



**CYTOTOXIC STUDIES AND THE**

**EXPLORATION OF ESSENTIAL OILS**

**IN SOME MEMBERS OF**

**MYRTACEAE**



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OF ESSENTIAL OILS IN SOME MEMBERS OF  
MYRTACEAE**

**Thesis**

*Submitted to the*

**UNIVERSITY OF CALICUT**

*for the Degree of*

**Doctor of Philosophy**

**in Botany**

*By*

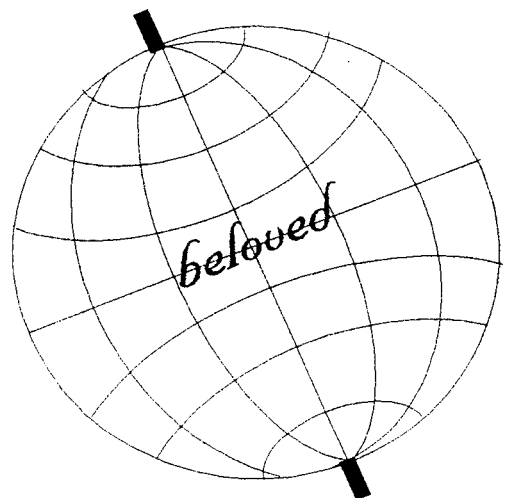
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**2003**

Dedicated to

*my*



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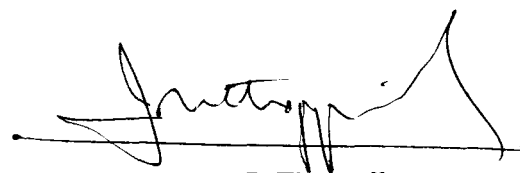
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*Place - C. U. Campus*

**CERTIFICATE**

**This is to certify that the thesis entitled "Cytotoxic studies and the exploration of essential oils in some members of Myrtaceae" is an authentic record of work carried out by Mr. Oomen P. Saj during 2000-2003 under my supervision and guidance and that no part thereof has been presented earlier for any other degree or diploma.**



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## DECLARATION

I here by declare that the thesis entitled **“Cytotoxic studies and the exploration of essential oils in some members of Myrtaceae”**, for the degree of Doctor of Philosophy in Botany of Calicut University, is a research work done under the guidance of Dr. John E. Thoppil, Senior Grade Lecturer, Genetics and Plant Breeding Division, Department of Botany. This has not been submitted earlier for any other degree or diploma.

Calicut University,

9 - 5 - 2003.

  
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Calicut University,

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# INTRODUCTION

Industrialization had created great problems to nature. Our environment is getting polluted drastically due to the deposition of industrial wastes. The unlimited usage of chemicals, synthetic products *etc.* as medicines and pesticides had made severe consequences on all forms of life. The equilibrium between abiotic and biotic components of global ecosystem has been depleting day after day. On this scenario, the usage of chemicals and synthetic products should be controlled or avoided. Hence, human interest should be concentrated on plants and plant-based products. The discoveries and usage of biomedicines, biopesticides and biodegradable products are the new trend and innovation among the world of scientists and researchers. Even now, about 75% of human population depends on plant extracts as a tool of traditional medicine. So, human interest is concentrated on biodegradable and nature friendly products.

In 1985, there are about a total of 3500 new chemical structures discovered, out of which 2619 were isolated from higher plants (Abelson, 1990; Kinghorn and Balandrin, 1993). According to Farnsworth and Morris (1976) higher plants are still “the sleeping giant of drug development”. The economic potential of various products obtained from them are not used satisfactorily even now. The potential for usage of higher plants is vast, since from an estimated total of approximately three lakh species, only about 500 have been studied extensively for medicinal and biological applications (Cordell, 1997). More formulations are hence developed in plant-based pharmaceuticals to produce anticarcinogenic drugs (Hartwell, 1967). One of the earliest reports is the use of extract of *Colchicum autumnale* L. bulb for reducing uncontrolled cell division. The property of preventing carcinogenesis has been reported on many plant extracts. (Haenszel *et al.*, 1972, 1976; Bjelke, 1974; Graham *et al.*, 1978).



Apart from these reports, antiseptic, antifungal, antibacterial and antibiotic activities of essential oils and leaf extracts were also reported from different plants. For instance, the Australian tea tree oil, Eucalyptus oil, Clove oil, Myrtle oil *etc.* have been used for various kinds of ailments. Cajeput oil obtained from *Melaleuca leucadendron* is used to cure laryngitis and bronchitis, as a carminative and as an anthelmintic (Carson and Riley, 1993; Rodriguez *et al.*, 1996; Perry *et al.*, 1997). The seeds of Jambolan/ Jamun tree is used in folk medicine to cure diarrhoea, dysentery and diabetes. The olenolic acid obtained from *Syzygium claviflorum* leaves was identified as anti HIV principle (Kashiwada *et al.*, 1998).

Myrtaceae is a large family comprising of 120 genera and over 3,375 species widely distributed in the warmer parts of the world (Singh and Jain, 1992). Australia and Tropical America are the chief centres of distribution of this family. In India, the family is represented by about 14 genera and 165 species occurring chiefly in Eastern India. The members of the family are trees or shrubs with aromatic fragrance. The species of *Eucalyptus* are giant trees attaining huge dimensions. Oil glands are found in the young stem, bark, leaves and floral parts. The members of the family are of considerable economic importance as they yield valuable timber, edible fruits, spices, oils and some species are grown as ornamentals.

Myrtaceae members are usually trees or shrubs. Leaves are opposite, rarely alternate, exstipulate and glandular punctate. The vascular bundles in the stem are bicollateral and lysigenous oil glands are present in young stem, leaves and floral parts (Hutchinson, 1959; Chopra, 1990; Shukla and Misra, 1991; Singh and Jain, 1992). Flowers are bisexual, regular, solitary or in heads or spikes or in cymes and epigynous. Calyx 4-5 lobed, often united with the ovary wall and thalamus, sometimes thrown off as a lid. Corolla 4-5, free or united in a cap sometimes thrown off also. Stamens indefinite, anther connectives often gland tipped, sometimes stamens united into bundles opposite to the petals. The anthers are small and usually versatile, dithecous and introrse. Carpels 2-5 or many,

syncarpous, inferior, showing axile placentation, style and stigma simple, fruit a berry, drupe, capsule or nut and seeds nonendospermic. The testa modified into wing or may be thorny, leathery or membranous. The embryo is curved or spirally twisted (Chopra, 1990; Shukla and Misra, 1991; Singh and Jain, 1992).

The family is divided into two subfamilies, chiefly based on the nature of the fruit (Briggs and Johnson, 1979; Shukla and Misra, 1991; Mabberley, 1997).

**Subfamily: I- Leptospermoideae-** Leaves opposite or alternate, fruit dry. This is further divided into two tribes:

**Tribe 1- Chamaelaucieae-** (ovary unilocular).

**Tribe 2- Leptospermeae -** (ovary multilocular).

**Subfamily: II - Myrtoideae-** Leaves opposite, fruits fleshy. It includes one tribe.

**Tribe 1- Myrteae-** (ovary 2-5 locular).



**Table 1** The details of myrtaceous plants collected during the present study.

Sl. No.	Name of the plant	Location	Altitude in metres	Herbarium Number
1	<i>Agonis flexuosa</i>	Ootty	2750	88001
2	<i>Beaufortia sparsa</i>	„	2750	88002
3	<i>Callistemon citrinus</i>	„	2750	88003
4	<i>Callistemon viminalis</i>	„	2750	88004
5	<i>Corymbia citriodora</i>	„	2750	88005
6	<i>Corymbia ficifolia</i>	„	2750	88006
7	<i>Eucalyptus globulus</i>	Kodaikanal	2840	88007
8	<i>Eucalyptus tereticornis</i>	Campus	50	88008
9	<i>Leptospermum nicholsii</i>	Ootty	2750	88009
10	<i>Melaleuca leucadendron</i>	Campus	50	88010
11	<i>Melaleuca styphelioides</i>	Ootty	2750	88011
12	<i>Syncarpia glomulifera</i>	Coonoor	2500	88012
13	<i>Acmena smithii</i>	Ootty	2750	88013
14	<i>Eugenia apiculata</i>	Ootty	2750	88014
15	<i>Eugenia uniflora</i>	SultanBattery	750	88015
16	<i>Feijoa sellowiana</i>	Coonoor	2500	88016
17	<i>Myrtus communis</i>	Ootty	2750	88017
18	<i>Pimenta dioica</i>	Kollam	40	88018
19	<i>Psidium guajava</i>	Vadakara	30	88019
20	<i>Syzygium aromaticum</i>	„	30	88020
21	<i>Syzygium cumini</i>	„	30	88021
22	<i>Syzygium jambos</i>	Campus	50	88022
23	<i>Syzygium malaccense</i>	„	50	88023
24	<i>Syzygium samarangense</i>	„	50	88024
25	<i>Syzygium zeylanicum</i>	Vadakara	30	88025
26	<i>Rhodomyrtus tomentosa</i>	Dodabetta	2850	88026

## Brief Profile of Plants Selected for the Present Study :

### Subfamily I - Leptospermoideae

#### 1. *Agonis flexuosa* Schau.

*Agonis* is a small genus of 11 species confined mainly to the Southwestern Australia. All plants are medium to large shrubs. But *A. flexuosa* is a small tree (Fig. 1).

The term *Agonis* is derived from the Greek word, *agon* = a cluster, referring to the arrangement of the fruits and *flexuosa* from the Latin word, *flexuosis*= bending or curving in a zig zag manner (Smith, 1963; Nayar, 1985). *Agonis flexuosa* is commonly called 'Willow Myrtle' or 'Weeping Myrtle' or 'Peppermint tree'. They produce small white flowers in sessile globular heads in the leaf axils. All species of *Agonis* appear to be pest free apart from an occasional webbing caterpillar. Propagation is easy from both seed and cuttings. This tree grows naturally in damp peaty soils, but cultivated usually. *A. flexuosa* is the mostly cultivated tree with graceful weeping foliage which reaches 15 metres or more in good conditions. Hence cultivated in most of the gardens. This is adaptable to a range of climates and soils. It is often smaller in cultivation and would take many years to reach its ultimate height. It has fibrous bark and lance shaped leaves. The flowers are white and small. There are five sepals and petals with many stamens. Flowers are produced on sessile globular heads. Carpels are three in number, syncarpous and epigynous. Fruits are produced in clusters and are dry with hard pericarp (Bentham and Mueller, 1866 ).

#### 2. *Beaufortia sparsa* R. Br.

*Beaufortia* is a flowering shrub named after Mary Somerset (1630-1714) Duchess of Beaufort, Patroness of Botany early in the 18<sup>th</sup> century (Smith,

1963). The red flowered species is called *sparsa* (Hereman, 1868). The word *sparsa* means few, far between (Smith, 1963).

The genus *Beaufortia* has 18 species of evergreen shrubs occurring mainly on poor soils, in scrubs and forests of warm temperate areas of Australia. They are cultivated for their terminal brush like heads of numerous small flowers each consisting of a tuft of coloured stamens. Leaves are small, simple, crowded, ovate, elliptical and borne in opposite pairs, in most species tightly packed and overlapping to conceal the stems. In frost prone regions, *Beaufortia sparsa* is grown in green houses, while in frost free climates they are planted in a border or at the base of warm sunny wall. They may also be used as low wind breaks (Brickell, 1997).

*Beaufortia sparsa* is commonly called 'Swamp Bottle Brush' (Fig. 2). It is an erect to spreading evergreen shrub with upright to recurved, oval, mid to deep green leaves which are many nerved. Leaves are 1 cm long at an average and elliptic. The bright orange-red flowers are produced in spikes. The spikes are very dense and oblong. The axis is usually growing out before flowering and attains 5-7 cm in length. They are produced from summer to autumn. The rachis and calyx are glabrous or slightly pubescent. Calyx tube is about 2.5 cm long, the claws slender, each with about five filaments at the end, scarcely  $\frac{1}{4}$  of the claw in length. Anther valves small and staminal disk glabrous. Ovule is solitary in each cell. Fruits are about 2.5 cm long (Bentham and Mueller 1866; Hereman, 1868).

### 3. *Callistemon citrinus* (Curtis) Skeels

Syn. *Callistemon lanceolatus* DC.

It is an evergreen shrub reaching to a height of about 3 - 4.5 m. It is usually cultivated in the gardens as an ornamental plant (Fig. 3).

The term *Callistemon* is originated from the Greek words, *Kalos* which means beautiful and *stemon* means stamen, referring to the scarlet colour of stamens (Smith, 1963; Nayar, 1985). The word *citrinus* means lemon coloured or

resembling citron (Smith, 1963). It is commonly called 'Lemon Bottle Brush', because the foliage smells lemony when crushed or bruised.

It is a native of Australia and New Zealand. There are about 25 species of *Callistemon* which are closely related to *Melaleuca* from the same part of the world. The young shoots of *C. citrinus* are pink or red and silky.

Leaves are simple, alternate, lanceolate and gland dotted. Inflorescence of loose cylindrical spikes are about 6 - 10 cm long. The spikes are like giant pipe cleaners and are bright crimson coloured. Calyx tube globose, five lobed, petals five, pinkish, stamens many, many seriate, filaments petaloid and projecting. Ovary is three celled and ovules many in each carpel. Capsule is dry and woody with an average size of 8 mm across. *C. citrinus* is a very drought resistant shrub, once established and they are sparsely branched.

#### 4. *Callistemon viminalis* Sol.Ex.Gaertner

It is commonly called 'Weeping Bottle Brush'. The word *viminalis* is from Latin, *vimen* which means a long flexible shoot (Smith, 1963). It is a beautiful tropical tree that boasts a spring time explosion of scarlet blossoms. It is an attractive tree even when not in bloom. This tree grows to a height of about 20 feet, forming wide rounded crown, if lower branches are pruned.

'Weeping Bottle Brush,' is a native of New South Wales, Australia. It is a common landscape item in South Florida and is also popular in Southern California. Now it is widely cultivated throughout the world. To enjoy the beauty of this tree, the lower branches are to be trimmed to show its distinctive weeping form. Plant at the edge of lake or pond, resembles a small but sporty weeping willow (Fig. 4).

The leaves are alternate, simple, lanceolate, gland dotted and drooping down. The leaves are growing up to 4 inches in length. They are very attractive bronze green when they emerge in spring, gradually turning dull green as they mature. Inflorescence is produced in loose spikes. Calyx and corolla are with



five segments, reddish in colour. The stamens are many and are the most attractive structures. Brilliant red stamens are arranged into 6 inch long structures that resemble the brushes used to clean bottles. These form at the tips of pendulous branches from which they wave seductively in the breeze. Ovary three celled and fruit a capsule. The capsules during maturation are woody, which is a distinction of this genus (Bentham and Mueller, 1866; Jackson, 1965).

##### **5. *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson \***

Syn. *Eucalyptus citrodora* Hook. (Lemon Gum).

The name *Corymbia* is derived from 'corymb', referring to floral clusters, where all flowers branch from the stem at different levels but ultimately terminate at about the same level (www.anbg.gov.au.2001). *Citriodora*, means 'lemon scented', referring to its smell (Smith, 1963; Jackson, 1965; Nayar, 1985).

This is a large tree believed to be originated in Central and Northern Coast of Queensland, Australia. The wood is good for saw timber and used for general construction, railway sleepers and tool handles. Bark may contain upto 12% tannin. The leaves are used to extract essential oil which has a strong and persistent fresh lemony aroma. This oil contains predominantly terpenoids, the main constituent being citronellol.

*Corymbia citriodora* is an evergreen tree, 24 – 40 m high with tall straight trunk which is 0.6 – 1.3 m in diameter and possesses thin graceful crown of drooping foliage (Fig. 5). Bark is smooth and grey, peeled off in thin irregular scales or patches and becoming mottled, exposing whitish or faintly bluish inner layer with powdery surfaces appearing dimpled. Twigs slender, slightly flattened and light green tinged with brown. Leaves are alternate, narrowly lance shaped, 10 –20 cm long and 1 –2.5 cm wide apically acuminate, basally acute, entire, glabrous, thin, light green on both surfaces with many fine parallel straight veins. Corymbs terminal 6 cm long and branched. Flowers many, 3-5 cm equal short stalks (umbels) from ovoid buds 8-12 mm long, 5-8 mm wide stamens, many thread like

white 6mm long anthers with long gland. Pistil is inferior with 3-celled ovary and also possesses long, stout and short style. Capsules a few, urn shaped or ovoid, narrowed into short neck, brown with scattered raised dots. Seeds a few, 4 – 5 cm long and are shiny black (Little, 1983).

#### 6. *Corymbia ficifolia* (F.Muell.) K.D. Hill & L.A.S. Johnson \*

Syn. *Eucalyptus ficifolia* Hook.(Crimson gum).

*Corymbia ficifolia* is commonly called as Crimson Gum Tree, Crimson Flowered Eucalyptus, Red Flowering Gum Tree, etc. The word *ficifolia*, means leaves like a fig, *Ficus* ( Smith, 1963).

*C. ficifolia* is an evergreen, compact round - headed tree having slow and moderate growth (Fig.6). The plant may grow up to 40 ft or more. The tree is very attractive during flowering season. The bark is rough-greyish brown in colour. Juvenile leaves are ovate to broadly lance shaped, mid to deep green above and paler beneath. Adult leaves are thick leathery, dark green and 7-15 cm long and 2 - 5cm wide. Flowers are produced in clusters of panicles. The colour may be light pink, salmon or most frequently light red. Blooming occurs all year, but usually during July and August. Sepals and petals are caducous. The filaments of the stamens are brightly coloured and attractive. Pistil is inferior and possesses three celled ovary with thick and stout style. The fruits are pendent woody capsules, 3 - 5 cm long and urn shaped. *C. ficifolia* is a native of Australia. Now it is widely cultivated in the gardens worldover. No significant pest problems are reported for this plant (Bentham and Mueller, 1866; Brickell, 1997).

#### 7. *Eucalyptus globulus* Labill.

The word *Eucalyptus* is derived from the Greek words, *eu* = well and *kalypto* = to cover, referring to the united calyx lobes and petals forming a lid. The word *globulus* means a small round ball, from the form of the flower heads of these trees (Smith, 1963; Nayar, 1985).

*Eucalyptus* has over 500 species of trees and shrubs found in all the driest habitats mainly in Australia, but also in Philippines, Malaysia, Indonesia, etc. They are commonly called 'Gum Trees', as they secrete aromatic resinous gum (Chopra, 1990). They are valued for their often aromatic foliage and their attractive bark. Young plants have generally opposite leaves, developing alternate ones as they mature. A number of plants are cultivated on road sides as avenue trees (Chopra, 1990; Singh and Jain, 1992). They are also called as 'Iron Bark'. Many species yield valuable timber. The wood is very hard and has various uses. Species of *Eucalyptus* are being successively cultivated in Nilgiri Hills and in other similar plains of India (Hooker, 1879). Eucalyptus oil is distilled from the leaves of numerous species. The oil is antiseptic and hence used commercially in various preparations.

*Eucalyptus globulus* is commonly called 'Blue Gum Eucalyptus', as it yields blue gum and oil of eucalyptus (Fig. 7). They are large trees with smooth bark. Seedlings and juvenile stems are glaucous. Seedling leaves are opposite, sessile, amplexicaul and bluish green. Where as adult leaves are alternate, petiolate and lanceolate, 10-30 cm in length, 3 – 4 cm in breadth and green. Inflorescence is a simple umbel with single or three flowers. Hypanthium is ribbed, angled, glaucous and operculum is long, flat, with a central knob shorter than the hypanthium. Flowers are sessile, globular to hemispherical, 4-ribbed and warty with a broad, convex disc and possess 3-5 valves. *E. globulus* plants are supposed to keep away malarial mosquito (Hooker, 1879; Mathew, 1995).

#### 8. *Eucalyptus tereticornis* Sm.

*Eucalyptus tereticornis* is commonly called as 'Forest Red Gum'. The word *tereticornis* means rounded horns, with reference to the calyptra, which is like rounded horns. It is usually known as *Eucalyptus* hybrid and is widely cultivated in India (Fig. 8). These trees grow up to a height of 15-20 metres. The bark is white and sometimes with patches. Leaves are alternate, petiolate, where as,

seedling leaves are opposite. When alternate, they are broad lanceolate to ovate or elliptic, 5-12.5 cm in length and 1.5 – 6 cm in breadth and bluish green in colour. Juvenile leaves are ovate and dull to bluish green. Intermediate leaves are broad, lanceolate, green concolourous where as adult leaves are lanceolate, 10-20 cm in length and 1-2 cm breadth, green concolourous, glabrous, subacute, cuneate, oblique at base, margin entire, apex acuminate and petiole about 2.5cm in length. Inflorescence is a simple umbel which is 7-11 fid. Flowers are white in colour. Hypanthium campanulate, rugose, operculum conical, horn shaped, apex acute, pedicellate, truncate and globular. Mature leaves are curved, linear, lanceolate and strongly scented, hence used for oil extraction. This tree is extensively planted in the plains and on lower slopes of hill for leaves and for timber (Mathew, 1995). Capsules are several, hemiglobose, 6-9 mm long and 8-10 mm in diameter. Seeds are many, tiny, 1mm long and broad and possess a shiny dark brown to black colour (Little, 1983).

### 9. *Leptospermum nicholsii* Dorrien Smith

*Leptospermum* Forst. (Tea Tree) is a genus of about 86 species distributed through out Australia and extending to Malaysia and New Zealand. Some are found growing in S.E. Asia. Some species are introduced into the Nilgiris. The word *Leptospermum* is coined from the Greek words, *leptos*=slender and *spermum*=a seed, in allusion to the small narrow seeds (Hereman, 1868). The word *Nicholsii* is after the collector named Nichols. They are cultivated for their usually aromatic neat foliage and their small, sometimes profusely borne flowers. The common name 'Tea Tree', derives from the practice of early settlers of soaking the leaves of several species of *Leptospermum* in boiling water to make a tea substitute.

*Leptospermum nicholsii* is a medium sized tree with whitish bark (Fig.9). The leaves are very narrow, lance shaped and dark green with about 0.7 to 1 cm long. Young leaves are purple tinged. They are cultivated for their usually aromatic neat foliage and their small and profusely borne flowers. The flowers are

produced from leaf axils in clusters of 2 or 3 with shallow cup shaped to star shaped structure. Each flower is with five crimson coloured, usually small, broadly ovate petals. Flowers are borne in late spring and early summer. Calyx tube broadly campanulate, stamens many, ovary inferior and fruits are dry and woody (Bentham and Mueller, 1866; Thompson, 1989; Graf, 1998 ).

#### 10. *Melaleuca leucadendron* L.

Syn. *Melaleuca quinquenervia* (Cav.) S.T.Blake

*Melaleuca leucadendron* is commonly called 'Cajaput Tree'. It is a native of Malacca islands (Hooker, 1879; Chopra, 1990). The word *Melaleuca* is coined from the Greek words, *Melos* = black and *Leukos* = white , referring to the black trunk and rather whitish branches. The word *leucadendron* is also from the Greek words, *Leukos* = white and *dendron* = a tree. This tree is also called as 'Paper Bark Tree', and cultivated in gardens as an avenue tree. This evergreen tree reaches a height of 12 m and has been planted extensively in reforestation projects. It invades open swampy areas (Fig.10).

Leaves are alternate, lanceolate, 3-7 nerved with anastomosing nerves. Flowers are produced in spikes, which are sessile, borne in the axil of the floral leaf, pentamerous; bracts deciduous; calyx tube subglobose, lobes 5, imbricate, glabrous; petals 5, spreading, deciduous; stamens many, more or less united at their bases to five bundles opposite to the petals and with versatile anthers. Ovary is perigynous, enclosed in the calyx tube and usually with many ovules in each cell. Fruit is a capsule, woody and opening by means of three valves. Seeds are dispersed by wind (Hooker, 1879).

It is an ornamental tree of tropical Asia. Cajaput oil is extracted from the leaves, which is used in various medicines. It is also used as a mosquito repellent. The tree yields valuable timber used in ship building (Singh and Jain, 1992).



### 11. *Melaleuca styphelioides* Sm.

*Melaleuca styphelioides* is commonly called as 'Prickly Paper Bark Tree', or 'Black Tea tree'. The word *styphelioides* means that some parts of the plant is constricted (Smith, 1963). It is a tall tree attaining sometimes 80 ft in height (Fig. 11). The young shoots and inflorescence are silky, pubescent or villous, otherwise glabrous. Leaves are alternate, ovate lanceolate, acuminate, pungent, pointed, mostly about 1.25 cm long, rigid and finely striate with many nerves. The leaves are pointed and spiny. This is in contrast with the white paper bark trunk. Creamy white bottle brush like flowers are produced in small cylindrical spikes during summer. The axis grows out before the flowering is over. The floral leaves are either like the foliage leaves and persistent or reduced to deciduous bracts. Calyx tube ovoid, lobes lanceolate, acuminate rigid, acute and pungent, as long as or longer than the tube. Petals are as long as the calyx lobes, but very deciduous. Staminal bundles are very long, but the claws are not much longer than the calyx tube and each with several filaments, shortly pinnate along the upper portion. Ovules are numerous, closely packed on a small placenta. Fruiting spikes are often leafy and the calyces are crowned by the rigid erect lobes (Bentham and Mueller, 1866; Thompson, 1989).

*M.styphelioides* is a native of Australia, but now it is widely cultivated in the gardens worldover. This tree is planted in the lawn and will tolerate wet soils.

### 12. *Syncarpia glomulifera* (Sm.) Niedz. \*

Syn. *Syncarpia laurifolia* Tenore

*Syncarpia glomulifera* is commonly called as 'Turpentine tree', 'Lustre Wood', etc. The word *Syncarpia* is derived from two Latin words, *Syn* = fused and *carpia* = fruit. The word *glomulifera* usually refers to the flowers in

glomerules or some parts of the plant in balls. The synonym *laurifolia* means bay leaved (Smith, 1963; Jackson, 1965). This is a large tree and is native to Queensland in Australia reaching to a height of 40 m. Now it is cultivated in certain gardens in India (Fig. 12).

The young shoots and the lower side of the leaves are more or less pubescent or glaucous. Leaves appear sometimes in whorls of four from 2 pairs being close together, broadly ovate to elliptical, oblong, obtuse or obtusely acuminate, glabrous above, 5-8 cm long on petioles which are 1.5 - 8 mm in length. Flowers are white, united, 6-10 together in globular heads on peduncles that are 2 - 2.5 cm long at the base of the new shoots, with 2-4 bracts close under the head, either short and scale like or leaf like and exceeding the flowers. Calyces connate at the base, the free parts broadly campanulate, softly pubescent, lobes short and broad. Petals are broadly ovate or orbicular and larger than sepals. Stamens are many arising from the flat disc and usually longer than the petals. Ovary is flat topped, tomentose, 3-celled with rather numerous ovules in each cell, erect on an oblong placenta. Fruiting heads 1.2 to 1.5 cm in diameter and the calyces connate to them. Bark fibrous and furrowed (Bentham and Mueller, 1866; Stanley and Ross, 1986).

## Subfamily II - Myrtoideae

### 13. *Acmena smithii* (Poir.) Merr. & Perry \*

Syn: *Eugenia smithii* Poir.

This is an evergreen tree found in the tropics and subtropics throughout the world. It is commonly called 'Lilly Pilly', (Fig. 13).

*Acmena* is a native of Queensland, Australia. It is usually found in subtropical rain forest areas or in dry rain forest areas near streams. It grows up to 20 ft in height. The subtropical climate is very fair for the flourished growth but protection should be given for long or hard freezes. The plants enjoy profuse

amounts of water but will grow in drier areas and grow best in areas of light sun or shade. It is a useful hedge and wind break plant.

*Acmena* is named after *Acmene*, a beautiful wood nymph from Greek, *acme*, the highest or best. The species name *smithii* is to renown Sir James Edward Smith (1759-1825) of Norwich, England, the purchaser of Linnaeus's collections and founder and the first president of Linnean Society of London (Smith, 1963).

Leaves are simple, opposite, evergreen, ovate to ovate-oblong and mostly 5 - 7.5 cm in length. The flowers are small, numerous and fluffy with creamy white colour, in panicles produced during spring season. Bracts minute and deciduous. Calyx tube turbinate above, long and free part very much broadened. Petals four, united in a small flat deciduous calyptra. Stamens many, long, anthers small, with very distinct globular divaricate cells. Carpels are two, ovules numerous. Fruits are purple coloured berries, globular with a diameter of 6 -12 mm. Fruits are crowned by circular prominent calyx rims. The endocarp is thick and hard. Large bunches of fruits, which ripen a couple of months later, attract different kinds of birds. Fruits can be eaten fresh or made into jams, jellies and drinks (Bentham and Mueller, 1866; Grieve, 2000).

#### 14. *Eugenia apiculata* DC.

Syn: *Luma apiculata*. Burret

The genus name *Eugenia* L. is given to honour Prince 'Eugene' of Savoy (1663-1736), a great patron of Botany, who spend 50 years of his active life in military campaigns and got wounded 13 times (Nayar,1985; Smith,1963). *Eugenia* are trees or shrubs, smooth or rarely tomentose or villous. Leaves opposite, rarely alternate, coriaceous or membranous and pinnate nerved. Bracts are usually small and deciduous. There are about 700 species inhabiting chiefly Tropical and Subtropical America, Tropical Asia, a few in Australia and Africa (Hooker,1879). 'Apiculata' means terminating abruptly in a short and often sharp point (Smith,1963).

*Eugenia apiculata* is a landscape shrub often cultivated for the edible fruits and for the beautiful foliage. Common name is 'Short Leaf Stopper', (Fig. 14). The leaves are simple, dark greenish brown and attractive. Flowers are wonderful, showy, fragrant and with white colour. Calyx lobes are often fused with the thalamus. Petals are white small and caducous. Stamens are many and carpels with numerous ovules. They produce dark purple black berries which are edible. Like other myrtaceous fruits, the fruits are with sweet flavour. The seeds are numerous and bitter to taste (www.anbg.gov.au. 2001).

### 15. *Eugenia uniflora* L.

*Eugenia uniflora* is a bush or small tree growing to the height of 8 m. It is commonly called as 'Surinam Cherry, Brazilian Cherry, Pitanga', etc. The country of origin is Israel. This plant is indigenous to South America, but widely cultivated worldwide, because it is adapted to both subtropical and tropical climates. Plants like full sun and are drought tolerant and need only moderate rainfall. This plant is useful in landscaping for its red coloured new foliage against the dark green older leaves along with its red to black ribbed fruits. It is also cultivated as a hedge plant in gardens (Fig. 15).

The species name 'uniflora', designates one flowered condition (Smith, 1963). They produce single axillary fragrant white flowers, which develop into sweet berries. Fruits develop and ripen in just three weeks after flowering. Sepals and petals five in number, whereas stamens are numerous. The fruit is crowned by the remains of the calyx lobes. The fruit can be excellent or only fair depending on the variety. The deep red almost black fruited varieties tend to produce sweeter fruit. Native areas extend from Surinam through Uruguay, hence called as "Surinam Cherry", (Graf, 1998).

### 16. *Feijoa sellowiana* O. Berg.

Syn. *Acca sellowiana* (O. Berg.) Burret

*Feijoa sellowiana* is a small tree or shrub with pineapple or guava flavoured fruits. Hence it is commonly called 'Pineapple Guava, Guavasteen, Feijoa' etc.

The name *Feijoa* is given to this plant to renoun a famous Brazilian Botanist, Don de Silva Feijoa of 19<sup>th</sup> century (Smith,1963; Nayar,1985). The species name *sellowiana* is in honour of Friedrich Sellow (1789-1831), a german traveller and naturalist who made extensive collections in Brazil and Uruguay. His family name was 'sello', but when in Brazil, he altered it to 'sellow', hence the varied spelling of epithets for South American plants commemorating him (Smith, 1963).

Subtropical Paraguay, Uruguay, N. Argentina and Southern Brazil are considered as the places of origin. This evergreen tree is a useful landscape plant, ornamental with its showy flowers and grey- green leaves. The flowers are produced in axillary cymose clusters. Petals are five, white with many purple coloured stamens. Ovary is inferior with many carpels. The fruit is a berry. It has green skin, covered with white powder. It is oblong and 8 cm in diameter. The fruit contains sweet/acidic white pulp. It should be harvested immediately after it falls or plucked a little early and allowed to ripen. The fruits have a delicious minty-pineapple flavour. It is eaten fresh, makes a good jam, jelly, syrups, chutney or preserved as such (Fig. 16).

### 17. *Myrtus communis* L.

*Myrtus communis* is commonly called Myrtle (Fig. 17). This plant, which was originated in West Asia, figures in the old testament and has long been naturalized in Europe. The leaves of the plant yield 'oil of Myrtle', (Chopra, 1956). The word *Myrtus* is derived from the Greek, 'myrton' which means perfume.



The Greeks considered this plant sacred to Aphrodite, the goddess of love and beauty. In biblical texts and ancient Christian paintings, it was associated with the virgin. This species represents love, chastity and virginity. In ancient Greece and Rome it was a symbol of youth, beauty and marriage. The word *communis* means common, general and growing in company. *M. communis* is usually cultivated in gardens for its aesthetic value (Smith, 1963; Nayar, 1985).

It is an attractive evergreen shrub or small tree. It grows wild in regions along the Mediterranean sea and temperate regions of Asia. It is cultivated as a garden plant in other parts of the world. The leaves are simple, opposite, entire, acuminate, dark green and shiny. The fragrant flowers are white and produced in cymes. The sepals and petals are five with numerous stamens, all are white in colour. The petaloid filaments are exerted and bear yellow coloured, bithecous, introrse anthers. The leaves, bark and blue black berries are fragrant. All these plant parts are used to make perfume. The bark is used in tanning industry of Southern Europe. In Greece it is used in festivals (Bentham and Mueller, 1866; Chopra, 1990).

#### 18. *Pimenta dioica* (L.) Merrill

Syn. *P. officinalis* Lindl.; *Myrtus dioica* L.; *M. pimenta* L.

*Pimenta dioica* is commonly called 'All Spice', as their fruits have a combination of flavours of clove, cinnamon and nutmeg. *Pimienta* is the Spanish name for peppercorns from which the name *Pimenta* originated. The word *dioica* means dioecious, having the male and female reproductive organs produced on separate plants. The word *officinalis* means sold in shops, applied to plants with real or supposed medicinal properties (Hereman, 1868; Smith, 1963; Jackson, 1965).

*Pimenta dioica* is an evergreen tree (Fig. 18), growing about 30 ft high, indigenous to the West Indian Islands and South America, extensively grown in Jamaica, where it flourishes best on lime stone hills near the sea. The tree begins to

flower in 3-4 years. The flowers appear in June - August and are quickly succeeded by the berries.

The slender upright trunk of the tree is covered with smooth grey bark. The shiny oval leaves have a pleasant odour when fresh. Petioles are 10-18 mm long, leaf blades 6-16 cm long and 3-6 cm wide. The leaves are oblong, lanceolate to elliptic; main veins 12-16 pairs and prominent beneath. Inflorescence is axillary pedunculate cymes and peduncles many, 3-7 cm long. The flowers are white, 6-10 mm wide. Calyx is four lobed, often fused with the inferior ovary. Sepals and petals four, stamens numerous, anthers bithecous and basifixed. Carpels two, syncarpous with one ovule in each chamber arranged on axile placenta. Style is short and stout. Fruit is crowned by the remains of calyx and is one seeded (Grieve, 2000; Griffie, 2000; Seigler, 2002).

The unripe fruits of this plant is collected and dried in sun to obtain the spice called AllSpice. The special qualities of the fruit reside in the rind of the berries. It loses its aroma on ripening, owing to loss of volatile oil and the berries are collected as soon as they attain their full size in July and August, but while unripe and green. They are either sun dried or dried in ovens. Sun dried fruits are of good quality, but it requires at least 12 days and needs protection from moisture. The dried fruits are reddish brown in colour. They contain 3 - 4.5% of volatile oil contained in the pericarp and obtained by distillation of the fruit. The oil of *Pimenta* is used for various kinds of preparations and as a medicine (Pandey, 1978; Grieve, 2000).

#### 19. *Psidium guajava* L.

*Psidium guajava*, commonly called 'Guava' is a small tree, native of Central America (Fig. 19). It was introduced to India by the Portuguese (Pandey, 1978). Now it is widely cultivated in India for their edible fruits. The Greek term, *Psidion* means Pomegranate and *Psidium* is the latin name (Nayar, 1985).

The bark of this tree is smooth, thin, greenish grey and wood is greyish brown in colour. The young branches are quadrangular and sometimes pubescent. Leaves are with very short petioles, opposite, 3-5 ribbed, ovate or oblong and

usually acuminate, softly pubescent beneath and with principal nerves prominent. Peduncles are axillary and 1-3 flowered. Flowers are large and white. Calyx obovate, limb undivided in aestivation and spreading valvately into 4-5 lobes when in flower. Petals 4-5, free and broad. Stamens are many, inserted in several series on a wide disk. Ovary many celled with numerous ovules in each cell, style subulate and stigma peltate or capitate. Fruit is a globose, ovoid or pyriform berry, usually crowned by the calyx-limb. Seeds are with a hard testa, a curved embryo and a long radicle. Many varieties are now under cultivation (Hooker, 1879; Gamble, 1935). The fruits are used to make guava jelly a type of jam. The bark and roots are astringent from the tannin which they contain (Pandey, 1978).

## 20. *Syzygium aromaticum* (L.) Merr. & Perry

Syn. *Eugenia aromatica* Baill.; *E. caryophyllata* Thunberg.;

*E. aromatica* L.; *Myrtus caryophyllatus* Sprengel.

The genus name *Syzygium* is originated from the Greek word, *Suzugos* which means jointed in allusion to the manner in which the branches and leaves are united in pairs in the Jamaican species *Calyptranthes suzygium*. The word *aromaticum* means fragrant (Smith, 1963). This is a small tree found in the tropics, usually cultivated for the clove of commerce, which are the unopened flower buds. The clove is highly esteemed as a flavouring material and as a culinary spice. They are aromatic, stimulant and carminative. The tree is a native of Moluccas and now it is widely cultivated in South India and Sri Lanka (Chopra, 1990). The chief clove producing countries are Tanzania, which grows 90% of the total output, Indonesia, Mauritius and West Indies (Pandey, 1978).

The tree is medium sized, cone shaped and evergreen (Fig.20). It attains a height of 10 to 12 m. The stem is usually forked near its base with two or three main branches. Smaller branches are slender, rather brittle and covered with grey bark. The leaves appearing in pairs are lanceolate, acute at both ends and have a

dark shining green colour. The aromatic nature of the leaves is due to numerous oil glands found on their under surfaces.

This tree is cultivated for its flower buds, which are greenish when fresh and are borne on ends of branches in small clusters. The unopened flower buds, which are picked green and dried in sun till they become dark brown form the "Clove" of commerce. The buds have a slightly cylindrical base and are crowned by the plump ball like unopened corolla which is surmounted by the four toothed calyx. If the bud is left unpicked, the flower develops after fertilization into a fleshy, purple and one seeded oval fruit known as 'Mother of Clove'. The fruit is about 2.5 cm long and 1.25 cm in width. The seed is oblong, rather soft in texture and grooved on one side. The leaves, unripe fruits and broken clove including the stalk are all aromatic and yield an essential oil, called 'Clove oil'.

The clove oil, which is highly aromatic is used for flavouring various food products. It is an ingredient of dentifrices, gargles and chewing gum. It is also used in perfumes and toilet preparations and as a clearing agent in histological works and as a mosquito repellent.

## 21. *Syzygium cumini* (L.) Skeels

Syn. *Eugenia jambolana* Lam.; *Myrtus cumini* L.

*Syzygium cumini* is a moderately sized tree commonly called as 'Jamun, Jambolan', etc. The word *cumini* is named after the collector Cumin. *Jambolana* is the Portuguese name for fruits from Jamun, the Indian one (Smith, 1963; Trimen, 1984; Nayar, 1985). This plant is originally from India and Indonesia.

It is an evergreen tree grown for its fruits (Fig. 21). Fruits and kernels are eaten in India. Dead bark is flaking off from the tree, but the live bark is brown to reddish when cut. Leaves are firm, shining, covered with white minute transparent dots, opposite, stalked, ovate or ovate-lanceolate, 7-15 cm long, entire, usually pointed with numerous parallel lateral veins, uniting to form a single vein running just within the margin and exstipulate. Inflorescence is in lateral panicles.

Flowers are small, pale green, subsessile, bisexual and epigynous. Calyx tube is adnate to the ovary, funnel shaped, limb shortly 4-5 lobed, petals 4-5, rounded, concave, carried up by the unfolding of stamens and falling off as flower expands. Stamens are numerous found in several series, much longer than petals, inserted on the calyx tube and folded in bud. Ovary contains 2-3 carpels, syncarpous, inferior with 2-3 cells. Style is simple, linear, stigma terminal, small, ovules many in each cell. Fruit is a dark purple berry with a single seed. In Indian folk medicine, the fruits and other plant parts of *S. cumini* is used as an astringent and to cure blisters in mouth, cancer, colic, diabetes, diarrhoea, digestion complaints, dysentery, piles, pimples, stomach - ache, etc. (Hooker, 1879; Gamble, 1935; Manilal, 1988; Mathew, 1995).

## 22. *Syzygium jambos* (L.) Alson

*Syzygium jambos*, commonly called 'Rose Apple', (Fig. 22) is a medium sized tree, native of South East Asia, now spread through out India and South Pacific Islands. It is cultivated for their edible fruits. It is a crisp, yellow 2.5-5 cm long fruit with the smell and taste of rose water. The rose apple is occasionally cultivated in the tropics but is rarely available in markets.

This tree is moderately hardy and will withstand extreme cold temperatures if fully grown. The tree will not flower or bear fruits in areas of frost. The leaves are decussate, oblong or lanceolate, 12-18 cm long and 1.5- 4 cm wide. The flowers are produced in cymes, which are large with hundreds of beautiful white stamens that attract nector loving insects. The stamens are inflexed in bud and during expansion the petals wither away. Carpels 2 - 3 and syncarpous. Fruit is a berry with 1 - 2 seeds. Flowering and fruiting usually follow regular cycles but these cycles vary through out tropics. Usually this tree will flower during midsummer with fruits ripening 2-3 months later (Hooker, 1879; Gamble, 1935).

## 23. *Syzygium malaccense* (L.) Merr. & Perry

Syn. *Eugenia malaccensis* L.

This tree is commonly called 'Malay Apple, Mountain Apple, Wax Apple', etc. It is a native of Malaysia and has been spread by human, through much



of South East Asia and Pacific Islands. Now it is commonly growing wild on Hawaiian Islands. The Malay apple was an important fruit of Polynesians and was later distributed to the America through one of Captain Bligh's voyages. In India it was believed to be brought by the early settlers. This tree is now cultivated in various gardens (Fig. 23).

*S. malaccense* is a medium to large sized tree growing to a height of 25-30 m. Malay apples thrive in tropical conditions. The leaves are very large, ovate - oblong with an average length of 20- 30 cm and breadth of 10-12 cm. Leaves are simple, exstipulate with smooth margin. The flower is axillary, solitary, large and rose-red in colour. Calyx lobes adnate to the inferior ovary. Petals are rose coloured and often fall off during the emergence of inflexed stamens. Stamens are numerous, often large and with rose coloured filaments. The flowers usually occur in early summer followed by fruit ripening three months later. Fruits are red - pear shaped and very beautiful with a waxy skin about the size of an apple. Flesh is crunchy often juicy, with mild sweet flavour. Some varieties have white or pink skin. It is always eaten fresh and chilled. Malay apples make thirst quenching snacks. Occasionally the fruit is used for wines (Hooker, 1879; Trimen, 1984).

#### 24. *Syzygium samarangense* (Blume) Merr. & Perry

*Syzygium samarangense* is a medium sized tree commonly called 'Java Apple, Wax Jambu', etc. The word *samarangense* is coined from the area of Samarang (Java) where it is originated. They grow up to 20- 25 m height. This tree is ultra tropical and will not survive in areas that receive frost. It needs adequate rainfall, some humidity and fertile soil for best growth (Fig. 24).

*S. samarangense* is a native of Malaysia and some Islands of Indonesia. Often cultivated in South-East Asia, but rarely grown else where. In India it is cultivated in house gardens for their edible fruits. The ripe fruits are available in the market during fruiting seasons. The fruits are occasionally exported to foreign countries like Canada, Europe, etc.

Leaves are simple, opposite, exstipulate, ovate with entire margin and submarginal veins are prominent. The flowers are large and produced in cymes,

usually red or cream in colour. The flower colour varies with varieties. Sepals four, adnate with the ovary wall and persistent. Petals four, red or cream in colour and often deciduous. Stamens many in different whorls fixed on the hypanthium, filaments petaloid and anthers bicelled. Ovary bicarpellary, syncarpous, bilocular with single ovule in each chamber on axile placentation. Fruit is a pear shaped berry with waxy and crispy flesh similar to *S. malaccense*. Fruit is often juicy with a subtle sweet taste, somewhat resembling common apple. Superior varieties are of excellent quality. Usually eaten fresh, but sometimes eaten with sugar sprinkled over the flesh, (Hooker, 1879; [www.cdfa.ca.gov/plant/agid.aid/2002](http://www.cdfa.ca.gov/plant/agid.aid/2002)).

#### **25. *Syzygium zeylanicum* DC.**

Syn. *Eugenia zeylanica* Wight

*Syzygium zeylanicum* is a handsome little tree with white flowers, often run wild in Western Ghats (Fig. 25). The species name *zeylanicum* refers to Ceylon, (Smith, 1963; Nayar, 1985). Young branches are often acutely quadrangular. Leaves are 2.5 - 12.5 cm long, varying in width from 1.25 – 5 cm, tapering at base and gives of aroma when bruised. Petioles are very short. Flowers are in terminal or axillary panicles of umbellules. Calyx tube is about 4 mm long, greyish and gland dotted. The pedicels of flowers are short, rough and often with glands. Petals are white and soon falling off. Stamens are many. Fruit is a very small white edible berry. The narrow leaved form is most abundant species (Hooker, 1879; Gamble, 1935).

#### **26. *Rhodomyrtus tomentosa* Wight**

Syn. *Myrtus tomentosa* Ait.

The term *Rhodomyrtus* is derived from two Greek words, *Rhodo*=red; *myrtos* = myrtle; from the rose coloured myrtle like flowers of these shrubs. The word *tomentosa* means densely woolly with matted hairs (Smith, 1963; Nayar, 1985).

*Rhodomyrtus tomentosa* is a thickly tomentose shrub (Fig. 26) with grey- tomentose foliage growing on hill stations like Nilgiri, Pulney Hills, Silent Valley, etc. (Gamble, 1935; Manilal, 1988). Lower leaves in three's and upper ones and those of branches opposite with 3-5 prominent nerves, starting from the base,

dark brown above, glabrous and shining, hoary beneath and rugose (Hooker,1879). Flowers are usually large, pink coloured and occur in axillary, 1-3 flowered cymes. Peduncles are about half the length of the leaves. Calyx tube turbinate, not or hardly produced beyond the ovary and with five persistent lobes. Petals are five, spreading, shortly clawed and pink coloured. Stamens are many and free in many series. Ovary 1-3 celled but appearing 2- 6 celled by spurious partition between the pairs of ovules which are arranged in vertical rows. Style filiform and stigma capitate. Fruit is a globose berry with numerous seeds. The fruit is about the size of a cherry, dark purple, pulp fleshy sweet and aromatic. The fruit is called 'Hill Goose Berry', and eaten raw or made into jam called "Thaonty", (Hooker,1879). Bark of the plant is thin, red and papery. The wood is dark red, close – grained and makes good walking-sticks (Gamble, 1935).

\*\*\*\*\*

Myrtaceae or Myrtle family is one of the families of angiosperms from which many economically important products such as essential oils, fruits, spices *etc.* are evolved. But the other potentialities of these plants are not used extensively. So far no work has been conducted to exploit the cytotoxicity of myrtaceous leaf extracts collectively. No collective attempt has been made yet to explore the essential oil composition in the members of Myrtaceae. Hence, the present study is an attempt to reveal the cytotoxic effects and phytochemical potentials of the leaf extracts and essential oils of the above mentioned plants of Myrtaceae. An attempt will also be made to find out the probable cause of the biological activities reported on these myrtaceous plants.

**\*Name Changes in Australian Plants :**

{[www.farrer.riv.csu.edu.au/ASGAP/changes.\(2000\)](http://www.farrer.riv.csu.edu.au/ASGAP/changes.(2000))}

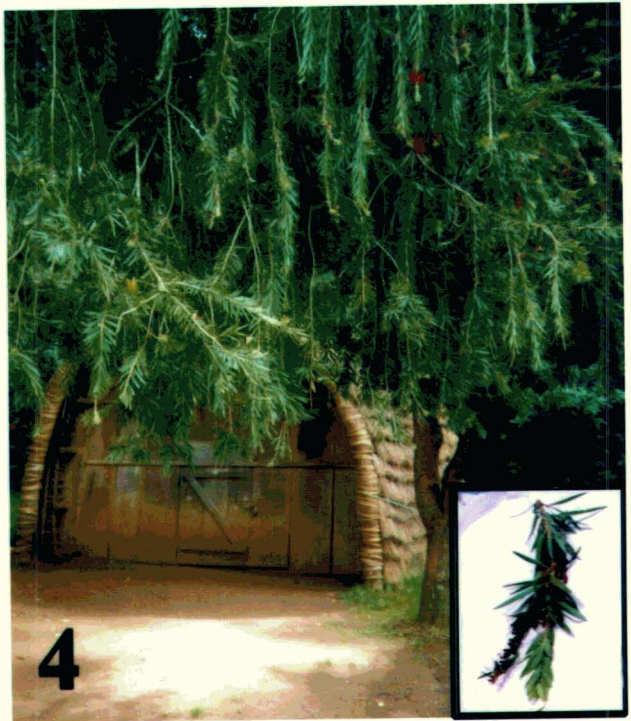
## Figure Legend

### Plate No. 1

Fig. No. 1	<i>Agonis flexuosa</i>	<b>Inset :</b>	A twig.
Fig. No. 2	<i>Beaufortia sparsa</i>	”	”
Fig. No. 3	<i>Callistemon citrinus</i>	”	”
Fig. No. 4	<i>Callistemon viminalis</i>	”	”
Fig. No. 5	<i>Corymbia citriodora</i>	”	”
Fig. No. 6	<i>Corymbia ficifolia</i>	”	”



# Plate 1



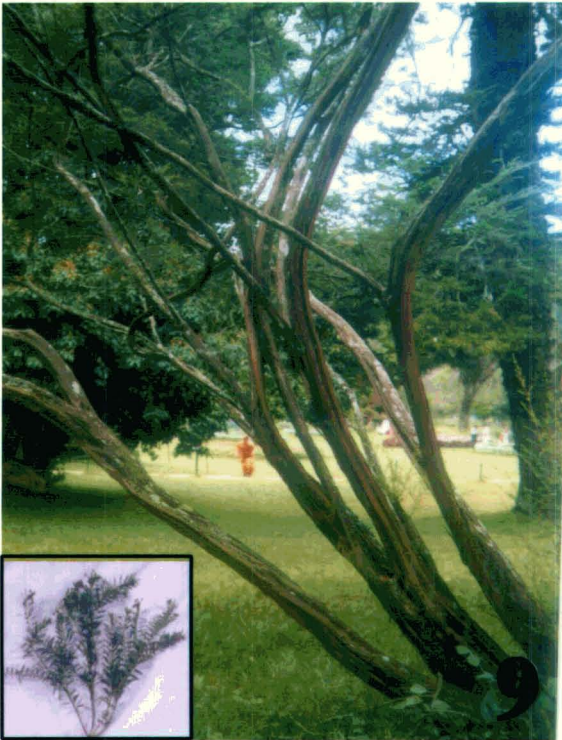
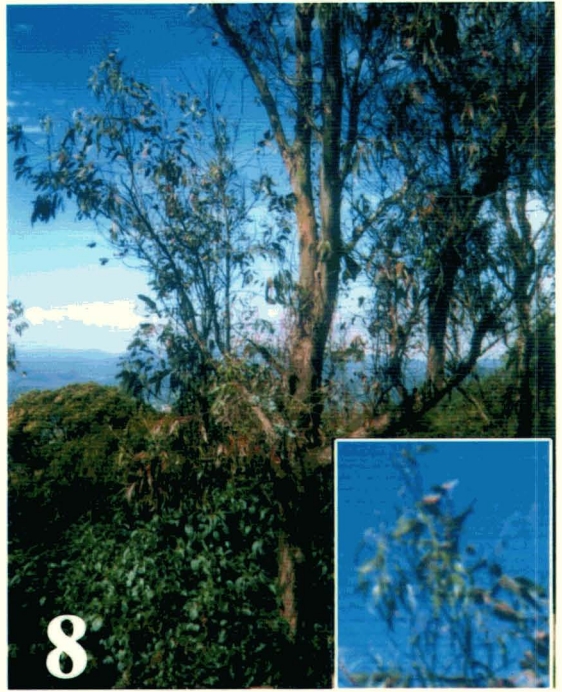
## Figure Legend

### Plate No. 2

Fig. No. 7 <i>Eucalyptus globulus</i>	Inset : A twig.
Fig. No. 8 <i>Eucalyptus tereticornis</i>	” ”
Fig. No. 9 <i>Leptospermum nicholsii</i>	” ”
Fig. No.10 <i>Melaleuca leucadendron</i>	” ”
Fig. No.11 <i>Melaleuca styphelioides</i>	” ”
Fig. No. 12 <i>Syncarpia glomulifera</i>	” ”



# Plate 2



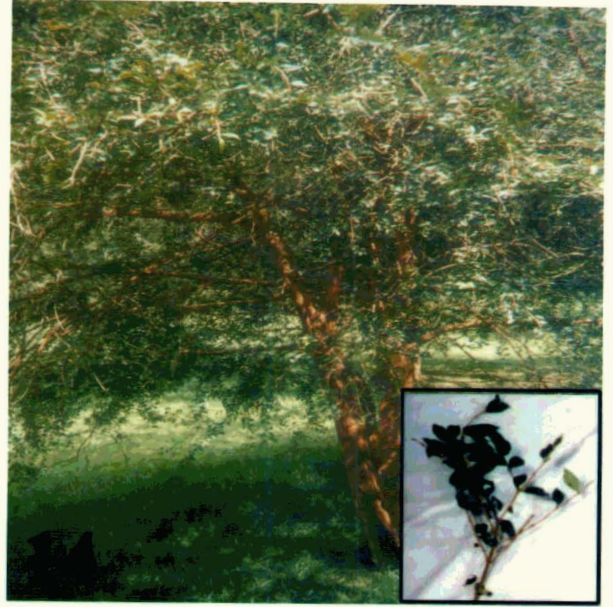
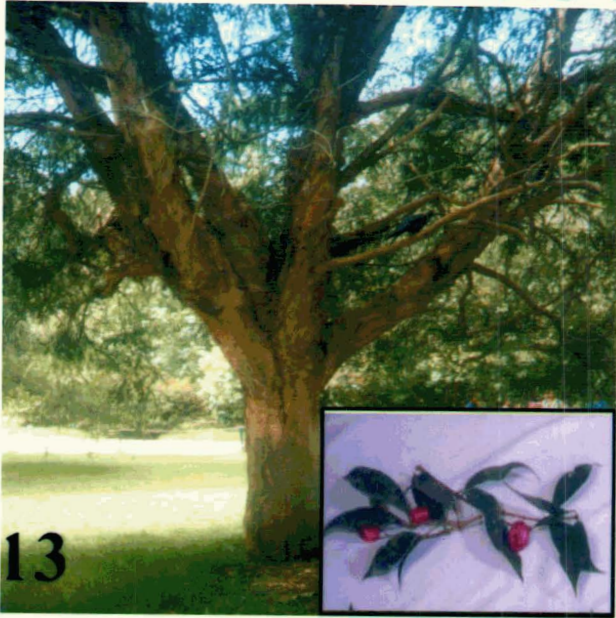
## Figure Legend

### Plate No. 3

Fig. No. 13 <i>Acmena smithii</i>	Inset : A twig
Fig. No. 14 <i>Eugenia apiculata</i>	„ „
Fig. No. 15 <i>Eugenia uniflora</i>	„ „
Fig. No. 16 <i>Feijoa sellowiana</i>	„ „
Fig. No. 17 <i>Myrtus communis</i>	„ A flower
Fig. No. 18 <i>Pimenta dioica</i>	„ „



# Plate 3



## Figure Legend

### Plate No. 4

Fig. No. 19 <i>Psidium guajava</i>	Inset : A twig
Fig. No. 20 <i>Syzygium aromaticum</i>	” ”
Fig. No. 21 <i>Syzygium cumini</i>	” ”
Fig. No. 22 <i>Syzygium jambos</i>	” ”
Fig. No. 23 <i>Syzygium malaccense</i>	” ”
Fig. No. 24 <i>Syzygium samarangense</i>	” ”



# Plate 4



## Figure Legend

### Plate No. 5

Fig. No. 25 *Syzygium zeylanicum*                      Inset : A twig

Fig. No. 26 *Rhodomyrtus tomentosa*                      „                      „



**Plate 5**



# REVIEW OF LITERATURE

## A. CYTOTOXIC EFFECTS

Research on phytochemicals have gained much importance even in the early 20<sup>th</sup> century. Owing to the advent of technologies and analytical methods the drastic effects of chemical pesticides are disclosed. Hence human interests are concentrated on biological and natural products. The cytotoxic studies gained much importance towards the later half of 20<sup>th</sup> century. Darlington (1942) studied about the karyotype, gene action, nucleic acid chemistry and metabolism and thus revealed the cause of chromosome abnormalities. Hadder and Wilson (1958) conducted a cytological assay to detect the action of C- mitotic chemicals and prophase poisons. They classified the abnormalities as prophase poisons and C-mitotic agents.

A lot of studies were done to reveal the response of dividing cells to plant extracts and essential oils. D'Amato and Avanzi (1949) had tested the effect of a number of essential oils and their components viz. eugenol, 1-8 cineole and turpentine on the mitotic cell division of *Allium* and proved their toxicity. The effect of vegetable oils and fats on the mitotic division of *Triticum* root tip cells were studied by Swaminathan and Natarajan (1956, 1957 and 1959) and detected chromosome fragmentation and C-mitosis as the frequent abnormalities. Kato (1957) observed chromosome bridges by the treatment with extracts of spinach and beet fruits.

Sharma and Sharma (1960) conducted experiments on spontaneous and chemically induced chromosome breaks. They detected that the chromosome breaks were caused by disruption of nucleic acid cycle of cell, together with non-synchronised nucleic acid metabolism at different gene sites. Betina and Murin (1964) detected the inhibition of mitotic activity in root tips of *Vicia faba* by the



antibiotic cyanine. The cytotoxic effects of mimosine was revealed by Pritchard and Court in 1966 and noticed an array of chromosome abnormalities.

The cytological effects of plant extracts were reported by many investigators (Ohno, 1960; Ohno and Tanihuzi, 1960; Palmer *et al.*, 1960). Das *et al.* (1968) studied the effect of asafoetida extract on root tip cells of *Vicia faba* and observed that it caused severe mitotic aberrations. They suggested that the chemicals in the extract can be used as a mutagen.

The cytological effects of lathyrogens in *Vicia faba* was investigated by Raj and Rao (1972). Chromosome abnormalities like scattering of chromosomes during metaphase, bridge formation during anaphase, *etc.* were observed by Bhalla *et al.* (1973) on *Allium cepa* root tip cells on treatment with different concentrations of Tobacco smoke concentrate. Sarkar (1974) studied the influence of plant extracts on chromosome metabolism of mature nuclei.

Abraham and Cherian (1978) noticed agglomeration of chromosomes, anaphase bridges, fragmentation of chromosomes, *etc.* during the treatment of water extract of betel leaves on onion root tip cells. Chromosome stickiness, C – mitosis, chromosome bridges, polyploidy and chromosomal breaks were observed by Shehab (1980) on treating onion root tip cells with water extracts of *Teucrium pilosum*. The effect of tobacco extract on *A. cepa* mitotic cells were observed by Bandhopadhyay and Sharma (1981). They have reported the activity of chemical constituents as cytotoxicants. Mallalah and Kabarity (1982) noticed the dissolution of the chromatin material in the interphase nuclei. The water extract treatment of *Anastatica hierochuntia* on the root tip cells of onion showed many chromosome abnormalities like despiralization, spindle disturbances, stickiness and lagging of chromosomes, bridge formation, fragmentation of chromosomes *etc.* The total percentage of abnormality increased with increase in time and concentration of the extract (Shehab and Zakaria, 1983). The effect of toxicants can be observed at the level of chromosomes through alteration in chromosome structure and number like aneuploidy and polyploidy (Sharma and Sharma, 1989).

The effect of toxicants present on plant extracts can be observed at the level of chromosomes (clastogenesis) through alteration of chromosome structure (chromosomal aberrations) and number (aneuploidy, polyploidy). A wide range of short term and long term procedures are available. The most common ones use higher plants like *Allium cepa*, *A. sativum*, *Vicia faba*, *Tradescantia virginiana* etc. or rodents like mice and rats *in vivo* as the simple and the most reliable test systems for monitoring chromosomal aberrations (Kihlman, 1971; Levan, 1949; Naismith, 1987; Sharma and Sharma, 1989). Low concentrations of tobacco leaf extract exerted a stimulating effect, where as high concentration acted as a mitodepressant, on root tip cells of *Allium sativum* (Sopova *et al.*, 1983). Stronger concentrations of extracts of immature *Solanum nigrum* fruits reduced the intensity of mitosis in *A. sativum*, where as, weaker concentrations stimulated it. The presence of a cytokinin like substance in the extract has been suggested to be responsible for this activity (Krivokapic *et al.*, 1970). Cellular damage including heavy pycnosis, clumping of chromosomes, fragmentation and spindle disturbances in *Allium cepa* root meristem were induced by leaf extract of *Ricinus communis* (George and Geethamma, 1990).

Antiviral, antimicrobial and cytotoxic activities of essential oil obtained from *Salvia fruticosa* was reported by Sivropoulou *et al.* (1997). High levels of viricidal activity against Herpes Simplex Virus was shown by 1,8 - cineole which was the major compound. Cytotoxic activity of Amaryllidaceae alkaloids was reported by Weniger *et al.* (1995). Popoca *et al.* (1998) recorded the cytotoxic activity of a few plants used as antitumourals in Mexican traditional medicine.

Cytotoxic properties including clastogenic and nonclastogenic effects of *Ocimum americanum* were reported by Tajo and Thoppil (1998) and they detected the most frequently occurred cytotoxic effects as chromosome clumping and stickiness. Miniya *et al.* (1999) observed mitoclastic abnormalities like clumped metaphase, anaphase bridge, sticky metaphase, ball metaphase and diagonal metaphase on onion root tip cells after treatment with *Mentha rotundifolia* extract.

Deena and Thoppil (2000) studied the cytotoxic properties of *Lantana camara* on *A. cepa* root tip cells. The major abnormalities observed were clumping, stickiness, diagonal orientation of chromosomes, etc. Mitoclastic effects of *Spilanthes ciliata* was reported by Sreeranjini and Thoppil (2001). The major abnormalities were chromosome bridges, diagonal and misorientation of chromosomes. In all these extracts the major chemical principles were found to be responsible for causing the clastogenic and nonclastogenic effects.

The leaf extracts of *Psidium guajava* showed cytotoxic effects against human cell lines (Villarreal *et al.*, 1992). The biological activity of 43 Jordanian medicinal plant extracts were examined for cytotoxicity, mutagenicity and antimicrobial activity by Alkofahi *et al.* (1997) and they found that the most effective extract was that of *Eucalyptus camaldulensis*.

Antitumour activity of leaf essential oil of Costa Rican black fruit, *Myricanthes* sp. (Myrtaceae) was reported by Setzer *et al.* (1999) on human cell lines. The ethanolic leaf extract of Malaysian traditional vegetable, *Eugenia polycantha* showed antitumour promoting activity (Mooi *et al.*, 1999). Cytotoxicity and antitumour activity were reported from phytochemicals extracted from *Syzygium cumini* (Bhattacharya *et al.*, 1992). Anticancer activity has been observed for the extracts of *Myrtus communis* and *Crataegus monogyna* (Alwan *et al.*, 1990; Sarkar *et al.*, 1996).

Water extracts of fruits of *Psidium guajava* and *Terminalia chebula* were found to be effective in reducing the mutagenic effects of two direct acting mutagens NPD and Sodium azide and an S<sub>9</sub> – dependent mutagen, 2-aminoflourine (Grover and Bala, 1992, 1993). Extracts of *Psidium guajava* and *Eucalyptus* spp., *Terminalia chebula*, *T. arjuna*, *Zizyphus jujuba*, *Aegilops* spp. and *Acacia arabica* were detected as antimutagenic against UV irradiation (Jain *et al.*, 1987).

Based on the cytotoxic studies conducted on the Australian tea tree plant (*Melaleuca alternifolia*), Faoagali *et al.* (1996) suggested that the compounds present in tea tree oil should not be used in burns and wounds, as it has cytotoxic effects on human fibroblast and epithelial cells. The tea tree oil had shown cytotoxic

and antitumour activity on hepatocellular carcinomic human cell line, human lymphoblastic leukaemia T- cell line, myeloid leukaemia, human chronic myelogenous leukaemia cell line, *etc.* (Hayes *et al.*, 1997). Strong cytotoxicity against leukaemia cells *in vitro* was shown by the chemicals obtained from the dried leaves of the myrtaceous member, *Baeckea frutescens* (Fujimoto *et al.*, 1996). *Eucalyptus grandis* extracts showed high cytotoxicity in mouse hepatoma cell line (Potgieter *et al.*, 1995).

The crude chloroform extract of the bark of *Syncarpia glomulifera* showed cytotoxic and antibacterial activity. It was detected that the major chemical compounds showed high degree of action (Setzer *et al.*, 2000).

The hydrolysable tannins from *Syzygium jambos* induced apoptosis on human leukaemia cells (Yang *et al.*, 2000). The extracts of several *Syzygium* spp. were found to exhibit cytotoxic activities (Horgen *et al.*, 2001). Kim *et al.* (2001), detected certain virus cell fusion inhibitory substances from *Eugenia caryophyllata* and isolated.

## **B. ESSENTIAL OIL ANALYSIS**

The phytochemical studies are of great importance now a days. Majority of our present medicines are derived directly or indirectly from higher plants, while several classic plant drugs have lost much ground to synthetic competitors. A few phytochemicals have gained a new investigational or therapeutical status in recent years. Several new plant derived substances have entered into world drug markets. Clinical plant based research has made particularly rewarding progress in the important fields of anticancer and antimalarial therapies. In addition to purified plant derived drugs, there is still an enormous market for crude herbal medicines. Natural product research can often be guided by ethnopharmacological knowledge and it can make substantial contributions to drug innovation by providing novel chemical structures and /or mechanisms of action (De Smet, 1997). One of the important drawbacks here is the extremely limited

knowledge about the ingredients in herbal medicines and their effects in humans, the lack of stringent quality control measures and the heterogenous nature of herbal medicines, all of which necessitates the continuous monitoring of the products (Chan, 1997). Hence phytochemical studies were conducted using all the various kinds of analytical methods in all fields of science. Several angiosperms are showing good or bad effects and there is a wide spectrum of literature. Since the family Myrtaceae is one with large number of medicinal and aromatic plants, this review is merely concentrated on that family only.

Essential oils and essential oil components have been reported to exhibit various kinds of biological activities especially antimicrobial effects. The extracts and essential oils from the leaves, bark and fruits exhibit a wide range of activities.

The components of essential oils obtained by hydrodistillation from the leaves of *Psidium guyanensis*, *Melaleuca alternifolia*, *M. cajaputi* ssp. *cajaputi*, *Kunzea ericoides* and *Leptospermum scoparium* showed antibacterial and antifungal activities against a number of disease causing bacteria and fungi (Santos *et al.*, 1997; Carson *et al.*, 1998; Porter and Wilkins, 1999 and Christoph *et al.*, 2000).

Similar activity of essential oil of *Melaleuca alternifolia* was reported by Carson and Riley (1993). Antimicrobial potential of the eight major components of essential oils of *M. alternifolia* was evaluated by Carson and Riley (1995). The major components were 1,8- cineole, terpinen – 4 - ol, p-cymene, linalool,  $\alpha$  – terpinene,  $\gamma$  – terpinene,  $\alpha$  – terpineol and terpinolene. Of these, terpinen – 4 - ol was active against all the test microbes, while the others showed varied activities.

The antimicrobial activity of three components of *Eucalyptus* essential oils, 1,8- cineole, linalool and eugenol on *Staphylococcus aureus* was determined. 1,8- cineole showed less significant activity, where as, eugenol was highly active, closely followed by linalool. The linalool – eugenol interaction showed an antagonistic effect between the two substances (Belaiche *et al.*, 1995; Dellacassa *et al.*, 1989 ).

The phytochemicals obtained from the leaves of *Psidium guajava* showed antimicrobial activity (Lutete, *et al.*, 1994). The aromatic essential oil

obtained from *Pimenta dioica* was found to be antimicrobial (Rodriguez *et al.*, 1996; Aureli *et al.*, 1992). The essential oil extracted from *Eucalyptus citriodora* shows similar activity as reported by Hajji *et al.* (1993) and Begum *et al.* (1997). The essential oil of *Syzygium aromaticum* showed a total inhibition of the growth of *Aspergillus flavus* in maize kernel (Belmont and Carvajal, 1998). Nematicidal activity of the essential oil obtained from the leaves of *Pimenta dioica* has been proved, against the parasitic nematode (*Meloidogyne incognita*). The essential oil and eugenol exhibited promising nematicidal activity (Leela and Ramana, 2000).

According to Santos and Rao (2000), 1,8- cineole, a terpenoid oxide present in many plant essential oils showed antiinflammatory and analgesic effects in rats. *Eucalyptus* oil and its major components, especially 1,8 – cineole and turpentine shows potential mosquito larvicidal property as reported by Corbert *et al.* (1995).

Similar activities were exhibited by extracts, essential oils and chemical components of all the myrtaceous plants. Previous reports on the essential oil composition of some of the important genera under study are reported below.

### ***Eucalyptus***

Mwangi *et al.* (1980) studied *Eucalyptus citriodora* essential oil composition. The chief constituents were citronellal (65-88%), citronellol (2-25%) and isopulegol (2-19%). They identified two chemical varieties, viz. the 1,8-cineole variety (18.7% cineole) and citronellol variety (32.1 - 51.8% citronellol) and these varieties did not differ morphologically from the normal variety.

Singh *et al.* (1983) conducted phytochemical studies on 17 species of *Eucalyptus* and suggested that *E. citriodora* may be a good source of citronellal and citronellol which are used in perfume industry, as well as a good timber species. Brophy *et al.* (1998a) found that the essential oils from the 13 species within the genus *Corymbia* can be categorized under two distinct phytochemical

groups. One with high amounts of  $\alpha$  - pinene, while the second was with lesser amounts of  $\alpha$  - pinene (5-34%).

According to Shiva *et al.* (1989) the leaves of *Eucalyptus tereticornis* showed higher percentage of pinene. Mehra and Shiva (1988) found that the essential oil obtained from the leaves of *E. tereticornis* was a rich source of both  $\alpha$  - and  $\beta$  - pinene and forms a valuable raw material for the manufacture of terpene based chemicals. Dayal and Maheswari (1985) analysed the terpenoids present in the essential oils of *Eucalyptus* species including *E. tereticornis*. On analysis with GLC, 23 components were obtained and 15 were identified, viz.  $\alpha$  - pinene,  $\beta$  - pinene, phellandrene, camphene, 1,8-cineole,  $\gamma$  - terpinene, p-cymene, limonene, citronellal, linalool, terpin -1-en-4-ol., citronellyl acetate, borneol,  $\alpha$  - terpineol and piperitone. Shiva *et al.* (1984) detected 7 important components from *E. tereticornis* essential oil, like  $\alpha$  - pinene,  $\beta$  - pinene,  $\alpha$  - terpinene,  $\beta$  - phellandrene, p-cymene, terpinyl acetate and 1,8-cineole.

Baslas and Saxena (1984) found that the mature fruits of *E. globulus* contain 1.8% oil of which the major constituent was 1,8 - cineole (72.5%). This oil also contain 28 other compounds. The chemical composition of the leaf essential oil obtained from *E. globulus* from Spain was estimated by Chalchat *et al.* (1995). The major compounds include 1,8-cineole (4.1-50.3%),  $\alpha$  - pinene (0.05- 17.85%), p- cymene (27.22%), cryptone (17.8%) and spathulenol (17%). Dellacassa *et al.* (1990) analysed the essential oils of 22 most common *Eucalyptus* spp. growing in Uruguay, including *E. globulus*, *E. tereticornis*, *E. citriodora*, etc. Even though 150 compounds were detected, 1,8-cineole, citronellal, p-cymene and benzaldehyde were the principal components. Dayal and Ayyar (1986) isolated major compounds from the leaf essential oil of *E. globulus* ssp. *bicostata*, which were 1,8-cineole (73.09%),  $\alpha$  - pinene, linalool,  $\alpha$  - terpineol and a new compound bicostol. Dayal and Maheswari (1985) analysed the leaf oil obtained from *Eucalyptus tereticornis*, *E. robusta*, *E. deglupta*, *E. camaldulensis*, *E. camaldulensis* X *E. tereticornis* hybrids and detected 23 components. The major components were  $\alpha$  - pinene,

$\beta$  - pinene,  $\alpha$ -phellandrene, camphene,  $\beta$  - phellandrene, limonene, cineole,  $\gamma$  -terpinene, p-cymene, citronellal, linalool, terpin-1-en-4-ol, citronellyl acetate, borneol,  $\alpha$  - terpineol and piperitone .

The leaf essential oil obtained from *E. globulus* and other 4 spp. acclimatized in Morocco showed 1,8-cineole (47.6 – 71%) and other terpenes like  $\alpha$  - and  $\beta$  - pinene, camphene ,  $\alpha$  - phellandrene, p-cymene , terpin-1-en-4-ol,  $\alpha$  - terpineol and globulol as the major constituents (Ahmadouch, *et al.*, 1985).

Oyedeji *et al.*(1999) analysed the volatile leaf oil constituents of 3 spp. of *Eucalyptus* from Nigeria. They detected sesquiterpenoids like nerolidol (34.8%) as the major component in *E. deglupta*. *E. alba* and *E. saliqua* contained monoterpenoids like  $\alpha$  - thujene (32.9 – 63.8%),  $\alpha$  - pinene (0.7- 24.7%), p-cymene (0.6- 12.9%) and 1,8-cineole (12.2 – 13.3%). Gupta *et al.* (1990) conducted GC /MS studies on the leaf essential oil of *E. sideroxylon* and *E. nitens*. They revealed the presence of 98 and 103 constituents respectively, of which 38 and 30 respectively were identified. 1,8- cineole was found in highest percentage in both the oils.

Antonov and Zhadanov (1986) studied the phytochemistry of *Eucalyptus macarthurii* and identified the following major compounds:  $\alpha$  - pinene (2.44%), camphene (0.26%),  $\beta$  - pinene (0.07%),  $\delta$ -3- carene (0.11%), n-cymene(0.18%), 1,8- cineole (30.21%),  $\alpha$  - terpineol (3.22%), terpenyl acetate (8.91%) terpene alcohol (15.93%), a sesquiterpene hydrocarbon (3.91%), palustrol (1.66%), a sesquiterpene alcohol (2.53%), globulol (8.62%) and ledol (3.68%). Gupta *et al.* (1988) analyzed the volatile constituents of the leaf essential oil of *Eucalyptus excreta*. A total of 68 peaks were recorded. 1,8- cineole (44.8%) and  $\alpha$  - pinene (37.14%) were identified as the major constituents. Singh *et al.*(1988) detected 48 components in the essential oil of *Eucalyptus urophylla*. The oil contained p-cymene (75%),  $\alpha$  - pinene(7%),  $\gamma$  - terpinene(4%), spathulenol (2%), terpinen-4-ol (1%) and  $\beta$  - caryophyllene (1%). The leaf essential oils of 10 species of *Eucalyptus* were analyzed with the help of GC and GC/MS by Molangui



*et al.*(1997) and about 60 chemical compounds were identified. The major constituents of these oils were monoterpenes like pinenes, 1,8- cineole, limonene, piperitone, terpinyl acetate, *etc.* which amounts to 65.3- 99%, except for *E. fastigata* which contain mainly sesquiterpenes (56.5%) in which the eudesmols (37%) make a large contribution.

Brophy *et al.* (1989b) detected that the main constituent of the leaf essential oil of *Eucalyptus bakeri* was 1,8-cineole (85-96%). In addition to the usual monoterpenes and sesquiterpenoids, Brophy and Lassak (1991) identified a phenolic ketone, torquatone in the volatile leaf oils of Western Australian species of *Eucalyptus*. Brophy *et al.* (1998b) analyzed the leaf essential oils of three rare *Eucalyptus* spp. (*E. curtisii*, *E. rubiginosa* and *E. tenuipes*). The major compounds were  $\beta$  – pinene,  $\alpha$  – pinene,  $\beta$  – ocimene,  $\alpha$  – terpineol and globulol. 1,8-cineole was found only in less amounts, *ie.* below 5%.

Menut *et al.* (1995) analyzed the chemical components of *Eucalyptus goniocalyx* and *E. patens* from Rwanda by GC and GC/MS. About 60 compounds were identified in both oil samples, most of which were monoterpenoids (*E. goniocalyx*- 72.8% and *E. patens*- 91.2%). The major components of *E. goniocalyx* were  $\alpha$  – pinene (29%), 1,8- cineole (18%), and p-cymene (17.2%). In *E. patens* oil, 1,8-cineole was the major constituent (39.6%), followed by limonene (32.5%) and  $\alpha$  – pinene (11.3%).

Singh *et al.* (1989) estimated the chemical constituents of essential oils in different *Eucalyptus* species grown in Kumon Hills, India, and classified the trees based on the constituents into 4 groups. (1) Cineole rich (2) Cineole and Pinene rich (3) Eudesmol rich and (4) Pinene rich.

Bignell *et al.* (1996), analyzed the essential oil of *Eucalyptus ficifolia* (*Corymbia ficifolia*) and 14 other species by GC and GC/MS and found that many of them contained  $\alpha$  – pinene (0.2 - 31.1%),  $\beta$  – pinene (0 - 12.5%), 1,8- cineole (0.2 - 76.8%), p-cymene (0 - 20.8%), aromadendrene (0 - 13.6%), bicyclogermacrene (0 - 43.4%) and spathulenol (0.1 - 15.2%) as the principal essential oil components. Bignell *et al.* (1997), studied the phytochemistry of

volatile leaf oils of South western and Southern Australian species of *Eucalyptus*. The leaf oils were isolated by vacuum distillation and the major components found were  $\alpha$  – pinene (0.08 - 33.1%), limonene (0 - 3.6%), 1,8- cineole (2.6 - 86.1%), p-cymene (0.3 - 14.2%), pinocarvone (0 - 9%), aromadendrene (0 - 29.9%) and spathulenol (0.1 - 15.2%).

Mizrahi *et al.* (1997), estimated the chemical composition of *Eucalyptus dunnii* growing in Argentina. The major compounds were 1,8- cineole (47.8% - 57.6%),  $\alpha$  – pinene (10 - 16.5%),  $\alpha$  – terpineol (2.9 - 8.2%) and globulol (5.3 - 6.6%). Chisowa (1997) studied the chemical composition of essential oils of three *Eucalyptus* spp. from Zambia (*E. globulus*, *E. smithii* and *E. radiata*) The major chemical components were 1,8- cineole (70.1 – 86.4%),  $\alpha$  – pinene (1.7 – 14%), limonene (3.7 - 5.7%), and  $\alpha$  – terpineol (1.9 - 6.4%).

Shieh and Shieh (1998), analyzed the essential oil components of *E. urophylla* from Taiwan and the main components were 1,8-cineole,  $\alpha$  – pinene, limonene, ocimene, 4-terpineol and  $\alpha$  – terpineol. Samate *et al.* (1998) detected the essential oil components of West African *Eucalyptus* spp. viz. *E. alba* and *E. camaldulensis*. The main compounds of *E. alba* were  $\beta$  – pinene (31%),  $\alpha$  – pinene (20.1 %) and limonene (16.8%), where as in *E. camaldulensis*  $\alpha$  – phellandrene (24.8%), 1,8-cineole (19.3%),  $\alpha$  – pinene (12.8%) and  $\gamma$  – terpinene (11.8%) predominates.

Dagne *et al.* (2000) analyzed the essential oil components of leaf oil of 12 *Eucalyptus* spp. from Ethiopia. They observed that *E. citriodora* (*Corymbia citriodora*) was rich in citronellal (73.8%) and citronellol (11.9%). In *E. globulus*, *E. tereticornis* and others the major compounds were  $\alpha$  – pinene (13.2 - 44.7%) and 1,8- cineole (34.5 - 57.5%).

### ***Leptospermum***

Ibrahim *et al.* (1995) studied the essential oil constituents of *Leptospermum javanicum* from peninsular Malaysia, which consist of  $\alpha$  – pinene (41.7%),  $\beta$  – pinene (12.4%), and  $\beta$  – caryophyllene (11.2%) in one sample, where as the

other two samples were characterized by terpinen - 4-ol (24.9% & 13.5% respectively) and low amount of pinenes (6.1% & 8.1% respectively). Perry *et al.* (1997) showed that Australian *Leptospermum* oils had significantly higher 1,8-cineole (mean 20%) content and total monoterpene levels (mean 51%) than the NewZealandian *L. scoparium* oils (1,8- cineole - mean 0.9% and total monoterpene content - mean 14%).

Porter and Wilkins (1999) identified the major components of essential oils of NewZealandian essential oils of *Leptospermum scoparium* and *Kunzea ericoides*. In *Leptospermum scoparium*, monoterpenes were present at low levels and sesquiterpene hydrocarbons were predominant and include groups possessing cubebene / copaene, selinene, calamene and cadinene skeletons. Oxygenated sesquiterpenes and triketones were also present. *Kunzea ericoides* was characterized by low levels of  $\alpha$  - pinene (>50%) and lower levels (>10%) of viridiflorol and viridiflorene.

Brophy *et al.* (1998c), detected the major compounds of the essential oils obtained from *Leptospermum brachyandrum*, *L. luehmanii*, *L. madidum*, *L. speciosum* and *L. pallidum*. All these species contain  $\alpha$  - pinene as the major compound, which was followed by lesser amounts of  $\beta$  - pinene,  $\beta$  - caryophyllene, aromadendrene, humulene and spathulenol. 1,8- cineole was found usually in small amounts (less than 10%). Brophy *et al.* (1998d) estimated the principal components of *L. blakelyi* and its allies and found that monoterpenes like  $\alpha$  - pinene was the major compound. But *L. divaricatum* and *L. microcarpum* exhibited chemotypes of monoterpenoid, sesquiterpenoid and mixed form. Brophy *et al.* (1999a) detected the leaf essential oils of 8 species of the genus *Leptospermum* in Eastern Australia and found that these oils were rich in sesquiterpenes,  $\alpha$  -,  $\beta$  - and  $\gamma$  - eudesmol. Brophy *et al.* (1999b) revealed the chemical constitution of *Leptospermum deanei* and its 8 allies and found that  $\alpha$  -,  $\beta$  - and  $\gamma$  - eudesmol was found only in *L. myrsinoides*, where as,  $\alpha$  - pinene was the principal component of oils of *L. deanei*, *L. trinervium* and *L. lamellum*. Bicyclogermacrene

was a principal component of oils of *L. deanei* and *L. trinervium*, while *L. laevigatum* was composed mainly of  $\beta$ -caryophyllene and aromadendrene.

Brophy *et al.* (1999c) analyzed the essential oils of *Leptospermum coriaceum*, *L. fastigiatum* and *L. nitens* growing in Australia. *L. coriaceum* contained  $\alpha$  - pinene (25.4%), 1,8- cineole (11.5%) and globulol (15.4%), whereas those of *L. fastigiatum* and *L. nitens* contained  $\alpha$  - pinene (82.8 % and 64.8-70.6% respectively) as the major component.

Brophy *et al.* (2000) studied the essential oil composition of *Leptospermum petersonii*, *L. liversidgei* and its allies. The principal compounds were  $\alpha$  - eudesmol,  $\beta$  - eudesmol,  $\gamma$  - eudesmol, citronellal, neral, geranial and some other sesquiterpenes like  $\beta$  - caryophyllene, globulol, viridiflorol and spathulenol. *L. rotundifolium* leaf oil contained the principal components, viz.  $\alpha$  - pinene (16-25%) and 1,8-cineole (21-28%). *L. wooroonooran* contained comparable amounts of mono and sesquiterpenes, the main monoterpenes were  $\alpha$  - pinene (4-11%),  $\beta$  - pinene (4-9%), sabinene (9-19%),  $\beta$  - caryophyllene (5-7%) and humulene (11-20%).

Brophy *et al.* (1993) studied the essential oils of the indigenous myrtacean members of Lord Howe Island. The fresh leaves of *Leptospermum polygalifolium* ssp. *howense* and *Melaleuca howeana*, endemic to Lord Howe Island (Australia), were found to contain 54 volatile constituents. In *L. polygalifolium*, the essential oil was dominated by  $\alpha$  - eudesmol,  $\beta$  - eudesmol and  $\gamma$  - eudesmol. 67 constituents were identified in *M. howeana*, including 1,8- cineole.

### ***Melaleuca***

Altman (1989) revealed the essential oil composition of *M. alternifolia*. It contains terpenes (viz. pinene, terpinene and cymene), cineole, terpineol and various other components including sesquiterpenes and their alcohols. Brophy *et al.* (1989a) analyzed the essential oils of sixteen *Melaleuca* spp. and three *Leptospermum* spp. The essential oils ranged from those containing almost exclusively terpenes (either mono, sesqui or both) to those that

contain exclusively other aromatic compounds. The species of *Melaleuca* viz. *M. citrolens*, *M. cajaputi* and *M. leucadendron* showed the existence of various chemotypes.

A comparative account of essential oils from three *Melaleuca* spp. growing in Egypt was studied by Aboutabl *et al.* (1991). The composition of *M. quinquenervia*, *M. armillaris* and *M. bracteata* was determined by GC/MS. Methyl eugenol was the main component in *M. bracteata* (97.7%). 1,8-cineole constituted 57.2% of the oil of *M. quinquenervia* and 33.7% of the essential oil in *M. armillaris*. Terpinen-4-ol (24.8%) was another major component in *M. armillaris*.

Motl *et al.* (1990) studied the chemical composition of Vietnamese cajaput leaf essential oil (*Melaleuca cajaputi*) and detected 61 components, of which 48 were identified. The major compounds were 1,8 -cineole, eugenol and methyl eugenol. Ramanoelina *et al.* (1994) detected the chemical composition of Madagascan *Melaleuca quinquenervia* leaf essential oil. The major components were 1,8- cineole (37%) viridiflorol (20%) and terpinolene (5%).

Carson and Riley (1995) separated 8 components with high antimicrobial activity from *M. alternifolia*. They include 1,8 -cineole, 1-terpinen-4-ol,  $\rho$ -cymene, linalool,  $\alpha$  - terpinene,  $\gamma$  -terpinene,  $\alpha$  - terpineol and terpinolene. Verghese *et al.* (1996) studied the constituents of the essential oil of Indian tea tree oil (*M. alternifolia*) by GC /MS. The main components were terpinen -4-ol (36.4%) and 1,8 -cineole (5.6%). This was compared with the tea tree oils of Australian origin and an inverse relationship with respect to the components was noticed.

Farag *et al.* (1998) analysed the essential oil of *M. ericifolia* by GLC. The oil contained 98.5% of ethyl eugenol as the single major component. 1,8-cineole (34.3%) and terpinen - 4- ol (39.1%) were the characteristic compounds of *M. armillaris*. High content of  $\alpha$  - terpineol (34.7%) was found in *M. leucadendron*, where as, caryophyllene (50%) and methyl eugenol (26.6%) were characteristic to *M. styphelioides*.

### *Callistemon*

Khanna *et al.* (1990) hydrodistilled the leaves of *C. polandi* and 19 components were identified by GLC. The major ones were 1,8-cineole (31.93%), myrcene (14.69%), methyl cinnamate (13.03%) and humulene (8.3%). Misra *et al.* (1997) estimated the essential oil components of *C. citrinus* leaves from Lucknow, India. The major components were 1,8 cineole (41.8%) and  $\alpha$  - pinene (11.6%).

Brophy *et al.* (1997a) analysed the leaf essential oils in *C. viminalis* as a clue to determine the validity of infraspecific taxa recognized in the taxon. All oils contained  $\alpha$  - pinene,  $\beta$  - pinene, myrcene and 1,8- cineole as their major components. Brophy *et al.* (1998e) analysed the essential oils of *Callistemon* species. The majority of essential oils had 1,8 - cineole as their major component (45-80%). The other compounds were  $\alpha$  - pinene (2 - 40%), limonene (2 - 9%) and  $\alpha$  - terpineol (1 - 13%). Ming *et al.* (1998) estimated the chemical composition of essential oil obtained from the leaves of *Callistemon citrinus*. The major components were 1,8 -cineole (68%),  $\alpha$  - pinene (12,8%) and  $\alpha$  - terpineol (10.6%).

### *Psidium*

Volatile constituents of *P. guajava* were obtained by supercritical fluid extraction and analysed with GC and GC/MS by Nieves *et al.* (1994). 17 compounds were identified, accounting for 86.1% of the aroma. They include  $\alpha$  - selinene (23.7%),  $\beta$  - caryophyllene (18.8%) and  $\delta$  - selinene (18.3%) as the major components.

The extract of Egyptian guava fruits was analysed using GC/MS and a total of 132, out of 160 compounds were identified. They comprised of 41 hydrocarbons (alkanes, cycloalkanes, alkenes, *etc.*), 9 aromatics, 3 monoterpenes, 15 sesquiterpene derivatives, 12 carbonyl compounds, 25 esters, 9 lactones, 13 alcohols and 5 miscellaneous compounds (Vernin *et al.* 1991).

Ekundayo *et al.* (1991) analysed the volatile compounds of guava fruits from Nigeria and detected a total of 25 compounds accounting for 80 % of the oil .

Free fatty acids (mainly lauric and myristic acids) were the most abundant group of constituents (34%). Large amounts of  $\beta$ -caryophyllene and oxygen containing sesquiterpenes (25%) were also typical for Nigerian guava.

Neto *et al.* (1994) analysed the chemical constituents of *P. pohliatum* and *P. guayanensis* from Brazil. The main constituents of both oils were 1,8-cineole (40.5%),  $\beta$ -pinene (8.6%), elemol (7.7%) and spathulenol (4.6%) in *P. guayanensis* and 1,8-cineole (63.8%), p-cymene (14.1%) and  $\alpha$ -terpinyl acetate in *P. pohliatum*.  $\beta$ -eudesmol (19.5% and 8.8%) and  $\gamma$ -eudesmol (5.2% and 2.8%) were found to be common in both oils.

Rossini *et al.* (1994) studied the comparative account of the chemical compounds of *P. luridum* and *P. incanum* from Uruguay. The major constituents of both oils were 1,8-cineole (36.56% and 30.96% respectively) and linalool (12.38% and 11.5% respectively).

Pino *et al.* (2001a) detected the chemical composition of leaf oil of *P. guajava* from Cuba, which showed 57 compounds of which  $\beta$ -caryophyllene (21.6%), E-nerolidol (19.2%) and selin-1-en-4 $\alpha$ -ol (13.4%) were the major ones. Pino *et al.* (2001c) identified 204 compounds from the aroma concentrate of strawberry guava (*P. cattleianum*).  $\alpha$ -pinene, (Z)-3-hexenol, (E)- $\beta$ -caryophyllene and hexa-decanoic acid were found to be the major components. The presence of many aliphatic esters and terpenic compounds contribute to the unique flavour of the strawberry guava fruit.

### ***Feijoa***

Rotman *et al.* (2001) studied the leaf essential oil constituents of pineapple guava, *Feijoa sellowiana* (*Acca sellowiana*). The major constituents were spathulenol (24.4%),  $\beta$ -cadinene (9.6%),  $\beta$ -terpineol (8.7%) and  $\beta$ -caryophyllene (8.1%).

### *Myrtus*

Weyerstahl *et al.* (1994) detected the constituents of the essential oil of *Myrtus communis* from Iran and the main constituents were the monoterpenes viz.  $\alpha$  - pinene (35%), 1,8- cineole (28%), limonene (11%), linalool (9%),  $\alpha$  - terpineol (5%) and linalyl acetate (2%). Bradesi *et al.* (1997) analyzed the essential oil components of *M. communis* from Corsica, France and noted the presence of  $\alpha$  - pinene (47.9- 59.5%) and 1,8- cineole (19.8-28.1%). Chalchat *et al.* (1998) compared the essential oils of leaves of *M. communis* from five Mediterranean regions. 47 compounds were detected by GC/MS. The major compounds include  $\alpha$  - pinene (35%-50%), limonene, 1,8-cineole and myrtenyl acetate. Ozek *et al.* (2000) analyzed the leaf essential oil of Turkish myrtle (*M. communis*) and the major compound was 1,8- cineole (18.2%). Asllani (2000) estimated the chemical composition of Albanian myrtle and over 100 compounds were detected. The main constituents were  $\alpha$  - pinene (11.4 - 22.5%), 1,8- cineole (13.8 - 21.8%), linalool (8.8 - 16.7%) and myrtenyl acetate (11.3 - 17.7%).

### *Pimenta*

Tucker and Maciarelo (1991) studied the essential oil components of *Pimenta dioica* of Jamaica and detected eugenol as the major compound. Bello *et al.* (1995) studied the phytochemistry of the leaf oil of *P. racemosa* of Western Cuba. Twenty six compounds were identified. The main constituents were 1,8- cineole (20.42%) and terpinen -4-ol (20.72%). Abaul *et al.* (1995) studied the chemical composition of the essential oils of three chemotypes of *Pimenta racemosa* var. *racemosa*. Major phenols like eugenol, chavicol and methyl eugenol were found to be present in two chemotypes, where as, acyclic oxygenated monoterpenes like geranial and neral were the major components in the third chemotype.



***Syncarpia* :**

Brophy *et al.* (1996) studied the leaf essential oils of three species of *Syncarpia*, all endemic to Eastern Australia. Yield of essential oils from fresh leaves of *S. glomulifera* ssp. *glomulifera*, *Syncarpia glomulifera* ssp. *glabra* and *S. verecunda* range from 0.12-0.4% and the major compound was  $\alpha$  - pinene (30-50%). *S. glomulifera* ssp. *glabra* also showed significant amounts of  $\alpha$  - thujene (11-27%). The major sesquiterpenes were aromadendrene (1-13%) and globulol (3-8%). Brophy *et al.* (1994) reported that the essential oil of *S. hilli* was dominated by hillayl acetate and hillone. These were accompanied by a range of mono and sesquiterpenes, usually in small amounts, of which the largest contributor was  $\alpha$  - pinene (2 - 22%).

***Rhodomyrtus* :**

The leaf essential oils of 7 members of the genus *Rhodomyrtus* were studied by Brophy *et al.* (1997b) and detected  $\alpha$  - pinene (20 - 23%),  $\beta$  - pinene (6 - 10%) and aromadendrene (12 - 17%) as the major components in *R. canescens*. In *R. effusa*, essential oil components were sesquiterpenes like globulol (11 - 22%), viridiflorol (8 - 10%) and spathulenol (15 - 18%). The major essential oil components in *R. macrocarpa* were  $\beta$  - caryophyllene (9 - 44%), aromadendrene (6 - 11%) and globulol (8 - 10%). The major components of *R. pervagata* were  $\alpha$  - pinene (27-35%) and  $\beta$  - pinene (18 -24%). In *R. psidioides*, monoterpenoids like  $\alpha$  - pinene (28 - 66%), and limonene (1 - 24% ) were found. *R. sericea* contained  $\alpha$  - pinene (28%),  $\beta$  - pinene (21%) and  $\beta$  - caryophyllene (13% ) as principal components. The oils of the two sub species of *R. trineura* were both essentially sesquiterpenoid in character. In *R. trineura* ssp. *trineura*, the major components were  $\beta$  - caryophyllene (16-29%), caryophyllene oxide, (2-12%) and globulol (7-10%) . While in *R. trineura* ssp. *capensis*,  $\alpha$  - pinene (26%), globulol (9-19%), viridiflorol (5-12%) and spathulenol (4-7%) were the principal compounds.

## *Eugenia*

The volatile oil of *Eugenia brasiliensis* leaves were analyzed by Vera *et al.* (1994). Seventy eight compounds were isolated. The main constituents are limonene (13.9%),  $\alpha$  - pinene (10.9%) 1,8- cineole (7.4%),  $\beta$  - pinene (6.1%) and linalool (6.0%). Dellacassa *et al.* (1997) studied the essential oil components of *E. uruguayensis* and 60 compounds were identified. The main compounds were limonene (17.6%), 1,8- cineole (17%),  $\alpha$  - pinene (10%) and caryophyllene oxide (8.3%). Pino *et al.* (2001b) analyzed the leaf oil of *E. cristata* and detected 35 compounds which constituted 99.7% of oil and the major components were limonene (45%),  $\gamma$  - terpinene (7.4%), linalool (6.2%), terpinen-4-ol (9.9%) and  $\alpha$  - terpineol (7.2%).

## *Syzygium*

Wong and Lai (1996) analyzed the volatile constituents from the fruits of four *Syzygium* spp. grown in Malaysia. They detected 43 compounds in *S. jambos*, 39 compounds in *S. samarangense* and 36 and 41 compounds in two cultivars of *S. malaccense* respectively. 3-phenyl-1-ol, (E) cinnamyl alcohol and other compounds with the C<sub>6</sub> - C<sub>3</sub> skeleton constituted about 60% of *S. jambos* volatiles. The volatiles of *S. samarangense* were characterized by the presence of large number of C<sub>9</sub> aldehydes as well as alcohols and those of *S. malaccense* was with very low terpenoid content. Djipa *et al.* (2000) correlates the tannin content present in *S. jambos* with antimicrobial activities.

Gopalakrishnan *et al.* (1988) detected the major essential oil components of *S. aromaticum* as eugenol, eugenyl acetate and  $\beta$  - caryophyllene. The percentage yield of clove bud was greater at a slightly immature stage than at the mature stage, but the clove yield was lower. It was suggested that oil from immature cloves may be suitable for perfumery and flavouring purposes but it was of less value for medicinal use. Gopalakrishnan and Narayanan (1988) analyzed the composition of clove (*Syzygium aromaticum*) leaf oil during leaf growth and

noticed that the content of caryophyllene ( 6.3 – 0.2 % )and the amount of eugenyl acetate (51.2 – 1.5%) decreased, where as, the eugenol content increased from 38.3 – 95.2%.

Menon and Narayanan (1992) studied the volatiles of dried clove buds and fresh green clove leaves and detected the chemical constituents. Apart from the aliphatic alcohols and monoterpene alcohols, eugenol, isoeugenol, farnesol and nerolidol were also identified.

Myint *et al.* (1996) studied the ethanolic extract of cloves (*S. aromaticum*), which was analyzed by gas chromatography directly and nine components were detected, of which the major compound was eugenol.

Khanna (1991) studied the physico-chemical constants and oil constituents of essential oil obtained from *Syzygium cumini*. The oil consisted of 59% hydrocarbons and 41% oxygenated derivatives. The major hydrocarbon derivatives were myrcene (14.62%),  $\beta$  – phellandrene (8.7%), terpinolene (7.1%),  $\gamma$  – terpinene (5.7%) and  $\beta$  – pinene (5.4%). The oxygenated derivatives included eugenol (7.8%),  $\alpha$  – terpineol (5.8%), cumin aldehyde (5.4%), methyl cinnamate (5.01%) and borneol (3.3%).

**Table: 2 PREVIOUS REPORTS ON THE BIOLOGICAL, MEDICINAL, PERFUMERY AND FLAVOURING ACTIVITIES OF SOME ESSENTIAL OIL COMPONENTS**

No.	Chemical Compounds	Activities	Reference
1.	$\alpha$ - thujene	Bioactive principle	Paramonov <i>et al.</i> , 2000. Usubillaga <i>et al.</i> , 2001. Jirovetz <i>et al.</i> , 2000.
2.	$\beta$ - thujene *		
3.	$\alpha$ - pinene	Anti-inflammatory Antiviral Bactericidal Flavour Herbicide, Larvicide Sedative, Tranquilizer Allelochemic, Allergenic Cancer preventive Insectifuge Irritant Antifungal, Antimicrobial Cytotoxic, Antitumourous	Sternberg and Duke, 1996. " " " " " " Mitchel and Rook, 1923. Stitt, 1990. Jacobson, 1990. Harborne and Baxter, 1983. Gallori <i>et al.</i> , 2001; Tzakou <i>et al.</i> , 2001; Singh <i>et al.</i> , 1988. Setzer <i>et al.</i> , 1999.
4.	Camphene	Insectifuge Flavour Spasmogenic Antioxidant	Jacobson, 1990. Sternberg and Duke, 1996. " "
5.	$\alpha$ - phellandrene	Dermal Irritant Perfumery Flavour Insectiphile	Harborne and Baxter, 1983. " " Sternberg and Duke, 1996. "

6.	$\beta$ - pinene	Allergenic Flavour Perfumery Herbicide Insectifuge Cytotoxic, Antitumourous	Mitchel and Rook, 1923. Sternberg and Duke, 1996. " Keeler and Tu, 1991. Jacobson, 1990. Setzer <i>et al.</i> , 1999. Bhattacharjee, 1998.
7.	Sabinene	Perfumery Bioactive principle	Sternberg and Duke, 1996. Paramonov <i>et al.</i> , 2000. Lis-Balchin and Roth, 2000.
8.	Myrcene	Allergenic Analgesic Antimutagenic Fungicidal Antioxidant	Mitchel and Rook, 1923. Kauderer <i>et al.</i> , 1991. " Keeler and Tu, 1991. Sternberg and Duke, 1996. Chaudhari <i>et al.</i> , 1989.
9.	$\beta$ - phellandrene	Antibacterial Expectorant Perfumery Insecticidal Antinemic	Senatore <i>et al.</i> , 2000. Harborne and Baxter, 1983. Sternberg and Duke, 1996. Ray <i>et al.</i> , 2000. "
10.	Citral	Allergenic Antiallergic Antihistamine Antishock Expectorant Cancer preventive Fungicidal	Mitchel and Rook, 1923. Huang, 1993. " " " Stitt, 1990. Sternberg and Duke, 1996.
11.	Cymene	Antimicrobial Antifungal	Gallori <i>et al.</i> , 2001. " Barnabas and Nagarajan, 1988.
12.	Linalool	Antifungal Insecticidal Anticancerous	Singh <i>et al.</i> , 2000. " Foray <i>et al.</i> , 1999.
13.	1, 8 - cineole	Antimicrobial Anti inflammatory Anticancerous Aromatic Repellent, Toxicant, Grain protectant Bactericidal, Fungicidal Nematicidal	Mazzanti <i>et al.</i> , 1998. Santos and Rao 2000. Foray <i>et al.</i> , 1999. Someya <i>et al.</i> , 2001. Ofori <i>et al.</i> , 1997. Saeed and Sabir, 1995. Chaudhari and Suri, 1991. Leela and Ramana, 2000.

14.	Limonene	Anticancer Antiflu Antiviral Enterocontractant Sedative Bioactive principle Antifungal Antimicrobial Cytotoxic-antitumourous	Sternberg and Duke, 1996. " " " Wagner and Wolff, 1977. Usubillaga <i>et al.</i> , 2001; Jirovetz <i>et al.</i> , 2000. Rao <i>et al.</i> , 2000. " Setzer <i>et al.</i> , 1999.
15.	Nerolidol	Aromatic Bioactive principle Bacteriostatic	Nagarajan <i>et al.</i> , 2001. " Ndounga <i>et al.</i> , 1991.
16.	Estragole	Antiaggregant Cancer preventive D.N.A. Binder Hypothermic Insectifuge Hepatocarcinogenic	Sternberg and Duke, 1996. Stitt, 1990. Harborne and Baxter, 1983. " Jacobson, 1990. Williamson and Evans, 1988.
17.	$\alpha$ - terpinene	Insectifuge Flavour Perfumery Antibacterial	Sternberg and Duke, 1996. " " Mosseiano <i>et al.</i> , 1995. Senatore <i>et al.</i> , 2000.
18.	Neral	Antifungal Insecticidal	Singh <i>et al.</i> , 2000. "
19.	$\alpha$ - terpinolene	Bioactive principle Antimicrobial	Jirovetz <i>et al.</i> , 2000. Carson and Riley, 1995.
20.	Eugenin	Inhibition to viral cell fusion, Viricidal Bioactive principle	Kim <i>et al.</i> , 2001. Masahiko <i>et al.</i> , 1998. Martin <i>et al.</i> , 1999.
21.	Linalyl acetate	Motor depressant Sedative Spasmolytic Flavour Perfumery Anticancerous	Sternberg and Duke, 1996. " " " " Foray <i>et al.</i> , 1999.
22.	$\beta$ - terpinene *		
23.	Neryl acetate	Antifungal	Kim <i>et al.</i> , 1995.
24.	Sabinyl acetate *		

25.	Methyl eugenin *		
26.	Citronellal	Allergenic Antiseptic Embryotoxic Insectifuge Flavour Bactericide Insecticidal	Mitchel and Rook, 1923. Wagner and Wolff, 1977. Sternberg and Duke, 1996. " " Spring, 1988. Bowers <i>et al.</i> , 2000.
27.	Citronellol	Allergenic Bactericide Candidicide Fungicide Flavour Perfumery Nematicide Herbicide Sedative	Mitchel and Rook, 1923. Spring, 1988. Sternberg and Duke, 1996. " " " " Keeler and Tu, 1991. Wagner and Wolff, 1977. Singh <i>et al.</i> , 1988.
28.	$\gamma$ - terpinene	Antioxidant Insectifuge Perfumery	Sternberg and Duke, 1996. " "
29.	Citronellyl acetate	Flavour	Sternberg and Duke, 1996.
30.	Methyl benzoate	Insecticidal	Miranda <i>et al.</i> , 1997.
31.	Terpinyl acetate	Bactericidal Flavour Perfumery Insectifuge	Sternberg and Duke, 1996. " " Jacobson, 1990.

32.	Borneol	Analgesic Antibronchitic Antiinflammatory Febrifuge Flavour Nematicidal Perfumery Spasmolytic Insectifuge Hepato- protectant Herbicide Antimicrobial Antifungal	Harborne and Baxter, 1983. " " Sternberg and Duke, 1996. " " " " " Jacobson, 1990. Lydon and Duke, 1989. " Faleiro <i>et al.</i> , 1999. Arambewela <i>et al.</i> , 1999.
33.	Methyl cinnamate	Insecticidal Nematicidal Perfumery Flavour	Sternberg and Duke, 1996. " " "
34	Carvacrol	Inhibitor Antidiuretic Antiinflammatory Antioxidant Antiseptic Bactericidal Carminative Nematicidal Vermifuge Allergenic Anaesthetic Anthelmintic Fungicidal	Sternberg and Duke, 1996. " " " " " " " " " " Mitchel and Rook, 1923. Harborne and Baxter, 1983. " "
35.	Citriodorol *		
36.	$\alpha$ - thujone	Insecticidal Larvicidal Anticancerous Insect repellent	Misra and Singh, 1986. " Foray <i>et al.</i> , 1999. Hwang <i>et al.</i> , 1985.
37.	Terpinen - 4 - ol	Antifungal Insecticidal	Singh <i>et al.</i> , 2000.
38.	$\beta$ - thujone	Insectifuge Anticancerous	Sternberg and Duke, 1996. Foray <i>et al.</i> , 1999.
39.	Globulol *		



40.	Chavicol	Fungicidal Nematicidal	Sternberg and Duke, 1996. "
41.	Eugenol	Antifungal Antibacterial, antifungal Allergic Nematicidal Antimitotic Antimutagenic Antitumour Anticonvulsant Cancer preventive Insectifuge Larvicide	Belmont and Carvajal, 1998. Aurora <i>et al.</i> , 1998. Frosch <i>et al.</i> , 1995. Leela and Ramana, 2001. Harborne and Baxter, 1983. Sternberg and Duke, 1996. " Pourgholami <i>et al.</i> , 1999. Stitt, 1990. Jacobson, 1990. Spring, 1988.
42.	Isoborneol	Insectifuge Motor stimulant Nematicidal Viricidal	Sternberg and Duke, 1996. " " Armaka <i>et al.</i> 1999.
43.	Methyl eugenol	Antibacterial, Antifungal	Aurora <i>et al.</i> , 1998, Kubo, 1993.
44.	Methyl chavicol	Hepato-carcinogenic Insecticidal Antibacterial, Antifungal	Bisset, 1994. Sternberg and Duke, 1996. Blewitt and Southwell, 2000; Garg, 2001, Aurora <i>et al.</i> , 1998.
45.	$\alpha$ - terpineol	Pediculocidal Scabicial Cytotoxic to human tumour cells	Oladimeji <i>et al.</i> , 2000. " Setzer <i>et al.</i> , 1999.
46.	$\beta$ - terpineol	Insectifuge Perfumery Antitumourous	Jacobson, 1990. Sternberg and Duke, 1996. Setzer <i>et al.</i> , 1999.
47.	Isoeugenol	Allergenic Antiaggregant Cancer preventive Motor depressant Motor stimulant Sedative Antioxidant Bacteriostat Antimicrobial	Mitchel and Rook, 1923. Laekeman <i>et al.</i> , 1990. Stitt, 1990. Sternberg and Duke, 1996. " " " Kang <i>et al.</i> , 1992. Ramanandraibe <i>et al.</i> , 2000.

48.	Bornyl acetate	Antifeedant Bactericidal Expectorant Flavour Insectifuge Sedative Spasmolytic Viricidal Insect repellent	Jacobson, 1990. Harborne and Baxter, 1983. " Sternberg and Duke, 1996. " " " " " Hwang <i>et al.</i> , 1985.
49.	Methyl isoeugenol	Anaesthetic Toxic Antihistaminic Herbicidal Bactericidal Expectorant Spasmolytic Cancer preventive Candidicidal Insect attractant Perfumery	Harborne and Baxter, 1983. " " " " " " Stitt, 1990. Sternberg and Duke, 1996. " "
50.	Iso bornyl acetate	Insectifuge Insect repellent	Sternberg and Duke, 1996. Hwang <i>et al.</i> , 1985.
51.	Eugenyl acetate	Antiaggregant Antiinflammatory Antispasmodic Spasmolytic	Sternberg and Duke, 1996. " " "
52.	Isoeugenyl acetate *		
53.	$\beta$ - elemene	Anticancer	Leeuwenberg, 1987.
54.	$\beta$ - bisabolene	Abortifacient Antirhinoviral Antiviral Antiulcer Antibacterial	Gen and Jong, 1991 . Denyer <i>et al.</i> , 1994 . " Yamahara, 1992 . Zhu <i>et al.</i> , 1999 .

55.	Aromadendrene	Antiseptic Cancer preventive	Harborne and Baxter, 1983. Stitt, 1990.
56.	$\beta$ – bourbonene *		
57.	$\alpha$ – selinene	Perfumery Bioactive	Sternberg and Duke, 1996. Palmeira <i>et al.</i> , 2001.
58.	Germacrene	Pheromonal	Sternberg and Duke, 1996.
59.	$\beta$ – caryophyllene	Antinemic Antibacterial	Srivastava <i>et al.</i> , 2000. Cobos <i>et al.</i> , 2001.
60.	$\alpha$ – humulene	Antitumour Perfumery	Zheng <i>et al.</i> , 1992. Harborne and Baxter, 1983.
61.	Piperitone	Antiasthmatic Herbicide Insectifuge	Lydon and Duke 1989. ” Bowers <i>et al.</i> , 1993.
62.	Piperitone oxide	Antifungal	Krishnamoorthy <i>et al.</i> , 2000.
63.	$\alpha$ – cadinene	Antifeedant	Jacobson, 1990.
64.	Farnesal	Allergenic Juvabional Nematicidal Perfumery Flavour	Mitchel and Rook, 1923. Russell 1986. Mitchel and Rook, 1923. ” ”
65.	$\gamma$ – selinene	Expectorant	Sternberg and Duke, 1996.
66.	$\beta$ – cadinene	Antifeedant	Jacobson, 1990.
67.	Isocaryophyllene	Bioactive principle Aromatic Perfumery	Nagarajan <i>et al.</i> , 2001. ” Harborne and Baxter, 1983.
68.	Bicyclogermacrene	Antifungal Antimicrobial	Gallori <i>et al.</i> , 2001. ”
69.	$\gamma$ – cadinene	Antifungal	Vila <i>et al.</i> , 2002.
70.	$\alpha$ – farnesene	Antimicrobial Antifungal	Arambewela <i>et al.</i> , 1999.

71.	Caryophyllene oxide	Antiedemic Anti inflammatory Antifeedent Insecticidal Antitumour Perfumery, Flavour	Shimizu, 1990. " Bettarini <i>et al.</i> , 1991. " Zheng <i>et al.</i> , 1992. Chawdhury and Kapoor, 2000. "
72.	$\beta$ -farnesene	Antimicrobial Antifungal Insect control	Arambewela <i>et al.</i> , 1999. Croteau <i>et al.</i> , 2001. "
73.	$\alpha$ -cadinol	Antifungal Insecticidal	Singh <i>et al.</i> , 2000. "
74.	$\alpha$ -farnesol *		
75.	$\beta$ -cadinol *		
76.	$\beta$ -farnesol *		
77.	$\gamma$ -cadinol *		

\* Previous reports not available.

## MATERIALS AND METHODS

Twenty six species belonging to sixteen genera of the family of Myrtaceae were collected from various parts of South India. They are deposited at the Herbarium of Botany Department, University of Calicut (CU No. 88001 to 880026; Table 1). Some specimens, viz. *Eucalyptus globulus*, *Leptospermum nicholsii*, *Corymbia citriodora* and *Melaleuca styphelioides* were authenticated by Dr. Lyn Craven, Principal Research Scientist, Australian National Herbarium, Centre of Plant Biodiversity Research, CSIRO Plant industry, Canberra, Australia. E- mail contacts were made with Researchers on Myrtaceae at Royal Botanical Gardens, Kew, U.K. for authentication and verification of binomials.

### Cytotoxic studies

Fresh leaves (100gms.) were collected and washed well to remove dirt. Moisture was removed by blotting with a cloth. Crude extracts were prepared by grinding the leaves with a mortar and pestle. From this crude extract 1%, 2% and 5% extracts were prepared in distilled water. Germinated bulbs of *Allium cepa* with large number of healthy roots (1-2 cm) were collected and washed thoroughly in distilled water at the time of peak mitotic activity (9 am to 10 am). The onion bulbs were kept at the rim of the bottle in which the extract is taken, in such a manner that the roots were completely immersed in the solution. A few root tips were cut from each sample at different time intervals like 2 hrs., 4 hrs., 6 hrs., 12 hrs. and 24 hrs. They were washed thoroughly with distilled water and immediately fixed in modified Carnoy's fluid (1 acetic acid : 2 alcohol) for 1 hour. After fixing, the root tips were transferred to 70% alcohol and kept under refrigeration.

Mitotic squash preparations were made with the help of improved techniques (Sharma and Sharma, 1990). The root tips were washed with distilled

water and hydrolyzed in 1N HCl for 1-2 minutes to separate the cells during squashing. The root tips were then washed thoroughly with distilled water and stained with 2% Acetocarmine for 3 hrs. After staining, the root tips were destained with 45% acetic acid, squashed and mounted on clean microslides.

All the slides were scanned under Quasmo Trinocular Pathological microscope and the photographs were taken with the Pentax camera system attached to the microscope.

The total number of cells showing divisions and the number of abnormal cells were counted for various phases from different microslides and the frequency of abnormal cells and the percentage of mitotic indices were calculated.

### **Extraction of Essential oil**

The leaves of each plant was cleaned and dried under shade. 50 gms. of each of the flaked and powdered material was hydrodistilled in a Clevenger Apparatus (Clevenger, 1928) at 100°C for 4 hours. The aromatic essential oils were collected and dried over anhydrous sodium sulphate. The pure oils were transferred into small amber coloured bottles and stored at 4 – 6 °C.

### **Gas Liquid Chromatography**

Gas liquid chromatographic analysis were carried out in Perkin Elmer HS - 40 Auto system Gas chromatograph, equipped with FID and connected with a chromatograph data processor PE Nelson 1022. Neat samples of the cooled essential oils were analyzed.

The GLC conditions used were as follows :

Column character: SS (Stainless Steel), SE-30 (Silicon E-30), solid phase; chemical in the column – 100% methyl silicon gum, Mesh size – 100/100, Column measurements: length 6 ft, Internal diameter: 2 mm Carrier gas: Nitrogen, Inlet pressure: 8 psi, Flow rate: 30 ml/minute. Temperature programme: from 80 °C (Initial temperature) to 220 °C (Final temperature) at a rate of 5 °C/minute. Injector temperature 200 °C and detector temperature 300 °C.

The percentage composition of the oil was computed from the GLC peak areas without using correction factor. The identity of the major components was assigned by comparing their GLC retention times with those of the standards, peak enrichment by co-injection with the standards and by comparison with literature data.

# RESULTS

## A. CYTOTOXIC EFFECTS

Cytotoxicity of plant extracts of various concentrations at different time intervals on *Allium cepa* root meristem were analyzed. Drastic cytotoxicity and mitotic inhibition were observed in all plant extracts, except two members. The extracts of plants, viz. *Rhodomyrtus tomentosa* and *Syzygium zeylanicum* in low concentrations and short duration of treatments showed slight stimulatory effects on the mitotic divisions. The cytotoxic effects of 26 specimens studied were tabulated (Table Nos. 4 – 29) and were compared with the control treatment in distilled water (Table 3).

The cytotoxic effects of all the plant extracts showed many clastogenic and nonclastogenic abnormalities. The major clastogenic abnormalities observed include nuclear lesions, chromosome stickiness, chromosome breakage and fragmentation, chromosome bridges, pycnosis and differential condensation of chromosomes. The nonclastogenic abnormalities detected were clumping of chromosomes, multipolarity, chromosome laggards, diagonal metaphase, anaphase and telophase, ball metaphase, scattered metaphase, sticky metaphase, anaphase and telophase, C-metaphase, binucleate, trinucleate, tetranucleate and polynucleate cells, polyploidy, misorientation of chromosome groups at metaphase and anaphase, early movement of chromosomes, nonsynchronised movement of chromosomes and disturbed metaphase and anaphase. The most frequent abnormalities observed were nuclear lesions, chromosome stickiness, binucleate and tetranucleate cells, diagonal orientation of chromosomes at metaphase, anaphase and telophase and micronuclei formation.



Mitotic indices in the various treatments with the myrtaceous plant extracts were found to be less than that of the control, except the earlier mentioned two leaf extracts (Table 28 and 29). In all other treatments mitotic indices showed an inverse relationship with an increase in the concentration of the extracts. The frequency of abnormalities was found to increase with the concentration of the extract and with the duration of treatment.

Among the 26 plants analyzed, the leaf extracts of *Callistemon citrinus*, *C. viminalis*, *Feijoa sellowiana*, *Psidium guajava*, *Syzygium cumini*, *S. jambos*, *S. malaccense* and *S. samarangense* showed mitotic divisions during all the five treatments, i.e. 2hr., 4hr., 6hr., 12hr. & 24hr., (Table Nos. 6, 7, 19, 22, 24, 25, 26 and 27). In cytotoxic assays with leaf extracts of *Agonis flexuosa*, *Eugenia uniflora* and *Myrtus communis*, divisional stages were observed only up to 12hr. Where as in 24 hr. treatment with these extracts, the root tip cells decayed and blackened (Table Nos. 4, 18 and 20). Treatment with the extracts of *Corymbia citriodora*, *Eucalyptus globulus*, *Leptospermum nicholsii*, *Acmena smithii*, *Eugenia apiculata*, *Syzygium aromaticum*, *Syzygium zeylanicum* and *Rhodomyrtus tomentosa* (Table Nos. 8, 10, 12, 16, 17, 23, 28 and 29) showed mitotic division stages only up to 6hr. treatments. After 12 hr. and 24 hr. treatment with these extracts the root tip cells were completely blackened and damaged. Treatment with the leaf extract of *Beaufortia sparsa*, *Corymbia ficifolia*, *Eucalyptus tereticornis*, *Melaleuca leucadendron*, *M. styphelioides*, *Syncarpia glomulifera* and *Pimenta dioica* showed division stages only up to 4 hr. treatment (Table Nos. 5, 9, 11, 13,14, 15 and 21). The other treatments viz. 6hr., 12hr., and 24hr., with these extracts showed complete damage of the root tip meristems.

The details of the results of cytotoxic studies conducted, under the two subfamilies of Myrtaceae – viz. *Leptospermoideae* and *Myrtoideae* were depicted separately.

## Subfamily: Leptospermoideae

### 1. *Agonis flexuosa*

The leaf extract of *Agonis flexuosa* showed mitotic divisions only up to 12hr. treatment, where as the 24 hr. treatment showed blackening and damage of root tip meristem of *Allium cepa*. The mitotic indices showed a gradual decrease with the increase in the concentration and increase in the time of treatment. The percentage of abnormal cells were found to be maximum in 5% extract during 2hr. treatment. The abnormal cells during the prophase stage was most frequent during short duration treatments *ie.* 2hr. and 4hr. Fragmentation of chromosomes, diagonal anaphase and sticky telophase were observed in all the treatments. C- metaphase cells were found to be more in low concentration treatments. Micronuclei formation were observed in majority of the treatments. The other cytotoxic effects like stickiness during metaphase and anaphase, clumping of chromosomes during metaphase, bridge formation, diagonal orientation at metaphase, disturbed metaphase, misorientation of chromosomes during anaphase and laggards were also found in various treatments. The 1% extract on 2hr. treatment showed a maximum mitotic index of 5.54% where as in 5% extract on 12hr. treatment it was reduced to a minimum, 0.47%. The frequency of abnormal cells ranges from 2.31% to 0.47% (Table 4).

### 2. *Beaufortia sparsa*

The leaf extract of *Beaufortia sparsa* showed mitotic divisions in *A. cepa* only up to 4hr. treatment. The 6hr., 12hr., and 24 hr., treatments showed blackening and complete damage of the root tip meristem. The maximum mitotic index, 2.27% was observed in 1% extract after 2hr. treatment. In 5% extract after 4hr. treatment it was reduced to 0.14%. Abnormalities in the prophase stage were found to be the most frequent anomaly observed in all treatments. The percentage of abnormalities range from 1.33% (1%, 2hr.) to 0.14% (5%, 4hr.). Stickiness and diagonal orientation of chromosomes during metaphase and anaphase, metaphase

clumping, chromosome bridges and sticky telophase were the common cytotoxic effects observed in all treatments. Fragmentation of chromosomes were observed only in higher concentration (5%, 4hr.) treatment (Table 5).

### 3. *Callistemon citrinus*

The leaf extract of *Callistemon citrinus* showed mitotic divisions in the meristematic cells of onion root tip in all the five treatments. The percentage of mitotic indices range from 5.84% (in 1%, 2hr.) to 1.44% (in 5%, 24 hr.). The maximum percentage of abnormality (3.69%) was observed in 2% extract after 4hr. treatment. Where as the minimum anomalies (1.44%) were observed in 5% extract during 24 hr. treatment. Nuclear lesions during prophase were the most prominent anomaly observed in all treatments. The most frequent aberrations include stickiness, metaphase clumping and diagonal orientation of chromosomes during metaphase, anaphase and telophase. Ball metaphase formations were quiet frequent during all treatments, but it was found to be maximum during 6hr. treatment. Metaphase disturbances, bridge formation, fragmentation of chromosomes and misorientation of chromosomes were the other cytotoxic effects observed on experimentation with the extracts of *C. citrinus*. No abnormal telophase cells were observed during 4hr. treatment, but they were found in all other treatments with the maximum effects during 6hr. duration (Table 6).

### 4. *Callistemon viminalis*

The leaf extract of *Callistemon viminalis* induced cytotoxic effects in *A. cepa* on all the five treatments. The mitotic index was found to be maximum in 2% extract after 2hr. treatment (7.79%). The lowest mitotic index value (0.41%) was found in higher concentration treatment (5%, 24hr.). The frequency of abnormal cells was found to be maximum during 6hr. treatments and minimum during 24hr. treatments. Nuclear lesions were found to be very prominent during prophase in all treatments. The other cytotoxic effects include disturbances

in metaphase, C- metaphase, stickiness, clumping, diagonal orientation, fragmentation, misorientation and bridge formation (Table 7).

### **5. *Corymbia citriodora***

The leaf extract of *Corymbia citriodora* showed cell division stages in *A. cepa* only up to the treatment of 6hr. duration. No divisional stages were observed in 12 hr. and 24 hr. treatments as the root tip cells get fully blackened and damaged. The mitotic index was reduced to 5.25% in the low concentration treatment (1%, 2hr.) and it was only 0.06% in higher concentration (5%, 6hr.). This showed a gradual decrease with increase in concentration and time duration. The frequency of occurrence of abnormal cells progressively increased from short duration to long duration and increase in the concentration of the extracts. A maximum of 45.55% abnormality was observed in the 5% extract after 6hr. treatment. Nuclear lesions were found to be quite frequent in all treatments, maximum in 5% extract during 6hr. treatment. C- metaphase and polyploid cells were found during 2hr. and 4hr. treatments. The frequent anomalies include stickiness, clumping, diagonal orientation, bridge formation, fragmentation, misorientation of chromosomes and disturbed metaphase. Abnormal cells in telophase were found to be lesser when compared to that in prophase (Table 8).

### **6. *Corymbia ficifolia***

The leaf extract of *Corymbia ficifolia* showed the maximum cytotoxic effects, as no division stages were observed in 5% extract during 4hr. treatment. In 6hr., 12hr., and 24 hr., treatments the root tips turn black and was fully damaged. The mitotic index even in low concentration and short duration treatment (1%, 2hr.) was 1.29%. The mitotic index was only 0.06% in 2% extract after 4hr. treatment. Clumping and diagonal orientation of chromosomes were observed during metaphase. Some abnormalities in prophase and telophase have also been found in most of the treatments. The most frequent cytotoxic effects were found to be nuclear lesions and abnormal nuclei during interphase.

Since all the cells in the interphase stage get damaged, the frequency of abnormal cells was increased to 91.10 % during 4hr. treatment with 5% extract( Table 9).

### 7. *Eucalyptus globulus*

The leaf extract of *Eucalyptus globulus* showed mitotic divisions in onion root tip meristem only up to the 6hr. treatment. The mitotic index was reduced to 2.63% in the low concentration treatment (1%, 2hr.). Where as, it was 0.08% in higher concentration treatment (5%, 6hr.). The frequency of abnormal cells range from 0.96% (in 2% extract after 2hr. treatment) to 0.08% ( in 5% extract after 6 hr. treatment). C- metaphase and micronuclei were found during the 4hr. treatment only. Other cytotoxic effects include stickiness, clumping, bridge formation, diagonal orientation, fragmentation, misorientation and disturbed metaphase. Prophase abnormalities were found to be higher than that in other divisional stages (Table 10).

### 8. *Eucalyptus tereticornis*

The treatments with the leaf extract of *Eucalyptus tereticornis* showed mitotic divisions in *A. cepa*, only up to 4hr. treatment. No division stages were observed after 4hr. treatment because the root tip cells showed severe damages.

The mitotic index was reduced to 0.78% in the lowest concentration experiment, ie. 1%, 2hr. Then it shows a gradual decrease to 0.14% in 5% extract after 4hr. treatment. The frequency of abnormal cells were found to be lesser, when compared with other plant extracts. It ranges from 0.58% to 0.14%. Micronuclei formation were observed only in 1% and 2% extracts after 2hr. treatment. Other cytotoxic effects noticed include stickiness, clumping, bridge formation, disturbed condition, fragmentation, misorientation and diagonal orientation of chromosomes (Table 11).

### 9. *Leptospermum nicholsii*

The leaf extract of *Leptospermum nicholsii* showed the mitotic division stages in the root tip meristem of *A. cepa* only up to 6hr. treatment. The root tip cells during 12 hr. and 24 hr. treatments get fully blackened and damaged. The mitotic index was observed to its maximum in 1% extract after 2hr. treatment, i.e. 3.68%. This shows retrogression to 0.18% in 5% extract after 6hr. treatment. The percentage of abnormalities range from 1.29% (in 5% extract after 2hr. treatment) to 0.18% (in 5% extract during 6 hr. treatment). C- metaphase cells were observed only during the short duration treatments. Micronuclei were found in all treatments of 6 hr. duration and in 5% extract after 4hr. treatment. The abnormalities of prophase cells were found to be the maximum during 4hr. treatment. Other frequent cytotoxic effects include stickiness, clumping, pulverization, diagonal orientation, disturbed condition, bridge formation, fragmentation and misorientation (Table 12).

### 10. *Melaleuca leucadendron*

The leaf extract of *Melaleuca leucadendron* showed the mitotic division stages in the root tip meristem of onion, only up to 4 hr. treatment. The root tip cells beyond 4hr. treatments showed severe damages and blackening. The mitotic index was reduced to 1.88% even in the 1% extract after 2 hr. Then it showed a gradual decrease to 0.52% in 5% extract after 4hr. The frequency of abnormal cells also showed a progressive increase from low concentration to high concentration treatments. Chromosome bridges were observed only in 1% extract. Only one micronuclei stage was found in 1% extract during 2hr. treatment. Fragmentation of chromosomes during anaphase, anomalies in prophase and telophase etc. were found in all treatments. Nuclear lesions were observed in all treatments and it showed an increase in frequency with respect to the increase in concentration and duration of time. The other cytotoxic effects include stickiness, diagonal orientation, clumping, misorientation, disturbed condition, ring chromosome, etc. (Table 13).

### 11. *Melaleuca styphelioides*

The cytotoxic experiments done with *Melaleuca styphelioides* leaf extract showed no mitotic division stages in *A. cepa* beyond 4hr. treatment. The root tip cells showed complete damage and blackening in the treatments with 6hr., 12hr. & 24hr. duration. The maximum percentage of mitotic division were observed in 1% extract after 2hr. (ie. 3.08%), where as the minimum in 5% extract after 4hr.(ie. 0.55%). The frequency of abnormal cells progressively increased from 5.43% ( 1% extract after 2hr.) to 23.88% (5% extract after 4hr.). Ball metaphase cells were observed in most of the treatments. The number of interphase nuclear lesions and abnormal cells progressively increased with respect to the increase in concentration and increase in duration of treatment. Stathmoanaphase, chromosome clumping, pulverization, disturbed condition, stickiness, bridges and micronuclei were observed in all treatments. Other major cytotoxic effects include diagonal orientation, misorientation and fragmentation of chromosomes. Abnormal cells in prophase were scored in all treatments, where as, abnormal cells in telophase were scored in majority of treatments(Table 14).

### 12. *Syncarpia glomulifera*

The leaf extract of *Syncarpia glomulifera* showed mitotic division stages in *A. cepa* root meristem only up to 4 hr. treatment. The maximum percentage of mitotic index was observed in 1% extract after 2hr. ie. 2.88%. This showed a retrogression to 0.18% in 5% extract after 4hr. The frequency of abnormal cells increase gradually with an increase in concentration as well as time duration from 1.63% to 15.51%. Polyploid cells were found in the treatments with 5% extract after 2hr. and also in 1% and 2% extracts after 4hr. treatments. The abnormalities in the prophase stage were found to be more when compared with other divisional stages. The other cytotoxic effects include disturbed metaphase, fragmentation in anaphase , misoriented anaphase, sticky telophase and pulverization of chromosomes. Other anomalies like nuclear lesions and

abnormalities like binucleate, trinucleate and tetranucleate cells in interphase were also showed a progressive increase with respect to the increase in concentration of extract and duration of treatment (Table 15).

## **Subfamily II Myrtoideae**

### **13. *Acmena smithii***

The leaf extract of *Acmena smithii* showed mitotic divisions in *A. cepa* root tip cells only upto 6hr treatment. No mitotic divisions were observed in 12hr. and 24hr. treatments as the root tip cells showed severe damages. The percentage of mitotic indices showed a retrogression from lower concentration and short duration treatments to higher concentration and long duration experiments. The maximum mitotic index was noticed in 1% extract after 2hr. (4.91%) and the minimum in 5% extract after 6hr. treatment (0.34%). The frequency of abnormal cells was found to be maximum in 2% extract after 4hr. treatment. Metaphase pulverization was found in 1% extract during 4hr. treatment only. Ball metaphases were frequently found in low concentration treatments. The scattering of metaphase chromosomes was a common anomaly in majority of experiments. Clumping and diagonal orientation were the other aberrations observed in metaphase stage. Early movement of chromosomes, sticky anaphase, bridge formation, non-synchronized movements of chromosomes and diagonal orientation were observed in anaphase stages. The telophase stages also showed bridges and stickiness of chromosomes. Abnormal cells in prophase stage were found to be of common occurrence in all treatments (Table 16).

### **14. *Eugenia apiculata***

The experiments done with the leaf extract of *Eugenia apiculata* showed mitotic divisions in *Allium cepa* root tip meristem only upto 6hr. treatment. The root tip cells during 12hr. and 24hr. treatments showed severe damages. The maximum percentage of mitotic index was noticed in 1% extract after 2hr.



treatment (*i.e.* 1.56%). This showed a gradual reduction to a minimum of 0.14% in 5% extract after 6hr. The frequency of aberrations range from 0.14% to 0.74%, the lower value in 5% extract after 6hr. treatment and higher value in 2% extract after 2hr. Chromosome pulverization, ball metaphase, sticky anaphase and bridge formation were the frequent cytotoxic effects noticed. Scattering of chromosomes, chromosome laggards and early movement of chromosomes have also been encountered. Abnormal cells in prophase stage were scored in all treatments. Micronuclei formation were found in all treatments of 2hr. and 4hr. durations (Table 17).

### 15. *Eugenia uniflora*

The experiments done with the leaf extract of *Eugenia uniflora* showed mitotic divisions in the onion root tip meristem only up to 12hr. treatments. The root tip cells of *A. cepa* in 24hrs. treatment showed severe damages. The maximum mitotic index (4.8%) was seen in the treatment with 1% extract for 2hr. and it decreased with respect to increase in concentrations and time duration. The minimum was found (0.08%) in 5% extract after 12hr. The percentage of abnormalities range from 1.54% to 0.08%, the maximum value being observed in 2% extract after 4hr. treatment and minimum in 5% extract after 12hr. The abnormality of prophase cells were found frequently in 2hr. and 4hr. treatments. Metaphase pulverization, clumping, ball metaphases, diagonal and scattered metaphases have also been analyzed in some treatments. Anomalies scored in anaphase include early movement of chromosomes, stickiness, non-synchronization, diagonal arrangement and bridge formation. Abnormality in telophase, *viz.* formation of bridges, stickiness and micronuclei formation were observed in some treatments (Table 18).

### 16. *Feijoa sellowiana*

The leaf extracts of *Feijoa sellowiana* induced cytotoxic effects in *A. cepa* root meristem in all the experiments *i.e.* up to 24hr. duration. The maximum

mitotic index was noticed in 1% extract after 2hr. treatment *i.e.* 14.27% . The minimum (0.28%) was observed in the case of 5% extract after 24hr. treatment. The frequency of abnormalities range from 2.83% to 0.28% . Scattered chromosomes during the metaphase stage was the common abnormality found in the low concentration and short duration treatments. Ball formation was observed in majority of the treatments. Where as pulverization, bridge formation, early movement of chromosomes stickiness, clumping, diagonal orientation and non-synchronized movement of chromosomes occur less frequently in the treatments. Prophase stage shows predominance of anomalies when compared with other divisional stages (Table 19).

#### 17. *Myrtus communis*

The experiments conducted with the leaf extract of *Myrtus communis* on *A. cepa* root meristem showed division stages only up to 12hr. duration. The root tips dipped for 24hrs. in leaf extracts for all concentrations showed severe damages. The maximum mitotic index (4%) was noticed in the 1% extract after 2hr. treatment. The percentage of mitotic indices showed a gradual decrease with respect to the increase in concentration and duration of treatment. The least was observed (0.71%) in the experiment done with 5% extract after 12hrs. The percentage of abnormality was found to be lower in all treatments when compared with other plants ranging from 0.32% to 0.83% .

Clumping, pulverization, scattering of chromosomes, stickiness, early movement and non-synchronized movement of chromosomes were the usual cytotoxic effects observed in the treatments. Diagonal anaphases were observed in higher concentration and long duration treatments. Bridges and star anaphases were not frequently observed in these experiments. Prophase anomalies were found to be lesser, when compared with the extract of other plants (Table 20).

#### 18. *Pimenta dioica*

The leaf extracts of *Pimenta dioica* showed mitotic divisions in *A. cepa* only up to 4hrs. in the experiments. The root tip cells get completely damaged after

6hr., 12hr. and 24hr. treatments. The maximum percentage of mitotic index (1.54%) and abnormality (0.91%) was noticed in 1% extract during 2hr. treatment and minimum was 0.16% on both parameters in 5% extract after 4hr. treatment. The anomalies like pulverization, clumping, ball formation, early movement of chromosomes, stickiness and scattering of chromosomes were observed. Stathmoanaphase, ring chromosome formation, star anaphase and diagonal orientation of chromosomes were found to be rare in these treatments. Nuclear lesions were found to be the most prominent prophase anomaly (Table 21).

### 19. *Psidium guajava*

The leaf extracts of *Psidium guajava* affected mitotic division in all the treatments *i.e.* upto 24hr. duration in all the concentrations. The maximum percentage of mitotic index was reduced to 3.91%, which was observed in the lowest concentration, *i.e.* 1% extract after 2hrs. The least was 0.16% in the highest concentration *i.e.* 5% extract after 24hrs. The frequency of abnormalities was found to be maximum (1.25%) in 1% extract after 4hr. treatment and minimum (0.16%) in 5% extract after 24hrs.

Pulverization, ball metaphase, scattered metaphase, stickiness, clumping, bridge formation and early movement of chromosomes during anaphase were found in most of the treatments. Normal division stages were not frequent beyond the 4hr. treatment. Diagonal orientation, non-synchronized movement of chromosomes and micronuclei were also scored in some treatments. Prophase anomalies were found to be predominant in short duration experiments (Table 22).

### 20. *Syzygium aromaticum*

The leaf extract of *Syzygium aromaticum* showed mitotic divisions in *A. cepa* only up to 6hr. in the experiments. No divisional stages were found beyond this duration, as the root tip meristems get severely damaged. The maximum percentage of mitotic index (4.29%) was observed in low concentration and short duration treatment *i.e.* 1% 2hr. and the minimum (0.13%) was noticed in 5%

extract during 6hr. treatment. The mitotic indices showed a decrease with respect to the increase in concentration of the extract. The percentage of anomalies range from 1.51% (in 1% extract after 4hr. treatment) up to 0.13% in (5% extracts during 6hr. treatment). Cytotoxic effects like prophase anomalies, chromosome pulverization, clumping, ball metaphase, diagonal metaphase and scattered metaphase were found to be in maximum frequency in 4hr. treatment than the 2hr. and the 6hr. treatments. The anaphasic irregularities observed include stickiness, bridge, non-synchronized movements, diagonal orientation and early movement of chromosomes. Sticky telophases were found only in 1% and 2% treatments for 2hrs. and 2% and 5% treatments for 4hrs. Micronuclei formations were observed in most of the treatments (Table 23).

### 21. *Syzygium cumini*

The leaf extracts of *Syzygium cumini* induced mitotic aberrations in all the five duration treatments and the percentage of mitotic index showed a gradual decrease. The maximum mitotic index was 8.41 in 1% extract after 2hr. treatment, whereas, the lowest is only 0.09% in 5% extract after 24hrs. The frequency of abnormal cells was found to be maximum (3.2%) in 1% extract after 4hr. treatment and minimum (0.09%) in 5% extract during 24hr. treatment. Prophase anomalies seem to be very much pronounced. Pulverization, clumping and scattering of chromosomes, stickiness, ball metaphase, bridges, early movement of chromosomes and diagonal orientation were the common cytotoxic effects observed in most of the treatments. Micronuclei were found only in higher concentrations of 4hr. and 6hr. treatments (Table 24).

### 22. *Syzygium jambos*

The extract of *Syzygium jambos* induced cytotoxic effects in *A. cepa* in all the different duration treatments. The maximum mitotic index was observed in 1% 2hr. (i.e. 10.11%) and the minimum was in the experiment with 5% extract

after 24hr. (*i.e.* 0.35%). The percentage of abnormality was found to be maximum (3.48%) in 1% extract after 6hr. treatment and minimum (0.35%) in 5% extract during 24hr. treatment.

Pulverization, clumping, ball formation and scattered metaphase, stickiness, early movement of chromosomes, non-synchronized movement, anaphase bridges and diagonal orientation were the commonly found cytotoxic effects in all the treatments. Prophase aberrations were found to be a unique feature in all the treatments (Table 25).

### 23. *Syzygium malaccense*

The experiments conducted with the leaf extracts of *Syzygium malaccense* showed divisional stages in *A. cepa* root meristem in all the treatments. The maximum mitotic index (7.71%) was noticed in 1% extract after 2hr. treatment and the minimum (0.49%) was in the 5% extract during 24hr. treatment. The mitotic index showed a gradual decrease with respect to increase in concentration and time of treatment. The maximum frequency of abnormal cells (1.31%) were observed in 2% extract after 6hr. treatment and minimum (0.33%) in 1% extract after 2hr. treatment.

Pulverization, clumping, ball metaphase and diagonal orientation of chromosomes, early movement of chromosomes, stickiness, scattered metaphase and bridge formation were the most frequent cytotoxic effects observed. Prophase anomalies occur commonly in all the treatments. Non-synchronized movement of chromosomes at anaphase was also scored in some treatments (Table 26).

### 24. *Syzygium samarangense*

The leaf extracts of *Syzygium samarangense* induced cytotoxic effects in *A. cepa* in all the treatments and the percentage of mitotic indices showed a gradual decrease from lower concentration to higher concentration. The maximum mitotic index was 8.80% in 1%, 2hr. treatment and minimum was 2.57% in 5%

24hr. treatment. The percentage of abnormality was found to be maximum (3.88%) in 1% extract after 6hr. treatment and minimum (2.47%) in 5% extract after 24 hr. treatment.

Pulverization, clumping, ball metaphase and scattered chromosomes during metaphase, early movement, stickiness, bridge formation, non-synchronized movement, diagonal orientation and micronuclei formation were the common cytotoxic effects observed. Micronuclei formation was frequently found in 4hr., 6hr., 12hr. and 24hr. treatments in all the experiments. Prophase anomalies were found to be very severe in all treatments when compared with that of other plants (Table 27).

## 25. *Syzygium zeylanicum*

The leaf extracts of *Syzygium zeylanicum* showed division stages in *A. cepa* only up to 6hr. treatment. The root tips after 12hr. and 24hr. treatments showed blackening and severe damages. In the short duration treatments the mitotic indices showed a slight increase with respect to the control treatments. Such an effect was noticed to the maximum in the case of 5% 4hr. treatment, where the mitotic index was increased to 19.84% (Table 28), when the control was only 13.79% (Table 3). A similar effect was detected in 5% 2hr. treatment also *i.e.* 18.76% . In 1% and 2% treatments after 2hrs. and 4hrs. also showed a slight stimulatory effect than the control experiments. In the 6hr. duration treatments the root tips showed a gradual decrease in the mitotic indices when compared to the control. The frequency of abnormality was found to be very less when compared to that in other plants. The percentage of anomalies ranges from 0.93% to 4.26% .

Eventhough the mitotic stimulatory effects were observed in short duration treatments, chromosome pulverization and clumping were noticed during metaphase. Early movement of chromosomes was noticed during anaphase. Polyploid cells were found quiet frequently during many of the treatments. Treatments with all these concentrations showed pulverization, clumping, ball metaphase, C-metaphase, diagonal orientation and scattering during metaphase.

Early movement, non-synchronized movement of chromosomes, diagonal orientation during anaphase and stickiness during anaphase and telophase stages were also observed. Anaphase bridges were observed in only one treatment. Prophase anomalies increase with an increase in concentration and time duration of treatments (Table 28).

#### **26. *Rhodomyrtus tomentosa***

The leaf extracts of *Rhodomyrtus tomentosa* showed a stimulatory effect of mitotic divisions in *A. cepa* during low concentration treatments for short duration. 21.09% mitotic index was found in 1% extract after 2hr. treatment. Similar effects were observed in 2% and 5% treatments. 4hr. treatments also showed a slight stimulatory effect. The mitotic indices showed a decrease than the control in 6hr. treatments. No division stages were found after 6hr. *i.e.* 12hr. and 24hr. treatments showed complete damages of root tip cells. The frequency of abnormality was found to be maximum (5.55%) in 5% extract after 4hr. treatment and the minimum (0.99%) in 1% extract after 2hr. C-metaphase stages were found in short duration and low concentration treatments. Pulverization was frequently found in 4hr. and 6hr. treatments. Scattering of chromosomes during metaphase and early movement of chromosomes during anaphase were the other common cytotoxic effects observed in all treatments. Stickiness of chromosomes during telophase was found to be common in long duration treatments. Drastic anomalies occur in prophase stages in all the treatments (Table 29).



Time	Total Cells	Prophase	Metaphase	Anaphase	Telophase	Mitotic Index(%)
2hr.	3125	95	132	128	118	15.14
4hr.	3075	86	98	102	138	13.79
6hr.	3216	198	22	18	134	11.57
12hr.	3327	188	16	20	92	9.50
24hr.	3215	60	58	63	64	7.62

**Table 3** Consolidated data of mitotic divisional stages of *Allium cepa* root tip cells and mitotic index(%) in various control treatments (Distilled water).

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase						Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Sticky	Clumping	Diagonal	C-Metaphase	Disturbed	Normal	Sticky	Diagonal	Bridge	Fragmentation	Misorientation	Lagards	Normal	Sticky			Micronuclei
1%2hr.	2728	40	20	18	2	4	1	2	1	24	1	1	1	3	1	2	21	9	-	1.76	5.54
2%2hr.	2794	32	27	10	3	2	-	1	2	13	2	3	1	3	2	1	13	7	2	2.04	4.48
5%2hr.	2812	24	30	6	2	5	-	-	3	5	3	1	2	5	2	3	8	8	1	2.31	3.84
1%4hr.	2932	22	28	4	1	2	-	1	2	3	2	2	1	6	1	-	5	7	1	1.84	3.00
2%4hr.	2878	16	29	4	1	3	1	2	3	3	1	1	1	4	2	1	3	8	2	2.05	2.95
5%4hr.	2892	10	27	3	2	4	-	-	2	3	2	1	1	5	1	2	3	11	1	2.01	2.66
1%6hr.	3018	14	16	2	3	3	-	2	1	3	1	2	1	7	2	1	1	7	2	1.56	2.22
2%6hr.	2992	8	17	-	1	2	-	1	-	1	-	1	1	7	2	1	1	4	1	1.14	1.47
5%6hr.	3008	4	11	-	1	1	1	1	-	-	-	2	-	4	2	-	-	3	2	0.93	1.06
1%12hr.	3059	4	12	-	2	3	-	1	2	-	1	1	-	3	1	2	-	3	1	0.98	1.11
2%12hr.	2918	1	6	-	1	3	-	1	3	-	1	-	3	1	2	-	-	2	-	0.79	0.82
5%12hr.	2955	-	4	-	-	-	-	-	3	-	-	1	1	2	-	-	-	1	2	0.47	0.47

Table 4 Consolidated data of cytotoxicity of leaf extract of *Agonis flexuosa*

Treatment (%) and time	Total cells counted	Prophase		Metaphase				Anaphase					Telophase		Frequency of abnormal cells (%)	Mitotic Index (%)
		Normal	Abnormal	Normal	Sticky	Clumping	Diagonal	Normal	Sticky	Diagonal	Bridge	Fragmentation	Normal	Sticky		
1%2hr.	2114	13	11	3	2	4	2	2	3	2	1	-	2	3	1.33	2.27
2%2hr.	2273	14	8	1	-	5	-	-	2	-	3	-	-	4	0.97	1.63
5%2hr.	2316	6	9	1	-	-	1	-	1	-	-	-	-	1	0.52	0.82
1%4hr.	1973	-	4	-	-	-	2	-	1	-	2	-	-	1	0.51	0.56
2%4hr.	2376	-	3	-	-	1	2	-	-	-	1	-	-	1	0.34	0.34
5%4hr.	2119	-	2	-	-	-	-	-	-	-	-	1	-	-	0.14	0.14

**Table 5 Consolidated data of cytotoxicity of leaf extract of *Beaufortia sparsa***

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Sticky	Clumping	Diagonal	Ball metaphase	Disturbed	Normal	Sticky	Diagonal	Bridge	Fragmentation	Misorientation	Normal	Sticky			Diagonal
1%2hr.	1985	35	28	16	4	2	-	-	3	12	3	4	1	-	1	4	2	1	2.47	5.84
2%2hr.	2138	31	23	20	7	3	1	1	2	10	5	6	-	3	-	6	2	3	2.62	5.75
5%2hr.	2297	28	34	14	6	4	2	1	4	11	7	2	-	4	3	5	2	2	3.09	5.62
1%4hr.	2389	23	41	8	12	6	3	-	7	5	4	3	5	2	3	8	-	-	3.60	5.34
2%4hr.	2790	18	45	11	14	8	5	2	8	8	7	4	2	4	4	10	-	-	3.69	5.38
5%4hr.	2889	19	25	6	17	5	4	5	10	4	5	5	1	3	4	13	-	-	2.91	4.36
1%6hr.	2245	12	26	4	3	2	8	4	12	5	2	8	2	4	2	3	4	4	3.61	4.68
2%6hr.	2183	8	32	3	5	4	6	2	3	3	-	5	-	3	1	4	4	3	3.12	3.94
5%6hr.	2397	9	29	2	8	2	3	4	2	6	1	4	2	3	3	4	4	4	2.88	3.76
1%12hr.	2163	5	15	3	1	1	3	1	5	2	1	1	3	4	3	2	3	3	2.03	2.59
2%12hr.	2479	4	18	2	4	3	2	2	6	3	1	1	4	2	4	1	4	4	2.22	2.62
5%12hr.	1867	2	13	1	3	4	3	4	3	1	-	4	2	4	2	1	1	1	2.36	2.63
1%24hr.	2439	2	11	-	2	3	1	-	4	-	2	5	3	2	1	-	3	2	1.60	1.68
2%24hr.	1993	-	8	-	4	-	2	1	3	-	1	-	4	3	2	-	2	2	1.61	1.61
5%24hr.	2291	-	10	-	5	1	2	1	5	-	2	-	2	1	1	-	2	1	1.44	1.44

Table 6 Consolidated data of cytotoxicity of leaf extract of *Callistemon citrinus*

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase						Telo-phase		Frequency of Abnormal cells (%)	Mitotic Index (%)
		Normal	Abnormal	Normal	Sticky	Clumping	Diagonal	C-metaphase	Disturbed	Normal	Sticky	Diagonal	Bridge	Fragmentation	Misorientation	Normal	Sticky		
1%2hr.	3358	120	34	32	1	-	-	-	-	36	1	-	-	-	-	28	-	1.07	7.51
2%2hr.	3413	133	38	45	1	2	1	-	-	19	3	-	-	-	-	23	1	1.35	7.79
5%2hr.	3246	136	29	36	-	2	2	-	3	20	-	2	-	-	-	18	-	1.17	7.64
1%4hr.	3427	118	42	23	4	3	5	-	2	28	4	3	1	4	2	21	5	2.19	7.73
2%4hr.	3239	89	58	18	5	4	8	2	1	12	8	6	2	3	2	11	10	3.37	7.38
5%4hr.	3193	69	53	11	6	2	5	1	3	9	3	7	2	2	1	7	14	3.16	6.11
1%6hr.	2838	52	64	8	3	4	2	1	2	6	4	3	2	6	1	3	8	3.52	5.96
2%6hr.	3017	44	73	6	4	5	4	-	4	5	4	7	-	-	2	2	6	3.61	5.50
5%6hr.	3345	39	82	7	5	3	6	-	2	3	7	5	1	2	1	2	5	3.56	5.08
1%12hr.	2965	8	24	2	3	4	2	1	2	1	3	2	-	1	2	1	5	1.65	2.06
2%12hr.	2892	6	30	1	4	2	5	-	-	-	4	1	-	-	-	-	2	1.66	1.90
5%12hr.	2739	3	19	-	5	3	1	-	1	-	6	-	-	-	1	-	2	1.39	1.50
1%24hr.	3018	-	16	-	4	1	-	1	-	-	2	4	-	-	1	-	1	0.99	0.99
2%24hr.	2978	-	12	-	3	-	-	1	-	-	1	3	-	-	1	-	-	0.71	0.71
5%24hr.	2711	-	6	-	2	1	-	-	-	-	-	1	-	-	-	-	1	0.41	0.41

Table No. 7 Consolidated data of cytotoxicity of leaf extract of *Callistemon viminalis*

Treatment (%) and time	Total cells counted	Inter-phase nuclear lesions & Abnormal nuclei	Pro-phase		Metaphase							Anaphase					Telophase		Frequency of abnormal cells (%)	Mitotic Index (%)			
			Normal	Abnormal	Normal	Sticky	Clumping	Diagonal	C-metaphase	Polyploid cell	Disturbed	Normal	Sticky	Diagonal	Bridge	Fragmentation	Misorientation	Normal			Sticky		
1%2hr.	2936	68	2	48	1	2	3	4	1	2	6	8	4	2	7	10	2	7	10	5.76	5.25		
2%2hr.	3124	123	6	61	2	5	2	4	1	2	8	3	6	2	3	11	1	3	11			7.62	4.58
5%2hr.	3094	165	1	52	4	4	5	2	1	1	5	1	8	1	2	8	1	2	8			8.47	3.65
			8		2																		
			1																				
			0																				
1%4hr.	2896	230	4	26	1	5	2	1	2	1	4	2	6	2	-	10	1	2	7	10.25	2.62		
2%4hr.	2974	318	2	28	-	4	3	2	1	1	3	1	7	-	-	14	2	-	5	13.05	2.46		
5%4hr.	2945	425	1	24	-	7	5	2	3	1	2	-	8	2	-	8	3	-	4	16.77	2.38		
1%6hr.	3118	675	-	5	-	-	1	-	-	-	-	2	-	3	-	-	3	-	2	22.16	0.51		
2%6hr.	3082	843	-	2	-	-	-	-	-	-	-	1	-	1	-	-	2	-	1	27.58	0.23		
5%6hr.	3328	1514	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	45.55	0.06		

Table 8 Consolidated data of cytotoxicity of leaf extract of *Corymbia citriodora*

Treatment (%) and time	Total cells counted	Inter-phase nuclear lesions & abnormal nuclei	Prophase		Metaphase			Anaphase		Telo-phase		Frequency of abnormal cells (%)	Mitotic Index (%)
			Normal	Abnormal	Normal	Clumping	Diagonal	Normal	Sticky	Normal	Abnormal		
1%2hr.	2875	1250	5	12	4	1	2	3	3	3	4	44.24	1.29
2%2hr.	3024	1528	2	15	2	-	2	2	5	-	4	51.39	1.06
5%2hr.	3076	2118	1	16	1	-	2	-	2	-	2	69.57	0.78
1%4hr.	2980	2130	-	5	-	3	-	-	-	-	2	71.81	0.34
2%4hr.	3125	2505	-	1	-	-	-	-	-	-	1	80.22	0.06
5%4hr.	3090	2815	-	-	-	-	-	-	-	-	-	91.10	0.00

**Table 9** Consolidated data of cytotoxicity of leaf extract of *Corymbia ficifolia*

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Sticky	Clumping	Diagonal	C-metaphase	Disturbed	Normal	Sticky	Diagonal	Bridge	Fragmentation	Misorientation	Normal	Abnormal			Micronuclei
1%2hr.	2738	36	18	5	1	1	-	-	-	4	-	-	-	1	1	4	1	-	0.84	2.63
2%2hr.	2917	43	21	4	-	-	1	-	1	3	1	-	1	-	1	2	2	-	0.96	2.74
5%2hr.	2597	19	13	5	1	1	-	-	-	4	1	3	1	-	-	2	3	-	0.89	2.04
1%4hr.	2096	9	11	2	2	-	2	-	1	1	-	2	-	-	-	-	-	2	0.95	1.53
2%4hr.	2178	10	12	1	-	1	-	1	-	-	-	1	2	-	1	-	-	1	0.87	1.38
5%4hr.	2216	8	14	1	-	-	-	2	-	-	1	-	-	1	1	-	-	-	0.86	1.26
1%6hr.	2237	2	3	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-	0.22	0.36
2%6hr.	2429	1	2	-	-	1	1	-	-	-	1	-	-	-	1	-	-	-	0.25	0.29
5%6hr.	2564	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	0.08	0.08

**Table 10 Consolidated data of cytotoxicity of leaf extract of *Eucalyptus globulus***



Treatment (%) and time	Total cells counted	Prophase		Metaphase				Anaphase					Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Sticky	Clumping	Disturbed	Normal	Sticky	Diagonal	Bridge	Fragmentation	Misorientation	Normal	Abnormal			Micronuclei
1%2hr.	2836	4	6	2	1	1	-	1	1	1	2	-	-	1	1	1	0.49	0.78
2%2hr.	2919	2	7	1	2	-	1	1	1	-	3	-	1	-	1	1	0.58	0.72
5%2hr.	2983	1	4	-	1	1	-	1	1	2	-	-	-	-	1	-	0.34	0.40
1%4hr.	2719	-	2	-	1	2	-	-	2	-	-	-	-	-	1	-	0.29	0.29
2%4hr.	2933	-	2	-	-	2	-	-	1	-	-	-	-	-	-	-	0.17	0.17
5%4hr.	2798	-	1	-	-	1	-	-	1	-	-	-	-	-	1	-	0.14	0.14

Table 11 Consolidated data of cytotoxicity of leaf extract of *Eucalyptus tereticornis*

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Pulverisation	Clumping	Diagonal	C-metaphase	Disturbed	Normal	Sticky	Diagonal	Bridge	Fragmentation	Misorientation	Normal	Abnormal			Micronuclei
1%2hr.	2718	52	8	13	-	-	-	1	2	9	-	-	-	3	1	10	1	-	0.59	3.68
2%2hr.	2693	47	11	9	-	-	1	2	3	6	-	-	1	2	1	7	3	-	0.89	3.45
5%2hr.	2787	39	14	5	-	-	-	3	5	4	-	-	2	5	2	3	5	-	1.29	3.12
1%4hr.	3018	24	19	3	-	1	-	-	2	2	-	1	1	4	1	-	3	-	1.06	2.02
2%4hr.	2993	16	19	1	1	-	-	-	1	-	-	1	-	5	-	-	4	-	1.04	1.60
5%4hr.	2825	9	14	-	2	-	-	-	3	-	-	-	-	6	2	-	2	1	1.06	1.38
1%6hr.	2679	2	3	-	2	-	-	-	-	1	2	-	-	-	1	2	1	2	0.41	0.60
2%6hr.	2813	-	2	-	2	-	-	-	-	-	2	-	1	-	-	1	-	1	0.32	0.32
5%6hr.	2829	-	1	-	-	-	-	-	-	-	1	-	-	-	1	1	-	1	0.18	0.18

Table 12 Consolidated data of cytotoxicity of leaf extract of *Leptospermum nicholsii*

Treatment (%) and time	Total cells counted	Interphase nuclear lesions & Abnormal nuclei	Prophase		Metaphase			Anaphase						Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)		
			Normal	Abnormal	Normal	Pulverization	Clumping	Disturbed	Normal	Sticky	Diagonal	Bridge	Ring chromosome	Fragmentation	Misorientation	Normal			Abnormal	Micronuclei
1%2hr.	3078	14	24	8	4	2	2	1	3	1	2	1	-	4	1	2	2	1	1.27	1.88
2%2hr.	3163	22	19	10	2	1	2	2	1	2	1	-	1	3	1	2	3	-	1.52	1.58
5%2hr.	2984	36	7	8	1	3	2	1	1	3	1	-	1	4	-	-	2	-	2.04	1.14
1%4hr.	3125	82	3	5	-	2	3	2	-	2	1	1	-	2	-	-	1	-	3.23	0.70
2%4hr.	3096	148	1	4	-	1	2	2	-	1	-	-	2	4	-	-	2	-	5.36	0.61
5%4hr.	3073	165	-	4	-	2	1	3	-	-	-	-	-	3	1	-	2	-	5.89	0.52

Table 13 Consolidated data of cytotoxicity of leaf extract of *Melaleuca leucadendron*

Treatment (%) and time	Total cells counted	Interphase lesions & abnormal nuclei	Prophase		Metaphase						Anaphase					Telophase			Frequency of abnormal Cells (%)	Mitotic Index (%)		
			Normal	Abnormal	Normal	Pulverization	Clumping	Diagonal	Ball-metaphase	Disturbed	Normal	Sticky	Diagonal	Bridge	Fragmentation	Misorientation	Stathmoanaphase	Normal			Abnormal	Micronuclei
1%2hr	2595	98	18	8	6	2	3	1	4	1	5	6	2	2	5	2	2	8	4	1	5.43	3.08
2%2hr	2838	140	12	10	4	2	2	2	5	1	2	4	1	2	4	1	1	3	5	2	6.41	2.22
5%2hr	2674	216	4	8	2	3	2	3	2	2	1	5	1	1	2	-	2	2	4	3	9.50	1.76
1%4hr	2790	345	4	6	2	2	1	1	1	1	1	6	1	3	1	1	4	2	4	1	13.55	1.51
2%4hr	2688	480	2	5	-	2	1	1	2	1	1	4	1	2	1	-	2	-	2	2	18.82	1.08
5%4hr	2935	685	-	2	-	1	2	-	-	2	-	5	-	1	-	-	2	-	-	1	23.88	0.55

Table 14 Consolidated data of cytotoxicity of leaf extract of *Melaleuca styphelioides*

Treatment (%) and time	Total cells counted	Inter phase nuclear lesions & abnormal nuclei	Prophase		Metaphase			Anaphase			Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)
			Normal	Abnormal	Normal	Pulverization	Disturbed	Normal	Fragmentation	Misorientation	Normal	Sticky	Polyploid cell		
1%2hr.	3190	32	35	10	1	-	5	1	2	1	10	2	-	1.63	2.88
2%2hr.	3232	55	23	16	5	-	2	2	3	2	5	4	-	2.54	2.14
5%2hr.	3182	88	8	12	8	-	8	6	2	2	2	3	4	3.74	1.41
1%4hr.	3260	175	4	14	2	-	2	2	2	2	2	3	4	6.35	0.98
2%4hr.	3245	252	4	14	1	1	2	1	5	-	-	2	2	8.54	0.77
5%4hr.	3295	505	2	8	-	2	6	-	8	-	-	-	1	15.51	0.18
			-	2	-	-	1	-	2	-	-	1	-		

Table 15 Consolidated data of cytotoxicity of leaf extract of *Syncarpia glomulifera*

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)		
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal	Normal	Sticky			Bridge formation	
1% 2hr.	3218	96	4	18	-	-	1	-	-	24	1	-	-	-	-	-	-	-	-	0.19	4.91
2% 2hr.	3378	102	16	16	-	1	1	-	1	12	2	-	1	2	-	14	-	-	0.77	4.83	
5% 2hr.	3198	63	24	6	-	2	1	-	2	8	1	1	1	3	-	2	1	1	1.22	3.69	
1% 4hr.	3363	48	19	2	1	3	1	1	2	3	2	1	4	1	2	2	1	1	1.16	2.80	
2% 4hr.	3313	36	24	2	-	2	-	4	3	2	1	1	2	-	1	2	1	1	1.24	2.48	
5% 4hr.	3476	27	18	1	-	1	-	1	2	2	-	-	4	-	-	-	3	1	0.86	1.73	
1% 6hr.	3520	8	16	2	-	-	-	2	3	1	-	-	-	3	-	-	1	1	0.74	1.05	
2% 6hr.	3416	4	9	-	-	2	-	-	2	-	1	-	-	-	-	-	1	-	0.53	0.64	
5% 6hr.	3219	-	3	-	-	2	-	-	1	-	-	-	2	1	-	-	1	1	0.34	0.34	

Table 16 Consolidated data of cytotoxicity of leaf extract of *Acmena smithii*

Treatment (%) and time	Total cells counted	Prophase		Metaphase			Anaphase					Telophase		Frequency of abnormal cells (%)	Mitotic index (%)	
		Normal	Abnormal	Normal	Pulverization	Ball metaphase	Scattered	Normal	Early movement	Sticky	Bridge formation	Laggards	Normal			Micronuclei
1% 2hr.	2893	15	11	5	1	2	1	3	2	1	1	1	2	1	0.69 0.74 0.64	1.56 1.18 1.18
2% 2hr.	2976	9	7	2	2	4	-	1	-	2	3	1	2	1		
5% 2hr.	2639	3	14	1	3	4	-	-	-	2	2	2	-	2		
1% 4hr.	2748	2	5	-	4	2	2	-	-	4	2	1	-	1	0.73 0.58 0.45	0.80 0.62 0.48
2% 4hr.	2921	1	4	-	2	3	1	-	-	3	2	1	-	2		
5% 4hr.	2898	1	2	-	3	2	1	-	-	2	2	-	-	1		
1% 6hr.	2583	-	2	-	2	1	-	-	-	1	2	-	-	-	0.31 0.29 0.14	0.31 0.29 0.14
2% 6hr.	2768	-	3	-	1	2	-	-	-	1	1	1	-	-		
5% 6hr.	2938	-	1	-	-	1	-	-	-	1	1	-	-	-		

Table no. 17 Consolidated data of cytotoxicity of leaf extract of *Eugenia apiculata*

Treatment (%) and Time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)		
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal	Normal	Bridge formation			Sticky	Micronuclei
1% 2hr	1939	29	11	12	-	1	2	-	1	16	-	-	-	-	1	17	1	2	-	0.98	4.80
2% 2hr	1879	23	8	9	-	-	3	1	4	12	1	-	1	4	-	8	1	1	-	1.28	4.05
5% 2hr	2049	33	14	5	1	6	-	-	-	5	3	2	-	-	2	6	1	2	-	1.51	3.90
1% 4hr	2134	12	18	2	2	3	-	-	-	1	2	-	2	-	1	2	2	2	-	1.50	2.30
2% 4hr	2079	8	21	3	4	1	-	-	-	2	4	-	-	-	-	-	1	1	-	1.54	2.17
5% 4hr	1993	4	6	-	2	-	1	-	1	-	2	-	-	1	-	-	1	1	2	0.85	1.05
1% 6hr	2231	-	4	-	1	1	1	-	-	-	-	1	1	1	-	-	-	1	2	0.58	0.58
2% 6hr	2094	-	2	-	-	1	-	-	1	-	-	1	1	1	-	-	-	1	1	0.43	0.43
5% 6hr	2248	-	1	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	1	0.18	0.18
1% 12hr	1924	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	2	0.16	0.16
2% 12hr	2418	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	1	0.12	0.12
5% 12hr	2549	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.08	0.08

Table 18 Consolidated data of cytotoxicity of leaf extract of *Eugenia uniflora*



Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase						Telophase		Frequency of abnormal cells(%)	Mitotic Index (%)
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal	Normal	Sticky		
1% 2hr.	2368	184	15	48	-	-	4	-	3	56	-	-	-	-	-	28	-	0.93	14.27
2% 2hr.	2413	198	18	36	-	-	3	-	5	42	-	-	1	-	-	21	-	1.12	13.43
5% 2hr.	2619	136	25	28	-	-	2	-	4	31	2	3	-	-	-	16	2	1.45	9.51
1% 4hr.	2296	78	39	12	2	3	5	-	8	13	2	1	-	-	1	9	4	2.83	7.71
2% 4hr.	2623	62	18	8	1	2	7	-	3	6	1	2	-	-	-	4	6	1.53	4.57
5% 4hr.	2198	43	20	4	3	2	4	1	2	3	4	1	2	1	1	3	4	2.05	4.46
1% 6hr.	2528	21	35	5	2	1	3	2	1	2	5	3	2	-	-	2	3	2.26	3.44
2% 6hr.	2493	17	29	2	3	-	2	-	2	2	1	2	3	-	1	2	4	1.89	2.81
5% 6hr.	2298	14	21	1	2	-	1	-	3	3	1	2	2	1	-	2	3	1.57	2.44
1% 12hr.	2192	9	19	-	2	-	-	1	1	1	2	4	1	-	-	-	3	1.51	1.96
2% 12hr.	2462	4	16	-	1	-	-	3	-	-	2	2	1	-	-	-	2	1.10	1.26
5% 12hr.	2618	4	13	-	-	-	2	1	-	-	1	3	1	-	-	-	2	0.88	1.03
1% 24hr.	2274	2	22	-	-	-	3	-	-	-	-	2	-	-	-	-	1	1.23	1.32
2% 24hr.	2467	-	11	-	-	-	3	-	-	2	-	-	1	-	-	-	-	0.69	0.69
5% 24hr.	2526	-	3	-	-	-	1	-	-	2	-	-	-	-	-	-	1	0.28	0.28

Table 19 Consolidated data of cytotoxicity of leaf extract of *Feijoa sellowiana*

Treatment (%) and Time	Total cells counted	Prophase		Metaphase			Anaphase					Telophase		Frequency of abnormal cells (%)	Mitotic Index (%)			
		Normal	Abnormal	Normal	Pulverization	Clumping	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal			Star anaphase	Normal	Sticky
1% 2hr	2478	38	4	14	-	1	1	16	-	1	-	-	-	-	23	1	0.32	4.00
2% 2hr	2672	28	4	12	-	1	1	13	-	2	-	-	-	2	17	2	0.45	3.07
5% 2hr	2516	17	4	10	-	2	2	9	1	3	-	1	-	-	14	3	0.64	2.62
1% 4hr	2708	8	8	3	1	2	1	3	2	2	-	1	-	1	2	4	0.81	1.40
2% 4hr	2526	3	5	2	2	1	-	3	3	5	-	-	1	1	1	2	0.79	1.15
5% 4hr	2495	2	5	1	2	3	1	2	1	2	-	-	1	-	1	4	0.76	1.00
1% 6hr	2898	3	3	2	1	4	2	2	-	3	-	1	1	2	2	3	0.69	1.00
2% 6hr	2926	3	3	2	2	3	1	1	-	4	-	1	2	-	2	5	0.72	0.99
5% 6hr	2739	2	4	1	1	5	1	1	1	2	1	-	2	-	1	4	0.77	0.95
1% 12hr	2483	1	3	1	1	3	2	1	1	3	-	-	2	-	1	4	0.77	0.93
2% 12hr	2513	-	3	-	1	3	3	-	1	2	-	-	3	-	-	5	0.83	0.83
5% 12hr	2396	-	2	-	1	4	1	-	1	3	-	-	2	-	-	3	0.71	0.71

**Table 20 Consolidated data of cytotoxicity of leaf extract of *Myrtus communis***

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase						Telophase		Frequency of abnormal cells (%)	Mitotic Index (%)		
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Stathmoanaphase	Diagonal	Ringchromosome	Star anaphase			Normal	Sticky
1% 2hr.	3176	12	15	4	-	-	1	2	3	2	2	3	-	1	-	1	2	2	2	0.91	1.54
2% 2hr.	3219	9	7	2	1	2	-	1	-	1	-	2	2	-	-	1	1	1	2	0.53	0.93
5% 2hr.	3373	5	6	1	2	1	-	-	-	-	1	-	1	-	1	1	1	-	2	0.36	0.53
1% 4hr.	2978	2	3	-	-	2	1	-	-	-	1	1	-	1	-	-	-	-	1	0.34	0.40
2% 4hr.	2992	-	3	-	-	2	3	-	-	-	-	-	1	-	-	-	-	-	1	0.37	0.37
5% 4hr.	3048	-	1	-	2	-	-	-	1	-	-	-	-	1	-	-	-	-	2	0.16	0.16

Table 21 Consolidated data of cytotoxicity of leaf extract of *Pimenta dioica*

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase			Frequency of abnormal Cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal	Normal	Sticky			Micronuclei
1% 2hr	2739	43	17	18	1	-	1	-	1	13	-	1	-	1	-	9	2	-	0.88	3.91
2% 2hr	2818	34	19	14	2	1	2	-	1	19	2	3	-	-	1	8	3	-	1.21	3.87
5% 2hr	2596	32	14	11	1	-	1	-	1	7	1	1	-	-	1	5	2	-	0.85	2.97
1% 4hr	2318	11	14	4	2	1	1	1	2	4	-	2	1	-	1	2	4	-	1.25	2.16
2% 4hr	2523	9	18	3	1	-	2	-	1	2	-	3	1	-	1	1	3	-	1.19	1.78
5% 4hr	2649	7	11	1	2	-	1	1	2	1	-	2	1	-	-	-	2	-	0.83	1.17
1% 6hr	1978	3	6	-	3	-	-	-	2	-	2	-	1	1	-	-	1	-	0.81	0.96
2% 6hr	2314	2	4	-	1	1	2	-	1	-	-	2	-	1	-	-	-	-	0.52	0.61
5% 6hr	1898	2	3	-	1	1	1	-	2	-	1	1	-	-	-	-	1	-	0.58	0.69
1% 12hr	2493	2	3	-	-	4	-	-	1	-	1	1	1	-	-	-	-	1	0.48	0.56
2% 12hr	1937	3	2	-	1	1	-	-	1	-	-	-	-	1	1	-	-	-	0.36	0.52
5% 12hr	1876	1	1	-	1	-	-	1	-	-	-	2	-	-	-	-	-	-	0.27	0.32
1% 24hr	2147	1	1	-	-	1	-	-	-	-	1	1	-	-	-	-	-	-	0.19	0.23
2% 24hr	2218	-	2	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	0.18	0.18
5% 24hr	2573	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-	-	1	0.16	0.16

Table 22 Consolidated data of cytotoxicity of leaf extract of *Psidium guajava*

Treatment (%) and Time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal	Normal	Sticky			Micronuclei
1% 2hr.	3149	48	7	28	-	-	2	-	1	22	1	3	1	1	-	17	3	1	0.64	4.29
2% 2hr.	3342	56	4	31	-	1	-	1	-	27	-	2	-	-	-	12	1	-	0.27	4.04
5% 2hr.	3298	39	4	21	-	-	1	-	1	18	-	1	-	-	1	9	-	1	0.27	2.91
1% 4hr.	2987	12	16	7	3	3	5	2	1	-	6	2	3	2	1	3	-	1	1.51	2.24
2% 4hr.	3018	6	22	4	4	3	2	1	2	-	2	1	5	1	-	2	1	1	1.49	1.89
5% 4hr.	3193	3	12	1	1	3	1	2	1	1	-	1	2	-	-	-	2	-	0.78	0.94
1% 6hr.	3329	-	4	-	-	-	2	-	2	-	-	3	-	-	-	-	-	1	0.36	0.36
2% 6hr.	3417	-	3	-	-	-	1	-	1	-	-	1	-	-	1	-	-	1	0.23	0.23
5% 6hr.	3197	-	1	-	-	1	-	-	-	-	-	-	-	-	1	-	-	1	0.13	0.13

Table 23 Consolidated data of cytotoxicity of leaf extract of *Syzygium aromaticum*.

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal	Normal	Sticky			Micronuclei
1% 2hr.	3259	143	8	43	2	3	-	-	4	51	-	2	-	-	-	18	-	-	0.58	8.41
2% 2hr.	3418	152	13	34	3	5	2	-	2	28	6	5	2	-	3	6	4	-	1.32	7.75
5% 2hr.	3394	108	29	18	5	5	4	-	3	14	3	7	4	-	2	4	3	-	1.92	6.16
1% 4hr.	3498	32	58	13	16	11	7	1	2	8	4	5	2	-	3	2	3	-	3.20	4.77
2% 4hr.	3563	26	43	5	15	6	4	2	1	3	5	8	3	1	2	-	4	1	2.67	3.62
5% 4hr.	3327	13	28	3	19	7	2	1	3	1	3	6	2	2	1	-	2	2	2.35	2.86
1% 6hr.	2988	4	17	2	16	5	3	1	2	1	2	8	1	1	2	1	3	-	2.04	2.31
2% 6hr.	3197	2	23	1	8	2	6	-	4	-	3	4	2	1	1	1	2	1	1.78	1.91
5% 6hr.	3424	3	14	-	5	1	3	1	2	1	1	5	-	-	-	-	2	2	1.05	1.17
1% 12hr.	3093	1	8	1	2	3	-	-	1	1	2	3	1	-	-	-	1	-	0.68	0.78
2% 12hr.	3478	1	4	-	3	2	1	-	-	-	5	4	-	-	-	-	-	-	0.55	0.58
5% 12hr.	3243	-	2	1	1	4	-	-	2	-	2	2	-	-	-	-	1	-	0.46	0.46
1% 24hr.	3318	-	1	-	-	-	1	-	1	-	-	2	-	-	-	-	2	-	0.21	0.21
2% 24hr.	3475	-	-	-	-	-	1	-	-	-	1	-	-	1	-	-	1	-	0.12	0.12
5% 24hr.	3218	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-	-	0.09	0.09

Table 24 Consolidated data of cytotoxicity of leaf extract of *Syzygium cumini*

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase		Frequency of abnormal cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal	Normal			Sticky
1% 2hr.	2129	80	16	39	-	-	3	-	-	41	-	-	-	2	-	34	-	0.99	10.11
2% 2hr.	2394	97	14	33	-	2	1	-	2	34	1	-	1	3	-	27	-	1.00	8.98
5% 2hr.	2276	67	21	23	2	4	5	-	1	18	4	5	2	1	-	14	9	2.37	7.73
1% 4hr.	2475	61	26	18	3	2	3	1	2	19	2	6	1	2	1	9	13	2.51	6.83
2% 4hr.	2488	53	34	15	2	4	3	-	4	16	4	5	2	3	2	6	15	3.14	6.75
5% 4hr.	2199	47	31	19	4	2	5	1	3	8	3	6	1	2	3	4	9	3.18	6.73
1% 6hr.	2210	29	36	4	5	6	2	1	-	3	5	7	2	4	2	2	7	3.48	5.11
2% 6hr.	2093	17	26	2	4	3	2	1	1	1	6	8	1	3	4	1	9	3.25	4.25
5% 6hr.	2478	12	38	1	7	6	4	2	2	1	9	2	-	1	1	1	7	3.19	3.79
1% 12hr.	2193	5	19	-	8	4	5	1	3	-	6	4	1	3	1	-	4	2.69	2.92
2% 12hr.	2314	4	22	-	7	2	8	-	1	-	7	2	-	2	2	-	3	2.42	2.59
5% 12hr.	2296	3	17	-	6	3	4	1	2	-	5	3	-	1	1	-	4	2.05	2.18
1% 24 hr.	2018	1	12	-	2	-	-	-	1	-	1	4	-	-	-	-	2	1.09	1.14
2% 24 hr.	2134	-	4	-	3	-	-	-	-	-	-	5	-	-	-	-	1	0.61	0.61
5% 24hr.	1987	-	2	-	1	-	-	-	-	-	-	2	-	-	-	-	2	0.35	0.35

Table 25 Consolidated data of cytotoxicity of leaf extract of *Syzygium jambos*

Treatment (%) and time	Total cells counted	Prophase		Metaphase						Anaphase					Telophase		Frequency of abnormal Cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal	Normal			Sticky
1% 2hr	2738	115	5	34	-	-	2	-	-	29	1	1	-	-	-	24	-	0.33 0.41 0.96	7.71 7.40 7.18
2% 2hr	2948	121	8	36	-	-	1	-	1	27	1	-	-	1	-	22	-		
5% 2hr	2813	116	12	24	-	2	2	-	2	18	3	2	1	1	-	17	2		
1% 4hr	2914	86	14	23	1	-	1	-	-	18	2	1	-	-	-	14	3	0.76 1.21 0.99	5.59 5.32 4.18
2% 4hr	2896	78	18	17	2	3	2	-	1	12	1	2	2	-	-	12	4		
5% 4hr	3018	63	11	14	1	1	3	1	1	11	3	4	-	1	2	8	2		
1% 6hr	2713	43	18	8	2	1	-	2	1	6	2	2	-	-	-	5	1	1.07 1.31 1.13	3.35 2.82 2.15
2% 6hr	2975	32	23	4	3	-	-	3	1	5	4	3	-	-	1	4	2		
5% 6hr	2934	22	10	3	2	3	4	-	-	3	3	4	2	-	2	2	3		
1% 12hr	2893	13	18	2	1	-	1	-	-	2	2	3	1	1	-	2	3	1.04 1.13 1.18	1.69 1.49 1.31
2% 12hr	3017	8	13	1	2	3	1	2	-	2	2	4	2	1	-	-	4		
5% 12hr	2973	4	14	-	2	2	2	3	2	-	1	3	1	-	2	-	3		
1% 24hr	3128	4	6	-	2	3	1	1	2	1	3	4	-	-	-	-	2	0.77 0.74 0.49	0.93 0.77 0.49
2% 24hr	2989	1	5	-	1	2	2	1	3	-	2	3	-	-	-	-	2		
5% 24hr	2877	-	4	-	2	1	1	-	1	-	1	2	1	-	-	-	1		

Table 26 Consolidated data of cytotoxicity of leaf extract of *Syzygium malaccense*



Treatment (%) and time	Total cells scored	Prophase		Metaphase						Anaphase						Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	Normal	Early movement	Sticky	Bridge formation	Nonsynchronized	Diagonal	Normal	Sticky	Micronuclei		
1% 2hr.	3137	100	59	24	3	2	5	1	6	32	8	5	2	2	3	16	8	-	3.32	8.80
2% 2hr.	3233	83	67	18	4	5	2	1	3	16	7	4	3	1	2	12	6	-	3.25	7.24
5% 2hr.	3096	73	48	13	6	7	8	3	7	20	4	3	2	4	1	15	7	-	3.23	7.14
1% 4hr.	3176	68	47	13	5	8	10	4	10	15	2	6	3	5	3	10	8	1	3.53	6.86
2% 4hr.	3043	64	53	11	4	6	12	2	8	13	4	5	4	4	5	8	9	1	3.85	7.00
5% 4hr.	3278	58	61	10	5	4	14	5	7	9	6	3	5	7	8	6	8	4	4.18	6.71
1% 6hr.	3193	54	69	8	3	2	8	4	3	10	8	4	3	5	4	6	9	2	3.88	6.33
2% 6hr.	3313	59	65	7	5	1	4	5	6	8	5	2	1	7	3	5	10	2	3.50	5.89
5% 6hr.	3217	54	66	6	4	3	5	3	8	7	3	6	3	2	1	5	10	1	3.58	5.81
1% 12 hr.	3133	28	43	2	3	5	8	4	10	3	7	8	5	6	1	2	5	2	3.42	4.53
2% 12hr.	3374	18	36	4	5	7	10	8	16	4	10	16	2	3	3	2	7	3	3.73	4.56
5% 12hr.	3218	19	31	2	8	10	11	4	12	2	8	11	4	5	6	3	6	2	3.67	4.48
1% 24 hr.	3319	4	16	-	10	5	14	3	18	1	2	15	5	8	4	-	8	2	3.31	3.47
2% 24 hr.	3279	2	20	-	8	4	11	2	13	-	4	8	2	7	3	-	7	2	2.78	2.84
5% 24 hr.	3118	3	13	-	5	2	8	1	8	-	8	4	6	3	6	-	9	4	2.47	2.57

**Table 27 Consolidated data of cytotoxicity of leaf extract of *Syzygium samarangense***

Treatment (%) and time	Total cells counted	Prophase		Metaphase							Anaphase					Telophase			Frequency of abnormal cells (%)	Mitotic Index (%)	
		Normal	Abnormal	Normal	Pulverization	Clumping	Ball metaphase	Diagonal	Scattered	C-metaphase	Normal	Early movement	Sticky	Bridge formation	Non-synchronized	Diagonal	Normal	Sticky			Polyploid cells
1% 2 hr.	3128	208	12	83	2	1	-	-	-	2	90	8	-	-	-	-	68	-	4	0.93	15.28
2% 2 hr.	3243	228	23	90	4	2	-	-	-	4	95	12	-	-	-	-	86	-	6	1.57	16.96
5% 2 hr.	3086	242	19	94	5	1	-	-	-	6	108	1	-	-	-	-	91	-	12	1.43	18.76
1% 4 hr.	3248	186	28	78	8	4	-	-	-	10	82	5	-	-	-	-	76	-	28	2.56	15.55
2% 4 hr.	3416	248	19	63	6	2	-	-	-	14	71	6	-	-	-	-	93	-	32	2.31	16.22
5% 4 hr.	3493	302	42	72	10	3	-	-	-	18	94	2	-	-	-	-	102	-	48	3.52	19.84
1% 6 hr.	3196	32	70	12	15	18	3	2	1	-	8	6	2	1	4	2	8	12	-	4.26	6.13
2% 6 hr.	3213	14	65	6	13	24	8	4	2	-	3	5	3	-	2	1	2	8	-	4.20	4.98
5% 6 hr.	3238	8	42	4	18	17	12	5	4	-	2	5	1	-	2	-	2	3	-	3.37	3.86

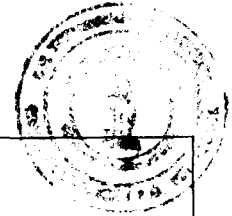
Table 28 Consolidated data of cytotoxicity of leaf extract of *Syzygium zeylanicum*

Treatment (%) and time	Total cells counted	Prophase		Metaphase				Anaphase		Telophase		Frequency of abnormal cells (%)	Mitotic Index (%)
		Normal	Abnormal	Normal	Pulverization	Scattered	C- metaphase	Normal	Early movement	Normal	Sticky		
1% 2 hr	2418	248	11	94	-	1	8	78	4	66	-	0.99	21.09
2% 2 hr	2534	218	23	68	-	4	6	52	8	53	-	1.62	17.05
5% 2 hr	2618	198	36	41	-	8	10	24	19	28	4	2.94	14.06
1% 4 hr	2476	146	68	48	-	16	3	38	7	34	2	3.88	14.62
2% 4 hr	2392	123	84	22	4	24	2	26	8	26	4	5.27	13.50
5% 4 hr	2398	116	92	18	6	16	1	14	10	18	8	5.55	12.47
1% 6 hr	2645	88	52	28	4	28	4	24	9	30	8	3.97	10.40
2% 6 hr	2590	64	58	20	8	14	-	18	12	20	10	3.94	8.65
5% 6 hr	2438	23	40	5	4	2	-	6	4	2	8	2.38	3.85

Table 29 Consolidated data of cytotoxicity of leaf extract of *Rhodomyrtus tomentosa*

NB 3227

**Table: 30 Comparison of clastogenic and nonclastogenic abnormalities observed in *A. cepa* root meristem induced by leaf extracts of members of Myrtaceae.**



<b>Subfamily : Leptospermoideae</b>			
<b>Sl.No.</b>	<b>Name of Plant</b>	<b>% of clastogenic anomaly</b>	<b>% of nonclastogenic anomaly</b>
1	<i>Agonis flexuosa</i>	81.67	18.33
2	<i>Beaufortia sparsa</i>	68.66	31.34
3	<i>Callistemon citrinus</i>	62.23	37.77
4	<i>Callistemon viminalis</i>	81.67	18.34
5	<i>Corymbia citriodora</i>	97.82	2.18
6	<i>Corymbia ficifolia</i>	99.93	0.07
7	<i>Eucalyptus globulus</i>	56.11	43.89
8	<i>Eucalyptus tereticornis</i>	82.31	17.69
9	<i>Leptospermum nicholsii</i>	79.13	20.87
10	<i>Melaleuca leucadendron</i>	94.63	5.37
11	<i>Melaleuca styphelioides</i>	96.62	3.38
12	<i>Syncarpia glomulifera</i>	97.01	2.99
<b>Sub family : Myrtoideae</b>			
13	<i>Acmena smithii</i>	72.49	27.51
14	<i>Eugenia apiculata</i>	71.96	28.04
15	<i>Eugenia uniflora</i>	66.01	33.99
16	<i>Feijoa sellowiana</i>	77.68	22.32
17	<i>Myrtus communis</i>	68.81	31.19
18	<i>Pimenta dioica</i>	71.38	28.62
19	<i>Psidium guajava</i>	75.58	25.42
20	<i>Syzygium aromaticum</i>	66.49	33.51
21	<i>Syzygium cumini</i>	70.75	29.25
22	<i>Syzygium jambos</i>	69.57	30.43
23	<i>Syzygium malaccense</i>	70.58	29.42
24	<i>Syzygium samarangense</i>	64.74	35.27
25	<i>Syzygium zeylanicum</i>	54.81	45.19
26	<i>Rhodomyrtus tomentosa</i>	70.08	29.92

## **Figure Legend**

### **Plate No. 6 ( Control )**

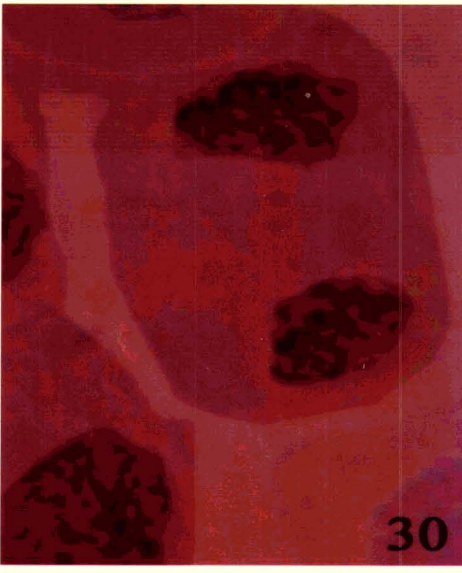
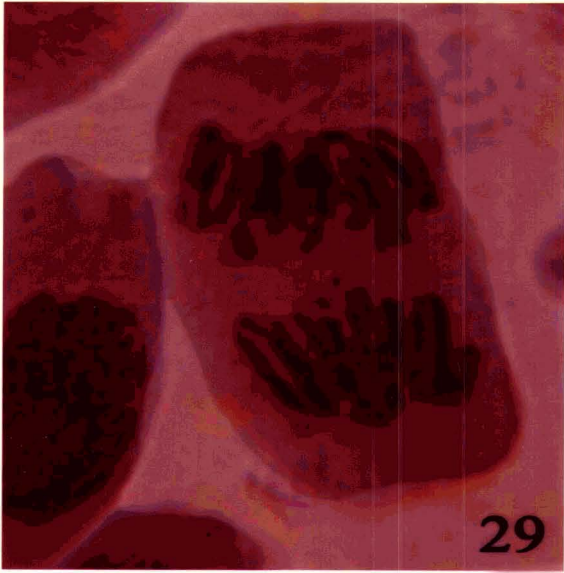
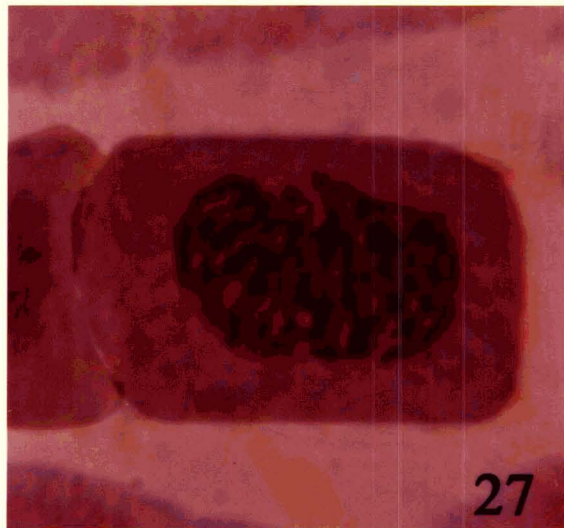
Fig. No. 27 Normal prophase.

Fig. No. 28 Normal metaphase (spread).

Fig. No. 29 Normal anaphase.

Fig. No. 30 Normal telophase.

Plate 6



## **Figure Legend**

### **Plate No. 7**

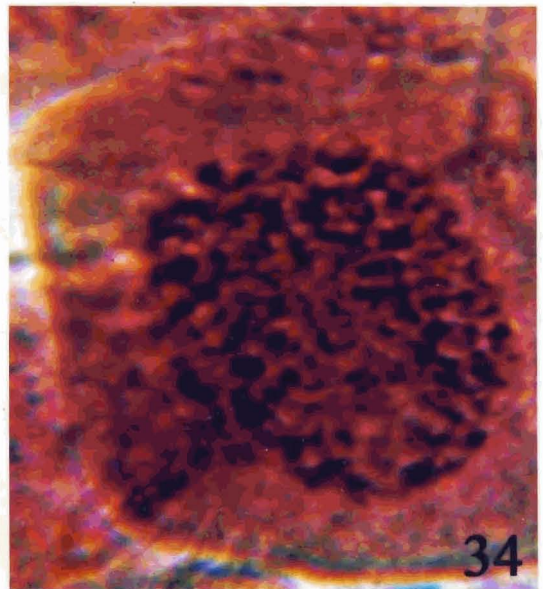
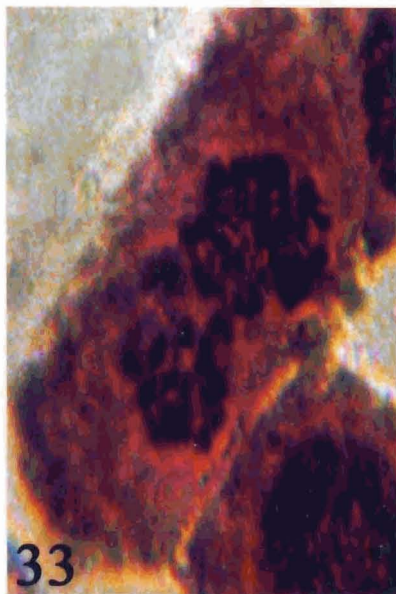
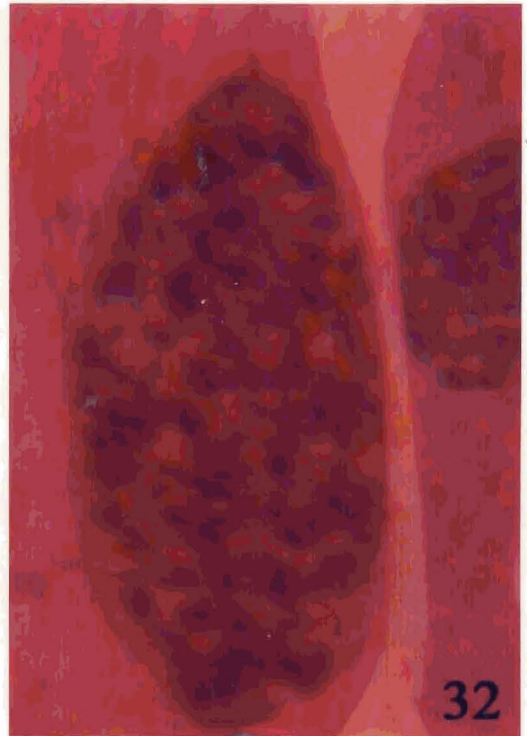
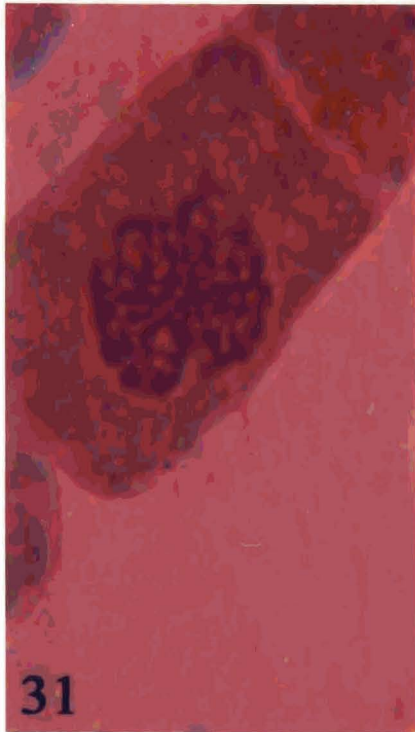
Fig. No. 31 Prophase nucleus showing stickiness.

Fig. No. 32 Abnormally enlarged prophase nucleus.

Fig. No. 33 Abnormal cell with distorted prophase nucleus.

Fig. No. 34 Pulverized nucleus during prophase.

# Plate 7





## Figure Legend

### Plate No. 8

Fig. No. 35 Complete stickiness of chromosomes and their diagonal orientation showing fragments at metaphase.

Fig. No. 36 Sticky and contorted metaphase.

Fig. No. 37 Sticky and diagonal metaphase.

Fig. No. 38 Disintegration of metaphase chromosomes.

Fig. No. 39 Diagonal and sticky metaphase.

Fig. No. 40 Irregular association of chromosomes and clumping at metaphase.

Fig. No. 41 Abnormal association of clumped metaphase chromosomes.

Fig. No. 42 Abnormal cell showing diagonal orientation of metaphase chromosomes and another cell with clumped ball metaphase.

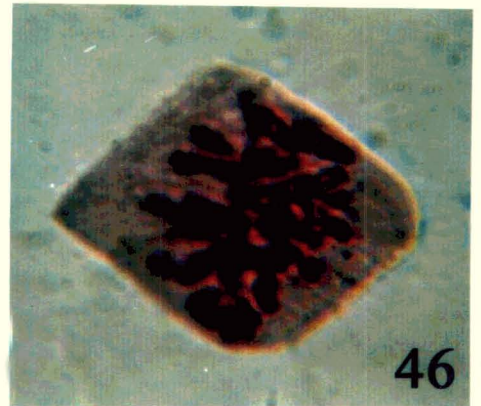
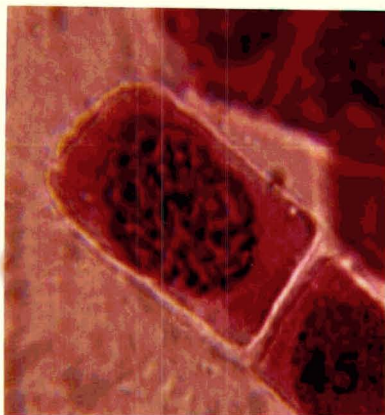
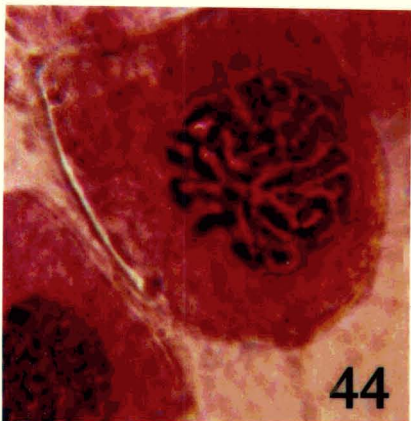
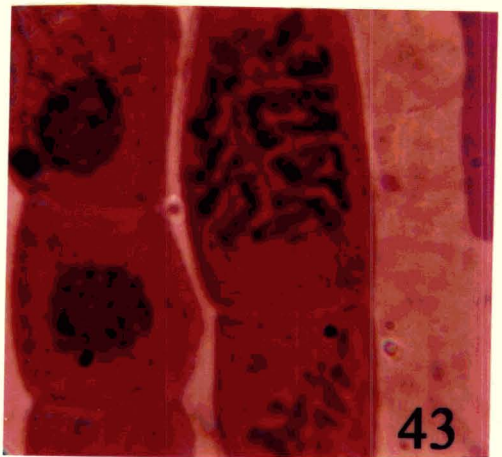
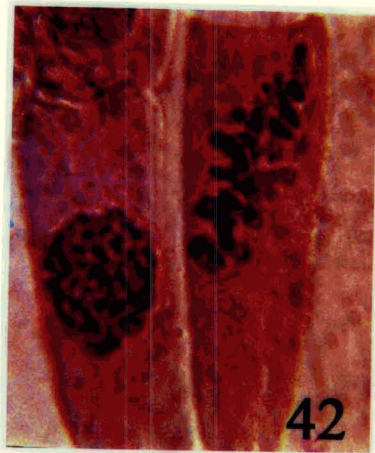
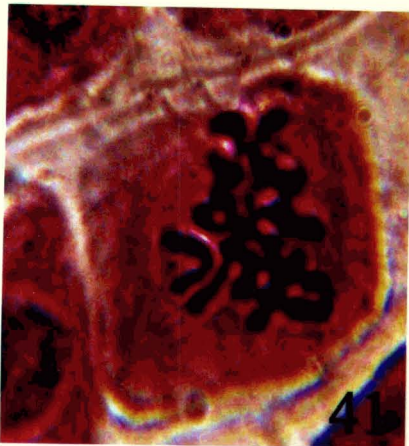
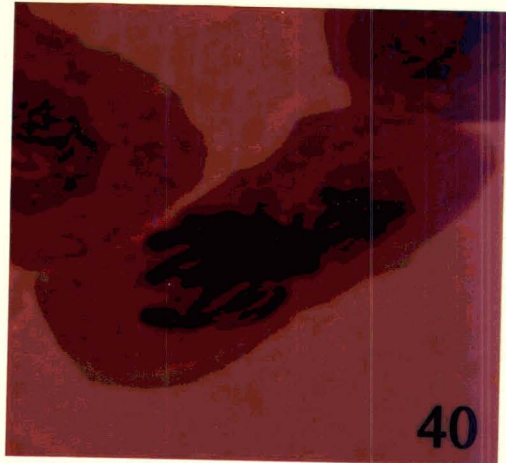
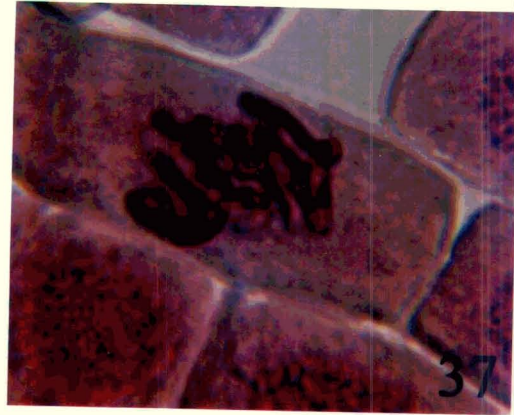
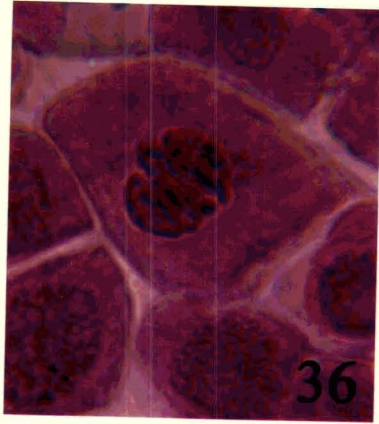
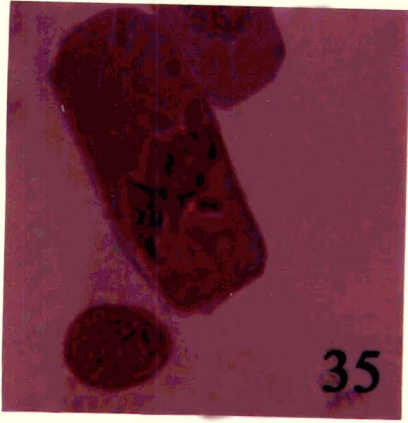
Fig. No. 43 Scattered metaphase with sticky chromosomes.

Fig. No. 44 Ball metaphase.

Fig. No. 45 Sticky ball metaphase.

Fig. No. 46 Diagonal C-metaphase showing abnormal condensation of chromosomes.

Plate 8



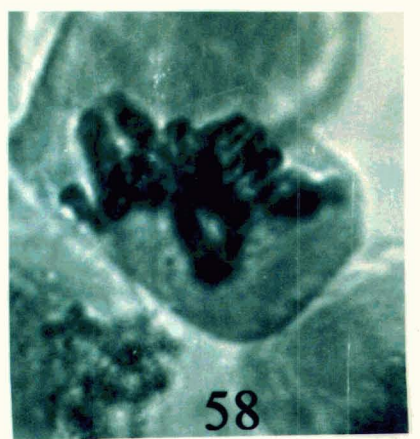
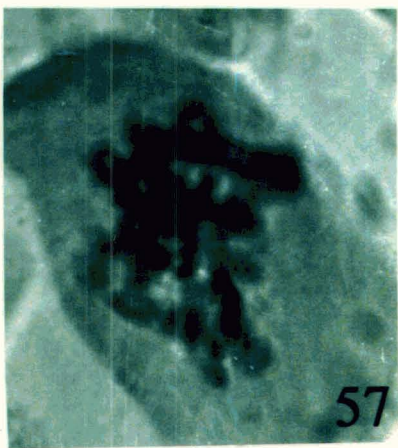
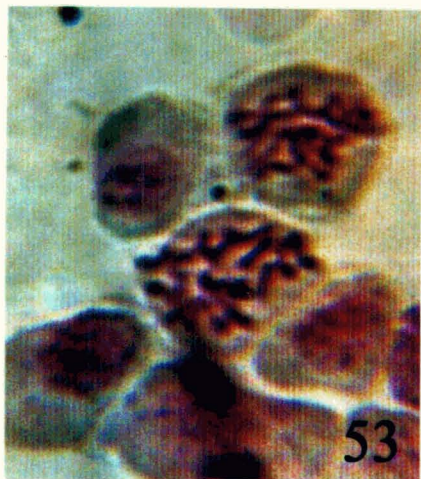
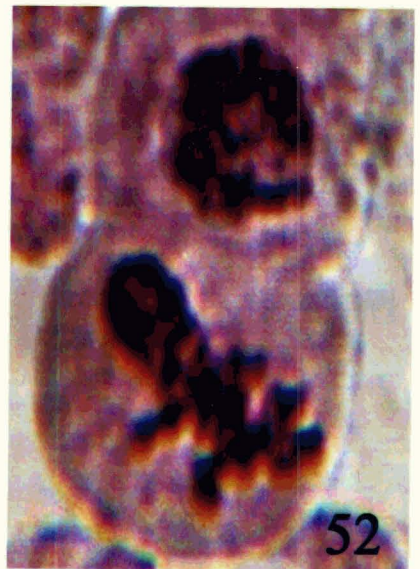
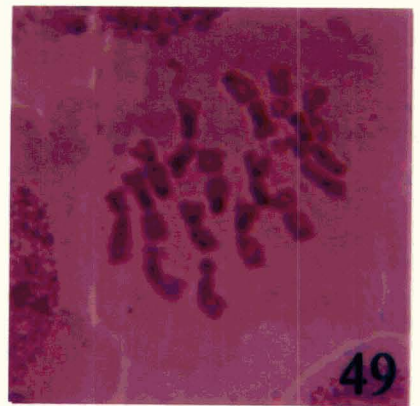
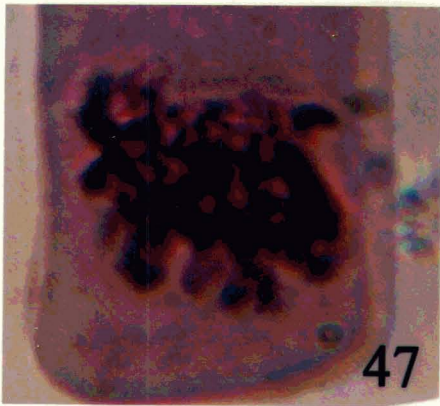
## Figure Legend

### Plate No. 9

- Fig. No. 47 Anomalous metaphase cell showing pulverized and differentially condensed chromosomes.
- Fig. No. 48 C- metaphase showing stickiness of chromosomes.
- Fig. No. 49 C- metaphase with scattered chromosomes.
- Fig. No. 50 C- metaphase chromosomes in an enlarged cell.
- Fig. No. 51 Abnormal metaphase cell showing differential condensation and stickiness of chromosomes.
- Fig. No. 52 Abnormal metaphase showing complete stickiness of chromosomes.
- Fig. No. 53 Irregular orientation of chromosomes at metaphase.
- Fig. No. 54 Misorientation of chromosomes during metaphase.
- Fig. No. 55 Chromosome pulverization at metaphase.
- Fig. No. 56 Two enlarged cells showing anomalous metaphase stages.
- Fig. No. 57 Abnormal association of pulverized chromosomes at metaphase.
- Fig. No. 58 Abnormal diagonal metaphase with 'deprotenized chromosomes'.



Plate 9



## Figure Legend

### Plate No. 10

Fig. No. 59 Sticky and diagonal metaphase showing pycnotic chromosomes.

Fig. No. 60 Abnormal metaphase cell with scattered chromosomes.

Fig. No. 61 Metaphase with a ring chromosome.

Fig. No. 62 A polyploid cell at metaphase stage.

Fig. No. 63 Disturbed anaphase showing non-synchronised movement of chromosomes.

Fig. No. 64 Enlarged cell with multipolarity of chromosomes.

Fig. No. 65 Diagonal and sticky anaphase with laggards.

Fig. No. 66 Anaphase with complete stickiness of chromosomes.

Fig. No. 67 Nonsynchronized multipolar movement during anaphase.

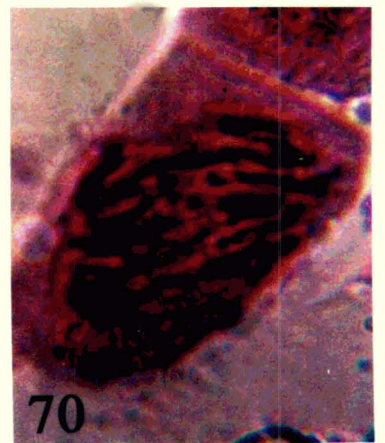
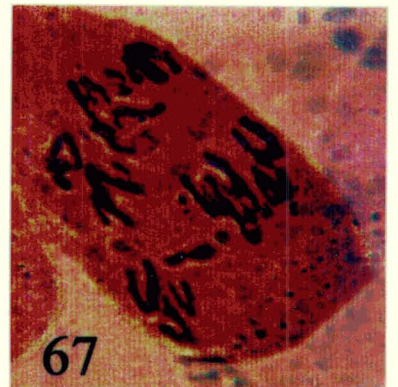
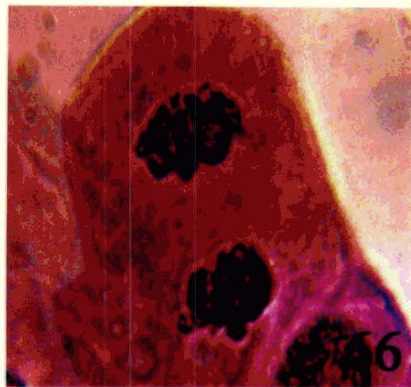
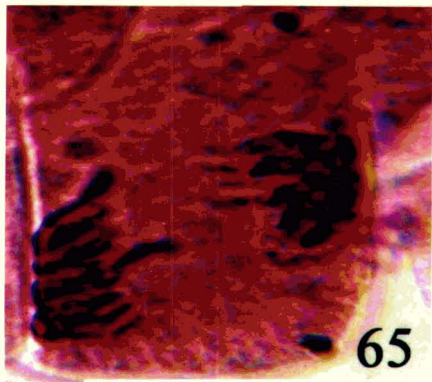
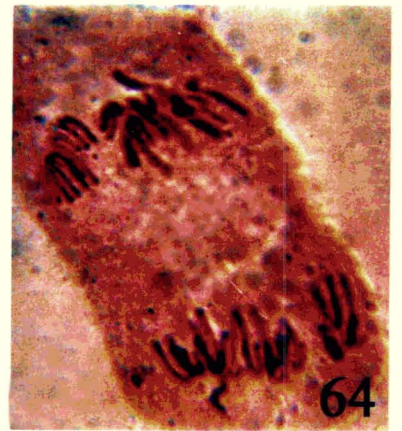
Fig. No. 68 Abnormal sticky anaphase showing early movement of chromosomes.

Fig. No. 69 Sticky star anaphase.

Fig. No. 70 A diagonal sticky anaphase with multiple bridge and fragments.



Plate 10



## Figure Legend

### Plate No. 11

Fig. No. 71 Anaphase cell with multiple bridge and early movement of chromosomes.

Fig. No. 72 Anaphase cell with multiple bridges and laggards.

Fig. No. 73 Late anaphase with multiple bridges.

Fig. No. 74 Abnormal anaphase showing chromosome fragments.

Fig. No. 75 Nonsynchronization in anaphase showing early movement of chromosomes.

Fig. No. 76 An anaphase cell with single bridge.

Fig. No. 77 Anaphase misorientation with lagging of chromosomes.

Fig. No. 78 Diagonal and sticky anaphase.

Fig. No. 79 Diagonal late anaphase in an abnormal cell.

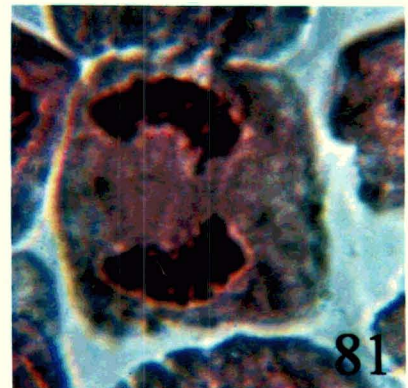
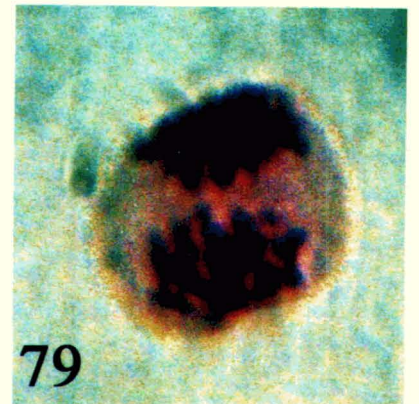
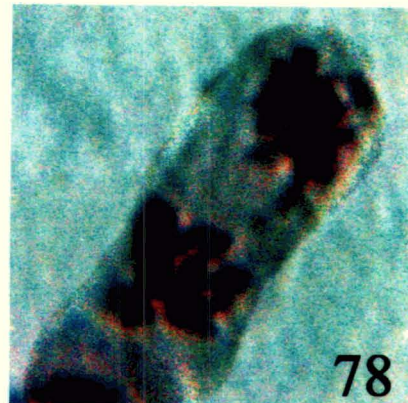
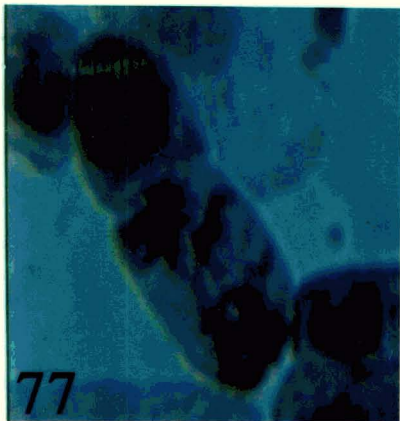
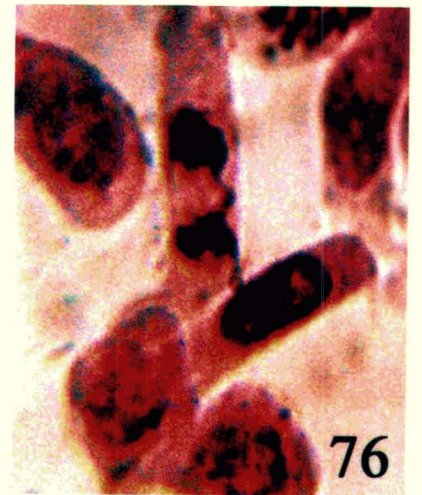
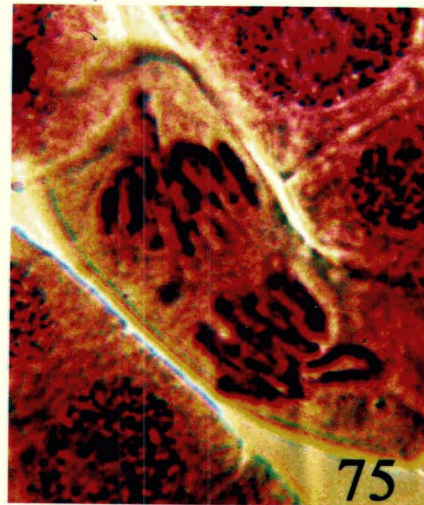
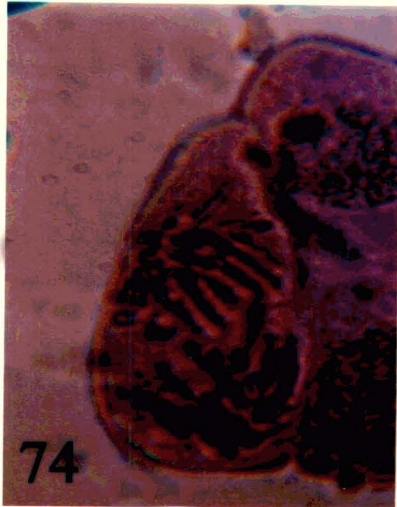
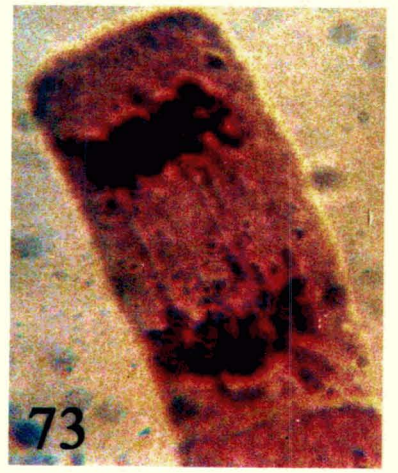
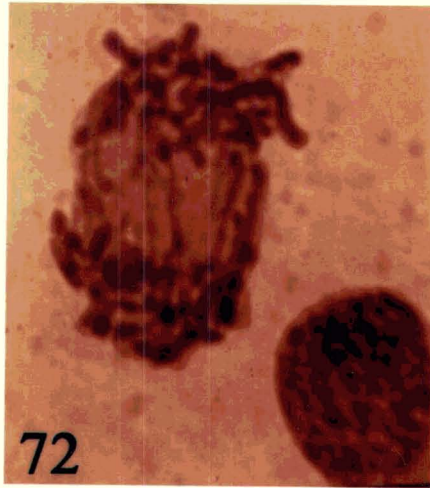
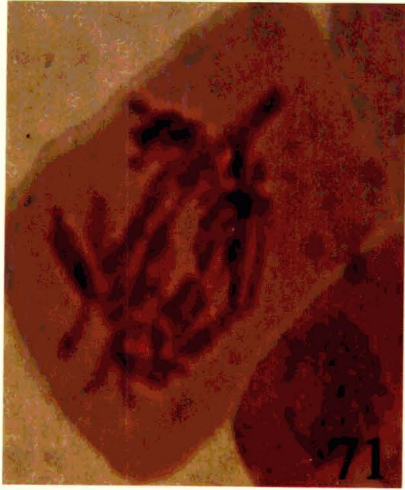
Fig. No. 80 Diagonal early anaphase and prophase in abnormal cells.

Fig. No. 81 Crescent shaped groups of chromosomes at anaphase.

Fig. No. 82 Sticky anaphase with nonsynchronous movement of chromosomes.



Plate 11



22



## Figure Legend

### Plate No. 12

Fig. No. 83 A cell with diagonal sticky anaphase stage with chromosome pulverization.

Fig. No. 84 Late diagonal anaphase with sticky and pulverized chromosomes.

Fig. No. 85 Misorientation of chromosomes at anaphase showing crescent shaped chromosome groups.

Fig. No. 86 Disintegration of chromosomes during late anaphase.

Fig. No. 87 Late sticky anaphase in an abnormal cell.

Fig. No. 88 Irregular, diagonal and partially sticky anaphase and sticky ball metaphase.

Fig. No. 89 Diagonal early anaphase in an enlarged cell and two daughter cells with one showing nuclear diminution.

Fig. No. 90 Diagonal movement of chromosomes during anaphase with laggards.

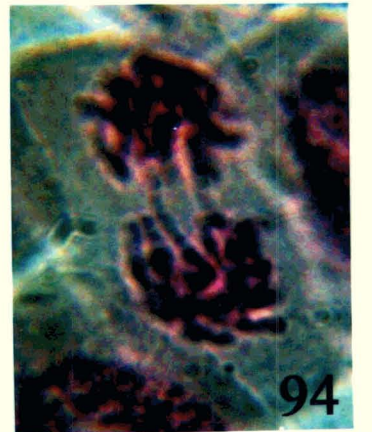
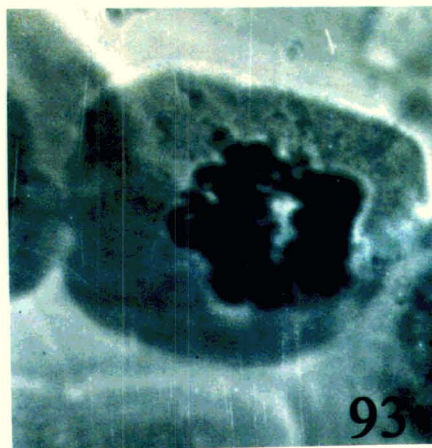
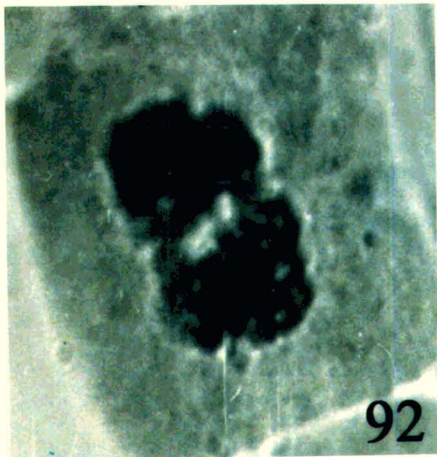
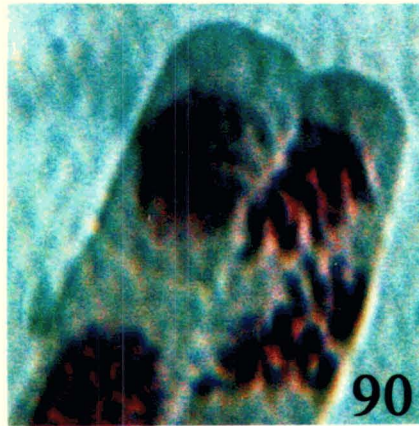
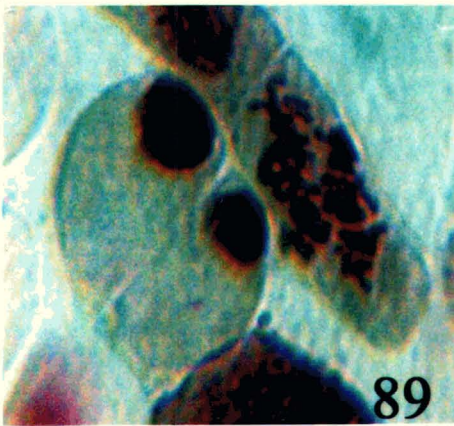
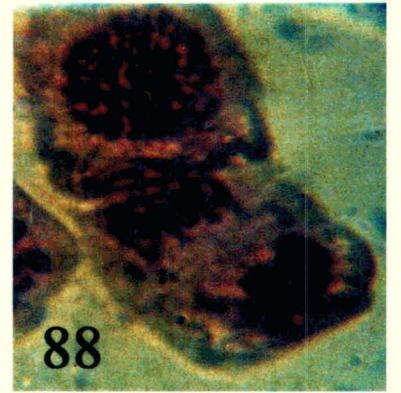
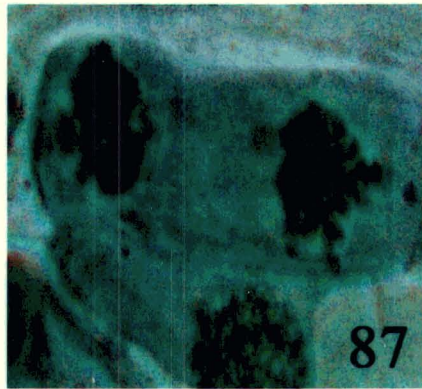
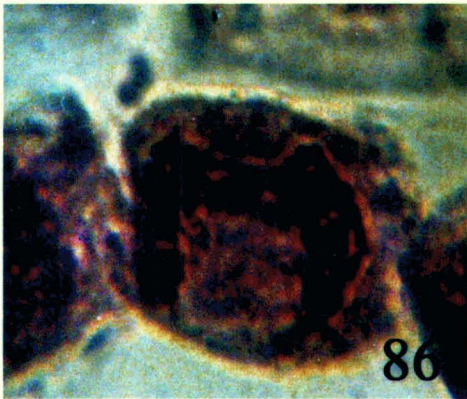
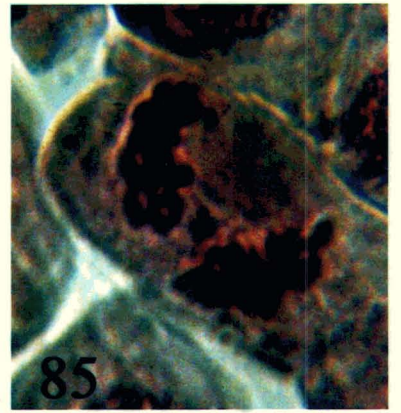
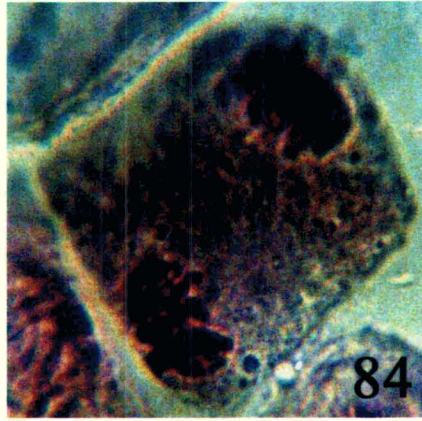
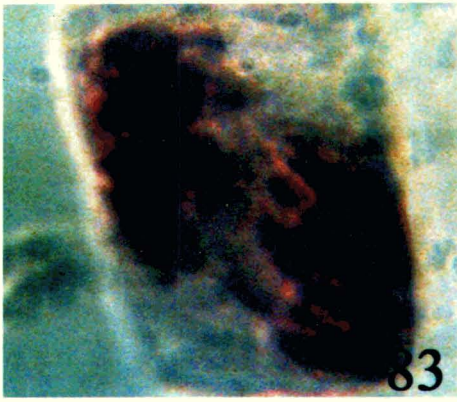
Fig. No. 91 Multiple bridge in a cell with diagonal and sticky anaphase chromosomes.

Fig. No. 92 Abnormal cell with late stathmoanaphase.

Fig. No. 93 Abnormal cell with early stathmoanaphase.

Fig. No. 94 Diagonal anaphase with chromosome bridges.

Plate 12



## Figure Legend

### Plate No. 13

Fig. No. 95 Abnormal cell with double bridge during anaphase stage.

Fig. No. 96 Two abnormal cells, one with single bridge at anaphase stage and other cell with disintegrated chromatin content.

Fig. No. 97 Anomalous cell with multiple bridge and early movement of chromosomes.

Fig. No. 98 Anaphase showing chromosome fragmentation.

Fig. No. 99 A cell with sticky telophase.

Fig.No.100 Abnormal sticky telophase showing disintegration of chromatin content.

Fig. No. 101 Diagonal and sticky early telophase.

Fig. No. 102 Abnormal cell with complete stickiness of chromosomes during telophase.

Fig. No. 103 Unequal groups of chromosomes at early telophase.

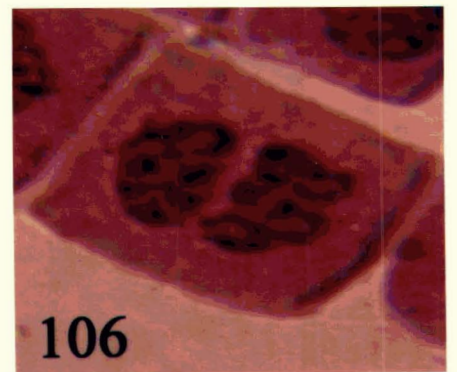
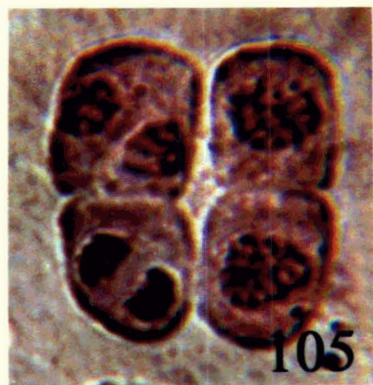
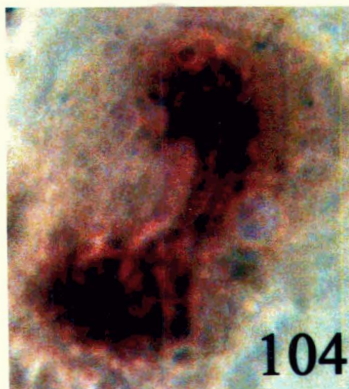
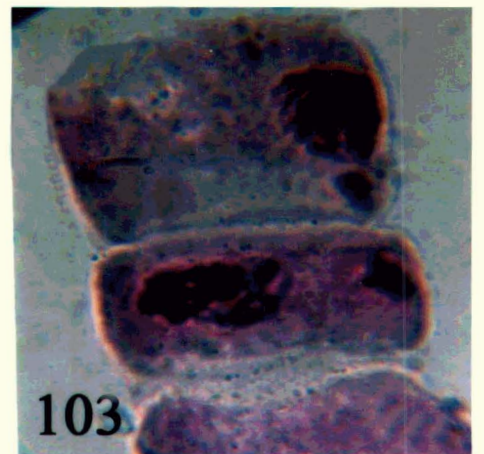
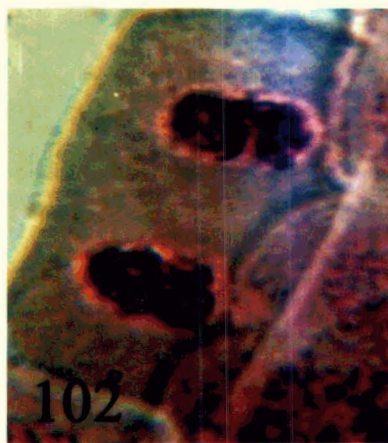
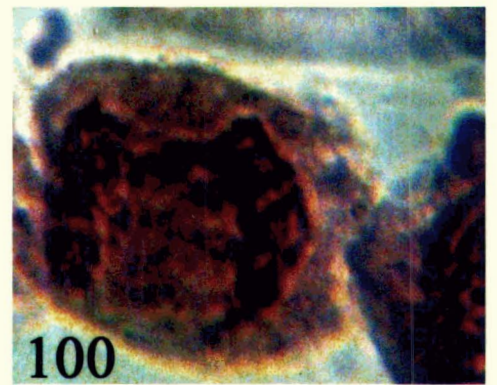
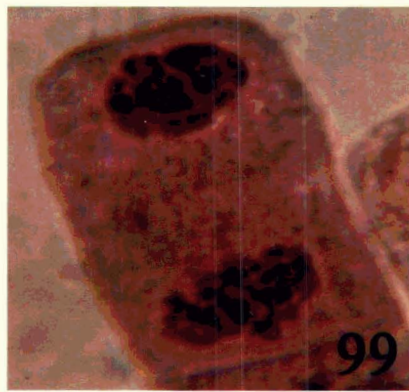
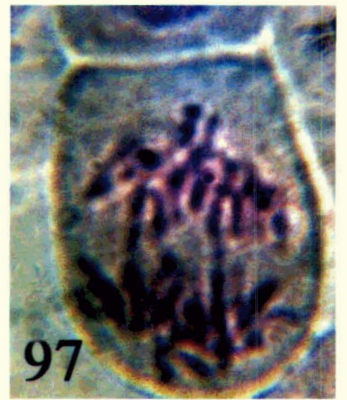
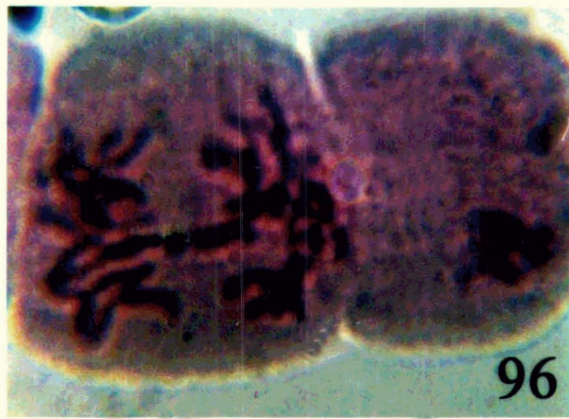
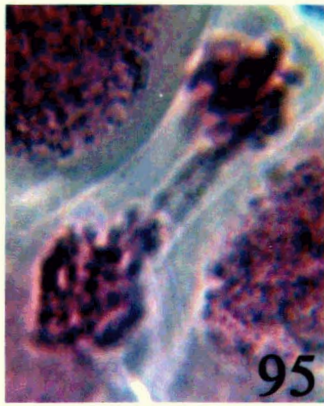
Fig. No. 104 Sticky telophase showing multiple bridges.

Fig. No. 105 Diagonal and sticky anaphase and telophase cells.

Fig. No. 106 Complete stickiness of early telophase chromosomes.



# Plate 13



## Figure Legend

### Plate No. 14

Fig. No. 107 Late telophase with unequal groups of chromosomes.

Fig. No. 108 Sticky crescent shaped chromosome groups at early telophase.

Fig. No. 109 Abnormal binucleate cell.

Fig. No. 110 Diagonally oriented binucleate cell and two daughter cells with distortion.

Fig. No. 111 Unequal cell division with one daughter cell showing bizarre form of nucleus.

Fig. No. 112 A cell with pycnotic crescent shaped micronuclei.

Fig. No. 113 Abnormal cell division with micro and macro cells.

Fig. No. 114 An abnormal cell showing two macronuclei and a micronucleus, (nuclear lesions are also found in one macronucleus).

Fig. No. 115 Interphase cell with a micronucleus.

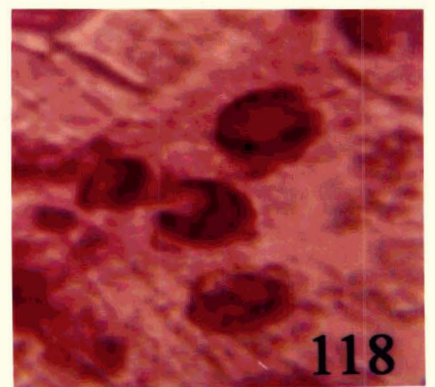
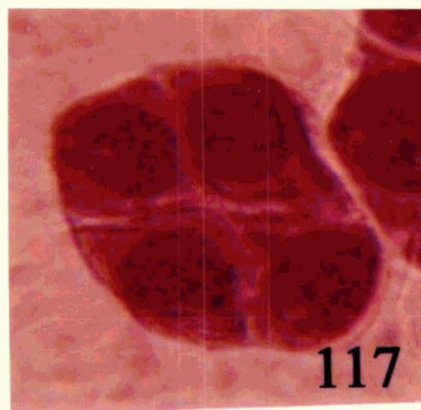
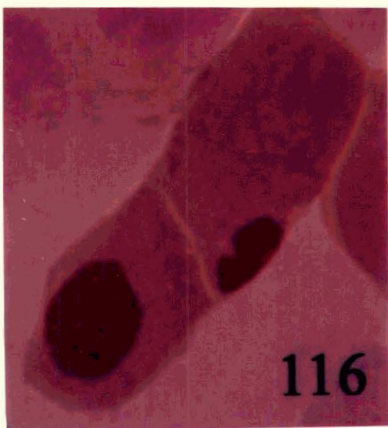
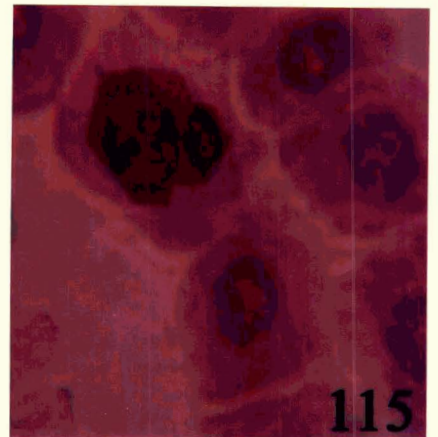
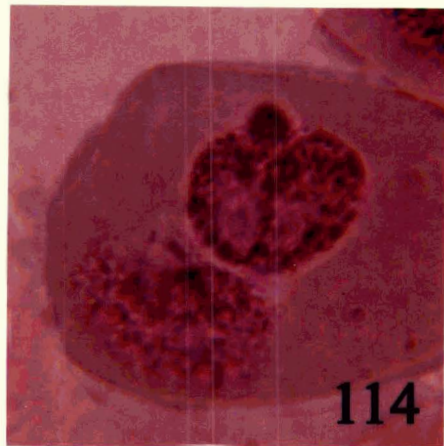
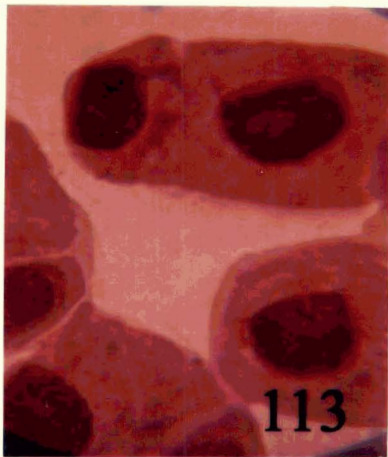
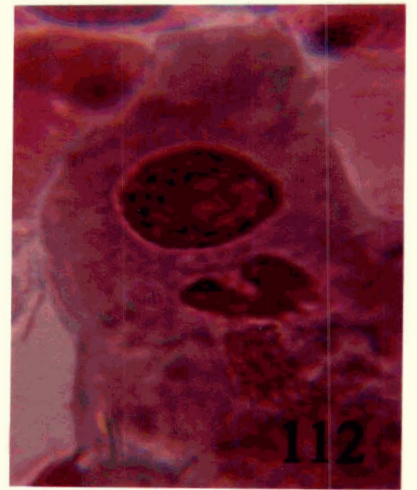
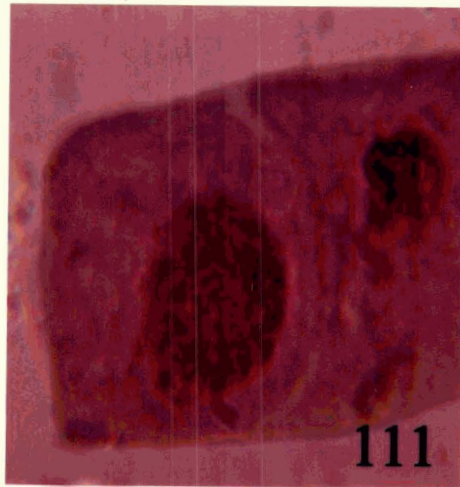
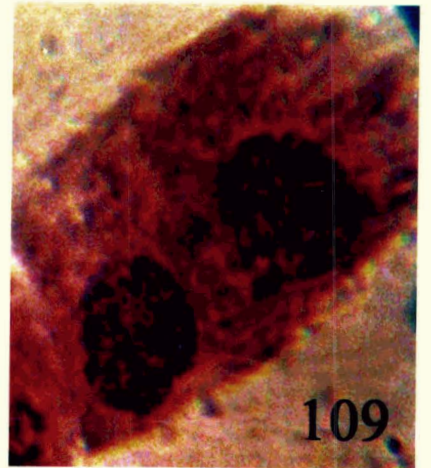
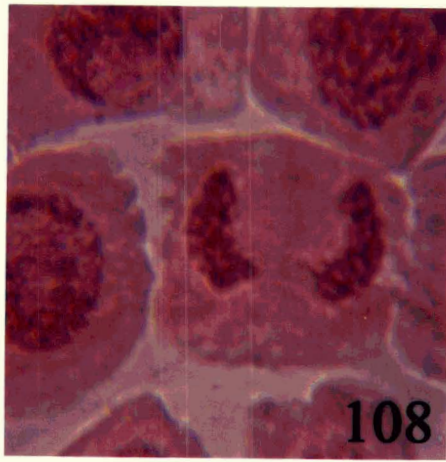
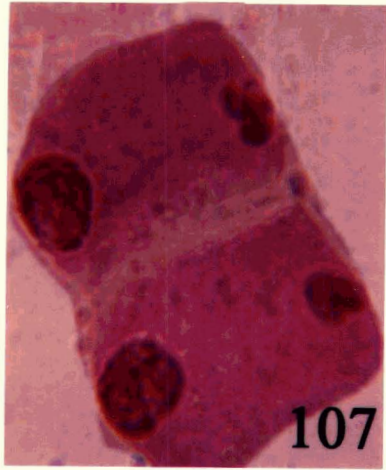
Fig. No. 116 Nuclear diminution and disintegration in a daughter cell.

Fig. No. 117 An abnormal cell showing non-equatorial partial separation of daughter cells.

Fig. No. 118 Crescent shaped nucleated cells.



Plate 14



## Figure Legend

### Plate No. 15

Fig. No.119 Multinucleated cells with five prophase nuclei and a metaphase plate.

Fig. No. 120 Unequal cell division with a macro and a micro cell.

Fig. No. 121 Abnormal division in an endomitotic cell.

Fig. No. 122 Mononucleate, binucleate, trinucleate and tetranucleate cells.

Fig.No. 123 Bizzare forms of chromosome groups, sticky anaphase and telophase.

Fig. No. 124 Abnormal scattering of chromosomes.

Fig. No. 125 Bizzare form of nucleus and nuclear disruption.

Fig. No. 126 Abnormal cells with disruption of nuclei and formation of lesions in the nuclei.

Fig. No. 127 Bizzare forms of nuclei at interphase stage and formation of macro and micronuclei.

Fig. No. 128 Nuclear diminution at interphase.

Fig. No. 129 Abnormal cell with multiple sticky metaphase plates.

Fig. No. 130 Abnormal mitotic divisional stages showing micronuclei and macro and micro cell formation.

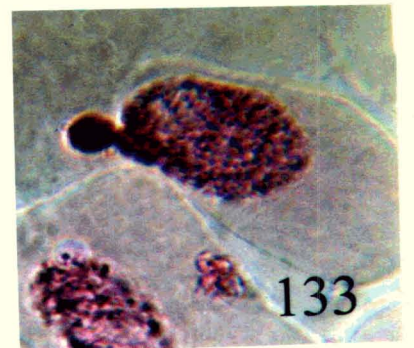
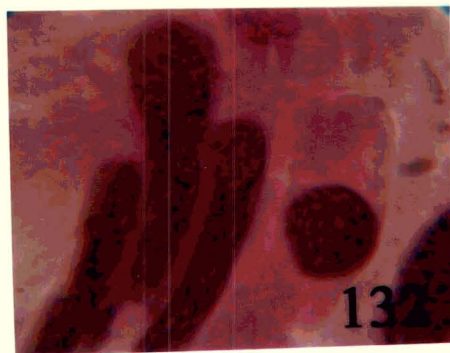
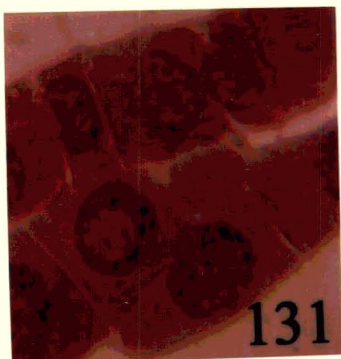
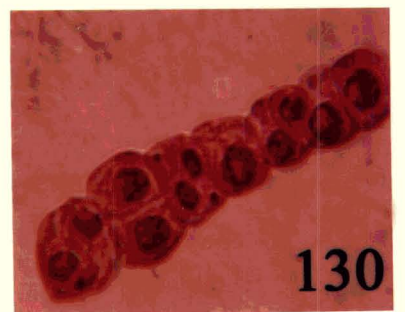
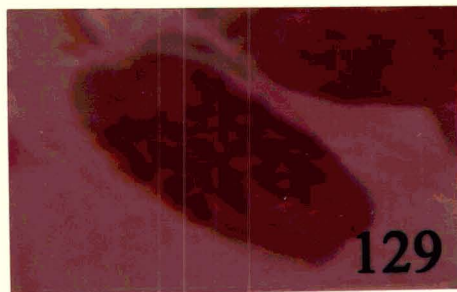
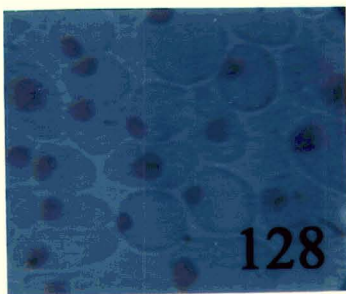
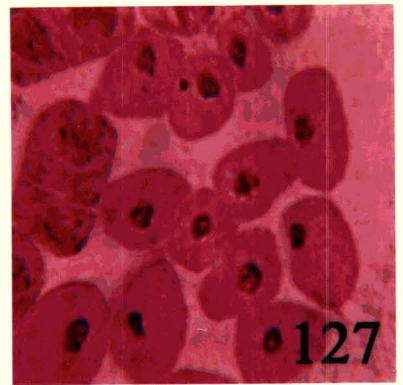
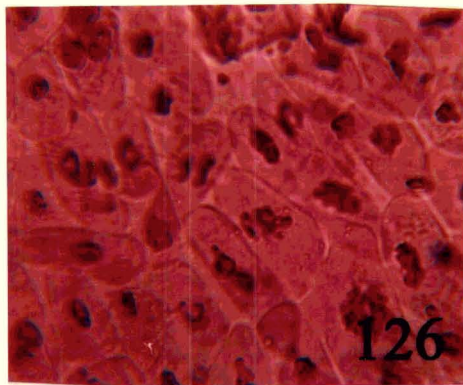
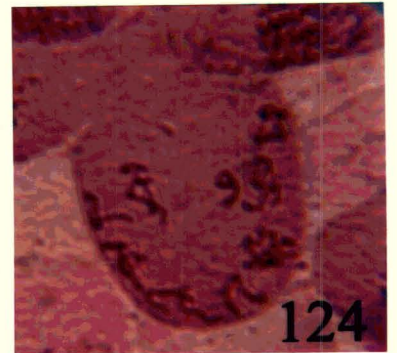
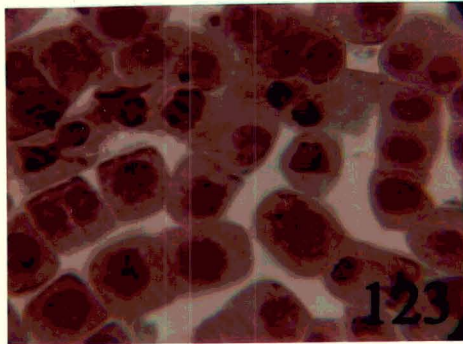
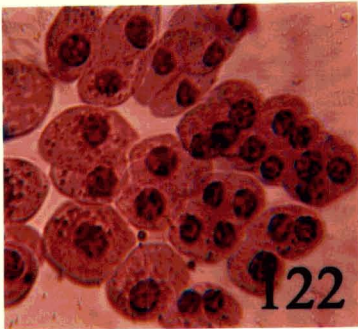
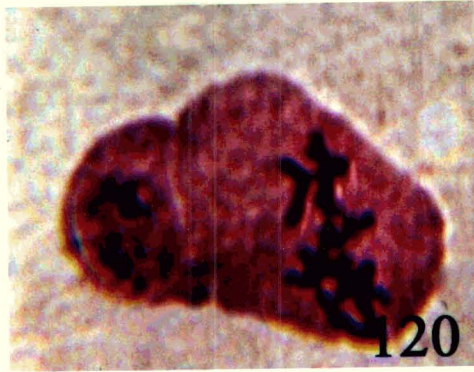
Fig. No. 131 A normal cell with nuclear lesion, a macro cell with nuclear lesion and a micro cell.

Fig. No. 132 Unusually elongated nuclei with lesions.

Fig. No. 133 A cell showing nuclear extrusion.



**Plate 15**





## B. ESSENTIAL OIL ANALYSIS

The volatile essential oils of 26 members of Myrtaceae showed varied chemical composition. Under the subfamily Leptospermoideae, of the 12 members studied, *Eucalyptus globulus* (7.35%) and *E. tereticornis* (6.62%) were found to be blessed with essential oil. The least percentage of oil content was detected in the crimson gum tree, *Corymbia ficifolia* (0.01%) and *Melaleuca styphelioides* (0.02%).

Under the Myrtoideae subfamily, of the 14 members studied, 3 members were found to contain high amount of leaf essential oil. They were clove or *Syzygium aromaticum* (6.6%), all spice or *Pimenta dioica* (6.4%) and myrtle or *Myrtus communis* (5.75%). The least oil content was observed in *Syzygium zeylanicum* and *Eugenia apiculata* with only 0.02%.

The detailed list of leaf essential oil yield in Myrtaceous taxa studied is shown in the Tables 31 and 32.

**Table : 31 Essential oil yield from the leaves of the plants studied under the subfamily – Leptospermoideae.**

Sl. No.	Name of the plant	Type	% of oil	Colour of oil
1	<i>Agonis flexuosa</i>	Oil rich	4.62	Pale yellow
2	<i>Beaufortia sparsa</i>	Oil rich	3.00	Yellow
3	<i>Callistemon citrinus</i>	Oil rich	2.73	Pale yellow
4	<i>Callistemon viminalis</i>	Oil rich	4.17	Pale yellow
5	<i>Corymbia citriodora</i>	Oil rich	5.00	Yellow
6	<i>Corymbia ficifolia</i>	Oil poor	0.01	Yellow
7	<i>Eucalyptus globulus</i>	Oil rich	7.35	Pale yellow
8	<i>Eucalyptus tereticornis</i>	Oil rich	6.62	Yellow
9	<i>Leptospermum nicholsii</i>	Oil rich	2.29	Pale yellow
10	<i>Melaleuca leucadendron</i>	Oil rich	2.50	Yellow
11	<i>Melaleuca styphelioides</i>	Oil poor	0.02	Yellow
12	<i>Syncarpia glomulifera</i>	Oil rich	2.94	Yellow

**Table 32 :** Essential oil yield from the leaves of the plants studied under the subfamily *Myrtoideae*.

Sl. No.	Name of the plant	Type	% of oil	Colour of oil
13	<i>Acmena smithii</i>	Oil poor	0.04	Yellow
14	<i>Eugenia apiculata</i>	Oil poor	0.02	Yellow
15	<i>Eugenia uniflora</i>	Oil poor	0.18	Yellow
16	<i>Feijoa sellowiana</i>	Oil poor	0.83	Greenish yellow
17	<i>Myrtus communis</i>	Oil rich	5.75	Reddish yellow
18	<i>Pimenta dioica</i>	Oil rich	6.40	Pale yellow
19	<i>Psidium guajava</i>	Oil poor	0.67	Pale yellow
20	<i>Syzygium aromaticum</i>	Oil rich	6.60	Pale yellow
21	<i>Syzygium cumini</i>	Oil poor	0.46	Pale yellow
22	<i>Syzygium jambos</i>	Oil poor	0.15	Yellow
23	<i>Syzygium malaccense</i>	Oil poor	0.03	Yellow
24	<i>Syzygium samarangense</i>	Oil poor	0.03	Yellow
25	<i>Syzygium zeylanicum</i>	Oil poor	0.02	Yellow
26	<i>Rhodomyrtus tomentosa</i>	Oil poor	0.11	Yellow

A wide range of chemical compounds were detected in the essential oils of the members of Myrtaceae in the present study.

Details of essential oil analysis of each taxa are shown below.

### **Subfamily : Leptospermoideae**

#### **1. *Agonis flexuosa***

Essential oil obtained from the leaves of *A. flexuosa* on GC analysis showed the presence of 17 major and trace chemicals (Table 33 ). Myrcene was found to be the dominant compound.  $\alpha$  - thujene, limonene,  $\gamma$  - terpinene, germacrene, sabinyl acetate,  $\alpha$  - phellandrene, isocaryophyllene,  $\alpha$  - terpinene and isoeugenol were found to be the other major chemical components. Seven other constituents were also detected in trace amounts (less than 1%).

The essential oil compounds detected were depicted in the Table 33.

Table : 33 Details of the GC analysis of leaf essential oil of *A. flexuosa*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - thujene	Monoterpenoid	12.53
2	$\alpha$ - phellandrene	"	3.47
3	myrcene	"	45.84
4	limonene	"	9.05
5	$\alpha$ - terpinene	"	1.84
6	$\beta$ - terpinene	"	0.42
7	sabinyl acetate	"	5.38
8	$\gamma$ - terpinene	"	7.66
9	citriodorol	Sesquiterpenoid	0.36
10	$\alpha$ - thujone	Monoterpenoid	0.35
11	methyl eugenol	Phenolic compound	0.32
12	iso eugenol	"	1.02
13	eugenyl acetate	"	0.31
14	$\beta$ - elemene	Sesquiterpenoid	0.42
15	aromadendrene	"	0.13
16	germacrene	"	5.43
17	iso caryophyllene	"	2.53

## 2. *Beaufortia sparsa*

The essential oil procured from the leaves of *Beaufortia sparsa* revealed 13 compounds and the dominant compound was  $\alpha$  - terpinene. Other major compounds include myrcene, 1,8 - cineole,  $\beta$  - caryophyllene, methyl benzoate,  $\delta$  - selinene and  $\beta$  - terpinene. Six trace components were also detected in the leaf essential oil (Table 34).

**Table : 34** Details of the GC analysis of leaf essential oil of *B. sparsa*

No.	Name of the compound	Class	Percentage yield
1	$\beta$ - pinene	Monoterpenoid	0.41
2	myrcene	"	5.96
3	1,8- cineole	"	2.28
4	limonene	"	0.19
5	$\alpha$ - terpinene	"	78.27
6	$\beta$ - terpinene	"	1.39
7	$\gamma$ - terpinene	"	0.25
8	citronellyl acetate	"	0.26
9	methyl benzoate	"	1.79
10	methyl cinnamate	"	0.61
11	eugenol	Phenolic compound	0.67
12	$\beta$ - caryophyllene	Sesquiterpenoid	2.52
13	$\delta$ - selinene	"	1.71

### 3. *Callistemon citrinus*

Essential oil obtained from the leaves of *Callistemon citrinus* showed some volatile compounds of which 12 were detected. The dominant compound identified was citral. Substantial amount of eugenol was detected together with other major oil components, viz. citronellol,  $\beta$  - elemene, isoeugenol,  $\beta$  - caryophyllene,  $\beta$  - thujene,  $\beta$  - pinene and citronellal. In addition to these, three compounds were detected in trace amounts.

The identified major and trace compounds and their yield are shown in the Table 35.

**Table : 35 Details of the GC analysis of leaf essential oil of *C. citrinus***

No.	Name of the compound	Class	Percentage yield
1	$\beta$ - thujene	Monoterpenoid	2.28
2	$\beta$ - pinene	"	2.03
3	citral	"	64.21
4	limonene	"	0.35
5	$\alpha$ - terpinene	"	0.36
6	citronellal	"	1.21
7	citronellol	"	4.89
8	eugenol	Phenolic compound	14.31
9	iso eugenol	"	2.89
10	eugenyl acetate	"	0.74
11	$\beta$ - elemene	Sesquiterpenoid	3.64
12	$\beta$ - caryophyllene	"	2.40

#### 4. *Callistemon viminalis*

Fifteen chemical compounds were detected from the essential oil of *Callistemon viminalis* leaves. The most important compound found in this oil is 1,8 - cineole. Substantial amount of citronellol together with other major components like  $\beta$  - pinene, sabinene, germacrene, citronellal,  $\alpha$  - pinene and  $\delta$  - selinene were also obtained. The yield of seven other constituents were in traces. Table 36 shows the details obtained after GC analysis.

Table : 36 Details of the GC analysis of leaf essential oil of *C. viminalis*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	3.53
2	$\beta$ - pinene	"	2.35
3	sabinene	"	2.85
4	1,8- cineole	"	61.37
5	$\beta$ - terpinene	"	0.37
6	citronellal	"	2.56
7	citronellol	"	14.43
8	methyl cinnamate	"	0.92
9	terpinen - 4 - ol	"	0.31
10	iso eugenol	Phenolic compound	0.10
11	eugenyl acetate	"	0.40
12	germacrene	Sesquiterpenoid	2.75
13	$\alpha$ - humulene	"	0.87
14	$\delta$ - selinene	"	2.01
15	$\gamma$ - cadinene	"	0.33

### 5. *Corymbia citriodora*

GC analysis of the essential oil obtained from the leaves of *C. citriodora* revealed 17 compounds, of which citronellal was detected as the dominant compound. Other major essential oil constituents obtained were  $\alpha$  - thujone, carvacrol, citronellol, 1,8 - cineole, isoborneol,  $\alpha$  - terpineol and methyl cinnamate. Nine other compounds were detected in trace amounts. The details are shown in the Table 37.

Table: 37 Details of the GC analysis of leaf essential oil of *Corymbia citriodora*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	0.19
2	$\alpha$ - phellandrene	"	0.19
3	sabinene	"	0.72
4	myrcene	"	0.75
5	linalool	"	0.95
6	1,8 - cineole	"	3.13
7	$\alpha$ - terpinolene	"	0.56
8	citronellal	"	61.40
9	citronellol	"	5.07
10	citronellyl acetate	"	0.35
11	methyl cinnamate	"	1.23
12	carvacrol	Phenolic compound	6.83
13	$\alpha$ - thujone	Monoterpenoid	7.06
14	iso borneol	"	2.68
15	$\alpha$ - terpineol	"	1.29
16	$\beta$ - elemene	Sesquiterpenoid	0.56
17	$\alpha$ - humulene	"	0.95

### 6. *Corymbia ficifolia*

The essential oil obtained from the leaves of *Corymbia ficifolia* revealed nineteen compounds, of which terpinyl acetate was detected as the dominant compound.  $\gamma$  - cadinene, chavicol, globulol, methyl cinnamate, bicyclogermacrene,  $\alpha$  - terpinolene, iso bornyl acetate,  $\beta$  - terpineol,  $\beta$  - farnesene and caryophyllene oxide were the other major components. Eight other compounds were present only in minor amounts. The details are shown in the Table 38.



Table: 38 Details of the GC analysis of leaf essential oil of *C. ficifolia*

No.	Name of the compound	Class	Percentage yield
1	sabinene	Monoterpenoid	0.11
2	linalool	"	0.41
3	$\alpha$ - terpinene	"	0.61
4	$\alpha$ - terpinolene	"	2.12
5	$\beta$ - terpinene	"	0.46
6	citronellal	"	0.28
7	citronellyl acetate	"	0.47
8	terpinyl acetate	"	55.33
9	methyl cinnamate	"	4.19
10	globulol	Sesquiterpenoid	5.21
11	chavicol	Phenolic compound	5.66
12	$\beta$ - terpineol	Monoterpenoid	1.36
13	iso bornyl acetate	"	1.44
14	$\beta$ - caryophyllene	Sesquiterpenoid	0.55
15	bicyclogermacrene	"	4.02
16	$\gamma$ - cadinene	"	8.10
17	caryophyllene oxide	"	1.25
18	$\beta$ - farnesene	"	1.35
19	$\beta$ - cadinol	"	0.90

### 7. *Eucalyptus globulus*

Twenty three compounds were detected from the essential oil obtained from the leaves of *E. globulus*. The essential oil showed  $\alpha$  - pinene, cymene, iso bornyl acetate,  $\gamma$  - terpinene, piperitone oxide,  $\alpha$  - humulene,  $\beta$  - terpinene,  $\alpha$  - terpinene, limonene, germacrene,  $\beta$  - farnesene, aromadendrene, isoeugenol,  $\beta$  - elemene, myrcene, camphene and methyl chavicol as the most important components. Six compounds were detected in traces. The detailed results are shown in the Table 39.

Table : 39 Details of the GC analysis of leaf essential oil of *E. globulus*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - thujene	Monoterpenoid	0.23
2	$\alpha$ - pinene	"	30.26
3	camphene	"	1.52
4	$\beta$ - pinene	"	0.53
5	myrcene	"	1.55
6	cymene	"	11.85
7	1,8 - cineole	"	0.22
8	limonene	"	2.14
9	$\alpha$ - terpinene	"	2.59
10	$\alpha$ - terpinolene	"	0.59
11	$\beta$ - terpinene	"	3.41
12	$\gamma$ - terpinene	"	6.80
13	terpinen 4 - ol	"	0.28
14	globulol	Sesquiterpenoid	0.43
15	methyl chavicol	Phenolic compound	1.10
16	iso eugenol	"	1.74
17	iso bornyl acetate	Monoterpenoid	7.75
18	$\beta$ - elemene	Sesquiterpenoid	1.64
19	aromadendrene	"	1.88
20	germacrene	"	1.92
21	$\alpha$ - humulene	"	3.68
22	piperitone oxide	Monoterpenoid	5.73
23	$\beta$ - farnesene	Sesquiterpenoid	1.89

### 8. *Eucalyptus tereticornis*

Sixteen chemical components were detected from the essential oil of *E. tereticornis*.  $\alpha$  - pinene and piperitone oxide were detected as the principal chemical compounds. Other major oil principles include linalool,  $\alpha$  - terpinolene, bornyl acetate,  $\beta$  - elemene, citronellyl acetate, iso caryophyllene, bicyclogermacrene and  $\beta$  - pinene. Six components were in traces (Table 40).

Table : 40 Details of the GC analysis of leaf essential oil of *E. tereticornis*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	34.32
2	$\beta$ - pinene	"	1.03
3	myrcene	"	0.49
4	linalool	"	4.85
5	estragole	"	0.57
6	$\alpha$ - terpinolene	"	3.27
7	citronellyl acetate	"	2.52
8	globulol	Sesquiterpenoid	0.17
9	$\alpha$ - terpineol	Monoterpenoid	0.69
10	bornyl acetate	"	2.78
11	$\beta$ - elemene	Sesquiterpenoid	2.74
12	aromadendrene	"	0.89
13	piperitone oxide	Monoterpenoid	36.19
14	iso caryophyllene	Sesquiterpenoid	2.24
15	bicyclogermacrene	"	1.08
16	$\alpha$ - farnesene	"	0.69

### 9. *Leptospermum nicholsii*

GC analysis of the hydrodistilled leaf essential oil of *L. nicholsii* showed 18 compounds.  $\alpha$  - pinene,  $\beta$  - phellandrene, citral and nerolidol were detected as the most important components. Other major oil components include farnesal, neral,  $\gamma$  - terpinene,  $\beta$  - bourbonene,  $\beta$  - caryophyllene,  $\beta$  - bisabolene, methyl eugenol and piperitone. Six oil components were observed in trace amounts. A detailed list of the components is depicted in the Table 41.

**Table : 41** Details of the GC analysis of leaf essential oil of *L. nicholsii*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	18.80
2	$\beta$ - pinene	"	0.26
3	sabinene	"	0.18
4	$\beta$ - phellandrene	"	15.17
5	citral	"	17.62
6	nerolidol	Sesquiterpenoid	17.48
7	neral	Monoterpenoid	4.27
8	$\beta$ - terpinene	"	0.35
9	methyl eugenol	Phenolic compound	1.52
10	$\gamma$ - terpinene	Monoterpenoid	3.39
11	terpinen-4-ol	"	0.12
12	$\beta$ - bisabolene	Sesquiterpenoid	1.69
13	$\beta$ - bourbonene	"	2.43
14	$\beta$ - caryophyllene	"	2.00
15	piperitone	Monoterpenoid	1.39
16	farnesal	Sesquiterpenoid	4.66
17	bicyclogermacrene	"	0.28
18	farnesol	"	0.12

#### 10. *Melaleuca leucadendron*

The essential oil obtained from the leaves of *M. leucadendron* on GC analysis showed the presence of several compounds. 25 compounds were detected.  $\beta$  - caryophyllene was found in substantial amount. Other major compounds include 1,8- cineole,  $\alpha$  - terpinolene,  $\gamma$  - cadinene, linalool, sabinene, myrcene,  $\alpha$  - pinene, methyl iso eugenol, iso caryophyllene,  $\beta$  - cadinene, farnesal, germacrene,  $\beta$  - elemene,  $\beta$  - bourbonene,  $\beta$  - terpinene, globulol and camphene. Seven oil components were also detected in trace amounts. The details are shown in the Table 42.

Table : 42 Details of the GC analysis of leaf essential oil of *M. leucadendron*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	4.30
2	camphene	"	1.27
3	sabinene	"	4.81
4	myrcene	"	4.77
5	linalool	"	5.86
6	1,8 - cineole	"	9.70
7	limonene	"	0.25
8	$\alpha$ - terpinolene	"	8.96
9	sabinyol acetate	"	0.21
10	$\beta$ - terpinene	"	1.53
11	citronellyl acetate	"	0.58
12	methyl benzoate	"	0.57
13	terpinyl acetate	"	0.51
14	citriodorol	Sesquiterpenoid	0.58
15	globulol	"	1.31
16	$\alpha$ - terpineol	Monoterpenoid	0.77
17	methyl iso eugenol	Phenolic compound	3.53
18	$\beta$ - elemene	Sesquiterpenoid	1.54
19	$\beta$ - bourbonene	"	1.50
20	germacrene	"	1.77
21	$\beta$ - caryophyllene	"	19.49
22	farnesal	"	1.88
23	$\beta$ - cadinene	"	2.01
24	iso caryophyllene	"	2.06
25	$\gamma$ - cadinene	"	7.97

### 11. *Melaleuca styphelioides*

The leaf essential oil of *M. styphelioides* on GC analysis showed the presence of several volatile compounds of which 17 were detected. The prominent ones include  $\gamma$  - cadinene,  $\alpha$  - cadinene,  $\beta$  - farnesene,  $\alpha$  - terpinene,  $\alpha$  - cadinol,  $\alpha$  - farnesene,  $\beta$  - caryophyllene and aromadendrene. Other major oil components include  $\alpha$  - terpineol, germacrene, terpinen - 4 -ol,  $\beta$  - elemene, sabinene and eugenyl acetate. Three compounds were detected in traces (Table 43).

**Table : 43** Details of the GC analysis of leaf essential oil of *M. styphelioides*

No.	Name of the compound	Class	Percentage yield
1	sabinene	Monoterpenoid	1.20
2	linalool	"	0.95
3	1,8 - cineole	"	0.34
4	estragole	"	0.28
5	$\alpha$ - terpinene	"	8.21
6	terpinen - 4 - ol	"	1.57
7	$\alpha$ - terpineol	"	3.79
8	eugenyl acetate	Phenolic compound	1.09
9	$\beta$ - elemene	Sesquiterpenoid	1.27
10	aromadendrene	"	4.22
11	germacrene	"	2.89
12	$\beta$ - caryophyllene	"	4.31
13	$\alpha$ - cadinene	"	16.64
14	$\gamma$ - cadinene	"	19.63
15	$\alpha$ - farnesene	"	6.64
16	$\beta$ - farnesene	"	8.52
17	$\alpha$ - cadinol	"	7.53

### 12. *Syncarpia glomulifera*

The essential oil obtained after the hydrodistillation of the leaves of *S. glomulifera* revealed several aroma compounds on GC analysis. out of which 14 compounds were detected.  $\alpha$  - terpinene and limonene were identified as the principal chemical compounds. Other major oil components like myrcene, methyl eugenol,  $\alpha$  - thujene,  $\beta$  - thujene,  $\beta$  - caryophyllene,  $\beta$  - phellandrene,  $\alpha$  - cadinene, globulol and 1,8 - cineole were also detected. Three compounds were detected in traces. The details are shown in the Table 44.

Table : 44 Details of the GC analysis of leaf essential oil of *S. glomulifera*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - thujene	Monoterpenoid	4.76
2	$\beta$ - thujene	"	4.42
3	myrcene	"	7.16
4	$\beta$ - phellandrene	"	3.74
5	1,8 - cineole	"	1.79
6	limonene	"	22.14
7	$\alpha$ - terpinene	"	28.26
8	methyl eugenol	Phenolic compound	6.18
9	globulol	Sesquiterpenoid	2.48
10	eugenol	Phenolic compound	0.53
11	methyl chavicol	"	0.54
12	$\alpha$ - terpineol	Monoterpenoid	0.93
13	$\beta$ - caryophyllene	Sesquiterpenoid	3.90
14	$\alpha$ - cadinene	"	3.25

### Sub family : Myrtoideae

#### 13. *Acmena smithii*

GC analysis of the leaf essential oil of *A. smithii* revealed 17 aromatic compounds in which the major components were  $\alpha$  - farnesol, piperitone, methyl iso eugenol, iso caryophyllene,  $\gamma$  - terpinene, cymene, methyl eugenol,  $\beta$  - bisabolene,  $\beta$  - elemene,  $\alpha$  - pinene,  $\beta$  - bourbonene,  $\alpha$  - terpineol, methyl eugenin and eugenol. Three oil components were detected in trace amounts. Details are shown in the Table 45.



Table : 45 Details of the GC analysis of leaf essential oil of *A. smithii*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	3.00
2	cymene	"	6.19
3	neral	"	0.60
4	eugenin	Phenolic compound	0.55
5	neryl acetate	Monoterpenoid	0.94
6	methyl eugenin	Phenolic compound	1.40
7	$\gamma$ - terpinene	Monoterpenoid	7.88
8	eugenol	Phenolic compound	1.11
9	methyl eugenol	"	3.96
10	$\alpha$ - terpineol	Monoterpenoid	1.82
11	methyl iso eugenol	Phenolic compound	9.34
12	$\beta$ - elemene	Sesquiterpenoid	3.58
13	$\beta$ - bisabolene	"	3.63
14	$\beta$ - bourbonene	"	2.62
15	piperitone	Monoterpenoid	13.58
16	iso caryophyllene	Sesquiterpenoid	9.19
17	$\alpha$ - farnesol	"	30.60

#### 14. *Eugenia apiculata*

The essential oil obtained from the leaves of *E. apiculata* on GC analysis showed the presence of several compounds of which 15 compounds were detected. The dominant compound was identified as methyl eugenin. Other major compounds viz.  $\alpha$  - thujone, carvacrol, citronellol, 1,8 - cineole and  $\alpha$  - terpineol were also detected. Majority of the compounds detected are in traces. The details are shown in the Table 46.

**Table : 46** Details of the GC analysis of leaf essential oil of *E. apiculata*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - thujene	Monoterpenoid	0.09
2	$\alpha$ - pinene	"	0.19
3	$\alpha$ - phellandrene	"	0.22
4	$\beta$ - pinene	"	0.72
5	linalool	"	0.96
6	1,8 - cineole	"	3.06
7	methyl eugenin	Phenolic compound	64.08
8	citronellol	Monoterpenoid	5.10
9	methyl cinnamate	"	0.98
10	carvacrol	Phenolic compound	6.90
11	$\alpha$ - thujone	Monoterpenoid	7.40
12	methyl eugenol	Phenolic compound	0.83
13	$\alpha$ - terpineol	Monoterpenoid	1.64
14	methyl iso eugenol	Phenolic compound	0.41
15	$\alpha$ - humulene	Sesquiterpenoid	0.84

**15. *Eugenia uniflora***

The essential oil obtained from the leaves of *E. uniflora* comprises several odorous compounds of which 28 compounds were identified. The oil contains substantial amounts of piperitone oxide. Other major components include  $\beta$  - phellandrene,  $\alpha$  - terpinene, iso caryophyllene,  $\beta$  - farnesol,  $\beta$  - farnesene, caryophyllene oxide,  $\delta$  - selinene,  $\alpha$  - cadinene,  $\alpha$  - cadinol, bicyclo germacrene, aromadendrene,  $\alpha$  - terpineol, iso eugenyl acetate,  $\alpha$  - farnesene, iso eugenol,  $\beta$  - caryophyllene,  $\beta$  - cadinol, limonene, myrcene and  $\beta$  - thujene. Moreover, seven oil components were detected in traces (Table 47).

Table : 47 Details of the GC analysis of leaf essential oil of *E. uniflora*

No.	Name of the compound	Class	Percentage yield
1	$\beta$ - thujene	Monoterpenoid	1.07
2	$\alpha$ - phellandrene	"	0.23
3	$\beta$ - pinene	"	0.21
4	myrcene	"	1.08
5	$\beta$ - phellandrene	"	6.80
6	limonene	"	1.16
7	$\alpha$ - terpinene	"	5.00
8	methyl eugenin	Phenolic compound	0.63
9	$\gamma$ - terpinene	Monoterpenoid	0.89
10	eugenol	Phenolic compound	0.21
11	$\alpha$ - terpineol	Monoterpenoid	2.60
12	iso eugenol	Phenolic compound	2.33
13	iso eugenyl acetate	"	2.52
14	aromadendrene	Sesquiterpenoid	2.74
15	$\beta$ - elemene	"	0.90
16	piperitone oxide	Monoterpenoid	24.35
17	$\beta$ - caryophyllene	Sesquiterpenoid	2.14
18	$\alpha$ - cadinene	"	3.53
19	$\delta$ - selinene	"	3.67
20	iso caryophyllene	"	4.71
21	bicyclogermacrene	"	3.02
22	$\alpha$ - farnesene	"	2.36
23	caryophyllene oxide	"	3.76
24	$\beta$ - farnesene	"	3.83
25	$\alpha$ - cadinol	"	3.19
26	$\alpha$ - farnesol	"	0.76
27	$\beta$ - cadinol	"	1.44
28	$\beta$ - farnesol	"	4.44

#### 16. *Feijoa sellowiana*

The GC analysis of essential oil obtained from the leaves of *F. sellowiana* showed many compounds of which 19 were identified. The oil shows the presence of limonene and  $\beta$  - caryophyllene as the principal components. It also contains substantial amount of citronellyl acetate. Other major oil components

detected include  $\alpha$  - pinene,  $\beta$  - pinene, iso caryophyllene and estragole. The essential oil also contains an array of compounds, which were detected only in trace amounts. The details of the GC analysis are shown in the Table 48.

**Table : 48** Details of the GC analysis of leaf essential oil of *F. sellowiana*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	8.70
2	$\beta$ - pinene	"	3.11
3	myrcene	"	0.16
4	limonene	"	29.29
5	estragole	"	1.35
6	neryl acetate	"	0.53
7	sabinyol acetate	"	0.60
8	$\gamma$ - terpinene	"	0.84
9	citronellyl acetate	"	17.08
10	$\beta$ - terpineol	"	0.33
11	methyl iso eugenol	Phenolic compound	0.79
12	eugenyl acetate	"	0.23
13	aromadendrene	Sesquiterpenoid	0.28
14	germacrene	"	0.39
15	$\beta$ - caryophyllene	"	27.38
16	$\beta$ - cadinene	"	0.54
17	iso caryophyllene	"	1.37
18	$\gamma$ - cadinene	"	0.97
19	farnesol	"	0.31

### 17. *Myrtus communis*

Twenty two essential oil components were detected from the leaves of *M. communis* by GC analysis. The oil contains substantial amount of  $\beta$  - thujene, which forms the prominent component.  $\beta$  - caryophyllene,  $\alpha$  - terpinene,  $\beta$  - phellandrene, limonene,  $\alpha$  - cadinene, methyl iso eugenol,  $\beta$  - elemene,  $\gamma$  - terpinene, myrcene, piperitone,  $\alpha$  - thujone, iso eugenol,  $\beta$  - terpinene and methyl chavicol were the other major essential oil components detected. Seven compounds

were detected only in trace amounts. The detailed account of GC analysis is given in Table 49.

**Table : 49 Details of the GC analysis of leaf essential oil of *M. communis***

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - thujene	Monoterpenoid	0.13
2	$\beta$ - thujene	"	21.73
3	$\alpha$ - pinene	"	0.62
4	$\beta$ - pinene	"	0.50
5	myrcene	"	3.11
6	$\beta$ - phellandrene	"	8.65
7	linalool	"	0.47
8	limonene	"	6.52
9	$\alpha$ - terpinene	"	9.28
10	$\alpha$ - terpinolene	"	0.73
11	$\beta$ - terpinene	"	1.36
12	$\gamma$ - terpinene	"	3.99
13	$\alpha$ - thujone	"	1.72
14	globulol	Sesquiterpenoid	0.47
15	eugenol	Phenolic compound	0.91
16	methyl chavicol	"	1.18
17	iso eugenol	"	1.41
18	methyl iso eugenol	"	5.52
19	$\beta$ - elemene	Sesquiterpenoid	4.28
20	$\beta$ - caryophyllene	"	9.55
21	piperitone	Monoterpenoid	3.01
22	$\alpha$ - cadinene	Sesquiterpenoid	6.52

### 18. *Pimenta dioica*

The essential oil from the leaves of *P. dioica* on GC analysis showed the presence of a few compounds, of which 11 compounds were detected. The major component was identified as iso eugenol. The major component, 1,8 - cineole was present in substantial amount. Other major oil principles viz. chavicol,  $\alpha$  - terpinene,  $\beta$  - caryophyllene and cymene were also detected. In addition to

these, the oil contains five chemical principles in traces. The details of GC analysis are shown in Table 50.

**Table : 50** Details of the GC analysis of leaf essential oil of *P. dioica*

No.	Name of the compound	Class	Percentage yield
1	sabinene	Monoterpenoid	0.47
2	cymene	"	1.47
3	1,8 - cineole	"	14.38
4	estragole	"	0.75
5	$\alpha$ - terpinene	"	4.38
6	$\beta$ - terpinene	"	0.84
7	citriodorol	Sesquiterpenoid	0.37
8	chavicol	Phenolic compound	8.60
9	iso eugenol	"	62.48
10	$\beta$ - caryophyllene	Sesquiterpenoid	3.15
11	bicyclogermacrene	"	0.80

### 19. *Psidium guajava*

Essential oil procured from the leaves of *P. guajava* showed several chemical principles, of which 19 were identified. The oil shows substantial amounts of  $\alpha$  - selinene, iso eugenol, myrcene and  $\delta$  - selinene.  $\beta$  - caryophyllene, eugenyl acetate, iso caryophyllene, piperitone,  $\beta$  - elemene,  $\gamma$  - cadinene, farnesol, 1,8 - cineole,  $\alpha$  - terpineol,  $\beta$  - bisabolene and  $\gamma$  - terpinene were the other major oil components detected. Four compounds in trace amounts were also detected from the essential oil. The detailed account of GC analysis is shown in the Table 51.

**Table : 51** Details of the GC analysis of leaf essential oil of *P. guajava*

No.	Name of the compound	Class	Percentage yield
1	$\beta$ - thujene	Monoterpenoid	0.54
2	$\alpha$ - pinene	"	0.70
3	$\beta$ - pinene	"	0.45
4	myrcene	"	13.65
5	1,8 - cineole	"	2.08
6	limonene	"	0.40
7	$\gamma$ - terpinene	"	1.46
8	$\alpha$ -terpineol	"	1.98
9	iso eugenol	Phenolic compound	16.47
10	eugenyl acetate	"	3.59
11	$\beta$ -elemene	Sesquiterpenoid	2.82
12	$\beta$ - bisabolene	"	1.92
13	$\alpha$ - selinene	"	18.54
14	$\beta$ - caryophyllene	"	7.28
15	piperitone	Monoterpenoid	3.03
16	$\delta$ - selinene	Sesquiterpenoid	11.05
17	iso caryophyllene	"	3.18
18	$\gamma$ - cadinene	"	2.64
19	farnesol	"	2.36

## 20. *Syzygium aromaticum*

Leaf essential oil of *S. aromaticum* on GC analysis revealed the presence of 16 compounds. The dominant compound was 1,8- cineole.  $\gamma$  - terpinene,  $\alpha$  - pinene, germacrene, citronellal,  $\beta$  - pinene, nerolidol and  $\delta$  - selinene were the other major oil principles detected. Eight components in trace amounts were also detected in the present study. Table 52 depict the details of GC analysis.



Table : 52 Details of the GC analysis of leaf essential oil of *S. aromaticum*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	7.67
2	$\beta$ - pinene	"	1.72
3	1,8 cineole	"	61.27
4	nerolidol	Sesquiterpenoid	1.40
5	$\beta$ - terpinene	Monoterpenoid	0.68
6	citronellal	"	2.16
7	$\gamma$ - terpinene	"	8.81
8	methyl benzoate	"	0.63
9	terpinen-4-ol	"	0.81
10	eugenol	Phenolic compound	0.29
11	$\alpha$ - terpineol	Monoterpenoid	0.25
12	iso eugenol	Phenolic compound	0.56
13	germacrene	Sesquiterpenoid	3.55
14	$\delta$ - selinene	"	1.06
15	iso caryophyllene	"	0.60
16	$\gamma$ - cadinene	"	0.30

### 21. *Syzygium cumini*

The GC analysis of essential oil obtained from the leaves of *S. cumini* revealed 22 compounds, of which sabinene and  $\beta$ -phellandrene were found in substantial amounts. Other major oil components include eugenyl acetate,  $\delta$  - selinene, linalool, isocaryophyllene,  $\beta$  - elemene,  $\alpha$  - terpinene, isoeugenol,  $\beta$  - caryophyllene,  $\beta$ -farnesene, myrcene, methyl chavicol, eugenol, limonene,  $\beta$  - thujene and caryophyllene oxide. Five compounds were also detected in trace amounts. The details of GC analysis is shown in the Table 53.

Table : 53 Details of the GC analysis of leaf essential oil of *S. cumini*.

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - thujene	Monoterpenoid	0.48
2	$\beta$ - thujene	"	1.18
3	$\alpha$ - phellandrene	"	0.68
4	$\beta$ - pinene	"	0.99
5	sabinene	"	15.92
6	myrcene	"	1.52
7	$\beta$ - phellandrene	"	14.62
8	linalool	"	6.68
9	limonene	"	1.36
10	$\alpha$ - terpinene	"	4.23
11	methyl eugenol	Phenolic compound	0.67
12	$\gamma$ - terpinene	Monoterpenoid	0.79
13	eugenol	Phenolic compound	1.44
14	methyl chavicol	"	1.45
15	iso eugenol	"	4.05
16	eugenyl acetate	"	9.38
17	$\beta$ - elemene	Sesquiterpenoid	4.72
18	$\beta$ - caryophyllene	"	2.82
19	$\delta$ - selinene	"	6.69
20	iso caryophyllene	"	6.16
21	caryophyllene oxide	"	1.04
22	$\beta$ - farnesene	"	2.01

## 22. *Syzygium jambos*

The essential oil obtained from the leaves of *S. jambos* showed the existence of several compounds of which 23 compounds were detected. The oil shows the presence of  $\alpha$  - terpinene and  $\gamma$  - cadinene as the principal compounds.  $\alpha$  - thujone, bicyclogermacrene, eugenyl acetate, aromadendrene, farnesal,

germacrene, sabinene, myrcene and  $\alpha$  - farnesene were the other main components detected. In addition to these half of the components detected, occur in traces in the essential oil. The detailed account of GC analysis is shown in the Table 54.

**Table : 54 Details of the GC analysis of leaf essential oil of *S. jambos***

No.	Name of the compound	Class	Percentage yield
1	sabinene	Monoterpenoid	1.84
2	myrcene	"	1.58
3	1,8 - cineole	"	0.22
4	linalool	"	0.09
5	$\alpha$ - terpinene	"	24.58
6	$\beta$ - terpinene	"	0.74
7	methyl eugenin	Phenolic compound	0.19
8	methyl benzoate	Monoterpenoid	0.28
9	methyl cinnamate	"	0.50
10	$\alpha$ - thujone	"	12.04
11	eugenyl acetate	Phenolic compound	4.06
12	$\beta$ - elemene	Sesquiterpenoid	0.30
13	aromadendrene	"	3.65
14	germacrene	"	2.39
15	piperitone	Monoterpenoid	0.70
16	piperitone oxide	"	0.98
17	$\alpha$ - cadinene	Sesquiterpenoid	0.70
18	$\gamma$ - cadinene	"	28.58
19	bicyclogermacrene	"	4.40
20	farnesal	"	3.56
21	$\alpha$ - farnesene	"	1.27
22	$\beta$ - farnesene	"	0.47
23	$\alpha$ - cadinol	"	0.68

### 23. *Syzygium malaccense*

The hydrodistilled essential oil from the leaves of *S. malaccense* on GC analysis showed the presence of an array of compounds, of which 18 compounds were detected. The main compounds were identified as piperitone,  $\beta$  -

caryophyllene and  $\alpha$  - humulene. Other main constituents include methyl iso eugenol,  $\beta$  -elemene, iso caryophyllene, bicyclogermacrene, linalool,  $\beta$ -bisabolene,  $\alpha$  - terpineol, citronellyl acetate and eugenyl acetate. Six oil components were also found to occur in trace amounts. The details of GC analysis are shown below (Table 55).

**Table : 55 Details of the GC analysis of leaf essential oil of *S. malaccense***

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	0.64
2	$\beta$ - pinene	"	0.40
3	linalool	"	4.17
4	$\alpha$ - terpinolene	"	0.88
5	$\beta$ - terpinene	"	0.90
6	$\gamma$ - terpinene	"	0.79
7	citronellyl acetate	"	1.34
8	globulol	Sesquiterpenoid	0.41
9	$\alpha$ - terpineol	Monoterpenoid	1.57
10	methyl iso eugenol	Phenolic compound	6.34
11	eugenyl acetate	"	1.15
12	$\beta$ - elemene	Sesquiterpenoid	5.93
13	$\beta$ - bisabolene	"	3.63
14	$\beta$ - caryophyllene	"	12.15
15	$\alpha$ - humulene	"	10.22
16	piperitone	Monoterpenoid	15.26
17	iso caryophyllene	Sesquiterpenoid	5.86
18	bicyclogermacrene	"	5.48

#### 24. *Syzygium samarangense*

GC analysis of essential oil obtained from the leaves of *S. samarangense* revealed 20 compounds. The major constituents include piperitone, methyl iso eugenol,  $\beta$ -elemene, eugenol,  $\beta$ -farnesene,  $\alpha$ -farnesol,  $\beta$ -farnesol,  $\beta$ -cadinol, linalool, bicyclogermacrene,  $\alpha$ -cadinol, neral,  $\gamma$ -cadinol and

$\beta$  - bourbonene. Few compounds were also detected in traces. Three major compounds were not detected due to the unavailability of pure standards. The details are shown in the Table 56.

**Table : 56** Details of the GC analysis of leaf essential oil of *S. samarangense*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	0.52
2	$\beta$ - pinene	"	0.57
3	myrcene	"	0.47
4	$\beta$ - phellandrene	"	0.58
5	linalool	"	2.45
6	neral	"	1.63
7	neryl acetate	"	0.70
8	citronellyl acetate	"	0.57
9	eugenol	Phenolic compound	3.81
10	methyl iso eugenol	"	6.36
11	$\beta$ - elemene	Sesquiterpenoid	4.12
12	$\beta$ - bourbonene	"	1.00
13	piperitone	Monoterpenoid	6.66
14	bicyclogermacrene	Sesquiterpenoid	2.98
15	$\beta$ - farnesene	"	3.61
16	$\alpha$ - cadinol	"	2.04
17	$\alpha$ - farnesol	"	3.42
18	$\beta$ - cadinol	"	2.54
19	$\beta$ - farnesol	"	3.08
20	$\gamma$ - cadinol	"	1.33

## 25. *Syzygium zeylanicum*

The essential oil obtained after hydrodistillation of leaves of *S. zeylanicum* showed several compounds on GC analysis. 17 compounds were identified, of which  $\alpha$  - phellandrene and  $\beta$  - thujene were the principal compounds.  $\beta$  - phellandrene, iso caryophyllene,  $\gamma$  - terpinene,  $\delta$  - selinene, linalyl acetate, neryl acetate,  $\beta$  - bisabolene,  $\alpha$  - terpinene, piperitone oxide,

$\beta$  - caryophyllene, citronellyl acetate and eugenyl acetate were the other major components. Only three compounds were detected in traces. The details are depicted in the Table 57.

**Table : 57** Details of the GC analysis of leaf essential oil of *S. zeylanicum*

No.	Name of the compound	Class	Percentage yield
1	$\beta$ -thujene	Monoterpenoid	22.01
2	$\alpha$ -pinene	"	0.71
3	$\alpha$ -phellandrene	"	32.08
4	$\beta$ - phellandrene	"	7.22
5	$\alpha$ -terpinene	"	1.73
6	linalyl acetate	"	3.15
7	neryl acetate	"	3.01
8	$\gamma$ - terpinene	"	4.19
9	citronellyl acetate	"	1.37
10	eugenol	Phenolic compound	0.83
11	iso eugenol	"	0.43
12	eugenyl acetate	"	1.12
13	$\beta$ -bisabolene	Sesquiterpenoid	2.26
14	$\beta$ -caryophyllene	"	1.48
15	piperitone oxide	Monoterpenoid	1.70
16	$\delta$ -selinene	Sesquiterpenoid	4.13
17	iso caryophyllene	"	4.65

## 26. *Rhodomyrtus tomentosa*

The essential oil obtained from the leaves of *R.tomentosa* shows a wide spectrum of compounds of which 16 were identified. The most important components were methyl chavicol and methyl eugenin. Other major components viz.  $\alpha$ -thujone,  $\delta$ -selinene, terpinen-4-ol, carvacrol, citronellal, methyl isoeugenol, 1,8-cineole,  $\alpha$  - humulene,  $\gamma$  - cadinene and aromadendrene were also detected. The essential oil also contains four compounds which were detected only in traces. The details of GC analysis are shown in Table 58.

Table : 58 Details of the GC analysis of leaf essential oil of *R. tomentosa*

No.	Name of the compound	Class	Percentage yield
1	$\alpha$ - pinene	Monoterpenoid	0.32
2	$\beta$ -pinene	"	0.49
3	sabinene	"	0.17
4	linalool	"	0.99
5	1,8-cineole	"	1.72
6	methyl eugenin	Phenolic compound	27.93
7	citronellal	Monoterpenoid	2.34
8	carvacrol	Phenolic compound	2.90
9	$\alpha$ -thujone	Monoterpenoid	9.88
10	terpinen-4-ol	"	3.19
11	methyl chavicol	Phenolic compound	28.56
12	methyl iso eugenol	"	1.85
13	aromadendrene	Sesquiterpenoid	1.02
14	$\alpha$ -humulene	"	1.45
15	$\delta$ -selinene	"	3.48
16	$\gamma$ -cadinene	"	1.39



**Table : 59 List of major essential oil components identified in the present investigation, arranged in order of their elution on SE 30 packed column.**

Sl.No.	Name of the essential oil component	Class
1.	$\alpha$ - thujene	Monoterpenoid
2	$\beta$ - thujene	"
3.	$\alpha$ -pinene	"
4	camphene	"
5	$\alpha$ -phellandrene	"
6	$\beta$ -pinene	"
7	sabinene	"
8	myrcene	"
9	$\beta$ -phellandrene	"
10	citral	"
11	cymene	"
12	linalool	"
13	1,8- cineole	"
14	limonene	"
15	nerolidol	Sesquiterpenoid
16	estragole	Monoterpenoid
17	$\alpha$ -terpinene	"
18	neral	"
19	$\alpha$ -terpinolene	"
20	eugenin	Phenolic compound
21	linalyl acetate	Monoterpenoid
22	$\beta$ -terpinene	"
23	neryl acetate	"
24	sabinyl acetate	"
25	methyl eugenin	Phenolic compound
26	citronellal	Monoterpenoid
27	citronellol	"
28	$\gamma$ -terpinene	"
29	citronellyl acetate	"
30	methyl benzoate	"
31	terpinyl acetate	"
32	borneol	"
33	methyl cinnamate	"
34	carvacrol	Phenolic compound
35	citriodorol	Sesquiterpenoid

36	$\alpha$ -thujone	Monoterpenoid
37	terpinen-4-ol	"
38	$\beta$ -thujone	"
39	globulol	Sesquiterpenoid
40	chavicol	Phenolic compound
41	eugenol	"
42	iso borneol	Monoterpenoid
43	methyl eugenol	Phenolic compound
44	methyl chavicol	"
45	$\alpha$ -terpineol	Monoterpenoid
46	$\beta$ -terpineol	"
47	iso eugenol	Phenolic compound
48	bornyl acetate	Monoterpenoid
49	methyl iso eugenol	Phenolic compound
50	iso bornyl acetate	Monoterpenoid
51	eugenyl acetate	Phenolic compound
52	iso eugenyl acetate	"
53	$\beta$ -elemene	Sesquiterpenoid
54	$\beta$ -bisabolene	"
55	aromadendrene	"
56	$\beta$ -bourbonene	"
57	$\alpha$ -selinene	"
58	germacrene	"
59	$\beta$ -caryophyllene	"
60	$\alpha$ -humulene	"
61	piperitone	Monoterpenoid
62	piperitone oxide	"
63	$\alpha$ -cadinene	Sesquiterpenoid
64	farnesal	"
65	$\delta$ -selinene	"
66	$\beta$ -cadinene	"
67	isocaryophyllene	"
68	bicyclogermacrene	"
69	$\gamma$ -cadinene	"
70	$\alpha$ -farnesene	"
71	caryophyllene oxide	"
72	$\beta$ -farnesene	"
73	$\alpha$ -cadinol	"
74	$\alpha$ -farnesol	"
75	$\beta$ -cadinol	"
76	$\beta$ -farnesol	"
77	$\gamma$ -cadinol	"

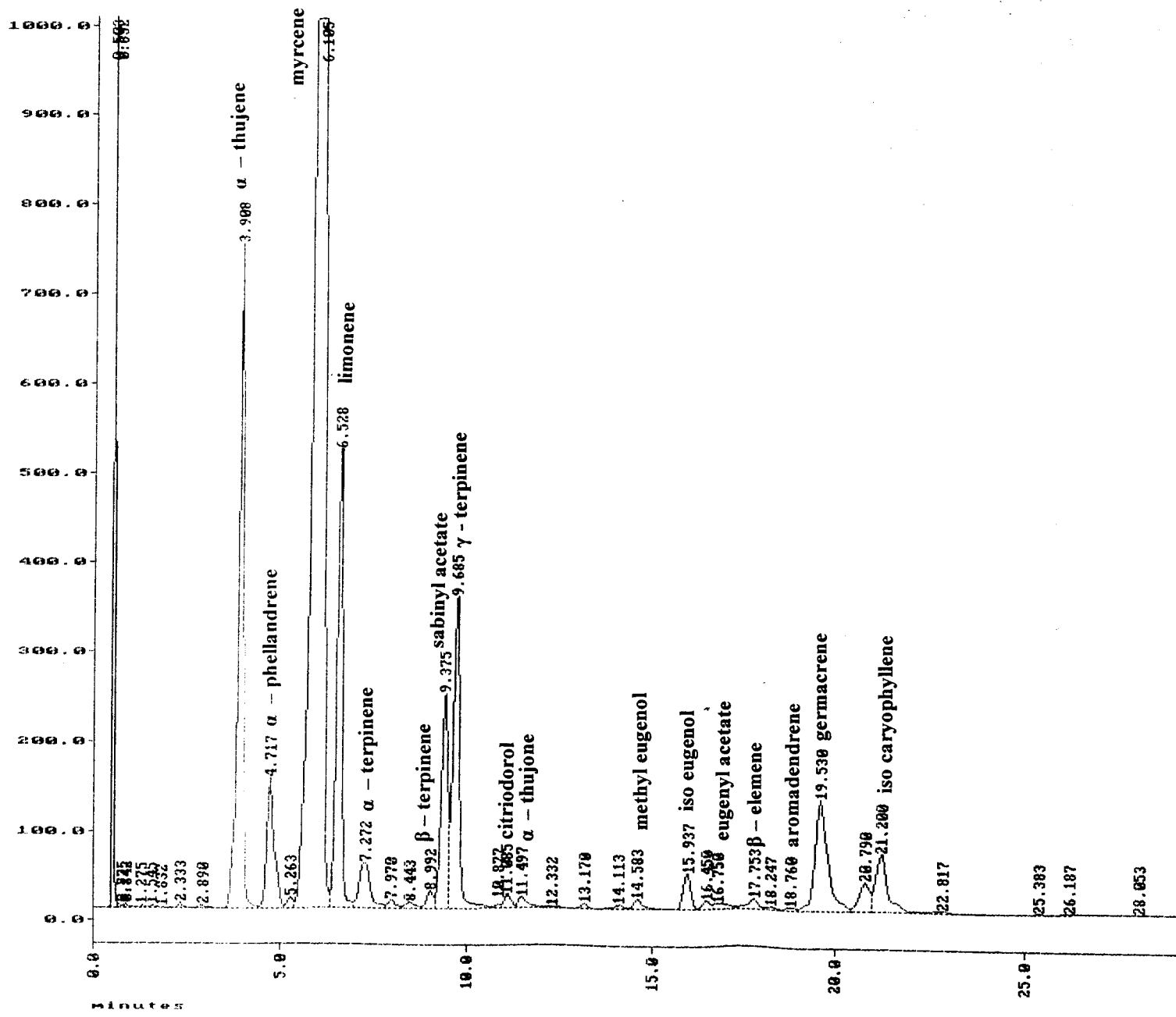


Fig. 134 Gas Liquid Chromatogram of the essential oil of *Agonis flexuosa*

87

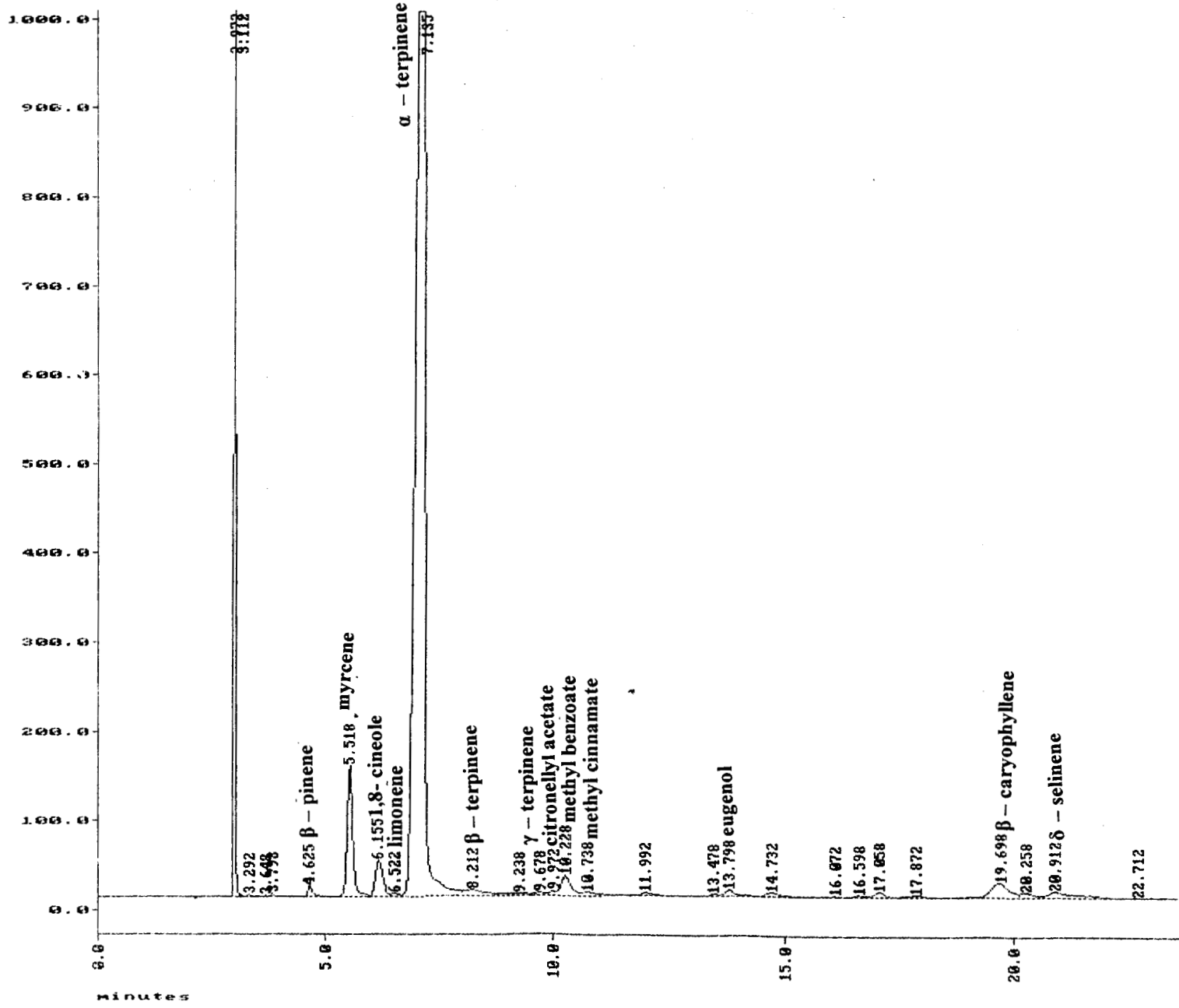


Fig. 135 Gas Liquid Chromatogram of the essential oil of *Beaufortia sparsa*

65

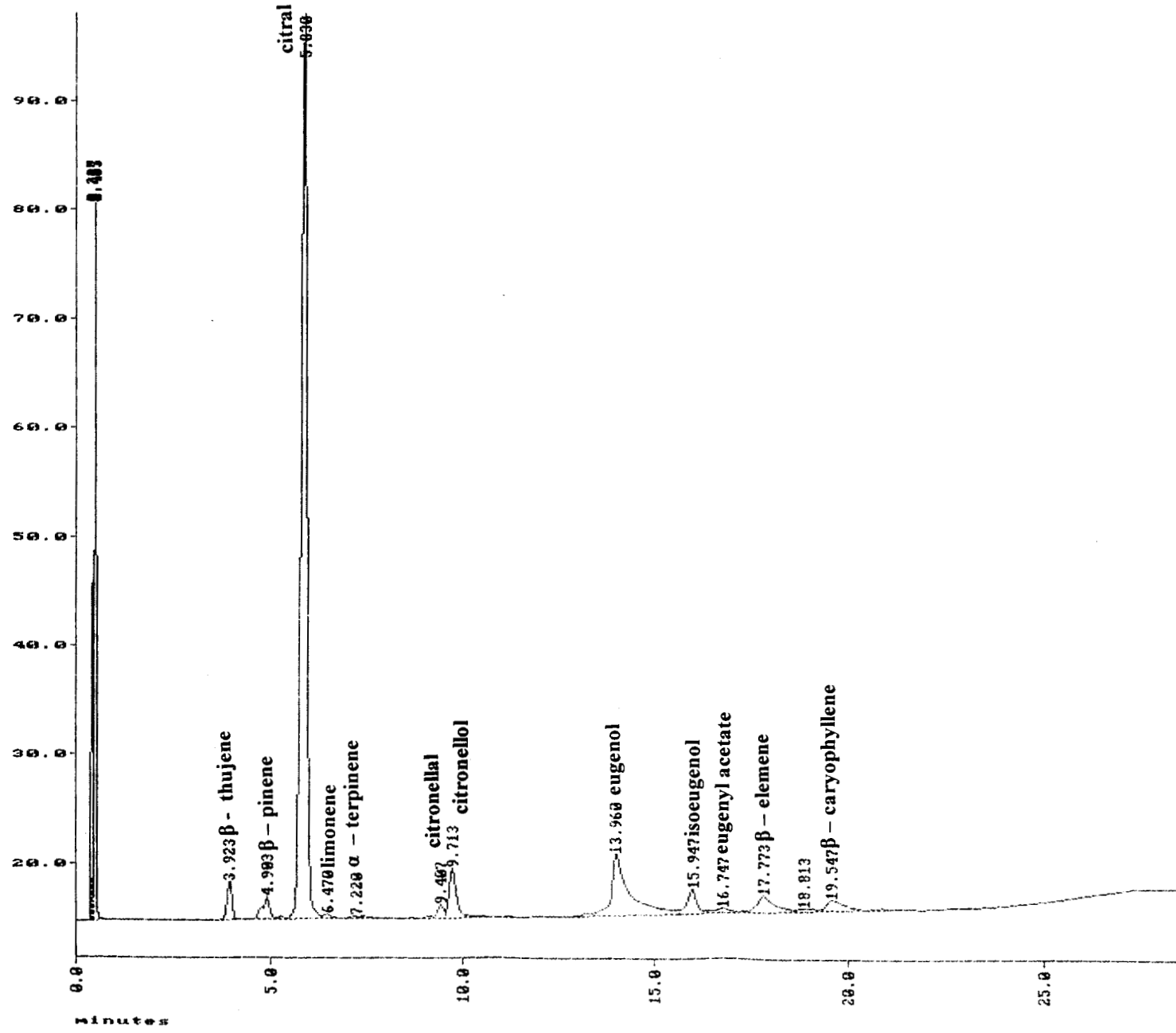


Fig. 136 Gas Liquid Chromatogram of the essential oil of *Callistemon citrinus*

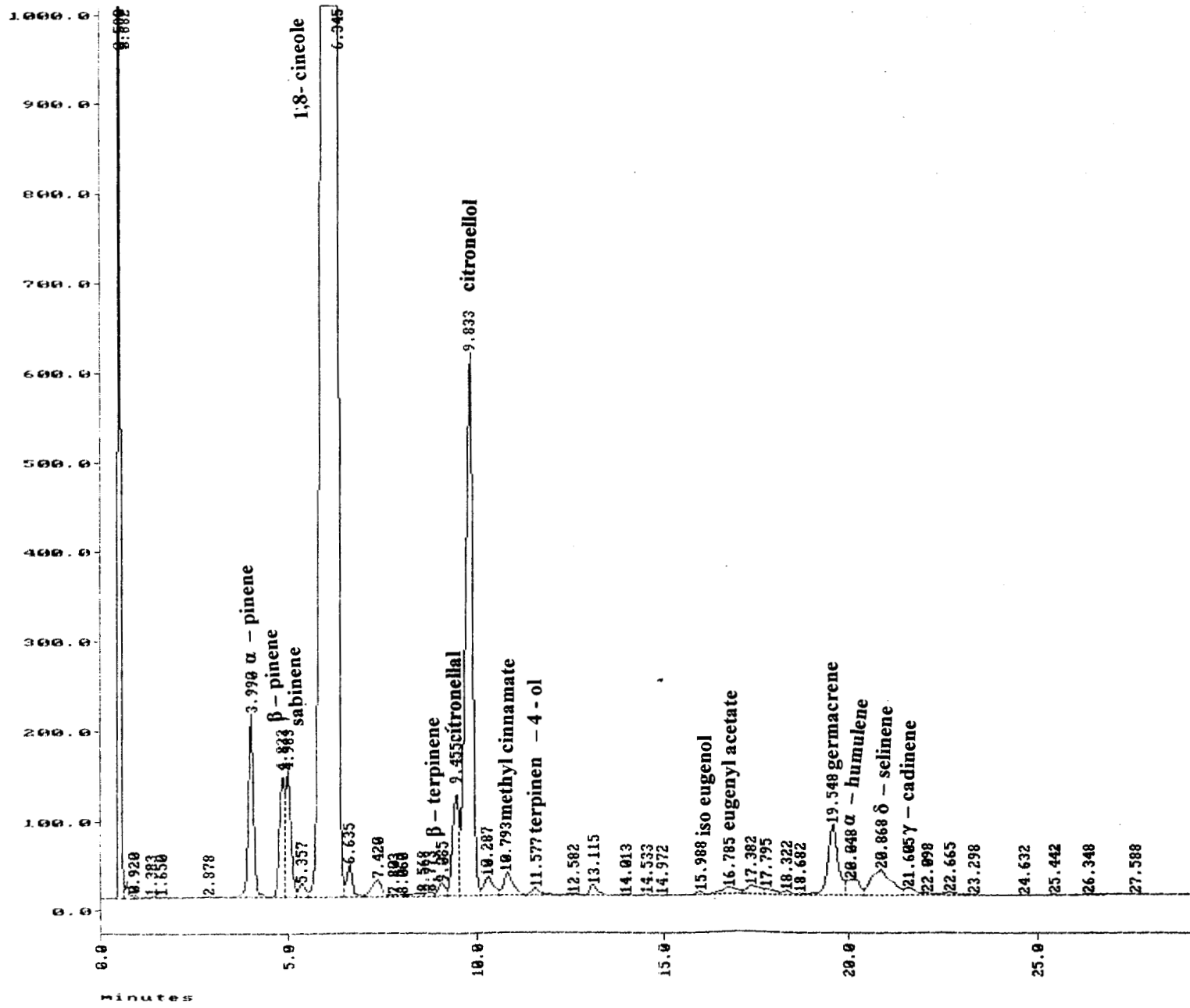


Fig. 137 Gas Liquid Chromatogram of the essential oil of *Callistemon viminalis*

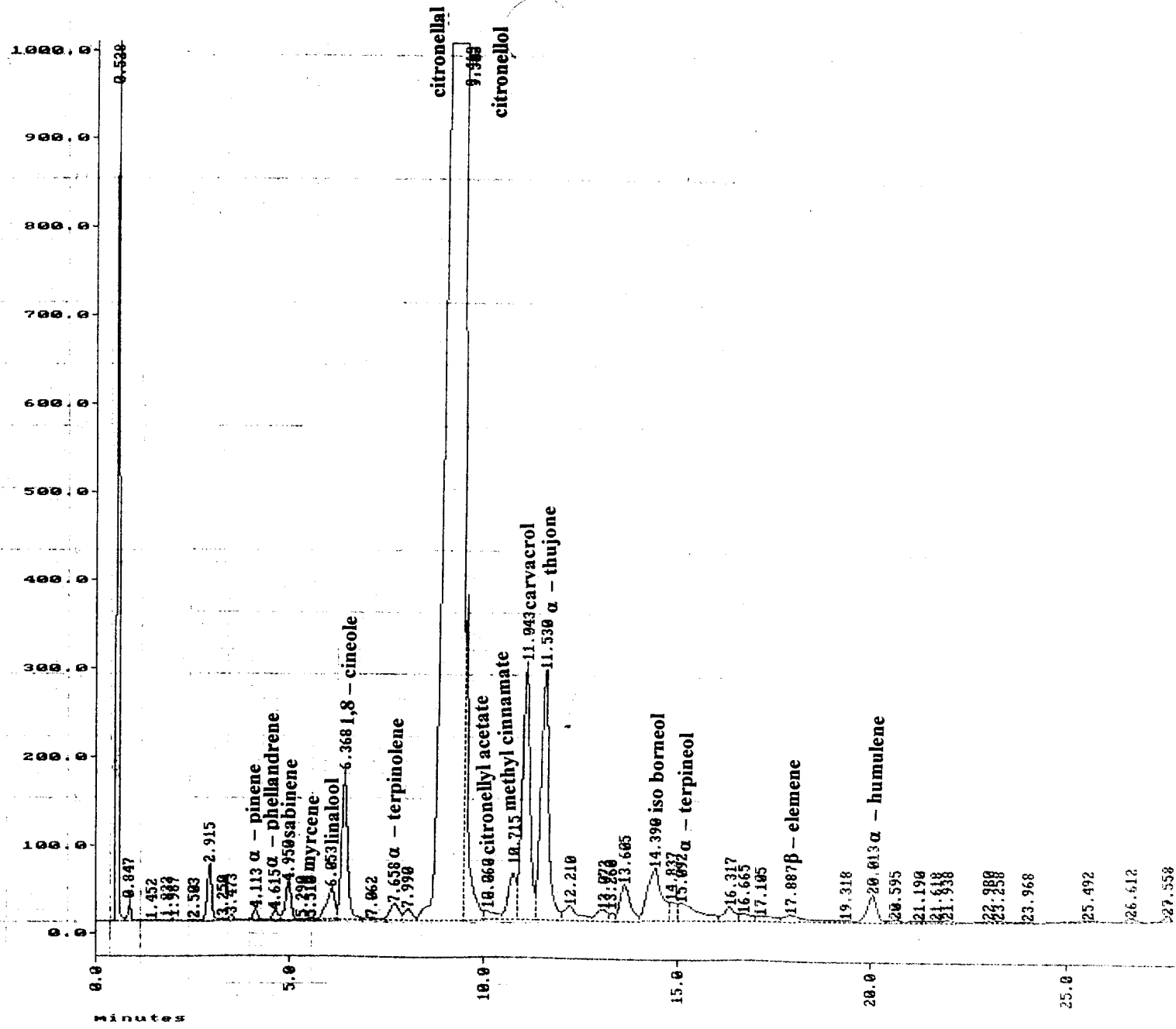


Fig. 138 Gas Liquid Chromatogram of the essential oil of *Corymbia citriodora*

2x

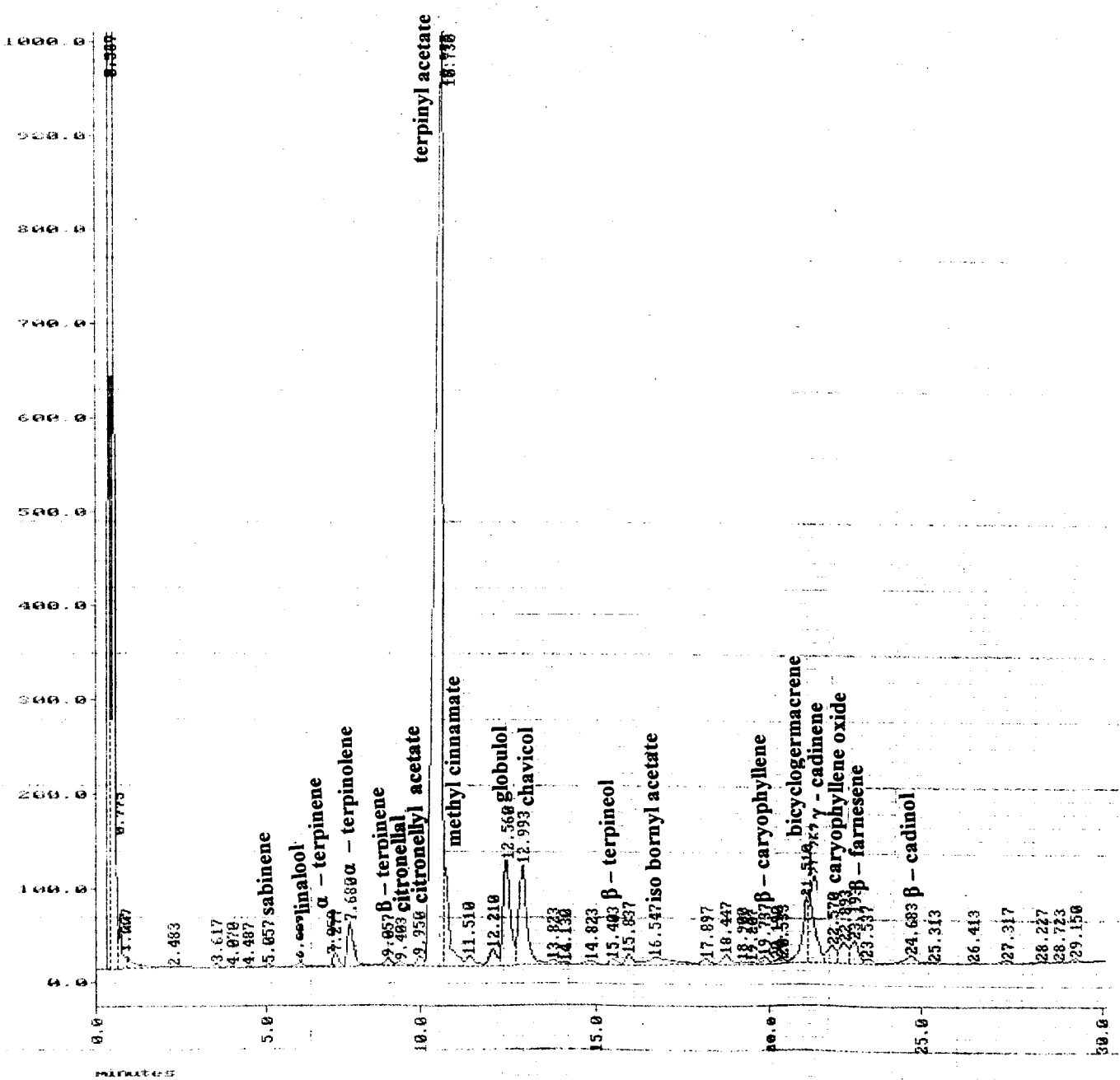


Fig. 139 Gas Liquid Chromatogram of the essential oil of *Corymbia ficifolia*

57



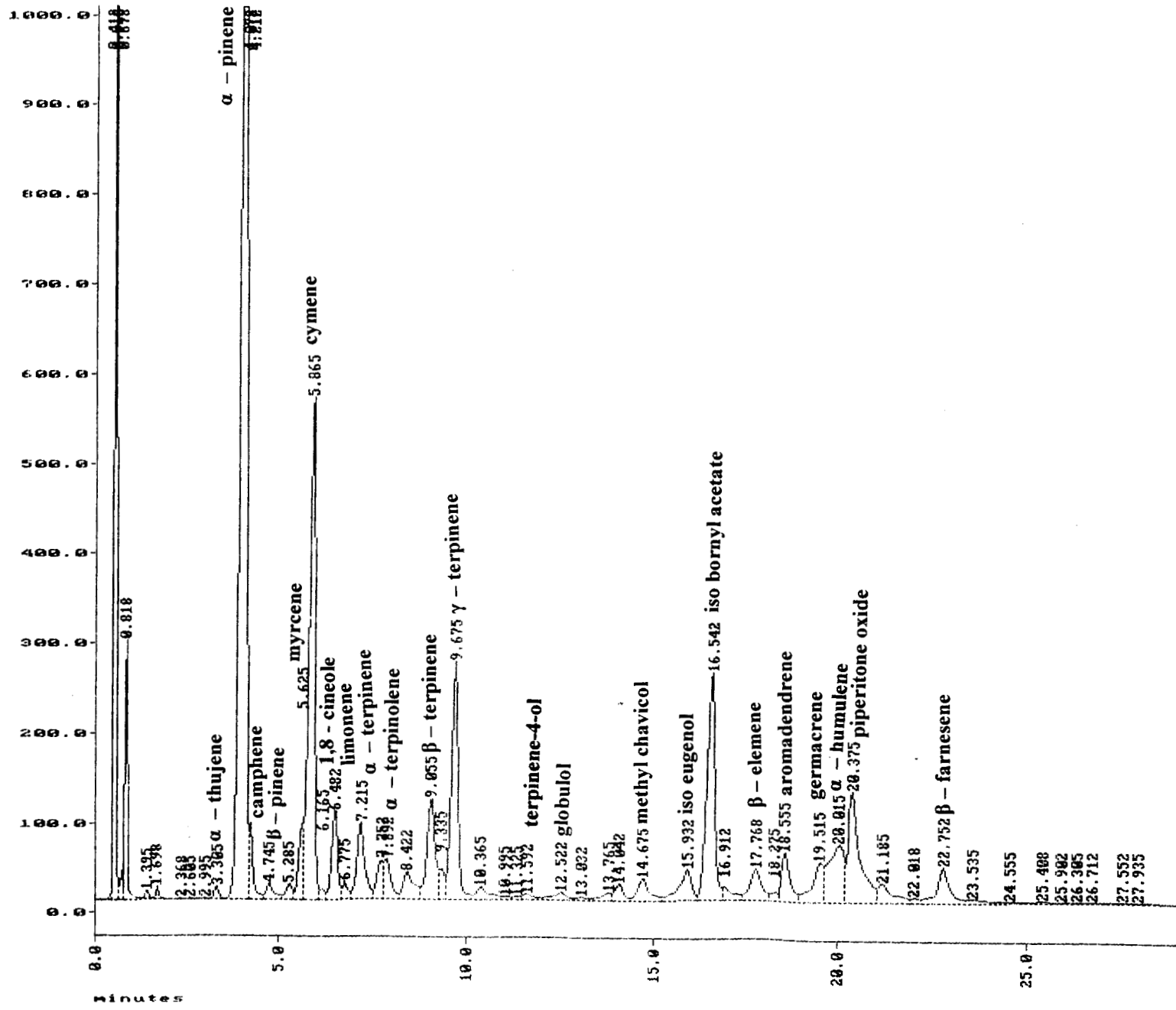


Fig. 140 Gas Liquid Chromatogram of the essential oil of *Eucalyptus globulus*

14

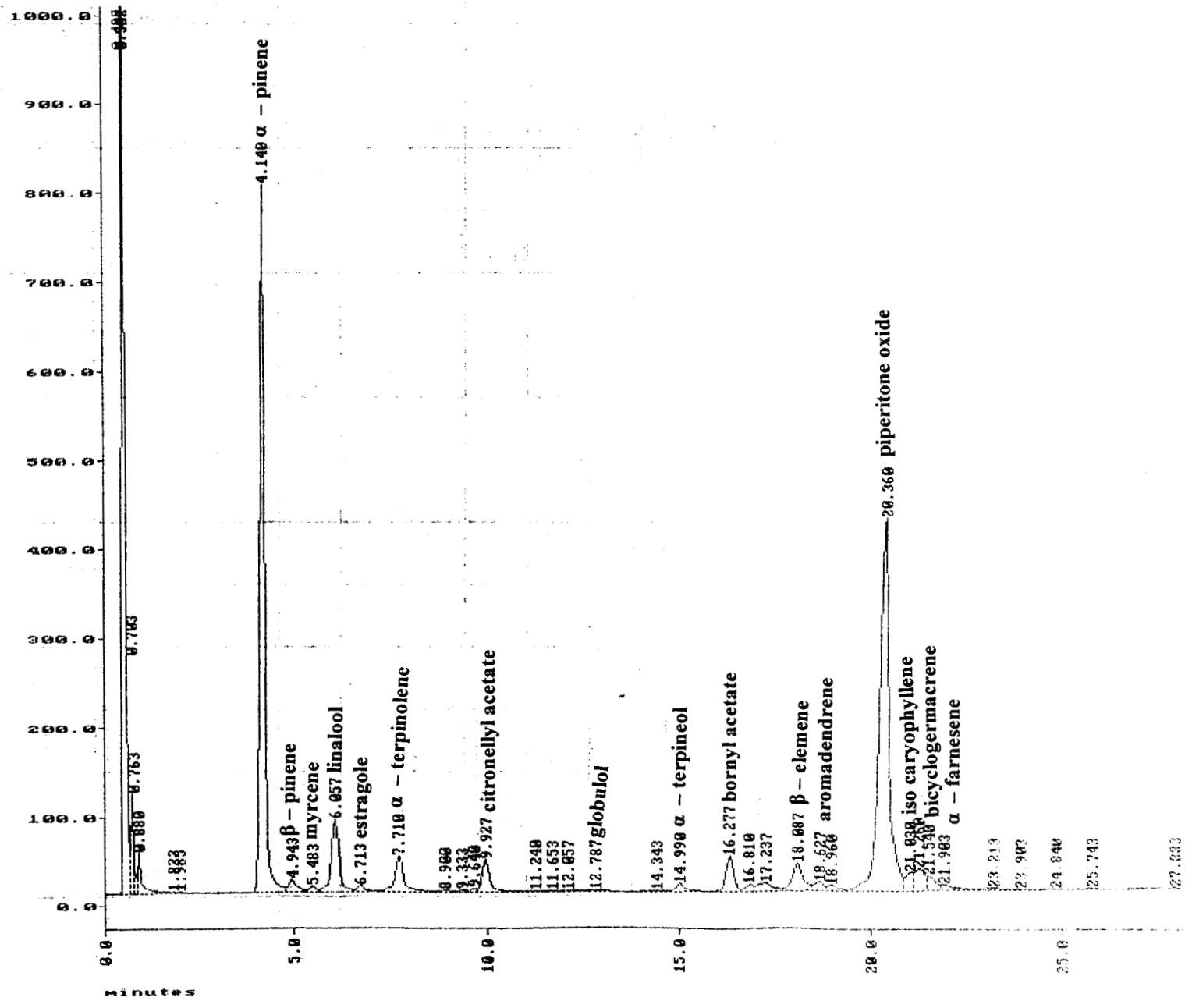


Fig. 141 Gas Liquid Chromatogram of the essential oil of *Eucalyptus tereticornis*

57

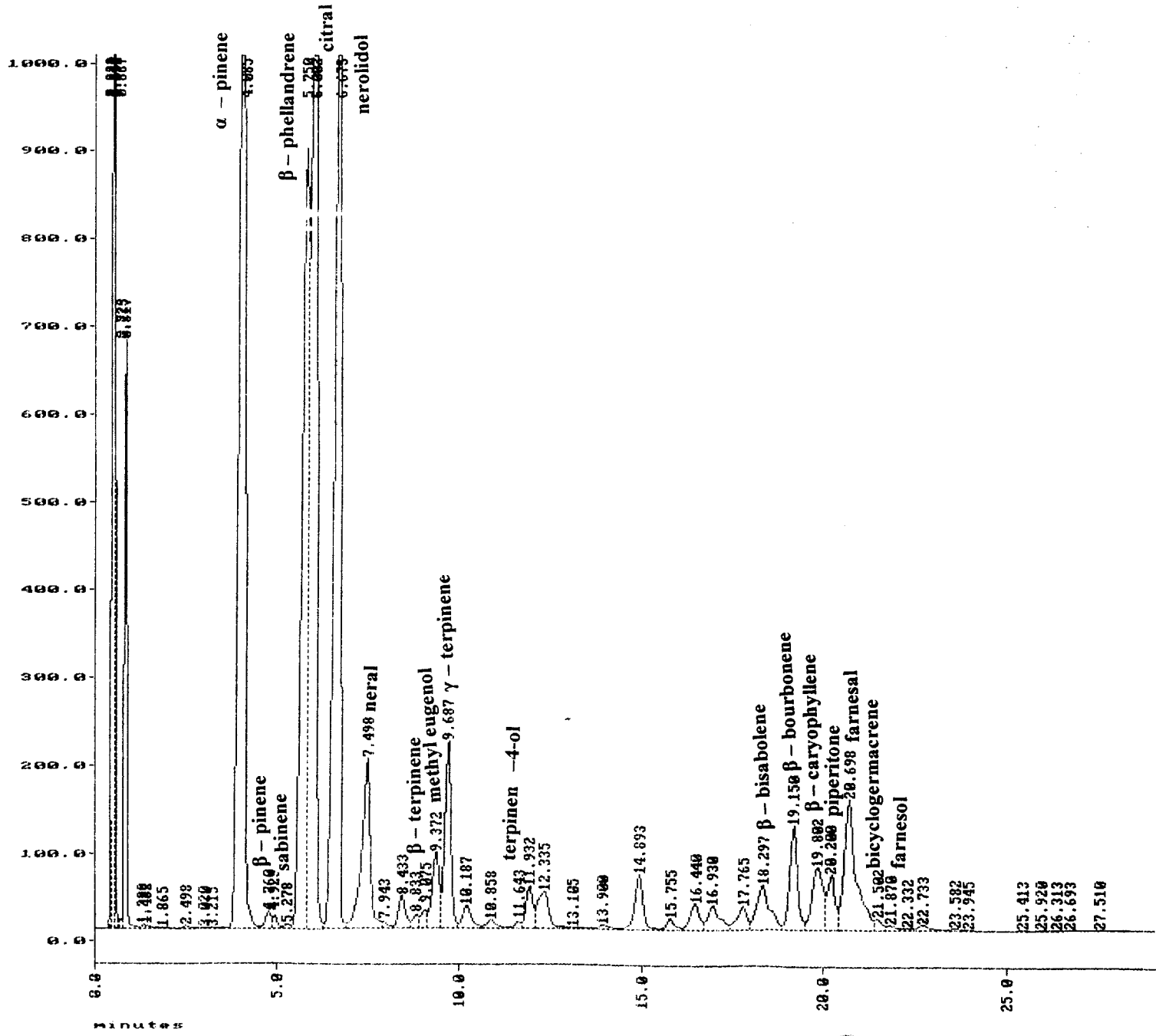


Fig. 142 Gas Liquid Chromatogram of the essential oil of *Leptospermum nicholsii*

9x

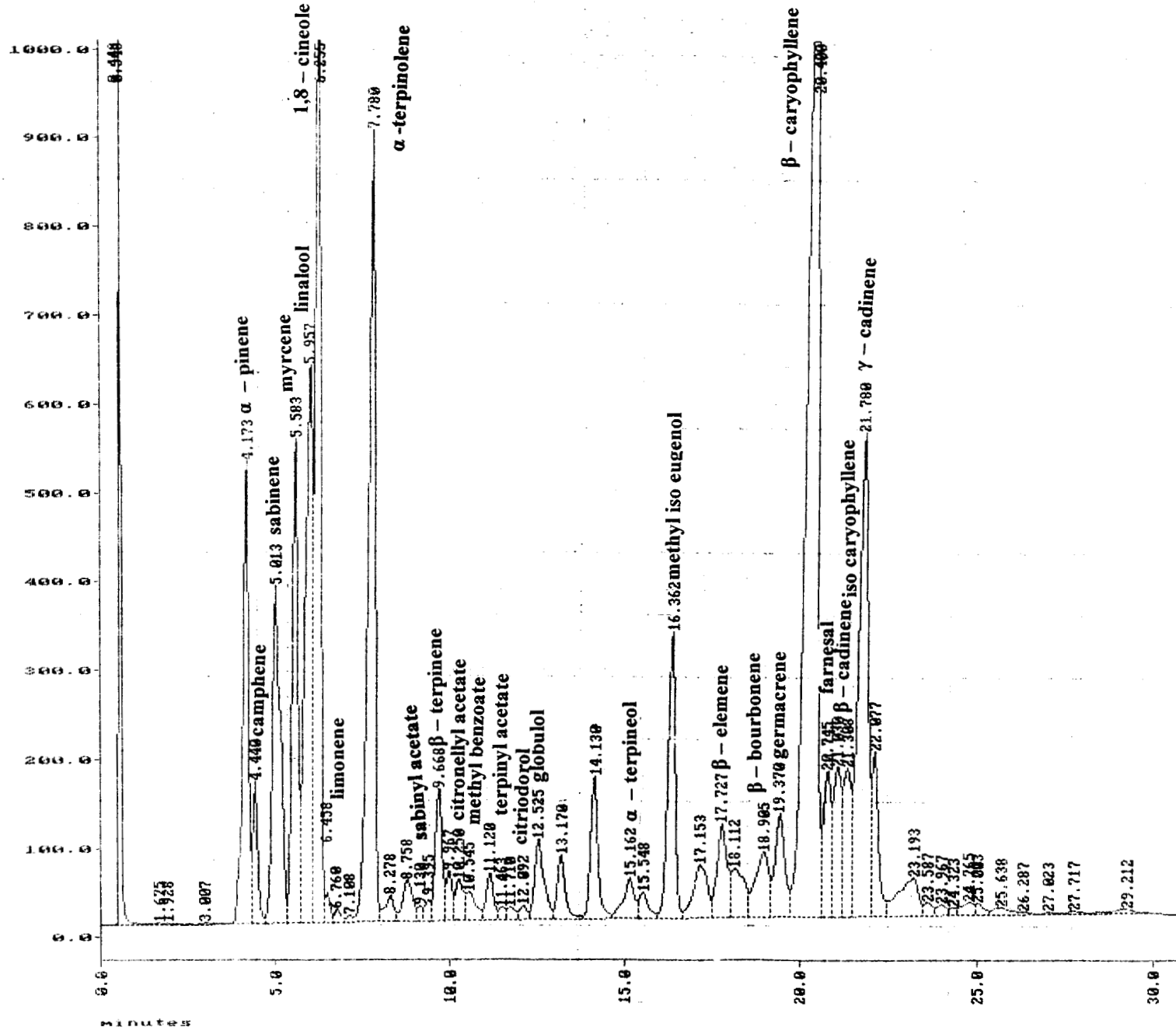


Fig. 143 Gas Liquid Chromatogram of the essential oil of *Melaleuca leucadendron*

FF

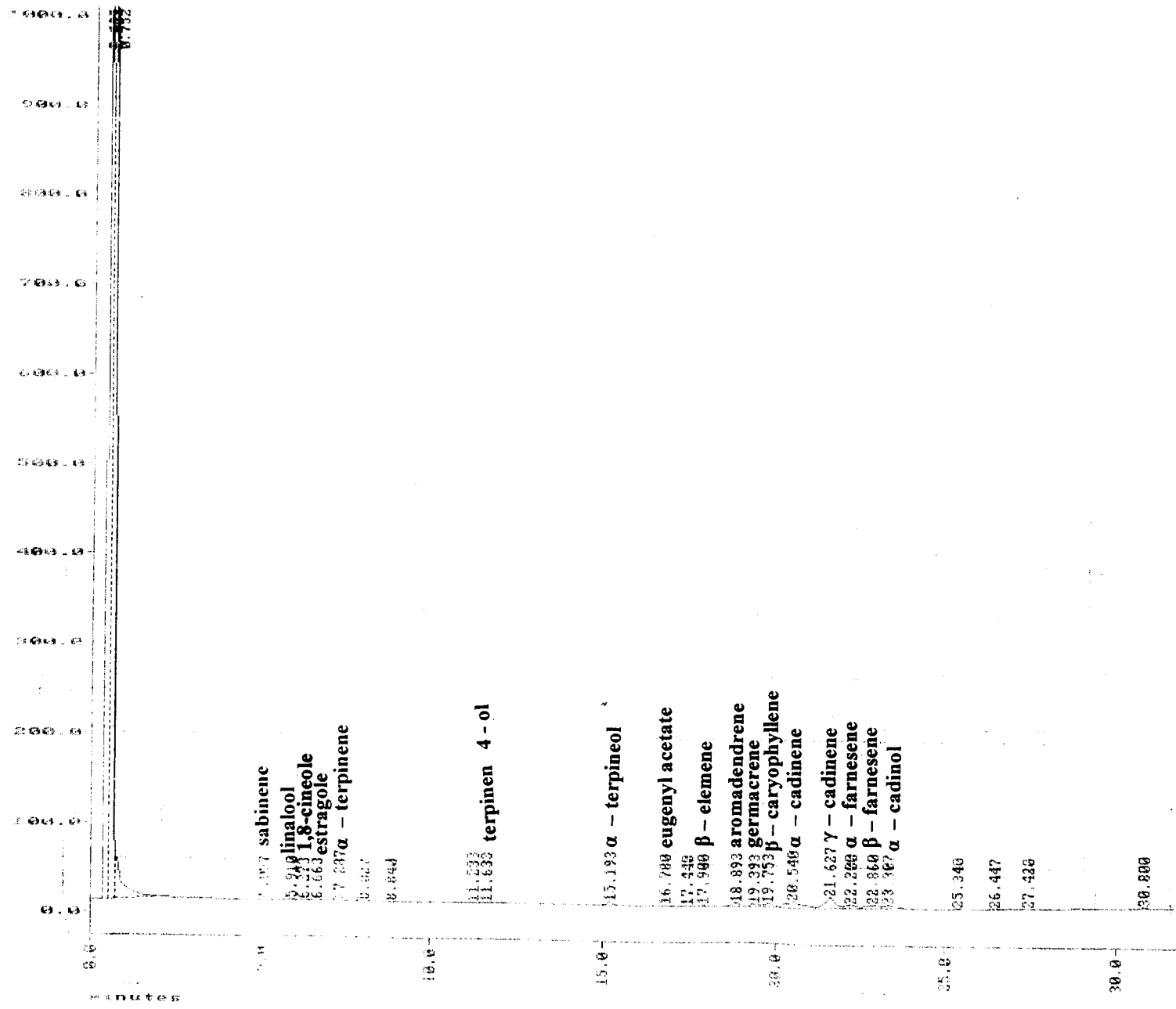


Fig. 144 Gas Liquid Chromatogram of the essential oil of *Melaleuca styphelioides*

48

1972

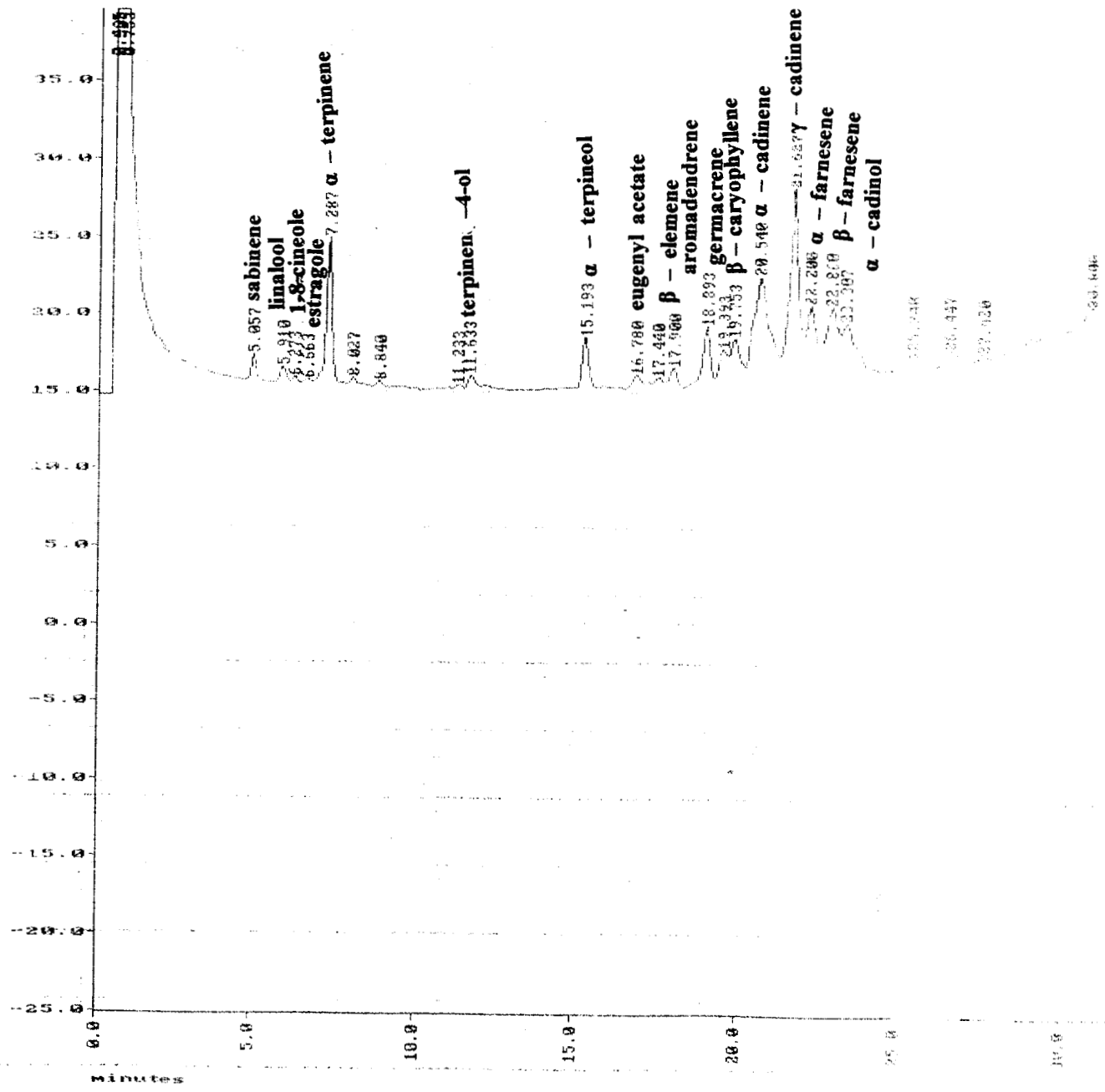


Fig. 144 (a) Gas Liquid Chromatogram of the essential oil of *Metaleuca stypelioides* (Enlarged).

67

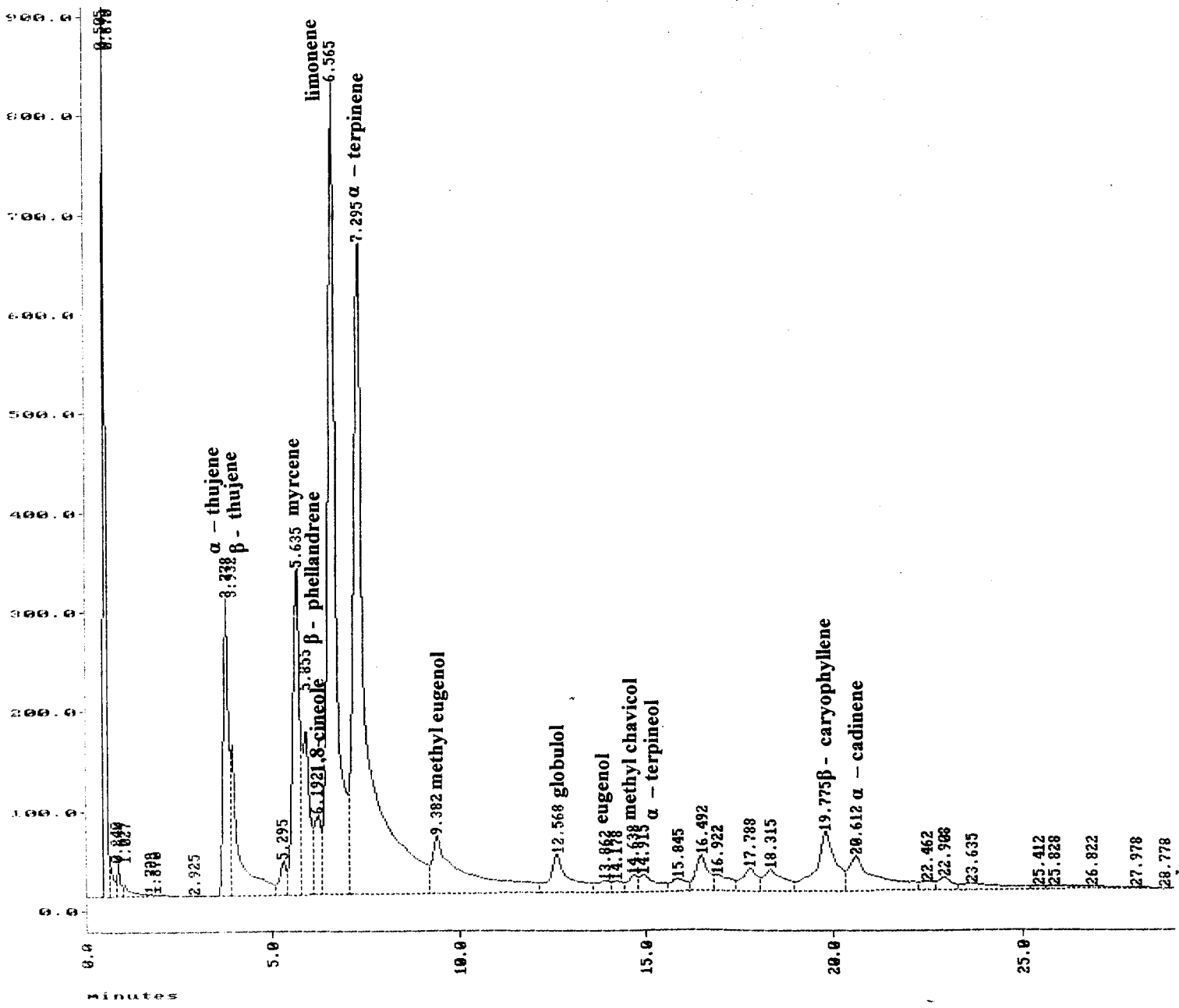


Fig. 145 Gas Liquid Chromatogram of the essential oil of *Syncarpia glomulifera*

R

151N

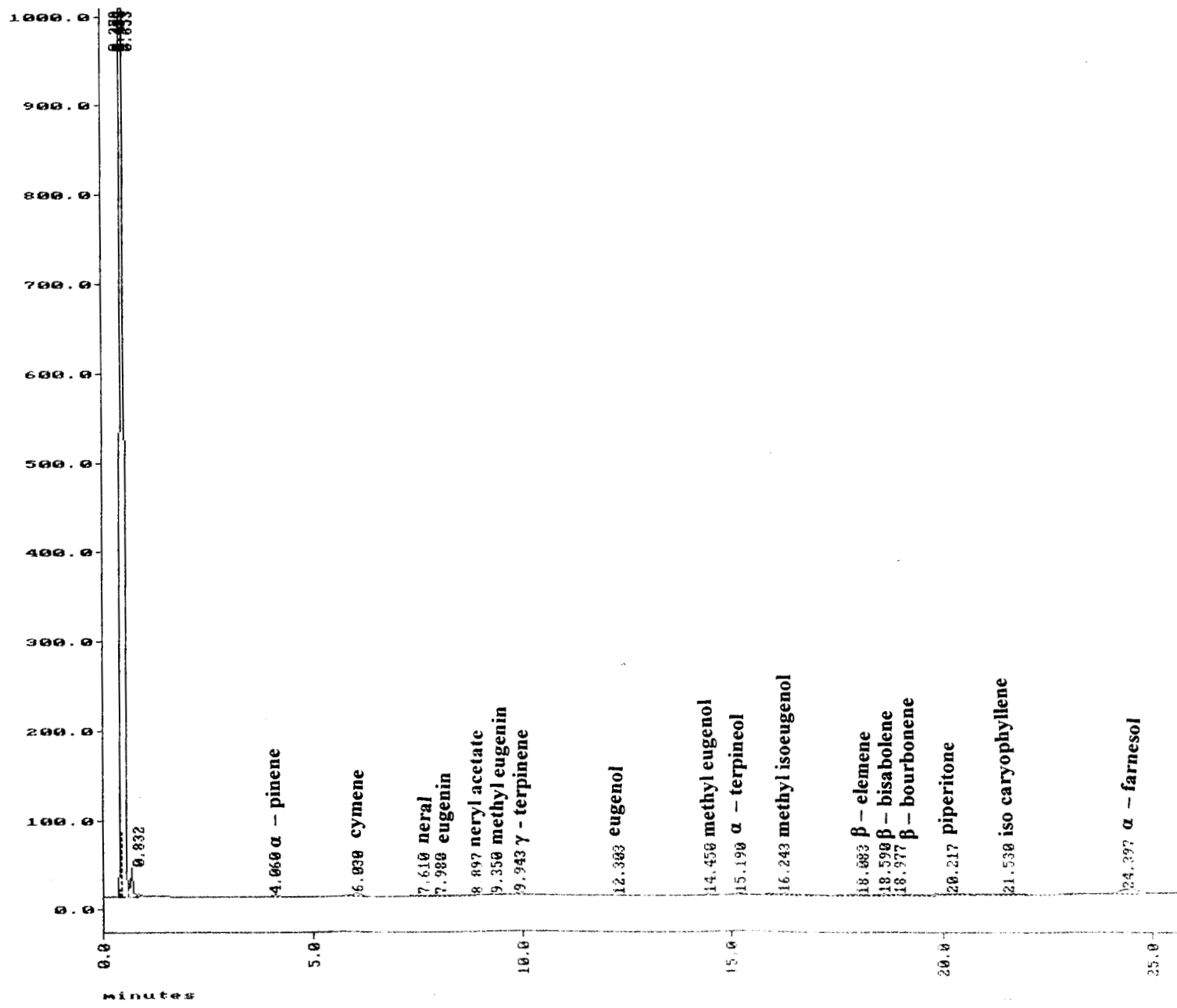


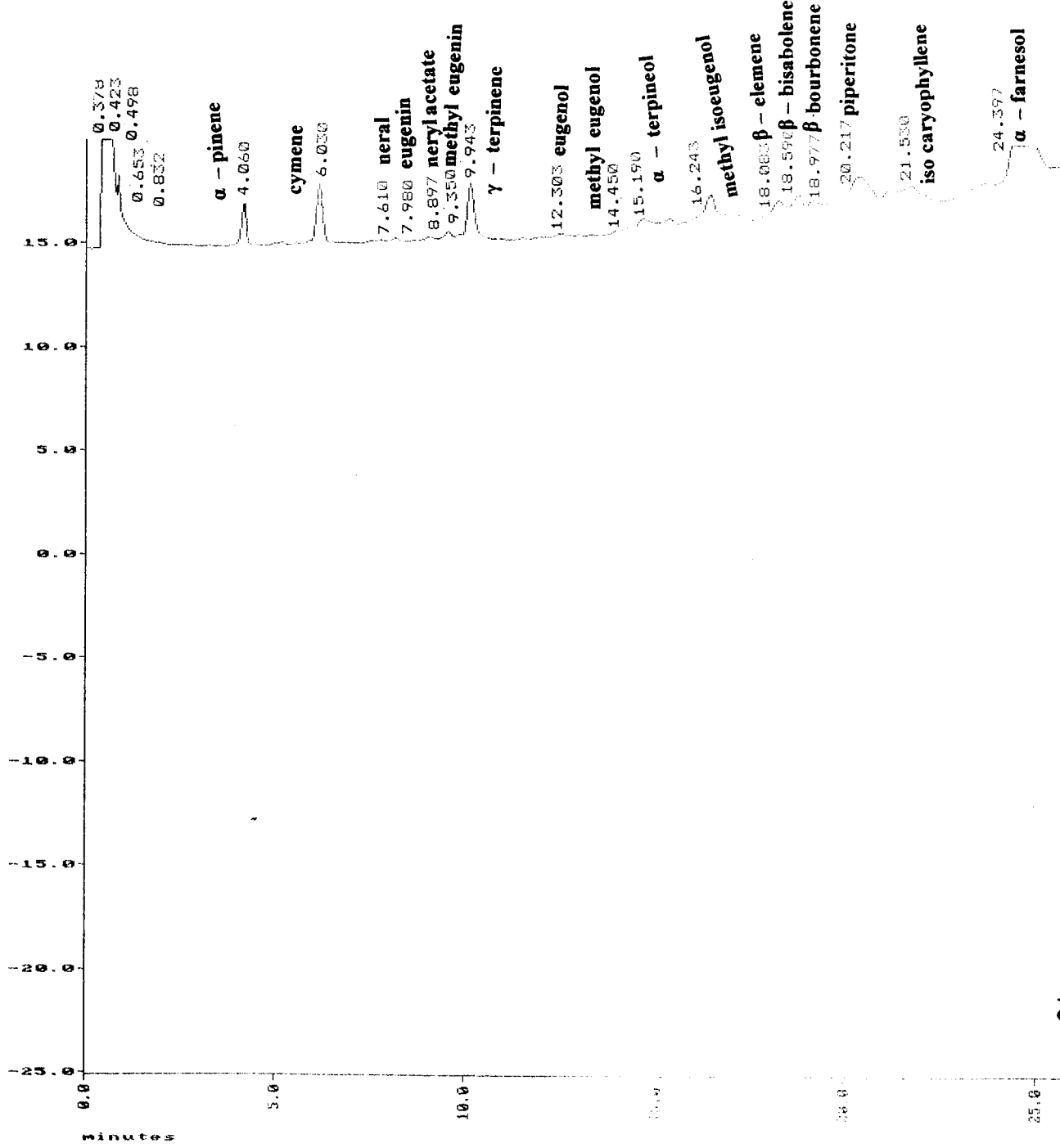
Fig. 146 Gas Liquid Chromatogram of the essential oil of *Acmena smithii*

15



1910

File : t.jz  
Run : 10 Type : Sample  
Collection : 16:44:23 Aug 02 2001 Method : THERMSTO [ 09:39:03 Aug 02 2001 ]



5

Fig. 146 (a) Gas Liquid Chromatogram of the essential oil of *Acmena smithii* (Enlarged).

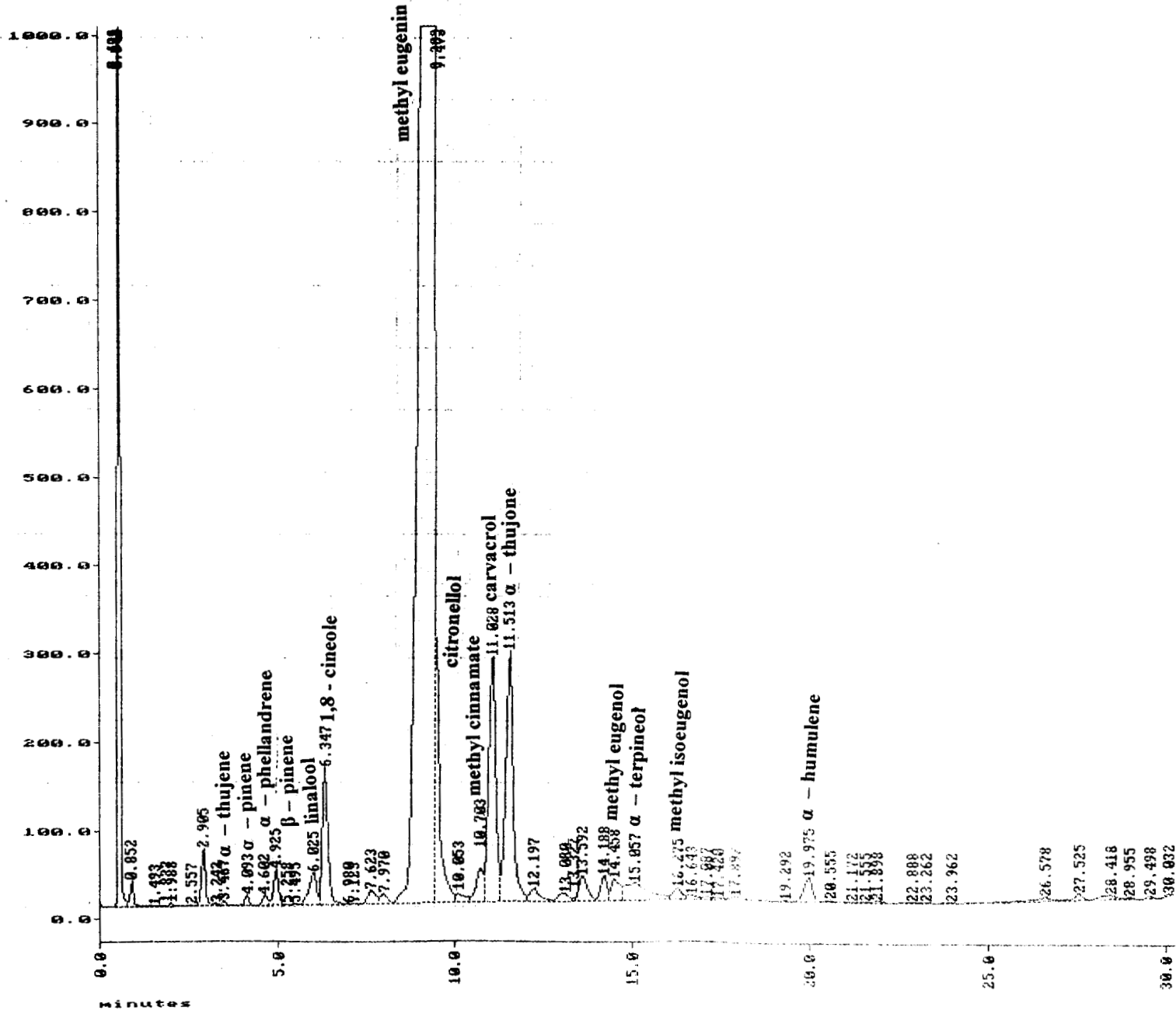


Fig. 147 Gas Liquid Chromatogram of the essential oil of *Eugenia apiculata*

5

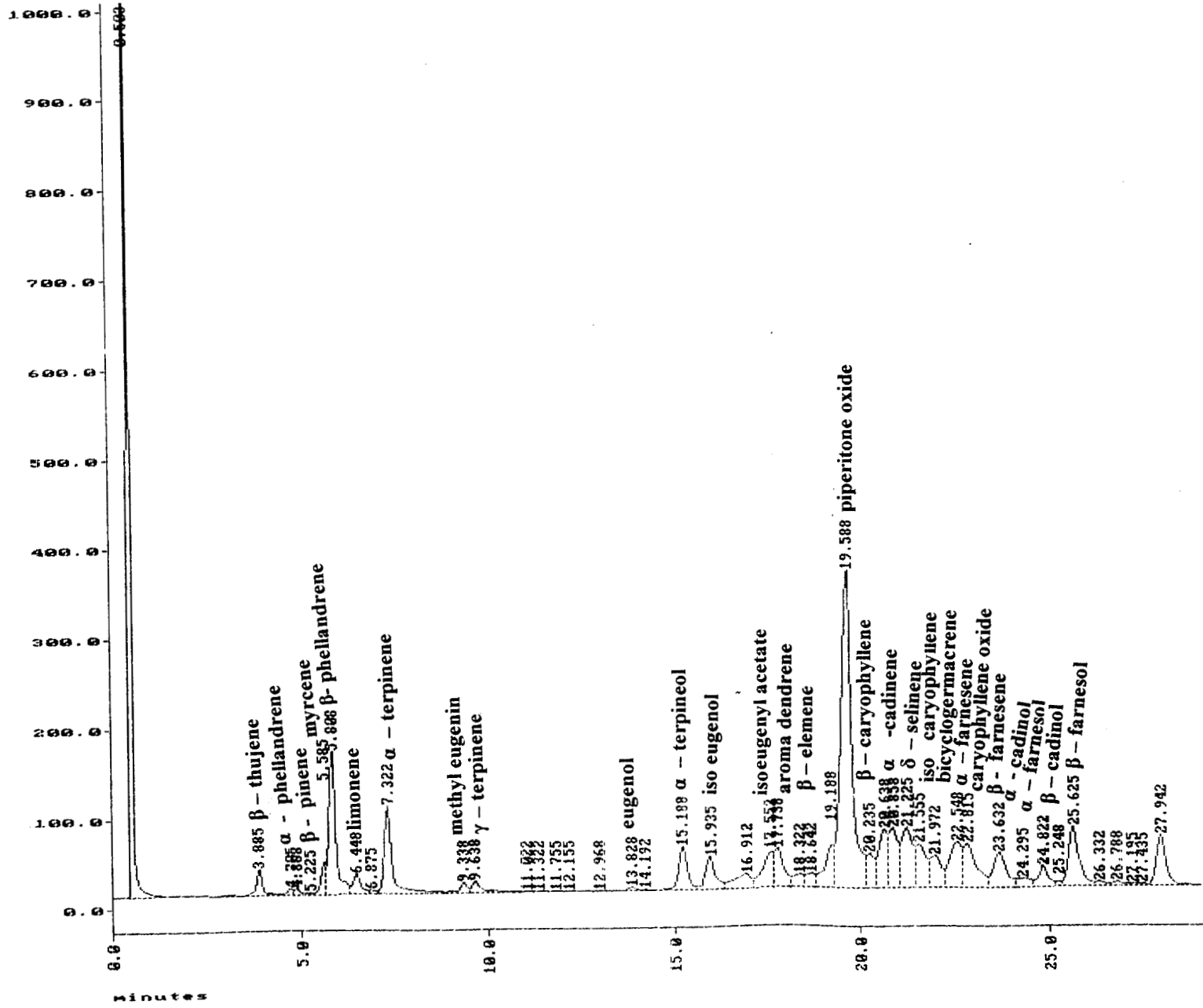


Fig. 148 Gas Liquid Chromatogram of the essential oil of *Eugenia uniflora*

24

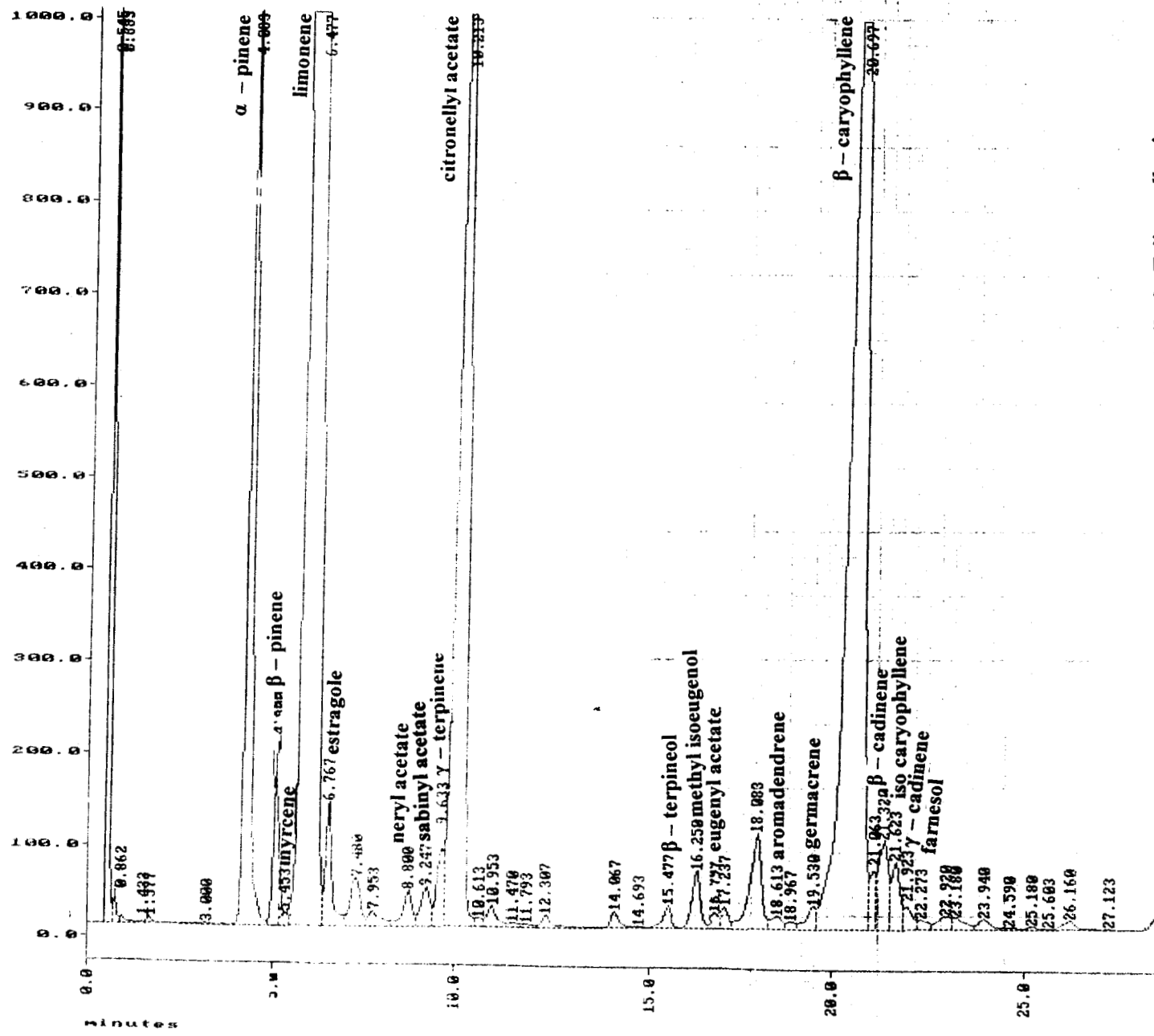


Fig. 149 Gas Liquid Chromatogram of the essential oil of *Feijoa sellowiana*

5

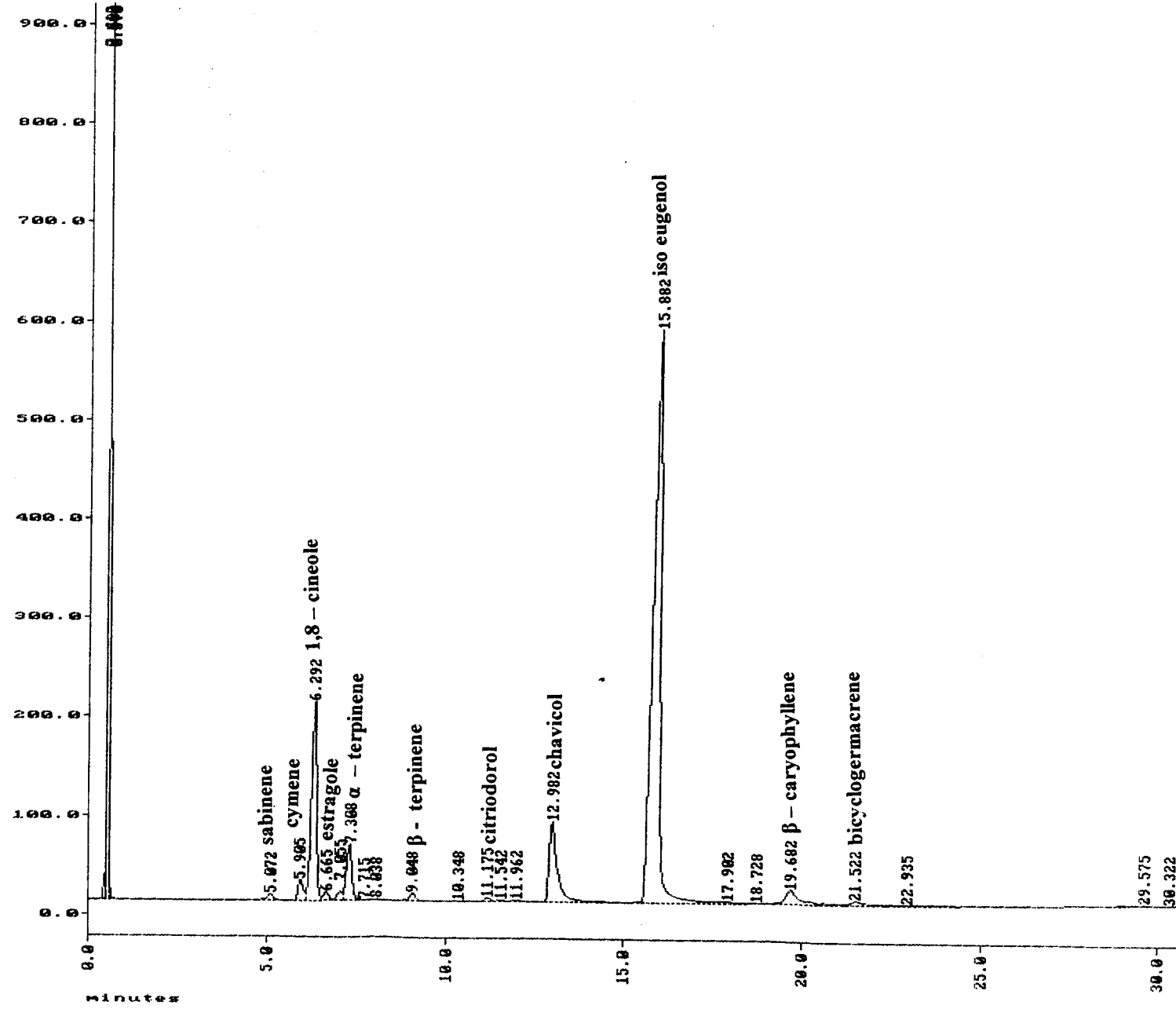


Fig. 151 Gas Liquid Chromatogram of the essential oil of *Pimenta dioica*

LS

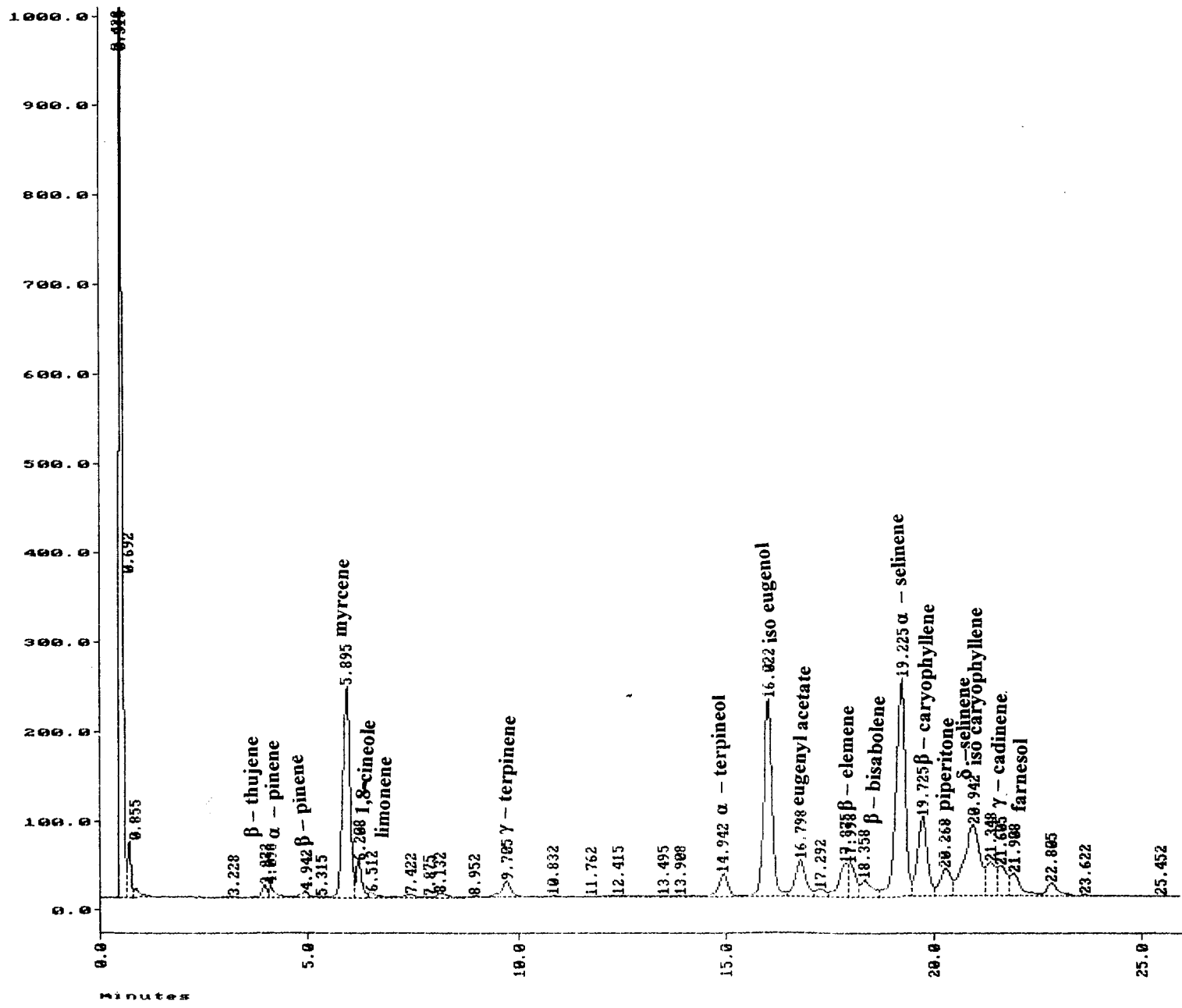


Fig. 152 Gas Liquid Chromatogram of the essential oil of *Psidium guajava*

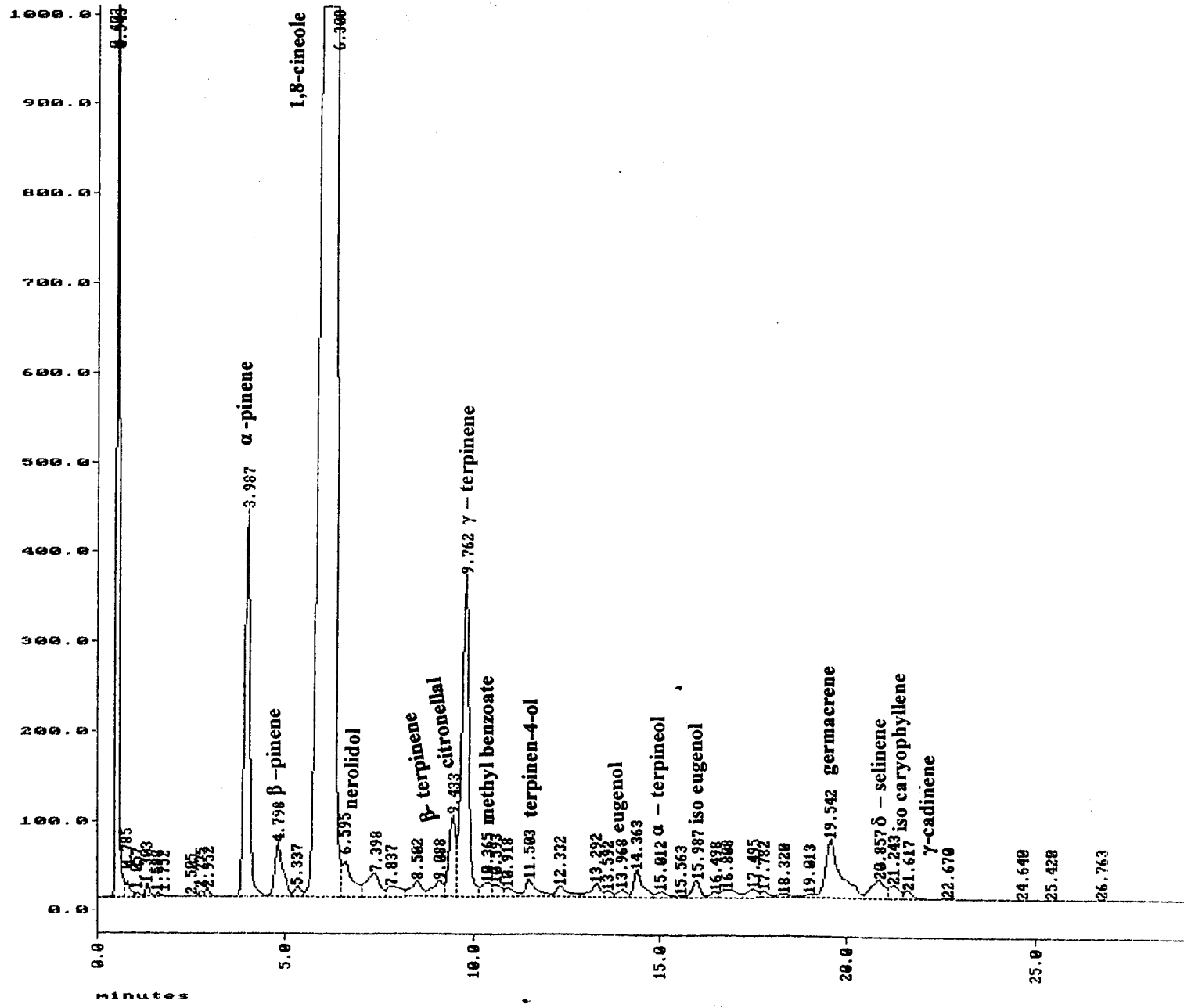


Fig. 153 Gas Liquid Chromatogram of the essential oil of *Syzygium aromaticum*

B.M.

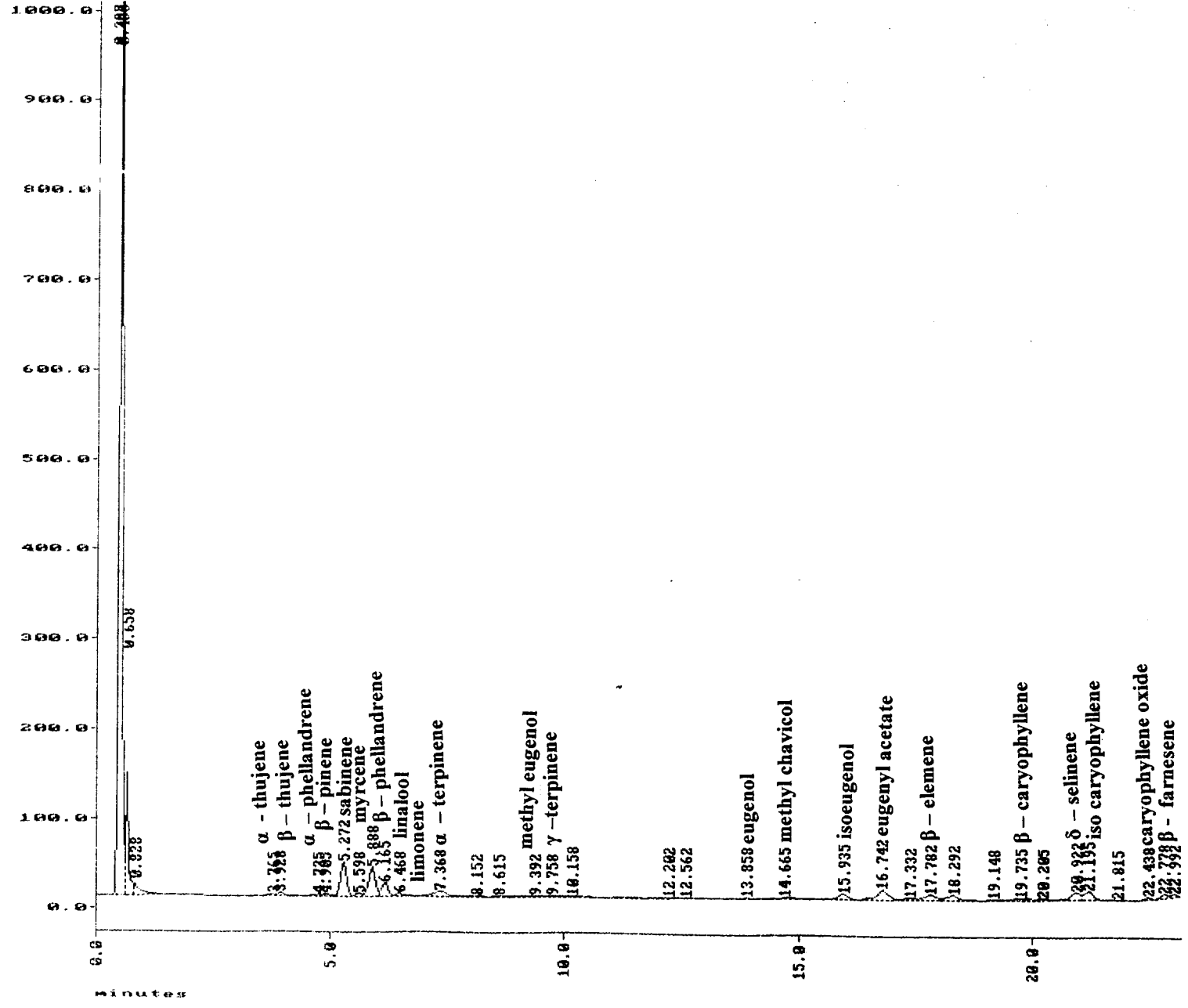


Fig. 154 Gas Liquid Chromatogram of the essential oil of *Syzygium cumini*



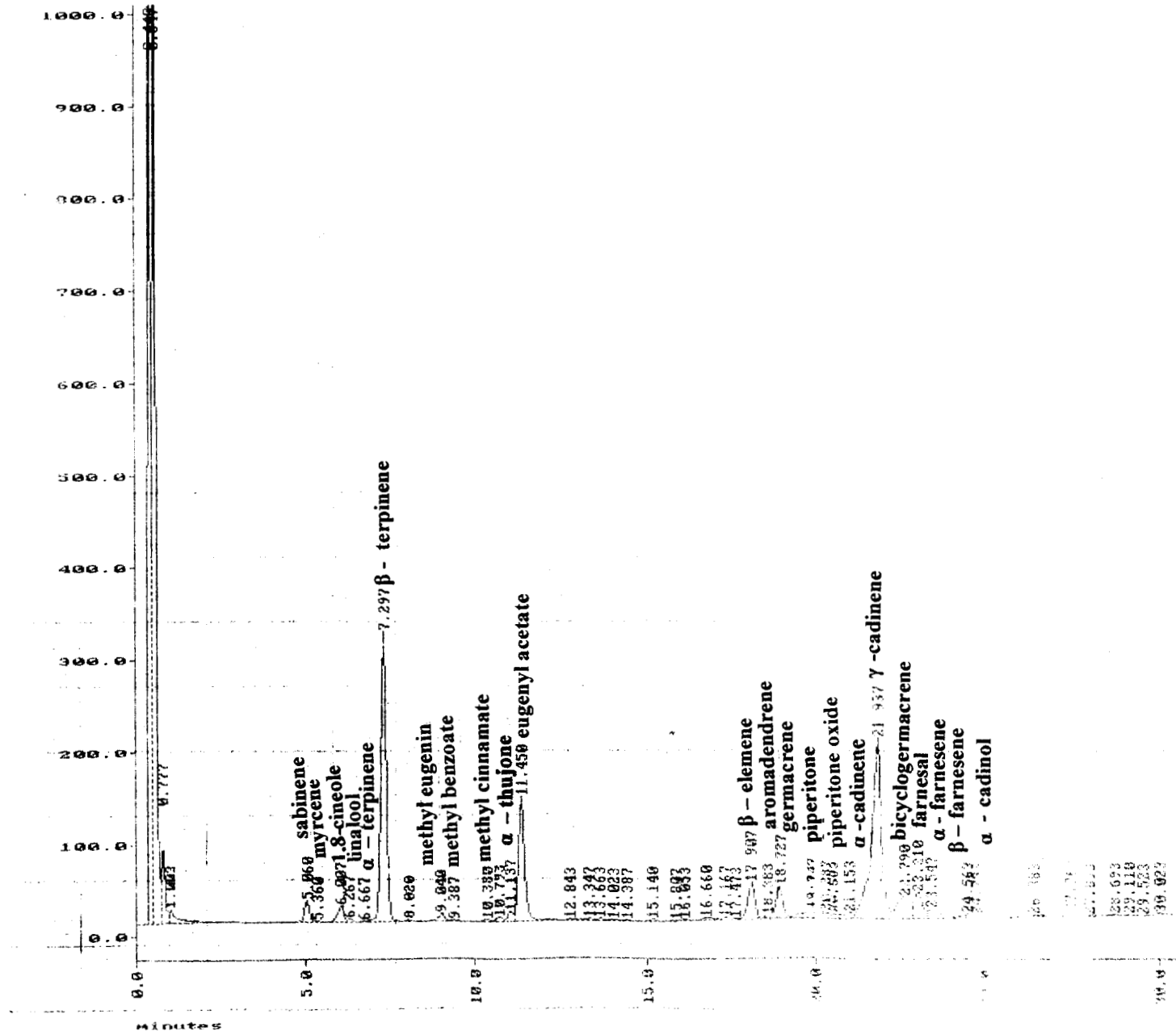


Fig. 155 Gas Liquid Chromatogram of the essential oil of *Syzygium jambos*

100

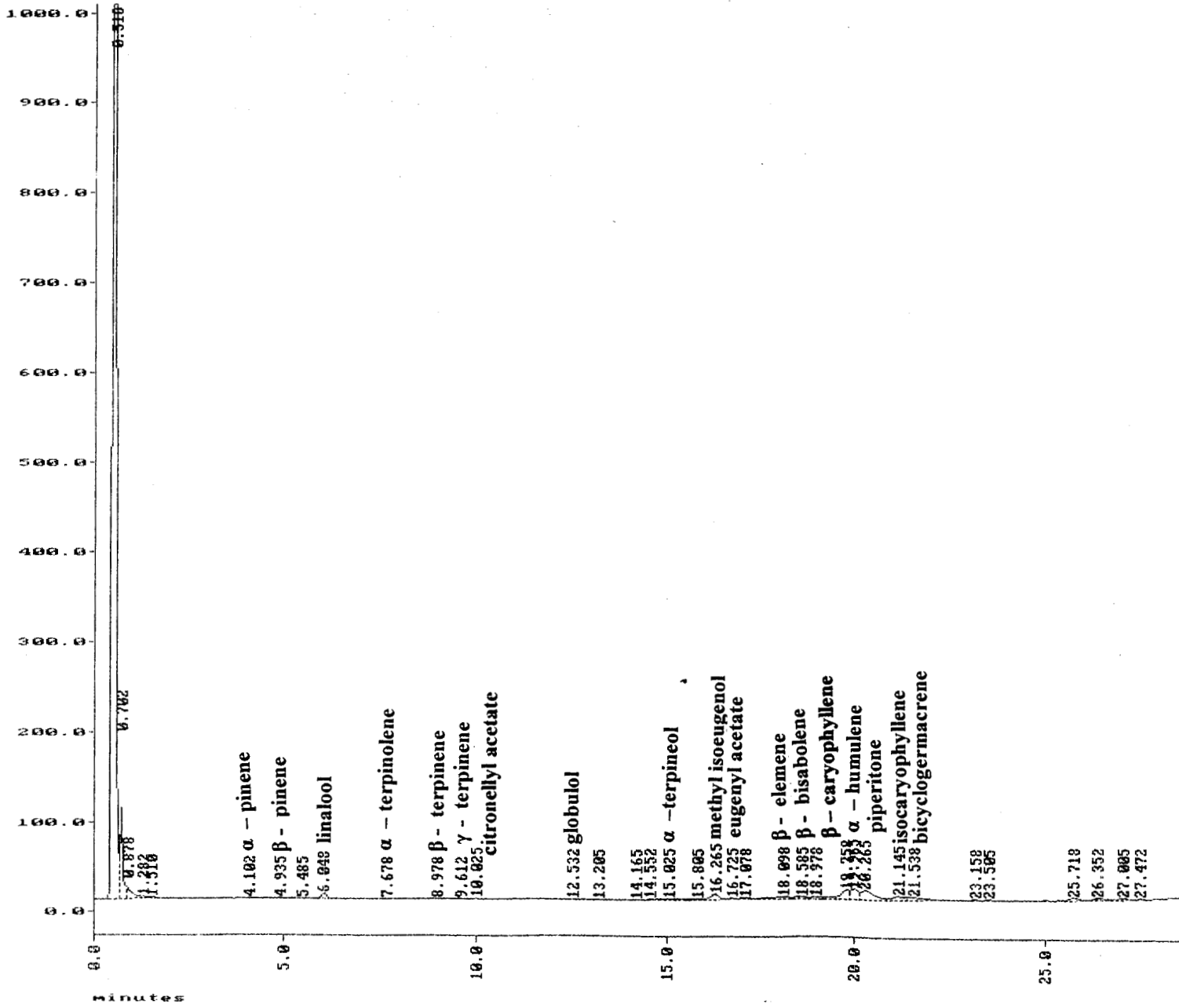


Fig. 156 Gas Liquid Chromatogram of the essential oil of *Syzygium malaccense*

29

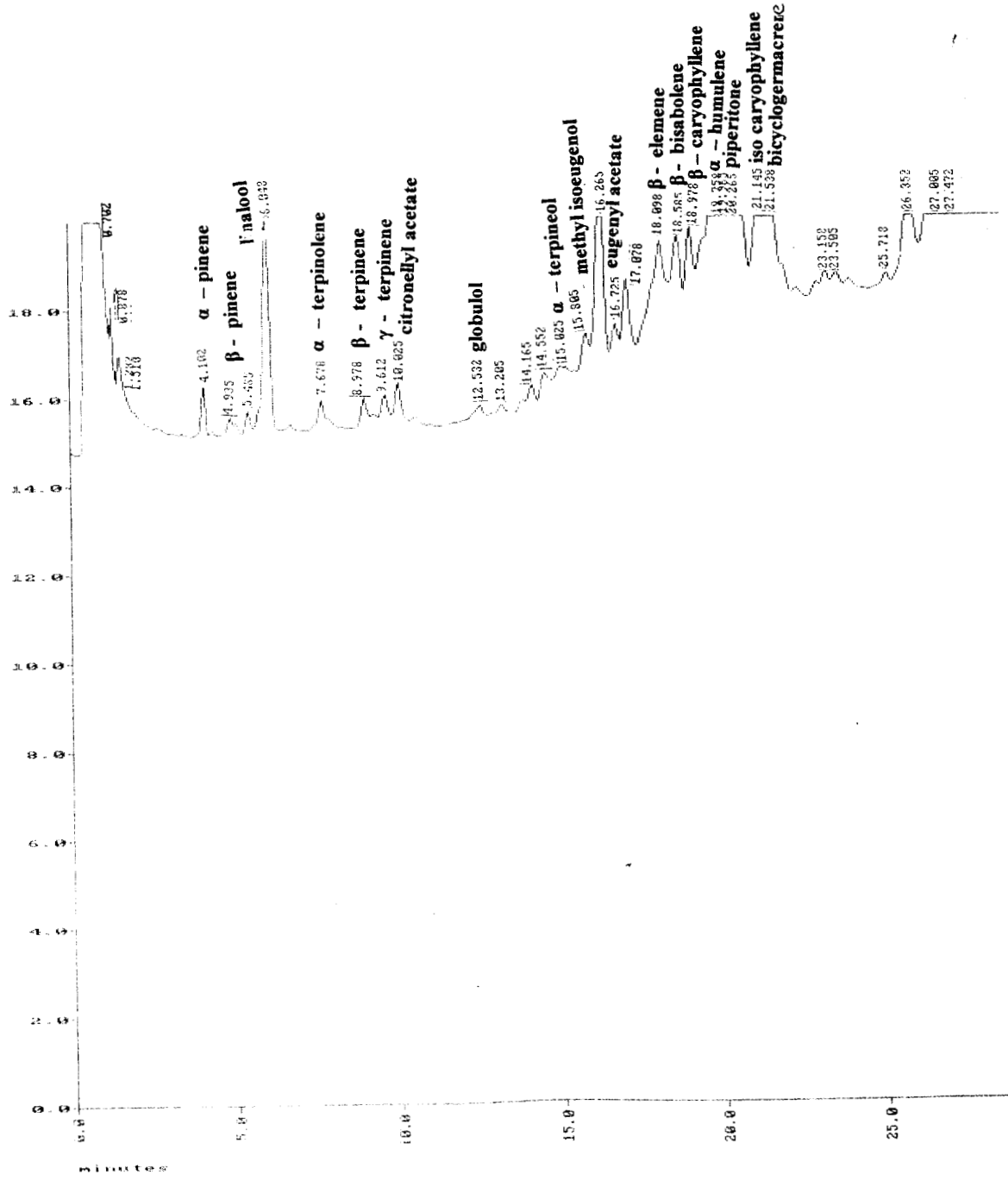


Fig. 156 (a) Gas Liquid Chromatogram of the essential oil of *Syzygium malaccense* (Enlarged).

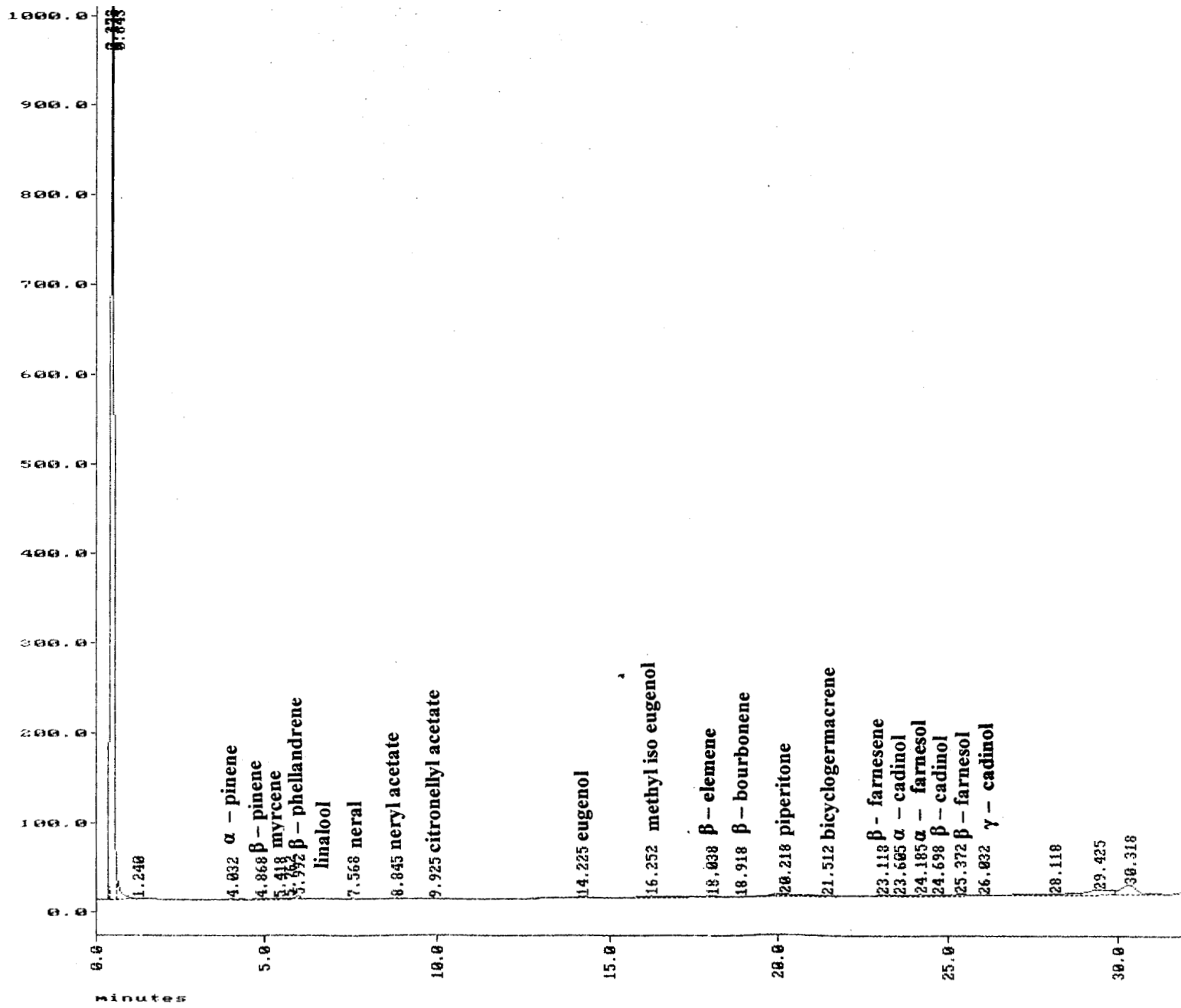


Fig. 157 Gas Liquid Chromatogram of the essential oil of *Syzygium samarangense*

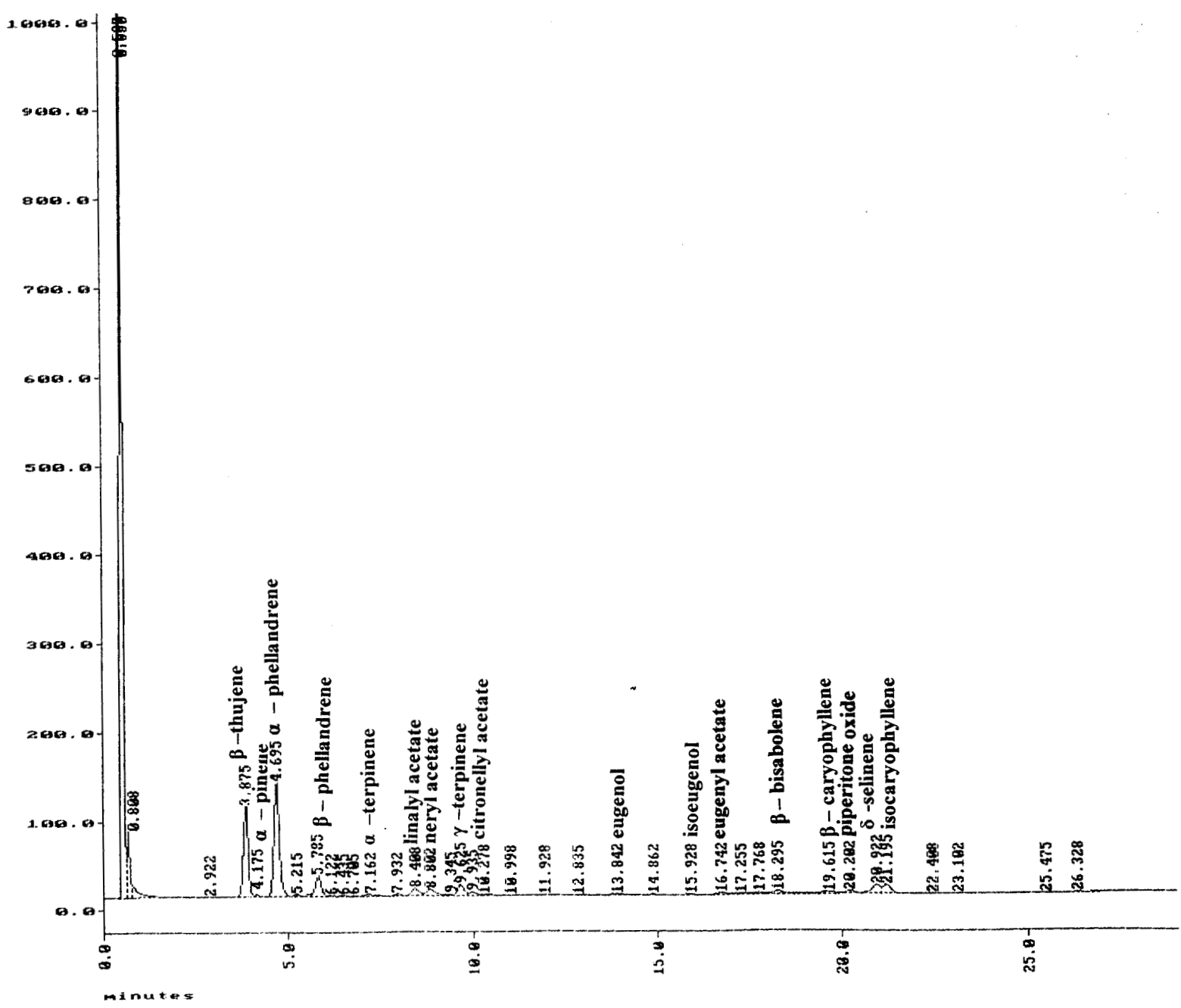


Fig. 158 Gas Liquid Chromatogram of the essential oil of *Syzygium zeylanicum*

59

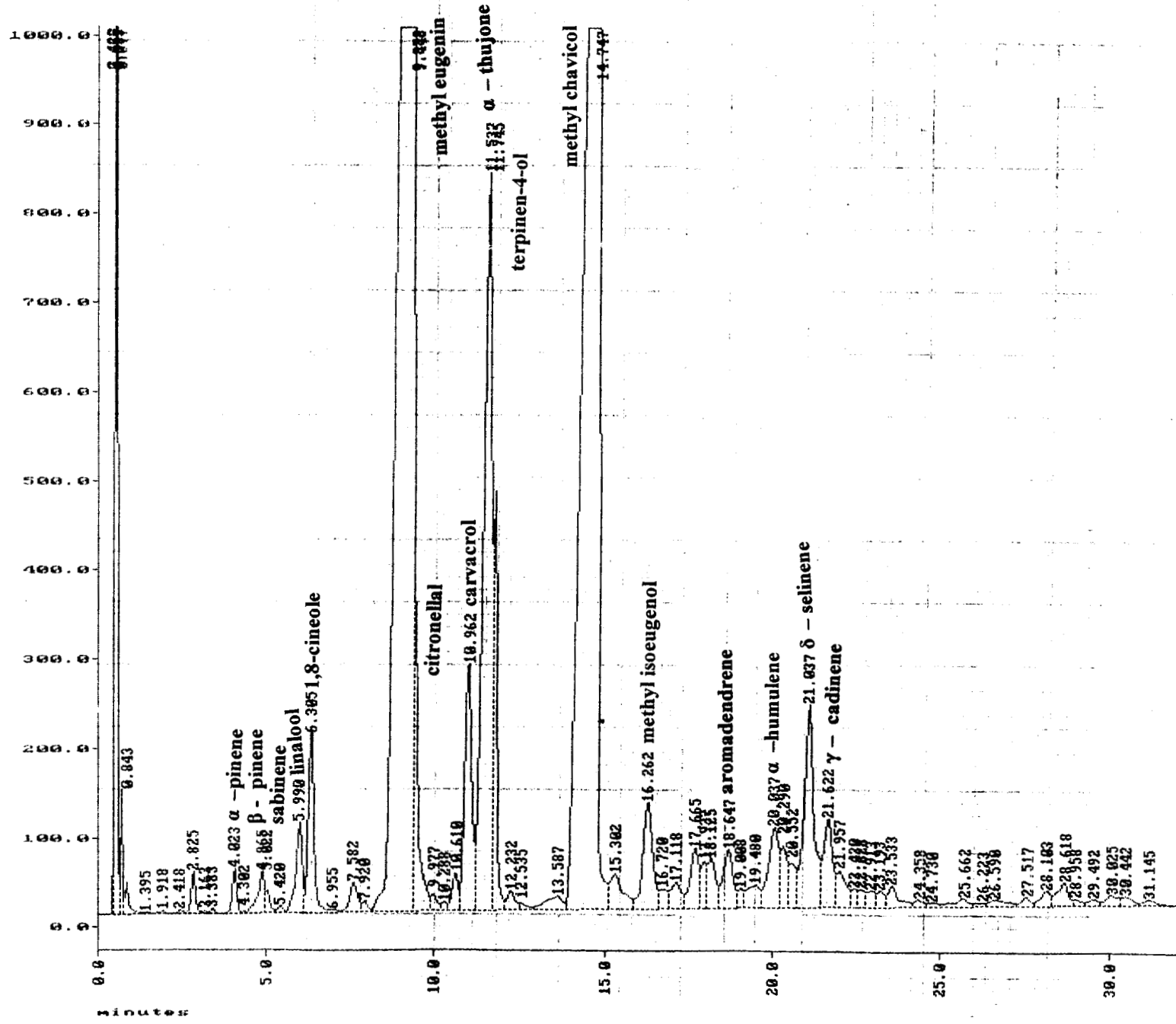


Fig. 159 Gas Liquid Chromatogram of the essential oil of *Rhodomyrtus tomentosa*

# DISCUSSION

## A. CYTOTOXIC EFFECTS

In the present investigation, the onion root tip cells showed normal mitotic divisions when treated with distilled water (control). The mitotic indices showed a gradual decrease from 2 hr. to 24 hr. treatment. But all the divisional stages observed were normal (Fig. 27, 28, 29 & 30 and Table 3).

A wide range of chromosome abnormalities were seen in *A. cepa* root tip cells after treatment with various types of myrtaceous leaf extracts and it increased with the duration and concentration of the treatment. Out of the 26 members investigated, 2 members viz. *Syzygium zeylanicum* and *Rhodomyrtus tomentosa* of the subfamily Myrtoideae showed a slight mitotic stimulatory effect in low concentration and short duration treatments. Such a stimulatory effect was noticed by Sopova *et al.* (1983), with low concentrations of tobacco leaf extract on the root tip cells of *Allium sativum* where as high concentrations acted as mitodepressant. In the present study also high concentrations and long duration of treatments showed a mitodepressant effect (Table 28 and 29). Similarly, the weaker solutions of *Solanum nigrum* immature fruit extract also stimulated the mitotic divisions in *A. sativum* where as the stronger concentrations of the extract reduced the mitotic indices. Krivokapic *et al.* (1970) suggested that it might be due to the presence of a cytokinin like substance in the extract. In the present investigation, the mitotic stimulatory effect exhibited by the leaf extracts of *S. zeylanicum* and *R. tomentosa* during low concentration and short duration may be due to the presence of mitostimulatory principles in them.

In all the other members of both the subfamilies, the mitotic indices showed a gradual decrease with respect to the increase in concentration of the extract and increase in the duration of treatment.

### **Subfamily : Leptospermoideae**

Of the 12 taxa studied under the subfamily Leptospermoideae, clastogenic anomalies like nuclear lesions and abnormally enlarged nuclei were observed only on experimentation with leaf extracts of *Corymbia ficifolia*, *C. citriodora*, *Melaleuca leucadendron*, *M. styphelioides* and *Syncarpia glomulifera*.

Under the nonclastogenic abnormalities, clumping of chromosomes, diagonal orientation of chromosomes, misorientation and early movement of chromosomes during anaphase were frequently found on treatment with leaf extracts of most of the members. C- metaphase cells were found only on experimentation with *Agonis flexuosa*, *Callistemon viminalis*, *Corymbia citriodora*, *Eucalyptus globulus* and *Leptospermum nicholsii* leaf extracts. Micronuclei formation was found only in *Eucalyptus globulus*, *E. tereticornis*, *Leptospermum nicholsii* and *Agonis flexuosa* leaf extract assays. Ball metaphase stages were observed in experimentation with *Callistemon citrinus* and *Melaleuca styphelioides* leaf extracts. Stathmoanaphase was observed only on treatment with *Melaleuca styphelioides* leaf extract. Polyploid cells were found on treatment with *Corymbia citriodora* and *Syncarpia glomulifera* leaf extracts.

The cells, which enter division from interphase after exposure to the treatment, showed structural changes in chromosomes and the cells, which were already in division at the time of the treatment, showed the physiological effects. The mitotic poison may cause metabolic imbalances which may interfere with the synthesis, state and structure of nucleic acids including physiological effects and clastogenic effects which may lead to mitotic delay and mitotic inhibition (Soni *et al.*,1982).

### **Subfamily: Myrtoideae**

Under the subfamily Myrtoideae, of the 14 taxa studied, both clastogenic and nonclastogenic abnormalities occur in varied frequencies. Pulverisation of metaphase chromosomes was found to be a common clastogenic anomaly. The other clastogenic abnormalities uniformly observed in all the experiments were anaphase



and telophase stickiness and bridges. Nuclear lesions were found to be less frequent in this subfamily while comparing to that in Leptospermoideae.

The nonclastogenic abnormalities usually found in all the treatments include clumping of chromosomes at metaphase, diagonal orientation, ball metaphase, early movement of chromosomes, nonsynchronous movement of chromosomes and scattering of chromosomes. Stathmo anaphases were observed only in the case of experiments with *Pimenta dioica* extract. Micronuclei formation was found in the leaf extract treatments with *Eugenia apiculata*, *E. uniflora*, *Psidium guajava*, *Syzygium aromaticum*, *S. cumini* and *S. samarangense*. Polyploid cells were found only in *S. zeylanicum* leaf extract treatment. Where as, C- metaphase stages were found in *S. zeylanicum* and *Rhodomyrtus tomentosa* treatments.

### **A Comparative Account Of Cytotoxicity**

Of the 26 taxa studied for cytotoxicity of leaf extracts, *Callistemon citrinus*, *C. viminalis*, *Feijoa sellowiana*, *Psidium guajava*, *Syzygium cumini*, *S. jambos*, *S. malaccense* and *S. samarangense* showed mitotic divisions during all the five treatments ie. 2 hr., 4 hr., 6 hr., 12 hr., and 24 hr. (Table No.6,7,19,22,24,25,26 and 27). The leaf extracts of *Agonis flexuosa*, *Eugenia uniflora* and *Myrtus communis* showed mitotic divisions only up to 12 hr. (Table No.4,18 and 20). Treatment with extracts of *Corymbia citriodora*, *Eucalyptus globulus*, *Leptospermum nicholsii*, *Acmena smithii*, *Eugenia apiculata*, *Syzygium aromaticum*, *S. zeylanicum* and *Rhodomyrtus tomentosa* (Table No.8,10,12,16,17,23,28 and 29) showed mitotic division stages only up to 6 hr. treatments. After 12 hr. and 24 hr. treatments with these extracts the root tip cells were completely blackened and damaged. The experiments with the leaf extracts of *Beaufortia sparsa*, *Corymbia ficifolia*, *Eucalyptus tereticornis*, *Melaleuca leucadendron*, *M. styphelioides*, *Syncarpia glomulifera* and *Pimenta dioica* showed division stages only up to 4hr. treatment (Table No. 5,9,11,13,14,15 and 21).

The other treatments viz. 6hr, 12 hr. and 24 hr. with these extracts showed complete damages of root tip cells.

On analysing the cytotoxic effects, the subfamily Leptospermoideae showed severe activity while comparing to the subfamily Mytoideae. The leaf extracts of the plant, viz. *Corymbia ficifolia* was identified as having the most severe activity, as the 5% extract during 4hr. treatment showed no dividing cells. The root tip cells during this treatment showed bizarre form of nuclei during interphase and divisional stages (Fig.111,123,125,126 and 127). Nuclear diminution was also observed (Fig. 89,128) during the various treatments with leaf extracts like *Beaufortia sparsa*, *Eucalyptus tereticornis*, *Melaleuca leucadendron*, *M. styphelioides*, *Syncarpia glomulifera* and *Pimenta dioica*. All these members except *Pimenta dioica* belongs to the subfamily *Leptospermoideae*.

The cytotoxic chemicals act on mitotic cells in three different manners (Ray and Barman, 1987).

1. Preprophase inhibitor.
2. Inhibitor of mitotic spindle formation and orientation, compounds being termed as mitoclastic agents.
3. Inhibitor of cell plate and cell wall formation between daughter nuclei resulting in binucleate and multinucleate cells. Different kinds of aberrations were classified into clastogenic aberrations attributable to the direct action in chromosomes and nonclastogenic or physiologic aberrations attributable to spindle abnormalities.

Results obtained during the present study revealed both the clastogenicity and nonclastogenicity of the plant extracts, which is evident from the direct actions on the chromosomes and the manifestation of spindle abnormalities. The reduction of mitotic indices might have been achieved by the inhibition of DNA synthesis at S-phase (Sudhakar *et al.*, 2001). It may be due to slowing of the rate of cell progression through mitosis (Sharma and Sahu, 1997) or due to the obstruction of the onset of prophase or due to the arrest of mitotic phases (Kabarity and Mallalah, 1980).

Shehab (1985) reported that chemical principles of plant extracts are capable of causing cytotoxic effect. The essential oil obtained from several plants have been shown to exhibit mitodepressive effects on the division stages of *A. cepa*, *A. sativum* and *V. faba* (Khandelwal, 1986; Beutler, *et al.*, 1993; Hayes, *et al.*, 1997; Mackeen *et al.*, 1997; Wilson, *et al.*, 1997 and Foray, *et al.*, 1999).

The investigations with leaf extracts of various concentrations act through various means, all of which finally disturb nucleic acid metabolism leading to hazards in DNA and protein synthesis. This resulted in an array of abnormalities both at nuclear and chromosome levels on *A. cepa* root meristems (George and Geethamma, 1990). Several plant derived antimetabolic chemicals have been reported to be acting upon microtubules and induce mitotic aberrations (Hilman and Ruthman, 1982; Chauhan, *et al.*, 1986). According to Sarma (1980), the changes brought about by the viscosity of the cytoplasm are primarily responsible for the chromosome abnormalities like unequal separation, formation of fragments during anaphase, polyploidy, chromatid separation, micronuclei formation, multinucleate cells *etc.*

### **CLASTOGENIC AND NONCLASTOGENIC ABNORMALITIES INDUCED BY MYRTACEOUS PLANT EXTRACTS**

A comparison of the clastogenic and nonclastogenic effects caused by myrtaceous plant extracts of the present study showed wide variations among themselves. In all the experiments the predominant type of cytotoxic anomalies observed, come under the clastogenic category. As quoted previously, the earlier workers were of opinion that the phytochemicals present in the leaf extracts might have a direct action on the chromosomes, DNA structure and protein synthesis. These results were manifested both at the nuclear and chromosome levels. Sometimes these chemicals might have been responsible for the changes in the viscosity of the cytoplasm also.

On the otherhand, the phytochemicals showed physiologic or nonclastogenic effects also on the *A. cepa* root meristem. This might have been caused due to spindle abnormalities or inhibition in spindle formation.

Out of the twenty six members studied presently, the percentage of clastogenicity was more pronounced in Leptospermoideae than Myrtoideae. All the members of Leptospermoideae showed high percentage of clastogenic anomalies with the least value in *Eucalyptus globulus* (56.11%) and highest in *Corymbia ficifolia* (99.93%). The nonclastogenic effects were found to be comparatively lesser in all the experiments ( Table 30 ). This indicates that the cytotoxic principles in the subfamily Leptospermoideae react directly on the chromosomes, DNA, protein and to a lesser extent affect the cytoplasm.

Under the subfamily Myrtoideae also, more pronounced cytotoxic effects were of the clastogenic category. Out of the fourteen taxa studied *Feijoa sellowiana* showed the maximum effect (77.68%) and the minimum was observed in the case of *Syzygium zeylanicum* (54.81%), [Table 30]. Eventhough the members of Myrtoideae showed high percentage of clastogenicity, it was lesser when compared to that of the members of Leptospermoideae. The phytochemicals present in the extracts of majority of the Myrtoideae members exerted a dual effect, which resulted in both clastogenic and nonclastogenic effects on the root meristems.

## CLASTOGENIC ABNORMALITIES

### Chromosome Stickiness

Chromosome stickiness is one of major abnormalities noticed in almost all the experiments in the present study (Fig.31, 35, 36, 37, 39, 45, 48, 65, 66, 69, 78, 82, 83, 87, 99, 100, 102, 104, 106 and 108). Darlington (1942) reported that stickiness is due to the disturbances in the nucleic acid metabolism of the cell. Stickiness of chromosomes has been interpreted to be the result of depolymerisation of DNA ( Darlington,1942 ), partial dissolution of nucleoproteins (Kaufmann,1956),

breakage and exchange of basic folded fibre unit of chromatids (Klasterska *et al.*, 1976) and / or stripping of protein covering of DNA in chromosomes (Stephen, 1979). Stickiness may be due to the action of the extracts on the protein ( El Sadek, 1972 ) which form an integral part of the chromosomes.

Stickiness may result from the enlargement of the chromatin fibre which fail to condense properly during the initial stages of mitosis (Mc Gill *et al.*, 1974). It has been suggested that stickiness is a type of physical adhesion involving mainly the proteinaceous matrix of the chromatin material (Stephen, 1979). Certain chemical substances present in the extracts, affect the DNA structure of chromosomes resulting in the depolymerisation of DNA. This together with or without partial dissolution of nucleoproteins (Mercykutty and Stephen, 1980) cause the stickiness of chromosomes.

Grundmann (1966) suggested that stickiness of chromosomes is probably due to heterochromatinisation of nucleic acid and thus making the chromosome contour adhesive. Induction of stickiness is sometimes manifested as cytotoxic effect of chemical substances (Panda and Sahu, 1985), while electron microscopic studies demonstrated stickiness as a chromatid type aberration (Mc Gill *et al.*, 1974; Klasterska *et al.*, 1976). In the present investigation, stickiness of chromosomes induced by leaf extracts of the members of Myrtaceae on *A. cepa* may be due to any of the above mentioned cytotoxic phenomena or a combination of many of them.

### **Nuclear lesions**

Nuclear lesions (Fig.126, 131 and 132) was the major abnormality noticed in the experiments with several plant extracts under Leptospermoideae, *viz.* *Melaleuca leucadendron*, *M. styphelioides*, *Corymbia ficifolia*, *C. citriodora* and *Syncarpia glomulifera*. Lesions were found in the nucleus at the interphase stage. Mercykutty and Stephen (1980) noticed the occurrence of nuclear lesions induced by plant derived chemicals in *A. cepa* root meristematic cells which may be due to the disintegration of portion of nuclear material by the action of plant extracts.

Under the light of the above sighted references it seems probable that the occurrence of nuclear lesions may be due to the disintegration of the chromatin content resulted from the action of the major chemical principles present in the plant extracts.

### **Chromosome bridges**

Chromosome bridge formation (Fig. 70,71,72,73,76,91,94,95,96,97 and 104) was a major clastogenic abnormality observed in several experiments conducted with the plant extracts obtained from the subfamilies, *viz.* Leptospermoideae and Myrtoideae.

Bridge formation may be due to the general stickiness of chromosomes at metaphase (El – Khodary *et al.*, 1990; Ahamed and Yasim, 1992) and / or due to the chromosome breakage and reunion (Ahamed and Yasim, 1992; Premjit and Grover, 1985).

According to Dempong and Maxwell (1973), the occurrence of chromosome bridges may be due to stickiness or due to the formation of dicentric chromosomes caused by breakage and reunion. Chromosome bridges are formed by the failure of terminalisation in bivalents especially from the chromosomes stretched towards poles. It is also probable that bridges result by stickiness of chromosome ends. The breaks might have been produced by the direct effect of the phytochemicals present in the extract on the structure of chromosomes. The cells with breaks undergo reciprocal translocation resulting in the formation of multivalents (Somasekhar and Gowda, 1984). According to Najjar and Soliman (1980), bridge formation may be due to chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to the unequal translocation or inversion of chromosomal segment. Formation of double and multiple bridges are due to the fusion between broken chromosomes (Young and Young, 1973). Breaking up of chromosomes followed by proximal chromatid reunion evidently results in dicentric anaphase bridge that can be attributed to the general stickiness of chromosomes (Abraham and Koshy, 1979; Anis and Aijaz, 1997; Gomurgn, 2000).

Thus it seems probable that the above mentioned causes play an important role in the formation of chromosome bridges observed in the present study.

### **Fragmentation of chromosomes**

Chromosome fragmentation is another frequently observed anomaly in the present study (Fig.70, 74 and 98). The occurrence of chromosome fragmentation and the increase of this anomaly with respect to increase in concentration and time was observed by D' Amato (1950). The chromosome fragmentation is the result of breaking of chromosomes at their fragile sites (Chauhan and Chauhan, 1999). The reduction in size of chromosomes is partly due to elimination of heterochromatin portion and partly due to strain caused on account of tighter coiling of nucleoprotein complex in the presence of high iron concentration of calcium salts. The changes that may occur in the viscosity of cytoplasm is one of the reasons for chromosome abnormalities like fragmentation, micronuclei formation, multinucleate cells and formation of unequal groups of chromosomes (Sarma, 1980; Sideroskii, 1984).

The chance for the occurrence of the above mentioned molecular effects or a combination of several effects seems to be the probable reason behind the occurrence of chromosome fragments in *A. cepa* root meristem in the present investigation.

### **Differential condensation of chromosomes**

Differential condensation of chromosomes was noticed with leaf extracts of several plants in the present study. According to Sakari *et al.* (1981) the differential condensation of chromosomes may be probably due to the failure of protein synthesizing mechanism of the affected cell or it may be due to the disintegration of histone proteins associated with the DNA leading to condensation and decoiling of chromatin fibrils or may be due to the action of chemicals found in the leaf extract on proteins present in the chromosome (Fig.47 and 51). The above mentioned cytotoxic phenomena may be probably responsible for the occurrence of

differential condensation of chromosomes in *A. cepa* on treatment with some myrtaceous leaf extracts.

### **Ring Chromosome**

Ring chromosome formation was noticed in the low concentration and short duration treatments of *Melaleuca leucadendron* and *Pimenta dioica*. This may be due to the breakage of chromosome ends and reunion of chromosomes (Fig. 61). According to Sax (1940) the rings may arise as a result of two breaks in the chromosome. Raghuvanshi and Singh (1976) are of opinion that the rings may arise due to telomeric losses and this type of ring may persist while those lacking centromere are lost. The cytotoxic chemicals present in the extracts of these plants act directly on the fragile sites of the chromosomes leading to breakage at the terminal region and reunion of the raw ends of the chromosome so as to form a ring.

## **NON – CLASTOGENIC ABNORMALITIES**

### **Chromosome clumping**

Clumping of chromosomes was one of the most frequently observed abnormality in most of the experiments (Fig. 40,41,42). Hadder and Wilson (1958) suggested that the scattered and clumped metaphases are the partial and full effect respectively of a C-mitotic agent. This may be because of the action of the chemicals present in the plant extracts on chromosome to chromosome spindles and pole to pole spindles. This resulted in the change of the distance between chromosomes, hence they were seen scattered or clumped together. Pritchard and Court (1966) reported that increased concentrations of cytotoxic chemicals was found to be hastening the onset of clumping.

Clumping of chromosomes, a prominent nonclastogenic abnormality encountered in most of the treatments of the present investigation seems to be due to the C-mitotic action of the phytochemicals present in the extracts.



### **Chromosome pulverization**

Pulverization of chromosomes was one of the major nonclastogenic abnormality observed in many of the members of the subfamily Myrtoideae and in a few members of the subfamily Leptospermoideae (Fig. 34,47,55,57,83 and 84 ).

According to Sakari *et al.* (1981), pulverization of chromosomes may be due to the premature condensation of chromosomes as a result of the action of chemical substances found in the leaf extracts.

In the present investigation pulverization of chromosomes observed in the treatments with extracts of several members of Myrtoideae and a few members of Leptospermoideae may be due to the reasons envisaged by earlier workers.

### **Laggard Formation**

Lagging of chromosomes (Fig. 65,72 and 77) was observed when the root tips were treated with extracts obtained from the leaves of *Agonis flexuosa*, *Corymbia citriodora* and *Eugenia apiculata*. Barthelmass (1957) and Nagpal and Grover (1994) reported that the lagging of chromosomes might be because of the hindrance of prometaphase movement of chromosomes, accompanied by adhesion of the centromere to the nuclear membrane or to the surrounding surface of the plasma membrane. According to Premjit and Grover (1985) laggards can be attributed to the delayed terminalisation, stickiness of chromosome end or because of the failure of chromosomal movements. Sagoo *et al.* (1991), suggested that the lagging of chromosomes is due to the abnormal activity of the spindle fibres. The failure of the normal organisation and function of spindle apparatus may lead to the formation of laggards (Patil and Bhat, 1992).

Chromosome laggards observed in some treatments with extracts of a few plants may be due to any one of the above mentioned reasons or due to a combined effect.

### **Ball metaphase**

Ball metaphase (Fig. 42,44 and 45) was observed in the treatments with leaf extracts of *Callistemon citrinus*, *Melaleuca styphelioides*, *Acmena smithii*, *Eugenia apiculata*, *Eugenia uniflora*, *Feijoa sellowiana*, *Pimenta dioica*, *Psidium guajava*, *Syzygium aromaticum*, *S. cumini*, *S. jambos*, *S. malaccense*, *S. samarangense* and *S. zeylanicum*. Ball metaphase is a form of C-mitosis with characteristically clumped chromosomes. Barber and Callan (1943) reported that the ball metaphase is followed by either a complete degeneration of the cell or a state similar to interphase. In the present study the ball formation observed during metaphase stage is supposed to be due to the localised activity of spindle apparatus induced by the phytochemicals present in the extracts, which is confirmed by earlier studies. Chromosomes were arranged in such a manner that their centromeres remain at the equator and arms radiating in different directions to form a "ball" configuration.

### **Disturbed metaphases and anaphases**

Both disturbed metaphases and anaphases (Figs. 38,42,43,52,53,56,57,58,60, 63 and 67) were observed in several experiments with extracts of members studied. Inhibition of spindle formation lead to disturbed metaphases and anaphases (Amer and Farah, 1983; El Khodary, 1989). According to Heaps *et al.* (1982) and Saleem *et al.* (1993), this may be due to the loss of activity of microtubules in spindle fibres. Disturbed metaphases and anaphases might be due to the disturbance of the spindle apparatus (Shehab *et al.*, 1978). Shehab (1979), suggested that the plant extracts are having an action on the spindle and hence they can be considered as stathmo – kinetic agents.

The occurrence of disturbed metaphases and anaphases in most of the treatments may be due to the stathmo - kinetic action exhibited by these plant extracts as has been visualized by earlier workers in other plant extracts as well.

### **Diagonal orientation of chromosomes**

Diagonal orientation of chromosomes during metaphases, anaphases and telophases (Figs.35,37,39,42,46,47,70,78,79,80,83,84,88,89,90,91,94,101,105 and 110) happens to be the much frequently observed physiological anomaly noticed during the present study. This may be due to the improper functioning of spindle apparatus induced by the chemicals found in the leaf extracts. According to Das *et al.* (1968), the improper functioning of spindle apparatus cause the diagonal orientation of chromosomes. This phenomenon has also been noticed by other workers (Tajo and Thoppil, 1998; Deena and Thoppil, 2000; Sreeranjini and Thoppil, 2001).

In the present investigation, it seems probable that the diagonal orientation of chromosomes during metaphase, anaphase and telophase may be due to a slight or a severe tilt in the normal orientation of the mitotic apparatus effected by the cytotoxic chemicals present in the extracts.

### **Multipolarity**

Multipolarity of chromosomes (Figs.64 and 67) was noticed in some leaf extract treatments. Occurrence of tripolar cells indicates the inhibition of cytokinesis (Somasekhar and Gowda, 1984). The error in spindle organization even leads to split and multipolar spindle (El - Khodary *et al.*, 1990).

In view of the above mentioned references the multipolar groups of chromosomes observed in some abnormal cells in a few treatments might have been due to the drastic effect of the spindle poison which may alter or affect the mitotic spindle apparatus leading to multipolarity.

### **C – metaphase**

C – metaphase (Figs. 46,48,49 and 50) is observed in treatments with the extracts of *Agonis flexuosa*, *Callistemon viminalis*, *Corymbia citriodora*, *Eucalyptus globulus*, *Leptospermum nicholsii*, *Syzygium zeylanicum* and *Rhodomyrtus tomentosa*. Levan (1938), named the scattering of chromosomes by spindle inhibition as C – mitosis or Colchicine mitosis. According to Nagl (1970), the spindle abnormalities lead to C – metaphase and at the same time even causes the breakage of metaphase chromosomes. Sharda *et al.* (1973), suggested that the C – mitosis is the secondary effect of a prolonged metaphase. The C – metaphase is produced as a result of inhibition of spindle fibre formation (El – Khodary *et al.*, 1989). The C- metaphase may lead to the formation of polyploid cells (Deysson, 1968) and such polyploid cells degenerate without further division.

The mitotic anaphase gets blocked due to the toxic effect of the chemicals found in the extracts, which leads to the C – mitotic divisions and ultimately results in polyploidy (Redei, 1998). C – mitosis is one of the consequences of inactivation of spindle apparatus associated with the delay in the division of centromere (Gomurgn, 2000). In C – metaphase chromosomes were found scattered and chromatids become very clear. The above mentioned causes seem to be responsible for the occurrence of C- metaphase in *A. cepa* root meristem, on treatment with some plant extracts.

### **Polyploidy**

Polyploid cells were noticed in the treatments with leaf extracts of *Corymbia citriodora*, *Syncarpia glomulifera* and *Syzygium zeylanicum* treatments (Fig. 62 ).

Polyploidy is a major numerical chromosomal abnormality observed with many plant extracts. Raj and Reddy (1971) and Minija *et al.* (1999), attributed polyploidy to the inhibition of spindle mechanism. Herichova (1973), noticed

polyploid cells in mitosis of barley after application of spindle destructing chemicals. The occurrence of hyperploid cells may be attributed to the spindle inhibition, lack of anaphase movement or failure of cell plate formation (Nagpal and Grover, 1994). According to Onfelt and Klasterska (1983), mitotic abnormalities are generally considered insignificant from the mutation point of view unless polyploid cells or aneuploid cells are induced.

Polyloid cells observed in *A. cepa* root meristem on treatment with extracts of some members of Myrtaceae may be due to inhibition of spindle mechanism, lack of anaphase movement or hindrance of cell plate formation resulted from the action of spindle destructing chemicals present in these plants.

### **Binucleate, Trinucleate, Tetranucleate and Multinucleate cells**

The formation of binucleate, trinucleate, tetranucleate and multinucleate conditions during interphase were observed in several experiments done with the leaf extracts of members of both the subfamilies (Figs. 109,110,119,121 and 122).

Oksala and Therman (1974) suggested that binucleated and multinucleated conditions are the peculiarities of cancer cells. Delay or failure of cytokinesis would account for the occurrence of binucleate and multinucleate cells (Ene-Obong and Amadi, 1987).

Binucleate cells are formed due to the inhibition of cell wall development during telophase. Trinucleate conditions may be the result of the endomitotic division that might have occurred inside the binucleate cells, *ie.* only one of the daughter nuclei undergoes mitotic division where as the other remained as such. The tetranucleate cells may be formed because of simultaneous endomitotic divisions occurred within a binucleate cell. The multinucleate conditions observed may be also due to the repeated endomitotic divisions.

The formation of the multinucleate cells may be the result of preceding multipolar mitosis or failure of cell plate formation (Grant, 1978). Binucleate cells

may arise due to the suppression of cell plate formation in the early telophase by the leaf extract treatment. Hence neither the cell plate nor the cell wall appeared at the equatorial plane in the treated cells (Sato and Tanaka, 1972).

Bi-, tri-, tetra-, and multinucleate cells frequently found in several treatments might be due to the inhibition of cell wall development at telophase. Several cyclic hydrocarbons, methylated oxypurines and plant extracts were known to inhibit cell plate formation (Amer *et al.*, 1971; Abraham and Cherian, 1976).

Mechanism of cytokinesis in plant cells involves the formation of new cell walls with the help of different organelles like microtubules, golgibodies and possibly mitochondria. However, accumulation of these organelles in the vicinity of phragmoplast was not observed in treated cells. Occasionally incomplete cell wall formation was also noticed. Such effects indicate that the chemicals found in the plant extracts affect either the function of microtubules or golgibodies or both. This might have led to the formation of multinucleate cells (Haur *et al.*, 1980; Heaps, 1967).

### **Early movement of chromosomes**

Early movement of chromosomes (Figs.67, 68 and 75) may be due to the disturbance in the spindle mechanism (Tajo and Thoppil, 1998). This kind of anomaly was observed in the treatments with majority of the leaf extracts. This may be due to the specific activity of some phytochemicals present in these extracts on the mitotic spindle apparatus leading to its malfunctioning.

### **Nonsynchronous movement**

Nonsynchronous movement of chromosomes was a frequently observed abnormality noticed during several experiments. According to Minija *et al.* (1999), nonsynchronous movement may be due to severe imbalances in the spindle mechanism. It may be due to the multipolar nature of the mitotic spindle apparatus

caused by the major chemical constituents of the plant extracts (Figs. 67, 75 and 82).

### **Star anaphase**

Star anaphase is the star shaped arrangement of chromosomes (Fig. 69) observed in the leaf extract treatment of *Pimenta dioica* and *Myrtus communis*. This kind of arrangement may be due to the clumping of daughter chromosomes into star like structures near the polar region of the cell. Disturbed spindle activity may be the probable cause of star anaphase.

### **Stathmo anaphase**

This was another abnormality observed in the treatment with leaf extracts of *Melaleuca styphelioides* and *Pimenta dioica*. Here, the daughter chromosomes do not separate fully, but they remain connected together by means of partial overlapping of the arms (Figs. 92 and 93). This may also be due to the abnormal functioning of spindle fibres. Since the extracts are having toxic activities on the spindle, they can be considered as stathmo-kinetic agents (Shehab, 1979).

### **Misorientation of chromosomes**

This was one of the major anomaly observed in several treatments during the present study (Figs. 54 and 85). Disturbed functioning of the spindle apparatus may be the reason for this kind of misoriented chromosome distribution. The disturbances can be the distortion of the spindle apparatus, a tilt in the equatorial organisation of metaphase chromosomes or a change in the direction of movement of daughter chromosomes during anaphase ( Das *et al.*, 1968; Saliem *et al.*, 1981 and Tajo and Thoppil, 1998).

### **Micronuclei formation**

Micronuclei formation was noticed in the leaf extract treatments of *Agonis flexuosa*, *Eucalyptus globulus*, *E. tereticornis*, *Leptospermum nicholsii*,

*Melaleuca leucadendron*, *M. styphelioides*, *Eugenia apiculata*, *E. uniflora*, *Syzygium aromaticum*, *S. cumini*, *S. samarangense* and *Psidium guajava* (Figs. 103, 107, 114 and 115).

The formation of micronuclei may be due to the action of the chemicals present in the extract on the spindle apparatus, leading to unequal separation of chromosomes at anaphase. The larger group of daughter chromosomes form a comparatively larger nucleus and the smaller group forms a micronucleus.

#### **Unequal cell division**

This kind of anomaly was observed in almost all the treatments, which showed the formation of micronuclei. The chromosomes after the unequal mitotic division form a large nucleus and a smaller nucleus. They separate to form two independent daughter cells, one with a large nucleus and other with small nucleus. Both these cells may become nonviable (Figs. 103, 107, 113, 114, 116, 117, 118, 120, 121, 123, 125, 126, 127, 129, 130 and 131).

#### **Interphase and Prophase anomalies**

The onion root tip cells after the treatment with many of the leaf extracts showed severe abnormalities both in prophase and interphase stages. They include nuclear transfer, bizzare nuclei formation, unusually enlarged nucleus, nuclear extrusion, pycnotic micronuclei formation, nuclear diminution and disintegration.

Due to the action of certain chemicals in the leaf extract, the division stages were arrested and nuclear extrusion occurred (Fig. 133). Raghuvanshi and Singh (1976) noticed such an effect on treating *Trigonella foenum-graecum* cells with Gamma rays. The cells showed nuclear extrusion during prophase. This may be due to karyokinetic activity of mutagenic agents. According to Raghuvanshi and Singh (1976), 'nuclear transfer' between PMC's known as 'cytomixis' is fairly common in a number of angiosperms. They suggested that in the somatic tissue cell wall is rather hard and 'nuclear transfer' does not appear to be common.

In the present study 'nuclear transfer' between somatic cells were observed that might be due to the dissolution activity of the chemicals on the cell walls, which resulted in nuclear extrusion.



The chemical constituents present in the leaf extracts of the family Myrtaceae showed a wide spectrum of toxic effects on *A. cepa* root tip cells. The unusually enlarged nuclei during interphase and prophase stages (Figs. 32 and 33) were the common effects observed in almost all treatments.

Nuclear extrusion (Fig.133), formation of pycnotic nuclei (Fig. 112), endomitotic cells (Fig. 119,120, 121 and 129), crescent shaped nuclei formation (Fig. 118), bizzare forms of nuclei at interphase (Figs. 123 and 127), nuclear diminution during interphase (Figs. 116 and 128), nonsynchronised divisions and endomitosis (Figs. 119, 120 and 121), abnormal cell division forming micro and macrocells (Figs. 114 and 131), abnormal scattering of chromosomes during division stages (Figs. 60 and 124), nuclear lesions (Figs. 131 and 132), and disintegration of chromosomes during divisional stages (Figs. 86, 116, 125, 128 and 132) were some other cytotoxic effects noticed during the present study. All these effects may be due to the direct action of chemicals present in the leaf extracts on the DNA associated proteins and mitotic spindle apparatus.

In nutshell, it can be stated that all the aberrations, result in the mitotic arrest directly or indirectly. Hence the final outcome, the reduction of mitotic indices. However, it is proved from the earlier experimentations that *A. cepa* assay has an excellent correlation with mammalian systems (Grant, 1982). Thus from the results of this study, it is suggested that the crude extract of various plants coming under the family Myrtaceae possess potential cytotoxic as well as cytostatic activities, which can be exploited only after a detailed analysis of the essential oils, the major secondary metabolites found in the leaves of the members of Myrtaceae.

## B. ESSENTIAL OIL ANALYSIS

The leaf essential oils of 26 taxa of Myrtaceae showed wide variation with respect to their yield on a dry weight basis (Tables 31 & 32) and essential oil composition (Tables 33 to 58).

### Oil Yield:

Under the subfamily Leptospermoideae, of the 12 members studied, *Corymbia ficifolia* and *Melaleuca styphelioides* were with the least herb oil content. The plants which contain high amount of leaf essential oil were *Eucalyptus globulus* and *Eucalyptus tereticorni*. The leaves of other members, viz. *Corymbia citriodora*, *Agonis flexuosa*, *Callistemon viminali*, *Beaufortia sparsa*, *Syncarpia glomulifera*, *Callistemon citrinus*, *Melaleuca leucadendron* and *Leptospermum nicholsii* were found to be with moderate oil content (Table 31).

Of the 14 members analysed for the leaf essential oil yield under the subfamily Myrtoideae, *Eugenia apiculata* and *Syzygium zeylanicum* were found to be with very low oil content. Where as, *Syzygium aromaticum*, *Pimenta dioica* and *Myrtus communis* show the abundance of herb oil. All other members studied presently, viz. *Acmena smithii*, *Eugenia uniflora*, *Feijoa sellowiana*, *Psidium guajava*, *Syzygium cumini*, *S. jambos*, *S. malaccense*, *S. samarangense* and *Rhodomyrtus tomentosa* possess less than 1% of essential oil yield on a dry weight basis (Table 32).

The oil yield from the leaves of *Corymbia citriodora* collected from various locations in Kenya ranged from 2.2 – 8.3% (Mwangi *et al.*, 1980). Singh *et al.* (1989) revealed that the oil yield of thirteen species of *Eucalyptus* growing in India ranged from 0.63 – 5.84%. Essential oil yield from dried leaves of *Corymbia citriodora* grown in different localities in Benin were analysed and it ranged from 2.3 – 5.9% (Moudachirou *et al.*, 1999). The leaf essential oil yield of *C. citriodora* growing in Greece varied from 1.77 – 4.5% on a dry weight basis (Mejdoub, 1995). Shiva *et al.* (1989) reported that the oil yield of *Eucalyptus tereticornis* ranged from 1.57% - 2.09% on a fresh weight basis.

Chisowa (1997) estimated the essential oil yield from *Eucalyptus globulus*, *E. radiata* and *E. smithii* and detected that it ranged from 6 – 9% on a dry weight basis.

The dried leaves of *Syzygium cumini* contained 0.18% essential oil with a sweet aroma (Khanna, 1991). The oil yield from *Callistemon citrinus* was analysed by Misra *et al.* (1997) and detected that it ranged from 0.2 – 0.6% in fresh leaves, flowers and fruits respectively. Brophy *et al.* (1997a) noticed the leaf essential oil yield of *Callistemon viminalis* and it ranged from 0.08 – 0.63% on a fresh weight basis with regards to their geographical distribution. Brophy *et al.* (1998 d) studied the essential oil content in fresh leaves of nine species of *Leptospermum* and noticed that it ranged from 0.7 – 0.1%. The oil yield from the fresh leaves of two subspecies of *Syncarpia glomulifera*, viz. *ssp. glomulifera* and *S. glomulifera ssp. glabra* was in the range of 0.1- 0.4% (Brophy *et al.*, 1996). Brophy *et al.* (1999e) isolated 0.05 – 0.1% volatile oil from *Melaleuca styphelioides* leaves.

Based on the present investigation, the leaves of majority of members of the subfamily Leptospermoideae were found to be “oil rich”. But, *Corymbia ficifolia* and *Melaleuca styphelioides* were found to be the “oil poor” taxa in this subfamily ( Table 31). In the case of the subfamily Myrtoideae, most of the taxa were found to be “oil poor”, baring *Myrtus communis*, *Pimenta dioica* and *Syzygium aromaticum* which were found to be “oil rich” (Table 32).

### **Chemical Composition**

The present study reveals that the major essential components of the 26 members of the family Myrtaceae belong to three classes of aromatic compounds, viz. monoterpenoids, sesquiterpenoids and phenolic compounds.

### a) Leptospermoideae

In this subfamily, the herb oil of *Agonis flexuosa* showed the dominance of monoterpenoid compound myrcene. The leaf essential oil of *Beaufortia sparsa* showed the dominance of the monoterpene,  $\alpha$ -terpinene. In *Callistemon citrinus* the monoterpenoid, citral was detected as the major compound in the leaves. Where as, in *Callistemon viminalis*, 1,8-cineole was detected as the principal leaf oil compound.

The monoterpenoid, citronellal was found as the main component of *Corymbia citriodora* leaf essential oil. Where as, terpinyl acetate was the main component in the volatile oil of *Corymbia ficifolia* leaves. In *Eucalyptus globulus* the leaf essential oil contain the monoterpenoid,  $\alpha$ -pinene as the principal compound. While in *Eucalyptus tereticornis* two monoterpenoids, viz.  $\alpha$ -pinene and piperitone oxide were detected as 'co-dominant' compounds in the leaf essential oil.

*Leptospermum nicholsii* leaf essential oil showed two monoterpenoids, viz.  $\alpha$ -pinene and citral and a sesquiterpenoid, nerolidol as the principal compounds.

*Melaleuca leucadendron* herb oil showed substantial amount of the sesquiterpenoid,  $\beta$ -caryophyllene. But *M. styphelioides* leaf oil showed a mixed chemical composition of terpenoids.

In the leaf essential oil of *Syncarpia glomulifera* two monoterpenoids, viz. limonene and  $\alpha$ -terpinene showed 'co-dominance'.

The chemical composition of leaf essential oil of 12 members of the subfamily Leptospermoideae is discussed in detail below (Tables 33 – 44).

#### 1. *Agonis flexuosa*

The major chemical component detected from the leaf essential oil of *Agonis flexuosa* was a monoterpenoid, myrcene (45.84%) followed by other major monoterpenoids,  $\alpha$ -thujene (12.53%), limonene (9.05%),  $\gamma$ -terpinene (7.66%), sabinyl acetate (5.38%),  $\alpha$ -phellandrene (3.47%) and

$\alpha$ -terpinene (1.84%). The major sesquiterpenoids were germacrene (5.43%) and iso caryophyllene (2.53%). The major phenolic compound was iso eugenol (1.02%) (Table 33 and Fig. 134).

With regards to herb oil composition of *A. flexuosa*, 86.54% was found to be monoterpenoids, 8.87% sesquiterpenoids, 1.65% phenolic compounds and 2.94% undetected chemical compounds. The findings of the herb oil analysis of *A. flexuosa* is a new report. So it seems probable that the essential oil of *A. flexuosa* belongs to a monoterpene class and the chemotype seems to be "Myrcene >  $\alpha$ -thujene".

## 2. *Beaufortia sparsa*

The leaf essential oil of *B. sparsa* showed the presence of the monoterpene compound,  $\alpha$ -terpinene (78.27%) as the dominant constituent. The major monoterpenoids detected include myrcene (5.96%), 1,8-cineole (2.28%), methyl benzoate (1.79%) and  $\beta$ -terpinene (1.39%). The sesquiterpenoids detected were  $\beta$ -caryophyllene (2.52%) and  $\delta$ -selinene (1.71%). The phenolic compound, eugenol was found in lesser amount (0.67%) (Table 34 and Fig. 135).

The herb oil of *B. sparsa* consists of 91.41 % monoterpenoids, 4.23 % sesquiterpenoids, 0.67 % of phenolic compounds and 3.69 % of undetected trace compounds. The results obtained from the chemical analysis of the leaf essential oil of *B. sparsa* is a new report. From these results it can be assumed that the herb oil of *B. sparsa* also belongs to the monoterpene class of essential oils and bears the distinct chemotype " $\alpha$ -terpinene".

## 3. *Callistemon citrinus*

*C. citrinus* leaf essential oil on GC analysis showed the dominance of the monoterpene, citral (64.21%). Other major monoterpenoids detected include citronellol (4.89%),  $\beta$ -thujene (2.28%),  $\beta$ -pinene (2.03%) and citronellal (1.21%). The sesquiterpenoids detected include  $\beta$ -elemene (3.64%) and  $\beta$ -caryophyllene

(2.40%). The phenolic compounds isolated were eugenol (14.31%), iso eugenol (2.89%) and eugenyl acetate (0.74%) (Table 35 and Fig. 136).

The herb oil of *C. citrinus* is composed of 75.33% of monoterpenoids, 6.04% of sesquiterpenoids, 17.94% of phenolic compounds and 0.69% of undetected trace chemicals. Previous reports (Misra *et al.*, 1997; Brophy *et al.*, 1998e; Ming *et al.*, 1998) confirm the occurrence of many of these chemical constituents in the leaf essential oil of *C. citrinus* grown in different geographical regions. The results obtained in the present investigation suggest that the herb oil of *C. citrinus* may come under the mixed class of essential oils and the chemotype seems to be “Citral > eugenol”.

#### 4. *Callistemon viminalis*

The most important compound detected from the leaf essential oil of *C. viminalis* was a monoterpenoid, 1,8-cineole (61.37%). The other monoterpenoids obtained were citronellol (14.43%),  $\beta$ -pinene (3.53%), sabinene (2.85%) and citronellal (2.56%). The major sesquiterpenoids were germacrene (2.75%) and  $\delta$ -selinene (2.01%) (Table 36 and Fig. 137).

The chemical composition of *C. viminalis* leaf essential oil can be summarised as monoterpenoids (88.69%), sesquiterpenoids (5.96%), phenolic compounds (0.5%) and undetected chemical compounds (4.85%).

Brophy *et al.* (1997a) identified the major component in the leaf essential oil obtained from *C. viminalis* as 1,8-cineole (60.82%). So it can be confirmed that the herb oil of *C. viminalis* belongs to the monoterpenoid class of essential oils and the chemotype is “1,8-cineole > citronellol”.

#### 5. *Corymbia citriodora*

The dominant chemical principle detected from the leaf essential oil of *C. citriodora* was citronellal (61.40%), a monoterpenoid. Other major monoterpenoids identified were  $\alpha$ -thujone (7.06%), citronellol (5.07%), 1,8-cineole

(3.13%), isoborneol (2.68%),  $\alpha$ -terpineol (1.29%) and methyl cinnamate (1.23%). The major phenolic compound identified was carvacrol (6.83%).

The chemical composition of essential oil of *C. citriodora* include 85.57% monoterpenoids, 6.83% phenolic compounds, 1.51% sesquiterpenoids and 6.09% undetected chemical compounds (Table 37 and Fig. 138). Earlier workers have confirmed the occurrence of citronellal as the dominant chemical component of the herb oil of *C. citriodora*, followed by citronellol in substantial amounts (Mwangi *et al.*, 1980; Moudachirou *et al.*, 1999; Dagne *et al.*, 2000). Thus it is confirmed that the leaf oil of *C. citriodora* belongs to the monoterpenoid class and the chemotype is "Citronellal".

## 6. *Corymbia ficifolia*

The herb oil of *C. ficifolia* showed the presence of terpinyl acetate, a monoterpenoid, as the dominant compound (55.33%). Other major monoterpenoids detected were methyl cinnamate (4.19%),  $\alpha$ -terpinolene (2.12%), iso bornyl acetate (1.44%) and  $\beta$ -terpineol (1.36%). The major sesquiterpenoids were  $\gamma$ -cadinene (8.10%), globulol (5.21%), bicyclogermacrene (4.02%),  $\beta$ -farnesene (1.35%) and caryophyllene oxide 1.25%). Chavicol (5.66%) was the major phenolic compound.

The chemical composition of essential oil of leaves was as follows: monoterpenoids 66.78%, sesquiterpenoids 21.38%, phenolic compounds 5.66% and trace compounds 6.18% (Table 38 and Fig. 139).

An earlier chemical report by Bignell *et al.* (1996) confirms the presence of many of these essential oil compounds in *C. ficifolia*. Thus in the light of the present study, it is assumed that the leaf essential oil of *C. ficifolia* belong to the monoterpenoid class of essential oils and the chemotype seems to be "Terpinyl acetate".

### 7. *Eucalyptus globulus*

GC analysis of the leaf essential oil of *E. globulus* revealed the presence of the monoterpene,  $\alpha$ -pinene (30.26%) as the major constituent. Other major monoterpenoids detected were cymene (11.85%), isobornyl acetate (7.75%),  $\gamma$ -terpinene (6.80%), piperitone oxide (5.73%),  $\beta$ -terpinene (3.41%),  $\alpha$ -terpinene (2.59%), limonene (2.14%), myrcene (1.55%) and camphene (1.52%). Iso eugenol (1.74%) and methyl chavicol (1.10%) were the phenolic compounds identified. The major sesquiterpenoids were  $\alpha$ -humulene (3.68%), germacrene (1.92%),  $\beta$ -farnesene (1.89%), aromadendrene (1.88%) and  $\beta$ -elemene (1.64%).

The chemical composition of the herb oil of *E. globulus* was as follows: Monoterpenoids (75.45%), Sesquiterpenoids (11.44%), Phenolic compounds (2.84%) and undetected chemical compounds (10.27%) (Table 39 and Fig. 140).

Several earlier reports (Baslas and Saxena, 1984; Dayal and Ayyar, 1986; Adikari *et al.*, 1992; Chisowa, 1997) confirm the presence of these essential oil components in the leaves of *E. globulus*. The peculiar essential oil composition revealed in the present investigation suggests that the herb oil of *E. globulus* may probably belong to the monoterpene class of essential oils and the chemotype seems to be a "Mixed monoterpene" one.

### 8. *Eucalyptus tereticornis*

The leaf essential oil of *E. tereticornis* shows the 'co-dominance' of the monoterpenoids, viz.  $\alpha$ -pinene (34.32%) and piperitone oxide (36.19%). Other major monoterpenoids were linalool (4.85%),  $\alpha$ -terpinolene (3.27%), bornyl acetate (2.78%), citronellyl acetate (2.52%) and  $\beta$ -pinene (1.03%). The major sesquiterpenoids detected were  $\beta$ -elemene (2.74%), iso caryophyllene (2.24%) and bicyclogermacrene (1.08%).

The chemical composition of essential oil of *E. tereticornis* can be summarised as follows: monoterpenoids 86.71%, sesquiterpenoids 7.81% and undetected compounds 5.48%. No phenolic compounds were detected during the present study (Table 40 and Fig. 141).



Previous reports ( Shiva *et al.*, 1984; Dayal and Maheshwari, 1985) confirm the presence of many of these aromatic principles in the leaf essential oil of *E. tereticornis*. So it seems probable that the herb oil of *E. tereticornis* may come under the monoterpenoid class of essential oils and the chemotype may be “ $\alpha$ -pinene – piperitone oxide”.

#### 8. *Leptospermum nicholsii*

The leaf essential oil of *L. nicholsii* showed the dominant monoterpenoid compounds like  $\alpha$ -pinene (18.80%), citral (17.62%),  $\beta$ -phellandrene (15.17%) and the sesquiterpenoid compound, nerolidol (17.48%). The other major monoterpenoid compounds were neral (4.27%),  $\gamma$ -terpinene (3.39%) and piperitone (1.39%). Other major sesquiterpenoids, *viz.* farnesal (4.66%),  $\beta$ -bourbonene (2.43%),  $\beta$ -caryophyllene (2.00%) and  $\beta$ -bisabolene (1.69%) were also detected. The only phenolic compound detected was methyl eugenol (1.52%).

61.55% of monoterpenoids, 28.66% of sesquiterpenoids, 1.52% phenolic compounds and 8.27% of undetected chemical compounds were found in the essential oil of *L. nicholsii* (Table 41 and Fig. 142). Several earlier workers (Brophy *et al.*, 1993, 1998c, 1999b; 1999d; Ibrahim *et al.*, 1995; Perry *et al.*, 1997) revealed the prevalence of many of these essential oil components in the genus *Leptospermum*. Results obtained from the chemical analysis suggest that the leaf essential oil of *L. nicholsii* may belong to the terpenoid class of essential oils and the chemotype seems to be “Mixed terpenoid”.

#### 10. *Melaleuca leucadendron*

In the leaf essential oil of *M. leucadendron* the sesquiterpenoid,  $\beta$ -caryophyllene (19.49%) was detected in substantial amount. The major monoterpenoids detected were 1,8-cineole (9.7%),  $\alpha$ -terpinolene (8.96%), linalool (5.86%), sabinene (4.81%), myrcene (4.77%),  $\alpha$ -pinene (4.3%),  $\beta$ -terpinene (1.53%) and camphene (1.27%). The other major sesquiterpenoids found were

$\gamma$ -cadinene (7.97%), iso caryophyllene (2.06%),  $\beta$ -cadinene (2.01%), farnesal (1.88 %), germacrene (1.77%),  $\beta$ -elemene (1.54%),  $\beta$ -bourbonene (1.5%) and globulol (1.31%). Methyl iso eugenol (3.53%) was the only phenolic compound detected. The essential oil of *M. leucadendron* was composed of 44.09% monoterpenoids, 40.11% sesquiterpenoids, 3.53% phenolic compounds and 12.27% of undetected chemical compounds (Table 42 and Fig. 143).

Earlier works conducted on the leaf essential oil of *M. leucadendron* (Ramanoelina *et al.*, 1994; Farag *et al.*, 1998) and related species (Brophy *et al.*, 1988, 1993) reveal the presence of many of these compounds. From the present study it can be suggested that the herb oil of *M. leucadendron* may belong to a mixed terpenoid class and the chemotype seems to be "Mixed terpenoid".

### 11. *Melaleuca styphelioides*

17 volatile compounds were detected from the leaf essential oil of *M. styphelioides*. The major monoterpenoid compound include  $\alpha$ -terpinene (8.21%) and the sesquiterpenoids were  $\gamma$ -cadinene (19.63%),  $\alpha$ -cadinene (16.64%),  $\beta$ -farnesene (8.52%),  $\alpha$ -cadinol (7.53%),  $\alpha$ -farnesene (6.64%),  $\beta$ -caryophyllene (4.31%) and aromadendrene (4.22%). Nine other constituents were found in traces. Eugenyl acetate (1.09%) was the only phenolic compound detected.

The herb oil of *M. styphelioides* was composed of 16.34% monoterpenoids, 71.65% sesquiterpenoids and 1.09 % phenolic compounds. 10.92% of the essential oil remains undetected (Table 43 and Fig. 144).

Previous works on the essential oil of *M. styphelioides* (Farag *et al.*, 1998; Brophy *et al.*, 1988, 1999e) also reveals the dominance of sesquiterpenoid compounds in this taxa.

Hence this oil belongs to the sesquiterpenoid dominant class of essential oils and the chemotype seems to be "Mixed sesquiterpenoid".

## 12. *Syncarpia glomulifera*

In the leaf essential oil of *S. glomulifera*, the monoterpenoids viz.  $\alpha$ -terpinene (28.26%) and limonene (22.14%) were identified as 'co-dominant' ones. Other major monoterpenoids include myrcene (7.16%),  $\alpha$ -thujene (4.76%),  $\beta$ -thujene (4.42%),  $\beta$ -phellandrene (3.74%) and 1,8-cineole (1.79%). The sesquiterpenoids that were found in major amounts were  $\beta$ -caryophyllene (3.9%),  $\alpha$ -cadinene (3.25%) and globulol (2.48%). The only one major phenolic compound detected was methyl eugenol (6.18%).

The essential oil of *S. glomulifera* was composed of 73.2% monoterpenoids, 9.63% sesquiterpenoids and 7.25% phenolic compounds and 9.92% of undetected chemical components (Table 44 and Fig. 145).

An earlier chemical analysis conducted by Brophy *et al.* (1996) support the presence of many of these essential oil components in the leaves of *S. glomulifera*. Thus the present investigation reports the presence of a probable monoterpenoid class of essential oil in the leaves of *S. glomulifera*. The chemotype seems to be " $\alpha$ -terpinene > limonene".

### b) Subfamily : Myrtoideae

The leaf essential oil composition of the 14 members analysed under the subfamily Myrtoideae showed wide variation in their chemical composition. The herb oil of *Eugenia apiculata* showed the dominance of phenolic compound, methyl eugenin (64.08%). The monoterpenoid, 1,8-cineole (61.27%) was the dominant constituent in *Syzygium aromaticum*. Iso eugenol, a phenolic compound was detected as the major chemical principle in the leaf essential oil of *Pimenta dioica*. All other members studied showed the lack of 'dominant' chemical constituents. Several terpenoids and phenolic compounds were found to be present in lesser amounts (Tables 45 to 58). Detailed discussion on the leaf essential oil composition of each taxa is given below.

### 13. *Acmena smithii*

The leaf essential oil of *A. smithii* showed the dominance of sesquiterpenoid compound  $\alpha$ -farnesol (30.60%), followed by the monoterpene piperitone (13.58%) and the phenolic compound methyl iso eugenol (9.34%). The other major monoterpenoids were  $\gamma$ -terpinene (7.88%), cymene (6.19%),  $\alpha$ -pinene (3.00%) and  $\alpha$ -terpineol (1.82%). The other major sesquiterpenoids include iso caryophyllene (9.20%),  $\beta$ -bisabolene (3.63%),  $\beta$ -elemene (3.58%) and  $\beta$ -bourbonene (2.62%). The phenolic compounds methyl eugenol (3.96%), methyl eugenin (1.40%) and eugenol (1.11%) were also found to be present in the oil.

The leaf essential oil of *A. smithii* was composed of 34.01% monoterpenoids, 49.63% sesquiterpenoids and 16.36% phenolic compounds (Table 45 and Fig. 146). All the chemical principles eluted on GC analysis were detected. The results obtained from the leaf essential oil of *A. smithii* is a new report. So it can be suggested that the leaf oil of *A. smithii* belongs to the terpenoid class of essential oils and the chemotype is "Mixed terpenoid".

### 14. *Eugenia apiculata*

The leaf essential oil of *E. apiculata* shows the dominance of the phenolic compound, methyl eugenin (64.08%). Other major compounds were monoterpenoids like  $\alpha$ -thujone (7.4%), citronellol (5.10%), 1,8-cineole (3.06%) and  $\alpha$ -terpineol (1.64%). The phenolic compound carvacrol (6.9%) was also detected in major amounts. No major sesquiterpenoids were detected (Table 46 and Fig. 147).

The essential oil composition of the leaves of *E. apiculata* is as follows: Monoterpenoids 20.36%, sesquiterpenoids 0.84%, phenolic compounds 72.22% and undetected chemical compounds 6.58%. The results obtained from the leaf essential oil of *E. apiculata* is a new report. So it seems probable that the herb oil of *E. apiculata* belongs to the phenolic class of essential oils and the chemotype may be "Methyl eugenin".

### 15. *Eugenia uniflora*

In the leaf essential oil of *Eugenia uniflora*, the monoterpenoid piperitone oxide (24.35%) was found in substantial amount. Other major monoterpenoids were  $\beta$ -phellandrene (6.80%),  $\alpha$ -terpinene (5%),  $\alpha$ -terpineol (2.6%), limonene (1.16%), myrcene (1.08%) and  $\beta$ -thujene (1.07%). The major sesquiterpenes detected include iso caryophyllene (4.71%),  $\beta$ -farnesol (4.44%),  $\beta$ -farnesene (3.83%),  $\delta$ -selinene (3.67%), caryophyllene oxide (3.76%),  $\alpha$ -cadinene (3.53%),  $\alpha$ -cadinol (3.19%), bicyclogermacrene (3.02%), aromadendrene (2.74%),  $\alpha$ -farnesene (2.36%),  $\beta$ -caryophyllene (2.14%) and  $\beta$ -cadinol (1.44%). Isoeugenyl acetate (2.52%) and Iso eugenol (2.33%) were the major phenolic compounds detected.

Of the different chemical compounds detected from the herb oil of *E. uniflora*, 43.39% was monoterpenoids, 40.49% sesquiterpenoids and 5.69% phenolic compounds. 10.43% of the essential oil remain undetected (Table 47 and Fig 148). The present study suggests the existence of a mixed terpenoid class of essential oil in the leaves of *E. uniflora* and the chemotype may be "Mixed terpenoid".

### 16. *Feijoa sellowiana*

The leaf essential oil of *F. sellowiana* showed the 'co-dominance' of the monoterpenoid, limonene (29.29%) and the sesquiterpenoid,  $\beta$ -caryophyllene (27.38%). Substantial amount of monoterpenoid, citronellyl acetate (17.08%) was also detected. Other major compounds detected were monoterpenoids like  $\alpha$ -pinene (8.7%),  $\beta$ -pinene (3.11%), estragole (1.35%) and the sesquiterpenoid, iso caryophyllene (1.37%).

*F. sellowiana* essential oil was composed of 61.99% monoterpenoids, 31.24% sesquiterpenoids and 1.02% phenolic compounds. 5.75% of the chemical principles remain undetermined during the present study (Table 48 and Fig. 149).

Previous reports (Johnshaw, 1989; Rotman *et al.*, 2001) confirm the presence of many of these essential oil constituents in *F. sellowiana*. So it can be suggested that the leaf essential oil of *F. sellowiana* may belong to the mixed terpenoid class of essential oils and the chemotype seems to be “Limonene >  $\beta$ -caryophyllene > citronellyl acetate”.

### 17. *Myrtus communis*

From the leaf essential oil of *M. communis*,  $\beta$ -thujene (21.73%), a monoterpenoid, was the compound detected in large amount. Other major monoterpenoids were  $\alpha$ -terpinene (9.28%),  $\beta$ -phellandrene (8.65%), limonene (6.52%),  $\gamma$ -terpinene (3.99%), myrcene (3.11%), piperitone (3.01%),  $\alpha$ -thujone (1.72%) and  $\beta$ -terpinene (1.36%). The major sesquiterpenoids detected were  $\beta$ -caryophyllene (9.55%),  $\alpha$ -cadinene (6.52%) and  $\beta$ -elemene (4.28%). The major phenolic compounds like methyl iso eugenol (5.52%), iso eugenol (1.41%) and methyl chavicol (1.18%) were also detected.

The leaf essential oil of *M. communis* was composed of 61.82% monoterpenoids, 20.82% sesquiterpenoids and 9.02% phenolic compounds. 8.34% chemical compounds were not detected (Table 49 and Fig. 150). Earlier workers (Rastogi, 1991; Weyerstahl *et al.*, 1994; Bradesi *et al.*, 1997; Chalchat *et al.*, 1998; Ozek *et al.*, 2000; Asllani *et al.*, 2000) have confirmed the occurrence of many of these essential oil components in *M. communis*. So the plant belongs to the mixed monoterpenoid class of essential oils and the chemotype may be “Mixed monoterpenoid”.

### 18. *Pimenta dioica*

The major leaf essential oil component of *P. dioica* was a phenolic compound, iso eugenol (62.48%). The monoterpenoid, 1,8-cineole was found in substantial amount (14.38%). Other important components were monoterpenoids like  $\alpha$ -terpinene (4.38%) and cymene (1.47%); phenolic compound like chavicol (8.6%) and sesquiterpenoid,  $\beta$ -caryophyllene (3.15%).

*P. dioica* leaf essential was composed of 22.29% monoterpenoids, 4.32% sesquiterpenoids and 71.08% phenolic compounds. 2.31% of chemical components remain undetected (Table 50 and Fig. 151). Previous studies conducted by several workers (Bello *et al.*, 1995; Abaul *et al.*, 1995; Bhattacharjee, 1998.) confirm the presence of many of these chemical constituents in the leaf essential oil of *P. dioica*. So the herb oil of *P. dioica* seems to belong to the mixed class of essential oils and the chemotype may be "Iso eugenol > 1,8- cineole".

#### 19. *Psidium guajava*

In the essential oil of *P. guajava* leaves, substantial amounts of monoterpenoid, myrcene (13.65%), phenolic compound, iso eugenol (16.47%) and the sesquiterpenoid compound,  $\alpha$ -selienene (18.54%) were detected. Other major monoterpenoids detected in the oil are piperitone (3.03%), 1,8- cineole (2.08%),  $\alpha$ -terpineol (1.98%) and  $\gamma$ -terpinene (1.46%). The other major sesquiterpenoids detected were  $\delta$ -selinene (11.05%),  $\beta$ -caryophyllene (7.28%), iso caryophyllene (3.18%),  $\gamma$ -cadinene (2.64%),  $\beta$ -elemene (2.82%), farnesol (2.36%) and  $\beta$ -bisabolene (1.92%).

The chemical composition of leaf essential oil of *P. guajava* can be summarised as : 24.29% of monoterpenoids, 49.79% of sesquiterpenoids, 20.06% of phenolic compounds and 5.86% of undetected chemical components (Table 51 and Fig. 152). Earlier workers (Ekundayo *et al.*, 1991; Nieves *et al.*, 1994) have confirmed the occurrence of many of the major essential oil components in *P. guajava* leaves. From the present investigation it can be stated that the herb oil of *P. guajava* belongs to the mixed class of essential oils and the chemotype may also be "Mixed".

#### 20. *Syzygium aromaticum*

From the leaf essential oil of *S. aromaticum*, the dominant compound detected was a monoterpenoid, 1,8- cineole (61.27%). Other major monoterpenoids were  $\gamma$ -terpinene (8.81%),  $\alpha$ -pinene (7.67%), citronellal (2.16%)

and  $\beta$ -pinene (1.72%). The major sesquiterpenoids were germacrene (3.55%), nerolidol (1.40%) and  $\delta$ -selinene (1.06%).

84% monoterpenoids, 6.91% sesquiterpenoids and 0.85% of phenolic compounds were detected from the leaf essential oil of *S. aromaticum*. 8.24% of chemical compounds were undetected (Table 52 and Fig. 153). Previous reports (Chopra *et al.*, 1956; Bhattacharjee, 1998) confirm the presence of many of these compounds in the essential oil of *S. aromaticum*. So it seems probable that the leaf oil of *S. aromaticum* belongs to the monoterpenoid class of essential oils and the chemotype is "1,8-cineole".

### 21. *Syzygium cumini*

In the leaf essential oil obtained from *S. cumini*, the major monoterpenoids like sabinene (15.92%) and  $\beta$ -phellandrene (14.62%) were found in substantial amounts. Other monoterpenoids were linalool (6.68%),  $\alpha$ -terpinene (4.23%), myrcene (1.52%), limonene (1.36%) and  $\beta$ -thujene (1.18%). The sesquiterpenoids detected were  $\delta$ -selinene (6.69%), iso caryophyllene (6.16%),  $\beta$ -elemene (4.72%),  $\beta$ -caryophyllene (2.82%),  $\beta$ -farnesene (2.01%) and caryophyllene oxide (1.04%). The phenolic compounds found were eugenyl acetate (9.38%), iso eugenol (4.05%), methyl chavicol (1.45%) and eugenol (1.44%).

Based on the present study, the leaf essential oil of *S. cumini* was composed of 48.45% monoterpenoids, 23.44% sesquiterpenoids, 16.99% phenolic compounds and 11.12% undetected chemical compounds (Table 53 and Fig. 154). An earlier worker (Vijayanand *et al.*, 2001), has confirmed the presence of many of these components in the leaf essential oil of *S. cumini*. Hence it can be assumed that the herb oil of *S. cumini* may belong to the mixed terpenoid class of essential oils and the probable chemotype is "Mixed terpenoid".



## 22. *Syzygium jambos*

The leaf essential oil of *S. jambos* showed the presence of the monoterpenoid,  $\alpha$ -terpinene (24.58%) and the sesquiterpenoid,  $\gamma$ -cadinene (28.58%) as the 'co-dominant compounds'. The other major monoterpenoids detected were  $\alpha$ -thujone (12.04%), sabinine (1.84%) and myrcene (1.58%). The major sesquiterpenoids like bicyclogermacrene (4.4%), aromadendrene (3.65%), farnesol (3.56%), germacrene (2.39%) and  $\alpha$ -farnesene (1.27%) and the major phenolic compound, viz. eugenyl acetate (4.06%) were also isolated (Table 54 and Fig.155).

The essential oil composition of *S. jambos* can be summarised as follows: Monoterpenoids (43.55%), sesquiterpenoids (46%), phenolic compounds (4.25%) and undetected chemical compounds (6.20%). Several workers (Rastogi and Mehrotra, 1990; Rastogi, 1991.) have confirmed the presence of many of these essential oil components in *S. jambos*. In the light of the present investigation and previous reports, it is suggested that the leaf oil of *S. jambos* may belong to a mixed terpenoid class and the chemotype also seems to be "Mixed terpenoid".

## 23. *Syzygium malaccense*

The main compounds isolated from the leaf essential oil of *S. malaccense* include monoterpenoids like piperitone (15.26%), linalool (4.17%),  $\alpha$ -terpineol (1.57%) and citronellyl acetate (1.34%); sesquiterpenoids like  $\beta$ -caryophyllene (12.15%),  $\alpha$ -humulene (10.22%),  $\beta$ -elemene (5.93%), iso caryophyllene (5.86%), bicyclogermacrene (5.48%) and  $\beta$ -bisabolene (3.63%) and phenolic compounds like methyl iso eugenol (6.34%) and eugenyl acetate (1.15%).

Only 77.12% of the herb oil components of *S. malaccense* were detected during the present study. The remaining 22.88% of components were undetermined due to unavailability of authentic samples. The detected components include: 25.95% monoterpenoids, 43.68% sesquiterpenoids and 7.49% phenolic compounds (Table 55 and Fig. 156).

An earlier study conducted by Wong and Lai (1996), confirm the occurrence of many of these essential oil components in *S. malaccense*. So it seems probable that the leaf oil of *S. malaccense* also belongs to the mixed terpenoid class of essential oils and the chemotype may also be "Mixed terpenoid".

#### 24. *Syzygium samarangense*

The major leaf essential oil components detected from *S. samarangense* were monoterpenoids like piperitone (6.66%), linalool (2.45%) and neral (1.63%); sesquiterpenoids like  $\beta$ -elemene (4.12%),  $\beta$ -farnesene (3.61%),  $\alpha$ -farnesol (3.42%),  $\beta$ -farnesol (3.08%), bicyclogermacrene (2.98%),  $\beta$ -cadinol (2.54%),  $\alpha$ -cadinol (2.04%),  $\gamma$ -cadinol (1.33%),  $\beta$ -bourbonene (1%); phenolic compounds like methyl iso eugenol (6.36%) and eugenol (3.81%).

Due to the lack of authentic samples only 48.44% of the essential oil components were detected during the present study. 51.56% of the chemical constituents remain undetected. Out of the detected compounds 14.15% were monoterpenoids, 24.12% sesquiterpenoids and 10.17% phenolic compounds (Table 56 and Fig. 157). A previous study done by Wong and Lai (1996) confirm the presence of these essential oil constituents in *S. samarangense*. Since a major portion of the leaf oil of *S. samarangense* remain undetected even after the present study, the herb oil of this plant could not be assigned to any class of essential oils and the chemotype remain undetermined.

#### 25. *Syzygium zeylanicum*

In the leaf essential oil of *S. zeylanicum* the monoterpenoids, viz.  $\alpha$ -phellandrene (32.08%) and  $\beta$ -thujene (22.01%) showed 'co-dominance'. Other major monoterpenoids detected were  $\beta$ -phellandrene (7.22%),  $\gamma$ -terpinene (4.19%), linalyl acetate (3.15%), neryl acetate (3.01%),  $\alpha$ -terpinene (1.73%), piperitone oxide (1.70%) and citronellyl acetate (1.37%). The sesquiterpenes detected include

iso caryophyllene (4.65%),  $\delta$ -selinene (4.13%),  $\beta$ -bisabolene (2.26%) and  $\beta$ -caryophyllene (1.48%). Eugenyl acetate (1.12%) was the major phenolic compound detected.

The herb oil of *S. zeylanicum* was composed of 77.17% monoterpenoids, 12.52% sesquiterpenoids and 2.38% phenolic compounds. 7.93% of the components remain undetected (Table 57 and Fig. 158). This chemical report on the leaf essential oil of *S. zeylanicum* is a new one. In the light of the present study, it can be stated that the leaf oil of *S. zeylanicum* belongs to the mixed monoterpenoid class of essential oils and the chemotype may also be "Mixed monoterpenoid".

#### 26. *Rhodomyrtus tomentosa*

The chemical composition of the leaf essential oil of *R. tomentosa* reveal the major phenolic compounds methyl chavicol (28.56%) and methyl eugenin (27.93%), which exhibit 'co-dominance'. Other major compounds include monoterpenoids like  $\alpha$ -thujone (9.88%), terpinen-4-ol (3.19%), citronellal (2.34%) and 1,8-cineole (1.72%); phenols like carvacrol (2.9%) and methyl iso eugenol (1.85%) and sesquiterpenoids like  $\delta$ -selinene (3.48%),  $\alpha$ -humulene (1.45%),  $\gamma$ -cadinene (1.39%) and aromadendrene (1.02%).

Monoterpenoids (19.10%), sesquiterpenoids (7.34%), and phenolic compounds (61.24%) were the different classes of chemical compounds detected in the leaf essential oil of *R. tomentosa*. 12.32% of chemical components were not detected (Table 58 and Fig. 159). Previous reports (Brophy *et al.*, 1997b) confirm the presence of many of these essential oil components in *Rhodomyrtus*. The present report on *R. tomentosa* is a new one. Hence it can be suggested that the herb oil of *R. tomentosa* may belong to a phenolic class of essential oils and the chemotype is "Methyl chavicol – methyl eugenin".

In conclusion it can be stated that the leaf essential oils of myrtaceous members analysed belong to six classes.

1. Monoterpenoid, 2. Sesquiterpenoid, 3. Terpenoid, 4. Phenolic, 5. Mixed and 6. Undetermined.

The chemotypes detected are :

- 1. Monoterpenoid chemotypes :** *Agonis flexuosa* (Myrcene >  $\alpha$  - thujene), *Beaufortia sparsa* ( $\alpha$  - terpinene), *Callistemon viminalis* (1,8 - cineole > citrcnellol), *Corymbia citriodora* (citronellal), *C. ficifolia* (Terpinyl acetate), *Eucalyptus globulus* (Mixed monoterpenoid), *E. tereticornis* ( $\alpha$  - pinene – piperitone oxide), *Syncarpia glomulifera* ( $\alpha$  - terpinene > limonene), *Myrtus communis* (Mixed monoterpenoid), *Syzygium aromaticum* (1,8 – cineole) and *S. zeylanicum* (Mixed monoterpenoid).
- 2. Sesquiterpenoid chemotype:** *Melaleuca styphelioides* (Mixed sesquiterpenoid).
- 3. Terpenoid chemotypes:** *Leptospermum nicholsii* (Mixed terpenoid), *Melaleuca leucadendron* (Mixed terpenoid), *Acmena smithii* (Mixed terpenoid), *Eugenia uniflora* (Mixed terpenoid), *Feijoa sellowiana* (Limonene >  $\beta$  – caryophyllene > citronellyl acetate), *Syzygium cumini* (Mixed terpenoid), *S. jambos* (Mixed terpenoid) and *S. malaccense* (Mixed terpenoid).
- 4. Phenolic chemotypes :** *Eugenia apiculata* (Methyl eugenin) and *Rhodomyrtus tomentosa* (Methyl chavicol – methyl eugenin).
- 5. Mixed chemotypes :** *Callistemon citrinus* (Citral > eugenol), *Pimenta dioica* (Iso eugenol > 1,8 – cineole) and *Psidium guajava* (Mixed).
- 6. Undetermined chemotype :** *Syzygium samarangense*.

The presence of several classes of leaf essential oils having several distinct chemotypes reveal the existence of chemical diversity in the investigated taxa of Leptospermoideae and Myrtoideae of the family Myrtaceae.

## CORRELATION OF BIOLOGICAL ACTIVITY AND ESSENTIAL OIL COMPOSITION

A thorough review of data shows that the percentage of cytotoxicity and mitotic inhibition caused by various plant extracts in *A. cepa* root meristem was different. Since Myrtaceae is a family with members having volatile components in their leaves, the leaf extracts definitely possess these essential oil constituents. On GC analysis, the volatile constituents and bioactive principles present in each and every essential oil was revealed.

A complete scanning of the previous reports on these essential oil components (Table 2) show a wide spectrum of important biological activities. Out of the 77 chemical compounds revealed in the present study (Table 59), majority show anticancerous and cytotoxic activities. That may be the probable reason for the inhibition of mitotic index and induction of cellular and chromosomal damages by the extracts of myrtaceous plants in the meristematic cells of *A. cepa*. Many of these chemical compounds present in the leaf essential oils are very effective bioactive principles, which may be used to promote or retard various vital functions in and out of the cells.

Foray *et al.* (1999) reported the anticancerous activities of 1,8-cineole, linalyl acetate,  $\alpha$ -thujone,  $\beta$ -thujone and linalool. Stitt (1990) showed citral, estragole, iso eugenol and methyl iso eugenol as cancer preventive principles. Leuwenberg (1987) noticed the anticancerous ability of  $\beta$ -elemene. Zheng *et al.* (1992) suggested that  $\alpha$ -humulene acts as an antitumourous principle. Mazzanti *et al.* (1998) and Santos and Rao (2000) recorded the antimicrobial and antiinflammatory activities of 1,8-cineole. Faleiro *et al.* (1999) showed the incidence of antimicrobial activities of borneol. Armaka *et al.* (1999) reported that isoborneol, another monoterpene found in several plant essential oils showed antiviral properties against herpes simplex virus I (HSV-I).

Setzer *et al.* (1999) recorded the cytotoxic activity to human tumour cells by  $\alpha$ -pinene,  $\beta$ -pinene, 1,8-cineole, limonene and  $\alpha$ -terpineol. Antitumourous activity of caryophyllene oxide was revealed by Zheng *et al.* (1992). Sternberg and Duke (1996) noticed the antiviral and anticancerous activities of limonene. Singh *et al.* (2000) suggested that linalool was antifungal and insecticidal. Gallori *et al.* (2001) noticed the effect of cymene as an antimicrobial and antifungal agent. The fungicidal activity of myrcene was noticed by Keeler and Tu (1991). Gallori *et al.* (2001) suggested that the antifungal and antimicrobial activity of  $\alpha$ -pinene.

Belmont and Carvajal (1998) noticed the antifungal activity of eugenol, where as Belaiche *et al.* (1995) recorded the antimicrobial property of eugenol. Ofori *et al.* (1997) reported that 1,8 - cineole and linalool were highly active antimicrobial agents. Antimicrobial and antifungal activities of farnesene were recorded by Arambewela *et al.* (1999).

Anticarcinogenic and antitumour activity of caryophyllene has been studied by Muroi and Kubo (1993) and Zheng *et al.* (1992). Sternberg and Duke (1996) noticed the anticarcinogenic property of  $\alpha$ -terpineol and limonene. Antitumour characteristic of eugenol was revealed by Zheng *et al.* (1992). Sternberg and Duke (1996) revealed the antiulcer and cancer preventive activities of eugenol.

Nerolidol, borneol, iso caryophyllene,  $\alpha$ -terpinyl acetate, linalyl acetate,  $\beta$ -bisabolene,  $\alpha$ -selinene,  $\alpha$ -thujene, myrcene, citronellol, citronellal, sabinene,  $\alpha$ - and  $\beta$ -pinene,  $\delta$ -cadinene and carvacrol were found as antibacterial, antifungal and bioactive principles in essential oils (Nagarajan *et al.*, 2001; Sternberg and Duke, 1996; Palmeira *et al.*, 2001; Paramonov, 2000; Cauladis *et al.*, 2001; Vernin *et al.*, 2001; Jirovetz, 2000).

$\gamma$ -terpinene and  $\beta$ - phellandrene were described as strong antibacterial agents (Senatore *et al.*, 2000). Terpeneol and  $\alpha$  - and  $\beta$ - pinenes were found to be lethal to body lice and head lice (Oladimeji *et al.*, 2000).

Insecticidal and antifungal properties of linalool, terpinen – 4 – ol,  $\gamma$ - terpinene,  $\alpha$  - cadinol, neral,  $\alpha$  - terpinene and cadinol were recorded by Singh *et al.* (2000). Blewitt and Southwell (2000) reported the insecticidal activities of methyl chavicol. Bowers *et al.* (2000) noted the insecticidal properties of citronellal. Camphor,  $\alpha$  - pinene and  $\beta$ - pinene were described as antifungal agents (Castellanos *et al.*, 2001). Antibacterial activity of bisabolene was described by Zhu *et al.* (1999). Methyl benzoate was described as a good insecticide by Miranda, *et al.* (1997).

Apart from these chemical principles, Larhsini *et al.* (2001) noticed that the aqueous extract of *Eugenia caryophyllata* (*Syzygium aromaticum*) was a most effective bactericidal agent. Aqueous extract of *Pimenta dioica* showed hypotensive activities in rats (Suarez, *et al.*, 1999, 2000). Fungicidal activity of clove oil was proved by Prasad *et al.* (1986).

Pesticidal activities of leaf powders of *Callistemon citrinus* (*C. lanceolatus*) at the rate of 20 kg / ha. against red pumpkin beetle *Aulacophora foveicollis* on musk melon (*Cucumis melo*) was reported by Rajak and Singh (2002). Yehoshua *et al.* (1995) noticed the antifungal properties of citral, which is the major constituent of *C. citrinus* of present study.

Palson and Jaenson (1999) reported that smoke of leaves of *Eucalyptus* showed 72.2% mosquito repellency. Misra *et al.* (1989a) applied the volatile oil obtained from the leaves of *Melaleuca leucadendron* on the fungi, viz. *Aspergillus* and *Fusarium moniliforma* and noticed the antifungal ability of the oil. The antifungal ability was unaffected by fluctuations in temperature, autoclaving and storage. Cox *et al.* (1998) studied the effect of the essential oil of *Melaleuca alternifolia* on bacteria and found that the oil principles disrupt the cell membrane function in bacteria and yeasts.

The oils obtained from three chemotypes of *Pimenta* sp. have been proved to possess antibacterial and antifungal activities. The chemotypes and the major components were neral / geranial; methyl chavicol / methyl eugenol and chavicol / eugenol (Aurora *et al.*, 1998). Nematicidal activity of the leaf essential oil of *Pimenta dioica* and eugenol has been proved (Leela and Ramana, 2000).

Potential antifungal activity has been reported for neryl acetate, citral (Kim, 1995) and for methyl eugenol (Kubo, 1993). Antibacterial potential of citral, carvacrol (Kim, 1995), methyl cinnamate (Southwell and Wilson, 1993) and methyl chavicol (Molino, 1993) have also been worked out. The bactericidal and fungitoxic activity of cineole (Saeed and Sabir, 1995) were also established.

The leaf extracts of *Psidium guajava* was found most active against seven pathogenic bacterial strains causing diarrhoea and dysentery (Lutete *et al.*, 1994). Mohammed *et al.* (1994) tested the leaf extracts of guava as an antimicrobial agent and found that guava leaf extract showed activity against all test organisms.

The Paraguayan natural medicine, 'Nangapiry', which is the water soluble extract from the leaves of *Eugenia uniflora* has been used as an antidiabetic agent (Matsumura *et al.*, 2000). Constant *et al.* (1997) reported the isolation of the anti-inflammatory principle of *Eugenia jambos*. This confirmed the anti-inflammatory action of ethnomedicine prepared from *E. jambos* which is used to reduce swellings in Guatemala.

Kim *et al.* (2001) isolated virus cell fusion inhibitory components from *Eugenia caryophyllata* (*Syzygium aromaticum*). Mansouri (1999) reported that the ethanolic leaf extracts of *Myrtus communis* showed highest activity (99%) against human pathogen, *viz.* *Staphylococcus aureus* isolated from nose and throat which were sensitive to the antibiotic Trimethoprim sulfamethoxazole.

Misra *et al.* (1989b) tested the essential oil obtained from *Callistemon citrinus* on pulse beetle (*Callosobruchus maculatus*) and noticed the insecticidal property of the oil. Ahmed and Eapen (1986) noticed the pesticidal activities of *Eucalyptus* and its major oil components like cineole and turpentine. They showed promising activity against insect pests, *viz.* *Musca domestica*, *Sitophilus oryzae*,



*Callosobruchus chinensis* and *Stegobium paniceum* and recommended that these can be used as botanical insecticides. Altman (1989) reported that the Australian tea tree oil (*Melaleuca alternifolia*) was a natural antiseptic, as the oil is composed of terpenes like pinene, terpinene, cymene, cineole, terpineol and various other components including sesquiterpenes and their alcohols.

Arora *et al.* (1999) reported that some bacteria showing resistance to antibiotics were sensitive to extracts of clove (*Syzygium aromaticum*). Begum *et al.* (1997) noticed the antimicrobial activity of essential oils extracted from the leaves of *Eucalyptus citriodora* (*Corymbia citriodora*) and *Eucalyptus camaldulensis*. Hajji *et al.* (1993) studied the antifungal and antibacterial effects of 21 species of *Eucalyptus* and suggested that moulds and yeasts were more sensitive to essential oil constituents than bacteria. Meena and Sethi (1994) studied the effect of spices and essential oils including clove and found that eugenol content in clove showed high activity against micro organisms.

Antibacterial action of essential oils of some Australian Myrtaceae members with special reference to the activity of chromatographic fraction of oils of *Eucalyptus citriodora* (*Corymbia citriodora*) was reported by Low *et al.* (1974). Kumar and Dutta (1987) recorded the toxicity of oils obtained from *Eucalyptus globulus*, *Syzygium aromaticum* and other indigenous Indian plants as larvicidal agents against *Anopheles stephensi* mosquitoes.

In the present investigation severe cytotoxic effects were noticed in *A. cepa* root meristem (Table 4 – 29). It can be considered that these aberrations at the chromosome level are caused by the volatile compounds present in the leaf extracts. Such compounds directly or indirectly affected the genomic constitution of *A. cepa* cells and reduced the mitotic indices. However, it is proved by Grant (1982) that *A. cepa* has an excellent correlation with mammalian system. Based on the observations of the present study and review of the other earlier experiments, it can be suggested that the leaf extracts and essential oils of the members of both the subfamilies of the family Myrtaceae can be utilised as

antitumour promoting substances and for the manufacture of therapeutic drugs for curing various kinds of cancers including leukaemia.

Various chemical components such as  $\alpha$ -pinene, camphene,  $\alpha$ -phellandrene,  $\beta$ -pinene,  $\beta$ -phellandrene, citral, cymene, linalool, 1,8-cineole, limonene, estragole,  $\alpha$ -terpinene, neral, citronellol, citronellal, borneol, carvacrol,  $\alpha$ -thujene, terpinen-4-ol,  $\beta$ -thujone, chavicol, iso borneol, methyl iso eugenol, iso eugenol, bicyclogermacrene, farnesene *etc.* possess antimicrobial, bactericidal, fungicidal, insecticidal and nematocidal properties (Table 2). Hence such components can be extracted from the leaf essential oil of various plants of Myrtaceae. These essential oil principles either singly or in combination can be used for the manufacture of antibiotics, bactericides, fungicides, insecticides, nematocides *etc.*

Since some of the constituents like  $\alpha$ -pinene and  $\beta$ -pinene (Sternberg and Duke, 1996), citronellal (Keeler and Tu, 1991), borneol (Lydon and Duke, 1989), methyl iso eugenol (Harborne and Baxter, 1983), piperitone (Lydon and Duke, 1989) and  $\alpha$ -cadinol (Singh *et al.*, 2000) showed herbicidal property, they can be utilised commercially for making biological herbicides and weedicides.

Methyl benzoate (Miranda *et al.*, 1997);  $\alpha$ -cadinol (Singh *et al.*, 2000), caryophyllene oxide (Bettarini *et al.*, 1991), methyl chavicol (Sternberg and Duke, 1996),  $\alpha$ -thujone (Misra and Singh, 1986), terpinen-4-ol (Singh *et al.*, 2000), citronellal (Bowers *et al.*, 2000), neral (Singh *et al.*, 2000), linalool (Singh *et al.*, 2000) showed insecticidal properties. Such compounds can be commercialised for the manufacture of herbal pesticides.

Viricidal property was exhibited by  $\alpha$ -pinene (Sternberg and Duke, 1996), the dominant principle found in *Eucalyptus tereticornis* in the present study. Hence, it can be used for producing herbal antiviral drugs. Pediculocidal activity was shown by  $\alpha$ -terpineol (Oladimeji, 2000). So the plants containing  $\alpha$ -terpineol can be exploited for making herbal pediculocides, hair oils to control pediculosis and dandruff.

Larvicidal and mosquito repellent properties of  $\alpha$ -pinene, 1,8-cineole, myrcene and eugenol were reported by many workers (Hwang *et al.*, 1985, Sternberg and Duke, 1996). Since, these compounds are dominant in many essential oils of the present study, it can be utilised to produce biological larvicides and mosquito repellents.

Many of the chemicals like  $\alpha$ -pinene,  $\beta$ -pinene, camphene, phellandrene, sabinene, 1,8-cineole,  $\alpha$ -terpinene, linalyl acetate,  $\gamma$ -terpinene, citronellyl acetate, terpinyl acetate, methyl cinnamate, eugenol, methyl iso eugenol,  $\alpha$ -selinene,  $\alpha$ -humulene, farnesol, iso caryophyllene and caryophyllene oxide (Sternberg and Duke, 1996) can be used as fragrance and flavour compounds. Thus the plants containing these chemical principles may be exploited to produce eco-friendly perfumery, cosmetic and flavour products.

The potentialities of the extracts of *Eugenia uniflora* (Matsumura *et al.*, 2000), *Syzygium cumini* (Aslam *et al.*, 1998) and *Psidium guajava* (Chang and Yang, 1983) can be used for the manufacture of drugs for diabetes. The chemical principles like  $\alpha$ -terpinene, linalool, borneol, citronellyl acetate, eugenol, myrcene, limonene and caryophyllene can be tested for the formulation of antidiabetic and contraceptive therapeutics (Wassel *et al.*, 1986).

The crude extracts of the leaves of these myrtaceous taxa may be used as a biodegradable herbal microbicide, fungicide, viricide, larvicide, nematocide and insecticide to save our environment.

## SUMMARY

In the present investigation twelve members of the subfamily Leptospermoideae and fourteen members of the sub family Myrtoideae were collected from various localities in South India. The cytotoxic potentials of leaf extracts and phytochemical aspects of essential oils were evaluated.

### Cytotoxic studies

Cytotoxicity of plant extracts of various concentrations (1%, 2% & 5%) at different time intervals (2 hr., 4 hr., 6 hr., 12 hr. & 24 hr.) on *Allium cepa* root meristem were analysed. Drastic cytotoxicity and mitotic inhibitions were observed in all plant extracts, except two members coming under the subfamily Myrtoideae. The extracts of plants viz. *Syzygium zeylanicum* and *Rhodomyrtus tomentosa* in low concentrations and short duration treatments showed slight stimulatory effects on the mitotic division in *A. cepa* root tip cells.

The cytotoxic effects of all plant extracts include many clastogenic and nonclastogenic abnormalities. The major clastogenic abnormalities observed include nuclear lesions, chromosome stickiness, chromosome breakage and fragmentation, chromosome bridges, pycnosis and differential condensation of chromosomes. The nonclastogenic abnormalities detected were clumping of chromosomes, multipolarity, chromosome laggards, diagonal metaphase, anaphase and telophase, ball metaphase, scattered metaphase, sticky metaphase, sticky anaphase and sticky telophase, C - metaphase, binucleate, trinucleate, tetranucleate and polynucleate cells, polyploidy, misorientation of chromosome groups at metaphase and anaphase, early movement of chromosomes, nonsynchronised movement of chromosomes and disturbed metaphase and disturbed anaphase. The most frequent abnormalities observed were nuclear

lesions, chromosome stickiness, binucleate and tetranucleate cells, diagonal orientation of chromosomes at metaphase, anaphase and telophase.

Mitotic indices in the various treatments with the myrtaceous leaf extracts were found to be less than that of the control, except the leaf extracts of *Syzygium zeylanicum* and *Rhodomyrtus tomentosa*. In all other treatments mitotic indices showed an inverse relationship with an increase in concentration of the extracts. The frequency of abnormalities was found to increase with the concentration of the extract and with the duration of the treatment. In the experiments with *S. zeylanicum* and *R. tomentosa* leaf extracts, in short duration treatments, the mitotic indices showed a slight increase with respect to the control experiments. With the increase in duration of treatments, both these plants showed severe cytotoxic effects.

In almost all experiments with the myrtaceous leaf extracts the nonclastogenic abnormalities showed predominance over the clastogenic effects.

### Essential Oil Analysis

In the present investigation the chemical analysis conducted on twenty six members of Myrtaceae show variations in both in the quantity and quality of essential oils.

Of the twelve members studied under the subfamily Leptospermoideae, *Eucalyptus globulus* (7.35%) and *E. tereticornis* (6.62%) were found to be blessed with essential oils. The least percentage of oil content was detected in *Corymbia ficifolia* (0.01%) and *Melaleuca styphelioides* (0.02%). Under the Myrtoideae subfamily, of the 14 members studied, three members were found to be oil rich. They are *Syzygium aromaticum* (6.6%), *Pimenta dioica* (6.4%) and *Myrtus communis* (5.75%). The least oil content was observed in *Eugenia apiculata* (0.02%) and *Syzygium zeylanicum* (0.02%).

On GC analysis, a wide range of chemical compounds was detected in the leaf essential oils of the members of Myrtaceae. The major essential oil

constituents belong to 3 classes of aromatic compounds, viz. monoterpenoids, sesquiterpenoids and phenolic compounds. However, the essential oils of these plants belong to 6 distinct classes of chemotypes. 1. Monoterpenoid, 2. Sesquiterpenoid, 3. Terpenoid, 4. Phenolic, 5. Mixed and 6. Undetermined.

Under the subfamily Leptospermoideae, *Agonis flexuosa* showed the monoterpenoid chemotype myrcene >  $\alpha$  - thujene. In *Beaufortia sparsa*,  $\alpha$  - terpinene (monoterpenoid chemotype) was identified as the dominant compound. Citral > eugenol was found to be the mixed chemotype detected in the leaf essential oil of *Callistemon citrinus*. 1,8 - cineole > citronellol was the monoterpenoid chemotype found in *Callistemon viminalis*. Citronellal in *Corymbia citriodora* and terpinyl acetate in *Corymbia ficifolia* were the other prominent monoterpenoid chemotypes detected. The leaf essential oil of *Eucalyptus tereticornis* ( $\alpha$  - pinene - piperitone oxide) and *Syncarpia glomulifera* ( $\alpha$  - terpinene > limonene) also belong to the monoterpenoid class of chemotypes. Whereas the leaf essential oil of *Eucalyptus globulus* (Mixed monoterpenoid), *Leptospermum nicholsii* (Mixed terpenoid), *Melaleuca leucadendron* (Mixed terpenoid) and *M. styphelioides* (Mixed sesquiterpenoid) do not possess a distinct chemotype.

Under the subfamily Myrtoideae, the leaf essential oil obtained from *Eugenia apiculata* showed the phenolic chemotype of methyl euginin. In *Syzygium aromaticum* leaf essential oil, 1,8 - cineole (monoterpenoid chemotype) was detected as the major constituent. Iso eugenol > 1,8 - cineole was detected as the mixed chemotype in the leaf essential oil of *Pimenta dioica*. The leaf essential oil of *Feijoa sellowiana* was found to belong to a terpenoid chemotype of limonene >  $\beta$  - caryophyllene > citronellyl acetate. Whereas the leaf essential oil of *Rhodomyrtus tomentosa* possess a distinct phenolic chemotype of methyl chavicol > methyl eugenin. The chemotypes of the leaf essential oils of *Myrtus communis* and *Syzygium zeylanicum* can be designated as mixed monoterpenoid. The leaf essential oils of *Acmena smithii*, *Eugenia uniflora*, *Syzygium cumini*, *S. jambos* and *S. malaccense* possess mixed terpenoid chemotypes. Similarly the leaf essential oil of *Psidium guajava* does not possess a distinct chemical composition and shows a

mixed chemotype. Where as, the leaf essential oil of *S. samarangense* cannot be classified under a specific chemotype since more than 50% of the essential oil components remain undetected due to the unavailability of some authentic chemical standards. So it is denoted as undetermined chemotype.

A total of 77 aromatic compounds were detected during the phytochemical studies of leaf essential oils of 26 myrtaceous plants under the present investigation. A wide spectrum of cytotoxic, antimicrobial, antifungal, insecticidal, nematocidal, scabicial, larvicidal and pediculocidal activities were reported for many of these components. Hence the crude leaf extracts of the myrtaceous members either alone or in combination can be used as herbal pesticide. Since many of the myrtaceous leaf extracts show severe mitotic inhibition on onion root tip meristematic cells, they can be effectively exploited as anticarcinogenic agents.

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