iii) Low yielding (less than 1000 fruits per year)

The biochemical variability in the primary and secondary metabolites of nutmeg, mace and leaf is studied.

1.9.2 Differentiation of Primary and Secondary Metabolites in Male, female and Bisexual Plants

Nutmeg is a dioecious plant, with the occasional occurrence of bisexual plants that may predominate in either male or female flowers. Sex determination is a problem in the seedling stage. Sex of the plant can be ascertained only after flowering, which takes six to eight years. Various investigators have studied different parameters in an attempt to identify the sex of the plant at an earlier stage. This study is an attempt to differentiate the primary and secondary metabolites of leaf in male, female and bisexual plants.

1.9.3 Biochemical Variability in *Myristica fragrans* and Related Taxa

The biochemical variability in the primary and secondary metabolites of *Myristica fragrans* is also studied with respect to the other related taxa. It includes the following:

i) *Myristica amygdalina*
ii) *Myristica andamanica*
iii) *Myristica beddomeii*
iv) *Myristica magnifica*
v) *Myristica malabarica*
vi) *Myristica prainii*
vii) *Knema andamanica*

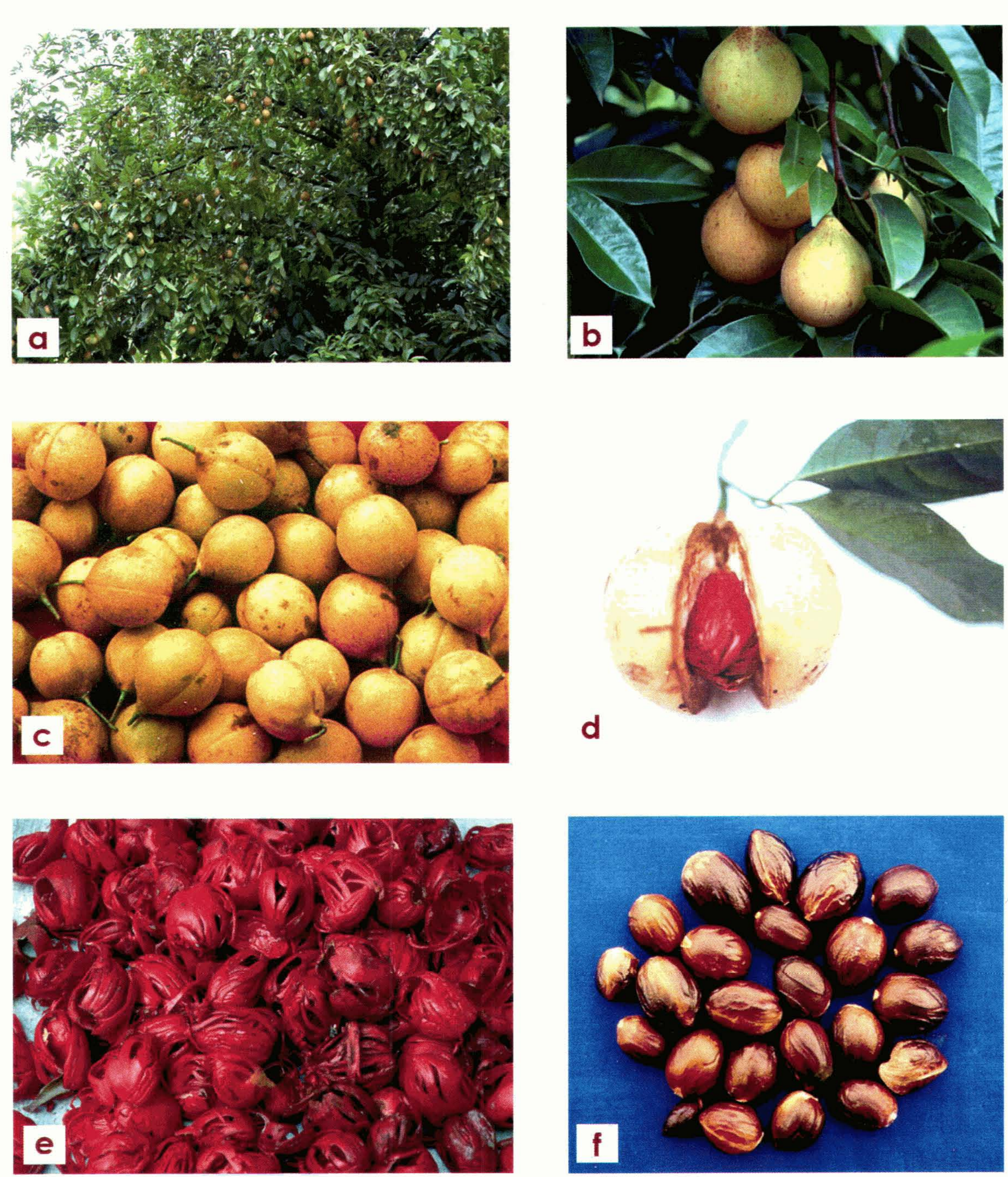

Plate 1 ***Myristica fragrans*** **a**. Plant **b**. a branch with fruits **c** Fruits **d**. a mature fruit with nut and mace exposed**e**. Mace **f** Nutmeg

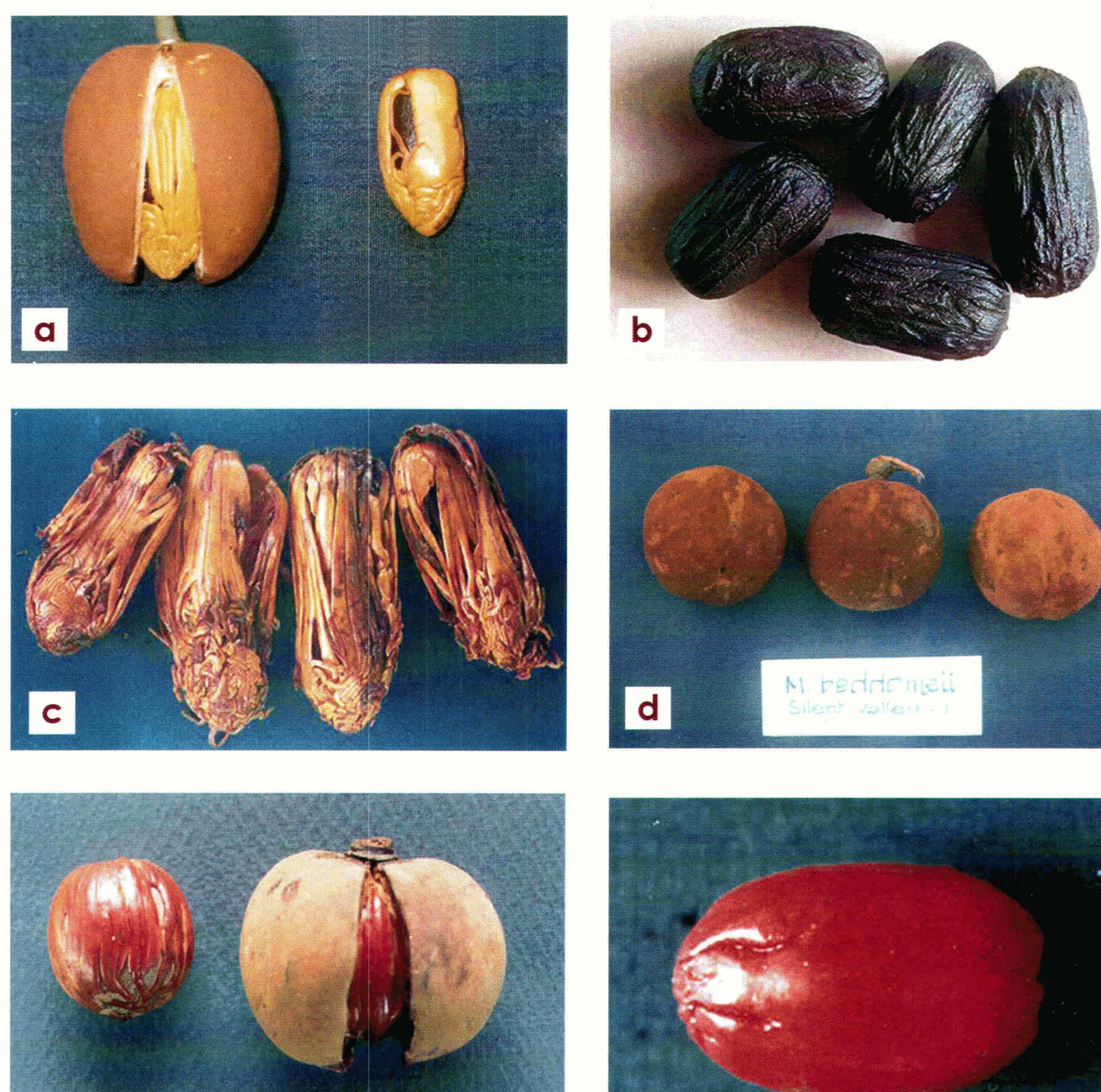

Plate 2 Fruits of *Myristica* species

a. *M. malabarica*- mature fruit and mace
b *M. malabarica* - nutmeg **c** *M.malabarica* – mace
d *M. beddomeii* – fruit **e** *M. beddomeii* –mature fruit with nut and mace exposed **f** *M. prainii* – mace covering the seed inside

Plate 3 Plants of *Myristica* species
a *M. fragrans* **b** *M. beddomeii* **c** *M. prainii* **d** *M. magnifica*
e *M. andamanica* **f** *M. malabarica*

Review of Literature

K.M. Maya "Biochemical variability in nutmeg (myristica fragrans) and related taxa" Thesis. Indian Institute of Spices Research, University of Calicut, 2005

Review of Literature

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Myristica fragrans is unique among spices as it produces two spices, nutmeg and mace. It belongs to the *Myristicaceae* family with about 18 genera and 300 species. It is native to the Moluccas in the East Indian Archipelago. Indonesia and Grenada are the major producers of nutmeg. Nutmeg was introduced in India for quite a long time. It is seen mainly in Kerala, Tamil Nadu and Karnataka (Purseglove *et al.*, 1981).

The nutmeg tree is a spreading evergreen tree, 4 to 10 meters high. Though it is dioecious, male and female flowers are sometimes seen on the same tree. All parts of the plant are aromatic. The root system is superficial with a surface plate of roots. The branches are numerous and spreading. It has a reddish sap. The leaves are alternate, glabrous and exstipulate. The lamina is 50-150 mm long and 20-70 mm broad. It is coriaceous, dark green above and light green beneath. The lower midrib is yellowish green, elliptic or oblong lanceolate. The petiole is about 10 mm long (Purseglove *et al.*, 1981).

2.1 FAMILY *MYRISTICACEAE*

It includes evergreen tree which are often stellately tomentose. The leaves are alternate, entire and often pellucid-punctate. It has no stipules. The flowers are small, dioecious, regular and in axillary or lateral panicles, cymes, umbels or fascicles and rarely in racemes. The bracteoles are persistent or caducous. The perianth is gamophyllous, inferior, with 3, sometimes 2 or 4, lobes valvate in bud. Three or more extrorse anthers are connate in a sessile or stipitate column ring or disk. The anthers are 2-celled, linear or ovate. The ovary is superior, free and 1-celled. There is one basal ovule which is erect and anatropous. The stigma is capitate, discoid or lobed. The fruit is more or less fleshy, splitting usually into 2, rarely 4, valves. The seeds are erect, enclosed in a thin or fleshy, entire or lacerate, coloured, often aromatic arillus. The testa is usually thick, albumen copious, hard and ruminate. The embryo is very small and basal with cotyledons divaricate, flat or crumpled. The radicle is short and inferior (Gamble, 1967).

Family *Myristicaceae* includes three major genera. They are *Gymnacranthera, Myristica* and *Knema.* Their characters are described briefly.

In ***Gymnacranthera,*** anthers are attached by their backs to a column formed of the connate filaments and anther-column is sessile with the apices of the anthers free.

The leaves are alternate, entire, evergreen and pergamaceous. The flowers are small and dioecious. The male flowers are in fascicles in axillary panicles and female flowers in short axillary racemes. The bracts are deciduous. The perianth is three to four lobed. The androecium is sessile and the connectives combined in an oblong thick column. The anthers are six to twelve in numbers and elongate. The lower parts are adnate to the column by their backs; the apices are free and often inflexed over the column. The ovary is ovoid. The stigmas are sessile, connate and scarcely bilobed. The fruit is globose or ovoid with thick fleshy pericarp. Arillus is laciniate almost to the base. The seed conforms to the fruit. The testa is woody and albumen is ruminate. The cotyledons are divaricate and connate at base (Gamble, 1967).

In ***Myristica,*** anther-column is stipitate and usually produced beyond the anthers which are completely attached to it.

The leaves are alternate, entire, evergreen, pergamaceous or chartaceous. The flowers are small and dioecious in cymes, umbels or fascicles. They arise from the axils of the leaves or of the scars of fallen leaves. The peduncles are usually thick with deciduous bracts. The bracteoles are persistent and usually oblique at the base of the perianth. The perianth is three-lobed. The androecium is stalked and the filaments and connectives connate in a column usually produced beyond the anthers. The anthers are twelve to thirty and elongate. The ovary is ovoid, stigmas are connate and two-lobed. The fruit is large, ovoid or oblong. It has a thick, succulent and rarely leathery pericarp.

Arillus is laciniate. Seed conform to the fruit which has a hard testa. Albumen is ruminate and cotyledons are connate (Gamble, 1967).

In ***Knema***, anthers are attached by their bases stellately to a peltate (usually stipitate) column formed of the connate filaments (Gamble, 1967).

The leaves are alternate, evergreen, coriaceous or chartaceous. The flowers are small and dioecious. The male flowers are in fascicles on thick peduncles from the axils of leaves or of the scars of fallen leaves. The female flowers are similar but fewer and rather longer. The bracts are deciduous and pedicles bracteolate. The perianth is three-lobed. The androecium is usually stalked with filaments and connectives connate in a peltate disk. The anthers are 8 to 20 in number, free, attached stellately to the margin of the disk and dehiscing downwards. The ovary is ovoid with a thick short style. There are two stigmas laciniate on the margins. The fruit is ovoid or oblong and pericarp is thick, fleshy and tomentose. The arillus is laciniate at the apex only. The seed conform to the fruit. Albumen is ruminate and cotyledons are divaricate or sub-erect (Gamble, 1967).

The different taxa included in the study have been described as follows.

2.1.1 *M. fragrans*, Houtt.

It is native of the Eastern Moluccas. It is cultivated in the Malayan Peninsula, Penang and the Malay Islands. It is a lofty tree with slender branches. The leaves are elliptic-lanceolate, acuminate which are 76 mm to 127 mm (3 to 5 inches) long and 38 mm to 50 mm (1.5 to 2 inches) broad. They are coriaceous and tip is caudate with acute base. They are pale yellow brown, paler with red-brown nerves beneath. *M. fragrans* has glabrous leaves which are 76 mm to 89 mm (3-3.5 inches) long. It is elliptic-oblong or lanceolate, acuminate, and glaucous beneath. The nerves are about 8 pairs and slender. The petiole is 6 mm to 12 mm (0.25 to 0.5 inch) long. The cymes have few flowers, at most 3 to 5 in the male and fewer in the female. The male racemes are 25 mm to 50 mm

(1 to 2 inches) long, and the flowers are 6 mm (0.25 inch) long, ellipsoid or urceolate and nodding. The flowers are bracteolate. The bracteole is a scale under the glabrate perianth. The anthers are 9-12 in number, connate in a cylindrical stipitate column. The fruit is ovoid, subglobose or pyriform and 38 mm to 50 mm (1½ - 2 inches) long. The perianth is strigose with appressed hairs, or quite glabrous. The perianth is 50 mm to 75 mm (2 to 3 inches) long, tawny-villous with drooping pedicels which are about 12.5 mm (0.5 inch) long. The pericarp is yellow with the arillus red and laciniate (Gamble, 1967; Hooker, 1973).

2.1.2 *M. andamanica*, Hook. F.

It is a slender handsome tree seen in the Andaman Islands with slender horizontal branches and often with curved stilt-like roots at the base. The branches are slender and quite glabrous with the youngest shoots rustily hoary. The bark is blackish green, and when cut, it is dark red with blood-red juice. The leaves are 76 mm to 102 mm (3 to 4 inches) in diameter and pale brown when dry on both surfaces. The leaves are pale silvery or coppery beneath. The base is acute with spreading nerves. *M. andamanica* has glabrous petioled leaves that are membranous, elliptic-oblong and sub acute. They are not glaucous beneath. The nerves are twelve to fifteen pairs and very slender. The petiole is strong and 25 mm to 38 mm (1 to 1.5 inches) long. The flowers are axillary and fasciculate, seen in the leaf axils. They are few, small, urceolate-globular and whitish. The male flowers are in sessile or peduncled clusters on the branches that are very shortly pedicelled. The bracteole is large with globose three-lobed perianth. The perianth is quite glabrous and smooth. The staminal column is oblong and obtuse. The fruit has the size and shape of a hen's egg. The pericarp is thick and brown with blood-red seed. It is apparently a very distinct species, but approaches *M. malabarica* (Parkinson, 1972; Hooker, 1973).

2.1.3 *M. malabarica*, Lamk.

It is seen in the evergreen forests of Western Ghats, up to 300 m. It is seen in the Konkan, Canara and North Malabar. It is a tree reaching 15 m in height with a diameter of 0.5 m. The bark is greenish-black and smooth; wood is yellowish-brown tinged with grey. It is moderately hard, not durable and of little use. It is sometimes used for building construction, tea boxes, light furniture, matchboxes and splints. The vernacular names are the following; Kanagi in Kannada, Patthiri in Tamil and Ponnam panu in Malayalam. It is nearly glabrous with slender flowering branches. The leaves are 100 mm to 200 mm x 38 mm to 100 mm and linear-oblong or elliptic-lanceolate. They are sub acute and glaucous beneath with eight to fourteen pairs of nerves. The leaves are thinly coriaceous on the flowering branches. They are thick and leathery on the fruiting and more or less shining above. The nerves are very slender with 19 mm to 26 mm (0.75 - 1-inch) petiole. The male panicles are 26 mm to 38 mm (1-1.5 inch) long, axillary and supra-axillary. The peduncle is naked below and sub umbellately cymose above. The male flowers are in subcymose panicles and bracteolate. The peduncles and pedicles are slender with globose perianth. The anthers are 10 to 15 in number. The bracteole is an orbicular scale with a three-toothed puberulous perianth 4.2 mm long. The anthers are connate in a cylindrical shortly stipitate column. Here the cymes are dichasioid with upto 20 male flowers and only 3 or 4 female flowers. The flowers are larger, dioecious and are seen in umbellate cymes. The perianth is 5 mm to 6.4 mm long, subglabrous with about 0.64 mm long pedicels. The ripe fruits are called Bombay nutmeg and Bombay mace that are used as adulterants of *Myristica fragrans*. They have no odour and taste. It is narrowly oblong and pubescent, 50 mm to 75 mm (2 to 3 inches) long and 19 mm (0.75 inch) broad. The seed is arillate, ovoid and slightly flattened on the side with a shining black testa. The aril is reddish yellow, irregularly lobed, laciniate and extending to the apex of the seed (Anonymous, 1962; Gamble, 1967; Hooker, 1973).

2.1.4 *M. magnifica*, Beddome Fl. Sylv.

It is found in the in swampy ground in evergreen forests of Western Ghats, Travancore and parts of Canara. It is an immense, gregarious tree, 30 m high. It is often buttressed and furnished with numerous aerial roots, which start from the trunk at 3 m to 6 m above the ground and spread along the ground, rising in loops above it. The bark is purplish-black and smooth. The wood is yellowish-white, soft and perishable. In Malayalam, it is called Kottha panu. The wood is light and used for making matchboxes and splints. The young parts are clothed with golden pubescene with thickly coriaceous leaves that are 250 mm to 600 mm (ten to twenty-four inches) long. The leaves are linear-oblong, acute or acuminate. They are densely stellate, tomentose beneath and glabrate with of 20 to 26 pairs of nerves. The petiole is stout, 12 mm to 25 mm (0.5 to 1 inch) long and channelled. The flowers are ovoid in short cymes, which are fascicles on thick woody peduncles. The male flowers are tomentose and densely crowded on very short and stout axillary bracteolate peduncles. The female flowers are slightly larger but less numerous than the male. The fruit is oblong, up to 100 mm (four inches) long, densely tomentose with a much deeply laciniate orange-red arillus. The red arils from seeds are used for dyeing purpose. The seeds yield oil that is used for burning and making candles (Anonymous, 1962; Gamble, 1967; Hooker, 1973).

2.1.5 *M. amygdalina*, Wall.

It is found in Tenasserim, Tavoy and Moulmein. It is a tall perfectly glabrous tree. The leaves are38 mm to 76 mm (1.5 - 2 inches) in diameter, coriaceous and pale brown on both surfaces with a 13 mm (0.5 inch) long petiole. They are 152 mm to 200 mm (six to eight inches) long, elliptic-lanceolate and acute at both ends. There are 8 to 12 pairs of nerves. The male panicles arise from the axils of fallen leaves and are 76 mm to 127 mm (three to five inches) long. They are branched from the base and quite glabrous. The flowers are slender, loosely clustered and the pedicels are as long as the perianth. The staminal column is globosely trigonous, fleshy and concave. The anthers are about 8 and combined. The fruit is shortly peduncled and 38 mm (1.5 inches) long. The

pericarp is rather thin and glabrous. The aril is yellow and lacerate at the tip only (Hooker, 1973).

2.1.6 *M. beddomeii*

It is seen in evergreen forests of Western Ghats up to 1500 m. It is a large tree reaching 27 m. in height with a diameter of about 0.8 m. The bark is blackish-green and rather smooth. The wood is yellowish brown, moderately hard and perishable. It is used for making tea boxes, matchboxes and splints. The vernacular names are Jajikai in Kannada, Kathu jathikai in Tamil and Pattha panu in Malayalam. The leaves are oblong or elliptic-lanceolate. They are glabrous beneath and usually glaucous and smooth above. The leaf nerves are transverse and nervules are conspicuous. They are 127 mm to 254 mm (five to ten inches) long and 6 mm to 100 mm (0.25 to 4 inches) broad. The flowers are in cymes and are dioecious. The fruit is globose, 50 mm to 64 mm (2 to 2.5 inches) in diameter with a fleshy pericarp. The arillus is orange-red and laciniate with their ends separate. This tree has been used as a rootstock for the vegetative propagation of *M. fragrans* (Gamble, 1967; Anonymous, 1962).

2.1.7 *M. prainii*

It is fairly frequent in semi-deciduous and evergreen forests. Here the leaves are not glaucous or pale beneath. They are 152 mm 300 mm (six to twelve inches) long, elliptic to oblong. The flowers are small and seen in branched panicles. It is a tall straight-stemmed tree with a high crown and slender branches. The bark is dark grey and smooth. When cut, it is reddish-brown with thin pinkish juice. The leaves have 15 to18 pairs of lateral nerves. The fruit is ovoid and 38 mm (1½ inch) long. It has a thick pericarp with a red laciniated seed (Parkinson, 1972).

2.1.8 *Knema andamanica*

It is also seen in the evergreen forests of Western Ghats up to 600 m. It is a moderate-sized tree with oblong-lanceolate acuminate leaves up to 200 m long,

50 mm to 76 mm broad. They are prominently and regularly nerved, glaucous, rusty and pubescent beneath. The flowers are stellately pubescent. The fruit is ovoid, 38 mm long and the aril has a brilliant crimson color. The bark is greenish-black and smooth. The wood is pale brown, moderately hard and of little value. The vernacular names include Rukt maru in Kannada, Chora patthiri in Tamil and Chora panu in Malayalam (Gamble, 1967).

2.2 *M. fragrans* Houtt.

Thus we have seen the botany of the various taxa studied. Now we will see the detailed botany of *M. fragrans*.

2.2.1 Inflorescence and Flowers

As the tree is dioecious, there is no method for determining the sex of the seedling. The male and female inflorescences are similar. They are glabrous, axillary and in umbellate cymes. One to ten flowers are seen in the male plant, and one to three in the female one. The main axis is 10-15 mm long and usually unbranched. The flowers are creamy yellow in colour, waxy, fleshy and fragrant. The calyx is bell-shaped, nectiferous with three reflexed triangular lobes with no petals. The male flowers are smaller than the female ones. The androecium is 7 mm long and glabrous with a 2 mm stock. It is acute at the apex with 8-12 stamens. The anthers are adnate to a central column and attached to each other by their sides. The female flowers are 10 mm long with a puberulous, superior, sessile, single celled, ovary. It is 7 mm long and has a very short white stigma (Purseglove *et. al.*, 1981).

2.2.2 Fruit

The fruit is a fleshy drupe usually pendulous, broadly pyriform, yellow and smooth. It is 60-90 mm long. When ripe, the yellow pericarp splits open into two halves, exposing the shiny brown testa surrounded by a laciniate red aril. The testa contains a brown kernel which is 20-30 mm long and 15-20 mm

broad. The kernel is wrinkled and contains a lighter coloured endosperm and a small embryo (Purseglove *et. al.*, 1981).

The chromosome number of the genus is believed to be seven, and the somatic number of *M. fragrans* $2n = 42$.

2.2.3 Pollination

The flowers are fragrant and secrete nectar. They are believed to be pollinated by small insects. An incompatibility mechanism is believed to be operating to ensure cross-pollination.

2.2.4 Climate and Soil

M. fragrans grows well in insular maritime climates in the tropics. They are believed to have originated in the Moluccas where the rainfall ranges from 2210-3667 mm per annum. Grenada receives an annual rainfall of 1524-2540 mm, with a dry season spanning two to three months.

In Banda and Amboina, nutmegs grow on rich volcanic soil. Grenada Island is entirely volcanic in origin, composed of pyroclastic rocks and some massive lava. The lavas are basalt and andesites; and the pyroclastic rocks are composed of andesitic material. Most of the nutmegs are grown on hill slopes where the soil is either Capitol Clay Loam or Belmond Clay Loam. They are well drained with good water retention and are only moderately erodible. In Penang, nutmegs grow on the exposed slopes of granite hills. This crop is cultivated mainly in the southern states of India. It requires a hot and moist climate with a rainfall of 150-300 cm per annum. It grows best at low elevations in alluvium soil (Anonymous, 1962; Purseglove *et al.*, 1981).

2.2.5 Cultivation

The plant is propagated from seeds. Fresh seeds with shells are dried for a day and are sown in nursery beds. The viability of fresh seeds is 98% in shade and 92% in the open while that of sun dried seeds stored for two months is only 7% in shade and almost nil in the open. The seeds are sown 300 mm apart and it takes up to three months to germinate. When the seedlings are 600-900 mm high, they are transplanted to the field. Young plants are grown in shade. Nutmeg is also cultivated as a mixed crop along with coffee, tea, coconut, arecanut and rubber plantations. Weeding the field and providing a mulch of dry leaves are the main post-planting operations usually carriedout. No much manuring is usually done. In plantations raised from seeds, there is no means of determining the sex until the plants begin to flower. (There are strong, but unsubstantiated claims that the sex can be known quite early.). The male plants in a plantation may be headed back and grafted with scions from female trees. Grafting may be made on seedlings of other species like *M. beddomeii* and *M. malabarica*. Vegetative propagation not only ensures female trees but also is useful for producing high-yielding and disease-resistant types (Anonymous, 1962).

Nutmegs are propagated by fresh seeds with their testa still attached. The seeds are planted in shaded nurseries at a spacing of about 300 mm apart and 25.5 mm deep. Germination takes one month or more. When the seedlings are 6 months old, they are transplanted to the field. As the plant is dioecious, seedling progeny will give about 50% of either sex. Vegetative propagation promotes the planting of the female tree with early bearing, and also provides the selection and propagation of high yielding varieties. Grafting may be made on seedling species other than those of *M. fragrans*. Thus, vegetative propagation has considerable attraction in nutmeg (Purseglove *et al.*, 1981). Grafting has the option of using different rootstocks other than *M. fragrans* like *M. beddomeii* and *M. malabarica* (Anonymous, 1962).

The botanical characteristics, ecology, propagation, cultural methods, harvesting, yield, products, active principles and processing methods used in various countries have been described by Ferrao (1993). The cultivation and trade of spices including nutmeg, cinnamon, cardamom, cloves, turmeric, ginger and pepper was studied. The prospects of cultivation of nutmeg, clove and cinnamon in Andaman and Nicobar Islands have been described by Rao (1991). He finds scope for cultivating tree spices in combination with other crops such as coconuts, arecanuts, coffee, pepper and forest trees. Different aspects of nutmeg investigation programme in Grenada including NPK manuring trials, observations on sex expression and fruit set, control of pest and production of nutmeg oil have been studied (Cruickshank, 1973).

Great progress was made in the tissue culture of tropical spices during the last three decades (Babu *et al.*, 1993). The two main areas of interest in it are the following. (i) The *in vitro* clonal propagation of cardamom, ginger, turmeric, black pepper and the tree spices which includes cinnamon, clove and nutmeg and (ii) Regeneration of plantlets from tissue culture and somaclonal variation.

Direct somatic embryos of nutmeg have been isolated. Enhanced embryogenic response was associated with broken zygotic embryos. Activated charcoal and light were the critical factors for induction of somatic embryogenesis in nutmeg. The somatic embryos synthesized chlorophyll, phenolics, etc and proved to be a stable source of secondary metabolites of nutmeg (Iyer *et al.*, 2000a). Viable mesophyll protoplasts were isolated from nutmeg leaves by a combination of the enzymes cellulase, hemicellulase and pectinase (Iyer *et al.*, 2000b).

2.2.6 Harvesting and Trade

A seedling tree begins to bear when five to eight years old. Vegetatively propagated trees bear fruits earlier. The yields increase up to 15 years and continue for 30-40 years. The fruits ripen in six to nine months with two seasons a year (Purseglove *et al.*, 1981). The main harvesting season is June-

October. The average annual yield per tree at Burliar is 1250 fruits per year (Anonymous, 1962).

The mature fruit is split open exposing the seed with a scarlet fibrous aril. The pericarp is removed after collection and the seed is separate from the aril and dried. When the drying is complete, the kernel rattles in the shell. After removing the shells, the kernels are sorted. The dried kernels are the nutmeg of commerce. In Indonesia, the kernel is usually limed before drying; it prevents insect attack and improves the shelf life of nutmeg. Mace is obtained by separating the arils and drying in the sun after flattening between boards. When the weather is cloudy, the mace is dried using artificial heat. Mace of superior quality is obtained by drying in specially constructed ovens. Sometimes salt water is sprinkled over the drying arils. This is believed to improve the shelf-life of mace (Anonymous, 1962).

The spices are imported from South East Asian countries for culinary and medicinal purposes (Anonymous, 1962). Smith (1986) has described the international trade in clove, nutmeg, mace, cinnamon, cassia and their derivatives. Indonesia is the largest producer of clove buds. The two nutmeg producing countries are Indonesia (75%) and Grenada (20%). Mace production is also dominated by Indonesia and Grenada.

There are mainly two types of spices: East Indian and West Indian. East Indian spice is obtained from Indonesia, Northern Celebes, Sangih Islands, West and North Sumatra where as West Indian spice is obtained mainly from the Island of Grenada (Anonymous, 1962).

East Indian spices are of better quality and are highly valued in the Indian trade. East Indian nutmeg is available in three grades. (i) Banda nutmeg is believed to be the finest with 8% essential oil, (ii) Siauw nutmeg almost as good as Banda but with 6.5% essential oil and (iii) Penang nutmeg is usually wormy and moldy and is used only for distillation purpose. Papua nutmeg is not

derived from *M. fragrans* but from *M. argentea* Warb. This is sometimes called as the fourth grade of East Indian nutmeg. But it can be distinguished by its longer size, peculiar shape, poor aroma and acrid taste. Bombay nutmeg is obtained from *M. malabarica*. It is long, narrow and devoid of aroma. It is thus an adulterant of true nutmeg (Anonymous, 1962).

Mace is yellowish red in colour, translucent and brittle. It has the odour and taste of nutmeg, but is softer and delicate. Three types of mace are found (Anonymous, 1962). (i) Banda mace is the finest, has a bright orange colour with fine aroma, (ii) Java Estate mace has golden yellow colour with brilliant crimson streaks and (iii) Siauw mace has lighter colour with less essential oil.

Papua mace is graded as the fourth class and is derived from *M. argentea*. It has lesser oil with undesirable aroma. Bombay mace is obtained from *M. malabarica*. It has dark red colour and devoid of aroma. It is used as an adulterant of Indian mace. West Indian mace is comparatively inferior in quality (Anonymous, 1962).

2.2.7 Pests and Diseases

A fruit rot of half ripe fruits caused by *Diplodia natalensis* Pole-Evans has been recorded. It is controlled by spraying Bordeaux mixture and eradicating the sources of infection (Anonymous, 1962).

2.2.8 Uses of Nutmeg and Mace

The spices are used as condiments and in medicine. Nutmeg is a stimulant, carminative, astringent and aphrodisiac. It is used in tonics and electuaries and forms a constituent of preparations for dysentery, stomach-ache, flatulence, nausea, vomiting, malaria, rheumatism, sciatica and early stages of leprosy. Higher doses have a narcotic effect. Delirium and epileptic convulsions are found to occur. Mace is also used similarly. It is chewed for masking foul breath (Anonymous, 1962).

Dried nutmeg and mace are used as spices. The essential oil and the oleoresin are the major products of interest from the spice (Purseglove *et al.*, 1981).

Nutmeg and mace are used as spice and also as medicine. They have applications in bakery products, soups, preserves, sauces and diary products. In India, both the spices are used as drugs. They are stimulants, carminative, astringent and aphrodisiac. These spices are used for the treatment of stomach-ache, dysentery, flatulence, nausea, malaria, rheumatism and vomiting. Mace is used as a mouth-freshener. It prevents dental caries. Alcoholic extracts of nutmeg have antimicrobial activity. The leaf essential oil has herbicidal properties and is used for preparing soaps and chewing gum. The rind of the fruit is used in pickles, jams and jellies.

Due to the strong aroma, the essential oil can be used as a natural flavouring extract (Krishnamoorthy & Rema, 2001). Nutmeg fat is used as a mild external stimulant (Purseglove *et al.*, 1981).

Alcoholic extracts of nutmeg have antibacterial activity against *Micrococcus pyogenes* var. *aureus.* Aqueous decoctions are toxic to cockroaches. Myristicin is used as an additive to pyrethrum to enhance its toxicity against house-flies. The leaf essential oil has weedicidal properties. It is also used for making soaps, dentifrices, chewing gums and tobacco. Nutmeg pericarp is used in pickles and jellies. Half-ripe fruits are candied in Malaya (Anonymous, 1962).

2.2.9 Medicinal Properties of Nutmeg and Mace

The *in vitro* inhibition of prostaglandin biosynthesis by essential oils and phenolic compounds was studied (Wagner *et al.*, 1986). Essential oils containing eugenol and thymol + carvacrol, eugenyl acetate, capsaicin, curcumin and carvacrol were found to inhibit prostaglandins.

β-caryophyllene, β-caryophyllene oxide, α-humulene, α-humulene epoxide and eugenol were found to induce Glutathione-S-Transferase (GST) in liver and small intestine of mouse. These sesquiterpenes induce the detoxifying enzymes and are thus potent anti-carcinogenic agents. Their activity is comparable with that of chemical carcinogens (Zheng *et al.*, 1992).

Eugenol and isoeugenol from nutmeg were the most active principles on rabbit platelet function (Rasheed *et al.*, 1984). Some activity was also shown by safrole, myristicin, elemicin, limonene, alpha-terpineol, terpinene-4-ol and linalool.

When the effects of dietary administration (3.9 mg/day for 17 months) of essential oil on the polyunsaturated fatty acid composition of the retina of aged rats were studied, their levels were increased (Recsan *et al.*, 1997). Thus there is a possible relationship between the antioxidant properties and the prevention of age related macular degeneration.

Sait & Satyaputhra (1995) have worked out the effect of deterpenation on the medicinal quality of nutmeg oil. Deterpenation was carried out by alcoholic extraction or chromatographic separation. Alcoholic extraction gave complete removal of monoterpene hydrocarbons whereas chromatographic separation gave only partial removal. Deterpenation caused a downgrading of the medicinal quality of the oil. This was due to an increase in the myristicin concentration.

Janssens *et al.*, 1990, have identified the most active constituent as inhibitors of platelet aggregation from nutmeg oil. It showed that, medicinally, nutmeg oil and nutmeg powder could be replaced by eugenol and isoeugenol.

Flavonoids from several plants including *M. fragrans* were found to have hypolipidaemic activity (Koshy & Vijayalakshmi, 2001).

The possible insulin function effects of several herbs, spices and medicinal plants were evaluated by Broadhurst *et al.* (2000). It was found that cinnamon was the most bioactive product followed by witch, hazel, green & black teas, allspice, bay leaves, nutmeg, clove, mushroom etc. This glucose-oxidation enhancing property was lost when treated with polyvinylpyrrolidone (PVP) treatment. This shows that the active compound may be phenolic in nature. This study shows the specific effects of plant extracts on insulin activity.

2.2.10 Antimicrobial Activity of Nutmeg and Mace

Two antimicrobial resorcinols, namely, Malabaricone B [1] & Malabaricone C [2] were isolated from mace. These compounds showed strong antifungal and antibacterial activities that were reduced by structural modifications by methylation (Orabi *et al.*, 1991). The phenylpropanoids including myristicin, elemicin and *trans*-isoelemicin also showed antifungal activity (Marston *et al.*, 1995). Nutmeg exhibited potent activity against *Bacillus subtilis, E.coli* and *Saccharomyces cerevisiae* (De *et al.*, 1999).

The ability of citronellal, alpha-terpinene and terpineol in the control of the mite, *Acarapis woodi* was reported by Calderone *et al.* (1991). The nematicidal activity of *Myristica fragrans* against *Meloidogyna incognita* was reported by Gotke *et al.* (1990). Their study revealed mace oil to be the most effective antimicrobial agent followed by nutmeg oil.

2.2.11 Antioxidant Activity of Nutmeg

When the antioxidant property of many essential oils was studied, the oil from *Myristica fragrans* was among the most effective antioxidants in both the egg-yolk assay and in the chick-liver assay (Dorman *et al.*, 1995).

Dorman *et al* (2000) studied the antioxidant property of nutmeg essential oil using an antioxidant assay and demonstrated an antioxidant capacity superior to the synthetic antioxidants (like Butylated Hydroxy Anisole, Butylated

Hydroxy Toluene, etc). When the antioxidant property of nutmeg was studied, it was shown to prevent the oxidative bleaching of β-carotene.

The total antioxidant status of essential oils from medicinal plants was studied for their free-radical scavenging capacity (Mantle *et al.*, 1998). The antioxidant capacity was determined by three different procedures. Their results demonstrate that the apparent antioxidant capacity depends entirely on the assay method employed and the particular free radical species generated. They also caution us against the use of a single assay method. The antioxidant activity of essential oil from various spices was also studied by Dang *et al.* (2001). In this study, the peroxide value of different spices increased during storage. They have demonstrated that the application of the sample in the form of extract produces better antioxidant effects in comparison with the use of essential oils. Eugenol was also shown to have antioxidant activity (Kumaravelu *et al.*, 1996). The antioxidant effect of eugenol on Carbon tetrachloride (CCl_4) – induced erythrocyte damage in rats was studied. When eugenol (10.7 mg/kg body weight/day) was administered along with CCl_4 to rats, it protected the functional integrity in erythrocyte. Eugenol from nutmeg inhibited the accumulation of lipid peroxidiation products in RBC. It also maintained the activity of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase, glutathione reductase and glucose-6-phosphate dehydrogenase at normal levels. Carbon tetrachloride induces an increase in membrane fluidity and alters the activities of membrane-bound enzymes like Na^+-K^+-ATPase, NADH-dehydrogenase and Ca^{2+}-ATPase. These effects were decreased by eugenol. Thus it protects the membrane from free radical attack.

2.2.12 Composition of Nutmeg and Mace

The following composition has been given for nutmeg (Anonymous, 1962). 14.3% moisture, 7.5% protein, 36.4% ether extracts, 28.5% carbohydrates and 11.6% fibre. It has a mineral content of 1.7% with 0.12% calcium, 0.24% phosphorous and 4.6 mg% of iron. The essential oil has a range of 6 to 16%

and a starch content of 14.6 to 24.2%, 2.25% pentosans, 1.5% furfural and 0.5% to 0.6% pectin. Thus the principal constituents of nutmeg are the fixed oil, essential oil and starch. The flavour and medicinal action are due to the essential oil. According to the specification of the Health Ministry, Government of India, nutmeg spice shall contain not greater than 5% of total ash, 10% crude fibre and not less than 25% of non-volatile ether extract. For medicinal use, it should contain not less than 5% essential oil and not more than 3% ash.

Mace is reported to have the following composition: 15.9% moisture, 6.5% protein, 24.4% ether extract, 47.8% carbohydrate and 3.8% fibre. It has 1.6% of mineral content with 0.18% calcium, 0.10% phosphorous and 12.6 mg% of iron. It has 4 to15% of essential oil, 25% amylodextrin, reducing sugars, pectin and resins. The mace oil resembles nutmeg oil in odour, flavour and composition. Thus no distinction is made between them in trade. According to the specification of the Health Ministry, Government of India, mace should contain not greater than 10% crude fibre, 3% total ash, 5% of foreign organic matter and deteriorated material and non-volatile ether extract content of 20 to 30% (Anonymous, 1962).

Nutritive value of nutmeg and mace per 100 g is given in Table 2.1.

Table 2.1. Nutritive value of nutmeg and mace (per 100 g) (Gopalan *et al.*, 1981).

Content	Nutmeg	Mace
Moisture	14.3. g	15.9 g
Protein	7.5 g	6.5 g
Ether extraction (fat)	36.4 g	24.4 g
Carbohydrate	28.5 g	47.8 g
Fibre	11.6 g	3.8 g
Minerals	1.7 g	1.6 g
Calcium	120 mg	180 mg
Phosphorus	140 mg	100 mg
Iron	4.6 mg	12.6 mg
Vitamin B_1	0.33 mg	0.35 mg
Vitamin B_2	0.01 mg	0.42 mg
Niacin	1.4 mg	1.4 mg

Nutmeg contains 6 to 16 % essential oil, 14.6 to 24.2 % starch, 0.5 to 0.6 % pectin, 1.5% furfural and 2.25% pentosans. The essential oil in mace has a range of 4 to 15 %. It has 25% of amylodextrin, reducing sugar, pectin and resins (Pruthi, 1979).

The chemical composition of nutmeg and mace was reported (Table 2.2. and Table 2.3) by Gopalakrishnan (1992).

Table 2.2. Recovery of nutmeg, mace and rind

Total Wt. (g)	Nut with shell		Mace		Rind		Mechanical loss	
	Wt. (g)	%	Wt. (g)	%	Wt. (g)	%	Wt. (g)	%
540	86.5	16.0	12	2.2	440.5	81.5	1.5	0.3

(Gopalakrishnan, 1992)

Table 2.3. Proximate analysis of nutmeg, mace and rind

Proximate composition %	**Nutmeg**		**Mace**		**Rind**	
	FWB	DWB	FWB	DWB	FWB	DWB
Moisture	40.00	-	40.00	-	88.00	-
Acidity	-	-	-	-	2.50	20.83
Essential oil (v/w)	6.60	11.00	9.20	15.30	0.10	0.83
Nonvolatile ether extract	20.20	33.60	13.19	21.98	0.45	3.75
Starch	18.10	30.20	26.43	44.05	0.05	0.42
Sugars						
a) glucose	0.06	0.10	0.10	0.17	0.90	7.50
b) fructose	0.04	0.07	0.06	0.10	0.68	5.66
Total reducing sugars	0.10	0.17	0.16	0.27	1.58	13.16
Sucrose	0.43	0.72	0.23	0.39	0.09	0.75
Total sugars	0.53	0.89	0.39	0.65	1.67	13.92
Protein	4.30	7.16	5.95	9.91	0.64	5.3
Crude fibre	7.00	11.70	2.36	3.93	3.31	27.58

Total ash	1.54	2.57	0.94	1.56	0.89	7.42
Ash insoluble in HCL	0.12	0.20	0.09	0.15	0.07	0.58
Polyphenols*						
Total tannins	1.50	2.50	-	-	0.29	2.42
True tannins	0.60	1.00	-	-	0.11	0.92
Pectin	-	-	-	-	1.69	14.10

FWB – Fresh weight basis, DWB – Dry weight basis *Lowenthal-Procter method as quercitannic acid ; (Gopalakrishnan 1992)

Fresh ripe pericarp has an acidic, astringent, aromatic juice. Fruit rind, on analysis, gave 86.8% moisture, 1% protein, 0.4% ether extract, 11.2% carbohydrates, 0.6% mineral matter with 0.04% calcium, 0.01% phosphorous, 2 mg iron and 8 i.u/100 g carotene (Anonymous, 1962).

M. malabarica kernel has high phenolics which can be used as an antioxidant for the protection of oils and fats against rancidity. The kernel has 41 to 43% ethyl acetate extract, half of which is soluble in oil. This oil-soluble resin gives effective protection against rancidity at a concentration of 0.008%. It has been proved that this resin is as effective as butylated hydroxy toluene (BHT), one of the best edible fat antioxidants in use. The ripe fruits are called Bombay nutmeg and Bombay mace that are used as adulterants of *M. fragrans.* They have no odour and taste. The following composition has been reported for Bombay nutmeg: 6.9% moisture, 40.76% fat and resin, 6.5% protein, 42.18% carbohydrate, 2.33% fibre and 1.33% ash content. The seeds are used in external application for indolent ulcers. Its crude fat is used as an embrocation in rheumatism, soars and pain. It is also used as an illuminant (Anonymous, 1962).

The following chemical composition has been reported for the mace of *M. malabarica ;* 4.07% moisture, 63.26% fat and resins, 7.31% protein, 20.80% carbohydrate, 3.06% fibre and 1.50% of ash content. It has an essential oil

content of 0 to 0.67%. Its fat is similar to that from the kernel. The resin has antioxidant properties, though less active than that of the kernel (Anonymous, 1962).

The following results were obtained when Khanum *et al.* (2001) studied the proximate composition and mineral content of spices. The moisture, protein, fat, total dietary fibre, carbohydrate, energy and essential oil contents of eight spices (aniseed, cardamom, cinnamon, cloves, cumin seeds, nutmeg, pepper and turmeric) were studied. The moisture content had a range from 5.42% in turmeric to 9.84% in cinnamon. The protein content ranged from 2.6% in pepper to 17.7% in cumin seeds. The fat content, on the other hand ranged from 2.2% in cinnamon to 24.35% in nutmeg. The carbohydrate content had a range from 48.1% (cumin seed) to 74.03% (pepper). There was a range of 5% (turmeric) to 7.91% (cinnamon) in the case of total dietary fibre content, whereas the essential oil content ranged from 0.89% (cinnamon) to 14.3% (cloves). Insoluble dietary fibre was found to bind larger proportions of sodium, potassium and iron when compared to the soluble fraction.

2.3. ESSENTIAL OIL

2.3.1 Extraction and Analysis of Essential Oil

Essential oils are traditionally extracted by steam distillation. Steam is passed through a container in which the sample is kept. As steam passes through the sample into a condenser, the oil that is volatile, will come along with steam, will be cooled and liquefied. Once the oil comes out of the sample, it becomes immiscible with water and can be separated. This method is relatively harsh and may result in a modification of the components in the oil.

The quality of essential oil depends upon several aspects of analysis. (i) Confirmation of identity and quality of source, (ii) Assessment of physical characteristics of the oil and (iii) Measurement of the concentration of a particular component in the oil for which lower or upper limits have been set and

for the qualitative or quantitative analysis of oil composition which is usually obtained by gas chromatography (Waterman, 1993).

Sound nutmeg on distillation retains some of the essential oil due to their high fat content. Thus it results in low yield. Defective nutmegs are infested with pests that consume much of the fixed oil, giving better oil yields (Purseglove *et al.*, 1981).

Ehlers *et al* (1998) had compared the composition of essential oil from nutmeg and mace by supercritical CO_2 extraction and steam distillation. They found that the oils obtained by the two different methods were similar in composition. An obvious difference was observed in the composition of East Indian, West Indian and Papuan oils. The most abundant aromatic ether was myristicin in East Indian, elemicin in the West Indian oil and safrole in the Papuan oils.

Jankovsky et al., 1993, described a new method for extracting essential oils and determining their constituents. They used a continuous distillation and extraction method with subsequent analysis by gas chromatography. The spices include basil, fennel, clove, nutmeg, caraway and anis. The results were comparable with the accepted methods. The extraction of essential oil, using liquid and dense carbon dioxide, from mace and other spices, were superior in quality when compared with the conventional steam distilled essential oils (Naik *et al.*, 1988). The influence of fruit ripeness and duration of distillation on the yield and quality of nutmeg oil was studied by Hayanis *et al.*, (1975). They have found that younger fruits produced more oil when compared to the older fruits.

2.3.2 Gas Chromatography

In gas chromatography, there are two phases, a stationary liquid phase and a mobile gaseous phase that is usually nitrogen, hydrogen or helium. When the sample is injected into the GC, the mobile phase takes the sample to the column packed with the stationery phase. The components of the oil emerge from the column depending upon their volatility. Nature of the stationary phase, temperature of the column and the programme of the run also affect the separation of the components. For a particular column and set of conditions, a compound will have exactly the same time to come out of the column called the retention time. Usually it is practical to inject a standard compound along with the oil and the retention time of compounds in the sample are calculated relative to the standard used.

Compounds are detected by a flame ionization detector (FID). Here, as the various components in the sample come out of the column, they pass through a hydrogen flame where it is burnt producing a charged particle that is detected by a pair of electrodes. The magnitude of the response produced is proportional to the number of oxidizable carbons in the molecule. The quantitative response from the detector is amplified and visualized as a series of peaks on the graph. The area of each peak will give a good indication of the percentage contribution of that particular component. Thus the gas chromatography is the most reliable method for confirming the oil composition.

The extraction of essential oils by a new solvent extraction technique was studied using 1, 1, 2-trichloro-1, 2, 2-trifluorethane. GC-MS analysis showed that the composition of the products obtained was similar to that of hydrodistillation (Bernard *et al.*, 1989).

The possibility of a liquid crystal stationary phase for the gas chromatographic analysis of essential oil constituents was studied by Betts (1990). It includes the use of a liquid crystal, bis-4-(4-methoxy benzylidineanil-2-chloroanilane) or (MBCA) 2. It has the potential for the analysis of essential

oils rich in aromatic constituents; and is believed to supplement conventional phase work (Betts, 1990).

2.3.3 GC-MS

When the compounds are unknown, they are identified by linking a gas chromatograph with a mass spectrometer (MS). In mass spectrometry, the compounds are bombarded with sub-atomic particles and the mass of the charged particles produced is determined. The bombardment causes a fragmentation pattern that is characteristic for a given compound. GC-MS systems have large libraries of fragmentation patterns, which facilitate easy identification of the component (Waterman, 1993).

Gas chromatography plays an important role if it is combined with any instrumental technique that can accept gaseous or volatile liquid samples and is compatible in speed. Both mass spectral and infrared instruments, which produce a spectrum in few, have been used in combination with gas chromatography. Both mass spectrometry and infrared spectrometry consists in recording a spectrum of the solute as it leaves the column.

In case of infrared spectrometry, effluent gas stream can be led directly through a gas cell in a rapid scan or interferometric spectrometer. Rapid scan technique has been used to yield the entire spectrum of a fraction during the brief period of its appearance at the end of the column.

The combination of gas chromatography with mass spectral techniques is especially useful. This is because the mass spectrometer is at its best when it is presented with small gas samples; the affluent from the GC consists of just such samples. It is useful for determining the molecular mass of compounds. It provides information regarding the fragmentation process. It is useful for determining the concentration of components in a mixture. The technique is very sensitive and has high precision (Sharma, 1995).

2.3.4 Nutmeg Essential Oil

The yield of essential oil has a range from 6 to 16% based on the origin and quality of the spice. Wormy nutmegs give a better yield of essential oil. The sample is comminuted, pressed to remove fixed oil and subject to steam distillation. The essential oil is colourless or pale yellow coloured with the characteristic spicy odour. The aroma of East Indian oils is believed to be more pronounced and characteristic of the spice than the West Indian oils. West Indian oils have lower specific gravity and refractive index with higher optical rotation. *d*-pinene, β-pinene, dipentene, *p*-cymene, *d*-linalool, terpinen-4-ol, *dl*-α-terpineol, geraniol, safrole, eugenol, isoeugenol, an aldehyde with citral odour, myristicin (3-methoxy-4, 5-methylenedioxy-1-allylbenzene), myristic acid and esters of myristic acid and other fatty acids are the major constituents of oil (Anonymous, 1962).

Nutmeg oils are classified as East Indian (Indonesia) or West Indian (Grenada and St. Vincent) based on the origin of nutmegs used. They are distinct in odour, flavour, chemical and physical properties (Purseglove *et. al.* 1981). When the composition of nutmeg oils from different locations was studied, they showed a variation in its composition (Baldry *et al.*, 1976). The specific toxicity of nutmeg essential oils was reported by Woolf (1999).

2.3.5 Mace Essential Oil

It has 4 to15% of essential oil. The mace oil resembles nutmeg oil in odour, flavour and composition. Thus no distinction is made between them.

2.3.6 Leaf Essential Oil

The essential oil from leaves have a yield of 0.41-0.62% with a pleasing spicy odour. It has a specific gravity of 0.8642 and an ester value 8.44. The yield of steam-distilled oil from dried East Indian leaves is 1.56%. It is colourless with a specific gravity of 0.8772, $[\alpha]_D^{27^\circ}$ of 3.5^0, n^{26° of 1.4742. It is reported to have 80% of α-pinene and 10% myristicin (Anonymous, 1962).

2.3.7. Rind Essential Oil

The rind oil composition was studied by gas chromatography-mass spectrometry (GC-MS) (Cheong *et al.*, 1999). Though the components were similar to those in nutmeg and mace, they differed substantially in concentrations. They have reported 16 monoterpenes (60%), 9 monoterpene alcohols (29%), 8 aromatic ethers (7%), 3 sesquiterpenes (1%), 6 esters (1%) and 8 other minor components. The concentrations of sabinene, myristicin and safrole were much lower while the terpinen-4-ol and α-terpineol contents were found to be higher when compared to nutmeg and mace oils.

2.3.8 Bark Essential Oil

The bark yields 0.14% of essential oil with the following properties. $d^{26^{o}}$, 0.871, $[\alpha]_D$ 12.2^0, and an ester value of 37.5. A variety of kino is obtained from injuries made in the bark (Anonymous, 1962). This oil appears to be devoid of aldehyde (Verghese, 2001c).

2.3.9 Flower Essential Oil

Flower oil from nutmeg has a pleasant fragrance that is distinctly different from other nutmeg oils (Verghese, 2001c). The oil is found to have α-pinene (9.51%), camphene (0.49%), sabinene + β-sabinene (14.05%), myrcene + α-phellandrene (4.42%), α-terpinene (3.67%), p-cymene (4.55%), limonene (4.4%), β-phellandrene + 1,8-cineol (4.41%), terpinolene (0.48%) and β-caryophyllene (0.1%). The alcoholic constituents in the flower oil included linalool (3.95 %), β-terpineol (0.36%), borneol (0.14%), terpinene-4-ol (11.63%) and 5.88% of α-terpineol (Verghese, 2001c).

2.3.10 Uses of Essential Oil

2.3.10.1 Confectionery

Essential oils are used for flavouring food products and liquors. It replaces ground nutmeg to avoid leaving particles in foods and beverages. Thus it is

used to flavour baked items, beverages, candies, meats and syrups.

2.3.10.2 Cosmetics

It is used for scenting soaps, tobacco, dental creams and perfumes. It is mildly counter irritant and is used in ointments, hair lotions and cosmetics. It is used in the cosmetic industry for a spicy odour.

2.3.10.3 Pharmaceuticals

Nutmeg oil is mainly used in the pharmaceutical industry. It is used to treat illnesses ranging from the nervous system to the digestive system. It is used for the treatment of urinary tract infections. Now the essential oil is used by many pharmaceutical companies in their formulations. In 1992, Procter and Gamble produced an alcohol free cough syrup with nutmeg oil as a major ingredient. This oil is used to clear congestion and in pain relieving ointments (Anonymous, 1962; http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/v4084e/v4084e07.htm)

2.3.11 Physical characteristics of essential oil

Most mono and sesquiterpenes have optical activity while phenylpropenes usually do not exhibit optical activity. But the oil as a whole has optical activity. Refractive index and specific gravity are also useful indicators of oil quality (Waterman, 1993).

Oil	*Specific rotation (^{0})*	*Refractive index*	*Weight (g) per ml*
Nutmeg (E. Indies)	+10-+25	1.475-1.488	0.885-0.915
Nutmeg (W. Indies)	+25-+45	1.472-1.477	0.860-0.880

Konig *et al.*, (1992) studied the enantiomeric composition of monoterpene hydrocarbons in the essential oil of *M. fragrans*.

2.3.12 Chemistry of Essential Oil

Essential oil is a complex mixture composed of terpenes and phenylpropenes. The constituents of the essential oil determine the characteristic odour and flavour properties of nutmeg and mace. Monoterpene hydrocarbons with small amounts of oxygenated monoterpenes and aromatic ethers are the major constituents of both nutmeg and mace oils (Purseglove *et al.*, 1981). Sabinene, myristicin, safrole and elemicin constitute the greater part, i.e., 80% of both these oils. East Indian and West Indian oils can be distinguished clearly on organoleptic ground. The organoleptic properties of essential oil depend upon the composition of various components, i.e., the relative proportion of individual monoterpenes and aromatic ethers. The West Indian oils have considerable amounts of α and β-pinene together with sabinene (40-50%). They are low in α-pinene, safrole and myristicin with higher amounts of sabinene whereas the East Indian oils have higher amounts of myristicin. (Purseglove *et al.*, 1981). The composition of the West Indian mace oil obtained by CO_2 extraction gave 13.2% of sabinene, 6.6% of α-pinene, 1.9% of myristicin and 4.2% of elemicin (Moyler, 1993). On the other hand, mace oil, as is reported by Variyar & Bandyopadhyay (1995a), contains 10.3% of α -pinene, 18.6% of β-pinene + sabinene, 18.5% of myristicin and 0.3% of elemicin. The odour of the West Indian oil is weaker, less characteristic and spicy. Hence they are considered to be inferior to the East Indian oils (Purseglove *et al*, 1981).

Table 2.4. Structure and physical properties of nutmeg oil components

NAME	STRUCTURE	Boiling Point /Melting Point ºC	DENSITY
d-Pinene	CH_3 CH_3 CH_3	155-156	0.8591

Myristicin	H_3CO … $CH_2CH{=}CH_2$	149.5	1.1437
Safrole	$CH_2CH{=}CH_2$	232-234	1.096
d-Linalool	$(CH_3)_2C{=}CH(CH_2)_2C(CH_3)(OH)CH{=}CH_2$	198-200	0.8733
d- Borneol	CH_3, CH_3, CH_3, H, OH	212	1.011
i-Terpinol	CH_3, OH, CH_3, CH_3	206-207	0.9338
Geraniol	CH_3, OH, CH_3, CH_3	229-230	0.8894

Dipentene	CH_3 … CH_3 $=CH_2$	175.5-176.5	0.8402
Eugenol	OH, OCH_3, $CH_2CH=CH_2$	225	1.0664
iso-Eugenol	OH, OCH_3, $CH=CHCH_3$	266	1.080
Camphene	CH_2 CH_3 CH_3	m.p.52	0.8486

(http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/v4084e/v4084e07.htm)

The gas chromatographic analysis of nutmeg and mace oils by Gopalakrishnan (1992) showed that α- pinene, β-pinene and sabinene together constituted 77.38% in nutmeg and 60.76% in mace oil. The constituents imparting the typical spice flavour, viz., the monoterpene alcohol, their esters and aromatic compounds were also found to be at appropriate levels in both the oils. He also noted that the concentration of myristicin and elemicin appears to be very high in Indian oils. But the study by Mallavarapu & Ramesh (1998) classifies Indian nutmeg oils to be intermediate in quality between East Indian and West Indian oils. Their investigation of essential oil by capillary GC and GC/MS showed that the nutmeg oil contained around 76.8% of monoterpenes,

12.1% of oxygenated monoterpenes and 9.8% of phenylpropanoid ether. They also suggested a mace oil composition of 51.2% monoterpenes, 30.3% of oxygenated monoterpenes and 18.8% of phenylpropanoid ethers. Since the essential oil composition of nutmeg and mace from various sources are not consistent, Verghese (2001a) has described a general compositional pattern for them. The pattern shows nutmeg oil with 85 to 93% of monoterpene hydrocarbons, 6.6 to 12% of oxygenated monoterpenes and 0.3 to 5% of aromatic ether. Mace oil, on the other hand, has 75 to 95% of monoterpene hydrocarbon, 4 to 18% of oxygenated monoterpenes and sesquiterpenes and 0 to 6% of aromatic ethers. The yield of essential oil from various spices including nutmeg was also described by Lewis *et al.* (1976).

In leaf essential oil, Madhavan *et al.* (1991) have described the myristicin and elemicin content to be low in the female when compared to the male tree. They also described myristicin to be the major hallucinogenic principle in nutmeg and mace whereas elemicin has been considered to be the major hallucinogenic compound in leaf and flower oils.

Myristicin has also been reported to be the major (70%) component of *Portenschlagia ramosissima.* This oil also includes elemicin, methyl eugenol, α- and β- pinenes, γ- terpinene, thymol and thujol (Bohannon & Kleiman, 1977). When the volatile constituents from parsley cultures were studied, myristicin along with p-mentha-1, 3, 8-triene and β-elemene were the chief constituents obtained in the callus culture (Gbolade & Lockwood, 1989).

The toxicity of spices containing methylene dioxybenzene derivatives was described by Buchanan (1978). Besides nutmeg and mace, the review includes black pepper, parsley, tarragon, fennel, basil, dill, cloves, cinnamon, chilly, paprika, anise and allspice. Archer (1988) has determined the safrole and myristicin content in nutmeg and mace by HPLC

Some of the uses of essential oil components are as follows.

1. d-Pinene is used in the manufacture of camphor, solvents, perfume bases and synthetic pine oil.
2. Myristicin is the most studied compound from nutmeg due to its pharmacological properties.
3. Safrole is used in perfumery, soap industry and as an antiseptic.
4. Geraniol is used in perfumery.
5. d-linalool, also called coriandrol, is used in perfumery.
6. Eugenol is used in the manufacture of vanillin. (http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/v4084e/v4084e04htm).
7. Myrcene from hop essential oil was found to accelerate the oxidation of α-acids during storage of beer (Hautake & Petricek, 1972).

2.3.13 Terpene

Plants have a wide range of isoprenoid compounds with a variety of structures and functions. Some of them like the steroids are primary metabolites whereas the majority is synthesized as secondary metabolites that are unique to plants. The high concentration of these compounds in turpentine oil gave the alternate name 'terpenoid' to these compounds. These compounds are known since antiquity as ingredients of soaps, perfumes, flavourings and as food colorants (Bramley, 1997).

The terpenoids are composed of C_5 isoprene units. The terpenoids are formedby polymerization of the isoprene by various cyclizations, rearrangements, additions and even deletions of carbon atoms from linear arrangements of isoprene units (Bramley, 1997).

2.3.13.1 General pathway of terpenoid biosynthesis

Plants produce chemicals that are characterized by a limited distribution which have no known function in the plant. These are called secondary metabolites.

The secondary metabolites are synthesized from one of the three pathways known as the acetate, mevalonate and shikimate pathways. The terpenes are synthesized by the mevalonate pathway while the phenylpropenes are produced by the shikimate pathway (Waterman, 1993).

The terpenoid pathway was originally elucidated from the studies in animals and yeast. But only in plants do the side branches occur to give the characteristic terpenoids (Bramley, 1997).

Two acetyl-CoA molecules combine to form a molecule of mevalonic acid (C_6), which combines to form the biologically active isoprene, isopentenyl pyrophosphate (IPP), which further isomerizes to dimethyl allyl pyrophosphate (DMAPP). DMAPP combines with IPP producing geranyl pyrophosphate (GPP). Another molecule of IPP adds on to GPP forming farnesyl pyrophosphate (FPP). Further addition of a molecule of IPP to FPP gives geranyl geranyl pyrophosphate (GGPP) (Bramley, 1997).

The **monoterpenoids** are the simplest class with a C_{10} skeleton. They are components of the essential oil and have applications in perfumes, food flavourings and pharmacology. Monoterpenoids are further classified as acyclic, cyclopentanoid, cyclohexanoid and irregular monoterpenes, e.g. Camphane, pinene, thujene, carene, fenchane etc (Bramley, 1997).

Sesquiterpenoids are formed from three isoprene units (C_{15}). They have a wide range of biological properties such as insect antifeedant, insect juvenile hormone, pheromones, phytoalexines, mycotoxins, antibiotics and plant growth regulators. Sesquiterpenes include curcumene, humulene, caryophyllene and α- cadinene. They are generally less volatile and less organoleptic than monoterpene. Yet, they are an essential part of the essential oils (Bramley, 1997).

Diterpenoids with C_{20} skeletons include gibberellins (GAs). About 90 GAs have been identified till now of which 79 are found in higher plants and 27 in fungi. Gibberellins can be divided into two groups. Those that retain the full C_{20} atoms of (–)-*ent-* kaurene (C_{20} GAs) e.g. GA_{13} and those that have lost one carbon atom with the formation of a 19, 10-γ-lactone structure (C_{19} GAs) e.g. GA_3 (Bramley, 1997).

The prefix '*ent*' is used for enantiomeric structures and it reverses the stereochemical designation at each chiral center. Gibberellins are produced in minute quantities with the highest concentration in embryos or endosperm. They act as regulators of developmental processes such as stem elongation, fruit and seed development, seed germination and dormancy. They control the internodal elongation in stems. The C_{20} GAs are more active than the C_{19} GAs.

The first committed step in the biosynthetic pathway is the conversion of geranyl geranyl pyrophosphate into *ent*-kaurene. This is a two-step cyclization reaction catalyzed by *ent*-kaurene synthase. It is then converted to *ent*-kaurenoic acid by three sequential oxidations at the C-19 of *ent*-kaurene. Ent-kaurenoic acid is hydroxylated to 7-hydroxy-kaurenoic acid catalyzed by kaurenoic acid hydroxylase. Ent-kaurenoic acid then gives rise to GA_{12} aldehyde. The steps till the formation of GA_{12} aldehyde are identical in both higher plants and fungi, but the pathways differ beyond GA_{12} in the number and position of the hydroxy groups introduced before oxidation of the C_{20} to give the G_{19} GAs (Bramley, 1997).

Triterpenoids have a C_{30} skeleton. They include phytosterols, saponins, cardenolides etc. They are formed by the head-to-head condensation of two FPP molecules forming squalene that is the precursor of all triterpenoids. About 4000 different molecules have been isolated with 40 skeleton types. Pentacyclic ring is the most common structure (Bramley, 1997).

Phytosterols are characterized by a 3 β-monohydroxy per hydro-1, 2-cyclopentanophenanthrene ring systems. These sterols are often esterified by a fatty acid at the C-3 hydroxyl group. This esterification process allows regulation of the amount of free sterols in membranes by sub-cellular compartmentation (Bramley, 1997). Most plants produce sterols that are alkylated at C-24, e.g. sitosterol, stigmasterol etc. Cholesterol is seen only in minute quantities. A combination of chromatography (GC, HPLC), mass spectrometry and NMR is used for the purification and identification of sterols. Sterols are synthesized from squalene. Formation of squalene from HMG-CoA involves the same steps in animals, plants and fungi. Squalene epoxidase catalyses the conversion of squalene into its 2, 3-epoxide. It then undergoes cyclization to polycyclic triterpenoids that are specific for different species. This reaction is catalyzed by squalene-epoxide cyclases. There are two types of cyclases (Bramley, 1997). (i) this group is responsible for cyclization of the chair-boat-chair-boat folded tetracyclic triterpenoids such as lanosterol and cycloartenol and (ii) here the cyclase produce β-amyrin through squalene epoxide having a chair-chair-chair-boat conformation.

Sterols have a role in plant membranes, analogous to cholesterol in animal cells. Plasma membrane has the highest sterol content as well as the highest sterol: phospholipid ratio. Chloroplast membranes have only a small amount of free sterol. Sterols are important for the growth of the plant. The cell has two sterol requirements, one for bulk sterol for new membrane production and the other for a 24-ethylsterol for a specific, stimulatory function. Cycloartenol is the plant equivalent of cholesterol and is believed to be the precursor of lanosterol (Bramley, 1997).

Saponins are water-soluble glycosidic triterpenoids abundant in the plant kingdom. They form stable foams in water. Saponins include three classes 1). Steroid glycosides, (2).Steroid alkaloid glycosides, (3). Triterpene glycosides. All these classes have one or more linear or branched carbohydrate chains attached to an aglycone called sapogenin. The sugar residues are later added

to the aglycone as ether or ester linkages. The function of saponins is not well understood. They protect the plant against fungal attack. They lyse the erythrocytes and this property is used for the detection. They have low oral toxicity to humans, probably due to low absorption (Bramley, 1997).

Cardenolides are C_{23} steroid derivatives with cardiac activity. They are also called cardiac glycosides. Some of them are seen in insects also. Digitoxin from *Digitalis* is used as a heart stimulant (Bramley, 1997).

Polyterpenes of high molecular weight are widely seen in the plant kingdom, e.g. natural rubber.

Sesterterpenoids include a group of compounds with C_{25} carbon atoms. They are formed by the extension of GGPP by condensation with IPP to form geranyl farnesyl pyrophosphate. This is the parent compound of this class. They are found along with diterpenoids in fungi, lichens, seaweeds and higher flowering plants (Bramley, 1997).

Sait & Hutajulu (1995) have tried to improve the quality of leaf essential oil by chemical deterpenation method. The leaf oil was deterpenated by two methods; i) a method based on the formation of water-soluble alkali–phenolates to separate the water insoluble non-phenolic constituents; and ii) a method based on the extraction of soluble oxygenated constituents in dilute ethanol to separate the insoluble terpenes from the oil. The former method gave better results.

2.3.13.2. Site of terpene biosynthesis

It occurs in the secretory cells at the base of the glandular trichomes in *Laviatae*. In *Umbelliferae,* oil production occurs in the oil ducts (Waterman, 1993).

The volume of the essential oil storage tissue (hypodermis) was largest under conditions of nutrient deficiency where as the essential oil content was highest at optimal nutrition. The soil type did not affect the essential oil content (Bernath *et al.*, 1975).

2.3.14 Phenylpropenes

They have a six-carbon aromatic ring with a 3-carbon side chain which always has a double bond e.g. cinnamaldehyde, safrole.

When the biosynthesis of eugenol and cinnamic aldehyde were studied, it suggested the synthesis of eugenol from phenylalanine. Phenylalanine, on the other hand, was incorporated in toto in the case of cinnamic aldehyde (Senanayake *et al.*, 1977).

The metabolic studies of volatile constituents in tissue cultures were carried out and the production of the principal volatiles was found to decline with repeated sub-culturing (Gbolade & Lockwood, 1990). β-elemene, myristicin and apiole were from the major volatiles of *Petroselinum crispum* culture. Increasing the medium sucrose concentration increased the synthesis of these compounds. Certain cultures of it were capable of reducing exogenous monoterpene aldehydes, citral and citronellal into geraniol, nerol, citronellol respectively.

The biosynthetic pathways of essential oil components were also studied by tracer experiments using ^{14}C – labelled sucrose in *Perilla frutescens* (Nishizawa *et al.*, 1989). The study was on 2 chemotypes; perillaketone and phenylpropanoid types. It was shown that perillaketone is not converted from isoegomaketone but it is synthesized directly from the hypothetical precursor egomaketone. It was also shown that elemicin is not synthesized via myristicin but from the possible common precursor, methyl eugenol.

2.3.14.1 Biosynthesis of phenylpropenes

The aromatic amino acid phenylalanine produced by the shikimate pathway is converted to trans-cinnamic acid by the enzyme, phenylalanine ammonia lyase (PAL). This pathway is common to many alkaloids, flavonoids and coumarins (Waterman, 1993).

2.4 MYRISTICIN

Myristicin is 5-allyl-1-methoxy-2, 3-methylenedioxy benzene and is about two thirds of the aromatic ether fraction. A 400-mg dose of myristicin, almost twice the amount present in 20 gm of nutmeg, produces only mild psychoactivity.

Myristicin ($C_{11}H_{12}O_3$, molecular weight 192.21) present in the oils of nutmeg and mace is identified in the following ways; on treatment with bromine, myristicin yields dibromomyristicin. Myristicin on oxidation with permanganate, gives myristinaldehyde and myristicinic acid. The same products are obtained by oxidation of isomyristicin (Guenther 1975). Myristicin, along with other volatiles, were isolated from parsley leaves by MacLeod *et al.* (1985).

Myristicin is believed to be responsible for the toxicity of nutmeg. 5 to 15 gm of nutmeg causes symptoms similar to atropine poisoning – flushing of skin, tachycardia, absence of salivation, excitation of central nervous system etc. As a methylenedioxyphenyl compound, myristicin produces type III spectrum with reduced cytochrome P-450 and can inhibit monooxygenations (http://www.erowid.org/plants/nutmeg/nutmeg_faq.shtml).

2.4.1 Toxicity of Myristicin

Jochen & Hans (2005) and Lee *et al* (2005) reported elemicin, myristicin and safrole as the major compounds responsible for toxicity

Ingestion of 5 or more grams of nutmeg in humans causes acute nutmeg poisoning. It includes giddiness, hallucinations and feeling of depersonalization. Symptoms usually appear 3-6 hours after ingestion. Recovery occurs within 24 hours. Sometimes the duration may extend to several days and even induce death(http://www.inchem.org/documents/pims/plant/pim355.htm#SubSectionTitle:7.2. 1%20%20Human%20data).

Hallstrom & Thuvander (1997) have given the toxicological evaluation of myristicin. Myristicin is also found in several members of *Umbelliferae*. Six to seven mg/kg of body weight is enough to cause psychopharmacological effects in man. Several intoxications have been reported after an ingestion of approximately 5g of nutmeg, corresponding to 1-2 mg myristicin/kg of body weight.

The structure-activity relationship of myristicin was studied by taking four chemicals structurally similar to myristicin. They are estragole, eugenol, safrole and dihydromyristicin. Here all the compounds except dihydromyristicin contain alkenyl side chain, which is responsible for the carcinogenic activity. Structural differences on the molecules at places other than the alkenyl side chain influence metabolic pathways and hence the carcinogenic potency (http://ntp-server.niehs.nih.gov/htdocs/Chem).

2.4.2 Beneficial Effects of Myristicin

2.4.2.1 Chemopreventive property

The mechanism of cancer prevention by a well-known group of enzymes called Glutathione S-Transferase (GST) was studied. It converts toxic peroxides to less toxic alcohols. It is capable of reversible binding and transport of several toxic compounds. Many insoluble toxic compounds are conjugated to 1-chloro-2, 4-dinitrobenzene to form soluble, less toxic, compounds by GST. Thus GST protects the body from harmful peroxides and toxic carcinogens. Myristicin has been proved to induce GST in many experimental animals like mouse and rat.

Myristicin is thus a potent phytochemical with chemopreventive properties (Ahmad, 2001).

2.4.2.2 Antioxidant property

Repeated administration (100 mg/kg/day) of myristicin and dehydrodisoeugenol (mono and dimeric phenylpropanoids from mace) suppressed lipid peroxidation and normalized superoxide dismutase activity in mice (Hattori *et al.*, 1993)

2.4.2.3 Antimicrobial and insecticidal property

Sarisan, an isomer of myristicin with antifungal properties was isolated from *Heteromorpha trifoliata* (Villegas *et al.*, 1988). Myristicin, apiol and dill-apiol were found to be toxic to insects and synergised the insecticidal activity of synthetic insecticides (Lichtenstein *et al.*, 1974). The insecticidal property of myristicin is believed to protect many plants from the insect attack (Stahl, 1981).

2.4.3 Metabolism of Myristicin

There are several possible pathways for its metabolism. It depends upon the dose, species, sex, extent of exposure, etc. One of the pathways is the cleavage of the methylenedioxyphenyl group through 4-oxidation producing a compound with two hydroxy substituents on the benzene ring. It then undergoes glucuronidation and excretion. The methylene group is exhaled as carbondioxide.

Methylenedioxyphenyl compounds interact with liver enzymes. Mixed function oxidation is briefly inhibited followed by the induction of cytochrome P-450 enzymes. These enzymes convert methylenedioxybenzene compounds to their reactive forms and they interact to form ligand complexes. The oxidation of the allyl chain to epoxy on hydroxy derivatives enhances their affinity for cytocrome P-448. The rate and extend of oxidation of the allyl side chain on myristicin determines its carcinogenic potential (http://ntp server.ni ehs.nih.gov/htdocs/Chem).

2.5 HALLUCINOGENS

They are a chemically diverse group of drugs that change the mood, thought, perception and brain function. Some of these drugs are synthetic while others are natural compounds. The main classes of hallucinogens are as follows. (i) the LSD Family: LSD is derived from ergot, a fungus and in morning-glory seeds, psilocybin, some mushrooms, etc., (ii) the Phenylethylamines: It includes mescaline, elemicin, MDA, PMA, TMA, MDMA. They are chemically related to amphetamines and (iii) the atropinic drugs: The atropinic drugs or belladonna alkaloids are found in potato family, in deadly night-shade and in jimson weed (http://members.aol.com/cocorc/hallucinogens.html). Nutmeg poisoning mimics an anticholinergic hyperstimulation (Demetriades *et al.*, 2005).

LSD, Acid, green or red dragon, white lightening, blue heaven, sugar cubes, micro dots, barrels, window pane, blotter, California sunshine, mellow yellow, purple haze etc. are some of the street names for hallucinogens.

Hallucinogens produce changes in thought, perception and emotions without serious effects on the central nervous system. Hallucinogenic effects include numbness, muscle weakness, twitching, rapid reflexes, impaired coordination, increased heart rate, blood pressure, high body temperature, tremors, dilation of pupils, reduced appetite, nausea, vomiting and abdominal discomfort. It may include severe emotional depression or panic. Extreme changes in behaviour may occur. There may be irregular breathing, sweating, trembling hands, changes in sense of light, hearing, touch, smell and time (http://members.aol.com/cocorc/hallucinogens.html).

There is a degree of structural resemblance between the chemical formula of myristicin and certain sympathomimetic amines. This suggests that myristicin acts as central monoamine oxidase (MAO) inhibiters. Experiments have proved that myristicin produces similar effects. It antagonizes reserpine ptosis, increases brain 5-hydroxytryptamine, both of which are changes induced by other MAO inhibiters. Myristicin and elemicin are believed to undergo

detoxication reaction similar to safrole to produce the psychomimetic drug, trimethoxy amphetamine. The recent description of a new synthetic hallucinoge n, 3 methoxy 4,5 methylene dioxyamphetamine (MMDA) is more suggestive of a psychotropic function for myristicin (http://www.erowid.org/plants/nutmeg/nut meg_journal1.shtml).

2.6 OLEORESIN

The natural isolates obtained by extracting plant material with suitable solvent and recovery of the solvent mainly by evaporation yields a residue called oleoresin. It contains all the volatile and non-volatile compounds soluble in the particular solvent. Various solvents can be used for the extraction of oleoresin. The solvents may be either polar or nonpolar in nature. All the polar constituents of the plant material will get solubilized in the polar solvent whereas non-polar compounds get extracted in the corresponding solvent (Boelens, 2000).

Oleoresin consists of both volatile and nonvolatile components. The volatile components include the essential oil constituents that are responsible for the flavouring and scent properties of the spice. The non-volatile components of the oleoresin include carotenoids, steroids, alkaloids, anthocyanins, glycosides etc. They are important for the taste, colour, texture and antioxidant properties of the product (Boelens, 2000).

The relative proportion of the essential oil and fatty oil depends upon the mode of extraction and solvent used. Oleoresins with high fat content are preferred for use in flavouring industry because they have greatest stability to heat. In perfume industry, oleoresins extracted with more polar solvents are preferred. Commercial nutmeg oleoresins have essential oil ranging from 10-19 % whereas mace oleoresins have essential oil content from 10-55 % (Purseglove *et al.*, 1981).

In vitro mutagenecity tests on nutmeg oleoresins along with capsicum pepper and shallot were carried out by Damhoeri *et al.* (1985). All the oleoresins were mutagenic with the highest activity in mace. Myristicin is believed to be responsible for the mutagenic effect.

2.7 FAT

2.7.1 *M. fragrans*-Nutmeg

Nutmegs contain 25-40 % of fixed oil otherwise called *oleum myristicaceae expressum.* It is a highly aromatic, orange-yellow coloured fat with the consistency of butter at room temperature. It has the odour and taste of nutmeg. It is composed of mainly trimyristin with a high proportion of essential oil. It is an ester of glycerol and tetradecanoic acid (myristic acid). The other major sources of trimyristin are coconut oil and palm kernel oil (Purseglove et. al., 1981, Anonymous, 1962, http://www.fao.org/documents/show_cdr.asp?url_ file= /docrep/v4084e/v4084e04.htm).

It is a semi solid with the smell and taste of nutmeg. It melts at 45^{0}C and has a density of 0.990 – 0.995, an iodine value of 33-65, acid value of 10-25, saponification value 154-190 and unsaponifiable matter 8-18%. It is completely soluble in hot alcohol and sparingly soluble in cold alcohol. It is freely soluble in ether and chloroform. This ether extractable fat contains glycerides, essential oil, resin and unsaponifiable matter. Commercially, nutmeg fat is extracted from damaged and worm-eaten nutmegs. The sample is powdered and cooked out or steamed before pressing. It may also be obtained by solvent extraction (Anonymous, 1962).

The fatty acid composition is reported to be 0.4% lauric acid, 71.8% myristic acid, 14.3 % palmitic acid, 1.2% stearic acid, 4.8% hexadecanoic acid, 5.2% oleic acid and 1.5% linoleic acid. The component glycerides reported are 71.3% fully saturated (41.7% of trymyristin, 29.6% of dimyristo-palmitin), 20.5%

di-saturated mono-unsaturated and 8.2% mono-saturated di-unsaturated (Anonymous, 1962).

These fixed oils have the same odour of nutmeg and hence used in perfumes. Nutmeg fat is used as a mild external stimulant in ointments, hair lotions, plasters. It is also used externally for sprains, paralysis and rheumatism. Trimyristin is used as a raw material for myristic acid. As a saturated fat, trimyristin is a possible substitute for cocoa fat. It is mixed with cottonseed oil and palm oil to produce an edible fat compound. It is also used as a tablet lubricant. Nutmeg fat is used in the manufacture of perfumes, soaps and candles. .It is sometimes substituted by fats from other *Myristica* species. (Anonymous, 1962; http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/v4084e/v4084e04.htm).

2.7.2 *M. fragrans*-Mace

The fat from mace is reported to be similar to nutmeg fat though in a much smaller amount. Twenty-six percent of fat is obtained from mace with the following characteristics; density of 0.9884, acid value of 3.4, saponification value of 108, an iodine value of 153-57 and 35% of unsaponifiable matter. The refined oil obtained after the removal of volatile and resinous matter had a density of 0.9769, saponification value of 161-62 and an iodine value of 118-19 (Anonymous, 1962).

Now we will see the nature of nutmeg fat obtained from two wild nutmeg species viz., *M. beddomeii* and *M. malabarica*.

2.7.3 *M. beddomeii*

M. beddomeii nutmeg, on extraction with benzene, gives a light yellow fat (25%) with a melting point of 40^{0}C. It has an iodine value of 43 and an unsaponifiable matter of 1%. The fat contains 89% fatty acids composed of 2% palmitic acid, 60% stearic acid, 35% oleic acid and 3% linoleic acid. It has 88% of di-

saturated mono-unsaturated fat and 10% of mono-saturated di-unsaturated fat. This fat differs from other *Myristica* fats in containing stearic acid as the predominant fatty acid (Anonymous, 1962).

2.7.4 *M. malabarica*

M. malabarica nutmeg contains 15-16% neutral fat with melting point 31-31.5^{0}C $n^{40°}$, 1.4580-1.4593. It has a saponification value of 189-191. The components of the mixed fatty acids are 39.2% myristic acid, 13.3% palmitic acid and 2.4% of other saturated fatty acids. It has an oleic acid content of 44.1% and 1% of linoleic acid. Analysis of a purified fat sample gave the following fatty acid composition: 69% myristic acid, 10% palmitic acid and 21% oleic acid. The fat has the following glyceride composition: 57% tri-saturated, 33% di-saturated mono-unsaturated, 8% mono-saturated di-unsaturated and 2% tri-unsaturated molecules (Anonymous, 1962).

2.8 PHENOLIC ACIDS

The functional aspects of phenolics must be considered while distinguishing primary and secondary compounds (Strack, 1997). By virtue of the secondary compounds, plants have evolved to a wide range of species in different habitats. Phenolics for example, form an integral part of polymeric materials like lignin and suberin that give protection against microbial attack (Strack, 1997). Anthocyanins along with flavones and flavonols as co-pigments attract animals for pollination and seed dispersal. Phenolics are also found to accumulate during stress. UV light has been found to induce the accumulation of UV light-absorbing compounds in plants. Phenolics are effective deterrents where they interfere with the digestion through interaction with the intestinal flora. Phytoalexins are induced by microbial attack in plants. Among the post-infectional phytoalexins, hydroxycoumarins and hydroxycinnamates are the major compounds (Strack, 1997). Secondary compounds also have

pharmacological value. They are also important for the astringent taste. Polyphenols are also used in leather industry (Strack, 1997).

Variyar & Bandyopadhyay (1995b) have estimated the phenolic acids in various spices including nutmeg and mace. Of the total phenolic acids in nutmeg, 16.5% was found to be ferulic acid and 32.1% of the total phenolics in mace was reported to be synapic acid.

When the effect of γ- irradiation on the phenolic acids of spices was studied, quantitative changes were observed in the phenolic acids in nutmeg. But, in the case of mace, there was no major qualitative or quantitative change in the phenolic acids upon irradiation (Variyar *et al.*, 1998).

The essential oil content, its composition and the thin layer chromatography profile of phenolic acids from nutmeg leaves was studied (Packiyasothy *et al.*, 1991). They have reported two additional phenolic compounds from the male plants when compared with that of the female. The essential oil content of female plants was reported to be higher when compared to that of male. The essential oil composition was also reported to be different. They have concluded that the TLC profile of phenolic acids could be used to determine the sex of *M. fragrans* seedlings, and the essential oil content and its composition would only be useful for determining the sex of the older plants.

2.9. CARBOHYDRATES, PROTEIN AND FATTY ACIDS

2.9.1 Carbohydrates

Carbohydrates are one of the most abundant groups of organic compounds in the plant kingdom. They exist as monosaccharides, disaccharides, oligosaccharides, polysaccharides and their derivatives. Carbohydrates form important energy reserves, which are utilized during growth and development of the plant (Avigad & Dey 1997).

2.9.1.1 Sucrose

This disaccharide is the principal product of photosynthesis. Sucrose has enormous importance as a nutrient to most living organisms. There has always been a great interest in the study of its chemistry, production, use as a raw material and as food and sweetener. But the study of its biochemistry in plants was very slow mainly because of the presence of the ubiquitous enzyme invertase that always hydrolyses any sucrose present in the medium (Avigad & Dey 1997).

Sucrose is a non-reducing highly soluble sweet sugar. It is chemically inert with proteins. Sucrose is the major product of photosynthesis and it is translocated from the photosynthetic tissues to non-photosynthetic tissues in this form. It provides energy and is the substrate for the synthesis of cellular materials and other storage forms of food. Excess sucrose accumulates in leaves and also in storage tissues. It is stored in the vacuole and forms 70% of the cell volume. It is mainly mobilized and utilized during germination and growth of the plant (Avigad & Dey 1997).

Sucrose has a role in controlling several important enzymes. Sucrose enhances the expression of a wound-inducible potato proteinase inhibitor II. It has been proved to induce the expression of several genes including β-amylase, sporamine etc. (Avigad & Dey 1997).

Sucrose esters, especially O-acylated compounds have been detected as components of the trichomes glycolipid fraction or the cuticular waxes of the leaf. A variety of acyl groups including acetyl, methylpentanoyl, methylbutyryl, methylhexanoyl etc. have been found in these structures. They are believed to be flavour precursors. These esters are also involved in plant-pest interactions (Avigad & Dey 1997).

2.9.1.2 α, α-Trehalose

This non-reducing disaccharide is widely distributed in nature. It is seen in insect hemolymph, in crustaceans, nematodes, bacteria, actinomycetes, fungi, yeast, mosses, ferns and algae. It serves as an energy reserve. It provides protection to cell viability during stress. It is found in significant levels in legume nodules. It is produced by a large number of bacterioids like *Bradyrhizobium*, *Rhizobium* and *Azotobacter* species. Trehalose is also synthesized by the mycorrhizal symbionts of vesicular plants. Its metabolism plays an important role in the process of reciprocal carbon mobilization between host and the symbiont. This interaction is modulated by many environmental and physiological factors (Avigad & Dey 1997).

2.9.1.3 Other oligosaccharides

There are mainly two classes of oligosaccharides in plants. Those that are synthesized *in vivo*, called primary oligosaccharides and those that are produced as a result of carbohydrate degradation, called secondary oligosaccharides. Maltose and its higher homologues are usually the products of starch degradation. Isomaltose and cellobiose are not part of primary oligosaccharides. Melibiose is found in the exudates, nectaries and tissues of several plants (Avigad & Dey 1997).

2.9.1.4 Trisaccharides

Raffinose, umbelliferose planteose are some of the trisaccharides studied in plants. Raffinose is seen widely in the leaves, stems and storage organs of higher plants. Its level is low in the leaves and it is translocated to the storage organs during plant growth. As the seeds mature and lose water, the concentration of raffinose increases (Avigad & Dey 1997).

Umbelliferose is an isomer of raffinose. It is ubiquitous among the storage organs, leaves and stem of umbelliferose. It is a reserve carbohydrate.

Planteose is another isomer of raffinose. It is deposited in the seeds during maturation. It is also a storage oligosaccharide that is utilized during germination (Avigad & Dey 1997).

2.9.1.5 Tetrasaccharides

The tetrasaccharides include stachyose, lychnose, isolychnose, sesamose etc. Stachyose is one of the abundant tetrasaccharides in plants. It is a higher homologue of raffinose. It is recognized as a major storage and transport sugar in woody plants, cucurbits and legumes. Stachyose present in the seeds is metabolized during germination. Stachyose and related oligosaccharides confer frost-hardiness to winter-hardy plants (Avigad & Dey 1997).

Lychnose and isolychnose are isomers of stachyose that are found in the vegetative storage organs and leaves of plants. Raffinose is the probable precursor of these tetrasaccharides. These oligosaccharides functions as reserve carbohydrates (Avigad & Dey 1997).

Sesamose is the higher homologue of the trisaccharide planteose (Avigad & Dey 1997).

2.9.1.6 Fructans

They are the most abundant storage carbohydrates in plants, next to starch and sucrose. The fructan polymers accumulate in roots, tubers and bulks of several family. Fructan oligo-and polysaccharides are also seen in fungi and bacteria (Avigad & Dey 1997).

They are linear or branched polymers of fructans. They accumulate in the vacuole and are seen as a mixture of chains of varying lengths depending on the physiological state of the tissue. Fructans are classified into the following basic structures. **Inulins** have exclusively the β-fructofuranosyl (2→1) -fructose linkage. **Levans** have mostly β-fructofuranosyl (2→6) fructose linkage. The term levan usually denotes bacterial fructans that are often branched molecules containing β-fructofuranosyl (2→1) fructose linkages. Phlein is the term used to

denote levans in plants with predominantly linear structures. The term Graminan denotes plant fructans that are highly branched containing both (2→1) and (2→6) type β-fructofuranosyl -fructose linkages. **Kestoses** are short chain fructans containing a sucrose unit (Avigad & Dey 1997).

An important function of fructans is its relationship to cold acclimatization especially in cereals and grasses. During prolonged exposure to cold temperature (3-15^{0}C) there seems to be a 20-30% of increase in fructan accumulation. They serve as an ancillary carbohydrate sink during cold season. It has also been observed that there is a reduction in the chain length of fructans during cold acclimatization (Avigad & Dey 1997).

2.9.1.7 Starch

Next to sucrose, starch is the principal reserve carbohydrate in plants. It is deposited in the plastids. Starch is an α-glucan composed of amylose with α (1→4) linkage and amylopectin with α (1→4) and α (1→6) linkages. Contrary to the classical concept, most native amylose molecules have a small number of (1→5) or α (1→6) branches clustered at the reducing end of the molecule. Starch accumulation occurs during periods of photosynthesis. When this store is filled, a major portion of it flows out of the plastid as a result of sucrose synthesis. During the dark period, chloroplast starch is rapidly broken down and the products exported into the cytoplasm with a majority getting converted into sucrose. Thus there is always an active flow and exchange of carbon between starch and sucrose (Avigad & Dey 1997).

Starch is accumulated in granules that differ in shape and size for different plant species. Structure of the granules is not well defined in the chloroplast, but they have distinct shape in the storage tissue. The starch in nutmeg resembles legume starches and the individual grains have a well-developed cracked hilum. The grains have an irregular shape and the size varies from 5 to 50μ. While in mace, the amylodextrin granules have a size of 5 to7μ. They are compound and irregular in shape with a distinct hilum (Anonymous, 1962). The

different properties of the starch granules are determined by the relative presence of amylose and amylopectin, by the length of the amylose chain, degree of branching in amylopectin etc (Avigad & Dey 1997).

2.9.1.8 Other reserve polysaccharides

Mannans, xyloglucans, α-glucan and β-glucan are the predominant reserve polysaccharides (Avigad & Dey 1997).

Mannans include four basic groups like pure mannans, glucomannans, galactomannans and galactoglucomannans. Pure mannans are composed of linear chains of 100-2000 residues of β (1→4) mannopyranosyls. They are the main reserve material in the seed endosperm of Palmae and Umbelliferae. Glucomannans contain 6-50% of β-glucosyl residues. They are present as storage material in many monocotyledons. Galactomannans are the largest and well studied group with α (1→6)-galactopyranosyl residues. Cold-water solubility increases with the increased number of galactosyl branches. In galactoglucomannans, α(1→6) galactosyl branches are linked to β-mannosyl backbone (Avigad & Dey 1997).

Xyloglucans have β(1→4) glucan cellulose type chain with short branches. These compounds are also called 'amyloid' because they stain blue with the iodine reagent for amylose. These polysaccharides are utilized during germination (Avigad & Dey 1997).

α-glucan starch is the most abundant α-glucan in plants. Floridean starch is a structural variant of amylopectin found in Rhodophyceae. It is accumulated in the cytoplasm and not in the plastid of the algae cells. Nigeran starch has alternate α (1→3) and α (1→4) glucopyranosyl linkages (Avigad & Dey 1997).

β-glucan are components of cell wall and are not used as a reserve material (Avigad & Dey 1997).

2.9.2 Protein

Saravana & Rose (2004) studied the extractability of proteins from a range of fruits with high levels of contaminating compounds such as tomato, banana, avocado and orange. When protein was extracted with different reagents like trichloroacetic acid, acetone and a phenol-based method, the phenol-based method gave higher protein yield.

Ying & Quan-Ying (2003) extracted protein from grape seeds with NaOH. Highest protein yield was obtained with NaOH at 1×10^{-1} mol/litre, at 40^0C with 40 minutes duration and a tissue: solvent ratio of 1:5.

A 24-hour dodecyl sulphate mediated protein extraction in a sonication bath followed by acetone precipitation was found to be optimal in Brassica *juncea* by Mounicou *et al.*, (2004).

Moure *et al.*, (2002) extracted proteins from defatted *Gevuina avellana* at different pH. Maximum protein extraction occurred at acidic and alkaline pH.

Wang-Wei e*t al.*, (2003) developed a protocol for isolating proteins from olive leaf utilizing acetone powder and sodium dodecyl sulphate. Various protein extraction studies were also carried out in coepea and guar (Abdalla *et al.*, 2001; Khalil, 2001) and other green plants (El-Baz *et al.*, 1999; Tangka, 2003; Gallegos-Tintore *et al.*, 2004).

2.9.2.1 Isozymes

Isozymes exist in multiple forms. They exhibit distinct chemical properties and developmental differences. Electrophoretic studies in 1950's led to the conclusive evidence that enzymes exist in multiple forms (McMillin, 1983). The term isozyme was described as different molecular forms of the enzyme with the same substrate specificity. Moreover, tissues differed in the nature of

isozymes expressed and also in the relative amount expressed. It was proved that they exhibited tissue, developmental and species specificity. By studying the changing isozyme expression during the course of a viral infection, it is possible to study the enzymes involved in the plant defence and also during cell damage (McMillin, 1983).

When genetic variability is studied in plant populations the following criteria are suggested (i) allelic expression should be distinguishable in individuals, (ii) the effect of each allelic substitution should be locus-specific, and distinguishable from substitutions at other loci, (iii) all base substitutions should be detectable and (iv) loci should be sampled at random, irrespective of their level of polymorphism (Brown & Weir, 1983).

The isozyme technique is believed to meet this criterion better than any preexisting method. Thus it is the most suited to study genetic variation.

The advantages of the isozyme technique are the following (i) allelic expression is usually co-dominant and free of epistatic or environmental effects, (ii) enzyme specificity allows attribution of alleles to loci and the comparability of loci in different populations of species, (iii) each allelic difference is detected as a mobility difference that is independent of the functional role of the enzyme and (iv) the loci sampled are frequently determined by the tissue expression, suitable extraction and availability of assays for zymograms.

Its disadvantages are the following (i) post-translational modifications of electrophoretic mobility (either genetic or environmental in origin) can occur, (ii) zymogram assays differ in specificity. Duplication of genetic material (as in polyploids) can lead to the inability to ascribe a particular variant to a single locus, (iii) only about one quarter of base substitutions result in amino acid replacements which alter the net charge on the protein and are readily detectable by routine electrophoresis, (iv) *trans* substitutions cannot be detected

and (v) the technique studies only a restricted class of proteins which may have a lesser tendency for genetic variability (Brown & Weir 1983).

2.9.2.2 Phylogenetic and systematic inferences from electrophoresis

Differences in the banding pattern are usually the result of changes in the structural genes coding for polypeptides; thus electrophoretic differences are the direct result of genetic differences. When compared with the study of morphology and the secondary chemistry, the equation between phenotype and genotype remains well defined in isozyme technique. The earlier studies with isozymes failed mainly because they studied enzymes with non-specific substrates like esterases and phosphatases (Crawford, 1983)

Allozymes (coded by alleles of the same gene) are almost always inherited as co-dominants, thus distinguishing homozygous individuals from heterozygous for a given loci (Crawford, 1983).

If the enzymes from different plants within a species appear identical under one electrophoretic condition, there is a high probability that they will remain identical under additional conditions. But identical electromorphs from different species have high chances to prove different with additional analyses (Crawford, 1983).

2.9.3 Fatty Acids

More than three hundred different fatty acids have been isolated in plants. But only a few are commonly used by these organisms as storage or membrane lipids. Saturated fatty acids with even numbered carbon atoms, like C-16, C-18 carbon atoms (palmitic and stearic acids) are predominant in plants. Most plants contain significant amounts of myristic acid and some others predominate in capric or lauric acids. Capric or lauric acids are important in the detergent and cosmetic industries and also for providing easily absorbed lipid for patients with digestive disorders. A fat with high stearate content is cocoa

fat which is important in determining the unique properties of chocolate (Harwood, 1997).

Plants being poikilothermic should contain membrane lipids that are fluid under the environmental conditions. Thus lipids should contain a large percentage of unsaturated fatty acids. Oleic acid is the most common monounsaturated fatty acid where as the polyunsaturated fatty acids like linoleic and linolenic acids are abundant as membrane lipids (Harwood, 1997).

In contrast to seeds, the fatty acid composition in leaf is highly consistent. They may also contain significant amount of unusual components. This is due to the fact that the structure and properties of membrane lipids should be carefully maintained whereas the energy provision allows some variability in the lipid composition in storage tissues. These lipids should be easily stored and broken down when required by the degradative enzymes. The structural requirements for these two properties are different and hence there is a variety of fatty acids in plants (Harwood, 1997).

2.9.3.1 Fatty acid composition of nutmeg and mace

The fatty acid composition of nutmeg and mace differs considerably in its composition (Table 2.5). Nutmeg fat contains 90.6% saturated and 8.7% unsaturated fatty acids. The fat from mace, on the other hand, has 38% of saturated and 62% unsaturated acids and thus has a lower melting point. Thus the two fats differ in the consistency and organoleptic properties. Nutmeg fat is used as a mild stimulant in ointments, in hair tonics and in plasters. It is used for treatment of rheumatism, paralysis and sprayings. It is also used for making soaps and candles (Verghese, 2001b).

Table 2.5. Fatty acid composition of nutmeg and mace lipids

Fatty acid	**% of the total lipids individual investigation**	
	Nutmeg	Mace
Lauric	1.6	-
Myristic	80.6	3.7
Palmitic	7.1	30.7
Hexadecenic	-	2.3
Stearic	0.2	1.7
Oleic	7.8	42.3
Linoleic	0.9	17.2
Linolenic	-	0.3
Arachinic	1.1	1.9
Saturated	90.6	38
Unsaturated	8.7	62.1
Saponification No. (found)	196	-
Calculated	227	198
Unsaponifiable (in the total fat)	7.8	10.9

(Verghese, 2001b).

When the lipid profile including fatty acid composition of mace was studied, it had 21.6% ether extract and 83% chloroform soluble lipids. The predominant fatty acids were palmitic and oleic acids (Prakashchandra & Chandrasekharappa, 1984).

2.10 CAROTENOIDS

They are an abundant group of naturally occurring pigments. They are responsible for the green colour of leaf and also for most of the yellow to red colours of flowers and fruits. More than 600 carotenoids have been studied.

Carotenoid hydrocarbons are called carotenes where as, their derivatives containing oxygen, are called xanthophylls. Carotenoids may be acyclic as in lycopene or contain 5- or 6-membered rings at one or both ends of the molecule, e.g., β-carotene, lutein, etc. By virtue of the extensive double bond system, these molecules can exist in a large number of isomers (*Cis/trans* isomers). Most carotenoids are found in the all-*trans* form although *cis* isomers do exist. Carotenoids are characterized by the presence of chromophore of conjugated double bonds. They vary from **3 in** the colourless phytoene to **13 in** canthaxanthin that is red. This conjugated double bond system makes them susceptible to isomerization and oxidative degradation (Bramley, 1997).

The four major carotenoids are β-carotene, lutein, violaxanthin and neoxanthin that are abundant in leaves. Minor carotenoids include α-carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin and lutein 5, 6-epoxide. During leaf senescence, the chloroplast disintegrates and esterification of the xanthophylls occurs (Bramley, 1997).

There are three main groups of carotenoids found in flower petals.
(1). Highly oxygenated carotenoids like auroxanthin and flavoxanthin,
(2). Carotenes e.g. β-carotenes,
(3). Species specific e.g. crocetin in the *Crocus*.
Flower carotenoids are frequently esterified.

In fruits, there is wide variation in the distribution of carotenoids. The biosynthesis of carotenoids in fruits is autonomous and continues even after the fruit has been removed from the plant. Unripe fruits contain the same pigments as other photosynthetic tissues. Upon ripening the chloroplasts differentiate into chromoplasts and *de novo* synthesis of carotenoids occurs. Fruits can be classified into eight groups based on the carotenoid contents. (1) With insignificant amount of carotenoid e.g. Strawberry. (2) With large amount of chloroplast carotenoids e.g. blueberry. (3) With large quantities of lycopene, its hydroxy derivatives and more saturated carotenoids e.g. Tomato. (4) With large

amounts of β-carotene and its hydroxy derivatives e.g. Peach. (5) With large amounts of carotenoid epoxides e.g. carambola. (6). With unusual carotenoids e.g. capsanthin in red pepper. (7) Poly-*cis* carotenoids e.g. prolycopene in tangerine tomatoes and (8) Apocarotenoids e.g. persicaxanthin in *Citrus* (Bramley, 1997).

The first committed step in the formation of carotenoids is the head-to-head condensation of two molecules of all-*trans* geranyl geranyl pyrophosphate via the cyclopropylcarbinyl pyrophosphate, prephytoene pyrophosphate to form phytoene. The formation of lycopene from phytoene involves four stepwise dehydrogenations. They occur alternatively to either side of the chromophore to form phytofluene, ζ-carotene, neurosporene and lycopene. The 15-15′ double bond is isomerized non-enzymatically from *cis* to *trans* configuration.

The most important function of carotenoids is photoprotection by quenching the triplet state of chlorophyll and scavenging for singlet oxygen. This is due to their ability to participate in photochemical reactions such as singlet-singlet energy, triplet-triplet energy, oxidation, reduction and isomerization. They also act as accessory light-harvesting pigments. Carotenoids are the precursors of abscisic acid (Bramley, 1997).

2.11 LYCOPENE

The scarlet colour of mace is due to the pigment lycopene. Lycopene is a carotenoid present in tomatoes, watermelon, apricots, pink grapefruit, red oranges & guava.

It is an acyclic isomer of beta-carotene with 11 conjugated and 2 unconjugated double bonds. It is mainly present in tissues as All Trans Lycopene (ATL). Lycopene is a phytochemical with potent nutraceutical properties. Its configuration enables it to inactivate free radicals. Free radicals formed during cell metabolism are highly aggressive and cause permanent damage. As an antioxidant, it is twice as potent as Vitamin A and ten times as effective as Vitamin E. Non-oxidative activity of lycopene is regulation of gap junction between cells. It participates in a host of chemical reactions that are believed to prevent carcinogenesis and atherosclerosis. Anguelova & Warthesen (2000a) have established the effectiveness of lycopene as an antioxidant. Thus the degradation of lycopene not only affects the attractive color, but also the nutritive value. Anguelova & Warthesen (2000b) have evaluated the chemical stability of lycopene in tomato powders during storage.

Materials and Methods

K.M. Maya "Biochemical variability in nutmeg (myristica fragrans) and related taxa" Thesis. Indian Institute of Spices Research, University of Calicut, 2005

Materials and Methods

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3.2.7. TLC of Flavonoids

Nutmeg, mace and leaf samples used for analysis were collected from IISR experimental farm Peruvannamuzhi, Calicut, India. The fruits were harvested at full maturity and dried. Dried nutmeg and mace were used for extracting various constituents.

3.1 PRIMARY METABOLITES

3.1.1 Carbohydrates

3.1.1.1 Phenol sulphuric acid method for total carbohydrate

Nutmeg, mace and leaf samples were homogenised in alcohol. To the dried residue, water and perchloric acid were added and centrifuged. The amount of carbohydrate present in the supernatant was estimated by phenol sulphuric acid method as described by Sadasivam & Manickam (1992).

3.1.1.2 Starch by Anthrone reagent

Starch is an important polysaccharide. It is the storage form of carbohydrate in plants abundantly found in roots, tubers, stems, fruits and cereals. Starch is a mixture of two types of components namely, amylose and amylopectin. Starch is hydrolysed into simple sugars by dilute acids and the quantity of simple sugars is measured colorimetrically (Sadasivam & Manickam, 1992).

Starch from nutmeg, mace and leaf was estimated from the alcoholic extract by the method of Sadasivam & Manickam (1992).

3.1.1.3 Reducing sugars (Nelson-Somogyi method)

Estimation of reducing sugars in nutmeg, mace and leaf samples were carried out from the alcoholic extract by Nelson-Somogyi method (Sadasivam & Manickam, 1992).

3.1.2 Protein

3.1.2.1 Kjeldahl's method

Protein was estimated from nutmeg, mace and leaf by the Kjeldahl's method as described by Sadasivam & Manickam (1992).

3.1.2.2 Lowry's method

Nutmeg, mace and leaf samples were extracted with 0.05M Tris HCl buffer, pH 7.4. Protein present in the samples was estimated by Lowry's method (Sadasivam & Manickam, 1992).

3.1.2.3 Ammonium sulphate fractionation of proteins

The solubility of proteins was markedly affected by the ionic strength of the medium. As the ionic strength is increased, protein solubility at first increases. This was referred to as 'salting in'. However, beyond a certain point the solubility begins to decrease and this known as 'salting out' (Sadasivam & Manickam, 1992).

At low ionic strengths the activity coefficients of the ionizable group of the proteins are decreased so that their effective concentration was decreased. This was because the ionizable groups become surrounded by counter ions that prevent interaction between the ionizable groups. Thus protein-protein interactions are decreased and the solubility was increased.

At high ionic strength, most of the water molecules become bound by the added ions. Thus there is not enough water molecules to hydrate the proteins. As a result, protein-protein interactions exceed protein-water interactions and the solubility decreases.

Because of difference in structure and amino acid sequences, proteins differ in their salting in and salting out behaviour. This forms the basis for the fractional precipitation of proteins by means of salt.

Ammonium sulphate was a particularly useful salt for the fractional precipitation of proteins. It was available in highly purified form, has great solubility allowing for significant changes in the ionic strength and was inexpensive. Changes in the ammonium sulphate concentration of a solution can be brought about either by adding solid ammonium sulphate or by adding a solution of known saturation, generally a fully saturated (100%) solution.

Leaf tissue was kept in 0.05M Tris buffer (pH 7.4) for one hour. The homogenized tissue was filtered and centrifuged. Ammonium sulphate was added slowly to the above filtrate. After one hour, it was again filtered and the residue dissolved in 1.5ml buffer. It was dialysed against 400ml of 0.05M Tris buffer. After centrifugation, the contents of the dialysing bag were subject to protein estimation.

3.1.2.4 SDS PAGE (Poly Acrylamide Gel Electrophoresis)

SDS (Sodium Dodecyl Sulphate) PAGE of the sample was carried out according to Hames (1994) with a 2.5% stacking gel and 7.5% separating gel. After the run, the gel was stained overnight in Coomassie Blue solution. The gel was destained in methanol, acetic acid, distilled water 3:1:6 solution and fixed in 7% acetic acid solution.

3.1.2.5 Isozyme analysis by PAGE (Poly Acrylamide Gel Electrophoresis)

Poly Acrylamide Gel Electrophoresis was carried out with the enzyme extract (Hames 1994) with a 2.5% of stacking gel and 7.5% of separating gel. After the run, the gel was stained for the specific enzyme.

i) Polyphenol oxidase (PPO)

80 mg of DOPA was dissolved in 50 ml of distilled water and the gel was incubated in the staining solution for 1-2 hours. The gel was fixed in 7% acetic acid (Holstein *et al.,* 1967).

ii) Peroxidase

The staining solution for peroxidase consists of 1.04 g of benzidine, 9 ml of acetic acid, 50 ml of 3% H_2O_2 in 80 ml of distilled water (Sadasivam & Manickam, 1992).

3.1.2.6 Total free amino acid estimation

Total free amino acid in nutmeg, mace and leaf samples was estimated by the Ninhydrin method of Yapinlee & Takahashi, (1966).

3.1.2.7 HPLC analysis of amino acids

Dry leaf sample was refluxed with alcohol; the extract was evaporated to dryness and then dissolved in amino acid diluent. The estimation was carried out in Shimadzu LC-10A HPLC Amino Acid Analyzer. The column used was Shim-pack ISC-07/S 1504 Na. The separating temperature used was 55^{0}C. The mobile phase was composed of three different buffers, mobile phase A (pH 3.2), mobile phase B (pH 10) and mobile phase C (which is alkali). The total run time was 1 hour. The mobile phase A passes through the column for the first 20 minutes when all the acidic amino acids will get separated. Then by gradient mixing, mobile phases A and B are mixed to get a range of pH and the respective amino acids will get separated based on the isoelectric pH. Towards the end (50-55minutes), mobile phase C was passed through the column to separate all the basic amino acids. The amino acids were detected only after derivatization by O-phthalaldehyde. As the amino acids come out of the column, they undergo post-column-derivatisation catalysed by two reaction solutions. They were then detected using fixed wavelength fluorescence detector, FLD 6A.

Preparation of mobile phase and reaction solution

The compositions of mobile phase and reaction solution are as given below:

Preparation of reaction reagent

Na_2CO_3	40.70g
H_3BO_3	13.576g
K_2SO_4	18.8g
Volume	1000.00 ml

Table 3.1 Composition of mobile phase and reaction solution

	Initial mobile phase (A)	Eluent (B)	Regenerator (C)
pH	3.2	10	
Sodium citrate $2H_2O$ (g)	13.72	23.52	---
Ethanol (99.5%) ml	49	---	---
Perchloric acid (60%) ml	11.67	---	---
Sodium hydroxide (g)	---	---	1.6
Boric acid	---	4.96	---
4N NaOH solution (ml)	---	12	---
Final volume	700 ml (adjust pH with perchloric acid)	400 ml (adjust pH with 4N NaOH)	200 ml

Amino acid diluent

Sodium citrate	1.96 g
Perchloric acid	1.6 ml
r-caprylic acid	10 µl

Adjust pH to 2.2, make up to 100 ml

3.1.3 Phenols

The alcohol extract of nutmeg, mace and leaf samples was used for the estimation of phenol by Folin-Ciocalteau reagent (Sadasivam & Manickam, 1992).

3.1.4 Lipid

3.1.4.1 Estimation of Fat

Fat from a known quantity of the seed was extracted with petroleum ether. The solvent was then distilled off completely, dried, weighed and the percentage of fat was calculated (Sadasivam & Manickam, 1992).

3.1.4.2 Extraction of fatty acids

Fatty acids present in nutmeg, mace and rind samples were extracted by incubating in NaOH-methanol at 70^{0}C for 2 hours. Free fatty acids were converted to fatty acid methyl esters (FAMEs) for GLC analysis by incubating in methanol-HCl (Hennessey *et al.*, 1983).

3.1.4.3 GLC of fatty acids

Methyl esters of fatty acids from fat were separated in a Perkin Elmer Autosystem gas chromatograph equipped with PE Nelson 1022 GC plus integrator. The detector used was FID. FID temperature was 300^{0}C and that of the injection port was 200^{0}C. The column used was Carbowax 20 M. The column oven temperature was programmed at 80-190^{0}C @ 24^{0}C/ minute with initial holding time of 1 minute. On reaching 190^{0}C, it ran isothermally.

3.1.5 Minerals

Minerals present in nutmeg, mace, rind and leaf were estimated by dry ashing (Tandon, 2001). Dry ashing was carried out usually at an ignition temperature of 550ºC to 600ºC followed by its extraction in dilute HCl or H_2SO_4 for determining various elements.

3.1.5.1. Phosphorus

Phosphorous was estimated by taking 30-50 ml of sample in a volumetric flask. Ten ml of vanadate-molybdate solution was added followed by 50 ml distilled water (Tandon, 2001).

3.1.6 Pectin

Pectin was extracted from rind and saponified. It was precipitated as calcium pectate by the addition of calcium chloride to an acid solution. After thoroughly washing to eliminate chloride ions, the precipitate was dried and weighed.

Fifty grams of the powdered sample was boiled for 30 minutes in 0.01 N HCl. The residue was washed with hot water and the filtrate was collected. To the residue, 0.05 N HCl was added and boiled for 20 minutes; washed and the filtrate collected. This residue was again boiled in 0.3 N HCl for 10 minutes washed and filtrate collected. The filtrates were pooled and made up to 500 ml. A suitable volume of 100-200 ml aliquot was taken and neutralised with 1 N NaOH after adding 250 ml distilled water. An excess of 100 ml 1 N NaOH was added and kept overnight. 50 ml of 1 N acetic acid was added followed by 25 ml 1 N calcium chloride solution. After one hour, it was boiled for 1-2 minutes, filtered, washed thoroughly with boiling water, dried and weighed (Sadasivam & Manickam, 1992).

3.2. SECONDARY METABOLITES

3.2.1 Essential Oil

3.2.1.1 Hydrodistillation of essential oil (Modified Clevenger Method)

The powdered sample (nutmeg, mace and leaf) was weighed and distilled with water in a round-bottomed flask to which a Clevenger trap was attached. The sample was distilled for 3 hours. The essential oil present in the sample comes out of it during boiling. It was immiscible with water. The essential oil being lighter than water forms a separate layer on the top, which can be quantified. It was then taken for further analysis (AOAC, 1975).

3.2.1.2 GLC of essential oil

The Gas Chromatographic separation of the oil samples were carried out in Perkin Elmer Autosystem Gas Chromatograph equipped with PE Nelson 1022 GC plus Integrator. The column oven was programmed as 70-220ºC @ 5ºC per min. The detector used was FID (Flame Ionization Detector) and the column was SE 30 (100% methyl silicone gum, 50-300ºC). The carrier gas was nitrogen @ 30 ml / min. The detector temperature was 300ºC and the injection port temperature was 200ºC. Identification of the compounds in the oil was done by comparing with Retention Time (Rt) of authentic standards.

3.2.1.3 GC-MS of leaf essential oil

Hewlett Packard GC attached with 5973 Mass Spectrometer was used for the analysis. The samples were analyzed in a capillary column DB 5 with 0.32 mm diameter and 0.25 µ film thickness. The carrier gas used was helium. The compounds were identified with the help of Wieley MS library.

3.2.2 Estimation of oleoresin

Oleoresin content of the sample was estimated by the method of ASTA (1968). The dry powdered sample was taken in a column; solvent was added and kept overnight. The extract was drained into a pre-weighed beaker and the solvent was evaporated to dryness. The difference in weight will give the amount of oleoresin present in the sample.

3.2.3 Estimation of non-volatile ether extract (NVEE)

The percentage of NVEE was estimated by AOAC (1975) method.

3.2.4 TLC of Phenolic acids

Five grams of the tissue was extracted with 20 ml of 2 N HCl for 20 minutes in a boiling water bath. The filtrate was then shaken with diethyl ether. The ether extract was mixed thoroughly with 5% Na_2CO_3 solution. The extract was

acidified to pH 3 with 5% H_2SO_4. After acidification, the solution was mixed thoroughly with diethyl ether. The ether extract was then evaporated to dryness and the phenolic acids present in the sample were dissolved in 0.5 ml alcohol (Smith, 1954).

Dry nutmeg leaves were extracted with 20 ml of 2N HCl and digested for 20 minutes. The extract was filtered and shaken thoroughly with diethyl ether (10 ml, 3 times). The ether layers were pooled and extracted with 10 ml of 5% Na_2CO_3 (3 times). The Na_2CO_3 layers were pooled and acidified with 5% H_2SO_4. It was again extracted with 10 ml ether (3 times) and the extract was evaporated to dryness. It was dissolved in 0.5 ml alcohol and applied to TLC plate (Smith, 1954).

The phenolic acid extract was applied to TLC plate of 0.2 mm thickness. The solvent system consists of toluene: acetic acid (4:1). After the run, the plate was first sprayed with 20% Na_2CO_3 followed by Folin water reagent (1:2). A standard mixture composed of ferulic acid, coumaric acid, caffeic acid, chlorogenic acid and synapic acid was also spotted.

3.2.5 Crude Fiber

The crude fiber was the organic matter in the dried residue remaining after digesting the sample with dilute sulphuric acid and sodium hydroxide.

Two grams of dry, powdered rind was taken in a crucible and boiled for 30 minutes with 200 ml of H_2SO_4. After boiling, the sample was thoroughly washed in boiling water and was again boiled for 30 minutes with 200 ml of NaOH. After digestion, the sample was washed thoroughly with boiling water, and rinsed in alcohol under vacuum. The weight of the dried crucible was taken and the difference in weight will give the weight of crude fibre present in the sample (ASTA, 1968).

3.2.6 Lycopene

100 mg of mace was extracted with acetone with the help of mortar and pestle. It was repeated till the sample became colourless. The acetone extracts were pooled and transferred to a separating funnel containing 20 ml of petroleum ether (40-60ºC). Another 20 ml of 5% sodium sulphate solution was added and the separating funnel was shaken thoroughly. The layers were allowed to separate. The upper petroleum ether layer was taken. The lower layer was re-extracted until it became colourless. The petroleum ether extracts were pooled, made up to 100 ml and the absorbance read at 503 nm (Sadasivam & Manickam, 1992).

3.2.7 TLC of flavonoids

Flavonoid pattern can be used for the recognition of different species in a genus and for the documentation of their natural hybrid. Flavonoids were extracted from mature leaves with 90% methanol and separated by TLC in chloroform: methanol (9:1) system. The plate was then visualised under UV light with and without ammonia (Harborne *et al.*, 1975).

Results and Discussion

K.M. Maya "Biochemical variability in nutmeg (myristica fragrans) and related taxa" Thesis. Indian Institute of Spices Research, University of Calicut, 2005

Results and Discussion

4.1 EVALUATION OF BIOCHEMICAL VARIABILITY IN NUTMEG (*Myristica fragrans*) GERMPLASM

4.1.1 Primary Metabolites

4.1.1.1 Carbohydrate

i) Total carbohydrate

iii) Reducing sugars

4.1.1.2 Protein

4.1.1.3 Phenol

4.1.1.4 Fat

4.1.1.5 Fatty acid composition of fat from nutmeg, mace and rind

4.1.1.6 Minerals

4.1.1.7 Pectin

4.1.2 Secondary Metabolites

4.1.2.1 Essential oil

i) Nutmeg oil

ii) Mace oil

iii) Leaf oil

iv) Rind oil

v) Yield of oil on storage of mace powder

4.1.2.2 Oleoresin

4.1.2.3 Non volatile ether extract (NVEE)

4.1.2.4 Pigments-Lycopene

4.1.2.5 Changes in lycopene on storage of mace powder

4.2 DIFFERENTIATION OF MALE, FEMALE AND BISEXUAL PLANTS BASED ON PRIMARY AND SECONDARY METABOLITES IN THE LEAF OF *Myristica fragrans*

4.2.1 Primary Metabolites

4.2.1.1 Carbohydrate

4.2.1.2 Protein

i) Total protein

ii) HPLC profile of amino acid

iii) Total free amino acid

4.2.1.3 Phenol

4.2.1.4 Minerals

4.2.2 Secondary Metabolites

4.2.2.1 Essential oil

4.2.2.2 Phenolic acids

4.2.2.3 Isozymes

4.3 BIOCHEMICAL VARIABILITY BETWEEN *Myristica fragrans* AND RELATED TAXA

4.3.1 Primary Metabolites

4.3.1.1 Carbohydrate

4.3.1.2 Protein

i) Total protein

ii) Amino acid

iii) Total free amino acids in leaf

4.3.1.3 Phenol

i) Fat extraction

ii) Fatty acid composition

4.3.1.4 Minerals

4.3.2 Secondary Metabolites

4.3.2.1 Essential oil

4.3.2.2 GC-MS of leaf essential oil

4.3.2.3 Phenolic acids

4.3.2.4 Crude Fibre

4.3.2.5 Pigments in wild nutmeg

4.3.2.6 Flavonoid analysis

4.1 EVALUATION OF BIOCHEMICAL VARIABILITY IN NUTMEG (*Myristica fragrans*) GERMPLASM

Biochemical variability in nutmeg accessions, which were grouped into 3 based on yield, was carried out. They are high yielding, medium yielding and low yielding. The biochemical variability within the accessions for primary and secondary metabolites was studied.

4.1.1 Primary Metabolites

4.1.1.1 Carbohydrate

i) Total carbohydrate

The total carbohydrate content of dried nutmeg, mace and leaf were calculated as described in section 3.1.1.1. The percentage of carbohydrate has been calculated (Table 4.1).

Table 4.1 Estimation of total carbohydrate in nutmeg, mace and leaf samples

No.	Accessions	Yield	Total carbohydrate (%)		
			Nutmeg	Mace	Leaf
1	A4 / 12	High	32.2	45.7	19.5
2	A9 / 4	High	31.3	44.1	18.5
3	A9 / 86	High	30.5	43.9	19
4	A9 / 18	Medium	31.4	44.2	18.5
5	A9 / 28	Medium	30.7	42.3	17.9
6	A9 / 71	Medium	33.1	43.2	19.1
7	A9 / 74	Low	30.4	43.7	18.5
8	A9 / 102	Low	32.4	44.1	18.2
9	A11 / 25	Low	31.5	42.5	18.6

The carbohydrate content in nutmeg and mace is reported to be 28.2% and 47.8% respectively (Gopalan *et al.*, 1981). The present study is in tune with the

earlier reports. Other researchers also have suggested the distinctive nature of Indian nutmeg compared to West Indian, East Indian, Papuan, etc.

ii) Starch

Starch present in the nutmeg, mace and leaf samples was estimated according to the procedure described in 3.1.1.2. The starch content of nutmeg has been reported as 14.6 – 24.2% (Pruthi, 1979) whereas that of mace is reported as 44.05% (Gopalakrishnan, 1992). Table 4.2 clearly illustrates the uniformity in values in the nine nutmeg, mace and leaf samples. The levels reveal the lack of correlation between the yield of nuts/plant and the concentration of metabolites.

Table 4.2 Estimation of starch in nutmeg, mace and leaf samples

No.	Accessions	Yield	Starch (%)		
			Nutmeg	Mace	Leaf
1	A4 / 12	High	27.6	38.1	17
2	A9 / 4	High	26.3	37.2	17.1
3	A9 / 86	High	28.1	36.3	16.8
4	A9 / 18	Medium	28.4	37.7	16.9
5	A9 / 28	Medium	27.5	37.2	15.8
6	A9 / 71	Medium	26.7	36.1	17.4
7	A9 / 74	Low	28.1	37.4	16.1
8	A9 / 102	Low	27.1	36.3	16.2
9	A11 / 25	Low	28.2	37.7	17.1

iii) Reducing sugars

Reducing sugars present in the dried nutmeg, mace and leaf samples were estimated according to section 3.1.1.3.

Gopalakrishnan (1992) has reported the amount of reducing sugars as 0.17% and 0.27% in nutmeg and mace respectively. In contrast to some of the

Indian reports, these values in Table 4.3 reflect the real field samples grown at a uniform place.

Table 4.3 Estimation of reducing sugars in nutmeg, mace and leaf samples

No.	Accessions	Yield	Reducing sugars (%)		
			Nutmeg	Mace	Leaf
1	A4 / 12	High	0.19	0.31	3.9
2	A9 / 4	High	0.17	0.3	2.96
3	A9 / 86	High	0.15	0.28	2.26
4	A9 / 18	Medium	0.17	0.27	3.39
5	A9 / 28	Medium	0.17	0.31	2.52
6	A9 / 71	Medium	0.16	0.3	3.04
7	A9 / 74	Low	0.2	0.28	2.6
8	A9 / 102	Low	0.16	0.29	2.31
9	A11 / 25	Low	0.18	0.3	3.14

4.1.1.2 Protein

Generally protein content is estimated employing the Lowry's method (Section 3.1.2.2.). The tissue is extracted in Tris buffer with pH 7.4. Attempts using 0.05 molar Tris buffer at pH 7.4 did not yield protein in leaf, nutmeg and mace. Attempts had been made with various buffers such as acetate buffer at pH 5, borate buffer at pH 8.8, sodium phosphate buffer at pH 6, pH 7.2 and at pH 8. But none of these could answer the actual protein content. The lack of dissolution of protein in various buffers is very interesting and needs further investigation.

When the sample/buffer ratio was decreased from 1g in 5ml to 0.25g in 5ml, the leaf protein recovery increased from 0.8% to 3.4%. In nutmeg kernel too, the protein percentage increased from 1% to 6.7% by decreasing the sample to buffer ratio from 1g in 5ml to 0.5 g in 5ml. When the proportion of tissue weight to buffer was increased to 0.25g/5 ml, recovery of protein was

about five times compared to 1g/5ml. This method of estimation gives a protein value of about 90% by Kjeldahl's method. This may attribute to some inhibiting factors in nutmeg protein extraction. There may be various factors like the presence of high myristicin and other phenolics that inhibit the extractability of protein. This will have great practical relevance in any future protein analysis. Zhang-YiShun *et al.,* (2003) reported a satisfactory result in *Litchi chinensis* Sonn, when the volume of Tris –HCl buffer was five times the fresh weight of the tissue. Attempts had also been made to concentrate proteins using ammonium sulphate (as per section 3.1.2.3.), 10% TCA solution, acetone powder, polyethylene glycol (PEG) and lyophilisation

Since Lowry's method of protein estimation was not successful, nitrogen was estimated (Table 4.4) by digestion technique as per section 3.1.2.1. The percentage of protein present in the sample was calculated based on its total nitrogen content.

Table 4.4 Estimation of protein in nutmeg, mace and leaf samples

No.	Accessions	Yield	Protein (%)		
			Nutmeg	Mace	Leaf
1	A4 / 12	High	5.3	6.1	3.5
2	A9 / 4	High	5.3	6.1	3.5
3	A9 / 86	High	5.3	6.1	3.4
4	A9 / 18	Medium	5.3	6.1	3.5
5	A9 / 28	Medium	5.3	6.1	3.2
6	A9 / 71	Medium	5.3	6.1	3.3
7	A9 / 74	Low	5.3	6.1	3.5
8	A9 / 102	Low	5.3	6.1	3.4
9	A11 / 25	Low	5.3	6.1	3.5

Electrophoretic separation of nutmeg leaf protein was carried out using Sodium Dodecyl Sulfate (SDS) in which varying the concentration of sample or

buffer did not give any clear-cut pattern (Section 3.1.2.4). This problem was confirmed by other investigators too. As protein extraction was not amenable in any buffer, SDS PAGE also did not give any positive result. Yield of nuts/plant as such do not reflect in the levels of primary metabolites.

Verghese (2000) gives a protein content of 7.5% in nutmeg and 6.5% in mace. The protein yield as described by Gopalakrishnan (1992) is 7.16% and 9.91% respectively in nutmeg and mace. Other investigators did not elaborate the method of protein estimation.

4.1.1.3 Phenol

The total phenol content of dried nutmeg, mace and leaf samples was calculated (Table 4.5) as per section 3.1.3. Level of total phenols did not show any variation between different nutmeg samples. Phenols form an integral part of terpenoids.

Table 4.5 Estimation of total phenols in nutmeg, mace and leaf samples

No.	Accessions	Yield	Phenol (%)		
			Nutmeg	Mace	Leaf
1	A4 / 12	High	2.6	0.4	0.21
2	A9 / 4	High	2.7	0.5	0.3
3	A9 / 86	High	2.5	0.33	0.27
4	A9 / 18	Medium	2.8	0.41	0.31
5	A9 / 28	Medium	2.4	0.4	0.26
6	A9 / 71	Medium	2.7	0.3	0.28
7	A9 / 74	Low	2.1	0.38	0.23
8	A9 / 102	Low	2.4	0.41	0.31
9	A11 / 25	Low	2.3	0.34	0.25

4.1.1.4 Fat

Table 4.6 gives the level of fat present in the nutmeg kernel and mace as per the procedure 3.1.4.1. Nutmeg kernel is reported to have 30 to 40% fat (Purseglove, 1981). Pest-attacked nutmegs will have less fat. Even though the fat has not much edible purpose, it has a lot of commercial applications. Mace has less fat compared to nutmeg. Since fat contains trace of essential oil, the fat never turns rancid like other vegetable fat. The variation in the fat content in the different groups has no direct correlation with the yield of nut in a plant.

Table 4.6 Estimation of fat in nutmeg and mace

No.	Accessions	Yield	Fat (%)	
			Nutmeg	Mace
1	A4 / 12	High	32.8	23.7
2	A9 / 4	High	30.9	24.8
3	A9 / 86	High	33.8	26.4
Mean ± Standard deviation			32.5±1.47	24.97±1.36
4	A9 / 18	Medium	34.9	23.4
5	A9 / 28	Medium	29.0	26.1
6	A9 / 71	Medium	28.9	24.1
Mean ± Standard deviation			30.93±3.44	24.53±1.4
7	A9 / 74	Low	28.2	23.9
8	A9 / 102	Low	32.2	24.1
9	A11 / 25	Low	38.1	25.1
Mean ± Standard deviation			32.83±4.98	24.37±0.64

4.1.1.5 Fatty acid composition of fat from nutmeg, mace and rind

Fatty acid compositions of fat from nutmeg, mace and rind were studied as per the procedure described in 3.1.4.2. and 3.1.4.3.

Table 4.7 Fatty acid compositions of nutmeg, mace and rind

	Lauric acid %	Myristic acid %	Palmitic acid %	Unidentified %	Stearic acid %	Unidentified %
M. fragrans – nutmeg	8.00	**55.10**	14.87	2.52	7.3	2.36
M. fragrans – mace	1.31	8.11	**52.56**	-	7.98	27.52
M. fragrans –rind	2.86	11.09	4.25	**50.00**	-	-

Nutmeg fat is mainly considered as trimyristin. Table 4.7 elaborates the percentage composition of fatty acids in the fat of nutmeg kernel, mace and rind of *Myristica fragrans*. Myristic acid was the predominant fatty acid in nutmeg followed by palmitic acid. In case of mace, palmitic acid was the dominant one followed by an unidentified fatty acid. In the rind, the highest concentration was observed for an unidentified fatty acid (Figures 4.1, 4.2 and 4.3).

Prakashchandra & Chandrasekharappa (1984) have studied the fatty acid composition of mace. It had palmitic and oleic acids as the predominant fatty acids.

In the nut of *Myristica fragrans*, myristic acid was the predominant fatty acid (55.1%) followed by palmitic acid (14.87%). The earlier report (Anonymous, 1962) gives the following fatty acid composition: lauric acid 0.4%, myristic acid 71.8%, palmitic acid 14.3%, stearic acid 1.2%, hexadecenoic acid 4.8%, oleic acid 5.2% and linoleic acid 1.5%. Verghese (2001b) reported 80.6% myristic acid, 7.8% oleic acid, 7.1% palmitic acid and 1.6% lauric acid in nutmeg. In nutmeg, 90.6% of the total fatty acids are saturated with only 8.7% of unsaturated fatty acids (Verghese 2001b).

In contrast to nutmeg, *M. fragrans* mace has palmitic acid (52.56%) as the predominant fatty acid followed by an unidentified fatty acid (27.52%).

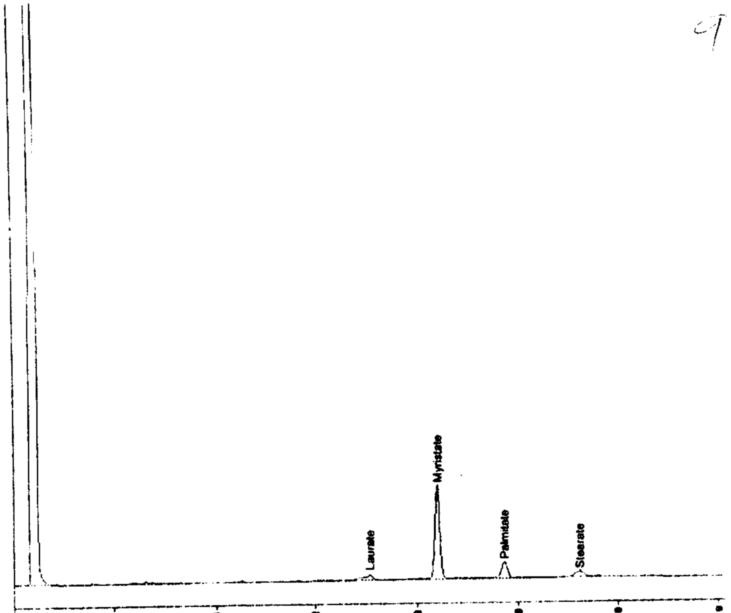


Figure 4.1 GC profile of FAMES of *M. fragrans*-nutmeg

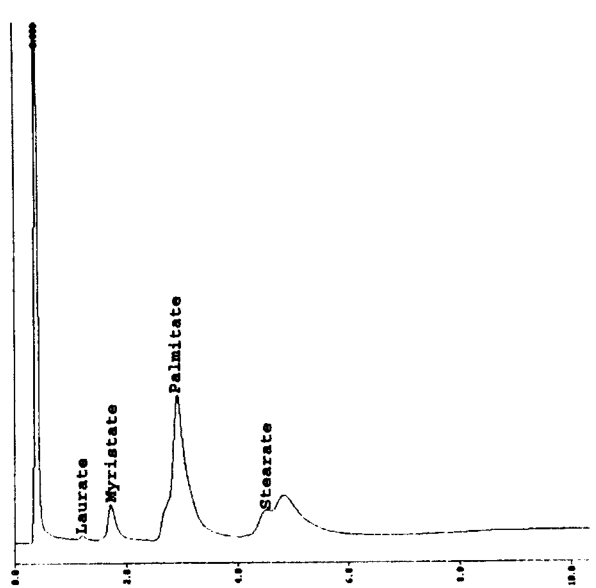


Figure 4.2 GC profile of FAMES of *M. fragrans*-mace

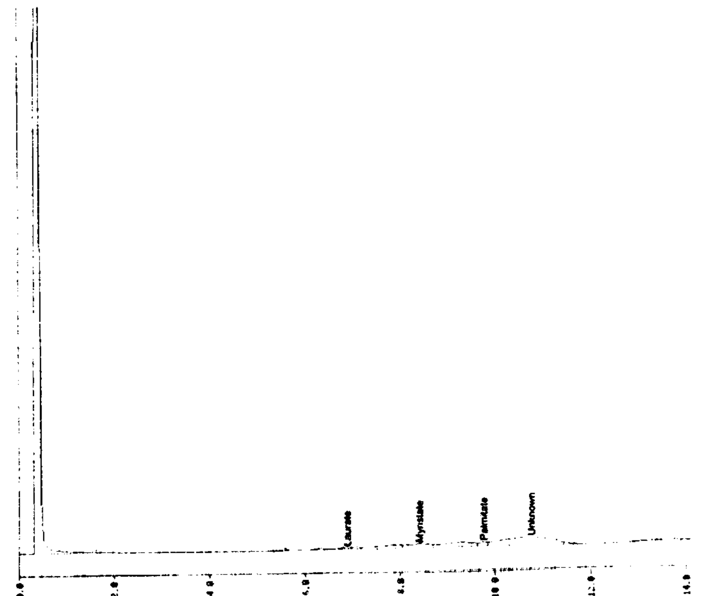


Figure 4.3 GC profile of FAMES of *M. fragrans*-rind

Verghese (2001b) has reported oleic acid (42.3%) as the predominant fatty acid in mace. It is followed by 30.7% of palmitic acid and 17.2% of linoleic acid. Thus it has 38% of saturated fatty acids and 62.1% of unsaturated fatty acids. However, previous report (Anonymous, 1962) describes a fatty acid composition similar to that of nutmeg. The differential composition of fatty acids in nutmeg and mace is reflected in the consistency and organoleptic properties of the fat. Mace fat, by virtue of its high unsaturated fatty acid content, has a lower melting point (Verghese 2001b).

4.1.1.6 Minerals

The minerals present in nutmeg, mace and rind were estimated (Table 4.8) as per section 3.1.5.

Verghese (2000) has given a total mineral content of 1.7g in nutmeg and 1.6 g in mace. It is composed of 0.12 % of calcium in nutmeg and 0.18% in mace. The iron content, on the other hand, is given as 4.6 mg % and 12.6 mg% respectively. The phosphorus content of nutmeg and mace is given as 0.14% and 0.1% respectively.

Table 4.8 Estimation of minerals in nutmeg, mace and rind of *Myristica fragrans*

	K %	Ca %	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Nutmeg	0.62	0.12	98	13.0	16	41
Mace	0.88	0.11	111	21.3	15	23
Rind	1.63	0.37	139	9.9	7	23

Ansari *et al.*, (2004) have reported 28 µg/g of zinc, 4.3 µg/g of manganese, 32.6 µg/g of copper and 222µg/g of iron in *Myristica fragrans*

4.1.1.7 Pectin

Pectin was extracted from the rind as given in section 3.1.6. The extraction was attempted from the fresh and dried samples. The yield of pectin was better (10%) in the dried sample when compared to the fresh sample (7%). The high pectin content makes the rind very suitable for jam, jelly etc. Generally, for other fruit-based jams, pectin is added additionally, which is not required here.

4.1.2 Secondary Metabolites

Secondary metabolites are unique compounds produced by the plant that have no immediate functions on the plant. They however, confer special advantages for the host. Secondary metabolites have great potential in the industrial point of view.

4.1.2.1 Essential oil

Essential oil contributes towards the aroma of spice. It is composed of terpenes and hydrocarbons. Major terpenic constituents in the oil are sabinene, myrcene, limonene, terpinene, myristicin, elemicin, safrole etc. The essential oil from the dried and powdered spice was estimated (Tables 4.9, 4.10 and 4.11) as per section 3.2.1.1. The essential oil thus obtained was then analyzed as described in section 3.2.1.2. (Figures 4.4, 4.5, 4.6 and 4.7)

i) Nutmeg oil

Studies carried out at IISR, Calicut (Maya *et al.*, 2004) indicate that essential oil content range from 3.9% to 16.5% in nutmeg and 6% to 26.1% in mace. They reported that some of the nutmeg accessions of IISR such as A9/18, A9/49 and A11/49 were rich in both nutmeg and mace oils.

Table 4.9. Major essential oil constituents of nutmeg

No.	Accessions	Yield	Essential oil (%)	α-pinene (%)	Sabinene (%)	Safrole (%)	Myristicin (%)	Elemicin (%)
1	A4 / 12	High	8.2	3.0	11.1	4.8	11.2	27.2
2	A9 / 4	High	7.1	7.5	35.9	0.1	12.5	13.7
3	A9 / 86	High	8.7	9.3	37.1	0.1	6.3	11.8
Mean ± Standard deviation			8 ± 0.82	6.6 ± 3.24	28.03 ± 14.68	1.67 ± 2.71	10 ± 3.27	17.57 ± 8.4
4	A9 / 18	Medium	16.5	12.2	35.6	0.1	15.1	4.6
5	A9 / 28	Medium	8.7	7.0	44.8	0.1	2.1	8.2
6	A9 / 71	Medium	5.0	4.1	45.0	0.1	1.6	0.8
Mean ± Standard deviation			10.07 ± 5.87	7.77 ± 4.1	41.80 ± 5.37	0.1 ± 0.0	6.27 ± 7.65	4.53 ± 3.70
7	A9 / 74	Low	6.4	9.4	34.2	0.1	4.0	0.6
8	A9 / 102	Low	7.5	23.5	39.6	0.3	1.5	1.1
9	A11 / 25	Low	10.9	3.5	11.8	20.2	2.3	15.1
Mean ± Standard deviation			8.27±2.35	12.13 ± 10.28	28.53 ± 14.74	6.87 ± 11.55	2.60 ± 1.28	5.60 ± 8.23

ii) Mace oil

Table. 4.10 Major essential oil constituents of mace

No.	Accessions	Yield	Essential oil (%)	α-pinene (%)	Sabinene (%)	Safrole (%)	Myristicin (%)	Elemicin (%)
1	A4 / 12	High	11.3	1.5	5.9	3.3	19.9	30.2
2	A9 / 4	High	7.1	7.7	19.7		22.0	20.8
3	A9 / 86	High	15.8	10.0	18.1	2.0	4.6	20.4
Mean ± Standard deviation			11.04 ± 4.35	6.4 ± 4.4	14.57 ± 7.55	2.65 ± 0.92	15.5 ± 9.5	23.8 ± 5.55
4	A9 / 18	Medium	26.1	10.4	21.8	0.2	27.4	1.2
5	A9 / 28	Medium	12.0	8.4	28.4	1.3	4.1	26.1
6	A9 / 71	Medium	16.2	8.3	41.9	3.2	1.1	1.0
Mean ± Standard deviation			18.1 ± 7.24	9.03 ± 1.18	30.7 ± 10.25	1.57 ± 1.52	10.9 ± 14.4	9.4 ± 14.4
7	A9 / 74	Low	12.1	15.4	37.4	0.3	1.6	0.4
8	A9 / 102	Low	7.6	10.0	24.7	0.7	10.7	2.3
9	A11 / 25	Low	15.5	5.1	21.8	13.1	0.7	2.2
Mean ± Standard deviation			11.73±3.96	10.17±5.15	27.97±8.3	4.7±7.28	4.33±5.53	1.63±1.07

Table 4.11 Major essential oil constituents of leaf

No	Accessions	Yield	Essential oil %	α-Pinene %	Sabinene %	Safrole %	Myristicin %	Elemicin %
1	A4/12	High	1.2	22.06	29.55	0.29	0.11	7.32
2	A9/4	High	0.9	15.39	14.14	0.15	10.33	8.77
3	A9/86	High	1.9	18.11	27.19	0.03	2.52	8.01
Mean ± Standard deviation			1.33 ± 0.51	18.52 ± 3.35	23.63 ± 8.3	9.73 ± 16.69	4.32 ± 5.34	8.03 ± 0.73
4	A9/18	Medium	1.1	20.37	28.64	0.05	5.71	0.74
5	A9/28	Medium	1.4	16.39	32.59	0.34	1.23	4.28
6	A9/71	Medium	1.05	16.02	31.41	0.15	1.00	1.5
Mean ± Standard deviation			1.18 ± 0.19	17.59 ± 2.41	30.88 ± 2.03	0.18± 0.15	2.65 ± 2.66	2.17 ± 1.86
7	A9/74	Low	0.85	19.63	30.01	0.67	1.43	0.39
8	A9/102	Low	1.6	28.50	32.65	0.67	0.39	0.27
9	A11/25	Low	0.75	20.82	33.76	0.12	0..29	0.92
Mean ± Standard deviation			1.07 ± 0.46	22.98 ± 4.81	32.14 ± 1.93	0.49 ± 0.32	0.7 ± 0.63	0.53 ± 0.35

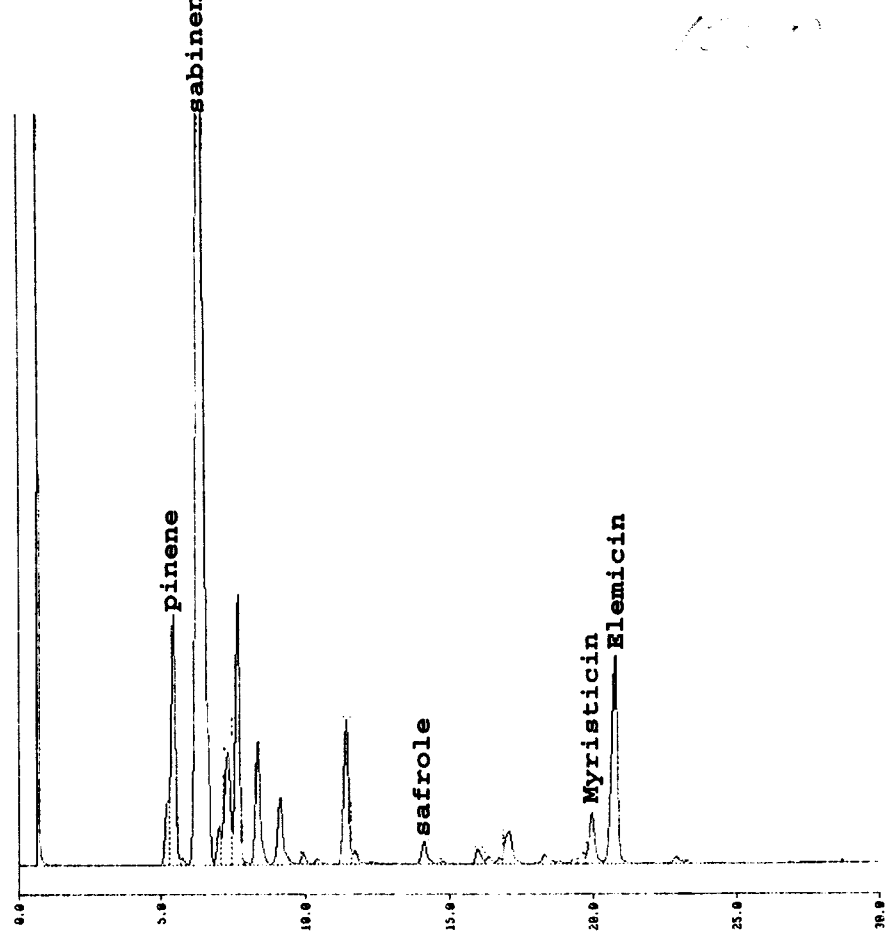


Figure 4.4 GC profile of nutmeg (*M. fragrans*) essential oil

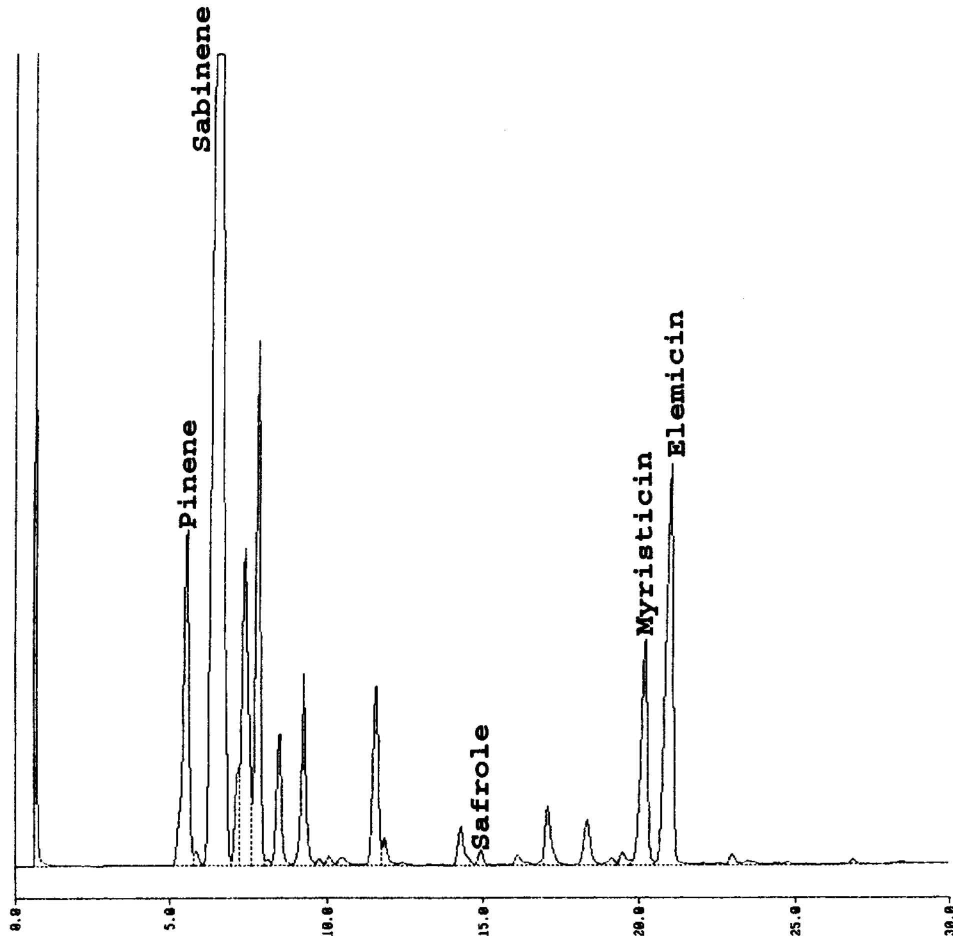


Figure 4.5 GC profile of mace (*M. fragrans*) essential oil

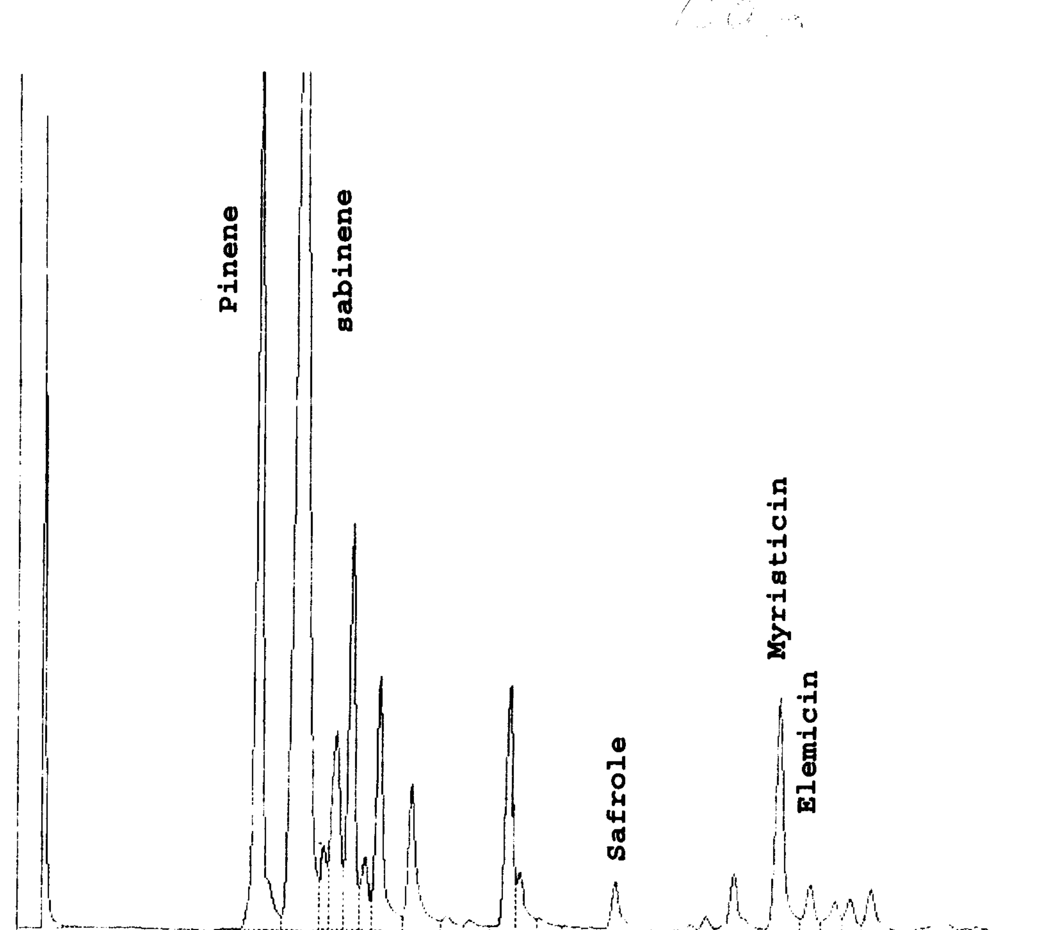


Figure 4.6 GC profile of leaf (*M. fragrans*) essential oil

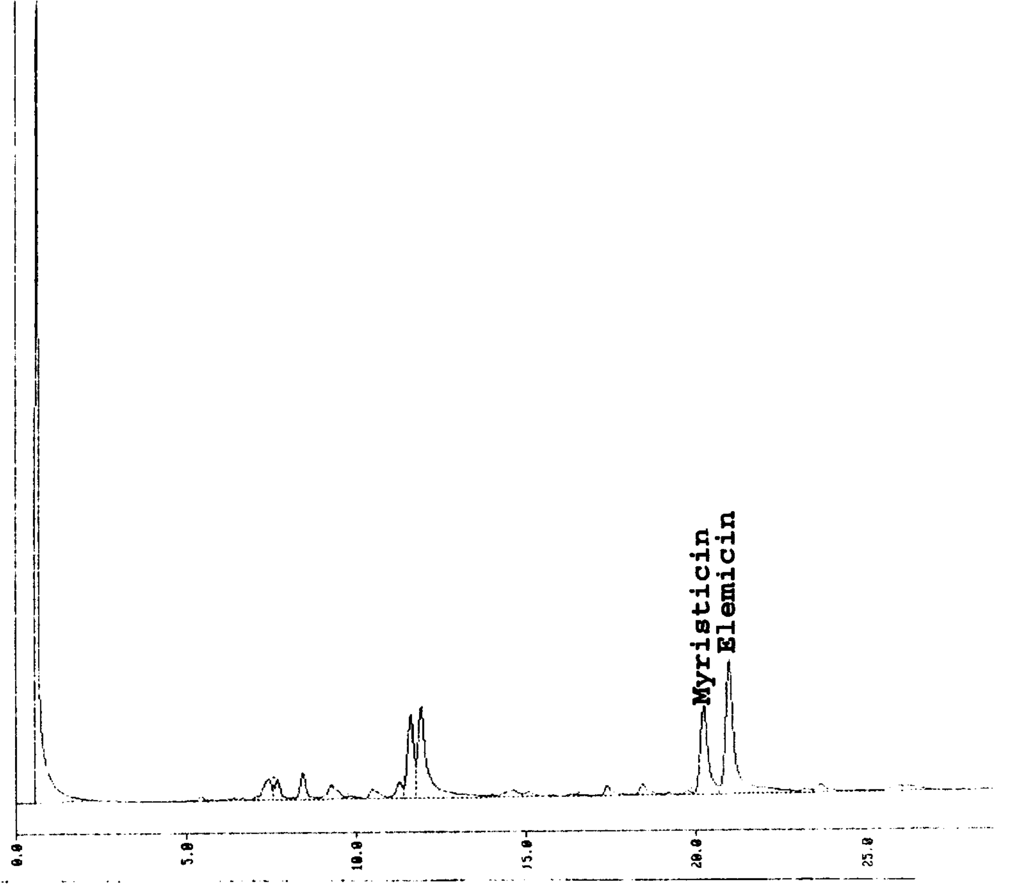


Figure 4.7 GC profile of rind (*M. fragrans*) essential oil

572.2 MAY/R3

Mallavarapu & Ramesh (1998) reported that nutmeg oil contained 76.8% monoterpenes, 12.1% oxygenated monoterpenes and 9.8% phenyl propanoid ether. They had reported that mace oil consists of 51.2% monoterpenes, 30.3% oxygenated monoterpenes and 18.8% phenyl propanoid ether.

Tables 4.9 and 4.10 indicate that the total yield of nutmegs from a tree do not indicate its oil recovery. However, medium yielders have a slight edge in oil yield compared to other groups. Similarly, the levels of important oil constituents such as myristicin and elemicin do not relate to yield of fruits per plant.

NB 4682

Myristicin or methoxy safrole is the major aromatic constituent of nutmeg and mace oils. Various clinical and toxicological studies indicate that 6 to 7 mg of myristicin per kg of body weight is enough to cause psychopharmacological effects. Woolf (1999) also reported the specific toxicity of nutmeg essential oils.

Nutmeg, mace and the essential oils extracted are generally used for preparation of confectionery items like cakes, cookies, doughnuts, fruit pies and puddings to give them a delicate smooth flavour. The oil is used in canned soups and stews and has an important application in neutralizing the unpleasant smell of cooked cabbage (Lewis, 1984). Purseglove *et al.*, (1981) reported myristicin, elemicin and safrole as the hallucinogenic principles in nutmeg oil. USA import large quantities of nutmeg and mace oils for various industrial, pharmaceutical and culinary applications. Every year India exports an average of 11 to 12 metric tones of nutmeg oil (Peter & Zachariah, 2000).

Another important observation in the study is the high level of sabinene in the oils. Studies by Maya *et al.*, (2004) have identified many accessions which can find specific industrial use. Table 4.12 is a list of nutmeg accessions available, categorized based on the industrial and culinary potential.

Various factors influence the oil recovery and the constituents in the oil. Lawrence (2000) who has compiled lot of information in nutmeg oil, indicate the variability in the oil content and its constituents. This is well illustrated in Tables 4.9 and 4.10.

Maya *et al.*, (2004) in their study, established that even though the West Indian nutmeg is low in myristicin, some nutmeg accessions available in India also have low myristicin in oil. The predominance of myristicin in some locations of cultivation may be a reflection of agroclimatic factors.

Myristicin being an antioxidant, studies conducted at various laboratories indicated that it has the property of scavenging cancer-causing free radicals.

iii) Leaf oil

Table 4.11 points to the fact that nutmeg leaf is also a potential source for oil and its constituents. Even though the concentration of various constituents varies in the leaf oil compared to nutmeg and mace oil, all the constituents are found in leaf oil too. Variability in the hallucinogenic principles such as myristicin, elemicin, safrole and the confectionery principle sabinene is found in leaf oil too.

As in the case of nutmeg and mace, leaf oil pattern also do not show any relation between the total yield of nuts per plant and its oil recovery.

Tables 4.9, 4.10 and 4.11 show some nutmeg accessions having low myristicin in the oil with comparatively high elemicin and vise versa. Khosla & Bhasin (2000) in their study establishes that myristicin and elemicin are synthesized separately in the plant.

iv) Rind oil

Dried nutmeg rind when subject to oil extraction had an oil yield of 0.123%. On analysis by GC, it was shown to contain myristicin (9.82%) and elemicin

(18.07%) (Figure 4.7). Essential oil extracted from the same accession (A9/41) had 7.4 % myristicin & 10.3 % elemicin in nutmeg and 18.8 % myristicin &19.2% elemicin in mace.

Cheong *et al.*, (1999) have studied rind oil composition by gas chromatography-mass spectrometry (GC-MS). The components were similar to those in nutmeg and mace, but differed substantially in concentration. They have reported 16 monoterpenes (60%), 9 monoterpene alcohols (29%), 8 aromatic ethers (7%), 3 sesquiterpenes (1%), 6 esters (1%) and 8 other minor components. The concentrations of sabinene, myristicin and safrole were much lower while the terpinen-4-ol and alpha-terpineol contents were much higher than in nutmeg and mace oils.

Based on the essential oil level of nutmeg and mace, germplasm accessions at IISR have been classified into various categories as given below.

Table 4.12 Classification of nutmeg germplasm accessions based on essential oil constituents.

No.	Accessions	Category	Remarks
1.	A4/12, A9/4	High myristicin & elemicin in nutmeg oil	Suitable for pharmaceuticals
2.	A4/12, A9/4	High myristicin & elemicin in mace oil.	
3.	A4/12, A9/4	High myristicin in both nutmeg and mace oils.	
4.	A9/71, A9/102	Low myristicin and elemicin levels in nutmeg oil	Suitable for preparing confectionery.
5.	A9/71, A9/74	Low myristicin and elemicin levels in mace oil.	
6.	A9/71	Low myristicin, low elemicin and low safrole coupled with high sabinene in both nutmeg and mace oils	

v) Yield of oil on storage of mace powder

The yield of oil on storage of powdered mace was studied for one month. The composition of mace oil was also studied by gas chromatography (section 3.2.1.2.). Eighty-five percent of the essential oil was retained at the end of one month. Among the five major components studied in oil, two of them showed an increase (pinene-33.25% & sabinene-45.78%) while the other three components had a decrease (safrole-27.69%, myristicin-63.5% and elemicin-57.39%) in its concentration (Figure 4.8). This increase in concentration of the monoterpene hydrocarbons (pinene & sabinene) may be relative while the decrease in the aromatic ethers (safrole, myristicin and elemicin) may be due to chemical transformations.

In case of turmeric rhizomes, the contents of essential oil and oleoresin were found to decline on storage (Goyal & Korla, 1993). There was 27.5% loss in the case of turmeric essential oil.

When the essential oil from ginger rhizomes was stored for three months, the neral and geranial content increased while the geraniol and geranyl acetate content was found to decrease. This is due to the conversion of geranyl acetate into geraniol, geranial and neral successively (Sakamura, 1987).

In case of immature cardamom, the level of essential oil remained almost constant on storage (Kumara *et al.*, 1985). But, its composition was found to vary. There was an increase in 1, 8-cineole and a decrease in α-terpinyl acetate content. Neither oil content, nor its composition varied significantly when the dried capsules were stored at 27^0-30^0C.

When the peel oil from *Citrus iyo* fruits was stored, the limonene content was found to increase while linalool decreased (Kobayashi *et al.*, 1983). However, limonene present in the orange essential oil was found to undergo autooxidation (Carmona *et al.*, 1976).

The quantitative and qualitative changes in the essential oil of dill plants were studied by Zlatev *et al.*, (1976). The essential oil content was found to increase during the first 48 hours of storage and then declined gradually. The phellandrene content increased and carvone content decreased during storage. There was 3 to 4 % of loss in the essential oil content when dry ripe coriander seeds were stored for two years (Luk'-yanov & Berestovaya, 1973).

4.1.2.2 Oleoresin

Oleoresin is the total extract of a spice. It can replace the spice for its aroma, flavour, taste etc. Nutmeg oleoresin is exclusive of the fat or fat it possesses. Mace oleoresin is inclusive of the fixed oil or fat also. Low value of oleoresin from nutmeg can be attributed to this phenomenon. If we do not separate fat first, the oleoresin received will be masked by the fat. Oleoresin is also used in industry similarly as oil. Oleoresin present in the accessions was estimated as per section 3.2.2.

Table 4. 13 Estimation of oleoresin in nutmeg and mace

No.	Accessions	Yield	Oleoresin (%)	
			Nutmeg	Mace
1	A4 / 12	High	7.7	21.5
2	A9 / 4	High	9.8	13.8
3	A9 / 86	High	8.4	17.7
Mean ± Standard deviation			8.63 ± 1.07	17.67 ± 3.85
4	A9 / 18	Medium	9.3	24.0
5	A9 / 28	Medium	3.6	26.3
6	A9 / 71	Medium	9.6	16.0
Mean ± Standard deviation			7.5 ± 3.38	22.1 ± 5.41
7	A9 / 74	Low	6.5	20.9
8	A9 / 102	Low	3.4	27.5
9	A11 / 25	Low	11.9	19.7
Mean ± Standard deviation			7.27 ± 4.3	22.7 ± 4.2

Generally oleoresin is extracted using acetone. However, in nutmeg this may lead to the extraction of fat also which will lead to false-high oleoresin values. Commercially fat is also important. In order to characterise the nutmeg accessions having genuine high fat and oleoresin, attempts were made to extract oleoresin using different solvents (Tables 4.14 and 4.15). This study revealed that in case of nutmeg, extracting fat first with petroleum ether from the kernel powder followed by extraction with acetone gives the true picture of oleoresin and fat content.

In the study, two different sequential extraction techniques were adopted. In the first pattern, fat was extracted with non-polar solvents like petroleum ether and hexane followed by polar solvents like acetone and alcohol. In the second pattern, the spice tissue was first treated with polar solvents like alcohol and acetone followed by non-polar solvents like petroleum ether and hexane.

Table 4. 14 Yield of oleoresin from nutmeg by different solvents.

No	Solvent 1	% of extract	Solvent 2	% of extract
1	Petroleum ether	28.26	Acetone	3.03
2	Petroleum ether	28.27	Alcohol	5.25
3	Acetone	23.59	Petroleum ether	8.42
4	Acetone	20.76	Ethyl acetate	10.54
5	Alcohol	11.64	Petroleum ether	19.23
6	Alcohol	11.31	Ethyl acetate	19.36
7	Ethyl acetate	**28.46**	Acetone	2.36
8	Ethyl acetate	**28.61**	Alcohol	2.52
9	Ethyl acetate	**27.72**	Petroleum ether	2.75
10	Petroleum ether	27.36	Ethyl acetate	3.08

Similar attempts were made for extracting fat and oleoresin from mace also. Since fat is not commercially important in mace, its extraction sequence is not very significant.

Table 4.15 Yield of oleoresin from mace by different solvents

No	Solvent 1	% of extract	Solvent 2	% of extract
1	Petroleum ether	16.06	Acetone	3.47
2	Hexane	16.18	Acetone	3.79

Boelens (2000) has reported various solvents for the extraction of oleoresins. They include benzene, diethyl ether, ethanol, acetone, trichloroethane, petroleum ether and dichloroethane. The solvents used are apolar or polar in nature. Since like dissolves like, apolar chemicals will easily dissolve in hexane and polar compounds in ethanol. Of this petroleum ether and hot ethanol were used for mace. The petroleum ether extract was 27 to 32% whereas ethanol gave 22 to 27% of oleoresin. The solvents used were benzene, ethanol and diethyl ether for nutmeg that gave 31 to 37%, 18 to 26% and 28 to 37% respectively (Boelens, 2000).

4.1.2.3 Non volatile ether extract (NVEE)

The Non-volatile Ether Extract (NVEE) of nutmeg and mace was estimated by AOAC method and found to be 33.77% and 18.65% respectively. Gopalakrishnan (1992) reports 33.6% of NVEE in nutmeg and 21.98% in mace while a previous study (Gopalan *et al.*, 1981) gives 36.4 % and 24.4% respectively in nutmeg and mace. NVEE is very critical in the industrial point of view. ISO standards and FAO standards recommend 25% NVEE for nutmeg and between 20 to 35% for mace. Considering this, Indian nutmeg has NVEE well above the international standard while mace NVEE is slightly low.

4.1.2.4 Pigments-Lycopene

The brilliant red colour of mace is due to the pigment lycopene (Gopalakrishnan *et al.*, 1979), which is a potent antioxidant. As an antioxidant, it is twice as

potent as Vitamin A and ten times as effective as Vitamin E. It participates in a host of chemical reactions that are believed to prevent carcinogenesis and atherosclerosis. The lycopene content in the various accessions was estimated (section 3.2.6.). The lycopene had a range of 82.4 mg% in A9/71 to 274 mg% in A9/74 (Table 4.16).

Table 4.16 Lycopene content of mace

No.	Accessions	Yield	Lycopene (mg %)
1	A4 / 12	High	127.9
2	A9 / 4	High	145.4
3	A9 / 86	High	136.4
4	A9 / 18	Medium	125.4
5	A9 / 28	Medium	92.9
6	A9 / 71	Medium	82.4
7	A9 / 74	Low	273.9
8	A9 / 102	Low	156.3
9	A11 / 25	Low	110.8

4.1.2.5 Changes in lycopene on storage of mace powder

As the degradation of lycopene not only affects the attractive color, but also the nutritive value, a study was undertaken to investigate the effect of storage of powdered mace on its lycopene content. Powdered mace was stored for a period of one month in six different storage systems. The six different storage systems used for the study were as follows. 1) Paper cover 2) Polyethylene cover with paper lining 3) Polythene cover with brown paper lining 4) Transparent bottle 5) Opaque bottle and 6) Amber-colored bottle. Frequent samples were drawn to estimate the lycopene content. It was estimated by standard procedure (Section 3.2.6.).

It was found that the extent of degradation was similar in all the six containers (Figure 4.9) and only 25% of lycopene was retained after 28 days. The degradation in lycopene can be attributed to isomerisation and oxidation. As mace is used in confectionery and pharmaceutical products, this change in configuration is a very critical factor in the value-addition of mace (Maya *et al.*, 2002).

4.2 DIFFERENTIATION OF MALE, FEMALE AND BISEXUAL PLANTS BASED ON PRIMARY AND SECONDARY METABOLITES IN THE LEAF OF *Myristica fragrans.*

Male and female plants are distinct in nutmeg. The actual sex of the plant is known only at the time of flowering, which happens after six years from the seedling stage. Many researchers had attempted to determine the sex of the plant at seedling stage. Except the study of Zachariah *et al.*, (1986) where they had succeeded in establishing a male-specific sterol compound, others could not pin point any particular chemical specific for male or female plant.

In this study, leaf analysis of male, female and bisexual plants was carried out pertaining to carbohydrate, starch and reducing sugars (Table 4.17) and protein. The study could not fix up any of these specific to male or female plant.

4.2.1 Primary Metabolites

4.2.1.1 Carbohydrate

Total carbohydrate, starch and reducing sugars present in the leaf of female, male and bisexual plants of *M. fragrans* were estimated as per sections 3.1.1.1., 3.1.1.2. and 3.1.1.3. respectively.

Table 4.17 Estimation of total carbohydrate, starch and reducing sugars in leaf

No	Accession	Sex	Total carbohydrate %	Starch %	Reducing sugars %
1	A9/2	Female	18.6	14.28	2.87
2	A9/20	Female	17.4	14.81	3.1
3	A9/28	Female	15.9	13.88	2.52
Mean ± Standard deviation			17.3 ± 1.35	14.32 ± 0.47	2.83 ± 0.29
4	A9/9	Male	16.2	13.98	3.4
5	A9/12	Male	17.8	12.86	2.9
6	A9/34	Male	18.1	14.46	3.4
Mean ± Standard deviation			17.37 ± 1.02	13.77 ± 0.82	3.23 ± 0.29
7	A4/44	Bisexual	17.6	12.69	3.1
8	A4/59	Bisexual	18.3	10.30	3.3
9	A4/64	Bisexual	18.1	12.83	2.9
Mean ± Standard deviation			18.00 ± 0.36	11.94 ± 1.42	3.1 ± 0.2

4.2.1.5 Protein

i) Total protein

As protein was not getting extracted in any buffers as per the standard procedure (section 3.1.2.2.), estimation was carried out by the Kjeldahl's method (section 3.1.2.1). In all the three groups, there was a protein yield of 3.5%

ii) HPLC profile of Amino acid

Studies aimed to determine the sex of nutmeg plant at seedling stage prompted to investigate the amino acid levels in male, female and bisexual plants (Figures 4.10, 4.10, 4.12 and 4.13) following the procedure described in section 3.1.2.7. Results of the study conducted to determine the amino acid levels have been listed in Table 4.18. Figures 4.14, 4.15 and 4.16. depict the graphic representation of the analytical data.

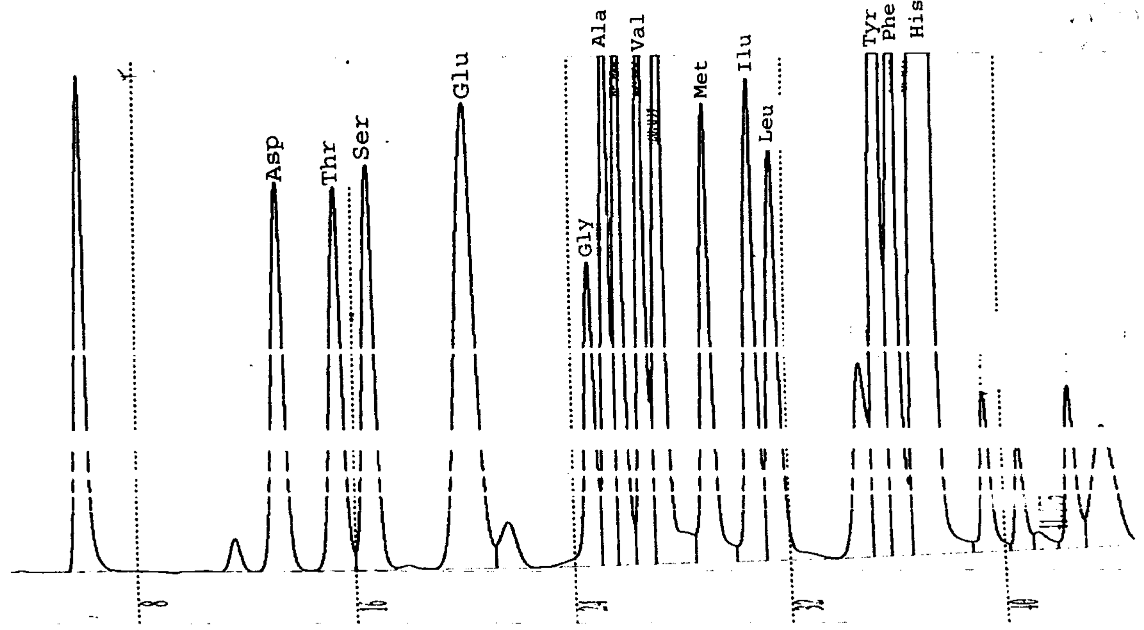


Figure 4.10 HPLC profile of amino acid standard mixture

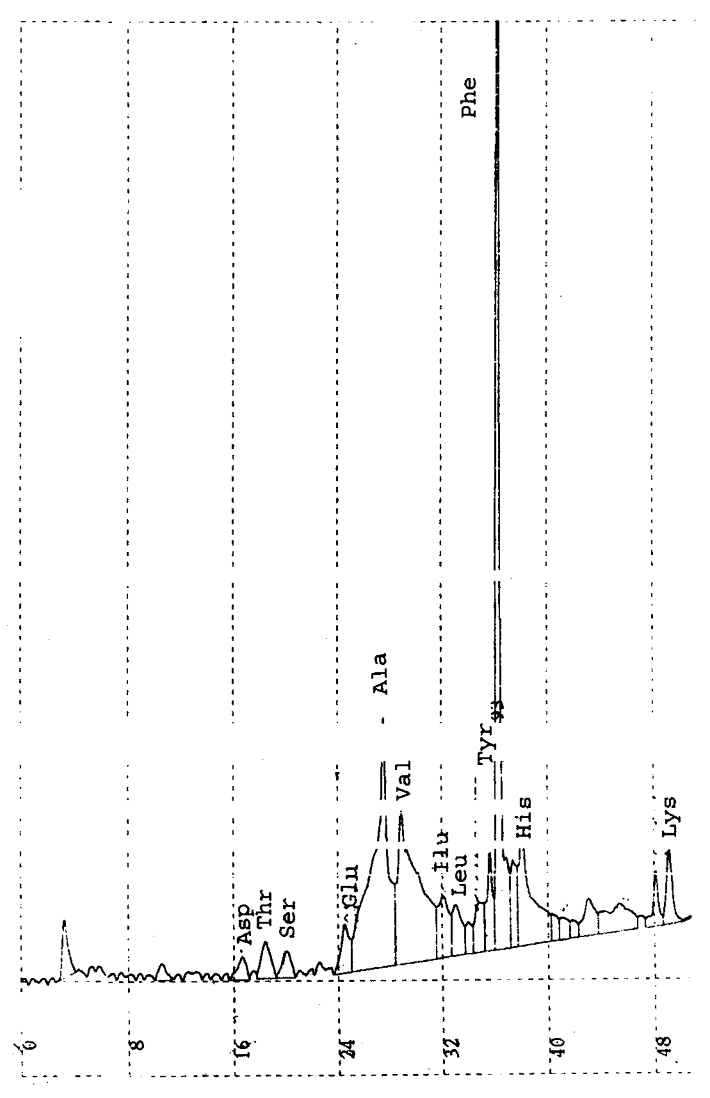


Figure 4.11 HPLC profile of amino acid in *M. fragrans* - male

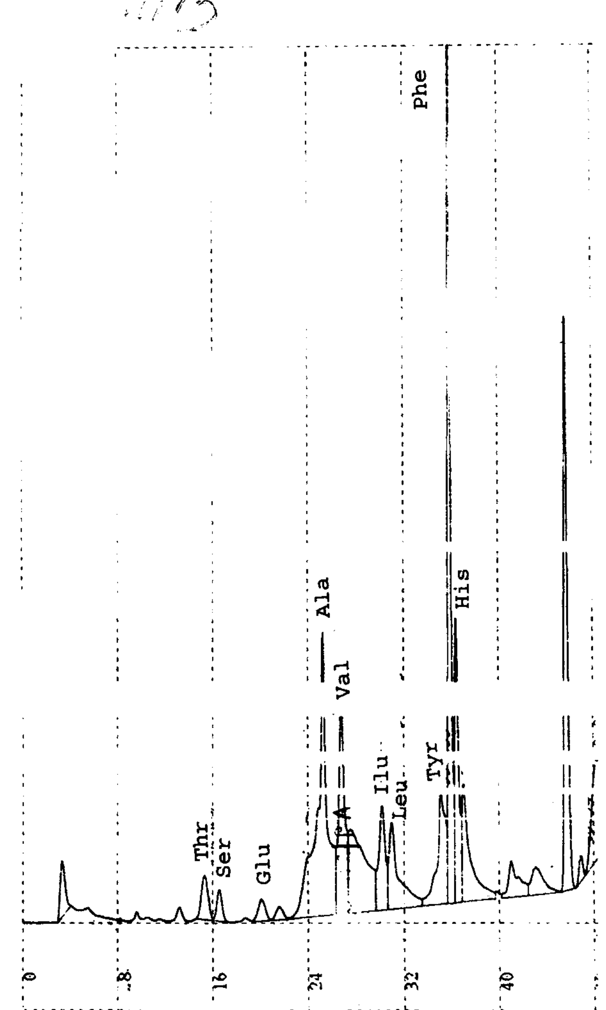


Figure 4.12 HPLC profile of amino acid in *M. fragrans* - female

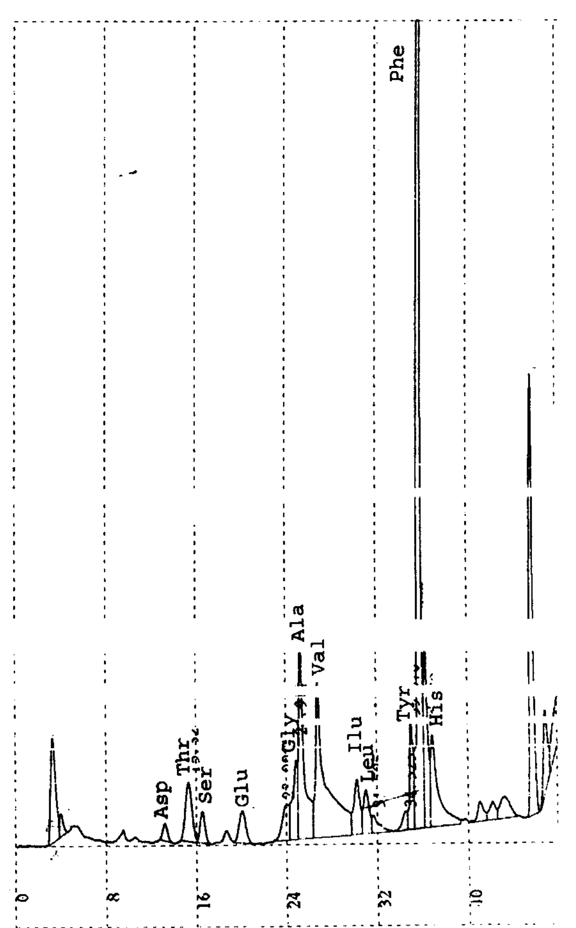


Figure 4.13 HPLC profile of amino acid in *M. fragrans* - bisexual

Table 4.18 Amino acid profile of the leaf of *M. fragrans*

Amino acid (µg/mg)	Asp	Thr	Ser	Glu	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys
A9/2 - female	1.9	7.6	2.8	2.3	46.3	0	9.2	11.2	6.7	54.3	4.9	0
A9/20 - female		6.2	3.3	1.7	60.1	22.4	12.4	16.9	8.3	44.9	4.3	11.1
A9/28 - female	0	13.1	4.9	2.6	67.8	52.0	13.0	19.4	8.1	114.5	7.5	0
A9/4 - female	12.3	29.1	21.1	8.1	59.5	80.3	37.4	36.3	0	442.9	1.3	11.4
Mean ± Standard deviation	6.1± 8.7	16.1± 11.7	9.8± 9.8	4.1± 3.4	62.4± 4.7	51.6± 28.9	18.0± 13.1	21.0± 10.8	5.7± 3.0	164.2± 188.3	4.5± 2.5	5.6± 6.5
A9/9 - male	1. 6	5.7	2.3	1.7	38.8	13.9	6.9	11.2	25.2	47.5	4.3	0
A9/12 - male	1.8	6.5	2.6	1.9	56.8	43.7	44.5	9.7	7.3	39.9	3.9	0
A9/34 - male	0	5.9	2.3	1.4	50.9	40.7	7.6	12.2	6.3	65.8	13.1	0
A9/9 - male	6.7	12.7	7.6	7.1	51.7	55.8	18.4	13.1	6.4	139.9	10.2	14.2
A9/9 - male	8.2	17.6	9.8	5.5	80.9	52.1	11.7	16.2	11.7	169.5	11.9	1.2
Mean ± Standard deviation	4.95± 4.35	12.08± 5.92	6.54± 3.89	4.68± 2.92	61.19± 17.1	49..54± 7.88	17.8± 15.6	12..5± 2.42	11..38 ± 8.05	92..53± 58.5	8.69± 4.33	3.06± 6.22
A4/64 bisexual	5.8	22.5	7.4	5.4	48.4	60.9	20.1	17.1	6.6	280.8	1.1	20.4
A4/59 bisexual	1.9	9.2	3.6	2.8	25.2	32.45	7	5.59	3.84	83.5	3.1	2.2
A4/44 bisexual	2.3	5.4	3.4	2.3	33.2	28.94	5.48	6.46	7.38	158.8	0.4	12
A4/64 - bisexual	2.8	9.1	4.4	2.4	44.6	32.06	8.11	9.59	7.88	108.0	0.3	0
Mean ± Standard deviation	2.33± 0.44	7.95±2. 01	3.8±0.5 6	2.46± 0.26	34.35± 9.72	31.15± 1.92	10.18± 6.72	9.69± 5.24	6.44± 1.8	157.8± 87.8	1.24± 1.29	8.65± 9.41

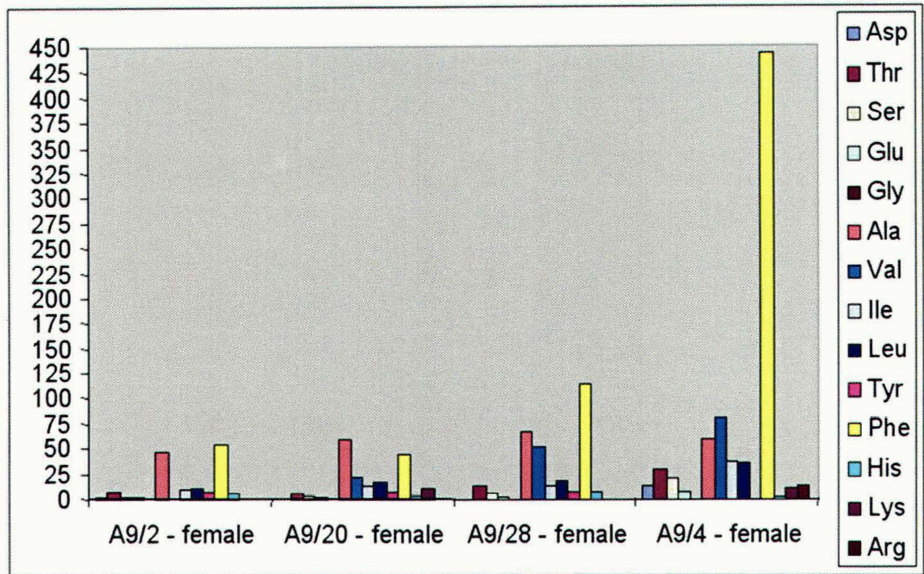


Figure4.14 Levels of leaf amino acids of *M. fragrans*- female

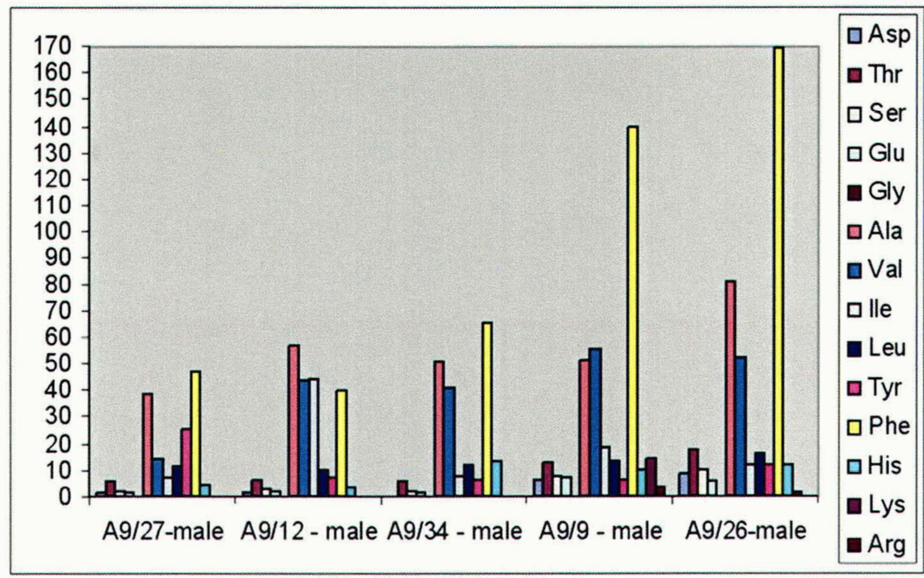


Figure 4.15 Levels of leaf amino acids of *M. fragrans*- male

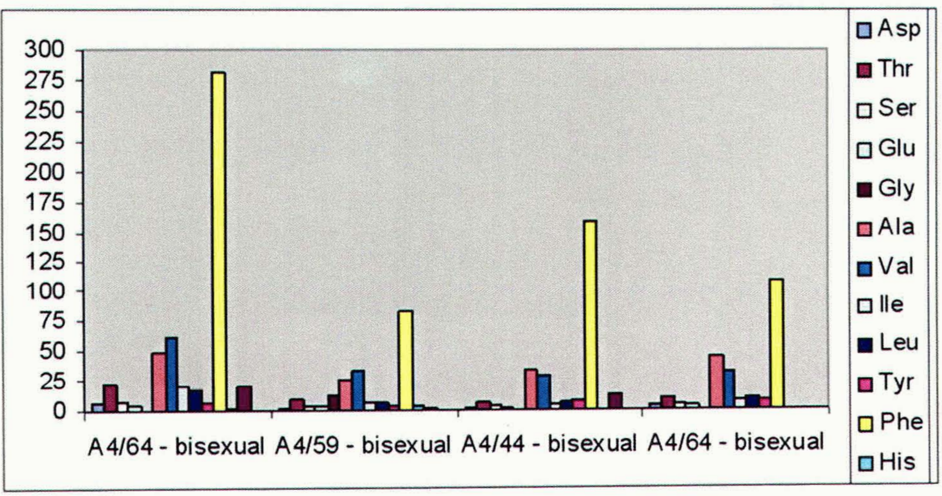


Figure 4.16 Levels of leaf amino acids of *M. fragrans*- bisexual

Table 4.19 Estimation of total free amino acids in the leaf

No	Accession	Sex	Free amino acid (μg/100mg)
1	A9/2	Female	87.31
2	A9/20	Female	95.41
3	A9/28	Female	119.72
Mean ± Standard deviation			100.81 ± 16.87
4	A9/9	Male	102.99
5	A9/12	Male	93.32
6	A9/34	Male	100.61
Mean ± Standard deviation			98.97 ± 5.04
7	A4/44	Bisexual	113.71
7	A4/59	Bisexual	106.27
9	A4/64	Bisexual	124.26
Mean ± Standard deviation			114.75 ± 9.07

Table 4.20 Estimation of minerals in the eaf

No	Accession	N %	K %	Ca %	Mg %	Fe (ppm)	Cu (ppm)	Zn (ppm)	P %	Mn (ppm)
1	A9/2-female	0.56	1.96	1.56	0.35	85	19.4	15	0.11	90.2
2	A9/20-female	0.56	1.02	1.68	0.34	51	33.8	17	0.11	119.7
3	A9/28-female	0.56	1.26	1.78	0.22	92	16.8	14	0.12	92.9
Mean ± Standard deviation		0.56± 0.0	1.41 ± 0.5	1.67± 0.1	0.3± 0.1	76.00± 21.9	23.33± 9.7	15.33 ±1.5	0.11± 0.01	100.93± 16.3
4	A9/9-male	0.56	1.14	1.39	0.35	232	38.3	19	0.08	150.8
5	A9/12-male	0.54	1.80	1.37	0.26	76	25.2	19	0.16	67.7
6	A9/34-male	0.54	0.56	2.14	0.33	77	37.9	20	0.08	221.2
Mean ± Standard deviation		0.55±0.01	1.17 ±0.62	1.63± 0.44	0.31± 0.05	128.33± 89.78	33.8±7.45	19.3± 0.58	0.11± 0.05	146.56± 76.84
7	A4/44-bisex	0.56	1.21	1.55	0.32	73	7.1	13	0.07	210.2
8	A4/59-bisex	0.56	1.34	1.15	0.28	51	24.5	17	0.08	66.8
9	A4/64-bisex	0.56	1.29	1.45	0.31	107	5.4	18	0.10	212.1
Mean ± Standard deviation		0.56± 0.00	1.28 ±0.27	1.38± 0.21	0.3± 0.02	77.0± 28.21	12.3±10.57	16.0± 2.65	0.08± 0.02	163.0± 83.35

Table 4.21 GC profile of the leaf essential oil from nutmeg plants

No	Accession	Sex	Essential oil %	α-Pinene %	Sabinene %	Safrole %	Myristicin %	Elemicin %
1	A9/28	Female	1.4	16.39	32.59	0.34	1.23	4.28
2	A9/71	Femlae	1.05	16.02	31.41	0.15	1.00	1.5
3	A9/18	Female	1.1	20.37	28.64	0.05	5.71	0.74
Mean ± Standard deviation			1.18± 0.19	17.59± 2.41	30.88± 2.03	0.18± 0.15	2.65± 2.66	2.17± 1.86
4	A4/64	Bisexual	1.4	23.38	28.43	0.10	1.37	2.16
5	A9/17	Bisexual	1.0	23.59	30.41	0.03	7.61	0.68
6	A4/59	Bisexual	0.8	21.81	29.34	0.07	8.37	5.55
Mean ± Standard deviation			1.07± 0.31	22.93± 0.97	29.39± 0.99	0.07± 0.04	5.78± 3.84	2.8± 2.5
7	A9/9	Male	0.8	21.14	26.26	0.25	0.74	0.82
8	A9/12	Male	1.6	15.57	29.91	0.15	1.30	3.1
9	A9/26	Male	1.0	21.41	36.79	0.12	0.76	0.95
Mean ± Standard deviation			1.13± 0.42	19.37± 3.3	30.99± 5.35	0.17± 0.07	0.93± 0.32	1.62± 1.28

From the study it can be concluded that there is no amino acid specific for male or female plant. Any approach using molecular markers might fix up any sex-linked molecule.

Levels of total free amino acid, total phenol and minerals of male, female and bisexual plants were also ascertained to fix up a distinct compound specific to any group (Table 4.19 and 4.20). Total free amino acids were found more in the bisexual plant compared to the other two groups. Among the minerals analysed, copper was found to be higher in the male lines.

iii) Total free amino acid

Total free amino acid present in the leaf of female, male and bisexual plants of *M. fragrans* were estimated (Table 4.19) as per section 3.1.2.6.

iv) Isozymes

Isozyme analysis from leaf samples was carried out for characterisation of nutmeg germplasm. Nutmeg accessions were subject to analysis for two enzymes, polyphenol oxidase (PPO) and peroxidase. On an average, about 8 to11 bands were obtained in most of the accessions while in 9 accessions we could find only 4 to 6 bands (Figure 4.17). Polyphenol oxidase isozyme patterns of all the accessions were similar with a single band of Rf value 0.5 (Figure 4.18). The results indicate that there is no clear-cut variability between the cultivated lines based on peroxidase and PPO.

4.2.1.3 Phenol

The phenol content of the leaf was estimated as per section 3.1.3. There was not much variation among the different groups. The phenol content of female and bisexual plants was 0.25 and that in the male plants was 0.26.

4.2.1.4 Minerals

Mineral content of leaf from female, male and bisexual plants of *M. fragrans* was studied as described as in section 3.1.5. The results obtained are given in

Table 4.20.

4.2.2 Secondary Metabolites

4.2.2.1 Essential oil

Some research groups (Madhavan *et al.*, 1991) claimed difference with respect to essential oil among the male, female and bisexual lines (as per section 3.2.1.1. and 3.2.1.2.). Table 4.21 clearly illustrates the fact that oil levels or constituents do not give any sexual identity in nutmeg. (Figures 4.19, 4.20 and 4.21)

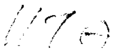
4.2.2.2 Phenolic acids

Phenolic acids from nutmeg (male, female and bisexual) leaves were extracted as per section 3.2.4.

A standard mixture composed of ferulic acid, synapic acid, coumaric acid, caffeic acid and chlorogenic acid was also spotted. The result showed that coumaric acid is the prominent phenolic acid in nutmeg leaf (Figure 4.22). There was no variability in the phenolic acid pattern in male, female and bisexual plants.

Compared to the reported dominance of ferulic and synapic acids in nutmeg and mace, the dominance of coumaric acid in leaf could be found in this study.

The phenolic acids of nutmeg and mace were studied by Variyar & Bandyopathyay (1998). Ferulic and synapic acids together formed 16.5% and 32.1% of the total phenolic acid of nutmeg and mace respectively.



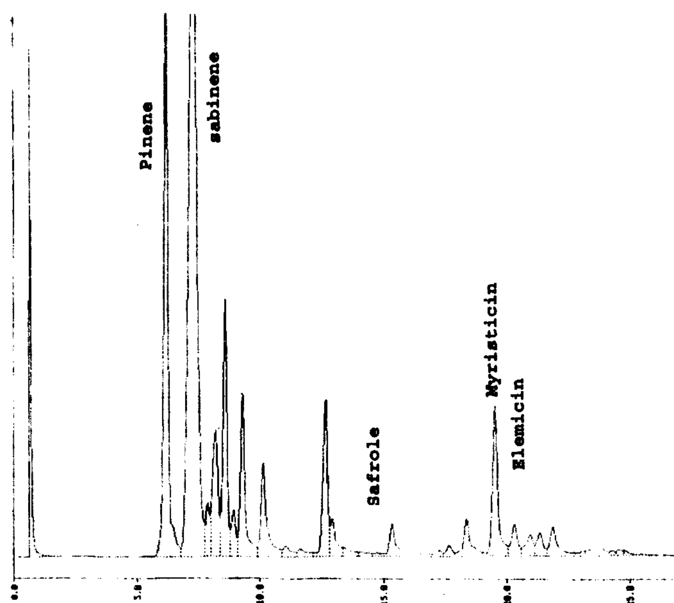


Figure 4.19 GC profile of leaf essential oil - female

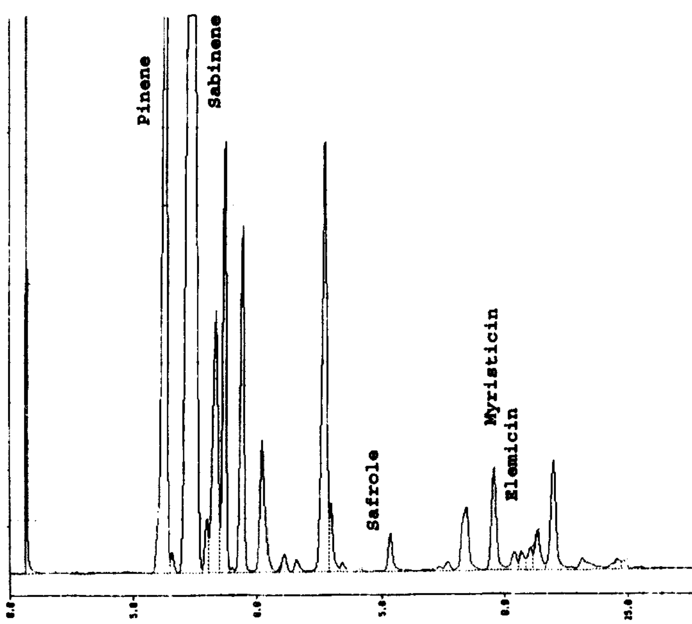


Figure 4.20 GC profile of leaf essential oil - male

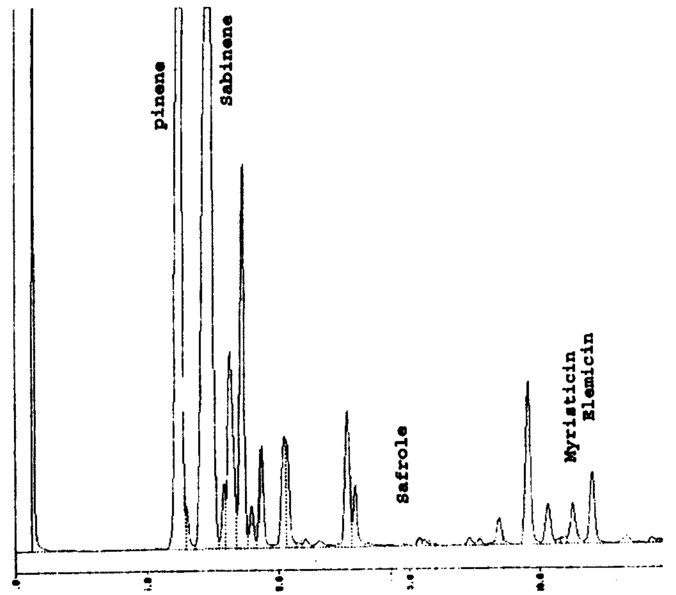


Figure 4.21 GC profile of leaf essential oil – bisexual

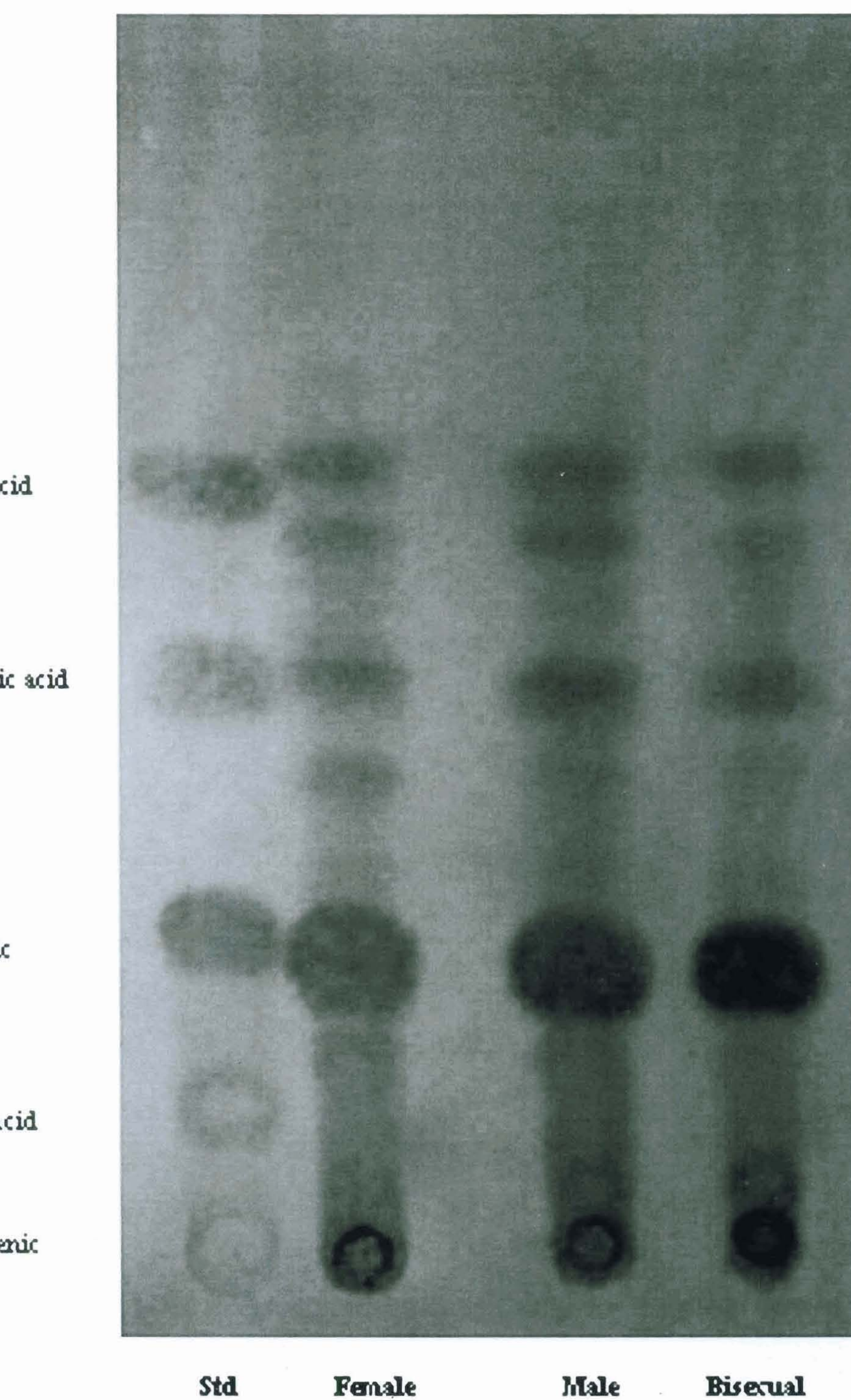


Figure 4.22 TLC profile of phenolic acids in *M. fragrans*

described in section 3.1.2.1.). There is no significant variation in leaf-protein content between *M. fragrans* and related taxa.

Table 4.23 Estimation of protein in the leaves of wild taxa

No	Species	%
1	*M. fragrans*	3.5
2	*Knema andamanica*	2.80
3	*M. amygdalina*	3.61
4	*M. andamanica*	3.38
5	*M. beddomeii*	2.92
6	*M. magnifica*	3.25
7	*M. malabarica*	2.88
8	*M. prainii*	3.42

ii) Amino acid

The amino acid profile of leaf samples of *M. fragrans* and related taxa was obtained as per section 3.1.2.7 (Figures 4.23 to 4.35). One common observation between *M. fragrans* and related taxa is both contain high phenylalanine (Table 4.24). *Knema andamanica* is very rich in threonine and alanine. In fact, its alanine content is very high compared to other wild plants.

Table 4.24 Amino acid profiles of the leaves of wild taxa

Species	**Asp** µg/mg	**Thr** µg/mg	**Ser** µg/mg	**Glu** µg/mg	**Ala** µg/mg	**Val** µg/mg	**Ile** µg/mg	**Leu** µg/mg	**Tyr** µg/mg	**Phe** µg/mg	**His** µg/mg
M. fragrans	3.8	11.6	5.8	3.5	51.1	39.6	15.5	14.2	8.1	134.7	5.1
Knema andamanica	40.6	139.2	16.1	38.5	269.1	58.5	20.8	18.5	17.4	88.6	5.5
M. amygdalina	15.8	21.0	4.8	5.5	52.7	39.8	12.5	11.9	5.4	118.4	3.3
M. andamanica	4.2	21.4	7.5	2.8	55.8	61.4	23.1	15.3	36.9	64.3	3.4
M. magnifica	2.3	11.3	3.7		49.1	26.6			33.6	6.4	2.3
M. malabarica	11.6	18.2	3.8	8.6	60.7	34.8	9.6	9.1	19.9	119.6	1.1
M. prainii	2.2	7.0	4.8	1.6	39.3	30.6	12.5	13.1	4.8	207.7	9.5

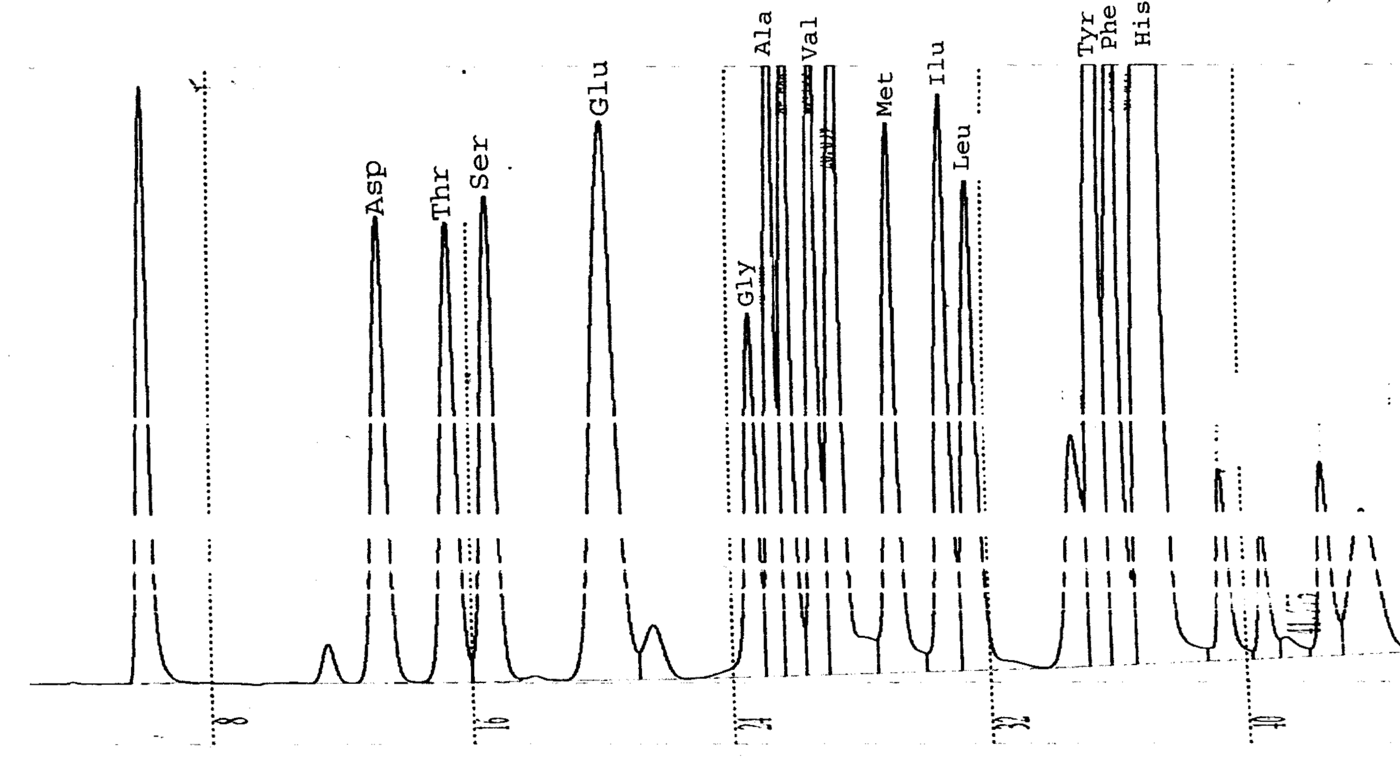


Figure 4.23 HPLC profile of amino acid standard mixture

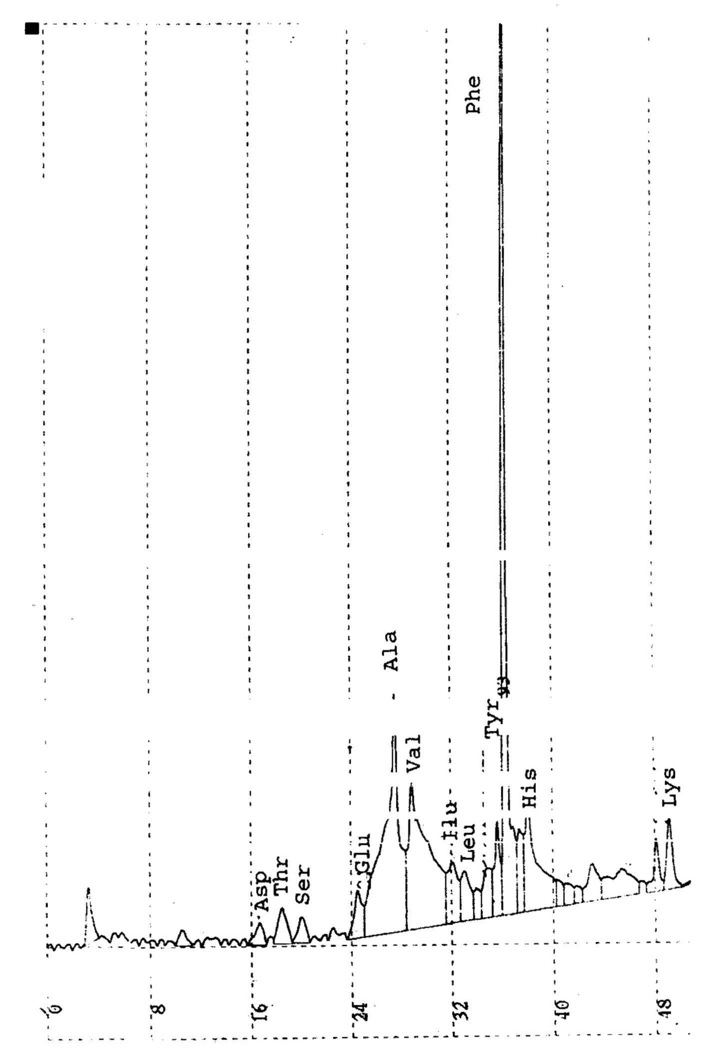


Figure 4.24 HPLC profile of amino acids in *M. fragrans*

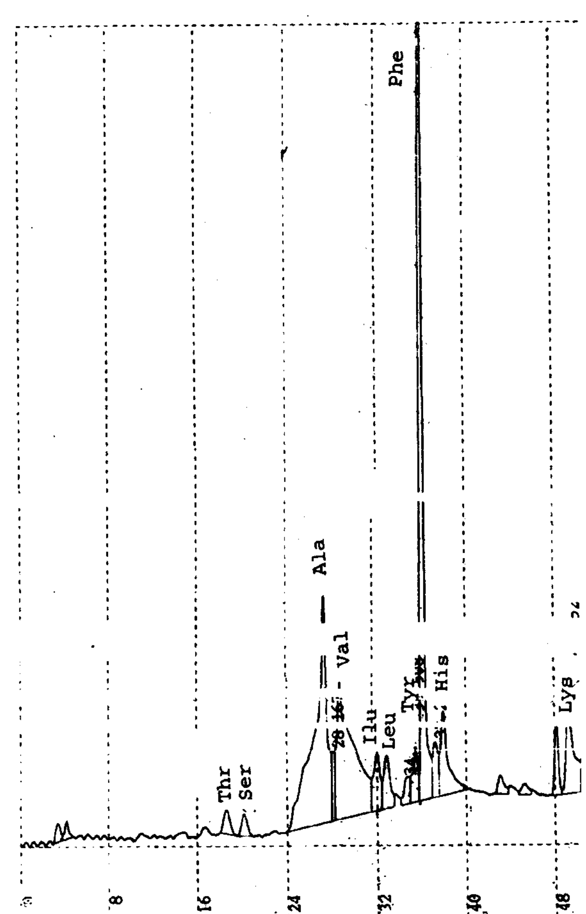


Figure 4.25 HPLC profile of amino acids in *M. prainii*

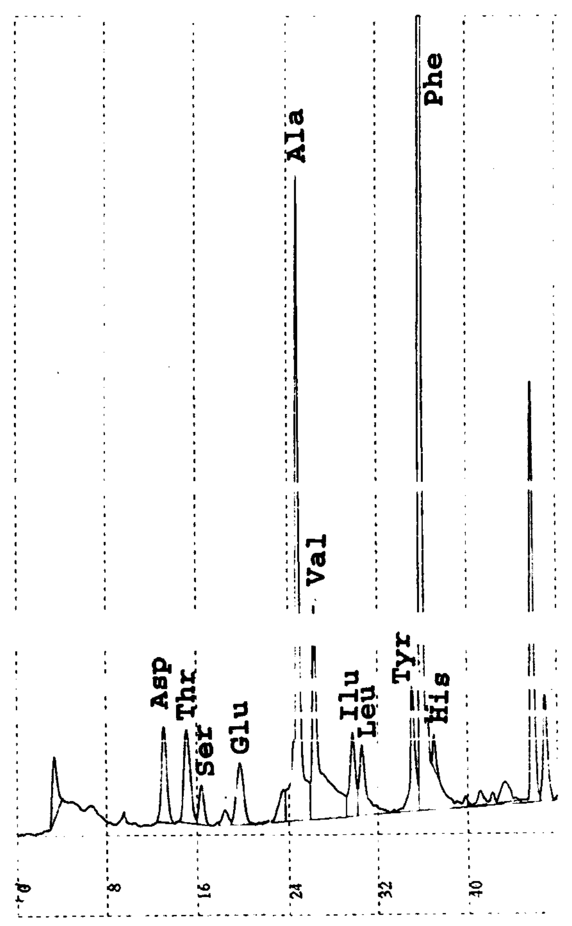


Figure 4.26 HPLC profile of amino acids in *M. amygdalina*

Figure 4.27 HPLC profile of amino acids in *M. malabarica*

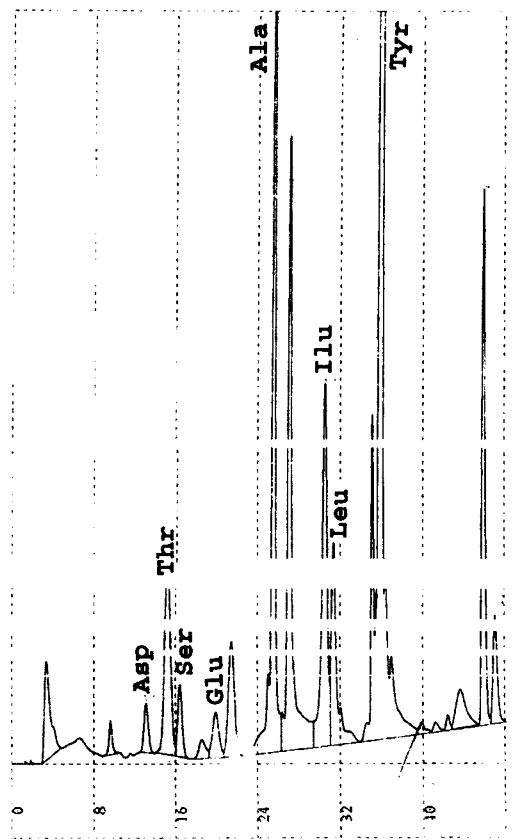


Figure 4.28 HPLC profile of amino acids in *M. andamanica*

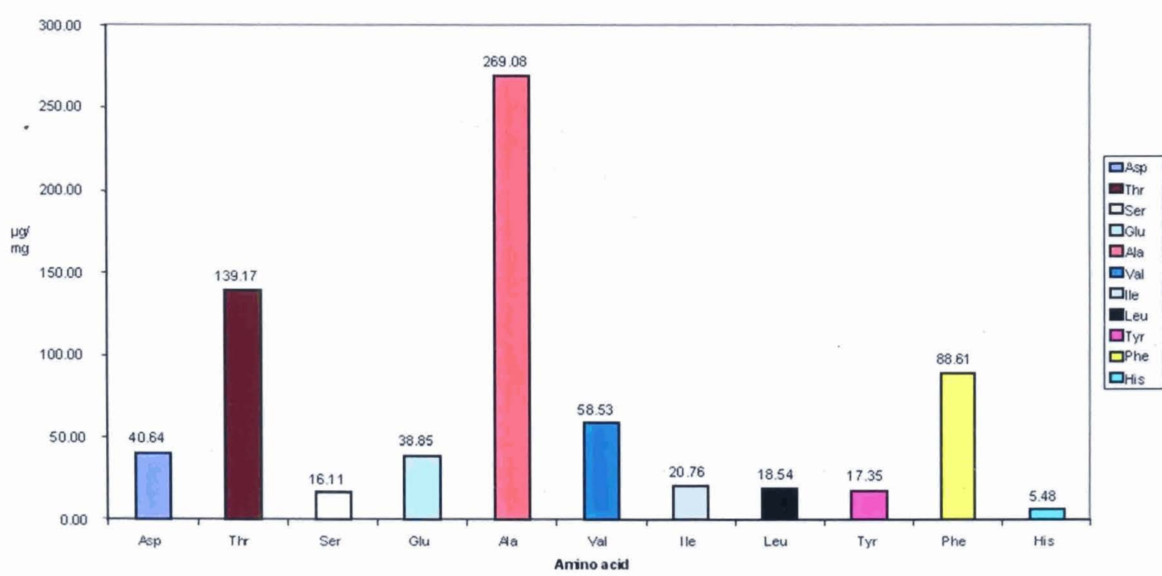


Figure 4.29 Levels of leaf amino acids in *Knema andamanica*

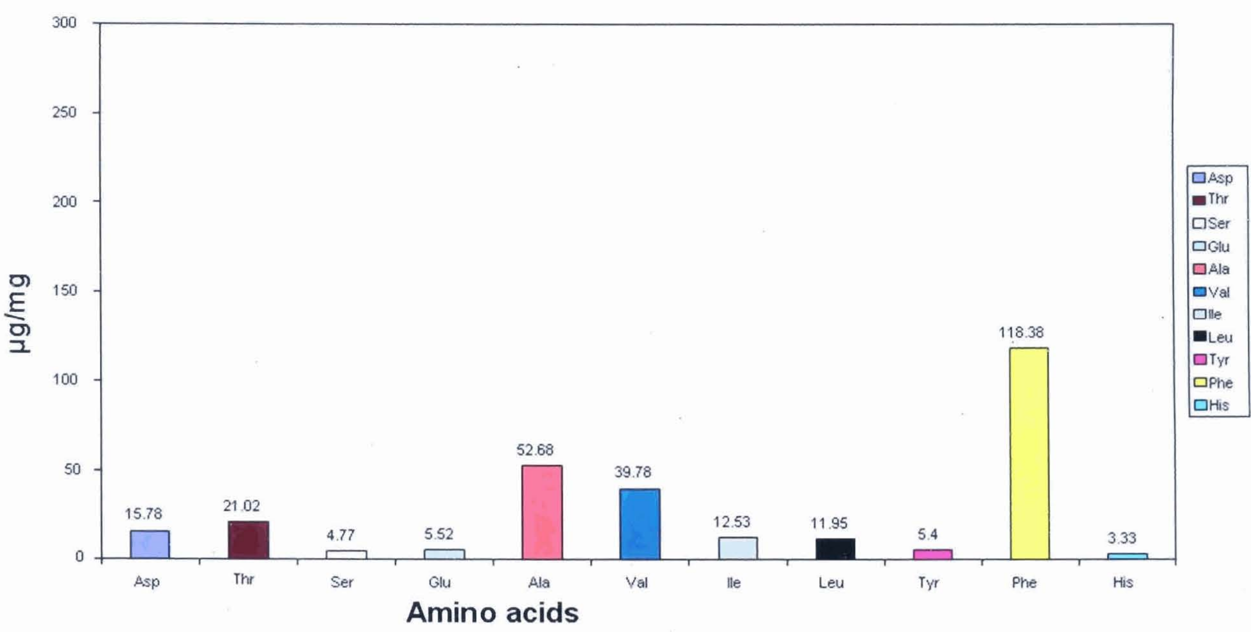


Figure 4.30 Levels of leaf amino acids in *M. amygdalina*

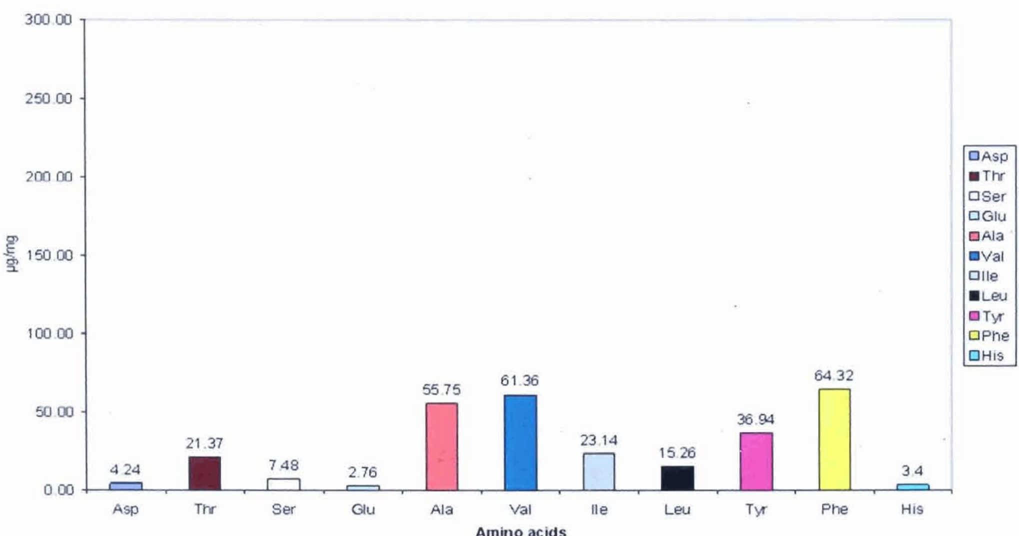


Figure 4.31 Levels of leaf amino acids in *M. andamanica*

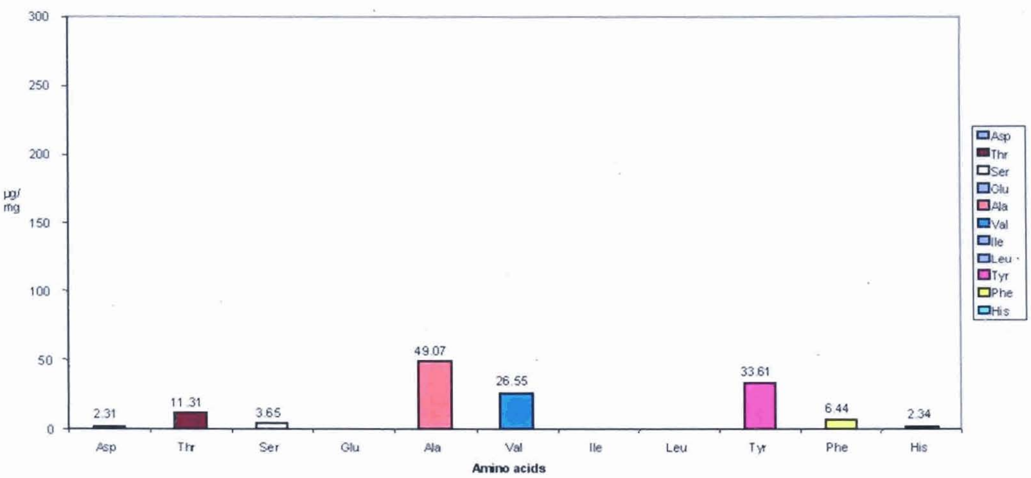


Figure 4.32 Levels of leaf amino acids in *M. magnifica*

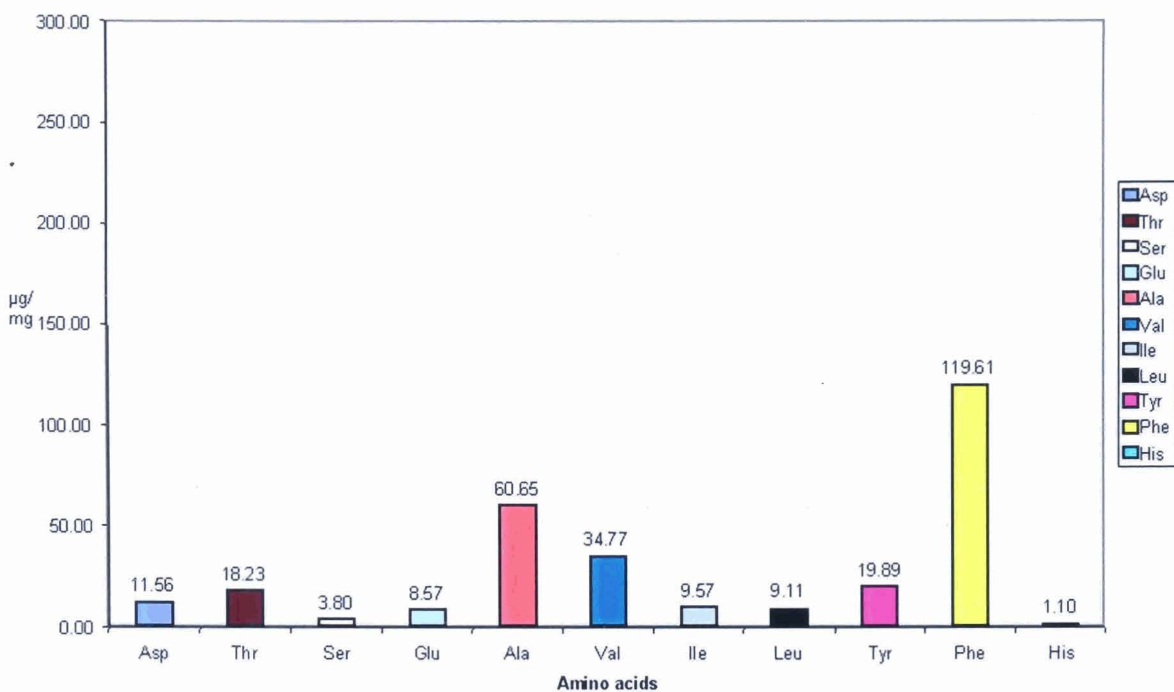


Figure 4.33 Levels of leaf amino acids in *M. malabarica*

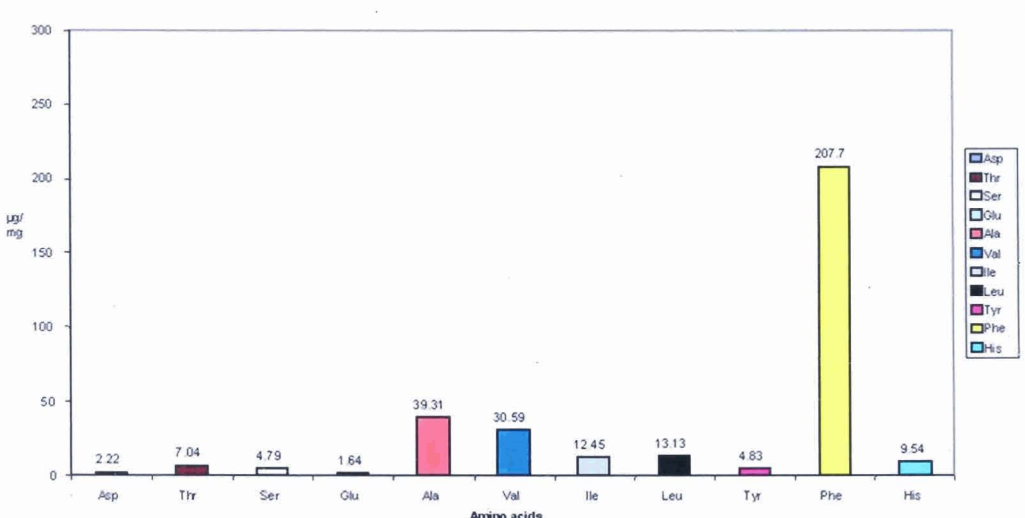


Figure 4.34 Levels of leaf amino acids in *M. prainii*

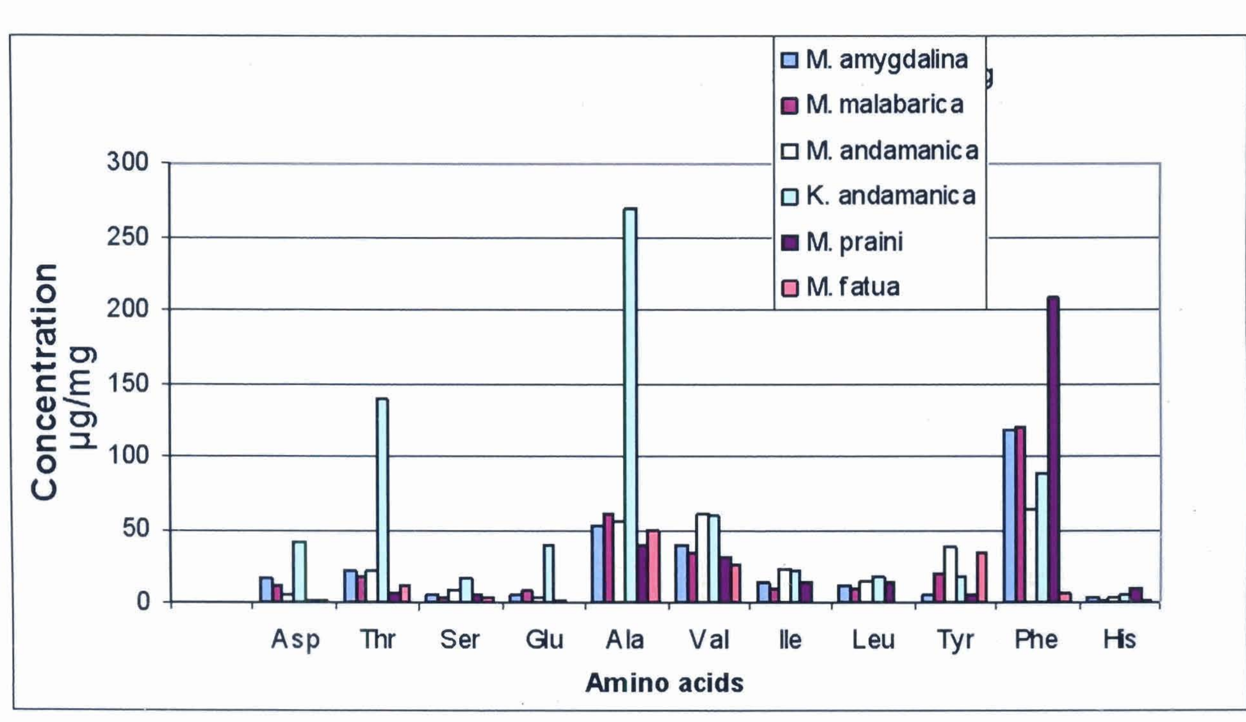


Figure 4.35 Comparative levels of amino acids in different taxa

iii) Total free amino acids in leaf

Total free amino acids were estimated as per the procedure in 3.1.2.6. They have a range from 127.65 µg/100mg to 215.21 µg/100mg (Table 4.25). The high free amino acid content of 198.43 µg/100mg in *Knema andamanica* is in similarity with the amino acid profile in Table 4.28. *M. prainii* and *Knema andamanica* had the highest amino acid content.

Table 4.25 Estimation of total free amino acids in the leaves of wild taxa

No	Species	Total free amino acids (µg/100mg)
1	*M .fragrans*	139.64
2	*Knema andamanica*	198.43
3	*M. amygdalina*	135.28
4	*M. andamanica*	179.01
5	*M. beddomeii*	174.66
6	*M. magnifica*	127.65
7	*M. malabarica*	148.39
8	*M. prainii*	215.21
Mean ± Standard deviation		164.78 ±31.90

iv) Isozymes

When isozyme analysis was carried out in the seven wild taxa using polyphenol oxidase (PPO) and peroxidase, no consistent isozyme pattern could be established.

4.3.1.3 Phenol

Phenol content of the leaf was estimated as per section 3.1.3. Similar to the

free amino content, phenol content also did not show any variation between the cultivated and wild taxa (Table 4.26).

Table 4.26 Estimation of phenol in the leaves of wild taxa

No	Species	Phenol %
1	*M. fragrans*	0.28
2	*Knema andamanica*	0.24
3	*M. amygdalina*	0.3
4	*M. andamanica*	0.24
5	*M. beddomeii*	0.3
6	*M. magnifica*	0.23
7	*M. malabarica*	0.21
8	*M. prainii*	0.22

4.3.1.4 Lipid

i) Fat extraction

Fat was extracted as described by 3.1.4.1. As stated earlier, fat content in nutmeg kernel is about 40% (Table 4.27). Only *M. prainii* had the nearest fat content compared to *M. fragrans. M. malabarica* kernel has 15 to16% of neutral fat (Anonymous, 1962). Among the mace samples, *M. fragrans* and *M. malabarica* had high fat compared to *M. prainii.* Cultivated and wild taxa had the same fat in rind in all the cases.

Table 4.27 Fat content of *Myristica* species

Species	Fat %		
	Nutmeg	Mace	Rind
M. fragrans	37.52	24.49	0.83
M. beddomeii	2.46		0.45
M. malabarica	9.75	29.54	
M. prainii	32.79	2.17	0.57

ii) Fatty acid composition

Fatty acids were estimated as per 3.1.4.2. and 3.1.4.3. (Figures 4.36 to 4.41) In the nutmeg of *Myristica fragrans*, myristic acid was the predominant fatty acid with (55.1%) followed by palmitic acid (14.87) (Table 4.28). The earlier report (Anonymous, 1962) on fatty acid composition was: lauric acid 0.4%, myristic acid 71.8%, palmitic acid 14.3%, stearic acid 1.2%, hexadecenoic acid 4.8%, oleic acid 5.2% and linoleic acid 1.5%. The composition of fatty acids as reported by Verghese (2001b) is 80.6% myristic acid, 7.8% oleic acid, 7.1% palmitic acid and 1.6% lauric acid. In nutmeg 90.6% of the total fatty acids is saturated with only 8.7% of unsaturated fatty acids (Verghese, 2001b).

In contrast to nutmeg, *M. fragrans* mace has palmitic acid (52.56%) as the predominant fatty acid followed by an unidentified fatty acid (27.52%). Considering the various reports available, the unidentified fatty acid may be oleic acid. Verghese (2001b) has reported oleic acid (42.3%) as the predominant fatty acid in mace. It is followed by 30.7% of palmitic acid and 17.2% of linoleic acid. Thus it has 62.1% of unsaturated fatty acids and 38% of saturated fatty acids. But the earlier report (Anonymous, 1962) gives a fatty acid composition similar to that of nutmeg. The differential composition of fatty acids in nutmeg and mace is reflected in the consistency and organoleptic properties of the fat. Mace fat, by virtue of its high content of unsaturated fatty acid, has a lower melting point (Verghese, 2001b).

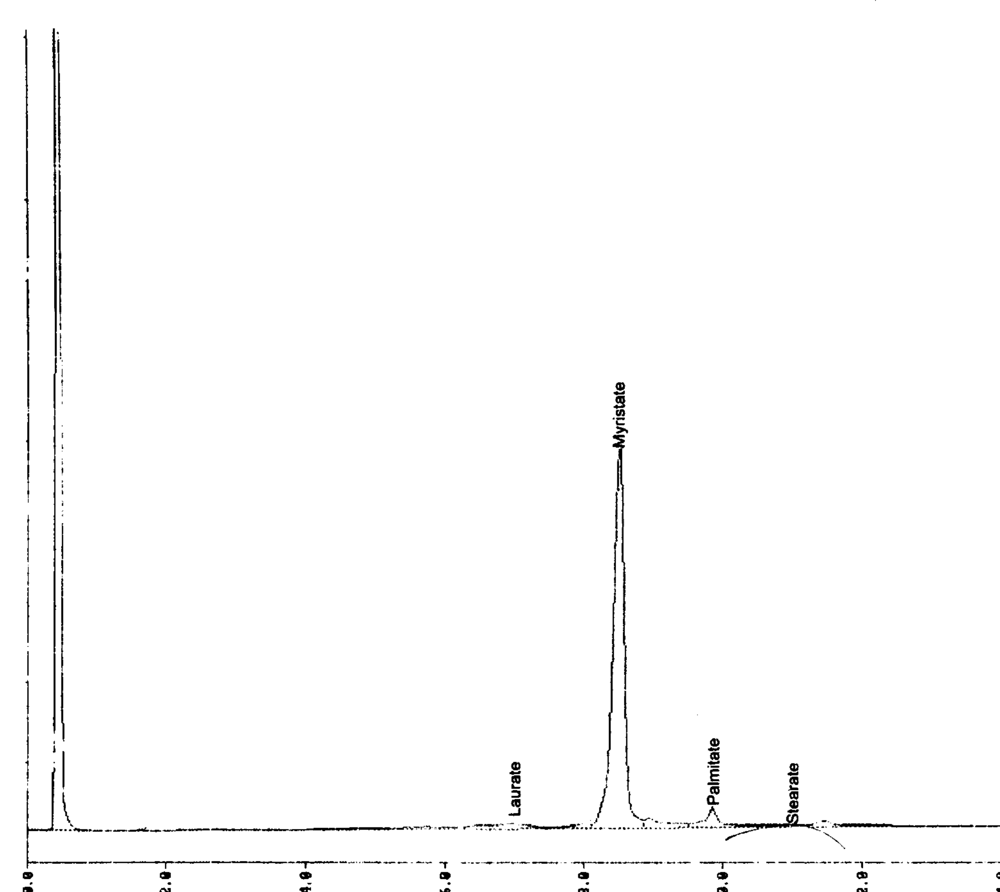


Figure 4.36 GC profile of FAMES of *M. beddomeii*-nutmeg

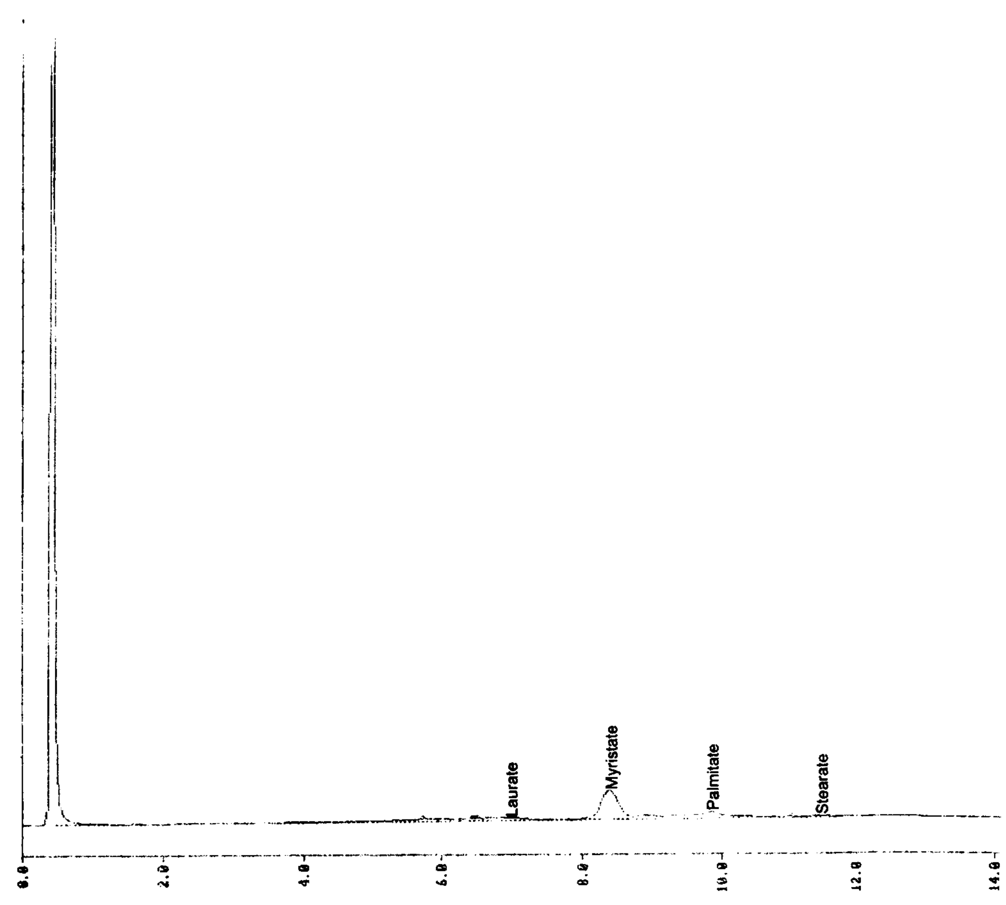


Figure 4.37 GC profile of FAMES of *M. beddomeii*-rind

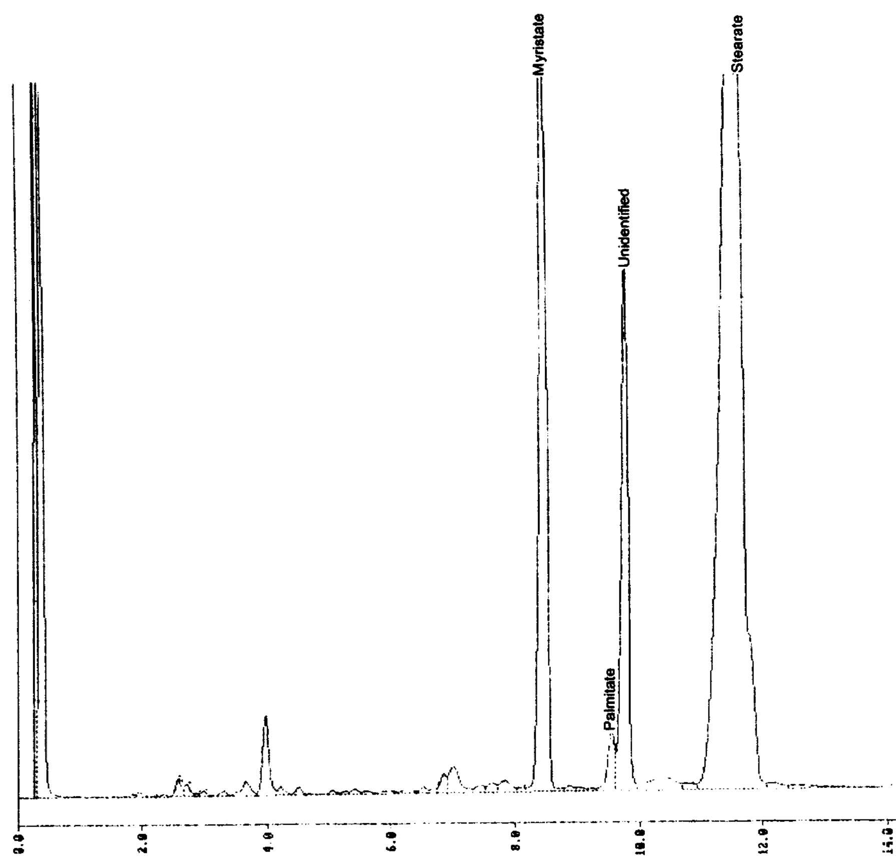


Figure 4.38 GC profile of FAMES of *M. malabarica*-nutmeg

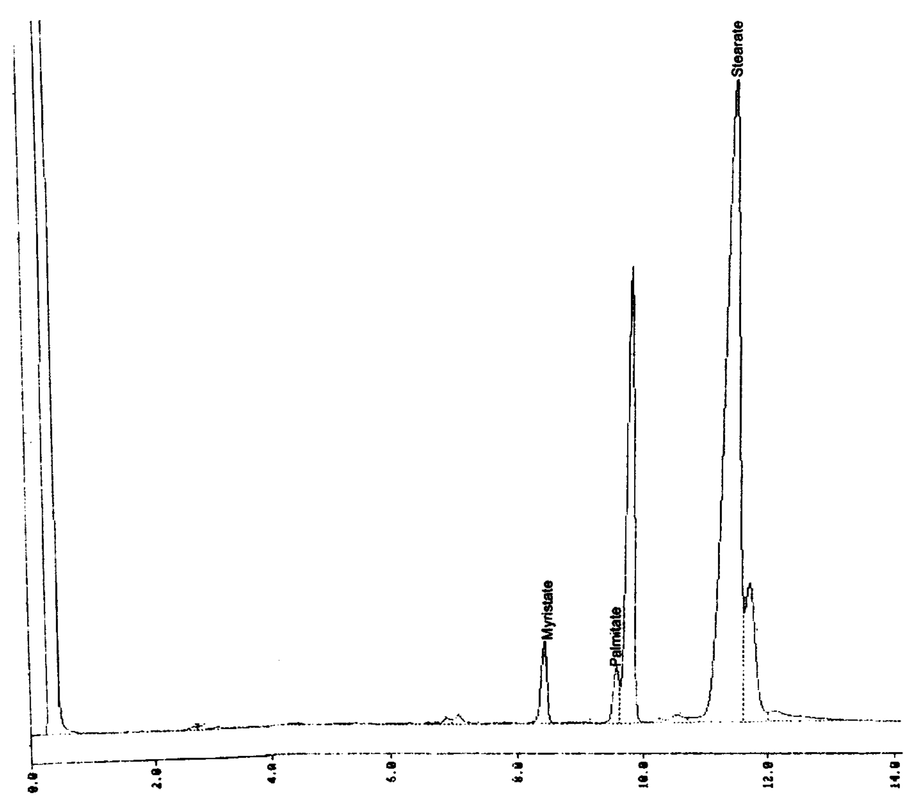


Figure 4.39 GC profile of FAMES of *M. malabarica*-mace

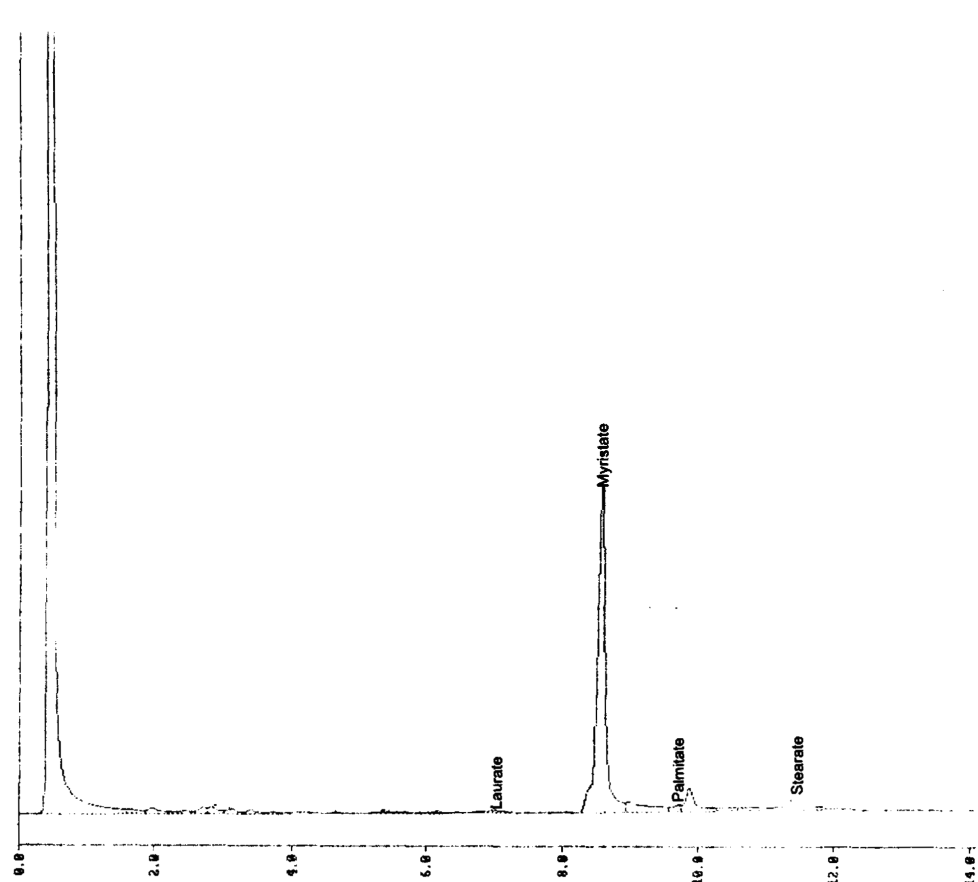


Figure 4.40 GC profile of FAMES of *M. prainii*-nutmeg

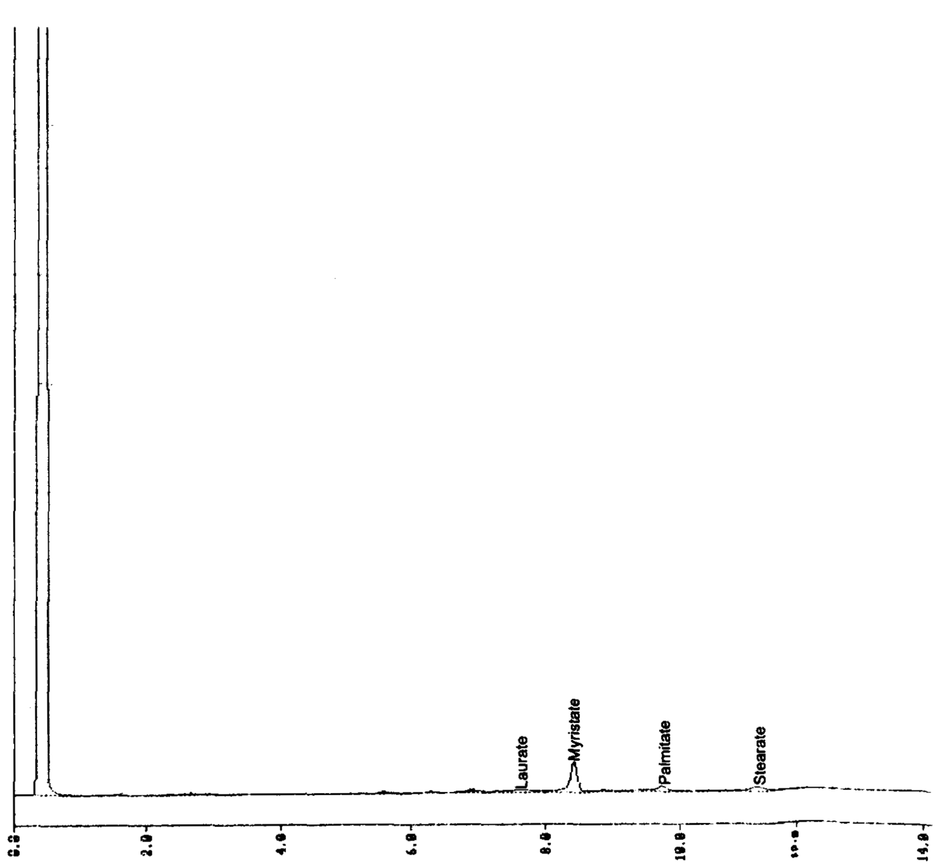


Figure 4.41 GC profile of FAMES of *M. prainii*-mace

Table 4.28 Fatty acid composition of *Myristica* species

Species	Lauric acid %	Myristic acid %	Palmitic acid %	Uniden-tified %	Stearic acid %	Uniden-tified %
M. fragrans – nutmeg	8.00	**55.10**	14.87	2.52	7.3	2.36
M. fragrans – mace	1.31	8.11	**52.56**	-	7.98	27.52
M. fragrans – rind	2.86	11.09	4.25	**50.00**	-	-
M. beddomeii - nutmeg	4.76	**78.20**	6.28	-	0.55	2.46
M. beddomeii - rind	9.07	**38.34**	22.17	2.91	5.40	-
M. malabarica – nutmeg	0.49	19.06	1.34	12.33	**57.64**	0.31
M. malabarica – mace	0.08	3.22	2.23	20.71	**59.39**	9.22
M. prainii – nutmeg	1.9	**61.39**	1.43	6.33	9.38	-
M. prainii – mace	12.08	**31.97**	16.95	5.38	9.14	1.42

Prakashchandra & Chandrasekharappa, 1984, have studied the lipid profile and fatty acid composition of mace. It had 21.6% ether extract, 83% chloroform soluble lipids and the predominant fatty acids were palmitic and oleic acids.

In *M. beddomeii* nutmeg, the fatty acid composition of the fat was reported (Anonymous, 1962) to contain stearic acid (60%) as the predominant fatty acid followed by oleic acid (35%). But the present study found myristic acid as the predominant fatty acid (78.20%) followed by palmitic acid (6.28%) (Table 4.28).

M. malabarica nutmeg contained stearic acid as the predominant fatty acid (57.64%) followed by myristic acid (19.06%). This is in contrast to the report (Anonymous, 1962), which describes myristic acid as the dominant fatty acid, followed by oleic acid.

M. malabarica mace also has stearic acid as the dominant fatty acid (59.39%) followed by an unidentified fatty acid (20.71%). As per the earlier report (Anonymous, 1962), mace has a fatty acid composition similar to nutmeg in *M. malabarica.*

Among the different species studied, *M. malabarica* differs from the rest of the group in having stearic acid as the predominant fatty acid in both nutmeg and mace.

4.3.1.6 Minerals

The total nitrogen content of leaf was found to be uniform in the various taxa (Table 4.29). Highest level of potassium was found in *M. fragrans* followed by *Knema andamanica* and calcium dominated in *M. magnifica* and *M. fragrans. M. malabarica* had high amounts of magnesium, iron and manganese, while, *M. amygdalina* and *M. prainii* were rich in magnesium and zinc.

A comparison had been made between cultivated and wild taxa of *Myristica* on the levels of various minerals in nut (kernel), mace and rind of *Myristica* species (Table 4.30). Among the minerals analyzed, nitrogen, copper, iron and zinc are found to be high in the kernel and mace of *M. fragrans.*

Among the wild taxa, calcium and manganese are high in the kernel of *M. beddomeii.*

The rind of *M. fragrans* contain significantly high amount of iron and that of *M. prainii* contain high amount of potassium.

Table 4.29 Profile of major and minor elements in the leaf

No	Species	N %	K %	Ca %	Mg %	Fe (ppm)	Cu (ppm)	Zn (ppm)	P %	Mn (ppm)
1	*M .fragrans*	0.56	1.24	1.65	0.33	90	28	16	0.1	110
2	*Knema andamanica*	0.45	1.22	1.23	0.223	29	35.2	14	0.099	274.2
3	*M. amygdalina*	0.58	0.78	1.26	0.379	17	4.4	30	0.085	164.9
4	*M. andamanica*	0.54	0.93	0.89	0.308	40	2.2	15	0.093	202.6
5	*M. beddomeii*	0.47	0.72	1.05	0.331	30	3.2	14	0.081	115.6
6	*M. magnifica*	0.52	0.66	1.96	0.358	38	10.2	34	0.077	173.9
7	*M. malabarica*	0.46	0.75	1.39	0.387	94	1.2	13	0.080	254.3
8	*M. prainii*	0.55	0.86	1.45	0.392	19	2.8	29	0.087	166.5

Table 4.30 Profile of major and minor elements in *Myristica* species

Species	N %	K %	Ca %	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
M. fragrans -nutmeg	0.85	0.62	0.12	98	13.0	16	41
M. fragrans -mace	0.98	0.88	0.11	111	21.3	15	23
M. fragrans-rind	0.56	1.63	0.37	139	9.9	7	23
M. beddomeii-nutmeg	0.54	0.89	0.75	22	0.8	8	64.2
M. beddomeii-mace	0.64	0.72	0.35	55	8.5	8	19.9
M. beddomeii-rind	0.42	1.93	0.45		12.6	1	3.3
M. malabarica-mace	0.53	2.05	0.73	102	2.5	9	11.9
M. prainii-mace	0.71	1.23	0.36	76	13.9	8	23.5
M. prainii-rind	0.42	3.95	0.37		6.1	6	6.5
Mean ± Standard deviation	0.62 ± 0.18	1.54 ±1.04	0.40 ± 0.22	86.14± 38.71	9.84 ± 6.3	8.6 ± 4.5	24.0 ± 18.72

4.3.2 Secondary Metabolites

4.3.2.1 Essential oil

Essential oil was estimated as described in 3.2.1.1. The essential oil is high (1.25%) in the leaf of *M. fragrans* when compared to wild taxa. Among the wild taxa, only *M. beddomeii* has significant content of oil (0.125%), others have only negligible oil content.

4.3.2.2 GC-MS of leaf essential oil

The leaf essential oil of *Myristica fragrans* and *Myristica beddomeii* were extracted and its GC profile was obtained (Figure 4.42 shows an overlay of *M. beddomeii* on *M. fragrans*). The samples were then subject to GC-MS.

Madhavan *et al.*, 1991, have reported sabinene as the predominant component followed by α-pinene in leaf essential oil of all the samples analyzed. Steam-distilled leaf essential oil from Indonesia has 80% α-pinene and 10% myristicin with poor flavour (Verghese, 2001c).

M. beddomeii leaf essential oil, when analyzed by GC-MS, revealed that β-caryophyllene was the predominant component in it. This sesquiterpene is known to have potent anti-carcinogenic property (Zheng *et al.*, 1992). β-caryophyllene along with β-caryophyllene oxide, α-humulene, α-humulene apoxide-I and eugenol were found to induce Glutathione-S-Transferase in the liver and small intestine of mouse. These natural anticarcinogens induce the detoxifying enzymes. Their activity is comparable with that of chemical carcinogens. Thus, this leaf oil is rich in the sesquiterpene that is a potent anticarcinogen. Table 4.31 illustrates that the wild species, *M. beddomeii* contains more than 40% of β-caryophyllene. It is interesting to note that the leaf oil of *M. fragrans* also contains 2.6% of β-caryophyllene.

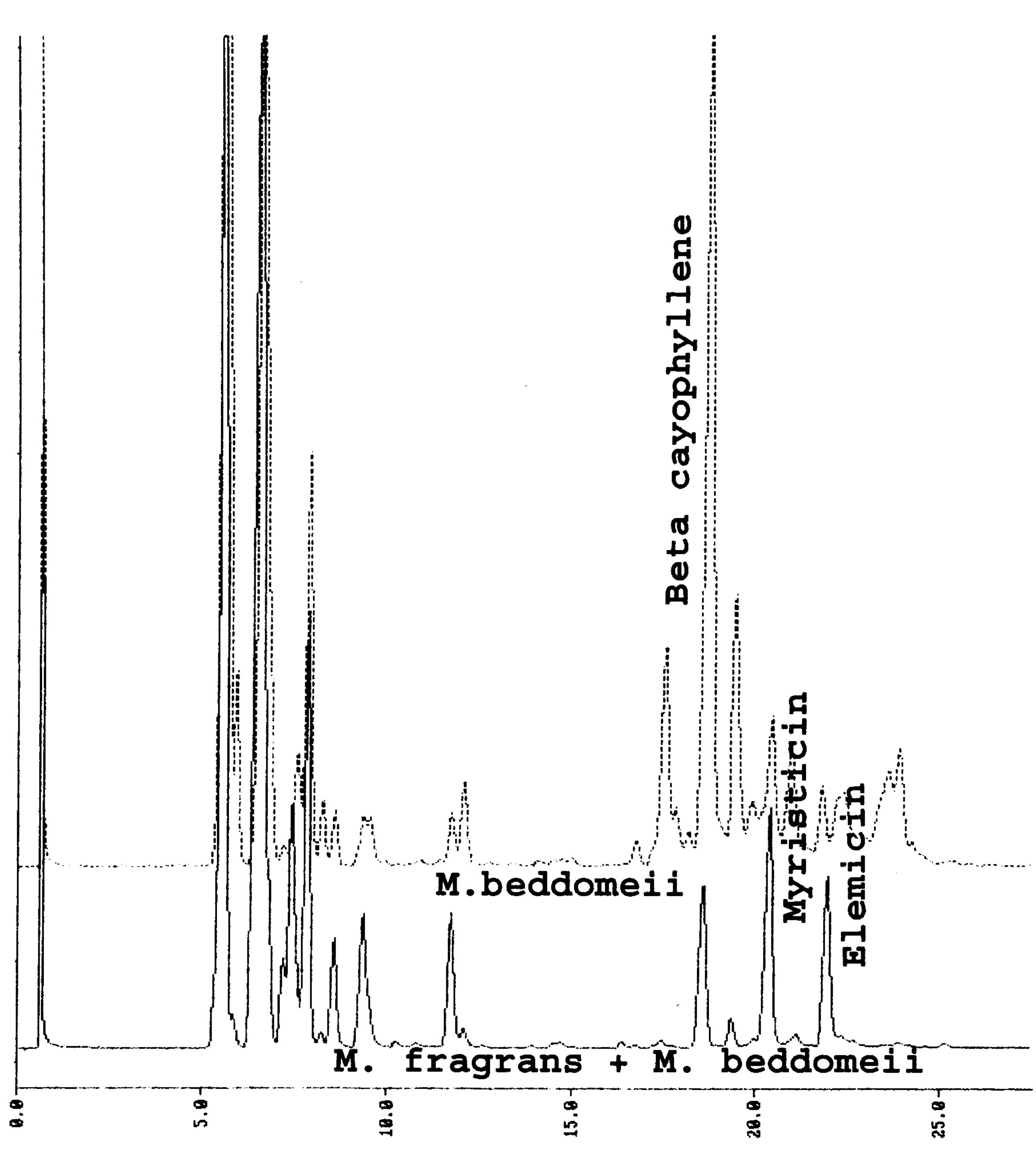


Figure 4.42 GC profile of leaf essential oil (Overlay of *M. beddomeii* on *M. fragrans*)

Table 4.31 GC-MS Profile of the leaf essential oil constituents

Rt	*Myristica fragrans*	Area %	Rt	*Myristica beddomeii*	Area %
3.25	Camphene	0.39			
3.79	2-β-Pinene	16.76			
4.02	Myrcene	2.32	3.98	Myrcene	0.05
4.28	α-phellandrene	2.12			
4.42	δ-3-carene	4.47			
4.54	α -terpinene	1.74			
4.72	o-cymene	0.14			
4.86	α -pinene	8.18			
5.58	γ-terpinene	2.83			
6.39	α -terpinolene	4.78			
6.76	Linalool	1.15	6.72	Linalool	0.27
7.33	p-menth-2-ene-1-ol	0.25			
9.73	β-fenchyl alcohol	1.85	9.65	β-fenchyl-alcohol	0.24
13..28	Safrole	0.61			
15.72	α-terpinyl acetate	0.38			

Continued

Table 4.31 GC-MS Profile of the leaf essential oil constituents

Rt	*Myristica fragrans*	Area %	Rt	*Myristica beddomeii*	Area %
16.57	α -copaene	0.66	16.68	α-copaene	4.72
17.06	Trans-β-damascenone	0.08	17.09	β-damascenone	0.18
			17.38	β -elemene	3.87
			17.91	(-)-lepidozene	0.16
18.26	β-caryophyllene	2.62	18.66	β-caryophyllene	44.26
			18.78	β -cubebene	0.15
			19.14	Trans-α-bergamotene	1.45
19.53	α -humulene	0.50	19.82	α -humulene	12.49
19.69	Cis-isoeugenol	0.83			
			19.94	Trans-β-farnesene	0.39
20.64	Germacrene-D	0.28	20.73	Germacrene-D	1.00
			20.91	β -selinene	1.12
			21.00	α -Curcumene	0.53
			21.13	β -ionone	0.25
			21.31	α -selinene	1.53
21.24	Bicyclo germacrene	0.32	21.36	Bicyclo germacrene	1.15

Continued

Table 4.31 GC-MS Profile of the leaf essential oil constituents

Rt	*Myristica fragrans*	Area %	Rt	*Myristica beddomeii*	Area %
			21.64	Germacrene A	0.9
			21.99	γ –muurolene	0.51
22.04	E,E-α- farnesene	0.06	22.11	E, E- α farnesene	0.16
			22.5	δ –cadinene	2.14
22.89	Myristicin	30.85			
			23.49	(+)- aromadendrene	0.89
			24.03	α-caryophyllene	0.28
24.10	Elemicin	1.32			
			24.29	d-nerolidol	2.48
			24.41	(+) spathulenol	0.28
24.56	Caryophyllene oxide	0.10	24.56	Caryophyllene oxide	1.4
25.68	Methoxy eugenol	0.12			
26.79	δ –cadinene	0.21	26.33	δ –cadinene	0.68
			27.09	β –eudesmol	0.99
27.25	α –cardinol	0.14			
			27.96	Cadalene	0.29

Continued

Table 4.31 GC-MS Profile of the leaf essential oil constituents

Rt	*Myristica fragrans*	Area %	Rt	*Myristica beddomeii*	Area %
			28.45	α -bisabolol	0.19
			30.10	Farnesol- 2	0.21
			38.57	Palmitic acid	1.59

A major dissimilarity observed between *M. fragrans* and *M. beddomeii* is that the latter lacks even traces of myristicin and elemicin in the leaf oil. The present GC-MS pattern revealed that the leaf oil of *M. fragrans* contained 30.85% of myristicin and 1.32% of elemicin. Compared to *M. fragrans*, the wild species lacks α- and β-pinene which contributes towards the woody note of spice oils.

4.3.2.3 Phenolic acids

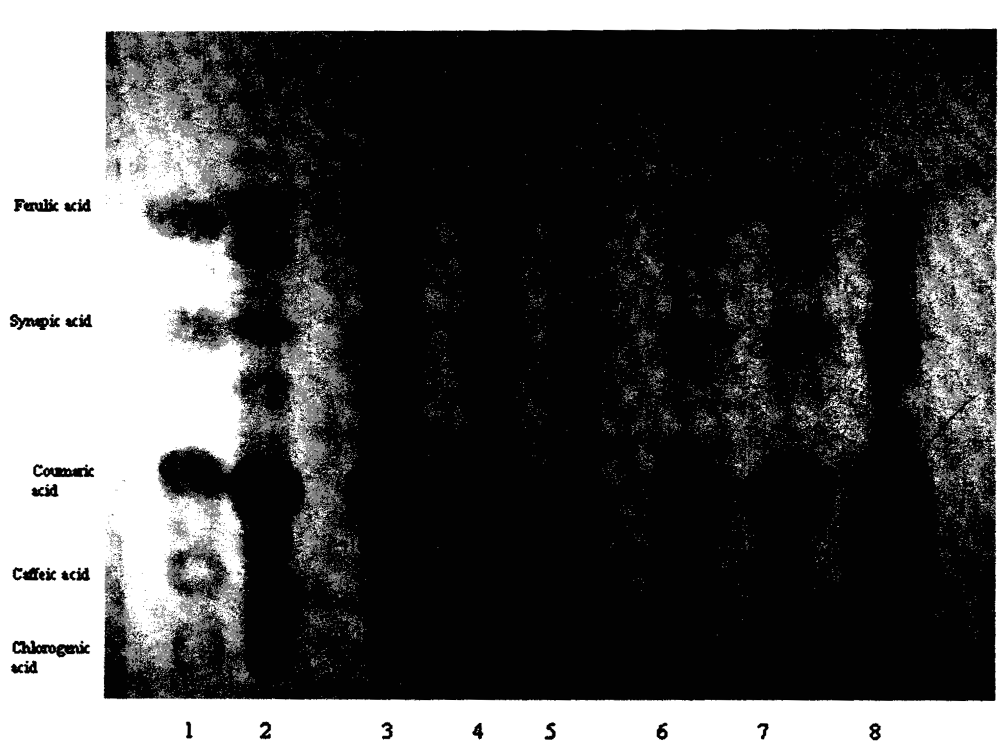
Phenolic acids from the leaves of the various taxa were extracted as per section 3.2.4.

A standard mixture composed of ferulic acid, synapic acid, coumaric acid, caffeic acid and chlorogenic acid was spotted along with the samples. The plate was visualized by first spraying with 20% Na_2CO_3 followed by Folin water reagent. Good resolution was obtained with the solvent system toluene: acetic acid (4:1). The major phenolic acids were found in all the taxa studied with the dominance of coumaric acid (Figure 4.43). The various spots had Rf values of 0.233, 0.267, 0.5, 0.533, 0.66, 0.733, etc. There was no variability in the phenolic acid pattern in the different taxa.

4.3.2.4 Crude Fibre

Table 4.32 Estimation of crude fibre in the rind

Species	Crude fibre %
M. fragrans	21.00
M. beddomeii	20.39
M. malabarica	20.60
M. prainii	22.12



1. Standard mixture
2. *M. fragrans*
3. *M. andamanica*
4. *M. amygdalina*
5. *M. prainii*
6. *M. magnifica*
7. *M. dactyloides*
8. *Knema andamanica*

Figure 4.43 TLC profile of phenolic acids in different taxa

The crude fibre yield in *M. fragrans*-rind is reported as 27.58% and 28.5% (Gopalakrishnan, 1992 and Verghese 2000). The crude fibre is estimated using Dosi fibre apparatus employing the Precision-Automated-Digestion-Technique. This may be one of the reasons for the reduction in the fibre content in this report and also due to the difference in location

4.3.2.5 Pigments in wild nutmeg

a. The pigment present in the mace of *M. prainii* is lycopene

b. In *M. beddomeii*, the pigment responsible for the colour of mace is not lycopene.

4.3.2.6 Flavonoid analysis

Flavonoids play an important role in the characterisation of different nutmeg species. Different attempts have been made to characterize the flavonoids in the wild and cultivated species of nutmeg. Promising results were obtained when the extract was subject to thin-layer-chromatography (TLC) using the solvent systems chloroform : acetic acid : water (30 : 15 : 2) and chloroform : methanol (9: 1). Among the two, the latter solvent system gave better resolution of flavonoids. As per the report of Harborne *et al* (1975), a dull yellow/orange fluorescence under UV light, which remains unchanged when exposed to ammonia vapour, is identified as flavonols. Based on these studies, the leaf extract of *M. fragrans* contains flavonols with a free 3-OH and with or without a free 5-OH group.

Summary and Conclusion

K.M. Maya "Biochemical variability in nutmeg (myristica fragrans) and related taxa" Thesis. Indian Institute of Spices Research, University of Calicut, 2005

Summary and Conclusion

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Myristica fragrans produces two spices, nutmeg and mace. It belongs to the *Myristicaceae* family with about 18 genera and 300 species. Though it is dioecious, male and female flowers are sometimes seen on the same tree. Under Family *Myristicaceae,* highly relevant species used for variability study are *M. fragrans, M. andamanica, M. malabarica, M. magnifica, M. amygdalina, M. beddomeii, M. prainii* and *Knema andamanica.* They are distinctly different in morphology, flowering pattern and related features.

M. fragrans, Houtt. is a native of the Moluccas in the East Indian Archipelago. *M. andamanica, Hook. F* is seen in the Andaman Islands with slender horizontal branches. They often have curved stilt-like roots at the base. *M. malabarica, Lamk,* from the Western Ghats yields fruits which have no odour and taste. They are called Bombay nutmeg and Bombay mace; they are used as adulterants of *Myristica fragrans. M. magnifica, Beddome Fl. Sylv.* is found in the swampy ground of Western Ghats. *Knema andamanica* is a moderate-sized tree seen in the evergreen forests of Western Ghats.

Nutmeg and mace are used for extracting essential oil by steam-distillation. The essential oil is colourless or pale yellow coloured with the characteristic spicy odour. Major constituents are sabinene, β-pinene, dipentene, *p*-cymene, *d*-linalool, terpinen-4-ol, *dl*-α-terpineol, geraniol, safrole, eugenol, isoeugenol, myristicin, myristic acid, esters of myristic acid and other fatty acids. Myristicin in the oils of nutmeg and mace is believed to be responsible for the toxicity of nutmeg. Oleoresin consists of both volatile and nonvolatile components.

Nutmeg is a dioecious plant, with the occasional occurrence of bisexual plants that may predominate in either male or female flowers. Sex determination is a problem in the seedling stage. Sex of the plant can be ascertained only after flowering, which takes 6-8 years.

Wide variability is observed between the cultivated and the wild plants of *Myristica.* Morphologically, leaves of *M. fragrans* are relatively very small compared to the wild plants. The shape of fruits, nutmeg and mace are distinctly different between wild and cultivated species. *M. fragrans* leaf yields 0.5 to 2% oil while the wild species yield only negligible oil. Nutmeg and mace of *M. fragrans* yields good amount (up to 16.5% and 26.1% respectively) of essential oil while the wild fruits are almost devoid of essential oil. The mace of *M. fragrans* is bright red by virtue of the lycopene present in it; it is not seen in *M. beddomeii.* Hence variability was observed between the wild and cultivated taxa of *Myristicaceae* with respect to morphological and levels of primary and secondary metabolites. A study was undertaken to establish the biochemical variability with the following objectives.

1. Evaluation of biochemical variability in nutmeg (*Myristica fragrans*) germplasm.
2. Differentiation of male, female and bisexual plants based on primary and secondary metabolites in the leaf of *Myristica fragrans.*
3. Biochemical variability between *Myristica fragrans* and related taxa.

Based on the study conducted, the results obtained can be illustrated as follows.

Biochemical variability in the nutmeg (*Myristica fragrans*) germplasm. Among the accessions analyzed, no variability was observed in some of the primary metabolites such as total carbohydrate (30.4% - 33.1% in nutmeg, 42.3% - 45.7% in mace and 17.9% - 19.5 % in leaf), starch (26.3% -28.4% in nutmeg, 36.3 % - 38.1 % in mace and 15.8 % - 17.4 % in leaf), reducing sugars (0.15% -0.2 % in nutmeg, 0.27% -0.31 % in mace and 2.26% - 3.9 % in leaf) and protein (5.3 % in nutmeg, 6.1% in mace and 3.5 % in leaf). The fat content ranged from 28.2% - 34.9% and 23.7% - 26.4% in nutmeg and mace respectively. When fatty acid methyl esters (FAMES) from the fat were identified in a Gas Chromatograph, myristic acid was found to be the dominant

(55.1%) fatty acid in nutmeg and palmitic acid (52.56%) dominated in mace fat. Rind had higher content of potassium, calcium and iron when compared with nutmeg and mace. Manganese was more in nutmeg while mace had higher copper content.

Distinct variability was observed in the nutmeg germplasm with respect to the essential oil composition. The essential oil content ranged from 5 % to 16.5% in nutmeg and 7.1% to 26.1% in mace. Some of the nutmeg accessions of IISR such as A9/18, A9/49 and A11/49 were rich in both nutmeg and mace oils. The present study has also revealed that the total yield of nutmegs from a tree do not indicate its oil recovery. However, medium yielders have a slight edge in the oil yield compared to the other groups. Accession A9/18, with a medium yield of nutmeg, had the highest oil recovery. Myristicin or methoxy safrole is the major aromatic constituent of nutmeg and mace oils. The study identified many accessions with low and high myristicin. The myristicin content was as low as 1.5 % (A9/102) and as high as 15.1% (A9/18). Another important observation in the study is the high level of sabinene (45% in A9/71) in the oils. Many accessions have been identified which can find specific industrial use. Nutmeg leaf is also a potential source for oil and its constituents. Even though the concentration of various constituents varies in the leaf oil compared to nutmeg and mace oil, all the constituents are found in leaf oil too. Variability in the hallucinogenic principles such as myristicin, elemicin, safrole and the confectionery principle sabinene is also found in leaf oil.

Extracting nutmeg oleoresin with acetone will extract fat also. Study conducted using different solvents indicate that extracting fat first with petroleum ether from the kernel powder followed by extraction with acetone gives the true picture of oleoresin and fat content. The oleoresin content of nutmeg had a range of 3.4 % - 11.9% while its fat content ranged from 28.2% - 34.9%. The Non-Volatile Ether Extract (NVEE) in nutmeg and mace was 33.77 and 18.65% respectively. Lycopene is the pigment responsible for the brilliant red colour of mace. It had a range of (82.4 mg % - 273.9 mg %). It is a nutraceutical with potent antioxidant properties (Maya *et al.*, 2002)

Primary and secondary metabolites of leaf from male, female and bisexual plants of *Myristica fragrans*

Some of the primary metabolites like total carbohydrate (15.9%-18.6%), starch (10.3%-14.81%), reducing sugars (2.52%-3.4%) and proteins (3.5%) did not show much variability within *Myristica fragrans*. When the amino acid profile was studied by HPLC, it revealed that there is no amino acid specific for male or female plant. Some of the male plants had high histidine and the female plant A9/4 had high phenylalanine. Total free amino acids were found more in the bisexual plant compared to the other two groups. The phenol content ranged from 0.21% - 0.31% within the species.

Among the minerals analysed, copper was found to be higher in the male lines. In contradiction to earlier reports, this study illustrates the fact that oil level or its constituents do not give any sexual identity in nutmeg. In case of phenolic acids, compared to the reported dominance of ferulic and synapic acids in nutmeg and mace respectively, the dominance of coumaric acid in leaf was established. Male, female and bisexual lines, however, did not show any variability. Based on polyphenol oxidase (PPO) and peroxidase isozymes, no variability was found between the cultivated lines of *Myristica fragrans*.

Biochemical variability among the various taxa. Levels of total carbohydrate, starch, reducing sugars and leaf-protein are all on par in cultivated and wild taxa. Fat content in nutmeg (*M. fragrans*) is about 40%. Only *M. prainii* had the nearest fat content compared to *M. fragrans*. In case of the mace samples, *M. fragrans* and *M. malabarica* had high fat compared to *M. prainii.* Cultivated and wild taxa had the same fat in the rind in all the cases. The rind of *M. beddomeii, M. malabarica* and *M. prainii* had crude fibre content similar to that of *M. fragrans* (21%).

Essential oil is high in the leaf of *M. fragrans* when compared to the wild taxa. Among the wild taxa, only *M. beddomeii* has significant content of oil (0.125%). Gas Chromatographic-Mass Spectral (GC-MS) study of leaf oils of

M. fragrans and *M. beddomeii* revealed some important findings. *M. beddomeii* is very rich in β-caryophyllene (40%). *M. fragrans* leaf oil contains 2.6% of β-caryophyllene, 30.85% of myristicin and 1.32% of elemicin. *M. beddomeii* lacks myristicin, elemicin, α- and β-pinene. Phenylalanine was found to be the dominant amino acid in all the different species except *Knema andamanica,* which is very rich in threonine and alanine. Its alanine content is very high compared to other wild plants. Total free amino acids ranged from 127.65 µg/100mg to 215.21 µg/100mg. *M. prainii* and *Knema andamanica* had the highest amino acid content. Nutmegs of both *M. fragrans* and *M. beddomeii* have myristic acid as the predominant fatty acid followed by palmitic acid. In *M. malabarica*, stearic acid is the predominant fatty acid followed by myristic acid. In contrast to nutmeg, *M. fragrans* mace has palmitic acid as the predominant fatty acid while the mace of *M. malabarica* dominates in stearic acid. Among the different species studied, *M. malabarica* differs from the rest of the group in having stearic acid as the predominant fatty acid in both nutmeg and mace. Among the minerals analyzed, nitrogen, copper, iron and zinc are found to be high in the kernel and mace of *M. fragrans.* Among the wild taxa, calcium and manganese are found to be high in the kernel of *M. beddomeii.* The rind of *M. fragrans* contains significantly high amount of iron and that of *M. prainii* contains high amount of potassium. The pigment present in the mace of *M. fragrans* and *M. prainii* is lycopene while, in *M. beddomeii*, it did not tally with the lycopene pattern.

From the study, it can be concluded that, biochemically, the cultivated and wild taxa are distinctly different. The dissimilarity is very profound in essential oil content, its profile, fat, minerals, amino acid profile, etc. The study also concludes the uniqueness of male and female lines having uniform oil and amino acid profile.

References

(1) **Abdalla** M H, Elkhalifa A O and Eltinay A H 2001 Factors affecting protein extraction from cowpea. *Journal of Food Science and Technology* **38** (5) 532-533.

(2) **Ahmad** H 2001. WWW. /Whey 2 health.com/Ahmad 2001.pdf.

(3) **Anguelova** T and Warthesen J J 2000a Degradation of lycopene, alpha-carotene, and beta-carotene during lipid peroxidation. *J. of Food Sci.* **65**:71-75

(4) **Anguelova** T and Warthesen J J 2000b Lycopene stability in tomato powders. *J. of Food Sci* **65**: 67-70.

(5) **Anonymous** 1962 The wealth of india – a dictionary of Indian Raw materials and Industrial Products. Volume VI. L-M, CSIR, New Delhi. pp. 473-480.

(6) **Ansari** T.M., Ikram N, Najam-ul-Haq M, Fayyaz I, Fayyaz Q, Ghafoor I and Khalid N 2004 Essential Trace Metal (Zinc, Manganese, Copper and Iron) Levels in Plants of Medicinal Importance. *Journal of Biological Sciences* **4** (2): 95-99.

(7) **AOAC** 1975 Official Methods of Analysis.. 12th Ed. Association of Official Analytical Chemists, Washington, D.C. p 1094

(8) **Archer** A W 1988 Determination of safrole and myristicin in nutmeg and mace by high-performance liquid chromatography. *Journal-of-Chromatography* **438** (1): 117-121.

(9) **ASTA** (American Spice Trade Association) 1968 Official Analytical Methods. 2nd edition. American Spice Trade Association, New York p. 53

(10) **Avigad** G and Dey P M 1997 Carbohydrate metabolism: Storage Carbohydrates. In: Plant Biochemistry Dey P M and Harborne J B (Eds.).Academic Press, New York pp. 143-204

(11) **Babu** K N, Regy Lukose, Ravindran P N, Lukose R and Vidhyasekaran P 1993 Tissue culture of tropical spices. In: Genetic engineering,

molecular biology and tissue culture for crop pest and diseases management. Daya Publishing House, Delhi pp 257-267.

(12) **Baldry** J, Dougan J, Matthews W S, Nabney J, Pickering G R and Robinson F V 1976 Composition and flavour of nutmeg oils. *International-Flavours-and-Food-Additives* **7**(1): 28-30

(13) **Bernard** T, Perineau F, Delmas M and Gaset A 1989 Extraction of essential oils by refining of plant materials. II. Processing of products in the dry state: *Illicium verum* Hooker (fruit) and *Cinnamomum zeylanicum* Nees (bark). *Flavour and Fragrance Journal.* **4**(2): 85-90.

(14) **Bernath** J, Foldesi D, Lassanyi Z and Zambo I 1975 The effect of nutrient supply and soil type on the common valerian (*Valeriana officinalis* ssp. Collina) II. Changes in the volatile oil and valepotriate contents. *Herba Hungarica.* **14**: 2-3, 37-46.

(15) **Betts** J J 1990 Possible value of a liquid crystal stationary phase for the gas chromatographic study of aromatic constituents of essential oils. *Journal of Chromatography.* **513**: 311-314.

(16) **Boelens** 2000 The chemical and sensory evaluation of edible oleoresins. *Perfumer and flavorist.* **25**: 10-23.

(17) **Bohannon** M B and Kleiman R 1977 Myristicin. A major volatile component in mature seed of Portenschlagia ramosissima. *Lipida.* **12** (3): 321-323.

(18) **Bramley** P M 1997 Isoprenoid Metabolism. In: Plant Biochemistry Dey P M and Harborne J B (Eds.). Academic Press, New York pp. 417-437

(19) **Broadhurst** C L, Polansky M M and Anderson R A 2000 Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. *J. Agricultural and Food Chemistry.* **48** (3): 849-852.

(20) **Brown** A H D and Wier B S 1983 Measuring genetic variability in plant population. In: Isozymes in plant genetics and breeding part A. Tanksley S D and Orton T J (Eds.) Elsevier Science Publishers B. V. pp. 219-239

(21) **Buchanan** R L 1978 Toxicity of spices containing methylenedioxybenzene derivatives: a review. *J. Fd-Safety.* **1** (4): 275-293.

(22) **Calderone** N W, Bruce W A, Allen Wardell G and Shimanuki H 1991 Evaluation of botanical compounds for control of the honey-bee tracheal mite, Acarapis woodi. *American Bee Journal.* **131** (9): 589-591.

(23) **Carmona** P, Bellanato J and Hidalo A 1976 Study of orange essential oils. II. Chemical modifications during aging. *Annales Technologie Agricole.* **25** (2): 159-174.

(24) **Cheong** Choo-Li, Wong-SiewMeng, Liew-KongYong, Choo-LC, Wong-SM and Liew-KY 1999 Essential oil of nutmeg pericarp. *Journal-of-the-Science-of-Food-and-Agriculture.* **79**: 13

(25) **Crawford** D J 1983 Phylogentic and systematic inferences from electrophoretic studies. In: Isozymes in plant genetics and breeding part A. Tanksley S D and Orton T J (Eds.)Elsevier Science Publishers pp. 257-287

(26) **Cruickshank** A M 1973 Some aspects of the nutmeg investigation programme in Grenada. Proceedings-of-the-Conference-on-Spices,-10th-14th-April,-1972. 1973; 105-111

(27) **Damhoeri** A, Hosono A, Itoh T and Matsuyama A 1985 In vitro mutagenicity tests on capsicum pepper, shallot and nutmeg oleoresins. *Agricultural-and-Biological-Chemistry.* **49**(5): 1519-1520

(28) **Dang** M N, Takacsova M, Nguyen D V: and Kristianova K 2001 Antioxidant activity of essential oils from various spices. *Nahrung* **45** (1): 64-66

(29) **De** Minakshi. Amith Krishna De and Arun Baran Banerjee 1999. Screening of spices for antimicrobial activity. *J. of Spices and Aromatic Crops* **8** (2): 135-144.

(30) **Demetriades** A K, Wallman P D, McGuiness A and Gavalas M C 2005 Low cost, high risk: accidental nutmeg intoxication *Emergency medicine journal* **22**: 223-225.

(31) **Dorman** H J D, Deans S G, Noble R C and Surai P 1995 Evaluation in vitro of plant essential oils as natural antioxidants *Journal-of-Essential-Oil-Research* **7**(6): 645-651

(32) **Dorman** H J D, Figueiredo A C, Barroso J G and Deans S G 2000 *In vitro* evaluation of antioxidant activity of essential oils and their components *Flavour-and-Fragrance-Journal* **15**(1): 12-16

(33) **Ehlers** D, Kirchhoff J, Gerard D and Quirin K W 1998 High-performance liquid chromatography analysis of nutmeg and mace oils produced by supercritical CO2 extraction - comparison with steam-distilled oils - comparison of East Indian, West Indian and Papuan oils. International-*Journal-of-Food-Science-and-Technology.* **33**(3): 215-223

(34) **El-Baz** F K, El-Fouly M M and Salama Z A 1999 Factors affecting protein extraction from green leaves. *Bulletin of the National Research Centre Cairo* **24**(4) 429-437.

(35) **Ferrao** J E M 1993 Spices: cultivation – technology – trade. In: Centro de Documentacao e Informacao, Instituto de Investigacao Cientifica Tropical; Lisboa; Portugal p. 413

(36) **Gallegos-Tintore** S, Pacheco- Aguirre J, Betancur- Ancona D and Chel-Guerrero L 2004 Extraction and characterization of soluble proteins from *Phaseolus lunatus* L seeds. *Archivos-Latinoamericanos-de-Nutrition* **54**(1): 81- 88

(37) **Gamble** J S 1967 Flora of the Presidency of Madras. Volume II. Botanical Survey of India. pp. 848-851.

(38) **Gbolade** A A and Lockwood G B 1989 Volatile constituents from parsley cultures. *Flavour-and-Fragrance-Journal* **4**(2): 69-71

(39) **Gbolade** A A and Lockwood G B 1990 Metabolic studies of volatile constituents in tissue cultures of Petroselinum crispum (Mill) Nyman. *Journal of Plant Physiology.* **136** (2): 198-202.

(40) **Gopalakrishnan** M 1992. Chemical composition of Nutmeg and Mace. *Journal of Spices and Aromatic Crops* **1**: 49-54.

(41) **Gopalakrishnan** M, Rajaraman K and Mathew A G 1979. Identification of the Mace pigment. *Journal of Food Science and Technology* **16**: 261-262.

(42) **Gopalan** C, Sastri B V and Balasubramanian S C 1981 Nutritive value of Indian foods. Indian Council of Medical Research, Hyderabad, India.

(43) **Gotke** N, Maheshwari M L and Mathur V K 1990 Nematicidal activity of *Myristica fragrans* against Meloidogyne incognita *Indian-Perfumer* **34**(2): 105-107

(44) **Goyal** R K and Korla B N 1993 Changes in the quality of turmeric rhizomes during storage. *Journal-of-Food-Science-and-Technology* **30**(5): 362-364

(45) **Guenther** E 1975 The essential oils, vol. 5 Robert E Krieger Publishing Company. pp. 59-81

(46) **Hallstrom** H and Thuvander A 1997 Toxicological evaluation of myristicin. *Natural-Toxins* **5**(5): 186-192

(47) **Hames** B D 1994 One dimensional polyacrylamide gel electrophoresis In: Gel electrophoresis of proteins: a practical approach (Hames B D and Rickwood D Eds.) The practical approach series, Oxford University Press, New York p 83

(48) **Harborne** J B, T.J. Mabry & Helga Mabry, 1975. The flavonoids part1 1975, Academic Press p 631

(49) **Harwood** J L 1997 Plant lipid metabolism. In: Plant Biochemistry Dey P M and Harborne J B (Eds.). Academic Press, New York. pp. 237-272

(50) **Hattori** M, Yang X W, Miyashiro H & Namba T 1993 Inhibitory effects of monomeric and dimeric phenylpropanoids from mace on lipid peroxidation *in vivo* and *in vitro*. *Phytotherapy Research* **7** (6): 395-401.

(51) **Hautke** P and Petricek D 1972 The importance of hop essential oils in beer flavour. *Chmelarstvi.* **45(**10): 154-156.

(52) **Hayanis**, Goutara and Ketaren S 1975 Influence of the degree of fruit ripeness and method and duration of distillation of nutmeg seed on the yield and quality of essential oil. *Bulletin-Penelitian-Teknologi-Hasil-Pertanian.* 14

(53) **Hennessey** T M, Andrew D and Nelson D L 1983 Biochemical studies of the excitable membrane of Paramecium tetraurelia VII Sterols and other neutral lipids of cilia. *Journal of Lipid Research* **24**(5): pp. 575-587.

(54) **Holstein** T, Burnett J and Quevedo W 1967 Genetic Regulation of Multiple Forms of Tyrosinase in Mice; Action of alpha and beta Loci. *Proc Soc Exp Biol Med* **126**: 415.

(55) **Hooker** J D 1973 Flora of British India. Periodicals experts, Vivek Vihar, New Delhi-32.p 102.

(56) http://members.aol.com/cocorc/hallucinogens.html.

(57) http://ntp-server.niehs.nih.gov/htdocs/Chem

(58) http://www.erowid.org/plants/nutmeg/nutmeg_faq.shtml.

(59) http://www.erowid.org/plants/nutmeg/nutmeg_journal1.shtml

(60) http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/v4084e/v4084e07.htm

(61) http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/v4084e/v4084e04htm

(62) http://www.inchem.org/documents/pims/plant/pim355.htm#SubSectionTitle:7.2.1%20%20Human%20data.

(63) **Iyer** R I, Jayaraman G, Gopinath P M and Sita G L 2000a Direct somatic embryogenesis in zygotic embryos of nutmeg (*Myristica fragrans* Houtt.). *Tropical Agriculture* **77**(2): 98-105.

(64) **Iyer** R I, Jayaraman R and Gopinath P M 2000b Isolation of protoplasts from leaves of nutmeg *Myristica fragrans* Houtt. *Biomedicine* **20**(3): 200-202.

(65) **Jankovsky** M, Perinova D and Petr J 1993 A new method for determining and isolating essential oils in spices and their constituents. *Zahradnictvi* **20**(4): 201-212.

(66) **Janssens** J, Laekeman G M, Pieters L A C, Totte J, Herman A G and Vlietinck A J 1990 Nutmeg oil: identification and quantitation of its most active constituents as inhibitors of platelet aggregation *Journal-of-Ethnopharmacology* **29**(2): 179-188

(67) **Jochen** Beyer and Hans Maurer *H* 2005 Abuse of Nutmeg (myristica Fragrans Houtt.): Identification of the Metabolites of its Ingredients Elemicin, Myristicin And Safrole In Rat And Human Urine By GC-MS: 9. *Therapeutic Drug Monitoring.* **27**(2): 212-213

(68) **Khalil** M M 2001 Biochemical and technological studies on the production of isolated guar protein. *Nahrung* **45**(1) 21-24.

(69) **Khanum** F, Sudarshanakrishna K R, Semwal A D, Vishwanathan K R and Khanum F 2001 Proximate composition and mineral contents of spices. *Indian Journal of Nutrition and Dietetics* **38**(3): 93-97.

(70) **Khosla** M K, Mala-Bhasin 2000 Biosynthetic correlation of major essential oil components in *Ocimum carnosum* L K Otto. *Indian Perfumer* **44**: 55-59

(71) **Kobayashi** A, Matsumoto M, Uchida K and Yamanishi T 1983 Aroma development in citrus iyo by pretreatment and storage. In: Proceedings of the International Society of Citriculture. Volume 2, 909-910.

(72) **Konig** W A, Kruger A, Icheln D and Runge T 1992 Enantiomeric composition of the chiral constituents in essential oils. Part 1:

monoterpene hydrocarbons. HRC,-*Journal-of-High-Resolution-Chromatography* **15**(3): 184-189

(73) **Koshy** A S and Vijayalakshmi N R 2001 Impact of certain flavonoids on lipid profiles - potential action of Garcinia cambogia flavonoids *Phytotherapy-Research* **15**(5): 395-400

(74) **Krishnamoorthy** B and Rema J 2001. Handbookof herbs and spices – K. V. Peter (Ed), Woodhead Publishing Ltd., Cambridge, England. 238-248.

(75) **Kumara** S J S, Packiyasothy E V and Jansz E R 1985 Some studies on the effect of maturity and storage and the chlorophyll content and essential oils of the cardamom fruit *(Ellettaria cardamom)*. *Journal of the Science of Food and Agriculture.* **36**(6): 491-498.

(76) **Kumaravelu** P and Subramaniyam S 1996 The antioxidant effect of eugenol on CC14-induced erythrocyte damage in rats. *Journal of Nutritional Biochemistry.* **7**(1): 23-28.

(77) **Lawrence** B M 2000 Progress in essential oils. *Perfumer & Flavorist* **25**: 66-68

(78) **Lee B K**, Kim J H, Jung J W, Choi J W, Han E S, Lee S H, Ko K H and Ryu J H 2005 Myristicin-induced neurotoxicity in human neuroblastoma SK-N-SH cells. *Toxicol Lett.* **157**(1): 49-56.

(79) **Lewis** Y S, Nambudiri E S, Krishnamurthy N and Amma B S K 1976 Essential oils in spices. *Arecanut and Spices Bulletin.* **8** (1): 5-8.

(80) **Lichtenstein** E P, Liang T T, Schulz K R, Schnoes H K and Carter G T 1974 Insecticidal and synergistic components isolated from dill plants. *Journal of Agricultural and Food Chemistry* **22**(4): 658-664.

(81) **Luk'-yanov** I A and Berestovaya M M 1973 Changes in the essential oil content of coriander seeds during storage. *Trudy VNII Efirnomaslichnykh 'tur.* **6**: 166-170.

(82) **MacLeod** A, Snyder C H and Subramanian G 1985 Volatile aroma constituents of parsley leaves. *Phytochemistry.* **24**(11): 2623-2627.

(83) **Madhavan** L, M Gopalakrishnan and C. S. Narayanan 1991. Chemical composition of nutmeg leaf and flower oils. *Indian Perfumers* **35**(3): 161-163.

(84) **Mallavarapu** G. R and Ramesh, S. 1998. Composition of essential oils of nutmeg and mace. *J. Medicinal and Aromatic Plant Science*, **20**: 746-748.

(85) **Mantle** D, Anderton J G, Falkous G, Barnes M, Jones P and Perry E K 1998 Comparison of methods for determination of total antioxidant status: application to analysis of medicinal plant essential oils. *Comparative-Biochemistry-and-Physiology-B,-Biochemistry-and-Molecular-Biology.* **121**(4): 385-391

(86) **Marston** A, Hostettmann K and Msonthi J D 1995 Isolation of antifungal and larvicidal constituents of Diplolophium buchanani by centrifungal partition chromatography. *Journal of Natural Products.* **58**(1): 128-130.

(87) **Maya** K M, Zachariah T J and Kishnamoorthy B 2002 Lycopene and volatile oil constituents-changes during storage of mace (*Myristica fragrans*) powder. In: National Seminar on Strategies for Increasing Production and Export of Spices.Abstracts of papers Zachariah *et al* (Eds.), 24-26 October 2002, Indian Society for Spices, Calicut, Kerala. pp 54-55

(88) **Maya** K M, Zachariah T J and Kishnamoorthy B 2004 Chemical composition of essential oil of nutmeg accessions. *J. of Spices and Aromatic Crops* **13**(2):135-139

(89) **McMillin** D E1983 Plant isozymes – historical aspects. In: Isozymes in plant genetics and breeding part A Tanksley S D and Orton T J (Eds.). Elsevier Science Publishers B. V. pp. 3-13

(90) **Mounicou** S, Meija J and Caruso J 2004 Preliminary studies on selenium-containing proteins in *Brassica junea* by size-exclusion

chromatography and fast protein liquid chromatography coupled to ICP-MS *Analyst* **129**(2): 116-123.

(91) **Moure** A, Rua M, Sineiro J and Dominguez H 2002 Aqueous extraction and membrane isolation of protein from defatted *Gevuina avellana* *Journal of Food Science* **67**(2): 688-696

(92) **Moyler** D A 1993 Extraction of essential oils with carbon dioxide. *Flavour-and-Fragrance-Journal* **8**(5): 235-247

(93) **Naik** S N, Maheshwari R C and Maheshwari M L 1988 Extraction of essential oils in liquid and dense carbon dioxide. *Indian Perfumer.* **32**(1): 74-85.

(94) **Nishizawa** A, Honda G and Tabata M 1989 Determination of final steps in biosynthesis of essential oil components in Perilla frutescens. *Planta-Medica.* **55**(3): 251-253.

(95) **Orabi** K Y, Mossa J S and El-Feraly F S 1991 Isolation and characterization of two antimicrobial agents from mace (*Myristica fragrans*). *Journal-of-Natural-Products* **54**(3): 856-859

(96) **Packiyasothy** E V, Jansz E R and Dharmadasa H M 1991 Studies on some chemical components of nutmeg *(Myristica fragrans* - Houtt) leaf directed at determination of sex of seedlings. *Journal-of-the-National-Science-Council-of-Sri-Lanka* **19**(2): 91-97

(97) **Parkinson** C E 1972 A forest flora of The Andaman Islands – an account of trees, shrubs and principal climbers of the Islands. layyed Press, Ballimaran, New Delhi. pp. 223-224.

(98) **Peter** K V and Zachariah T J 2000 Spice oils and oleoresins: challenges and opportunities. *Journal of Medicinal and Aromatic Plant Sciences.* **22**: 247-252.

(99) **Prakashchandra** K S and Chandrasekharappa G 1984 Lipid profile and fatty acid composition of fat extracted from arils (mace) of *Myristica fragrans* and seeds of *Artocarpus hirsutus. Journal of Food Science and Technology* **21**(1), 40-42

(100) **Pruthi** J S 1979 Spices and condiments. National Book Trust, New Delhi. pp. 166-172.

(101) **Purseglove** J W, Brown E G, Green C L and Robbins S R J 1981 Spices-Vol-1 New York, USA, UK: Longman Group Ltd. p 439

(102) **Rao** M R N 1991 Prospects of nutmeg, clove and cinnamon cultivation in Andaman & Nicobar Islands. *Indian Cocoa, Arecanut and Spices Journal.* **14**(3): 118-120.

(103) **Rasheed** A, Laekeman G M, Vlietinck A J, Janssens J, Hatfield G, Totte J and Herman A G 1984 Pharmacological influence of nutmeg and nutmeg constituents on rabbit platelet function. *Planta Medica* **50**(3): 222-226.

(104) **Recsan** Z, Pagliuca G, Piretti M V, Penzes L G, youdim K A, Noble R C and Deans S G 1997 Effect of essential oils on the lipids of the retina in the ageing rat: a possible therapeutic use. *Journal of Essential oil Research.* **9**(1): 53-56.

(105) **Sadasivam** S and Manickam A 1992 Biochemical methods for agricultural sciences. Wiley Eastern Limited, Madras.pp. 1-246.

(106) **Sait** S and Hutajulu T F 1995 Improving clove leaf oil quality for export using chemical deterpenation process. *Warta IHP.* **12**(12): 74-77.

(107) **Sait** S and Satyaputra I 1995 The effect of deterpenation process on the medicinal quality of nutmeg oil. *Warta-IHP.* **12**: 1-2, 41-43.

(108) **Sakamura** F 1987 Changes in volatile constituents of *Zingiber officinale* rhizomes during storage and cultivation. *Phytochemistry.* **26**(8): 2207-2212.

(109) **Saravana** R S and Rose J K C 2004 A critical evaluation of sample extracton techniques for enhanced proteomic analysis of recalcitrant plant tissues. *Proteomics* **4**(9): 2522-2532.

(110) **Senanayake** U M, Wills R B H & Lee T H 1977 Biosynthesis of eugenol and cinnamic aldehyde in Cinnamomum zeylanicum. *Phytochemistry* **16**(12): 2032-2023.

(111) **Sharma** B K 1995 Instrumental Methods of Chemical Analysis. GOEL Publishing House, Meerut. pp 427-483

(112) **Smith** A E 1986 International trade in cloves, nutmeg, mace, cinnamon, cassia and their derivatives. Report,-Tropical Development and Research Institute, U. K. p 161

(113) **Smith** Bate E C 1954 Astringency in foods. *Foods* **23**: 124.

(114) **Stahl** E 1981 Variation of myristicin content in cultivated parsnip roots (Pastinaca sativa ssp. Sativa var. hortensis). *Journal of Agricultural and Food Chemistry.* **29**(4): 890-892.

(115) **Strack** D 1997 Phenolic metabolism. In: Plant biochemistry Dey and Harborne (Eds.). Academic press.pp 387-416.

(116) **Tandon** H L S 2001 Methods of analysis of soils, plans, waters and fertilizers Fertiliser development and consultant organisation, New Delhi p 143.

(117) **Tangka** J K 2003 Analysis of ths thermal energy requirements for leaf protein concentrate from some plants *Biosystems Engineering* **86**(4): 473-479

(118) **Variyar** P. S. and Bandyopadhyay C 1995a. On the volatiles of clove, cardamom, nutmeg and mace. *PAFAI* **17**(1): 19-25.

(119) **Variyar** P S and Bandyopadhyay C 1995b Estimation of phenolic acids in cinnamon, clove, cardamom, nutmeg and mace by high performance liquid chromatography. *Journal of Spices and Aromatic Crops.* **4**(2): 129-134.

(120) **Variyar** P S, Bandyopadhyay C and Thomas P 1998 Effect of gamma-irradiation on the phenolic acids of some Indian spices. *International Journal of Food Science and Technology.* **33**(6): 533-537

(121) **Verghese** J 2000 Nutmeg and Mace – I General background *Spice India* **13**(12): 2-4

(122) **Verghese** J 2001a Nutmeg and Mace – IV Essentil oils of *Myristica fragrans* Houtt. (and *Myristica argenea* Warb)-5 *Spice India*, **14**(10): 7-11

(123) **Verghese** J 2001b Nutmeg and Mace – III. Nutmeg and mace butter *Spice India*, **14**(6): 14-16.

(124) **Verghese** J 2001c Nutmeg and Mace – IV Essential oils of *Myristica fragrans* Houtt-1 *Spice India*, **14**(5): 2-4.

(125) **Villegas** M, Vargas D, Msonthi J D, Marston A and Hostettmann K 1988 Isolation of the antifungal compounds falcarindiol and sarisan from *Heteromorpha trifoliata*. *Planta Medica*. **54**(1): 36-37.

(126) **Wagner** H, Wierer M and Bauer R 1986 In vitro inhibition of prostaglandin biosynthesis by essential oils and phenolic compounds. *Planta-Medica*, **3**: 184-187.

(127) **Wang-Wei**, Scali M, Vignani R, Spadafora A, Sensi E, Mazzuca S and Cresti M 2003 Protein extraction for two-dimensional electrophoresis from olive leaf, a plant tissue containing high levels of interfering substances. *Electrophoresis* **24**(14): 2369-2375

(128) **Waterman** P G 1993 The chemistry of volatile oils. In: Volatile oil crops: their biology, biochemistry and production (eds) Robert K M Hay and Peter G. Waterman (Eds.) Longman Scientific and Technical, Longman Group UK Ltd. pp. 47-61

(129) **Woolf** A 1999 Essential oil poisoning. *Journal-of-Toxicology,-Clinical-Toxicology* **37**(6): 721-727

(130) **Yapinlee** and Takahashi Tunekazu (1966). Estimation of free amino acids, *Analytical Biochemistry*, **14**: 71-77.

(131) **Ying** Li-Feng and Quan-Ying 2003 Study on grape seeds. *Journal of Herbei Vocation Technical College* **17**(3): 26-28.

(132) **Zachariah** T J, Gopalam A, Krishnamoorthy B and Ravindran P N 1986 Steroid degradation compound associated with sex expression in nutmeg (*Myristica fragrans*). Proc. Indian natn. Sci. Acad. **B 52**(5): pp. 685-688.

(133) **Zhang-YiShun**, Xiang-Xu, Huang-ShangZhi and Fu-JiaRui 2003. A method of extracting embryo proteins in *Litchi chinenesis* Sonn. *Journal of Tropical and Subtropical Botany* **11**(2): 174-176.

(134) **Zheng** G Q, Kenney P M and Lam L K T 1992 Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. *Journal of Natural Products.* **55**(7): 999-1003.

(135) **Zlatev** S, Balinova A and Zlateva M 1976 Changes in the essential oil of dill plants during post-harvest storage. *Rasteniev dni-Nauki.* **13**(9): 51-57.

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