Systematic studies on selected members of the subfamily Lamioideae (Lamiaceae) using molecular phylogeny and phytochemical profiling

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GEETHIKA K.

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UNIVERSITY OF CALICUT
DEPARTMENT OF BOTANY
Calicut University P.O. 673 635, Kerala, India

Dr. SUNOJKUMAR. P.
Assistant Professor
Angiosperm Taxonomy and Floristics Division
Principal Investigator, DST & DBT Projects

Purayidathkandy House
Elathur P.O.
Calicut 673 303
Kerala, India.

CERTIFICATE

As the Adjudicators have not mentioned any corrections in the thesis entitled "Systematic studies on selected members of the subfamily Lamioideae (Lamiaceae) using molecular phylogeny and phytochemical profiling", it is certified that the thesis being submitted without any corrections.

CU Campus

Dr. Sunojkumar P

Phone: 0494 2401144 - 406, 407 (Off.); 09446891708 (Mob.); Email: drsunoj@gmail.com

DECLARATION

I, Geethika, K., hereby declare that the thesis entitled **"Systematic studies on selected members of the subfamily Lamioideae (Lamiaceae) using molecular phylogeny and phytochemical profiling"** submitted to the **University of Calicut** in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy** in **Botany** is a bonafide record of the original research work carried out by me under the supervision and guidance of **Dr. P. Sunojkumar**, Assistant Professor, Department of Botany, University of Calicut and that it has not been submitted earlier either in part or full for the award of any degree or diploma to any candidate of any University.

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Dedicated to My Family

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Plants have always been an attractive source of drugs that forms the ingredients in traditional systems of medicine, nutraceuticals, folk medicines, modern medicines, food supplements, pharmaceuticals intermediates bioactive principles and lead compounds in synthetic drugs (Nayak *et al*., 2010). Currently 25% of drug molecules are obtained from plant sources. Traditional knowledge and use of plants are exploited as a source of base information to isolate chemotherapeutic agents and drugs from plants.

In the field of new drugs research and development, natural products play crucial role based on their accessibility and cost effectivity. Studies on natural product by exploration of existing scientific knowledge, traditional uses and discovery of potential chemotherapeutic agents are aimed to determine medicinal values of plants.

Phytochemicals are used as motifs for lead optimization programs, which are intended to make effective and safe drugs (Balunas and Kinghorn, 2005). The primary metabolites such as amino acids, nucleotides, chlorophyll, simple carbohydrates or membrane lipids, have a recognized roles in processes like solute transport, translocation, photosynthesis, differentiation, respiration and nutrient assimilation (Taiz and Zeiger, 2006). Secondary metabolites include alkaloids, terpenoids, phenolics etc. for which no role has yet been found in growth, photosynthesis, reproduction or other primary functions. Secondary metabolites are synthesized as part of the defense system of plants (Phan *et al.,* 2001). The presence of components like sugar, steroids, glycosides, triterpenoids and a small quantity of phosphoric acid were proven to exhibit anti-fungal, anti-bacterial, anti-viral and anti-carcinogenic properties (Neamsuvan *et al*., 2012).

More than 4,000 phytochemicals have been documented and about 150 phytochemicals have been studied in detail (American Cancer Society, 2000) and these phytochemicals are categorized by physical characteristics, chemical characteristics and protective function (Meagher and Thomson, 1999). Plants produce these chemicals to protect themselves. According to recent studies, many phytochemicals can also protect human against diseases and promote health (Rao, 2003). Studies suggested that phytochemicals may reduce the risk of coronary heart disease by various activities such as preventing the oxidation of low density lipoprotein (LDL) cholesterol, normalizing blood pressure and clotting, reducing the synthesis or absorption of cholesterol and also by improving arterial elasticity (Mathai, 2000). Phytochemicals also play an important role in the detoxification of substances that cause cancer. They neutralize free radicals, activate enzymes that detoxify carcinogens and also hinder enzymes that activate carcinogens. Phytochemicals have been encouraged for the prevention and treatment of high blood pressure, diabetes and macular degeneration (American Cancer Society, 2000).

Phenolics: Phenolics are a class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. They are the largest category and most widely distributed phytochemicals in the plant kingdom. They have been considered as the large and complex group of chemical constituents found in plants (Walton *et al*., 2003). They have an important role in the defense mechanisms of plants and their antioxidant properties determine its important role in protection against free radical-mediated disease processes. Flavonoids, phenolic acids and polyphenols are the three most important groups of dietary phenolics (Saxena *et al*., 2013)**.** Phenol is considered as the simplest class of this group and flavonoids are the largest and most studied group of plant phenols (Dai and Mumper, 2010).

Flavonoids: Flavonoids are a group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects**.** They are the polyphenolic molecules containing 15 carbon atoms and are soluble in water. Many of these molecules are found in variety of vegetables, fruits and beverages like tea, coffee and fruit drinks (Pridham, 1960). Flavonoids can be divided into six major subtypes, which include flavones, isoflavonoids, flavanones, chalcones, anthoxanthins and anthocyanins. Flavonoids are important sources of antioxidants and promote several health effects. Besides antioxidant activity, these molecules have been stated to possess many useful properties like, anti- viral and anti-inflammatory activity, antimicrobial activity, enzyme inhibition, anti-allergic activity, vascular activity, oestrogenic activity and cytotoxic antitumor activity (Tapas *et al*., 2008).

Alkaloids: Alkaloids are a class of naturally occurring compounds that mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. The name alkaloid is derived from "alkaline" which is used to describe any nitrogen-containing base (Mueller-Harvey and McAllan, 1992) and they react with acids to form salts. They are found primarily in plants and are especially common in certain families of flowering plants. An alkaloid contains at least one nitrogen atom in an amine-type structure, one derived from ammonia by replacing hydrogen atoms with hydrocarbons. This or another nitrogen can be active as a base in acid-base reactions. About 2000 different alkaloids have been isolated of which some are of pharmacological interests. Important alkaloids include morphine, strychnine, atropine, colchicines, ephedrine, quinine and nicotine (Pallardy, 2010). The medicinal properties of alkaloids are quite diverse. Alkaloids have many pharmacological activities including analgesic properties (Codein), antihypertensive effects (many indole alkaloids), anesthetic properties (Cocaine), antimalarial effect (Quinine),

anticancer actions (dimeric indoles, vincristine, vinblastine) and antiarrhythmic effect (quinidine, spareien) (Wink *et al.*, 1998).

Terpenoids: Terpenoids are a large class of naturally occurring organic chemicals derived from terpenes. Most of them are multicyclic structures with oxygen containing functional groups. Terpenes, basically consist of five carbon isoprene units assembled to each other by thousands of ways. Depending on the number of carbon units, terpenoids are divided into monoterpenes, diterpenes, sesquiterpenes, triterpenes and tetraterpenes. Terpenoids are structurally most diverse group among plant secondary metabolites. Sometimes they act as phytoalexins in plant direct defense or as signals in indirect defense responses (McCaskill and Croteau, 1998). Many terpenoids such as Taxol and its derivatives are used as anticancer drugs and artemisinin and its related compounds are used as antimalarial drugs. Terpenes play an important role as growth regulators and signal compounds of plants. In addition, terpenoids have medicinal properties such as anticarcinogenic, anti-ulcer, hepaticidal, antimicrobial or diuretic activity (Langenheim, 1994; Dudareva *et al*., 2004).

Fatty acids (FAs) are important biomolecules that act as energy reserves, plays major role in cell signaling and maintaining membrane fluidity. According to the researches FA composition play crucial role in enduring various biotic and abiotic stresses, specifically in chilling stress, salt stress, heat stress and wound healing (Guschina and Harwood, 2006; Nishida and Murata, 1996; Upchurch, 2008; Zhang *et al*., 2012; Yaeno *et al*., 2004; Walley *et al*., 2013). Since the FAs with unusual structure act as precursors for various synthetic products, they bear great demand for pharmaceutical and chemical industries. Moreover, FAs have been exclusively profiled for chemotaxonomic perspectives in plants (Mongrand *et al*., 2001, 2005; Wolff *et al*., 2001; Dussert *et al*., 2008; Dogru-Koca *et al*., 2016), in fungi (Mishra

et al., 2010), in bacteria (Malviya *et al*., 2011), in microalgae (Dunstan *et al*., 2005) and in cyanobacteria (Shukla *et al*., 2012).

Phytochemistry is very important for the determination of active ingredients of medicinal plants, their quantification and analysis have beneficial and harmful effects to human health. Various phytochemical technique are used in the quality control of Chinese medicines or herbal medicines of various chemical components, such as saponins, alkaloids, volatile oils, flavonoids and anthraquinones. But it is very difficult to determine the nature of biologically active compounds in herbal medicine. Therefore, phytochemical methods are important to screen and analyze bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms.

Subfamily Lamioideae

Lamiaceae, commonly called as Mint family, is one of the largest families among dicotyledons, being composed of more than 236 genera and 7173 species (Harley *et al*., 2004). Many species belonging to the family are aromatic, due to the presence of external glandular structures that produce volatile oil. The typical secondary metabolites of Lamiaceae include various terpenoids and phenolic compounds. The family is of outstanding importance in its use as a source of indigenous medicinal plants, used by people world over particularly in Indian cultures and tradition. Mint plants are one of the two major sources of culinary herbs used by people, the other being Apiaceae.

Although Lamiaceae is usually thought of as an aromatic family, there are numerous genera which appear not to accumulate or sequester essential oils. The oil-rich and oil-poor genera have a distribution which corresponds remarkably well with Erdtman's two subfamilies. The oil-rich genera are

mainly in the subfamily Nepetoideae and the oil-poor genera are mainly in the Lamioideae. This study focused on the oil-poor subfamily Lamioideae to study the taxonomic significance of phytochemicals within the subfamily based on the plants found in South India.

Lamioideae, is characterized by the fact that its species are very polymorphic which means that there are several morphoforms for each species. Species belonging to this sub-family are also well-known to make interspecific hybrids among each other. The name Lamioideae is derived from the Greek term “laimos” meaning jaws. These species are annual or perennial, herbaceous with tap and rhizomatous roots. The stems are erect and mostly reddish at the base which also exists as branchy. The flowers are petiolate, opposite and alternated. They are zygomorphic and constituted by a rigid and hairy calyx with mostly five sepals. The corolla is formed by two lips which are well developed and has variable coloration. The upper one has a double central lobe to protect the reproduction organs. There are four fertile didnamous stamens. The anthers have rounded glabrous or woolly lobes. The ovary is almost superior with two carpels and a bifid stigma. The inflorescence is constituted by sessile and pubescent flowers which are in several close verticillasters superimposed along the stem. There is no endosperm in fruit. The pollination takes place through bees and butterflies (Conti *et al*., 2007; Pignatti, 1982).

Subfamily Lamioideae is strongly represented in warm temperate to subtropical regions, (Harley *et al*., 2004) but also occurs in some tropical and cold temperate regions and consists mainly of herbs, subshrubs or shrublets. In south India, the subfamily is represented by six genera; *Anisomeles, Colebrookea, Gomphostemma, Leonotis, Leucas* and *Pogostemon*. Genera like *Colebrookea* and *Leonotis* represents single species only. The genera which include more number of species are *Leucas* (31), followed by

Pogostemon (21) whereas *Anisomeles* and *Gomphostemma* are represented by four species, each with one variety.

***Anisomeles*:**The genus *Anisomeles* R.Br. (Lamiaceae: Lamioideae) with 26 species (Bean, 2015) are short-lived perennial shrubs distributed in tropical Africa, South and South-East Asia and North-East Australia (Harley *et al.,* 2004). The members of this genus are characterized by the presence of camphor-scented flowers possessing anti-inflammatory and antiseptic properties and are widely used in folk medicine in India to cure various diseases like fever, psoriasis, skin diseases, abdominal pain etc. Four species of *Anisomeles*, viz; *A. heyneana, A. malabarica, A. indica* var. *indica* and *A.indica* var. *albifora* are found in South India.

***Colebrookea*:** Genus *Colebrookea* represents only a single species; *Colebrookea oppositifolia.* It is commonly called as Indian squirrel tail (English), Binda (Hindi), Pansara (Bengali), Bosiki (Oriya), Jolidi (Telungu) and Dhusure (Nepali) (Madhavan *et al*., 2011).In India, the plant grows wild on hills and plains throughout. The plant possesses hepatoprotective, cardioprotective and anti-inflammatory properties (Singh *et al.,* 1983). The essential oil of *Colebrookea oppositifolia* possesses fungitoxic property (Venkateshappa and Sreenath, 2013). *Colebrookea* has anthelmintic properties which is employed in the treatment of dermatitis, bleeding, nose bleeds, dysentery and bloody coughs and also it is used in the management of ringworms (Venkateshappa and Sreenath, 2013). It is also used to cure diseases like epilepsy, fever and urinary problems (Gupta *et al.,* 2001). Because of its anti-inflammatory effects, it is used for treating sore eyes, headache, corneal opacity or conjunctivitis (Torri, 2012).

***Gomphostemma*:** The genus *Gomphostemma* Wall. ex. Benth. is widely distributed in Burma and east India through Indo-China, with around 8 species in Malesia, mostly in Sumatra and Peninsula Malaysia, and not

extending east of the Philippines, Celebes and Bali with about 30 species in south east Asia. They usually grow as rainforest sub-shrubs or herbs, mostly below 1000 m altitude, occurring in lower montane rainforest areas (Walsingham and Bramley, 2010). The genus was used in the treatment of dysentery, diarrhoea, arthritis and in curing inflammation by insect stings (Prasad *et al*. 2013, Dutta, 2014). Few species of *Gomphostemma* in north-eastern states of India are used for alleviating malarial fever (Dutta, 2014). According to earlier research reports, *Gomphostemma* is a rich repository of secondary metabolites like phenols, alkaloids, flavonoids, terpenoids *etc*. (Dutta, 2014) and also reported to possess antioxidant properties (Shyama *et al*., 2012). Members of *Gomphostemma* reported from south India are endemic to Western Ghats and they are; *G. heyneanum* var. *heyneana, G. heyneanum* var. *rottleri*, *G. eriocarpum* and *G. kerelensis*.

Leonotis*:Leonotis*(Pers.) R.Br. is a striking member of the Lamioideae, with dense verticils of predominantly orange-haired flowers on instantly recognizable flowering stems. Only a single species, *L. nepetifolia* (L.) R.Br. is found in India as an annual weed and it has a pan-tropical distribution (Iwarsson and Harvey, 2003). Commonly, *L. nepetifolia* is known as Klip Dagga or Lion's Ear. In Trinidad it is known as shandilay. The leaves are brewed as a tea for fever, coughs, malaria and womb prolapse. The leaves were traditionally used for rheumatic problems and also serve as a tonic and the floral heads are used against scalds, ringworm, burns and some skin diseases. Crushed root is used in the treatment of rheumatism, headache, rickets and wounds and also they were applied locally for facilitating breast milk (Ayanwuyi *et al*., 2009). The plant has been evaluated for its anticancer, anti-inflammatory, antioxidant, anti-diarrheal, antibacterial properties (Imran *et al.,* 2012). It has been used to treat fever, bronchial asthma, malaria and influenza in Ayurvedic system (Gnaneswari and Venkatraju, 2012).

***Leucas*:** The genus *Leucas* R.Br. consists of more than 100 species are found in warm and tropical regions of Africa, Asia and some extending to Australia. (Singh, 2001; Mabberley, 2008). The distribution pattern of *Leucas* in India reveals that about 85 percent of the total species are found in south India (Singh, 2001). Furthermore, many of them are strictly restricted to Western Ghats. Plants of the genus *Leucas* are generally shrubs, subshrubs, perennial herbs or annual herbs with woody root or stem base. The plants are known as '*Tumba*' in regional languages in southern India and used widely as an essential ingredient in many folk medicines. Medicinal and therapeutic effects of *Leucas* are clearly described in the Bhavaprakasa Nighaṇṭu, written based on Indian Materia Medica of Bhava Misra (c 1600-1600 CE) and in Sodhala Nighaṇṭu. The name '*Kutumbika*' used in the *Charaka Saṃhita* is identified as *Leucas*. The plants are known as Droṇapushpi in Ayurveda literature and are recorded as having sweet, salty, pungent, bitter taste along with heavy and dry properties. It is known to have antibiotic, antipyretic, antiseptic, anthelmintic, germicidal and insecticidal properties (Kiritikar and Basu, 1918; Rastogi and Mahrotra, 1993). Its penetrating character and hot potency aggravates *pitta* and alleviates *vata-kapha* stages of the diseases. It promotes taste sensation and increases the digestive fire and also increases the intellectual capacity. *Leucas* is capable of expelling out waste products from the body, helps in subsiding edemas, relieves piles, diabetes and it is beneficial against diseases such as fever, jaundice and in respiratory diseases. *Leucas* species reported in south India includes; *L. angularis, L. aspera, L. beddomei, L. biflora, L. chinensis, L. ciliata, L. eriostoma* var. *eriostoma, L. eriostoma* var. *lanata, L. helianthimifolia, L. hirta, L. lamiifolia, L. lanata, L. lanceaefolia, L. lavandulifolia, L. lavandulifolia* var. *deccipiens*, *L. lavandulifolia* var. *nagalapuramiana*, *L. marrubioides, L. martinicensis, L. montana, L. nepetifolia, L. prostrata, L. pubescence, L. rosmarinifolia, L.*

sebaldiana, L. sivadasniana, L. stelligera, L. stricta, L. suffruticosa, L. urticifolia, L. wightiana and *L. zeylanica*.

***Pogostemon*:** The characteristic feature of the genus *Pogostemon* Desf. is the well exerted stamens which bears monoliform hairs. Globally *Pogostemon* is represented by 96 species and is represented in south and southeast Asia by 79 taxa. India has the highest number of *Pogostemon* species in the world, represented by 56 taxa (53 species and 3 varieties), of which 22 taxa (19 species and 3 varieties) are endemic. The major centre of *Pogostemon* diversity in peninsular India is the Western Ghats. Their main habitat is open forest areas and the rockey surfaces in grasslands (Shinoj *et al*., 2016). *Pogostemon* has a long history of use in southern Asia and the far-east as incense, body and garment perfume. Traditionally, the dried leaves of several *Pogostemon* species are placed between stored clothing to repel insects and to impart a pleasant smell. Patchouli oil steam distilled from *P. cablin* leaves is almost universally used as a fixing agent in perfumery, blending beautifully with an exceptionally wide range of fragrance and body-care materials. A decoction of the leaves is used in Chinese medicine with other drugs to treat diarrhea, nausea, vomiting, colds and headache. *Pogostemon* species reported in south India includes; *P. atropurpureus, P. auricularius, P. benghalensis, P. cruciatus, P. deccanensis, P. gardneri, P. heyneanus, P. mollis, P. myosuroides, P. nilagiricus, P. paludosus, P. paniculatus, P. peethapushpam, P. plectranthoides, P. purpurascens, P. quadrifolius, P. rotundatus, P. speciosus, P. travancoricus* var. *travancoricus, P. travancoricus* var. *devicolamensis, P. wightii* and *P. raghavendranii.*

The study also attempt to study the evolutionary relationships of the members of the subfamily Lamioideae using molelcular phylogenetic tools by employing the sequences generated and downloaded from gen banks. Molecular phylogenetics applies a combination of molecular and statistical

techniques to infer evolutionary relationships among organisms or genes. It uses the structure and function of molecules and how they change over time to infer these evolutionary relationships. The evolutionary nature and origin of diversity of Lamiacean plants in India was not studied comprehensively so far. Correlating the phytochemical data and phylogenetic inferences may reveal the evolutionary nature of phytochemical diversity in this subfamily.

Here we attempt to trace the evolutionary relationship within subfamily Lamioideae using DNA sequence and correlate the presence and absence of various phytochemicals within the dendrogram.

Importance of the study

The Lamiaceae or Mint family are best known for the essential oils common to many members of the family, although a wide range of other compounds have also been reported (Richardson, 1992). Throughout the world, hundreds of Lamiaceaen species are used as medicinal and aromatic plants (Zielinska and Matkowski, 2014). The medicinal and aromatic properties of majority of the members are attributed to the presence of variable chemical constituents in their plant body. Different plants sometimes possess different phytochemicals and the analysis of these chemicals may provide characters for taxonomic groupings as well as to trace the relationships among them.

Within the family Lamiaceae, subfamily Lamioideae are considered as an oil poor subfamily and studies were scare. Hence, this study is attempted to profile the volatile compounds and fatty acids in this subfamily to identify the chemical diversity possessed by these plants in India and to test their usefulness in chemotaxonomy.

Chemotaxonomic studies focus on the distribution of chemical compounds or groups of biosynthetically related compounds. The family

Lamiaceae is rich in flavanoids of different structures and has often proved useful for chemotaxonomic purposes (Tomas-Barberan and Gil, 1992). A special chemical feature of this family is the presence of lipophilic external flavanoid aglycones, which are often highly methylated (Tomas-Barberan and Wollenweber, 1990). These compounds are found on the surface of the plants (leaves, stems, inflorescence) and there is evidence that they, like essential oils, are stored in special glandular hairs.

Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical technique that combines the features of mass spectrometry to identify different substances within a test sample (Sparkman *et al*., 2011). GC-MS is used to fragment the analyte to be identified on the basis of its mass; whereas GC separates the volatile and thermally stable substitutes in the sample.

Here we employ GC-MS analysis of essential oil as well as fatty acids of the leaf samples to profile their phytoconstituents within the subfamily. Also attempts were made to use it as a chemotaxonomic tool to assess relationship of Indian members of subfamily Lamioideae in South India.

Studies of phylogenetic relationships within the Lamioideae were based mostly on morphological and anatomical data. A molecular phylogenetic study of the subfamily Lamioideae illuminates the evolutionary relationship within the subfamily. Correlating the evolutionary tendencies of the subfamily with the distribution of secondary metabolites may reveal the evolution of phytochemicals within the subfamily. The present study envisages the phytochemical and molecular analysis of selected members of the family Lamiaceae (subfamily Lamioideae) of south India with the following objectives.

Objectives of the proposed study

1. To analyze the phytochemical constituents present in selected taxa through GC-MS analysis.
2. To find out the correlation between flavonoids, terpenoids, diterpenoids and taxonomy.
3. To use phytochemical data as a marker for taxonomic analysis.
4. To find out the evolutionary significance of distribution of phytochemical constituents using phylogenetic tools.
5. To construct phenograms based on chemical constituents.
6. To study the correlation of phytochemical data and phylogenetic inferences with an aim to reveal the evolutionary tendencies within the subfamily Lamioideae.

REVIEW OF LITERATURE

Phytochemistry of Lamiaceae

Nature is a huge reservoir of variable living components possessing useful, harmful and inactive chemical constituents. Almost all organisms produce natural products, but they are mostly known from plants, algae, insects, fungi and prokaryotes. Chemistry of natural products plays a vital role in the coexistence and interaction of these organisms in the ecosystem (Reynolds, 2007; Larsen *et al.,* 2007). Many natural products have been used as traditional medicines and as natural poisons for thousands of years since they have bioactive constituents. Plants are the important contributors of natural products. Chemotaxonomy is truly the modern and applied approach to classify plants based on diversity of phytochemicals and natural products (Singh, 2016).

Lamiaceae or Labiatae, also called as the mint family, is a family of flowering plants and is regarded as the second largest source of culinary herbs (Richardson, 1992). It has been acknoweledged for centuries as a group having considerable culinary and pharmaceutical interests (El-Gazzar and Watson 1970b). Chemical constituents like flavonoids play a crucial role in chemotaxonomy within infra family and infra generic level and the Lamiaceae members are rich in these constituents (Thomas-Barberan and Gil, 1992). Lamiaceae is also rich in terpenoids and this may be the core reason for their anti bacterial, anti insect and anti fungal activities (Cole, 1992).

A wide variety of compounds with much taxonomic significance have been identified and isolated from the family Lamiaceae. These include the discovery of lithospermic acid, which is a derivative of

rosmarinic acid, found only in Lamiaceae and Boraginaceae (Molgaard and Ravan, 1986). According to Cole (1992), in the division of Lamiaceae subfamilies like Lamioideae and Nepetoideae, the taxonomic significance of terpenoids is much relevant. Nepetoideae are essential oil rich group whereas Lamioideae are essential oil poor and less studied group.

Since long, many attempts were there on the isolation, identification and classification of chemical constituents in Lamiaceae. Abdalla *et al.* (1983) isolated and identified 13 flavonoids from *Salvia triloba* and also identified 7-glucosides and 7-glucuronides of apigenin, luteolin, 6-methoxyapigenin and 6-methoxyluteolin and chrysoeriol 7-glucuronide.

Al-Hazimi *et al.* (1984) characterized Isocarnosol, a novel diterpene from the leaves of *Salvia lanigera.* As a continuation, Al-Hazimi (1986) attempted the isolation of a new natural methyl ester of Carnosic acid from *Salvia lanigera* and the structure of this ester was elucidated by spectroscopic methods.

Harborne *et al*. (1986) conducted a survey of aerial tissues of 42 European taxa of the genus *Teucrium* andhas revealed the widespread presence of five surface flavonoids: cirsiliol, cirsilineol, salvigenin, cirsimaritin and 5-hydroxy-6,7,3',4'-tetramethoxyllavone. Salvigenin is characteristic of species of section *Polium,* whereas S-hydroxy-6,7,3',4'-tetramethoxyflavone is completely confined to species of the other 5 sections surveyed. According to the results obtained, chemical results are correlated with sectional classification and usefully indicate that atleast one taxon, *T. compactum,* misplaced within the genus.

Marin *et al.* (1991) studied fatty acid composition of nutlet lipids in species from different subfamilies of family Lamiaceae. They used 62 species from Saturejoideae, seven species from Ajugoideae and four species from

Scutellarioideae for the study. Linolenic acid was found to be the major fatty acid in all species studied from Saturejoideae but in Ajugoideae linoleic and linolenic acid were the major fatty acids. Linoleic acid dominated in Scutellarioideae and the authors concluded that the fatty acid composition of nutlet lipids and their linolenate/linoleate ratios could be used as taxonomic markers for delimiting genera belonging to different sub families of Lamiaceae.

Tomas-Barberan *et al.* (1991) studied the correlations between flavonoid composite and infrageneric taxonomy of some European *Galeopsis* species and found the existence of hypolaetin, isoscutellarein and hypolaetin 4'-methyl ether 7-*O*-*β*-D- (2'*O*-*β*-D-allolosyl) glucosides as well as their mono-*O*-acetylated and di-*O*-acetylated derivatives in the sugar moiety. They concluded that subgenus *Galeopsis* accumulates luteolin, apigenin and scutellarein 7-*O*-*β*-D-glucuronides and p-coumaroyl glucosides of apigenin and luteolin whereas 8-hydroxyflavone glycosides are confined to subgenus *Ladanum*.

Angers *et al*. (1996) studied seed fatty acid composition and glyceride profile of 7 *Ocimum* chemotypes belong to the species; *O. basilicum, O. canum, O. gratissimum* and *O.sanctum*. The oil contents found to be 18% to 26% and among the total neutral lipids extracted 94% to 96% were triglycerides. The major fatty acids identified were linolenic, oleic and palmitic acid, and the linolenic acid was found to be similar among the four species studied. A higher quantity of linolenic acid were found in *O. canum* and lowest in *O. sanctum*

Baser *et al*. (1996) studied the essential oil composition of 13 *Nepeta* species of Turkey and found that 1-8-Cineole is the major compound in *N. sulfuriflora*, *N. nuda* subsp. *albiflora*, *N. nuda* subsp. *Nuda* and in *N. italic*. Geijerene is the major compound in *N. nuda* subsp. *nuda*, Caryophyllene

oxide in *N. conferata*, *N. isaurica*, *N. cilicia*, *N. nuda* subsp. *glandulifera*; β-pinene in *N. phyllochlamys*, α-terpineol in *N. viscid*; Linalool in *N. flavida* and 4 aα, 7 α, 7aα-nepetalacetone in 4 *N. cadmea* and in *N. caesarea*.

Kurkcuoglu *et al*. (2001) analyzed the essential oil constituents of *Satureja boissieri* from Turkey by GC/MS and 45 components were characterized representing 97% of the oil. The main components were identified as carvacrol (40.8%), g-terpinene (26.4%), and p-cymene (14.5%).

Vieira *et al*. (2001) studied the genetic diversity of *Ocimum gratissimum* L. based on volatile oil constituents, flavonoids and RAPD marker. The major volatile oil constituents found in *O. gratissimum* were eugenol, thymol and geraniol. Cluster analysis revealed three groups that are distinct genetically based on volatile oil constituents.

Grayer *et al*. (2002) surveyed 31 accessions of nine species belonging to three subgenera of *Ocimum* for flavonoid glycosides and found that the major flavonoids in var. *americanum* and in all other species investigated for subgenus *Ocimum*, were flavonol 3-*O*-glucosides and 3-*O*-rutinosides. In glass house grown plants, the level of flavonol glycosides produced was reduced significantly, but levels of flavone glycosides were unaffected. In all 9 species of *Ocimum* studied, Luteolin 5-*O*-glucoside was found and is considered to be a key character for the genus.

Valant-Vetschera *et al*. (2003) analyzed several newly studied species and further accessions of Lamiaceae for their exudate flavonoid profiles and found that the chemodiversity was relatively low, with only some 15 derivatives being found. Among the species studied, only *Salvia arizonica* yielded a rare diterpene quinone, demethylfruticulin A.

Azcan *et al*. (2004) screened 12 *Salvia* species from turkey for identifying the fatty acid composition of seed oil. The species studied were *S.*

albimaculata,*S. candidisima, S. cedronella, S. cryptantha, S. forskahlei.*,*S. fruticosa, S. halophila*, *S. hypargeia*, *S. sclarea., S. tomentosa, S. tchihatcheffii* and*S. virgate.*The hexane extract yields in these species were found to be 2.0% to 20.9%. Fatty acid compositions were determined by GC-MS followed by the esterification of oils.The main fatty acid components of *S. halophila*, *S. hypargeia*, and *S. sclarea*are unsaturated oleic, linoleic, and linolenic acids. In others except *S. candidissima*, the dominant acids are oleic, linoleic and palmitic acids.

Citoglu *et al.* (2005) investigated 16 taxa of *Ballota* by analyzing the contents of diterpenoid and flavonoid compositions, and the relationships were compared with their morphological properties. The analysis of clusters revealed no concordance between chemical data and other features such as distributions, habitats, anatomic and morphologic characters.

Akcin (2006) studied the similarities of some species of the genus *Thymus* L. from Turkey, using the morphological and chemical characters and the resultened dendrograms obtained from morphological and chemical data were similar.

Vilijoen *et al*. (2006) studied the essential oil composition and chemotaxonomy of *Salvia stenophylla* and its allies *S. repens* and *S. runcinata.* GCMSresult showed thatthe dominant compounds in the *S. stenophylla* oils were α-bisabolol (46.5%), (E)-nerolidol (53.6%), limonene (38.1%), δ-3-carene (24.9%), γ-terpinene (20.3%) and p-cymene (18.4%). In *S. repens* oil, major compounds were (E)-nerolidol (25.2%), ledol (25.4%), camphor (12.7%), β-caryophyllene (13.6%) and β-phellandrene (22.2%) whereas *Salvia runcinata* oils had (E)-nerolidol (72%), α-pinene (45%), α-bisabolol (41.1%), limonene (24.1%) and β-pinene (15%) and 26% of guaiol in high percentages. According to the observed results, *S. stenophylla* and *S. repens* are the closest allies within the complex.

Urwin and Mailer (2008) studied the fatty acid composition of 39 species of the genus *Lavandula*and observed that qualitatively fatty acid profiles were similar in all species of Lavender analysed. The major fatty acid in all species being a-linolenic acid.

Li *et al*. (2010) investigated the distribution of tanshinones, a group of biologically active diterpenes, in some Chinese members of the genus *Salvia*. Tanshinones in various *Salvia* species were determined using high-pressure liquid chromatography with a diode array detector and the distribution supported the circumscription of sect. *Drymosphare* in the original sense of Bentham.

Salimpour*et al*. (2011) studied the chemotaxonomy of six *Salvia* species viz; *S. atropatana*, *S. oligophylla*, *S. aethiopis*, *S. sclarea*, *S. reuterana* and *S. macrosiphon* using essential oil composition markers. The results showed that due to the presence of higher amount of occidentalol, iso-Longifolene, β- Acoradiene, the species *S. oligophylla* got separated from *S. aethiopis*. Similarly *S. aethiopis* and *S. sclarea* are grouped to one sub cluster due to the similarity in essential oil composition.

Cicek *et al.* (2011) analyzed the essential oil composition of three species; *Scutellaria diffusa, S. heterophylla* and *S. salviifolia* from Turkey and the main components in the oil of *S. diffusa* were determined as hexadecanoic acid (30%) and caryophyllene oxide (9%) whereas Germacrene D (21%), hexadecanoic acid (16%) and caryophyllene (13%) were found as major components in the oil of *S. heterophylla and* germacrene D (40%), bicyclogermacrene (14%) and caryophyllene (11%) in *S. salviifolia*.

Radulovic and Blagojevic (2012) analysed the essential oil obtained from above-ground parts of *Micromeria dalmatic* by GC and GCMS analysis andidentified 116 components, comprising 93.6% of the total oil composition.

The major compounds being 3-oxygenated p-menthane monoterpenes consists of pulegone (29.6%), menthone (11.7%) and piperitenone (10.8%).

Kharazian (2012) investigated the flavonoid constituents in some of endemic *Salvia* L. species in Iran and concluded that hydroxylation, methoxylation and glucosylation patterns may be considered to be specific to the *Salvia* species and their presence could be significant in chemotaxonomy and pharmaceutical researches.

Delange *et al*. (2012) studied the fatty acid composition of the seed oil of *Salvia coccinea* L. growing in Cuba. Seed oils of the species were extracted with hexane and analyzed by GC and followed by GCMS after trimethylation reaction and the major fatty acids found were linolenic acid (18:3; 33.1%), linoleic acid (18:2; 25.2%), oleic acid (18:1; 13.3%) and stearic acid (18:0; 12.5%), whereas other fatty acids were found in minor proportions.

Kilic (2013) studied two *Satureja* L. species from different localities of Turkey (*S. hortensis* and *S. boissieri* to determine their taxonomic classification based on chemical characters and found out that γ-terpinene (30.4%, 26.5% and 32.1%), carvacrol (26.4%, 25.2% and 23.3%) and *p*-cymene (10.5%, 13.2% and 10.4%) were the main compounds of *S. boissieri,* species collected from Sanliurfa, Bingol and Malatya accessions, respectively. Carvacrol (25.0%, 34.1% and 32.1%), thymol (28.2%, 20.2% and 28.1%) and γ-terpinene (10.1%, 11.3% and 9.4%) were found as main constituents of *S. hortensis* in Adiyaman, Diyarbakir and Elazig samples, respectively.

Kilicand Bagci (2013) analyzed the essential oil of the aerial parts of *Ziziphora clinopodioides* Lam., *Z. persica* Bunge and *Z. tenuior* L. by GC and GCMS. Altogether 37, 45 and 36 compounds were identified representing 90.20%, 93.12% and 92.69% of the oil, respectively in the species. The study

revealed that pulegone and piperitenone derivatives are characteristic and represent excellent chemotaxonomical markers for *Ziziphora* taxa.

Okach *et al.* (2013) analyzed the phytochemicals of plants of Lamiaceae family (*Calamintha nepeta*, *Becium obovatum*, *Fuestia africana*, *Hyptis pectinata*, *Hoslundia opposita*, *Leonotis nepetifolia*, *Leucas calostachys*, *Ocimum kilimandscharicum*, *Plectranthus barbatus* and *Satureja biflora*) commonlyused in traditional medicine in Uriri District of Kenya. The study concluded that various pharmacological and biological properties of the phytochemicals determine the medicinal value of the plant species of Lamiaceae family as useful sources of drugs in ethnomedicine.

Topcu *et al*. (2013) studied the hexane extract of *Salvia fruticosa* Mill. and the major fatty acids obtained are oleic acid (29%), palmitoleic acid (29%) and stearic acid (23.20%), which exhibited high anticholinesterase activity.

Zielinska and Matkowski (2014) conducted a review on recent advances in phytochemical, pharmacological, biotechnological and molecular research on the genus *Agastache* and reported that the phytochemical profile of all *Agastache* species consisted of two main metabolic classes; phenylpropanoids and terpenoids. The essential oil of most populations of different *Agastache* species contain over 50% of a phenylallyl compound; estragole. Also two unique lignans; agastenol and agastinol were also isolated.

Kharazian (2014) studied the chemotaxonomy and flavonoid diversity of *Salvia L.* (Lamiaceae) in Iran and found that flavonoids are appropriate indicators to determine the taxonomic position of *Salvia* species. His studies was based on 14 species of *Salvia* and the flavonoids identified were flavones, chalcones, flavonols, flavanones, isoflavones and dihydroflavonols.

Shanayda (2015) identified and analysed the flavonoids in the aerial parts of three *Nepetoideaen species*; *Ocimum americanum*, *Lophanthus anisatus* and *Satureja hortensis*. The highest content of rutin was found in the grass *Ocimum americanum* ($62{,}06 \cdot 10^{2}$ %) and *Satureja hortensis* ($28{,}56 \cdot 10^{-2}$ %). *Lophanthus anisatus* possess the maximum content of apigenin and its derivatives ($30{,}71 \cdot 10^{-2}$ % и $26{,}79 \cdot 10^{-2}$ %, respectively).

Verma *et al.* (2015) studied the essential oil composition of *Mentha longifolia* which is collected from Garhwal Region of Western-Himalaya and identified total of 55 constituents, forming 97.5% of the total oil composition. The oil was characterised by high quantity of oxygenated monoterpenes (74.0%) and sesquiterpene hydrocarbons (18.0%). The most characteristic constituents of the oil were *trans*piperitone epoxide (48.7%), piperitenone oxide (21.2%), germacrene D (9.8%), (*E*)-caryophyllene (2.3%), 2- hydroxy piperitone (1.6%), α-humulene (1.5%), thymol (1.4%) and α-longipinene (1.0%).

Lukas *et al*. (2015) investigated the essential oil diversity of European *Origanum vulgare* (Lamiaceae) and found that content of essential oil compounds of European *O. vulgare* ranged between 0.03% and 4.6%. The monoterpenes were primarily made up of sabinene, myrcene, carvacrol methyl ether, linalyl acetate, p-cymene, 1,8-cineole, sabinene hydrate, β-ocimene, γ-terpinene, linalool, a-terpineol, thymol and carvacrol. Among the sesquiterpenes b-caryophyllene, caryophyllene oxide, germacrene D, spathulenol, germacrene D-4-ol and oplopanone were often present in higher amounts.

Kharazian and Hashemi (2017) investigated morphological characters, chemotaxonomy and the flavonoid compounds of five *Marrubium* species and formulated that flavonoid profiles and morphological characters were found

to be appropriate markers to detect the variation and taxonomic status in this genus.

Dogan (2017) analysed the chemical composition of the essential oils of dried aerial parts of four *Phlomis* species; *P. rigida*, *P. oppositiflora*, *P. linearis* and *P. kurdica* by GC and GCMS. Epizonaren (14.3%), δ-cadinene (11.0%), spathulenol (10.7%), α-copaene (7.5%) and β-bourbonene (5.4%) were the main compounds determined in *P. rigida*; germacrene D (17.3%), chrysanthenyl acetate (5.9%), trans-chrysanthenol (5.8%) and 2-pentadecanone (5.2%) in *P. linearis*; germacrene D (22.7%), germacrene B (15.0%), bicyclogermacrene (9.0%), camphor (5.9%) and caryophyllene oxide (5.4%) in *P. oppositiflora* and germacrene D (36.5%), β-farnesene (14.5%), β-pinene (10.5%) and bicyclogermacrene (7.2%) in *P. kurdica*.

Toplan *et al.* (2017) studied the composition and biological activities of *Salvia veneris* in Cyprus and a total of 36 constituents, representing 99.8% of the essential oil were identified. GC-FID and GCMS analysis of the essential oil revealed the existence of 1, 8-cineole (51.0%), camphor (9.3%), camphene (6.3%), α-pinene (5.8%) and β-pinene (5.4%) as main constituents. According to LCMS/MS result, rosmarinic acid was found to be the major compound in the methanol extract whereas total phenol content was determined as 19±0.20 mg gallic acid equivalents (GAE) in 100 mg in the extract. According to these results, *S. veneris* could be considered as a new potential plant for food, pharmaceutical and cosmetic industries.

Cacan *et al*. (2018) investigated the leaf fatty acid composition of *Satureja hortensis*, *S. boissieri*, *Thymus kotschyanus* var. *glabrescens*, *T. kotschyanus* var. *kotschyanus, T. hausknechtii*, *T. pubescens* var. *pubescens*, *T. fallax*, *Origanum vulgare* subsp. *gracile* and *O. acutidens* using GCMS. In this study, the plants showed the presence of both saturated and unsaturated fatty acids. The major fatty acids found were palmitic acid methyl ester

(13.49-27.71%), linoleic acid methyl ester (10.85-19.47%) and linolenic acid methyl ester (40.68-56.53%). The other fatty acids were found in minor proportions. The highest unsaturated fatty acid determined in *Satureja hortensis* (73.19%) and the lowest amount found in *Thymus hausknechtii* (39.05%). Palmitic acid methyl ester was found the major saturated fatty acids in all taxa studied.

Phytochemistry of the subfamily Lamioideae

Lamioideae, is the second largest of seven Lamiaceae subfamilies with over 1260 species in 63 genera (Harley *et al*., 2004). This subfamily is frequently occurring in warm temperate to subtropical regions, although some genera are also present in tropical and cold temperate regions and consist mostly of plants with shrubby or herbaceous habits. Lamioideae have a near-cosmopolitan distribution though concentrated in northern to tropical Africa and Eurasia (Roy and Lindqvist, 2015).

Compared to the subfamily Nepetoideae, Lamioideae is the less studied subfamily in chemical aspects. It has been regarded as the volatile oil poor subfamily. Eventhough many attempts were taken place to innovate the chemical nature of the members of the subfamily. Some of the studies and their major outcomes were discussed here.

Khalil *et al*. (1996) isolated and identified three labdane-type diterpenes; 3-oxo-marrubiin and a mixture of two related C-15 epimeric diterpenes based on 9α,13α,15,16-bisepoxy-15-hydroxy-3-oxo-labdan-6β, 19-olide, and the acylated flavone apigenin 7-O-[6"-O-(p-hydroxy-trans-cinnamoyl)glucoside] from the areal parts of *Leucas neufliseana* and it was the first report on the isolation of epimeric diterpenes from a natural source.

Aitzetmuller *et al*. (1997) studied the seed fatty acid content of *Phlomis tuberosa, Leonurus sibiricus* and *Panzerina canescens* of family

Lamiaceae and subfamily Lamioideae using GCMS method. The percentages of fat in seeds of these species are 11.8%, 16%, 28.5% respectively. The authors reported the occurance of new allenic fatty acid; phlomic acid , 7,8-eicosadienoic acid or 20:2A 7,8ol ene, in the seeds of the genus *Phlomis* and several other genera of subfamily Lamioideae. Phlomic acid is a unusual fatty acid produced by chain-elongation of the major seed oil fatty acid, laballenic acid or 18:2A5,6 allene.

Bankova *et al*. (1999) supported the view that *Stachys* and *Betonica* are well separated genera. For this, the above-ground parts and roots from 4 *Stachys* species; *S. germanica*, *S. sylvatica*, *S. thracica* and *S. plumosa* as well as of three *Betonica* species; *B. officinalis*, *B. bulgarica* and *B. scardica* were screened for phenols (phenylethanoid glycosides, flavonoid glycosides and the phenolic diterpene betolide). A flavonoid glycoside, three phenylethanoid glycosides and the phenolic diterpene betolide were isolated and identified, most of them for the first time in the investigated species.

Skaltsa *et al*. (2001) studied the composition of essential oils of *Stachys* subsect. Swainsonianeae by GCMS analysis and the statistical analysis of oil componenets resulted in the separation of *S.ionica*, due to the high amount of *(E)*-nerolidol, α Cadinol and low amount of (+)-*(E)*-caryophyllene.

Al-Yousuf *et al*. (2002) studied the analgesic activity of *Leucas inflata* using methanol and acetone as extraction medium. Various analgesic activities were evaluated in mice using various experimental models and it was concluded that the crude methanol and acetone extract of *L. inflata* has CNS depressant properties, manifested as antinociception and sedation. Both extracts have anti-inflammatory and antipyretic actions.

Bure and Sellier (2004) analysed the essential oil of Indonesian patchouli (*Pogostemon cabin* Benth.) using GCMS (EI/CI). Altogether 41 compounds were separated and 28 of them (92.9% of the total oil) were identified, using different ionization techniques in mass spectrometry (EI, NCI and PCI with ammonia and deuterated ammonia as reagent gases). Four new compounds were found in this oil: germacrene D (0.2%), γ-gurjunene (2.2%), 7-epi-α- selinene (0.2%) and aciphyllene (3.4%).

Isolation and identification of the flavonoid constituents of flowering aerial parts *of Leonotis leonurus* were done by El-Ansari *et al*. (2009). They also evaluated hepatoprotective, anti-inflammatory and cytotoxic activities of the aqueous alcoholic and chloroform extracts. Among the ten flavonoid compounds isolated, two were identified as methylated flavones, six as flavone glycosides, and other two as flavone aglycons. It was also noticed that 70% methanol and chloroform extracts showed strong hepatoprotective and anti-inflammatory activity.

Sadeghi-Nejad and Deokule (2010) evaluated the in vitro antifungal activity of leaf extracts of *Pogostemon parviflorus* against three different genera of dermatophytes including *Trichophyton, Microsporum* and *Epidermophyton*, using the agar dilution method. They found that with the minimum inhibitory concentration (MIC) values between 2.5-10 mg/mL, the ethanolic extract of leaf completely prevented the growth of tested dermatophytic species and the minimum fungicidal concentration (MFC) values were also in the range of 2.5-10 mg/mL. The presence of saponins, reducing sugars, tannins, phenols and proteins and the absence of glycosides, anthraquinones, alkaloids or flavonoids were confirmed by preliminary tests. According to the Thin Layer Chromatography (HPTLC) studies, ethyl acetate extract of *Pogostemon parviflorus* leaves included triterpenes, as 10 and 14 peaks of ultra violet (UV) absorption that were observed in 254 nm and 366

nm, respectively. Hence antidermatophytic activity of this plant may be due to the triterpenes.

Meghashri *et al*. (2010) studied the antioxidant potential of crude methanolic extract of *Leucas aspera* leaves. Different antioxidant assays like DPPH and super oxide radical- scavenging activity ensures the strong antioxidant ability of the sample. Further fractionation of methanolic extract by silica gel column yielded a compound which posses' great antioxidant potentials. Characterization of this compound using UV, IR, 1H NMR, 13C NMR and mass spectroscopy, the bioactive compound isolated was found to be a novel flavonoid 5,7-dihydroxy-2-[14- methoxy-15-propyl phenyl]-4H-chromen-4-one or leucasin.

Preliminary pharmacognostic and phytochemical analysis of roots and leaves of *Leonotis nepetifolia* were done by Trivedi *et al*. (2011). The major finding of the study was that the physiochemical parameters, qualitative, quantitative and HPTLC profile together may be used for quality parameters of *Leonotis nepatifolia* to obtain genuine and standard drug for therapeutic purpose.

Piozzi and Bruno (2011) reviewed the chemistry of diterpenoids from roots and aerial parts of the genus *Stachys* and the presence of these diterpenoids in other taxa and their biological properties have been also reviewed.

Das *et al*. (2011) conducted phytochemical screening and antioxidant activity of different extracts (n-hexane, ethyl acetate and ethanol) of *Leucas aspera* and the results revealed the presence of significant amounts of alkaloids, glycosides, tannins and flavonoids in ethanol extract while the other two extracts contain moderate amount of the chemical constituents and also

the ethanol extract possesses more anti-oxidant activity than ethyl acetate and n-hexane extracts.

Ushir and Patel (2011) studied the essential oil composition from aerial parts (stem, leaves, flowers and fruits) of *Anisomeles indica* and *Anisomeles malabarica* and found that linalyl acetate (15.3%) and α-thujone (11.9%) were the major compounds in *A. indica* and α-thujone (17.6%), terpenyl acetate (16.45%) and *δ*-cadinene (11.5%) were the major compounds in *A. malabarica*.

Phytochemical analysis of the acetone extract of aerial parts of *A. heyneana* has led to the isolation of new phyllocladanediterpene acid, phyllocladan-16a,17-dihydroxy- 19-oic acid, along with known phyllocladanediterpene, phyllocladan-16a,19-diol, cembranediterp-eneovatodiolide, sitosteryl-3-O-b-D-glucoside, and verbascoside (Roshan *et al.* 2012).

Goren *et al*. (2012) analysed the fatty acid composition of the seed oil of 23 *Stachys* taxa using GCMS and the major compounds found to be linoleic (27.1-64.3%), oleic (20.25-48.1%), palmitic (4.3-9.1%), stearic (trace to 5.2%) and 6-octadecynoic (2.2-34.1%) acids. And they concluded that these major fatty acids can be used as chemotaxonomic markers for the genus *Stachys.* They also performed a cluster analysis to compare and characterize the fatty acid composition among the 23 species studied.

Imran *et al*. (2012) investigated the preliminary phytochemical analysis of *Leonotis nepetifolia* and the results reveals the presence of bioactive constituents comprising alkaloids, flavonoids, phenolics, glycosides, steroids, tannins and saponins in different solvents. They also summarized that the presence of these phytochemicals can be correlated with the medicinal potential of this plant.

Ramya *et al*. (2012) assessed the phytochemical components and antimicrobial activity of two important medicinal plants *Coleus aromaticus* and *Leucas aspera* and revealed that the two plant extracts (methanol and ethanol) contained tannins, alkaloids and glycosides and also both of the plants leaf exhibited antibacterial activity against enteric pathogens such as *Shigella* sp*., Salmonella typhi* and *Escherichia coli.*

Antioxidant and antibacterial activity of different parts of *Leucas aspera* were studied by Chew *et al*. (2012). The result showed that the root extract demonstrated the strongest antioxidant activity with IC50 value of 6.55 µg/mL. The crude extract of root, flower, leaf and stem showed notable antibacterial activity and with inhibition zone ranging from 9.0 to 11.0 mm, root extract showed the highest cativity, at concentration of 100 mg/mL.

Rahman and Islam (2013) investigated the antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* ethanolic extract. With IC50 value of 99.8±1.22 µg/ml, the result showed potential radical scavenging effect, which was significant ($P<0.01$) in comparison to ascorbic acid with IC50 value of 1.25±0.95 µg/ml. The extract also showed notable antibacterial effect against both gram positive and gram negative bacterial strains.

Anjana and Thoppil (2013) evaluated the chemical composition of the essential oils of four *Pogostemon* species (*P. deccanensis, P. heyneanus,P. benghalensis* and *P. auricularius*) and their larvicidal activity against *Aedes albopictus* (Diptera: Culicidae) and found that all the essential oils showed high larvicidal activity in 24 hour exposure tests. *Pogostemon deccanensis* showed maximum activity with an LC90 of 21.72 ppm and LC50 of 6.00 ppm, among the species studied.

Ulhe and Narkhede (2013) studied the anatomical and phytochemical characters of leaves of *Anisomeles indica* and revealed the presence of alkaloids, tannins, glycosides, carotenoids and saponins. In the leaf powder of *A. indica*, aromatic oil is found in 6% in 3 gm of dry weight. The study concluded that the leaves of *A. indica* contains double percent amount of aromatic oil and it contains more alkaloids, followed by tannins, saponins, glycosides, carotenoids and polyuronoids in the decreasing order.

Bhoria and Kainsa (2013) given a detailed review on the phytochemical and pharmacological studies carried out on *Leucas cephalotes*. According to the results, the *L. cephalotes* contains new labdane, nor labdane and abietone-type, diterpenes named leucasdins A (1), B (2), C (3), five sterols and eight flavones.

Rai *et al*. (2013) carried out the preliminary qualitative and quantitative phytochemical screening of methanolic, ethanolic and chloroform extracts of *Leucas linifolia, Coleus aromaticus* and *Pogostemon patchouli.* The result showed the presence of steroids and absence of terpenoids, amino acids in all the leaf extracts. Among the 3 solvents used, methanol seems to be good for almost all chemical constituents. The results concluded that the use of appropriate solvent for extraction and purification of the specific phytochemical from plants is one of the crucial steps in all phytochemical studies.

A chemical analysis of essential oil of *Anisomeles malabarica* have shown the presence of anisomelic acid, malabaric acid, anisomelolide, 2-acetoxymalabaric acid, anisomelyl acetate and anisomelol (Preethy *et al.* 2013).

Larvicidal and pupicidal activities of crude methanol extract of *Anisomeles malabarica* were studied by Ramaraj and Unpaprom (2013) and

found that the plant extract have the potential to be used as an ideal control for mosquito vector with an eco-friendly approach. They opined because of the highest larval and pupal mortality was found in the methanol extract of *A. malabarica* against the first to fourth instars larvae and pupae of *Anopheles stephensi*.

Ramani *et al*. (2013) explored the antiparkinson's and antioxidant activity of *Leuacslanata*. They used ethyl acetate fraction of ethanolic extract for the study. Initially they screened *L. lanata* for in vitro radical scavenging activity and further evaluated the effect of its extract on rotenone induced parkinson's disorder in mice and they found that the oxidative damage induced by rotenone was reduced by the administration of *L. lanata* extract in all the treatments. They concluded that the free radical scavenging activity of *L. lanata* might be the reason behind its protective effect on the oxidative damage induced by rotenone.

Kamalam *et al*. (2013) carried out the evaluation of *Leonotis nepetifolia* for its phytochemical and heavy metal analysis. Neutraceutical studies revealed the presence of proteins, carbohydrates and aminoacids. Presence of flavonoids, diterpene, phenolic class of compounds were confirmed by HPTLC studies and the presence of lead, cadmium, chromium and copper were confirmed by heavy metal analysis.

Analysis of essential oil composition of the endemic Soqotraen *Leucas virgata* and its antimicrobial and antioxidant activities were studied by Mothana *et al*. (2013) using GC and GCMS methods. A total of 43 constituents, representing 93.9% of the total essential oil were identified. The major components identified were, high content of oxygenated monoterpenes (50.8%). Camphor (20.5%), exo-fenchol (3.4%), fenchon (5.4%) and borneol (3.1%) were the other constituents identified. Among oxygenated sesquiterpenes β-Eudesmol (6.1%) and caryophyllene oxide (5.1%) were the

major compounds. The oil exhibited a high antibacterial activity against the tested *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. No activity was found against *Psuedomonas aeruginosa* and *Candida albicans*. A moderate antioxidant activity (31%) was exhibited in DPPH-radical scavenging assay.

Antony *et al*. (2013) investigated the antimicrobial potential of *Leucas aspera* engineered silver nanoparticles against *Aeromonas hydrophilia* in infected *Catla catla*. The green synthesized silver nanoparticles were characterized using UV-visible spectroscopy (UV-vis), dynamic light scattering (DLS), transmission electron microscopy (TEM), Fourier transform infra-red spectroscopy (FTIR) and inductively coupled plasmon optical emission spectroscopy (ICP-OES). The wave length of the sample was found to be 429 nm in the visible range which is specific for silver nanoparticles and the particle size was found to be in the range of 29- 45 nm with an average diameter of 189.3 nm. FTIR prediction showed the presence of possible polyphenol and protein encapsulates on the silver nanoparticles. The in vivo antimicrobial analysis and various biochemical and histochemical parameters showed that *Leucas aspera* engineered silver nanoparticles is a good antimicrobial candidate against the pathogen *Aeromonas hydrophilia.*

Veerabadran *et al*. (2013) investigated the antioxidant and antiproliferative potential of *Leonotis nepetifolia* leaves.With inhibition percentage of 60.57%, the DPPH assay showed methanol extract of *L. nepetifolia*leavesto be more significant in scavenging free radicals. Quantitative estimation of phytochemicals such as phenols and flavonoids were done and the quantity is represented as 0.107% and 0.089%. The study concluded that the leaves of *L. nepetifolia*were significant in scavenging free radicals and causing damage to proliferative cells.

Kundu *et al*. (2013) studied the antioxidant and antifungal properties of the essential oil of *Anisomeles indica* from India and a total of 26 compounds were identified, representing 90.70% of the essential oil and the major components were eugenol (17.63%), α-terpeneol (α–terpineol, 14.17%), β-pinene (8.11%), bornyl acetate (5.61%) etc.

Latha *et al*. (2013) evaluated the in-vitro phytochemical analysis on aqueous ethanolic extract of whole plant of *Leucas aspera* and confirmed the presence of sterols, alkaloids, flavonoids, galactose, oleanolic acid, ursolic acid, steroids, cardiacglycosides, saponins, tannins and β-sitosterol in the plant and the aerial parts contain α-sitosterol and β-sitosterol. The total phenolic contents and antioxidant capacity of ethanol extract were found to be 15.36 ± 0.512 GAE/g dry weight of extract and 190.00± 7.95 mg/g and respectively.

Pranoothi *et al*. (2014) studied the qualitative and quantitative screening, phytochemical analysis and screening of in vitro biological activities of *Leucas indica* var. *nagalapuramiana*, a high value medicinal plant of Seshachalam hill range of Eastern Ghats, Andhra Pradesh.Thestudy revealed the presence of alkaloids, phenols, tannins, saponins, flavonoids, steroids and reducing sugars. Quantitative analysis of the total flavonoids was 62.34 µg Rutin/µg and total phenol was 105 µg GAE/µg. A moderate antimicrobial activity was found in methanolic extract.

Antibacterial activity of *Anisomeles malabarica* were done by Packialakshmi and Nisha (2014) and found that, in non-polar studies the maximum zone of inhibition were found in *Pseudomonas aeruginosa* and in polar studies the maximum zone of inhibition were found in *Staphylococcus aureus*.

Dharmadasa *et al*. (2014) conducted screening of local and introduced varieties of *Pogostemon heyneanus* Benth. for superior quality physical, chemical and biological parameters. Altogether 26 morphological characters were assessed. Among this plant height, leaf margin, leaf apex, leaf base and leaf shape were polymorphic characters. All phytochemicals tested were identical to both varieties. However the presence of prominent spots of Rf 0.20 (rose color spot), 0.12 (dark brown spot), 0.45 (dark green spot) were characteristic for local variety. Introduced variety was reported to have higher oil content (0.52%), higher total ash content (12.32%), higher number of compounds in essential oil, patchouli alcohol content (57.0%) and antioxidant capacity (108.53±2.5 mh Trolox equivalent per g of extract). According to the results, introduced variety could be recommended for establishment of commercial cultivation.

Shirsat *et al*. (2014) studied the preliminary phytochemistry and antimicrobial activity of *Salvia plebeia*and *Colebrookea oppositifolia* and observed that both plants are rich in chemical composition containing alkaloids, flavonoids, tannins, phenolics, glycosides, saponins and steroids. The result concluded that leaf extracts of both the plants showed significant antimicrobial activity, however the highest antifungal and antibacterial activity was observed in methanolic extracts.

The primary phytochemical analysis of aqueous, methanolic and acetone extract of *Pogostemon benghalensis* were done by Naise and Bhadange (2014) and the results revealed the presence of various secondary metabolites like alkaloids, tannins, carbohydrates, sterol, terpenoids, quinon and flavonoids.

Babu *et al.* (2014) studied the phytochemistry and antimicrobial activity of *Leucas indica* and the presence of alkaloids, flavonoids, carbohydrates, glycosides, steroids, saponins, fixed oils, tannins, phenolic

compounds, proteins and amino acids were confirmed by preliminary qualitative tests. It was suggested that *Leucas indica* is useful in the treatment of infections caused by some of the bacteria since the crude extract of the leaf parts shows a wide range of antimicrobial activity.

Pullagummi *et al*. (2014) compared the hexane, methanol and ethanol extracts of Patchouli (*Pogostemon cablin*) and Geranium (*Pelargonium graveolens*) for their potential antibacterial activity against four bacterial species (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Enterobacter aerogenes*) using disc diffusion assayed method. The minimum inhibitory concentration (MIC) was ranged from 60 to 80 µl in Geranium ethanol and methanol extract whereas MIC ranged from 40 to 80 µl for Patchouli hexane extract. The study reveals that both plant leaves have potential broad spectrum antibacterial activity.

Dutta (2014) screened *Gomphostemma niveum* for secondary metabolites such as alkaloids, terpenoids, phenols, flavonoids, tannins and saponins and total phenol, flavonoid and alkaloid contents and proved that crude extract of the plant species contains medicinally important bioactive compounds. The total phenol, alkaloid and flavonoid contents were found 75.04 mg/g, 0.083 mg/g and 28.23 mg/g in terms of gallic acid, colchicines and quercetin equivalent respectively.

Venditti *et al.* (2015) studied the chemical composition of the flowering aerial parts of*Stachys annua* (L.) L. subsp. *annua*. Analysis of hydrodistilled volatile oil by GCMS, showed sesquiterpenoids as the major fraction (42.5%); phytol (9.8%), germacrene D (9.2%) and spathulenol (8.5%) were the most abundant constituents.

Murthy *et al.* (2015) studied the ethnobotany, chemistry and pharmacology of an aromatic genus *Anisomeles* in India. Anisomelic acid and

ovatodiolids are the more abundant bioactive compounds which show greater biological significance among the wide range of constituents isolated. And also these show a wide range antimicrobial, anti-inflammatory, anticancer, insecticidal, antipyretic, antioxidant, cytoprotective and herbicidal properties.

Chemical composition and biological properties of *Anisomeles indica* essential oil were studied by Basappa *et al*. (2015) and a total of 17 constituents identified. Farnesyl acetone, nootkatone and jasmatone were the major components identified. With IC50 value 9.86 µL/mL, essential oil exhibited strong antioxidant potential.

Muthuraj *et al.* (2015) determined the antibacterial activity and photochemical constituents through Gas Chromatography Mass Spectroscopy Analysis (GCMS) and qualitative phytochemical screening of *Pogostemon mollis* Benth. Totally 47 compounds were isolated by their retention indices (RI), retention time (RT) and mass spectra. Various secondary metabolites like phenol, flavonoids, terpenoids, pyosteroids, cardic glycosides, quinine and coumarians were detected in preliminary phytochemical screening.

Cheriyamundath *et al.* (2015) studied the DPPH radical scavenging property of methanol leaf extract from *Pogostemon quadrifolius*. Sequential extraction of leaf was carried out in three solvents, viz; petroleum ether, chloroform and methanol and only methanol extract has exhibited the DPPH scavenging property with IC_{50} 14.5±1.05 µg mL^{-1}. The DPPH radical scavenging property of the extract may be due to the presence of phenolic compounds.

Melkani *et al*. (2016) analysed essential oil from *Anisomeles indica* collected from Central Himalayan region using GC and GCMS. Among the 43 compounds identified representing 88.1% of the oil, abietadiene (20.5%), *β*-caryophyllene (8.8%), (*E,E*)-*α*-farnesene (5.5%), linoleic acid (8.7%),

trans-ferruginol (8.1%) and abietol (6.1%) were the major constituents. The oil was found to be rich in oxygenated diterpenes and diterpene hydrocarbons (54.7%).

Ishtiaq *et al*. (2016) carried out the macro and microscopic evaluation, phytochemical and physicochemical properties of leaf, stem and inflorescence of *Colebrookea oppositifolia.* Phytochemical analysis of methanolic extract indicated the presence of alkaloids, glycosides, triterpenoids, flavonoids, sterols and tannins. Histochemistry of the T.S. of leaf and stem gave positive results with conc. HCI, ferric chloride, phloroglucinol and Sudan III which indicated the presence of Ca^{2+} oxalate crystals, tannins, lignin and volatile oils respectively.

Kalpana and Rajeswari (2016) carried out the phytochemical and pharmacological investigation of *Leucas aspera*. The crude extracts of the leaves of *L. aspera* showed statistically significant anti-inflammatory activity and anti-helmintic effect. The aqueous extract of *L. aspera* showed a very good anti-arthritic activity. The study concluded that the *L. aspera* can be formulated in broad spectrum antibiotics and also confirms the traditional uses in pathogenic disease.

Kamaleswari and Nandagopalan (2016) carried out the phytochemical screening of *Pogostemon auricularis* and the resultconfirmed the presence of alkaloids, tannin, phenolic, flavonoids, glycosides, saponins, flavon glycosides, phytosterols, cardiac glycosides, fixed oils and fats in leaves. Cardiac glycosides phytosterols showed lowest amount while alkaloids flavonol glycosides showed moderate scores phenolics, flavonoids. Fixed oils and fats showed high scores but saponin and glycosides were absence. Oil was found in 9.9 % in 2 gm of dry weight of powder of leaves of *Pogostemon auricularis*.

Kaur and Kumar (2016) studied the phytochemical composition and in vitro antioxidant activity of *Leucas aspera* leaves and the presence of flavonoids, phenols, saponins, carbohydrate, tannins, alkaloid and terpenoids were confirmed by phytochemical screening. Total phenol and total flavonoid quantity was found to be 16 µg and 43.08 µg equivalents of gallic acid and quercetin per milligram of plant extract, respectively. The plant shows significant antioxidant activity with the IC50 value for DPPH radical scavenging activity, 20 µg as compared to ascorbic acid which was having IC50 as 9.34 IC50.

Choudhary *et al*. (2017) analyzed the seed fatty acid composition of 26 species and 5 varities of *Leucas* from various localities of India. The fatty acid compositions of seed oils were determined by GCMS analysis and they found that there is significant variation in the fatty acid profiles among species and their varients. The study revealed major fatty acids as palmitic, stearic, oleic, linoleic and laballenic acid; whereas myristic, palmitoleic, cis-vaccenic, linolenic, eicosanoic, eicosenoic, phlomic and docosanoic acid as minor fatty acids. The work also proved the presence of an unusual fatty acids laballenic acid a potent pharmaceutical candidate in all the *Leucas* species studied. Significant quantity of laballenic acid were found in *L. helianthimifolia*, *L. ciliata* var. *vestita* and *L. hirta* and can act as potential source for isolation of pharmaceutical compounds.

Brari and Thakur (2017) evaluated the bioefficacy of four essential oils (*Zanthoxylum armatum*, *Rabdosia rugosa*, *Artemisia maritima* and *Colebrookea oppositifolia* against *Callosobruchus analis* (Coleoptera: Bruchidae) and found that *R. rugosa* and *A. maritima* oil were most effective in reducing the egg hatchability to 48.00 ± 3.2 and 49.52 ± 2.2% respectively at a lowest dose of 10 µl/ml and also found that egg hatching inhibition percentage increased with an increase in concentration of all the treatments.

Kamaleswari and Nandagopalan (2017) attempted to compare the preliminary phytochemical constituents of the leaf extracts of two extreme environments such as: xeric (*Anisomeles malabarica*) and hydrophytic (*Pogostemon auricularis*) plants. In the methanolic extract of both *P. auricularius*, and *A. malabarica* the maximum yield of extract was recorded as (28% W/W) and (20% W/W) respectively. And the total phenolic content in methanol extract of *P. auricularis* was found to be 54.59 ± 0.5 mg GAE/g extract and that of *A. malabarica* was 48.28 ± 0.5 mg GAE/g extract were identified respectively.

Dechayont *et al*. (2017) investigated the antioxidant and antimicrobial activities of *Pogostemon cablin* leaf extracts. High levels of total flavonoid content and highest antioxidant activities were found in ethanolic extracts 280.12 ± 2.04 mg quercetin equivalent/g of dry plant extract and (IC50 = 18 ± 0.90, 20 ± 0.24 μg/mL) by DPPH and ABTS scavenging assays, respectively. Also the ethanolic extract had the greatest activity against methicillin resistant *Staphylococcus aureus*, methicillin sensitive *S. aureus* and *Streptococcus pyogenes* with zone diameters of 11.67 ± 1.53, 10.33 ± 2.52 and 10.33 ± 1.15 mm, respectively.

Sardar and Manik (2017) carried out the GCMS analysis of acetone extract of *Colebrookea oppositifolia* and found that the major compounds were Phytol (41.28%), n-Hexadecanoic acid (27.52%), Octanoic acid tridec-2eny ester (5.1%), 9,12,15 Octadecatrienoic acid (9.9%), 2-Dodecen-1-ny,succinic anhydride(4.4%).

Kusuma and Mahfud (2017) carried out the GCMS analysis of essential oil of *Pogostemon cablin* growing in Indonesia extracted by microwave-assisted hydrodistillation. Representing approximately 97.97% of the oil, a total of 19 compounds were identified. Oxygenated terpene patchoulol (26.32%) was identified as the main compound of the oil. δ-

guaiene (14.69%), α-guaiene (12.18%), α-Gurjunene (11.13%), seychellene (8.42%), viridiflorol (5.93%), β-caryophyllene (4.63%) and β-patchoulene (2.87%) were the other major compounds. 2(1H)-naphthalenone, octahydro-1-methyl-1-(2-propenyl)-, (1a,4ab,8aa)- (2.64%); iso-.aplha.-cedren-15-al (0.35%); 7-oxabicyclo[4.1.0]heptane, 1,3,3-trimethyl-2-(3-methyl-1,3-butadienyl)-, [1a,2b(Z),6a]- (0.12%) and hexahydrothunbergol (0.11%) were the four new compounds found in the oil.

Bincy *et al.* (2017) investigated the larvicidal potential of different solvents (Petroleum ether, chloroform, acetone, methanol and aqueous) extracts of *Pogostemon quadrifolius* tested against the fourth instar larvae of *Culex quinquifasciatus.* In petroleum ether extract, the maximum larval mortality was detected (LC50 0.112mg/ml) followed by acetone extract (LC50 0.0234 mg/ml). Presence of alkaloids, flavonoids, saponins and terpenoids were confirmed by the phytochemical tests.

Rahman *et al*. (2018) carried out the preliminary screening of *Pogostemon quadrifolius,* itsantimicrobial activity and TLC profiling of the crude plant extract. The presence of diverse classes of bioactive components was confirmed by phytochemical analysis. The quantitative analysis of alkaloids, flavonoids and total phenolic content in different extracts varied from each other. According to the results of antibacterial studies, methanolic extract and ethyl acetate extract showed activity against standard and MDR strains respectively. The ethanolic extract also showed fungicidal activity.

Huang *et al*. (2018) investigated the potential hepatoprotection of Patchouli oil (PO) through an ethanol-induced hepatotoxicity rat model. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in serum was decreased by PO pretreatment. PO could enhance the activities of glutathione (GSH), glutathione reductase (GR) and superoxide dismutase (SOD) as well

as the ratio of glutathione to oxidized glutathione (GSH/GSSG) in liver and suppress the content of reactive oxygen species (ROS), tumor necrosis factor alpha (TNF-a), free fatty acid (FFA) and triglyceride (TG). The results concluded that by relieving oxidative stress and preventing lipid accumulation, PO showed potent effect against ethanol-induced hepatotoxicity.

Ajaib *et al*. (2018) studied the phytochemical, antibacterial and antifungal activities of leaves and bark of *Colebrookea oppositifolia* and the phytochemical screening indicated the presence of alkaloids, flavonoids, steroids, glycosides and saponins whereas terpenoids, tannins, and cardiac glycosides are showed only in bark extract. The maximum antifungal potential exhibited by petroleum ether extract of leaves (15 ±0.3 mm) against *Aspergillus nigar* and petroleum ether extract showed maximum effectiveness against *Psuedomonas aeruginosa* with bark extract (18±2.2 mm).

Sajitha and Thoppil (2018) carried out the phytochemical evaluation and in vitroantioxidant studies of selected species of *Gomphostemma* (*G. heyneanum* var. *heyneana*, *G. heyneanum* var. *rottleri* and *G.eriocarpum)* from Western Ghats. Presence of many secondary metabolites like phenols, flavonoids, alkaloids, terpenoids and steroids were detected through preliminary screening. Quantitative estimation results revealed that *G. heyneanum* var. *heyneana* has the highest concentration of flavonoids and phenols. Antioxidant potential determined in terms of inhibition percentage shows that *Gomphostemma* species have significant radical scavenging activity.

Bilusic Vundac (2019) has given a detailed review on the taxonomical and phytochemical characters of 10 *Stachys* taxa from Balkan Peninsula. This review summaries the phytochemistry of *S. alpina*, *S. germanica*, *S.*

menthifolia, *S. oblique*, *S. officinalis, S. palustris*, *S. recta* subsp. *recta*, *S. recta*. subsp. *crenata*, *S. salviifolia*, and *S. sylvatica*.

Kambrath and Thoppil (2019) studied the anti inflammatory activity of selected species of *Gomphostemma* (*G. heyneanum* var. *heyneana*, *G. heyneanum* var. *rottleri* and *G. eriocarpum)* from Western Ghats and found that *G. heyneanum* var. *heyneana* proved to be more effective with the potential to inhibit cyclooxygenase and inducible nitric oxide synthase activity, among the three extracts studied.

Vasudha *et al*. (2019) evaluate the phytochemical screening, antimicrobial and antioxidant activities from the roots and leaves of *Leucas aspera* and the presence of carbohydrates, proteins, amino acids, steroids flavonoids, terpenoids, saponins, alkaloids, tannins and phlobatannins were confirmed by preliminary phytochemical tests. Methanolic extracts of the leaves of *L. aspera* exhibited highest antibacterial activity against *Staphylococcus aureus* (12.8±0.31 mm), followed by *Bacillus subtilis* (11.4±0.3 mm), *Escherichia coli* (9.8±0.21 mm), and *Pseudomonas aeruginosa* (7.3±0.29 mm). Also methanolic leaf extracts showed effective scavenging activity, (38.39 and 36.85%, respectively, against DPPH and H_2O_2 free radicals with half maximal inhibitory concentration values of 136.17 and 142.42 µg/ml) compared with root extracts.

Molecular phylogeny of Lamiaceae

Phylogenetic classification of Lamiaceae was started with the effort of El-Gazzer and Watson (1970a) by the introduction and popularization of numerical phenetical system of classification to this family.

According to Harley *et al.* (2004) the family Lamiaceae had traditionally been considered closely related to Verbenaceae, but the phylogenetic studies by Cantino *et al*., (1992) and Wagstaff *et al*., (1998) showed that many of the genera yet classified in Lamiaceae belongs to Verbenaceae.

Steane *et al*. (2004) studied the phylogenetic relationships between *Clerodendrum* (Lamiaceae) and other Ajugoid genera inferred from nuclear and chloroplast DNA sequence data. The process of circumscribing taxa has become increasingly analytical with the development of molecular methods for systematic research. The authors suggested that the molecular methods can be used to test the hypothesis objectively when morphology signals the possibility that taxa are closely related. *Clerodendrum* is morphologically similar to *Amasonia, Huxleya, Aegiphila*and *Kalaharia*. Nuclear ribosomal ITS and chloroplast ndhF sequence data were used to clarify the positions of these 4 genera relative to *Clerodendrum.*They showed that Australian monotypic genus Huxleya were evolved from within *Clerodendrum*and accordingly, *Huxleya*and *Clerodendrum* were sinked and make a new combination, *Clerodendrum linifolium*.

Oliveira *et al.* (2007) carried out a study on molecular phylogeny, biogeography and systematics of the genus *Dicerandra*, which is endemic to the South eastern United States. All the nine species of the genus are threatened or endangered and are restricted to sand hill vegetation and a mosaic of scrub habitats. Molecular phylogenetic analysis identified two

strongly supported clades, corresponding to the four annuals and to the five perennials in separate lineages. The nuclear and plastid trees were incompatible in their placement of two perennial taxa, *D. cornutissima* and *D. immaculata* var. *savannarum*, due to ancient hybridization or to lineage sorting. Based on the analyses, the widespread *D. linearifolia* is not monophyletic, with populations of *D. linearifolia*var. *linearifolia* and grouped together into Western or Eastern clades. The western clade, occurs in an area drained by rivers flowing toward the Gulf of Mexico comprising populations of *D. linearifolia* var. *linearifolia* and var. *robustior* whereas the eastern clade occupies a region drained by rivers flowing to the Atlantic Ocean comprising populations of *D. linearifolia* var. *linearifolia*, *D. densiflora*, *D. odoratissima*, and *D. radfordiana*. This pattern of genetic differentiation was reported in the plants for the first time.

Maki *et al.* (2010) examined the phylogenetic relationships between 14 taxa of *Isodon* (Lamiaceae) occurring in Japan and South Korea using sequence variations in 11 chloroplast DNA regions. The resulted phylogenetic tree consists of three main clades comprised of different sections. Complex phylogeny of the taxa may be due to the lineage sorting of chloroplast DNA variations following rapid divergence of the genus.

Li *et al*. (2012) suggested that *Wenchengia,* which was assigned to be a monotypic subfamily should be more appropriate to treat the genus as a member of Scutellarioideae. The phylogenetic position of *Wenchengia alternifolia* were inferred by therediscovery of the species from its type locality, using two plastid DNA regions (*rbcL*, *ndhF*), morphological data, anatomical and cytological characters. The molecular data as well as the combination of the molecular and the morphological data suggested a close relationship of the genus to a clade consisting of *Scutellaria, Holmskioldia* and *Tinnea* representing Scutellarioideae.

Krawczyk *et al.* (2013) reviewed the taxonomic status of *Galeobdolon luteum* from historical and contemporary perspectives. It was showed that *G. luteum* should be included in the genus *Lamium*. The testing of hypothesis was done by a comparative analysis between the representatives of the genera *Galeobdolon* and *Lamium* using four DNA regions like ITS, *accD*, *rpoC1* and *trnH-psbA*.The analysis suggested that *G. luteum* is not genetically distant enough from *Lamium* and integration of *Galeobdolon* and *Lamium* is legitimate.

Molecular phylogeny of the subfamily Lamioideae

The majority of the Lamioideaen genera are endemic to temperate-subtropical Eastern Asia and has centers of diversity in the Old World. Examples include *Ajugoides, Bostrychanthera, Chelonopsis, Comanthosphace, Eriophyton, Matsumurella, Rostrinucula,* and *Suzukia*. Genera like *Gomphostemma, Craniotome, Microtoena, Colebrookea, Colquhounia* and *Roylea* are distributed in tropical Asia. A few genera like *Moluccella* and *Eremostachys* are confined to Central Asia whereas others like *Phlomoides*, *Leonurus* and *Lamium* are distributed across Eurasia. Some genera such as *Acrotome*, *Leonotis* and *Otostegia* are endemic to sub-Saharan Africa and tropical Arabia while in others like *Achyrospermum, Leucas* and *Pogostemon* shows disjunct distributions between tropical Africa and tropical East Asia (Roy and Lindqvist, 2015).

Barber *et al*. (2002) carried out molecular studies on the genus *Sideritis* which comprises approximately 150 species of annuals and perennials and distributed chiefly in the Mediterranean region.*Sideritis* species were divided into two perennial *viz*; *Sideritis*and*Empedoclea* and two annual *viz*; *Hesiodia*and *Burgsdorfia*sections. Independent phylogenies comprising sequence data from both chloroplast and nuclear markers were constrcucted to determine the continental origin of the insular group. They included 25 continental taxa representing all four sections of subgenus *Sideritis* and sevenisland taxa drawn from the Macaronesian subgenus *Marrubiastrum.* The

study proved that the annual sections are not monophyletic in any analysis. All analyses demonstrated *Sideritis cossoniana*, as the closest continental relative of the Macaronesian group. The phylogenies also had an implication that a distinct increase in woodiness among the Macaronesian species relative to their continental congeners, give further support for the secondary nature of woodiness character in island plants.

Scheen and Albert (2009) studied molecular phylogenetics of the genus *Leucas* of subfamily Lamioideae. The genus included more than 100 species distributed in east Africa, Indian subcontinent to Australia. The phylogenetic analysis using three plastid DNA loci, the *trnL-F* region, *trnS-G* spacer, and the *rps16* intron supported Asian species to form a monophyletic group whereas Afro- Arabian species form a paraphyletic group with other genera like *Acrotome, Isoleucas, Leonotis* and *Otostegia.*

Bendiksby *et al.* (2011) presented a taxonomic update of the subfamily Lamioideae, based on earlier published data as well as 71 new DNA extracts from four chloroplast regions such as *rps16, matK*, *trnL* intron and *trnL-F* spacer. By this study 10 out of 13 previously unplaced small or monotypic Asian lamioid genera were phylogenetically placed and additionally 37 lamioid species have been identified and the updation of classification were done accordingly. A new tribe Paraphlomideae Bendiksby was identified to includes*Ajugoides*, *Matsumurella* and *Paraphlomis*. *Acanthoprasium* has been given a genus status. The study proposed to remain 10 genera non-monophyletic (*Ballota, Lagopsis*, *Microtoena*, *Phlomoides*, *Leonotis*, *Leonurus*, *Leucas, Sideritis*, *Stachys* and *Thuspeinanta*). By the transfer of some genera, *Lamium* and *Otostegia* could be made monophyletic. *Eriophyton* and *Stachyopsis* were placed in *Lamieae, Loxocalyx* in Leonureae and *Hypogomphia* in Stachydeae whereas some genera *Betonica*, *Colquhounia*, *Galeopsis*, and *Roylea* were remain unclassified at the tribal level. Three East Asian *Galeobdolon* species and *Lamium chinense* are transferred to *Matsumurella* whereas four *Otostegia* speciesand *Sulaimania*

were transferred to *Moluccella*. Three *Lamium* species along with *Alajja* were transferred to *Eriophyton*. They made 14 new combinations, 13 at species rank and one at the rank of subgenus.

Dundar *et al*. (2013) conducted morphological revision and phylogenetic analysis of *Stachys* L. sect. *Eriostomum* based on nuclear ribosomal internal transcribed spacer (nrITS) sequences. Morphological analysis suggested some status changes to the previous arrangements of subsections and the phylogenetic analysis suggested two subsections, instead of three.The tree obtained clearly displayed monophyly of *Eriostomum*, andnew status of the subgenus *Betonica* (L.) R.Bhattacharjee.

Salmaki *et al.* (2013) conducted a study on molecular phylogeny of tribe Stachydeae of the subfamily Lamioideae. Maximum parsimony and Bayesian phylogenetic analyses of nuclear *ITS* and plastid *trnL* intron, *trnL-trnF* spacer, *rps16* intron were conducted. To identify major evolutionary lineages and to test taxonomic hypotheses within these largest lamioid tribes, bulk amounts of DNA sequence data from a taxonomically and geographically broad sampling of the tribe were performed. This represents both old and new world species and all 12 recognized genera of tribe Stachydeae. Altogether 143 accessions corresponding to 121 species were included. Phylogenetic reconstructions showed that nuclear and plastid data corroborate monophyly of the tribe, with *Melittis* as sister to all remaining Stachydeae. The authors suggested a phylogenetic name Eurystachys to the other well supported clade. And within Eurystachys, the majority of recognized taxa appear to be para or polyphyletic eventhough both nuclear and plastid data for several named and unnamed groups support monophyly. The study suggests that the impact of hybridization in the evolution of Stachydeae may be the cause of plastid-nuclear incongruence.

Salmaki *et al.* (2015) conducted a study on molecular phylogeny of *Stachys persepolitana* and placed the same in genus *Lamium*. The main purpose of this study was to test the hypothesis that *Stachys persepolitana* is irrelevantly placed in *Stachys* tribe Stachydeae of subfamily Lamioideae. Phylogenetic position of *S. persepolitana* were investigated using plastid such as *rps16* intron, *trnL-F* and *matK* region and nuclear (*nrITS*) DNA sequence data with both Maximum parsimony and Bayesian inference approaches. According to the plastid and nuclear datas, *S. persepolitana* should be included in the genus *Lamium* and not in *Stachys*. Morphological characters also strongly support its placement in *Lamium*.

Yao *et al*. (2016) studied the phylogenetic relationships, character evolution and biogeographic diversification of the genus *Pogostemon*. Due to morphological capriciousness and putative convergent evolution within the genus, the infrageneric taxonomy of the genus was found to be problematic. *Pogostemon* is the only genus in Lamiaceae in which some species of the genus are following aquatic life. Phylogenetic analyses were carried out using the nuclear *ITS* and five plastid regions such as *matK*, *trnH-psbA*, *trnL-F*, *rbcL*, *rps16* and firmly confirmed the monophyly of *Pogostemon* and its sister relationship with the genus *Anisomeles*. Genus *Pogostemon* was resolved into two major clades, and it could be clearly seen that none of the three morphologically defined subgenera of *Pogostemon* were supported as monophyletic. A new infrageneric *Pogostemon* classification consisting of two subgenera is proposed. According to biogeographic diversification analyses and molecular dating, *Pogostemon* split from its sister genus in southern and South East Asia in the early Miocene. The findings suggest that from Asia to Africa transoceanic long-distance dispersal of *Pogostemon* happened at least twice, once in the late Miocene and again during the late-Miocene or early-Pliocene.

AREA OF PRESENT STUDY

For the present study specimens were collected from the South Indian states of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana. The study covers an area of 635780 sq. km and bounded by the Bay of Bengal in the east, the Arabian Sea in the west, Indian ocean in the south and Vindhya and Satpura ranges by the north.

The Deccan and the Malabar are the two major floristic regions of South India. The largest plateau in India, the Deccan stretching from mountain ranges of central India viz; Aravallis, the Malva, the Vindhyas, the Satpura and the Chota Nagpur hills in the north and almost right down to Kanyakumari in the South. The Deccan consists of three distinctive physiographic subdivisions. (a) North Deccan Plateau (b) South Deccan Plateau (c) East Deccan Plateau. South Deccan Plateau and East Deccan Plateau fall within the present study area. The South Deccan plateau is drained by the Kaveri River, which rises in the Western Ghats of Karnataka and bends south to break through the Nilgiri Hills at the island town of Shivanasamudra and then falls into Tamil Nadu at Hogenakal falls and finally emptying into the Bay of Bengal. The Eastern Deccan plateau, called Telangana and Rayalaseema, is made of vast sheets of massive granite rock, which effectively traps rainwater. It is very sparsely populated rugged terrain. It is characterized by the tropical deciduous forest and in the open plains it is replaced with thorny bushes and drought resistant species. Having once constituted a segment of the ancient continent of Gondwanaland, this land is the oldest and most stable in India. The Deccan plateau consists of dry tropical forests that experience only seasonal rainfall.

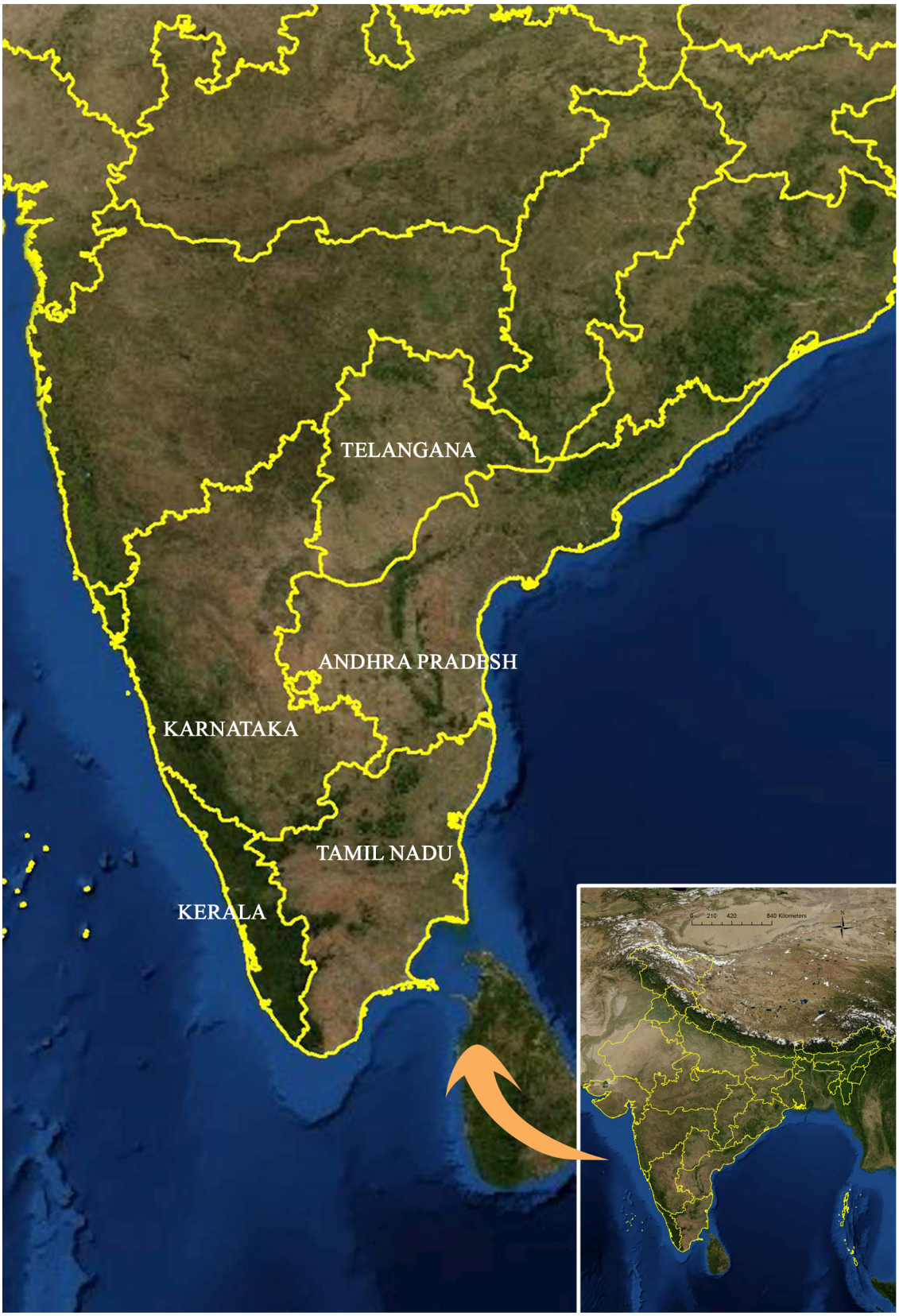


Figure 3.1: Area of study

The Malabar Coast is a long, narrow coastline on the southwestern shore line of the mainland Indian subcontinent. Geographically, it comprises the wettest regions of southern India, as the Western Ghats intercept the moisture-laden monsoon rains, especially on their westward-facing mountain slopes. This coast is over 845 km long and stretches from the coast of southwestern Maharashtra, along the region of Goa, through the entire western coast of Karnataka and Kerala, and up to Kanyakumari. This area is floristically very rich and includes coastal plains and a series of hill ranges of the Western Ghats. It is flanked by the Arabian Sea on the west and the Western Ghats on the east. The southern part of this is referred to as the South Western Ghats moist deciduous forests.

South India mainly encompasses two mountain regions Western Ghats and Eastern Ghats. Western Ghats also known as Sahyadri, are a mountain range that covers and area of 140000 square kilometres in a stretch of 1600 kilometre running parallel to the Western coast of Indian peninsula travels in the states of Kerala, Tamil Nadu, Karnataka, Goa, Maharashtra and Gujarat. It is regarded as one among eight "hottest hotspots" of biological diversity in the world. The range runs north to south along the Western border of Deccan Plateau and separate the plateau from narrow Coastal Plains called Konkan overlooking the Arabian Sea. Western Ghats blocks southwest monsoon winds from reaching the Deccan Plateau (Vijayan, 2013). The average elevation is around 1200 m. It support the most luxurious growth of tropical wet evergreen, semi evergreen moist deciduous forest, by the virtue of its geographical location topography and rainfall from south west and north east monsoon.

Eastern Ghats run along the eastern border of Deccan Plateau extends over a length of 1750 km width of 200 km in the north and 100 km in the south. Eastern Ghats run from the northern Odisha through Andhra Pradesh to

Tamil Nadu in the south passing some parts of Karnataka and in Wayanad district of Kerala.The Nilgiri Hills marks the Southern boundary of Eastern Ghats whereas the Mahanati basin marks the northern boundary of Eastern Ghats.The Eastern Ghats are discontinuous range of mountains and the rivers Godavari and Krishna cuts across it. The climate and vegetation of these areas are often spoken as of temperate type.

Phytogeographic divisions

Eleven phytogeographic zones were recognizes in India (Balakrishnan, 1996). They are, (1) North-west Himalayas, (2) Indo Gangetic Plains, (3) Eastern Himalayas; Sikkim and Arunachal Pradesh, (4) North eastern India and North Bengal, (5) Central India, (6) Arid Zone (7) Northern Western Ghats and Northern West Coast, (8) South Western Ghats, West Coast and Lakshadweep, (9) Deccan, (10) Eastern Ghats Coromandel Coast and (11) Andaman and Nicobar Islands.

Rain

South-west monsoon and the north-east monsoon are the two rain bearing winds in South India. In the states of Kerala and Karnataka, south-west monsoon is dominant. The north-east monsoon is comparatively weaker and comes in wake of south-west monsoon. There are intermittent pre-monsoon showers in April-May and afterwards the south-west monsoon open in full force. In the distribution of rain in South India, Western Ghats plays a crucial role.

In the beginning of June, the south-west monsoon begins on the west coast of South India. The rain fall is arbitrary, uncertain and it varies from place to place and year to year. Andhra Pradesh, Tamil Nadu and Telengana receives a lesser rain fall (100-200 cm) whereas West coast of South India receives the heaviest rainfall (more than 220 cm).

Forests

The forest of South India include tropical and moist deciduous forests in the Southern Karnataka and Kerala; tropical thorn forest in Deccan Plateau; tropical, wet and semi evergreen forest in Western Ghats and montane and wet temperate forest in higher parts of Tamil Nadu and Kerala. According to the exposure to wind, soil and climatic conditions, the southern tropical wet evergreen forests were subdivided into two groups; the southern hilltop tropical evergreen forests and west coast tropical evergreen forest.

Soil

Soils are formed from the withering of rocks due to different catastrophic events occurred on the crust of earth. It is a mixture of minerals and organic matter. Geographically Indian soils are categorized into three major groups

(i) Mature soils of Peninsular India (Red, Black and Lateritic soils)

(ii) Alluvial soils of Indo-Gangetic Plains

(iii) Scanty soils of Himalayas

The major types of soil met in South India are;

a) Red soil: It is mainly found in Karnataka, Andhra Pradesh and Telengana. In valleys and plains, it is dark and fertile. It is made up of silica and aluminium with free quarts and sand. They are characterized by the absence of lime nodules. They are less in bases, humus and nutrients.

b) Black soil: It is mainly found in Tamil Nadu, parts of Karnataka and Andhra Pradesh. The presence of double hydrated ferrous and aluminiun silicates makes the black color of this soil. The soil contains a high proportion of calcium and magnesium carbonates and are clayey

or loamy, fine grained and argillaceous. Black soil is poor in phosphorous and rich in organic matter and nitrogen.

c) Lateritic soil: They occur as reddish or yellowish red and becomes black on exposure to sunlight. This kind of soil is mainly occurring in Tamil Nadu, and in Eastern and Western Ghats. The hydrated oxides of aluminium and iron with minor quantities of manganese and titanium oxides are the major constitution of this soil. They are characterized by rich humus content. The soil is much fertile and contains a good quantity of organic matter. At higher altitudes in Kerala, the lateritic soil supports plantation of crops.

d) Aluvial soil: This is the most productive type of soil in India, also present in the fringes of Peninsular India. It constitutes a high proportion of clay, and are more sticky and the texture may vary from sandy loam to clay. Its color varies from grey to reddish brown. The quantity of potassium is higher in this soil, whereas nitrogen is deficient.

Climate

The region has a tropical climate and depends on monsoons for rainfall. The homoclimatic region of India is classified into five categories on the basis of the ratio of the rainfall to evapotranspiration; arid region, semi-arid region, sub-humid region, humid regime and super-humid regime (Chowdhury and Sarwade., 1982). The coastal districts of Andhra Pradesh, Telengana, interior areas of Karnataka and some districts in Tamil Nadu come under semi-arid climate. Northern coastal Telengana, southern districts of Karnataka and north Tamil Nadu have dry sub-humid climate whereas coastal Karnataka and northern parts of Kerala have moist sub-humid climate. Southern districts of Kerala and high altitude locations of Tamil Nadu predominate in humid regime. Super humid regime occurs in the smallest area of Tamil Nadu, Kodaikanal.

MATERIALS AND METHODS

The present work included 45 taxa from the subfamily Lamioideae, giving more importance to the genus *Leucas* and representations from other genus like *Pogostemon, Anisomeles, Leonotis, Gomphostemma* and *Colebrookea.* Collection of plant samples were purely based on availability criteria, since a bulk amount of leaf samples was needed for the essential oil extraction. Most of the narrow endemic species were also not included because of its low population size. Thorough field studies were conducted and a total of 30 taxa, which includes 20 species of *Leucas*, 5 species of *Pogostemon*, 2 species of *Anisomeles*, 1 species of each *Leonotis*, *Gomphostemma* and *Colebrookea* were collected from different parts of South India. Leaves were collected from three different accessions or sometimes from adjacent populations for extraction. Experimentations were repeated at least three times for minimizing errors. Occurrence of species was identified primarily from earlier works of Singh (2001), Sunojkumar (2005), Vimal (2017) and Shinoj (2019). Information obtained from herbarium specimens was also very useful for field collection. Details regarding materials and methods adopted are given below;

MATERIALS

One important aspect of the work was phytochemical studies of the subfamily Lamioideae (Lamiaceae) in South India. Apart from this, molecular phylogeny of the subfamily been studied to know whether there is any relation between chemical systematics and molecular systematics. Lamiaceaen members are rich source of volatiles and subfamily Lamioideae is an oil-poor subfamily, when compared to subfamily Nepetoideae. Due to poor quantity of oils, bulk quantity of leaves was needed for the extraction of essential oils. Since it requires only lesser quantity of leaves, for fatty acid

profiling we could add more taxa. These were also done in triplicates. All phytogeographical regions of South India were visited for the specimen collection. Altogether 45 taxa were included in the overall study and the details of the specimens collected are given in table 4.1. Details such as taxa name, locality of collection, collection number and GPS reading are mentioned. The first 30 taxa mentioned in the table were taken into consideration for volatile profiling whereas all the 45 taxa studied for fatty acid profiling. Altogether 48 taxa were taken for molecular studies (including outgroup).

Field collection trips were conducted during all flowering season throughout the period. Quantity ranging from 200 g to 500 g of fresh leaves was taken from a single accession for the extraction of essential oil. Freshly plucked leaves were used for fatty acid profiling to avoid degradation of fatty acids. Liquid Nitrogen cylinder was carried to the field and the plucked leaves were quickly transferd to the Liquid Nitrogen kept in the cylinder after proper labelling. This will help the leaf to prevent the degradation of leaf fatty acids. Two to four leaves were preserved in Liquid Nitrogen. Tender leaves were handpicked and kept in plastic zip bags with silica gel for DNA extraction and further molecular studies. Proper labeling was done on each collection bag as well as on zip bags. The labeling include collection locality, collection number, date of collection and name of species collected.

Table 4.1: List of plants collected, Voucher specimen information and locality

Sl No	Name of Taxa	Locality	Collection No.	GPS Reading
1	*Anisomeles heyneana* Benth.	Kodachadri hills	CU No.148844	11º 8’ 2” N 75º 53’ 18” E
2	*Anisomeles malabarica* (L.)R. Br.ex Sins	Tirupathi, Andra Pradesh	CU No.148818	13º 37’ 44” N 79º 25’ 28” E
3	*Colebrookea oppositifolia* Sm.	Sholayar forest	CU No.148842	10º 18’ 33” N 76º 23’ 47” E
4	*Gomphostemma heyneanum* Wall. ex Benth. var. *heyneana*	Sholayar forest	CU No.148825	10º 18’ 33” N 76º 23’ 47” E
5	*Leonotis nepetifolia* (L.)R. Br.	Srikalahasti, Andra Pradesh	CU No.148848	13º 45’ 9” N 79º 42’ 13” E
6	*Leucas angularis* Benth	Kodagu, Karnataka	CU No.148822	11º52’53” N 76º 02’ 30” E
7	*Leucas aspera* (Willd.) Link	Thirunelveli, Tamil Nadu	CU No.148868	8º 25’ 33” N 77º 39’ 35” E
8	*Leucas biflora* (Vabl) Sm.	Elathur, Calicut	CU No.148874	11º 15’ 31” N 75º 46’ 49” E
9	*Leucas ciliata* Benth.	Kurishumala, Vagamon	CU No.148849	9º 50’ 45” N 76º 57’ 52” E
10	*Leucas eriostoma* Hook.f.	Thalacaury, Karnataka	CU No.148819	12º 28’ 0” N 75º 50’ 0” E
11	*Leucas helianthimifolia* Desf.	Royal Valley Estate, The Nilgiris	CU No.148837	11º 29’30” N 76º 31’10” E
12	*Leucas hirta* (B.Heyne ex Roth) Spreng.	Kalvari mount, Idukki	CU No.148812	9º 50’ 45” N 76º 57’52” E
13	*Leucas lanata* var. *candida* Haines	Marappalam, Berliar road, The Nilgiris	CU No.148815	11º 33’98” N 76.84’36” E

14	*Leucas lanceaefolia* Desf.	Doddapetta, Ooty	CU No.148814	11º 24’ 43” N 76º 44’ 2” E
15	*Leucas lavandulifolia* Sm.	Kaduvakkuzhi, Wayanad	CU No.148808	10º 14’ 49” N 77º 10’ 40” E
16	*Leucas lavandulifolia* var. *nagalapuramiana* Chandrab. & S.R. Sriniv.	Srikalahasti, Andra Pradesh	CU No.148817	13º 45’ 9” N 79º 42’ 13” E
17	*Leucas marrubioides* var. *puleneynsis* Hook.f.	Nadukani, The Nilgiris	CU No.148852	11º 30 ‘ 40” N 76º 19’ 58” E
18	*Leucas martinicensis* (Jacq.) R.Br.	Marayur, Idukki	CU No.148859	10º 14’ 49” N 77º 10’ 40” E
19	*Leucas montana* (Roth) Spreng.	Talakona waterfalls, Andra Pradesh	CU No.148821	13º 63’ 48” N 79º. 42’ 59” E
20	*Leucas prostrata* (Hook.f.) Gamble	Naduvattom, The Nilgiris	CU No.148813	11º 28’ 12” N 76º 33’ 38” E
21	*Leucas rosmarinifolia* Benth	Coonoor, Ooty, The Nilgiris	CU No.148809	11º 22’ 57” N 76º 44’ 25” E
22	*Leucas stelligera* Wall.ex Benth.	Valur, Kodachadri hills	CU No.148870	13º 53’ 81” N 74º 51’ 368” E
23	*Leucas stricta* Benth.	Srikalahasti, Andra Pradesh	CU No.148875	13º 45’ 9” N 79º 42’ 13” E
24	*Leucas urticifolia* (Vahl) Sm.	Thirunelveli, Tamil Nadu	CU No.148893	8º 25’ 33” N 77º 39’ 35” E
25	*Leucas wightiana* Wall.ex Benth.	Thirunelveli, Tamil Nadu	CU No.148896	8º 25’ 33” N 77º 39’ 35” E
26	*Pogostemon benghalensis* (Burm.f.) Kuntze	Vagamon meadow	CU No.148801	9º 68’ 61” N 76º 90’ 52” E
27	*Pogostemon mollis* Benth.	Chembra peak, Wayanad	CU No.148823	11º 54’ 66” N 76º 08’ 38” E

28	*Pogostemon quadrifolius* (Benth.) F. Muell.	Calicut University Campus	CU No.148891	11º 8’ 2” N 75º 53’ 18” E
29	*Pogostemon speciosus* Benth.	Doddapetta, Ooty	CU No.148888	11º 24’ 44” N 76º 43’ 53” E
30	*Pogostemon wightii* Benth.	Royal Valley Estate, The Nilgiris	CU No.148855	11º 28’ 35” N 76º 32’ 46” E
31	*Anisomeles indica* (L.) Kuntze	Mathilakam, Kodungallur	CU No.148871	10º 22’ 0” N 76º 22’ 0” E
32	*Gomphostemma heyneanum* Wall. ex Benth. var. *rotleri*	Vellanippacha, Trissur	CU No.148851	10º 31’ 49” N 76º 12’ 53” E
33	*Gomphostemma keralensis* Vivek., Gopalan & R.Ansari	Periya, Wayanad	CU No.148857	11º 83’ 26” N 75º 85’ 33” E
34	*Leucas beddomi* (Hook.f.) Sunojk. & P.Mathew	Chembra peak, Wayanad	CU No.148886	11º 32’ 14” N 76º 04’ 97” E
35	*Leucas chinensis* f. *riukiuensis* (Ohwi) T.Yamaz.	Nelliyampathy, Palakkad	CU No.148889	10º 32’ 56” N 76º 71’ 45” E
36	*Leucas eriostoma* var. *lanata* Hook.f.	Mullayanagiri	CU No.148810	13º 23’ 26” N 75º 43’ 18” E
37	*Leucas helianthimifolia* Desf.	Royal Valley Estate, The Nilgiris	CU No.148853	11º 28’ 35” N 76º 32’ 46” E
38	*Leucas lavandulifolia* var. *decipiens* (Hook.f.) Chandrab. & S.R.Sriniv.	Paikkara lake, Ooty	CU No.148816	11º 40’ 51” N 76º 72’ 51” E
39	*Leucas nepetifolia* Benth.	Maruthwamala, kanyakumari	CU No.148860	08º 13’ 11” N 77º 50’ 49” E
40	*Leucas sebldiana* Sunojk.	Chembra peak, Wayanad	CU No.148866	11º 54’ 66” N 76º 08’ 38” E
41	*Leucas sivadasaniana* Sunojk.	Kodachadri hills	CU No.148807	13º 51’ 39” N 74º 87’ 47” E

42	*Leucas zeylanica* (L.) W.T.Aiton	Calicut University Campus	CU No.148824	11º 8’ 2” N 75º 53’ 18” E
43	*Pogostemon heyneanus* Benth.	Calicut University Campus, Botanical Garden	CU No.148872	11º 8’ 2” N 75º 53’ 18” E
44	*Pogostemon myosuroides* (Roth) Kuntze	Srikalahasti, Andhra Pradesh	CU No.148856	13º 45’ 9” N 79º 42’ 13” E
45	*Pogostemon paniculatus* (Wild.) Benth.	Calicut University campus	CU No.148878	11º 8’ 2” N 75º 53’ 18” E

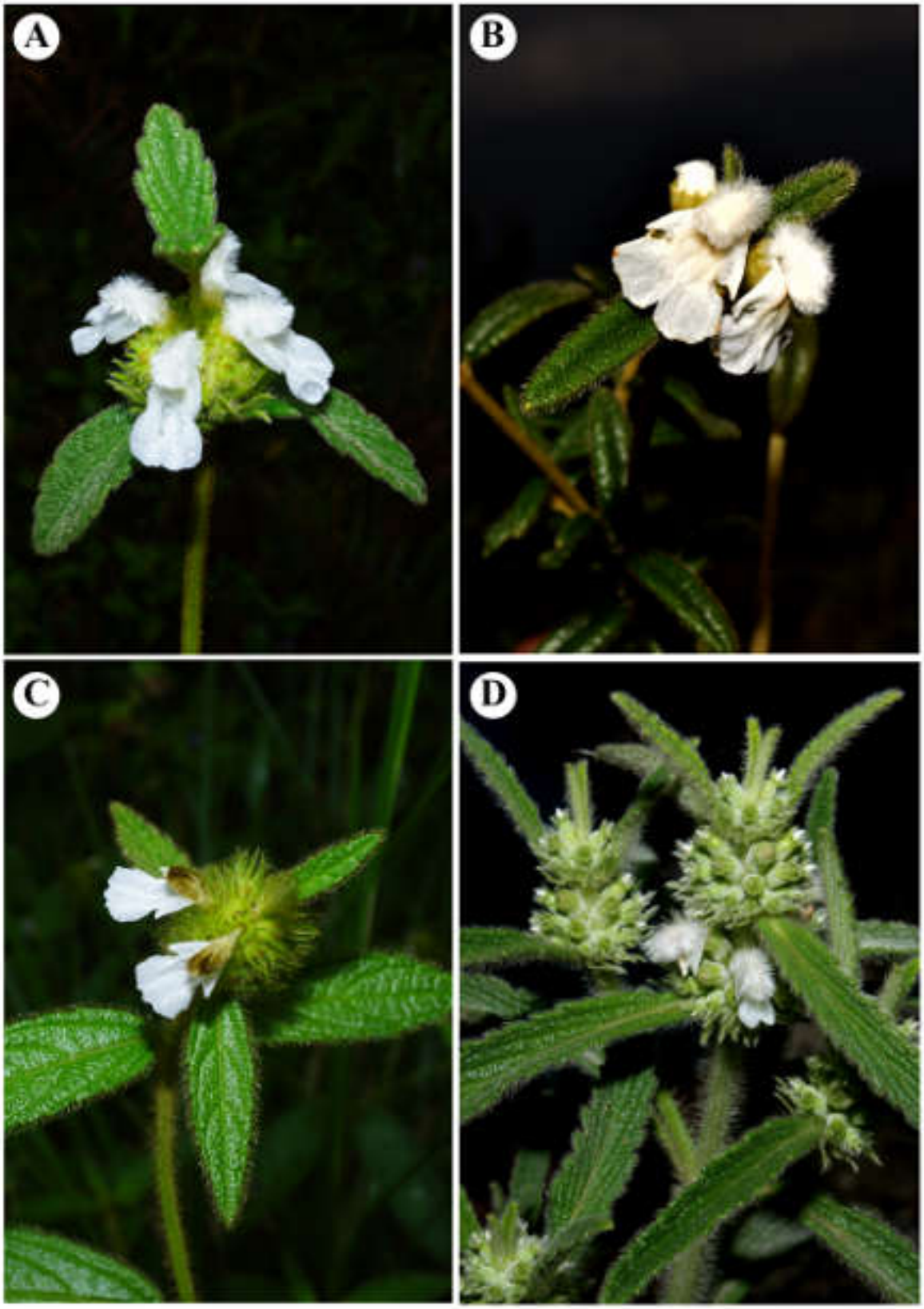


Plate. 1: ***Leucas*** **Sec.** ***Astrodon*****: A.** ***Leucas hirta.*****; B.** ***Leucas helianthimifolia.*****; C.** ***Leucas ciliata.*****; D.** ***Leucas eriostoma***

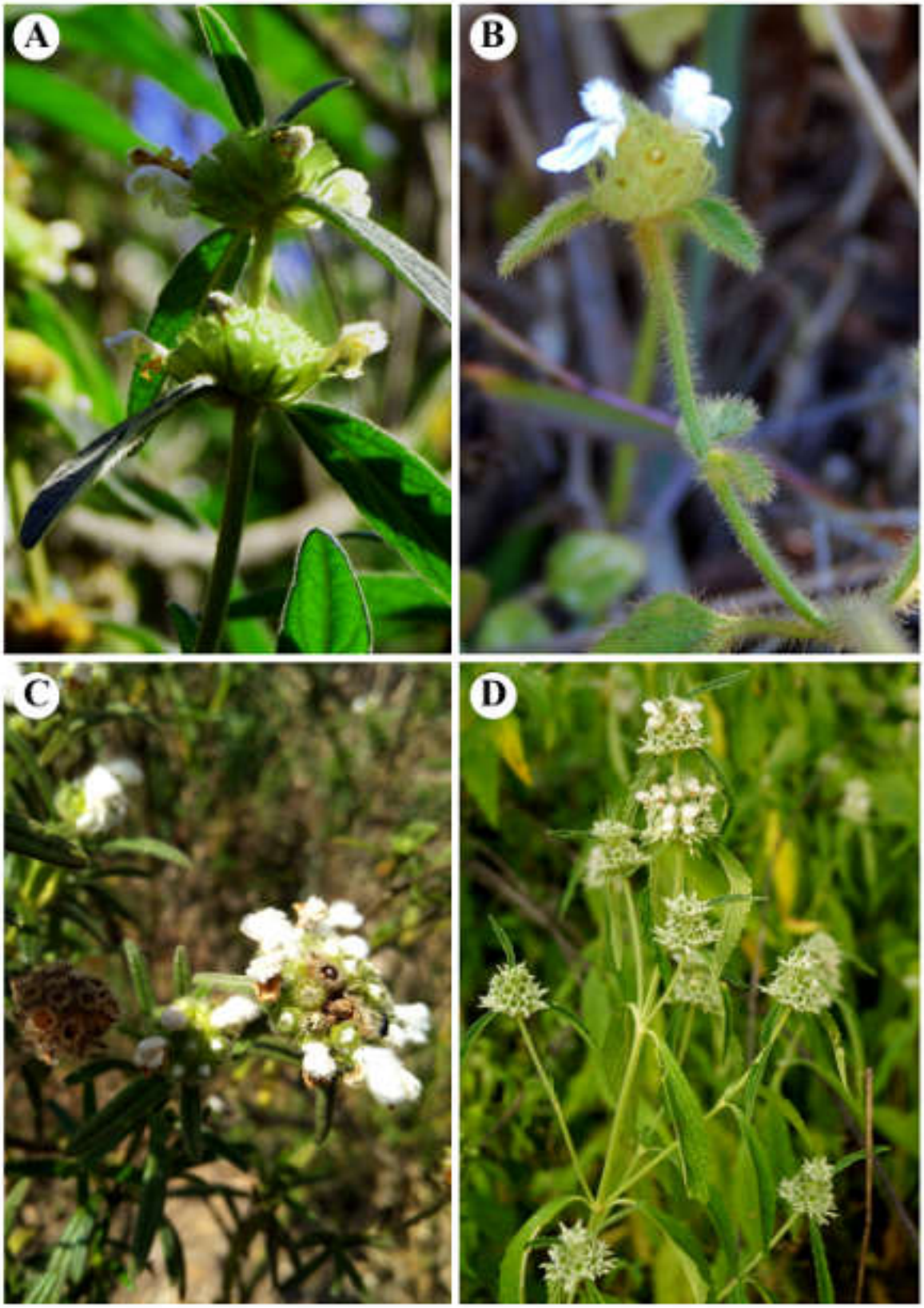


Plate. 2: ***Leucas* Sec. *Astrodon*: A. *Leucas lanceaefolia.*; B. *Leucas prostrata.*; C. *Leucas rosmarinifolia.*; D. *Leucas stelligera***

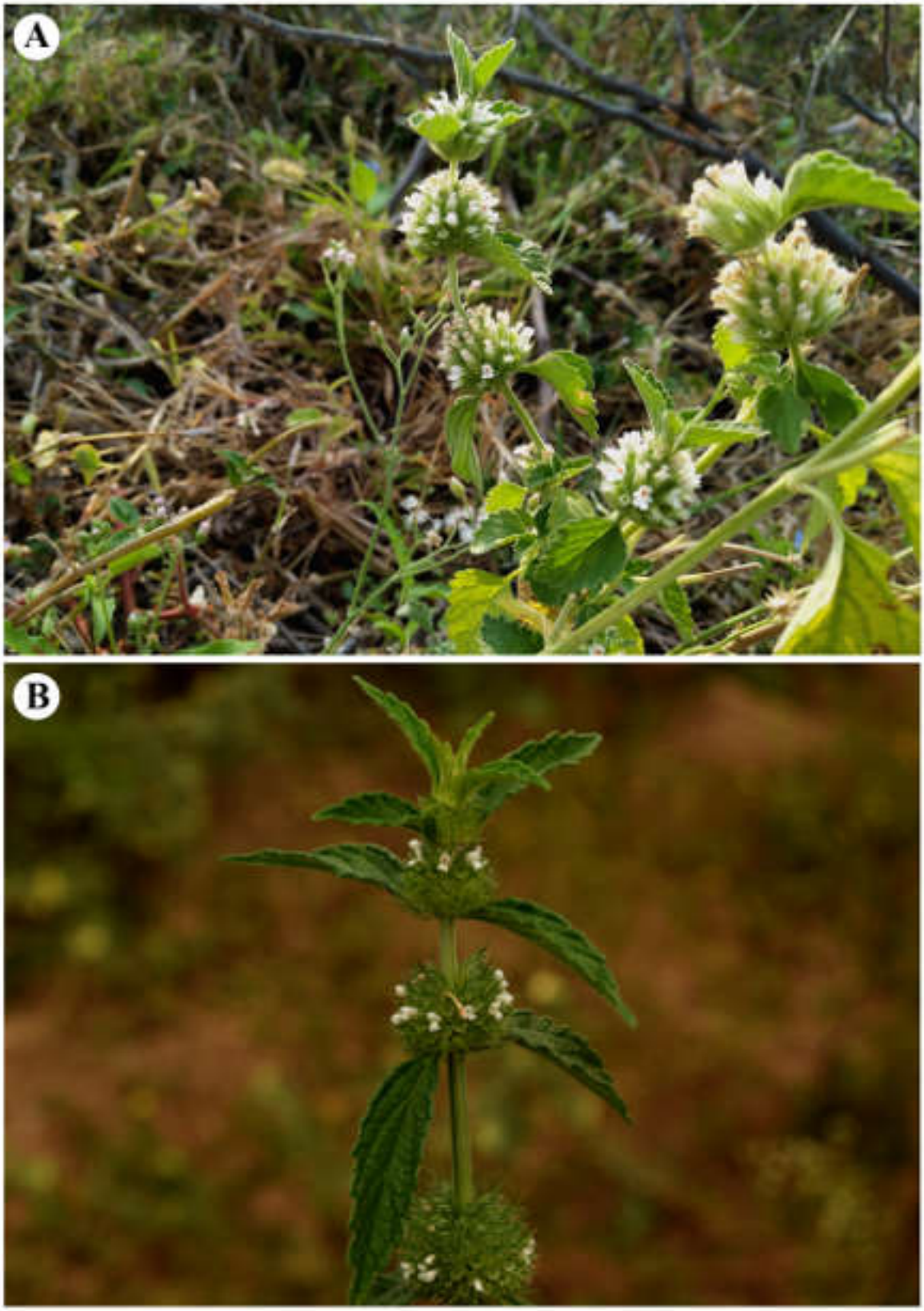


Plate. 3: ***Leucas*** **Sec.** ***Hemistoma*****: A.** ***Leucas urticifolia.*****; B.** ***Leucas martinisencis***

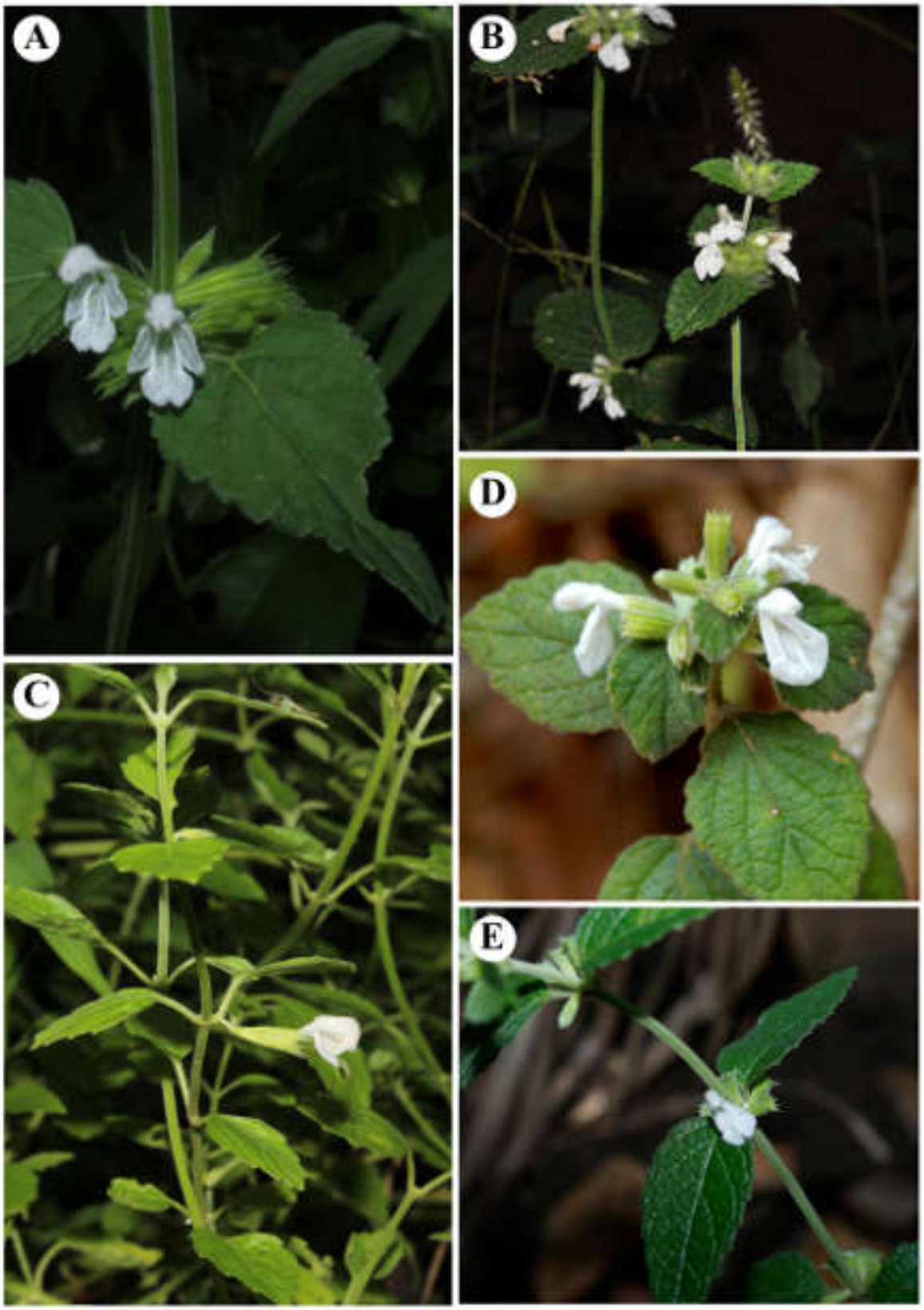


Plate. 4: ***Leucas*** **Sec.** ***Ortholeucas*****: A.** ***Leucas angularis*****.; B.** ***Leucas marrubioides*** **var.** ***pulneyensis*** **.; C.** ***Leucas biflora*****.; D.** ***Leucas lanata*** **var.** ***candida*** **.; E.** ***Leucas montana***

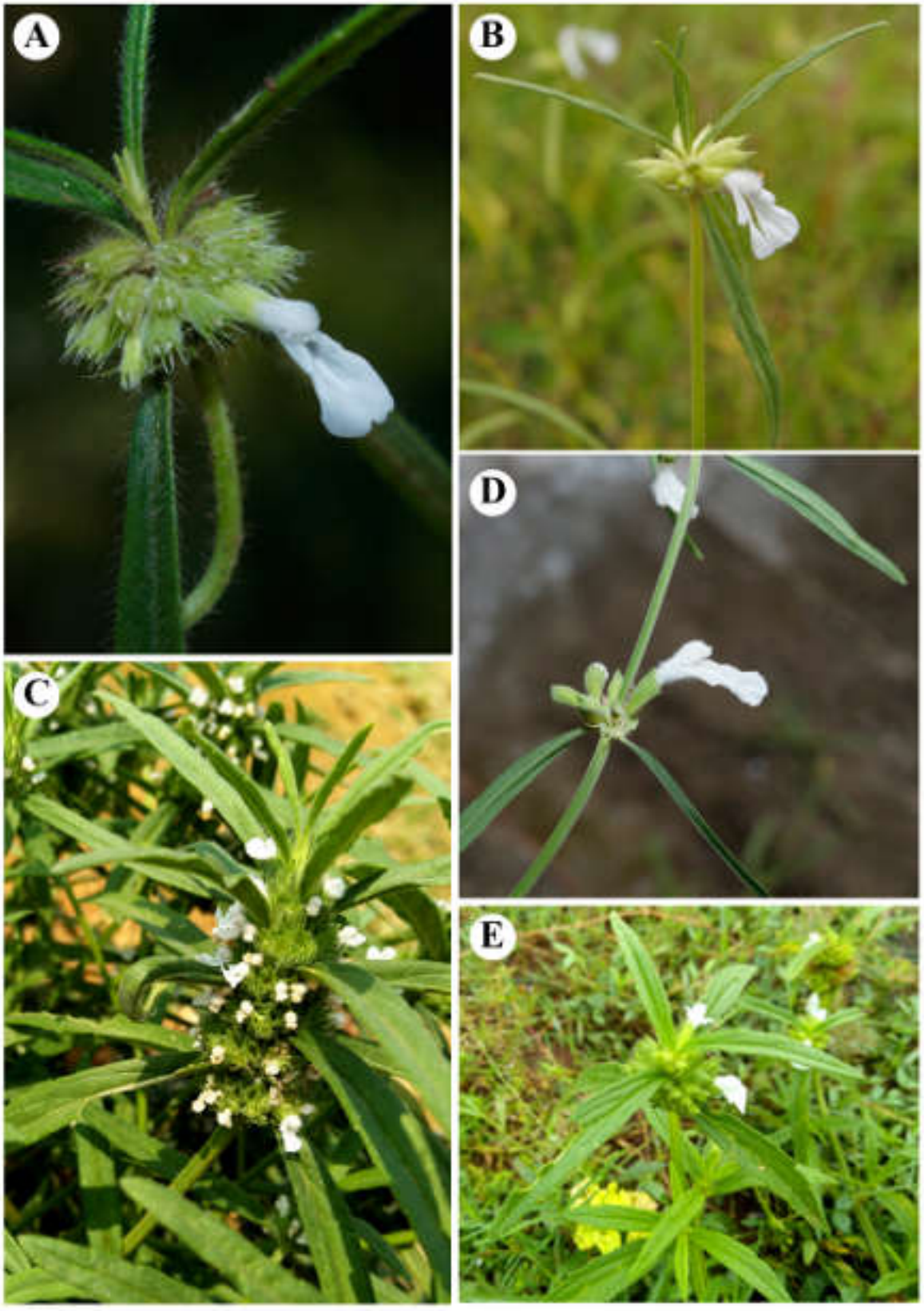


Plate. 5: ***Leucas*** **Sec.** ***Plagiostoma*****: A.** ***Leucas stricta.*****; B.** ***Leucas lavandulifolia.*****; C.** ***Leucas wightiana.*****; D.** ***Leucas lavandulifolia*** **var.** ***nagalapuramiana.*****; E.** ***Leucas aspera***

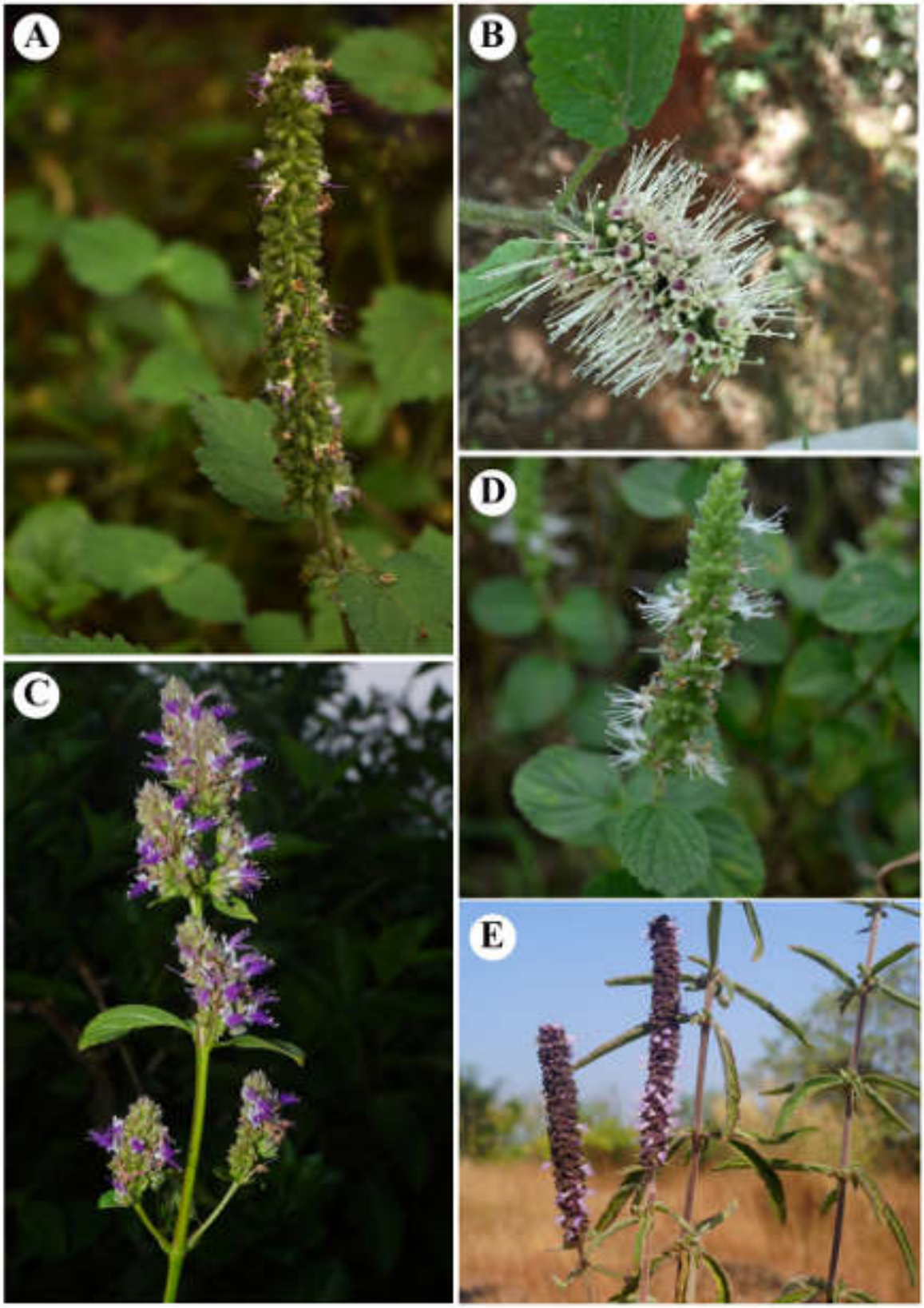


Plate. 6: A. *Pogostemon wightii*.; B. *Pogostemon speciosus*.; C. *Pogostemon benghalensis*.; D. *Pogostemon mollis*.; E. *Pogostemon quadrifolius*

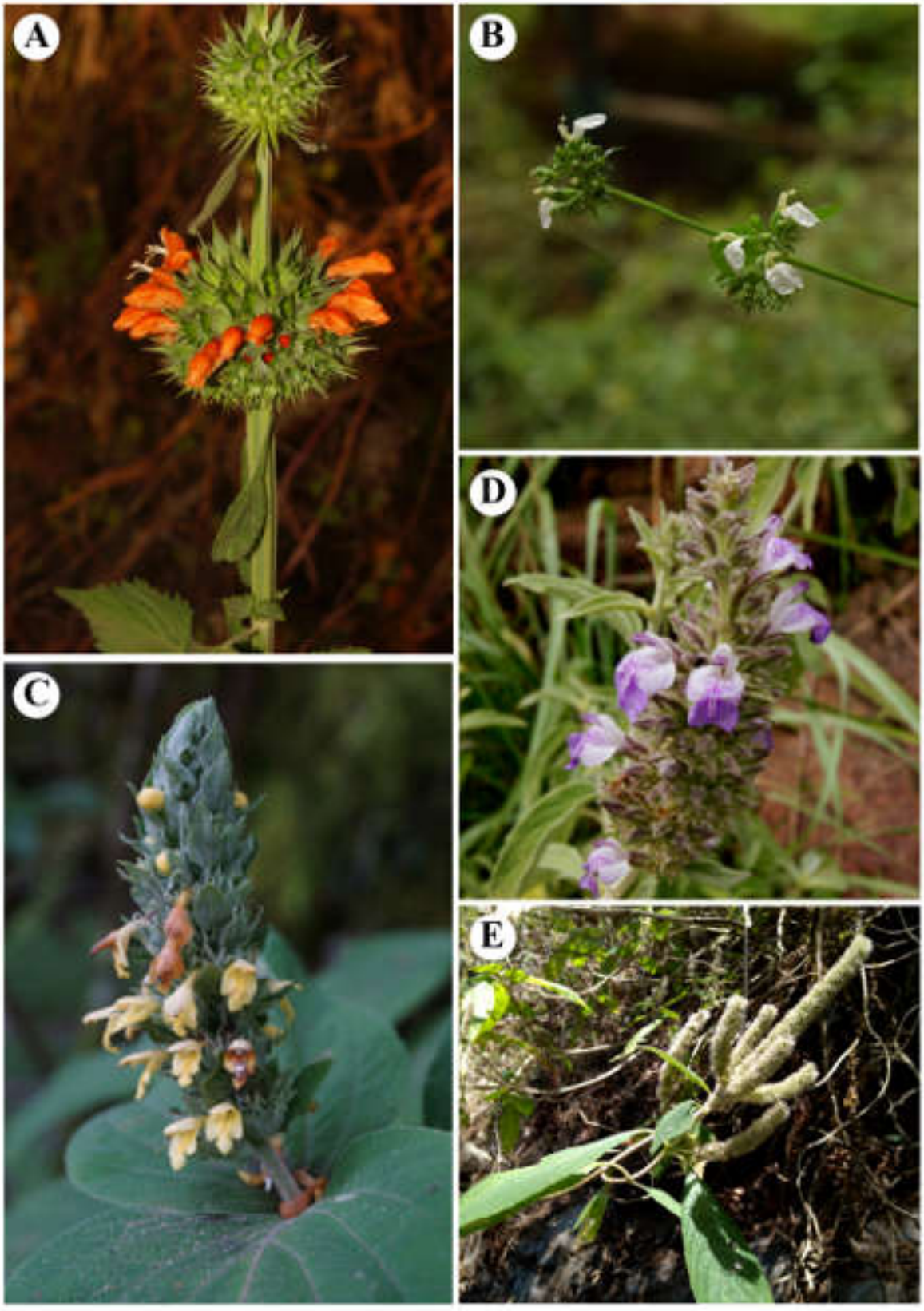


Plate. 7: A. *Leonotis nepetifolia*.; B. *Anisomeles heyneana*.; C. *Gomphostemma heyneanum* var. *heyneana*.; D. *Anisomeles malabarica*.; E. *Colebrookea oppositifolia*

METHODS

4.1 VOLATILE PROFILING OF SELECTED TAXA

4.1. A. Preparation of samples

The oil from fresh leaves of plant was obtained by hydrodistillation for 3 hours, using a Clevenger-type apparatus according to the method recommended in the Pharmacopoeia (European Pharmacopoeia, 2004). The oil was dissolved in GC graded n-hexane since the quantity of essential oil obtained is very less and was kept at +4 ºC till analysis.

4.1. B. GC and GC-MS Conditions

GC-MS: The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

GC*:* The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 5.1.

Identification of the components: Identification of the essential oil components were carried out by comparison of their relative retention times

with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial data bases (Wiley GC/MS Library, MassFinder Software 4.0) (McLafferty and Stauffer, 1989; Hochmuth, 2008) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils were also carried out.

4.1. C. Cluster Analysis

Dendrogram using chemical data were constructed using the software PAUP 4b10 version (Swofford, 2003). The statistical analysis was performed to study the relationship within the members of the subfamily Lamioideae on the basis of their leaf essential oil composition. Unweighted Pair Group Method with Arithmetic mean (UPGMA) with distance matrix method was used for the hierarchical clustering analysis.

4.2 FATTY ACID PROFILING

4.2. A. Chemicals and reagents

Analytical grade chemicals and GC grade solvents were used for all analyses. The chemicals and glass/plastic ware used were, sodium chloride (Merck), methanol (Sigma), hexane (Sigma), conical glass tube (Pyrex), teflon coated cap (Pyrex), glass pasteur pipette (Fisher scientific) and nitrogen gas.

4.2. B. Fatty acid methyl ester analyses

Fatty acid methyl esters were prepared using method reported by Christie (1993) with minor modifications. 1 to 2 leaves were transferred to teflon lined screw capped glass tube to which 1 ml of 2% (w/v) HCl in methanol was added and the solution was incubated at 80°C for 1 hour. FAMEs were extracted twice by adding 1 ml of 0.9% NaCl and 2 ml of

hexane, vortexing for 40 s and centrifuged for phase separation. The collected hexane layer was dried under stream of nitrogen gas and resuspended in 100 µl of hexane for GC-MS analysis.

4.2. C. Gas Chromatography and Mass Spectrometry

The fatty acid methyl esters were analyzed using Agilent Technologies gas chromatography-mass spectrometer (5977A MSD coupled with 7890B GC series) equipped with a DB-wax capillary column (30 m × 0.25 mm × 0.25 mm). The inlet pressure of carrier gas (Helium- 99.9%) was kept at 20.90 Psi with 1.8 ml/min of column flow rate and oven temperature of 150ºC. The column temperature program was 50°C for 3 min, followed by a 25ºC/min ramp to 230°C then hold at 23°C for 18 min was used. The injection volume of the sample was kept at 1 µl with split ratio of 20:1. FAMEs were identified through comparison of mass spectral data to NIST library by examination of spectral data.

4.2. D. Statistical Analysis

The statistical analysis was performed to study the relationship among the members of the subfamily Lamioideae on the basis of their leaf fatty acid composition. Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) were conducted with Euclidean distance similarity index of fatty acid composition using XLSTAT software. PCA is a multivariate statistical technique that generates new sets of orthogonal axes (or variable) known as principal components from original data set. The eigenvalues and coefficient loading matrices were also obtained from PCA.

4.3 MOLECULAR PHYLOGENY

4.3. A. DNA extraction

In order to extract DNA, silica dried leaves collected during field investigation were used following the procedure of Doyle and Doyle (1987)

with slight modifications. Extractions were carried out using DNAeasy plant mini kit (Qiagen, Hilden, Germany) for getting better result and sufficient amount of DNA from the specimen according to manufacturer's instructions.

Isolation and purification of DNA from plant specimen were made quicker and easier by using the DNAeasy plant mini kits. Approximately 25 mg of lyophilized tissue and 100 mg of tissue material were used for processing. Purification of the isolated DNA involves only a less amount of time and requires no chloroform extraction or phenol or alcohol precipitation. Thus DNAeasy plant mini kit is highly suitable for simultaneous processing of multiple samples. Purified DNA is eluted in low salt buffer or water and thus ready for use in other various applications.The size of the DNA isolated is up to 40 kb with fragments of 20-25 kb predominating. The removal of all inhibitors of PCR and other enzymatic reactions were ensured by DNAeasy membrane. This DNA is highly suitable for use in all downstream applications including sequencing.

Table 4.2:- The composition of buffers are shown below

2 X CTAB Buffer	(Per 200 ml)
100 mM Tris-HCl (pH 8.0)	20 ml 1M Tris- HCl pH 8.0
1.4 M NaCl	56 ml 5 M NaCl (or 16.4 g)
20 mM EDTA	16 ml 0.5 M EDTA
2% CTAB	4.0 g powder
2% PVP40	4.0 g
0.2% β-mercaptoethanol	Add right before use

The pH was adjusted to 5 using HCl and made upto 200 ml using distilled water.

The mortars and pestles were chilled in -20ºC freezer overnight before starting the extraction. The 2 X CTAB solutions was incubated and the water

bath was set to a temperature of 65ºC. β-mercaptoethanol buffer was added to CTAB Buffer, right before use. (10 ml pre- 2X CTAB + 20 µl β-mercaptoethanol per sample).

4.3. A.1. Standard procedure for DNA isolation using DNeasy plant mini kit

1. Disrupt samples (</= 100 mg wet weight or </=20 mg lyophilized tissue) using a mortar and pestle.

2. Added 400 µl Buffer AP1 and 400 µl RNase A. Vortex and incubated for 10 min at 65ºC. Inverted the tubes 2-3 times during incubation. Do not mix buffer AP1 and RNase A before use.

3. Added 130 µl Buffer P3. Mixed and incubated for 5 min on ice,

4. Centrifuged the lysate for 5 min at 20,000 × g (14000 rpm).

5. Pipette the lysate into a QIshredder spin coloumn placed in a 2 ml collection tube. Centrifuged for 2 min at 20,000 × g. (remaining cell debris and salt precipitates were removed by centrifugation through a QIAshredder spin coloumn. The preparation of a cleared lysate was essential to prevent clogging of the DNeasy spin column in the following step).

6. Transferred the flow-through into a new tube without disturbing the pellets. Added 1.5 volumes of Buffer AW1, and mixed by pipetting.

7. Transferred 650 µl of the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuged for 1 min at ≥ 6000 × g (≥8000 rpm).

8. Placed the spin column into a new 2 ml collection tube. Added 500 µl Buffer AW2, and centrifuged for 1 min at ≥6000 × g. discarded the flow-through.

9. Added another 500 µl Buffer AW2. Centrifuged for 2 minutes at 20,000 × g. Removed the spin column from the collection tube carefully so that the column does not come into contact with the flow-through.

10. Transferred the spin column to a new 1.5 ml or 2ml microcentrifuge tube.

11. Added 100 µl buffer AE for elution. Incubated for 5 minutes at room temperature (15-25°C). Centrifuged for 1 minute at ≥ 6000 × g.

12. Repeat the step 11.

The DNA was visualized and quantified on 1% agarose gel using standard commercial DNA ladders using a transilluminator.

4.3. B. PCR amplification, agarose gel electrophoresis and sequencing

The *trnL-trnF* intergenic spacer were amplified using universal primers of Taberlet *et. al.* (1991) either as one fragment (hereafter referred to as the *trnL-F* region) using primers c and f or as two separate fragments using primers c and d and e and f respectively. The *rps16* intron was amplified using the primers *rpsF* and *rpsF2R* (Oxelman *et al*., 1997).

4.3. B.1. Primer dilution

The *trnL-F* forward lyophilized primer of 29.30 nm concentration was diluted with 293 microlitre of Milli- Q water. In the same way 27 nm of *trnL-F* reverse primer added with 270 microlitre of Mill- Q water, *rps16* forward primer 31.6 nm of *rpsF* with 316 microlitre and *rpsF2R* of 29.80 nm

with 298 microlitre of Milli- Q water. Overnight incubation was done for the diluted primers. Water was added to each tube in order to convert the primer concentration to 100 µm.

After overnight incubation take 1 µl from each primer tube and make up to 10 µl as working stock (1:10, primer:milli-Q water). This is to be done in order to dilute the concentration to 10 µm solution. This is the working stock.

Table 4.3:- Primers for amplification of cpDNA regions.

	Region	Primer used	Reference
1	*trnL-trnF* intergenic spacers	Forward (Name:-e) GGTTCAAGTCCC TCTATCCC	Taberlet *et al.* (1991)
		Reverse (Name:-f) ATTTGAACTGGTGACACGAG	
2	*rps16* intron	Forward GTGGTAGAAAGCAACGTGCGACTT	Oxelman *et al*. (1997)
		Reverse TCGGGAT GAACATCAATTGCAAC	

Polymerase Chain Reactions (PCR) were done in 25 µL using GT PCR Master Mix (TAKARA BIO INC, Seta 3-4-1, Otsu, Shiga 520-2193, Japan), 1µM of each primer, and 1 µL unquantified genomic DNA. The DNA quantifications were performed using Master Cycler (Nexus gradient, Eppendorf, Germany) using a programme consisting of an initial denature step of 4 min at 94ºC followed by 30 cycles of 30 seconds duration, (94ºC), 30 sec annealing (60ºC), and 1 min extension (72ºC) ending with final 4 min extension (72ºC).

4.3. B.2. Agarose Gel Electrophoresis of PCR products

The PCR products were checked in 1.2 % of agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/mL Ethidium Bromide. 1 µL of 6X gel

loading dye (Himedia) was mixed with each samples and run the gel using Electrophoretic unit (Enduro, Labnet International inclusive) at 75 V power supply with 0.5 X TBE as electrophoretic buffer for about 1-2 hours, until the Bromophenol blue dye front had migrated to such an extent i.e, almost at the bottom of the gel. The 100 bp DNA ladder (Invitrogen 100 bp DNA ladder of 0.1 µg/ µL concentration was used to analyse the size of the amplified DNA. Gels were visualized in the UV transilluminator and the particular images were documented using the gel Documentation system.

4.3. B.3. DNA Sequencing

Sanger's sequencing using the Big Dye Terminator v3.1; amplified product later on subjected to the Sangers sequencing which was carried out using the PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, USA) followed by manufacturer's instructions.

Table 4.4:- Composition of DNA sequencing solution

Particulars used	**Amount**
PCR Product (ExoSAP treated)	10-20 mg
Primer	3.2 pM (either forward or Reverse)
Sequencing Mix	0.28 µl
DMSO	0.30 µl
5X Reaction buffer	1.86 µl
Sterile distilled water	Make up to 10 µl

Sequencing PCR was carried out. It consists of first and foremost denaturation step of 96^{o}C for 2 minute, followed by 30 cycles of 96^{o}C denaturation for 30 seconds, 50^{o}C annealing for 40 seconds and elongation at 60^{o}C for 4 minutes.

4.3. B.4. Procedure for Post Sequencing PCR clean up

The products after sequencing were cleaned up using Master Mix-I, Master Mix-II and 70% ethanol. For each sequenced reaction product, 12 µl Master Mix-I and 52 µl Master Mix-II were needed.

Composition of the Master Mix

Master Mix-I - 2 µl of 125 mM EDTA in 10 µl Milli Q Water.

Master Mix-II - 2 µl of 3M Sodium Acetate at pH 4.6 in 50 µl ethanol.

To the 10 µl of each and every reaction product 12 µl of Master Mix-I was added. Thoroughly mixed and to this 52 µl of Master Mix-II was added. The contents were mixed by inverting the tubes many times during incubation at room temperature for 30 minutes. After the incubation period, proper centrifugation has been carried out at 14,000 rpm for 20 minutes. Later on the supernatant was discarded and the 70% ethanol wash was repeated. The supernatants were decanted and 70% of ethanol wash were repeated. After decanting the supernatant the resultant pellet was kept for air drying. The cleaned air dried products were sequenced in ABI 3730/3500 Genetic Analyzer (Applied Biosystems) at Vision Scientific Services, Ernakulam.

4.3. B.5. Editing of Sequence and Multiple Sequence Alignment.

Quality of the sequence data was checked using quality value (QV) i.e., a widely accepted and established metric for determining quality of the standard sequence data. The QV> 20, means the probability that the base was miscalled is not greater than 1%, is acceptable standard for a good sequence reaction. For all the samples sequenced, the quantity value was >20. Thus practically all samples sequenced could be used for further studies.

4.3. B.6. Multiple Sequence Alignment

Forward and reverse sequences were combined using Bioedit (Hall, 1999) software. Sequences were aligned using Clustal W (Thompson *et al.,*1994) incorporated within MEGA 6 (Tamura *et al.,* 2013) software. Alignments were thoroughly checked and manually edited before analysis.

4.3. C. Phylogenetic Analysis

To know whether the genes can be concatenated or not, Partition Homogeneity Test i.e, Incongruence Length Difference (ILD) (Lee *et al*., 2001) was done for each data set. The two different genes were concatenated depending upon the credibility of values. Partition finder V1.1.0 (Lanfear *et al*., 2012) were used for calculating the model of sequence evolution of two different genes. The model of sequence evolution was TVM+ G for *rps16* introns and TrN+G for *trnL-F* intergenic spacer. Maximum Likelihood and Bayesian analysis were performed using the concatenated dataset using RAxML version 8: (Stamatakis, 2014) and MrBayes version 3.2 (Ronquist and Huelsenbeck, 2003) softwares respectively to get the standard phylogenetic reconstruction of DNA sequence data. In the analysis gaps were coded as 5^{th} character.

4.3. C.1. Partition Homogeneity Test (ILD Test)

PAUP 4b10 version (Swofford, 2003) was used for conducting Partition Homogeneity Test using heuristic search. Number of replicates of 100 was used and parsimony was used as the optimality criterion. Total of 919 characters of 48 taxa were used. All the characters were set to un-ordered. All the characters had equal weights, 434 characters were constant and 439 variable characters were found to be parsimony un-informative. Number of parsimony informative character was 148. Gaps were treated as 5^{th} character. Tree Bisection Reconnection (TBR) was used as the Branch Swapping

Algorithm and random sequence addition was followed. If the branch length is zero, then branches get collapsed. The P value obtained should be less than 0.5.

4.3. C.2. Partition Finder v1.1.0

Partition schemes and model of molecular evolution for nucleotides alignment was done by using partition finder v 1.1.0 software. This program is mainly used for three purposes.

1. In order to find out the best-fit partitioning schemes for a given dataset.
2. To compare any number of user-defined partitioning schemes.
3. In order to find out the best-fit models of molecular evolution for each subset in any partitioned dataset.

It could be seen that *rps16* followed the same evolutionary model whereas *trnL-F* followed a different substitution model. Results obtained using the software was found to be accurate and much more robust.

4.3. C.3. RAxMLGUI 1.3- Randomized Axelerated Maximum Likelihood

Commonly **RAxML** (Randomized Axelerated Maximum Likelihood) (Stamatakis, 2014) is regarded as a sequential and parallel Maximum Likelihood based inference program to construct large phylogenetic trees. Analysis started with importing 48 taxa contained 919 characters into RAxML GUI 1.3 software. Settings had been changed according to the data sets: ML + thorough bootstrap were used; number of replications was set to 1000, bootstrap per branch length was selected, edited the set/edit partitions and changed to DNA, p1 = 1-324 and p2=325-919, evolutionary model was set to GTRGAMMA and run the analysis. After a couple of hours, trees with robust topology and good bootstrap supporting values was obtained.

4.3. C.4. Bayesian analysis using MrBayes version 3.2

Bayesian analysis is the most reliable method for tree construction to get a good and robust topology. We used MrBayes version 3.2 (Ronquist and Huelsenbeck, 2003), the command based software for conducting Bayesian analysis of the data sets. Convergence has been obtained using Metropolis coupled Monte Carlo Markov Chain (MCMCMC or MC^3), in order to get the best posterior probability support for the nodes in Bayesian analysis. The idea of the method was to introduce a series of Markov chains samples from the heated posterior probability distributions. For getting an appropriate convergence, the Potential Scale Reduction Factor (PSRF) (Gelman and Rubin, 1992) was analysed. PSRF compares variance among runs with the variance within the runs. When PSRF value approaches 1.0 the chain converges and a proper tree will be obtained. For 10,00,000 generations, two simultaneous independent runs with 4 Marcov chains were performed and tree sampling was done in every hundred generation resulting in 10000 trees. The first 25 of the trees were discarded after the analysis since it is considered to be in burnin face. A majority- rule consensus tree based on remaining 7500 trees were computed and the posterior probability of the branches were shown at each nodes. Trees were observed in FigTree v1.4.2 (Rambaut, 2014).

Various steps used to construct a Bayesian tree

Getting data into MrBayes:- MrBayes only accept Nexus file format of an aligned nucleotide or morphological data. The concatenated DNA data set of two different genes *trn L-F* intergenic spacer and *rps16* introns of 919 bp length of 48 taxa were converted into Nexus file formats. The file was imported into the software using the command **execute <filename>**. The commands were typed at the MrBayes command prompt.

Specifying the model:- The command **'Lset'** is used to define the structure of the model and **'prset'** is used to define the prior probability distributions on the parameters of the model. The two commands were commonly used to specify the evolutionary models. Usually specified models can be obtained using the command **'showmodel'**.

The general structure of the substitution model is determined by settings of the 'Nst' (Number of substitution types) value. This value may change based on evolutionary models. Here evolutionary model obtained was TVM+G for *rps16* introns data set. TrN+G model was selected for *trnL-F* intergenic spacer. A value of Nst =6 has been set for the two different datasets. Rates at each side is found to be gamma distributed and the command used was rates=gamma.

Setting up of priors:- Next step is to setup the priors for specified model. There are six types of parameters in the model.

1. The topology
2. The branch lengths
3. The four stationary frequencies of the nucleotides.
4. The six different nucleotides substitution rates.
5. The proper proportion of invariable sites.
6. The shape parameter of a gamma distribution of the rate variation.

Commonly, by default; priors will work well for most of analysis. By typing **'help prset'** command we got the default settings for the parameter in our models.

Iset applyto=(2) nst=6 rates=gamma; prset applyto=(2) revmatpr = dirichlet (1,1,1,1,1,1,1) statefreqpr=dirichlet(1,1,1,1) shapepr=uniform (0.1, 50); This commands were used for the model=**TVM+G;**

Iset applyto=(4) nst=6 rates=gamma; prset applyto=(4) revmatpr = dirichlet (1,1,1,1,1,1,1) statefreqpr=fixed (equal) shapepr= uniform(0.1, 50)

pinvarpr=uniform(0,1); This commands were used for the model=**TrN+G.**

Checking the model:- The command 'showmodel' was used to check the model before the analysis. Here I checked and reconfirmed the model and gave a nod to continue to the next step.

Setting up the analysis:- Usually analysis starts with **mcmc** command. Before starting the analysis one has to look back to the run settings by typing the command **help mcmc**. Here number of generations by default was found to be 1000000. So no need to change the settings and analysis was run for 1000000 generations. Number of chains were set to be 2 (Nchains=2).

Running the analysis: - To start the analysis the command **mcmc** was executed. The analysis was run for few hours to 2 days depend upon complexity of data set.

When to stop the analysis:- At the end of the run MrBayes asks "whether or not you want to continue the analysis?" Before typing a reply command **NO** the average standard deviation of split frequencies have to be checked if it tends to 0.01, sampling was good enough to stop analysis. Then type the command **NO** to stop the run.

Summarizing the samples of substitution model parameters:- During each Run samples of substitution model parameters were written to the .p files for every sample frequency generation.

The command **'sump'** was used to summarize sample parameter values. Burn in percentage was set to 25%. Here 10000 trees were generated of which 2500 has been discarded by the system.

Summarizing samples of trees and branch lengths:- Trees and branch lengths were printed to '.t' files. The command **sumt burnin=2500** was used to summarize the tree and branch length information. Sumt command also provide output summary statistics of the dataset bipartitions, trees with clade credibility values, and a phylogram. Execution of this command also generate additional files. One among them is the '.parts' file. It contains list of taxation bipartitions, their posterior probabilities and branch lengths. The second tree gives only branch lengths. These tree were viewed in FigTree (Rambaut, 2014) package.

4.3. D. Distribution of phytochemical constituents in phylogenetic tree

Trees obtained from the analyses were saved for comparisons. The combined analysis trees were examined in Mesquite v.3.0.3 (Maddison and Maddison, 2015). The distribution of the major and specific phytoconstituents was examined on the trees. The test uses the phylogenetic information to test whether changes among one character are associated with the changes in another character. In all examinations, taxa that were not chemically samples were excluded from the analysis.

RESULTS

5. A. VOLATILE PROFILING OF SELECTED TAXA

The essential oil from 30 taxa of the subfamily Lamioideae, with multiple accessions was subjected to GCMS analysis. Species with abundant availability were collected in multiple accessions and others were collected in a single accession. The GCMS results shows a wide range of chemical components in all plant samples studied. Identification of the oil constituents was accomplished by comparison of their mass spectra and retention indices with those of standard samples and literature (Adams, 1989; Joulain and Koenig, 1998). All the essential oils are complex mixtures of chemical constituents (Table 5.1). Altogether, a total of 176 chemical constituents were identified. Among them, the sesquiterpenes consist the main proportion in all taxa studied. Some compounds are very specific to some species only. Table 5.2 displays the specific compounds in plants. GCMS chromatograms of all the studied samples are illustrated in Figure 5.1.

Lamioideae is generally an oil poor subfamily and hence the oil yield calculated for majority of plants was only trace in their amounts. All the investigated taxa contain essential oil range from 0.01 to 0.6 % based on dry weight (Table 5.1). Genus *Pogostemon* is having comparatively a higher oil yield with a pale yellow to brown colored oil, in which *Pogostemon benghalensis* had the highest oil yield (>0.5%). All other species yield only a trace amount of oil with a colorless to pale yellow colored oil.

Table 5.1: Volatile constituents of essential oil

RRI	COMPONENT	*Anisomeles heyneana*			*Leonotis nepetifolia*			*Anisomeles malabarica*			*Gomphostemma heyneanum* var. *heyneana*	*Colebrookea oppositifolia*
	Accession	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**1**
1032	*α*-Pinene	-	-	-	-	-	-	11.0	7.0	5.5	-	-
1035	*β*-Pinene	-	-	-	-	-	-	2.7	1.8	1.8	-	-
1203	Limonene	-	-	-	-	-	-	1.1	0.5	-	-	-
1272	Styrene	-	-	-	-	-	-	0.8	0.4	0.3	-	-
1285	Isoamylisovalerat	-	-	-	-	-	-	1.4	0.6	1.7	-	-
1443	Dimethyltetradecane	-	-	-	-	-	-	1.4	1.0	-	-	-
1452	1-Octen-3-ol	-	-	-	-	-	-	-	-	-	0.9	9.8
1497	*α*-Copaene	-	-	-	-	-	-	-	-	-	0.8	-
1535	*β*-Bourbonene	-	-	-	1.3	1.5	1.2	-	-	-	-	-
1549	*β*-Cubebene	-	-	-	-	-	-	-	-	-	1.5	-
1553	Linalool	0.9	0.9	0.5	-	-	-	-	-	-	-	2.0
1572	*α*-Bergamotene	-	-	-	-	-	-	-	-	-	-	1.5
1577	*α*-Cedrene	-	-	-	-	-	-	-	-	-	1.5	-
1594	*β*-Funebrene	-	-	-	-	-	-	-	-	-	2.2	-
1600	*β*-Elemene	-	-	-	1.4	1.1	1.0	-	-	-	1.5	-
1612	*β*-Caryophyllene	1.5	3.3	0.8	13.5	14.3	11.8	10.4	11.7	8.3	36.1	24.7
1613	*β*-Cedrene	-	-	-	-	-	-	-	-	-	3.3	-
1687	*α*-Humulene	-	-	-	6.4	6.4	5.6	2.1	2.5	1.9	7.1	4.2
1726	Germacren D	-	-	-	48.2	54.0	48.6	-	-	-	1.5	1.1
1740	*α*-Muurolene	-	-	-	1.0	0.4	0.4	-	-	-	-	-
1745	Selina-4(15)-7(11)-diene	-	-	-	-	-	-	-	-	-	2.1	-
1755	Bicyclogermacren	-	-	-	1.4	1.8	1.7	-	-	-	-	-
1773	*δ*-Cadinene	1.8	3.1	1.3	0.6	0.4	0.4	-	-	-	-	-
1785	7-*epi*-*α*-Selinene	-	-	-	-	-	-	-	-	-	1.7	-
1929	Bp 123 M+208	-	-	-	-	-	-	-	-	-	-	11.8
1992	Neophytadiene	9.0	23.8	23.1	-	-	-	-	-	-	-	-
1993	Neophytadieneisomer	2.4	5.2	5.8	-	-	-	-	-	-	-	-

1933	Tetradecanal	-	-	-	1.7	-	0.7	-	-	-	-	-
1957	Cubebol	-	-	-	0.8	0.7	0.7	-	-	-	-	-
2001	Isocaryophylleneoxide	-	-	-	3.8			-	-	-	1.6	-
2008	Caryophylleneoxide	-	-	-	3.8	3.2	3.1	3.7	3.2	3.0	-	2.7
2030	Neophytadieneisomer	3.6	8.0	8.6	-	-	-	-	-	-	-	-
2041	Bp 191 M+272	-	-	-	-	-	-	-	-	-	-	4.4
2050	(*E*)-Nerolidol	-	-	-	-	-	-	1.3	1.7	1.5	1.8	-
2071	Humuleneepoxide II	-	-	-	1.1	0.9	0.8	0.6	0.5	0.5	2.1	-
2080	1,10-diepi-Cubenol	-	-	-	-	-	-	1.0	0.5	0.4	-	-
2131	Hexahydrofarnesylacetone	-	-	-	-	-	-	3.4	3.7	6.0	-	-
2143	Cedrol	-	-	-	-	-	-	-	-	-	1.2	-
2187	T-Cadinol	-	-	-	-	-	-	-	-	-	2.1	-
2255	*α*-Cadinol	-	-	-	1.0	0.8	0.6	-	-	-	1.7	-
2278	Neocembrene A	-	-	-	-	-	-	11.0	13.1	10.0	-	-
2287	Bp 119 M+272	-	-	-	-	-	-	-	-	-	-	9.8
2296	Isophytol	1.2	1.7	2.0	-	-	-	-	-	-	-	-
2308	Bp 95	-	-	-	-	-	-	-	-	-	-	1.7
2312	9-Geranyl-*p*-cymene	-	-	-	-	-	-	-	-	-	-	5.0
2380	epi –Manoyloxide	-	-	-	-	-	-	2.0	1.8	1.7	-	-
2450	Bp 191, M+290Diterpene	-	-	-	-	-	-	-	-	-	-	2.3
2478	Bp 204 M+272	-	-	-	-	-	-	-	-	-	-	4.5
2500	Bp 109 M+272	-	-	-	-	-	-	6.8	10.0	8.7	-	-
2575	Diterpene	-	-	-	-	-	-	1.0	-	1.3	-	1.0
2620	Diterpene	-	-	-	-	-	-	2.0	-	2.0	-	2.1
2622	Phytol	65.0	33.0	47.7	12.1	7.4	14.5	3.0	4.5	4.3	-	-
2835	M+286 Diterpene	-	-	-	-	-	-	11.7	15.3	14.7	-	-
2920	Diterpene	-	-	-	-	-	-	6.0	6.9	8.4	-	-
2931	Hexadecanoicacid	0.4	-	0.2	-	-	-	4.0		8.0	-	-
	Total	**85.8**	**79**	**90**	**98.1**	**95.1**	**91.1**	**88.4**	**86.7**	**90**	**70.7**	**88.6**
	Yield (%v/dry wt)	tr	tr	tr	0.01	0.02	0.02	tr	tr	tr	0.05	0.05

RRI	COMPONENT	*Leucas ciliata*			*Leucas hirta*			*Leucas montana*			*Leucas helianthimifolia*			*Leucas martinicensis*			*Leucas lavandulifolia*		
	Accession	**1**	**2**	**3**	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
1032	*α*-Pinene	-	-	-	-	-	-	-	-	2.6	4.3	61.0	61.1	-	-	-	-	-	-
1035	*β*-Pinene	-	-	-	-	-	-	-	-	-	-	Tr	2.3	-	-	-	-	-	-
1132	Sabinene	-	-	-	-	-	-	-	-	-	-	1.7	1.3	-	-	-	-	-	-
1203	Limonene	-	-	-	-	-	-	-	-	-	-	5.3	5.0	-	-	-	-	-	-
1246	*(Z)*-*β*-Ocimen	-	-	-	0.1	Tr	0.1	-	-	-	-	-	-	-	-	-	-	-	-
1266	(*E*)-*β*-Ocimen	-	-	-	1.1	0.3	0.8	-	-	-	-	-	-	3.7	4.2	3.3	-	-	-
1290	Terpinolene	-	-	-	-	-	-	-	-	-	1.0	2.9	2.5	-	-	-	-	-	-
1452	1-Octen-3-ol	3.4	-	0.3	1.9	0.8	1.5	-	-	-	-	-	-	-	-	-	1.2	2.0	2.1
1492	Cyclosativene	2.2	1.6	1.4	1.2	1.3	1.2	-	-	-	-	-	-	-	-	-	-	-	-
1500	Pentadecane	-	-	-	-	-	-	-	-	-	2.0	0.4	0.4	-	-	-	-	-	-
1535	*β*-Bourbonene	-	-	-				-	-	-	-	-	-	-	1.0	1.0	-	-	-
1594	*trans*-*β*-Bergamotene	-	-	-	-	-	-	1.0	-	0.8	-	-	-	-	-	-	-	-	-
1600	*β*-Elemene	-	-	-	4.9	4.7	4.4	1.5	-	0.4	-	-	-				-	-	-
1612	*β*-Caryophyllene	33.7	27.7	28.0	50.0	51.2	48.4	52.2	67.8	64.0	36.4	10.7	10.2	9.3	10.0	10.0	71.5	71.3	70.0
1668	*(Z)*-*β*-Farnesene	-	-	-	-	-	-	-	-	-	1.3	0.2	0.3	-	-	-	-	-	-
1687	*α*-Humulene	5.2	4.5	4.5	8.6	8.8	8.2	11.0	14.4	14.3	5.2	1.5	1.4	4.3	4.6	4.8	13.6	13.5	13.0
1688	Selina 4,11-diene	-	-	-	-	-	-	-	-	-	1.2	0.2	0.3	-	-	-	-	-	-
1694	Drima-7,9(11)-diene	-	-	-	-	-	-	-	-	-	-	-	-	3.0	3.0	3.0	-	-	-
1705	Zizanene	-	-	-	-	-	-	-	-	-	5.3	1.6	1.6	-	-	-	-	-	-
1719	1-Heptadecene	5.3	6.0	4.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1726	Germacrene D	-	-	-	0.9	0.9	0.9	-	-	-	-	-	-	58.2	56.2	57.0	0.7	-	0.5
1742	*β*-Selinene	-	-	-	-	-	-	2.6	2.3	3.0	8.0	2.0	1.9	-	-	-	-	-	-
1744	*α*-Selinene	-	-	-	-	-	-	2.0	2.8	2.4	-	-	-	-	-	-	-	-	-
1745	Selina-4(15)-7(11)-diene	-	-	-	-	-	-	1.2	1.3	0.5	-	-	-	-	-	-	-	-	-
1755	Bicyclogermacren	-	-	-	-	-	-	-	-	-	-	-	-	1.3	1.1	1.2	-	-	-
1758	(*E,E*)-*α*-Farnesene	-	-	-	-	-	-	-	-	-	1.0	Tr		-	-	-	-	-	-
1765	Geranylacetate	-	-	-	-	-	-	-	-	-	1.6	0.3	0.3	-	-	-	-	-	-
1957	Cubebol	-	-	-	-	-	-	-	-	-	-	-	-	2.3	2.4	2.1	-	-	-
1993	Neophytadieneisomer	1.3	0.6	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2008	Caryophylleneoxide	-	-	-	3.6	3.2	4.1	-	-	-	3.8	1.3	1.5	-	-	-	0.8	1.0	1.5

2050	(*E*)-Nerolidol	1.2	1.4	2.4	-	-	-	-	-	-	1.9	0.2	0.2	-	-	-	0.4	-	0.6
2071	Humuleneepoxide II	-	-	-	-	-	-	-	-	-	1.1	0.1	0.2	-	-	-	-	-	-
2096	Elemol	-	-	-	1.2	-	1.1	-	-	-	-	-	-	-	-	-	-	-	-
2100	(*E*)-Sesquilavandulylacetate	-	-	-	7.1	8.0	7.6	-	-	-	-	-	-	-	-	-	-	-	-
2183	(*E*)-Sesquilavandulol	-	-	-	1.7	1.5	1.6	-	-	-	-	-	-	-	-	-	-	-	-
2185	γ-Eudesmol	-	-	-	-	-	-	-	-	-	-	-	-	0.9	0.9	3.0	-	-	-
2204	Eremoligenol	-	-	-	-	-	-	-	-	-	-	-	-	3.0	3.0	-	-	-	-
2202	Bp 273, M+288 Diterpene	-	-	-	-	-	-	2.2	-	-	3.9	0.6	0.6	-	-	-	3.0	2.8	2.9
2232	α-Bisabolol	-	-	-	-	-	-	-	-	-	0.8	0.1	0.1	-	-	-	-	-	-
2255	α-Cadinol	-	-	-	-	-	-	-	-	-	-	-	-	0.8	0.8	1.0	-	-	-
2257	β-Eudesmol	-	-	-	-	-	-	0.9	1.0	1.0	-	-	-	-	-	-	-	-	-
2264	Intermedeol	-	-	-	1.1	1.1	1.1	-	-	-	-	-	-	-	-	-	-	-	-
2272	14-Acetoxy-β-Caryophyllene	-	-	-	1.1	1.1	1.1	-	-	-	-	-	-	-	-	-	-	-	-
2273	Selin-11-en-4α-ol	-	-	-	2.4	2.2	2.1	-	-	-	-	-	-	-	-	-	-	-	-
2324	(6S,7R)-Bisabolene	-	-	-	-	-	-	-	-	-	2.0	Tr	0.2	-	-	-	-	-	-
2450	Bp 191, M+290Diterpene							12.4	6.2	2.4	2.2								
2400	Pentacosane	-	-	-	-	-	-	-	-	-	2.3	0.3	0.2	-	-	-	-	-	-
2470	Bp 191 M+270Diterpene	-	-	-	-	-	-	3.3	1.0	0.7	-	-	-	-	-	-	-	-	-
2478	Bp 204 M+272Diterpene	-	-	-	-	-	-	3.4	1.5	-	-	-	-	-	-	-	-	-	-
2496	Bp 119M+288 Diterpene	-	-	-	-	-	-	3.4	-	-	-	-	-	-	-	-	1.7	1.5	1.6
2600	Hexacosane	-	-	-	-	-	-	-	-	-	3.8	0.2	0.3	-	-	-	-	-	-
2622	Phytol	16.2	23.0	21.4	1.0	1.8	0.9	-	-	-	-	-	-	2.9	3.0	3.8	1.2	1.0	1.0
2700	Heptacosane	2.0	2.5	1.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2931	Hexadecanoicacid	1.0	2.6	2.0	-	-	-	-	-	-	-	-	-	-	-	-	1.4	0.5	1.4
	Total	**71.5**	**69.9**	**66.4**	**87.9**	**86.9**	**85.1**	**97.1**	**98.3**	**92.1**	**89.1**	**90.6**	**91.9**	**89.7**	**90.2**	**90.2**	**95.5**	**93.6**	**94.6**
	Yield (%v/dry wt)	0.05	0.03	0.03	0.04	0.04	0.04	Tr	tr	Tr	0.02	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.02

RRI	COMPONENT	*Leucas lavandulifolia* var. *nagalapuramiana*		*Leucas marrubioides* var. *pulneyensis*		*Leucas biflora*		*Leucas rosmarinifolia*	
	Accession	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
1452	1-Octen-3-ol	-	-	1.5	1.4	-	-	-	-
1612	*β*-Caryophyllene	2.0	3.7	66.4	47.2	3.1	6.8	3.0	11.1
1687	*α*-Humulene	-	-	8.2	5.8	2.3	4.0	0.7	1.6
1992	Neophytadiene	-	-	-	-	14.0	14.2	-	-
1993	Neophytadieneisomer	-	-	-	-	3.6	3.0	-	-
2030	Neophytadieneisomer	-	-	-	-	5.8	4.5	-	-
2041	Pentadecanal	-	-	-	-	1.7	5.4	-	-
1742	*β*-Selinene	-	-	2.2	1.4	-	-	-	-
1745	Selina-4(15)-7(11)-diene	-	-	-	-	-	-	1.1	3.7
1688	Selina 4,11-diene	-	-	2.7	1.9	-	-	-	-
1726	Germacrene D	-	-	2.0	1.0	-	-	-	-
2008	Caryophylleneoxide	-	-	2.2	1.2	-	-	1.4	4.0
2050	(*E*)-Nerolidol	-	-	2.0	1.7	1.1	0.6	-	-
2051	Gleenol	-	-	-	-	-	-	-	-
2131	Hexahydrofarnesylacetone	-	-	-	-	3.8	4.8	-	-
2202	Bp 273 M+288	-	-	-	-	-	-	14.0	15.2
2232	*α*-Bisabolol	-	-	0.9	0.3	-	-	-	-
2264	Fattyacid	-	-	-	-	4.0	6.3	-	-
2296	Isophytol	-	-	-	-	5.0	4.1	-	-
2324	Fattyacid	-	-	-	-	17.1	23.0	-	-
2400	Tetracosane	-	-	-	-	-	-	2.5	1.8
2450	Bp 191 M+290	65.1	63.2	-	-	-	-	-	-
2460	Bp 137 M+272 Diterpene	-	-			-	-	4.0	3.0
2470	Bp 191 M+288	8.6	8.5	-	-	-	-	5.2	3.0
2478	Bp 204 M+272	13.4	13.1	-	-	-	-	3.1	2.9
2485	Bp 137 M+290	1.5	1.3	-	-	-	-	-	-
2496	Bp 119, M+288	-	-	-	-	-	-	7.3	6.5
2500	Pentacosane							3.0	1.9

2615	Bp 107 M+290	3.0	2.8	-	-	-	-	-	-
2633	Phytol	-	-	3.8	32.7	35.1	20.1	-	-
2670	Bp 121 M+286	1.3	1.2	-	-	-	-	-	-
2950	Bp 177 M+290	2.1	1.8	-	-	-	-	-	-
2700	Heptacosane	-		-	-	-	-	7.7	4.4
2830	Diterpene	-	-	-	-	-	-	6.0	2.6
2931	Hexadecanoicacid	-	-	-	-	-	-	11.0	11.3
2950	Diterpene	-	-	-	-	-	-	23.4	19.1
	Total	**97**	**95.6**	**91.9**	**94.6**	**96.6**	**96.8**	**93.4**	**92.1**
	Yield (%v/dry wt)	0.15	0.12	Tr	tr	tr	tr	tr	tr

RRI	COMPONENT	*Leucas stricta*	*Leucas lanata* var. *candida*	*Leucas prostrata*	*Leucas lanceaefolia*	*Leucas eriostoma*	*Leucas urticifolia*	*Leucas wightiana*	*Leucas aspera*	*Leucas stelligera*	*Leucas angularis*
	Accession	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032	*α*-Pinene	-	-	5.0	3.0	3.0	-	-	-	6.0	-
1174	Myrcene	-	-	-	-	-	-	2.0	-	-	-
1203	Limonene	-	-	-	-	-	-	-	-	1.0	-
1290	Terpinolene	-	-	-	-	-	-		-	1.7	-
1393	3-Octanol	-	-	-	-	1.8	-	-	-	1.5	-
1452	1-Octen-3-ol	-	-	-	1.7	10.0	-	3.1	-	18.7	3.4
1479	*δ*-Elemene	-	-	-	-	-	0.8	-	-	-	-
1492	Cyclosativene	-	-	1.0	-	-	-	-	-	-	-
1497	*α*-Copaene	1.3	2.5	-	-	-	-	-	-	-	1.5
1535	*β*-Bourbonene	-	-	-	-	-	1.7	-	-	-	-
1553	Linalool	-	-	-	1.4	-		2.0	-	-	-
1600	*β*-Elemene	3.0	-	-		-			-	-	7.3
1612	*β*-Caryophyllene	71.0	39.0	16.3	28.4	7.0	14.8	34.0	37.5	29.4	49.5
1661	Alloaromadendrene	-		-	-	-	-	0.7	-	-	-
1668	*(Z)-β*-Farnesene	1.7	3.2	-	-	-	-		-	-	1.0
1687	*α*-Humulene	5.6	7.6	-	33.0	1.0	3.2	3.7	3.6	4.1	6.3
1726	Germacrene D	2.5		-	-	-	33.0	0.8	-	-	1.0
1740	Valencene	-	2.3	-	-	-	-	0.5	-	-	-
1742	*β*-Selinene	-	13.4	1.0	-	-	-	1.9	2.4	-	-
1744	*α*-Selinene	-	5.9	-	-	-	-	1.1	1.6	-	-
1745	Selina-4(15)-7(11)-diene	-	2.9	-	-	-	1.0	13.6	7.2	-	-
1773	*δ*-Cadinene	-	2.7	1.3	-	-	-	1.2	-	-	2.5
1776	*γ*-Cadinene	2.2	-	-	-	-	-	-	-	-	
1783	*β*-Sesquiphellandrene	-	-	-	-	-	-	-	-	-	0.8
1785	7-*epi*-*α*-Selinene	-	1.0	-	-	-	-	2.3	2.7	-	-
1788	Selina 4,11-diene	1.0	-		-	-	-	0.7	-	-	-
1854	Germacren B	-	-	-	-	-	1.9	-	-	-	-
1957	Cubebol	-	-	-	-	-	-	1.3	-	-	-
1993	Neophytadieneisomere I	-	-	-	-	-	-		-	-	0.8
2001	Isocaryophylleneoxide	-	-	-	-	-	1.4	-	-	-	-
2008	Caryophylleneoxide	1.6	1.5	4.8	-	-	5.5	1.0	1.0	1.0	1.5
2050	(*E*)-Nerolidol	-	5.1	-	-	-	-	-	-	-	-

2071	Humuleneepoxide II	-	-	2.0	1.0	-	1.4	-	-	-	-
2100	(*E*)-Sesquilavandulylacetate	-	-	-	-	-	-	-	-	-	1.5
2122	Hedycaryol	-	-	1.7	-	-	-	-	-	-	-
2125	Bp 135 M+220	-	-	3.5	-	-	-	-	-	-	-
2185	*γ*-Eudesmol	-	-	1.0	-	-	-	-	0.8	-	-
2201	Bp159, M+ 288 Diterpene		-	-	-		-	4.8	-	-	-
2202	Bp 273, M+288 Diterpene			-	-		-		2.9	-	-
2209	T-Muurolol	0.8	-	-	-	-	-	3.4	-	-	0.8
2257	*β*-Eudesmol	-	1.3	2.7	-	-	-	-	-	-	-
2264	Intermedeol	-	-	11.1	-	-	-	-	-	-	-
2273	Selin-11-en-4*α*-ol	0.8	1.1	23.6	-	-	-	-	-	-	0.8
2340	Bp107, M+220		-	-	-	-	-	11.3	15.7		
2400	Pentacosane	-	-	-	2.0	-	-	-	-	-	-
2450	Bp 191 M+290	-	-	-	-	49.3	6.3	-	-	3.5	0.8
2460	Bp 137 M+272 Diterpene	-	-	-	-	1.8	-	-	-	5.0	-
2470	Bp 191 M+288	-	-	-	1.5	4.2		-	-	-	-
2478	Bp 204 M+272	-	-	-	-	9.0	1.5	-	1.9	6.7	-
2496	Bp 119 M+288	-	-	-	-	-	-	-	2.4	-	-
2615	Bp 107 M+290 Diterpene	-	-	-	-	2.7	-	-	-	-	-
2622	Phytol	-	0.7	6.9	-	-	21.1	4.2	10.3	2.7	2.5
2670	Bp 121 M+286 Diterpene	-	-	-	-	1.0	-	-	-	-	-
2671	Bp 167	-	-	-	1.9	-	-	-	-	-	-
2680	Bp 167	-	-	-	2.0	-	-	-	-	-	-
2750	Diterpene	-	-	-	4.6	-	-	-	-	-	-
2790	Diterpene	-	-	-	5.8	-	-	-	-	-	-
2870	Diterpene	-	-	-	2.5	-	-	-	-	-	-
2930	Bp 189 M+270 Diterpene	-	-	-	-	-	-	-	-	5.9	-
2931	Hexadecanoicacid	-	-	-	-	-	3.2	-	-	-	-
2950	Bp 177 M+290 Diterpene	-	-	-	-	4.3	-	-	2.1	1.5	-
	Total	**91.5**	**90.2**	**81.9**	**88.8**	**95.1**	**96.8**	**93.6**	**92.1**	**88.9**	**82**
	Yield (%v/dry wt)	0.05	0.03	tr	0.02	0.1	0.03	tr	0.05	tr	tr

RRI	COMPONENT	*Pogostemon benghalensis*			*Pogostemon mollis*			*Pogostemon wightii*			*Pogostemon speciosus*	*Pogostemon quadrifolius*		
	Accession	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**1**	**2**	**3**
1032	*α*-Pinene	-	-	-	3.1	1.5	3.2	0.4	0.3	0.2	0.1	0.1	-	-
1035	*α*-Thujene	-	-	-	1.1	0.5	1.4	0.2	0.1	0.1	0.1	0.1	-	-
1118	*β*-Pinene	-	-	-	4.8	2.8	4.9	0.2	0.2	0.1	-	-	-	-
1132	Sabinene	-	-	-	6.7	3.8	6.0	0.5	0.5	0.4	-	-	-	-
1188	*α*-Terpinene	-	-	-	0.2	0.2	0.3	-	-	-	-	0.1	-	-
1203	Limonene	-	-	-	0.2	0.2	0.2	tr	0.2		-	-	-	-
1255	*γ*-Terpinene	-	-	-	0.7	0.5	0.9	-	-	-	-	-	-	-
1266	(*E*)-*β*-Ocimene	1.5	2.4	2.0	-	-	-	-	-	-	-	-	-	-
1280	*p*-Cymene	-	-	-	0.5	0.5	0.5	0.1	-	0.1	-	0.2	tr	-
1290	Terpinolene	-	-	-	0.3	0.2	0.3	-	-	0.5	-	-	-	-
1304	1-Octen-3-one	-	-	-	-	-	-	0.6	0.8		-	-	-	-
1391	(*Z*)-3-Hexen-1-ol	-	-	-	-	-	-	0.2	0.2	0.1	0.2	-	-	-
1393	3-Octanol	-	-	-	-	-	-	0.8	0.8	0.8	-	-	-	-
1452	1-Octen-3-ol	-	-	-	0.5	0.7	0.4	14.0	13.9	14.0	0.4	0.4	tr	0.2
1466	*α*-Cubebene	-	-	-	9.0	8.8	7.6	tr	tr	tr	0.2	0.4	0.2	0.3
1479	*δ*-Elemene	-	-	-	-	-	-	-	-	-	tr	-	-	-
1493	*α*-Ylangene	0.6	1.0	2.4	-	-	-	-	-	-	-	-	-	-
1497	*α*-Copaene	-	-	-	0.7	1.0	0.8	0.5	0.7	0.6	1.6	-	-	-
1535	*β*-Bourbonene	-	-	-	-	-	-	0.3	0.3	0.3	1.3	-	-	-
1544	*α*-Gurjunene	-	-	-	1.2	1.4	1.1	-	-	-	-	-	-	-
1549	*β*-Cubabene	-	-	-	1.9	1.8	1.5	-	-	-	-	-	-	-
1550	*α*-Bergamotene	-	-	-	0.7	0.6	0.6	-	-	-	-	-	-	-
1553	Linalool	-	-	-	-	-	-	0.3	0.3	0.3	-	-	-	-
1589	Aristolene	-	-	-	-	-	-	-	-	-	-	0.2	0.2	0.3
1589	Isocaryophyllene	-	-	-	-	-	-	-	-	-	-	0.5	0.5	0.5
1596	*α*-Guaiene	0.7	2.2	2.8	-	-	-	0.2	0.2	0.2	-	-	-	-
1597	*β*-Copaene	-	-	-	-	-	-	-	-	-	0.2	-	-	-
1600	*β*-Elemene	0.2	0.3	0.9	-	-	-	-	-	-	tr	-	-	-

1610	Calarene	-	-	-	-	-	-	-	-	-	-	0.2	0.2	0.3
1611	Terpinen-4-ol	-	-	-	2.1	2.3	2.4	0.1	0.1	0.1	-	-	-	-
1612	*β*-Caryophyllene	0.3	1.0	1.2	6.1	5.2	4.9	1.3	1.7	1.8	15.7	19.0	21.0	22.0
1617	6,9-Guaiadiene	0.2	0.4	0.5	-	-	-	-	-	-	-	-	-	-
1639	Cadina-3,5-diene	-	-	-	3.7	3.5	4.4	0.1	0.1	0.1	-	-	-	-
1650	*γ*-Elemene	0.2	0.5	0.6	-	-	-	-	-	-	-	-	-	-
1661	Alloaromadendrene	0.3	0.7	1.2	-	-	-	0.4	0.5	0.4	1.3	-	-	-
1677	*epi*-Zonarene	-	-	-	0.4	0.4	0.5	-	-	-	0.1	-	-	-
1687	*α*-Humulene	0.8	2.4	3.3	0.3	0.3	0.3	0.4	0.4	0.3	1.3	4.8	5.5	5.7
1704	*γ*-Muurolene	-	-	-	-	-	-	-	-	-	0.2	-	-	-
1706	*α*-Terpineol	-	-	-	0.1	0.2	0.2	0.4	0.4	0.3	-	0.1	tr	tr
1709	*α*-Terpinylacetate	-	-	-	-	-	-	0.2	0.2	0.1	-	-	-	-
1718	4,6-Guaiadiene	1.2	2.2	2.8	-	-	-	-	-	-	-	-	-	-
1719	Borneol	-	-	-	-	-	-	-	-	-	-	0.1	tr	tr
1722	Bicyclosesquiphellandrene	-	-	-	1.2	1.3	1.3	-	-	-	tr	-	-	-
1726	Germacrene D	2.2	4.5	6.8	1.0	0.8	1.0	-	-	-	4.5	-	-	-
1740	*α*-Muurolene	-	-	-	-	-	-	0.4	0.5	0.4	0.4	-	-	-
1741	*β*-Bisabolene	-	-	-	1.0	1.0	1.0	0.4	0.5	0.6	0.4	-	-	-
1755	Bicyclogermacrene	0.8	1.6	2.0	-	-	-	-	-	-	tr	0.1	0.1	0.1
1773	*δ*-Cadinene	0.4	0.8	1.0	2.6	2.9	2.7	0.7	0.6	0.7	2.7	0.1	0.1	0.1
1776	*γ*-Cadinene	-	-	-	-	-	-	-	-	-	tr	4.0	4.7	4.5
1783	*β*-Sesquiphellandrene	-	-	-	3.8	3.2	3.9	-	-	-	-	-	-	-
1799	Cadina 1,4-diene	-	-	-	5.8	6.5	6.5	-	-	-	tr	-	-	-
1807	*α*-Cadinene	-	-	-	-	-	-	-	-	-	-	0.4	0.3	0.3
1849	Calamenene	-	-	-	9.2	12.0	9.0	0.1	0.1	0.2	0.1	0.2	0.1	0.1
1854	Germacrene B	2.8	6.3	8.6	-	-	-	-	-	-	0.2	-	-	-
1860	Isochamaecyrone*	0.4	0.5	0.5	-	-	-	-	-	-	-	-	-	-
1871	Hexanoicacid	-	-	-	-	-	-	0.5	0.4	0.5	-	-	-	-
1900	*epi*-Cubebol	-	-	-	0.2	0.2	0.2	1.8	2.0	1.8	2.2	-	-	-
1957	Cubebol	0.2	tr	0.5	0.6	0.6	0.5	8.3	8.4	7.1	13.0	-	-	-
2001	Isocaryophylleneoxide	-	-	-	-	-	-	-	-	-	-	0.6	0.7	0.1

2008	Caryophylleneoxide	-	-	-	1.1	1.2	0.8	0.7	1.0	0.8	1.0	11.1	13.4	13.4
2050	(*E*)-Neronidol	-	-	-	0.3	0.2	0.3	1.8	2.0	2.0	-	tr	tr	tr
2051	Gleenol	-	-	-	-	-	-	-	-	-	0.6	-	-	-
2057	Ledol	-	-	-	0.7	0.5	0.7	1.5	1.5	1.4	0.9	-	-	-
2069	Germacrene D-*4*□-ol	0.3	0.7	0.6	-	-	-	-	-	-	1.3	-	-	-
2071	Humuleneepoxide II	-	-	-	-	-	-	-	-	-	-	2.7	3.4	3.4
2080	Cubenol	-	-	-	1.0	1.1	1.0	0.7	0.7	0.7	0.5	-	-	-
2080	1,10-d*iepi*-Cubenol	0.1	0.3	1.2	-	-	-	-	-	-	-	3.0	3.3	3.3
2088	1-*epi*-Cubenol	-	-	-	0.7	0.8	0.8	1.4	1.1	1.1	0.8	-	-	-
2096	Elemol	-	-	-	1.3	1.2	1.2	-	-	-	-	-	-	-
2103	Guaiol	0.5	1.0	0.8	-	-	-	-	-	-	-	-	-	-
2122	Hedycaryol	-	-	-	-	-	-	1.6	1.7	1.5	-	-	-	-
2144	Spathulenol	0.3	1.0	1.0	0.2	0.2	0.2	-	-	-	-	-	-	-
2156	α-Bisabololoxide B	-	-	-	-	-	-	0.7	1.5	1.1	-	-	-	-
2183	Selina-6-en-4-ol	-	-	-	-	-	-	-	-	-	0.2	-	-	-
2185	γ-Eudesmol	-	-	-	1.0	1.2	1.0	0.6	0.6	0.6	-	-	-	-
2187	T-Cadinol	-	-	-	-	-	-	-	-	-	1.5	32.5	35.0	33.2
2191	Zingiberenol	-	-	-	0.3	0.4	0.4	-	-	-	-	-	-	-
2202	1(10),5-Germacradien 4α-ol	1.4	2.1	1.8	-	-	-	-	-	-	0.7	-	-	-
2209	T-Muurolol	-	-	-	1.1	1.2	1.1	0.7	0.7	0.6	0.6	0.8	0.8	0.9
2210	Copaborneol	-	-	-	-	-	-	0.5	0.4	0.4	0.7	-	-	-
2219	δ-Cadinol	-	-	-	0.3	0.3	0.4	0.7	0.7	0.6	0.5	-	-	-
2232	α-Bisabolol	-	-	-	0.3	0.1	0.1	51.0	50.0	53.2	34.0	0.7	0.5	0.5
2239	Carvacrol				-	-	-	-	-	-		8.4	0.4	1.0
2250	α-Eudesmol	-	-	-	0.2	0.4	0.5	tr	tr	tr	-	-	-	-
2255	α-Cadinol	0.6	1.2	1.1	1.6	1.8	1.8	1.1	0.8	0.9	1.0	-	-	-
2257	β-Eudesmol	-	-	-	-	-	-	1.1	0.8	0.9	-	-	-	-
2264	Intermediol	-	-	-	-	-	-	-	-	-	3.0	-	-	-
2270	7-Isopropyl-1,4-dimethyl-2-azulenol	71.5	43.6	28.3	-	-	-	-	-	-	-	-	-	-

2316	Caryophylladienol I	-	-	-	-	-	-	-	-	-	-	0.9	0.9	0.8
2324	Caryophylladienol II	-	-	-	-	-	-	-	-	-	0.2	2.0	2.5	2.4
2349	Isopimaradiene	-	-	-	-	-	-	-	-	-	0.2	-	-	-
2350	6-Methoxy-2-(1-buten-3-yl)naphthalene	1.3	1.4	1.0	-	-	-	-	-	-	-	-	-	-
2356	Isopimara-8,15-diene	-	-	-	0.6	0.9	0.5	-	-	-	-	-	-	-
2357	14-Hydroxy-*β*-caryophyllene	-	-	-	-	-	-	-	-	-	-	0.2	1.8	1.7
2392	Caryophyllenol II	-	-	-	-	-	-	-	-	-	-	1.2	1.5	1.4
	Total	**88.8**	**78.1**	**72.9**	**80.4**	**76.4**	**79.3**	**97.5**	**97.9**	**97.9**	**93.9**	**95.1**	**97.1**	**96.5**
	Yield (%v/dry wt)	0.6	0.5	0.5	0.08	0.08	0.08	0.09	0.12	0.12	0.08	0.12	0.12	0.12

RRI- Relative retention indices calculated against n-alkanes, % calculated from FID data. tr-Trace (< 0.1 %)

Table 5.2. Specific compounds in essential oil of plant leaves

Plant Name	Component	Area percentage
Pogostemon benghalensis	α- Ylangene	1.33
	6,9- Guaiadiene	0.37
	γ-Elemene	0.43
	4,6- Guaiadiene	2.07
	Isochamaecyrone	0.47
	Guaiol	0.77
	7- Isopropyl-1,4-dimethyl-2-azulenol	47.8
	6- Methoxy-2-(1-bten-3-yl)naphthalene	1.23
Pogostemon mollis	γ-Terpinene	0.7
	α- Gurjunene	1.23
	α-Bergamotene	0.63
	Bicyclosesquiphyllandrene	1.27
	Cadina 1,4-diene	6.27
	Zingeberenol	0.37
	Isopimara-8,15-diene	0.67
Pogostemon wightii	1-Octen-3-one	0.7
	α- Terpinylacetate	0.17
	Hedycaryol	1.6
	α Bisabololoxide B	1.1
	Hexanoic acid	0.47
	β Eudesmol	0.93
Pogostemon speciosus	β Copanene	0.2
	γ Muurolene	0.2
	Gleenol	0.6
	Selina-6-en-4-ol	0.2
	Isopimaradiene	0.2
Pogostemon quadrifolius	Aristolene	0.23
	Isocaryophyllene	0.5
	Calarene	0.23
	14-Hydroxy-β-caryophyllene	1.23
	Borneol	0.1
	α- Cadinene	0.33
	Carvacrol	3.23
	Caryophylladienol I	0.83
	Caryophyllenol II	2.3
Leucas ciliata	1-Heptadecene	5.2
Leucas hirta	(E)-Sesquilavandulylacetate	7.57
	(E)-Sesquilavandulol	1.6
	14-Acetoxy-β-Caryophyllene	1.1
Leucas montana	trans-β-Bergamotene	0.9
	Bp 191 M+288 Diterpene	1.67
	(Z)-β-Farnasene	0.6
	Zizanene	2.83
	Pentadecane	0.93

Leucas helianthimifolia	(E,E),α- Farnasene	11
	Geranylacetate	0.73
	(6S,7R)-Bisabolene	1.1
	Hexacosane	1.43
Leucas martinicensis	Drima-7,9(11)-diene	3
	Erimoligenol	3
Leucas prostrata	Bp 135 M+220	3.5
Leucas lanceaefolia	Bp 167	1.9
	Unknown Diterpene	4.6
	Unknown Diterpene	5.8
	Unknown Diterpene	2.5
Leucas urticifolia	δ- Elemene	0.8
Leucas wightiana	Myrcene	2
	Bp 159 M+ 288 Diterpene	4.8
Leucas stelligera	Bp 189 M+ 270 Diterpene	5.9
Leucas biflora	Pentadecanal	3.55
	Unknown fatty acid	5.15
	Unknown fatty acid	20.05
Leucas rosmarinifolia	Tetracosane	2.15
	Pentacosane	2.45
	Unknown Diterpene	4.3
	Unknown Diterpene	21.25
Leucas lavandulifolia var. *nagalapuramiana*	Bp 137 M+ 290	1.4
Anisomeles malabarica	Styrene	0.5
	Dimethyltetradecane	1.2
	Neocembrene A	11.36
	epi- Manoyloxide	1.83
	Bp109 M+272	8.5
	Unknown Diterpene	1.13
	Unknown Diterpene	2
	Unknown Diterpene	7.1
	M+286 Diterpene	13.9
Gomphostema heyneanum var. *heyneana*	α Cydrene	3.3
	β Cydrene	3.3
	β Funebrene	2.2
	Cedrol	1.2
Leonotis nepetifolia	Tetradecanal	1.2
Colebrookea oppositifolia	α Bergamotene	1.5
	Bp 123M+208	11.8
	Bp 191M+272	2.3
	Bp 95	1.7
	Bp119M+272	9.8
	9-Geranyl-p-cymene	5

5. A.1. Major compounds

The results show that 7-Isopropyl-1,4-dimethyl-2-azulenol, Calamenene and T-Cadinol were the major componunds in *Pogostemon benghalensis* (47.8%), *Pogostemon mollis* (10.06%) and *Pogostemon quadrifolius* (33.57%), respectively. Whereas *α*-Bisabolol was the major compound in both *Pogostemon wightii* (51.4%) and *Pogostemon speciosus* (34%). The shrub *Pogostemon benghalensis* was recorded as the most essential oil yielding plant compared to the other species. The component 7-Isopropyl-1,4-dimethyl-2-azulenol is specific to only *P. benghalensis*.

Interestingly β- Caryophyllene was the major compound in many of *Leucas* species such as *L. stricta* (71%), *L. lavandulifolia* (70.93), *L. montana* (61.33%), *L. marrubioides* var. *pulneyensis* (56.8%), *L. hirta* (49.86%), *L. angularis* (49.5%), *L. lanata* var. *candida* (39%), *L. aspera* (37.5%), *L. wightiana* (34%), *L. ciliata* (29.8%) *L. stelligera* (29.4%), *L. helianthimifolia* (19.1%). Whereas Germacrene D was the major compound in *L. martinicensis* (57.12%) and *L. urticifolia* (33%). Selin-11-en-4α –ol was the major compound in *L. prostrata* (23.6%); α- Humulene was the major compound in *L. lanceaefolia* (33%); Bp 191 M+ 290 was the major compound in *L. eriostoma* (49.3%); Bp 191 M+ 290, an unknown compound was the major compound in *L. lavandulifolia* var. *nagalapuramiana* (64.15%). In *L. biflora*, Phytol (27.6%) was the major one. Fatty acid, Hexadecanoic acid was the major compound in it (21.25%) in *L. rosmarinifolia*.

Among two *Anisomeles* species studies, Phytol was the major compound in *A. heyneana* (48.56%) whereas M+ 286 Diterpene, an unknown compound was the major compound in *A. malabarica* (13.9%). In *Colebrookea oppositifolia* and *Gomphostemma heyneanum* var. *heyneana* , β-

Caryophyllene was the major compound. In *Leonotis nepetifolia*, Germacrene D was the major compound (50.26%) (Table 5.3).

Table. 5.3. Major compounds in the essential oil

Sl No	Name of Taxa	Major compound
1	*Anisomeles heyneana* Benth.	Phytol
2	*Anisomeles malabarica* (L.)R. Br.ex Sins	M+ 286 Diterpene
3	*Colebrookea oppositifolia* Sm.	β- Caryophyllene
4	*Gomphostemma heyneanum* Wall. Ex Benth var. *heyneana*	β- Caryophyllene
5	*Leonotis nepetifolia* (L.)R. Br.	Germacrene D
6	*Leucas angularis* Benth	β- Caryophyllene
7	*Leucas aspera* (Willd.) Link	β- Caryophyllene
8	*Leucas biflora* (Vabl) Sm.	Phytol
9	*Leucas ciliata* Benth.	β- Caryophyllene
10	*Leucas eriostoma* Hook.f.	Bp 191 M+ 290
11	*Leucas helianthimifolia* Desf.	β- Caryophyllene
12	*Leucas hirta* (B.Heyne ex Roth) Spreng.	β- Caryophyllene
13	*Leucas lanata* var. *candida* Haines	β- Caryophyllene
14	*Leucas lanceaefolia* Desf.	α- Humulene
15	*Leucas lavandulifolia* Sm.	β- Caryophyllene
16	*Leucas lavandulifolia* var. *nagalapuramiana* Chandrab. & S.R. Sriniv.	Bp 191 M+ 290
17	*Leucas marrubioides* var. *pulneyensis* Hook.f.	β- Caryophyllene
18	*Leucas martinisencis* (Jacq.) R.Br.	Germacrene D
19	*Leucas montana* (Roth) Spreng.	β- Caryophyllene
20	*Leucas prostrata* (Hook.f.) Gamble	Selin-11-en-4α –ol
21	*Leucas rosmarinifolia* Benth	Hexadecanoic acid
22	*Leucas stelligera* Wall.ex Benth.	β- Caryophyllene
23	*Leucas stricta* Benth.	β- Caryophyllene
24	*Leucas urticifolia* (Vahl) Sm.	Germacrene D
25	*Leucas wightiana* Wall.ex Benth.	β- Caryophyllene
26	*Pogostemon benghalensis* (Burm.f.) Kuntze	7-Isopropyl-1,4-dimethyl-2-azulenol
27	*Pogostemon mollis* Benth.	Calamenene
28	*Pogostemon quadrifolius* (Benth.) F. Muell.	T-Cadinol
29	*Pogostemon speciosus* Benth.	*α*-Bisabolol
30	*Pogostemon wightii* Benth.	*α*-Bisabolol

5.A.2. GC-MS Analysis

Leaf essential oil of five *Pogostemon* species were studied. In *P. benghalensis* 19 components were characterized, representing 79.93 ± 8.11% of the total oil. In *P. benghalensis*, 7-Isopropyl-1,4-dimethyl-2-azulenol (47.8%) was the major compound. This is a unique compound seen in *P. benghalensis* only and thus 7-Isopropyl-1,4-dimethyl-2-azulenol can be regarded as a chemical marker for *P. benghalensis.* The other unique compounds in *P. benghalensis* was α- Ylangene, 6,9- Guaiadiene, γ-Elemene, 4,6- Guaiadiene, Isochamaecyrone, Guaiol and 6- Methoxy-2-(1-bten-3-yl)naphthalene. In *P. speciosus* 44 components were characterized, representing 93.9% of the total oil whereas 45 components were characterized, representing 97.76±0.23 % of the total oil in *P. wightii*. The major compound in both *P. wightii* and *P. speciosus* was α-Bisabolol which was a natural monocyclic sesquiterpene alcohol. Another major component present in both the species was Cubebol, which was also a sesquiterpene alcohol. *P. mollis* was rich in Calamenene, a sesquiterpene. The unique compounds present only in *P. mollis* were γ- Terpinene, α- Gurjunene, α-Bergamotene, Bicyclosesquiphyllandrene, Cadina 1,4-diene, Zingiberenol and Isopimara 8,5-diene. On the basis of chemical data, *P. mollis* appears closely related to *P. speciosus* and *P. wightii*. A total of 31 components were characterized in *P. quadrifolius*, representing 96.23±1.02% of the total oil in which T-Cadinol, a sesquiterpene was the major compound. Aristolene, Isocaryophyllene, Calarene, 14-Hydroxy-β-caryophyllene, Borneol, α-Cadinene, Carvacrol, Caryophylladienol I, Caryophyllenol II were the unique compounds in *P. quadrifolius.*

In the genus *Leucas*, the leaf essential oil of 20 taxa were analyzed. Many unknown compounds were found and a majority of them are diterpenes. GCMS results show a wide variety of components in genus *Leucas.* In *L.*

ciliata and *L. marrubioides* var. *pulneyensis*, 10 compounds were characterized representing 69.26±2.60% and 93.25±1.90% of the total oil respectively. In both species β- Caryophyllene was the major compound and 1-Heptadecene was the unique compound in *L. ciliata.* Monocyclic sesquiterpene like α-Humulene was present in both species in a higher quantity. In *L. lavandulifolia,* 10 compounds were characterized representing 94.56±0.95% of the total oil in which β- Caryophyllene was the major compound.

An endemic variety, *L. lavandulifolia* var. *nagalapuramiana* where taxonomy was in confusion was also studied and it was found that an unknown compound, Bp 191 M+ 290 was the major compound in it. A total of 8 compounds, representing 96.3±0.98% of the total oil were found, among them majority of the compounds were unknown and they are yet to be identified. In *L. biflora*, a total of 12 compounds, which includes some unknown compounds also, representing 96.7±0.14% of the total oil were found. Phytol was found to be the major compound.

In *L. montana*, 14 compounds, representing 95.83±3.28% of the total oil were found. Similarly in *L. aspera* also a total of 14 compounds were found, representing 92.1% of the total oil. In both species some unknown compounds were noticed and β- Caryophyllene was the major compound. In the endemic species *L. lanceaefolia*, α- Humulene was the major component, consist of 13 compounds, including some unknowns. This represent 88.8% of the total essential oil. α- Humulene was followed by the compound β-Caryophyllene.

L. eriostoma and *L. stelligera* were coming under same section when their morphological characters were considered. Similarly chemically also they show much similarity in their profiles. But they were different in their major components. In *L. eriostoma,* an unknown compound called Bp 191

M+290 was the major compound whereas in *L. stelligera,* β- Caryophyllene was the major compound. Including some unknown compounds, a total of 12 compounds representing 95.1% of the total oil was found in *L. eriostoma* whereas 14 compounds representing 88.9% of the total oil was found in *L. stelligera*. Alkenyl alcohol like oct-1-ene was present in both species in an optimum quantity.

In *L. stricta* and *L. angularis,* β- Caryophyllene was the major compound. Similarly α- Humulene was the second largest component in both taxa treated. And many of the other compounds present in both species were somewhat similar. A total of 9 compounds were characterized, representing 91.5% of total oil and 16 compounds with some unknowns were identified representing 82% of the total oil, in *L. stricta* and *L. angularis* respectively. In *L. hirta* also β- Caryophyllene was the major compound which was followed by α- Humulene. In this species, 16 compounds were characterized representing 86.63±1.41% of the total oil.

In *L. rosmarinifolia*, A total of 15 compounds, with some unknown compounds, representing 92.7±0.84% of the total oil were identified and found that Hexadecanoic acid, a fatty acid was the major compound, followed by an unknown compound called Bp 273 M+288. The essential oil of *L. rosmarinifolia* was different in having unique compounds like Tetracosane, Pentacosane and some unknown diterpenes. Altogether 14 compounds including some unknowns were identified in *L. urticifolia* representing 96.8% of the total oil. A sesquiterpene, Germacrene D was regarded as the major compound. Phytol and β- Caryophyllene were the other major components in both species.

In the African species *L. martinicensis*, Germacrene D was the major compound followed by β- Caryophyllene. A total of 12 compounds were characterized in *L. martinicensis* representing 90.03±0.28% of the total oil. In

L. prostrata, Selin-11-en-4- α-ol was found as the major compound, which was followed by β- Caryophyllene. A total of 14 compounds representing 81.9% of the total oil were found which include some unknown compounds also. Bp 135 M+220 was very specific to *L. prostrata* only.

In the endemic *L. lanata* var. *candida* and *L. wightiana* the major compound was β- Caryophyllene. Altogether 15 compounds, representing 90.2% of the total oil and 20 compounds including some unknown compounds, representing 93.6% of the total oil were characterized in *L. lanata* var. *candida* and *L. wightiana* respectively. In *L. lanata* var. *candida* β- Caryophyllene was followed by the compound β- Selinene whereas in *L. wightiana* β- Caryophyllene was followed by the compound Selina-4(15)-7(11)-diene. In *L. helianthimifolia,* a total of 23 compounds including some unknown compounds, representing 90.53±1.40% of the total oil were found. *L. helianthimifolia* was also rich in β- Caryophyllene like majority of *Leucas* species. Compounds like (Z)-β-Farnasene, Zizanene, Pentadecane, (E,E),α-Farnasene, Geranylacetate, (6S,7R)-Bisabolene and Hexacosane were found specific to *L. helianthimifolia.*

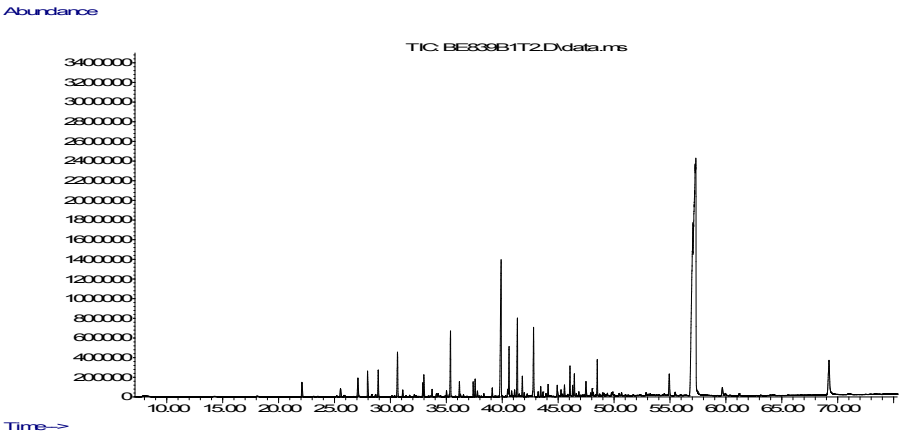
The genus *Leonotis* is represented by a single species in South India. The leaf essential oil of *Leonotis nepetifolia* was analyzed and found that Germacrene D was the major compound, followed by β- Caryophyllene. A total of 15 compounds including some unknowns were found, representing 94.76±3.51% of the total oil. Tetradecanal was found to be the specific compound in *Leonotis nepetifolia.*

Leaf essential oil of two species of *Anisomeles*; *A. heyneana* and *A. malabarica* were studied. An acyclic diterpene alcohol, Phytol was the major compound in *A. heyneana* whereas an unknown compound M+ 286 Diterpene was the major compound in *A. malabarica.* Almost 9 compounds including some unknown compounds were recognized in *A. heyneana* representing

84.93±5.55% of the total oil and 22 compounds including some unknown compounds were found in *A. malabarica*, representing 88.36±1.65% of the total oil. Styrene, Dimethyltetradecane, Neocembrene A, epi- Manoyloxide, Bp109 M+272 and some unknown diterpenes were the specific compounds identified in *A. malabarica.*

In the present study, in *Colebrookea oppositifolia* and *Gomphostemma heyneanum* var. *heyneana* were the only taxa represented in this genus, the compound β-Caryophyllene was found to be the major constituent. A total of 16 compounds including some unknowns representing 88.6% of the total oil and 18 compounds including some unknowns representing 70.7% of the total oil were identified in *Colebrookea oppositifolia* and *Gomphostemma heyneanum* var. *heyneana* respectively. The compound α Bergamotene, unknowns like Bp 123M+208, Bp 191M+272, Bp 95, Bp119M+272 and 9-Geranyl-p-cymene were found to be specific component in *Colebrookea oppositifolia* whereas in *Gomphostemma heyneanum* var. *heyneana* the specific compounds were α Cydrene, β Cydrene, β Funebrene and Cedrol.

Figure 5.1. GCMS Chromatogram of species studied



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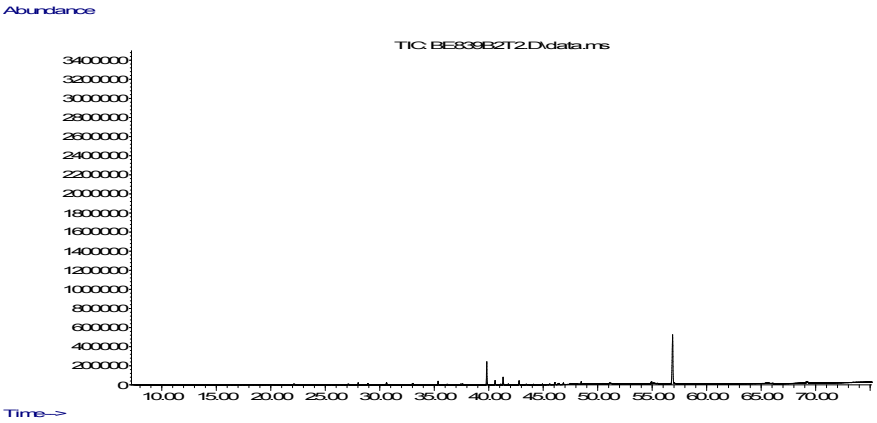

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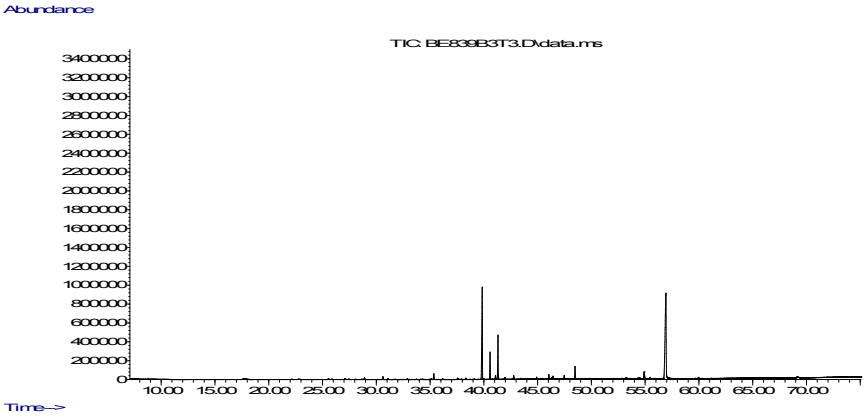
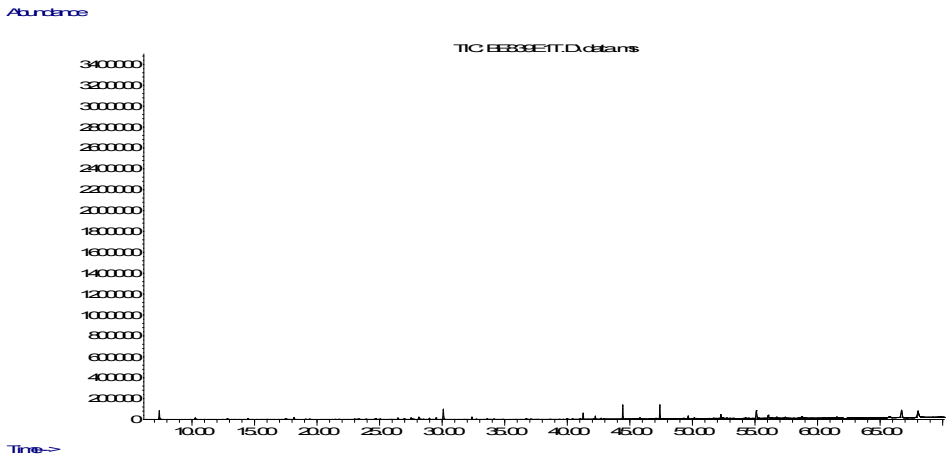


Anisomeles heyneana 3



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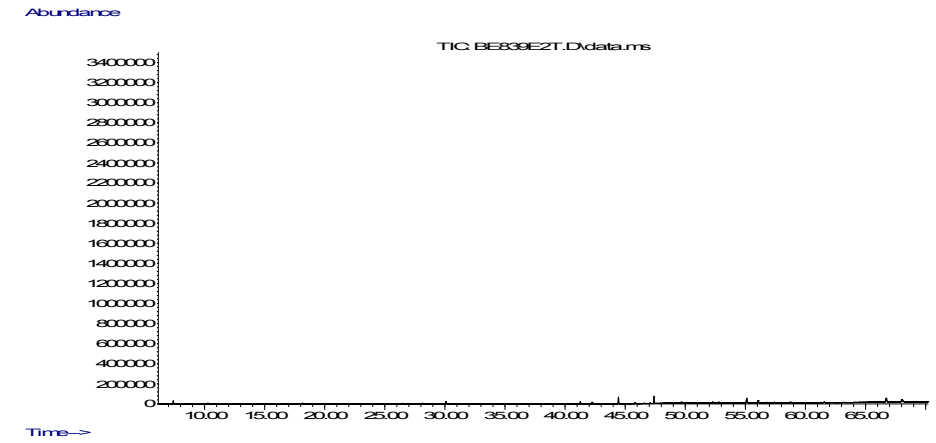

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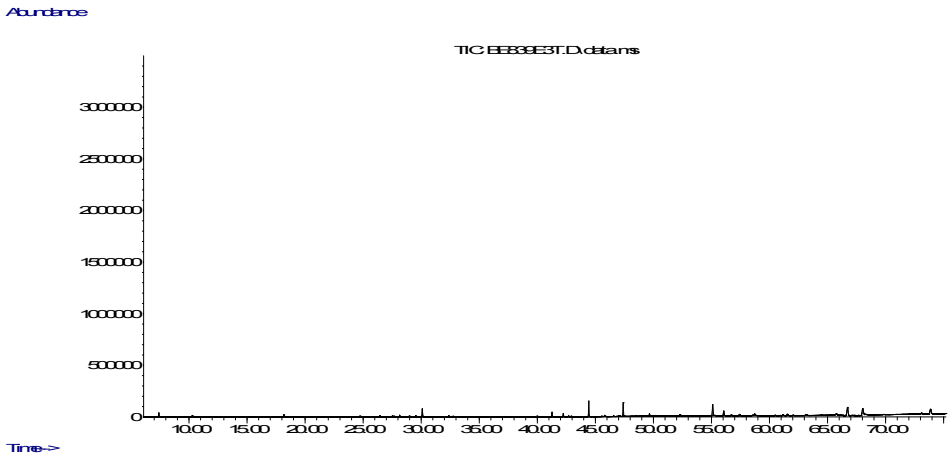
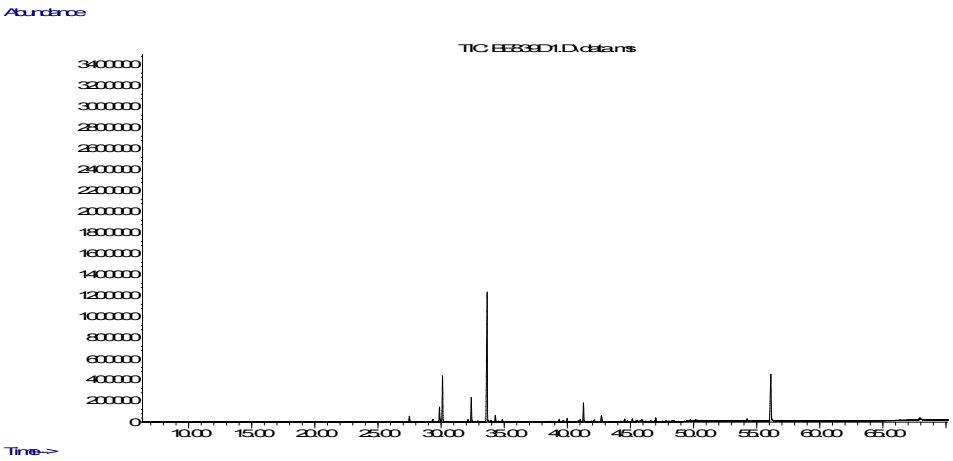
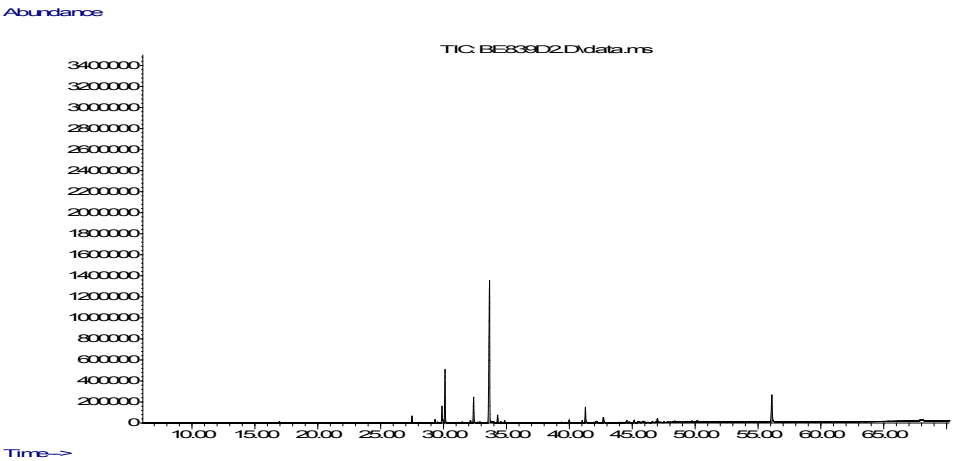


Anisomeles malabarica 3



Leonotis nepetifolia 1



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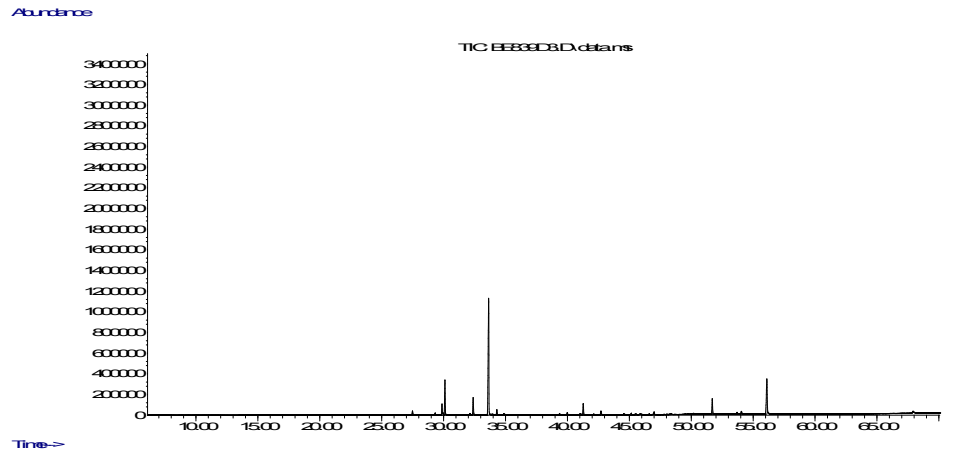

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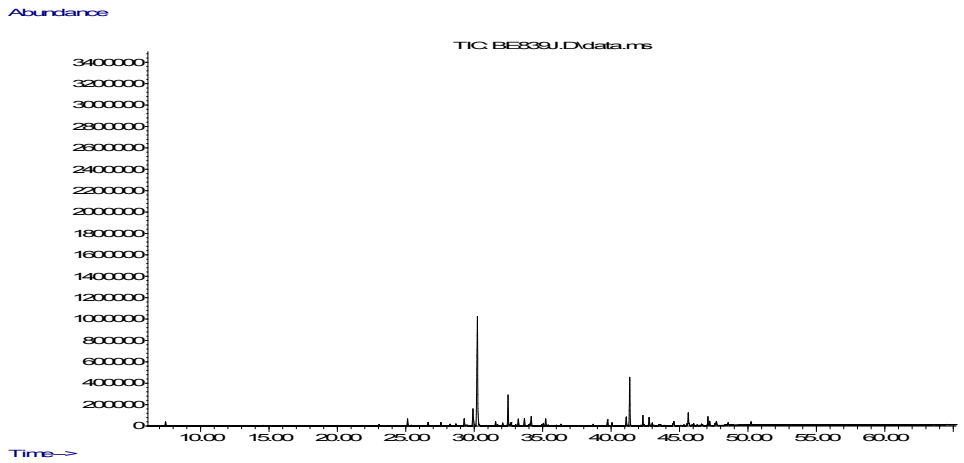
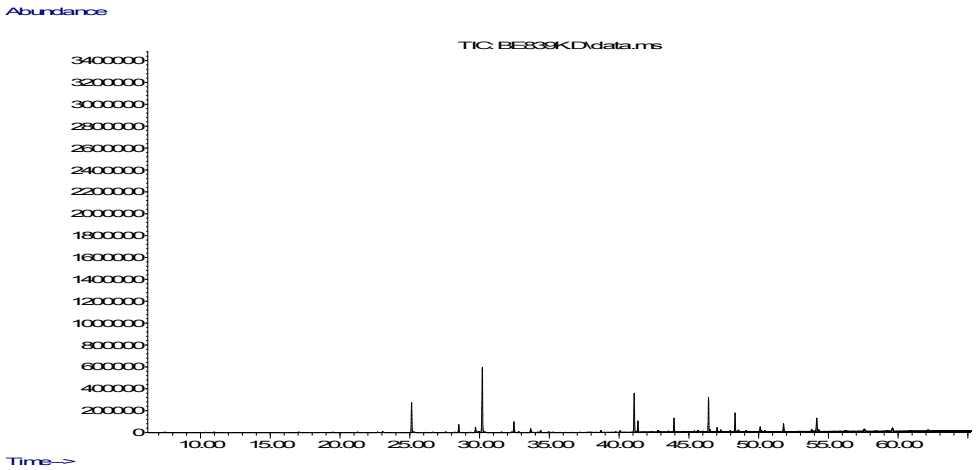
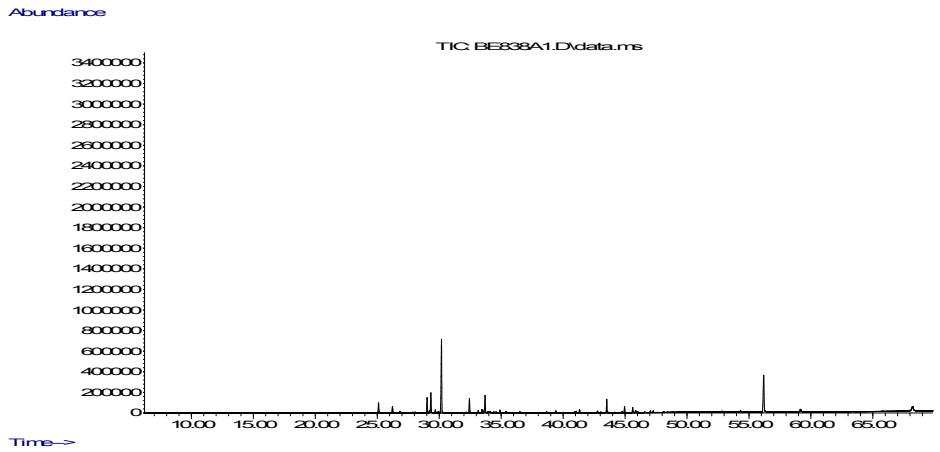


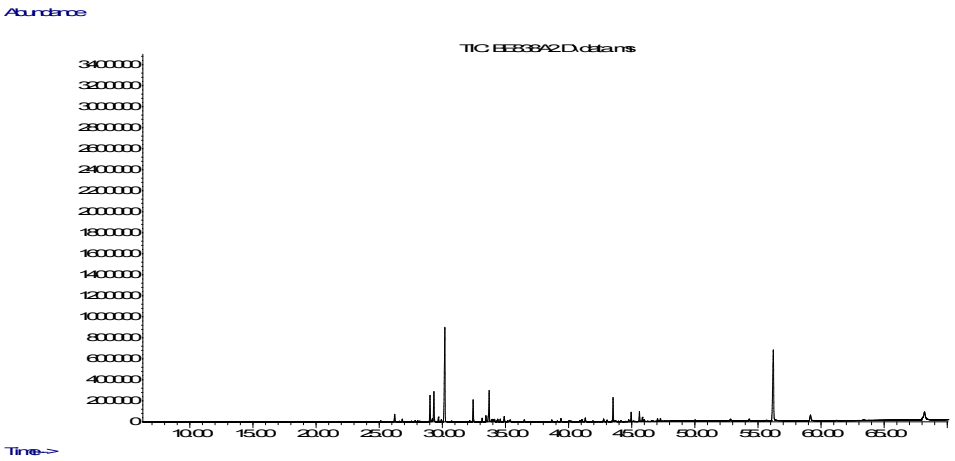
Gomphostemma heyneanum* var. *heyneana 1



Colebrookea oppositifolia 1



Leucas ciliata 1



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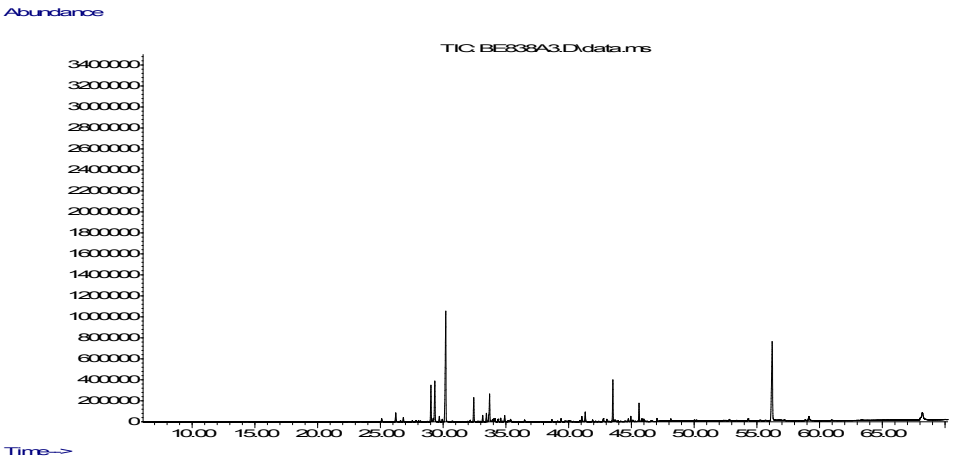

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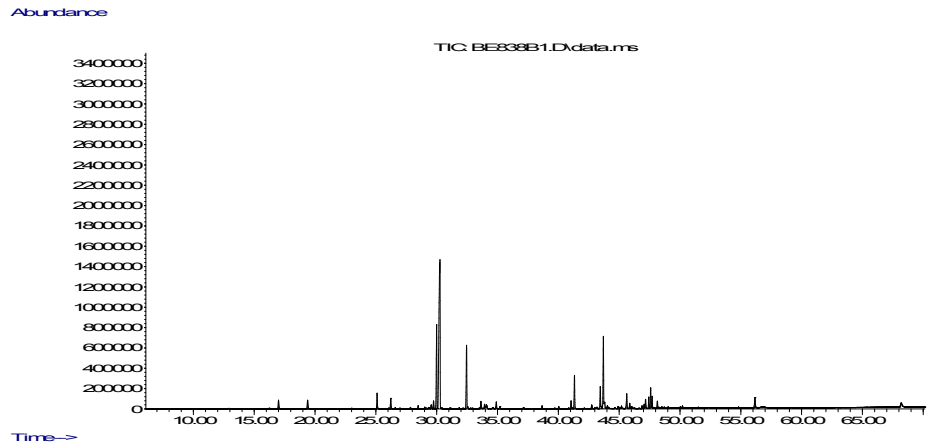
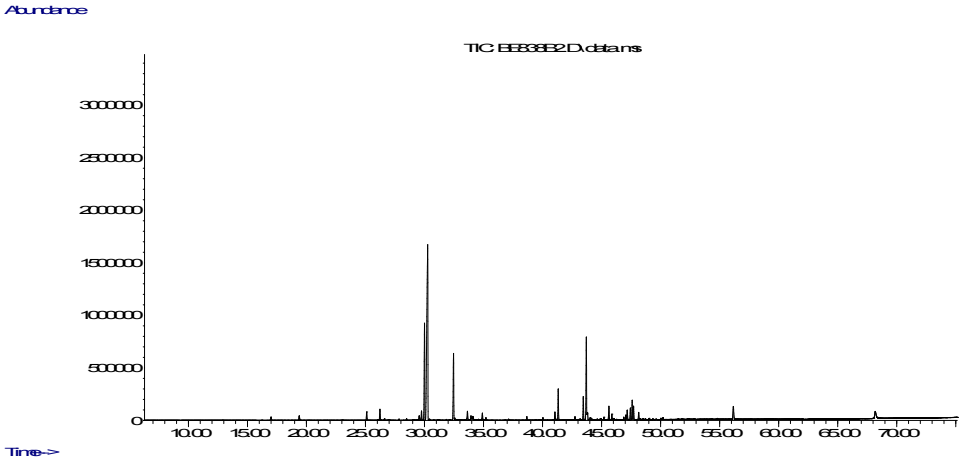


Leucas hirta 1



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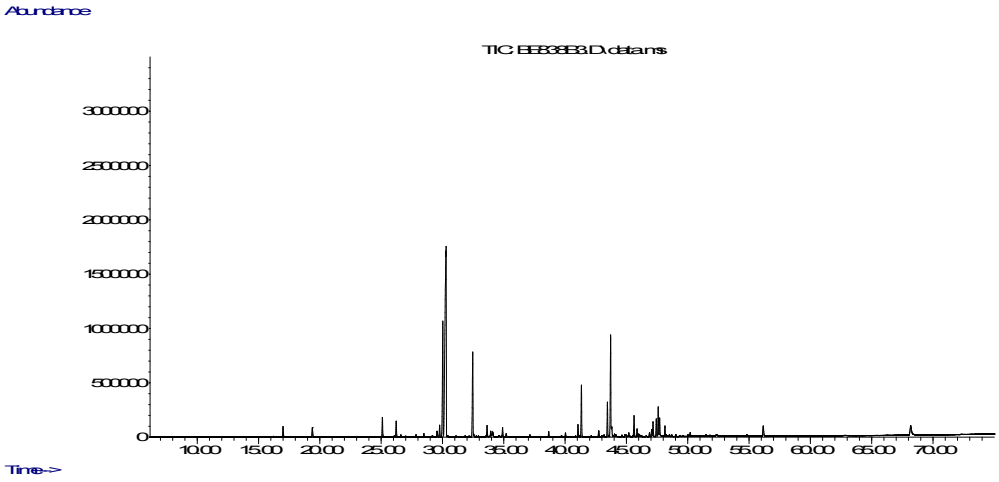

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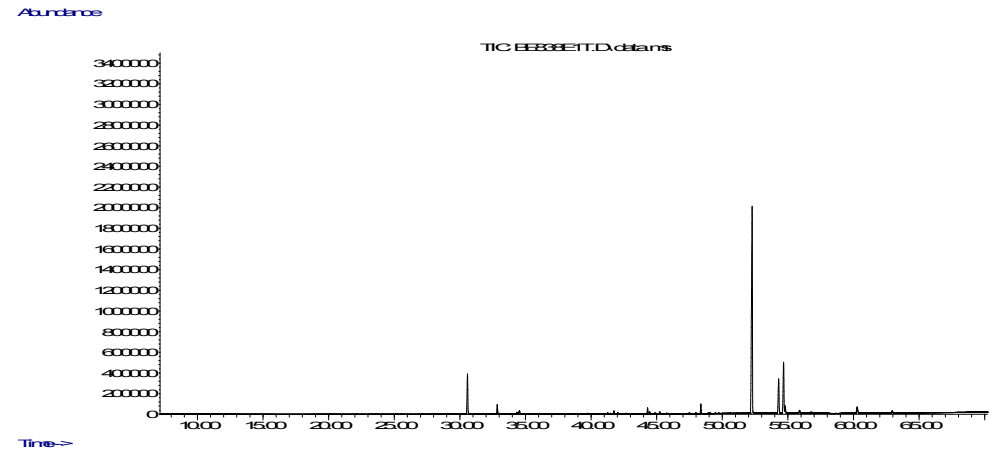
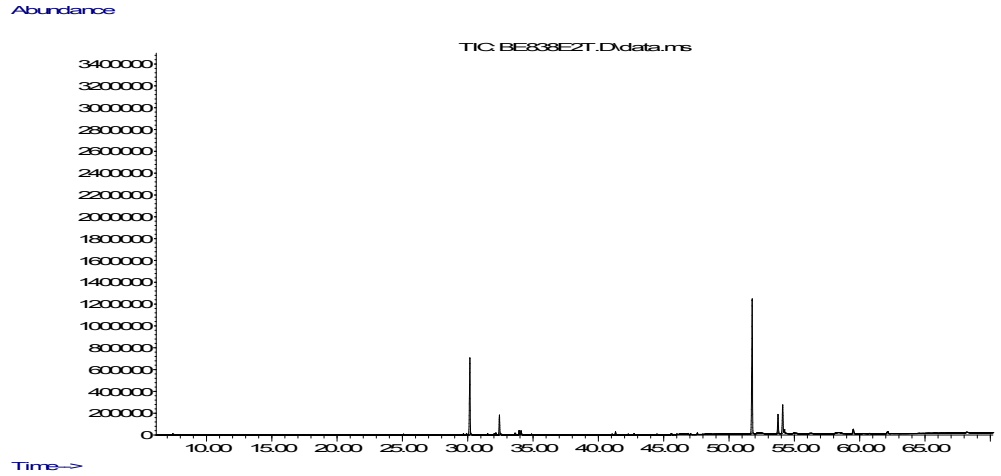


Leucas montana 1



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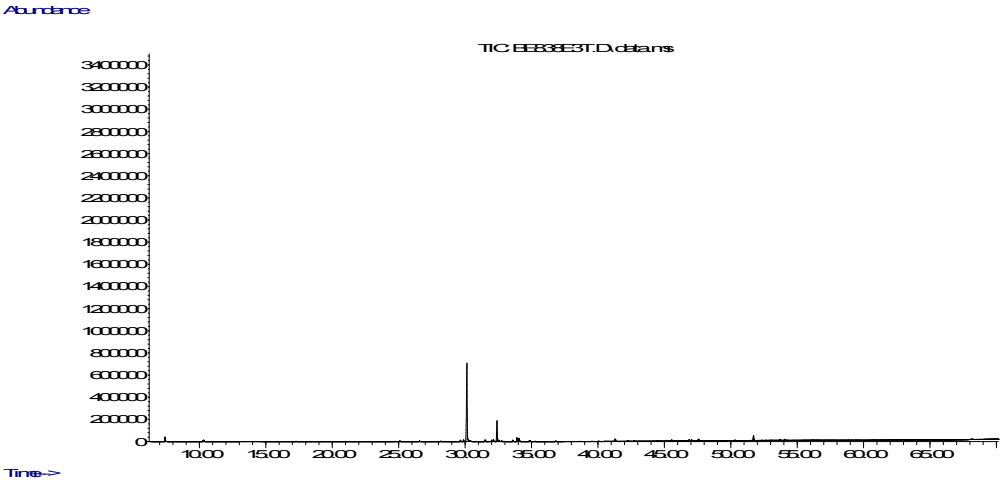

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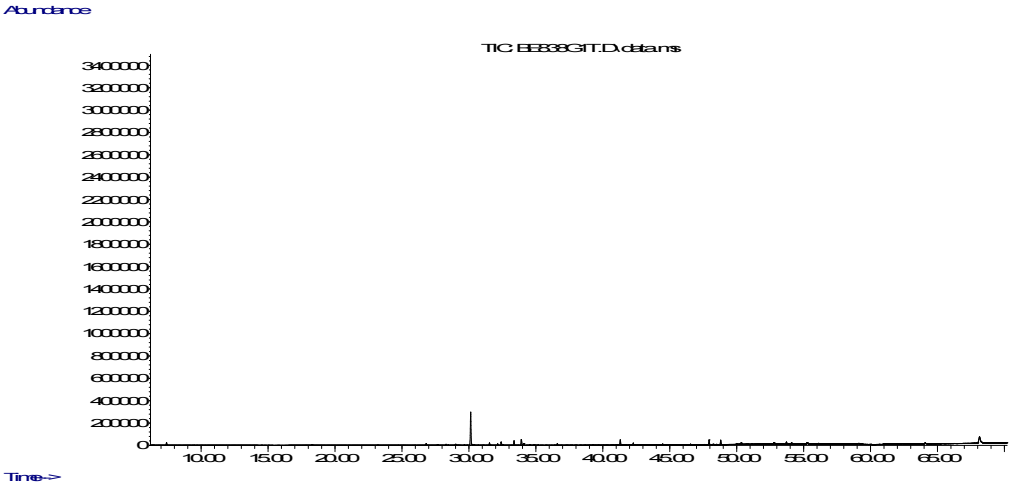
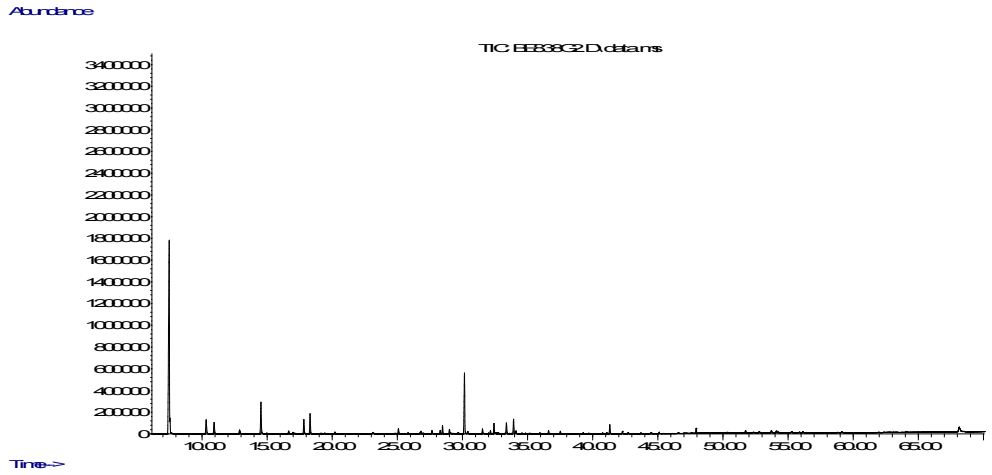


Leucas helianthimifolia 1



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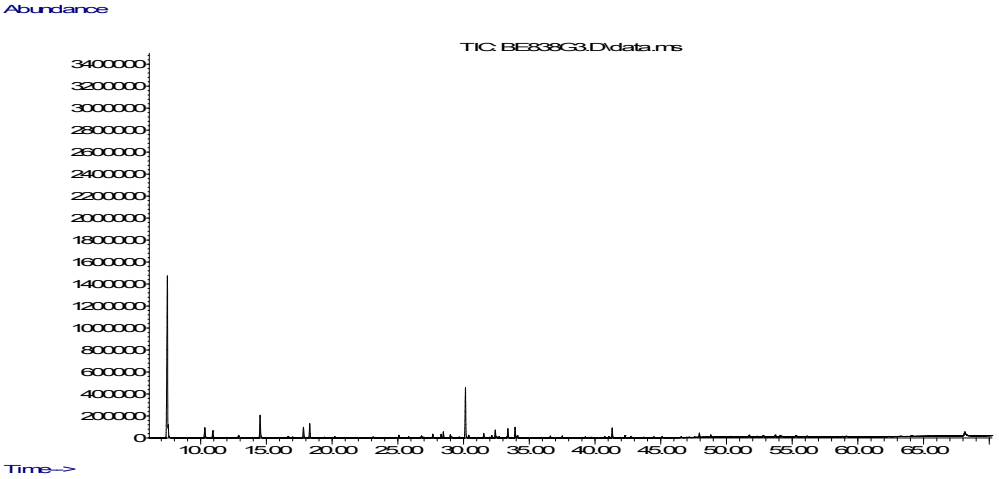

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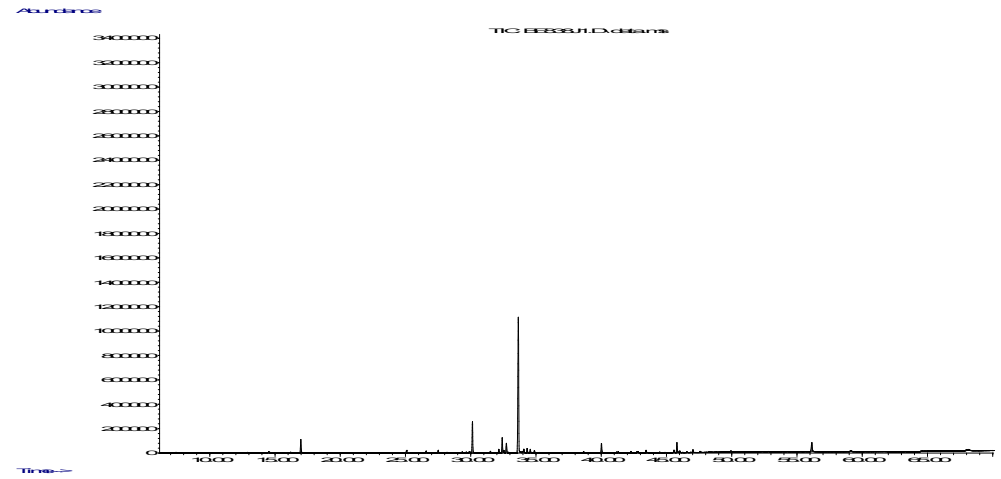
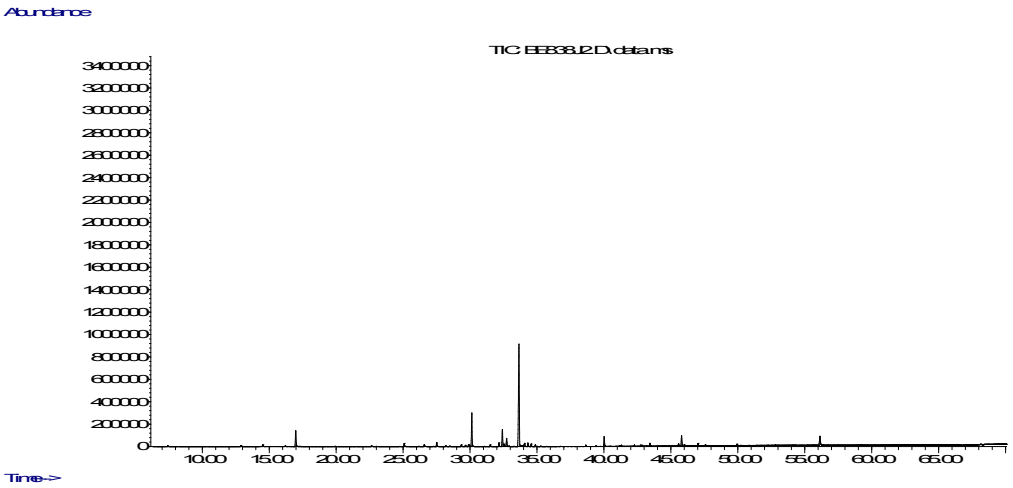


Leucas martinicensis 1



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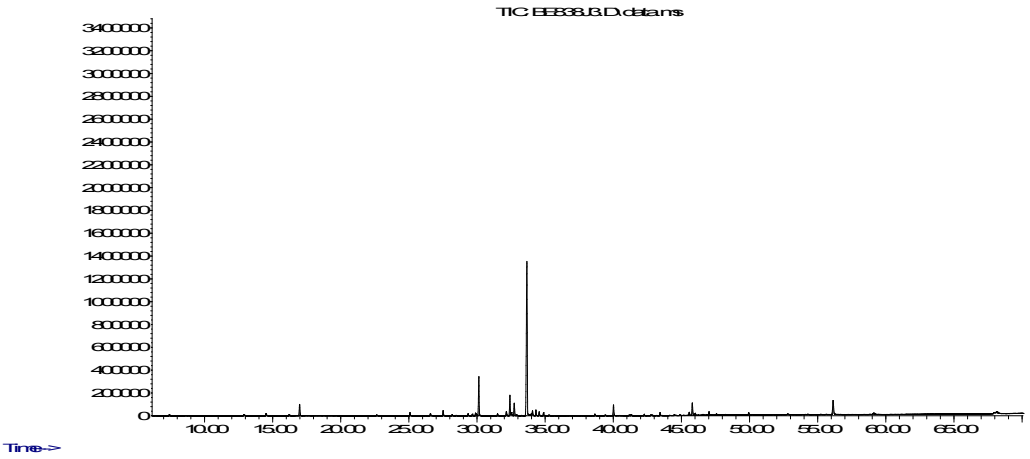

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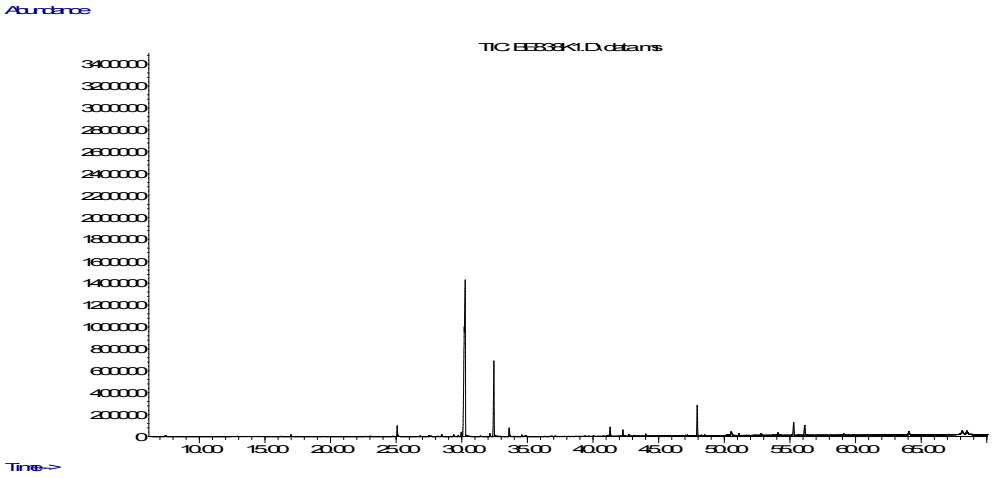

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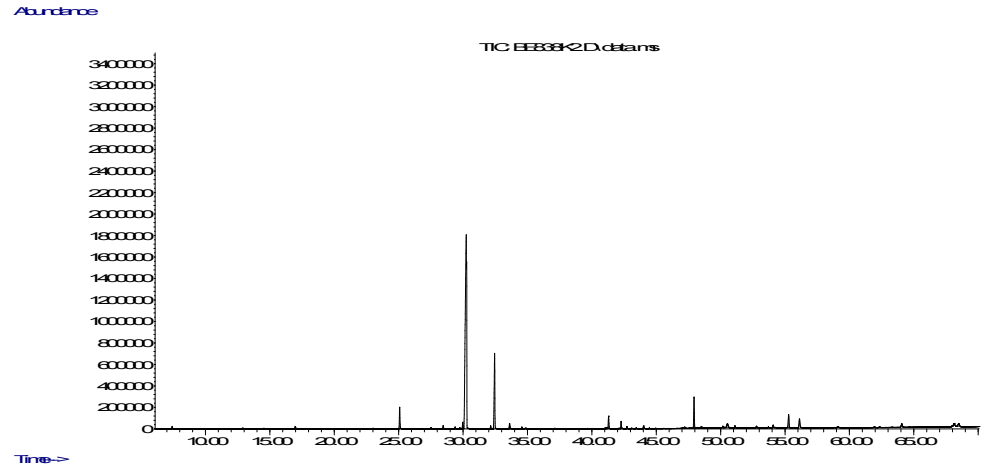

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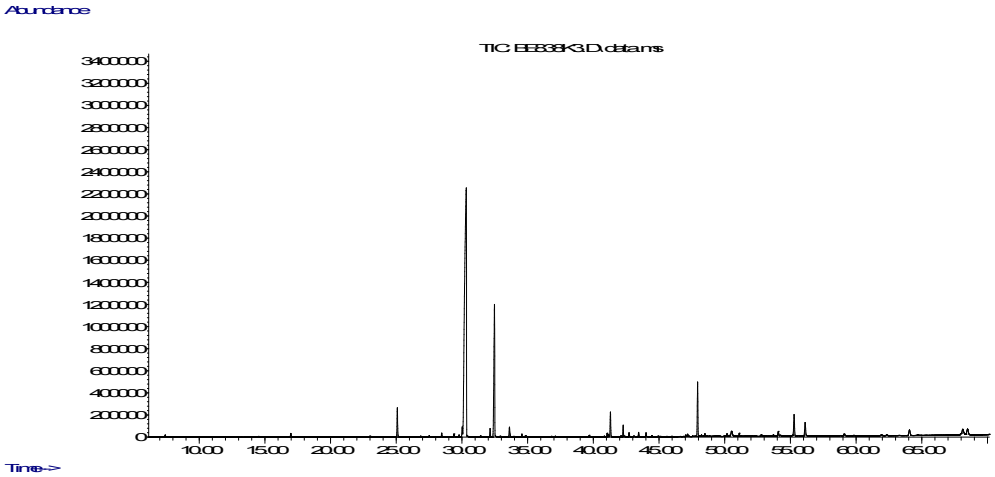

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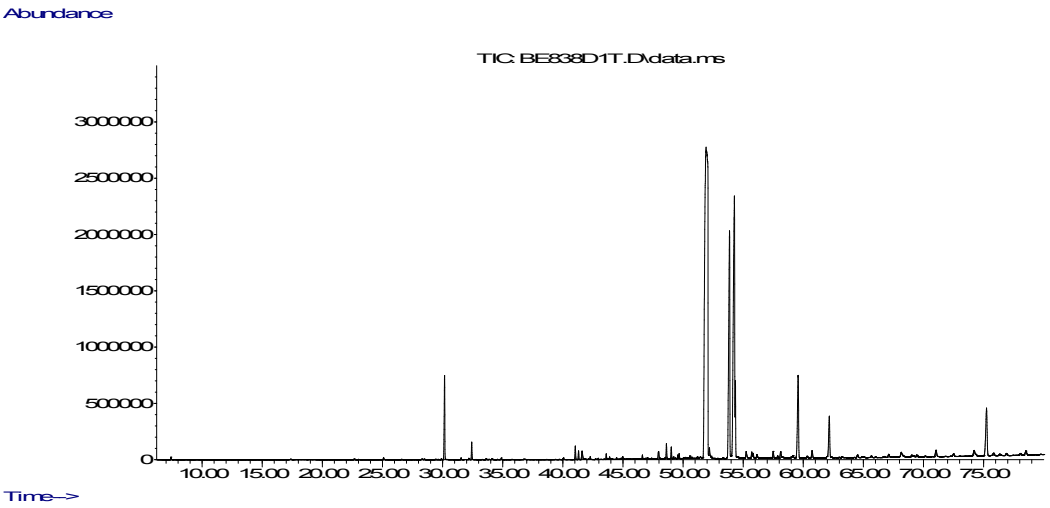

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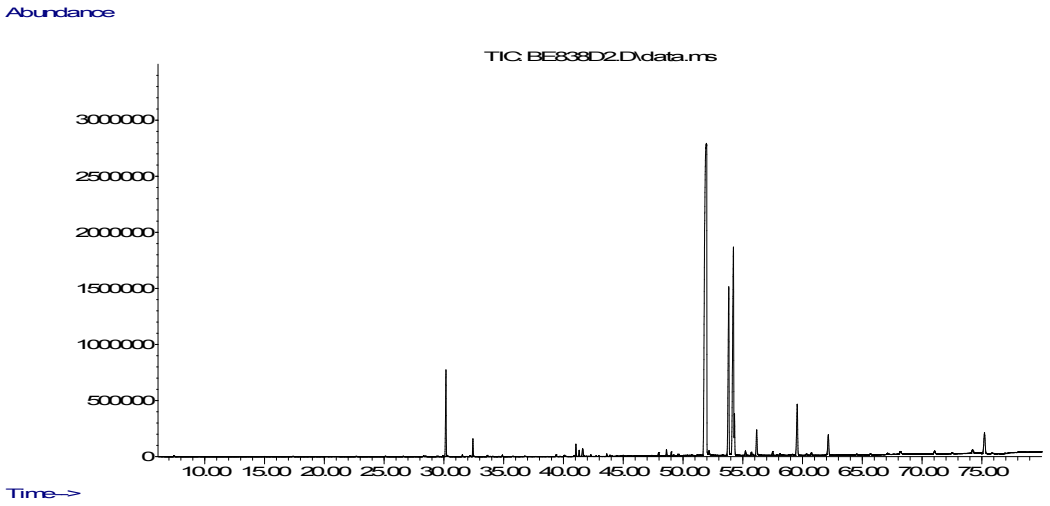
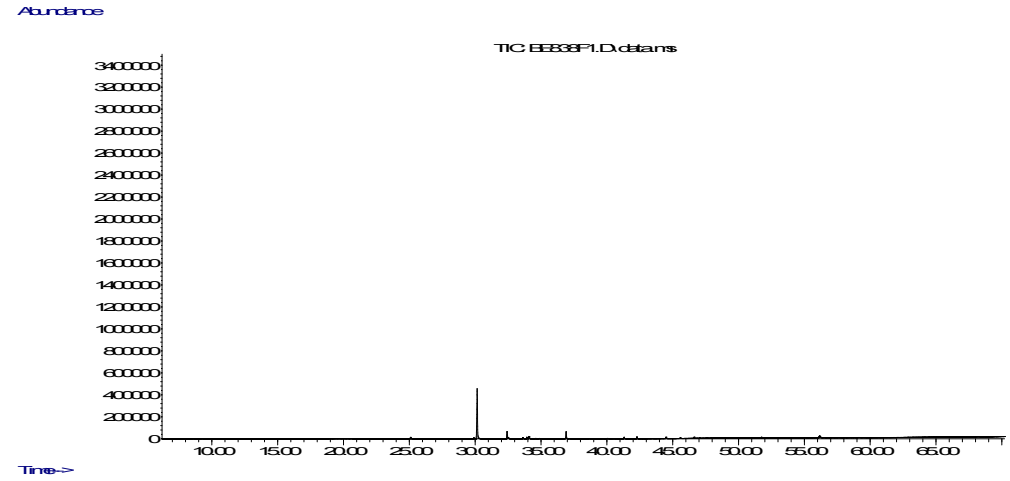
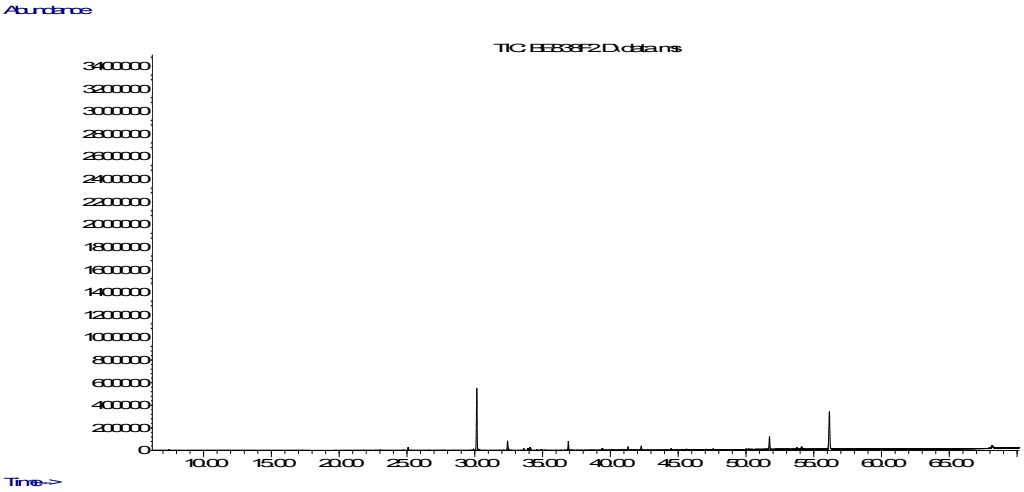


Leucas lavandulifolia* var. *nagalapuramiana 2



Leucas marrubioides* var. *pulneyensis 1



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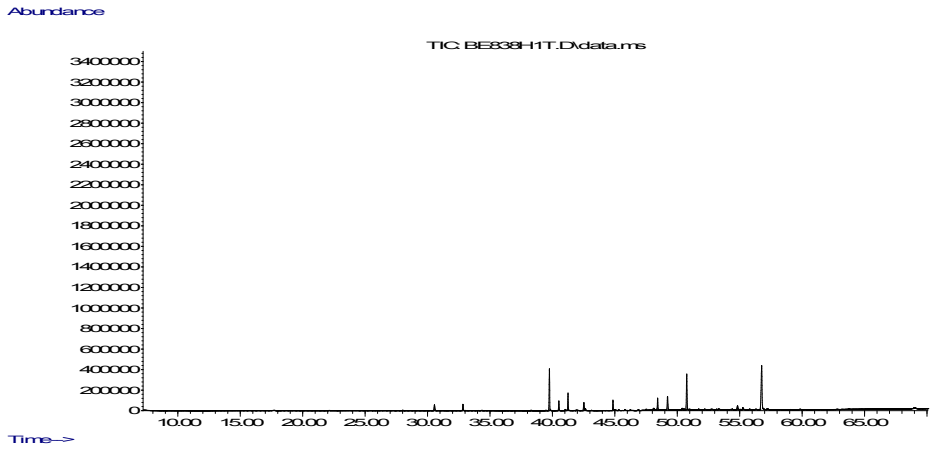

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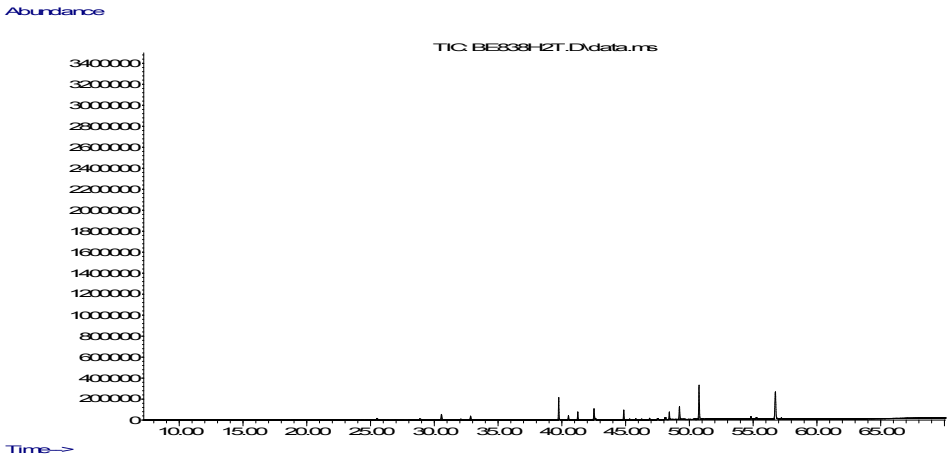

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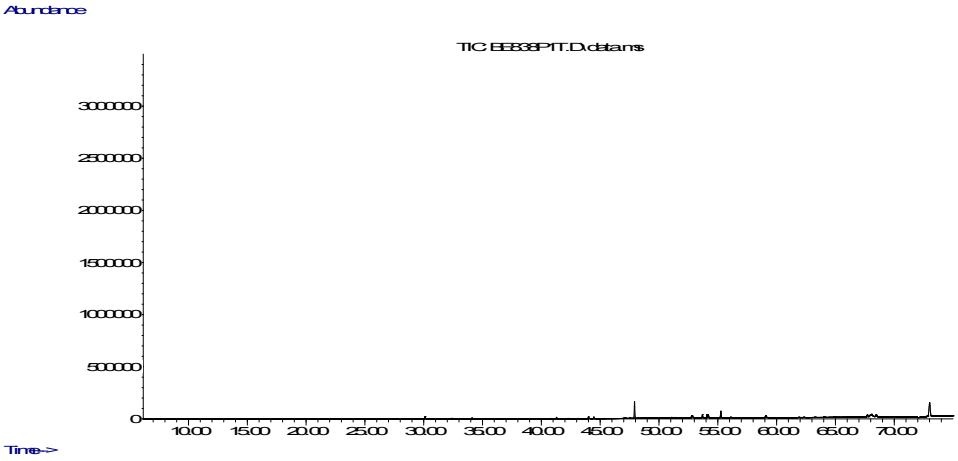

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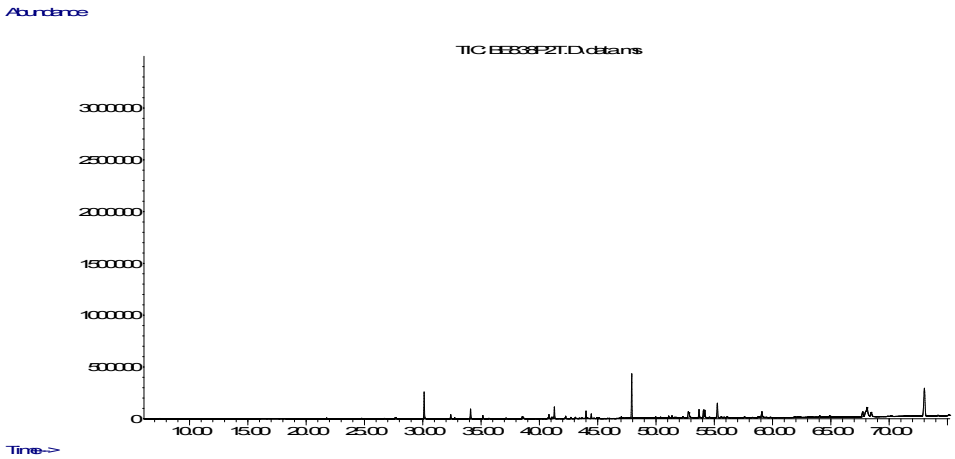
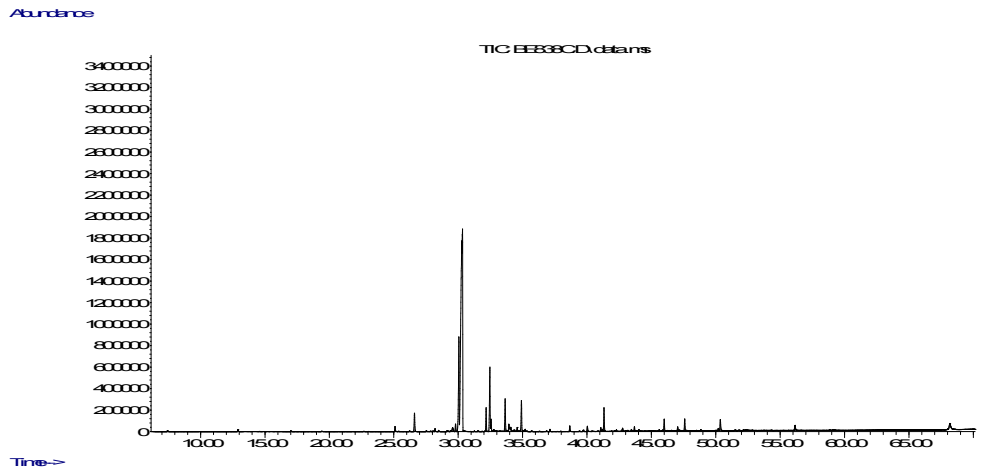


Leucas rosmarinifolia 2



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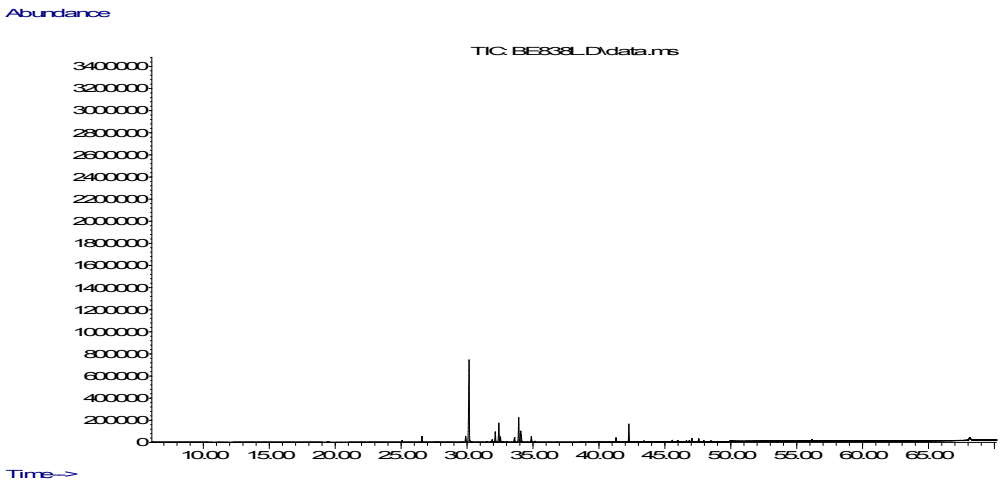
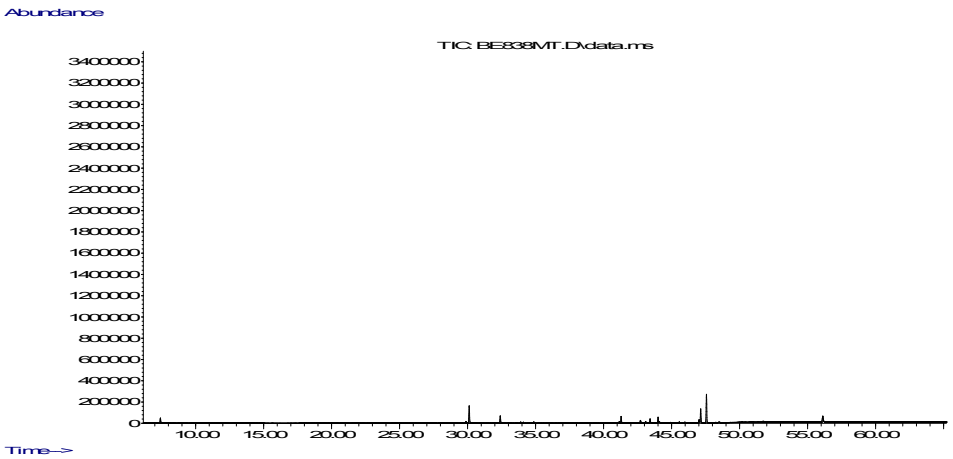
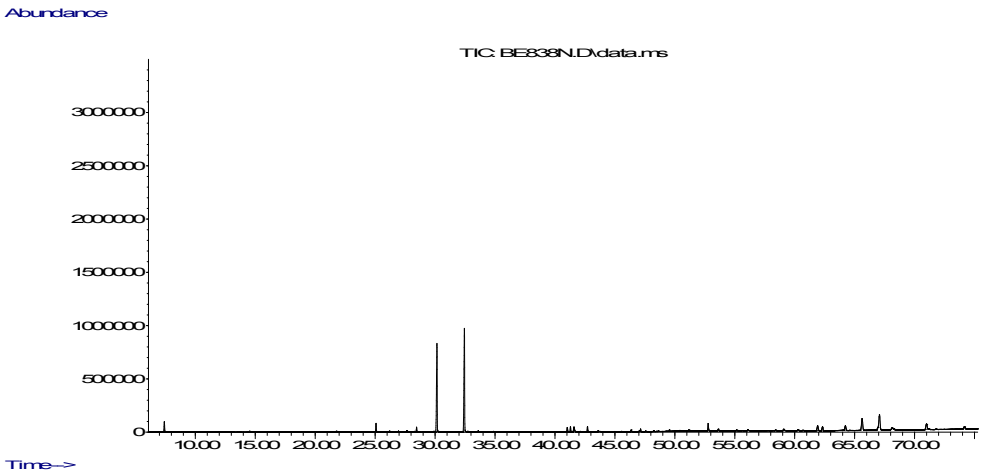


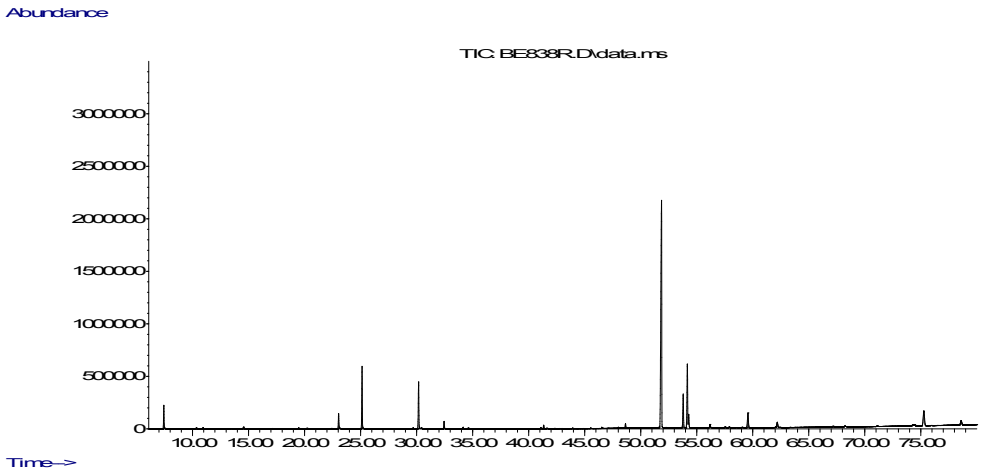
***Leucas lanata* var. *candida* 1**



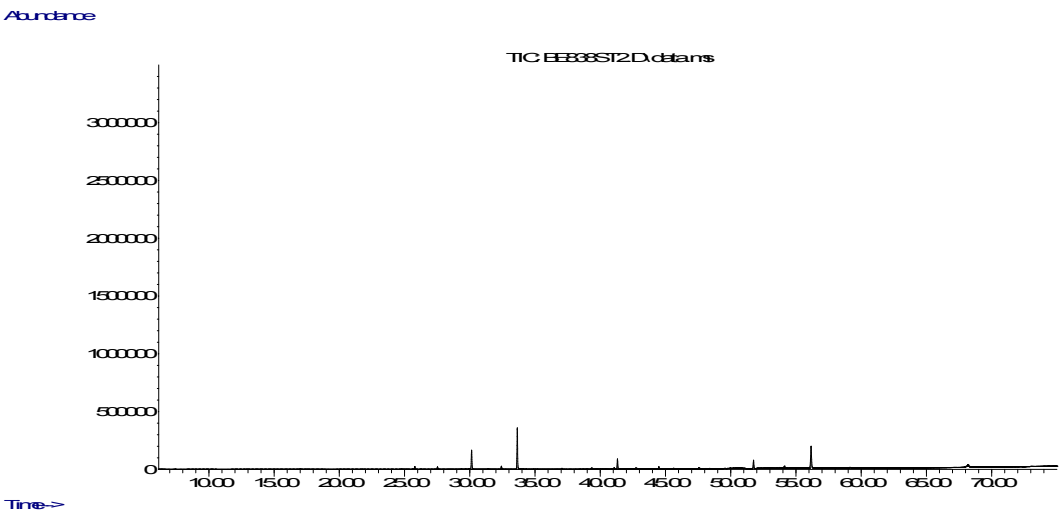
***Leucas prostrata* 1**



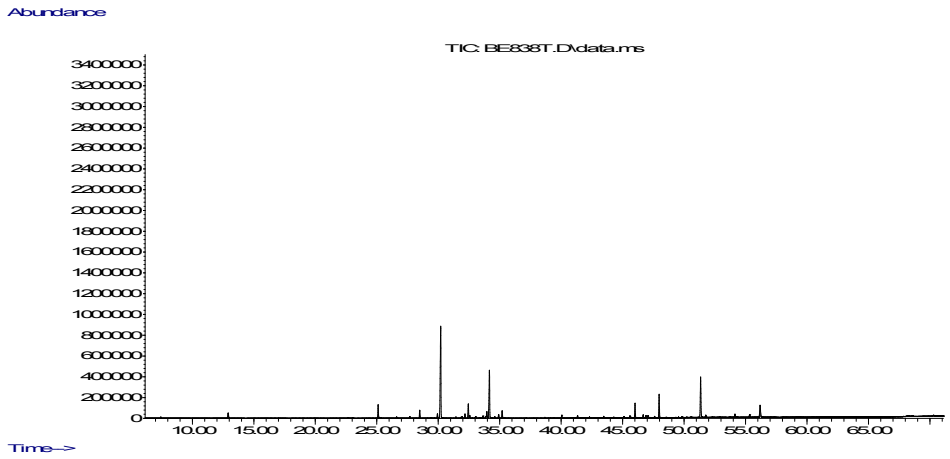
Leucas lanceaefolia 1



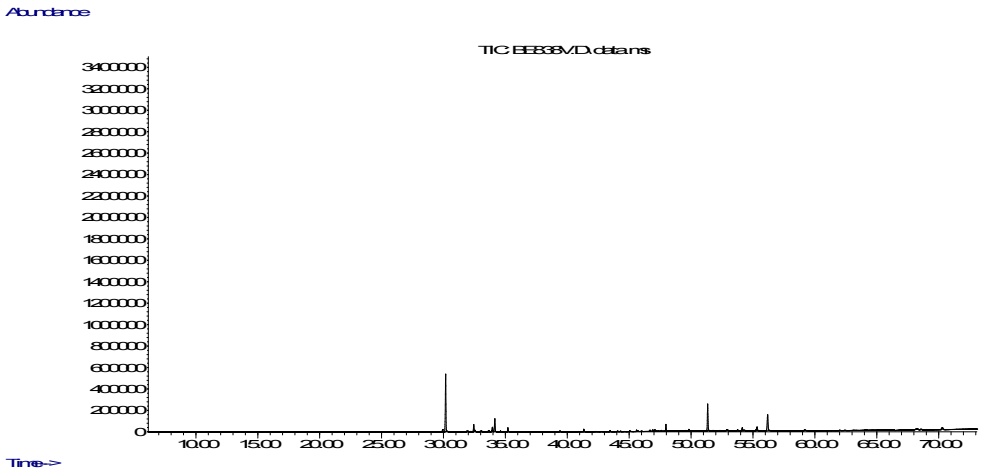
Leucas eriostoma 1



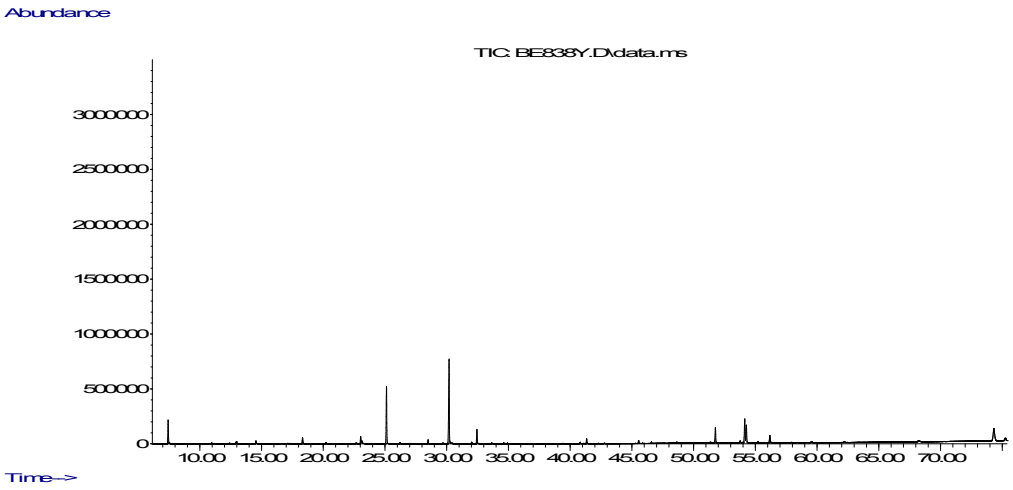
Leucas urticifolia 1



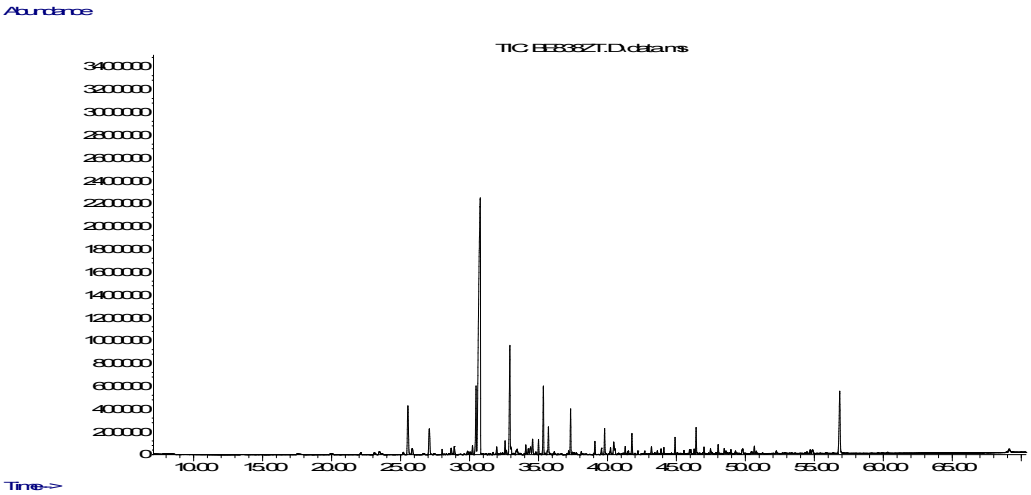
Leucas wightiana 1



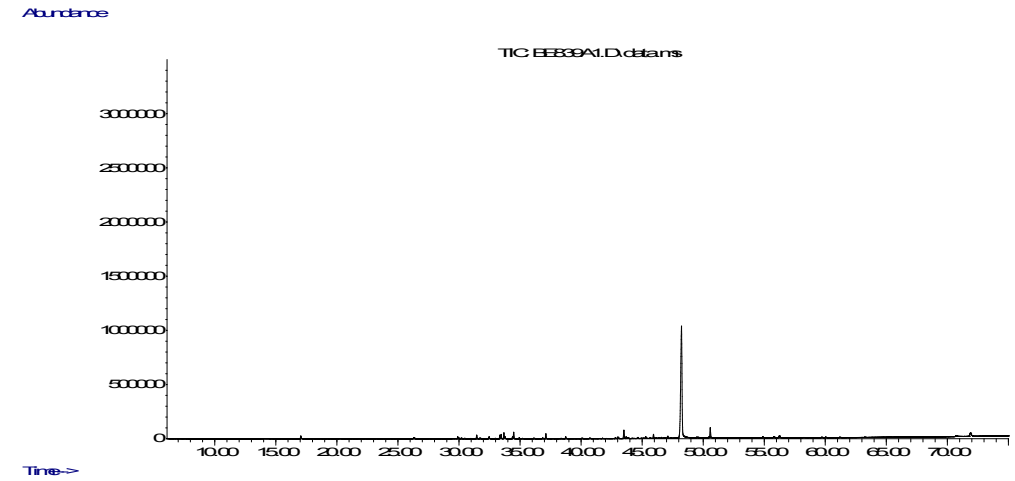
Leucas aspera 1



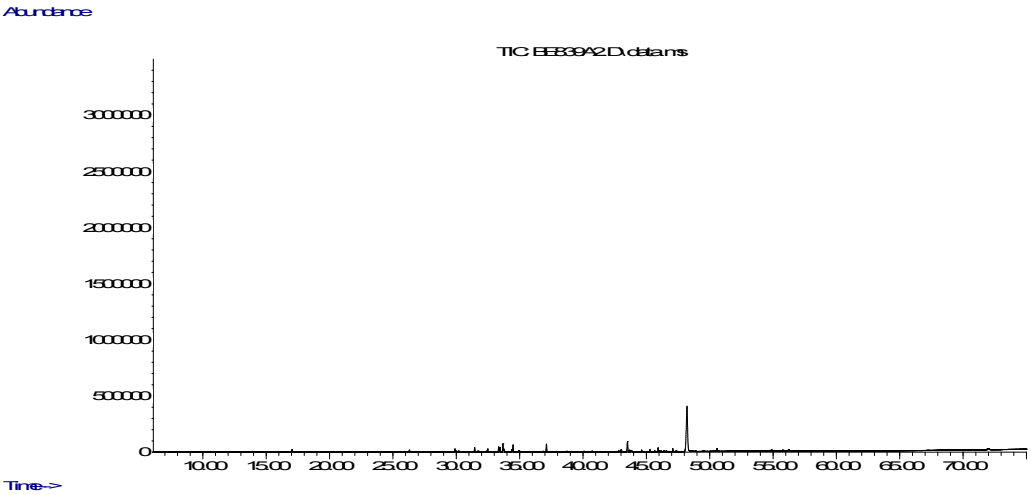
Leucas stelligera 1



Leucas angularis 1



Pogostemon benghalensis 1



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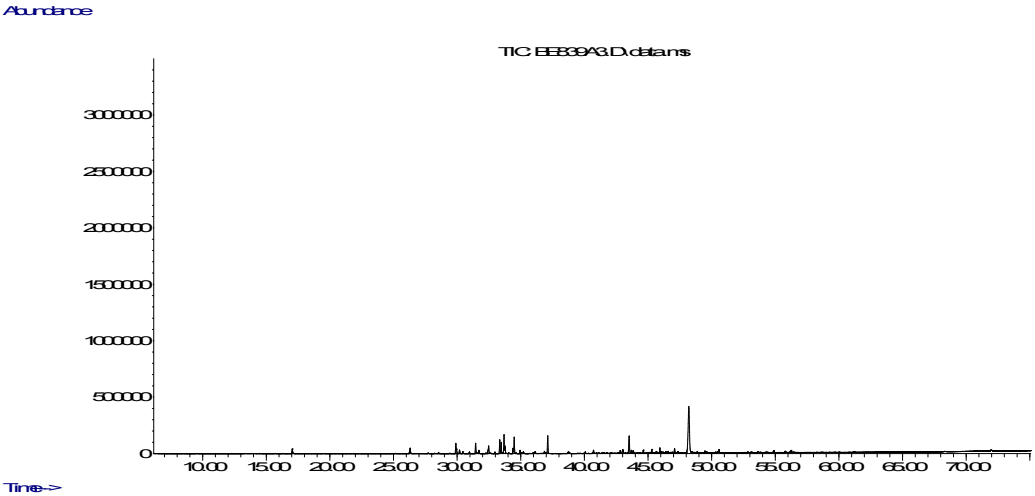
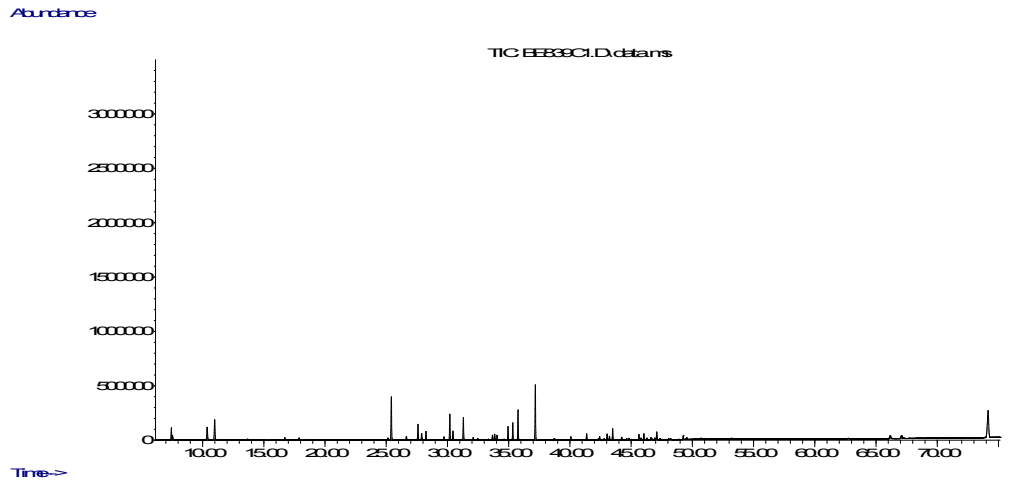
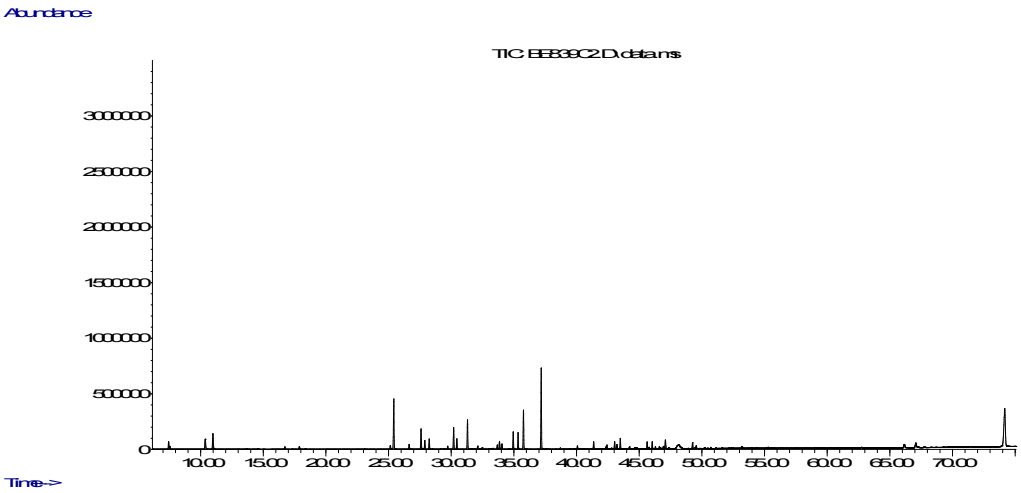


Pogostemon benghalensis 3



Pogostemon mollis 1



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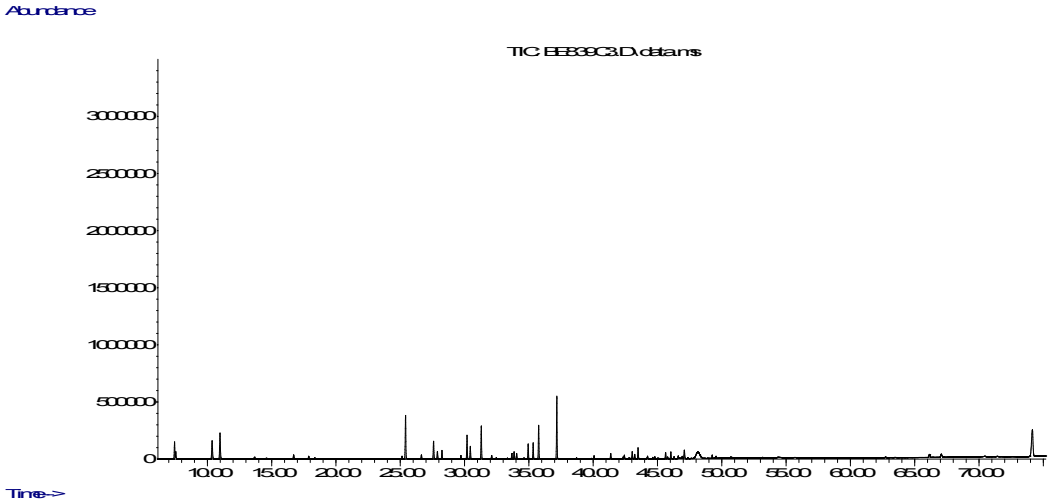

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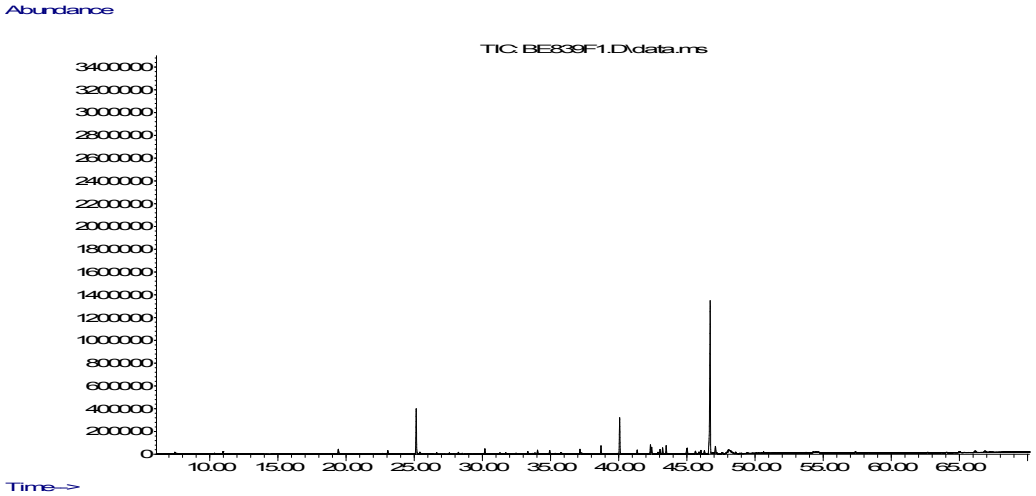
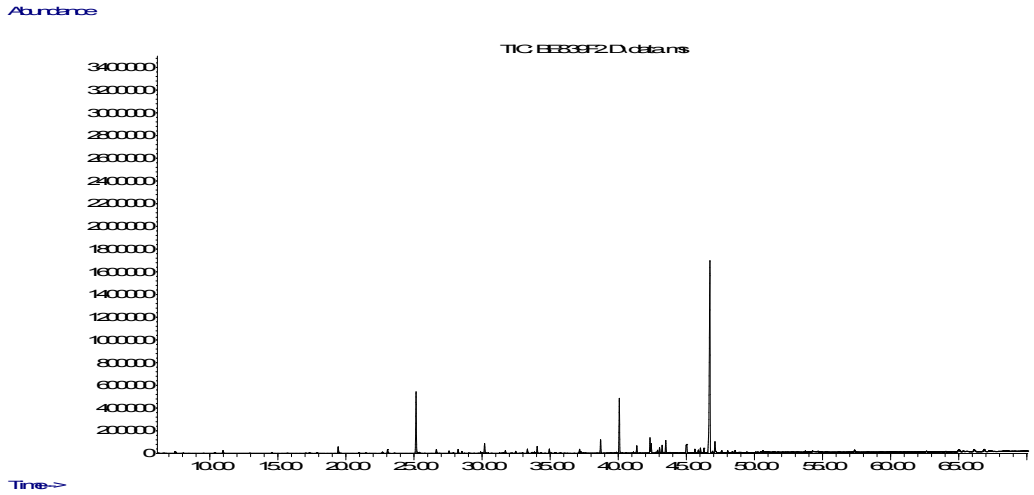


Pogostemon wightii 1



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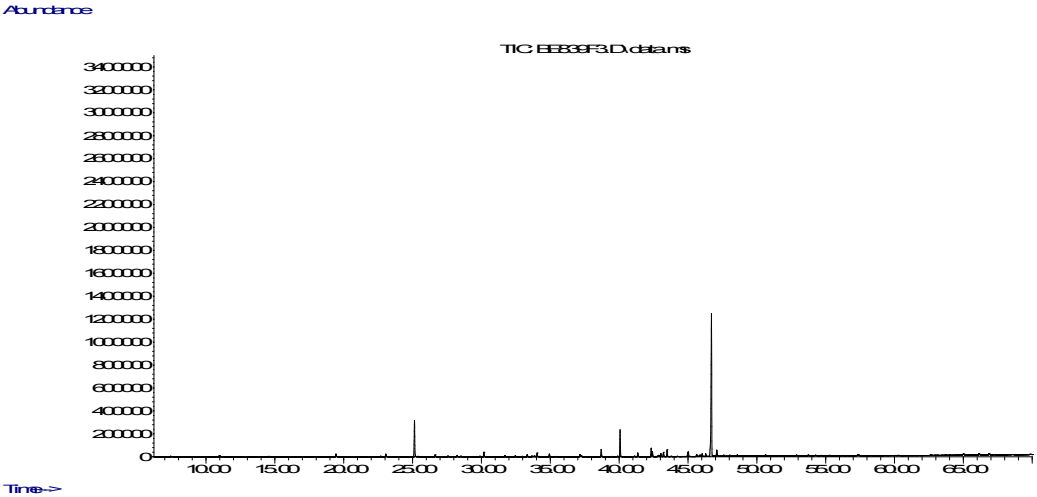

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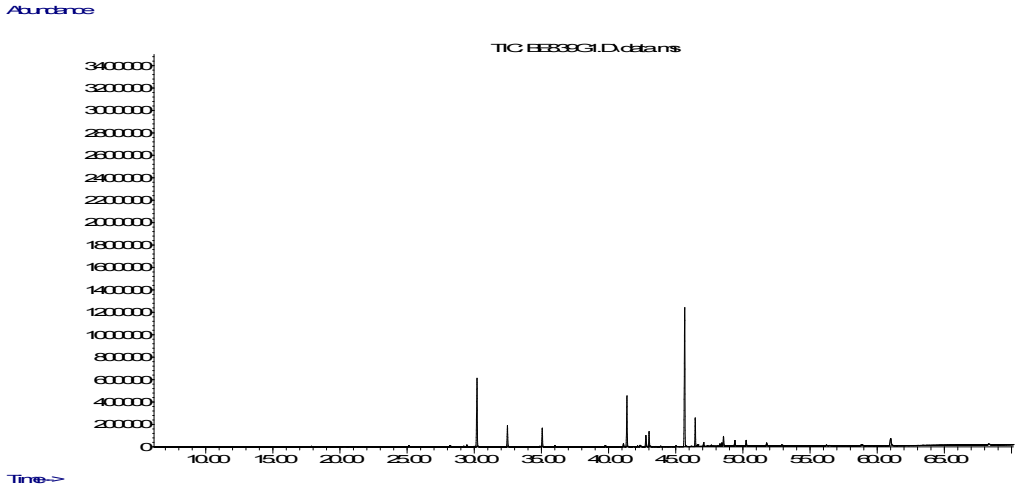
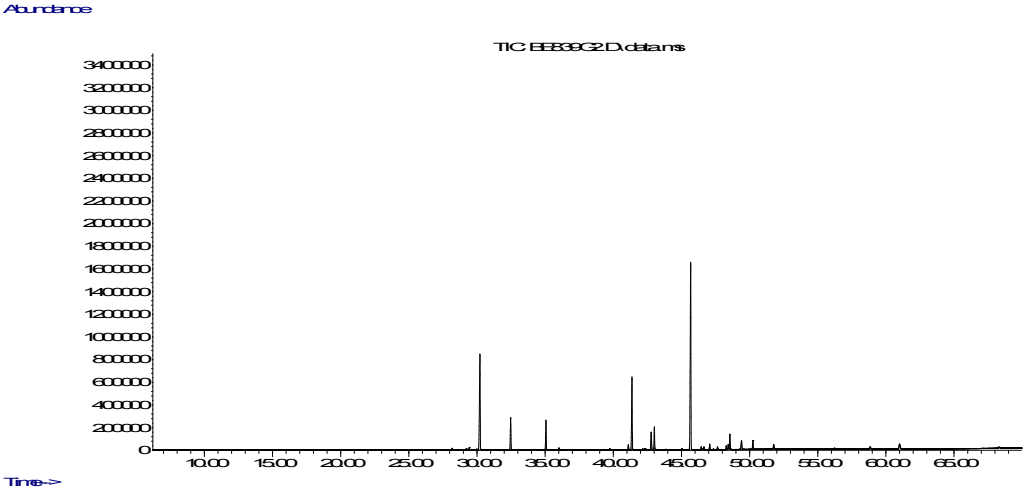


Pogostemon quadrifolius 1



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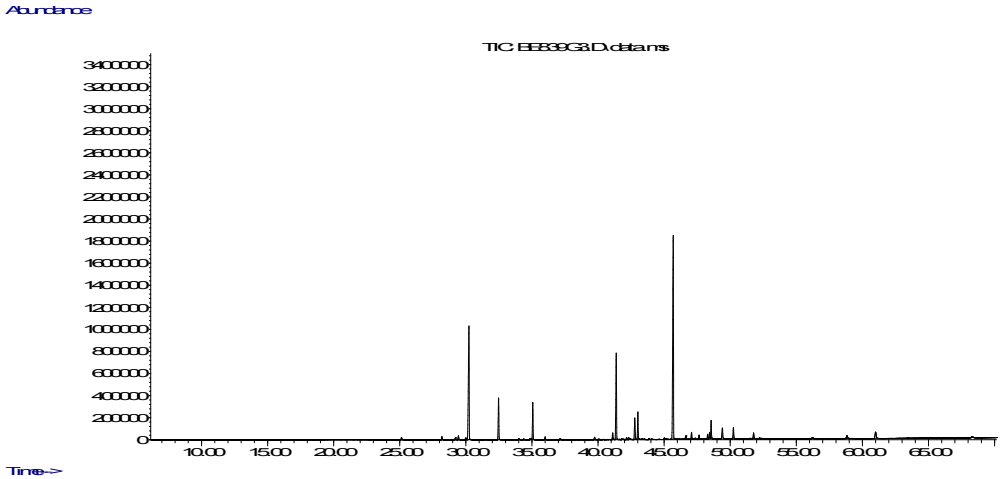

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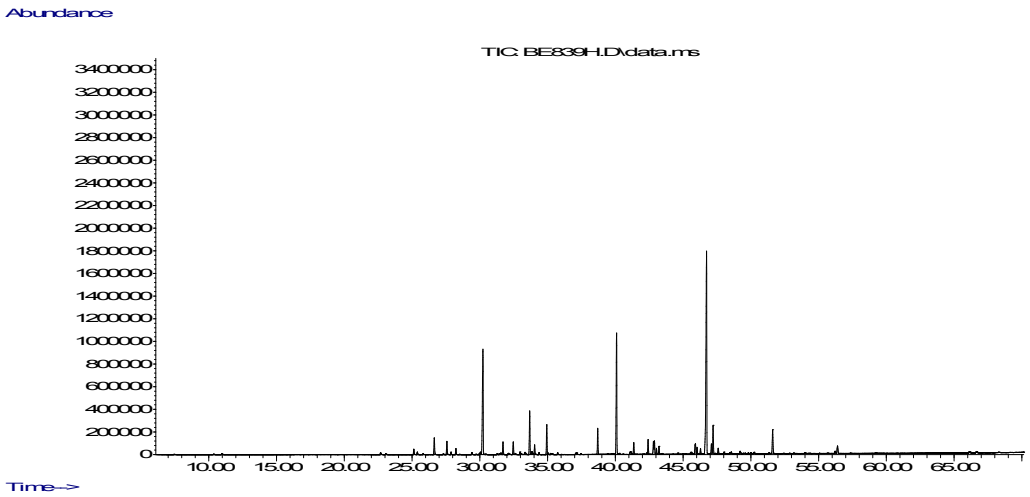


Pogostemon speciosus 1

5. A.3 Cluster Analysis

To what extent the composition of volatile oils correlates the morphologic similarity in the subfamily Lamioideae was attempted here. All the components of table 5.1 were subjected to cluster analysis, to evaluate whether the identified constituents group according to the taxonomic similarities in the subfamily Lamioideae. The distance matrix method, UPGMA (The Unweighted Pair-Group Method with Arithmetic mean) (Sneath and Sokal, 1973) was used for the Cluster analysis. The resulted dendrogram appears in Figure 5.2.

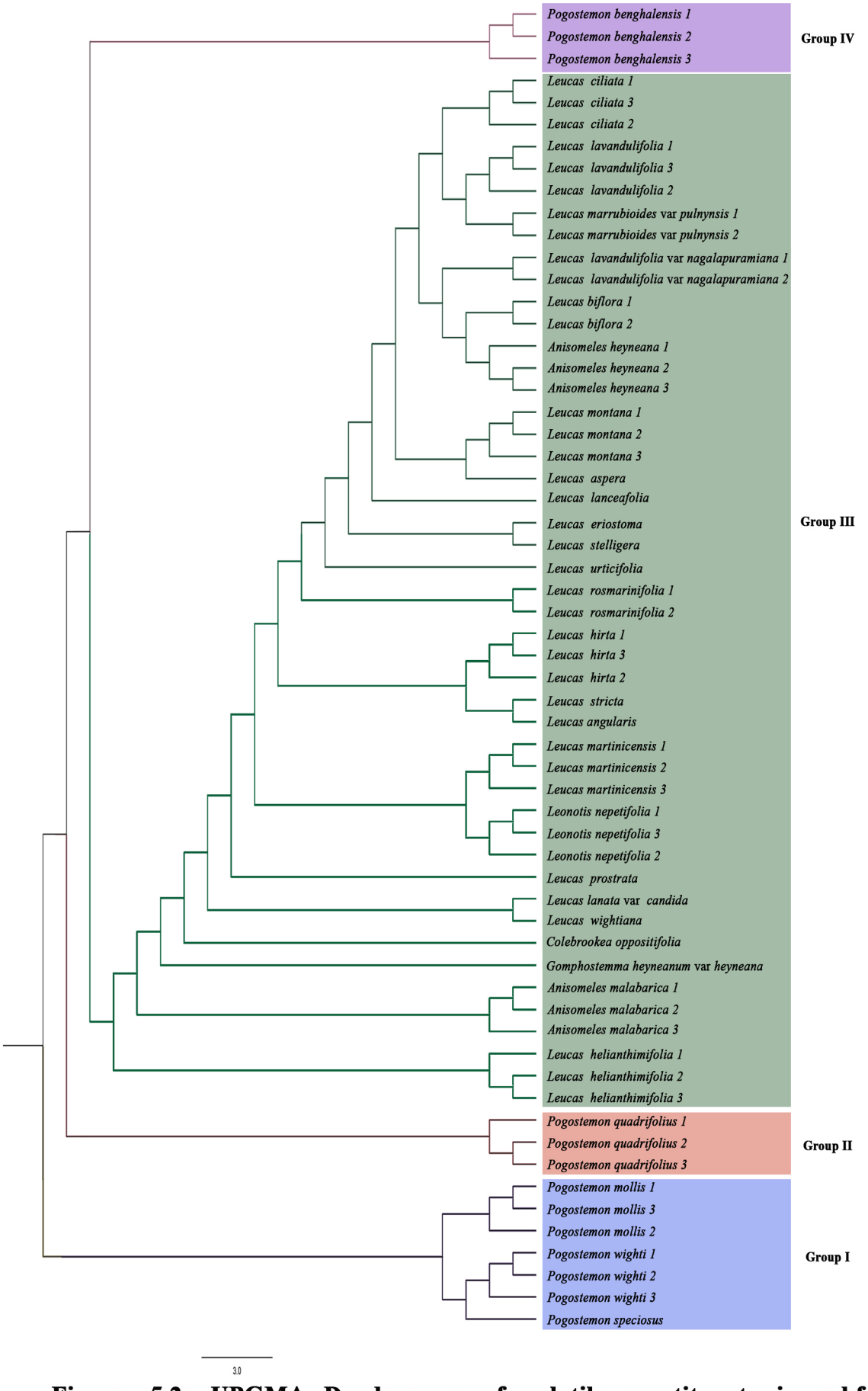


Figure. 5.2: UPGMA Dendrogram of volatile constituents in subfamily Lamioideae

5. B. FATTY ACID PROFILING

Mature leaf samples of 38 species and seven varieties of the subfamily Lamioideae were collected from wide a range of agro-climatic zones across South India. Apart from 30 species selected for essential oil analysis, additionally 15 taxa were also included in the study (Table 4.1).

5. B.1. Fatty acid composition

Total of 14 Fatty acids; five saturated, four monounsaturated, three polyunsaturated and two unusual fatty acid with allenic double bonds were observed in the leaves of Lamioideae members. The concentration of these fatty acids and prevalence of saturated, monounsaturated, polyunsaturated and unusual fatty acid in the investigated Lamioideae members are presented in Table 5.4. Representative GCMS chromatogram is illustrated in Figure. 5.3. Based on concentration of fatty acid in leaves, four major and ten minor fatty acids have been identified in this present study. Out of 14 fatty acids, laballenic (C18:2Δ5,6) and phlomic acid (C20:2Δ7,8) were two unusual fatty acids reported in this study. The mass spectra and structures for those unusual fatty acids have been showed in Figure. 5.4. and Figure 5.5 respectively.

5. B.2. Major Fatty acids

Palmitic, stearic, linoleic and linolenic acid were found to be the major FAs in Lamioideae species. Palmitic (C16:0), stearic (C18:0), linoleic acid (C18:2Δ9,12) and linolenic acid (C18:3Δ9,12,15) were found to be in the range of 17.93 ± 0.51 – 47.54 ± 0.98 %, 2.44 ± 0.64 – 12.75 ± 0.27 %, 6.93 ± 0.94 – 23.63 ± 0.31 % and 12.77 ± 0.81 – 57.10 ± 1.33 % of the total fatty acid contents respectively. Saturated fatty acid mainly palmitic acid predominated in all collected species of Lamioideae and their varities and highest percentage was observed in *Leucas sebaldiana* (47.54±0.98%) and lowest percentage in *Pogostemon speciosus* (17.93±0.51%). Similarly stearic

Table 5.4. Percentage of leaf fatty acid compositions of Lamioideae species. Values are given as mole percentage. nd = not detectable; SFA = Saturated fatty acid; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid, UFA = Unusual fatty acid

Sl. No.	Plants	C14:0	C16:0	C18:0	C20:0	C22:0	SFA	C16:1Δ9	C18:1Δ9	C18:1Δ11	C20:1Δ11	MUFA	C18:2Δ9,12	C18:3Δ9,12,15	C20:2Δ11,14	PUFA	MUFA/PUFA	C18:2Δ5,6	C20:2Δ7,8	UFA
1	***Anisomeles heyneana***	0.77 ± 0.17	24.48 ± 2.29	4.29 ± 0.41	1.80 ± 1.47	1.99 ± 0.20	33.33 ± 2.29	4.11 ± 0.17	12.03 ± 0.93	0.73 ± 0.24	nd	16.87 ± 0.93	13.03 ± 1.02	36.78 ± 4.44	nd	49.81 ± 4.44	0.34	nd	nd	0
2	***Anisomeles indica***	0.86 ± 0.13	24.49 ± 3.34	3.11 ± 0.29	0.74 ± 0.17	1.48 ± 0.85	30.68 ± 3.34	4.77 ± 0.54	11.93 ± 0.76	0.77 ± 0.04	nd	17.47 ± 0.76	11.22 ± 0.77	40.63 ± 4.53	nd	51.85 ± 4.53	0.34	nd	nd	0
3	***Anisomeles malabarica***	2.84 ± 0.21	24.40 ± 0.28	5.15 ± 0.35	2.91 ± 1.03	nd	35.3 ± 1.03	4.64 ± 0.70	5.19 ± 1.00	1.86 ± 0.52	nd	11.69 ± 1.00	19.92 ± 0.08	29.07 ± 0.21	nd	48.99 ± 0.21	0.24	3.92 ± 3.42	nd	3.92 ± 3.42
4	***Colebrookea oppositifolia***	1.96 ± 1.09	20.69 ± 2.26	2.44 ± 0.64	1.95 ± 0.71	nd	27.04 ± 2.26	3.14 ± 0.36	5.98 ± 4.22	1.10 ± 0.46	nd	10.22 ± 4.22	12.46 ± 0.63	50.35 ± 4.62	nd	62.81 ± 4.62	0.16	nd	nd	0
5	***Gomphostemma heyneanum* var. *heyneana***	0.73 ± 0.07	22.68 ± 3.04	6.66 ± 0.52	0.87 ± 0.16	0.31 ± 0.18	31.25 ± 3.04	1.97 ± 0. 12	5.92 ± 0.21	0.71 ± 0.09	0.81 ± 0.20	9.41 ± 0.21	21.41 ± 1.22	36.31 ± 2.23	0.47 ± 0.19	58.19 ± 2.23	0.16	0.63 ± 0.18	0.54 ± 0.25	1.17 ± 0.25
6	***Gomphostemma heyneanum* var. *rotleri***	0.61 ± 0.24	18.94 ± 3.67	10.44 ± 1.83	1.04 ± 0.34	0.88 ± 0.39	31.91 ± 3.67	1.67 ± 0.24	9.37 ± 0.78	2.35 ± 1.34	nd	13.39 ± 1.34	17.63 ± 1.16	37.17 ± 1.72	nd	54.8 ± 1.72	0.24	nd	nd	0
7	***Gomphostemma keralensis***	1.13 ± 0.55	22.50 ± 0.37	6.52 ± 0.08	1.54 ± 0.37	0.13 ± 0.00	31.82 ± 0.55	3.68 ± 0.18	3.90 ± 0.07	0.43 ± 0.04	nd	8.01 ± 0.18	17.48 ± 0.55	36.56 ± 2.05	nd	54.04 ± 2.05	0.15	4.77 ± 1.46	nd	4.77 ± 1.46
8	***Leonotis nepetifolia***	1.39 ± 0.25	21.34 ± 0.77	3.74 ± 0.54	0.48 ± 0.06	nd	26.95 ± 0.77	5.60 ± 0.39	1.35 ± 0.07	0.22 ± 0.07	nd	7.17 ± 0.39	8.55 ± 0.20	57.10 ± 1.33	nd	65.65 ± 1.33	0.11	0.16 ± 0.10	nd	0.16 ± 0.10
9	***Leucas angularis***	1.12 ±0.29	38.20 ± 1.87	6.23 ± 1.61	0.72 ± 0.09	0.74 ± 0.24	47.01 ± 1.87	2.39 ± 0.13	3.17 ± 0.43	0.63 ± 0.20	nd	6.19 ± 0.43	12.33 ± 1.62	34.30 ± 2.60	nd	46.63 ± 2.60	0.13	0.16 ± 0.11	nd	0.16 ± 0.11
10	***Leucas aspera***	0.96 ± 0.03	22.35 ± 1.34	5.00 ± 0.32	0.61 ± 0.13	nd	28.92 ± 1.34	2.13 ± 0.16	3.26 ± 0.62	0.28 ± 0.05	nd	5.67 ± 0.62	15.89 ± 0.19	46.44 ± 4.23	nd	62.33 ± 4.23	0.09	3.08 ±1.76	nd	3.08 ±1.76
11	***Leucas beddomi***	1.28 ± 0.20	22.63 ± 3.47	4.82 ± 1.08	1.58 ± 1.25	0.70 ± 0.64	31.01 ± 3.47	3.29 ± 1.01	8.82 ± 0.61	0.49 ± 0.29	nd	12.6 ± 1.01	17.85 ± 1.08	34.19 ± 0.88	nd	52.04 ± 1.08	0.24	4.36 ± 2.76	nd	4.36 ± 2.76
12	***Leucas biflora***	0.48 ± 0.04	26.42 ± 1.96	2.89 ± 0.09	0.66 ± 0.14	0.47 ± 0.08	30.92 ± 1.96	2.84 ± 0.06	3.22 ± 0.34	0.47 ± 0.02	nd	6.53 ± 0.34	16.65 ± 0.84	45.37 ± 2.88	nd	62.02 ± 2.88	0.11	0.52 ± 0.04	nd	0.52 ± 0.04
13	***Leucas chinensis***	1.06 ± 0.17	35.86 ± 3.14	4.77 ± 0.46	0.77 ± 0.03	0.61 ± 0.17	43.07 ± 3.14	1.70 ± 0.55	3.37 ± 0.35	0.61 ± 0.10	nd	5.68 ± 0.55	18.73 ± 2.71	32.17 ± 2.27	nd	50.9 ± 2.27	0.11	0.35 ± 0.08	nd	0.35 ± 0.08
14	***Leucas ciliata***	1.04 ± 00	34.14 ± 0.48	6.54 ± 0.27	0.63 ± 0.06	0.38 ± 0.04	42.73 ± 0.48	2.58 ± 0.07	7.57 ± 0.48	0.57 ± 0.07	nd	10.72 ± 0.48	13.49 ± 0.07	19.94 ± 0.44	nd	33.43 ± 0.44	0.32	13.49 ± 0.03	nd	13.49 ± 0.03
15	***Leucas eriostoma***	1.57 ± 0.20	30.46 ± 2.01	12.28 ± 2.07	1.88 ± 0.65	nd	46.19 ± 2.07	5.68 ± 0.59	4.32 ± 1.46	nd	nd	10 ± 1.46	13.66 ± 0.11	29.00 ± 3.36	nd	42.66 ± 3.36	0.23	1.15 ± 0.26	nd	1.15 ± 0.26
16	***Leucas eriostoma* var. *lanata***	1.44 ± 0.27	25.06 ± 0.89	11 ± 1.34	2.25 ± 0.16	nd	39.75 ± 1.34	3.83 ± 0.71	3.21 ± 0.28	nd	nd	7.04 ± 0.71	17.36 ± 0.99	34.26 ± 0.89	nd	51.62 ± 0.99	0.14	1.58 ± 0.21	nd	1.58 ± 0.21
17	***Leucas helianthimifolia***	1.64 ± 0.55	18.70 ± 1.95	3.24 ± 0.83	1.47 ± 0.60	2.13 ± 1.48	27.18 ± 1.95	1.84 ± 0.69	5.71 ± 2.34	0.54 ± 0.14	nd	8.09 ± 2.34	12.54 ± 1.54	50.70 ± 5.81	nd	63.24 ± 5.81	0.13	1.39 ± 0.57	nd	1.39 ± 0.57
18	***Leucas hirta***	0.35 ± 0.08	28.36 ± 0.63	5.09 ± 0.78	0.76 ± 0.19	0.27 ± 0.02	34.83 ± 0.78	2.57 ± 0.02	7.76 ± 1.10	1.02 ± 0.01	nd	11.35 ± 1.10	11.66 ± 0.70	41.68 ± 3.26	nd	53.34 ± 3.26	0.21	0.48 ± 0.08	nd	0.48 ± 0.08
19	***Leucas lanata* var. *candida***	0.78 ± 0.23	19.09 ± 0.16	5.51 ± 0.66	0.95 ±0.17	1.14 ± 0.16	27.47 ± 0.66	2.32 ±0.04	3.76 ± 0.43	0.37 ± 0.02	nd	6.45 ± 0.43	11.15 ± 0.71	54.93 ± 2.25	nd	66.08 ± 2.25	0.10	nd	nd	0
20	***Leucas lanceaefolia***	2.64 ± 0.06	28.99 ± 2.04	4.53 ± 1.14	2.76 ± 1.55	nd	38.92 ± 2.04	2.36 ± 0.77	1.77 ± 1.03	0.53 ± 0.42	nd	4.66 ± 1.03	7.52 ± 1.19	47.26 ± 3.48	nd	54.78 ± 3.48	0.09	1.28 ± 0.56	nd	1.28 ± 0.56
21	***Leucas lavandulifolia***	0.80 ± 0.07	28.51 ± 1.79	4.87 ± 1.16	0.43 ± 0.13	0.50 ± 0.13	35.11 ± 1.79	1.92 ± 0.23	4.39 ± 0.02	0.38 ± 0.11	nd	6.69 ± 0.23	16.30 ± 0.26	41.23 ± 3.16	nd	57.53 ± 3.16	0.12	0.67 ± 0.05	nd	0.67 ± 0.05
22	***Leucas lavandulifolia* var. *decipiens***	0.87 ± 0.12	21.42 ± 2.31	8.32 ± 0.49	0.84 ± 0.10	0.72 ± 0.12	32.17 ± 2.31	3.40 ± 0.11	2.13 ± 0.07	0.28 ± 0.05	nd	5.81 ± 0.11	15.61 ± 1.07	46.24 ± 2.06	nd	61.85 ± 2.06	0.09	0.17 ± 0.06	nd	0.17 ± 0.06
23	***Leucas lavandulifolia* var. *nagalapuramiana***	4.36 ± 0.56	28.23 ± 0.23	4.97 ± 2.17	nd	nd	37.56 ± 2.17	5.21 ± 2.89	7.07 ± 0.45	nd	nd	12.28 ± 2.89	16.85 ± 0.77	32.35 ± 0.94	nd	49.2 ± 0.94	0.25	0.93 ± 1.23	nd	0.93 ± 1.23
24	***Leucas marrubioides* var. *pulneyensis***	0.94 ± 0.28	21.40 ± 0.81	5.26 ± 0.63	1.12 ± 0.10	1.61 ± 0.27	30.33 ± 0.81	2.03 ± 0.31	4.04 ± 1.00	0.36 ± 0.03	nd	6.43 ± 1.00	12.76 ± 0.57	45.75 ± 2.51	nd	58.51 ± 2.51	0.11	4.74 ± 0.97	nd	4.74 ± 0.97
25	***Leucas martinicensis***	3.87 ± 0.34	27. 68 ± 1.22	3.43 ± 0.62	1.69 ± 0.91	nd	36.67 ± 1.22	5.44 ± 0.30	1.96 ± 0.68	0.95 ± 0.77	nd	8.35 ± 0.77	9.26 ± 0.67	45.68 ± 2.41	nd	54.94 ± 2.41	0.15	nd	nd	0
26	***Leucas montana***	3.56 ± 0.66	26.39 ± 1.04	3.91 ± 0.11	0.78 ± 1.03	1.4 ± 0.21	36.04 ± 1.03	3.20 ±0.89	6.83 ± 0.40	0.62 ± 0.06	nd	10.65 ± 0.89	9.49 ± 0.37	46.13 ± 2.61	nd	55.62 ± 2.61	0.19	nd	nd	0
27	***Leucas nepetifolia***	0.73 ± 0.14	32.21 ± 0.78	6.84 ± 1.04	0.83 ± 0.13	0.55 ± 0.12	41.16 ± 1.04	1.91 ± 0.24	3.27 ± 0.16	0.36 ± 0.07	nd	5.54 ± 0.24	18.98 ± 0.60	34.01 ± 1.00	nd	52.99 ± 1.00	0.10	0.30 ± 0.16	nd	0.30 ± 0.16
28	***Leucas prostrata***	1.31 ± 0.24	31.99 ± 1.24	7.65 ± 1.50	1.40 ± 0.39	2.34 ± 1.98	44.69 ± 1.98	4.19 ± 0.24	3.73 ± 0.51	0.47 ± 0.17	nd	8.39 ± 0.51	17.99 ± 2.93	27.65 ± 1.63	nd	45.64 ± 2.93	0.18	1.28 ± 0.36	nd	1.28 ± 0.36
29	***Leucas rosmarinifolia***	0.57 ± 0.02	22.38 ± 2.96	3.42 ± 0.80	1.02 ± 0.21	nd	27.39 ± 2.96	3.01 ± 0.03	6.18 ± 1.36	0.29 ± 0.02	nd	9.48 ± 1.36	11.65 ± 3.46	47.87 ± 7.35	nd	59.52 ± 7.35	0.16	3.63 ± 1.06	nd	3.63 ± 1.06
30	***Leucas sebaldiana***	0.51 ± 0.29	47.54 ± 0.98	12.75 ± 0.27	2.18 ± 0.05	0.39 ± 0.07	63.37 ± 0.98	6.62 ± 0.50	3.68 ± 0.10	0.24 ± 0.03	nd	10.54 ± 0.50	8.16 ± 0.66	12.77 ± 0.81	nd	20.93 ± 0.81	0.50	5.17 ± 0.66	nd	5.17 ± 0.66
31	***Leucas sivadasiniana***	0.87 ± 0.18	26.23 ± 1.73	6.89 ± 0.35	1.08 ± 0.22	nd	35.07 ± 1.73	2.88 ± 0.07	9.30 ± 0.76	0.53 ± 0.09	nd	12.71 ± 0.76	15.96 ± 1.45	28.78 ± 1.53	nd	44.74 ± 1.53	0.28	7.48 ± 0.45	nd	7.48 ± 0.45
32	***Leucas stelligera***	3.04 ± 0.21	40.46 ± 0.59	5.70 ± 3.39	2.80 ± 0.74	nd	52 ± 3.39	4.07 ± 6.36	6.64 ± 1.15	4.25 ± 0.49	nd	14.96 ± 6.36	12.75 ± 0.11	14.19 ± 4.15	nd	26.94 ± 4.15	0.56	3.58 ± 0.57	nd	3.58 ± 0.57
33	***Leucas stricta***	2.08 ± 1.20	26.55 ± 6.58	5.10 ± 1.66	1.22 ± 0.88	1.82 ± 0.48	36.77 ± 6.58	3.76 ± 0.99	5.23 ± 1.93	0.70 ± 0.20	nd	9.69 ± 1.93	6.93 ± 0.94	46.25 ± 6.65	nd	53.18 ± 6.65	0.18	0.31 ± 0.27	nd	0.31 ± 0.27
34	***Leucas ternifolia***	0.53 ± 0.05	19.78 ± 0.85	4.75 ± 0.44	0.67 ± 0.14	0.20 ± 0.03	25.93 ± 0.85	2.37 ± 0.23	3.78 ± 0.41	0.38 ± 0.05	nd	6.53 ± 0.41	16.89 ± 0.66	48.39 ± 1.37	nd	65.28 ± 1.37	0.10	2.27 ± 0.24	nd	2.27 ± 0.24
35	***Leucas urticifolia***	3.68 ± 1.01	32.31 ± 5.95	5.63 ± 0.37	1.08 ± 0.11	nd	42.7 ± 5.95	5.18 ± 0.72	3.12 ± 1.02	2.49 ± 2.12	nd	10.79 ± 2.12	9.27 ± 2.23	37.42 ± 4.75	nd	46.69 ± 4.75	0.23	1.93 ± 0.22	nd	1.93 ± 0.22
36	***Leucas wightiana***	1.28 ± 0.30	24.87 ± 1.41	4.00 ± 0.37	0.68 ± 0.11	nd	30.83 ± 1.41	4.17 ± 1.15	6.28 ± 0.77	0.58 ± 0.18	nd	11.03 ± 1.15	14.02 ± 4.75	42.97 ± 1.39	nd	56.99 ± 4.75	0.19	0.76 ± 0.19	nd	0.76 ± 0.19
37	***Leucas zeylanica***	1.10 ± 0.06	22.50 ± 1.85	4.48 ± 0.20	0.65 ± 0.03	0.74 ±0.06	29.47 ± 1.85	2.73 ± 0.13	2.86 ± 0.26	0.38 ±0.09	nd	5.97 ± 0.26	11.31 ± 0.41	51.84 ± 0.84	nd	63.15 ± 0.84	0.09	1.41 ± 0.23	nd	1.41 ± 0.23
38	***Pogostemon benghalensis***	1.17 ± 0.48	39.70 ± 1.43	6.26 ± 1.70	1.30 ± 0.09	0.29 ± 0.08	48.72 ± 1.70	4.14 + 1.18	5.62 ± 3.03	0.78 ± 0.62	nd	10.54 ± 3.03	16.36 ± 4.83	23.78 ± 2.16	nd	40.14 ± 4.83	0.26	0.57 ± 0.30	nd	0.57 ± 0.30
39	***Pogostemon heyneanum***	1.71 ± 0.06	30.89 ± 1.00	4.20 ± 0.11	1.33 ± 0.08	0.75 ± 0.03	38.88 ± 1.00	1.65 ± 0.20	5.04 ± 0.26	0.70 ± 0.09	nd	7.39 ± 0.26	22.19 ± 0.67	31.54 ± 0.78	nd	53.73 ± 0.78	0.14	nd	nd	0
40	***Pogostemon mollis***	1.60 ± 0.03	26.98 ± 5.45	6.82 ± 0.70	1.05 ± 0.05	0.93 ± 0.24	37.38 ± 5.45	2.73 ± 0.42	12.37 ± 0.63	0.77 ± 0.05	0.48 ± 0.15	16.35 ± 0.63	24.78 ± 4.96	18.29 ± 2.04	nd	43.07 ± 4.96	0.38	3.20 ± 0.47	nd	3.20 ± 0.47
41	***Pogostemon myosurioides***	3.98 ± 0.33	32.67 ± 3.02	4.51 ± 0.59	2.09 ± 1.47	nd	43.25 ± 3.02	6.15 ± 0.71	7.73 ± 0.42	0.90 ± 0.16	nd	14.78 ± 0.71	9.17 ± 0.75	32.74 ± 4.48	nd	41.91 ± 4.48	0.35	nd	nd	0
42	***Pogostemon paniculatus***	0.80 ± 0.05	26.05 ± 0.99	3.51 ± 0.86	0.59 ± 0.07	0.61 ± 0.02	31.56 ± 0.99	2.64 ± 0.02	5.48 ± 0.33	0.62 ± 0.01	nd	8.74 ± 0.33	23.63 ± 0.31	36.08 ± 0.54	nd	59.71 ± 0.54	0.15	nd	nd	0
43	***Pogostemon quadrifolius***	0.28 ± 0.08	34.67 ± 0.72	6.94 ± 0.30	0.86 ± 0.10	0.26 ± 0.10	43.01 ± 0.72	5.06 ± 1.36	11.93 ± 0.76	0.60 ± 0.30	0.24 ± 0.08	17.26 ± 1.36	15.70 ± 1.77	23.71 ± 1.16	nd	39.41 ± 1.77	0.44	0.33 ± 0.09	nd	0.33 ± 0.09
44	***Pogostemon speciosus***	4.3 ± 0.31	17.93 ± 0.51	3.03 ± 0.38	2.29 ± 0.80	nd	27.55 ± 0.80	3.23 ± 0.09	4.62 ± 0.37	1.11 ± 0.26	nd	8.96 ± 0.37	13.28 ± 0.70	50.19 ± 0.99	nd	63.47 ± 0.99	0.14	nd	nd	0
45	***Pogostemon wightii***	4.49 ± 3.00	20.58 ± 0.63	3.79 ± 0.22	nd	nd	28.86 ± 3.00	3.41 ± 0.25	5.77 ± 0.51	1.14 ± 0.52	nd	10.32 ± 0.52	15.09 ± 0.71	45.70 ± 2.73	nd	60.79 ± 2.73	0.17	nd	nd	0

acid is present in an optimum quantity in all species studied and the highest percentage was observed in *Leucas eriostoma* (11±1.34%) and lowest quantity in *Colebrookea oppositifolia* (2.44±0.64%). Essential fatty acids like linoleic acid and linolenic acid are found as the major fatty acid in the species studied whereas linolenic acid or omega-3-fatty acid predominated in all the species with highest percentage is observed in *Leonotis nepatifolia* (57.10±1.33%) and lowest percentage in *Leucas sebaldiana* (12.77±0.81%). Linoleic acid or omega-6-fatty acid is moderately present in al the species studied with highest quantity in *Pogostemon mollis* (24.78±4.96%) and lowest quantity in *Leucas stricta* (6.93±0.94%). The ratio of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) is lower (≤1) in all the species studied.

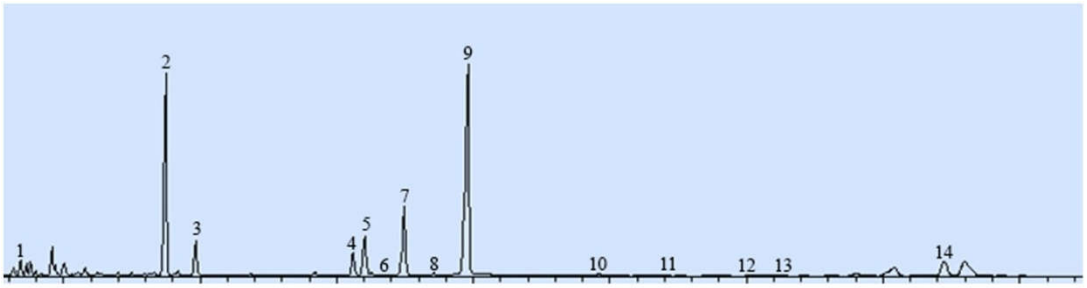


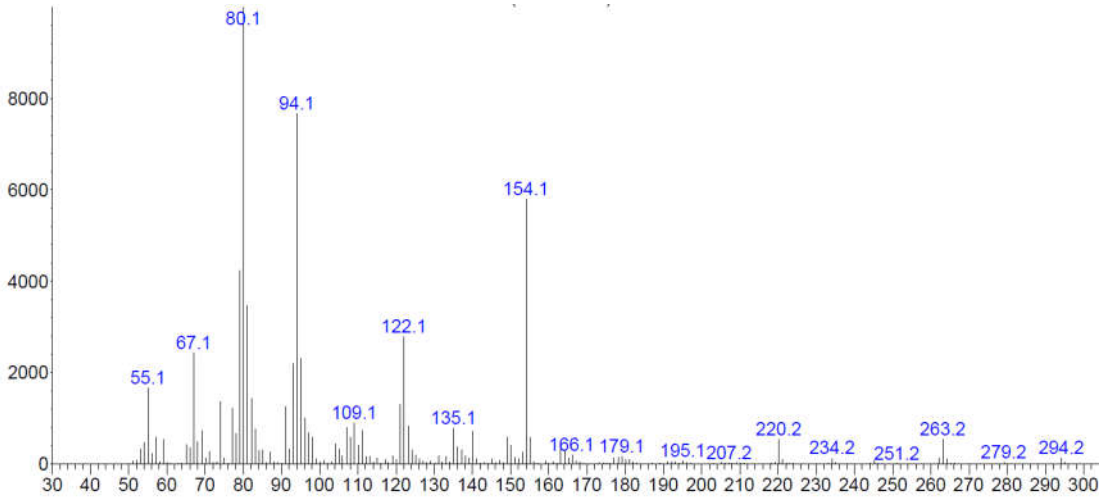
Figure 5.3. Representative GC chromatogram of fatty acid methyl esters (FAMEs) of Lamioideae species. [peak 1, myristic acid (C14:0); 2, palmitic acid (C16:0); 3, palmitoleic acid (C16:1Δ9); 4, stearic acid (C18:0); 5, oleic acid (C18:1Δ9); 6, cis-vaccenoic acid (C18:1Δ11); 7, linoleic acid (C18:2Δ9,12); 8, laballenic acid (C18:2Δ5,6); 9, linolenic acid (C18:3Δ9,12,15); 10, eicosanoic acid (C20:0); 11, eicosenoic acid (C20:1Δ11); 12, eicosadienoic acid (C20:2Δ11,14); 13, phlomic acid (C20:2Δ7,8); 14, docosanoic acid (C22:0)].

5. B.3. Unusual fatty acids

Usual fatty acids are those accumulate through the action of several pathways, such as fatty acid or triacylglycerol synthesis, transport, degradation and usually found in triacylglycerol. Unusual fatty acids are obtained by the enzymatic modifications of usual fatty acids to produce compounds that are not naturally synthetized in the host (Ledesma-Amaro and Nicaud, 2016). Unusual fatty acid, laballenic acid (C18:2Δ5,6) was observed

in most the species collected (Table 5.4). Laballenic acid is found as a minor fatty acid with highest concentration in *Leucas ciliata* (13.49±0.03%). Laballenic acid was totally absent in some species like *Anisomeles heyneana*, *Anisomeles indica*, *Colebrookea oppositifolia, Gomphostemma heyneanum* var. *rotleri, Leucas lanata* var. *candida*, *Leucas montana*, *Leucas martinicensis*, *Pogostemon wightii, Pogostemon speciosus, Pogostemon paniculatus, Pogostemon heyneanum* and *Pogostemon myssurioides*. Phlomic acid (C20:2Δ7,8), another allenic fatty acid is identified in the leaf sample of only one species; *Gomphostemma heyneanum* var *heyneana* with the concentration (0.54±0.25%). Phlomic acid has been reported in several other members of Lamiaceae with highest concentration in *Phlomis tuberosa* (2.9 %) (Aitzetmuller *et al*.,1997).

A.



B.

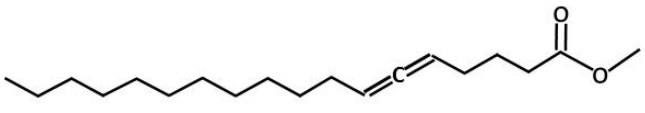
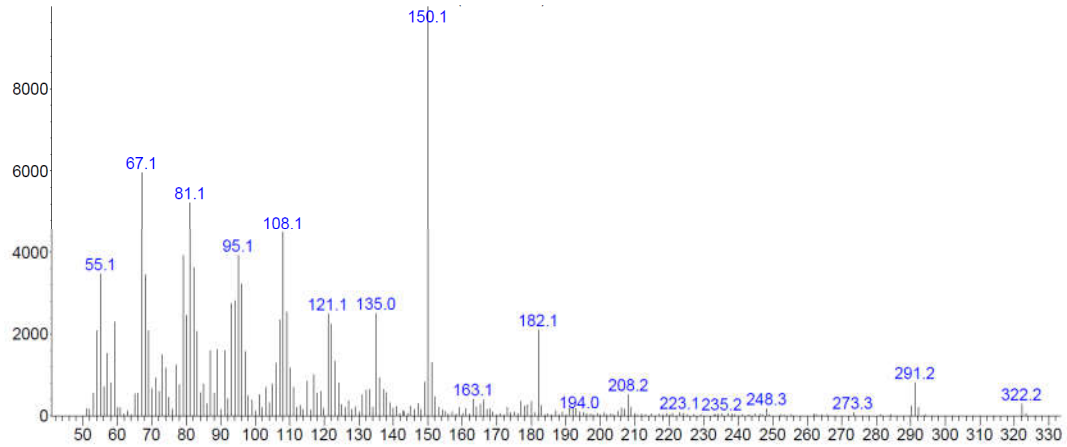


Figure 5.4. A. Mass spectrum and B. Structure of Laballenic acid

A.



B.

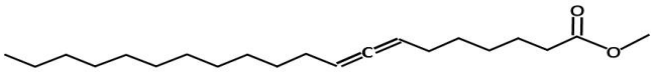


Figure 5.5. A. Mass spectrum and B. Structure of Phlomic acid

5.B.4. Minor Fatty acids

In the leaf samples of Lamioideae members, 10 minor fatty acids were observed (Table 4). Myristic (C14:0) palmitoleic (C16:1Δ9), oleic (C18:1Δ9), cis-vaccenoic (C18:1Δ11), laballenic (C18:2Δ5,6), eicosanoic (C20:0), eicosenoic (C20:1Δ11), eicosadienoic (C20:2Δ11,14), phlomic (C20:2Δ7,8) and docosanoic acid (C22:0) were estimated to be in the range of 0.28 ± 0.08 - 4.49 ± 3.00%, 1.65 ± 0.20 - 6.62 ± 0.50%, 1.35 ± 0.07 - 20.88 ± 2.06%, 0.01 - 4.25 ± 0.49%, 0.01 - 7.48 ± 0.45%, 0.01 - 2.91 ± 1.03%, 0.01 - 0.81 ± 0.20%, 0.01 - 0.47 ± 0.19% , 0.01 - 0.54 ± 0.25% and 0.01 - 2.34 ± 1.98% of the total fatty acids. Among the minor fatty acids, oleic acid predominated in *Pogostemon mollis* (12.37 ± 0.63%), *Anisomeles heyneana* (12.03 ± 0.93%), *Anisomeles indica* (11.93 ± 0.76 %) and *Pogostemon quadrifolius* (11.93 ± 0.76 %). Oleic acid possesses anti-inflammatory and anticancer properties and can reduce the risk of cardiovascular diseases (Sales-Campos *et al.,* 2013).

Similarly palmitoleic acid predominated in *Leucas sebaldiana* (6.62 ± 0.50%), *Pogostemon myosurioides* (6.15 ± 0.71%) and *Leucas eriostoma* (5.68 ± 0.59%). Minor FA like eicosadienoic acid (C20:2Δ11,14) and unusal FA phlomic acid (C20:2Δ7,8) were reported in only one species studied; *Gomphostemma heyneanum* var *heyneana* with the concentration (0.47 ± 0.19%) and (0.54 ± 0.25%) respectively. Eicosenoic (C20:1Δ11) was detected only in 3 species studied; *Gomphostemma heyneanum* var *heyneana* (0.81 ± 0.20%), *Pogostemon mollis* (0.48 ± 0.15%) and *Pogostemon quadrifolius* (0.24 ± 0.08%). Similarly docosanoic acid (C22:0) were present in 28 taxa studied with higher concentration in *Leucas prostrata* (2.34 ± 1.98%).

5. B.5. Multivariate analysis

Inorder to test the relationship among the different Lamioideae species, Principal Component Analysis (PCA) was carried out for 14 Fatty acids as variables and were further analysed using PCA software. Principal component analysis (PCA) is a statistical method which reduces the dimentionality of original data set and uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components (Ringner, 2008). The correlation between each fatty acid with Lamioideae species were demonstrated and plotted on a bi-plot graph. For bi-plot graph, the Pearson correlation data matrix of fatty acid variables was used. Eigenvalues are indicators of relative importance of each dimension. The first five PCs had 3.142, 2.783, 1.870, 1.343 and 1.045 eigenvalues respectively (Table 5.5). The first PC explained 22.44% and second PC recorded 19.88% of total variations (figure 5.6 and Figure 5.7). Other three PCs marked 13.36%, 9.59% and 7.46% of total variations respectively. The PC1-PC2 and PC1-PC3 score plots elucidated 42.32% and 35.80% of total variance respectively. From PC1-PC2 score plot, it is clear that all fatty acid vectors were distributed

within the correlation circle (figure 5.5). PC1 accounted for high positive loading factor for C18:2Δ9,12 (0.54), C20:2Δ11,14 (0.862), C20:2Δ7,8 (0.862), C20:1Δ11 (0.832) and negative high loading factor for only minor fatty acid; palmitoleic acid (C16:1Δ9; -0.482). PC2 exhibited positive correlation with high loading factor for only major fatty acid, linolenic acid, (C18:3Δ9,12,15; 0.926), while negative correlation and high loading factor for C16:0 (-0.708) and C18:0 (-0.609). Moreover, PC3 contributes only high positive loading factor for C14:0 (0.718) and C18:1Δ11 (0.491). Hierarchial clustering analysis (HCA) and PC1-PC2 score plot revealed that *Leucas lavandulifolia, Leucas biflora, Leucas wightiana, Leucas hirta, Leucas lanceaefolia, Leucas martinicensis, Leucas montana, Leucas stricta, Leucas lanata* var. *candida, Leucas zeylanica, Leucas helianthimifolia, Leucas ternifolia, Leucas aspera, Leucas lavandulifolia* var. *decipiens, Leucas rosmarinifolia, Leucas marrubioides* var. *pulneyensis, Anisomeles indica, Anisomeles heyneana, Leonotis nepetifolia, Colebrookea oppositifolia, Pogostemon wightii* and *Pogostemon speciosus* grouped together on the basis of higher linolenic acid concentration among all collected species (figure 5.8).

5. B.6. Chemotaxonomy

Algorithmic hierarchical clustering (AHC) dendrogram generated by using Euclidian distance and ward method is presented in Figure 5.8. The dendrogram is divided into three major subgroups on the basis of similarity of fatty acids; the first group represent species grouped on the basis of lower linolenic acid (less than 25%) and higher quantity of major fatty acids, while second on the basis of presence of (25-40%) of linolenic acid and third group on the basis of abundance of major fatty acid, linolenic acid (more than 40%). The first group is smaller and consists of only less members when compared to second and third groups.

Table 5.5: Correlation matrix loading, eigenvalue, variance and cumulative values of the significant principal components (PCs).

	F1	F2	F3	F4	F5
C14:0	-0.388	-0.018	0.718	0.006	-0.151
C16:0	-0.316	-0.708	-0.154	-0.109	0.188
C16:1Δ9	-0.482	-0.366	0.310	-0.373	0.351
C18:0	0.011	-0.609	-0.409	-0.387	0.133
C18:1Δ9	0.011	-0.325	0.069	0.738	0.259
C18:1Δ11	-0.231	-0.362	0.491	0.480	-0.180
C18:2Δ9,12	0.514	-0.203	-0.344	0.264	-0.372
C18:2Δ5,6	-0.076	-0.385	-0.406	-0.028	-0.441
C18:3Δ9,12,15	0.109	0.926	0.229	-0.196	-0.017
C20:0	-0.356	-0.429	0.314	-0.041	-0.030
C20:1Δ11	0.832	-0.316	0.221	0.015	0.108
C20:2Δ11,14	0.862	-0.252	0.341	-0.168	0.058
C20:2Δ7,8	0.862	-0.252	0.341	-0.168	0.058
C22:0	0.131	0.287	-0.325	0.316	0.628
Eigenvalue	3.142	2.783	1.870	1.343	1.045
Variability (%)	22.441	19.879	13.358	9.592	7.463
Cumulative %	22.441	42.320	55.678	65.270	72.734

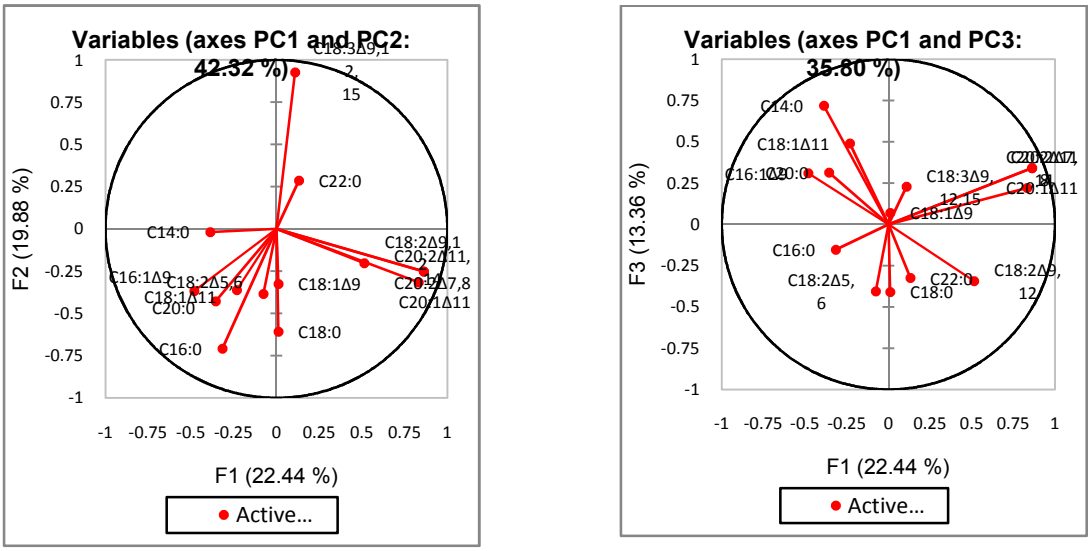


Figure. 5.6. Loading plot for principal component analysis of 14 fatty acids from collected Lamioideae species ;PC1-PC2 and PC1-PC3.

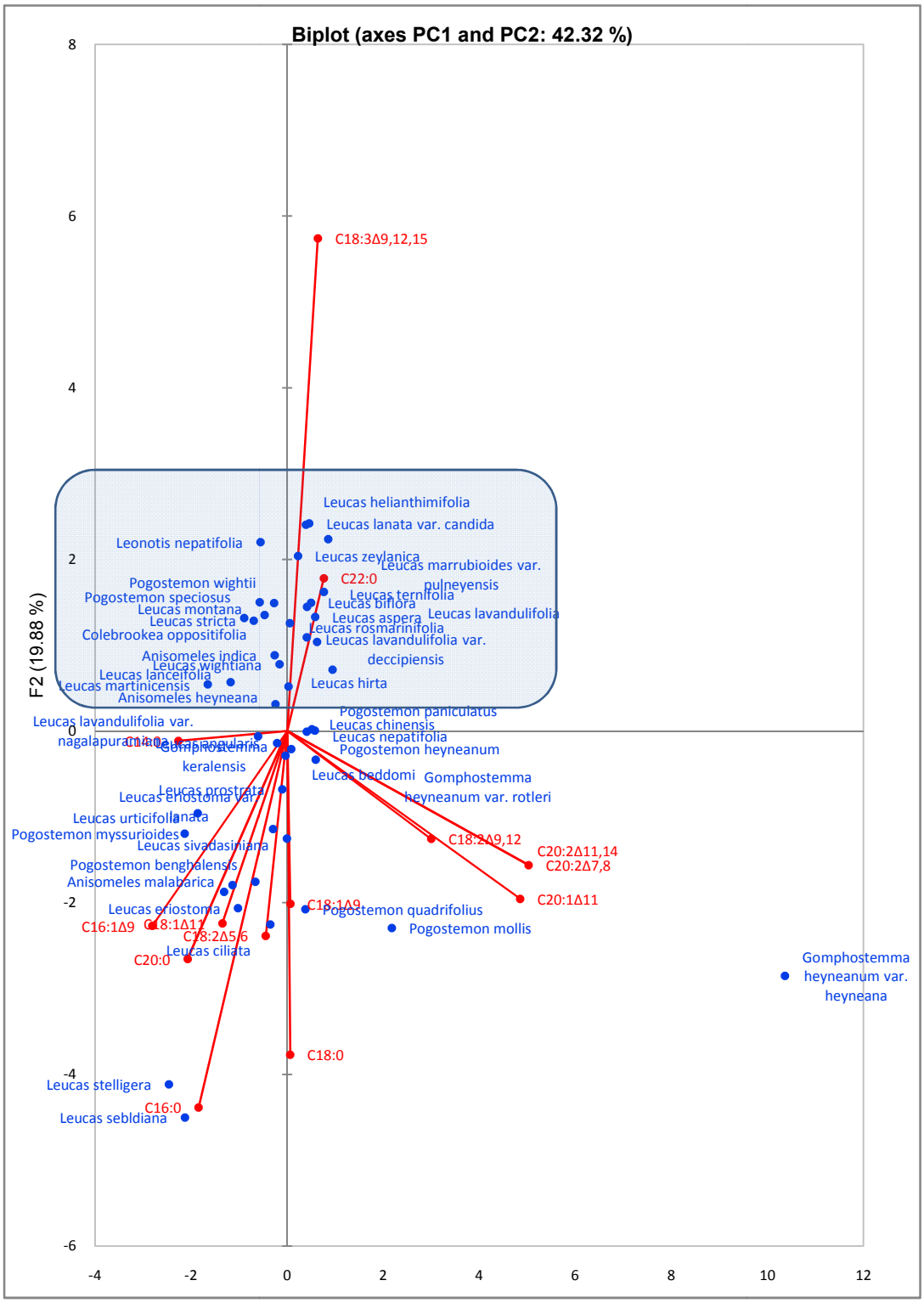


Figure 5.7. Scatter biplot diagram of leaf fatty acid profiles of Lamioideae species according to Principal component 1 (PC1) and Principal component 2 (PC2) axes of Analysis

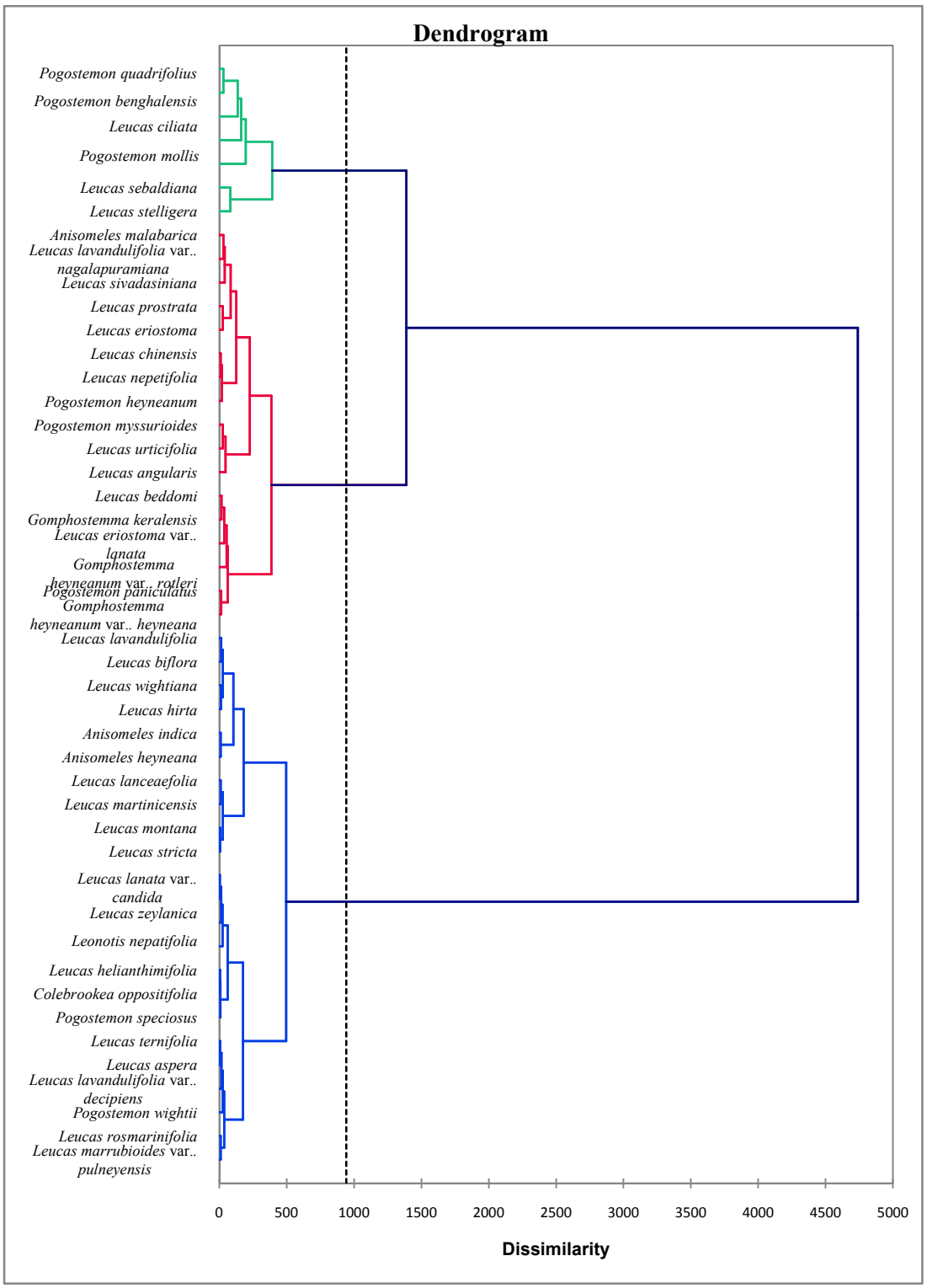


Figure 5.8: Dendrogram obtained by Hierarchial Clustering Analysis of collected Lamioideae species and varieties

5.C MOLECULAR PHYLOGENY

5.C. 1. Agarose Gel Electrophoresis

The amplified products of the gene regions *trnL-F* intergenic spacer and *rps16* introns were subjected to 1.2% agarose gel electrophoresis. The size of the amplicons were visualized and photographs were also taken using the Gel Documentation System (Labnet, Enduro GDS, aplegen).

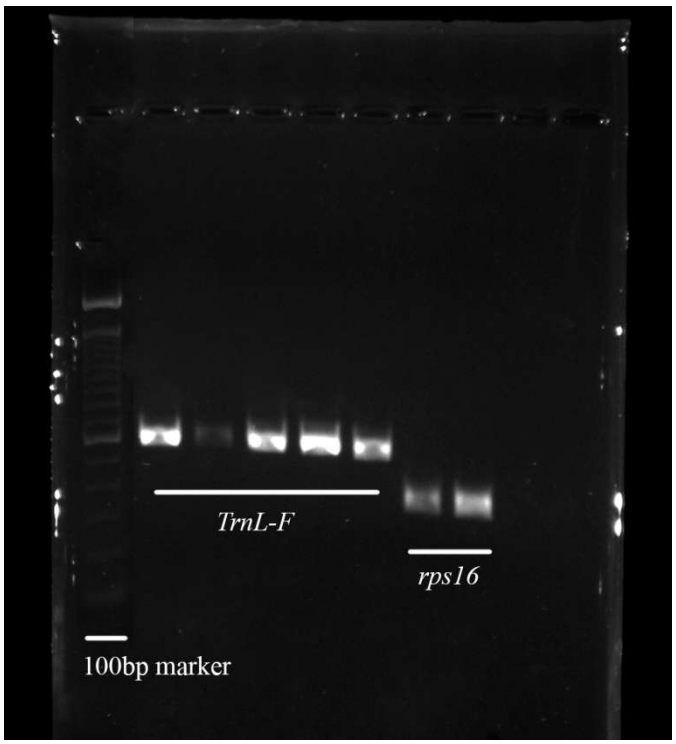


Figure 5.9:- Agarose Gel Electrophoresis images of two genes

DNA ladder of 100 bp (Invitrogen 100 bp DNA ladder of 0.1 µg/µl concentration) was used as reference. It was observed that the size of the *trnL-F* intergenic spacer was in between 600-700 and *rps16* intron was in between 900-1000 base pairs.

5.C. 2. Partition Homogeneity test

Partition Finder v1.1.0 was used to find out the different model of sequence evolution for the *trnL-F* and *rps16* intergenic spacer. The best-fit model of sequence evolution according to the Partition Finder v1.1.0 Windows analysis for *trnL-F* region was found to be TrN+G and *rps16* intergenic spacer region was TVM+G

Concatenated data set of *trnL-F* and *rps16* intergenic spacer based on DNA sequenced data

The chloroplast DNA data set consisted of one chloroplast region *rps16* and *trnL-F* intergenic spacer. The concatenated dataset had 919 characters of which 434 was conserved sites, 439 variable, 148 parsimony informative (Pi) sites and 284 singletons. Gaps were treated as 5 th character.

Table 5.6: Nucleotide Frequencies of concatenated data set of *trnL-F* intergenic spacer and *rps16* intron based on DNA sequenced data

Sl. No.	Name of the Taxa	Percentage of				Total length of the base pair
		T	C	A	G	
1	*Gomphostemma heyneanum* var.*heyneana*	30.6	17.8	32.4	19.2	1303
2	*Anisomeles heyneana*	31.6	17.4	33	18	1307
3	*Pogostemon wightii*	30.8	17.4	33.4	18.3	1155
4	*Pogostemon benghalensis*	31.7	19.7	31.9	16.5	1259
5	*Pogostemon mollis*	31.4	17.5	32	18.9	1343
6	*Colebrookea oppositifolia*	31.3	17.5	31.9	19.3	1297
7	*Pogostemon quadrifolius*	31.3	17.4	32.3	19	1304
8	*Leonotis nepetifolia*	30.7	17.8	31.4	19.1	1280
9	*Leucas marrubiodes* var. *pulneyensis*	30.9	17.6	32.3	19.2	1297
10	*Leucas lavandulifolia* var. *nagalapuramiana*	31.3	17.9	31.6	19	1295
11	*Leucas stricta*	31.4	17.4	31.5	19.5	1368
12	*Leucas stelligera*	31.3	17.8	31.6	19.1	1287
13	*Anisomeles malabarica*	31.9	17.7	32.4	17.9	1282
14	*Leucas angularis*	31.1	17.7	32.4	18.6	1208
15	*Leucas biflora*	31.2	17.8	32.3	18.7	1215
16	*Leucas eriostoma*	31.8	17.2	32.2	18.8	1156
17	*Leucas helianthimifolia*	31.5	17.6	32.3	18.6	1196

18	*Leucas hirta*	31.5	17.6	32.2	18.7	1215
19	*Leucas lanceaefolia*	31.7	17.4	32.2	18.7	1217
20	*Leucas lavandulifolia*	31.4	17.6	32.2	18.8	1217
21	*Leucas aspera*	31.5	17.5	32.2	18.5	1216
22	*Leucas ciliatq*	31.6	17.4	32	18.8	1226
23	*Leucas montana*	31.6	22	29.2	17.2	332
24	*Leucas lanata* var. *candida*	31.1	16	33.8	19.1	852
25	*Leucas martinicensis*	30	17	34	19	1672
26	*Leucas rosmarinifolia*	30.4	16.4	34.2	19	1674
27	*Leucas wightiana*	30.3	17	34.2	18.5	1711
28	*Leucas urticifolia* var. *urticifolia*	31	17.9	31.7	19.4	1210
29	*Otostegia fruticosa*	30.85	17.87	32.6	18.66	1141
30	*Otostegia modesta*	30.9	17.82	32.57	18.70	1139
31	*Otostegia tomentosa* subsp. *ambigens.*	30.88	17.76	32.54	18.81	1143
32	*Leucas oligocephala*	30.36	17.61	32.97	19.04	1113
33	*Leucas densiflora*	30.87	17.67	32.54	18.9	1137
34	*Leucas bracteosa*	30.74	17.99	32.17	19.07	1106
35	*Leucas grandis*	30.83	17.75	32.77	18.63	1132
36	*Leonotis nepetifolia* var. *Africana*	30.67	17.79	32.77	18.75	1141
37	*Leonotis myrcifolia*	30.98	17.86	32.39	18.75	1136
38	*Leucas calostachys*	30.88	17.69	32.56	18.84	1130
39	*Leonotis myrothamnifolia*	31.04	17.67	32.54	18.73	1137
40	*Leonotis leonurus*	30.30	18.01	32.84	18.83	1099
41	*Leucas capensis*	30.78	17.83	32.65	18.72	1127
42	*Acrotome anguistifolia*	30.72	18.08	32.66	18.52	1139
43	*Acrotome inflate*	30.56	17.93	32.59	18.90	1132
44	*Acrotome fleckii*	30.26	18.05	32.47	18.93	1130
45	*Acrotome hispida*	30.68	18.16	32.53	18.60	1134
46	*Scutellaria sieberi*	30.9	16.9	33.5	18.7	1279
47	*Scutellaria hirta*	30.9	16.9	33.6	18.5	1280
48	*Ocimum americanum*	32	16.4	33.1	18.5	1089

5.C. 3. Phylogenetic Analysis

Altogether 48 taxa were used in the analysis. Analyses were conducted for concatenated data only.

5.C. 4. Maximum Likelihood Analysis

Maximum Likelihood + Thorough bootstrap were used with 1000 bootstrap replicates. Bootstrap per branch length was selected for the Maximum Likelihood analysis using RAxML.

5.C. 4.a. Phylogeny based on concatenated data set of *trnL-F* and *rps16*

A concatenated plastid DNA of 48 taxa with data set of two regions *trnL-F* and *rps16* were prepared in MEGA. The concatenated dataset had 919 characters of which 434 was conserved sites, 439 variable, 148 parsimony informative (Pi) sites and 284 singletons. Gaps were treated as 5th character.

The combined analysis shows a better resolved phylogenic tree with *Anisomeles, Pogostemon* and *Colebrookea* clustered on one side with a BS of 95 (Clade A). On the opposite end *Gomphostemma, Leucas, Leonotis, Otostegia* and *Acrotome* form a cluster (Clade B) with a boot strap of 99. Other *Lamiaceaen* members, *Ocimum* and *Scutellaria* form a group on re-rooting the tree as outgroups.

Three subclades were recognized in Clade A; *Anisomeles* clade (BS 100), *Pogostemon* clade (BS 98) and *Colebrookea* clade (BS 95). *Colebrookea* clade which is represented by a single species; *Colebrookea oppositifolia*, was sister to a large clade consisting of *Anisomeles* and *Pogostemon* which again bifurcated to form *Pogostemon* clade (*P. benghalensis*, *P. mollis*, *P. quadrifolius* and *P. wightii*) and *Anisomeles* clade (*A. heyneana* and *A. malabarica*).

The clade B splits into two, the *Gomphostemma* clade, which is represented by a single species; *G. heyneanum* var. *heyneana* and clade consists of all the *Leucas* species, *Leonotis, Otostegia* and *Acrotome.* And the later bifurcated to form two subclades with most of the African *Leucas* together with *Acrotome*, *Leonotis* and *Otostegia* clustered on one side with a BS of 82 (African Clade). On the opposite end, all the Asian *Leucas* species form a cluster (Asian *Leucas* Clade) with a support value of 36.

In Asian *Leucas* Clade, two subclades could be recognized; Clade I and Clade II. The Clade I (BS 50) consisting of 5 species: *Leucas montana*, *L.*

marrubioides var *pulneyensis*, *L. lanata* var *candida*, *L. angularis*, *L. biflora*. Potential synapomorphic characters of this section are non-cilliated calyx mouth and non-grooved stems. This subclade posses the characters of the classical taxonomical section *Ortholeucas* proposed by Bentham (1832).

The Clade II sister to clade I, forms a polytomy with a poor BS value (36) consist of members of section section *Plagiostoma* (*L. lavandulifolia, L. lavandulifolia* var. *nagalapuramiana*, *L. aspera, L. wightiana and L. stricta*) and section *Astrodon* (*L. hirta, L. helianthimifolia, L. ciliata, L. rosmarinifolia, L. stelligera, L. lanceaeolia* and *L. eriostoma*). The members of the section *Plagiostoma* clustered together with a BS of 90. Potential synapomorphies of this clade is annual herbaceous nature, linear lanceolate leaves. The members of the section *Astrodon* share potential synapomorphies include they are spreading herbs, usually the length of the calyx teeth is 2-3 mm, calyx teeth spreading out, fan shaped corolla were also present.

Section *Hemistoma* consists of Arican *Leucas*. *L. urticifolia* and *L. martinicensis* were the two taxa from India, which belongs to this section. *Leonotis nepetifolia* also clustered with this group. It was found that these taxa collected from India were nested within African lineages.

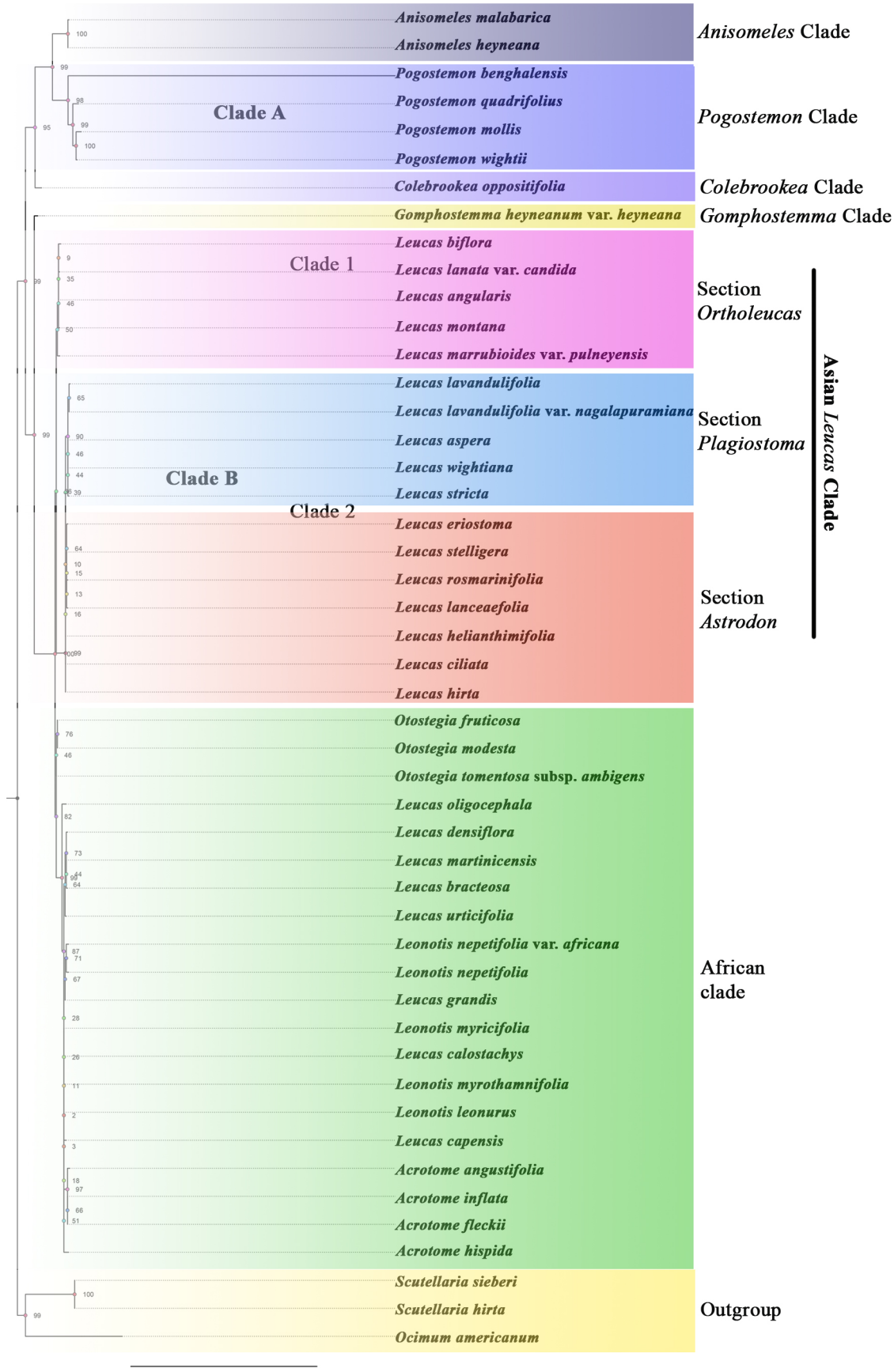


Figure 5.10: The 50% majority rule consensus phylogram from a partitioned RAxML analysis of two regions of chloroplast genome (*trnL-F* & *rps16*)

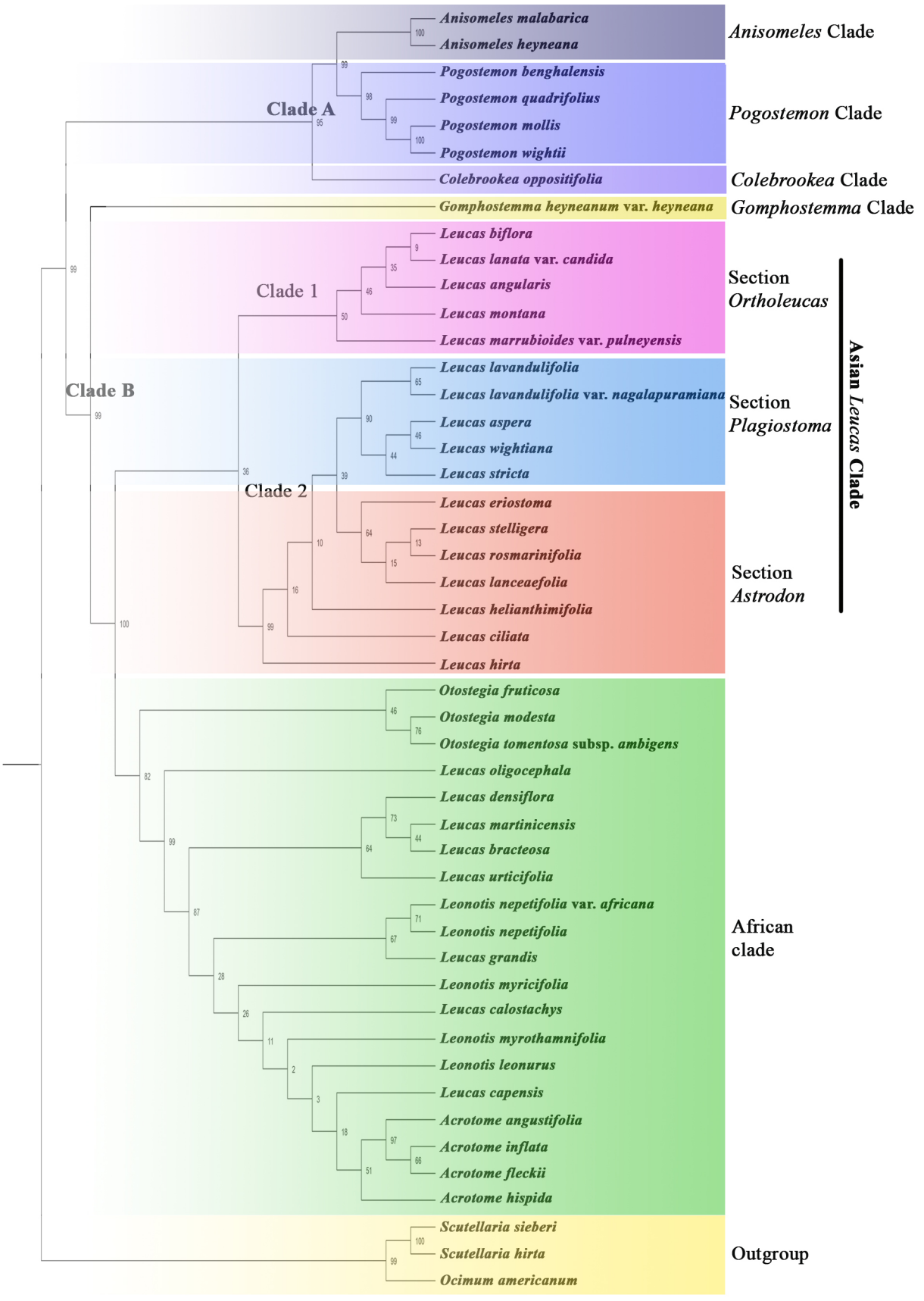


Figure 5.11: The 50% majority rule consensus cladogram from a partitioned RAxML analysis of two regions of chloroplast genome (*trnL-F* & *rps16*)

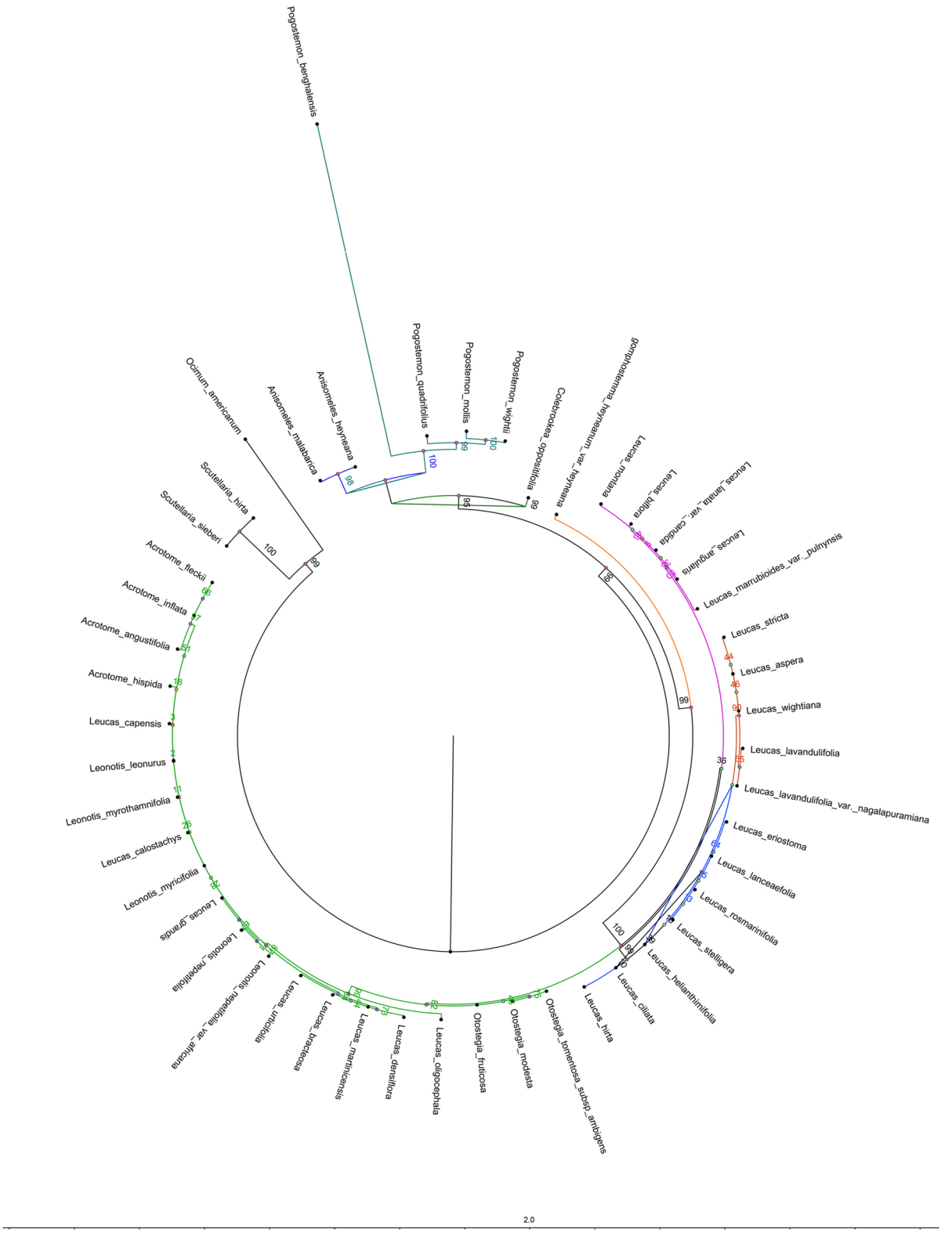


Figure 5.12: The 50% majority rule consensus polar diagram from a partitioned RAxML analysis of two regions of chloroplast genome (*trnL-F* & *rps16*)

5.C. 5. BAYESIAN ANALYSIS

5.C. 5.a Phylogeny based on concatenated data set of *trnL-F* and *rps16*

As in the previous analysis, here also we used 48 taxa for assessing phylogenetic relationship using MrBayes package. The concatenated plastid DNA dataset consists of two chloroplast regions *trnL-F* intergenic spacer and *rps16* intron. The concatenated dataset had 919 characters of which 434 was conserved sites, 439 variable, 148 parsimony informative (Pi) sites and 284 singletons. Gaps were treated as 5th character.

The HPD values obtained in the Bayesian analysis are given below;

Table 5.7: 95% HPD Interval

			95% HPD Interval					
Parameter	Mean	Variance	Lower	Upper	Median	min ESS*	avg ESS	PSRF+
TL{all}	0.854505	0.002054	0.767747	0.939053	0.852531	417.41	419.69	0.999
r(A<->C){all}	0.143794	0.000280	0.111477	0.174812	0.143254	321.45	378.80	1.009
r(A<->G){all}	0.256524	0.000428	0.215552	0.295583	0.256185	308.24	366.77	0.999
r(A<->T){all}	0.142197	0.000258	0.112236	0.174605	0.141163	311.21	391.95	1.000
r(C<->G){all}	0.078291	0.000256	0.047742	0.109291	0.077369	170.43	308.62	0.999
r(C<->T){all}	0.249860	0.000451	0.212676	0.295530	0.249190	441.59	448.60	1.007
r(G<->T){all}	0.129334	0.000255	0.102374	0.163917	0.128962	430.70	451.70	1.001
alpha{all}	3.706745	15.072765	1.261219	7.754150	2.816873	347.27	472.04	0.999

* Convergence diagnostic (ESS = Estimated Sample Size); min and avg values correspond to minimal and average ESS among runs.
ESS value below 100 may indicate that the parameter is undersampled.
\+ Convergence diagnostic (PSRF = Potential Scale Reduction Factor; Gelman and Rubin, 1992) should approach 1.0 as runs converge.

Table 5.8 :Mr Bayes Model and partition settings

```
Model settings:

   Settings for partition 1 --
      Datatype  = DNA
      Nucmodel  = 4by4
      Nst       = 6
                  Substitution rates, expressed as proportions
                  of the rate sum, have a Dirichlet prior
                  (1.00,1.00,1.00,1.00,1.00,1.00)
      Covarion  = No
      # States  = 4
                  State frequencies are fixed to be equal
      Rates     = Gamma
                  The distribution is approximated using 4 categories.
                  Likelihood summarized over all rate categories in each generation.
                  Shape parameter is uniformly distributed
                  on the interval (0.10,50.00).

   Settings for partition 2 --
      Datatype  = DNA
      Nucmodel  = 4by4
      Nst       = 6
                  Substitution rates, expressed as proportions
                  of the rate sum, have a Dirichlet prior
                  (1.00,1.00,1.00,1.00,1.00,1.00)
      Covarion  = No
      # States  = 4
                  State frequencies have a Dirichlet prior
                  (1.00,1.00,1.00,1.00)
      Rates     = Gamma
                  The distribution is approximated using 4 categories.
                  Likelihood summarized over all rate categories in each generation.
                  Shape parameter is uniformly distributed
                  on the interval (0.10,50.00).
```

Table 5.9: MrBayes parameter settings

```
Active parameters:

                     Partition(s)
   Parameters        1  2
   ----------------------
   Revmat            1  1
   Statefreq         2  2
   Shape             3  3
   Ratemultiplier    4  4
   Topology          5  5
   Brlens            6  6
   ----------------------

   Parameters can be linked or unlinked across partitions using 'link' and 'unlink'
```

```
   1 --  Parameter  = Revmat{all}
         Type       = Rates of reversible rate matrix
         Prior      = Dirichlet(1.00,1.00,1.00,1.00,1.00,1.00)
         Partitions = 1 and 2

   2 --  Parameter  = Pi{all}
         Type       = Stationary state frequencies
         Prior      = Fixed
         Partitions = 1 and 2

   3 --  Parameter  = Alpha{all}
         Type       = Shape of scaled gamma distribution of site rates
         Prior      = Uniform(0.10,50.00)
         Partitions = 1 and 2

   4 --  Parameter  = Ratemultiplier{all}
         Type       = Partition-specific rate multiplier
         Prior      = Fixed(1.0)
         Partitions = 1 and 2

   5 --  Parameter  = Tau{all}
         Type       = Topology
         Prior      = All topologies equally probable a priori
         Partitions = 1 and 2
         Subparam.  = V{all}

   6 --  Parameter  = V{all}
         Type       = Branch lengths
         Prior      = Unconstrained:GammaDir(1.0,0.1000,1.0,1.0)
         Partitions = 1 and 2
```

All other Lamiacean members like *Scutellaria* and *Ocimum* are clustered as outgroup.

Close observation of the Bayesian tree shows two major clades; Clade A and Clade B. Clade A contain the genus *Anisomeles, Pogostemon* and *Colebrookea* with a pp value of 0.98 and in this clade three sub clades were observed; *Anisomeles* clade (pp value 0.95), *Pogostemon* clade (pp value 0.95) and *Colebrookea* clade (pp value 0.98). The *Anisomeles* clade is represented by genus *Anisomeles* with two species *A. heyneana* and *A. malabarica* noticed as the recently evolved clade. *Pogostemon* clade contains the genus *Pogostemon* with four species *P. beghalensis, P. quadrifolius, P. mollis* and *P. wightii* (PP value 0.95) and this clade again forms a bifurcation with a pp value of 0.95. Here *P. beghalensis* and *P. quadrifolius* clusterd on one side and the remaining species sister to it clustered on another side. In this cluster *P. wightii* is noticed as recently evolved species. *Colebrookea* clade is represented by the genus *Colebrookea* with a single species *Colebrookea oppositifolia* supported by posterior value of 0.98 and this clade forms the primitive clade in this major clade.

Clade B (PP value 0.99) bifurcated to form two clades, *Gomphostemma* clade which is represented by a single species; *G. heyneanum* var. *heyneana* and clade consists of all the *Leucas* species, *Leonotis, Otostegia* and *Acrotome.* The later clade bifurcated to form two subclades with genus like *Acrotome*, *Leonotis*, *Otostegia* and most of the African *Leucas* clustered on one side with a pp value of 0.96 (African Clade). On the other end, all the Asian *Leucas* species clustered to form Asian *Leucas* Clade with a posterior probability value of 0.96. The Asian *Leucas* clade bifurcated to form two subclades; Clade I and Clade II.

Clade I

Clade I (pp 0.96) consists of 5 species; *L. biflora*, *L. angularis*, *L. lanata* var. *candida*, *L. montana* and *L. marrubioides* var. *pulneyensis*. This clade bifurcated to form *L. angularis*, *L. lanata* var. *candida*, *L. montana* and *L. biflora* on one side and *L. marrubioides* var. *pulneyensis* on the other side which forms the primitive group.

Clade II

Clade II (pp 0.95) forms the largest clade with 12 taxa consisting of members of section *Astrodon* (*L. ciliata, L. hirta, L. helianthimifolia, L. eriostoma, L. lanceaefolia, L. rosmarinifolia, L. stelligera*) and section *Plagiostoma* (*L. lavandulifolia* var. *nagalapuramiana*, *L. lavandulifolia*, *L. wightiana*, *L. aspera* and *L. stricta*). The members of the section *Plagiostoma* clustered together with a pp value of 0.99. *L. ciliata* form the primitive species in this clade and *L. stricta* is the recently evolved species.

Both the trees obtained by ML and Bayesian analysis shows similar topology with fairly good bootstrap and posterior probability values. A further analysis including more number of taxa from the subfamily Lamioideae representing species from different parts of the world (data obtained from genebank) were also carried out (figure 5.16 and 5.17). The ML tree topology more or less support earlier observations by Scheen and Albert (2007) and it agrees the monophyletic origin of subfamily Lamioideae and the paraphyletic evolution of the genus *Leucas*.

Thus the Lamioideae subfamily in South India shows monophyletic evolution with five distinct clade represents 5 different lines of evolution in *Gomphostemma, Leucas, Anisomeles, Pogostemon* and *Colebrookea.*

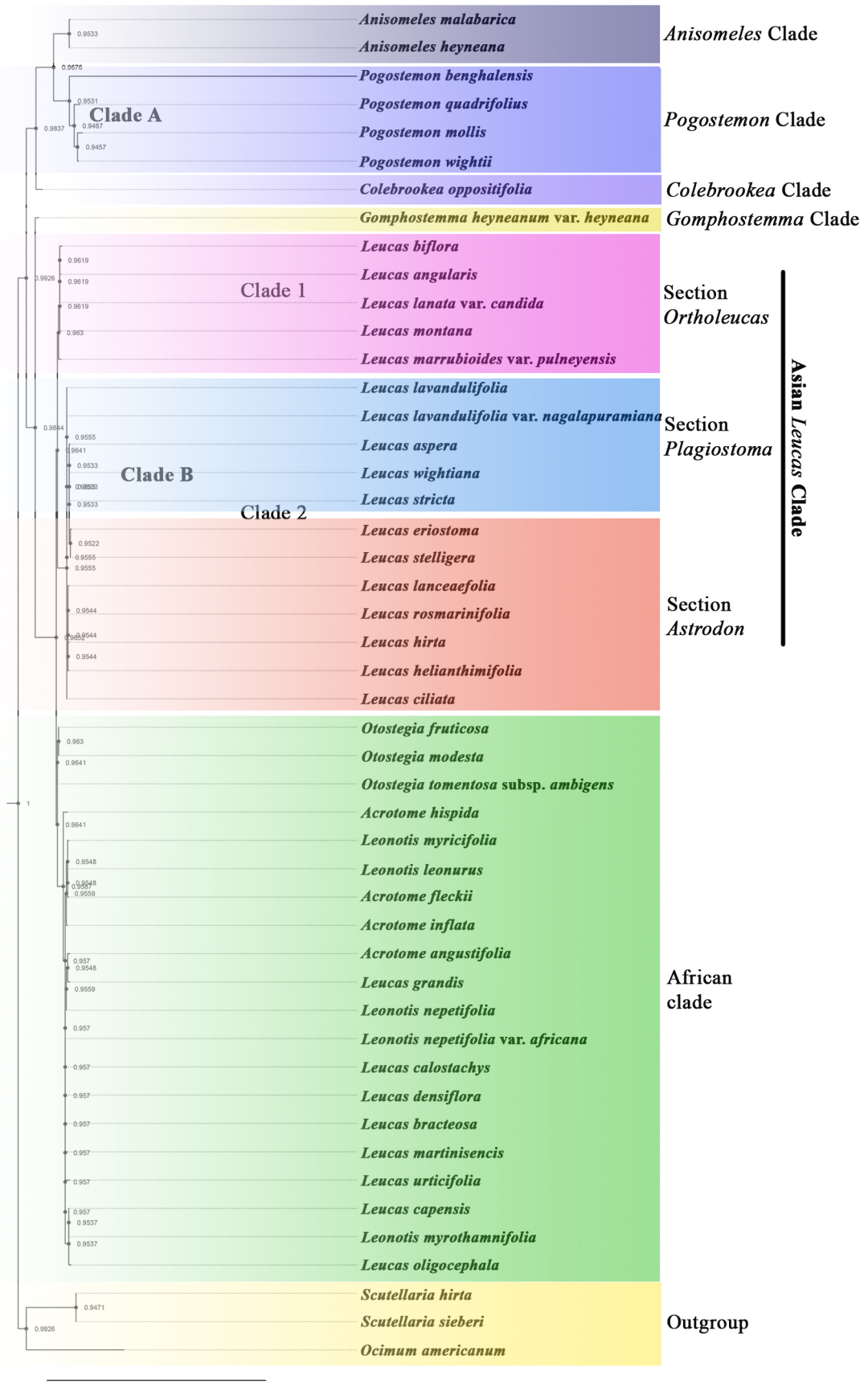


Figure. 5.13: The 50% majority rule consensus phylogram from a partitioned Bayesian analysis of two regions of chloroplast genome (*trnL-F* & *rps16*)

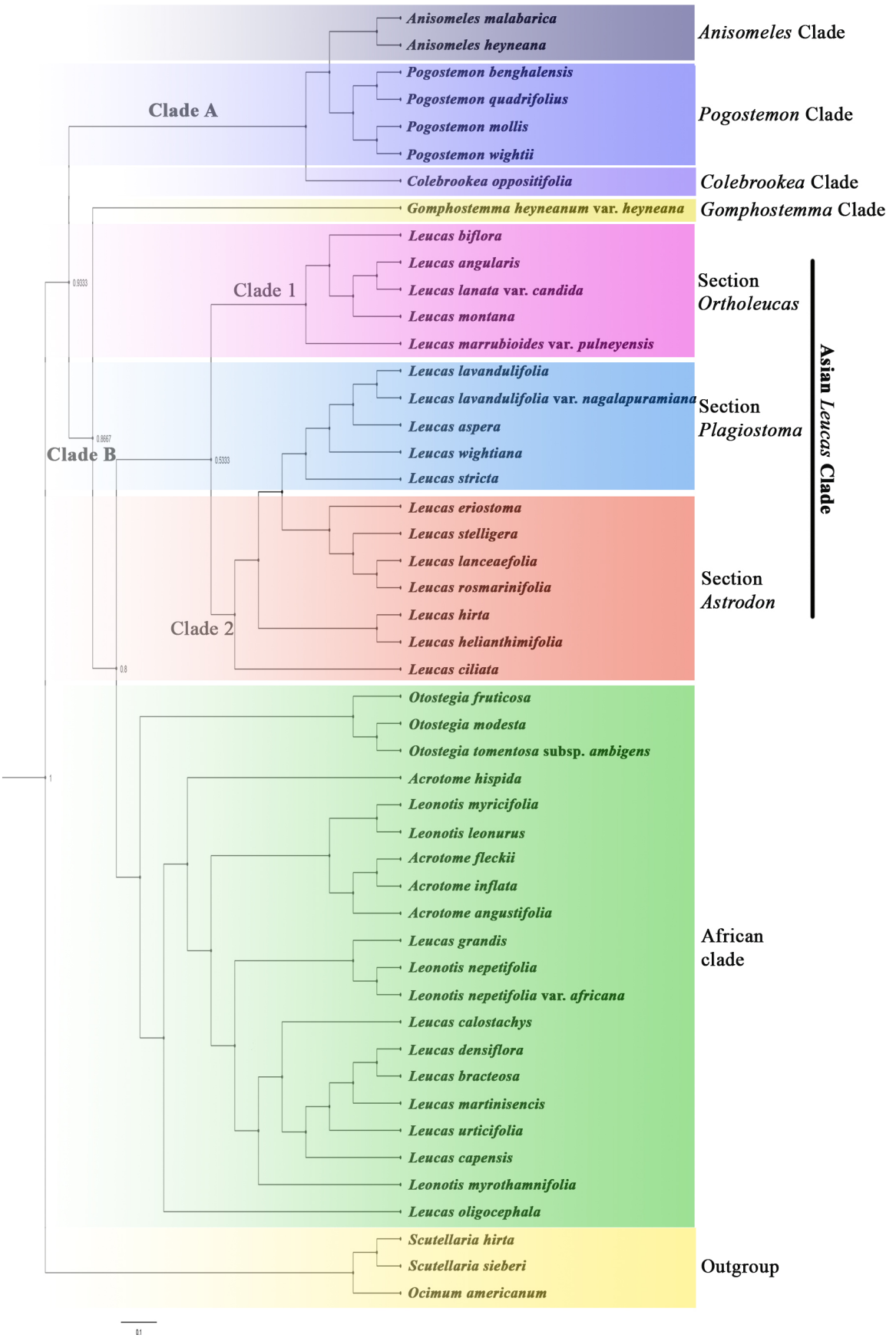


Figure 5.14: The 50% majority rule consensus cladogram from a partitioned Bayesian analysis of two regions of chloroplast genome (*trnL-F* & *rps16*)

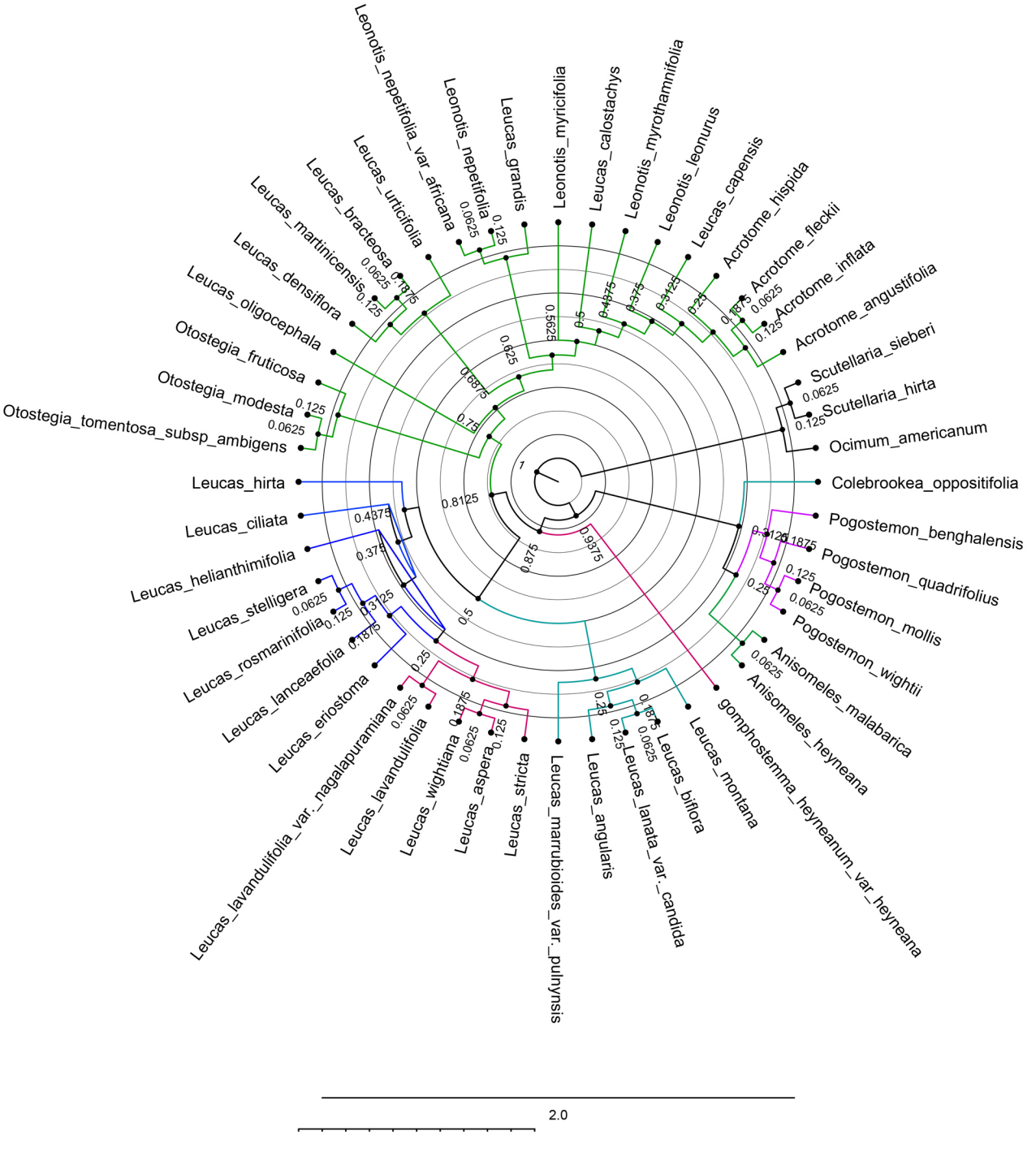


Figure 5.15: The 50% majority rule consensus polar diagram from a partitioned Bayesian analysis of two regions of chloroplast genome (*trnL-F* & *rps16*)

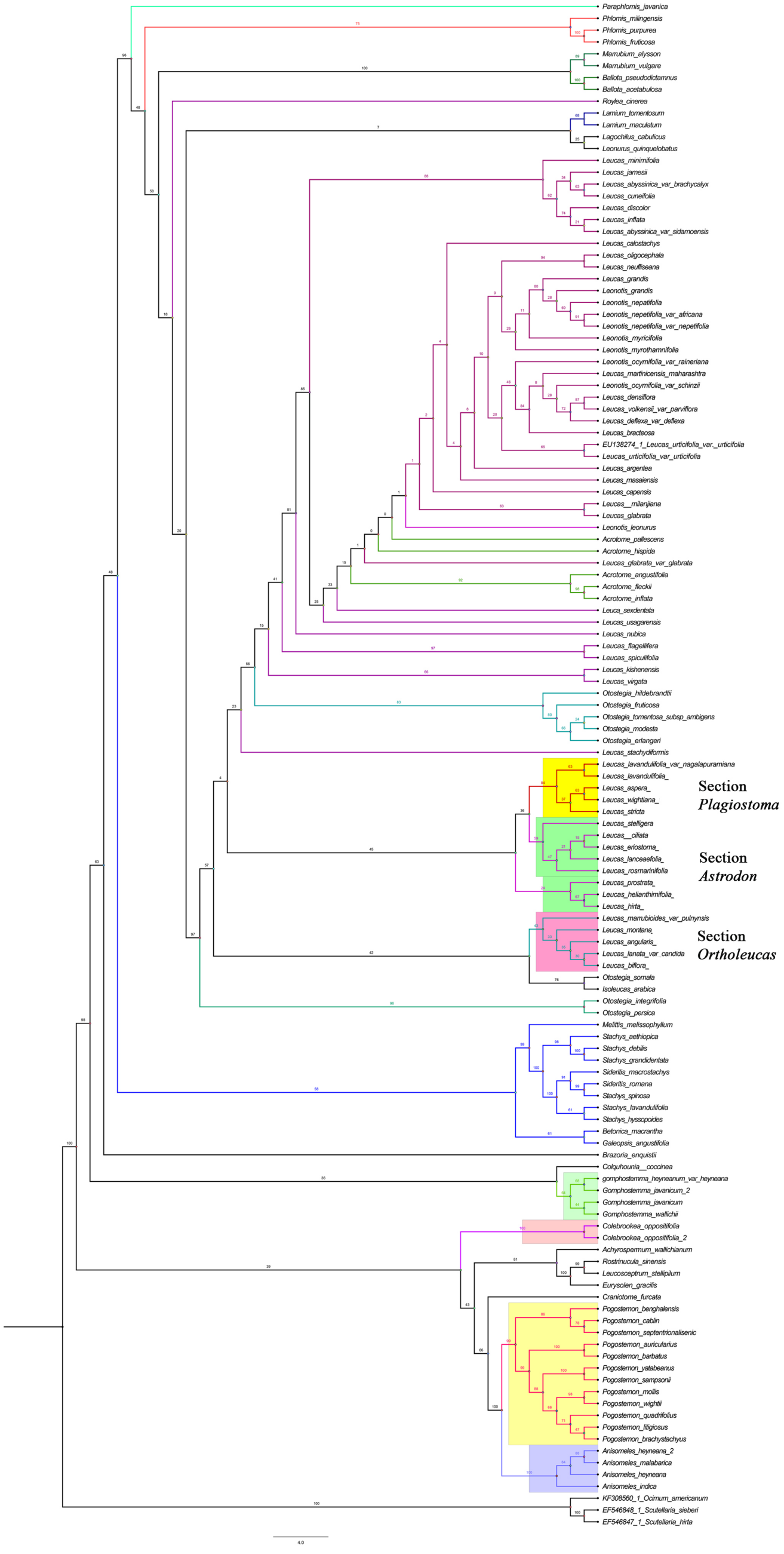


Figure 5.16: The 50% majority rule consensus cladogram from a partitioned RAxML analysis of two regions of chloroplast genome (*trnL-F* & *rps16*) of the subfamily Lamioideae

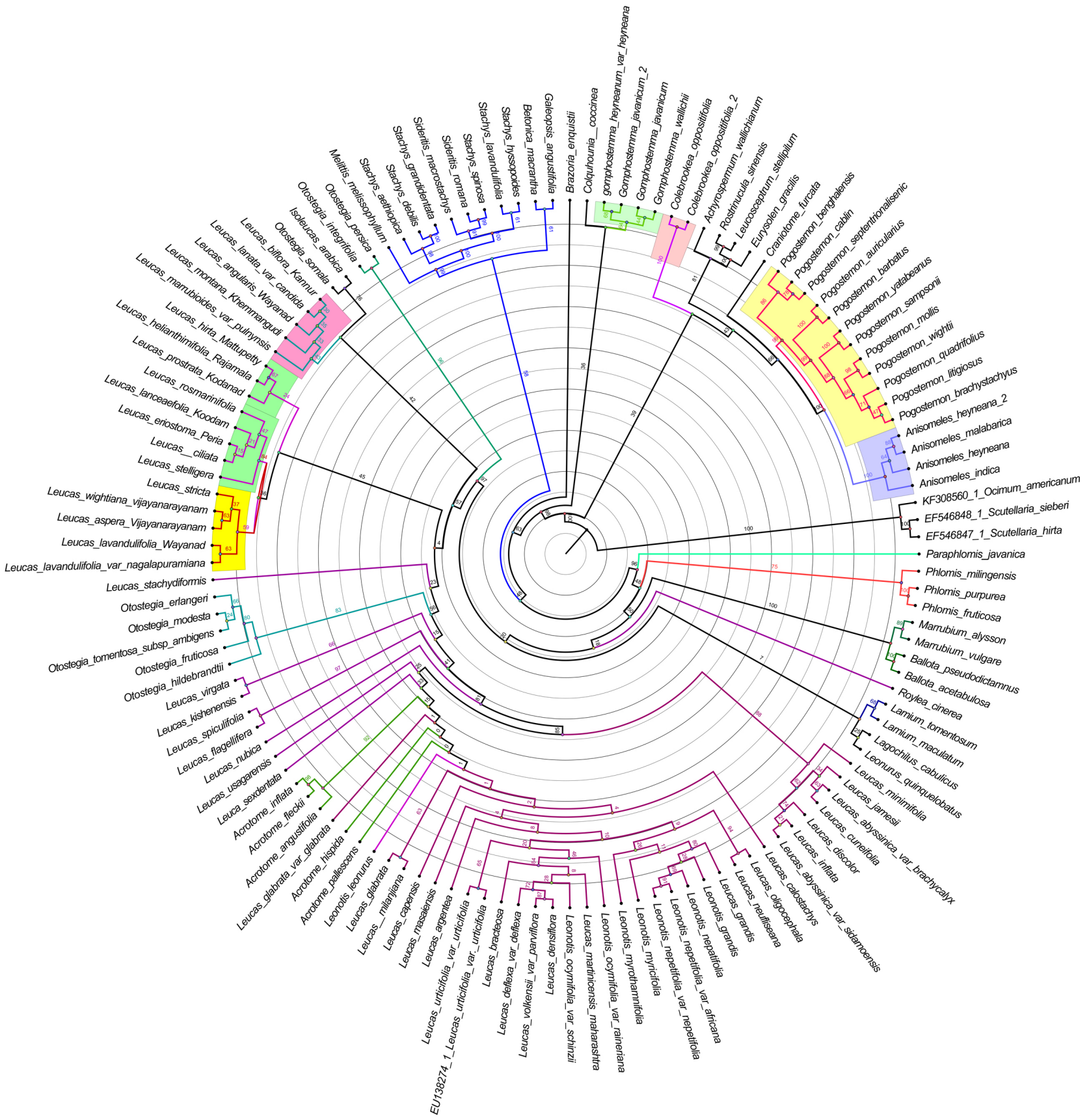


Figure 5.17: The 50% majority rule consensus polar diagram from a partitioned RAxML analysis of two regions of chloroplast genome (*trnL-F* & *rps16*) of the subfamily Lamioideae

5.C.6 Distribution of phytochemical constituents in phylogenetic tree

12 major compounds (Linolenic acid, Hexadecanoic acid, β-caryophyllene, α-bisabolol, Germacrene D, α-Humulene, T- Cadinol, Selin-11-en-4-α-ol, Phytol, M+ 286 Diterpene, Bp 191 M+ 290, 7-isopropyl-1,4-dimethyl-2-azulenol) and 1 specific compound (Laballenic acid) were selected for the present analysis. Maximum Likelihood tree obtained through the Bayesian analysis were used for studying the distribution pattern of the compounds selected. All the 13 characters were coded in a matrix based on their presence or absence among the 28 taxa studied and the analysis were performed by tracing the 13 characters in the ML tree. 13 different trees were obtained with similar topology which shows the distribution pattern of each compounds and their probable evolutionary pattern in their respective trees.

All the compounds were classified into 5 classes; Fatty acids, Diterpenes, Sesquiterpenes and two unknown compounds (BP 191 M+ 20 and 7-isopropyl-1,4-dimethyl-2-azulenol). All the 13 trees obtained in the analysis were combined and represented in a single ML tree (figure 5.18). This tree shows the distribution pattern of each class of compounds.

Both Linolenic acid and Laballenic acid shows similar distribution patter in all 28 taxa studied except in *P. wightii*, *L. martinicensis* and *L. montana*. The fatty acid Hexadecanoic acid is limited to four taxa only (*L. urticifolia, Leonotis nepetifolia, L. ciliata, L. rosmarinifolia*). The sesquiterpenes β-Caryophyllene, α- Humulene and the fatty acid linolenic acid shows similar distribution pattern. The diterpene phytol is sparsely distributed in the subfamily Lamioideae and an unknown diterpene M+ 286 Diterpene, is unique to *L. urticifolia* only. The compound 7-isopropyl-1,4-dimethyl-2-azulenol was found only in *P. benghalensis.*

From the present study, it was concluded that both fatty acids and sesquiterpenes shows similar distribution pattern and the diterpenes were sparsely distributed in the subfamily Lamioideae.

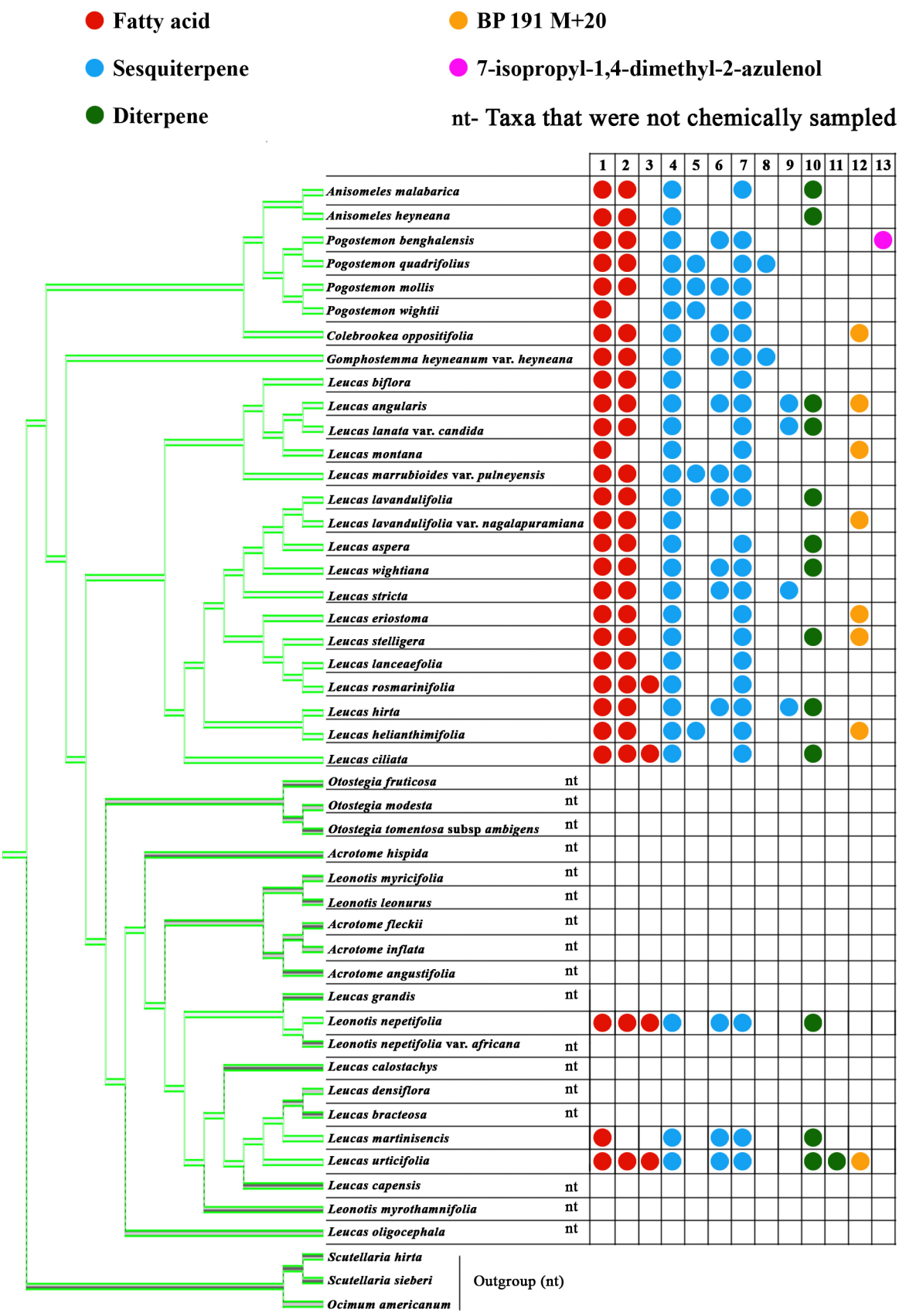


Figure 5.18: cladogram of Lamioideae illustrating the distribution of major and specific compounds; 1. Linolenic acid; 2. Laballenic acid; 3. Hexadecanoic acid; 4. β-caryophyllene; 5. α-bisabolol; 6. Germacrene D; 7. α-Humulene; 8. T-Cadinol; 9. Selin-11-en-4-α-ol; 10. Phytol; 11. M+ 286 Diterpene; 12. Bp 191 M+ 290; 13. 7-isopropyl-1,4-dimethyl-2-azulenol.

DISCUSSION

The works carried out here aimed at the phytochemical profiling and molecular phylogeny of the subfamily Lamioideae in south India. Plants studied here were collected from different localities in south India. A total of 30 taxa were selected for the volatile profiling and 45 taxa were selected for fatty acid profiling. Altogether 28 taxa were newly sequenced here and available DNA sequences of African and Arabian taxa were downloaded from the genbank for analysis. A total of 48 taxa were used for phylogenetic analysis.

The volatile profiles reveal that the populations of *Pogostemon wightii*, *P. speciosus* and *P. mollis* form a group and they clearly stand out forming a separate cluster (group I) in the analysis. Thus chemically, these three species appears to be different having different metabolic pathways leading to compounds in its essential oil, not significantly shared with rest of the species. High content in α-Bisabolol (>30% in all populations, <0.8% in all other taxa) and relatively high amounts of Cubebol (>7%, generally <2% in other taxa) appear to be useful chemotaxonomic markers for *P. wightii* and *P. specious*. In the presence of compounds like α- Copaene, β- Bisabolene, epi-cubebol, Ledol, Cubenol,1-epi-Cubenol, T-Muurolol and δ-Cadinol, *P. mollis* appears closely related to both *P. wightii* and *P. speciosus*. Previous report on the GCMS analysis of the methanolic extract of *P. mollis* showed that 2-furancarboxaldehyde was the major compound (Muthuraj *et al.*, 2015). But, there are no previous reports on the GC-MS analysis of essential oil of *P. wightii.* Hence, this is the first report which gives the volatile profile of *P. wightii*. Murugan and Mallavarapu (2013) investigated the essential oil of *P. speciosus* and reported that the main component of the oil was the sesquiterpene alcohol α-bisabolol (40.8%), which is of high value from the

therapeutic and perfumery point of view and the other major components of the essential oil were (*E*)-caryophyllene (4.5%), cubebol (5.3%) and δ-cadinene (4.5%). Their result is almost matching with the result obtained in the present study. The in-vivo and in-vitro studies of bisobolol and bisobolol rich oil has identified several pharmacological properties like anti-inflammatory, anti-irritant, non-allergic, anti-microbial and anticancer activities (Kamatou and Viljoen, 2010). Bisobolol and bisobolol rich oil have proven to be an important ingredient in future cosmetic and skin care products (Kamatou and Viljoen, 2010). The presence of α-bisabolol makes *P. speciosus* and *P. wightii* an alternative source of this compound for commercial utilization.

For the rest of the taxa, resolution provided by the cluster analysis is low. *P. quadrifolius* is deviated from other taxa by the presence of relatively high amounts of T-Cadinol (>30%), β- Caryophyllene (>19%), Caryopylleneoxide (>11%), Carvacrol and α- Humulene (>5). The three populations of *P. quadrifolius* individually forms group II. Recently, T-Cadinol was reported as the major compound in *Astericus maritimus* (Benomari *et al.,* 2019). Data on the GCMS profile of essential oil of *P. quadrifolius* was not reported earlier but GCMS analysis of different extracts (petroleum ether, acetone and methanol) of leaves and flowers of *P. quadrifolius* were reported (Jisha *et al*., 2016). The report suggests that 2,4,6 Trimethoxy acetophenone was the major phytoconstituent in petroleum ether (25.95%) and acetone extracts (77.96%) of leaves where 1-Methoxy-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone (66.65%) was the major constituent in methanol extract of leaves. The 3 methyl pentane (57.39%) was the major phytoconstituent in petroleum ether extract of flowers, where n-butane (53.26%) and geranyl vinyl ether (44.64%) were the major compounds in acetone and methanol extracts. In the UPGMA dendrogram *P. quadrifolius* alone lies as the second group.

The group IV consists of *P. benghalensis* accessions alone and forms a separate group in the dendrogram, deviated from the other *Pogostemon* species. Presence of a unique compound 7-Isopropyl-1,4-dimethyl-2-azulenol separates *P. benghalensis* from other species of *Pogostemon*. Thus 7-Isopropyl-1,4-dimethyl-2-azulenol can be regarded as a chemical marker for *P. benghalensis.* This compound has been found as a major constituents in the essential oil of *Eupatorium odorata* (Joshi, 2013).

The composition of leaf essential oil of *P. benghalensis* was previously studied by some researchers and reported that *P. benghalensis* contains limonene, a-phellandrene, b-caryophyllene, c-cadinene, b-bisabalol, a-elemene, b-elemene, a-murolene, a- copane, a-patchulene, c-patchulene and d-guaiene (Dhananjaya and Pant, 2001). According to Bhuiyan *et al*., (2011) leaf oil is rich in cadinene isomer (2.615%), elemol (1.458%), a bulnesene (2.184%), c-elemene (2.118%) and germacrene D (1.190%). They also pointed out some unidentified compounds as the major constituent in their results. Devendra *et al*., (2014) reported that 7-Isopropyl-1,4-dimethyl-2-azulenol is the major compound in the leaf essential oil of *P. benghalensis,* which is very much matching with our findings. Similar to *P. quadrifolius,* the three populations of *P. benghalensis* alone cluster as the fourth group in dendrogram.

The common features shared among the group III members in the dendrogram was the presence of higher quantity of the compound β-Caryophyllene. This is a large group representing different populations of 25 taxa. In majority of the members, β-Caryophyllene was the major compound and in rest of the other members it is one among the leading compounds. In the essential oils of many different spices and food plants, the major plant volatile compound was the sesquiterpene (E)-β-caryophyllene [(E)-BCP] which is found in large amount. Usually, (E)-BCP is found together with

small quantities of its isomers (Z)-β-caryophyllene [(Z)-BCP or isocaryophyllene] and α-humulene (formerly α-caryophyllene) or in a mixture with its oxidation product, BCP oxide (Gertsch *et al*., 2008). (E)-BCP is commercially used as a food additive and in cosmetics because of its weak aromatic taste (Skold *et al*., 2006).

Only a few species in this group were previously studied whereas others are attempted here for the first time. This is an important aspect with regard to this work. A few studies on the GCMS analysis of essential oil of the species *L. lavandulifolia* were carried out previously. The β-caryophyllene was reported as the major compound in *L. lavandulifolia* among the other 93 compounds identified (Rosamma, 2002). Joshi (2014) also reported β-caryophyllene as the major compound in the essential oil of *L. lavandulifolia*. The chemical composition of *L. stelligera* was studied earlier and reported β-caryophyllene as the major compound (Joshi, 2015), as our study indicates. Studies on the chemical composition of essential oil of *Leonotis nepetifolia* and *Leucas martinicensis* were done previously and found that the main components of *Leonitis nepetifolia* and *Leucas martinicensis* leaf oils were germacrene D, followed by β-caryophyllene (Muhayimana *et al*., 1998). This result is well agreeing with the result obtained in the present study. Germacrene D was reported as the major compound in the leaf and flower essential oil of *Leonotis leonurus* and *Leonotis ocymifolia* (Oyedeji *et al*., 2005). Prasad *et al*., (2017) reported Germacrene D as the major compound in the essential oil of *Leucas mollisima*. It has been reported that germacrene D plays a crucial role as a precursor of various sesquiterpenes such as selinenes and cadinenes (Bulow and Konig, 2000; Telascrea *et al*., 2007). But according to Oyedeji (1999), the major compound in the essential oil of *Leonotis nepetifolia* was β-caryophyllene, which is followed by germacrene D.

While comparing the obtained results with the previously done research works, Ushir and Patel (2011) studied the essential oil composition from aerial parts (stem, leaves, flowers and fruits) of *A. indica* and *A. malabarica* and found that linalyl acetate (15.3%) and *α*-thujone (11.9%) were the major compounds in *A. indica* and *α*-thujone (17.6%), terpenyl acetate (16.45%) and, *δ*-cadinene (11.5%) were the major compounds in *A. malabarica*. Phytochemical analysis of the acetone extract of aerial parts of *A. heyneana* has led to the isolation of new phyllocladanediterpene acid, phyllocladan-16a,17-dihydroxy- 19-oic acid, along with known phyllocladanediterpene, phyllocladan-16a,19-diol, cembranediterpene-ovatodiolide, sitosteryl-3-O-b-D-glucoside, and verbascoside (Roshan *et al.,* 2012).

Leucas aspera is a well studied species with regards to its phytochemical aspects. The main constituent identified in *L. aspera* is β-caryophyllene (Joshi, 2015). Correlation of the compounds from plants growing in Nepal revealed high amount of β-caryophyllene, 1-octen-3-ol and caryophyllene oxide, whereas in this study, 1-octen-3-ol is found to be totally absent and caryophyllene oxide found to be relatively low amount and no relative changes to quantity of β-caryophyllene. It is interesting to note that the compound 3-octanol was detected in trace amount from Nepal, and is found to be completely absent in the present study. The compounds like linalool, (E)-nerolidol, (Z)-caryophyllene, β-eudesmol and humulene epoxide II those identified from plants of Nepal (Satyal *et al*., 2012) were not detected even in trace amount in this study. Furthermore, α-farnesene and menthol identified from other parts of south India in significant amount (Mangathayaru *et al*., 2006) were neither detected in this study. Moreover, the GC-MS analysis of the volatile oil of *L. aspera* identified carvone, carvacrol, menthol, phellandral, and farnesene as major components (Gerige *et al*., 2007) which is not at all promising with the obtained result. The changes in

the geographical, climatic and soil conditions may be the reason for the quantitative and qualitative divergence which in turn may affect the composition and other secondary metabolites of the plants (Joshi, 2013; Joshi, 2014).

The presence of β-caryophyllene in essential oils impart the antimicrobial, antifungal and cytotoxic activity (Fernandez-Ocana *et al*., 2004; Stoyanova *et al*., 2006; Silva *et al*., 2008) to the plants possessing it. Also β-caryophyllene has anticancer activity (Legault and Pichette, 2007), local anaesthetic (Ghelardini *et al*., 2001) and peripheral effects (Kuwahata *et al*., 2012). Hence, these Lamioideaen plants could be a good source of β-caryophyllene, which is the main constituent of the oil.

Out of 30 species studied, only a few species (eight) were previously studied in phytochemical aspects, whereas all others (twenty two) are considered for the first time in our study.

Secondary metabolites like fatty acids and essential oils have proven chemotaxonomic importance at the generic level in Lamiaceae. They probably act as defense against herbivores, viruses, microbes or competing plants and as signal compounds to attract pollinators. Moreover, many species of Lamiaceae are aromatic and often used in folk medicines. (Wink, 2003).

The leaf fatty acid composition of studied Lamioideae members showed different saturated and unsaturated fatty acid concentrations. Palmitic acid methyl ester was found as main saturated fatty acid components in all studied samples and linolenic acid methyl esters was found as main unsaturated fatty acid components. The leaf fatty acid composition of nine Lamiacaeae taxa were analyzed and 13 Fatty acids were identified. The main fatty acids found were palmitic acid methyl ester (13.49-27.71%), linoleic acid methyl ester (10.85-19.47%) and linolenic acid methyl ester (40.68-

56.53%); while other fatty acids were found in minor proportions (Cacan *et al*., 2018). In the present study also linoleic acid and linolenic acid were found in abundance along with stearic and palmitic acid (Table 5.4). Linoleic acid has peculiarly a crucial role as precursors for the biosynthesis of omega-3 and eicosanoids (prostaglandins, thromboxanes and leukotrienes) (Youdim *et al*., 2000). Linolenic and oleic acids were reported to have insecticidal activity against *Aedes aegyptii* larvae (DeLany *et al*., 2000).

The study completed by me is the first comprehensive and comparative analysis of leaf fatty acid composition of Lamioideae species. Previously, studies on seed oil fatty acid composition of some species of the genus *Leucas* were undertaken. Verma *et al* (2017) identified 19 fatty acids in all parts of *Leucas cephalotes* in which palmitic acid, oleic acid, linoleic and linolenic acids were the major fatty acids. Similarly the seed oil fatty acid contents of *L. urticifolia* (11.1% palmitic, 5.32% stearic, 29.7% oleic, 24.01% linoleic, 5.77% a-linolenic and 24% laballenic) and *L. cephalotes* (13% palmitic, 3.9% stearic, 41.6% oleic, 13.5% linoleic and 28% laballenic) were reported (Sinha *et al*.,1978; Nasirullah and Osman,1983). The seed fatty acid compositions of 26 species and five varieties of *Leucas* were studied and reported major fatty acids as palmitic, stearic, oleic, linoleic and laballenic acid, whereas myristic, palmitoleic, cis-vaccenic, linolenic, eicosanoic, eicosenoic, phlomic and docosanoic acid were detected in minor quantities (Choudhary *et al*., 2017).

Some studies on the fatty acid compositions of the species *Leonotis nepetifolia* were carried out previously. Oliveira *et al*. (2015) identified 16 compounds in the leaf fatty acid of *Leonotis nepetifolia*, totaling 95.13% and methyl linoleate (46.98%) was the major compound. Bagby *et al.* (1965) reported a new allenic fatty acid; laballenic fatty acid (16%) from the seed oil of *Leonotis nepetifolia*.

It is noteworthy that, the unusual fatty acid, phlomic acid and the unsaturated fatty acid, eicosadienoic acid was found only in *Gomphostemma heyneanum* var. *heyneana*. Total saturated fatty acid of studied species was between 26.95 ± 0.77% and 63.37 ± 0.98 %. *Leonotis nepetifolia* has the lowest level of total saturated acid and *Leucas sebaldiana* the highest amount of total saturated fatty acid concentrations. Total monunsaturated fatty acids of studied species were between 4.66 ± 1.03% and 17.47 ± 0.76%. *Leucas lanceaefolia* has the lowest level of total monunsaturated acid and *Anisomeles indica* the highest amount of total monunsaturated fatty acid concentrations. In the case of polyunsaturated fatty acid concentration, *Leucas sebaldiana* (20.93 ± 0.81% has the lowest level whereas *Leucas lanata* var. *candida* (66.08 ± 2.25%) has highest amount of total polysaturated fatty acid concentrations. *Leucas ciliata* (13.49 ± 0.03) has the highest amount of total unusual fatty acid concentration.

While comparing the leaf fatty acid composition concentration with that of seed oil fatty acid concentration, the results showed variations. The total saturated fatty acid in the seed oil of *Leucas sebaldiana* was found to be 26. 61 ± 0.98% (Choudhary *et al*., 2017) whereas its concentration is very high in leaves. The concentration of total and polyunsaturated fatty acid is comparatively lesser and monounsaturated fatty acid and unusual fatty acid are comparatively higher in seed oil. The unusual allenic fatty acid, laballenic acid was found to be a major fatty acid in the seeds of *Leucas* with higher concentration in *Leucas helianthimifolia* (44.88 ± 0.87%) (Choudhary *et al*., 2017) whereas its concentration is very less (1.39 ± 0.57%) in leaves. The first known unusual fatty acid, laballenic acid, is proven to have anti-inflammatory properties (Patel *et al*., 2015). Laballenic acid significantly minimizes the manufacture of lipopolysaccharides (LPS) induced tumor necrosis factor (TNF)-a and interleukin (IL)-1b in rats (Patel *et al*., 2015). So

that, such novel therapeutic act of laballenic acid can be employed for the development of anti-inflammatory drugs.

Similarly, phlomic acid, another allenic fatty acid which is very scarcely found in seeds are completely absent in leaves except *Gomphostemma heyneanum* var. *heyneana*. In seeds, the concentration of phlomic acid is higher in *Leucas ciliata*, whereas phlomic acid is found to be totally absent in the leaves of *Leucas ciliata*. Formerly, Phlomic acid has been reported in some other Lamiaceaen members with highest concentration in *Phlomis tuberosa* (2.9%) (Aitzetmuller *et al*., 1997).

Out of 236 genera in the family Lamiaceae, laballenic acid has been reported from 13 genera. In the seed oil of Lamiacean members, the presence or absence of these unusual fatty acids could be useful for chemotaxonomic and evolutionary studies (Aitzetmuller *et al*., 1997). Moreover, according to the previous reports, combining data from the relative abundance of unusual and usual fatty acids is useful for taxonomic purpose (Pujadas-Salva and Velasco, 2000; Ozcan, 2013). The selection of specific plant genus or species for pharmaceutical, nutritional and industrial usages depends mainly on the significant variation in seed oil fatty acids composition.

Monounsaturated fatty acid mainly oleic acid (OA) is moderately present in all collected species and their varieties and highest percentage was observed in *Pogostemon mollis* (12.37 ± 0.63%). Oleic acid owns anti-inflammatory and anticancer properties and also it can lower the risk of cardiovascular diseases (Sales-Campos *et al*., 2013).

The result shows significant variations in the leaf fatty acid composition amongst Lamioideae species and varieties studied (Table 5.4). Some of the collected species are endemic to particular habitat and it suggests that these species are habituated to specific climatic condition (Table 4.1).

The geographical location and climatic conditions can regulate the fatty acid composition of several species (Johansson *et al*., 2000). Several factors like nutrient availability, salinity, moisture, latitude and average day light have significant impact on fatty acid constitution (Angelini *et al*., 1997; Ghebretinsae *et al*., 2008; Wu *et al*., 1998; Hrastar *et al*., 2012). An unexpected significant variation in predominated unusual medium chain FAs (lauric acid and myristic acid) and regular fatty acid (oleic acid and linoleic acid) in *Cuphea* species have been reported due to speciation, adaptive radiation and habitat conditions (Graham *et al*., 2016).

Hierarchical clustering did not allow a clear-cut distinction to be made between the species of Lamioideae. The clustering differentiated by HCA displays chemical similarities and differences that are not seen otherwise (Custodio *et al*., 2003). In the case of genus *Leucas*, the fatty acid profiles do not provide a clear cut proof to hold the separation of Asian *Leucas* from its African relatives as suggested by Scheen and Albert (2007, 2009). Similarly, at infrageneric level, the species did not show similar clusters identified using morphology and molecular methods. Studied taxa assembled many similar constituents in their fatty acid composition that could be documented by the same ecological conditions of their habitat but differences were also detected. We need to evaluate whether the fatty acid composition could be effected by the pedoclimatic circumstances and cause chemical convergence.

Phylogenetic analysis was conducted jointly using concatenated sequences. Maximum Likelihood and Bayesian approaches were performed in RAxML and MrBayes packages respectively. According to Scheen and Albert (2009) and Vimal (2017), *Leucas* is not monophyletic and consists of two clades which correspond to an Asian group and a heterogenous Afro-Arabian group and these two sister clades were well supported in both Likelihood and Bayesian methods. The present study also supports this statement. The genera

Acrotome, Leonotis and *Otostegia* nested within the African species clade along with African *Leucas* species to form a paraphyletc group. This is in accordance with the earlier works on morphology (Sebald, 1980; Ryding, 1998) and molecular phylogeny (Scheen *et al*., 2009, 2010).

According to Scheen *et al*, (2010) seven genera; *Ballota*, *Leonotis*, *Leonurus*, *Leucas*, *Phlomis*, *Sideritis*, and *Stachys*, were shown to be para- or polyphyletic. The new molecular results confirm that Asian monotypic genera, *Colebrookea* belongs to the tribe Pogostemoneae, a relationship previously suggested based on morphology and limited unpublished DNA-sequence data (Scheen *et al*., 2010). The very distinctive monotypic genus *Colebrookea* is resolved as the phylogenetic sister to the subclade of *Anisomeles* and *Pogostemon.* This is in agreement with the updated phylogeny of the subfamily Lamioideae propose by Bendiksby *et al*, (2011). Some morphological traits of *Colebrookea* such as small nutlets not much longer than broad, with a very distinctive sclerenchyma region, and lack of glands, although the condition of having eglandular hairs on the nutlets are similar to the genera in its sister clade.

From the phylogram of combined data, it is clear that Asian *Leucas* is genetically distict from other genera included. It also demonstrates that there are genetic variations within Asian *Leucas* and three distinct groups could be identified corresponding to three groups identified morphologically. In all analysis using combined data set, almost similar topology is obtained and this suggests the robustness of these groups, irrespective of slight differences in support values.

Genera like *Scutellaria* and *Ocimum* included in the analysis served as the outgroups. The inclusion of more taxa will provide an increased phylogenetic resolution and stronger support for most of the clades within Lamioideae.

The present investigation showed distinct molecular groups within the genus *Leucas*. Based on molecular similarities, morphologists considered different infrageneric sections within genus *Leucas* (Bentham, 1830, 1834, 1848; Hooker, 1885; Briquet, 1896; Singh, 2001; Sunojkumar, 2005). The three clades recognized in *Leucas* represent three molecular groups, as three sections. The section *Astrodon* Benth., the section *Plagiostoma* Benth., the section *Ortholeucas* Benth. (*Leucas*).

Based on the present molecular phylogenetic analysis three infrageneric sections are recognized in *Leucas s.str.* They are;

1. Section *Astrodon* Benth.

The section consists of species like *L. ciliata, L. hirta, L. helianthimifolia, L. eriostoma, L. lanceaefolia, L. rosmarinifolia* and *L. stelligera*

2. Section *Plagiostoma* Benth.

This section consists of *L. lavandulifolia* var. *nagalapuramiana*, *L. lavandulifolia*, *L. wightiana*, *L. aspera* and *L. stricta.*

3. Section *Leucas* (Benth.) Singh

The section consists of *L. angularis*, *L. lanata* var. *candida*, *L. montana* and *L. marrubioides* var. *pulneyensis*

The comparison of between the molecular data and the chemoprofiles of the analyzed species showed rather a complex situation. The phylogenetic tree based on chloroplast genes, *rps16* intron and *trnL-F* intergenic spacer regions of the selected Lamioideae members principally justified their division based on the morphology but seem to be different in the case of their chemical profiles. The phytochemical evolution might have occurred in a

different line or perhaps the phytochemistry may be controlled by certain factors such as soil, climate and other geographical location aspects. Similar results were reported by Wink (2003) during the comparison of distribution and types of secondary metabolites with molecular phylogeny data in Lamiaceae. He reported that change in genetic expression of the corresponding genes, which probably evolved earlier in evolution of Lamiaceae could be the reason for the absence of iriodes in most but not all the members of the Nepetoideae.

This study recommends that the distribution and type of secondary metabolites have some importance for taxonomy. Their distribution more likely reflect adaptations to ecological conditions and particular life strategies ingrained in a given phylogenetic frame work and therefore they have to be investigated carefully and critically for assessing them as chemotaxonomic markers.

SUMMARY AND CONCLUSION

The present study aims to reveal the phytochemical profile as well as the phylogeneetic relationships between the selected members of the subfamily Lamioideae. A total of 30 taxa were selected based on certain criteria, which were already discussed in methodology part. Volatile profiles as well as fatty acid profiles of the selected taxa were analysed carefully. This study provides useful information on the distribution of secondary metabolites in species of the subfamily Lamioideae. Major conclusions drawn out of this study are;

a) The present study provides the first comprehensive analysis of essential oil composition of 30 taxa as well as leaf fatty acid composition of 45 taxa of the subfamily Lamioideae.

b) A total of 176 chemical constituents were identified. Among them, the sesquiterpenes consist the main proportion in all studied taxa.

c) β-Caryophyllene, a sesquiterpene is the major compound in many of the selected species especially in the genus *Leucas* and this compound is present in the essential oil of all the species.

d) 7-Isopropyl-1,4-dimethyl-2-azulenol can be regarded as a chemical marker for *P. benghalensis.*

e) The UPGMA tree divides the 30 taxa into 4 groups based on their similarity of chemical compounds in essential oil.

f) Out of 30 taxa studied, only a few species (eight) were previously studied in phytochemical aspects, whereas all others (22) are considered as the new report.

g) Total 14 Fatty acids; five saturated, four monounsaturated, three polyunsaturated and two unusual fatty acid with allenic double bonds were observed in leaf of Lamioideae members

h) Palmitic acid, stearic acid, linoleic acid and linolenic acid were the major fatty acids whereas myristic acid, palmitoleic acid, oleic acid, cis-vaccenoic acid, laballenic acid, eicosanoic acid, eicosenoic acid, eicosadienoic acid, phlomic acid and docosanoic acid were the minor fatty acids.

i) Unusual fatty acid, laballenic acid is found as a minor fatty acid with highest concentration in *Leucas ciliata*. According to Choudhary *et al*, 2017, the concentration of laballenic acid is very higher in seed oil.

j) Another unusual fatty acid, phlomic acid is present only in the leaves of *Gomphostemma heyneanum* var. *heyneana.*

k) The dendrogram is divided into three major subgroups on the basis of similarity of fatty acids; the first group represent species grouped on the basis of lower linolenic acid (less than 25%) and higher quantity of major fatty acids, while second on the basis of presence of (25-40%) of linolenic acid and third group on the basis of abundance of major fatty acid, linolenic acid (more than 40%).

l) The UPGMA dendrograms constructed based on the volatile profile as well as fatty acid profile were quite different from phylogenetic trees.

m) Three molecular groups are present in *Leucas s.str.* which correspond to three infrageneric lineages. These lineages represent morphological sections within the genus. The molecular phylogenetic approach supports classical botanists (Bentham, 1834 and Hooker 1885) in the infragenric classification of *Leucas*.

n) Lamioideaen subfamilies in south India are monophyletic in the evolution with five distinct clade represented by 5 different lines of evolution in *Gomphostemma, Leucas, Anisomeles, Pogostemon* and *Colebrookea.*

o) Both fatty acids and sesquiterpenes shows similar distribution pattern in the phylogenetic tree and the diterpenes were sparsely distributed in the subfamily Lamioideae.

Analysis of phytochemical constituents through GC-MS analysis was our primary objective. We have successfully fulfilled this objective by providing a detailed report on the volatile profile as well as fatty acid profiles with their chromatogarms in the results.

One of the major objectives of this work was to check whether the phytochemical data can be used as a marker for taxonomic purpose. From the results it could be clearly seen that we cannot fully rely on the phytochemical data for the classification of plants. On using chemical data for classification, the plants with similar chemical profile have shown close similarity and they clusterd together in the dendrogram. It seems to be quite different from the classification using morphological characters. Similar situation happened in the present study also. So according to this study the chemical data canot be used as a tool for taxonomic purpose. But for the identification purpose, chemical data sometimes act as a marker; for example 7-Isopropyl-1,4-dimethyl-2-azulenol can be regarded as a chemical marker for *P. benghalensis*, since it is present only in *P. benghalensis* in large quantity. Moreover, we could not find any correlation between these phytochemicals and taxonomy and hence the second and third objectives were also got fulfilled.

Another objective was to construct the phenograms based on chemical constituents. We have constructed UPGMA tree based on volatile constituents as well as the dendrogram based on fatty acid profiles in our study. Both the trees appear to be different from their phylogenitic tree since the line of evolution for chemical constituents are different. We tried to find the distribution patteren of phytochemical constituents in the phylogenic tree using character evolution approach.

It was very difficult to compare the molecular data and the chemoprofiles of the analyzed species. The phylogenetic tree based on chloroplast genes, *rps16* intron and *trnL-F* intergenic spacer regions of the selected Lamioideae members principally corrobated their division based on the morphology but seems to be different in the case of their chemical profiles. This also point out that phytochemical evolution occurred in a different line than that of morphological evolution. So it could be concluded that evolution of phytochemical constituents in subfamily Lamioideae might be controlled by different factors. This could be soil, climate and geographical location as opined by Wink (2003).

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PUBLICATIONS
PRODUCED DURING THE STUDY

1. **Geethika K** and P Sunojkumar. 2017. Preliminary phytochemical screening of 6 members of *Leucas* (Lamiaceae). International *Journal of Pharmaceutical Sciences Review and Research*. 47(1):60-64.

2. **Geethika K** and P Sunojkumar. 2017. Phytochemical screening and High-Performance Thin Layer Chromatography profile of three species of *Leucas* (Lamiaceae). *Ancient Science of Life*. 37(2): 102-107

Papers under Review

1. **Geethika K** and Sunojkumar P. 2019. Antioxidant activity of methanol extract of three endemic species of *Leucas* (Lamiaceae) having restricted distribution in South India. [Under review in the journal "*Indian journal of Traditional knowledge*"– impact factor 0.92]

2. **Geethika K,** Kemal Husnu Can Baser, Betul Demirci, Gozde Orturk and Sunojkumar P. 2019. Essential oil composition of two species of *Anisomeles* (Lamiaceae) from Peninsular India. [Communicated to the journal "*Chemistry of natural compounds*-Springer]

Papers presented in National / International Symposia/ Conference

1. **Geethika K** and M Sabu. 2016. *Pollination Biology of Syzygium Caryophyllatum* (L.) Alston. XXVI Annual Conference of Indian Association for Angiosperm Taxonomy and International Seminar on Conservation and Sustainable Utilization of Biodiversity. November, Department of Botany,Shivaji University, Kolhapur, India.

2. **Geethika K** and Sunojkumar P, 2017. Phytochemical Screening and HPTLC Fingerprint profile of *Leucas stelligera*, *Leucas eriostoma* and *Leucas ciliata* (Lamiaceae). XXVII Annual conference of Indian

Association for Angiosperm Taxonomy and International symposium on "Plant Systematics: Priorities and Challenges. November, Department of Botany, University of Delhi, India.

3. **Geethika K** and Sunojkumar P, 2017. Phytochemical Screening and HPTLC Fingerprint profile of four species of *Leucas* (Lamiaceae). GMF National Seminar on Modern Trends in Conservation, Utilisation and Improvement of Plant Genetic Resources. November, Department of Botany, University of Kerala and Gregor Mendal Foundation, Calicut University, India.

4. **Geethika K**, Grish Mishra and Sunojkumar P, 2018. Phytochemical screening, HPTLC and Fatty acid profiles of *Leucas eriostoma* Hook. f. (Lamiaceae). International seminar on phytochemistry. March, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Trivandrum, India.

5. **Geethika. K**, Ashish Chowdhary, Shaweta Arora, Girish Mishra and sunoj Kumar. P, 2018. Fatty acid profiling in the genus *Leucas* (Lamiaceae) and its medicinal importance. XXVIII Annual Conference of Indian Association for Angiosperm Taxonomy and International Symposium on Conservation of Angiosperm Diversity: Hidden Treasure of Today and Tomorrow. November. Department of Botany, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India.

6. **Geethika K,** Girish Mishra and Sunojkumar P, 2019. Fatty acid profiles and Antioxidant properties of two endemic species of *Leucas* (Lamiaceae) in the western Ghats. National seminar on Plant Sciences: Current Challenges & Perspectives. March, Department of Botany, University of Calicut, Kerala, India.