2.1 Introduction

Plants are the essential part of our life and it is impossible about life without plants. The researchers are focusing on the plant based products and their importance as nutraceuticals for solving pharmacological issues for development of new drugs and for other applications. Since ancient time medicinal and aromatic plants are an important source of medically beneficial compounds and also used to treatment of different diseases. Newer pharmaceuticals are developed by the novel bioactive molecules which are isolated from the plants with the help of systemic analysis.

India is best known with 45,000 plants species and medicinal properties are assigned to several thousands. The medicinal properties of about 2000 species are recorded in literature. Indigenous system of medicine commonly employ about 500-700 species [35]. About 2000 plants based formulation are used in Indian system of medicine [36]. However excessive and unscientific exploitation of flora is leading to threat of extinction of many plant species. Government of India has already published a red list of plant under threat of extinction [37].

The total plant wealth of India is about 4500 species, which is about 12% of the global wealth, of which 33% are endemic [38]. Out of 15000 flowering plant species belonging to 315 different families and to 2250 genera, 5000 are endemic (47 families and 140 genera). Samant et al (1999) [39] documented that, 1748 species are recorded for their medicinal application from Indian Himalayan region. The list includes angiosperms 191 families, 878 genera and 1685 species, gymnosperms 4 families, 6 genera and 12 species and pterodophytes 28 families, 31 genera and 51 species. The richness of species is maximum in herbs (1020 species), trees (399 species), shrubs (338 species) and pterodophytes (51 species). Family Asteraceae includes 129 species, Fabaceae includes 107, Lamiaceae includes 63 species, Rubiaceae includes 55 species and Euforbiaceae includes 51 species. Other families include less than 50 species. Lamiaceae (syn. Labiatae) includes 252 genus and 7000 species [40]. Some of the species are used in Mediterranean region since ancient times for their culinary and medicinal properties [41].

Use of plants in Indigenous system of medicine in ancient civilization and like Ayurveda, Unani, Greece and oriental system indicates that the plant and plant originated product were used for maintaining the human health and cure many alments. However the conservation of the germ plasm of plant species and their cultivation is a subject of serious concern among the researchers.

Different plants species from different families have been reported for their various application in the daily functioning of human life. *Thymus linearis* and *Micromeria biflora* belonging to the family Lamiaceae and genus *Thymus* a group of perennial, aromatic herbs comprising more than three
hundred species found predominantly in Mediterranean region, Asia, Southern Europe and North Africa. Genus *Micromeria* widely distributed throughout the world some species having medicinal value, only 3 species are reported to growing wildly in Himalayan region of India. The plants are erect-ascending, perennial herbs with leaves subsessile, ovate lanceolate, base sub acute [42]. *Callicarpa macrophylla* is a medicinally important plant belonging to family Verbenaceae. This comprises 17 genera and about having over 96 species in India. It is distributed in Southern and Western part of India [42]. Different parts of this plant used to cure rheumatic pains, stomach and wounds [43].

### 2.2 Use of Plants in traditional system of medicine

Various plants species used as tinctures, tea, poultices and other preparation in different traditional system of medicine are under current interest for their different pharmacological studies which includes antifungal, antibacterial, antidiabetic and anti-inflammatory activities are extensively studied by advanced scientific techniques reported in last few decades [44-49].

Tribal people used medicinal plants as folk medicine for the treatment of insect poisoning and to treat inflammation as well as diseases like diabetes mellitus, rheumatism, abdominal disorders and inflammation. Many compounds have been isolated from the medicinal plants which include saponins, flavonoids, terpenoids, triterpenoids and phenolic compounds etc. Such compounds are reported to having protective effects against various hazardous disease produce by pathogens due to their chemical properties [50]. Different floras having medicinal and aromatic properties are distributed in India, indicating a rich source of these plant species in the word [51].

It has been reported that medicinal plants are used to control the diverse diseases such as bronchitis, pneumonia, ulcer, diarrhea and catarrh [52]. Wildly growing 2500 plant species in Indian sub continent have been reported as medicinal plant species and 300 species of them are used as drug manufacturing unites in India [53]. It has been reported that 701 species out of 1748 medicinal plant species of Indian Himalayas occurs in the Uttarakhand state However only 20% of the flora has yet been screened for their pharmacological properties which provides good opportunity to the researcher for development of novel drug molecules [54].

### 2.3 Natural products

Natural products are obtained from animal and plant kingdom, which are fine biochemical factories for the biosynthesis of primary and secondary metabolites (Fig.2.1).
Fig 2.1: Outline of biosynthesis of natural products

Natural products include plant secondary metabolites, which apparently do not have any role in primary metabolic processes however they have forbidden physiological effects on other organisms. Secondary metabolites can be differentiated into three broad categories; terpenes and terpenoids, alkaloids, and phenolic compounds. Terpenes offer much potential in industrial and medicinal applications [55].

Essential oils from different aromatic plant species are also extensively studied throughout the world because of their safe use as perfumery materials, food flavors and food preservation. The importances of conducting studies need to understand the bioactive functional and preventive effect of essential oil. Now it is broadly accepted that research on essential oil is strong need and basic requirement for the development of Natural product research. Application of essential oil implicated by their counter acting against free radicals and preventive property to oxidative damages [56].

According to earlier literature plant extracts and essential oils have received great interest due to the presence of important natural products which are used in the treatment of various diseases. They have also served as alternative remedies for the treatment of dangerous disease [57].
Aromatic oils have also been included into alternative medicines for combating against bacteria and fungi infections, including athletes’ foot, boils, acne, gingivitis and vaginal candidacies [58]. Today industrialized countries have been providing promising market for medicinal and aromatic plant products. Due to their use in aromatherapy and medicinal attributes either by ingestion, or by inhalation of vapors their demand is increasing daily day. The aromatic oils are an important in cosmetic industries, as main flavouring ingredients viz. in toothpastes, shampoos, body washes and soaps [59]. The essential oils are also used as natural food preservatives, antioxidants and for aroma. They are preferred over synthetic chemicals because of their biosafety. The essential oils find their usage in protecting the food materials by inhibiting the growth of food born pathogens and also find extensive application in pharmaceutical and food industry [60-61].

2.3.1 Terpenoids

Terpenoids constitute a major group of natural products which are present in free and combined forms in many organisms. These may be volatile monoterpenes and sesquiterpenes, di terpenes, triterpenes, steroids structures, carotenoids and polymers, like rubber [62]. Essential oils are made up of terpenoids and are derived from isoprene (C$_5$H$_8$) units, are universally present in many living organisms and are synthesized biologically (Fig. 2.1).

In addition to their commercial applications, terpenes play important biological role in plants. Some terpene metabolites are essential for plant growth and development (e.g. gibberellin phytohormones) and also play important role in host parasite and ecological interactions [63]. Volatile and non-volatile terpenes play important role in interaction of pollinators and predators of herbivores, protection against photo oxidative reactions and in mediating thermo tolerance. These are defense agents against microbes and insects [64]. The basic building block for biosynthesis of terpenoids (isoprenoids), isopentenyl diphosphate is generated in plants by two different pathways. These are cytosolic mevalonate pathway (MVA) and the plastidial methylerythritol pathway (MEP). The cytosolic pathway provides the precursors for mono sesqui, di, tri and tetra terpenoids and steroids [65].

Medicinal plants are capable of synthesizing the vide variety of low molecular weight organic compounds with unique and complex structures and have characteristic odour due to the presence of volatile and lipophilic natural constituents generally known as monoterpenoids. These possess a basic skeleton of 10 carbon units and compounds accumulate in resin ducts, secretary cavities and epidermal glands [66]. They are synthesized through geranyl pyrophosphate (GPP) [67]. Sesquiterpenoids are widely distributed, aliphatic or cyclic, isoprenoid C$_{15}$ compound. Sesquiterpenoids compounds form the largest class of terpenoids. These are derived from farnesyl pyrophosphate (FPP) [68-69]. Diterpenoids comprise four C$_5$ isoprenoid units leading to C$_{20}$ compounds. They are large and
ubiquitous family of isoprenoid natural products. In general diterpenes are non-volatile and are found in resins of higher plants. Diterpenoids are formed by cyclisation of GGPP [70].

Sesterpenoids are a novel class of C_{25} terpenes and constitute a very small group of secondary compounds found mainly in phytopathogenic fungi and insect protective waxes. These are formed by linear extension of GGPP by condensation with C_5 moiety derived from IPP to yield GFPP. Five isoprene units are linked head to tail or from the phenyl residue by addition of cis-isoprene unit [71].

Triterpenoids are composed of six C_5 isoprene units and constitute a large diverse group of natural products. Triterpenes occur most frequently as O-glycosides. These arise by cyclisation of squalene or the 3 S-isomer of 2, 3 epoxy 2, 3-dihydrosqualene. Squalene is formed by linkage of two FPP residue joined tail to tail [72].

Tetraterpenoids comprise eight C_5 isoprenoid units leading to C_{40} compounds. The carotenoids are predominant class of tetraterpenoids. Photosynthetic bacteria synthesize carotenoids of acyclic type, while fungal carotenoids are frequently acidic. These are formed by dimerization of GGPP to form phytone [73].

Polyterpenoids occur in the form of latex, in a network of interconnected latex cells and are found in single thin walled parenchyma cells as droplets. Over 2000 species of higher plants have been shown to contain rubber and gutta [74]. Rubber molecule is considered to be derived from dimethylallyl diphosphate (DMAPP), which is formed by isomerisation of IPP. Natural rubber is of isoprene units, linked together by 1,4-addition to give cis-1,4-linkage [75]. C_{35} compounds, C_{45} and C_{50} homocarotenoids and polyprenols (C_{45}-C_{115}) are minor terpenoids. They are constructed by continuation of chain elongation by addition of C_5 units in head to tail fashion [76].

2.3.2 Biosynthesis of Terpenoids

Biosynthesis of terpenoids (Monoterpenoids, Sesterpenoids Diterpenoids, Triterpenoids, Tetraterpenoids and Polyterpenoids) illustrated in Fig. 2.2.
Fig. 2.2: Biosynthesis of terpenoids
2.4 Traditional and Allopathic medicine

Since the gradual development of modern allopathic medicine, the use of medicinal plants as traditional medicine declined to a considerable extent. However, the interest in traditional medicine, rejuvenated in recent years for a variety of reasons. These include side-effects and toxicity of modern synthetic drugs, development of multi-drug resistant microorganisms and incapability of modern drugs to find effective cures for multiple numbers of diseases. A major proportion of the world lives in poor and under developed countries still relay on traditional medicinal system, also known as complementary or alternative systems of medicine [77]. The study plants used in traditional medicinal may lead to the development of novel pharmaceuticals [78].

2.5 Protective effect of Antioxidants

Synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) are used to retard the oxidation process in food, beverages and other materials. However; the use of synthetic antioxidants must be under strict regulation due to potential health hazards [79]. The search for novel natural antioxidants as alternative to the synthetic chemicals is therefore intensified in recent year. It has been reported that flavonoids, terpenoids and phenolic compounds etc. have protective antioxidant effects due to their chemical properties. Free radicals are highly toxic and generate more oxidative stress in plants and animals. Many plant originated compounds have also been reported as inhibitory agent against free radical reaction [80]. Newly the use of plant-based natural antioxidants which have reducing and antiradical properties such as phenols, flavonoids, phenolic acids and tocopherols in foods and preventive and therapeutic nutraceuticals is being advocated. These are also believed to posses anticarcinogenic potential and diverse health-promoting effects [81-83].

Toxigenic fungi present in foods and grains pose a potential hazard to human and animal health. Dermatomycoses and fungal infections are probably the most common communicable disease infecting humans. These are also a serious threat in immune system in compromised patients [84]. Fungal infections are becoming incurable problem because of antibiotics resistance and residual toxicity. Because of increased number of reported cases of food-associated infections food safety has become a fundamental issue for food industry and food consumers. Aromatic plants, herbs and spices have been used for ages both as flavoring and as preservatives agents. These are biodegradable antifungal agents with no harmful side effects [85].

2.6 Ethnobotanical approach for pharmacological studies

Approximately 265,000 species of seed plants survive on earth [86] and a large number of these are yet to be screened for their chemical composition and medicinal importance [87].
According to the WHO [88] medicinal plants could be an affordable source for developing medicines for primary healthcare needs [89]. The, putative medicinal plants should be investigated to understand their properties, safety, and efficacy [90, 81]. Plant originated products such as essential oils and extracts have been used since ancient time as natural treatment of disease [91-92]. In a world with imperfect financial resources it seems at present not possible to screen all the species for their biological activity and isolation of their bioactive compounds. Hence, in order to save document on pharmaceutical and medicinal usability’s of plants and conservation of biodiversity some reasonable and efficient survey plan is needed.

The ethnobotanical approach is one such way which meets this requirement and can be applied to choose plants for pharmacological studies. It has been suggested by researchers that use of plant extracts to treat diseases is a therapeutic modality, which has stood the test of time [93]. At present, maximum population of the word is involved in traditional medicine and primarily medicinal plants, to cure illnesses and ailments [94]. The various botanical sources, particularly metabolites and their biological activities, alongside the different practices of preparing and applying herbal remedies devised by certain ethnic groups throughout the millennia, have became the most useful basis for the development of new pharmaceuticals [95]. Ethnopharmacology has played a significant role in the progress of conventional medicine and is likely to become increasingly important in the years to come. A cooperative approach by ethnobotanists, ethnopharmacologists, physicians and phytochemists is thereby essential to spur the progress of medicinal plants research [93].

It is a well known fact that the chemical composition of plants in active compounds may differ with their geographic origin due to ecological (e.g., climatic conditions) and evolutionary-historic reasons. It has been reported that these variations are genetic, phenological status and environmental conditions which can influence the regulation of the biosynthesis of secondary metabolites. Hence, it is not surprising that the folkloric use of plants vary among geographic regions [96]. Consequently, the phytochemical properties and medicinal value of a plant species only can be exhaustively addressed by considering the geographically determined component of variation. With about 6340 different vascular plant species reported, the Balkans, compared to 10,500 species accepted in the Flora Europaea, is one of the most important biodiversity centers of Europe [97]. According to Samant et al., 1998 [98] out of the total species of vascular plants, 1748 species are medicinal. The Himalayas have a great wealth of medicinal plants and traditional medicinal awareness. The Indian Himalayan region alone supports about 18,440 species of plants Angiosperms: 8000 spp., Gymnosperm: 44 spp., Pteridophytes: 600 spp., Bryophytes: 1736 spp., Lichens: 1159 spp. and Fungi: 6900 spp.) of which about 45% are having medicinal properties.
2.7 Family Lamiaceae

It has been reported that the dicotyledonous family Lamiaceae is an incredibly big natural group of aromatic and medicinal plants [99]. According to the earlier researcher the enlarged Lamiaceae contains about 236 genera and 6,900 to 7,200 species and also reported that the largest genera are *Salvia* (900), *Scutellaria* (360), *Stachys* (300), *Plectranthus* (300), *Hyptis* (280), *Teucrium* (250), *Thymus* (220), and *Nepeta* (200) with a very wide range of morphological diversity [100-101]. Majority of these plants are annual and perennial herbs, cosmopolitan, growing under a great variety of soil and climate and constitute a scrub type of vegetation [102]. The members of this family have a global distribution [101]. Plants of family Lamiaceae are distinctive in being herbs or shrubs. They are often aromatic with ethereal oils. They have usually 4-sided stems, opposite leaves, a verticillaster or thyrse in florescence, and zygomorphic, usually bilabiate flowers having a superior ovary, often deeply 4-lobed with a gynobasic style, the fruit a schizocarp of usually 4 nutlets or a berry or drupe. The plants of this family are worldwide in distribution. Economically important species include culinary herbs like *Ocimum* (basils), *Mentha* (mint), *Rosmarinus* (rosemary), *Salvia* (sage) and *Thymus* (thyme), fragrance plants, and a number of cultivated ornamentals [103].Various plant species of family Lamiaceae have been credited within folk medicine as antiseptic, antifungal and as antimicrobial agents. [104-109].

It is well known that plant of family Lamiaceae (Labiatae) used as a frame work to evaluate the occurrence of typical primary and secondary metabolites. The aromatic oils from plant of family Lamiaceae has been reported for their importance as curing and treatment of different diseases like intestinal disorder and bronchitis [110].

Large number of species of family Lamiaceae produce essential oils whose compounds are largely used in food (as flavorings), cosmetics (fragrances and aftershaves), and medicine [19]. In India, the family Lamiaceae is represented by about 69 genera and 425 species of which 261 are endemic. The temperate Himalayas, the Deccan, North Western India and South India are the chief centers of concentration. South India is one of the major centers of distribution since it supports over 139 species [32]. Himalayan region is a big source of the plants of family Lamiaceae. Himachal Pradesh represents 42 genera and 111 species [111], while Uttarakhand region possess 49 genera and 151 species of Lamiaceae [112] were reported.

In addition various aroma chemicals, perfume products, medicinal, pot herbs and honey pasturage, the family yield a number of choice ornamentals. Many members of Lamiaceae find use in cosmetic industry, for the preparation of detergents, fabric conditioning mixtures deodorants and bath preparations long lasting fragrant preparations and perfumes, herbal preparations, on-irritating cleansers, hair gels and in baking industry. Some species find use in the preparation of dentifrices,
mouthwashes, toothpowders, chewing gum and lozenges, which act as anti-plaque and anti-tartar agents of teeth. Many species showed antifungal and antibacterial activities and these properties are due to their volatility, is of much importance since herbal fungicides are non-toxic and easily biodegradable [113]. The Lamiaeceae members are characterized by their peculiar morphology and aromaticity. The family Lamiaceae is of great importance from a commercial standpoint [114].

2.7.1 Thymus linearis

Genus Thymus belonging to the family Lamiaeceae which comprising of about 350 species of perennial, aromatic evergreen or semi evergreen herbs and sub shrubs is mostly growing in Mediterranean region, Asia, Southern Europe, northern temperate region and North Africa [115]. Thymus is the ancient Greek name for these aromatic plants, which are commonly known as Thyme [116]. It has been reported that different biological properties of Thymus species are considered it as medicinal rich herbaceous plants. Numerous application of Thymus oils and extracts in pharmaceutical, cosmetic and perfume industry also for flavoring and preservation of several food products have been reported [117]. In native medicine, flowering parts and leaves of Thymus species have been extensively used as herbal tea, tonic, carminative, antitussive and antiseptic, as well as for treating colds [118-121]. Many species and varieties of genus have been studied for their essential oil composition [122]. The chemical variability in the essential oil of plants of genus Thymus has been characterized by great morphological and physiochemical properties [123-124]. Stahl-Biskup and Laakso suggested that Northern species of genus Thymus have widespread phenomenon, covering the essential oil by chemical polymorphism [125].

Plants of the genus Thymus distributed throughout the world and have made it one of the most popular plants species due to presence of aromatic medicinal and culinary properties in different Thymus species. The pharmacological properties of the plants of genus Thymus have due to the presence of different volatile compounds. Therefore, there is a considerable research interest towards the compositional analysis of Thymus essential oils. Thymus species are commonly used as herbal tea, flavoring agents (condiment and spice) and medicinal plants [126]. Thyme oils find wide use in dietary supplementation due to their antioxidant capacity [127]. Methyl rosmarinate a new compound in sweet marjoram was isolated for the first time from Thyme [128].

It has been reported that essential oil from T. repolutus (from Turkey), T. pubescens, T. serpyllum, T. kotschyanus T. persicus were exhibited good to moderate antibactericidal activities [118-119,129]. The composition and antioxidant activity of the oils T. caespititius, T. camphorates and T. mastichina from Portugal have been reported [130]. It has been reported that leaves of Thymus caramanicus effective against microorganism infection and used in the treatment of harmful diseases such as rheumatism, skin disorders and as an antibacterial agent [131].
Thymol (66.62-71.49 %), p-cymene (5.52-7.12 %), β-caryophyllene (3.91-4.09 %), δ-terpinene (3.22-4.3 %) and carvacrol (2.64-2.77 %) have been reported as main components in the essential oil of *Thymus daenensis* from Iran. It has also been reported that extracts from *Thymus daenensis* have good free radical scavenging activity and antifungal activity against pathogenic fungi viz. *Alternaria solani, Fusarium solani* and *Rhizoctonia solani* [132].

Different spectroscopic techniques such as HPLC combined with diode array detection and atmospheric pressure chemical ionization mass spectrometry have been used to analyzed 40 Macedonian species and infra-specific taxa of *Thymus* belonging to sections *Marginati, pseudothymbra* and *serpyllum*. 5, 6- Dihydroxy -7, 3, 4- trimethoxy was the most common identified falvone among nineteen different flavones. Most of the species also provided surface flavanones, including criodictyal, naringenin and isosakuranetin were reported. The flavonoid profiles found in species of *Thymus* provide useful additional taxonomic characters at various different levels of classification [133].

The essential oils of *Thymus pubescens* and *Thymus serpyllum* collected at pre and post flowering stages were found to possess bactericidal activities. [125]. Nickavar et al. (2005) characterized the essential oils of *Thymus daenensis* and *Thymus kotschyanus*. Both the essential oils were found to be rich in thymol and carvacrol [134]. Thymol (52.28-66.65%) as the major compound besides p-cymene (1.81-21.60%) and γ-terpinene (1.94-12.48%) have also been reported in the essential oil of *T. linearis* collected from western Himalaya (India) [135].

The essential oil from *T. spathulifolius* has been reported as effective volatiles against growth of microorganisms while polar and non-polar sub fractions of the methanol extract showed moderate antibacterial activity in comparison to the essential oil was reported. The polar subfraction of the methanol extract from *Thymus spathulifolius* was able to reduce the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Inhibition values of linoleic acid oxidation were calculated as 92% and 89% for the oil and the polar sub fraction, respectively. Thymol (36.5%), carvacrol (29.8%), p-cymene (10.0%) and e-terpinene (6.3%) were reported as the major compounds in the essential oil of *T. spathulifolius* [136].

Diverse pharmacological properties, such as spasmolytic, antiseptic, antitussive, expectorant, antispasmodic activities, antibacterial, antifungal, antiviral, antiparasitic and antioxidant activities are associated with the plants of genus *Thymus* [122, 137-139]. Thymol, carvacrol and geraneol have been reported as major compound of group present in the *Thyme* oil and these compounds responsible for antiseptic, antioxidative, insecticidal, preservative and anesthetic properties [140]. Polyphenols rich *Thymus* is an important source of natural and safe antioxidants. Earlier studies have demonstrated that polyphenols, which are the most abundant natural antioxidants in our diet, inhibit oxidative processes
which influence by free radicals and other reactive species [141]. It has been reported that *Thymus daenensis* used in medicine by the Greeks, the Egyptians and the Romans. Antioxidant properties of the essential oils from different species of Genus *Thymus* mainly due to the major compounds thymol and carvacrol were reported [142].

Different compounds have been reported in the essential oil of wild thyme which showed chemotype variation in various species. Essential oil from wild thyme growing in Estonia contained (E)-nerolidol, caryophyllene oxide, myrcene and borneol chemotypes [143]. 1,8-Cineole, caryophyllene oxide, borneol, caryopyllene, germacrene D, camphor, camphene and myrcene were reported as major constituents of essential oils from wild thyme growing wild in Lithuania and also suggested that 1,8-cineole-caryophyllene oxide chemotype was determined in Lithuania [144].

Earlier workers explained rosmarinic acid, was the dominant phenolic acid besides phenolic acids includes ferulic acid, caffeic acid and its other derivatives, chlorogenic acid and p-coumaric acids) which were reported in *Thymus* species. Flavanones: naringenin, eriodictyol and dihydroquercetin; flavonoids: apigenin and apigenin-7-glucoside, flavonols: quercetin and rutin were reported in *Thymus* species. Five native Hungarian *Thymus* species (*T. glabrescens* Willd., *T. pannonicus* All., *T. praecox* Opiz, *T. pulegioides* L., and *T. serpyllum* L.) was characterized by means of polyphenolic pattern [145]. Pervious reports described that thymole exhibited antiseptic and carvacrol exhibited antifungal bioactivities [146]. Non-volatile antioxidants, such as flavonoids and vitamin E were also reported from the extracts of *T. vulgaris* [147-148]. Pharmacological important compounds (thymol) are those which are produced in large quantities in the essential oils of *Thymus* species [149]. Numerous species of genus *Thyme* (*Thymus* L.) have been reported as aromatic and medicinal plants and their essential oils also reported for inhibiting the oxidation process and microorganism attack [118, 125,150].

### 2.7.2 *Micromeria biflora*

About 100 species of genus *Micromeria* widely distributed throughout the globe are herbs; a few are of medicinal value. 3 species are reported from India especially the Himalayan region. The plants of *Micromeria biflora* are erect-ascending with a woody, creeping base, aromatic, tufted, perennial herbs. Stems arise in plenty from the base, simple or branched and short-hairy. Leaves are subsessile, ovate lanceolate, base, subacute, with a thickened margin, glabrous, gland-punctate, beneath, 0.5-0.8×0.2-0.4 cm. Bracts subulate, 0.1-0.15 cm long [42]. Various species of *Micromeria* are effective against heart disorders, headache, wounds, skin infections in cattle and treating cold [151]. Pulegone, isomenthone, p-menthone, limonene, linalol, α-pinene, β-pinene, p-cymene, α-terpinene, ð-terpinene, α-terpineol, camphene, β-bourbonone and borneol are the most encountered
essential components in *Micromeria* species [152]. Essential oil composition of *Micromeria* species such as *Micromeria fruticosa* spp., *Micromeria serpillifolia* have been studied [153-154].

Several species of the genus *Micromeria* have been used as herbal tea and also used as remedies for different medical problem. Trans- caryophyllene (43.7%), caryophyllene oxide (18.0%), spathulenol (8.5%), α-humulene (4.6%), α-myrcene (3.1%) and germacrene- D (3.1%) as major constituents in the essential oil of *Micromeria biflora* ssp. arabica K. Walth were reported [155].

The essential oils contained mostly monoterpene hydrocarbons and oxygenated monoterpenes. The main constituents were neral (25.3-32.2%) and geranial (26.7-41.3%) [156]. The essential oil of *Micromeria biflora* from leaf has played a significant role against dental caries causing bacteria, especially its showed strong activity against *Streptococs mutans* [157]. *Micromeria biflora* and some other Chinese medicinal plants species reported as rich source of terpenes (geranial, naginataketone, p-cymene, beta- himanchalene) or phenol ethers (apoile, elemicin,1,2,3,4-tetramethoxy- 5-allyl-benzene) were identified in the oil [158]. Ultrasonic-assisted solvent extraction can effectively extract oleanolic acid and ursolic acid from *Micromeria biflora* with advantages of simple operation and high efficiency [159]. It has been reported that essential oil from *Micromeria cicalica* (*Labiatae*) contained pulegone (66.55, 64.10%), cis-p-menthone (21.71, 25.31%), trans-p-menthone (9.59, 5.59%), nerol (0.35, 2.49%) and 3-octonol (0.81, 0.25%), as major constituents.

The extracts and pulegone rich essential oil of *Micromeria cicalica* exhibited a significant antibacterial and antifungal activity and effective against *Candida albicans* and *Salmonella typhimurium* [152].

### 2.8 Family Verbenaceae

Verbenaceae is economically most important family of plant Kingdome. Various genera, such as *Callicarpa* and *Lantana*, have been reported as an important ornamental species. Seventy five genera and 3,000 species of family Verbenaceae in tropics and subtropics: herbs, shrubs or trees, some are climbers: a few furnish valuable timber, some yield aromatic oils, others are grown as ornamentals. 17 genera with over 96 species distributed in Southern and Western India and tropical and subtropical Himalayas; a few naturalized throughout India [42].

Several plants of the family Verbenaceae have been reported to possess bio-active properties, which explain the importance of Verbenaceae plants and validate their use in the folk medicinal system of Bangladesh. *Clerodendrum inerme* has been reported to have significant antinociceptive activity [160], which might explain its use for rheumatoid arthritis, which has quite intensive pain as one of its symptoms. Anti-inflammatory properties have also been reported for *Clerodendrum trichotomum* [161].
Previous studies suggested that *Callicarpa* is medicinally important genus and plant *Callicarpa macrophylla* from Calcutta is used in traditional medicinal to relieve stomach disorders and rheumatism [162]. Several bioactive compounds have been isolated from the roots of *Clerodendrum eriophyllum* Gürke [163].

### 2.8.1 *Callicarpa macrophylla*

Current pharmacological reports described that different pharmacological properties viz., anti-inflammatory, hemostatic, neuroprotective, anti-amnesic, antitubercular, antioxidant, antimicrobial and analgesic effects showed by extracts of *Callicarpa* and which correlates with activating blood, promoting Qi circulation, relieving swelling, easing pain in addition to correcting menstrual disorder and alleviating anemofrigid cold in ancient Chinese works. According to the earlier literature the plants of genus *callicarpa* showed good medicinal properties as well as different pharmacological activities which might be due to the presence of terpenoids and flavonoid. It has also been reported that pharmacological results have validated the use of *Callicarpa* species in the traditional medicine [164]. Few essential oil constituents from *C. americana* were isolated and reported as bioactive compounds and oil selectively toxic to *Oscillatoria perornata*, a cyanobacterium, and was only slightly phytotoxic or antifungal [165].

From 140 tropical and subtropical species of *Callicarpa macrophylla*; 10 are shrub or tree species in India; chiefly in Eastern and Central Himalayas (3630 m). The plants of *Callicarpa macrophylla* are erect, stellate-pubescent, perennial under shrubs; leaves in sub or unequal pairs on 0.3 -1.5 cm long petioles, elliptic-dentate with gland-tipped teeth, glabrescent or so above, 10-20 (-25) ×4-6 (-8) cm; flowers in axillary, 1-3 cm long, peduncled, cymose, corymbose panicles [42]. About twenty species from *Callicarpa* genus are distributed in China and South Asia [166].

*Callicarpa macrophylla* (*Verbenaceae*) is and erect shrub, commonly known as Priyangoo globally distributed across Indo-gangetic region and sub-Himalayan tracks of India up to an altitude of 2000 m [167-169]. Flowering and fruiting during the month August-December [170]. Its leaves are used in the treatment of diarrhoea, dysentery and arresting bleeding. Leaves juice from *C. macrophylla* used in gastric, headache and bleeding troubles [171]. It has medicinal properties and grows wildly in the lower warm valleys of the Himalaya. It is a perennial, deciduous shrub attaining 2.5 m height. Essential oil is obtained from different parts of Priyangoo revealed oil content in young leaves and tender stem (0.04%) panicles (0.03%) and seed (0.13%) [172].

The parts of *Callicarpa macrophylla* are used to cure many diseases such as rheumatic pains and stomach. The bark is used to heat cut and wounds [43]. Seed and roots are used for digestion and abdominal problem by the Natives of Gori Valey in district Pithoragarh [173]. The methanol extracts of the deposit of the water extracts obtain after distillation of the essential oil of the leaves of
Calllicarpa macrophylla, yielded 16 α, 17-isopropylidene-3-oxophyllocladane (iso propylidino calliterpenone) along with calliterpenone and its monoacetate [174]. Ethanolic extracts of leaves of Calllicarpa macrophylla shows good anti-inflammatory activity [175].

The leaves of C. macrophylla are reported to contain triterpenes flavonoids 7-O-glucuronides of luteolin and apigenin and 5, 4-dihydroxy-3, 7, 3-trimethoxy-flavone in addition to L (+)-α-Amino-β-(p-metoxynphenyl)-propionic acid, calliterpenone and its monoacetate, β-sitosterol, β-sitosterol-β-D-glucoside, ursolic acid, 2α-hydroxyursolic acid, crategolic acid [176]. New clerodane-type diterpenoids (pentandranolic acids A–C and pentandralactone) isolated from the leaves of C. pentandra [177]. It has been reported that ethanolic extract of Calllicarpa macrophylla flower showed anti-arthritic activity and also exhibited anti-inflammatory, analgesic, antipyretic and antifungal activities [178]. Alcoholic extracts of C. macrophylla have glycoside, flavanoids, tannins carbohydrates and steroid content. Glycosides saponins, flavanoids tannins and carbohydrates have been reported in the aqueous extracts from stem of C. macrophylla [178].

Pharmacology, cytological mechanism and clinical curative effect have been reported on C. nudiflora [179]. Earlier researcher investigated the efficiency of C. nudiflora tablets on antibiosis, antiphlogosis, and hemostasis [180]. C. nudiflora is also used as antimicrobial and antiphlogistic drug with wide pharmacological activities [181]. GC-MS analysis of the C. nudiflora revealed presence of humulene epoxide (17.28%), α-bisabole epoxide (10.51%), (+)-2-carene (9.24%), terpinolene (8.70%), farnesol (6.65%), longipinene epoxide (4.24%), o-cymene (3.93%), sabinene (3.62%), β-terpineol (2.95%), and linalool (2.05%) [182]. Bacterostasis and hemostasis activities of the extracts from C. kwangtungensis Chun. were reported [183]. β-sitosterol-β-D-glucoside have been isolated from plants of Callicarpa macrophylla [163]. The main constituents of the essential oil were spathulenol (18.1%), germacrene B (13.0%), bicyclogermacrene (11.0%), globulol (3.3%), viridiflror (2.6%), α-guaiene (2.3%), and g-elemene (2.0%) were reported as main components in the essential oil of Calllicarpa japonics [184].

Oxygen-containing monoterpenes are the predominant class in T. dreatensis oil. The major compounds reported are linalool (30.4%), thymol (20.2%) and geranial (19.6%). Carvacrol (44.4-57.7%) besides p-cymene (10.3-17.3%) and γ-terpinene (10.8-14.2%) have been reported in the essential oil of T. pallescens [185]. Thymol was the major component present to the tune of 52.28-66.65% in the essential oil of T. linearis collected from different location of western Himalaya (India) [135]. About 43.7% of trans-caryophyllene was reported in the essential oil of Micromeria biflora ssp. arabica K. Walth collected from Pakistan [155]. Neral (25.3-32.2%) and geranial (26.7-41.3%) have been reported as major compounds in the essential oil of Micromeria biflora (Buch. Ham ex D. Don) Benth. collected from South India [156]. Similarly, the content of selinene derivatives, β-selinene
(41.6-29%) and α-selinene (6-1.7%) has been reported as major compounds in the essential oils from leaves and fruits of *C. macrophylla* Vahl. collected from India [186]. This chemical heterogeneousness observed would rise for certain genetic and ecological factors [187].

Literature study has revealed that various works pertaining to the composition of essential oils of selected plants has been conducted. Such plants were collected from various other regions and habitats.

However few studies were conducted on the essential oils of *Thymus linearis* and *Callicarpa macrophylla* but no literature exists on the antioxidant, anti-inflammatory, analgesic, antipyretic and other pharmacological activities of the essential oils from aerial parts of selected plants. Anti-inflammatory [175], analgesic, antipyretic [188], antibacterial [189] and antifungal activities of methanolic extracts *Micromeria biflora* (collected from Himanchal Pradesh) have been reported [157].

### 2.9 Research Gaps

Although various scattered literature exists on the composition and pharmacological activities of major components in the essential oils of selected medicinal plants, but it is observed firstly that such plant species were not collected from the Kumaun region of India. It is observed from literature that the composition of various important components of a plant affecting the medicinal properties depends on secondary variants like place and time of collection, soil type, ecological niches and other factor like humidity and stress conditions. The genetic character of a plant also decides its chemical makeup which is responsible for a specific Chemo type. Since the chemical makeup varies with different chemo type of a plant species, it is obvious that it might affect its biological activities also. Hence it becomes necessary to analyze the variation in chemical make up of the essential oil and to evaluate the biological activity because biological activity depends on the chemical composition of minor and major constituents.

With this aim and also for the fact that minimal work has been executed on the composition, antioxidant activity, pharmacological activity and phenolic assay of essential oil and extracts from selected plants of family Lamiaceae and Verbenaceae from the Kumaun region, hence it was well thought-out of interest to screen the plants of this regions for evaluating their biological activities with essential oil composition.

After study of earlier literature the following research gaps are suggests for further study.

1. The reported essential oil analysis of selected plants only from single place (South India and Western Himalya) but the composition of essential oil varies with climatic condition so plant materials will collected from multiple places (Tarai of Kumaun Regions) for their essential oil analysis
2. The whole plants were taken for essential oil analysis by earlier researcher but we took different parts of plants for isolation of essential oils and their variation in constituents of the oils will be studied.

3. Antioxidant and other pharmacological activities of essential oil from *M. biflora*, *T. linearis* and *C. macrophylla* were also evaluated which were not done earlier.

4. Few pharmacological like antibacterial and antifungal activities were reported on essential oil and extracts. Antibacterial and antifungal activity could be evaluated against other bacteria and fungi not workout earlier.

5. No report exist on estimation of phenolic contents and physiochemical analysis of essential oil and extracts of selected plants.

6. Antioxidant properties of essential oil of *Thymus linearis* were reported from Pakistan but it was not reported on *T. linearis* growing in India, Antioxidant activity and chemical composition could be determined from this region using different methods. Although essential oil composition of *M. biflora*, *T. linearis* and *C. macrophylla* were reported from another region but there was no report on variation in chemical composition and their antioxidant and pharmacological activities. Keeping in view the above facts work will be undertaken in following line.

(i) The essential composition of plants under investigation growing in Kumaun region have not been investigated.

(ii) The difference in chemical composition of different plants parts from plants under investigation has not previously been investigated.

(iii) The antifungal and antibacterial properties of plant under investigation against the bacteria and fungi not yet investigated are proposed to be investigated.

(iv) The phenolic contents of the plants under investigation and their antioxidant potential has be investigated which not earlier has investigated from this region.