Chemical Characterization, Enantiomeric Distribution, and Bioactivity Analysis of Essential Oil from the Selected Lamiaceae Plants of Nepal and Their Application in Topical Formulation

A Dissertation Submitted for

the Partial Fulfilment of the Requirements for the Award of the Degree of

Doctor of Philosophy (Ph.D.)

In

Chemistry

By

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DECLARATION

I, “**Prem Narayan Paudel**”, hereby declare that the entire work presented herein is genuine work done originally by me and has not been published or submitted elsewhere for the requirement of a degree program. Any literature, data, or works done by others and cited within this dissertation have been given due acknowledgement and listed in the reference section.

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RECOMMENDATION

The undersigned hereby certify that we have read and recommended to the School of Science for the acceptance of the dissertation entitled **"Chemical Characterization, Enantiomeric Distribution, and Bioactivity Analysis of Essential Oil from the Selected Lamiaceae Plants of Nepal and Their Application in Topical Formulation"** by Mr. Prem Narayan Paudel in the partial fulfilment of the degree requirements of a Ph.D. in Chemistry.

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This is to certify that **Mr. Prem Narayan Paudel** worked under our supervision for his Ph.D. degree studies on the title **“Chemical Characterization, Enantiomeric Distribution, and Bioactivity Analysis of Essential Oil from the Selected Lamiaceae Plants of Nepal and Their Application in Topical Formulation"**. His work, as embodied in this dissertation, is original. **Mr. Prem Narayan Paudel,** with KU Regd. Number **015475-13,** has fulfilled all the formalities before submission of this dissertation. His work and conduct have been satisfactory. The dissertation is recommended for the award of the degree of Ph.D. in Chemistry.

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LIST OF ABBREVIATIONS

EOs	:	Essential Oils
% (w/v)	:	Percentage Weight by Volume
µg	:	Microgram
^{0}C	:	Degree Celsius
GC-MS	:	Gas Chromatography-Mass Spectrometry
FID	:	Flame Ionization Detector
C GC-Ms	:	Chiral Gas Chromatography-Mass Spectrometry
RI	:	Retention Index
RT	:	Retention Time
ATCC	:	American Type Culture Collcetion
DMSO	:	Dimethyl Sulphoxide
BHT	:	Butylated Hydroxytoluene
DCM	:	Dichloromethane
GMS	:	Glycerylmonostearate
DPPH	:	2, 2-diphenyl-1-picrylhydrazyl
ABTS	:	2, 2’-azinobis-3-ethylbenzothiazoline-6-sulfonic acid
FRAP	:	Ferric Reduction Antioxidan Power
DMEM	:	Dulbecco’s Modified Eagle Medium
CAMHB	:	Cation-adjusted Muller Hinton Broth
PBS	:	Phosphate Buffered Saline
TEA	:	Triethanol Amine
CFU/ml	:	Colony Forming Units/milliliter
RPM	:	Revolutions Per minute
GIS	:	Geographical Information System
HCA	:	Hierarchial Cluster Analysis
PCA	:	Principal Component Analysis
IC$_{50}$	:	Inhibitory Concentration at 50%
SD	:	Standard Deviation
µL	:	Microliter
µg	:	Microgram
MIC	:	Minimum Inhibitory Concentration
RSA	:	Radical Scavenging Activity
UV-Visible	:	Ultra Violet-Visible spectrophotometer
WHO	:	World Health Organization
MTT	:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

ABSTRACT

Essential oils (EOs) are complex mixtures of biologically active volatile compounds that have been utilized for a long time as flavoring agents, preservatives, and natural ingredients in many commercial products. In recent years, they have drawn great attention due to their increasing demand for food, cosmetics, and pharmaceuticals. Since many studies have revealed the good antimicrobial, antioxidant, and cytotoxic activities of essential oils, it is very important to characterize them based on their chemical profiles.

In the present study, some selective plants from the Lamiaceae family of Nepal were collected to determine the chemical composition, enantiomeric distribution, and biological activities. The hydro-distilled essential oils were characterized for volatile compounds by Gas Chromatography and Mass Spectrometry (GC-MS), GC-Flame Ionization Detection (GC-FID), and enantiomeric composition by Chiral GC-MS. The chemometric analysis was applied to identify the chemotaxonomic relationship among Lamiaceae essential oils. The antimicrobial property was evaluated by the microbroth dilution method using some ATCC bacterial and fungal strains. The antioxidant activity was determined by DPPH and ABTS radical-scavenging assays. The i*n vitro* cytotoxicity was evaluated in human breast cancer (MCF-7) and fibroblast (NIH-3T3) cell lines by using the Cell Counting Kit-8 kit assay. The antioxidant-rich essential oils were blended into the cream formulation.

The results revealed the variation in the yield of essential oils with harvesting seasons. The species showed higher essential oil yield in the summer season and tropical regions than others. *Ocimum tenuiflorum* L. had the highest concentration (1.68±0.13%), and *Leucosceptrum canum* Sm. had the lowest yield among the samples (0.15±0.05%). The average essential oil yield obtained from the Lamiaceae plant species in Nepal was about 0.76%.

Oxygenated monoterpenes were the dominant class of terpenoids present in most of the essential oil samples, with concentrations above 49%. *Mentha pulegium* L. showed the highest proportion of oxygenated monoterpenes (91.63%), followed by *Mentha spicata* L. *(*85.3%) and *Perilla frutescens* (L.) (83.05%). The lowest yield of oxygenated monoterpenes was found in *Colebrookea oppositifolia* Sm. at 0.89%. The single compound, carvone, was detected in the highest concentration for *Mentha spicata* L. oil (68.51%). The seasonal variations in the chemical composition of essential oils were also detected among the Lamiaceae samples.

Origanum majorana L. comprises linalool and terpinen-4-ol as major compounds, with 13.8% and 32.1% in spring, and 15.37% and 33.35% in summer. Similarly, the carvone of *M. spicata* L. was not much influenced by seasonal variation, with 51.96% in winter and 68.51% in summer. Major constituents of *O. tenuiflorum* L., eugenol (32.15 to 34.95%), and *trans*-β-elemene (29.08 to 32.85%) were not much influenced by seasonal variation except minor ones. The same result was also detected in *O. basilicum* L., where methyl chavicol (62.16–64.42%) and linalool (26.92–27.09%) were not variable during the winter and summer seasons. A minor seasonal variation in the major compounds was observed for *M. pulegium* L. In contrast, the major constituents of *L. canum* Sm. were highly influenced by seasonal variations. The leading compound, β-pinene, of *L. canum* Sm. was found at 29.07% in winter, which decreased to 15.21% during summer. Similarly, another leading compound, β-caryophyllene, of *L. canum* Sm. was found at 13.29% in winter, which increased to 33.51% during summer, thereby indicating the seasonal variation in the chemical composition of Lamiaceae essential oils.

In the chemometric analysis, agglomerative hierarchical cluster analysis for Lamiaceae essential oil showed two main groups of volatile classes: the first dominated by oxygenated monoterpenes and the second by sesquiterpene hydrocarbons. Both principal component analysis and clustered heatmaps confirmed the two distinct groups of volatile components as assigned by HCA. The chiral GC-MS revealed several chiral compounds in the essential oil samples. The α-thujene, α-pinene, sabinene, β-pinene, camphene, limonene, 1-octen-3-ol, linalool, α-terpineol, *cis*-sabinene hydrate, menthone, β-caryophyllene, terpinen-4-ol, germacrene D, borneol, β-bisabolene, δ-cadinene, (*E*)-β-ionone, and (*E*)-nerolodol were common chiral compounds in the EO, dominating in the levorotatory form (62.4%).

In the DPPH assay, *O. tenuiflorum* L. exhibited relatively good antioxidant activity (IC_{50} 69.23-82.99 µg/mL), when compared to the standards (ascorbic acid, IC_{50} 6.37 µg/mL and BHT, IC_{50} 12.46 µg/mL), and in the ABTS aasay, this essential oil exhibited the strongest activity (IC_{50} 5.88-17.69 µg/mL), when compared to the standards (ascorbic acid, IC_{50} 1.98 µg/mL and quercetin, IC_{50} 7.79 µg/mL). The least activity was noted in *M. pulegium* L. from Nuwakot during the summer with an IC_{50} value of 646.58 µg/mL in the DPPH assay and with an IC_{50} value of 145.35 µg/mL in the ABTS assay. The antioxidant activity *M. spicata* L., *P. frutescens* (L.), *L. canum* Sm., and *O. majorana* L. The essential oils obtained in the summer was higher as compared to the essential oils collected during the winter. In contrast, *O. ameicanum* L., *O.*

basilicum L., and *O. tenuiflorum* L. The essential oils collected during winter exhibited higher antioxidant activity than those from the summer. The present study also revealed that the seasons and environmental conditions may influence the phytochemistry of plants, thereby affecting their antioxidant properties. Samples from tropical zones had better antioxidant activities than the other parts.

The effect of these essential oil samples on the viability of human breast cancer (MCF-7) and fibroblast NIH-3T3 cell lines was also found to be significant. Among the Lamiaceae essential oils, *P. frutescens* (L.) (IC_{50} 7.41 and 8.14 µg/mL), *C. umbrosum* (M. Bieb.) C. Koch (IC_{50} 21.70 and 12.53 µg/mL), *M. longifolia* L. (IC_{50} 23.76 and 12.12 µg/mL), and *O. tenuiflorum* L. (23.43 µg/mL) samples were highly cytotoxic as compared to other species against both cell lines. While *M. pulegium* L., *M. spicta* L., *P. glabar* Benth., and *O. basilicum* L. essential oils displayed the least toxicity, with the IC_{50} values varying from 99.64 to 90.56 µg/mL. Among the essential oil studied against fungal strains, *O. majorana* L., *M. pulegium* L., and *O. tenuiflorum* L. were more effective against *Candida albicans* and *Aspergillus niger* (MIC, 78.1 µg/mL). *O. majorana* L. essential oil exhibited a broad spectrum of antimicrobial activity, with a MIC value of at least 156.3 to 312.5 µg/mL for all tested organisms. For anti-bacterial activity, only slight inhibition of these oils was found against all the tested bacterial strains. Similarly, the essential oils of *O. majorana* L., *O. tenuiflorum* L., and *O. basilicum* L. were utilized for the formulation of cream, which showed the retention of their best efficacy after extensive investigation of several pharmacological parameters. Hence, the present study concluded that the chemotaxonomic profiles of many essential oil samples from the Lamiaceae family of Nepal were prepared. They also exhibited excellent and varied biological efficacies, which can be useful in bioprospecting for the benefit of human health.

Keywords: Lamiaceae, Essential Oil, GC-MS, Chemical Composition, Enantiomer, Antioxidant, Antimicrobial, Cytotoxicity, Formulation.

CHAPTER 1

1.1 INTRODUCTION

1.1.1 Background

Plants and their essential oils have had potential applications in the health care system and treating mankind's diseases since ancient times. One of the most rapidly expanding fields of research is the investigation of natural plant products, including essential oils, as potential sources of novel chemical entities. The medicinal and aromatic medicinal plants (MAPs) are special kinds of plants like herbs and spices that have the characteristics of fragrances and several therapeutic values (Chang, 2000; Raquel & Fernando, 2016). They are regarded as abundant sources of bioactive secondary metabolites and bionutrients that have exhibited crucial functions in the treatment of several chronic diseases like cancer, diabetes, coronary heart problems (Saxena *et al*., 2013; Alamgir, 2017; Stephane & Jules, 2020). The discovery of pharmaceutical drugs based on the ethnobotanical knowledge of medicinal plants may also contribute to identifying novel bioactive compounds. It is commonly known that plants create these substances to defend themselves, but they also defend the plants from harm and diseases and enhance their flavor, color, and perfume (Narasinga Rao, 2003; Khade *et al*., 2023). The essential oils, which are recognized as a significant class of secondary metabolites generated by aromatic medicinal plants, are partially responsible for the medicinal qualities of aromatic plants. Several studies carried out in recent years have also emphasized the beneficial aspects of EOs and their major compounds, like terpenes and terpenoids (mostly monoterpenes and sesquiterpenes), along with their biological properties. Although EOs have been primarily used in food, cosmetics, and perfumes due to their fragrance, the research also highlights the potential application of volatile compounds to treat and prevent many other human diseases (Caputi & Aprea, 2012; Djilani & Dicko, 2012). EOs from aromatic plants have gained increased attention in medicine and aromatherapy. Scientists are now more interested in advanced research, particularly on their anticancer properties. Chemically, EOs are made up of several terpene and phenylpropanoid compounds, which are responsible for their biological activities (Astani *et al*., 2010; De Almeida *et al*., 2011; Kumari *et al*., 2014a). Since the scope of essential oils has intensively increased after the pandemic, many people from the developed countries have fascinated towards the Ayurvedic herbal medicines made from naturally occurring herbs (Gyawali *et al*., 2020). The attraction towards the herbal medicine is mainly due to potentially safe drugs that are free from side effects and affordable to a large number of people (Baral & Kurmi, 2006; Kumari *et al*., 2014b).

Nepal Himalaya has a globally significant and biologically diverse ecosystem with altitude variation, which is probably the largest altitude variation in the world within a narrow width of merely 130 km and is home to a wide range of unique medicinal plants (Baral & Kurmi, 2006). More than 13067 plant species have been found in Nepal, among of which 312 plant species are endemic (Tiwari *et al*., 2019). Therefore, the rich plant biodiversity of Nepal offers a greater possibility to explore new bioactive components for the development of new drugs. The government is also supporting the researchers through several channels, including those who are involved in the field of plants. So, the country can engage them for the national revenue through the development of quality medicines. The importance of medicinal and aromatic plants can be clearly understood from the higher dependency of a large population from around the world as well as people from the developing countries on traditional medicines for primary health care (WHO, 2000). Such herbs are of high significance in various aspects because they are regarded as richest bio-resources of modern and traditional medicinal system, food supplements, nutraceuticals, and chemical entities for synthetic drugs (Hammer *et al*., 1999; Balunas & Kinghorn, 2005). Researchers are adopting for the participation of traditional practitioners in the entire process of drug development to obtain quick results and also to gain the trust of the market. Traditional healing systems such as Homeopathy, Ayurveda, Unani, and Siddha have been in common practice since ancient times, and all healing systems use a large number of medicinal plants and their products (Gewali, 2008; Ghimire, 2008). Therefore, the demand for Himalayan medicinal plants is rapidly growing due to improving livelihoods and the health of the world's growing population (Joshi *et al*., 2016).

Lamiaceae (also known as Labiatae) is an important herb family that contains over 250 genera and more than 7000 plant species (Hedge, 1992) and also represents the richest EO-bearing plant family. This family is also recognized for the wealth of species with therapeutic values that have been consumed since ancient times. These plants are more common in the Mediterranean regions (Shaiq Ali *et al*., 2000). The Lamiaceae family includes nearly twenty-four plant species with entire aromatic parts, which come under several widely used culinary herbs like sage, thyme, rosemary, oregano, basil, mint, lavender, marjoram, savory, and perilla. (Celiktas *et al*., 2007; Hussain *et al*., 2008). The essential oils are primarily found in the leaves of aromatic plants, which emerge oppositely, with each pair positioned at just right angles to the previous one as a decussate arrangement (Hussain, 2009). Most of these aromatic plants possess a complex mixture of bioactive compounds, which are responsible for their overall biological efficacy in both *in vitro* and *in vivo* conditions. These secondary metabolites are actually crucial to .their potent antioxidant, anti-inflammatory, antimicrobial, and anti-cancer efficacy (Silva *et al*., 2021). Moreover, plants belonging to this family are also valuable in diverse fields like food, cosmetics, flavoring, fragrance, perfumery, pesticide, and pharmaceutical industries. Owing to these immense applications of Lamiaceae plants, they are widely cultivated as a vital source of functional foods. These facts force many researchers to conduct the studies on the different aspects of Lamiaceae plant species. The importance of many Lamiaceae family members in regard to the culinary and essential oil industries has been explored for more than 100 years (Lawrence, 1993; Manosroi *et al*., 2006). The U.S. Food and Drug Administration has recommended herbs like oregano, rosemary, sage, and thyme as a safe (GRAS) list (Kaefer & Milner, 2008). The Lamiaceae EOs have also been used in the treatment of different diseases, like intestinal disorders and bronchitis (Burt, 2004; Baris *et al*., 2006). Nowadays, there has been a rapid increase in the use of synthetic flavoring, fragrance, preservatives, and other antimicrobial compounds. But such synthetic chemicals are highly hazardous and detrimental to health if they exceed the permissible level of intake (Bhavaniramya *et al*., 2019).

Therefore, essential oils could be a suitable alternative to ensure food safety and maintain the nutritional content and quality of food, thereby overcoming the risks to human health. Because of the dominant oxygenated monoterpenes in the EOs, they have potential antibacterial activity, and hence they are being used as flavorings legally (Burt, 2004; Dhifi *et al*., 2016; Benali *et al*., 2020). The Lamiaceae plants are widely used as drugs in traditional and modern systems of medicine (Vukovic *et al*., 2009; Nieto, 2017; Borges *et al*., 2019). Some Lamiaceae plant species, such as *Perilla frutescens* (L.), *Pogostemon cablin* Benth., *Rosmarinus officinalis* L., *Lavandula angustifolia* Mill., and *Agastache rugosa* Kuntze, are ubiquitously used to diminish fever, expel superficial evils, induce diuresis, promote blood circulation, and lessen edema (Guo *et al*., 2019; Luo *et al*., 2019). For a long time, studies on the biological activities of Lamiaceae EOs have been carried out continuously, such as their antitumor, antioxidant, antimicrobial, and anti-inflammatory efficacy (Nieto, 2017; Guo *et al*., 2019; Karpinski *et al*., 2020). Therefore, EOs from aromatic plants have drawn great attention in recent years due to their multifaceted biological activities and diverse chemical compositions (Santos & Rao, 2000; El-Sayed *et al*., 2014). The Lamiaceae plants rich in EOs are very important and valuable in natural medicine, cosmetology, and aromatherapy.

In Nepal, nearly 49 genera and 171 species from the Lamiaceae family have been recorded, according to the Annotated Checklist of the Flowering Plants of Nepal in the last update (ACFPN, 2023b). Therefore, many plant species belonging to different genera of the

Lamiaceae family could be found in diverse geographical regions, from the lower altitude-tropical climate to the higher altitude-temperate climate to the subalpine climate of Nepal. Some species of this family are found to be cultivated for their own utilization, while a large number of plant species from this family, growing in wild states, are also distributed in different geoclimatic regions of Nepal. Among them, *Mentha arvensis*, *Mentha piperita*, *Ocimum basilicum*, *Ocimum sanctum*, *Thymus vulgaris*, and *Thymus linearis* are being cultivated as crops. While the rest of the other plant species grow in wild states, commonly in hilly regions at different altitudes.

1.1.2 Essential Oils

Essential oils are hydrophobic liquids containing a mixture of different natural, volatile organic components that have a strong characteristic flavor and fragrance. These EOs are the final products of secondary metabolism, and most of their compounds are terpenoids, generally monoterpenes and sesquiterpenes, as well as sometimes diterpenes and other aromatic compounds. The terpenes are the unsaturated hydrocarbons, which have a distinct chemical relationship to the simple isoprene molecule as a building unit. They are made up of two isoprene units joined by a head-to-tail union for the chemical formula, C_{10} H_{16}. In addition to the monoterpene, EOs may comprise more finished hydrocarbons with the same composition but with a greater molecular mass. The general formula $(C_5H_8)_n$ can be used to describe the composition of monoterpenes, diterpenes, and sesquiterpenes, which are shown in Figures 1.1 and 1.2 (Sharmeen *et al*., 2021; Silva *et al*., 2021). Although EOs contain a variety of chemicals, monoterpenes are the most common constituents.

Essential oils, also known as ethereal oils, can usually be extracted from different parts of aromatic plant materials through hydro-distillation or steam-distillation methods. A mixture of fragrant liquid substances is generated by different plant parts like flowers, peels, rhizomes, buds, seeds, leaves, twigs, bark, herbs or grass, wood, fruits, roots, and the entire plant from a single plant species (Burt, 2004; Skocibusic *et al*., 2006; Palazzolo *et al*., 2013; Kumari *et al*., 2014a). However, essential oils with specific characteristics such as distinct chemical properties and biological efficacies could generally be acquired from a single botanical source when the age of the plant, the climate, and the edaphic and harvesting periods are fairly similar (Sirousmehr *et al*., 2014). Therefore, the name of the source plant is usually used to identify an essential oil. The EOs are often found to be liquid, fragrant, and have a nice odor and essence. A fragrance substance is a chemically pure compound, and it is volatile under normal conditions owing to its aroma. It could be very useful for society in multiple facets.

Essential oils are transparent, mostly pale yellow in color, soluble in non-polar or weakly polar organic solvents, and of lighter density than water, with few exceptions (Gupta *et al*., 2010). These EOs are usually colorless in fresh condition, but a few may also be pale yellow, blue (*Matricaria chamomilla*), orange (*Citrus sinensis*), or green (*Citrus bergamia*) (Martin *et al*., 2010). However, they may be easily oxidizable with time by light, heat, or air, resulting in a dark color (Skold *et al*., 2006).

In the cosmetics and perfume industries, the term ‘essential oil’ is frequently used as ’perfume oil’ or ‘compound’ oil. EOs with standardized content of compounds must have certain chemicals that contribute to their therapeutic values. Therefore, EOs have been extensively utilized in perfumes, cosmetics, soaps, and other products for flavoring food and beverages, pharmaceutical uses, disinfectants, insecticides, fungicides, etc.

1.1.3 Recent Trends for Applications of Essential Oils

Essential oils from different plants have drawn great attention and deep interest from all the scientific and common people in recent years due to their high demands for food, cosmetics, and pharmaceutical industries as potential active natural ingredients (Dreger & Wielgus, 2013). Many findings have revealed the significant antimicrobial, antioxidant, and cytotoxic properties of EOs. It is foremost important to characterize them based on their chemical profiles and pharmacological properties. Medicinal plants with strong aromas are used for the extraction of EOs. They have great importance in pharmacy, perfumery, flavor for food and drinks, preservatives, agricultural insecticides, and aromatherapy. Nowadays, the significance of EOs has also been recognized in plant chemotaxonomy (Khan *et al*., 2023).

Currently, the cosmetic industries utilize essential oils or different mixtures of their compounds, either as active components or as preservatives, in various ranges of products. The roles of their benefits are associated with the unique chemical profiles of essential oils. However, it is difficult to interpret their potential utilization in cosmetics and personal care products without proper chemical characterizations. Similarly, it often requires marvellous attempts at different formulations in order to seek the appropriate mixtures of EOs to achieve the specific benefits of the desired final products. Flavors and fragrances have actually made their way into everyday life for various purposes, either as personal care products, food items, or pharmaceutical formulations. Here, flavors and scents are added to these products to fascinate them or to remove the unpleasant taste or smell of the products (Sharmeen *et al*., 2021). Moreover, EOs have contributed massively to stimulating demand for natural ingredients in new cosmetics and wellness industries. Therefore, the popular cosmetics industries are forced to endorse natural fragrances and allow minimally processed natural ingredients. Because artificial fragrance chemicals have potentially adverse health effects, which are the main elements of cosmetics. EOs are primarily used in the preparation of perfumes and cosmetics because they can help to suppress oxidative stress in skin, delay skin ageing, protect against UV radiation, and prevent the degradation of collagen.

Common Monoterpenes of the Lamiaceae

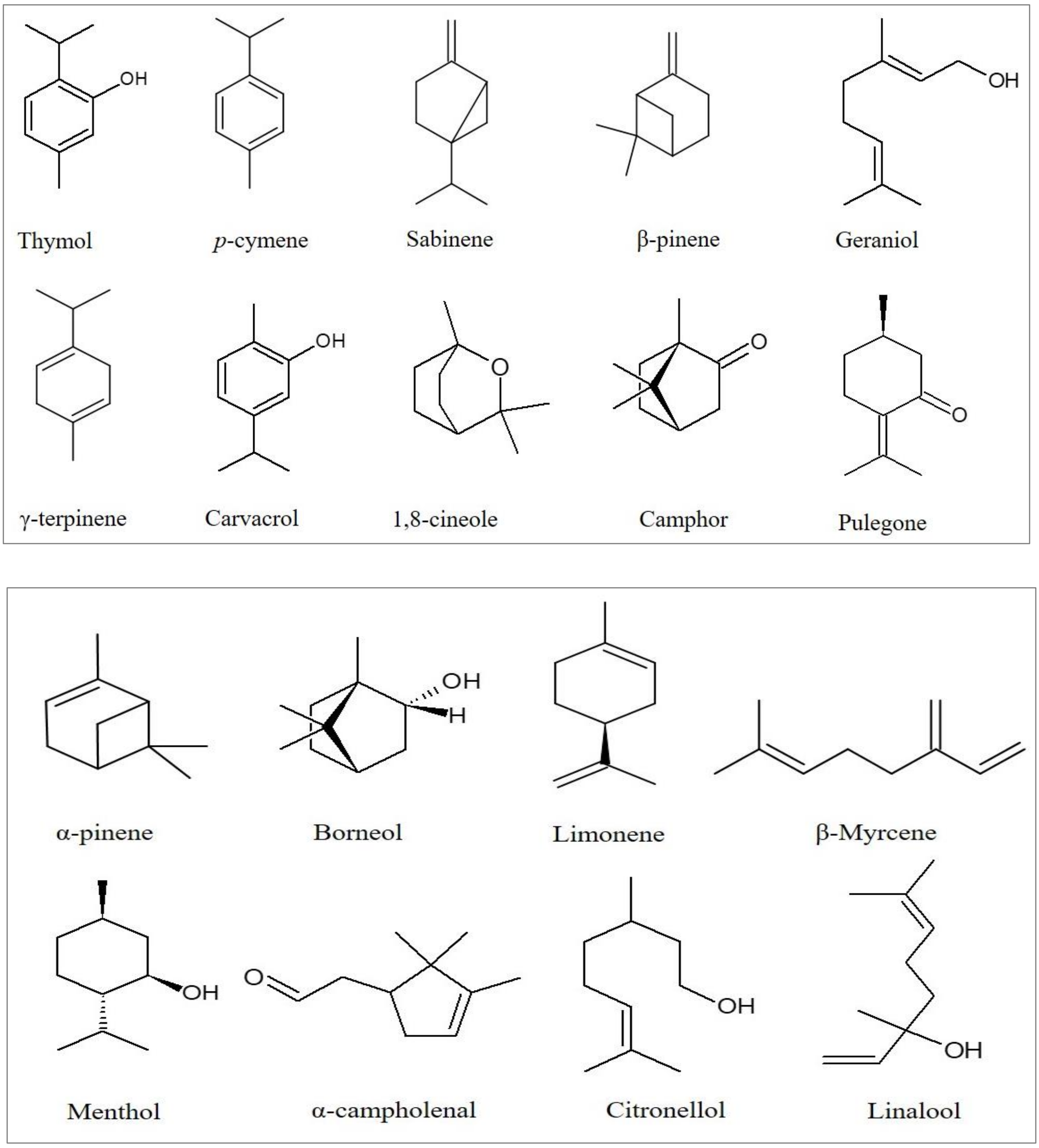


Figure 1. 1 Chemical structure of some major monoterpenes of Lamiaceae essential oils.

Common Sesquiterpenes of the Lamiaceae

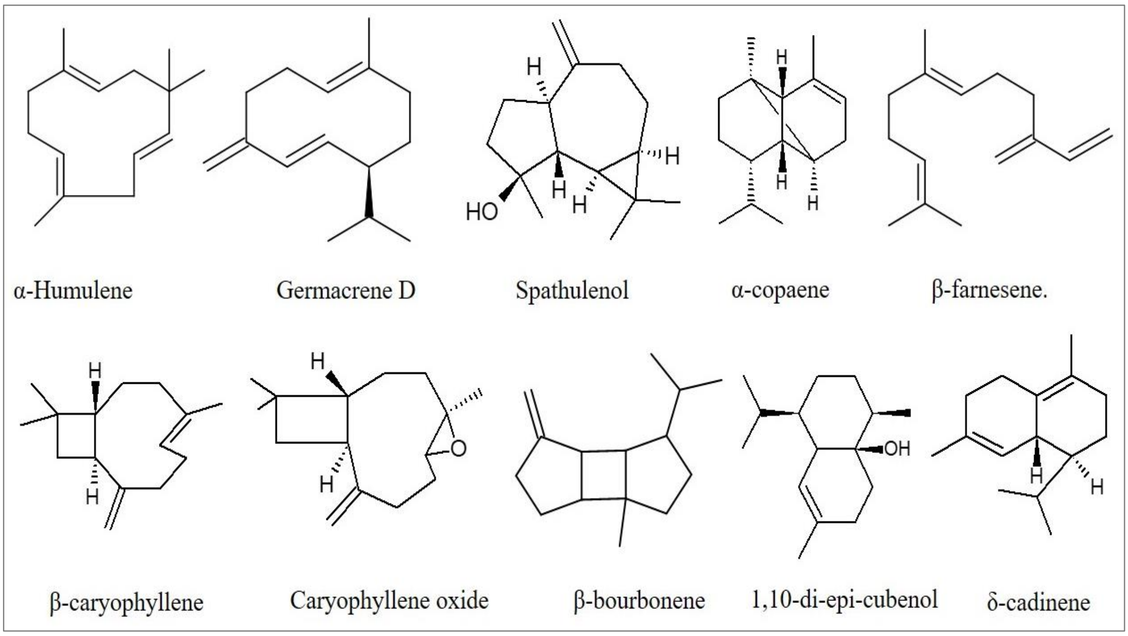


Figure 1. 2 Chemical structure of some major sesquiterpenes of Lamiaceae essential oils.

Nowadays, consumers express their serious concern as well as a great attraction towards formulation products containing natural ingredients rather than any artificial ingredients or chemical compounds (Masih & Singh, 2012). Plant secondary metabolites found in skincare products help to maintain the strength, integrity, texture of skin, moisturize, skin flexibility, photoprotection and collagen degradation (Kumar *et al.*, 2016a; Kumar *et al.*, 2016b). Many research studies have also found that adding plant-based active compounds, like α-hydroxy acid, retinoic acid, coenzymes Q10, etc., to skin care products has several therapeutic benefits (Knott *et al.*, 2015). The ability to get precise therapeutic values from the blending of natural compounds is now regarded as a crucial component of modern herbalism and cosmetics (Koo & Desai, 2014). A new product formulation for skincare products that consisted primarily of antioxidant-rich EOs of different plant species relating to Lamiaceae species was based on their medicinal properties.

1.1.4 Antioxidant Properties of Essential Oils

Regarding their biological properties, antioxidants are defined as substances having the capability of delaying or inhibiting the oxidation of substrates significantly at low concentrations (Halliwell, 2000). The oxidative damage to the biological molecules results in a variety of degenerative or metabolic disorders, including diabetes, inflammation, arthritis, Parkinson’s, Alzheimer’s disease, etc. (Mimica-Dukic *et al.*, 2004; Kashihara *et al.*, 2010).

The term ‘oxidative stress’ simply indicates the alteration of the antioxidant status of cells and tissues upon exposure to oxidants. There is a depletion of antioxidants during oxidative stress. The reactive oxygen species (ROS) and reactive nitrogen species (RNS) comprise the diverse reactive entities like superoxide ($O_2^{\,-}$), hydroxyl ($OH.^-$), peroxyl (ROO**.**) peroxynitrite (.ONOO**.**) and nitric oxide (NO**.**) radicals, as well as non-free radical species such as hydrogen peroxide (H_2O_2), nitrous acid (HNO_2), and hypochlorous acid (HOCl) (Yildirim *et al*., 2004; Mosquera *et al.*, 2007). The stimulating fact is that aerobic organisms develop antioxidant defense mechanism, which arrest the damage caused by ROS and RNS entities. Here, the defense mechanisms may be both enzymatic and non-enzymatic. During the enzymatic mechanism, several enzymes like superoxide dismutase, catalase, glutathione reductase, peroxidase, and nitric oxide synthase are found to be involved. While the non-enzymatic mechanism is prohibited for antioxidants and trapping agents such as ascorbic acid, α-tocopherol, β-carotene, glutathione, flavonoids, uric acid, cysteine, vitamin K, serum albumin, bilirubin, and trace elements such as zinc and selenium (Mosquera *et al*., 2007). Therefore, antioxidant substances can be classified regarding their mode of action as free radical terminators, chelators of metal ions involved in catalyzing lipid oxidation, or oxygen scavengers that react with oxygen-closed systems.

Essential oils are recognized to be highly potent antioxidants, which can reduce oxidative damage (Yanishlieva-Maslarova & Heinonen, 2010). Besides this activity, volatile compounds in EO can also act as pro-oxidants, thereby altering cellular redox status and damaging cellular macromolecules, mostly proteins and DNA (Bakkali *et al*., 2008). It must be taken into consideration when we focus on the antioxidant capabilities of EOs. Therefore, antioxidants may have a significant role in the prevention of several diseases by inhibiting the oxidation of oxidisable substances via free radical-scavenging and suppressing oxidative stress (Durackova, 2010). Antioxidant substances have the ability to prevent the delay of oxidative damage to lipids, nucleic acids, and proteins by ROS (Pokorny *et al*., 2001).

Essential oils obtained from different plant species are known to exhibit varying degrees of antioxidant potential, as explored by several recent studies (Descalzo & Sancho, 2008; Tabata *et al*., 2008). Some of the EOs have been explored as highly efficient antioxidants compared to some of the synthetic antioxidants (Mimica-Dukic *et al*., 2004; Hussain *et al*., 2008). EOs from different herb species, like *Mentha*, *Origanum*, *Rosemary,* and *Melissa*, have been reported to be as good antioxidant potential (Ivanova *et al*., 2005; Singh *et al*., 2005; Venskutonis *et al*., 2005). The antioxidant potential of EOs and other plant extracts may be associated with the presence of hydroxyl groups in their chemical compounds' structure (Hou *et al*., 2007).

In the last two decades, people have expressed their great concern and awareness regarding the safety of synthetic antioxidants in food preservation, in addition to their health implications. There are some of the most commonly used synthetic antioxidants, like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), butylated hydroquinone, etc. But these synthetic antioxidants are known to have toxic and other carcinogenic effects on human health and food systems (Descalzo & Sancho, 2008; Paradiso *et al*., 2008). They may also cause liver swelling, influence liver system functions, and cause cerebrovascular diseases (Choi *et al*., 2007). Therefore, there is a dire need for the isolation and characterization of several natural antioxidants without any side effects to use in food or medicinal purposes in order to replace synthetic antioxidants. Natural antioxidant sources could play a vital role in helping endogamous antioxidants to neutralize the oxidative damage (Govindarajan *et al*., 2003).

Many previous findings have revealed that the study on the exploration of natural sources of antioxidants and their potent novel compounds is being carried out continuously (Descalzo & Sancho, 2008; Paradiso *et al*., 2008). The plants are regarded as huge sources of natural antioxidants, along with potential novel compounds exhibiting good antioxidant activities (Mosquera *et al*., 2007). Many previous studies have also established the fact that plant-based antioxidants have valuable effects on the prevention of several chronic diseases, as discussed earlier. Therefore, there has been a great enthusiasm among the researchers for finding the role of bioactive components in aromatic medicinal plants to reduce the risk of many diseases.

In the present study, the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical-scavenging technique is used to study the antioxidant properties of essential oils obtained from the aerial parts of plant materials. The chemical 2, 2-diphenyl-picrylhydrazyl, is characterized as a stable free radical. by virtue of the delocalization of electron over the molecule as a group so that the molecule does not dimerize, as it would be like other most free radicals. The delocalization gives rise to the deep violet color, characterized by an absorption band in the sample solution at about 517 nm. When a solution of DPPH is mixed with that of a substance, which can donate a hydrogen atom, it gives a reduced form with a loss of violet color. Finally, a pale color may be seen at the end due to the presence of picryl residue. This transformation is shown in Figure 1.3 (Ionita, 2005).

Similarly, another method for evaluating the antioxidant activity is the 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS method. In this technique, blue-green-colored ABTS chromophores could absorb at different wavelengths, like 645, 734, 815, and 415 nm. However, many researchers have followed the wavelength of 734 nm because possible interferences can be removed and sample turbidity would be reduced at that wavelength (Opitz *et al.*, 2014). During this process, the blue-green color will change into a pale blue color when the unstable form of the ABTS radical takes an electron from the AO. This indicates the regeneration of a stable form of ABTS. Regarding the reaction time, the previous studies have reported different times, varying from one to thirty minutes.

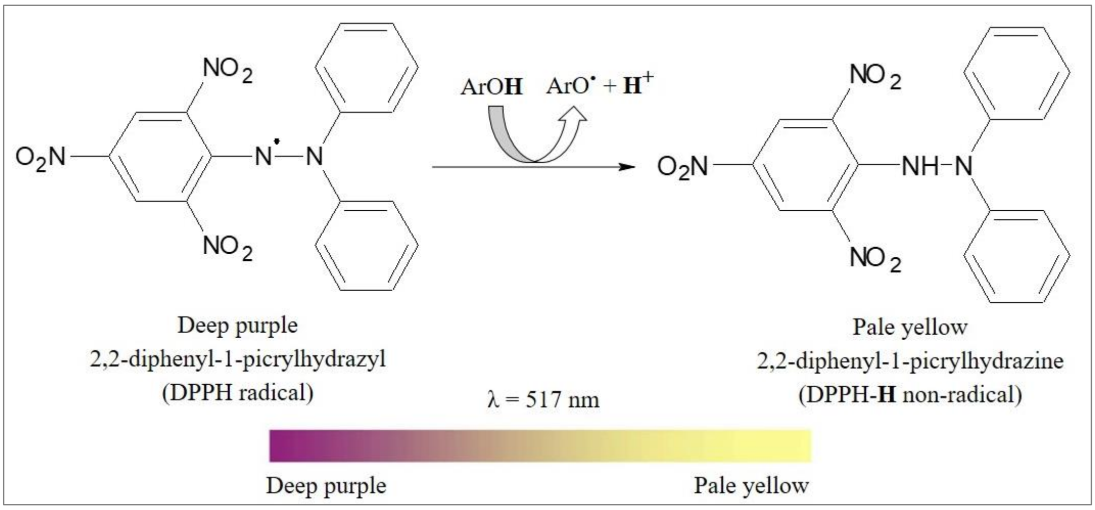


Figure 1. 3 Transformation of 2,2-diphenyl-1-picrylhydrazyl to 2,2-diphenyl-1-picrylhydrazine from purple to yellow color during the reaction.

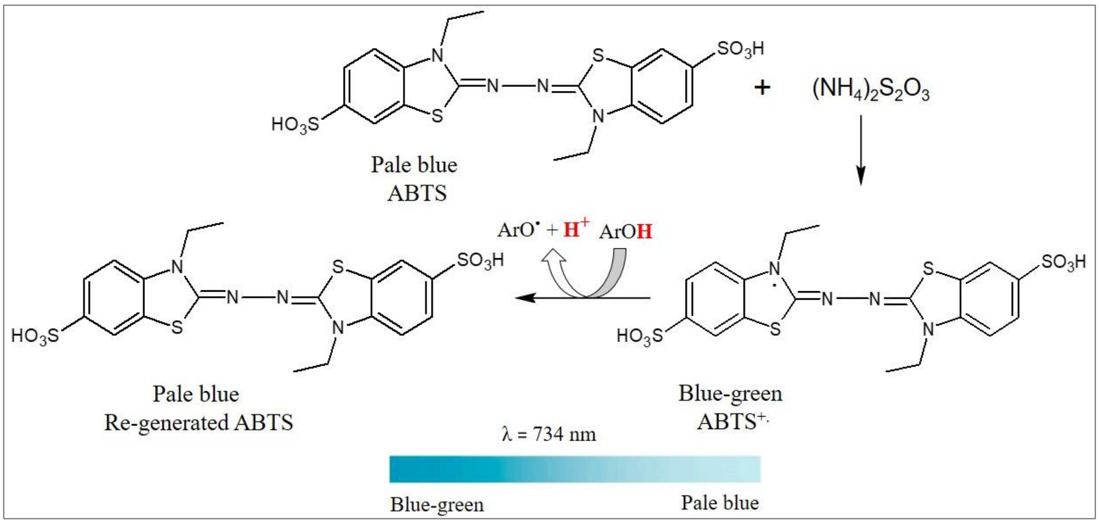


Figure 1. 4 Transformation 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) from pale blue to blue-green and finally to pale blue color during the reaction.

Figure 1.4 clearly shows the transformation of ABTS during the reaction with a distinct color change band (Sadeer *et al.*, 2020). The protocol for this method was initially proposed by Miller (Miller *et al*., 1993).

Although phenolic chemicals are thought to be responsible for antioxidant activity, new research has shown that volatile components, either alone or in combination, can also contribute to overall antioxidant activity. Lemon balm essential (*Melissa offinalis* L.) was reported to have higher antioxidant potential as compared to BHT. The primary components contributing to the antioxidant activity were citronella, neral, and geranial, with percentage yields of 13.7, 16.5, and 23,4, respectively, according to the Gas Chromatography-Mass Spectrometry study (Mimica-Dukic *et al*., 2016).

1.1.5 Antimicrobial Properties of Essential Oils

Essential oils are well-known to have promising antimicrobial activities, in accordance with many previous studies. The antibacterial activity depends not only on the presence of major active constituents but also on the interaction between large numbers of the associated compounds, which can have synergistic or antagonistic effects. There are several other contributing factors for their activity, like content, concentration, interaction between major active components, etc., and the susceptibility of microbial agents is also responsible for their antimicrobial property (Bassole & Juliani, 2012). The presence of some inactive components might impact the resorption rate, rate of reaction, and biological potentials of active constituents. Thus, the resultant of both major and minor compounds could just change the efficacy to produce noteworthy synergistic or antogonistic actions (Pandey *et al.*, 2014; Nascimento *et al*., 2018).

Several previous studies reported in the literature have revealed that essential oils have prominent antibacterial and antifungal activity against different pathogenic microorganisms (Pandey *et al*., 2003; Singh *et al*., 2005; Rahman *et al.*, 2011; Zore *et al.*, 2011; Assiri *et al.*, 2016). Essential oils from cinnamon, oregano, and thyme had important antibacterial activity against *Escherichia coli*, *Brochothris thermosphacta*, *Listeria monocytogenes*, and *Pseudomonas flurescens* (Mith *et al.*, 2014). EOs from *Thymus* and *Origanum* were also reported to exhibit potent antibaterial and antifungal efficacy (Burt, 2004; Sokovic & Van Griensven, 2006). The antibacterial activities of essential oils from four Lamiaceae plant species: wild oregano (*Origanum minutiflorum*) endemic in Turkey, oregano (*Origanum onites*), black thyme (*Thymbra spicata*), and wild savory (*Satureja cuneifolia*) were determined, which showed strong antibacterial potential against the strains tested (Baydar *et* *al*., 2004). Many previous studies have already been carried out on the biological potentials of *Rosmarinus officinalis* essential oils and their major compounds, which showed their prominent antibacterial activities (Oluwatuyi *et al*., 2004). Similarly, the antimicrobial activities of marjoram oils have also been described in the literature. Among several essential oils as potential antimicrobial resources, marjoram oil (*Origanum majorana* L.) may have the highest potential from the industrial applications point of view.

Essential oils from dietary herbal plant species in the Lamiaceae family, including thyme, have been utilized as major sources of medicine. and food preservatives for over 4000 years (Burt, 2004; Rota *et al*., 2008). Nowadays, there has been a great attraction towards essential oils and other naturally occurring antimicrobials in the food industry. Because they have exhibited significant antimicrobial activities as well as imparting pleasing flavor to foods (Burt, 2004). Some essential oils have shown a promising capacity for food safety when added to processed and raw foods. The most effective natural antimicrobials could also be isolated from several spices, herbs, essential oils, and different plant families (Burt, 2004; Bakkali *et al*., 2008). To the best of our knowledge, there are no earlier reports yet available for the detailed chemical profiling, evaluation of the biological efficacy, and antioxidant potentials of essential oils from the plants of the Lamiaceae family originating in Nepal. However, a large number of plant species from other families have been investigated for their essential oils as potential biological efficacies.

1.1.6 Cytotoxicity of Essential Oils

Essential oils consist of a large number of chemical compounds, and hence they have no specific cellular targets. They can readily pass via the cytoplasmic membrane and disrupt the structure, thereby making it permeabilized. Therefore, cytotoxicity can produce such damage to the cell membrane. Actually, essential oils have the tendency to coagulate the cytoplasm, thereby causing injury to the lipids and proteins (Ultee *et al*., 2000; Burt, 2004). The damage to the cell wall and cell membrane may lead to lysis and thereby the leakage of macromolecules (Oussalah *et al*., 2006). Essential oils are also accountable for stimulating the depolarization in the mitochondrial membrane of eukaryotic cells by diminishing the membrane potential. They also affect the ionic Ca^{++} cycle and other ionic channels, thereby reducing the pH gradient (Bakkali *et al*., 2008). The permeabilization of the outer and inner mitochondrial membranes results in cell death through necrosis and apoptosis. These findings explore prooxidant activity that is similar to phenol (Burt, 2004; Barbehenn *et al*., 2005).

The cytotoxicity of essential oils can be evaluated using different techniques, as reported in the literature. Among all possible assays, MTT is the simplest, most reliable, and extensively reported method to measure the viability of cells with the application of antiproliferative agents. MTT is a tetrazolium salt, and it is transformed into formazan by the succinate dehydrogenase system. It is only active in viable cells and is connected to the mitochondrial respiratory chain. Here, yellow-colored tetrazolium salt gets reduced to water-insoluble purple formazan by mitochondrial succinate dehydrogenase. The quantity of dye can be evaluated by using a microplate reader at 540 nm after the solubilization of formazan.

There is another technique in which the viability of cells can also be determined in the presence or absence of the test compound by using a cell counting kit. Here, cell counting kit-8 provides an expedient and robust way of carrying out a cell viability assay. The kit simply uses a water-soluble tetrazolium salt to enumerate the number of living cells by developing an orange formazan dye upon bio-reduction in the presence of an elctron carrier. The WST-8 kit solution is directly transferred into the test cells without the pre-mixing of the required components. Then, WST-8 tetrazolium salt is reduced by cellular dehydrogenase to an orange formazan, which is soluble in a tissue culture medium. The quantity of formazan formed is directly proportional to the number of living cells, and it can be measured by taking the absorbance at 460 nm using a multiscan high plate reader. This kit solution is very useful for cytotoxicity assays due to its excellent stability and very small cytotoxic effects, even after a long incubation period. The detection sensitivity is much better than that with other tetrazolium salt-based assays.

The cytotoxic effects of essential oils may be mostly supported due to the presence of alcohols, phenols, and aldehyde groups in the volatile compounds (Bruni *et al.*, 2004). The cytotoxic activity is an important aspect of the immense worth of EOs regarding their application, not only in the preservation of agricultural or marine products but also against certain pathogens. EOs, along with some dominant volatile compounds, are highly effective against several organisms. Carvacrol, a dominant compound in the essential oils of *O. majorana* and *M. officinalis,* has been reported to lessen the fluidity of the membrane by altering its fatty acid profiles (Ultee *et al.*, 2000; Di Pasqua *et al.*, 2006). This indicates the importance of EOs and their major compounds in various aspects of application fields.

1.1.7 Topical Formulation Using Essential Oils

Topical formulations are optimized concentrations of base by considering a particular site of the body or type of skin condition. The product may be considered for moisturizing or to maximize the penetration of an active ingredient into or via the skin. The topical formulations may be in different forms, such as ointments, gels, creams, lotions, solutions, suspensions, foams, and shampoos. The most commonly used topical formulations are semisolid dosage forms, which include ointments, creams, lotions, and gels. Among them, the present study will focus on topical creams with enriched antioxidant properties that incorporate the Lamiaceae essential oils.

Topical formulations are very useful in suppressing oxidative damage in the skin, delaying skin ageing, shielding from UV radiation, and preventing collagen degradation. The various cosmetic products loaded with natural ingredients have shown higher efficacy and display without any side effects, along with greater intrinsic acceptability as compared to synthetic products (Gyawali *et al*., 2020a). Nowadays, the use of formulations with medicinal plant-based products is more common due to the awareness of consumers regarding synthetic products (Masih & Singh, 2012). Many previous reports have explored the importance of aromatic medicinal plants as the best alternatives for cosmetics and cosmeceutical products because they show valuable therapeutic properties. Moreover, the plant secondary metabolites present in the skin care products would also support the strength, elasticity, and texture of the skin. The addition of active plant-based ingredients would result in several therapeutic benefits from the topical formulations (Knott *et al*., 2015; Gyawali *et al*., 2016; Krishnan *et al*., 2017).

Many of the traditional medicines utilized in developing countries have not been explored for their quality, safety, or potential efficacy for proper application to the same level as compared to those in developed countries (Joshi *et al*., 2016). However, there are some amazing claims made about the effectiveness of herbal medicine in the practice of traditional medicinal systems. In the context of Nepal, there is a dire need for proper documentation and determination of the biological potentials of essential oils from several other aromatic medicinal plants collected in different regions, with a special reference to their ability to fight against various diseases. Here, essential oils of selective aromatic medicinal plants from Lamiaceae that were collected from different parts of Nepal based on ethnopharmacological information have been subjected to their different analyses, as mentioned. Therefore, a large number of aromatic medicinal plants in Nepal are still uncertain and unknown for their biological activities as well as chemical profiles, thus leading to different product formulations by applying essential oils. The present study has focused extensively on documenting the plant species belonging to the Lamiaceae family in Nepal and educating people about the importance of such valuable aromatic medicinal plants. This study can provide deep-level insights for conservation, exploration, sustainable utilization, chemical characterization, biological activities, and the formulation containing essential oils for topical use of such a valued aromatic plant from Nepal.

Therefore, this study has been taken as potential research in the development of products by employing traditional knowledge with modern technology. The extraction of plant species belonging to this family has revealed several important active compounds from their biological aspects. This emphasizes the urgent need for an inclusive study to explore additional information on the medicinal importance of several other possible Lamiaceae plant species. Therefore, the present research work supports providing some inclusive information about the medicinal values of the plant species from the Lamiaceae family of Nepal.

1.2 RATIONALE AND SIGNIFICANCE OF THE STUDY

The research work has the great intent of providing some exclusive information about the plant species of the Lamiaceae family in the context of Nepal. There are nearly 171 species of this family under identification, but less than fifty percent of species are being subjected to chemical profiling. A plethora of studies have found that most species have not been widely investigated for chemical characterization, enantiomeric distribution, biological activities, or product formulation. Many recent studies have mentioned that essential oils with active components possess several pharmacological potentials, like antimutagenic, angiogenic, antiparasitic, antiplatelet, antielastase, and antihepatotoxic (Can Baser, 2008). Most of these plant species are aromatic in their characteristics and have a complex mixture of volatile bioactive constituents, which can contribute to the overall biological potential in both *in vitro* and *in vivo* analyses. They are also known as potential sources for the isolation of various bioactive molecules like terpenes, phenols, flavonoids, etc. The following key points highlight why we chose this research, mainly focusing on the Lamiaceae plants of Nepal.

- The rich plant biodiversity of Nepal offers a greater possibility of exploring some new aromatic medicinal plants with their potent bioactive molecules for drug discovery.
- In Nepal, there are nearly 49 genera and 171 species of the Lamiaceae family on record. However, less than 50% of species are subjected to chemical and pharmacological profiling, indicating the importance of this family.
- The distribution of secondary metabolites is variable with the season and geographical regions, which has been less focused on the Lamiaceae family plants of Nepal. This study will help in the advancement of knowledge on chemical variation and its application.

- This study also provides some key information on the enantiomeric composition of Lamiaceae species from Nepal, which has not been reported previously.
- Because of the serious concern and awareness of people for cosmetics and cosmeceutical products containing plant-based natural ingredients, the formulation and evaluation of skin care products by utilizing several EOs are of great importance.

1.3 RESEARCH QUESTIONS

In recent years, researchers have conducted several activities on medicinal and aromatic plants (MAPs) from different geo-climatic zones of Nepal, where indigenous knowledge has been considered a primary step in developing and identifying therapeutically valuable natural products. *Origanum majorana* showed the best antibacterial activity (Joshi *et al*., 2009). *O. vulgare* has high antimicrobial, anti-hyperglycemic, antioxidant, anti-inflammatory, and anticancer properties due to the presence of rich thymol and carvacrol compounds (Gewali, 2008). Essential oils from *O. majorana* and *O. vulgare* from Pakistan were also reported to have good antioxidant, antibacterial, and cytotoxic potentials, while *O. vulgare* also showed potential antimalarial efficacy (Hussain *et al*., 2011). The extensive literature review showed that plant species of the Lamiaceae family are potential natural resources for therapeutic uses, flavoring agents, natural preservatives, perfumery, and aromatherapy from worldwide perspectives. However, there is very limited research on the comparative phytochemistry and bioactivities of plants belonging to the Lamiaceae family from Nepal. The use of Lamiaceae plants as herbal drugs in the treatment and prevention of several diseases is foremost important to validate as therapeutic beneficials. Therefore, these plant species from different geographical locations have been collected and subjected to chemical characterization and biological studies. The research questions for the present study were set as follows:

1. What are the distributions of aromatic Lamiaceae plant species in Nepal?
2. What are the major volatile compounds of Lamiaceae essential oils based on seasonal and geographical variation?
3. What are the patterns of the distribution of chiral compounds in the essential oils of Lamiacea family plants?
4. What are the biological efficacies of different Lamiaceae plant essential oils?
5. What are the valuable applications of essential oils in the development of value-added products?

1.4 OBJECTIVES OF THE RESEARCH STUDY

Through the extensive review of the literature, there are no such studies available regarding the chemical characterization, enantiomeric distribution, biological activities, and formulation of creams using EOs from the plants of the Lamiaceae family available in Nepal. Therefore, the present study is chosen to explore some new information regarding the Lamiaceae plant species of Nepal.

The main objectives of the present research study were as follows:

1.4.1 General Objectives

- To assess the impacts of seasonal and geographical variation on the chemical composition of essential oils of Lamiaceae plant species from different regions of Nepal and to incorporate the essential oils into skin care products.

1.4.2 Specific Objectives

- To develop the chemical profiles of volatile organic compounds present in the Lamiaceae essential oil samples.
- To determine the chemical relationships among the Lamiaceae essential oil samples.
- To evaluate the enantiomeric distribution of chiral compounds present in the essential oil samples.
- To investigate their antimicrobial, cytotoxic, and antioxidant properties.
- To develop the skin protective product and evaluate it for topical use.

CHAPTER 2

2.1 REVIEW OF LITERATURE

2.1.1 Essential Oils

Essential oils are composed of mixtures of volatile organic compounds with a strong fragrance. They are highly volatile, insoluble in water, but soluble in organic solvents, and they can be obtained through the distillation of aromatic plant materials (Burt, 2004; Kumari *et al.*, 2014). A highly fragrant compound is a chemically pure substance, and it can be very beneficial to mankind because of its pleasing aroma.

2.1.2 Sources of Essential Oils

The literature review revealed that among the 400,000 known flowering plant species, including both aromatic and medicinal plants, from the 295 families in the world, there are about 2000 species from nearly 60 families of essential-oil-bearing plants (Singh *et al.*, 2014). The plant families that include the majority of the economically significant essential oil-bearing species are not restricted to any particular taxonomic group but can also be found dispersed among all plant classes (Baser & Buchbauer, 2009). Approximately 3000 essential oils from the 2000 aromatic plants are of great value and are utilized in diverse fields of applications (Bakkali *et al.*, 2008; Raut & Karuppayil, 2014). Though the plants belonging to both Gymnosperms and Angiosperms have a higher ability to accumulate essential oils, the plants of Angiosperms are the major sources of the most commercially important essential oils. In terms of the global market, Lamiaceae comes under the three major families for the main sources of aromatic plants and essential oils (Burt, 2004; Hussain *et al.*, 2008). Principally, most of the plants have a tendency to generate volatile compounds quite often, but the quantity is only in traces. The plants that can produce essential oils of commercial interest are commonly called essential oil plants.

Essential oils can be extracted from the various parts of aromatic plants. However, the parts of the plant that may serve as the major source of EOs could be quite different. These are the flowers and inflorescences (e.g., chamomile, lavender, rose), leaves (e.g., basil, laurel, lemongrass, peppermint, rosemary), fruits (e.g., black pepper, nutmeg), peels (e.g., orange, lemon, tangerine, bergamot), seeds (e.g., cumin, cardamom, anise, funnel), beriies (e.g., juniper, allspice), bark (e.g., cinnamon, cassia, sassafras), wood (e.g., cedarwood, camphor, sandalwood), root/rhizomes (e.g., ginger, vetiver, turmeric), and resin (e.g., myrrh, frankincense) (Cava *et al.*, 2007; Hussain *et al.*, 2008; Tongnuanchan & Benjakul, 2014).

2.1.3 Lamiaceae Family Bearing Essential Oils

The plant family Lamiaceae is an important herb family that comprises over 250 genera and greater than 7173 species (Hedge, 1992; Sun *et al*., 2022) and also represents one of the richest essential oil-bearing families. The Lamiaceae family is also recognized as a group of plant species with complete medicinal values (Kallunki & Heywood, 1994). Nearly twenty-four plant species from this family endure aromatic nature in whole parts (Hussain *et al*., 2008). The six most well-known common names for spices that have aromatic properties are thyme, basil, oregano, rosemary, sage, and lemon balm (Bekut *et al*., 2018). Numerous species in this family are abundant in terpenes and flavonoids, with diterpenoids being the most prevalent. Many of the Lamiaceae plant species are aromatic and possess several bioactive compounds, which are responsible for their medicinal properties (Harley, 2012). Some examples include the species like *B. officinalis, G. hederacea, H. pectinata, Lavandula, Lamium, M. officinalis, Mentha, M. vulgare, Origanum, Ocimum, R. officinalis, Salvia, S. hortensis, S. lavandulifolia, S. lateriflora, Sideritis, Teucrium, Tymus*, and *Ziziphora tenuior* (Uritu *et al*., 2018).

Furthermore, the plants belonging to the Lamiaceae family are also applicable for other purposes, such as in food, cosmetics, flovoring, fragrance, perfumery, and pharmaceuticals (Nieto, 2017; Borges *et al*., 2019). Because of their broader applications in different fields, the plants of this family are widely cultivated in various regions of the world. Therefore, several studies have been conducted on the different facets of Lamiaceae plant species, including their biology, ecology, and application sites.

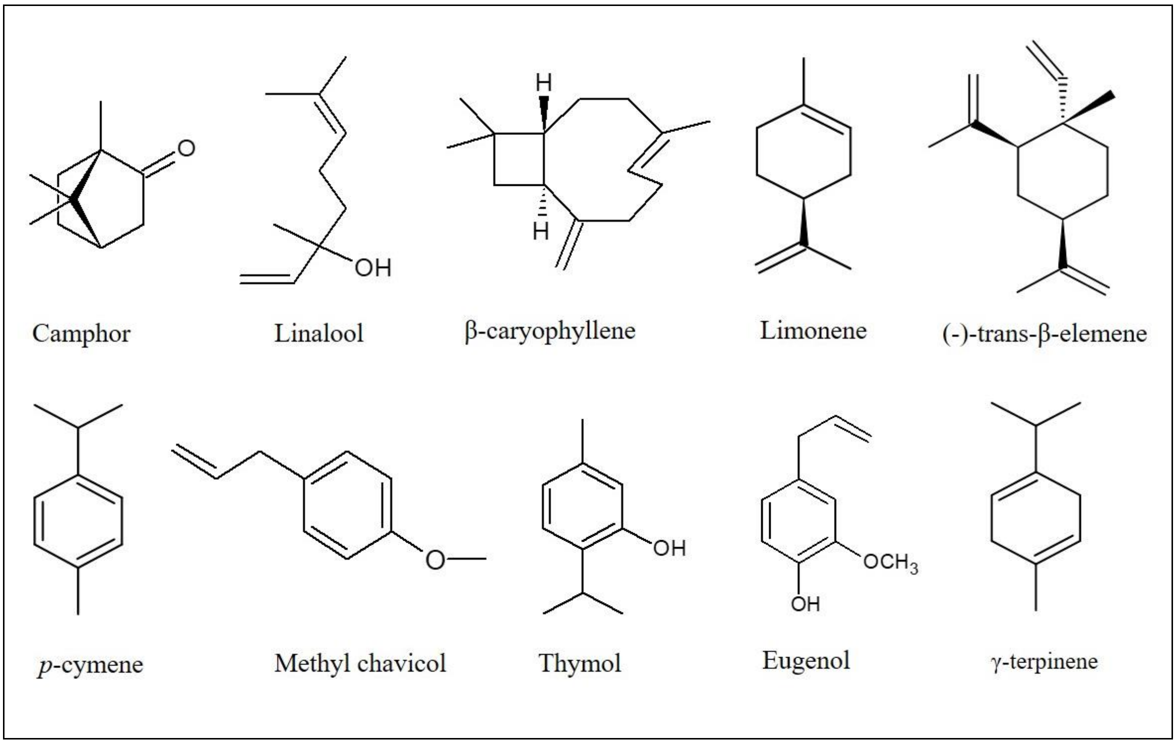
In the context of Nepal, it has been reported that a large number of plant species belonging to the Lamiaceae family have been distributed in different regions of the country. Some species of this family are found to be cultivated for their own utilization. While a variety of plant species from this family have been found in wild-growing states in different parts of the country. There are nearly 49 genera and 171 species from the Lamiaceae family on record, according to the Annotated Checklist of the Flowering Plants of Nepal in the last update (ACFPN, 2023c). This study focuses on extensively documenting the plant species belonging to the Lamiaceae family in Nepal and raising awareness among people about the importance of such a valuable medicinal plant.

In this present study, the following plant species from the Lamiaceae family growing in different regions of Nepal were taken into consideration for their extensive studies like chemical characterization, biological activities, and product formulation containing EOs for topical use of such valued medicinal plants of Nepal.

2.1.3.1 The *Ocimum* species

The genus *Ocimum* L., comprising more than 150 species, has been reported to grow widely throughout temperate regions of the world (Pandey *et al*., 2014). *Ocimum* species are widespread in tropical areas such as Asia, Africa, India, and Nepal (Stefan *et al*., 2013). The best-known species are strongly aromatic herbs like *Ocimum tenuiflorum, Ocimum basilicum, Ocimum gratissimum,* and *Ocimum americanum.* They are also recognized as holy basil, sweet basil, wild basil, clove basil, American basil, lime basil or hoary basil, respectively. These species are often cultivated in many countries in East Asia, Europe, America, and Australia for the production of essential oils (Zheljazkov *et al*., 2008). Traditional aspects: EOs of *Ocimum* species have been extensively utilized as high-value aromatic chemicals in food (as flavoring agents), perfumery, cosmetic, and pharmaceutical preparations, and as spices in abundant ways. *Ocimum* species are known for their diverse uses in folk medicine for the treatment of various gastric and urinary diseases, insomnia, inflammation, and constipation due to their diverse biological actions, such as carminative, stimulant, antiseptic, antimicrobial, antioxidant, antipyretic, insecticidal, and antispasmodic activities (Perez-Gonzalez *et al*., 2019). The aerial parts of the plant species are deliberated antispasmodic, stomachic, and carminative in native medicine (Sajjadi, 2006). *O. basilicum* and *O. gratissimum* have been reported for their antiemetic activities (Ahmed *et al*., 2019). Currently, the potential applications of *O. sanctum*, *O. gratissimum*, and *O. basilicum* essential oils, mainly as antioxidant and antimicrobial agents, have also been explored (Hussain *et al*., 2008).

In the context of Nepal, at least four plant species of this genus are reported by the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023d). They are *Ocimum tenuiflorum*, *Ocimum basilicum, Ocimum gratissimum,* and *Ocimum americanum.* The chemical structures of the major compounds of essential oils from *Ocimum* species are given in Figure 2.1. To determine the structural activity association, it is important to distinguish the chemical variations among the possible volatile compounds of various *Ocimum* species. By considering this fact, the present study has been conducted for the chemistry of some *Ocimum* essential oil samples from two different geographical locations. Also, the aim of the present investigation is to explore the biological activities of EOs as potential sources for further beneficial applications.



Camphor Linalool β-caryophyllene Limonene (-)-trans-β-elemene

p-cymene Methyl chavicol Thymol Eugenol γ-terpinene

Figure 2.1 Chemical structures of some major chemical compounds in the essential oils of *Ocimum* species.

2.1.3.2 The *Mentha* species

The genus *Mentha* includes more than 25-30 species all around the world. Many of these species are perennial herbs bearing essential oils and are widely cultivated as industrial crops for the production of EOs. These species are widely distributed throughout the world's temperate zones (Gelluce *et al*., 2007). *Mentha arvensis*, *Mentha piperita*, *Mentha longifolia*, and *Mentha spicata* are commonly known as menthol mint, peppermint, wild mint, and spearmint, respectively, and they are often cultivated in several countries in East Asia, Europe, America, and Australia for the purpose of essential oil production (Pandey *et al*., 2003; Gelluce *et al*., 2007). The entire herb of these species has been used to isolate many compounds that have been explored as antifungal, antiviral, antimicrobial, insecticidal, antioxidant, antiamoebic, antihemolytic, antiallergenic, and antitumoral agents. The plants of *Mentha* species are generally utilized in commercial spice mixtures for many processed foods and herbal teas. However, EOs from *Mentha* species are commonly used for flavor liqueurs, breads, salads, soups, cheese, and cosmetics (Yadegarinia *et al*., 2006). Additionally, they have also been used traditionally for the treatment of many digestive tract diseases due to their carminative, antiemetic, spasmodic, analgesic, and anti-inflammatory potentials (Gelluce et al., 2007). The essential oils from some *Mentha* species, like *M. arvensis*, *M. piperita*, *M. longifolia*, and *M. spicata*, are potential sources for displaying antimicrobial, antioxidant, and radical-scavenging potentials (Pandey *et al*., 2003; Gulluce *et al*., 2007).

These activities are mainly contributed due to the presence of phenolic compounds (Hosseinimehr *et al*., 2007).

In the context of Nepal, at least three plant species of this genus are described by the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023e). They are *Mentha arvensis*, *Mentha longifolia*, and *Mentha spicata*. The chemical structures of the major compounds of essential oils from *Mentha* species are shown in Figure 2.2. To determine the structural activity connection, it is essential to identify the chemical differences among the possible volatile compounds of several *Mentha* species. Knowing this fact, the present study has been carried out on the chemistry of some *Mentha* essential oil samples from two geographical sites. Furthermore, the goal of the present study is to establish the biological activities of essential oils as potential sources for beneficial uses.

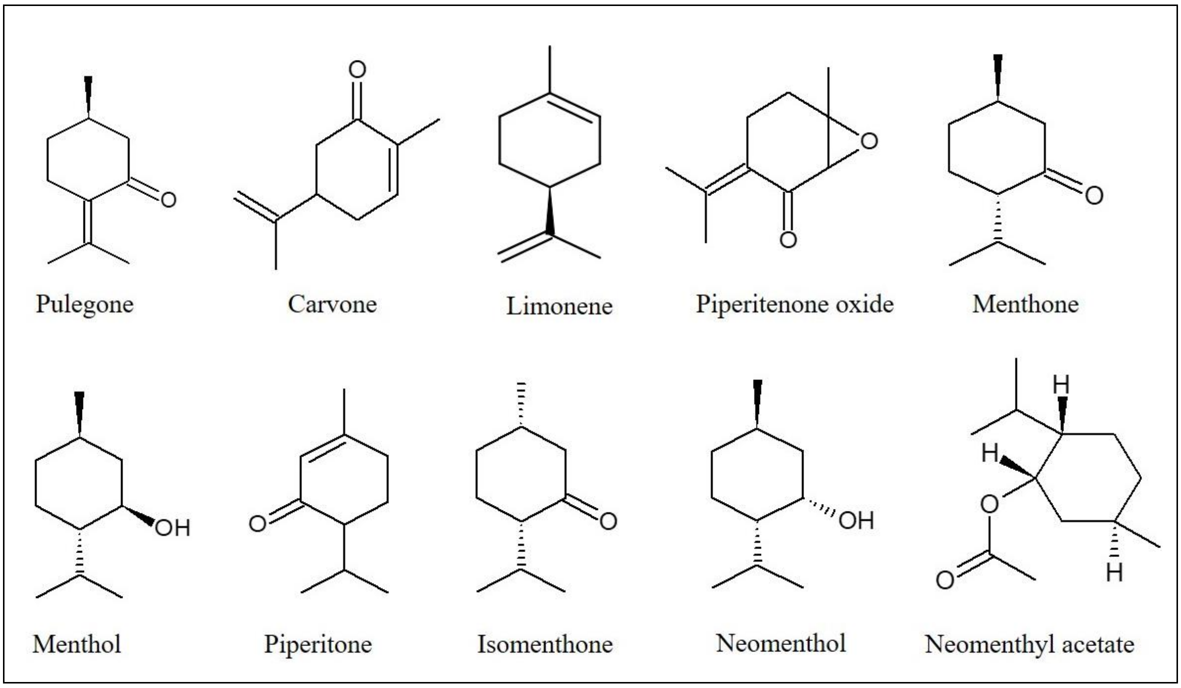


Figure 2.2 Chemical structures of some major chemical compounds in the essential oils of *Mentha* species.

2.1.3.3 The *Origanum* species

The genus *Origanum* is a very important plant group in the family Lamiaceae, which consists of about 900 species of annual, perennial, and shruby herbs. The *Origanum* species is prevalent throughout the world (Kordali *et al*., 2008) and has strong aromatic leaves. This genus also comprises some essential culinary herbs like oregano (*O. vulgare* L.) and sweet marjoram (*O. majorana* L.). The plants of this genus are widely used for the flavoring of alcoholic beverages, food products, and perfumery for their spicy fragrance. In addition to their commercial importance, these plants have also been utilized for a long time as condiments and spices for foods such as salads, soups, sausages, and meats (Baydar *et al*., 2004). Both academia and the food industry have been attentive in the biological activities of *Origanum* extracts and essential oils due to their antimicrobial and antioxidant potentials (Baydar *et al*., 2004).

In Nepal, at least two plant species of this genus are informed by the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023f). They are *Origanum majorana* L., and *Origanum vulgare* L. The chemical structures of the major compounds of essential oils from *Origanum* species are shown in Figure 2.3. To examine the structural activity association, it is needed to detect the chemical variation among the possible volatile compounds of *Origanum* species. Considering this fact, the present study has been carried out for the chemistry of *Origanum* EO samples from three geographical locations. Also, the objective of the present research is to evaluate the biological activities of EOs as possible sources for further valuable utilization.

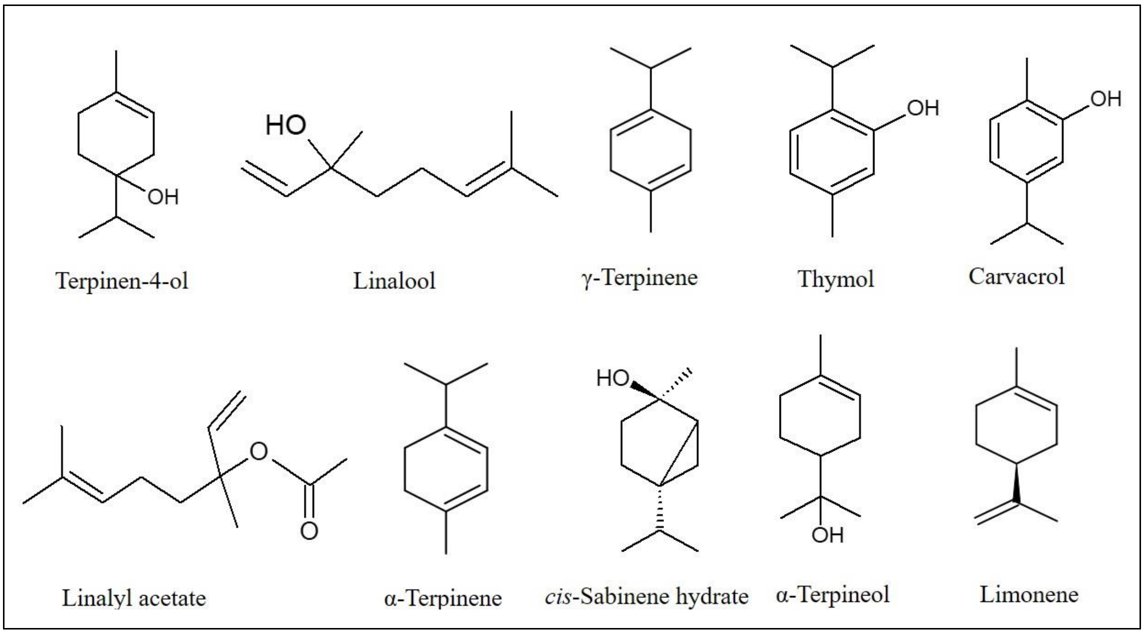


Figure 2.3 Chemical structures of some major chemical compounds in the essential oils of *Origanum* species.

2.1.3.4 The *Perilla* species

The genus *Perilla* is an annual herbaceous plant native to Asia, and its leaves are commonly utilized in many Asian gourmet dishes. This genus includes *Perilla frutescens* L. as an important plant herb. The young, huge raw leaves are frequently used to wrap and eat cooked food in addition to being used as garnish, in sushi, and as a component in soups (Kim *et al*., 2007). The leaves are used medicinally to cure food poisoning. Basically, the anti-allergic, anti-inflammatory, and anti-tumor-promoting compounds found in perilla plants have drawn a lot of attention (Makino *et al*., 2003; Banno *et al*., 2004). *Perilla* extract and rosmarinic acid, a significant polyphenolic component, prevented the liver damage brought by D-galactosamine and lipopolysaccharide (Osakabe *et al*., 2002).

In Nepal, only a single plant species of this genus is described by the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023g). It is *Perilla frutescens* (L.). The chemical structures of the major compounds of EOs from *Perilla* species are shown in Figure 2.4. To explore the structural activity association, it is important to identify the chemical differences among the possible volatile compounds of *Perilla* species. Knowing this fact, the present study has been conducted on the chemistry of *Perilla* essential oil samples from two geographical locations. Likewise, the objective of the present work is to establish the biological assessment of EOs as possible sources for further utilization.

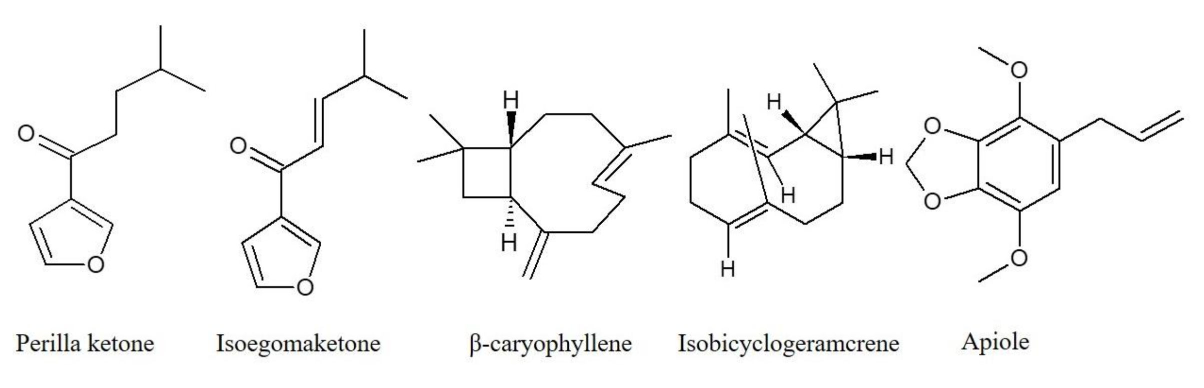

Perilla ketone Isoegomaketone β-caryophyllene Isobicyclogeramcrene Apiole

Figure 2.4 Chemical structures of some major chemical compounds in the essential oils of *Perilla* species.

2.1.3.5 The *Elsholtzia* species

Elsholtzia is a genus including about 42 species under the family Lamiaceae in the world (Liu *et al*., 2007; Chen *et al*., 2022). They are widely distributed in East Asia, Africa, North America, and Europe, especially in China, Korea, Japan, India, and Nepal. The genus *Elsholtzia* plants are mostly aromatic, frequently used as domestic folk medicine, herbal tea, food, spices, beverages, perfumeries, cosmetics, aromatherapies, and the source of honey manufacture. It has been reported that at least 33 plant species of this genus are found in China (Flora of China, 1994). Most of the *Elsholtzia* species have a distribution at an altitude of 1000 m to 3000 m and grow in hilly grassland, waste areas, forests, thickets, or valleys in warm areas. The plants belonging to the *Elsholtzia* genus are commonly used as domestic folk medicines for the treatment of colds, headaches, fever, diarrhoea, rheumatic arthritis, and nephritis in China (SACM, 1999).

Regarding Nepal, at least 12 plant species of this genus are described by the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023a). They are *E. blanda, E. ciliate, E. densa, E. concinna, E. eriostachya, E. flava, E. fruticosa, E. pilosa, E. stachyodes,* and *E. strobilifera.* The chemical structures of the major compounds of essential oils from *Elsholtzia* species are given in Figure 2.5. To determine the structural activity relationship, it is vital to distinguish the chemical variations among the possible volatile compounds of various *Elsholtzia* species. Considering this point, this study has been conducted for the chemistry of two *Elsholtzia* essential oil samples. Further, the target of this present investigation is to support the biological efficacy of EOs as potential sources for beneficial utilization.

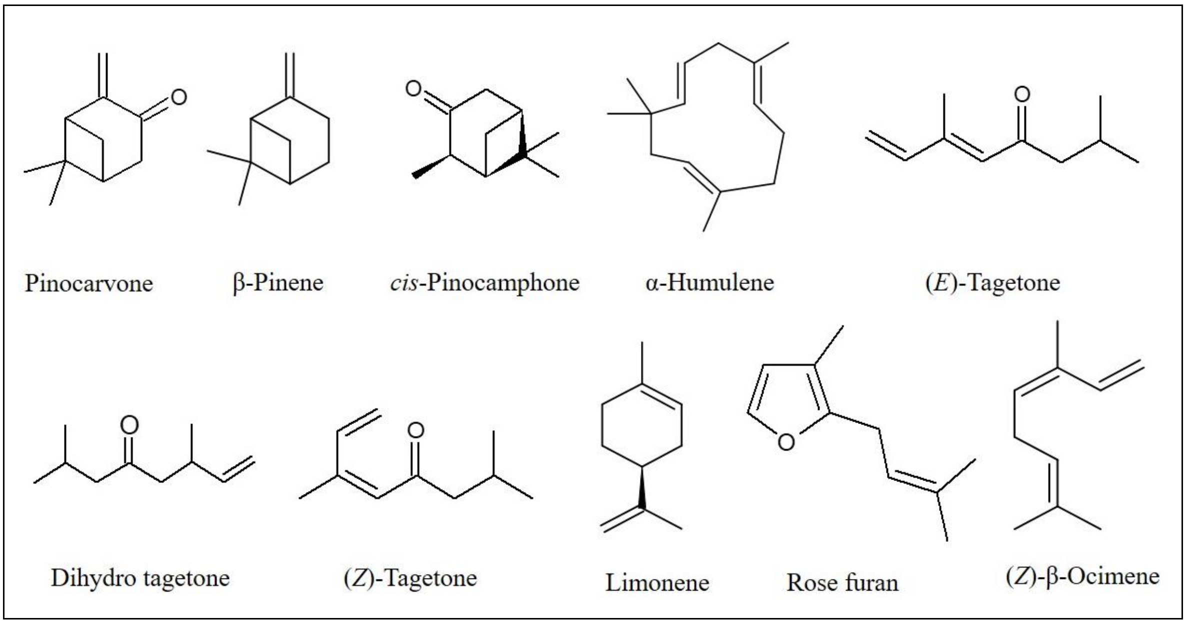


Figure 2.5 Chemical structures of some major chemical compounds in the essential oils of *Elsholtzia* species.

2.1.3.6 The *Pogostemon* species

Pogostemon is a large genus from the family Lamiaceae, first described as a genus in 1815. It is native to warmer parts of Asia, Africa, and Australia. The best-known member of this genus is patchouli, *Pogostemon cablin,* widely cultivated in Asia for its scented foliage used for perfume, incense, insect repellent, herbal tea, etc. Some members of the genus (i.e., *Pogostemon erectus*, *Pogostemon stellatus,* and *Pogostemon helferi*) are grown ornamentally in the aquarium hobby and are used for aquascaping. The most famous member of this genus, Pogostemon cablin (Patchouli), has been used in traditional medicine as an antifungal and anti-cold remedy (Wu *et al*., 2004).

Regarding Nepal, at least five plant species of this genus are mentioned in the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023h). They are *P. amarantoides, P.*

auricularius, *P. benghalensis, P. glaber,* and *P. tuberculosus.* The chemical structures of the major compounds of essential oils from *Pogostemon* species are shown in Figure 2.6. To determine the structural activity connection, it is necessary to distinguish the chemical diversity among the possible volatile compounds of various *Pogostemon* species. Considering this fact, this study has been conducted for the chemistry of *Pogostemon* essential oil samples. Further, the aim of this present investigation is to back up the biological efficacy of EOs as potential sources for beneficial utilization.

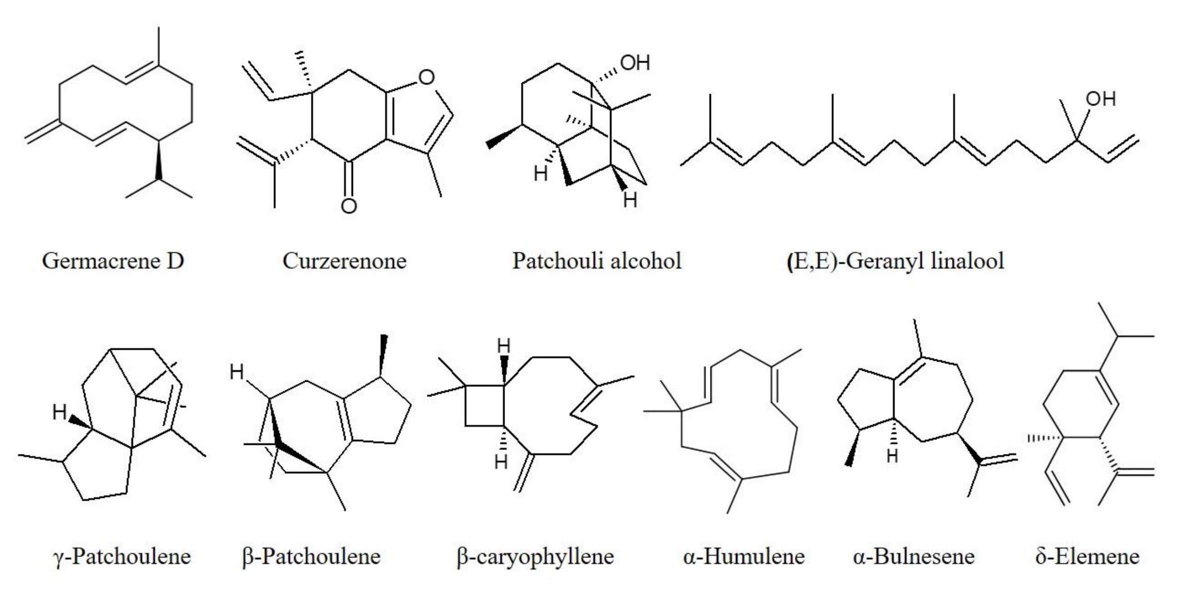


Figure 2.6 Chemical structures of some major chemical compounds in the essential oils of Pogostemon species.

2.1.3.7 The *Colebrookea* species

Colebrookea oppositifolia Smith is the only single species in the *Colebrookea* genus (Lamiaceae). This plant, commonly known as “Indian squirrel tail”, is widely distributed in subtropical regions of the world such as India, Nepal, Pakistan, Myanmar, Thailand, and China, where it grows in hills and plains at altitudes of 250–1700 m (Yadav, 2019). This plant is evergreen, densely woolly shrubs or small trees, 1.2 to 3.6 m. It has been extensively used in the traditional Indian medical system to treat a variety of conditions, including headache, fever, dysentery, peptic ulcer, dermatitis, wounds, hemostasis, antifungal, and anti-fertility agents. However, the plant's roots have been used the most frequently to treat epilepsy (Rubiaceae *et al*., 2011; Viswanatha *et al*., 2018). In Nepal, *C. oppositifolia* is traditionally recognized as dhusure, dosul, dhulsu, or dhursuli, and its leaves are used mainly to treat ailments of the ocular region such as cataracts, corneal opacity, and keratoconjunctivitis and as an anthelmintic (Joshi *et al*., 2000).

In the context of Nepal, at least a single plant species of this genus is stated by the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023i). It is *Colebrookea oppositifolia* Sm. The chemical structures of the major components of essential oils from *Colebrookea* species are shown in Figure 2.7. To determine the structural activity connection, it is necessary to know the chemical variations among the possible volatile compounds of *Colebrookea* species. Considering this fact, this study has been carried out here for the chemistry of *Colebrookea* essential oil samples. Further, the goal of this present investigation is to establish the biological efficacy of EOs as potential sources for beneficial utilization.

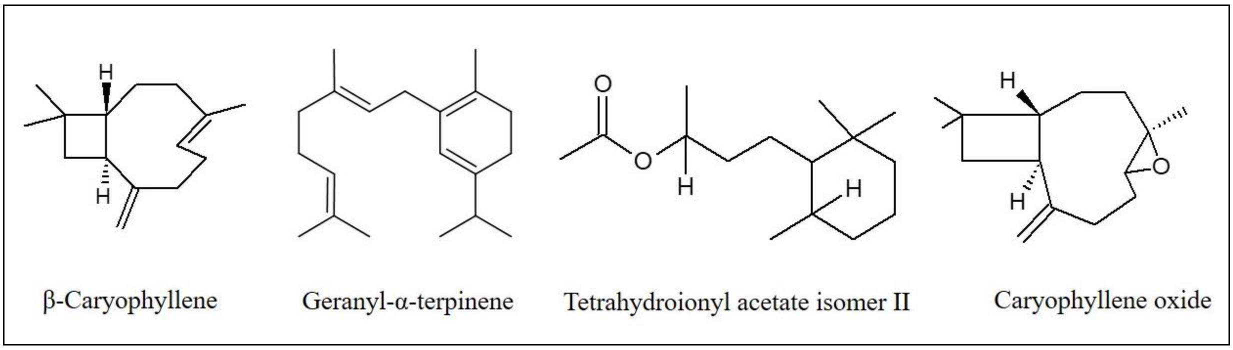


Figure 2.7 Chemical structures of some major chemical compounds in the essential oils of *Colebrookea* species.

2.1.3.8 The *Colquhounia* species

Colquhounia is a genus of about six species of evergreen or semi-evergreen shrubs or subshrubs in the Lamiaceae, first described in 1922. They are native to the Himalayas and southwestern China, south to Peninsular Malaysia. They are shrubs growing to 1 to 3 m tall, rarely to 4 m. Their leaves are aromatic in nature and are long, 1 to 6 cm, finely toothed, and borne in opposite pairs on the square stems. The flowers are tubular, two-lipped, and carried on terminal spikes.

In Nepal, at least five plant species of this genus are listed in the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023j). They are *Colquhounia coccinea* Wall. *Colquhounia coccinea* var. *coccinea* Wall., *Colquhounia coccinea* var. mollis Wall., *Colquhounia coccinea* var. parviflora Wall., and *Colquhounia coccinea* var. vestita Wall. *The chemical structures of the major components* of essential oils from *Colquhounia* species are shown in Figure 2.8 (Bhatt *et al*., 2009). To investigate the structural activity association, it is important to identify the chemical diversities among the possible volatile compounds of various *Colquhounia* species. Knowing this fact, this study has been carried out here for the chemistry of *Colquhounia* essential oil samples. Further, the aim of this investigation is to establish the biological efficacy of EOs as potential sources for further valuable uses.

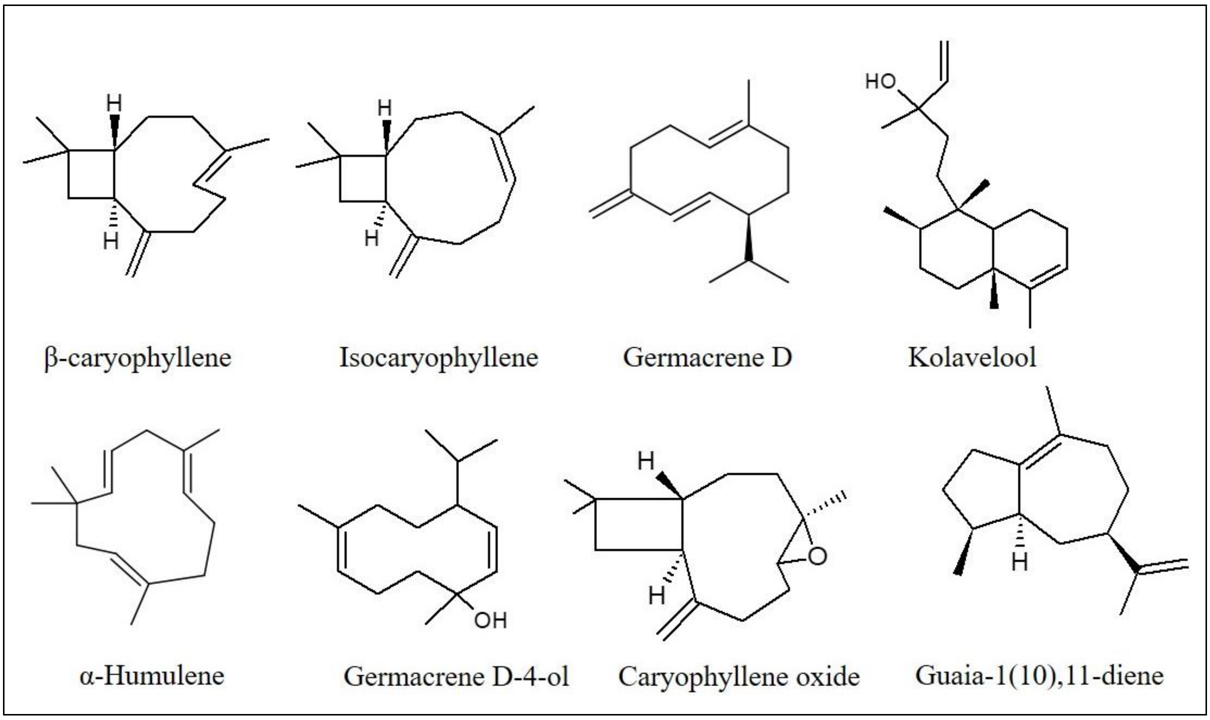


Figure 2.8 Chemical structures of some major chemical compounds in the essential oils of *Colquhounia* species.

2.1.3.9 The *Leucosceptrum* species

Leucosceptrum is a genus of flowering plants in the family Lamiaceae, first described in 1806. It contains only one known species, *Leucosceptrum canum*, native to south-western China (Sichuan, Tibet, and Yunnan), the eastern Himalayas (Nepal, Bhutan, Assam, Nagaland, and Bangladesh), and northern Indochina (Myanmar, Thailand, Laos, and Vietnam).

Regarding Nepal, only a single species of this genus is reported by the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023k). It is *Leucosceptrum canum* Sm. The chemical structures of the major components of EOs from this species are shown in Figure 2.9. To explore the structural activity connection, it is important to identify the chemical variation among the possible volatile compounds of *Leucosceptrum* species. Considering this fact, this study has been carried out for the chemistry of *Leucosceptrum* essential oil samples. Moreover, the aim of this study is to establish the biological activities of EOs as potential sources for further beneficial uses.

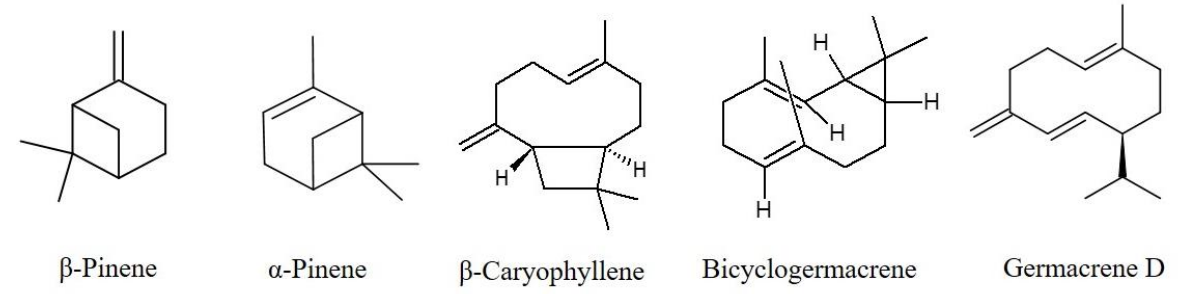


Figure 2.9 Chemical structures of some major chemical compounds in the essential oils of *Leucosceptrum* species.

2.1.3.10 The *Clinopodium* species

Clinopodium is a genus of flowering plants in the family Lamiaceae. It is in the tribe Mentheae of the subfamily Nepetoideae, but little else can be said with certainty about its phylogenetic position. *Clinopodium* species are used as food plants by the larvae of some Lepidoptera species, including *Coleophora albitarsella*. Various *Clinopodium* species are used as medicinal herbs. For example, *C. laevigatum* is used in Mexico as a tea under the name poleo or yerba de borracho to cure hangovers, stomach aches, and liver disease. *Clinopodium* has been defined very differently by different authors. Some people have limited it to just 13 species, all closely related to the type species, *Clinopodium vulgare.*

In Nepal, at least three species of this genus are listed in the Annotated Checklist of the Flowering Plants of Nepal (ACFPN, 2023l). They are *Clinopodium piperitum (*D. Don), *Clinopodium umbrosum (M. Bieb),* and *Clinopodium vulgare* L. The structures of the major compounds of EOs are shown in Figure 2.10. It is important to know the chemical variations among the volatiles of this species from a structural activity point of view. This study has been run for the chemistry of a *Clinopodium* EO to find their efficacy for beneficial uses.

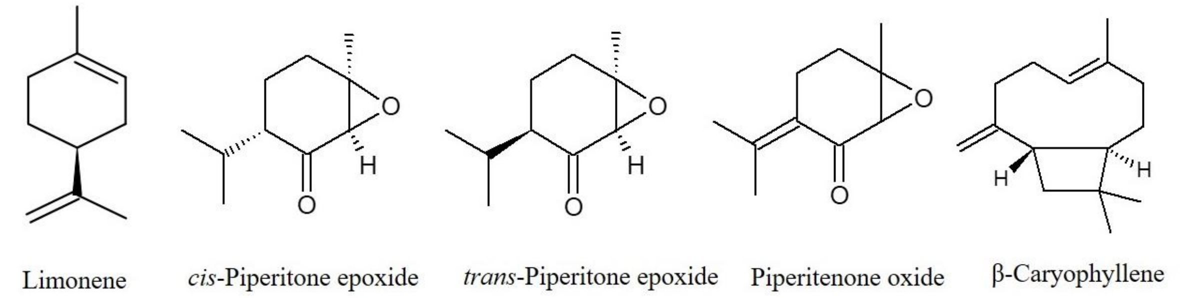


Figure 2.10 Chemical structures of some major chemical compounds in the essential oils of *Clinopodium* species.

2.1.4 Status of Essential Oils in Nepal and the Global Scenario

In Nepal, there are many industries producing personal care products, cosmetics, beverages, and ice cream products, along with other daily uses, which have been established all over the country. These industries excessively utilize essential oils as raw materials, which have also been imported from outside the country. The report of the customs department and trade and export promotion centre in Nepal shows that essential oils with a price of nearly NRS. 11 crore were exported abroad from 2009 to 2016 AD. At the same time, essential oils with a cost of nearly NRS. 66 crore were imported from abroad in order to be used in food, drinks, cosmetics, and other industries (TEP, 2024). Another report also mentioned that nearly 55 tons of essential oils are exported abroad annually (Gurung, 2010). These data highlight the significance of essential oils and their market status in the context of Nepal.

The current status of the essential oil industry in Nepal has not flourished in encouraging and productive ways. Therefore, the essential oil industry in the country has not been developed properly in the absence of modern agricultural and horticultural practices, as well as a lack of understanding of their utilization in value-added products. However, the trend looks to improve in the future scenario.

From a worldwide market perspective, nearly one lakh ton of essential oils are expected to be produced globally from 160 types of aromatic plants. Most of them are agro-based productions. Approximately, these EOs are classified into two categories: the first category of essential oils produced in larger quantities and the second category of EOs produced in smaller quantities. In the global market, ten types of EOs, consumed to a greater extent, would cover 80% of the market, and the rest of the 150 types of EOs, consumed in lower quantities, would cover 20% of the market in the world (Shukla, 2015; ITC, 2016). The production of EO is increasing annually due to the fascination of people with natural products. Because people are more aware of health and well-being, food safety, and environmental concerns. Therefore, the demand for natural products like EOs is rapidly increasing day by day. According to the report of the International Trade Centre, the market for EOs and allied products has been rising by 8% annually since 2001 AD (ITC, 2016). The price of world exports of EOs and allied products was about 8 billion US dollars in 2001 AD, which had just increased by 3.1 times to about 27 billion US dollars by 2015 AD. At the same time, the import of these substances has increased from 8.3 billion US dollars to 26.5 billion US dollars (ITC, TMIS, 2024). The major countries that produce essential oils are Brazil, China, the United States of America, Egypt, India, Mexico, Indonesia, etc. The United States of America is the main country for the highest consumption of essential oils of the total world‘s production, accounting for about 40%, followed by European countries (30%) and Japan (7%).

Lamiaceae essential oils possess several types of bioactive compounds, with the most dominant role. These major compounds are of great value and have a wide application in diverse fields, as shown in Annex 8.

2.1.5 Factors Influencing the Yield and Composition of Essential Oil

Several factors could influence the yields and composition of essential oils obtained from the different plant species. Sometimes, it is very difficult to differentiate the factors because they may be interrelated and can influence one another. These factors can be categorized into: geographical origin, seasonal and maturity variation, plant developmental stage, parts of the plant used, storage period, postharvest drying period, and genetic variation as well (Hussain *et al*., 2008). Here, some variables or factors are described briefly in the following ways:

2.1.5.1 Variation in geographical origin of plants

Many previous reports in the literature have shown the disparities in the yields and chemical composition of essential oils according to the geographical regions (Celiktas *et al*., 2007; Van Vuuren *et al*., 2007). According to one study, essential oils from *Mentha longifolia* (L.) and *Tagetes minuta*, which were collected from different regions, showed variations in yields and chemical composition (Hussain *et al*., 2008). Another significant environmental component that appears to affect the quantity of essential oils and their chemical composition is altitude. There were considerable differences in the yield and chemical composition of the *Origanum vulgare* essential oils from the 23 locations, which were dispersed throughout Greece. Similar to this, another study revealed that the amount and content of essential oils varied greatly according to the environments in which the plants were grown. Furthermore, the plant's affinity for these conditions suggests that the genetic makeup of the plant could have a higher impact on the chemical composition of the essential oils produced than the type of soil where it is grown (Milos *et al*., 2001).

2.1.5.2 Variation in seasonality and maturity of plants

Because the precise ontogenic growth stage will vary with changes in the season, these two elements are related to one another. There are several reports in the literature about how the chemical composition of essential oils varies from different plants collected during various seasons (Celiktas *et al*., 2007; Van Vuuren *et al*., 2007). The microenvironment in which the plant was growing, had an impact on the essential oil production, which varied significantly from month to month. *Santolina rosmarinifolia*'s essential oil content and yield varied from month to month (Pala-Paul *et al*., 2001), and these fluctuations could be attributable to temperature and precipitation.

2.1.5.3 Variation in genetic type

Genotype is commonly defined as the genetic make-up of an organism, as determined by its physical appearance or phenotype. While chemotype is generally defined as "a collection of organisms that produce the same chemical profile for a given class of secondary metabolites". The alterations in the chemical profiles were detected in essential oils from the same population and region. This indicates the presence of different chemotypes within the same species. The essential oil composition and yields of the 24 wild and 19 cultivated caraway populations grown by Galambosi and Peura (1996) were significantly different from each other under identical conditions. One of the most significant factors affecting the content of a plant's essential oils is its genetic makeup.

2.1.5.4 Variation in the growth stage of plant, plant parts used, and post-harvest drying period

Besides the variables discussed earlier, there are other prevailing factors for the variations in yields and chemical composition of essential oils from the plant species. These factors are the parts of the plant applied, the developmental stage of plants, and post-harvest drying time. Another previous study described the variation in the chemical composition of essential oils obtained from the stems, leaves, and flowers of *Salvia officinalis* L. plants harvested in Northern Portugal (Santos-Gomes & Fernandes-Ferreira, 2001). There were significant variations in the major components of the essential oils from shoot samples collected throughout the year at two different locations in Northern Portugal.

In the process of essential oil production, post-harvest drying of plant material is a common practice. The post-harvest drying is considered to enhance the essential oil yield and enhance the distillation by refining heat transfer, in addition to providing.increased loading capacity due to the loss of plant moisture. Other benefits are the suppression of various biochemical reactions and the reduction of microbial development in the dried samples (Soysal & Oztekin, 2001). But the essential oils may be lost during the post-harvest treatment because there may be volatilization and physical damage to the essential oil glands. Terpenoids and other compounds of essential oils are typically found in their free form, but they can also be attached to sugar molecules.

2.1.6 Techniques for the Extraction of Essential Oils

The isolation of essential oils is an important aspect of improving their quality and overall yields. They can be extracted from the crude materials using different methods. They are hydrodistillation, stean distillation, solvent extraction, expression under pressure, microwave-assisted extraction, supercritical fluid extraction, and subcritical water extraction (Vila Verde *et al.*, 2018). Hydrodistillation is the conventional method of essential oil extraction that is both the earliest and simplest. Here, the principle of extraction is centered on isotropic distillation. During the boiling process, the plant sample is soaked up in water, and the essential oils in the oil cells could diffuse through the cell walls via osmosis. The amount of time needed for distillation varies depending on the nature of the plants being used. In the hydrodistillation method, the vapors are allowed to condense before separating the essential oil from the aqueous phase. The more volatile essential oil should not be lost, and hence, sincere attention should be drawn to guarantee the proper condensation of steam.

According to the steam distillation principle, when two non-miscible liquids are combined, every single component creates a vapor pressure, as in the case of their purity. Hence, the total vapor pressure of the boiling mixture is equal to the sum of the partial vapor pressure of each of its components present there. The mixture begins to boil when the total vapor pressure reaches the atmospheric pressure. This suggests that the mixture's boiling point is attained at a lower temperature than the boiling points of its constituent parts. As a result, steam distillation can separate volatile from non-volatile components while lowering the boiling point and avoiding extremely high temperatures (Donelian *et al.*, 2009). Steam distillation acquires the advantages of the volatility of compounds to evaporate when heated with steam and the hydrophobicity of compounds to separate into an oil phase during the condensation process. Moreover, this technique has another positive aspect that the displacement of atmospheric oxygen by the steam could protect the volatile components from oxidation. Though steam distillation is a popular method for the extraction of essential oils on a commercial level, it has not been extensively preferred in research laboratories.

2.1.7 Chemistry of Essential Oils

Terpenes are the most common compounds present in essential oils. They are comprised of several 5-carbon-base (C5) building units, which are known as isoprene (Gunther, 1952). These isoprene units can form the building blocks by connecting together in a ‘head-to-tail’ arrangement to from monoterpenes, sesquiterpenes, diterpenes, and larger sequences of compounds (Pinder, 1960). The common terpenes are monoterpenes (C10) and sesquiterpenes (C15). In some cases, they also exist in hemiterpenes (C5), diterpenes (C20), triterpenes (C30), and tertraterpenes (C40). A terpene having oxygen is commonly named ‘terpenoid’.

Monoterpenes are commonly formed by the connection of two isoprene building units. They are the most representative molecules, comprised of 80-90% of the essential oils, and generate different structures. They also have several functional groups such as olefins (ocimene, myrcene, terpenenes, phellandrenes, pinenes, etc.), aldehydes (geranial, citronellal, etc.), ketones (menthones, pulegone, carvone, fenchone, .pinocarvone etc.), alcohols (geraniol, citronellol, nerol, menthol, carveol, etc.), esters (linalyl acetate, citronellyl acetate, isobornyl acetate, etc.), and ethers (1,8-cineol, menthofurane, etc.).

The previous findings have characterized several bioactive compounds, like thymol, eugenol, carvacrol, β-caryophyllene, linalool, 1,8-cineole, and pulegone, in the Lamiaceae essential oils (Barbosa *et al*., 2017; Miura *et al*., 2021; Rojas-Olivos *et al*., 2018). They are characterized by two or three primary components at relatively high concentrations (20-70%) as compared to other components in trace amounts. For example, carvacrol (30%) and thymol (27%) are the major components of the *O. compactum* EO, linalol (68%) of the *Coriandrum sativum* essential oil, α- and β-thujone (57%) and camphor (24%) of the *Artemisia herba-alba* essential oil, 1,8-cineol (50%) of the *Cinnamomum camphora* EO, α-phellandrene (36%) and limonene (31%) of leaf and carvone (58%) and limonene (37%) of seed *Anethum graveolens* essential oil, menthol (59%) and menthone (19%) of *Mentha piperita* essential oil. Generally, these major components could determine the overall biological potential of EOs.

2.1.8 Biological Activities of Essential Oils

2.1.8.1 Antioxidant activities

Most of the Lamiaceae essential oils have exhibited excellent antioxidant activity (Farouk *et al*., 2016; Bouyahya *et al*., 2017; Bardaweel *et al*., 2018; Santos *et al*., 2020). Natural antioxidant compounds show their antioxidant property through several mechanisms, like (1) chain breaking by the donation of hydrogen atoms or electrons that convert free radicals into more stable species; (2) chelating metal ions, which are involved in the generation of reactive oxygen species; (3) decomposing lipid peroxides into stable final products; and (4) inhibiting the deleterious action of prooxidant enzymes. Because of the complexity of the composition of plants and plant-based foods, it is very tough to separate each antioxidant component and study it individually. Scientists are searching for advanced methods that can be reliable for measuring the antioxidant activity of foods and other biological systems. However, the antioxidant potential of essential oils has been identified in several *in vitro* studies.

Over the last twenty years, there has been a significant increase in public concern regarding the safety of synthetic antioxidants (such as BHT, BHA, and TBHQ) in food preservation, in addition to their potential health effects. It is well recognized that these synthetic antioxidants have harmful and carcinogenic impacts on humans and food systems. The literature review displays several previous studies on the extracts from natural resources, which have established their strong antioxidant potential (Descalzo & Sancho, 2008; Paradiso *et al*., 2008). Many natural sources of antioxidants have been identified, and several studies are still being carried out continuously. Some of these essential oils and plant extracts have been reported to be more effective than some other synthetic antioxidants. The antioxidant potential of essential oils and plant extracts is primarily due to the presence of hydroxyl groups in their chemical structures.

2.1.8.2 Antimicrobial activities

Essential oils from Lamiaceae plants have demonstrated remarkable antimicrobial activity in various previous studies (Alimpic *et al*., 2015; Ruiters *et al*., 2016; Piras *et al*., 2018). To determine the antimicrobial activity of essential oils and plant-based ingredients, many assays, including the disc-diffusion method, well-diffusion method, microbroth dilution technique, and evaluation of minimum inhibitory concentration, are frequently applied. The results of a method may be affected by several factors, including the technique used to separate the plant's essential oils, the growth phase, the amount of inoculum utilized, pH of the culture medium and temperature, and the incubation period; hence, the comparison of published results is very complicated (Burt, 2004).

The most significant technique for measuring the antimicrobial efficacies of essential oils is the evaluation of the minimum inhibitory concentration (MIC), which provides accurate, exact, and reproducible results. Sometimes, minimum bactericidal (MBC) or bacteriostatic concentration is considered, both terms agreeing closely with the MICs. The strength of the antimicrobial efficacy can be evaluated by the dilution of essential oils in agar or broth. Here, the microbroth dilution technique is used to measure the antimicrobial activity of different essential oil samples in terms of the minimum inhibitory concentration (MIC).

2.1.8.3 Cytotoxicity of essential oils

Many Lamiaceae essential oils have shown effective cytotoxicity against cancer cell lines as an alternative natural product for cancer treatment (Russo *et al*., 2015; Sharifi-Rad *et al*., 2017; Mesquita *et al*., 2019). Cytotoxicity can be assessed using a variety of techniques, according to the literature. MTT is the most straightforward, dependable, and widely documented test for determining cell viability when looking for anti-proliferative drugs. An undamaged mitochondrial membrane and an intact mitochondrial respiratory chain are necessary for viable cells. The use of mitochondrial dehydrogenases in living cells can be utilized to determine the toxicity of bioactive components. The succinate dehydrogenase system, which is only active in living cells, is a part of the mitochondrial respiratory chain that converts MTT, a salt of tetrazolium, into formazan. The mitochondrial succinate dehydrogenase reduces the yellow tetrazolium salt to purple formazan, which is water-insoluble. A microplate can be used to measure the amount of dye. A Cell Counting Kit-8 kit (Dojindo Molecular Technologies, Inc., Rockville, MD, USA) can also be used to assess the viability of the cells in the presence or absence of the test substances. It is another reliable and practical method for carrying out a cell viability experiment provided by Cell Counting Kit 8 (WST-8 / CCK8) (Phan *et al*., 2023a), and this method has been used here.

2.1.9 Formulation of Cream Using Essential Oils

Due to greater demand for natural, unadulterated products in various sectors, essential oils are being widely used all around the world and are continuing to increase their massive acceptance. Therefore, a huge quantity of essential oils is produced all around the world to support cosmetics, aromatherapy, and phytomedicine businesses (Masih & Singh, 2012; Baser & Buchbauer, 2015).

Due to increasing consumer awareness of health issues, the desire for organic and natural hygiene goods has also been driving growth in the global market for essential oils. Additionally, the demand for natural flavors and fragrances in cosmetics, relaxation, and dermal applications is probably going to increase rapidly with the consumption of essential oils (Kumar *et al*., 2016b; Gyawali *et al*., 2020b). Essential oils and oleoresins are essential to the fragrance and flavoring industries, in addition to being utilized in the food processing and seasoning industries. Flavor and fragrance producers are among the top importers of essential oils on a global scale. Their sales provide a window into market trends and the ensuing demand for essential oils. Here, the formulation of cream has been developed by using suitable Lamiaceae essential oils.

CHAPTER 3

3.1 MATERIALS AND METHODS

The present research work was confined to a study of some selective Lamiaceae species originating in Nepal for their chemical characterization, different biological activities, and product formulation using essential oils. The entire research analysis was carried out in the laboratories of the Department of Pharmacy, the Department of Chemical Science and Engineering, Kathmandu University, Nepal; the Aromatic Plant Research Centre, Utah, USA; the Division of Natural Drug Discovery, University of Toyama, Japan; and the Department of Chemistry, University of Alabama in Huntsville, USA.

3.1.1 Description of Sampling Sites

Considering the significance of valuable Nepalese aromatic medicinal plants from the Lamiaceae family, a credible, holistic, and transparent methodology has been followed that is widely applied as the standard method in this study. Sampling sites have been assigned by extensive literature surveys carried out on the availability of plant species in various selective regions of Nepal. This study has considered the following sampling sites: tropical region (0-1000 m): Bardiya and Kapilvastu; sub-tropical region (1000-2000 m): Gorkha, Kavre, Kathmandu, Lalitpur, and Bhaktapur; temperate region (2000-3000 m): Nuwakot and Sinddhupalchowk, and alpine region (4000-5000 m): Rasuwa, in a preliminary field survey depending on location access and convenience.

3.1.2 Collection of Aromatic Medicinal Plants, Identification, and Vouchering

The different Lamiaceae plants from Nepal were selected based on an intensive review and ethnopharmacological information. The aerial parts of plant materials (stems, leaves, and flowers) from the selective Lamiaceae plant species were collected from different regions of Nepal, as shown in Figure 3.1. Finally, they were brought to the laboratory and processed for extraction, herbarium specimen preparation, and identification as per guidelines. The plant materials were dried in the shade and stored at room temperature below 35 ℃ for essential oil extraction. The plant samples were identified and authenticated by Ms. Rita Chhetri at the National Herbarium and Plant Laboratories, Godawari, Nepal, using floristic literature and herbariums (Polunin and Stantion, 1984; Stantion, 1988), and voucher specimens were deposited. Plants from different geographical locations and seasonal variations were considered to understand the quality variations. The details of plant materials collected in this study are presented in Annex 9, and photographs of all plant materials taken during sample collection are shown in Annex 15. Similarly, Annex 14 shows the distribution of Lamiaceae plant species across different geographical locations in Nepal, along with our sampling points, indicating their wild occurrence in diverse ecosystems.

3.1.3 Materials

3.1.3.1 Chemical reagents and standards as references

All the chemicals and standards used in this research work are of analytical grade and are presented in Table 3.1.

Table 3. 1 List of Chemical reagents used during the research work

Chemicals

- Acetic Acid
- Aluminium chloride
- Amphotericin B
- Ammonium thiocyanate
- Anhydrous sodium sulphate
- Ascorbic acid
- B-Dex 325 capillary column
- Butylated hydroxytoluene
- Carbopol-940
- CAMHB
- Cetyl alcohol
- Cyclopentasiloxane
- 3-[4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide]
- Dichloromethane
- Dimethylsulfoxide
- 2, 2,-diphenyl-1-picrylhydrazyl
- Ferrous Sulphate
- Ferric chloride
- Ferrous chloride
- Fetal bovine serum
- Gallic acid
- Gemcitabine
- Gentamicin
- Glycerin
- Glycerylmonostearate
- Homologous series of C8-C40 *n-alkanes*
- Hydrogen gas
- Helium gas
- Linoleic acid
- Phosphate buffered saline
- Potassium fericyanate
- Potassium sorbate
- Potassium ferricyanide
- Resazurin
- Sodium benzoate
- Sodium bicarbonate
- Sodium dodecyl sulfate
- Sodium nitrite
- Steric acid
- Thiobarbituric acid
- Trichloro-acetic Acid
- Triethanolamine
- Triethanol amine
- Tryptic soya agar
- White soft paraffin
- Yeast-nitrogen base
- ZB-5 MS capillary column
- Zinc oxide

3.1.3.2 Equipment/instrumental apparatus

The instruments used for different analyses during the course of research, along with their company identification, are given in Table 3.2.

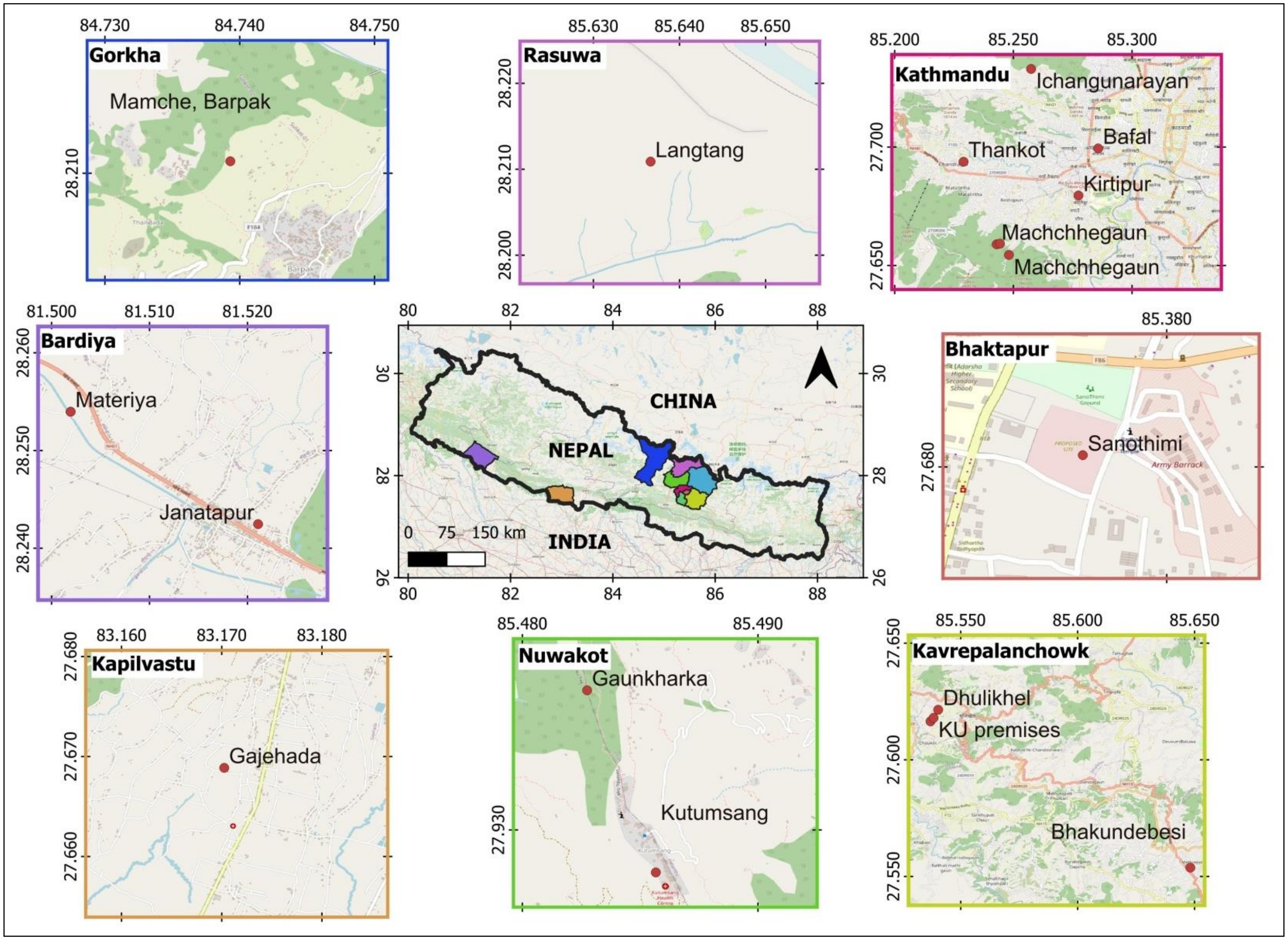


Figure 3. 1 The geographical locations for collection of Lamiaceae plants on ethnobotanical basis from the different parts of Nepal.

Table 3. 2 List of Instruments required

Name of instruments	Uses
Clevenger's apparatus (Jain scientific Glass Works, India)	Extracting essential oil by hydro-distillation
GC-MS	Identification of volatile components
UV-vis Spectrophotometer U-2001 Hitachi, Japan	Absorbance measurement
Micro-plate reader, Epoch2, Bio-Tek Instrument, Inc., USA	Absorbance measurement
Incubator	Control the temperature
MultiScan SkyHigh plate reader,Thermo Fisher	Absorbance measurement
Laminar flow	To prevent contamination
Hot air oven	To dry and to control the temperature.
pH meter	To measure the pH
Brookfield viscometer	To evaluate the viscosity
Two parallel plate	To determine the preadability of a product
Franz cell diffusion	To measesure DPPH activity of creams
Electric Balance MP-300 Ohyo, Japan	To weigh out
96-micro-titer plate	Samples loading
Heating mantle (Biobase 1000 mL capacity, Germany)	Heating purposes
Centrifuge machine	To centrifuge

3.1.4. Microbial Strains Utilized to Assess Antimicrobial Activity

The microbial strains were purchased from ATTC (Lines 199-203), and cells were harvested from freshly cultured plates for further analyses. Some bacterial and fungal strains were also used during analysis at the Zest Laboratory and Research Center, Bhaktapur. The bacterial and fungal strains obtained from different sources were applied to determine the antimicrobial efficacy of selected Lamiaceae plant essential oils.

Bacterial Strains:

(i) *Bacillus cereus (B. cereus)* (ATCC 14579)

(ii) *Staphylococcus aureus (S. aureus)* (ATCC 29213)

(iii) *Staphylococcus epidermidis (S. epidermidis)* (ATCC 14990)

(iv) *Bacillus cereus (B. cereus)* (ATCC 11778)

(v) *Escherichia coli (E. coli)* (ATCC 8739)

(vi) *Staphylococcus aureus (S. aureus)* (ATCC 6538)

(vii) *Pseudomonas aeruginosa (P. aeruginosa)* (ATCC 9027)

Fungal Strains:

(i) *Aspergillus niger (A. niger)* (ATCC 16888)
(ii) *Candida albicans (C. albicans)* (ATCC 18804)
(iii) *Trichophyton mentagrophytes (T. mentagrophytes)* (ATCC 18748)
(iv) *Aspergillus fumigatus (A. fumigatus)* (ATCC 96918)
(v) *Cryptococcus neoformans (C. neoformans)* (ATCC 32045)
(vi) *Microsporum canis (M. canis)* (ATCC 11621)
(vii) *Microsporum gypseum (M. gypseum)* (ATCC 24102)
(viii) *Trichophyton rubrum* (*T. rubrum*) (ATCC 28188)
(ix) *Candida albicans* (*C. albicans*) (ATCC 10231)

3.1.5 Different Cell Lines Used to Assess the Cytotoxicity

All these cell lines were procured from Prof. Shiro Watanabe, Natural Drug Discovery Laboratory, Toyama, Japan. They were preserved in standard Dulbecco's modified Eagle's medium with 10% fetal bovine serum supplemented with 0.1% sodium bicarbonate and 1% antibiotic–antimycotic solution. They are: breast cancer (MCF-7) and fibroblast (NIH-3T3) cell lines.

3.1.6 Experimental Protocol

3.1.6.1 Seasonal variation analysis in essential oils

The direct influence of the harvesting season can be observed for the composition, antioxidant, antimicrobial, and cytotoxic efficacies of essential oils from the Lamiaceae plant. The seasonal variations in the amount and quality of essential oils were investigated during this study by using different standard protocols, as discussed in the following sections below. The herbarium specimens of the collected plant samples were prepared for their identification. The rest of the plant samples were washed with water and then dried at room temperature for one week.

3.1.6.2 Geographical variation analysis in essential oils

The composition, antioxidant, antimicrobial, and cytotoxic properties of essential oils from selective Lamiaceae plant species may be affected by the origin of plants in different geographical regions. The geographical variations in the amount and quality of essential oils were investigated using different standard protocols as discussed in the following sections below.

The plant samples, collected in clean polythene bags, were taken to the laboratory and washed with water, followed by shade drying at room temperature for one week. The herbarium specimens of the collected plant samples were also prepared for their identification.

3.1.7 Extraction of Essential Oils

The air-dried and chopped aerial parts of plant samples (ca. 100 g, n = 3) were employed for hydrodistillation for 3 h with 500 mL of distilled water applying a Clevenger-type apparatus (Jain Scientific Glass Works, JSGW, India) adopting the standard method (Chu *et al*., 2011; Kasrati et al., 2015). Then, the essential oils so achieved were dried over anhydrous sodium sulfate following the filtration and stored at 4 ℃ until further analysis. The entire process for the extraction of essential oils is shown in the flow chart (Figure 3.2). Finally, the yields of essential oils were determined based on the volume-to-weight proportion of essential oils and plant materials.

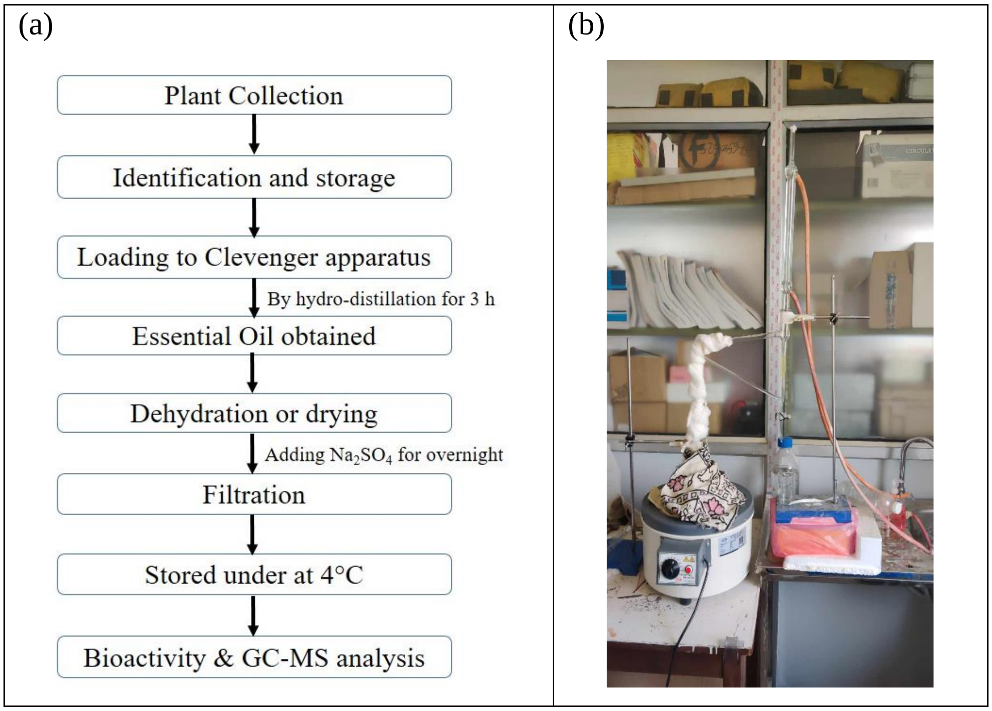


Figure 3. 2 Extraction of essential oil (a) hydrodistillation process, and (b) Clevenger-apparatus.

3.1.8 Chromatographic Analysis of Essential Oils

3.1.8.1 Gas Chromatography-Mass Spectrometry analysis

The essential oils of Lamiaceae plant species were analysed by GC-MS using a Shimadzu GC-MS-QP2010 (Shimadzu Scientific Instruments, Columbia, MD, USA), conducted in the elctron impact mode (electron energy = 70 eV) with a scan range of 40-400 amu, scan rate of 3.0 scans/s, and the GC-MS solution software version 4.5 (Shimadzu Scientific Instruments, Columbia, MD, USA). The GC column was a ZB-5MS fused silica capillary column with a (5% phenyl)-polymethyl siloxane stationary phase and a film thickness of 0.25 µm. The carrier gas was helium, with a column head pressure of 552 kPa and a flow rate of 1.37 mL/m. The injector temperature was 250 ℃, and the ion source temperature was 200 ℃. The GC oven temperature was programmed with a 50 ℃ initial temperature, and the temperature increased at a rate of 2 ℃/min to 260 ℃. A 5% w/v solution of the EO sample in dichloromethane (DCM) was prepared, and 0.1 µL was injected with a splitting mode (30:1) (Satyal *et al*., 2017).

3.1.8.2 Volatile compound identification

The identification of the individual compounds of the essential oils was performed by the comparison of the retention indices obtained by reference to a homologous series of n-alkane (C8-C40), either with those of standard ones or with literature data, and the comparison of the mass spectral fragmentation pattern (above 80% similarity) with those reported in the literature (Adams, 2007) and from the in-made library at the Aromatic Plant Research Center (Satyal, 2015) using the GC-MS solution software version 4.45 (Shimadzu Scientific Instruments, Columbia, MD, USA). The results (composition) were reported as a relative percentage of the total ion peak area. The relative percentages of the individual components are listed in the table under the results and discussion section, and the overall steps involved in the identification of components are shown in Figure 3.3.

3.1.8.3 Gas Chromatography-Flame Ionization Detection

The GC-FID analysis was performed by using Shimadzu GC-MS-QP2010 (Shimadzu Scientific Instruments, Columbia, MD, USA) connected with a flame ionization detector in the same conditions, a split/splitless injector, and Shimadzu autosampler AOC20i (Shimadzu Scientific Instruments, Columbia, MD, USA) with a ZB-5 capillary column (Phenomenex, Torrance, USA), as previously reported (DeCarlo *et al*., 2018). The carrier gas was hydrogen gas, which is typically the fuel of choice because of the small number of ions produced during combustion. When components ionize, the flame becomes more electrically conductive, which triggers a measurable electrical signal in the detector. The percentage composition of essential oils was determined by computing the GC-FID total ion peak area.

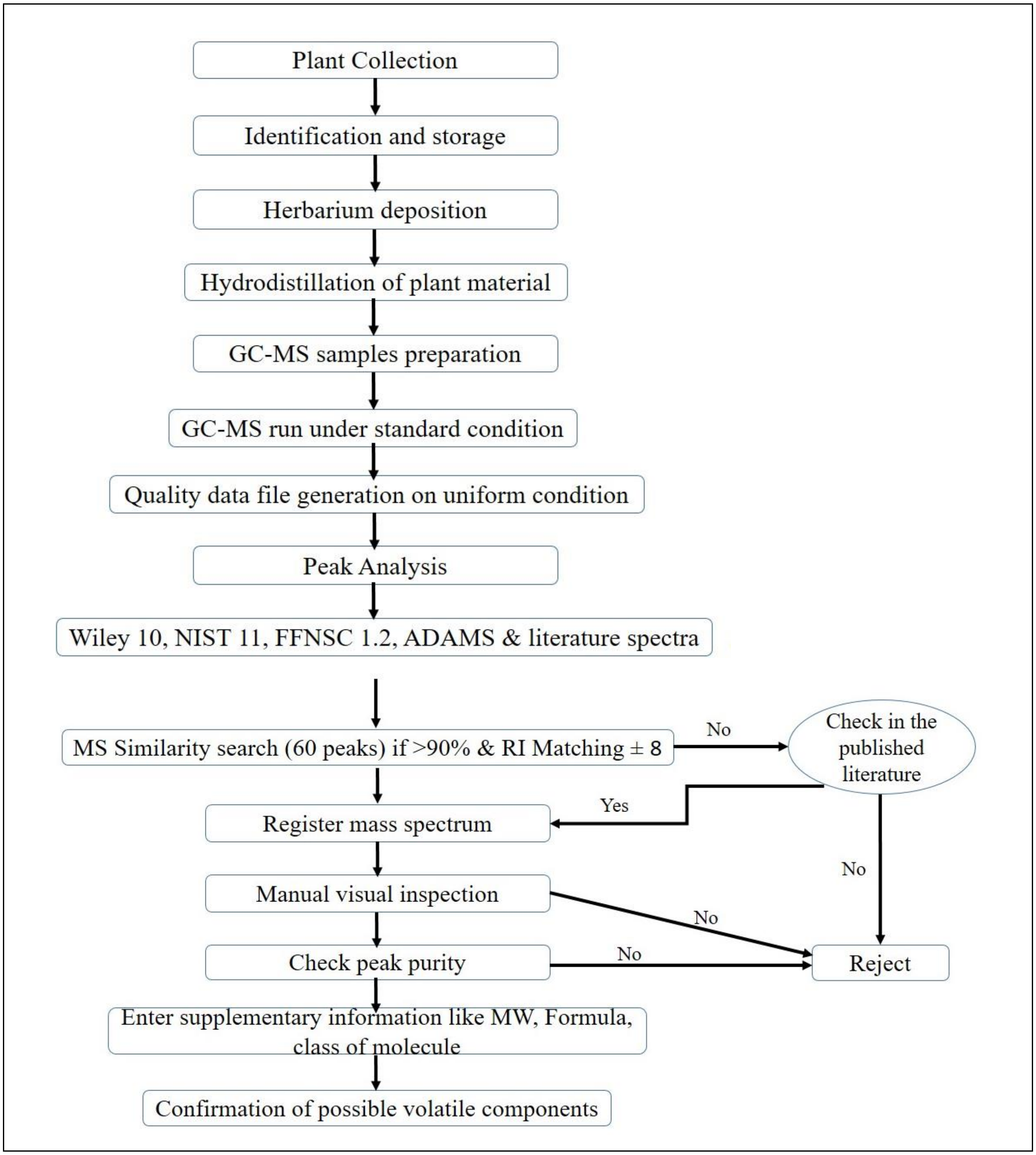


Figure 3. 3 Flow chart diagram for identification of volatile compounds in essential oil sample.

3.1.8.4 Chiral GC-MS analysis for enantiomeric compounds

The enantiomeric analysis for all the essential oil samples was performed using a Shimadzu GC-MS-QP2010S with EI mode (70 eV) and a B-Dex 325 chiral capillary GC-column. This analysis helped to characterize and quantify the chiral compounds present in different EO samples. The scan rate was 3.0 scans/s, and the scan range was 40-400 m/z. The column temperature was set at

50 °C which increased by 1.5 °C/min until it reached 120 °C and then by 2 °C/min until it reached 200 °C. The final temperature of the column was 200 °C and it was kept constant. Helium was used as the carrier gas, .with a constant flow rate of 1.8 mL/m. A 3% w/v solution in dichloromethane (DCM) was prepared for the essential oil sample, and 0.1 µL was injected at a split ratio of 1:45 (Satyal *et al*., 2017; DeCarlo *et al*., 2019).

The percentage composition of chiral compounds was determined from the total ion peak area. They were detected by comparing retention times and mass spectral fragmentation patterns with standard samples provided from Sigma-Aldrich (Milwaukee, WI, USA). The enantiomeric distributions of compounds for each essential oil sample are given in the table under the results and discussion section.

3.1.8.5 Multivariate analyses for the composition of Lamiaceae essential oils

A total of 30 Lamiaceae species essential oil compositions for each sample were taken into consideration for the analysis. The multivariate statistical analysis was performed to distinguish the association among all Lamiaceae essential oils. Total percentages of the monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpene hydrocarbons (SH), and oxygenated sesquiterpenes (OS) classes of every essential oil were obtained from the entire data set and are presented in Annex 2. The 30×6 data matrix was applied for different variables. The matrix was standardized for the multivariate analysis by subtracting the mean and then dividing it by the standard deviation.

The percentages of the most abundant essential oil components (such as carvone, limonene, methyl chavicol, linalool, camphor, germacrene D, terpinen-4-ol, γ-terpinene, perilla ketone, isoegomaketone, pinocarvone, β-pinene, pinocamphone, eugenol, *trans*-β-elemene, β-caryophyllene, menthone, pulegone, *iso*-menthone, piperitenoneoxide, and *cis*-piperitone epoxide) were used to provide chemical relationships between the EO samples through the application of agglomerative hierarchical cluster (AHC) analysis using the OriginPro 2016 64Bit (Origin version 9.3, OriginLab Corporation, USA). Dissimilarity was found by using Euclidean distance, and clustering was defined using Ward’s method. The principal component analysis (PCA) was adopted to verify the interrelation (Origin version 9.3, OriginLab Corporation, USA). A clustered heatmap was obtained by using Euclidean distance via the Ward linkage (Origin version 9.3, OriginLab Corporation, USA).

3.1.9 Biological Activities of Essential Oils

3.1.9.1 Evaluation of antimicrobial activities

The *in vitro* antimicrobial properties of essential oils were determined in terms of their minimum inhibitory concentration using the microbroth dilution method. The bacterial strains applied in this study have been described in Section 3.4. All bacterial strains were cultured on tryptic soy agar. The solution of each essential oil was prepared at a concentration of 5000 µg/mL using dimethyl sulfoxide (DMSO). Then, 50 µL of solution was diluted with 50 µL of cation-adjusted Mueller Hinton broth (CAMHB) (Sigma-Aldrich, St. Louis, MO, USA), which was transferred to the top well of a 96-well plate. The solution of the essential oils thus prepared was serially two-fold diluted in fresh CAMHB, resulting in the final concentrations of 2500, 1250, 625, 312.5, 156.3, 78.1, 39.1, and 19.5µg/mL. The bacterial strains were obtained from a fresh culture and added to each well of 96-well plates (~0.1 µL) at a concentration of nearly 1.5×10^8 CFUs/mL (determined using the McFarland standard). Then, they were incubated at 37 ℃ for 24 h. Gentamicin (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive antibiotic control, and DMSO was used as a negative control (50 µL DMSO diluted in 50 µL broth medium and then serially diluted as mentioned above). This analysis was conducted using the standard method (Sahm & Washington, 1991; Decarlo *et al.*, 2020).

The fungal strains used in this study have been described in Section 3.4. All fungi were cultured on a yeast-nitrogen-base growth medium (Sigma-Aldrich, St. Louis, MO, USA). All the stock solutions for essential oils were prepared using the method discussed earlier. The freshly cultivated fungi strains, with approximately 7.5×10^7 CFUs/mL final concentrations, were added to each well of 96-well micro-dilution plates, which were then incubated at 35 ℃ for 24 hours. Here, DMSO was used as a negative control, and amphotericin B (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control (Sahm & Washington, 1991; Decarlo *et al*., 2020). All the cells were harvested from freshly cultured plates for further analysis. The antibacterial and antifungal activities of EOs are listed in the table under the results and discussion section. The flow chart diagram for carrying out the antimicrobial activities of EOs is shown in Figure 3.4.

In microbroth dilution studies, there are several protocols to evaluate the MIC values. The most frequently adopted techniques are the measurement of optical density and the listing of colonies by viable count.

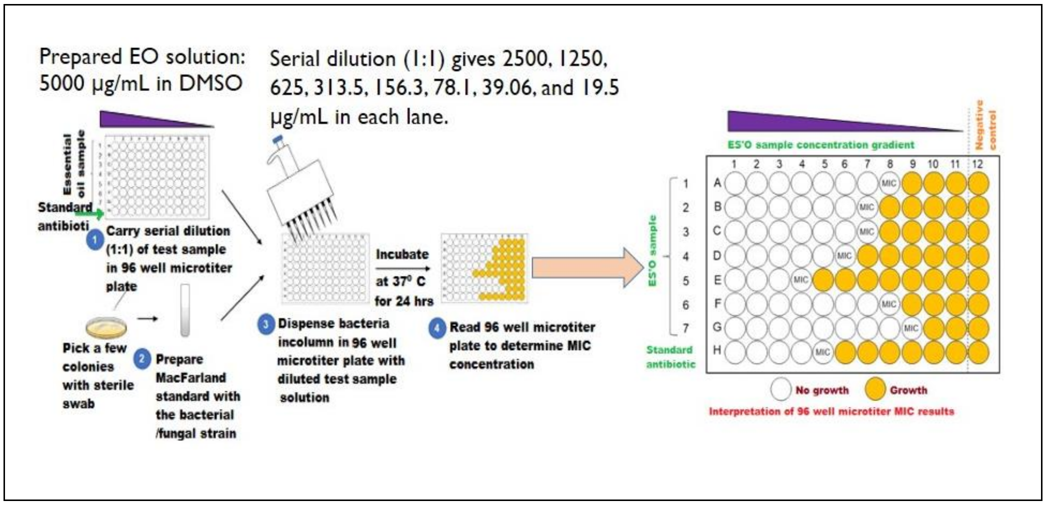


Figure 3. 4 A flow chart diagram for *in vitro* anti-microbial activity by micro-broth dilution technique.

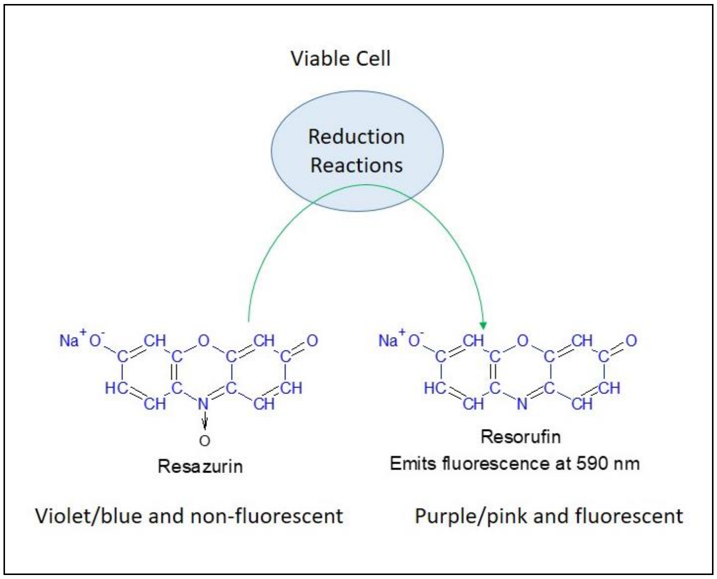


Figure 3. 5 Reduction of resazurin to resorufin from viable cells.

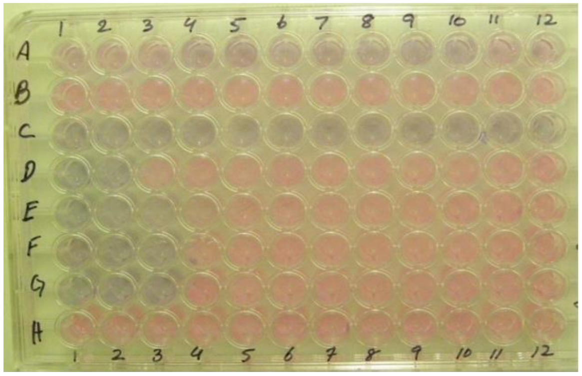


Figure 3. 6 A 96 well-plate displaying the color change under the influence of antibacterial efficacy of essential oils (D-G).

A new microdilution technique for evaluating the MIC values of essential oil-based components involves the use of resazurin as a visual indicator (Sarkar *et al*., 2007). These results compare fairly with those achieved by the viable counting and optical density methods, and the technique is more sensitive than the agar-dilution one.

Resazurin is applied for the evaluation of cell growth. This is a violet/blue non-fluorescent and non-toxic dye that becomes purple/pink and fluorescent when reduced to resorufin by the oxidoreductase enzyme within viable cells, as shown in Figure 3.5. The color change was then observed visually. The growth was shown by the color change from purple to pink or colorless. The MIC values were recorded for the lowest concentration, in which a sharp color change was observed by the alteration of violet/blue to purple/pink. A 96-well plate during the assay can clearly show the variation in color due to the antibacterial impacts of essential oils (Figure 3.6).

3.1.9.2 Evaluation of antioxidant activities

The evaluation of the antioxidant potentials of different Lamiaceae essential oils was performed by following a standard protocol with different assay techniques.

3.1.9.2.1 DPPH free radical-scavenging assay

The antioxidant potential of different Lamiaceae essential oil samples was determined depending on their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl stable free radicals (Sigma-Aldrich, St. Louis, MO, USA). This assay was performed using the colorimetric method with slight modifications following the standard method (Kim *et al*., 2007). In this method, 2 mL of different concentrations of essential oil were mixed with 2 mL of DPPH solution (100 µM). Then, the mixture was employed to stand at room temperature in the dark for 30 minutes to complete the reaction, and the absorbance was measured at 517 nm using a UV spectrophotometer (UV-1800, Shimadzu, Japan). Ascorbic acid and butylated hydroxytoluene (BHT) were used as the standards, and methanol (2 mL each) was used as a negative control. The DPPH radical-scavenging capacity was computed as a percentage of DPPH discoloration using the equation:

$$\text{DPPH scavenging percentage (\%)} = [1- (A_{\text{sample}} – A_{\text{sample blank}})/A_{\text{control}}] \times 100\ldots\ \ldots\ldots..(3.1)$$

Where A_{control} is the absorbance of the DPPH solution without an essential oil sample, A_{sample} is the absorbance of the DPPH solution with an oil sample, and $A_{\text{sample blank}}$ is the absorbance of the essential oil sample without DPPH.

The antiradical activity was measured in terms of IC_{50} values (µg/mL). It indicates the essential oil concentration required to scavenge 50% of free radicals. A lower IC_{50} value corresponds to higher antioxidant activity in the essential oils. A similar process was performed for the standards, ascorbic acid and BHT. The experiments were carried out in triplicate.

3.1.9.2.2 ABTS radical-scavenging assay

The ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging potential of essential oils was determined by using the standard protocol as reported previously (Min Yang & Rice-Evans, 1999). A sufficient volume of 7 mM ABTS (38.402 mg of ABTS in 10 mL distilled water) (Glentham Life Sciences, United Kingdom) was mixed in a 1:1 ration with potassium persulfate (6.6 mg) (10 mL, 2.45 mM) and allowed to stand at room temperature for 14-16 hours to form a stable oxidized ABTS radical. This working ABTS stock solution was diluted with methanol until the absorbance at 734 nm was 0.70±0.02. Now 2 mL of ABTS stock solutions were added to each 1 mL of sample preparation, incubated for 10 minutes at dark room temperature, and triplicate absorbance was measured at 734 nm using a 96-microplate reader (Bio Tek, EPOCH). Using the given equation (Shah & Modi, 2015), the linear % inhibition concentration (IC%) was calculated, and an IC_{50}% value was compared with the standard.

$$IC\% = [(\text{mean Abs. of control} - \text{mean Abs. of sample})/\text{mean Abs. of control}] \times 100 \ldots\ldots(3.2)$$

As a control sample, 1 mL of ethanol was mixed with 2 mL of ABTS stock solutions. Ascorbic acid (Fischer Scientific, Bengaluru, India) was used as a standard.

3.1.9.3 Cytotoxicity assay of essential oils using cell lines

The cytotoxicity assay for the Lamiaceae essential oils was carried out using 3T3 and MCF-7 cancer cell lines. The cell viability was measured using a cell counting Kit-8 kit (Dojindo Molecular Technologies, Inc., Rockville, MD, USA) (Nguyen *et al*., 2020; Phan *et al*., 2023b). The 3T3 and MCF-7cell lines were a kind gift from Prof. Shiro Watanabe and were maintained to standard Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) supplemented with 0.1% $NaHCO_3$ and 1% antibiotic-antimycotic solution. For the cytotoxicity tests, exponentially growing cells were harvested and plated in 96-well plates (1×10^4 cells/well) in DMEM at 37 °C under humidified 5% CO_2 and 95% air for 24 h. After the cells were washed with phosphate-buffered saline (PBS), the medium was changed to serially diluted test samples in Dulbecco's Modified Eagle Medium (DMEM), with the control and blank in each plate. After 72 h of incubation, cells were washed twice with PBS, and 100 µL of DMEM containing 10%

WST-8 cell counting kit solution was added to each well. After 3 h of incubation, the absorbance at 450 nm was measured using a MultiscanSkyHigh plate reader (Thermo Fisher Scientific). Cell viability was determined from the mean values from three wells using the following equation:

Cell viability (%) = $[(\text{Abs}_{\text{test sample}})-(\text{Abs}_{\text{blank}})]/[(\text{Abs}_{\text{control}})-(\text{Abs}_{\text{blank}})] \times 100$..................(3.3)

Gemcitabine (GEM) was utilized as a positive control. The different concentrations of essential oil samples were tested, while essential oil concentrations were taken as 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL with three replications. Now, IC_{50} values were determined from the non-linear regression of the mean values ± SD of the entire data set. Figure 3.7 shows color change due to the cytotoxicity effect of EOs.

In this case, only active samples were normally taken just before adding CCK-8 during the experiment. The morphological evaluation was determined just before adding CCK-8. Advanced judgement was carried out using different concentrations for samples with a percentage inhibition of 50% and above to calculate the IC_{50}. The cell growth suppression rates are predicted in terms of IC_{50} values. Cell viability can be calculated using the ratio of total live/total cells (live and dead cell). Staining also facilitates the visualization of overall cell morphology. Here, EVOS FL (10 × magnification) was used for this study. The term cell index (%) was also used to show the capability of essential oils to kill cells at very high concentrations (100 µg/mL). But they activate cancer cell growth at low concentrations. Therefore, its application as an anticancer agent would be minimal. However, the EOs that show aggressive cell growth at low concentrations (cell index, %) could be good wound healing agents.

Cell Counting Kit 8 provides a convenient and robust way of performing a cell viability assay. It uses a water-soluble tetrazolium salt to quantify the number of live cells by producing an orange formazan dye upon bio-reduction in the presence of an electron carrier. WST-8 solution is added directly to the test cells, with no pre-mixing of components required. Here, WST-8/CCK8 tetrazolium salt is reduced by cellular dehydrogenases to an orange formazan product that is soluble in a tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells and is measured by absorbance at 460 nm.

The excellent stability and little cytotoxicity of the WST-8/CCK8 solution make the kit useful for assays that require long incubation (such as 24 to 48 hours). The detection sensitivity is higher than with other tetrazolium salt-based assays such as MTT, XTT, or MTS, etc. Here, the IC_{50} values (concentration at which 50% of cells were killed) were calculated from the graphplotted log concentration against percent cell viability, as shown in the figure of results and discussion section.

Figure 3. 7 A typical 96 well-plate assay displaying the color change due to cytotoxic impacts of essential oil.

3.1.10 Formulation of Cream Utilizing Essential Oils

3.1.10.1 Optimization of the formulation base: Base formula

The optimization of the base formula was conducted as previously described (Rowe *et al.*, 2006). Finally, the physical properties were observed visually, like cream texture, washability, and emulsification strength between the oil/water (o/w) phases.

3.1.10.2 Formulation of Skin Care Cream

For the formulation of cream, some essential oils were chosen based on their primary results of better antioxidant and antimicrobial properties and also considering the fragrance of the essential oils. Here, the samples of *O. tenuiflorum*, *O. majorana,* and *O. basicilum* were considered as the best combination for the formulation of topical cream.

Oil phase: The oil phase was prepared by taking the optimized concentration of cetyl alcohol, paraffin, and glyceryl monostearate, heating it at 70-80 ºC with continuous stirring, and allowing it to cool at 50 ºC. Then previously weighted essential oils of *O. tenuiflorum*, *O. majorana,* and *O. basicilum* (0.6 mg, 3.5 mg, and 4.5 mg) were added to the oil phase, respectively, to preserve its antioxidant capacity (Gyawali *et al.*, 2016; Tan *et al.*, 2022).

Water phase: The glycerin and sodium benzoate (as preservative) were heated with q.s. DW (in a closed container) at 70-80 ºC with continuous stirring and cooled to 50 ºC.

Cold phase: The cold phase was prepared by mixing the optimized concentration of dimethicone, triethanolamine, cyclopentasiloxane, potassium sorbate, carbopol-940, and zinc oxide using a homogenizer (Colloid Mill 60-100). Before mixing, they were soaked overnight.

Finally, the heated oil phase was added slowly to the water phase at a temperature of 50 ºC with continuous stirring at 1500-2000 rpm for 25 minutes, and the cold phase ingredients were added during this time with increasing speed to 2500 rpm for 10 minutes. The homogenizer was run again for 20 minutes while mixing at last. Then, finally, the prepared cream was allowed to stand at room temperature (27 ºC) for 1-2 hours with stirring. The different compositions of formulated cream are presented in Table 3.3.

Table 3. 3 The composition of formulated creams using essential oils.

Ingredients	Quantity for 100 g cream (%)
Water phase	
Glycerin	9.0
Sodium benzoate	0.4
Distilled water	q.s.(69.39)
Oil phase	
Cetyl alcohol	3.0
White soft paraffin	4.0
Glyceryl monostearate	5.0
Essential Oil	
O. majoarana	3.5 mg (0.0035g)
O. basilicum	4.5 mg (0.0045g)
O. tenuiflorum	0.6 mg (~ 1mg, 0.001g)
Cold phase	
Dimethicone	3.0
Triethanol amine	0.3
Cyclopentasiloxane	5.0
Potassium sorbate	0.4
Carbopol-940	0.4
Zinc oxide	0.1

3.1.10.3 Evaluation of pharmaceutical parameters

3.1.10.3.1 pH

The pH of a 10% w/v cream suspension in distilled water was measured three times at 25 ºC over three months using a pH meter (HI 2210 pH meter, Italy), which was previously calibrated using standard buffers with pH 4, 7, and 10, respectively.

3.1.10.3.2 Rheology profile study

For the rheological profile study, the formulated cream was directly transferred into the Brookfield viscometer (DV III Ultra, Italy) with an LV-64 spindle, initially with an adjusted rpm of 1 to 10 at room temperature and later with a shear rate of 17-102 s^{-1} (Gyawali *et al*., 2020b), where the shear rate was 1.7 times the adjusted spindle rpm. The final results were represented as a graph of viscosity (Pascal-second) vs. shear rate (s^{-1}). The measured dial readings were multiplied by factor 10 to give viscosities in centipoise (cP) or miliPascal-second (mPa.s.), where 1cP = 1mPa.s.

3.1.10.3.3 Cream spreadability

The spreadability of the cream was evaluated by the parallel-plate method. In this study, two glass slides measuring 20 by 20 cm were selected. About 0.5 g of the formulated cream was placed over one of the slides for 2 minutes at room temperature. Another slide was employed on top of the cream. Now, it was like being sandwiched between the slides, and 200 g of weight was placed on the upper slide. Then, the cream between the two slides was pressed uniformly to form a thin layer. Finally, the weight was removed, and the spread diameter was measured in triplicate for three months (Garg *et al*., 2002).

3.1.10.3.4 Sensitivity

A portion of cream was applied to the forearms of five volunteers and left for 20 minutes. After 20 minutes, any kind of irritation that occurred was noted.

3.1.10.3.5 Washability

A portion of cream was applied over the skin of the hand and allowed to flow under the force of flowing tap water for 10 minutes. The time when the.cream was completely removed was noted.

3.10.3.6 Organoleptic characteristics

The organoleptic characteristics (color, odor, and homogeneity) of formulated cream were evaluated visually.

3.1.10.3.7 Type of emulsion test

Two grams of cream were centrifuged at 5000 rpm for 10 minutes at 25 ºC and emulsion instability (%) was calculated using the following equation (Restu *et al*., 2015) for three months.
Emulsion instability (%) = [Emulsion separation (cm)/Total emulsion height, cm] ×100…....(3.4)
An ideal unstable cream emulsion has 100% emulsion instability (Restu *et al*., 2015; Tan *et al*., 2022). This experiment was repeated three times.

3.1.10.3.8 Physical compatibility test

Essential oils, when loaded with the base cream, showed no characteristic change (NCC) in color, preserving homogeneity, viscosity, and texture. When the cream base was in contact with EO for months in separate vials, there was a good compatibility test between the EO and the base. No changes in color, preserving homogeneity, viscosity, and texture, were observed physically. Physical compatibility testing of excipients is required because it is important in product development and commercialization, allowing for the selection of the best excipients and maximizing shelf life (Tan *et al*., 2022). Different sets of formulations were carried out using: EO + base 1, base 2, and base 3.

3.1.10.4 Permeability study

An *in vitro* permeability study was carried out using a Franz diffusion cell (Electrolab India Pvt. Ltd., India). A cellophane membrane was applied to separate the donor compartment and the receiver compartment. The donor compartment was filled with 0.3 g of cream sample, and the receiver .compartment was filled with phosphate buffer solution (pH 7.40). The temperature of the diffusion medium was thermostatically controlled at 32 ± 1 °C by surrounding water in the jacket, and the medium was stirred by a magnetic stirrer at 100 rpm. The sample (1.0 mL) was withdrawn as per predetermined (0-, 1-, 2-, 3-, 4-, 5-, 6-, 7-h, and 24-h) intervals and replaced by an equal volume of fresh fluid (Inoue *et al*., 2014). The sample withdrawn was studied for its percentage DPPH scavenging activity with a phosphate buffer solution as a blank. The efficacy was converted to a percentage release using the formula, as given below:

Percentage release = [(% activity of sample)/(% activity of cream)] × 100 …..…………(3.5)

3.1.10.5 Antioxidant activity of the formulated cream

DPPH radical-scavenging potential: The DPPH radical-scavenging efficacy of formulated cream was determined by following the standard protocol as described previously with slight modification (Paudel *et al*., 2022; Phosri *et al*., 2022). This experiment was conducted in triplicate. FRAP assay: The FRAP assay for the cream was carried out using the standard method as described previously with a slight modification (Park *et al*., 2008). The experiment was performed in triplicate. ABTS radical-scavenging assay: The ABTS assay was conducted to evaluate the antioxidant activity of cream using the standard protocol as described previously with slight modification (Re *et al*., 1999). The experiment was run in triplicate. The IC_{50}% value was determined by using the equation (Shah & Modi, 2015).

3.1.10.6 Stability study

The formulated cream was subjected to ICH climatic zone 30±2ºC and relative humidity (RH) 75±5% for 3 months. Also, the freeze-thaw cycling test for accelerated stability was carried out for 48 hours at 5-8 ºC and 48 hours at 40-42 ºC for successive three months. The cream's properties, like spreadability, pH, centrifugation, and antioxidant activities, were determined three times using the previous method (Pinto *et al*., 2021; Plyduang *et al*., 2022).

3.1.11 Statistical Analyses

The entire data sets received from the research work were processed and analyzed using differrent softwares. They were Microsoft Excel, OriginPro 2016 64Bit (Origin version 9.3, OriginLab Corporation, USA), and IBM SPSS STATISTICS VERSION 8.5.5, USA, and GIS software (ArcGIS software, ESRI, California, USA). GIS was used to creat a GIS map of the sampling sites. The analyses of chemical composition, antioxidant, antimicrobial, and cytotoxic efficacies were performed, and the data were expressed as the mean±standard deviation (SD) of the three replicates. Data were also analyzed by analysis of variance (ANOVA) using OriginPro 2016 64Bit (Origin version 9.3, OriginLab Corporation, US) at a 5% significance level. Similarly, multivariate analysis was performed to identify the relationships among Lamiaceae essential oil samples. Here, the experiment for cytotoxicity was performed in three replicates, and IC_{50} values were obtained from non-linear regression of the mean values±SD of the entire data set.

CHAPTER 4

4.1 RESULTS AND DISCUSSION

4.1.1 Percentage Yields and Organoleptic Properties of Essential Oils

The yields of essential oil (v/w %) for different Lamiaceae species, along with their organoleptic properties, are presented in Annex 1. The average yield of the essential oil obtained from Lamiaceae in Nepal was about 0.76%, which seems to be considerable. The minimum and maximum essential oil yields were obtained from *L. canum* (S-20) (0.15%) and *O. tenuiflorum* (S-26) (1.68%), respectively. Among the three *Mentha* species, greater essential oil contents were found from the leaves of *M. spicata* (S-25) (1.62%), and the lowest was from the leaves of *M. pulegium* (S-2) (0.63%). Within *Ocimum* species, leaves of *O. tenuiflorum* (S-26) provided higher essential oil yields (1.68%), whereas *O. americanum* (S-9) provided minimum yields (0.35%). *O. majorana* showed essential oil yields ranging from 0.50-1.16%. Of the two species of *Elsholtzia*, the highest essential oil yield was found in *E. strobilifera* (0.90%), followed by *E. blanda* (0.88%). Similarly, *P. frutescens* had a better essential oil content, ranging from 0.78% to 0.87%. Among the other four Lamiaceae plants, relatively better EO contents were obtained from *P. glaber* (0.45%), followed by *C. oppositifolia* (0.34%), *C. coccinea* (0.23%), and *C. umbrosum* (0.20%). Figure 4.1 shows the comparative trends of variations in the yields of different Lamiaceae plants. With respect to several Lamiaceae species, the variations in the contents of EOs were significant ($p < 0.05$).

Similarly, the organoleptic properties of all Lamiaceae essential oils were evaluated based on their odor, color, and appearance. The majority of essential oils are clear to viscous liquid, light-yellow in color, and have a pleasant smell. Due to their pleasing odor, they can be used in different cosmeceutical products. This study also emphasizes the formulation of new products, like creams for topical uses.

Our results are far better or in agreement with those of the yields of Lamiaceae essential oils, as reported previously by several researchers (Kofidis *et al*., 2004; Kicel *et al*., 2005), though no detailed study has been reported for many other Lamiaceae plants in the literature. A previous study performed by Kicel *et al*. investigated the yield of essential oil from *O. tenuiflorum* ranging from 0.59- 0.83%, during different vegetation stages, which is smaller than the current results (Kicel *et al*., 2005)**.** The yield of the essential oils obtained from the aerial parts of *M.*

spicata was reported to be 0.1%-1.8% (Kofidis *et al*., 2004**)**. A little variation in the essential oil yields among different Lamiaceae plants across the countries could be attributed to the changes in the agroclimatic conditions in the different regions.

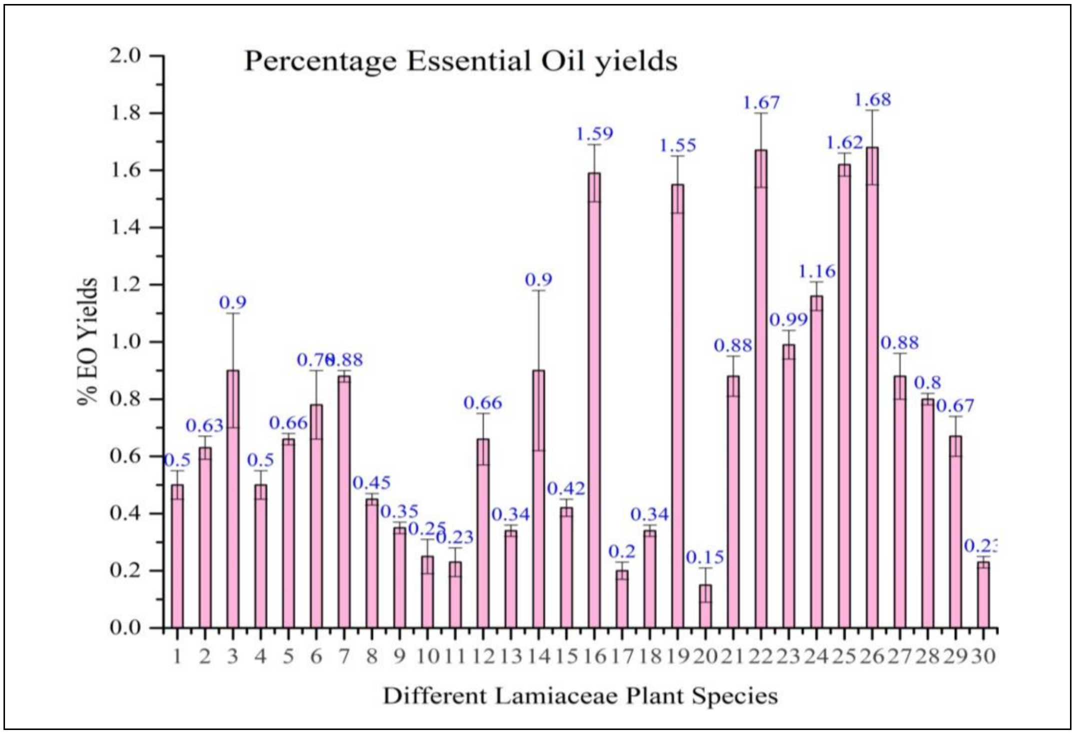


Figure 4. 1 Comparative yields of essential oil among the Lamiaceae species.

4.1.2 Seasonal Variation in the Yields of Essential Oils

The results revealed that the variation in the yields of essential oil relating to harvesting seasons was significantly different ($p < 0.05$). The *Ocimum* species showed the maximum essential oil content in autumn, when they found mature and fully bloomed. Among the *Ocimum* species, *O. tenuiflorum*, *O. basilicum,* and *O. americanum* produced 1.68, 0.88, and 0.42% essential oil yields when harvested during autumn, while 0.5, 0.66, and 0.35% when collected in summer, respectively. Likewise, the greater essential oil yield of *E. strobilifera* was found to be 0.9% during the autumn season, which may be explained by the fact that *E. strobilifera* was in a time of full vegetation during the autumn. Conversely, the highest amounts of essential oils from *M. spicata*, *M. longifolia*, *O. majorana,* and *M. pulegium* were found to be 1.62, 1.55, and 1.16% yields during the summer season, respectively, while lower in the other seasons. Figure 4.2 shows that greater essential oil yield was observed for *M. spicata, P. frutescens,* and *O. americanum* during the summer than in the winter season. Whereas, *O. basilicum* and *L. camum* were found to have higher essential oil yields during the winter than in the summer. The fact behind this result might be attributed to the influence of seasons on plant maturity and full blooming time. The present results showed that the majority of Lamiaceae plants had better essential oil yields in the summer than in the winter.

Many previous studies reported in the literature have revealed that the essential oil yields of different species of Lamiaceae were found to be quite variable depending on the changes in seasons. Kofidis *et al*. described greater essential oil yields of *M. spicata* from the summer period, which is in agreement with the present results (Kofidis *et al*., 2004). Other studies have also explored that the growing season has a crucial impact on the essential oil content of *O. majorana*. In contrast to current findings, the study of de Vasconcelos Silva *et al*. reported the maximum percentage of basil essential oil from Brazil from the end of autumn to the beginning of winter and the minimum during the summer period (de Vasconcelos Silva *et al*., 2003).

Several other studies in the literature found that as the seasons changed, plants' essential oil contents varied significantly (Van Vuuren *et al*., 2007; Hussain *et al*., 2008; Kamatou *et al*., 2008; Hussain *et al*., 2010; Rathore *et al*., 2022). There were no earlier extensive investigations reported in the literature on the seasonal variations, which made it tough to compare the findings of the present analysis with them.

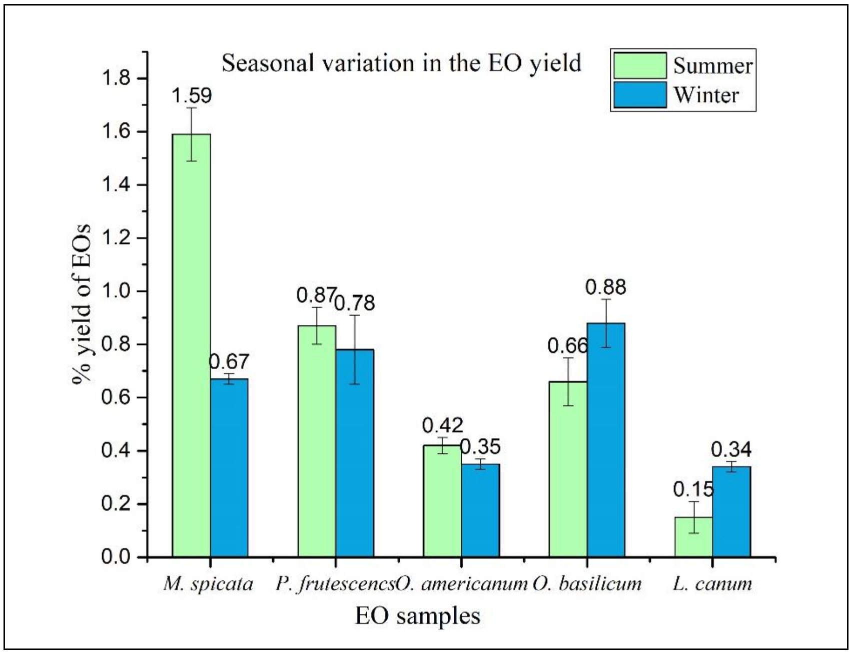


Figure 4. 2 Seasonal variation in the yields of essential oils for Lamiaceae species.

4.1.3 Geographical Variation in the Yield of Essential Oils

Figure 4.3 displays the fluctuations in the yields of Lamiaceae essential oils regarding different geographical locations (from tropical to sub-tropical). The essential oil yield of *O. majorana* obtained from the Sanothimi region was 0.90% (v/w), whereas from Bafal it was 1.16%, respectively. Here, small variations were obtained in the yield of marjorum essential oils relating to geographical areas (altitude variation). The essential oil yields of *M. spicata*, and *P. frutescens* achieved from Dhulikhel (sub-tropical) were 1.59% and 0.87% respectively, whereas from Bansgadhi (tropical region) of Nepal, they were 1.62% and 0.80%, respectively. The contents of the essential oils were slightly changed based on regions for the current results. The reason behind this fact may be due to several influencing factors like temperature, relative humidity, sunshine duration, air movement, rainfall, etc. (Hussain *et al*., 2010). Similarly, in the yield of *M. pulegium* essential oil collected from Nuwakot and Sindhupalchowk in Nepal, there were only slight variations in the yield of both essential oils (0.63 to 0.66%) in the two regions. Likewise, the essential oil yield of *C. coccenea* from Rasuwa was 0.23%, while from Kave, it was also about 0.23%.

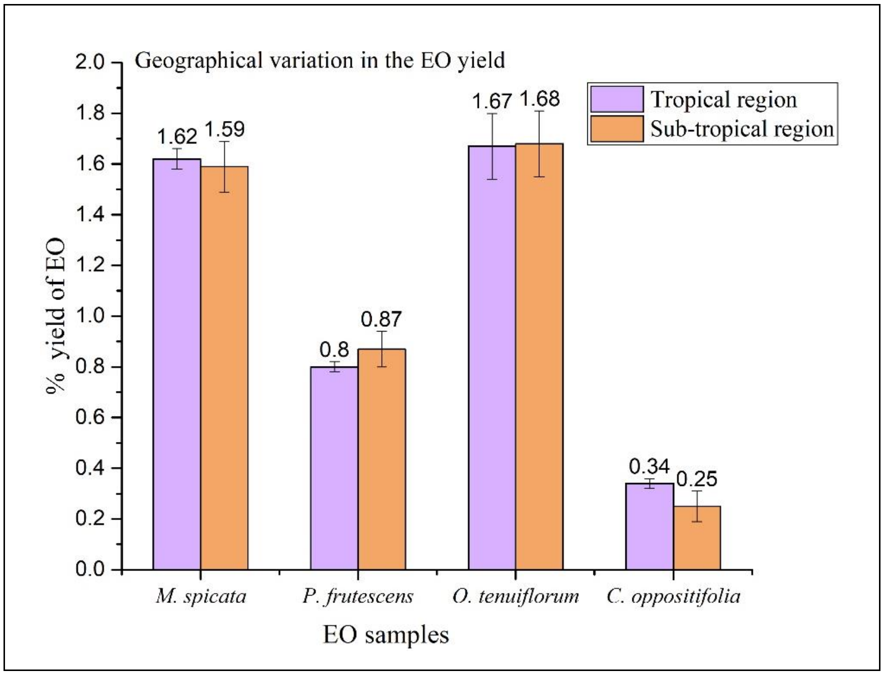


Figure 4. 3 Geographical variation in the yields of essential oils for Lamiaceae species.

In comparison, the highest yields of essential oil were found for the plant samples taken from subtropical regions compared to that collected from tropical regions. The low essential oil yields from the plain region could be ascribed due to the high temperature, and hence the partial evaporation of EOs may be anticipated because the tropical regions in Nepal are very hot, with an elevation of 30 to 42 °C. However, the plants from hilly regions were also in full bloom as a result of excessive rainfall. Several previous studies have reported that EO yields depend not only on temperature and relative humidity. But it also depends on the duration of sunshine, air movement, and rainfall, as described previously. The overall results showed that a marginal variation in the yields of essential oils was observed concerning the geographical regions.

4.1.4 Chemical Composition of Essential Oils

A total of fifteen Lamiaceae essential oil samples were employed for the chemical characterization of their volatile compounds. The whole results obtained for their chemical composition are presented in Tables 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 4.11, 4.12, 4.13, and 4.14, respectively.

4.1.4.1 The Genus *Origanum*

The GC-MS analysis of essential oils from three *O. majorana* samples explored the characterization of 42 to 50 chemical compounds, representing 91.11% to 99.81% of the total essential oil (Table 4.1). The *O. majorana* essential oil was characterized by the most abundant component of terpinen-4-ol (32.1 to 34.59%). Other major compounds in the *O. majorana* EO were linalool (13.8-15.37%), γ-terpinene (5.6-9.5%), linalyl acetate (5.9-6.67%), *cis*-sabinene hydrate (3.48-5.34%), and p-cymene (1.8-6.9%).

All three samples of *O. majorana* were dominant in their oxygenated monoterpene fraction. The contents of this fraction were 67.57 to 77.25%, respectively. *O. majorana* also contained a considerable proportion of monoterpene hydrocarbons (9.34 to 27.6%), and the main monoterpene hydrocarbons were γ-terpinene, *p*-cymene, sabinene, and α-terpinene, respectively, whereas *O. majorana* had very low amounts of sesquiterpene hydrocarbons. The GC-MS chromatogram of *O. majorana* essential oil displays the separation of volatile compounds, which is given in Annex 10. The flactuations in the major components of *Origanum majorana* essential oils from Sanothimi and Bafal locations can be observed in Figure 4.4

The findings of this study are in accordance with those investigated by several researchers previously. Because they also detected terpinen-4-ol as the most dominant compound, along with other minor compounds in the marjoram essential oil (Busatta *et al*., 2008; Jelali *et al*., 2011;

Raina & Negi, 2012; Tahmasebi *et al.*, 2016; Amor *et al.*, 2019; Abhasi-Maleki *et al.*, 2020). Likewise, a study conducted on the essential oil of marjoram from Nepal identified terpinen-4-ol (22.42%), γ-terpinene (14.69%), and linalool (11.61%) as the major compounds (Shrestha *et al.*, 2013). Several previous findings described that terpinen-4-ol, either single or in association with *cis*-sabinene hydrate, linalyl acetate, γ-terpinene, etc., was detected, along with other components like α-terpineol, α-terpinene, linalyl acetate, and linalool in the *O. majorana* essential oil (Arnold *et al*., 1993; Tabanca *et al*., 2004; Calin-Sanchez *et al*., 2015; Soliman *et al*., 2016). Moreover, several previous findings showed that thymol or carvacrol were the predominant components in *O. majorana* (Banchio *et al*., 2008). Similarly, *O. majorana* essential oil from Turkey displayed carvacrol (78.27- 79.46%) as the dominant compound (Baser *et al*., 1993).

Generally, *O. majorana* essential oil is dominated by terpinen-4-ol, *cis*-sabinene hydrate, γ-terpinene, α-terpinene, α-terpineol, *p*-cymene, and linalool. Here, the disparities in the chemical quantity and composition of *O. majorana* essential oil could be recognized by several factors, like the diverse agro-climatic conditions of the areas, the extraction method, type of plant, the adaptive metabolism of plants, and the plant parts being utilized (Sellami *et al*., 2009). Indeed, three *O. majorana* essential oils from Nepal displayed some minor differences in volatile constituents. Even though these results were consistent with the previous findings, there were some slight distinctions due to the environmental and climatic conditions. The greater contents of terpinen-4-ol could be attributed to the rearrangements of compounds during the distillation period, which were described in the studies conducted previously, and *cis*-sabinene hydrate is also present in Nepalese marjoram samples (Raina & Negi, 2012). The present analysis of the chemical composition highlights the broader information regarding marjorum essential oils from Nepalese land for the new researchers.

Table 4. 1 Chemical composition of *Origanum majorana* essential oil.

		Composition						
		Nagarjun (Spring)			**Sanothimi (Summer)**			**Bafal (Summer)**
RI	**Components**	**(%)**	**RI**	**Components**	**(%)**	**RT**	**Components**	**(%)**
Monoterpene hydrocarbons		**27.22%**			**9.34%**			**21.19%**
920	Tricyclene	0.05	-	-	-	-	-	-
923	α-Thujene	0.21	924	α-Thujene	0.05	12.516	α-Thujene	0.21
930	α-Pinene	0.50	931	α –Pinene	0.15	12.945	α -Pinene	0.46
947	Camphene	0.32	948	Camphene	0.09	13.872	Camphene	0.25
971	Sabinene	**3.61**	972	Sabinene	1.29	15.225	Sabinene	**3.25**
975	β-Pinene	0.32	978	β-Pinene	0.12	15.490	beta-Pinene	0.30
987	Myrcene	1.31	989	Myrcene	0.16	16.119	Myrcene	0.90
1003	*p*-Mentha-1(7),8-diene	0.05	-	-	-	17.042	*p*-Mentha-1(7),8-diene	0.03
1005	α-Phellandrene	0.10	-	-	-	17.190	α-Phellandrene	0.05
1016	α-Terpinene	**5.10**	-	-	-	17.913	α-Terpinene	2.10
1023	*p*-Cymene	1.80	1024	*p*-Cymene	**6.90**	18.468	*p*-Cymene	4.63
1027	Limonene	1.41	1028	Limonene	0.52	18.716	Limonene	1.19
1029	β-Phellandrene	1.11	1029	β-Phellandrene	0.06	18.824	β-Phellandrene	0.80
1033	(*Z*)-β-Ocimene	0.13	-	-	-	-	-	-
1043	(*E*)-β-Ocimene	0.12	-	-	-	19.735	(*E*)-β-Ocimene	0.03
1058	γ-Terpinene	**9.52**	-	-	-	20.673	γ-Terpinene	**5.60**
1084	Terpinolene	**2.51**	-	-	-	22.423	Terpinolene	1.39
1088	*p*-Cymenene	tr	-	-	-	-	-	-
1329	δ-Elemene	0.05	-	-	-	-	-	-
Oxygenated monoterpenoids		**69.56%**			**77.25%**			**67.57%**
1030	1,8-Cineol	0.10	1031	1,8-Cineol	0.12	18.888	1,8-Cineol	0.14
1070	*cis*-Sabinene hydrate	**4.41**	1071	*cis*-Sabinene hydrate	**3.48**	21.542	*cis*-Sabinene hydrate	**5.34**
-	-	-	1087	*trans*-Linalool oxide	0.52	-	-	-
1102	Linalool	**13.83**	1099	Linalool	**15.37**	23.723	Linalool	**15.21**
1103	*trans*-Sabinene hydrate	1.62	1102	*trans*-Sabinene hydrate	0.71	23.833	trans-Sabinene hydrate	2.36
1124	*cis*-*p*-Menth-2-en-1-ol	1.92	1124	*cis*-*p*-Menth-2-en-1-ol	1.35	25.219	*cis*-*p*-Menth-2-en-1-ol	1.78
1142	*trans*-*p*-Menth-2-en-1-ol	1.01	1142	*trans*-*p*-Menth-2-en-1-ol	0.78	26.476	*trans*-*p*-Menth-2-en-1-ol	0.95
1154	Menthone	0.10	-	-	-	-	-	-
1174	Borneol	0.20	-	-	-	28.723	Borneol	0.19
1186	Terpinen-4-ol	**32.11**	1180	Terpinen-4-ol	**33.35**	29.508	Terpinen-4-ol	**34.59**

1186	Terpinen-4-ol	**32.11**	1180	Terpinen-4-ol	**33.35**	29.508	Terpinen-4-ol	**34.59**
-	-	-	1187	*p*-Cymen-8-ol	0.53	29.583	*p*-Cymen-8-ol	0.18
-	-	-	-	-	-	36.855	Terpinen-4-ol acetate	0.29
-	-	-	1190	1,4-Hydroxy cineol	**3.35**	-	-	-
1196	α-Terpineol	**3.72**	1195	α –Terpineol	**2.63**	30.233	α-Terpineol	3.49
1197	*cis*-Piperitol	0.40	1197	*cis*-Piperitol	0.12	30.300	*cis*-Piperitol	0.31
1208	*trans*-Piperitol	0.62	1208	*trans*-Piperitol	0.26	31.037	*trans*-Piperitol	0.46
1214	*cis*-Sabinene hydrateacetate	0.05	-	-	-	-	-	-
1222	Nerol	0.22	-	-	-			
-	-	-	1224	Isoascaridole	0.15	32.016	Isoascaridole	0.15
1236	Pulegone	0.05	-	-	-	-	-	-
1248	Linalyl acetate	**5.91**	1252	Linalyl acetate	**6.67**	-	-	-
-	-	-	1255	*p*-menthane-1,2,3-triol	0.69	-	-	-
1273	*trans*-Ascaridol glycol	0.12	1276	*trans*-Ascaridol glycol	1.15	35.501	trans-Ascaridol glycol	0.14
1282	Bornyl acetate	**2.43**	1282	Bornyl acetate	**2.83**	36.128	Bornyl acetate	2.03
-	-	-	1291	Terpinen-4-ol acetate	0.31	-	-	-
-	-	-	1297	Carvacrol	0.08	-	-	-
-	-	-	1354	Terpen-diol	0.46	-	-	-
1293	Terpin-1-en-4-yl acetate	0.22	-	-	-	-	-	-
1355	Neryl acetate	0.21	1361	Neryl acetate	0.14	40.937	Neryl acetate	0.06
1375	Geranyl acetate	0.31	1377	Geranyl acetate	0.48	42.223	Geranyl acetate	0.13
-	-	-	1486	Hydroxy linalyl acetate	1.12	-	-	-
-	-	-	1488	*p*-Menthane-1,2,4-triol	1.44	-	-	-
Sesquiterpene hydrocarbons		**2.98%**			**0.13%**			**1.78%**
1417	β-Caryophyllene	**2.42**	1418	β –Caryophyllene	0.13	44.977	β-Caryophyllene	1.62
1452	α-Humulene	0.10	-	-	-	47.166	α-Humulene	0.08
1492	Bicyclogermacrene	0.41	-	-	-	49.638	Bicyclogermacrene	0.08
1500	(*E*,*E*)-α-Farnesene	0.05	-	-	-	-	-	-
Oxygenated sesquiterpenoids		**0.15%**			**2.71%**			**0.45%**
1573	Spathulenol	0.05	1579	Spathulenol	0.14	54.532	Spathulenol	0.16
1578	Caryophyllene oxide	0.10	1580	Caryophyllene oxide	**2.54**	54.841	Caryophyllene oxide	0.29
-	-	-	1612	Humulene epoxide II	0.13	-	-	-
Others		**0.25%**			**1.68%**			**7.51%**
849	(3*Z*)-Hexenol	0.05	-	-	-	-	-	-
-	-	-	1124	Cyclooctanone	0.47	25.611	Cyclooctanone	0.06
-	-	-	-	-	-	29.666	Cryptone	0.07

1042	Benzene acetaldehyde	0.05	-	-	-	-	-	-
-	-	-	1188	3-*cis*-Hexenyl butyrate	0.13	-	-	-
1203	*p*-Cumenol	0.05	-	-	-	30.651	*p*-Cumenol	0.09
-	-	-	-	-	-	33.792	Linalool acetate	6.51
-	-	-	-	-	-	35.793	*cis*-Linalool oxide acetate (pyranoid)	0.07
-	-	-	1345	2-Methyl-2-(para-tolyl) propionaldehyde	0.24	39.920	2-Methyl-2-(para-tolyl) propionaldehyde	0.07
2047	Abietatriene(DT)	0.05	-	-	-	-	-	-
-	-	-	-	-	-	90.233	n-Tricosane	0.06
-	-	-	-	-	-	94.340	n-Tetracosane	0.09
-	-	-	-	-	-	98.294	n-Pentacosane	0.10
-	-	-	-	-	-	102.099	n-Hexacosane	0.09
-	-	-	-	-	-	105.782	n-Heptacosane	0.07
Total	**50**	**99.81**	**-**	**42**	**91.11**		**49**	**98.50**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

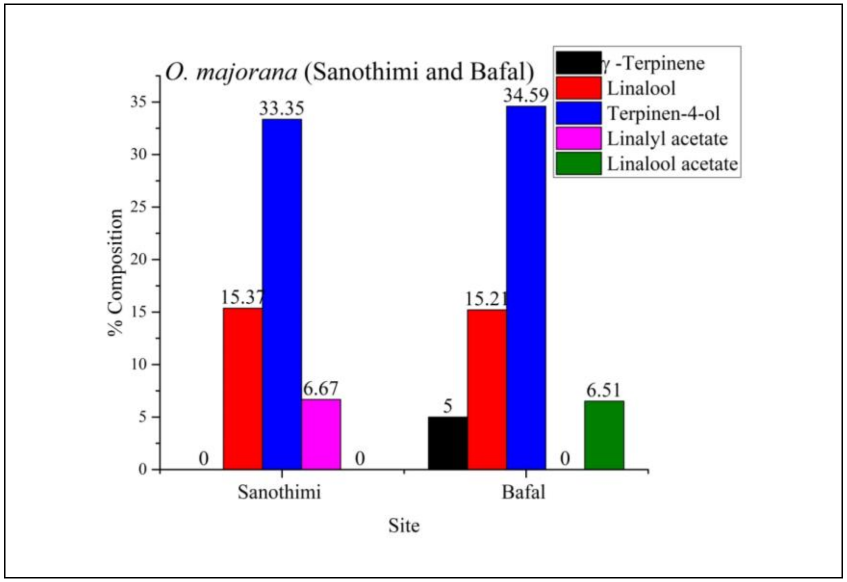


Figure 4. 4 The variation in the leading compounds of *Origanum majorana* essential oils collected from Sanothimi and Bafal areas.

4.1.4.2 The Genus *Mentha*

The chemical compounds identified in the essential oils of three *Mentha* species (*Mentha spicata*, *M. longifolia*, and *M. pulegium*) are reported in Tables 4.2, 4.3, and 4.4. A total of 54 to 60, 52, and 39-61 components were detected in the EOs of *M. spicata*, *M. longifolia*, and *M. pulegium*, representing 97.47-99.58, 93.85, and 91.4-99.98% of total essential oils, respectively. The most leading compounds (> 5%) in *M. spicata* were found to be carvone (51.96-71.56%), limonene (4.12-9.63%), and *neo*-dihydro carveol (3.15-6.72%). Piperitenone oxide (67.43%), limonene (6.44%), piperitenone (2.33%), germacrene D (1.70%), and *trans*-piperitone epoxide (1.67%) were the main components in *M. longifolia*. Whereas, *M. pulegium* had menthone (41.64-66.57%), pulegone (7.12-38.31%), isomenthone (2.23-6.01%), and piperitone (2.20-2.33%) as major compounds. Furthermore, the *Mentha* essential oils also contained considerable amounts of several other minor constituents.

Mentha spicata, *M. longifolia,* and *M. pulegium* are mainly composed of oxygenated monoterpenes, 74.9-85.3%, 76.32%, and 85.69-91.93%, respectively. Carvone, piperitenone oxide, and menthone were the dominant oxygenated monoterpenes in the essential oils of *M. spicata*, *M. longifolia*, and *M. pulegium*, respectively. *M. spicata* and *M. longifolia* also found a substantial amount of monoterpene hydrocarbons (6.31-13.47, 10.98%) and sesquiterpene hydrocarbons (3.21-9.31, 3.8%), respectively, while *M. pulegium* had a very small amount of these compounds. The GC-MS chromatograms of *Mentha* essential oils are shown in Annex 10, displaying the separation of chemical components.

There are several studies reported in the literature on the composition of *Mentha* essential oils. Here, in *M. spicata* from Palestine, the EO was characterized with 31 compounds with oxygenated monoterpenes (90%) as the most abundant components, followed by sesquiterpene and monoterpene hydrocarbons, and carvone chemotype (65%) as the major component (Ali-Shtayeh *et al*., 2019). Similarly, *M. spicata* from Algeria explored 44 unique compounds, with oxygenated monoterpenes (67.2%), followed by monoterpene hydrocarbons (20.8%) as the most abundant chemical components, along with a prominent carvone chemotype (49.5%) (Bardaweel *et al*., 2018). In *M. spicata* from Morocco, carvone (53.69-57.11%), limonene (27.77-31.13%), and *trans*-carveol (1.79-3.90%) were reported as the main components (Bensabah *et al*., 2013). In another study, the major compounds of *M. spicata* from Pakistan during the summer and winter seasons were identified to be carvone (59.50% and 63.24%), limonene (10.44% and

9.09%), and 1,8-cineol (6.36% and 4.51%) (Hussain *et al*., 2010). The major compound of *M. spicata* from Algeria was carvone (59.40%), along with considerable amounts of limonène (6.12%), 1,8-cineol, germacrene D (04.66%), β-caryophyllene (2.969%), β-bourbonene (2.796%), α-terpineol (1.986%), and terpinene-4-ol (1.120%) (Boukhebti *et al*., 2011). Sokovic and Griensven also reported that carvone (49.52%), menthone (21.92%), limonene (5.77), and 1,8-cineol (3.06%) were the major compounds identified in the EO of *M. spicata* from Montenegro (Sokovic & Van Griensven, 2006). Therefore, current results are in agreement with many previously published results on *M. spicata* EOs, as discussed earlier.

It is interesting to note that the results of some of the studies reported in the literature on *M. longifolia* are in contrast to the current results. The analysis of *M. longifolia* from Saudi Arabia revealed 46 compounds, and it was characterized by a high percentage of oxygenated monoterpenes (30.40%) with piperitone as the major one (30.77%), followed by sesquiterpene hydrocarbons (26.08%), in which caryophyllene (5.58%) was the main, and monoterpene hydrocarbons (23.90%) with γ-terpinene (1.36%) as the main (Burham *et al*., 2019). In *M. longifolia* EO from Tunisia, pulegone (26.92%), 1,8-cineol (21.3%), and L-menthone (10.66 %) were detected as their major compounds in the winter season. In the spring EO, the major components were pulegone (38.2 %) and oleic and palmitic acids (23.79 % and 15.26%, respectively). Oxygenated monoterpenes were predominant in the two studied samples (Zouari-Bouassida *et al*., 2018). In contrast to the previous results, the major compounds in *M. longifolia* from China were reported to be carvone, limonene, *(E)*-caryophyllene, and α-terpineol, along with the identification of 39 compounds (Bai *et al*., 2020). In the *M. longifolia* EOs from Saudi Arabia, *trans*-dihydrocarvone, 1,8-cineol, β-caryophyllene, β-bourbonene, germacrene D, and bicyclosesquiphellandrene were identified in significant amounts (Anwar *et al*., 2017). The main constituents of *M. longifolia* from Bosnia and Herzegovina were piperitone oxide (63.58%), 1,8-cineol (12.03%), piperitenone oxide (4.81%), and caryophyllene oxide (4.33%), with the dominant being oxygenated monoterpenes (Niksic *et al*., 2012). The analysis of *M. longifolia* from Tunisia identified 41 compounds, along with the dominance of pulegone (47.15), 1,8-cineol (11.54%), menthone (10.7%), α-pinene (3.57%), α-terpineol (3.17%), and δ-cadinene (3.53%) (Hajlaoui *et al*., 2009). However, the results of the present study are consistent with those found by some other researchers. Vijoen *et al*. described piperitenone oxide (15-66%) as the main compound detected in the essential oils and the piperitenone oxide chemotype of *M. longifolia* (Viljoen *et al*., 2006). Gulluce *et al*. mentioned *cis*-piperitone epoxide, pulegone, and piperitenone oxide as the major compounds of essential oil from *M. longifolia* of Turkey (Gulluce *et al*., 2007). Rabab Ez-Zriouli et al. reported pulegone (45.48%), menthone (14.2%), piperitone (8.15%), isomenthone (7.18%), dihydrocarbon (5.16%), and carvone (5.2%) in *M. pulegium* from Morocco (Ez-Zriouli *et al*., 2022). In *M. pulegium* from Algeria, 29 compounds were detected, and the major component was pulegone (38.82%) along with the appreciable contents of menthone (19.24%), piperitenone (16.53%), piperitone (6.348%), isomenthone (6.10%), limonene (4.29%), and octaan-3-ol (1.85%) (Boukhebti *et al*., 2011). The analysis of *M. pulegium*, the essential oil from Tunisia, revealed 41 compounds, along with pulegone (61.11%), isomenthone (17.02%), menthone (5.92%), and piperitone (2.63%) as the dominant components (Hajlaoui *et al*., 2009). In *M. pulegium* from Portugal,.it was reported that pulegone was the major compound (88.64%) (Luís & Domingues, 2021). The present results showed some similarity with the previous reports published in the literature, as discussed above, and were found to have menthone, pulegone, and isomenthone as the major components. The minor variations in the chemical compositions of different *Mentha* essential oils could be attributed to the different factors like agroclimatic, seasonal, or geographical conditions of the regions, extraction methods, and adaptive metabolism of plants.

Table 4. 2 Chemical composition of *Mentha spicata* essential oil.

		Composition						
		Dhulikhel (Winter)			**Dhulikhel (Summer)**			**Bardiya (Summer)**
RT	**Components**	**(%)**	**RT**	**Components**	**(%)**	**RT**	**Components**	**(%)**
Monoterpene hydrocarbons		**13.47%**			**14.39%**			**6.31 %**
11.701	α-Pinene	0.36	12.948	α-Pinene	0.38	12.963	α-Pinene	0.26
-	-	-	12.250	Hashishene	0.02	-	-	-
12.542	Camphene	0.04	13.874	Camphene	0.05	13.893	Camphene	0.04
13.757	Sabinene	0.30	15.169	Sabinene	0.34	15.183	Sabinene	0.20
14.038	β-pinene	0.63	15.492	β-pinene	0.63	15.496	β-pinene	0.03
14.681	Myrcene	**1.58**	15.530	Myrcene	2.26	16.114	Myrcene	**1.05**
17.161	Limonene	**9.55**	18.819	Limonene	9.63	18.676	Limonene	**4.12**
17.467	(*Z*)-β-Ocimene(cis)	0.65	19.079	(*Z*)-β-Ocimene	0.84	19.104	(*Z*)-β-Ocimene	0.25
18.108	(*E*)-β-Ocimene (trans)	0.32	19.742	(*E*)-β-Ocimene	0.19	19.742	(*E*)-β-Ocimene	0.32
-	-	-	19.079	γ-Terpinene	0.03	-	-	-
-	-	-	22.361	Terpinolene	0.04	-	-	-
19.861	α -Pinene oxide	0.04	-	-	-	19.861	α -Pinene oxide	0.04
Oxygenated monoterpenoids		**79.04 %**			**74.99 %**			**85.3 %**
17.274	1,8-Cineol	**1.53**	18.959	1,8-Cineol	2.32	18.872	1,8-Cineol	**1.04**
21.571	Linalool	0.38	23.345	Linalool	0.30	23.348	Linalool	0.38
23.742	*cis*-Limonene oxide	0.02	-	-	-	23.742	*cis*-Limonene oxide	0.02
24.038	*trans*-Limonene oxide	0.04	25.922	*trans*-Limonene oxide	0.04	25.938	*trans*-Limonene oxide	0.04
-	-	-	28.268	δ-Terpineol	0.04	-	-	-
26.399	Borneol	0.13	28.399	Borneol	0.20	28.409	Borneol	0.13
-	-	-	28.967	Terpinen-4-ol	0.07	-	-	-
27.731	*p*-Cymen-8-ol	0.08	-	-	-	28.731	*p*-Cymen-8-ol	0.08
28.284	*cis*-Dihydro carvone	**4.14**	30.200	*cis*-Dihydro carvone	0.33	30.284	*cis*-Dihydro carvone	**2.14**
28.447	*neo*-Dihydro carveol	**6.72**	-	-	**-**	30.447	*neo*-Dihydro carveol	**3.15**
28.580	Dihydro carveol	**3.25**	-	-	**-**	31.580	Dihydro carveol	**1.25**
28.689	*trans*-Dihydro carvone	0.13	-	-	-	31.689	*trans*-Dihydro carvone	0.13
30.010	*trans*-Carveol	0.56	32.233	*trans*-Carveol	0.38	32.240	*trans*-Carveol	0.46
31.147	*cis*-Carveol	1.31	-	-	-	33.147	*cis*-Carveol	1.31
31.898	Carvone	**51.96**	34.168	Carvone	**68.51**	34.186	Carvone	**71.52**
-	-	-	35.159	Isopiperitenone	0.02	-	-	-
33.501	*trans*-Carvone oxide	0.04	35.598	*trans*-Carvone oxide	0.03	35.501	*trans*-Carvone oxide	0.04

35.387	neo-Dihydro carveol acetate	0.06	-	-	-	36.705	neo-Dihydro carveol acetate	0.06
36.774	Dihydro carvyl acetate	**4.20**	36.774	Dihydro carvyl acetate	0.12	36.874	Dihydro carvyl acetate	**1.62**
37.225	trans-Carvyl acetate	0.06	-	-	-	37.225	*trans*-Carvyl acetate	0.06
37.549	Piperitenone	0.22	39.674	Piperitenone	0.2	39.649	Piperitenone	0.22
-	-	-	40.571	Eugenol	0.06	-	-	-
38.998	cis-Carvyl acetate	**4.21**	41.048	cis-Carvyl acetate	0.38	41.146	cis-Carvyl acetate	**1.65**
-	-	-	41.247	Piperitenone oxide	1.93	-	-	-
Sesquiterpene hydrocarbons		**5.05 %**			**9.31 %**			**3.21 %**
-	-	-	42.117	α-Copaene	0.06	-	-	-
40.496	β-Bourbonene	**1.24**	42.676	β-Bourbonene	0.99	42.672	β-Bourbonene	**1.24**
40.659	α-Bourbonene	0.09	42.848	α-Bourbonene	0.08	42.859	α-Bourbonene	0.09
40.871	β-Elemene	0.12	43.006	β-Elemene	0.22	43.041	β-Elemene	0.12
-	-	-	44.140	α-Gurjunene	0.15	-	-	-
41.952	Sibirene	0.09	-	-	-	43.952	Sibirene	0.09
42.738	β-Caryophyllene	0.83	44.977	β-Caryophyllene	1.51	45.113	β-Caryophyllene	0.53
43.374	β-Copaene	0.18	45.571	β-Copaene	0.13	45.567	β-Copaene	0.18
-	-	-	46.565	cis-Muurola-3,5-diene	0.26	-	-	-
44.259	Aromadendrene	0.11	-	-	-	46.859	Aromadendrene	0.11
44.879	(*E*)-β-Farnesene	0.26	47.001	(*E*)-β-Farnesene	0.57	47.118	(*E*)-β-Farnesene	0.26
44.955	α-Humulene	0.11	47.185	α-Humulene	0.20	47.190	α-Humulene	0.11
45.381	*cis*-Muurola-4(14),5-diene	0.39	47.616	*cis*-Muurola-4(14),5-diene	0.66	47.681	*cis*-Muurola-4(14),5-diene	0.19
45.741	9-epi-(*E*)-Caryophyllene	0.07	-	-	-	47.741	9-epi-(*E*)-Caryophyllene	0.07
-	-	-	47.989	*trans*-Cadina-1(6),4-diene	0.07	-	-	-
46.562	Germacrene D	**1.11**	48.877	Germacrene D	3.51	48.862	Germacrene D	**1.11**
47.416	Bicyclogermacrene	0.08	49.671	Bicyclogermacrene	0.39	49.616	Bicyclogermacrene	0.08
-	-	-	50.721	γ-Cadinene	0.10	-	-	-
-	-	-	51.020	δ-Cadinene	0.16	-	-	-
48.822	β-Cadinene	0.08	-	-	-	49.822	β-Cadinene	0.08
48.909	*trans*-Calamenene	0.22	51.148	*trans*-Calamenene	0.2	51.152	trans-Calamenene	0.12
49.897	α-Cadinene	0.07	52.121	α-Cadinene	0.11	52.151	α-Cadinene	0.07
Oxygenated sesquiterpenoids		**0.39 %**			**0.20 %**			**0.17 %**
54.466	1,10-Di-epi-cubenol	0.22	-	-	-	-	-	-
-	-	-	54.505	Germacrene D-4-ol	**0.07**			

56.735	α-Cadinol	0.17	59.024	α-Cadinol	0.13	56.735	α-Cadinol	0.17
Others		**1.34%**			**0.69%**			**1.24%**
14.123	1-Octen-3-ol	0.20	15.530	1-Octen-3-ol	0.1	14.123	1-Octen-3-ol	0.20
15.100	3-Octanol	0.31	16.569	3-Octanol	0.17	15.100	3-Octanol	0.31
16.816	Hexyl acetate	0.11	-	Hexyl acetate	-	16.816	Hexyl acetate	0.11
20.306	1-Nonen-3-ol	0.06	-	-	-	20.306	1-Nonen-3-ol	0.06
21.897	*n*-Nonanal	0.06		*n*-Nonanal		21.897	*n*-Nonanal	0.06
22.081	1-Octen-3-yl acetate	0.05		1-Octen-3-yl acetate		22.081	1-Octen-3-yl acetate	0.05
22.884	3-Octanol acetate	0.20	24.648	3-Octanol acetate	0.04	22.884	3-Octanol acetate	0.20
-	-	-	23.669	3-Oxa-2,2,4-trimethyl-4-vinyl-cyclohexanone	0.02	-	-	-
34.612	Dihydroedulan	0.22	36.748	Dihydroedulan	0.06	36.612	Dihydroedulan	0.12
41.057	(*E*)-Jasmone	0.13	-	-	-	43.257	(*E*)-Jasmone	0.13
-	-	-	43.203	*(Z)*-Jasmone	0.14	-	-	-
-	-	-	94.341	Tetracosane	0.05	-	-	-
-	-	-	98.293	Pentacosane	0.05	-	-	-
-	-	-	102.097	Hexacosane	0.04	-	-	-
Total	**56**	**99.29**		**60**	**99.58**		**54**	**97.47**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

Table 4. 3 Chemical composition of *Mentha longifolia* essential oil.

	Composition	
RT	**Components**	**Area (%)**
Monoterpene hydrocarbons		**10.98 %**
12.519	α-Thujene	0.03
12.961	α-Pinene	**1.02**
13.876	Camphene	0.20
15.176	Sabinene	0.58
15.506	β-Pinene	**1.26**
16.122	Myrcene	1.01
17.053	*p*-Mentha-1(7),8-diene	0.04
17.862	α-Terpinene	0.02
18.369	*p*-Cymene	0.09
18.786	Limonene	**6.44**
18.850	β-Phellandrene	0.05
19.046	*(Z)*-β-Ocimene	0.09
19.734	*(E)*-β-Ocimene	0.03
20.538	γ-Terpinene	0.05
22.358	Terpinolene	0.07
22.697	*p*-Cymenene	0.10
Oxygenated monoterpenoids		**76.32 %**
18.918	1,8-Cineol	0.61
21.358	*cis*-Sabinene hydrate	0.03
23.340	Linalool	0.24
24.881	*trans*-*p*-Mentha-2,8-dien-1-ol	0.03
28.287	δ-Terpineol	0.03
28.428	Borneol	0.76
28.975	Terpinen-4-ol	0.25
29.469	*p*-Cymen-8-ol	0.85
30.002	α-Terpineol	0.26
31.404	Coahuilensol methyl ether	0.72
33.330	Carvone	0.07
33.800	*cis*-Piperitone epoxide	0.06
34.083	*trans*-Piperitone epoxide	**1.67**
35.116	Isopiperitenone	0.95
37.305	Carvacrol	0.13
39.769	Piperitenone	**2.23**
39.885	*cis*-3-Isopropenyl-2-methylene cyclohexyl acetate	0.36
42.083	Piperitenone oxide	**67.43**
Sesquiterpene hydrocarbons		**3.8 %**
42.768	β-Bourbonene	0.08
43.119	β-Elemene	0.12
45.037	β-Caryophyllene	1.27
45.620	β-Copaene	0.04
47.007	*(E)*-β-Farnesene	0.38
47.221	α-Humulene	0.12
48.854	Germacrene D	**1.70**
51.032	δ-Cadinene	0.09
Oxygenated sesqyiterpenoids		**0.15 %**

54.852	Caryophyllene oxide	**0.15**
Others		**2.6 %**
14.561	Benzaldehyde	0.03
16.610	3-Octanol	1.05
19.974	(2*E)*-Octen-1-al	0.09
19.974	(2*E)*-Octen-1-al	0.09
22.615	3-Methyl-1,2-cyclohexanedione	0.04
24.664	3-Octanol acetate	0.44
32.694	3-*cis*-Hexenyl 3-methyl butanoate	0.02
36.687	Dihydroedulan	0.09
37.323	6-Hydroxy carvotone lactone	0.26
46.681	Geranyl acetone	0.03
Total	**52**	**93.85**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

Table 4. 4 Chemical composition of *Mentha pulegium* essential oil.

	Composition							
	Nuwakot (Summer)			Nuwakot (Autumn)			Sindhupalchowk (Autumn)	
RT	**Components**	**%**	**RT**	**Components**	**%**	**RI**	**Components**	**%**
Monoterpene hydrocarbons		**1.04 %**			**0.89 %**			**3.3 %**
11.694	α-Pinene	0.20	12.963	α-Pinene	0.15	930	α-Pinene	0.6
12.538	Camphene	0.06	-	-	-	946	Camphene	0.1
13.748	Sabinene	0.06	15.183	Sabinene	0.07	969	Sabinene	0.3
14.027	β-pinene	0.29	15.498	β-Pinene	0.18	975	β-Pinene	0.6
-	-	-	15.873	3-Octanone	0.04	981	3-Octanone	0.2
-	-	-	16.118	Myrcene	0.02	986	Myrcene	0.05
-	-	-	-	-	-	1002	p-Mentha-1(7),8-diene	0.05
-	-	-	-	-	-	1014	α-Terpinene	0.05
16.753	*p*-Cymene	0.06	18.378	*p*-Cymene	0.02	1022	p-Cymene	0.05
17.042	Limonene	0.37	18.683	Limonene	0.41	1027	Limonene	1.2
-	-	-	-	-	-	1028	β-Phellandrene	0.05
-	-	-	-	-	-	1055	γ-Terpinene	0.05
Oxygenated monoterpenoids		**85.69 %**			**91.63 %**			**87.65 %**
-	-	-	-	-	-	1029	1,8-Cineol	0.05
18.425	Dihydro tagetone	0.29	-	-	-	-	-	-
25.785	Menthone	**66.57**	27.719	Menthone	**41.64**	1164	Menthone	54.8
-	-	-	27.925	Menthofuran	0.02	-	-	-
26.149	Isomenthone	**6.01**	28.083	Isomenthone	2.23	1168	Isomenthone	4.9
26.395	Neomenthol	**1.81**	28.328	Neomenthol	0.13	1171	Neomenthol	1.5
25.948	Pinocarvone	0.07	-	-	-	-	-	-
26.498	Borneol	0.19	28.495	Borneol	0.07	1173	Borneol	0.2
26.725	*trans*-Isopulegone	**0.32**	28.720	*trans*-Isopulegone	0.86	1176	*trans*-Isopulegone	0.7
26.870	Menthol	0.13	-	-	-	1177	Menthol	0.2
27.038	**Terpinen-4-ol**	0.05	29.035	Terpinen-4-ol	0.04	1180	Terpinen-4-ol	0.1
28.003	α-Terpineol	0.05	30.163	α-Terpineol	0.03	1195	α-Terpineol	0.05
28.787	Verbenone	0.07	-	-	-	1207	Verbenone	0.05
31.018	Pulegone	**7.12**	33.436	Pulegone	**38.31**	1243	Pulegone	22.2
31.297	Carvone	0.08	33.520	Carvone	0.02	-	-	-
-	-	-	32.504	Bicyclo[3.3.1]nonan-3-ol 7-methylene	0.05	-	-	-

-	-	-	33.914	*cis*-Piperitone epoxide	0.25	1250	*cis*-Piperitone oxide	0.1
-	-	-	34.239	trans-Piperitone epoxide	5.34	-	-	-
32.008	Piperitone	**2.33**	-	-	-	1254	Piperitone	2.2
32.406	Pulegone oxide A	0.09	34.569	Pulegone oxide A	0.03	1259	Pulegone oxide A	0.05
33.993	*neo*-Isopulegyl acetate	0.29	-	-	-	-	-	-
34.200	Pulegone oxide B	0.39	-	-	-	1281	Bornyl acetate	0.1
-	-	-	35.095	Isopiperitenone	0.04	-	-	-
-	-	-	36.347	Pulegone oxide B	0.09	-	-	-
-	-	-	36.537	Thymol	0.09	-	-	-
-	-	-	36.955	Buchu camphor	0.02	-	-	-
-	-	-	-	-	-	1270	Neomenthyl acetate	0.1
37.493	Piperitenone	0.12	39.748	Piperitenone	1.18	1336	Piperitenone	0.3
-	-	-	41.207	Piperitenone oxide	1.20	1358	Piperitenone oxide	0.1
-	-	-	39.946	*cis*-3-Isopropenyl-2-methylene cyclohexyl acetate	0.20	1339	*cis*-3-Isopropenyl-2-methylenecyclohexyl acetate	0.3
44.415	1-Acetoxy-ortha-menth-3-one	0.08	-	-	-	-	-	-
Sesquiterpene hydrocarbons		**0.31 %**			**3.88 %**			**5.55 %**
39.938	α-Copaene	0.15	42.143	α-Copaene	0.34	1373	α-Copaene	0.6
40.443	β-Bourbonene	0.16	42.663	β-Bourbonene	0.08	1380	β-Bourbonene	0.2
-	-	-	42.900	β-Cubebene	0.03	-	-	-
-	-	-	43.022	β-Elemene	0.17	1386	β-Elemene	0.3
-	-	-	44.979	β-Caryophyllene	1.03	1416	β-Caryophyllene	1.3
-	-	-	46.999	*(E)*-β-Farnesene	0.11	1449	(*E*)-β-Farnesene	0.1
-	-	-	47.204	α-Humulene	0.07	1452	α-Humulene	0.1
-	-	-	48.838	Germacrene D	1.61	1479	Germacrene D	2.4
-	-	-	49.481	γ-Amorphene	0.01	-	-	-
-	-	-	49.684	Bicyclogermacrene	0.17	1492	Bicyclogermacrene	0.2
-	-	-	50.183	*(E,E)*-α-Farnesene	0.04	1500	(*E*,*E*)-α-Farnesene	0.05
-	-	-	51.045	δ-Cadinene	0.22	1514	δ-Cadinene	0.3
Oxygenated sesquiterpenoids		**1.58 %**			**0.66%**			**1.3 %**
-	-	-	53.573	*(E)*-Nerolidol	0.09	1557	(*E*)-Nerolidol	0.1
52.266	Spathulenol	0.33	54.560	Spathulenol	0.17	1589	Spathulenol	0.3
52.546	Caryophyllene oxide	1.04	54.869	Caryophyllene oxide	0.28	1579	Caryophyllene oxide	0.3
-	-	-	-	-	-	1603	α-Oplopenone	0.05

-	-	-	-	-	-	1606	Humulene epoxide I	0.05
			57.778	*iso*-Spathulenol	0.08	1629	*iso*-Spathulenol	0.1
-	-	-	-	-	-	1636	*allo*-Aromadendrene epoxide	0.1
-	-	-	-	-	-	1638	Hinesol	0.05
-	-	-	-	-	-	1640	τ-Muurolol	0.05
-	-	-	-	-	-	1652	α-Cadinol	0.05
-	-	-	-	-	-	1711	Pentadecanal	0.1
-	-	-	-	-	-	1859	Platambin	0.05
57.750	Mustakone	0.21	-	-	-	-	-	-
-	-	-	59.834	Dihydro germacrene D	0.04	-	-	-
Others		**2.52 %**			**2.93 %**			**2.15 %**
8.110	3-Methyl cyclopentanone	0.07	-	-	-	**1117**	3-Octyl acetate	0.5
-	-	-	31.919	Coahuilensol methyl ether	0.03	-	-	-
-	-	-	33.485	2,4-Diisopropyl-1,1-dimethyl cyclohexane	0.05	-	-	-
12.666	3-Methyl cyclohexanone	0.10				-	-	-
14.413	3-Octanone	0.13						
15.104	3-Octanol	0.65	16.606	3-Octanol	0.74	995	3-Octanol	0.9
15.199	2,6-Dimethyl-3-heptanone	0.20	-	-	-	-	-	-
22.887	3-Octanol acetate	0.52	24.690	3-Octanol acetate	0.14			
-	-	-	36.955	Buchu camphor	0.02	1270	Neomenthyl acetate	0.1
-	-	-	37.351	6-Hydroxy carvotone lactone	0.11			
-	-	-	40.556	Eugenol	0.06	1349	Eugenol	0.05
33.213	*neo*-Menthyl acetate	0.17	-	-	-	-	-	-
-	-	-	45.535	1-Acetoxy-*o*-menth-3-one	0.19	-	-	-
-	-	-	46.593	1-Acetoxy-*p*-menth-3-one	0.10	-	-	-
-	-	-	-	-	-	1487	Phenylethylisovalerate	0.1
43.425	Pileric acid	0.13	49.361	Phenyl ethyl 3-methyl butanoate	0.08	-	-	-
-	-	-	50.913	α-Isomethyl ionone	0.04	1564	1,5-Epoxysalvial-4(14)-ene	0.05
-	-	-	-	-	-	1589	Salvial-4(14)-en-1-one	0.05

-	-	-	-	-	-	1881	(*Z*)-Hexadecatrienal	0.05
-	-	-	-	-	-	1886	(*E*)-Hexadecantrienal	0.1
-	-	-	86.006	Docosane	0.08	-	-	-
-	-	-	90.287	Tricosane	0.17	-	-	-
-	-	-	94.396	Tetracosane	0.23	-	-	-
-	-	-	98.347	Pentacosane	0.26	-	-	-
66.064	*cis*-Thujopsenic acid	0.18	102.154	Hexacosane	0.24	-	-	-
-	-	-	105.838	Heptacosane	0.20	-	-	-
Total	**39**	**91.4**		**61**	**99.98**		**61**	**99.97**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

4.1.4.3 The Genus *Elsholtzia*

The GC-MS analysis of two *Elsholtzia* essential oils revealed 55 compounds representing about 98.21% of total *E. strobilifera* oil and 41 compounds representing about 94.29% of total *E. blanda* oil, respectively (Table 4.5). In the *E. strobilifera* EO, pinocarvone (40.73%) and β-pinene (9.97%) were the most prominent compounds, followed by *cis*-pinocamphone (7.93%), α-humulene (7.29%), sabinene (4.74%), humulene epoxide II (2.31%), and verbenone (2.18%) as other important identified components. Among the major classes of terpenes, the oxygenated monoterpenes were the most dominant in this essential oil, accounting for nearly 57.5%. Pinocarvone, *cis*-pinocamphone, and verbenone were the major oxygenated monoterpenes in *E. strobilifera*. It also contained a considerable quantity of monoterpene hydrocarbons (20.84%), and sesquiterpene hydrocarbons (12.37%), respectively, whereas this EO had very low amounts of oxygenated sesquiterpenes. The GC-MS chromatogram of *E. strobilifera* essential oil displays the separation of several volatile compounds, which is presented in Annex 10.

A study carried out by Murari and Mathela identified the presence of β-pinene (12.1%), diterpene (10.4%), and carvone (10.5%) as important components in *E. strobilifera* (Murari & Mathela, 1980). Whereas, one other study showed that *E. strobilifera* growing in India contained pinocarvone (51.9%) and β-pinene (9.7%) as the main compounds with yields of 0.3% (Bisht *et al*., 1985). In contrast to the above results, a subsequent report on *E. strobilifera* identified the presence of pinocarvone (7.4%), geranial (citral b) (29.9%), neral (citral a) (18.3%), humulene epoxide II (6.3%), and *trans*-calamenene (3.9%) (Melkani *et al*., 2005). However, the present study has shown the dominance of pinocarvone and β-pinene, along with 55 other compounds. This is a highly prominent result as compared to the previous study (Murari & Mathela, 1980; Melkani *et al*., 2005), and current results are in agreement regarding the major components described by Bisht *et al*. (Bisht *et al*., 1985).

Similarly, *E. blanda* essential oil contained dihydrotagetone (49.08%) and (Z)-tagetone (15.55%) as the most dominant compounds, followed by (*E*)-tagetone (7.94%), (*Z*)-β-ocimene (3.65%), benzaldehyde (2.85%), and β-caryophyllene (2.78%) compounds in smaller amounts. Regarding the major classes of terpenes, the oxygenated monoterpenes were the most dominant in this EO, accounting for nearly 74.18% of the total essential oil. Dihydrotagetone, (*E*)-tagetone, and (*Z*)-tagetone were the major oxygenated monoterpene compounds in *E. blanda*. This essential oil also exhibited a significant amount of sesquiterpene hydrocarbons (7.98%), whereas it had very low amounts of monoterpene hydrocarbons (4.95%) and oxygenated sesquiterpenes (2.15%). The GC-MS chromatogram of *E. blanda* essential oil displays the separation of several volatile compounds, which is shown in Annex 10.

E. blanda essential oil from Vietnam was mostly made up of monoterpenoids, with camphor (25.14%), camphene (22.64%), α-pinene (11.53%), and 1,8-cineol (9.89%) as the four dominant components (Ngoc & Binh, 2022). *E. blanda* from India was characterized with geranyl acetate (71.38%), linalool (5.27%), geraniol (3.77%), (*E*)-β-ocimene (2.96%) (Kotoky *et al*., 2017), β-caryophyllene (1.75%), α-bergamotene (1.70%), (*Z*)-β-ocimene (1.38%), acetophenone (1.13%), and linalyl acetate (1.11%) as being the most abundant compounds (Bestmann *et al*., 1992). Likewise, two types of *E. blanda* essential oils from India were also described, with linalool as the major component in both the inflorescence parts (77.3-80.2%) and vegetative aerial parts (57.9-62.9%) (Kotoky *et al*., 2017). *E. blanda* essential oil from Vietnam was also found to contain linalool as the dominant component (Lesueura *et al*., 2007). Several previous reports on the composition of *E. blanda* EO showed that *E. blanda* presents great variability as a major chemical compound depending on its availability in different countries. Moreover, *E. blanda* essential oil from Nepal exhibited dihydrotagetone and (*Z*)-tagetone as major constituents, which were not mentioned in the previous studies. The reason behind this might be due to the environmental and climatic conditions because the plant species may adopt changes to the biosynthetic pathways of the secondary metabolites. The most abundant components were oxygenated monoterpenoids and monoterpene hydrocarbons, followed by sesquiterpene hydrocarbons, which had more similar patterns to those of other species of the *Elsholtzia* genus as described previously (Phetsang *et al*., 2019). Here, Figure 4.5 shows the distinct variation in the major components of *E. strobilifera* and *E. blanda* essential oils, though both species are from the same genus, *Elsholtzia*.

Table 4. 5 Chemical composition of essential oils of *Elsholtzia strobilifera* and *Elsholtzia blanda.*

	Composition *(E. strobilifera)*			**Composition** *(E. blanda)*	
RT	**Components**	**Area (%)**	**RT**	**Components**	**Area (%)**
Monoterpene hydrocarbons		**20.84 %**			**4.95 %**
11.315	α-Thujene	0.17	-	-	-
11.696	α-Pinene	0.48	11.658	α-Pinene	0.1
12.538	Camphene	0.14	-	-	-
12.736	Thuja-2,4(10)-diene	0.2	-	-	-
13.802	Sabinene	**4.74**	13.714	Sabinene	0.08
14.125	β-Pinene	**9.97**	13.992	β-Pinene	0.22
14.667	Myrcene	0.93	14.618	Myrcene	0.45
16.759	*p*-Cymene	0.44	-	-	-
17.059	Limonene	1.07	17.007	Limonene	0.08
17.153	β-Phellandrene	0.65	-	-	-
17.223	1,8-Cineol	1.19	-	-	-
17.442	*(Z)*-β-Ocimene	0.09	17.451	*(Z)*-β-Ocimene	**3.65**
18.124	*(E)*-β-Ocimene	1.88	18.085	*(E)*-β-Ocimene	0.27
18.422	Dihydrotagetone	0.16	-	-	-
18.839	γ-Terpinene	0.08	-	-	-
19.618	*cis*-Sabinene hydrate	0.16	-	-	-
19.724	Pinol	0.13	-	-	-
Oxygenated monoterpenoids		**57.50 %**			**74.13 %**
-	-	-	17.171	1,8-Cineol	0.1
-	-	-	18.743	Dihydrotagetone	**49.08**
-	-	-	21.225	α-Pineneoxide	0.08
21.645	Linalool	0.47	21.547	Linalool	0.9
-	-	-	22.404	Epoxyocimene	0.11
-	-	-	23.421	*allo*-Ocimene	0.1
23.362	α-Campholenal	0.09	-	-	-
-	-	-	23.714	Epoxyocimeneisomer	0.22
24.414	*trans*-Pinocarveol	0.55	-	-	-
-	-	-	24.697	*(E)*-Tagetone	**7.94**
24.894	*trans*-Verbenol	0.84			
-	-	-	25.167	(*Z*)-Tagetone	**15.55**
26.153	Pinocarvone	**40.73**	-	-	-
26.870	*cis*-Pinocamphone	**7.93**	-	-	-
-	-	-	28.168	Methyl chavicol	0.15
27.068	Terpinen-4-ol	0.86	-	-	-
27.482	*p*-Cymen-8-ol	0.16	-	-	-
28.030	Myrtenal	0.77	-	-	-
28.045	α-Terpineol	0.52	-	-	-
28.095	Myrtenol	0.41	-	-	-
28.888	Verbenone	**2.18**	-	-	-
29.632	*trans*-Carveol	0.12	-	-	-
31.790	*trans*-2-Hydroxy pinocamphone	0.08	-	-	-
33.964	Bornyl acetate	0.24	-	-	-
36.528	*trans*-Myrtenyl acetate	0.11	40.165	Geranyl acetate	0.81
Sesquiterpene hydrocarbons		**12.37 %**			**7.98 %**

-	-	-	39.92	α-Copaene	0.28
-	-	-	40.426	β-Bourbonene	0.1
-	-	-	40.834	β-Elemene	0.18
42.715	β-Caryophyllene	0.89	42.723	β-Caryophyllene	**2.78**
-	-	-	43.34	β-Copaene	0.16
44.905	*(E)*-β-Farnesene	0.52	-	-	-
45.052	α-Humulene	**7.29**	44.921	α-Humulene	0.44
-	-	-	45.193	*allo*-Aromadendrene	0.12
45.378	*cis*-Muurola-4(14),5-diene	0.31	-	-	-
-	-	-	46.528	GermacreneD	1.35
46.553	Germacrene D	1.43	-	-	-
47.208	*iso*-bicyclogeramcrene	0.29	-	-	-
-	-	-	47.178	Farnesene isomer	0.12
47.414	Bicyclogermacrene	0.44	47.397	Bicyclogermacrene	0.32
-	-	-	47.609	α-Muurolene	0.07
48.041	*(E,E)*-α-Farnesene	0.79	-	-	-
-	-	-	48.473	γ-Cadinene	0.13
48.505	γ-Cadinene	0.6			
48.850	δ-Cadinene	0.13	48.799	δ-Cadinene	0.46
48.925	*trans*-Calamenene	0.99	-	-	-
Oxygenated sesquiterpenes		**3.93 %**			**2.15 %**
-	-	-	51.359	*(E)*-Nerolidol	0.31
-	-	-	52.229	Spathulenol+GermacreneD-4-ol	0.44
52.261	Spathulenol	0.92	-	-	-
52.528	Caryophyllene oxide	0.44	52.518	Caryophylleneoxide	1.05
			52.92	Allohedycaryol	0.16
53.506	Humulene epoxide I	0.11			
54.184	Humulene epoxide II	**2.31**	54.11	Humulene epoxide II	0.09
-	-	-	54.816	10-*epi*-γ-Eudesmol	0.15
55.462	*iso*-Spathulenol	0.15	-	-	-
-	-	-	55.969	*epi*-α-Cadinol	0.22
-	-	-	56.078	*epi*-α-Muurolol	0.08
Others		**3.57 %**			**5.71 %**
14.419	3-Octanone	0.31	13.18	Benzaldehyde	**2.85**
15.091	3-Octanol	0.2	19.394	Acetophenone	2.41
19.305	Acetophenone	0.8	20.476	2-*cis*-Hexenaldiethylacetal	0.29
22.077	1-Octen-3-yl acetate	0.27	25.382	Dehydromevalonic lactone	0.23
22.878	3-Octanol acetate	0.07	-	-	-
34.732	*trans*-Pinocarvylacetate	0.42	-	-	-
Total	**55**	98.21		**44**	94.92%

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

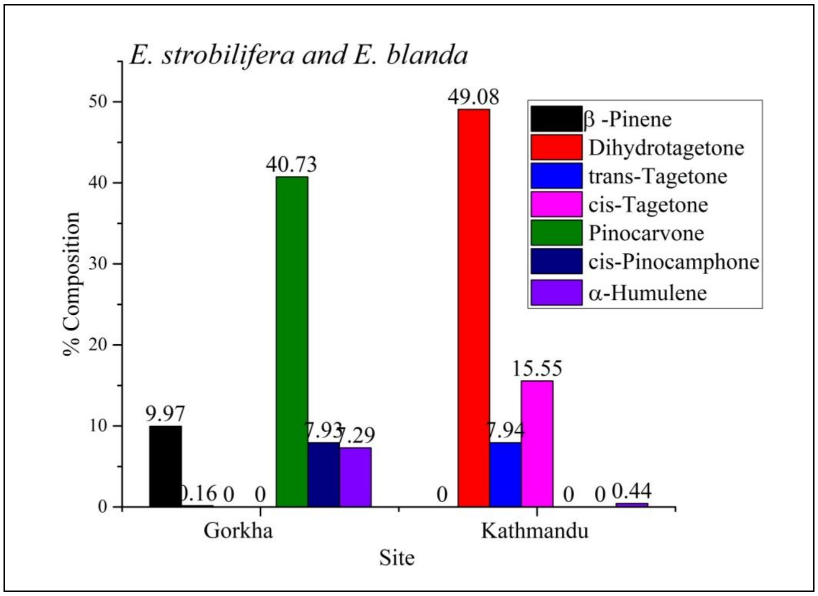


Figure 4. 5 The variation in the major components of *E. strobilifera* and *E. blanda* essential oils.

4.1.4.4 The Genus *Ocimum*

The volatile compounds detected in the essential oils of *O. tenuiflorum*, *O. americanum*, and *O. basilicum* are shown in Tables 4.6, 4.7, and 4.8. A total of 45-53, 57-60, and 51-54 compounds, representing 93.42-99.71%, 99.84-99.89%, and 96.59-99.74%, were identified in *O. tenuiflorum*, *O. americanum*, and *O. basilicum*. The major compounds (> 5%) in *O. tenuiflorum* essential oil were detected as eugenol (26.15-34.95%), *trans*-β-elemene (25.55-32.85%), β-caryophyllene (19.22-21.64%), and caryophyllene oxide (0.75-3.75%). The major constituents identified in *O. americanum* were camphor (51.3-65.88%), linalool (9.72-9.91%), germacrene D (1.99-7.75%), β-caryophyllene (3.97-6.35%), and limonene (3.96-4.4%). Methyl chavicol (estragole) was the main constituent isolated in *O. basilicum* (62.16-64.42%). Linalool (26.92-27.05%) was the second.major component found in *O. basilicum*, followed by *trans*-γ-bisabolene (1.84-2.38%). Besides, *Ocimum* essential oils also had a considerable amount of minor constituents.

O. tenuiflorum essential oil was dominant in its sesquiterpene hydrocarbon fraction. The contents were about 53.17-57.11%, whereas *O. americanum*, and *O. basilicum* contained mainly oxygenated monoterpenes. The concentration of oxygenated monoterpenes in *O. americanum* was 68.57-82.38%, while in *O. basilicum*, it was 90.81-92.51%. The major oxygenated monoterpenes found in *Ocimum* essential oil were camphor and methyl chavicol. The GC-MS chromatograms of three *Ocimum* essential oils are shown in Annex 10, displaying the separation of chemical components.

Several previous findings have revealed the composition of essential oils extracted from *Ocimum* species growing under the diverse climates in different countries. In *O. tenuiflorum* essential oil from Bangladesh, it was reported to contain eugenol (41.7%) as the key constituent (Mondello *et al*., 2002). Similarly, *O. tenuiflorum* essential oil from India was reported to have eugenol (1.94–60.20 %), methyl eugenol (0.87–82.98 %), β-caryophyllene (4.13–44.60 %), and β-elemene (0.76–32.41 %) as major constituents (Raina *et al*., 2013). Whereas, Hikmawanti et al. reported that *O. tenuiflorum* stem EO from Indonesia was found to have α-copaene (5.56%), caryophyllene (17.28%), germacrene-D (9.29%), and methyl eugenol (56.72%) as the major compounds (Hikmawanti *et al*., 2019). de Vasconcelos Silva *et al*. also reported that eugenol (41.70%), limonene (3.80%), and β-caryophyllene (24.40%) were the main components identified in *O. tenuiflorum* from north-eastern Brazil (de Vasconcelos Silva *et al*., 2003). These results showed similarity with *O. tenuiflorum* EO from India, which contained eugenol (46.2%), (*E*)-caryophyllene (27.6%), and β-elemene (16.3%) as main compounds. The comparison of the chemical composition of EO showed some variations due to climatic, seasonal, geographical, and genetic differences.

Essential oil of *O. americanum* from India identified camphor (33.87%), limonene (7.22%), longifolene (6.73%), caryophyllene (5.50%), and isoledene (5.47%) as the most abundant chemical components (Mahendran & Vimolmangkang, 2023). *O. americanum* from Bangladesh was reported to have high amounts of camphor (38.6%), and limonene (10.6%) as the major compounds (Mondello *et al*., 2002). The current results of *O. americanum* are in accordance with the previous study (Smitha & Tripathy, 2016).

In *O. basilicum* stem essential oil from Indonesia, methyl eugenol (52.60%), caryophyllene (18.75%), and germacrene-D (9.19%) were reported as the major compounds (Hikmawanti *et al*., 2019). Current findings are in good agreement with those of Mahendran and Vimolmangkang, who reported citral (19.56%), estragole (18.58%), linalool (17.61%), and camphor (9.22%) as the main constituents in Indian *O. basilicum* (Mahendran and Vimolmangkang, 2023). In *O. basilicum* essential oil from Armenia, methyl-chavicol (57.3%) was reported as the most dominant component (Avetisyan *et al*., 2017). In the next study, the essential oil of *O. basilicum* from Djibouti was reported to contain linalool (41.2%) and estragole (30.1%) as the major constituents. Whereas, *O. basilicum* from Bangladesh was reported to contain linalool as the chief component (Mondello *et al*., 2002). According to the another report led by Gurbuz, linalool (41.2%) was identified as the leading compound in *O. basilicum* EO from Turkey (Gurbuz *et al*., 2006). While Purkayastha and Nath described that camphor, limonene, and β-selinene were the key components in *O. basilicum* EOs from north-east India (Purkayastha & Nath, 2006). The slight variations in the chemical profiles of the EOs from different countries could be associated with many environmental factors.

Table 4. 6 Chemical composition of *Ocimum tenuiflorum* essential oil.

		Composition						
		Bardiya (Winter)			**Bardiya (Autumn)**			**Kathmandu (Autumn)**
RT	**Components**	**(%)**	**RT**	**Components**	**(%)**	**RT**	**Components**	**(%)**
Monoterpene hydrocarbons		**0.20 %**			**0.31 %**			**0.33 %**
11.693	α -Pinene	0.02	12.962	α-Pinene	0.12	12.962	α -Pinene	0.21
-	-	-	13.893	Camphene	0.06	-	-	-
13.748	Sabinene	0.03	15.182	Sabinene	0.02	13.748	Sabinene	0.01
14.025	β-Pinene	0.08	15.496	β-Pinene	0.05	15.182	β-Pinene	0.05
14.652	Myrcene	0.01	-	-	-	-	-	-
17.037	Limonene	0.04	18.671	Limonene	0.06	18.671	Limonene	0.06
17.441	*(Z)*- β-Ocimene	0.01	-	-	-			
18.099	*(E)*-β-Ocimene	0.01	-	-	-			
Oxygenated monoterpenoids		**1.15 %**			**0.99%**			**1.16%**
17.202	1,8-Cineol	0.01	18.872	1,8-Cineol	0.06	18.872	1,8-Cineol	0.36
18.419	Dihydro tagetone	1.02	-	-	-	-	Dihydro tagetone	-
21.544	Linalool	0.02	23.348	Linalool	0.52	23.348	Linalool	0.60
21.983	*cis*-Thujone	0.03	-	-	-			
25.710	Pinocarvone	0.04	-	-	-	26.105	Pinocarvone	0.05
-	-	-				28.409	Borneol	0.20
-	-	-	28.409	Borneol	0.41	-	-	-
Sesquiterpene hydrocarbons		**57.11 %**			**62.54 %**			**53.17 %**
40.461	*cis*-β-Elemene	1.72	42.648	*cis*-β-Elemene	1.92	42.648	*cis*-β-Elemene	2.61
41.198	*trans*-β-Elemene	**29.08**	43.433	*trans*-β-Elemene	**32.85**	43.433	trans-β-Elemene	**25.55**
41.727	Methyl eugenol	1.21	43.903	Methyl eugenol	1.43	43.703	Methyl eugenol	1.08
41.871	β -Longipinene	0.04	-	-	-	44.105	β -Longipinene	0.01
-	-	-	44.126	*cis*-Caryophyllene	0.02	-	-	-
42.487	α -Barbatene	0.19	44.802	α-Barbatene	0.20	44.800	α -Barbatene	0.31
43.001	β -Caryophyllene	**19.85**	45.309	β-Caryophyllene	21.64	45.001	β -Caryophyllene	**19.22**
-	-	-	43.549	epi-Cubebene isomer	0.02	-	-	-
-	-	-	-	-	-	43.901	Isobazzanene	0.10
-	-	-	46.014	*cis*-Thujopsene	0.03	-	-	-
-	-	-	46.148	Isobazzanene	0.08	-	-	-
44.544	β -Barbatene	0.22	46.891	β-Barbatene	0.23	45.364	β -Barbatene	0.23
-	-	-	47.015	*(E)*-β-Farnesene	0.06	-	-	-

44.996	α -Humulene	1.12	47.283	α-Humulene	1.18	45.990	α -Humulene	1.18
46.081	β -Chamigrene	0.24	48.326	β-Chamigrene	0.20	46.081	β -Chamigrene	0.20
-	-	-	48.830	γ-Gurjunene	0.03	-	-	-
47.051	β -Selinene	1.06	49.347	β-Selinene	0.81	47.051	β -Selinene	0.97
47.253	Valencene	0.04	49.522	Valencene	0.04	47.253	Valencene	0.04
47.488	α -Selinene	1.01	49.779	α-Selinene	0.92	47.488	α -Selinene	0.91
-	-	-	50.188	α-Cuprenene	0.03	51.188	α-Cuprenene	0.03
48.204	Germacrene A	**1.19**	50.503	Germacrene A	0.69	48.204	Germacrene A	**0.69**
48.828	γ -Cadinene	0.07	-	-	-	49.505	γ -Cadinene	0.07
-	-	-	51.061	δ-Cadinene	0.01	-	-	-
-	-	-	51.148	7-epi-α-Selinene	0.04	-	-	-
-	-	-	51.333	1,4-Dihydro cuparene	0.03	-	-	-
49.335	*(E)*-γ-Bisabolene	0.04	51.555	*(E)*-γ-Bisabolene	0.07	49.935	*(E)*-γ-Bisabolene	0.06
53.518	Bisabolenol isomer	0.03	-	-	-	53.518	Bisabolenol isomer	0.01
-	-	-	52.037	γ-Cuprenene	0.03	-	-	-
Oxygenated sesquiterpenoids		**7.28 %**			**1.15 %**			**7.87 %**
45.674	Dehydro sesquicineole	0.02	-	-	-	45.674	Dehydro sesquicineole	0.02
-	-	-	48.133	,5-Di-epi-aristolochene	0.02	-	-	-
49.516	10-epi-Cubenol	0.07	51.814	10-epi-Cubenol	0.02	49.516	10-epi-Cubenol	0.02
52.606	Caryophyllene oxide	**3.37**	54.897	Caryophyllene oxide	0.75	54.897	Caryophyllene oxide	**3.75**
54.124	Humulene epoxide II	0.35	56.484	Humulene epoxide II	0.03	56.484	Humulene epoxide II	0.19
54.337	β-Atlantol	0.04	-	-	-	56.736	β-Atlantol	0.04
54.590	Intermedeol isomer	0.15	56.938	Intermedeol isomer	0.04	56.983	Intermedeol isomer	0.15
-	-	-	58.846	elina-3,11-dien-6-α-ol	0.02	-	-	-
-	-	-	59.237	neo-Intermedeol	0.19	-	-	-
55.706	Caryophylla-4(12),8(13)-dien-5-β-ol	0.14	-	-	-	57.505	Caryophylla-4(12),8(13)-dien-5-β-ol	0.14
56.243	*llo*-Aromadendrene epoxide	0.05	-	-	-	-	-	-
56.909	Selin-11-en-4-α-ol	**1.45**	-	-	-	58.846	Selin-11-en-4-α-ol	**1.12**
57.551	Isospathulenol	0.97	59.854	Isospathulenol	0.06	59.345	Isospathulenol	1.94
58.435	epi-α-Bisabolol	0.04	-	-	-	-	-	-
59.661	Sesquiterpineol	0.63	-	-	-	59.661	Sesquiterpineol	0.46
Others		**33.53 %**			**35.49 %**			**31.20 %**
8.284	(3*Z*)-Hexenol	0.07	9.162	(3*Z*)-Hexenol	0.07	9.162	(3*Z*)-Hexenol	0.07
-	-	-	10.992	,5-Diethyl tetrahydro furan	0.01	15.525	1-Octen-3-ol	0.02
14.112	1-Octen-3-ol	0.02	15.525	1-Octen-3-ol	0.02	21.887	n-Nonanal	**0.02**
-	-	-	16.577	3-Octanol	0.01	-	-	-

28.188	Methyl chavicol	0.01	-	-	-	-	-	-
34.214	Dihydroedulan	0.03	-	-	-	-	-	-
38.943	Eugenol	**32.15**	**41.183**	Eugenol	**34.95**	41.183	Eugenol	**26.15**
-	-	-	-	-	-	60.572	Sesquiterpinyl alcohol	5.16
-	-	-	-	-	-	67.814	Phytone	0.03
21.887	n-Nonanal	0.02	-	-	-	68.733	Neophytadiene	0.10
43.876	Isobazzanene	0.08	-	-	-	-	-	-
60.572	Sesquiterpinyl alcohol	1.16	-	-	-	-	-	-
66.719	Phytone	0.03	68.733	Neophytadiene	0.10	-	-	-
-	-	-	70.901	Phytadiene isomer	0.03	-	-	-
-	-	-	90.280	Tricosane	0.03	-	-	-
-	-	-	94.388	Tetracosane	0.04	-	-	-
-	-	-	98.338	Pentacosane	0.05	-	-	-
-	-	-	102.139	Hexacosane	0.04	-	-	-
-	-	-	105.826	Heptacosane	0.03		-	-
Total	**49**	**98.28**		**53**	**99.71**		**45**	**93.42**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

Table 4. 7 Chemical composition of *Ocimum americanum* essential oil.

		Composition			
		Thankot (Winter)		**Thankot (Summer)**	
RT	**Components**	**(%)**	**RI**	**Components**	**(%)**
Monoterpene hydrocarbons		**11.35%**			**6.64 %**
11.304	α-Thujene	0.03	-	-	-
11.682	α-Pinene	0.35	12.937	α-Pinene	0.19
12.533	Camphene	**1.44**	13.873	Camphene	0.72
13.735	Sabinene	0.09	15.155	Sabinene	0.14
14.013	β-Pinene	0.38	15.471	β-Pinene	0.24
14.642	Myrcene	0.58	16.090	Myrcene	0.37
15.636	α-Phellandrene	0.12	-	-	-
16.273	α-Terpinene	0.06	-	-	-
16.741	*p*-Cymene	0.18	18.360	*p*-Cymene	0.69
17.067	Limonene	**4.40**	18.697	Limonene	3.96
17.140	β-Phellandrene	0.04	18.780	β-phellandrene	0.02
17.430	(*Z*)- β-Ocimene	0.12	-	-	-
18.108	(*E*)- β-Ocimene	**2.43**	19.726	(*E*)-β-Ocimene	0.28
18.828	γ-Terpinene	0.25	-	-	-
20.585	Terpinolene	0.88	22.354	Terpinolene	0.03
Oxygenated monoterpenoids		**68.57%**			**82.38%**
17.201	1,8-Cineol	0.31	18.862	1,8-Cineol	0.31
18.409	Dihydro tagetone	0.15	20.075	Dihydro tagetone	0.09
19.612 **-**	*cis*-Sabinene hydrate **-**	1.20	21.388	*cis*-Sabinene hydrate	1.30
21.728	Linalool	**9.91**	23.587	Linalool	9.72
23.359	α-Campholenal	0.18	-	-	-
25.016	Camphor	**51.30**	27.083	Camphor	**65.88**
-	-	-	27.333	*trans*-β-Terpineol	0.41
25.290	Camphene hydrate	0.30	-	-	-
25.340	Menthone	0.04	-	-	-
25.742	Pinocarvone	0.16	-	-	-
26.113	exo-Acetoxy camphene	0.06	-	-	-
26.382	Borneol	0.46	28.416	Borneol	0.26
27.006	Terpinen-4-ol	**1.50**	29.020	Terpinen-4-ol	1.64
-	-	-	29.426	*p*-Cymen-8-ol	0.28
28.041	α-Terpineol	2.54	30.048	α-Terpineol	1.60
28.186			30.107	Myrtenol	0.73
28.522	epi-Borneol	0.18	30.549	epi-Borneol	0.08
26.868	*p*-1,8-Menthadien-4-ol	0.10	28.866	*p*-1,8-Menthadien-4-ol	0.13
-	-	-	33.316	Carvone	0.08
-	-	-	40.759	Terpenediol	0.04
Sesquiterpene hydrocarbons		**18.54%**			**6.75%**
39.925	α-Copaene	0.56	42.086	α-Copaene	0.29
40.427	β-Bourbonene	0.20	42.611	β-Bourbonene	0.13
40.712	β-Cubebene	0.16	**42.870**	β-Cubebene	**0.10**
40.834	(E)-β–Elemene	0.38	42.976	(E)-β-Elemene	0.19
42.758	β–Caryophyllene	**6.35**	44.970	β–Caryophyllene	3.97

43.337	β-Copaene	0.11	45.548	β-Copaene	0.03
44.914	α--Humulene	0.37	47.163	α-Humulene	0.27
46.603	Germacrene D	**7.75**	48.772	Germacrene D	1.38
47.203	*iso*-bicyclogeramcrene	1.60	49.305	*iso*-bicyclogeramcrene	0.30
47.399	Bicyclogermacrene	0.59	49.644	Bicyclogermacrene	0.04
47.606	α-Muurolene	0.08	-	-	-
48.471	γ-Cadinene	0.05	-	-	-
48.795	δ-Cadinene	0.34	50.997	δ-Cadinene	0.05
Oxygenated sesquiterpenoids		**0.57%**			**2.58 %**
-	-	-	52.997	Isocaryphyllene oxide	0.09
-	-	-	54.532	Spathulenol	0.29
52.489	Caryophyllene oxide	0.24	54.856	Caryophyllene oxide	1.91
-	-	-	56.437	Humulene epoxide II	0.07
-	-	-	58.102	*allo*-Aromadendrene epoxide	0.16
53.432	(*E*)-β-Elemenone	0.04	-	-	-
55.956	*epi*-α-Cadinol	0.11	-	-	-
56.066	*epi*-α -Muurolol	0.08	-	-	-
56.701	α-Cadinol	0.10	59.009	α-Cadinol	0.06
Others		**0.96 %**			**1.49 %**
8.280	(3*Z*)-Hexenol	0.11	9.145	(3*Z*)-Hexenol	0.27
-	-	-	10.400	2-Butyl furan	0.04
-	-	-	14.562	Benzaldehyde	0.03
14.105	1-Octen-3-ol	0.10	15.510	1-Octen-3-ol	0.07
15.078	3-Octanol	0.15	16.560	3-Octanol	0.06
15.540	Hexenyl acetate	0.10	-	-	-
21.902	n-Nonanal	0.06	-	-	-
	Methyl chavicol	0.13	-	-	-
-	-	-	29.261	4-Methyl acetophenone	0.06
27.431	*p*-Cymen-8-ol	0.06	-	-	-
27.730	Methyl salicylate	0.07	29.739	Methyl salicylate	0.06
30.372	(3Z)-Hexenyl 2-methyl butanoate	0.04		-	-
38.370	Eugenol	0.21	-	-	-
-	-	-	39.897	2-Methyl-2-(para-tolyl) propionaldehyde	0.18
-	-	-	59.799	Dihydro germacrene D	0.12
46.194	*trans*-Cadina-1(6),4-diene	0.06	-	-	-
51.903	3-cis-Hexenyl benzoate	0.05	-	-	-
-	-	-	90.237	*n*-Tricosane	0.05
-	-	-	94.344	*n*-Tetracosane	0.07
-	-	-	98.294	*n*-Pentacosane	0.08
-	-	-	102.097	*n*-Hexacosane	0.07
-	-	-	105.780	*n*-Heptacosane	0.06
Total	**60**	**99.89**		**57**	**99.84**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). “–“ = not detected; % = percent composition. **RT:** identification based on retention time.

Table 4. 8 Chemical composition of *Ocimum basilicum* essential oil.

				Composition	
	Kapilvast (Summer)			**Kapilvast(Winter)**	
RT	**Components**	**(%)**	**RT**	**Components**	**(%)**
Monoterpene hydrocarbons		**0.71 %**			**0.3 %**
12.342	α-Thujene	0.02	12.516	α-Thujene	0.02
12.757	α-Pinene	0.19	12.945	α-Pinene	0.06
14.956	Sabinene	0.03	15.155	Sabinene	0.03
15.261	β-Pinene	0.20	15.471	β-Pinene	0.04
15.890	Myrcene	0.04	16.119	Myrcene	0.04
18.423	Limonene	0.02	18.635	Limonene	0.05
-	-	-	19.046	*(Z)*-β-Ocimene	0.02
19.508	*(E)*-β-Ocimene	0.21	19.734	*(E)*-β-Ocimene	0.04
Oxygenated monoterpenoids		**28.09 %**			**28.65 %**
18.604	1,8-Cineol	0.05	18.918	1,8-Cineol	0.60
19.857	Dihydro tagetone	0.12	29.075	Dihydro tagetone	0.03
21.136	*cis*-Linalool oxide (furanoid)	0.13	21.136	*cis*-Linalool oxide (furanoid)	0.14
22.216	*trans*-Linalool oxide (furanoid)	0.13	22.216	*trans*-Linalool oxide (furanoid)	0.18
-	-	-	22.358	Fenchone	0.02
23.450	Linalool	**26.92**	23.327	Linalool	**27.05**
25.279	Limona ketone	0.02	25.678	Limona ketone	0.03
29.825	α-Terpineol	0.01	-	-	-
32.655	Neral	0.24	32.655	Neral	0.28
33.593	Chavicol	0.02	33.693	Chavicol	0.01
-	-	-	33.810	*p*-Anis aldehyde	0.02
34.661	Geranial	0.31	34.566	Geranial	0.11
35.929	*(E)*-Anethole	0.12	35.929	*(E)*-Anethole	0.16
40.220	Eugenol	0.02	40.495	Eugenol	0.02
Sesquiternepe hydrocarbons		**5.6 %**			**4.09 %**
41.797	α-Copaene	0.08	42.086	α-Copaene	0.08
42.578	β-Cubebene	0.05	42.870	β-Cubebene	0.09
42.690	*trans*-β-Elemene	0.07	42.998	*trans*-β-Elemene	0.07
43.357	Methyl Eugenol	0.02	43.357	Methyl Eugenol	0.11
43.820	α-Gurjunene	0.02	43.820	α-Gurjunene	0.02
44.621	β-Caryophyllene	0.79	45.245	β-Caryophyllene	0.24
45.446	*trans*-α-Bergamotene	0.58	45.446	*trans*-α-Bergamotene	0.68
45.909	(*Z*)-β-Farnesene	0.08	46.064	(*Z*)-β-Farnesene	0.12
46.665	*(E)*-β-Farnesene	0.25	47.018	*(E)*-β-Farnesene	0.14
46.856	α-Humulene	0.13	47.297	α-Humulene	0.44
47.641	*epi*-Caryophyllene	0.03	47.831	*epi*-Caryophyllene	0.03
48.463	Germacrene D	**0.79**	48.826	Germacrene D	0.14
48.607	*trans*-β-Bergamotene	0.10	-	-	-
49.334	Bicyclogermacrene	0.09	49.684	Bicyclogermacrene	0.03
50.073	β-Bisabolene	0.10	50.122	β-Bisabolene	0.02
50.705	δ-Cadinene	0.04	51.066	δ-Cadinene	0.04

52.071	*trans*-γ-Bisabolene	2.38	52.071	*trans*-γ-Bisabolene	1.84
Oxygenated sesquiterpenes		**0.44 %**			**1.06 %**
50.392	Sesquicineole	0.01	-	-	-
53.250	*(E)*-Nerolidol	0.07	53.573	*(E)*-Nerolidol	0.07
54.220	Germacrene D-4-ol	0.04	54.256	Germacrene D-4-ol	0.02
54.505	Caryophyllene oxide	0.22	54.852	Caryophyllene oxide	0.64
56.120	Humulene epoxide II	0.02	-	-	-
58.703	β-Eudesmol	0.02	59.043	β-Eudesmol	0.21
60.389	α-Bisabolol	0.06	61.089	α-Bisabolol	0.12
Others		**64.9 %**			**62.49 %**
9.085	*(2E)*--Hexenal	0.01	-	-	-
14.409	Benzaldehyde	0.01	14.562	Benzaldehyde	0.05
15.626	6-Methyl-5-hepten-2-one	0.04	15.726	6-Methyl-5-hepten-2-one	0.01
16.767	n-Octanal	0.11	16.767	n-Octanal	0.16
16.844	Hexenyl acetate	0.03	16.844	Hexenyl acetate	0.03
23.552	6-Methyl-3,5-heptadien-2-one	0.01	23.552	6-Methyl-3,5-heptadien-2-one	0.01
23.660	1-Octen-3-yl acetate	0.01	-	-	-
30.475	Methyl chavicol	**64.42**	30.188	Methyl chavicol	**62.16**
30.777	Octyl acetate	0.06	30.676	Octyl acetate	0.01
42.310	*(3Z)*--Hexenyl-(3Z)-hexenoate	0.15	42.410	(3*Z*)-Hexenyl-(3*Z*)-hexenoate	0.04
53.696	(*E*)-*p*-Methoxy cinnamaldehyde	0.05	53.710	(*E*)-*p*-Methoxy cinnamaldehyde	0.02
Total	**54**	**99.74**		**51**	**96.59**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

4.1.4.5 The Genus *Perilla*

The GC-MS analyses of hydro-distilled essential oils of three *P. frutescens* samples revealed the identification of 50 to 54 chemical components, representing 91.98 to 99.67% of the oils, respectively (Table 4.9). *P. frutescens* essential oil was characterized by the most dominant component, perilla ketone (42.26 to 56.26%). Other important components in *P. frutescens* were found to be isoegomaketone (23.68-23.85%), β-caryophyllene (1.33-7.33%), isobicyclo geramcrene (3.29-5.27%), and apiole (9.16%).

All three samples of *P. frutescens* essential oil were dominated by their oxygenated monoterpene fraction, and the contents were about 69.46 to 83.05%. Perilla ketone and isoegomaketone were the major oxygenated monoterpene compounds in this EO. The *P. frutescens* also contained a considerable proportion of sesquiterpene hydrocarbons (7.41 to 15.42%), and the main sesquiterpene hydrocarbons were β-caryophyllene and isobicyclogermacrene, respectively, whereas this essential oil had very low amounts of monoterpene hydrocarbons and oxygenated sesquiterpene. Annex 10 shows the GC-MS chromatogram of *P. frutescens* EO, displaying the separation of chemical components.

These results show some sort of similarity with the previous findings of Ahmed and Al-Zubaidy, who also identified perilla ketone (0.17-97.9%), perilla aldehyde (0.45-82.15%), and β-dehydro-elsholtzia ketone (58.03-67.75%) as the major components in *P. frutescens* from Iraq (Ahmed & Al-Zubaidy, 2020). Similarly, *P. frutescens* from England revealed limonene (14.21%), perilla aldehyde (50.64%), and *trans*-caryophyllene (20.58%) as the most abundant components, dominating oxygenated monoterpenes (Eldeghedy *et al*., 2022). However, the present findings of the research work are in agreement with those investigated by Gwari *et al*. who reported perilla ketone (44.7-69.2%), isoegomaketone (7.3-27.6%), *trans*-caryophyllene (0.1-17.8%), and linalool (0.3-5.0%) as the most abundant volatile compounds from India (Gwari, 2015). In contrast to the above results, Huong revealed that myristicin (43.90%), elemicin (28.79%), α-caryophyllene (8.33%), perillaldehyde (7.97%), (*Z, E*)-farnesene (2.81%) and limonene (1.04%) were major components in *P. frutescens* EO from Vietnam (Huong *et al*., 2020). Hu *et al*. reported that 1-(2-furyl)-1-hexanone (25.79%), amylphenol (20.24%), apiol (10.13%), and o-xylene (9.33%) were identified as major components in *P. frutescens* from China (Hu *et al*., 2014).

Table 4. 9 Chemical composition of *Perilla frutescens* essential oil.

				Composition				
	Kavre (Summer)			**Kavre (Winter)**			**Bardiya (Summer)**	
RT	**Components**	**(%)**	**RT**	**Components**	**(%)**	**RT**	**Components**	**(%)**
Monoterpene hydrocarbons		**0.09 %**			**0.51 %**			**0.32 %**
-	-	-	12.957	α-Pinene	0.05	12.657	α-Pinene	0.05
13.760	Sabinene	0.02	15.181	Sabinene	0.04	14.980	Sabinene	0.04
14.037	β-Pinene	0.05	15.500	β-Pinene	0.05	15.400	β--Pinene	0.05
-	-	-	16.114	Myrcene	0.04	-	-	-
17.050	Limonene	0.02	18.676	Limonene	0.31	18.447	Limonene	0.18
-	-	-	20.556	γ-Terpinene	0.02	-	-	-
Oxygenated monoterpenoids		**76.59 %**			**69.46 %**			**83.05 %**
-	-	-	18.872	1,8-Cineol	0.01	-	-	-
18.433	Dihydro tagetone	0.04	20.096	Dihydro tagetone	0.02	20.096	Dihydro tagetone	0.06
21.591	Linalool	1.23	23.390	Linalool	0.86	23.390	Linalool	1.86
-	-	-	27.277	Menthone	0.04	26.277	Menthone	0.04
-	-	-	27.718	Pinocarvone	0.02	27.718	Pinocarvone	0.02
28.004	α-Terpineol	0.05	30.010	α-Terpineol	0.02	30.010	α-Terpineol	0.02
-	-	-	30.188	Methyl chavicol	0.02	28.605	Methyl salicylate	0.10
32.320	Perilla ketone	**50.79**	34.359	Perilla ketone	**42.26**	34.359	Perilla ketone	**56.26**
-	-	-	34.485	*trans*-Piperitone epoxide	0.02	34.985	*trans*-Piperitone epoxide	0.02
-	-	-	36.720	Egomaketone	1.96	37.708	*iso*-egomaketone	**24.40**
36.530	Methyl geranate	0.11	38.606	Methyl geranate	0.12	38.606	Methyl geranate	0.21
35.519	*iso*-egomaketone	**23.85**	37.708	*iso*-egomaketone	**23.68**	-	-	-
38.472	Eugenol	0.60	40.623	Eugenol	0.50	40.473	Eugenol	0.30
-	-	-	41.188	Piperitenone oxide	0.05	-	-	-
Sesquiternepe hydrocarbons		**13.39 %**			**15.42 %**			**7.41 %**
37.376	δ-Elemene	0.07	39.502	δ-Elemene	0.04	39.606	δ-Elemene	0.09
39.967	α-Copaene	0.10	42.144	α-Copaene	0.04	42.356	α-Copaene	0.14
40.187	*trans*-β-Damascenone	0.03	42.356	*trans*-β-Damascenone	0.01	42.456	*trans*-β-Damascenone	0.05
40.474	β-Bourbonene	0.11	42.672	β-Bourbonene	0.03	42.674	β-Bourbonene	0.13
40.882	*trans*-β-Elemene	0.19	43.041	*trans*-Elemene	0.12	43.041	*trans*-β-Elemene	0.12
41.796	*(Z)*-Caryophyllene	0.02	-	-	-	-	-	-
42.822	β-Caryophyllene	**6.56**	45.113	β-Caryophyllene	7.33	45.343	β-Caryophyllene	1.33
43.379	γ-Elemene	0.05	45.567	γ-Elemene	0.02	45.467	γ-Elemene	0.07

44.100	6,9-Guaiadiene	0.03	46.310	6,9-Guaiadiene	0.01	46.510	6,9-Guaiadiene	0.01
-	-	-	47.018	*(E)*-β-Farnesene	0.73	-	-	-
44.970	α-Humulene	0.88	47.242	α-Humulene	0.83	47.242	α-Humulene	0.90
46.563	Germacrene D	1.19	48.826	Germacrene D	0.75	48.726	Germacrene D	0.78
47.280	Isobicyclogeramcrene	3.73	49.477	Isobicyclogeramcrene	5.27	49.677	Isobicyclogeramcrene	3.29
48.047	*(E,E)*-α-Farnesene	0.24	50.197	*(E,E)*-α-Farnesene	0.18	50.297	*(E,E)*-α-Farnesene	0.28
48.832	δ-Cadinene	0.14	51.066	δ-Cadinene	0.06	51.166	δ-Cadinene	0.22
51.162	Germacrene B	0.05	-	-	-	-	-	-
Oxygenated sesquiterpenoids		**1.09 %**			**10.02 %**			**1.2 %**
51.379	*trans*-Nerolidol	0.24	53.576	*trans*-Nerolidol	0.23	53.456	*trans*-Nerolidol	0.23
52.531	Caryophyllene oxide	0.46	54.872	Caryophyllene oxide	0.31	54.664	Caryophyllene oxide	0.51
-	-	-	55.008	6-Methoxy elemicin	0.03	-	-	-
53.464	*trans*-β-Elemenone	0.13	55.641	*trans*-β-Elemenone	0.19	55.941	*trans*-β-Elemenone	0.11
54.135	Humulene epoxide II	0.05	56.485	Humulene epoxide II	0.03	56.485	Humulene epoxide II	0.07
55.203	iso-Spathulenol	0.04	-	-	-	56.860	iso-Spathulenol	0.04
55.990	epi-α-Cadinol	0.03	-	-	-	57.212	epi-α-Cadinol	0.06
56.100	epi-α-Muurolol	0.03	-	-	-	57.435	epi-α-Muurolol	0.05
56.739	α-Cadinol	0.11	-	-	-	58.066	α-Cadinol	0.13
-	-	-	60.349	Apiole	9.16	-	-	-
Others		**8.51 %**			**2.46 %**			
8.246	(2*E*)-Hexenal	0.04	9.115	(2*E*)-Hexenal	0.03	9.115	(2*E*)-Hexenal	0.06
8.296	(3*Z*)-Hexanol	0.06	9.161	(3*Z*)-Hexenol	0.08	9.461	(3*Z*)-Hexenol	0.09
8.799	n-Hexanol	0.02	9.710	n-Hexanol	0.02	-	-	-
			9.563	(2*E*) -Hexenol	0.04	10.563	(2*E*)- -Hexenol	0.04
13.183	Benzaldehyde	0.28	14.629	Benzaldehyde	1.30	14.629	Benzaldehyde	1.10
14.143	1-Octen-3-ol	0.83	15.563	1-Octen-3-ol	0.52	15.563	1-Octen-3-ol	0.62
15.099	3-Octanol	0.09	16.578	3-Octanol	0.09	15.865	3-Octanone	0.06
-	-	-	15.850	6-Met-5-hepten-2-one	0.01	-	-	-
-	-	-	-	3-Octanone	0.02	-	-	-
17.949	Benzene acetaldehyde	0.02	-	-	-	18.860	Benzene acetaldehyde	0.02
21.906	*n*-Nonanal	0.05	-	-	-	23.680	*n*-Nonanal	0.05
22.889	5-hydroxy-4,6-dimethyl-6-Hepten-3-one	0.02	-	-	-	-	-	-
25.124	2-*trans*-6-cis-Nonadienal	0.02	-	-	-	25.124	(2*E*,6*Z*) -Nonadienal	0.02
27.749	Methyl salicylate	0.05	-	-	-			
28.834	1-(3-furanyl)-4-methyl-2-Pentanone	0.59	-	-	-	30.335	1-(3-furanyl)-4-methyl-2-Pentanone	0.69

3.32

30.207	1-(3-furanyl)-4-methyl-2-Penten-1-one	3.89	-	-	-	30.680	1-(3-furanyl)-4-methyl-2-Penten-1-one	
34.656	1H-Pyrazole-4-carboxylic acid	2.32	-	-	-	34.656	1H-Pyrazole-4-carboxylic acid	
-	-	-	48.612	*(E)*-β-Ionone	0.02	-	-	-
49.706	Dodec-5-en-11-olide	0.04	-	-	-	-	-	-
51.940	(3Z) -Hexenyl benzoate	0.03	-	-	-	-	-	-
-	-	-	51.169	Myristicin	0.12	-	-	-
-	-	-	52.721	Elemicin	0.06	-	-	-
-	-	-	53.731	Isoelemicin	0.03	-	-	-
-	-	-	56.905	Dill apiole	0.07	-	-	-
95.899	Pentacosane	0.05	-	-	-	95.899	Pentacosane	0.06
103.319	Heptacosane	0,03	-	-	-	103.319	Heptacosane	0.05
Total	**52**	**99.67**		**54**	**97.87**		**50**	**91.98**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

4.1.4.6 The Genus *Pogostemon*

Table 4.10 shows the chemical composition of *P. glaber* essential oil. The GC-MS analysis confirmed the presence of 40 compounds, representing 83.81% of the total essential oil. Germacrene D (16.70%), curzerenone (12.58%), δ-elemene (10.93%), curzerenone B (9.76%), curzerene (5.17%), and germacrene B (3.59%) were the major compounds found in *P. glaber*. As shown in Table 4.10, the *P. glaber* EO sample was found to be rich in sesquiterpene hydrocarbons (43.64%), followed by oxygenated sesquiterpenes (37.52%), oxygenated monoterpenes (2.19%), and monoterpene hydrocarbons (0.35%). The major sesquiterpene hydrocarbons were germacrene D, δ-elemene, germacrene B, and bicycle-germacrene. The main oxygenated sesquiterpenes were curzerenone, curzerenone B, and curzerene. Besides these compounds, *P. glaber* also contained substantial amounts of several other minor constituents. The GC-MS chromatogram of *P. glaber* essential oil displays the separation of volatile chemical components, which is shown in Annex 10.

There were no detailed previous studies reported on the composition of *P. glaber* essential oil. However, *P. glaber* was dominated by sesquiterpene hydrocarbons analogous to other species of the *Pogostemon* genus (Satyal *et al*., 2018; Srivastava *et al*., 2022). But it is remarkable to observe that *P. glaber* mainly contained sesquiterpene hydrocarbons, followed by oxygenated sesquiterpenes in exceptional contrast to the other Lamiaceae essential oils.

Table 4. 10 Chemical composition of *Pogostemon glaber* essential oil.

RT	Composition	
	Components	**Area (%)**
Monoterpene hydrocarbons		**0.35 %**
14.021	β-Pinene	0.05
18.089	*(E)*-β-Ocimene	0.30
Oxygenated monoterpenoids		**0.36**
21.541	Linalool	0.36
Sesquiternepe hydrocarbons		**43.64 %**
37.109	Bicylcoelemene	1.83
37.456	*trans*-δ-Elemene	**10.93**
38.873	α-Cubebene	0.10
40.464	β-Bourbonene	1.70
40.626	α-Bourbonene	0.10
40.724	β-Cubebene	0.26
40.848	β-Elemene	0.74
42.730	β-Caryophyllene	2.67
43.376	γ-Elemene	2.05
44.236	Aromadendrene	0.15
44.929	α-Humulene	0.42
45.722	*trans*-Cadina-1(6),4-diene	0.10
46.719	Germacrene D	**16.70**
46.928	*cis*-β-Guaiene	0.11
47.515	Bicyclogermacrene	3.22
47.645	α-Muurolene	0.16
48.033	*(E,E)*-α-Farnesene	0.64
51.517	Germacrene B	3.59
Oxygenated sesquiterpenoids		**37.52 %**
47.436	Curzerene	**5.17**
48.624	epi-Cubebol	0.27
48.812	δ-Cadinene	0.39
49.937	*(Z)*-Nerolidol	0.27
52.240	Germacrene D-4-ol	1.09
52.516	Caryophyllene oxide	0.16
52.984	Spathulenol	0.11
53.832	Curzerenone	**12.58**
54.408	Isocurzerenone	1.82
55.112	Ledol isomer	0.22
55.832	*iso*-Spathulenol	0.42
56.108	*epi*-α-Muurolol	0.16
56.739	α-Cadinol	0.51
58.303	Khusinol	0.88
63.992	Curzerenone A	3.59
64.743	Curzerenone B	**9.76**
Others		**0.11 %**
8.290	(3*Z*)-Hexenol	0.04
14.105	1-Octen-3-ol	0.07
Total	**40**	**83.81**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

4.1.4.7 The Genus *Colebrookea*

C. oppositifolia essential oil showed an extensively diversified chemical composition (Table 4.11). Two samples of *C. oppositifolia* were identified as having 46 and 48 chemical constituents, representing 87.8% to 83.8% of total EO. They were dominant in geranyl-α-terpinene (31.90%) and β-caryophyllene (22.16%) in one sample, while β-Caryophyllene (39.99%) and geranyl-α-terpinene (27.79%) in another sample. *C. oppositifolia* essential oil contained a considerable quantity of sesquiterpene hydrocarbons (26.67 to 47.51%) and oxygenated sesquiterpenes (4.5 to 15.92 %). However, this essential oil had very low amounts of monoterpene hydrocarbons (0.31 to 1.22%), and oxygenated sesquiterpenes (0.89 to 1.92%), unlike the other Lamiaceae essential oils. The major sesquiterpene hydrocarbons were β-caryophyllene (22.16 to 39.99%) and α-humulene (4.22 to 7.11%). Tetrahydroionyl acetate isomer II (10.41%) and caryophyllene oxide (3.61-4.72%) were the main oxygenated sesquiterpene compounds in these samples. The GC-MS chromatogram of *C. oppositifolia* essential oil displays the separation of several volatile chemical components (Annex 10).

The literature review showed very few results reported on the composition of *C. oppositifolia* essential oils. However, *C. oppositifolia* from India was dominated by δ-cadinene (24.38%), β-sesquiphellandrene (16.15%), and torreyol (15.93%) as the main compounds (Lal *et al.*, 2022). Moreover, *C. oppositifolia* essential oil from Nepal exhibited β-caryophyllene and geranyl-α-terpinene as major constituents, which were not reported in the previous studies.

Table 4. 11 Chemical composition of *Colebrookea oppositifolia* essential oil form different geographical regions.

	Composition				
	Kathmandu (Winter)			**Kavre (Summer)**	
RT	**Components**	**(%)**	**RT**	**Components**	**(%)**
Monoterpene hydrocarbons		**0.31 %**			**1.22 %**
11.683	α-Pinene	0.22	12.941	α-Pinene	0.11
-	-	-	13.872	Camphene	0.02
-	-	-	15.160	Sabinene	0.10
14.011	β-Pinene	0.05	15.476	β-Pinene	0.19
-	-	-	16.092	Myrcene	0.07
-	-	-	17.853	α-Terpinene	0.05
-	-	-	18.350	*p*-Cymene	0.04
17.020	Limonene	0.04	18.651	Limonene	0.34
-	-	-	18.760	β-Phellandrene	0.01
-	-	-	19.031	*cis*-β-Ocimene	0.01
-	-	-	19.727	*(E)*-β-Ocimene	0.02
-	-	-	20.534	γ-Terpinene	0.07
-	-	-	22.356	Terpinolene	0.02
Oxygenated monoterpenoids		**1.92 %**			**0.89 %**
-	-	-	18.849	1,8-Cineol	0.03
18.400	Dihydro tagetone	0.06	20.075	Dihydro tagetone	0.05
21.581	Linalool	1.52	23.327	Linalool	0.22
-	-	-	26.642	Camphor	0.03
-	-	-	27.247	Menthone	0.06
-	-	-	27.687	Pinocarvone	0.05
-	-	-	28.951	Terpinen-4-ol	0.03
32.435	Geraniol	0.20	34.424	Geranial	0.06
Sesquiternepe hydrocarbons		**26.67 %**			**47.51 %**
40.844	*trans*-β-Elemene	0.12	42.998	*trans*-β-Elemene	0.17
-	-	-	43.979	*(Z)*-Caryophyllene	0.13
41.754	β-Longipinene	0.10	-	-	-
42.958	β-Caryophyllene	**22.16**	45.245	β-Caryophyllene	**39.99**
45.014	α-Humulene	**4.22**	47.298	α-Humulene	**7.11**
48.792	β-Cadinene	0.07	-	-	-
-	-	-	51.010	δ-Cadinene	0.11
Oxygenated sesquiterpenoids		**15.92 %**			**4.15**
-	-	-	48.572	*(E)*-β-Ionone	0.12
47.418	*epi*-Cubebol	0.11	49.670	*epi*-Cubebol	0.12
51.255	Tetrahydroionyl acetate isomer II	**10.41**	-	-	-
52.616	Caryophyllene oxide	**4.72**	54.905	Caryophyllene oxide	**3.61**
54.121	Humulene epoxide II	0.43	56.454	Humulene epoxide II	0.30
55.668	Caryophylla-4(12),8(13)-dien-5-beta-ol	0.11	-	-	-
67.995	*(Z)*-Lanceol acetate	0.91	-	-	-
Others		**42.98 %**			**33.68 %**
8.765	*n*-Hexanol	0.04	-	-	-
9.889	Cyclohexanone	0.04	-	-	-

14.101	1-Octen-3-ol	0.24	-	-	-
15.071	3-Octanol	0.21	-	-	-
19.267	Acetophenone	0.07	21.024	Acetophenone	0.06
-	-	-	30.156	Methyl chavicol	0.05
34.192	Dihydroedulan	0.07	-	-	-
34.858	*cis*-Theaspirane	0.05	-	-	-
35.931	*trans*-Theaspirane	0.07	-	-	-
38.369	Eugenol	0.07	40.495	Eugenol	0.22
44.513	Geranyl acetone	0.12	-	-	-
45.396	Tetramethyl 4,6,8,10-Tridecane	0.63	-	-	-
46.385	*(E)*-β-Ionone	0.08	-	-	-
60.087	Pentadecanal	0.14	-	-	-
66.725	Phytone	0.76	68.959	Phytone	0.08
68.957	*(Z)*-Hexadecatrienal	0.12	-	-	-
69.195	*(E)*-Hexadecantrienal	0.63	-	-	-
71.326	Bioformene	0.11	-	-	-
-	-	-	74.180	δ-Iraldiene	0.11
72.267	Geranyl-α-terpinene	**31.90**	74.552	Geranyl-α-terpinene	**27.79**
73.311	Kolavenol	0.58	-	-	-
73.405	Pentylcurcumene	0.39	75.180	Pentylcurcumene	**3.24**
73.614	*cis-cis*-Geranyl linalool	**2.03**	-	-	-
-	-	-	75.947	*trans-cis*-Geranyl linalool	1.00
-	-	-	76.675	*(Z,E)*-Geranyl linalool	0.18
74.307	*p*-Camphorene	0.13	-	-	-
77.962	Manool	2.28	-	-	-
78.561	Annonene	0.32	-	-	-
80.610	*cis*-Abienol	0.41	83.099	*cis*-Abienol	0.13
81.899	Neryl linalool isomer	0.39	-	-	-
-	-	-	85.969	Docosane	0.07
-	-	-	94.356	Tetracosane	0.20
87.867	Tricosane	0.17	90.256	Tricosane	0.20
-	-	-	98.311	Pentacosane	0.23
-	-	-	102.114	Hexacosane	0.21
103.295	Heptacosane	0.26	105.797	Heptacosane	0.23
Total	**46**	**87.8**		**48**	**83.3**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). “–“ = not detected; % = percent composition. **RT:** identification based on retention time.

4.1.4.8 The Genus *Colquhounia*

The GC-MS analysis of the essential oils of two *Colquhounia coccinea* samples revealed the identification of 54 to 57 chemical components, representing 96.6 to 98.48% of the total EOs, respectively (Table 4.12). *C. coccinea* EO was characterized by the most abundant component of isocaryophyllene (28.17 to 32.17%). Other vital components in *C. coccinea* EO were found to be β-caryophyllene (19.76-20.77%), germacrene D (9.85-12.85%), kolavelool (7.54-8.44%), and α-isocomene (2.66-3.65%).

The samples of *C. coccinea* essential oil were dominated by the sesquiterpene hydrocarbon fraction. The contents of this fraction were about 76.88 to 79.91%. *C. coccinea* also exhibited a considerable proportion of oxygenated sesquiterpenes (5.48 to 6.01%), and the main oxygenated sesquiterpenes were caryophyllene oxide and *allo*-aromadendrene epoxide, whereas *C. coccinea* had very low amounts of monoterpene hydrocarbons and oxygenated monoterpenes. The GC-MS chromatogram of *C. coccinea* essential oil displays the separation of several volatile chemical components (Annex 10).

There are very few reports in the literature regarding the chemical composition of *C. coccinea* essential oil. However, current results showed some similarities with previous reports published in the literature. *C. coccinea* from India reported that β-caryophyllene (44.1%), germacrene D (15.8%), cadina-1-4-diene (11.1%), α-humulene (8.7%), and caryophyllene oxide (5.7%) were the major constituents, which showed some similarity to our results (Bhatt *et al.*, 2009).

Table 4. 12 Chemical composition of *Colquhounia coccinea* essential oils from different geographical regions.

	Composition				
	Rasuwa (Winter)			**Kavre (Winter)**	
RT	**Components**	**(%)**	**RT**	**Components**	**(%)**
Monoterpene hydrocarbons		**0.24 %**			**0.21 %**
11.683	α-Pinene	0.12	11.680	α-Pinene	0.16
14.037	β-Pinene	0.02	14.009	β-Pinene	0.05
Oxygenated monoterpenoids		**2.48 %**			**2.64 %**
-	-	-	34.437	Thymol	0.50
21.591	Linalool	0.55	21.556	Linalool	0.75
28.004	α-Terpineol	0.05	27.957	α-Terpineol	0.07
29.560	β-Cyclocitral	0.05	29.551	β-Cyclocitral	0.09
33.864	Isothymol	0.87	33.864	Isothymol	0.77
34.192	Dihydroedulan	0.62	34.206	Dihydroedulan	0.32
34.437	Thymol	0.20	-	-	-
Sesquiternepe hydrocarbons		**76.88 %**			**79.91 %**
34.885	*cis*-Theaspirane	0.22	34.870	*cis*-Theaspirane	0.18
35.931	*trans*-Theaspirane	0.31	35.953	*trans*-Theaspirane	0.19
37.102	Bicylcoelemene	0.10	37.087	Bicylcoelemene	0.10
37.376	δ-Elemene	0.38	37.320	δ-Elemene	0.38
39.530	α-Ylangene	0.37	39.520	α-Ylangene	0.27
39.967	α-Copaene	0.86	39.939	α-Copaene	0.26
40.474	β-Bourbonene	1.02	40.497	β-Bourbonene	2.02
40.598	α-Isocomene	**3.65**	40.594	α-Isocomene	**2.66**
40.679	α-Bourbonene	0.33	40.679	α-Bourbonene	0.13
40.715	β-Cubebene	0.05	40.715	β-Cubebene	0.10
40.990	*trans*-β-Elemene	0.34	40.867	*trans*-β-Elemene	0.74
41.770	β-Longipinene	0.14	41.770	β-Longipinene	0.24
42.822	β-Caryophyllene	**19.76**	42.966	β-Caryophyllene	**20.77**
43.397	γ-Elemene	0.22	43.383	γ-Elemene	0.71
43.741	Aromadendrene	0.25	43.741	Aromadendrene	0.45
44.256	Isogermacrene	0.13	44.256	Isogermacrene	0.13
44.970	α-Humulene	**3.88**	45.005	α-Humulene	**2.88**
46.104	Isocaryophyllene	**28.17**	46.096	Isocaryophyllene	**32.17**
48.877	Germacrene D	12.85	46.751	Germacrene D	9.85
49.103	Viridiflorene	0.87	47.003	Viridiflorene	0.57
-	-	-	47.090	β-Selinene	0.15
49.504	Guaia-1(10),11-diene	**2.00**	47.504	Guaia-1(10),11-diene	**3.00**
49.842	α-Bulnesene	0.21	47.842	α-Bulnesene	0.21
50.509	α-Muurolene	0.32	48.509	α-Muurolene	0.12
50.721	γ-Cadinene		48.838	γ-Cadinene	0.98
51.167	Germacrene B	0.45	51.167	Germacrene B	0.65
Oxygenated sesquiterpenoids		**6.01 %**			**5.48 %**
50.905	Hedycaryol	0.30	50.605	Hedycaryol	0.30
52.377	*(E)*-Nerolidol	0.24	51.377	*(E)*-Nerolidol	0.24
54.268	Spathulenol	0.87	52.264	Spathulenol	0.37
54.534	Caryophyllene oxide	1.00	52.566	Caryophyllene oxide	1.79
56.437	Humulene epoxide II	0.08	54.116	Humulene epoxide II	0.18
56.582	Sesquiterpeneol	0.21	54.582	Sesquiterpeneol	0.24

57.102	Ledol	0.29	54.778	Ledol	0.29
57.006	Eremoligenol	0.46	55.406	Eremoligenol	0.16
58.102	*allo*-Aromadendrene epoxide	1.85	55.989	*allo*-Aromadendrene epoxide	0.85
58.193	epi-α-Muurolol	0.15	56.093	epi-α-Muurolol	0.12
59.009	α-Cadinol	0.16	56.730	α-Cadinol	0.16
59.536	Pogostol	0.18	56.836	Pogostol	0.48
59.980	14-Hydroxy-(*E*)-caryophyllene	0.22	58.010	14-Hydroxy-(*E*)-caryophyllene	0.30
9.880	Cyclohexanone	0.04	9.878	Cyclohexanone	0.07
14.105	1-Octen-3-ol	0.06	14.096	1-Octen-3-ol	0.16
-	-	-	21.872	n-Nonanal	0.07
38.472	Eugenol	0.14	38.378	Eugenol	0.12
45.554	Tetramethyl 4,6,8,10-tridecane	0.16	45.547	Tetramethyl 4,6,8,10-tridecane	0.11
Others		**10.99 %**			**10.24 %**
47.295	Methyl-γ-ionone	1.02	46.295	Methyl-γ-ionone	1.09
48.905	Selina-4,11-diene	0.04	46.805	Selina-4,11-diene	0.06
68.959	Phytone	0.08	66.699	Phytone	0.14
76.686	Annonene	1.15	76.681	Annonene	1.00
77.576	Kolavelool	8.44	77.570	Kolavelool	7.54
Total	**54**	**96.6**		**57**	**98.48**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

4.1.4.9 The Genus *Leucosceptrum*

The volatile chemical compounds determined in the *L.* canum essential oil during the analysis are presented in Table 4.13. The GC-MS analysis identified nearly 56 to 57 volatile chemical components, representing 97.41 to 98.75% of the total EO. The major constituents in *L. canum* were found to be α-pinene (5.11-22.11%), β-pinene (15.21-29.02%), β-caryophyllene (13.29-33.51%), germacrene D (4.2-10.81%), linalool (1.13-6.13%), and bicyclogermacrene (3.79-5.05%). *L. canum* also contained a significant amount of various minor constituents. Regarding the major classs of chemical constituents, *L. canum* essential oil mainly consisted of sesquiterpene hydrocarbons (31.09-60.08%), followed by monoterpene hydrocarbons (23.12-56.04%). Annex 10 shows the GC-MS chromatogram of *L. canum* EO, displaying the separation of several volatile chemical components. To the best of our knowledge, there have been no detailed studies reported previously regarding the essential oil composition of this genus.

Table 4. 13 Chemical composition of *Leucosceptrum canum* essential oil.

	Composition				
	Lalitpur (Winter)			**Lalitpur (Summer)**	
RT	**Components**	**(%)**	**RT**	**Components**	**(%)**
Monoterpene hydrocarbons		**56.04 %**			**23.12 %**
12.365	α-Thujene	2.01	12.494	α-Thujene	0.33
12.882	α-Pinene	**22.11**	12.940	α-Pinene	5.11
14.997	Sabinene	1.19	15.143	Sabinene	0.43
15.420	β-Pinene	**29.07**	15.510	β-Pinene	15.21
15.911	Myrcene	0.36	16.075	Myrcene	0.22
16.981	α-Phellandrene	0.40	17.171	α-Phellandrene	0.10
17.640	α-Terpinene	0.07	-	-	-
18.134	*p*-Cymene	0.22	18.330	*p*-Cymene	0.13
18.458	Limonene	2.14	18.635	Limonene	1.29
20.313	γ-Terpinene	0.17	20.519	γ-Terpinene	0.13
22.123	Terpinolene	0.31	22.335	Terpinolene	0.17
Oxygenated monoterpenoids		**1.73 %**			**7.11 %**
23.110	Linalool	1.13	23.379	Linalool	6.13
-	-	-	27.229	Menthone	0.23
-	-	-	27.668	Pinocarvone	0.07
28.682	Terpinen-4-ol	0.16	28.935	Terpinen-4-ol	0.19
29.700	α-Terpineol	0.25	29.952	α-Terpineol	0.35
-	-	-	33.296	Carvone	0.14
33.882	*cis*-Verbenyl acetate	0.14	-	-	-
Sesquiternepe hydrocarbons		**31.09 %**			**60.08 %**
-	-	-	36.995	*cis*-Theaspirane	0.12
-	-	-	38.072	*trans*-Theaspirane	0.12
38.920	δ-Elemene	0.48	39.197	δ-Elemene	0.23
-	-	-	41.667	α-Ylangene	0.56
41.799	α-Copaene	0.24	42.085	α-Copaene	0.22
-	-	-	42.497	*cis*-β-Elemene	0.18
42.333	β-Bourbonene	0.96	42.618	β-Bourbonene	0.92
-	-	-	43.008	*trans*-β-Elemene	3.57
-	-	-	44.122	α-Gurjunene	0.12
42.722	*trans*-β-Elemene	1.89	-	-	-
43.828	β-Maaliene	0.43	-	-	-
44.761	β-Caryophyllene	**13.29**	45.094	β-Caryophyllene	33.51
45.262	β-Copaene	0.17	-	-	-
45.440	*trans*-α-Bergamotene	0.16	-	-	-
-	-	-	45.563	β-Copaene	0.14
46.680	*(E)*-β-Faresene	0.11	-	-	-
46.876	α-Humulene	1.09	47.196	α-Humulene	2.86
-	-	-	48.404	*trans*-Cadina-1(6),4-diene	0.12
47.965	Selina-4,11-diene	0.29	-	-	-
48.523	Germacrene D	4.20	48.847	Germacrene D	10.81
48.624	Selinadiene	0.12	48.942	Selinadiene	0.27
-	-	-	49.268	β-Selinene	0.12
-	-	-	49.466	Valencene	0.11
49.115	Viridiflorene	0.23	-	-	-

49.422	Bicyclogermacrene	5.05	49.687	Bicyclogermacrene	3.79
49.532	α-Muurolene	0.12	49.829	α-Muurolene	0.16
50.102	β-Bisabolene	1.31	50.383	β-Bisabolene	0.55
50.416	γ-Cadinene	0.18	50.720	γ-Cadinene	0.20
50.732	δ-Cadinene	0.66	51.018	δ-Cadinene	0.67
50.770	7-epi-α-Selinene	0.29	51.099	7-epi-α-Selinene	0.62
51.036	β-Sesquiphellandrene	0.11	-	-	-
-	-	-	53.440	Germacrene B	0.11
Oxygenated sesquiterpenoids		**4.99 %**			**3.56 %**
54.268	Spathulenol	2.20	54.535	Spathulenol	1.25
54.534	Caryophyllene oxide	1.11	54.848	Caryophyllene oxide	2.03
57.469	*iso*-Spathulenol	0.20	-	-	-
57.970	epi-α-Cadinol	0.14	-	-	-
58.115	epi-α-Muurolol	0.34	-	-	-
58.548	Hinesol	0.46	-	-	-
58.739	α-Cadinol	0.54	59.029	α-Cadinol	0.28
Others		**3.56 %**			**4.92 %**
8.978	(2*E*)- Hexenal	0.53	-	-	-
9.031	(3Z)- Hexenol	0.37	9.130	(3Z)-Hexenol	0.09
9.432	(2*E*)-Hexenol	0.68	9.520	(2*E*)-Hexenol	0.28
9.572	*n*-Hexanol	0.18	9.665	*n*-Hexanol	0.14
10.820	Cyclohexanone	0.09	-	-	-
16.364	3-Octanol	0.17	16.559	3-Octanol	1.98
23.437	*n*-Nonanal	0.09	-	-	-
29.893	Methyl chavicol	0.19	-	-	-
-	-	-	36.315	Dihydroedulan	0.50
41.622	*trans*-3-Isopropenyl-2-methylene cyclohexyl	0.11	-	-	-
-	-	-	48.590	*trans*-β-Ionone	0.23
-	-	-	74.562	3-*cis*-Cembrene A	0.12
-	-	-	83.074	Cembrenol	0.18
89.900	Tricosane	0.09	90.243	Tricosane	0.19
94.004	Tetracosane	0.16	94.355	Tetracosane	0.28
97.949	Pentacosane	0.21	98.308	Pentacosane	0.34
101.743	Hexacosane	0.25	102.111	Hexacosane	0.33
105.406	Heptacosane	0.20	105.795	Heptacosane	0.26
Total	**57**	**97.41**		**56**	**98.75**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

4.1.4.10 The Genus *Clinopodium*

The chemical components determined in *C. umbrosum* essential oil are given in Table 4.14. A total of 47 compounds in *C. umbrosum*, representing 97.96% of the total EO, were detected. The dominant constituents of *C. umbrosum* were found to be piperitenone oxide (36.46%), *cis*-piperitone epoxide (22.21%), *trans*-piperitone epoxide (13.89%), β-caryophyllene (4.91%), caryophyllene oxide (3.94%), (*E*)-β-farnesene (2.66%), and linalool (2.66%). *C. umbrosum* also found to have significant amounts of several minor compounds. Concerning to the different classes of volatile constituents, *C. umbrosum* mainly consisted of oxygenated monoterpenes (77.66%), followed by sesquiterpene hydrocarbons (8.31%). The GC-MS chromatogram of *C. umbrosum* essential oil displays the separation of several volatile chemical components, which is presented in Annex 10.

To the best of our knowledge, there have been few reports on the composition of *C. coccinea* essential oils in the literature. One of them, Kumar *et al*. reported that β-caryophyllene (13.9%), germacrene D (11.6%), and spathulenol (10.6%) were the major constituents in *C. umbrosum* from India (Kumar *et al*., 2014b). The results of several previous studies reported in the literature have suggested the variation in essential oil composition may be due to climatic (Lakusic *et al.*, 2012; Aissi *et al*., 2016), edaphic (Rahimmalek *et al.*, 2013), altitudinal (Ray *et al.*, 2019; Rawat *et al*., 2020), genetic (Holm *et al*., 1997; Ju *et al.*, 2021), or phenological (Moghaddam *et al.*, 2015; Afshari & Rahimmalek, 2018).

Table 4. 14 Chemical composition of *Clinopodium umbrosum* essential oil.

RT	**Composition**	
	Components	**Area (%)**
	Monoterpene hydrocarbons	**4.31 %**
12.938	α-Pinene	0.21
15.156	Sabinene	0.04
16.090	Myrcene	0.16
18.349	*p*-Cymene	0.08
18.673	Limonene	3.64
19.031	*(Z)*-β-Ocimene	0.08
19.725	*(E)*-β-Ocimene	0.10
	Oxygenated monoterpenoids	**77.66 %**
21.353	*cis*-Linalool oxide (furanoid)	0.08
23.363	Linalool	2.66
24.868	*trans-p*-Mentha-2,8-dien-1-ol	0.05
25.622	*cis*-Limonene oxide	0.06
25.892	*cis*-Mentha-2,8-dien-1-ol	0.09
27.246	Menthone	0.10
28.956	Terpinen-4-ol	0.11
29.412	*p*-Cymen-8-ol	0.08
29.974	α-Terpineol	0.32
31.404	Coahuilensol methyl ether	0.20
34.014	*cis*-Piperitone epoxide	**22.21**
34.217	*trans*-Piperitone epoxide	**13.89**
35.047	Isopiperitenone	0.27
36.615	Thymol	0.65
39.646	Piperitenone	0.43
41.454	Piperitenone oxide	**36.46**
	Sesquiterpene hydrocarbons	**8.31 %**
42.644	β-Bourbonene	0.09
43.001	trans-β-Elemene	0.22
44.968	β-Caryophyllene	**4.91**
46.981	*(E)*-β-Farnesene	2.66
47.172	α-Humulene	0.17
48.761	Germacrene D	0.16
48.919	*trans*-β-Bergamotene	0.10
	Oxygenated sesquiterpenoids	**4.51%**
54.871	Caryophyllene oxide	3.94
53.541	*(E)*-Nerolidol	0.30
54.549	Spathulenol	0.27
	Others	**3.17 %**
15.524	1-Octen-3-ol	0.81
15.843	3-Octanone	0.18
16.554	3-Octanol	0.19
19.603	Benzene acetaldehyde	0.07
23.828	1-Octen-3-yl acetate	0.29
24.648	3-Octanol acetate	0.20
31.350	4,7-Dimethyl benzofuran	0.05
37.310	6-Hydroxy carvotone lactone	0.58
85.970	*n*-Docosene	0.06

90.243	*n*-Tricosane	0.13
94.350	*n*-Tetracosane	0.16
98.298	*n*-Pentacosane	0.17
102.107	*n*-Hexacosane	0.15
105.791	*n*-Heptacosane	0.13
Total	**47**	**97.96**

Note: RI = retention indices; compounds are listed in order of elution (increasing RI). "–" = not detected; % = percent composition. **RT:** identification based on retention time.

4.1.4.11 Seasonal variation in the chemical composition of essential oils

The variations in the contents of most of the volatile compounds in the essential oils investigated in the present study concerning to the species and harvesting season are presented in Tables 4.1, 4.2, 4.4, 4.6, 4.7, 4.8, 4.9, and 4.13, which were found to show quantitative differences. Figure 4.6 also shows the variation in the major compounds of essential oils from *M. spicata*, *M. pulegium*, *O. tenuiflorum*, *O. americanum*, *O. basilicum*, *P. frutescens*, and *L. camum* with respect to the seasons. Table 4.1 and Figure 4.4 show the alterations in the volatile composition of *O. majorana* essential oil considering the different seasons. Mostly the variations in the EO components of two *O. majorana* were seen in terpinen-4-ol (32.1-33.35%), linalool (13.8-15.37%), γ-terpinene (not detected-9.5%), linalyl acetate (5.9-6.67%), *cis*-sabinene hydrate (4.4-3.48%), and *p*-cymene (1.8-6.9%). The results indicate that the essential oil obtained from summer herbs included significant concentrations of the important antioxidant components linalool and terpinene-4-ol. In two *M. spicata* essential oil samples from Dhulikhel, the concentrations of carvone, limonene, and *neo*-dihydro carveol from winter and summer crops were found to be 51.96-68.51%, 9.55-9.63%, and 6.72%-not detected, respectively (Tables 4.2 and Figure 4.6-a). The prominent variations in the components of *M. pulegium* EO from autumn to summer at Nuwakot include menthone (41.64-66.57%), pulegone (38.31-7.12%), isomenthone (2.23-6.01%) piperitone (not detected-2.33%), and *trans*-piperitone epoxide (5.34%- not detected) (Table 4.4 and Figure 4.6-f).

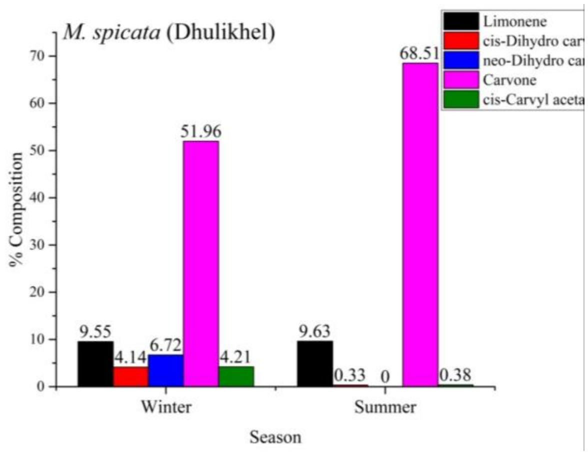
The volatile components explored in the essential oils of *O. tenuiflorum* during two seasons are presented in Table 4.6. Most of the components of *O. tenuiflorum* essential oil showed quantitative variations when the contents of the components were examined during harvesting seasons. Major fluctuations in the essential oil components include eugenol (32.15-34.95%), *trans*-β-elemene (29.08-32.85%), β-caryophyllene (19.85-21.64%), and caryophyllene oxide (3.75-0.75%) with respect to both seasons, winter and autumn (Figure 4.6-g). While sesquiterpinyl alcohol, which was present in the winter, was not detected in the autumn. The highest amount of eugenol, a valuable antioxidant component, was detected in the winter season, while lower amounts were present in the EOs collected during the autumn season. Table 4.7 and Figure 4.6 (c) demonstrated how the composition of *O. americanum* EO, which includes camphor (51.3-65.88%), linalool (9.72-9.91%), germacrene D (1.99-7.75%), β-caryophyllene (3.97-6.35%), and limonene (3.96-4.4%), was affected differently by the seasons. In *O. basilicum* EO from winter to summer, the major variations were observed in methyl chavicol (estragole) (62.16-64.42%), linalool (26.92-27.05%), and *trans*-γ-bisabolene (1.84-2.38%) (Table 4.8 and Figure 4.6-d).

The compositions of *P. frutescens* essential oil determined at two different seasons (winter and summer) are presented in Table 4.9 and Figure 4.6-b. The variations in the chemical components of this essential oil were identified as perilla ketone (42.26-50.97%), isoegomaketone (23.68-23.85%), β-caryophyllene (7.33-6.56%), isobicyclo geramcrene (5.27-3.29%), and apiole (9.16%-not detected) from winter to summer season. The components detected in *C. oppositifolia* essential oil in two different seasons (winter and summer) are shown in Table 4.11. The variations in the components of *C. oppositifolia* were investigated as geranyl-α-terpinene (31.90-27.79%), β-caryophyllene (22.16-39.99%), α-humulene (4.22-7.11), and tetra-hydroionyl acetate isomer II (10.41%-not detected). The variation in the major constituents of *L. canum* essential oil was found to be α-pinene (22.11-5.11%), β-pinene (29.02-15.21%), β-caryophyllene (13.29-33.51%), germacrene D (4.2-10.81%), linalool (1.13-6.13%), and bicyclogermacrene (3.79-5.05%) from winter to summer (Table 4.13 and Figure 4.6-e). So, most of the leading compounds fluctuated in a reverse manner in *L. canum* with respect to the harvesting season.

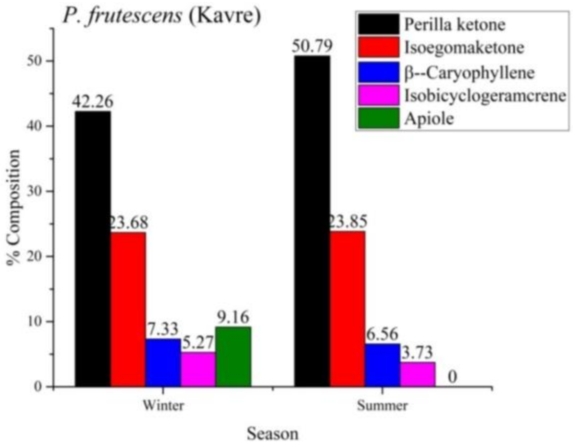
The seasonal fluctuations in the volatile compositions of essential oils could be attributed to phenological status, and environmental factors may have an impact on how essential oil biosynthesis is regulated (Kamatou *et al*., 2008; Aissi *et al*., 2016). Many previous studies have shown that the harvesting seasons can vary the volatile composition of *M. spicata* and *M. pulegium* EOs (Kofidis *et al*., 2004; Gulluce *et al*., 2007). The variation in the composition of *P. frutescens* essential oil is also in agreement with the previous finding (Ju *et al*., 2021). Similarly, the results of variations in the chemical composition of essential oils from *O. americanum* and *O. basilicum* are in accordance with the previous studies reported in the literature (Hussain *et al*., 2008; Smitha & Tripathy, 2016).

The present results further contribute to this understanding by demonstrating that the composition of the essential oils of *P. frutescens*, *L. canum*, *O. majorana*, *M. spicata*, *O. tenuiflorum*, *O. basilicum*, and *O. americanum* can vary with the harvesting season. This study establishes the fact that there have been crucial impacts of seasonal variations on the quality and quantity of volatile chemical compounds present in essential oils.

(a)



(b)



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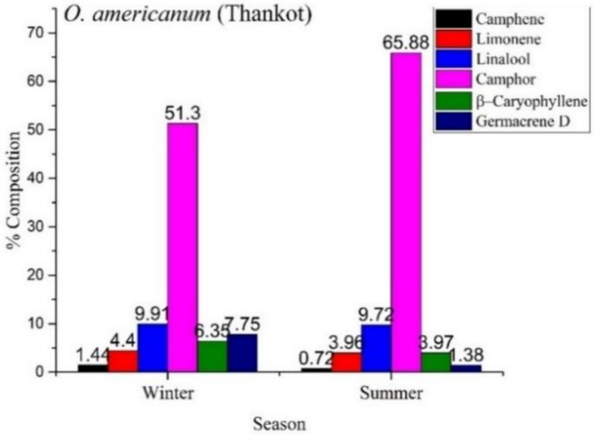

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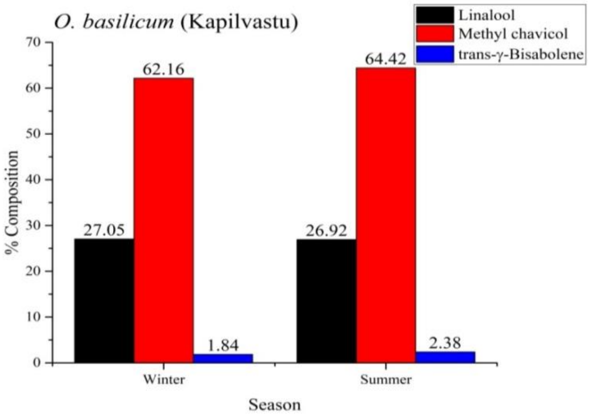

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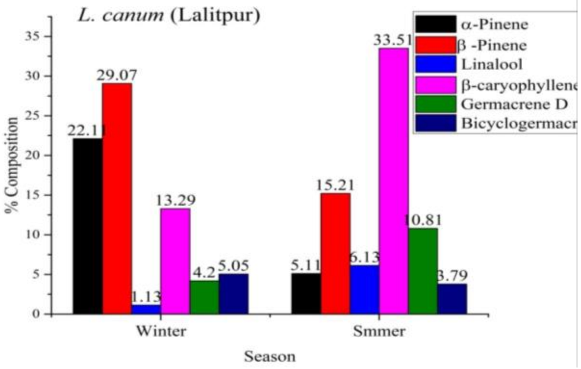

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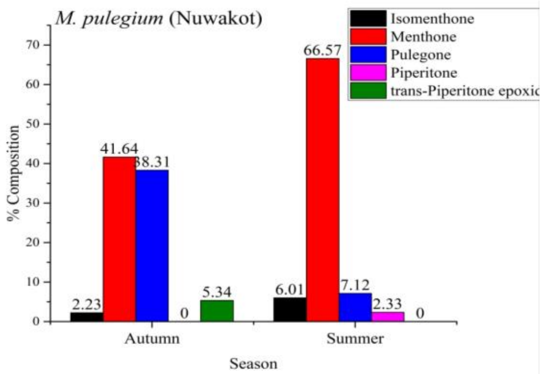


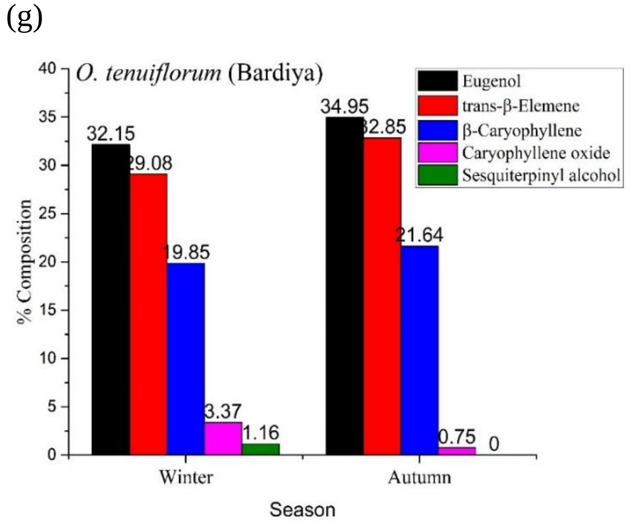


Figure 4. 6 Seasonal variation in the major components of essential oil for Lamiaceae plants.

Several reports regarding the qualitative and quantitative examination of *Mentha* essential oils from various nations can be found in the literature (Pandey *et al*., 2003; Singh *et al*., 2005; Viljoen *et al*., 2006; Gulluce *et al*., 2007). The current findings are consistent with a report that demonstrated the significant seasonal variations in the essential oil composition extracted from *O. gratissimum* leaves (Smitha & Tripathy, 2016).

Additionally, there are several other studies reported in the literature regarding qualitative and quantitative variation in essential oil from different countries (Celiktas *et al*., 2007; Bousbia *et al*., 2009). Finally, these results were consistent with many previous findings, as discussed earlier. An interesting fact can be observed from our results that the most prominent compounds were in the leading position even after the change in season, besides the variation in concentration. The compositions of the major compounds of essential oils during the summer were greater than those from the winter, indicating their commercial importance in diverse fields.

4.1.4.12 Geographical variation in the chemical composition of essential oils

The variations in the volatile composition of *O. majorana*, *M. spicata*, *M. pulegium*, *O. tenuiflorum*, *P. frutescens*, and *C. coccinea* essential oils with respect to geographical locations are summarized in Tables 4.1, 4.2, 4.4, 4.6, 4.9, and 4.12. Figure 4.7 also shows the distinctive variation in the major compounds of EOs with geographical regions. Here, both qualitative and quantitative variations were noticed in the EOs collected from different geographical areas. The amounts of terpinen-4-ol, linalool, linalyl acetate, and γ-terpinene in *O. majorana* EOs collected from Bafal were 34.59%, 15.21%, 6.51%, 5.60%, and from Sanothimi were 33.35%, 15.37%, not detected, and not detected, respectively. A marginal variation was observed in the major constituents. While the amounts of oxygenated terpenes were higher in essential oil extracted from the Santhothimi. In *M. spicata*, geographical variation was observed in the major components, along with minor variation for other compounds. The major components, carvone and limonene, varied from 51.96% and 9.63% at Dhulikhel (sub-tropical) to 71.52% and 4.12% at Bardiya (tropical). The *neo*-dihydro caeveol acetate was missing in the essential oil from Dhulikhel, but it was detected in the essential oil from Bardiya (Figure 4.7-a). The major variations were observed in the contents of menthone (41.64 to 54.80%), pulegone (38.31 to 22.20%), and isomenthone (2.23 to 4.9%) in *M. pulegium* essential oil collected from Nuwakot and Sindhupalchowk, respectively (Figure 4.7-e). The *trans*-piperitone epoxide was detected in the EOs extracted from Nuwakot samples, while it was not detected in the Sindhupalchowk.

In *O. tenuiflorum* essential oil, the major variations were noted in the contents of eugenol, *trans*-β-elemene, β-caryophyllene, caryophyllene oxide, and sesquiterpinyl alcohol from plants of Kathmandu (sub-tropical) and Bardiya (tropical), respectively. Eugenol was determined in higher concentrations in the essential oil obtained from the Bardiya (34.95%) than from the Kathmandu sample (26.15%). The sesquiterpinyl alcohol was explored in the essential oil extracted from Kathmandu, while it was missing in the essential oil from Bardiya (Figure 4.7-c). *O. tenuiflorum* samples from hilly and plain regions consisted of 53.17% and 62.54% sesquiterpene hydrocarbons, while 26.55% and 35.24% were oxygenated monoterpenes, respectively. The concentration of eugenol, which possesses good antioxidant potential, in the EO from plain areas was significantly higher than that from hilly sites. The greater the amounts of eugenol in the EOs might be associated with their higher potential for the activity. The principal constituent of the *P. frutescens* essential oils was perilla ketone, with a contribution of 50.79.2% and 56.26% from plants of the Dhulikhel and Bardiya regions, respectively (Figure 4,7-b). Similarly, *P. frutescens* essential oil from the subtropical and tropical zones comprised of 76.59 and 83.05% oxygenated monoterpenes and 13.79 and 7.41% sesquiterpene hydrocarbons, respectively.

The essential oils of *C. coccinea* from Kavre and Rasuwa also showed considerable variation in the contents of major constituents, like isocaryophyllene, β-caryophyllene, germacrene D, and kolavelool, respectively (Figure 4.7-d). Isocaryophyllene was observed in higher proportions in the EO achieved from Kavre (32.17%) than from the Rasuwa sample (28.17%).

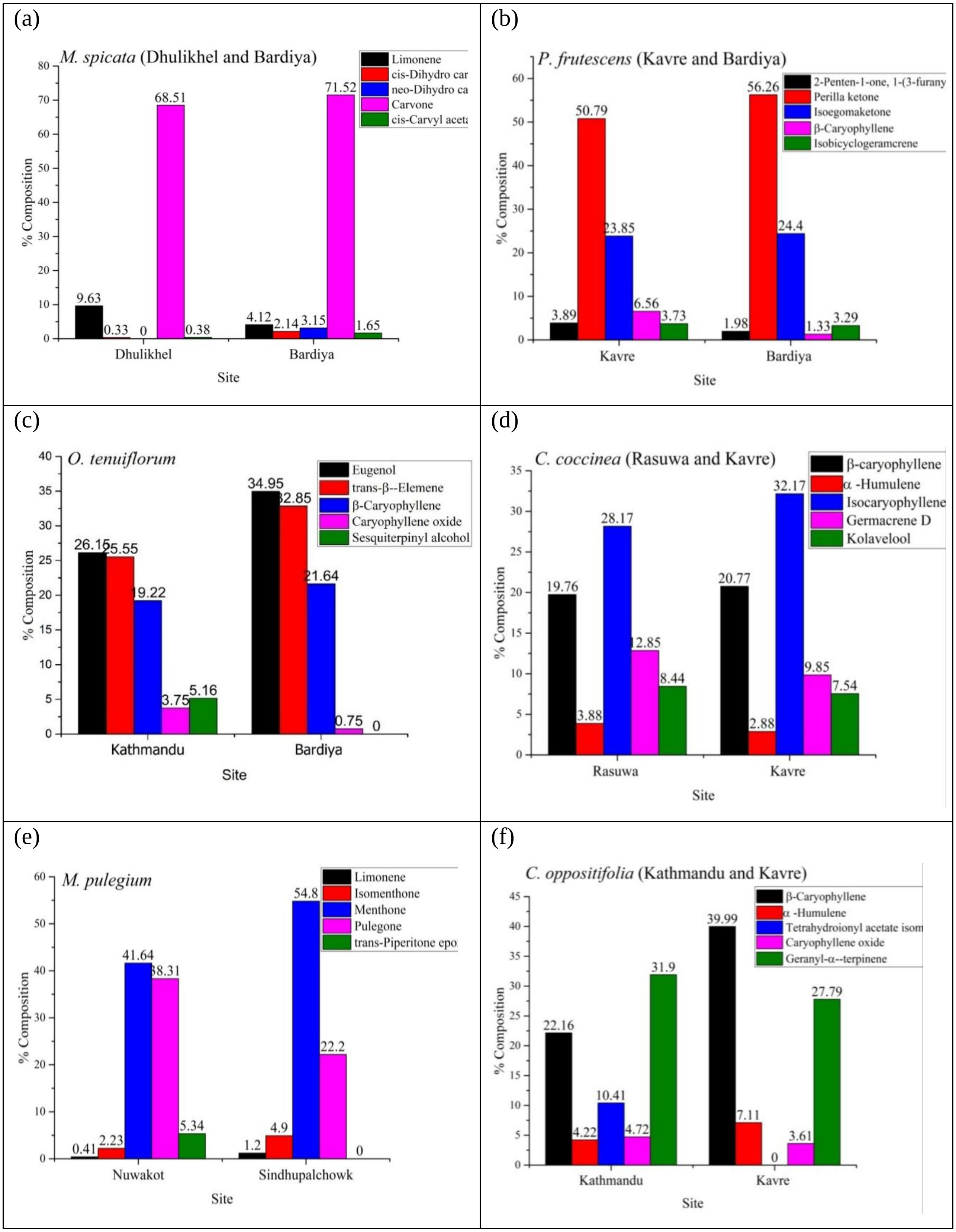


Figure 4. 7 Geographical variation in the major components of essential oils for Lamiaceae species.

Similarly, the essential oils of *C. oppositifolia* from Kavre and Kathmandu also showed variation in the contents of major constituents, like β-caryophyllene, geranyl-α-terpinene, and tetrahydroionyl acetate, respectively (Figure 4.7-f). The present results showed a marginal variation in the essential oil composition of *C. coccinea* and *M. pulegium*, while there was a significant change in the leading compounds of *C. oppositifolia* with the geographical variation. There are some previous findings that have shown that the geographical variation could influence the qualitative and quantitative composition of essential oils to some extent (Ray *et al*., 2019; Rawat *et al*., 2020).

Meanwhile, as there are no extensive previous reports described in the literature regarding the effects of geographical discrepancies on the volatile composition of Lamiaceae essential oils, together with *P. frutescens*, *O. majorana,* and *C. coccinea*, it could not possible to compare the present results with earlier work. However, the geographical variations in the volatile composition of *M. spicata and O. tenuiflorum* EOs from different countries have been published in the literature (de Vasconcelos Silva *et al*., 2004; Mondal *et al*., 2007), which are consistent with the present results.

4.1.5 Enantiomeric Composition of Essential Oils

The results for the enantiomeric distributions of chiral compounds of Lamiaceae essential oils are presented in Tables 4.15, 4.16, 4.17, 4.18, 4.19, 4.20, 4.21, 4.22, 4.23, 4.24, 4.25, 4.26, 4.27, and 4.28. The chiral GC-MS analysis identified the distribution of enantiomeric compounds in the various essential oils of Lamiaceae species, varying from 7 to 17, with the lowest by *O. tenuiflorum*, *E. blanda*, *C. coccinea,* and *P. glaber* and the highest by *L. camum* (Annex 12). Annex 12 clearly shows the total number of chiral compounds, dextro form, levo form, dextro enantiomerically pure, and levo enantiomerically pure, for each essential oil sample. The results also showed that Lamaiaceae essential oils were found to be the dominant levorotatory. Nearly 20 common chiral compounds were determined in the Lamiaceae essential oil samples from Nepal (Annex 12).

The determination of the enantiomeric composition of chiral terpenoids is a powerful tool to authenticate and identify the essential oils because they obtained from different plants may be adulterated due to the addition of several carrier oils and other foreign components (Ojha *et al*., 2022; Dangol *et al*., 2023). However, geographical location and distillation time do not affect the enantiomeric distribution of chiral compounds present in the essential oils (Chanotiya & Yadav, 2009). The (+)- and (–)-enantiomers have distinctly different biological and organoleptic properties, although they have the same physicochemical properties. Hence this tool is useful to detect (+)- and (–)-enantiomers with distinctly different biological efficacy.

4.1.5.1 *O. majorana*

The chiral GC-MS analysis was performed for the identification of enantiomeric compounds that are present in the essential oil samples. The relative percentages of the dextrorotatory and laevorotatory compounds identified in the *O. majorana* essential oils are presented in Table 4.15. There are altogether 12 chiral compounds detected in majorana essential oils, namely, α-pinene, camphene, sabinene, β-pinene, limonene, linalool, *cis*-sabinene hydrate, terpinen-4-ol, linalyl acetate, bornyl acetate, α-terpineol, and β-caryophyllene. To the best of our knowledge, this is a detailed analysis of the enantiomeric distribution of chiral compounds present in *O. majorana* essential oil, especially those belonging to Nepalese origin. The terpinen-4-ol is the major oxygenated monoterpene found in the essential oils of three samples, and it is a nearly racemic mixture in the essential oils from Nagarjun and Bhaktapur, whereas the (+)-terpinen-4-ol enantiomer is the most predominant chiral compound in the marjoram essential oil from Bafal. This also shows that *O. majorana* has a nearly racemic mixture of α-pinene. Other components, such as (–)-β-caryophyllene, (–)-bornyl acetate, and (–)-linalyl acetate, were detected as enantiomerically pure in all marjoram samples. Similarly, dextrorotatory enantiomers of sabinene, limonene, and *cis*-sabinene hydrate were dominant in marjoram, whereas camphene, β-pinene, and linalool were dominant as the levorotatory enantiomers. Terpinen-4-ol in majorana essential oil from Israel was not optically pure, and the enantiomeric composition was about (+)-terpinen-4-ol (73.0 %) and (–)-terpinen-4-ol (27.0 %) (Ravid *et al*., 1987). The enantiomeric distribution of linalool was reported only in a sample of marjoram oil with (–)-linalool (82.0 %) and (+)-linalool (18.0 %) (Casabianca & Graff, 1996). These previous results were found to agree with the chiral distribution of linalool and terpinen-4-ol as compared to current results, although there is not any detailed information from the previous study on the enantiomeric distribution of other components.

Finally, it is concluded that the (+)/(−) ratios of each of the terpenoids remain relatively constant in the present samples, regardless of the geographical location of the oil source. However, some variations in the enantiomeric distribution may be seen for the chiral components detected in minor amounts, but it should not be considered evidence of adulteration for a particular case unless further extensive investigations are carried out.

Table 4. 15 Enantiomeric distributions of chiral compounds in the essential oil of *Origanum majorana.*

Compounds	RT	RT	Nagarjun		Bhaktapur		Bafal	
	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)
α-Thujene	13.92	13.99	-	-	-	-	70.62	29.38
α-Pinene	16.40	15.92	55.81	44.19	50.81	49.2	48.71	51.29
Camphene	18.30	17.73	2.31	97.87	3.18	96.8	3.6	96.4
Sabinene	19.74	20.60	95.63	4.37	91.6	8.4	84.92	15.08
β-Pinene	20.27	20.62	24.31	75.69	29.3	70.7	29.17	70.83
Limonene	25.99	25.06	66.48	33.52	66.82	33.2	62.3	37.7
cis-Sabinene hydrate	40.70	41.25	88.52	11.48	86.8	13.2	92.58	7.42
Linalool	44.69	45.30	31.43	68.57	29.6	70.4	64.86	35.14
trans-Sabinene hydrate	46.15	46.84	-	-	-	-	100	0
Terpinen-4-ol	54.64	54.93	58.31	41.69	52.6	47.4	97.98	2.02
Linalyl acetate	56.54	NA	0.0	100.0	0.0	100.0	0.0	100.0
Borneol	59.11	58.59	-	-	-	-	0.0	100.0
Bornyl acetate	NA	59.46	0.0	100.0	0.0	100.0	0.0	100.0
α-Terpineol	60.58	59.73	75.3	24.7	72.4	27.6	83.18	16.82
β-Caryophyllene	NA	69.33	0.0	100.0	0.0	100.0	0.0	100.0

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and'-'= not detected.

4.1.5.2 *M. spicata*

The chiral GC-MS analysis allowed for the identification and composition of various chiral components in *M. spicata* essential oils (Table 4.16). The essential oils of *M. spicata* were found to have 11 chiral terpenoid components in total, namely, α-pinene, sabinene, β-pinene, limonene, 1-octen-3-ol, linalool, borneol, carvone, β-caryophyllene, germacrene D, and δ-cadinene for their enantiomeric distributions. To the best of our knowledge, this is an extensive analysis of the enantiomeric distribution of chiral compounds present in *M. spicata* essential oil, especially those belonging to Nepalese origin.

This study shows that *M. spicata* essential oil has a nearly racemic mixture of sabinene and β-pinene. Similarly, components like borneol, carvone, β-caryophyllene, and germacrene D were detected in enantiomerically pure levorotatory form (100%) in three EO samples of *M. spicata*, whereas (+)-1-octen-3-ol was a pure enantiomer found only in the samples from Dhulukhel (winter) and Bardiya. (+)-δ-cadinene (100%) was only detected in the samples from Dhulukhel (summer). The (–)-carvone is the major oxygenated monoterpene found in all essential oil samples, and it is in nearly pure form, whereas the (–)-limonene enantiomer is the most predominant chiral compound in this essential oil. The linalool detected in *Mentha* oil was dextrorotatory, and the α-pinene chiral terpenoid was levorotatory. Since there is no previous study regarding the enantiomeric distribution of chiral compounds present in *M. spicata* EOs, these are the detailed results. Therefore, this study highlights volatile chiral terpenoids as the representive components of this essential oil.

Table 4. 16 Enantiomeric distributions of chiral compounds in the essential oil of *Mentha spicata.*

Compounds	**RT**	**RT**	***M. spicata*** **(Dhulikhel-winter)**		***M. spicata*** **(Dulikhel-summer)**		***M. spicata*** **(Bardiya-summer)**	
	(+)	**(–)**	**(+)**	**(-)**	**(+)**	**(-)**	**(+)**	**(-)**
α-Pinene	16.40	15.92	36.92	63.08	38.19	61.81	39.18	60.82
Sabinene	19.74	20.60	45.79	54.21	51.63	48.37	50.63	49.37
β-Pinene	20.27	20.62	47.53	52.47	42.4	57.6	45.4	54.6
Limonene	25.99	25.06	1.06	98.94	1.1	98.9	1.09	98.91
1-Octen-3-ol	33.95	NA	100	0	-	-	100	0
Linalool	44.69	45.30	93.47	6.53	90.02	9.98	92.47	7.53
Borneol	59.11	58.59	0	100	0	100	0	100
Carvone	61.74	NA	0	100	0	100	0	100
β-Caryophyllene	69.33	NA	0	100	0	100	0	100
Germacrene D	73.48	73.73	0	100	0	100	0	100
δ-Cadinene	77.33	76.50	-	-	100	0	-	-

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and’-‘= not detected.

4.1.5.3 *M. longifolia*

Table 4.17 shows chiral GC-MS reports for the various chiral components identified in *M. longifolia* essential oils. In total, 15 chiral terpenoid compounds were determined for their .enantiomeric distributions in the essential oils of *M. longifolia*, namely, α-thujene, α-pinene, camphene, sabinene, β-pinene, limonene, .β-phellandrene, linalool, terpinen-4-ol, borneol, bornyl acetate, α-terpineol, β-caryophyllene, germacrene D, and δ-cadinene.

This analysis shows that *M. longifolia* essential oil has a nearly racemic mixture of sabinene, .β-pinene, and terpinen-4-ol. Similarly, components like borneol, bornyl acetate, α-terpineol, β-caryophyllene, germacrene D, and δ-cadinene were found to be enantiomerically pure levorotatory form (100%) in this essential oil sample, except for δ-cadinene, which exists in dextrorotatory. (–)-Camphene enantiomer is the most dominant chiral component detected in these essential oils. Most of the enantiomers are levorotatory except linalool, which exists in dextrorotatory. There are no previous studies regarding the enantiomeric .distribution of chiral compounds present in *M. longifolia*. So, this is an extensive analysis of *M. longifolia*. Therefore, this study highlights chiral terpenoids as the representative components of these oils in a detailed way.

Table 4. 17 Enantiomeric distributions of chiral compounds in the essential oil of *Mentha longifolia*.

Compounds	**RT**	**RT**	***M. longifolia* (Bardiya-summer)**	
	(+)	**(–)**	**(+)**	**(-)**
α-Thujene	13.92	13.99	14.82	85.18
α-Pinene	16.40	15.92	28.95	71.05
Camphene	18.30	17.73	3.34	96.66
Sabinene	19.74	20.60	52.3	47.7
β-Pinene	20.27	20.62	48.19	51.81
Limonene	25.99	25.06	3.3	96.7
β-Phellandrene	26.88	26.15	12.55	87.45
Linalool	44.69	45.30	94.92:	5.08
Terpinen-4-ol	54.64	54.93	42.91	57.09
Borneol	59.11	58.59	0	100
Bornyl acetate	NA	59.46	0	100
α-Terpineol	60.58	59.73	0	100
β-Caryophyllene	NA	69.33	0	100
Germacrene D	73.48	73.73	0	100
δ-Cadinene	77.33	76.50	100	0

Note: RT = Retention time (min), dextrorotatory (+), and levorotatory (-), and'-'= not detected.

4.1.5.4 *M. pulegium*

The chiral GC-MS analysis revealed several chiral components in *M. pulegium* essential oil, whcis is presented in Table 4.18. The essential oils of *M. pulegium* were detected to have 13 chiral terpenoid in total, namely, α-pinene, β-pinene, limonene, menthone, isomenthone, neomenthone, menthol, borneol, pulegone, piperitone, β-caryophyllene, germacrene D, and δ-cadinene for their enantiomeric distributions. To the best of our knowledge, this is the first detailed analysis of the enantiomeric .distribution of chiral compounds present in the *M. pulegium*, belonging to Nepalese origin.

This study shows that *M. pulegium* essential oil has a nearly .racemic mixture of α-pinene, β-pinene, and piperitone. Similarly, components like menthone, menthol, borneol, β-caryophyllene, and germacrene D were found to be enantiomerically pure levorotatory form in three essential oil samples of *M. pulegium*, whereas isomenthone, neomenthone, pulegone, and δ-cadinene were detected as pure dextrorotatory enantiomers in these samples. The (–)-limonene compound is the most predominant chiral component in these essential oils. Since there are no such previous studies regarding the enantiomeric distribution of chiral compounds present in *M. pulegium* essential oils, this is a new report. Therefore, this study reveals the volatile chiral terpenoids as representive components of this essential oils in a more detailed way.

Table 4. 18 Enantiomeric distributions of chiral compounds in the essential oil of *Mentha pulegium.*

Compounds	**RT**	**RT**	***M. pulegium* (Nuwakot-autumn)**		***M. pulegium* (Nuwakot -summer)**		***M. pulegium* (Sindhu. -autumn)**	
	(+)	**(–)**	**(+)**	**(-)**	**(+)**	**(-)**	**(+)**	**(-)**
α-Pinene	16.40	15.92	39.55	60.45	38.41	61.59	40.55	59.45
β-Pinene	20.27	20.62	59.11	40.89	46.37	53.63	52.37	47.63
Limonene	25.99	25.06	6.36	93.64	5.8	94.2	6.8	93.2
Menthone	47.41	NA	0	100	0	100	0	100
Isomenthone	49.05	49.65	100	0	100	0	100	0
Neomenthone	55.29	55.68	100	0	100	0	100	0
Menthol	58.44	NA	-	-	0	100	0	100
Borneol	59.11	58.59	0	100	0	100	0	100
Pulegone	59.69	59.88	100	0	100	0	100	0
Piperitone	63.01	63.45	-	-	37.02	62.98	40.48	59.52
β-Caryophyllene	69.33	NA	0	100	-	-	0	100
Germacrene D	73.48	73.73	0	100	-	-	0	100
δ-Cadinene	77.33	76.50	100	0	-	-	100	0

Note: RT = Retention time (min), dextrorotatory (+), and levorotatory (-), and'-'= not detected.

4.1.5.5 *E. strobilifera*

The chiral GC-MS analysis report showed the composition of various chiral components in essential oils. This analysis was performed for the identification of enantiomeric compounds of *E. strobilifera* (Table 4.19). *E. strobilifera* EO was found to have 12 chiral terpenoids for the enantiomeric distributions in this essential oil. This study is an attempt to capture the *Elsholtzia* species of Nepalese origin because the same plant species from any origin produce the essential oils with the same enantiomeric ratio. The (+)- and (–)-enantiomers have distinctly different biological and organoleptic properties, although they have the same physicochemical properties. In this study, all the 12 chiral terpenoids detected in *E. strobilifera* oil were levorotatory. Similarly, (–)-α-thujene was the predominant chiral compound (97.1%) in *E. strobilifera* oil. Furthermore, levorotatory (–)-β-caryophyllene, and (–)-germacrene D were detected in pure form (100% pure enantiomer) in the essential oil. Through the extensive literature review, there was no elaborative previous study with regard to the enantiomeric distribution of chiral compounds for *Elsholtzia* species. As far as our knowledge is concerned, this study provides new information on the enantiomeric composition of this essential oil. Hence, this study presents additional information on volatile terpenoids as typical components of *Elsholtzia* species.

4.15.6 *E. blanda*

The chiral GC-MS analysis was performed for the identification of enantiomeric compounds of *E. blanda* (Table 4.19). The essential oil of *E. blanda* was found to have 7 chiral terpenoids components in total for their enantiomeric distributions. In this study, the chiral terpenoid, α-pinene detected in *E. blanda* oil was dextrorotatory, and the other six chiral terpenoids were levorotatory. Similarly, (–)-sabinene was the predominant enantiomer (76.62%) in *E. blanda* oil. Furthermore, the levorotatory (–)-β-caryophyllene and (–)-germacrene D were detected in enantiomerically pure form (100%) in this essential oil. Through the extensive literature review, there are no elaborative previous studies about the enantiomeric distribution of chiral compounds for *Elsholtzia* species. This study covers new information on the enantiomeric composition of this essential oil. Therefore, this study presents additional information on the volatile terpenoids as representative of this essential oil.

Table 4. 19 Enantiomeric distributions of chiral compounds in the essential oils of *Elsholtzia strobilifera* and *Elsholtzia blanda*.

Compounds	**Enantiomeric distribution, dextrorotatory (+), and levorotatory (-)**					
	RT	**RT**	***E. strobilifera***		***E. blanda***	
	(+)	**(-)**	**(+)**	**(-)**	**(+)**	**(-)**
α-Thujene,	13.92	13.99	2.9	97.1	-	-
α-Pinene,	16.40	15.92	28.8	71.2	64.7	35.3
Camphene	18.30	17.73	7.5	92.5	-	-
Sabinene	19.74	20.60	10.9	89.1	23.4	76.6
β-Pinene,	20.27	20.62	6.1	93.9	43.9	56.1
Limonene	25.99	25.06	6.6	93.4	29.8	70.2
β-Phellandrene	26.88	26.15	12.6	87.4	-	-
Linalool	46.69	46.17	29.3	70.7	43.6	56.4
α-Terpineol	60.58	59.73	9.1	90.9	-	-
Verbenone	62.73	61.70	6.1	93.9	-	-
β-Caryophyllene	NA	69.33	0	100	0	100
Germacrene D	73.48	73.73	0	100	0	100

Note: RT = Retention time (min), NA = Reference enantiomer not available, and '-'= not detected.

4.1.5.7 *O. tenuiflorum*

Table 4.20 shows chiral GC-MS reports for the identification and composition of various chiral components in *O. tenuiflorum* essential oil. In total, 7 chiral terpenoid components were evaluated for their enantiomeric distributions in the leaf essential oil of *O. tenuiflorum*, like α-pinene, camphene, sabinene, β-pinene, limonene, borneol, and β-caryophyllene.

This analysis shows that components like borneol and β-caryophyllene were detected as enantiomerically pure forms of levorotatory enantiomers in *O. tenuiflorum*. Sabinene, β-pinene, and limonene detected in the essential oil were levorotatory enantiomers, whereas α-pinene was present in dextrorotatory eneatiomers. (–)-Camphene was the most dominant enantiomer in these oils. There were no previous studies regarding the enantiomeric distribution of chiral compounds in *O. tenuiflorum* essential oil. Therefore, this study highlights the chiral terpenoids as the representative components of these oils in an elaborate way.

Table 4. 20 Enantiomeric distributions of chiral compounds in the essential oil of *Ocimum tenuiflorum.*

Compounds	**RT**	**RT**	***O. tenuiflorum* (Bardiya-winter)**		***O. tenuiflorum* (Bardiya-autumn)**		***O. tenuiflorum* (Kath -autumn)**	
	(+)	(–)	(+)	(-)	(+)	(-)	(+)	(-)
α-Pinene	16.40	15.92	64.32	35.68	53.62	46.38	62.43	47.53
Sabinene	19.74	20.60	20.8	79.2	-	-	25.82	75.18
Camphene	18.30	17.73	-	-	2.72	97.28	-	-
β-Pinene	20.27	20.62	33.56	66.44	19.66	80.34	18.65	81.35
Limonene	25.99	25.06	17.14	82.86	38.12	61.88	25.40	74.60
Borneol	59.11	58.59	-	-	0	100	0	100
β-Caryophyllene	NA	69.33	0	100	0	100	0	100

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and’-‘= not detected.

4.1.5.8 *O. americanum*

The chiral GC-MS analysis for the identification and composition of various chiral components in *O. americanum* essential oil is presented in Table 4.21. In total, 12 chiral terpenoid components were evaluated for their enantiomeric distributions in *O. americanum*, namely, α-pinene, camphene, β-pinene, limonene, *cis*-sabinene hydrate, linalool, camphor, terpinen-4-ol, borneol, α-terpineol, β-caryophyllene, and germacrene D for their enantiomeric distributions.

This study shows that β-caryophyllene and germacrene D were detected in enantiomerically pure levorotatory to dextrorotatory forms in *O. americanum*. (+)-Camphor enantiomer is the most predominant chiral compound in this essential oil. The α-pinene, camphene, β-pinene, limonene, *cis*-sabinene hydrate, and terpinen-4-ol detected in essential oil samples were dominant in dextrorotatory forms, whereas the linalool, borneol, and α-terpineol chiral terpenoids were dominant in levorotatory forms. This is a comprehensive report because there has been no previous research on the enantiomeric distribution of chiral chemicals in *O. americanum.* As a result, this study explores the volatile chiral terpenoids as parts of this oil broadly.

Table 4. 21 Enantiomeric distributions of chiral compounds in the essential oil of *Ocimum americanum.*

Compounds	RT	RT	***O. americanum*** **(Thankot-winter)**		***O. americanum*** **(Thankot-summer)**	
	(+)	**(–)**	**(+)**	**(-)**	**(+)**	**(-)**
α-Pinene	16.40	15.92	71.59	28.41	67.83	32.17
Camphene	18.30	17.73	91.39	8.61	93.19	8.81
β-Pinene	20.27	20.62	64.97	35.03	55.41	44.59
Limonene	25.99	25.06	82.63	17.37	75.56	23.44
cis-Sabinene hydrate	40.70	41.25	-	-	91.23	8.77
Linalool	44.69	45.30	10.27	89.73	1.36	98.64
Camphor	50.12	49.31	99.33	0.67	100	0
Terpinen-4-ol	54.64	54.93	79.85	20.15	76.04	23.96
Borneol	59.11	58.59	-	-	7.4	92.6
α-Terpineol	60.58	59.73	34.59	65.41	44.27	55.73
β-Caryophyllene	NA	69.33	0	100	0	100
Germacrene D	73.48	73.73	0	100	0	100

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and'-'= not detected.

4.1.5.9 *O. basilicum*

Table 4.22 shows chiral GC-MS reports for the identification and composition of various chiral components in *O. basilicum* essential oil. In total, 8 chiral terpenoid components were evaluated for their enantiomeric distributions in the leaf essential oil of *O. basilicum*, namely α-pinene, β-pinene, limonene, linalool, α-terpineol, β-caryophyllene, germacrene D, and β- bisabolene.

This study shows that β-caryophyllene and germacrene D were detected in enantiomerically pure levorotatory to dextrorotatory forms in *O. basilicum* samples. (–)-Linalool enantiomer is the most predominant chiral compound in both essential oils of *O. basilicum.* α-Pinene and limonene were detected in nearly racemic form in the essential oil. β-Pinene and β- bisabolene detected in these essential oils were dominant in dextrorotatory forms, whereas the linalool, borneol, and α-terpineol chiral terpenoids were dominant in levorotatory forms. Among the chiral terpenoids, linalool is the most dominant enantiomer existing in levorotatory form. This is a detailed analysis of the enantiomeric distribution of chiral chemicals found in *O. basilicum* essential oils because there have been no such previous studies on this part. As a result, the present study provides further information regarding the volatile chiral terpenoids as representive components of *O. basilicum* essential oil.

Table 4. 22 Enantiomeric distributions of chiral compounds in the essential oil of *Ocimum basilicum.*

Compounds	**RT**	**RT**	***O. basilicum* (Kapilvastu-summer)**		***O. basilicum* (Kapilvastu-winter)**	
	(+)	**(–)**	**(+)**	**(-)**	**(+)**	**(-)**
α-Pinene	16.40	15.92	55.74	44.26	56.08	43.92
β-Pinene	20.27	20.62	78.89	21.11	57.35	42.65
Limonene	25.99	25.06	49.05	50.95	49.45	50.55
Linalool	44.69	45.30	0.14	99.86	2.12	97.88
α-Terpineol	60.58	59.73	21.75	78.25	25.32	74.68
β-Caryophyllene	69.33	NA	0	100	0	100
Germacrene D	73.48	73.73	0	100	0	100
β- Bisabolene	75.55	75.73	91.71	8.29	92.20	7.80

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and'-'= not detected.

4.1.5.10 *P. frutescens*

The chiral GC-MS analysis for the identification and composition of various chiral components in *P. frutescens* essential oils is presented in Table 4.23. In total, 12 chiral terpenoid components were evaluated for their enantiomeric distributions in the essential oil of *P. frutescens*, namely, α-pinene, sabinene, β-pinene, limonene, 1-octen-3-ol, linalool, α-terpineol, β-caryophyllene, germacrene D, δ-cardinene, (*E*)-β- ionone, and (*E*)-nirolidol.

This analysis displays that 1-octen-3-ol was detected in enantiomerically pure form (100%) as dextrorotatory in all samples of *P. frutescens*, whereas α-terpineol and β-caryophyllene were in pure form in the samples from Kavre (summer) and Bardiya (summer). Similarly, δ- cardinene, *(E)*-β-ionone, and *trans*-nirolidol were in the optically pure form in the samples from Kavre (winter) and Bardiya (summer). The α-pinene, sabinene, and germacrene D were nearly in racemic forms for all essential oil samples. The β-pinene, limonene, and linalool chiral terpenoids were dominant in levorotatory forms. This is about the detailed reports on the enantiomeric distribution of chiral chemicals as representative found in *P. frutescens* essential oils because there have been no previous reports on it.

Table 4. 23 Enantiomeric distributions of chiral compounds in the essential oil of *P. frutescens.*

Compounds	**RT**	**RT**	***P. frutescens* (Kavre-summer)**		***P. frutescens* (Kavre- winter)**		***P. frutescens* (Bardiya-summer)**	
	(+)	**(–)**	**(+)**	**(-)**	**(+)**	**(-)**	**(+)**	**(-)**
α-Pinene	16.40	15.92	-	-	47.08	52.92	48.20	51. 80
Sabinene	19.74	20.60	-	-	58.8	41.2	-	-
β-Pinene	20.27	20.62	22.48	77.52	57.35	42.65	54.08	45.92
Limonene	25.99	25.06	20.2	79.8	4.08	95.92	10.69	89.31
1-Octen-3-ol	33.95	NA	100	0	100	00	100	0
Linalool	44.69	45.30	15.85	84.15	8.74	91.26	15.80	84.20
α-Terpineol	60.58	59.73	0	100	-	-	100	0
β-Caryophyllene	NA	69.33	0	100	-	-	0	100
Germacrene D	73.48	73.73	54.01	45.99	54.81	45.19	54.11	45.89
δ- Cardinene	77.33	76.50	-	-	100	0	-	-
(*E*)-β- Ionone	80.10	80.25	-	-	100	0	100	0
(*E*)-Nirolidol	83.40	83.59	-	-	0	100	-	-

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and'-'= not detected.

4.1.5.11 *P. glaber*

The chiral GC-MS analysis for the identification and composition of various chiral components in *P. glaber* essential oil is shown in Table 4.24. In total, 7 chiral terpenoid components were evaluated for their enantiomeric distributions in the essential oil of *P. glaber* namely,α-pinene, β-pinene, limonene, 1-octen-3-ol, linalool, β-caryophyllene, and germacrene D.

This analysis displays that 1-octen-3-ol, β-caryophyllene, and germacrene D were detected in enantiomerically pure (–)-levorotatory forms in the essential oil samples. The β-pinene, limonene, and linalool chiral terpenoids were dominant in (–)-levorotatory form, while α-pinene was dominating in (+)-dextrorotatory form. To the best of my knowledge, there is no previous study on the enantiomeric distribution of chiral compounds present in *P. glaber* essential oils.

Table 4. 24 Enantiomeric distributions of chiral compounds in the essential oil of *Pogostemon glaber.*

Compounds	**RT**	**RT**	***P. glaber* (winter)**	
	(+)	**(–)**	**(+)**	**(-)**
α-Pinene	16.40	15.92	72.07	27.93
β-Pinene	20.27	20.62	41.61	58.39
Limonene	25.99	25.06	37.37	62.63
1-Octen-3-ol	33.95	NA	100	0
Linalool	44.69	45.30	16.47	83.53
β-Caryophyllene	NA	69.33	0	100
Germacrene D	73.48	73.73	0	100

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and'-'= not detected.

4.1.5.12 *C. oppositifolia*

The chiral GC-MS analysis revealed various chiral components in *C. oppositifolia* essential oil, which are shown in Table 4.25. A total of 11 chiral terpenoid components were evaluated for their enantiomeric distributions in *C. oppositifolia* essential oil, namely α-pinene, sabinene, β-pinene, limonene, 1-octen-3-ol, linalool, α-terpineol, β-caryophyllene, germacrene D. δ-cardinene, and (*E*)-β-ionone.

This analysis clearly shows that 1-octen-3-ol, β-caryophyllene, germacrene D. δ-cardiene, and (*E*)-β-ionone were detected in enantiomerically pure levorotatory form in *C. oppositifolia*. α-Pinene and β-pinene were detected nearly in racemic forms in essential oils from Kavre (summer), whereas α-terpineol was detected in the essential oil from Kathmandu (winter). The α-pinene, limonene, and linalool chiral terpenoids were dominant in levorotatory form, whereas β-pinene was dominant in dextrorotatory forms in essential oil from Kathmandu. Similarly, limonene and linalool chiral terpenoids were dominating in levorotatory form, whereas sabinene was dominating in (+)-dextrorotatory form in essential oil from Kavre. To the best of our knowledge, this is a detailed analysis of the enantiomeric distribution of chiral compounds as representative found in *C. oppositifolia* essential oil.

Table 4. 25 Enantiomeric distributions of chiral compounds in the essential oils of *Colebrookea oppositifolia*.

Compounds	**RT**	**RT**	***C. oppositifolia* (KTM-winter)**		***C. oppositifolia* (Kavre- summer))**	
	(+)	**(–)**	**(+)**	**(-)**	**(+)**	**(-)**
α-Pinene	16.40	15.92	15.4	84.6	48.78	51.22
Sabinene	19.74	20.60	-	-	65.41	34.59
β-Pinene	20.27	20.62	75.62	24.38	54.08	45.92
Limonene	25.99	25.06	22.14	77.86	10.69	89.31
1-Octen-3-ol	33.95	NA	100	0	-	-
Linalool	44.69	45.30	31.58	68.42	38.02	61.98
α-Terpineol	6.58	59.73	47.32	52.68	-	-
β-Caryophyllene	NA	69.33	0	100	0	100
Germacrene D	73.48	73.73	0	100	-	-
δ-Cardiene	77.33	76.50	-	-	100	0
trans-β-Ionone	80.10	80.24	100	0	100	0

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and’-‘= not detected.

4.1.5.13 *C. coccinea*

The chiral GC-MS analysis exhibited the chiral components in *C. coccinea* essential oil, which are shown in Table 4.26. A total of 7 chiral terpenoid components were evaluated for their enantiomeric distributions in the essential oil of *C. coccinea,* namely α-pinene, β-pinene, 1-octen-3-ol, linalool, α-terpineol, β-caryophyllene, and germacrene D.

This analysis clearly displays that 1-octen-3-ol and β-caryophyllene were detected in enantiomerically pure dextrorotatory and levorotatory forms in the samples. The β-pinene and α-terpineol were detected nearly in racemic forms in essential oil collected from both locations. The α-pinene and linalool chiral terpenoids were dominant in levorotatory forms, whereas germacrene D was dominant in dextrorotatory forms in both samples. Through the extensive literature review, there are no elaborative previous studies on enantiomeric distribution for *C. coccinea*. As far as we are aware, this study covers new information about the enantiomeric composition of these essential oils. Therefore, this study highlights the volatile terpenoids as the representative chiral components of this essential oil in more detail,

Table 4. 26 Enantiomeric distributions of chiral compounds in the essential oil of *Colquhounia coccinea.*

Compounds	**RT**	**RT**	***C. coccinea* (Rasuwa)**		***C. coccinea* (Kavre))**	
	(+)	**(–)**	**(+)**	**(-)**	**(+)**	**(-)**
α-Pinene	16.40	15.92	10.52	89.48	9.34	90.66
β-Pinene	20.27	20.62	45.47	54.53	40.17	59.83
1-Octen-3-ol	33.95	NA	100	0	100	0
Linalool	44.69	45.30	32.07	67.93	30.29	69.71
α-Terpineol	60.58	59.73	51.1	48.99	50.1	49.9
β-Caryophyllene	NA	69.33	0	100	0	100
Germacrene D	73.48	73.73	75.71	24.29	73.41	26.59

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and’-‘= not detected.

4.1.5.14 *L. canum*

The chiral GC-MS analysis for the identification and composition of various chiral components in *L. canum* essential oils is presented in Table 4.27. In total, 17 chiral components were evaluated for their enantiomeric distributions in the essential oil of *L. canum*, namely α-thujene, α-pinene, sabinene, β-pinene, α-phellandrene, limonene, 1-octen-3-ol, linalool, menthone, terpinen-4-ol, α-terpineol, (*E*)-β-elemene, β-caryophyllene, germacrene D, β-bisabolene, δ-cardinene, and (*E*)-β-Ionone.

This analysis clearly shows that (+)-β-pinene enantiomer is the most predominant chiral compound in both essential oil samples of *L. canum*. The α-pinene, sabinene, limonene, terpinen-4-ol, and α-terpineol detected in these essential oils were dextrorotatory, whereas the linalool and germacrene D chiral terpenoids were existed in levorotatory forms. The β-bisabolene was detected nearly in racemic forms in essential oil collected during winter, whereas it was not detected in essential oil collected during summer. The chiral terpenoids α-thujene and β-caryophyllene were detected to be enantiomerically pure, existing in levorotatory forms. Whereas, chiral terpenoids like menthone, β-elemene, δ-cardinene, and *trans*-β-Ionone were detected as enantiomerically pure only in this sample collected during the summer season. The α-phellandrene and 1-octen-3-ol were enantiomerically pure only in the essential oil sample collected during the winter season. The results showed that the distribution of chiral terpenoids in these essential oils was not in a regular manner. To the best of our knowledge, there is no previous study regarding the enantiomeric distribution of chiral compounds present in *L. canum*, and this study highlights the volatile terpenoids as the representative chiral components in this essential oil in an elaborative way.

Table 4. 27 Enantiomeric distributions of chiral compounds in the essential oil of *Leucosceptrum canum.*

Compounds	**RT**	**RT**	***L. canum* (Winter)**		***L. canum* (Summer)**	
	(+)	**(–)**	**(+)**	**(-)**	**(+)**	**(-)**
α-Thujene	13.92	13.99	0	100	100	0
α-Pinene	16.40	15.92	71.92	28.08	80.21	19.79
Sabinene	19.74	20.60	84.96	15.04	100	0
β-Pinene	20.27	20.62	99.47	0.53	98.35	1.65
α-Phellandrene	22.81	22.59	100	0	16.81	83.19
Limonene	25.99	25.06	68.49	31.51	47.76	52.24
1-Octen-3-ol	33.95	NA	100	0	-	-
Linalool	44.69	45.30	17.07	82.94	14.75	85.25
Menthone	47.41	NA	-	-	0	100
Terpinen-4-ol	54.64	54.93	64.2	35.8	65	35
α-Terpineol	60.58	59.73	85.06	14.94	68.81	31.19
β-Elemene	66.02	NA	-	-	0	100
β-Caryophyllene	NA	69.33	0	100	0	100
Germacrene D	73.48	73.73	2.71	97.29	7.36	92.64
β-Bisabolene	75.55	75.73	45.25	54.75	-	-
δ-Cardinene	77.33	76.50	-	-	100	0
trans-β-Ionone	80.10	80.25	-	-	100	0

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and'-'= not detected.

4.1.5.15 *C. umbrosum*

The chiral GC-MS analysis detected various chiral components in *C. umbrosum* essential oil, which is presented in Table 4.28. *C. umbrosum* essential oil was found to have 12 chiral components in total, namely, α-pinene, β-pinene, limonene, .1-octen-3-ol, linalool, menthone, terpinen-4-ol, α-terpineol, β-caryophyllene, germacrene D, δ-cadinene, and *trans*-nerolidol for their enantiomeric distributions. This study shows that 1-octen-3-ol, menthone, .β-caryophyllene, germacrene D, and δ-cadinene were detected in enantiomerically pure levorotatory to dextrorotatory forms in *C. umbrosum*. The essential oil had nearly a racemic mixture of α-pinene, β-pinene, and terpinen-4-ol. (–)-Limonene enantiomer is the most.dominant chiral compound in this sample. The linalool, α-terpineol, and trans-nerolidol chiral terpenoids detected in this oil were dominant in their levorotatory forms. Since there is no such previous study regarding the enantiomeric distribution of chiral compounds present in *C. umbrosum* essential oil and hence this is a detailed report regarding chiral compounds. Therefore, this study highlights the chiral compounds of this essential oil, providing concrete information for new researchers.

Table 4. 28 Enantiomeric distributions of chiral compounds in the essential oil of *Clinopodium umbrosum.*

Compounds	**RT**	**RT**	***C. umbrosum***	
	(+)	**(–)**	**(+)**	**(-)**
α-Pinene	16.40	15.92	39.21	60.79
β-Pinene	20.27	20.62	55.65	44.35
Limonene	25.99	25.06	1.5	98.5
1-Octen-3-ol	33.95	NA	100	0
Linalool	44.69	45.30	21.31	78.69
Menthone	47.41	NA	0	100
Terpinen-4-ol	54.64	54.93	58.83	41.17
α-Terpineol	60.58	59.73	14.84	85.16
β-Caryophyllene	69.33	NA	0	100
Germacrene D	73.48	73.73	0	100
δ-Cadinene	77.33	76.50	100	0
(E)-Nerolidol	83.40	83.59	25.19	74.81

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-).

4.1.6 Multivariate Analysis for the Chemical Composition of Essential Oils

The entire data set shows that the components of essential oils vary significantly between essential oil samples. A multivariate statistical analysis .was utilized to differentiate the chemical relationship between the volatile class of terpenes and Lamiaceae EO samples, which includes hierarchical cluster analysis (HCA), principal components analysis (PCA), and clustered heat map. Here, the different volatile classes of terpenes were determined based on the volatile composition of Lamiaceae essential oil samples, such as monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and other compounds for the multivariate analyses, and is presented in Annex 2. The hierarchical cluster analysis (HCA) was used to establish chemical associations between the essential oil samples. The principal component analysis is a widely utilized tool for obtaining a diagram of an unpredictable informational index and has been adopted for reducing the measurements and uncovering the relations among the information items. Similarly, clustered heatmap has been used to correlate between the different clusters of volatile classes and essential oil samples.

The hierarchical cluster analysis (Figure 4.8) shows the formation of two broad clusters with the dominance of major chemotypes. It includes clusters with dominant oxygenated monoterpenes (57.50-92.51%) and sesquiterpene hydrocarbon (26.67-79.91%). However, two clusters can be further classified into 10 sub-groups, representing their major chemotypes. The first cluster comprises ten samples: S-4,-8, -10,-11, -13, -18, -20, -22, -26, and -30. The second cluster comprises twenty samples: S-1, -2, -3, -5, -6, -7, -9, -12, -14, -15, -16, -17, -19, -21, -23, -24, -25, -27, -28, and -29 samples, indicating their major chemotypic groups of different volatile class of terpenes.

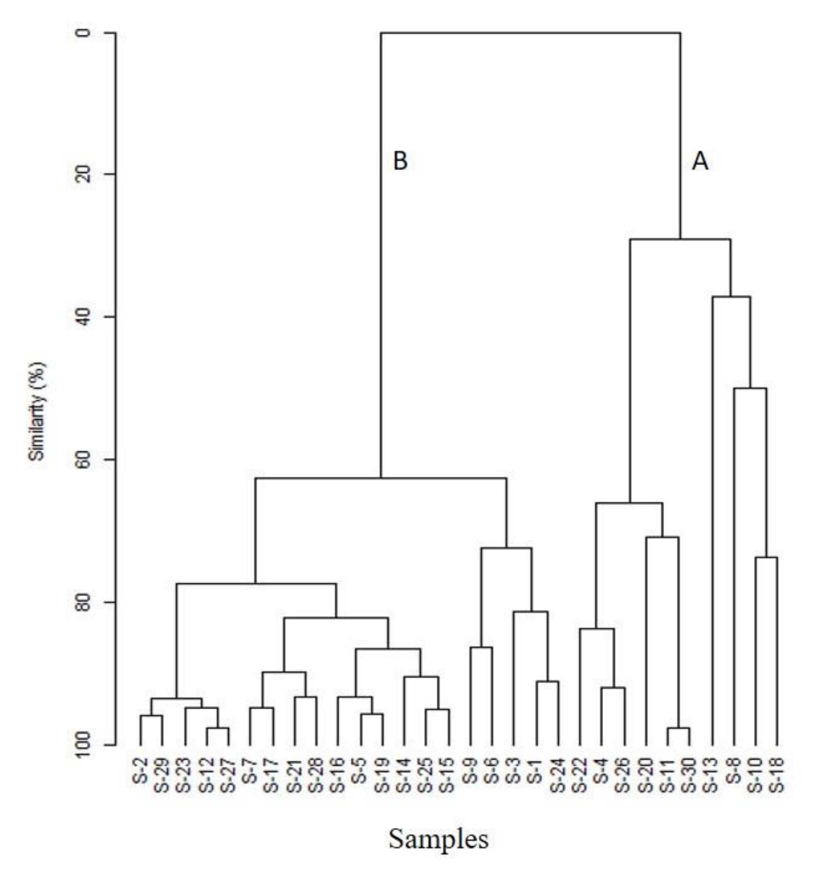


Figure 4. 8 A dendrogram obtained from the agglomerative hierarchical cluster analysis based on chemical composition of Lamiaceae species essential oils.

The principal component analysis was applied to determine the variations in five classes of volatile compounds in the 30 essential oil samples of Lamiaceae species. The PCA of the essential oil samples data resulted two principal components with eigenvalues ≥1 (Figure 4.9), accounting for 71.22% of the total variance across the entire dataset. The PCA Figure 4.10-a shows the score plot for the .main variation of essential oils compositions among the Lamiaceae species.

The first and second principal components exhibited 49.13% and 22.10% of the total variance, respectively. In the biplot (Figure 4.10-b), nine Lamiaceae EO samples, S-4, S-8, S-10, S-13, S-18, S-20, S-22, S-26, and S-30 have large positive loadings on PC1. So, the variables have the greater impact. on each component primarily. The samples S-2, -6,-11, -12, -14, -15, -17, -21, -23, -25, -27, -28, and -29 have large negative loadings on PC2. Again, PC1 showed positive correlations with sesquiterpene hydrocarbon, oxygenated sesquiterpene, and other compounds. Meanwhile, PC1 displayed negative correlations with monoterpene hydrocarbon and oxygenated monoterpene. Similarly, the second component exhibited positive correlations with monoterpene hydrocarbon and sesquiterpene hydrocarbon, as well as negative correlations with .oxygenated monoterpene, oxygenated sesquiterpene, and other compounds. Here, as with HCA, the PCA analysis also established the formation of two major clusters.

The first cluster was characterized by the maximum amounts of .sesquiterpene hydrocarbon (26.67-79.91%), followed by monoterpene hydrocarbon (0.2-56.04%), other compounds (0.11-42.98%), oxygenated sesquiterpene (1.15-37.52%) and finally oxygenated monoterpene (0.89-35.24%). Similarly, the second group was characterized by the maximum amounts of oxygenated monoterpenes (57.50-92.51%), followed by monoterpene hydrocarbon (0.09-27.6%), and minor amounts of sesquiterpene hydrocarbon (0.13-18.54%), oxygenated sesquiterpene (0.1-10.02%), and other compounds (0.25-8.51%).

Finally, applying the multivariate analyses: heatmap analysis combined with HCA of terpene classes, the color pattern changed with color intensity and was found to increase gradually, indicating the highest to the lowest amounts of different volatile classes. Among the five terpene classes, the first vertical column in the clustered heatmap showed a larger portion of intense red color indicating the dominance of the chemotypic group for Lamiaceae essential oil samples i.e. oxygenated monoterpene. While the second vertical column in the clustered heatmap showed a larger portion of intense red color indicating the dominance of another chemotypic group for

Lamiaceae essential oil samples i.e. sesquiterpene hydrocarbon. Therefore, the clustered heatmap shown in Figure 4.11 also supported the results of HCA and PCA.

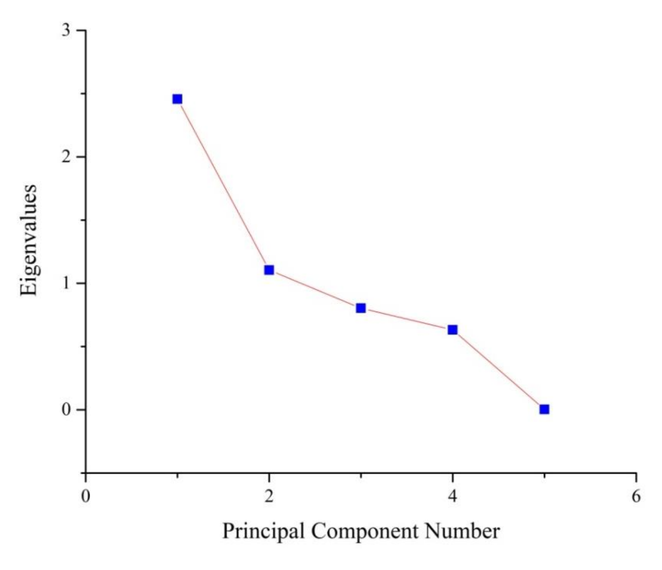


Figure 4. 9 Scree plot for possible Principal Component Number.

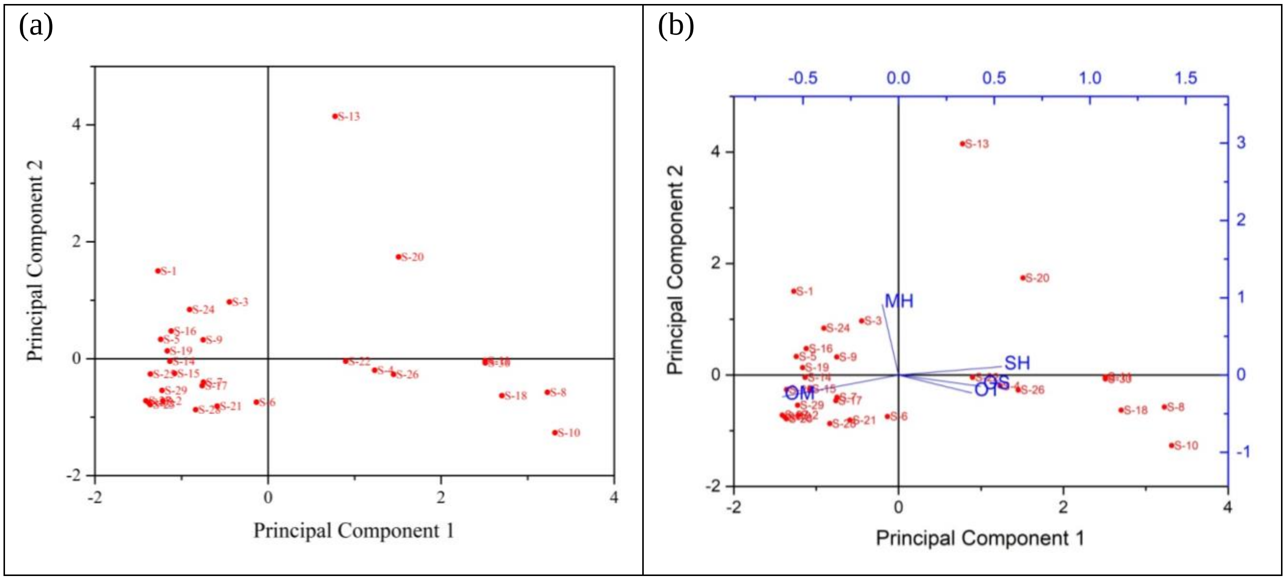


Figure 4. 10 Principal component analysis (a) PCA score plot showing the variation in the composition across the Lamiaceae species change and (b) Biplot for PCA of volatile components of Lamiaceae species essential oils showing the variation in PC1 and PC2 axes.

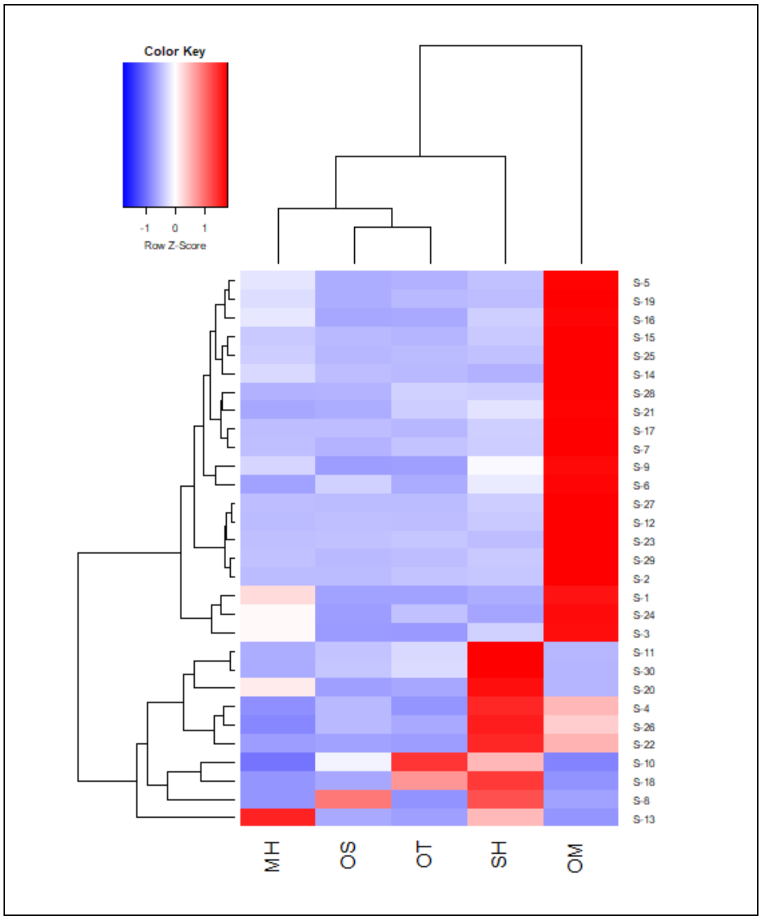


Figure 4. 11 Clustered heatmap of Lamiaceae species essential oils representing different classes of terpenes.

4.1.7 Antimicrobial Properties of Essential Oils

The Lamiaceae essential oil samples from *O. majorana*, *M. pulegium*, *M. spicata*, *M. longifolia*, *E. strobilifera*, *E. blanda*, *O. tenuiflorum*, *O. americanum*, *O. basilicum*, *P. frutescens*, *P. glaber*, *C. oppositifolia*, *C. coccinea*, *L. canum*, and *C. umbrosum* showed moderate antimicrobial activity in reference to the previous study (Duarte *et al.*, 2007). These essential oils were tested against some bacterial strains: *B. cereus*, *S. aureus*, *S. epidermidis*, *E. coli*, *S. aureus*, *P. aeruginosa*, and fungal strains: *A. niger*, *C. albicans*, *T. mentagrophytes*, *A. fumigatus*, *C. neoformans*, *M. canis*, *M. gypseum*, and *T. rubrum*. The antimicrobial potentials (based on IC_{50} values) of Lamiaceae essential oils are presented in Tables 4.29, 4.30, 4.31, 4.32, 4.33, 4.34, 4.35, 4.36, 4.37, 4.38, 4.39, 4.40, 4.41, and 4.42, respectively. Some of the prominent antimicrobial activities exhibited by the essential oil samples along with standards are given in Figure 4.12. Figure 4.12 (a). shows MIC values of essential oil against Gram–negative bacterial strain, which varies beyond 316.3 µg/mL for most of the essential oil samples, indicating the poorer (weaker) antibacterial efficacies. Here, *P. aeruginosa* was more sensitive strain towards *L. canum* essential oil, while both strains were the most resistant strains towards the remaining essential oils. Similarly, Figure 4.12 (b) shows MIC values of essential oils against Gram–positive bacterial strains, which vary beyond 156.3 µg/mL, indicating moderate antibacterial efficacies. *S. aureus* was a highly sensitive strain against *O. majorana* and *E. blanda* as compared to other essential oils. Likewise, *S. epidermidis* was also sensitive towards *E. strobilifera* and these strains were weaker sensitive towards the remaining essential oils though the MIC value of standard, gentamicin was lower for these strains than those of EO samples. The higher resistance of Gram-negative bacteria towards EOs could be ascribed by the convolution of double membrane cell structure than that of single membrane cell structure of Gram-positive (Bagamboula *et al*., 2004). These findings are in agreement to the previous studies that Gram-positive more .sensitive than Gram-negative ones (Cantore *et al*., 2004; Gulluce *et al*., 2007).

Figure 4.12 (c) shows the MIC values of different essential oils against fungal strains which varies beyond the 78.1 µg/mL. Among the fungal strains, *M. gypseum* was the .highly resistant strain while *C. albicans* was the highly sensitive strain against the essential oil samples. Among the essential oil samples studied, those of *O. majorana*, *M. pulegium*, and *O. tenuiflorum* are more effective against *Candida albicans* and *Aspergillus niger* with a very low minimum inhibitory concentration (MIC, 78.1 µg/mL). While *O. majorana* had a strong antifungal potential against the fungal strains, with MIC value varying upto 312.5 µg/mL. 13 of 15 Lamiaceae essential oils had some selective antifungal activity with MIC value varying from 78.1 to 312.5 µg/mL. This shows that the essential oils, rich in antifungal secondary metabolites, were strong against selected fungi. Compounds like terpinen-4-ol, linalool, linalool acetate, pulegone, menthone, carvone, etc. are responsible for this activity. Despite this, many other essential oils also possessed antibacterial constituents in high concentrations. However we found only slight inhibitory effects of these oils against all the tested bacterial strains during the evaluation of bactericidal activity. The broth micro-dilution assay exhibited strong to moderate antimicrobial activity for the tested essential oils. Some of the MIC values obtained for the present samples were many times higher than the previous findings. The major deviations concern with the essential oils and their major metabolites, which were to be responsible for the antimicrobial property. In the previous study, it has been shown that the whole essential oil shows better efficacy than its lead constituents, suggesting synergistic effects.

4.1.7.1 The Genus *Origanum*

Antifungal susceptibility testing has shown that the essential oil of the genus *Origanum* has strong inhibitory activity. *O. majorana* has shown strong inhibitory activity against *C. albicans*. In comparison to many samples, the essential oils obtained from Bhaktapur and Bafal showed strong inhibition against the fungi *A. niger* and *C. albicans* (Table 4.29). While we correlated this activity with the chemical composition, it was found to be quite relevant to the major compounds, i.e., terpinen-4-ol (Candelaria-Duenas *et al*., 2021), linalool (Setzer *et al*., 2006), and linalool acetate (Reichling *et al*., 2006), of *O. majorana*. These two compounds were found in relatively higher concentrations in both the Bhaktapur and Bafal samples but in relatively lower concentrations in the Nagarjun samples. A plethora of research shows that essential oils, which are rich in these two compounds, are strong against fungi (Caprari *et al*., 2023). According to the data, linalool already demonstrated fungicidal activity, even at a lower concentration (MIC 0.11± 0.03%) for the *C. albicans*, whereas 2% lavender essential oil with 32.75 % linalool killed 100% of the *C. albicans* (D'Auria *et al*., 2005).

Therefore, the present results are very similar in comparison to the previous findings. In a previous report, the antibacterial property of the essential oil .from the leaves of *O. vulgare* was reported. The lowest value of the MIC was 800–900 µg/mL against *S. aureus*, but in the present sample, it was 156.3-625 µg/mL. However, the greater amount of phenolic compounds was described as the cause of inhibition (Martucci *et al*., 2015).

Table 4. 29 Minimum inhibitory concentration of *O. majorana* essential oil against bacteria and fungi.

Name of micro-organism	**MICs (µg/mL)**		
	Nagarjun	**Bhaktapur**	**Bafal**
Bacillus cereus (ATCC 14579)	312.5	312.5	312.5
Staphylococcus aureus (ATCC 29213)	312.5	156.3	625.0
Staphylococcus epidermidis (ATCC 14990)	312.5	312.5	312.5
Aspergillusniger (ATCC 16888)	156.3	78.1	78.1
Candida albicans (ATCC 18804)	156.3	78.1	156.3
Trichophytonmentagrophytes (ATCC 18748)	156.3	156.3	78.1
Aspergillusfumigatus (ATCC 96918)	312.5	156.3	312.5
Cryptococcus neoformans (ATCC32045)	312.5	312.5	312.5
Microsporumcanis (ATCC11621)	312.5	312.5	312.5
Microsporumgypseum (ATCC24102)	312.5	312.5	312.5
Trichophytonrubrum (ATCC28188)	312.5	312.5	312.5

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

4.1.7.2 The Genus *Mentha*

Among the essential oils of *Mentha* species, *M. pulegium* an exhibited inhibitory effect against *C. albicans* and *A. niger* fungi with a MIC of 78.10 µg/mL (Table 4.32). In comparison among the different geographical origins, the samples collected from Nuwakot and Sindhupalchowk during the autumn and from Nuwakot during the summer exhibited strong efficacy against the fungi *A. niger* and *C. albicans*. Interestingly, the Nuwakot sample from the summer season was inactive against *A. niger*. A similar pattern was found with another sample as well. It indicates that, due to seasonal variation, the therapeutic properties of these oils are also variable. In the present finding, the activity level of essential oil from the Lamiaceae family was seasonal-dependent, which was in accordance with a previous study. While comparing this property with the chemical composition, it was found to be strongly relevant to its major compounds, i.e., pulegone, which is responsible for the inhibition of *A. niger*. It is known that (*R*)-pulegone at a concentration of 800 µg/mL completely inhibits the growth of *A. flavus* Link (Gonzalez-Chavez *et al*., 2011). Many of the leading compounds, such as menthone and pulegone, have been recognized as least-sensing molecules intricated in the synchronization of activities among the groups of single-celled organisms (Alem *et al*., 2006). It was interesting that the essential oil of *M. pulegium* having menthone greater than 54% in an additive manner increased the antifungal effectiveness against *C. albicans*, whereas the same oil having less than 41% of menthone was not effective with the same fungi. Similarly, the essential oil of *M. spicata*, which is rich in carvone (51.96-68.51%), was not effective against the tested microorganisms (Table 4.30). Previously, carvone was identified as an antifungal constituent against various fungi strains, including *Candida* spp., *Aspergillus* spp., and many mycotoxigenic fungi (Bouyahya *et al*., 2021). The effect of carvone was also found against a strain of bacteria, including *S. aureus*. It penetrates the bacterial cells and thus increases cell permeability. In a previous study, the antibacterial effect of *M. spicata* oil was evaluated to be highly sensitive, with a MIC of 10 µL/mL (Shahbazi, 2015). Despite having 79.04% oxygenated monoterpenes in this result, no effective result was noticed, contrasting with previous findings in *M. spicata* (Snoussi *et al*., 2015). Essential oils that contain high amounts of oxygenated monoterpenes, such as *O. majorana* (65.2%) and *M. pulegium* (85.69%), generally have the highest antimicrobial activity, while essential oils containing a greater quantity of other compounds, such as monoterpene hydrocarbons, oxygenated monoterpenes, and others, are usually less effective as antimicrobials.

Table 4. 30 Minimum inhibitory concentrations of *M. spicata* essential oil against bacteria and fungi.

Name of micro-organism	MICs (µg/mL)		
	(Dhulikhel winter)	(Dulikhel-summer)	(Bardiya-summer)
Bacillus cereus (ATCC 11778)	1325	1325	1325
Staphylococcus aureus (ATCC 6538)	1325	1325	1325
Pseudomonas aeruginosa (ATCC 9027)	662.5	662.5	1325
Escherichia coli (ATCC 8739)	1325	1325	1325
Candida albicans (ATCC 10231)	1325	662.5	662.5
Aspergillus niger (ATCC 16888)	331.25	1325	331.25

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

Table 4. 31 Minimum inhibitory concentrations of *M. longifolia* essential oil against bacteria and fungi.

Name of micro-organism	MICs (µg/mL)
	(Bardiya-summer)
Bacillus cereus (ATCC 11778)	1257
Staphylococcus aureus (ATCC 6538)	2514
Pseudomonas aeruginosa (ATCC 9027)	1257
Escherichia coli (ATCC 8739)	2514
Candida albicans (ATCC 10231)	1257
Aspergillus niger (ATCC 16888)	628.5

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

Table 4. 32 Minimum inhibitory concentrations of *M. pulegium* essential oil against tested bacteria and fungi.

Name of micro-organism	MICs (µg/mL)		
	Nuwakot-autumn	Nuwakot - summer	Sindhu. - autumn
Bacillus cereus (ATCC 14579)	312.5	312.5	312.5
Staphylococcus aureus (ATCC 29213)	312.5	312.5	312.5
Staphylococcus epidermidis (ATCC 14990)	312.5	312.5	625
Aspergillusniger (ATCC 16888)	78.1	156.3	156.3
Candida albicans (ATCC 18804)	156.3	78.1	78.1
Trichophytonmentagrophytes (ATCC 18748)	156.3	156.3	156.3
Aspergillusfumigatus (ATCC 96918)	156.3	156.3	312.5
Cryptococcus neoformans (ATCC32045)	312.5	312.5	312.5
Microsporumcanis (ATCC11621)	312.5	312.5	312.5
Microsporumgypseum (ATCC24102)	312.5	312.5	312.5
Trichophytonrubrum (ATCC28188)	312.5	312.5	625

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

Likewise, it was reported that *M. citrate* essential oil displayed antibacterial property against all eight tested bacteria of Gram-positive strains, namely *Staphylococcus aureus*, *Staphylococcus epidermis,* and *S. mutans,* as well as Gram-negative strains, namely *P. aerugenosa*, *K. pneumoniae, E. coli* (DH5α), *E. coli* (MTCC 723), and *S. typhimurium*, with MIC values of 250–1000 µg/mL (Verma *et al*., 2016). *M. longifolia* has previously been known to have better activity against fungal species than against bacteria (Table 4.31). *M. longifolia* from Iran was active against *E. coli* and *S. aureus* (Rasooli & Rezaei, 2002). In the present study*, S. aureus* and *E. coli* were found to be the most resistant strains. While other strains were highly resistant to *M. longifolia* essential oil. This finding is not in close line with previous research due to various contributing factors associated with them (Hajlaoui *et al*., 2008).

4.1.7.3 The Genus *Elsholtzia*

The essential oils of *E. strobilifera* and *E. blanda*, composed of pinocarvone (40.73%) and dihydrotagetone (49.08%), also showed higher activity against tested organisms. *E. strobilifera* exhibited mild activity against *S. aureus* with a MIC of 650 µg/mL and strong activity against *S. epidermidis* species with a MIC value of 156.3 µg/mL (Table 4.33). *E. blanda* also exhibited strong activity to moderate against *S. aureus* and *S. epidermidis*, with MIC values of 156.3 µg/mL and 650 µg/mL, respectively. The antimicrobial efficacy of other organisms, like *B. cereus*, *A. niger*, *A. fumigatus*, and *C. albicans,* was very strong, with MICs of 312.5 µg/mL. According to the literature, pinocarvone, dihydrotagetone, β-caryophyllene, β-pinene, camphor, etc. were reported to have significant antimicrobial activity. In addition, many other constituents that are present in the oil as minor compounds, such as pinene, eugenol, menthol, and linalool, are well-known compounds with strong antimicrobial activity (Nhan & Huyen, 2017).

Table 4. 33 Minimum inhibitory concentrations of *E. strobilifera* and *E. blanda* essential oils against tested bacterial and fungal strains.

Name of micro-organism	**MICs (µg/mL)**	
	E. strobilifera	***E. blanda***
Bacillus cereus	1250	312.5
Staphylococcus aureus	650	156.3
Staphylococcus epidermidis	156.3	650
Aspergillus niger	156.3	156.3
Candida albican	312.5	312.5
Aspergillus fumigatus	312.5	312.5

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

4.1.7.4 The Genus *Ocimum*

The essential oils of the genus *Ocimum* also showed the variable strength of their antimicrobial activities. *O. tenuiflorum* samples from Bardiya and Kathmandu exhibited strong results against *C. albicans*, with MICs ranging from 81.25 to 162.5 µg/mL (Table 4.34). The results showed that the major bioactive compounds such as eugenol (26.15-34.95%), *trans*-β-elemene (25.55-32.85%), β-caryophyllene (19.22-21.64%) were found in high concentrations in EO samples. All three EOs contained a small proportion of several antimicrobial compounds, such as camphor, linalool, methyl chavicol, and caryophyllene oxide. *O. americanum* EO from the Thankot site also exhibited strong efficacy against *C. albicans*, with a MIC value of 350 µg/mL (Table 4.35). Camphor (56.88%) is the leading constituent, which is responsible for inhibiting *C. albicans*. Camphor has already been proven to be an antifungal constituent that induces the expression of the CDR1 gene in *C. albicans* (Ivanov *et al*., 2021). Moreover, *O. tenuiflorum* was found to be very active against *C. albicans*. In comparison to many EOs, the samples collected from Kathmandu exhibited strong activity against the fungus *C. albicans*, whereas samples from Bardiya also showed good results. It was reported previously that the activity level of the Lamiaceae family EOs is normally seasonally dependent. While we compared this property with the chemical composition, it was found to be strongly relevant to the major compounds of the EO. Eugenol is responsible for the inhibition of *C. albicans*. It is known that eugenol inhibits the growth of this fungus through DNA fragmentation in the cell (Jianhua & Hai, 2009). A study showed that *O. basilicum* from Turkey inhibited the weak growth of some bacterial strains in the genera *Bacillus*, *Micrococcus*, *Escherichia*, and *Staphylococcus* with inhibition, whereas only *Acinetobacter* was inhibited strongly (Adiguzel *et al*., 2005). However, the present *O. basilicum*, which originated from Kapilvastu, exhibited MICs of 1314-2628 µg/mL (Table 4.36). The leading compound was also methyl chavicol in the samples from Bangladesh (36.7 and 29.9%), which is proportionally higher in the Nepalese sample (62.16–64.42%). The sample from Bangladesh was strong against the food-borne pathogens of the genera *Bacillus*, *Staphylococcus*, *Listeria*, *Escherichia*, *Shigella*, *Vibrio*, and *Salmonella* with MICs of 62.5–500 µg/mL (Hussain *et al*., 2008). It indicates that methyl chavicol alone may not be sufficient for antimicrobial activity against the tested organisms. These compounds, together, are accountable for the antimicrobial activities of the leaf extracts (Yamani *et al*., 2016). One of the leading compounds, eugenol, has an inhibitory effect against *A. niger* (Meena & Sethi, 1994). Similarly, linalool and eugenol are also well-known against food-borne pathogens such as *E. coli*, *S. typhimurium*, *Listeria monocytogenes*, and *Vibrio vulnificus*.

Table 4. 34 Minimum inhibitory concentrations of *O. tenuiflorum* essential oil against tested bacteria and fungi.

Name of micro-organism	**MICs (µg/mL)**		
	(Bardiya-winter	**(Bardiya-autumn)**	**(Kath - autumn)**
Bacillus cereus (ATCC 11778)	650	1300	650
Staphylococcus aureus (ATCC 6538)	1300	650	2600
Pseudomonas aeruginosa (ATCC 9027)	1300	650	1300
Escherichia coli (ATCC 8739)	650	1300	1300
Candida albicans (ATCC 10231)	162.5	325	81.25
Aspergillus niger (ATCC 16888)	650	1300	650

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

Table 4. 35 Minimum inhibitory concentrations of *O. americanum* essential oil against tested bacteria and fungi.

Name of micro-organism	**MICs (µg/mL)**	
	(Thankot-summer)	**(Thankot-winter)**
Bacillus cereus (ATCC 11778)	650	1300
Staphylococcus aureus (ATCC 6538)	650	1300
Pseudomonas aeruginosa (ATCC 9027)	650	650
Escherichia coli (ATCC 8739)	1300	2600
Candida albicans (ATCC 10231)	350	650
Aspergillus niger (ATCC 16888)	650	1300

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

Table 4. 36 Minimum inhibitory concentrations of *O. basilicum* essential oil against tested bacteria and fungi.

Name of micro-organism	**MICs (µg/mL)**	
	Kapilvastu-summer	**Kapilvastu-winter**
Bacillus cereus (ATCC 11778)	1579	1314
Staphylococcus aureus (ATCC 6538)	1257	1579
Escherichia coli (ATCC 8739)	2628	1530
Aspergillusniger (ATCC 16888)	2600	2557
Candida albicans (ATCC 10231)	1314	1579

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

It was confirmed that eugenol exhibits a dose-dependent rise in the zone of inhibition against the strains used for analysis. Here, linalool displayed a similar influence against all tested strains except for *L. monocytogenes*. It is also well known to inhibit the growth of *L. monocytogenes*, but the alteration in the zone size between the test concentrations was not significant (Kim *et al*., 1995; Pattnaik *et al*., 1997). β-Caryophyllene also possesses antimicrobial activity. It was also explored the antimicrobial properties of EOs extracted from the leaves of *O. syriacum*, which had β-caryophyllene at a concentration of 12.6% (Alma *et al*., 2003). *O. syriacum* was shown to have efficacy against *S. aureus*, *E. coli*, and *P. aeruginosa*. These findings suggested that β-caryophyllene might be responsible for having this antimicrobial potential against these three species of bacteria that were demonstrated in the current study. It was reported that essential oil at concentrations of 4.5 and 2.25% completely inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*, while *P. aeruginosa* showed higher resistance to the antibacterial treatment with tulsi oil compared to the three other bacteria used during the test (Yamani *et al*., 2016).

4.1.7.5 The Genus *Perilla*

P. frutescens essential oil also displayed good results against fungi, Gram-positive, and Gram-negative bacteria (Table 4.37). Varying degrees of inhibition were found strong against *E. coli*, *C. albicans*, and *A. niger*, with MICs varying from 156.3 to 625 µg/mL. The result was also good against *P. aeruginosa*, with a MIC value of 625 µg/mL. In contrast, all samples showed the lowest inhibition against *B. cereus* and *S. aureus*. In a previous antimicrobial study of this plant, there was no inhibition against *Salmonella* spp., *S. aureus*, and *E. coli* (Ahmed & Al-Zubaidy, 2020). Similarly, the MICs on *S. aureus* and *E. coli* were 500 µg/mL and 1250 µg/mL, respectively (Qunqun, 2003). Perilla aldehyde, the most prevalent terpene-type compound, moderately inhibits a broad range of bacteria in the range of 0.125–1.00 µg/mL (Kang *et al*., 1992). The primary components are terpenoids, including perilla ketone (42.26–56.26%), β-caryophyllene (1.33–7.33%), which exert potent antifungal properties (Ahmed & Al-Zubaidy, 2020; Li *et al*., 2022). Similar antimicrobial properties of a few compounds from perilla were also recently confirmed in *S. aureus* and *C. albicans* (Nguyen *et al*., 2022). This activity of essential oils is probably due to the presence of active constituents that could suppress or inhibit the growth of bacterial strains either individually, synergistically, or antagonistically.

Table 4. 37 Minimum inhibitory concentrations of *P. frutescens* essential oil against tested bacteria and fungi.

Name of micro-organism	MICs (µg/mL) (Kavre-summer)	MICs (µg/mL) (Kavre-winter)	MICs (µg/mL) (Bardiya-summer)
Bacillus cereus (ATCC 11778)	1250	1250	1250
Staphylococcus aureus (ATCC 6538)	1250	1250	1250
Pseudomonas aeruginosa (ATCC 9027)	625	625	1250
Escherichia coli (ATCC 8739)	1250	625	625
Candida albicans (ATCC 10231)	312.5	156.3	625
Aspergillus niger (ATCC 16888)	312.5	156.3	312.5

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

4.1.7.6 The Genus *Pogostemon*

Essential oil of the genus *Pogostemon* also demonstrated moderate inhibitory properties against fungi *C. albicans* and *A. niger* with MICs of 628.5 and 657µg/mL (Table 4.38). The essential oil showed mild efficacy against bacterial strains, with a MIC of 1314 µg/mL. Germacrene D (16.70%), curzerenone (12.58%), and δ-elemene (10.93%) are the dominant compounds that can be responsible for inhibitory activity. The principal component of the essential oil was germacrene D (Setzer *et al.*, 2006). The literature revealed that the germacrene D-rich essential oil showed good antimicrobial and cytotoxic activity (Joshi *et al.*, 2011). Germacrene D was also dominant in the Indian essential oil (Murugan & Mallavarapu, 2012).

Table 4. 38 Minimum inhibitory concentrations of *P. glaber* essential oil against tested bacteria and fungi.

Name of micro-organism	MICs (µg/mL) (Kath-winter)
Bacillus cereus (ATCC 11778)	1314
Staphylococcus aureus (ATCC 6538)	1314
Pseudomonas aeruginosa (ATCC 9027)	1314
Escherichia coli (ATCC 8739)	2628
Candida albicans (ATCC 10231)	328.5
Aspergillus niger (ATCC 16888)	657

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

4.1.7.7 The Genus *Colebrookea*

The essential oil of *C. oppositifolia* also displayed inhibitory effects, mainly against *C. albicans* and *A. niger* (Table 4.39), *B. cereus* and *S. aureus* were also sensitive to essential oil originating from Kathmandu. Antibacterial compounds, linalool (1.52%), caryophyllene oxide (4.72%), and geranyl-α-terpine (31.9%), were comparatively higher in the Kathmandu sample. Manool (2.8%) and (*Z*, *Z*)-geranyl linalool (2.03%) were present in the sample from Kathmandu but missing in the Kavre. Geranyl-α-terpinene, β-caryophyllene, and α-humulene are some major constituents that may synergistically contribute to antimicrobial activity.

Table 4. 39 Minimum inhibitory concentrations of *C. oppositifolia* essential oil against tested bacteria and fungi.

Name of micro-organism	**MICs (µg/mL)**	
	(Kathmandu-winter)	**(Kavre-summer)**
Bacillus cereus (ATCC 11778)	657	1314
Staphylococcus aureus (ATCC 6538)	657	657
Pseudomonas aeruginosa (ATCC 9027)	1314	2628
Escherichia coli (ATCC 8739)	1314	2628
Candida albicans (ATCC 10231)	328.5	328.5
Aspergillus niger (ATCC 16888)	657	657

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

4.1.7.8 The Genus *Colquhounia*

C. coccinea essential oil also showed antimicrobial properties, particularly antifungal activity against organisms (Table 4.40). Though some bioactive compounds such as isocaryophyllene (28.27–32.17%), β-caryophyllene (19.76-20.77%), α-humulene (2.88-3.88%), etc. are present in all samples, their antibacterial activity is found to be weak. However, moderate antifungal properties were detected against *C. albicans*, and *A. niger* with MICs of 632.5 µg/mL. The sample from Rasuwa was found to be better than the sample from Kavre.

Table 4. 40 Minimum inhibitory concentrations of *C. coccinea* essential oil against bacteria and fungi.

Name of micro-organism	**MICs (µg/mL)**	
	(Rasuwa – winter)	**(Kavre-winter)**
Bacillus cereus (ATCC 11778)	1265	1265
Staphylococcus aureus (ATCC 6538)	1265	2530
Pseudomonas aeruginosa (ATCC 9027)	632.5	1265
Escherichia coli (ATCC 8739)	2530	2530
Candida albicans (ATCC 10231)	632.5	1265
Aspergillus niger (ATCC 16888)	632.5	1265

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

4.1.7.9 The Genus *Leucosceptrum*

L. canum essential oil also showed some inhibitory properties against selected microorganisms. Strong inhibitory properties were shown against *P. aeruginosa* and *C. albicans*, with a MIC of 316.25 µg/mL (Table 4.41).

Similarly, essential oil was also active against the bacteria *S. aureus* and both fungi *C. albicans* and *A. niger*, with MICs of 632.5 µg/mL. The β-pinene (29.07%), β-caryophyllene (13.29%), and germacrene D (4.20%) were the major compounds in this EO, which may be responsible for the antimicrobial property. The plant possesses potent to moderate activity against *Bacillus subtilis*. *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas agarici*, *Streptococcus minor*, and *S. ferus* (Devkota *et al*., 2010).

Table 4. 41 Minimum inhibitory concentrations of *L. canum* essential oil against bacteria and fungi.

Name of micro-organism	**MICs (µg/mL)**	
	(Lalitpur - winter)	**(Lalitpur- summer)**
Bacillus cereus (ATCC 11778)	1265	2530
Staphylococcus aureus (ATCC 6538)	1265	632.5
Pseudomonas aeruginosa (ATCC 9027)	316.25	632.5
Escherichia coli (ATCC 8739)	1265	2530
Candida albicans (ATCC 10231)	316.25	632.5
Aspergillus niger (ATCC 16888)	632.5	632.5

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

4.1.7.10 The Genus *Clinopodium*

Essential oils of *C. umbrosum* also showed moderate inhibitory properties against *B. cereus*, *P. aeruginosa*, and *C. albicans* with a MIC of 789.5 µg/mL (Table 4.42). This essential oil was slightly active against the other bacteria. Oxygenated monoterpenes, such as piperitenone oxide (36.46%), *cis*-piperitenone epoxide (22.21%), and *trans*-piperitenone epoxide (13.89%), were major constituents. The main fraction was comprised of oxygenated monoterpenes. According to a previous report, monoterpenoid fractions were also the major compounds found in the essential oils of related *Clinopodium* species (Dunkic *et al*., 2017).

The bioactivity of essential oils was determined by their composition, the functional groups present in active compounds, and their synergetic interactions. Also, the mechanism varies with the type of essential oil and the microbial strains used. It is known that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria. This could be attributed to the rigid, rich in lipopolysaccharide, and more complex nature of the outer membrane in Gram-negative bacteria. This results in the prevention of the diffusion of hydrophobic components via the membrane. While such a complex membrane is not found in Gram-positive bacteria. They are actually encircled by a thick peptidoglycan wall. This is not dense enough to resist small antimicrobial molecules, which could facilitate access to the cell membrane (Huang *et al*., 2014). Moreover, Gram-positive bacteria may ease the infiltration of hydrophobic compounds of essential oils due to the lipophilic ends of lipoteichoic acid present in the cell membrane (Jianhua & Hai, 2009).

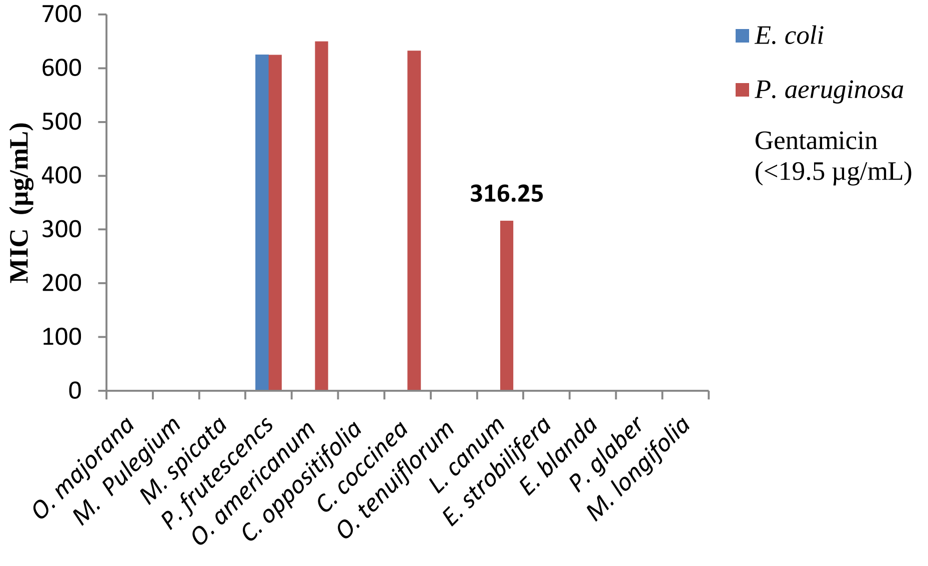
Table 4. 42 Minimum inhibitory concentrations of *C. umbrosum* essential oil against bacteria and fungi.

Name of micro-organisms	MICs (µg/mL)
Bacillus cereus (ATCC 11778)	789.5
Staphylococcus aureus (ATCC 6538)	1579
Escherichia coli (ATCC 8739)	1579
Pseudomonas aeruginosa (ATCC 9027)	789.5
Candida albicans (ATCC 10231)	789.5

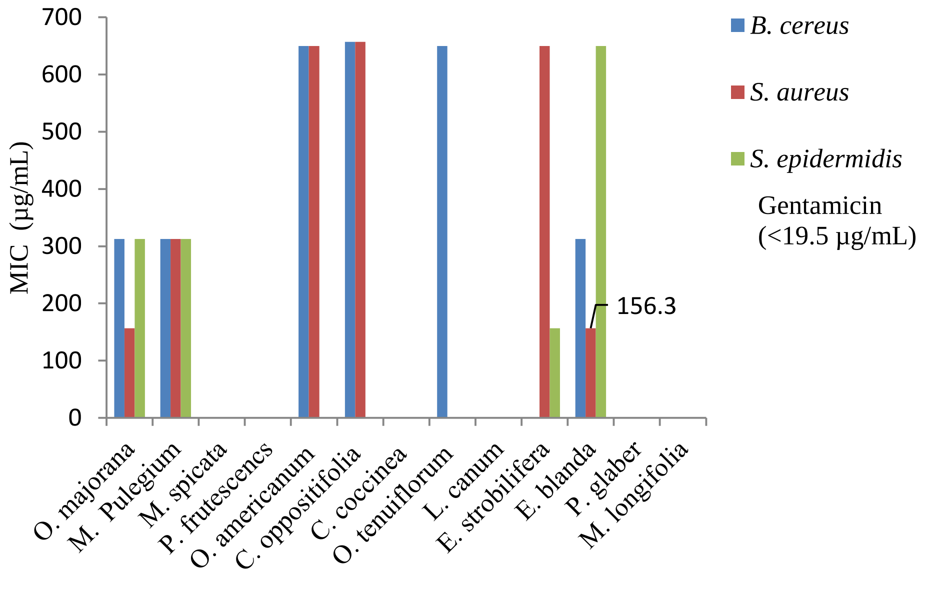
Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

It was observed that plants growing in the middle range, i.e., the subtropical region, had better antifungal activity, while those growing at tropical altitudes had better antibacterial activity. Since the antimicrobial efficacy may be directly connected to the specific composition of the essential oil, its power could be altered. Generally, altitude change affects terpenoid biosynthesis and oxygenated monoterpenes and is greater in sub-tropical plant species. While sesquiterpene components were maximum at lower altitudes. The effect of altitude appears to be a vital factor in the chemical profile of essential oils. Therefore, the location of the plant must be taken into consideration regarding the particular intended use (Sanli & Karadogan, 2017). Recent research shows a relationship between the chemical constituents of extracted essential oils from the .Himalayan zone and their antimicrobial efficacy, indicating their potential to be used as antibacterial agents (Rathore *et al.*, 2023). The major compounds of the Lamiaceae family, thymol and carvacrol, interfere with cellular metabolism after penetrating the cell (Marino *et al*., 2001). The present result concludes that the plant *O. majorana* is strong enough to inhibit a wide range of bacteria and fungi in the tested strains.

(a) MIC value against Gram-negative bacterial species



(b) MIC value against Gram-positive bacteria species



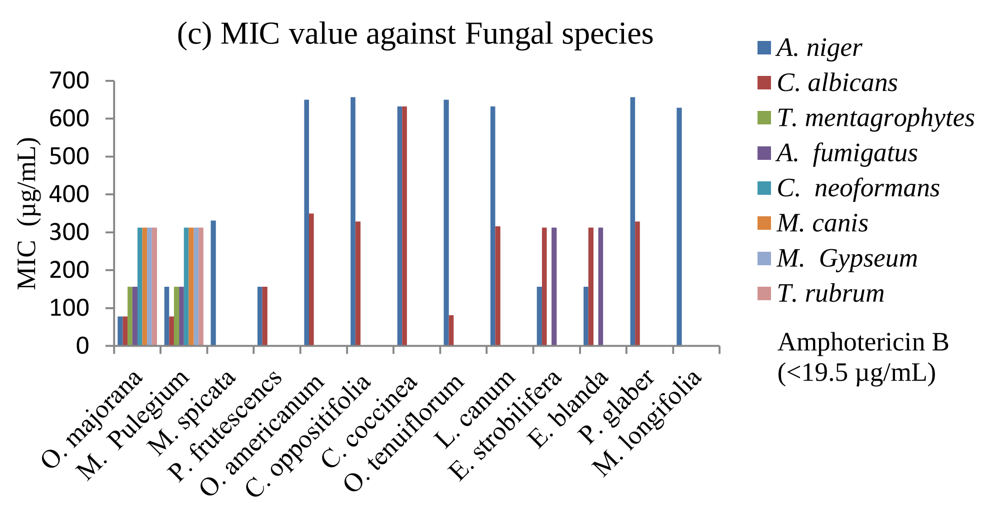


Figure 4. 12 A comparative antimicrobial activity of Lamiaceae essential oil against bacterial and fungal strains, in terms of MIC values.

4.1.8 Antioxidant Activities of Essential Oils

The evaluation of the antioxidant potentials of the Lamiaceae plant species under this study was carried out by employing the following *in vitro* assays:

4.1.8.1 DPPH radical-scavenging activity

The antioxidant potentials of Lamiaceae essential oils and standards were investigated by the DPPH radical-scavenging method. They were expressed in terms of 50% scavenging (IC_{50}) and are presented in Annex 3. The free radical-scavenging potential of the EOs along with standards was observed to be enhanced in a concentration-dependent way, as shown in Annex 11. The comparative antioxidant capacity of essential oil samples in terms of IC_{50} values from the DPPH method is shown in Figure 4.13 (a). Here, all the essential oil samples showed the ability to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH-H to a different extent. The highest antioxidant and radical-scavenging activity was observed for *O. tenuiflorum* (IC_{50} 69.23-82.99 μg/mL), though it was weaker than the standards used (ascorbic acid, IC_{50} 6.73 μg/mL, and BHT, IC_{50} 12.46 μg/mL). The least antioxidant potential was noted for *M. pulegium* essential oil from Nuwakot during the summer (IC_{50} 646.58 μg/mL). The *C. coccinea* essential oil collected from Rasuwa showed relatively better radical-scavenging capacity with IC_{50} values of 174.7 μg/mL than that of Kavre with IC_{50} of 192.42 μg/mL, respectively. Among *majorana* essential oil samples, *O. majorana* essential oil from Bafal, Kathmandu, exhibited good radical-scavenging potential with an IC_{50} value of 187.44 µg/mL, followed by essential oils from Bhaktapur (IC_{50} of 225.61 µg/mL), and Nagarjun, Kathmandu (IC_{50} of 468.7 µg/mL). The *O. basilicum* essential oil also displayed substantial free radical-scavenging efficacy with an IC_{50} value of 236.14 µg/mL, which was collected during the winter season. *O. tenuiflorum* collected from plain and hilly areas exhibited the maximum radical-scavenging activity (IC_{50} 69.23-78.96 µg/mL), followed by *P. frutescens* (IC_{50} 334.26-359.16 µg/mL) and *M. spicata* (IC_{50} 343.26.1-448.2 µg/mL) with marginal activity. *P. glaber*, *M. longifolia*, *C. umbrosum,* and *C. oppositifolia* EOs exhibited lower radical-scavenging capacity than other Lamiaceae plants employed for the analysis. The IC_{50} values of these EOs were greater than 450 µg/mL. In overall, the radical-scavenging potential showed a significant variation among the tested Lamiaceae essential oil samples ($p < 0.05$).

One of the previous studies reported that essential oils with major components like linalool, menthone, and piperitenone oxide exhibited excellent radical-scavenging capacity (Sharopov *et al*., 2015). However, the better antioxidant potential and maximum radical-scavenging activity of *O. tenuiflorum*, *C. coccinea*, *O. majorana*, and *O. basilicum* essential oils may be correlated with the high percentages of eugenol, terpinen-4-ol, isocryophyllene, linalool, and methyl chavicol components, respectively (Gulcin, 2011; Oliveira *et al*., 2021; Badr *et al.*, 2023). Though it looks challenging to predict the radical-scavenging and antioxidant capacity of the whole EO to one or a few major components. Because the EO is a complex mixture of several active components. Generally, the antioxidant potential of the whole EO exhibited better antioxidant and radical-scavenging capacity than the individual components. This is probably an indication of synergistic interaction between different EO constituents.

There are no detailed previous findings described in the literature regarding the DPPH radical-scavenging capacity of many Lamiaceae essential oils. However, one previous study explored that *M. longifolia* oil had better radical-scavenging potential (Baris *et al*., 2006). Another study also recognized that different *Mentha* species were capable of scavenging DPPH radicals in the following decreasing order: *M. piperita* > *M. dalmatica* and *M. spicata* (Dorman *et al.*, 2003). Literature also revealed the mild radical-scavenging capacity of *Mentha* EOs (Mimica-Dukic *et al*., 2016; Yadegarinia *et al*., 2006). These results are in agreement with some previous studies, which reported that the essential oils of *O. tenuiflorum* and *O. basilicum* disclosed superior antioxidant capacity than individual components, signifying the possible some sort of synergism of the EO components (Joshi, 2013; Politeo *et al*., 2007). Similarly, another study described *O. majorana* essential oil as a potential source of antioxidants (IC_{50} of 16.83 µg/mL) (Chaves *et al*., 2019). There are some exclusive findings on the radical-scavenging potential of Lamiaceae essential oils (Mimica-Dukic *et al*., 2004; Dastmalchi *et al*., 2008), which show the importance of Lamiaceae plant species.

4.1.8.2 ABTS radical-scavenging activity

The antioxidant potentials of Lamiaceae essential oils were determined by the ABTS radical-scavenging method, expressed in terms of IC_{50} values, which are presented in Annex 3. The radical-scavenging capacity of the essential oils was also observed to increase in a concentration-dependent way, and the trends of radical-scavenging with change in concentration in the ABTS assay are shown in Annex 11. The comparative antioxidant capacity of essential oil samples in terms of IC_{50} values from the ABTS method is presented in Figure 4.13 (b). The highest antioxidant property was exhibited by *O. tenuiflorum* (IC_{50} 5.88-17.69 µg/mL), which was more effective as compared to other samples and was even stronger than the standard used (quercetin, IC_{50} 7.79 µg/mL, and ascorbic acid, IC_{50} 1.98 µg/mL). The least effect was noted for *M. pulegium* essential oil from Nuwakot during the summer (IC_{50} 145.35 µg/mL) and *O. americanum* from Thankot during the summer (IC_{50} 145.66 µg/mL). Among the *Mentha* species, *M. spicata* EO displayed better radical-scavenging activity with an IC_{50} value (IC_{50} 34.15-80.89 µg/mL) followed by *M. longifolia* (IC_{50} 53.49 µg/mL), and the lowest *M. pulegium* (IC_{50} 61.44-145.35 µg/mL). *O. majorana* essential oils collected from three locations showed relatively better scavenging capacity with lower IC_{50} values (IC_{50} 34.74-49.03 µg/mL).

The *E. strobilifera* essential oil also displayed good radical-scavenging efficacy, with an IC_{50} value of IC_{50} of 34.74 µg/mL. The *C. coccinea* and *O. basilicum* essential oils displayed substantial radical-scavenging activity, with IC_{50} values ranging from 38.13-52.05 µg/mL and 44.38-61.4 µg/mL, respectively. The ABTS scavenging potential also varied significantly among the Lamiaceae essential oil samples ($p < 0.05$). According to the literature review, there haven't been any previous in-depth findings on the ability of certain EOs from the Lamiaceae family to scavenge ABTS radicals. However, one researcher investigated that *O. tenuiflorum* essential oil had a good radical-scavenging capacity (Zheljazkov *et al*., 2008). As expected, the better antioxidant capacity of *O. tenuiflorum* EO could be due to the maximum percentage of eugenol contents (Sharopov *et al*., 2015).

The IC_{50} values obtained from the ABTS technique were found to be lower as compared to those achieved by the DPPH technique. The disparities in IC_{50} values between these two techniques could be attributed to different mechanisms of activity. The previous findings have revealed that the e^- transfer reaction proceeds at a much faster rate during the ABTS assay in comparison to the reaction occurring in the DPPH assay. The reaction occurring in the DPPH assay might be responsible for the hydrogen-donating ability of various components present in the essential oil samples (Platzer *et al*., 2021).

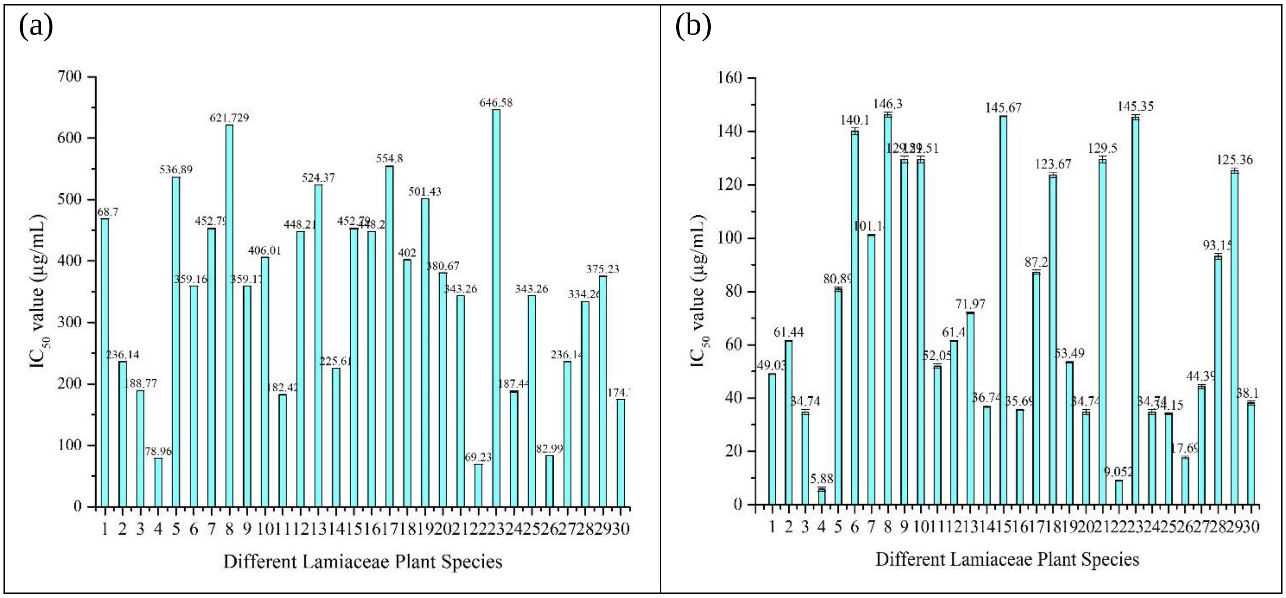


Figure 4. 13 Anioxidant activity of Lamiaceae essential oil in terms of IC_{50} values, (a) DPPH assay and (b) ABTS assay

4.1.8.3 Seasonal variation in the antioxidant activities

In both assays, essential oils showed some variation in their antioxidant potential with changes in harvesting seasons as shown in Figure 4.14 (a, b). The present findings explored that the antioxidant capacity of *M. spicata*, *P. frutescens*, *L. canum*, and *O. majorana* EOs achieved during the summer was greater as compared to those obtained during the winter season. These results may be associated with the variation in leading compounds like carvone, perilla ketone, isocaryophyllene, terpinen-4-ol, etc. from the winter to the summer season. In contrast, *O.*

americanum and *O. basilicum* essential oils collected during the winter season revealed higher activity than those from the summer one. Similarly, *O. tenuiflorum* essential oil displayed its best antioxidant property when harvested during the winter than that in the autumn season. These results may be correlated with the variation in the dominance of their prominent compounds like camphor, methyl chavicol, eugenol, etc., or some difference in synergistic effects with the harvesting seasons. The variations in the antioxidant properties of the Lamiaceae essential oils with respect to the seasons showed a significant difference ($p < 0.05$).

The variation in the antioxidant potential of essential oils might be due to the fluctuation of active constituents regarding the seasons. Thus, some of the previous reports in the literature also showed that seasonal and environmental conditions can directly influence the phytochemistry of a plant species (Lakusic *et al*., 2012; Aissi *et al*., 2016). There was a notable fluctuation in the antioxidant activity of the same plant when it was gathered throughout different seasons of the year. It might be due to the fluctuations in the quantity of the active constituents in individual plant species (Guimaraes *et al*., 2023). The disparity in the chemical profiles could be reinforced by the thermoregulatory action of hydrophobic compounds in the essential oils (Kamatou *et al*., 2008). Overall, the present results also support the fact that the antioxidant potential of essential oils varies with the harvesting seasons, and most of the Lamiaceae essential oils from the summer had better antioxidant activity than those from the winter.

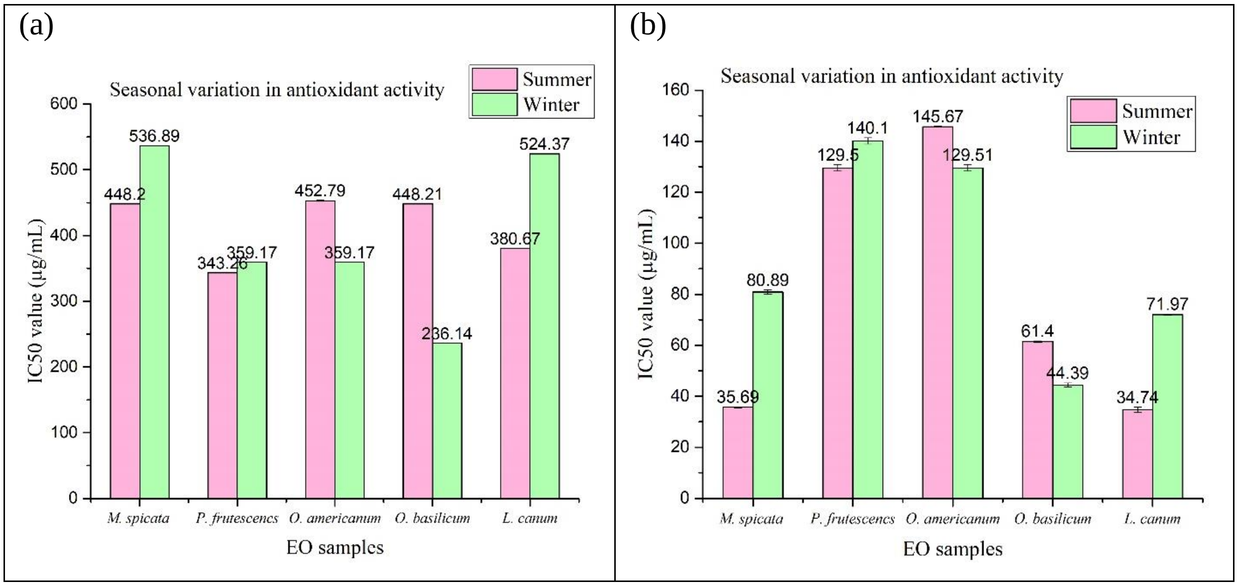


Figure 4. 14 Seasonal variation in antioxidant activity of Lamiaceae essential oils (IC_{50} values) in (a) DPPH assay and (b) ABTS assay

4.1.8.4 Geographical variation in the antioxidant activities

The geographical variations in the antioxidant potentials of some of the Lamiaceae essential oils are shown in Figure 4.15 (a, b). *O. tenuiflorum* oil showed better antioxidant activity collected from the Bansgadhi (tropical) than that from the Kirtipur (sub-tropical). This may be due to high amounts of eugenol in the *O. tenuiflorum* collected from Bansgadhi. Similarly, the *P. frutescens* and *M. spicata* essential oils obtained from the Bansgadhi region revealed better antioxidant activity compared to that from Dhulikhel (sub-tropical). This may be attributed to the high percentage of perilla ketone in *P. frutescens* and the high amounts of carvone in *M. spicata*, both produced from the Bansgadhi. Moreover, *C. coccinea* essential oil obtained from the Langtang region exhibited better antioxidant potential as compared to that from Bethanchowk, which may be associated with variations in the amounts of isocaryophyllene and β-caryophyllene or may be due to some synergistic effects of the compounds. A significant alteration in the scavenging power of Lamiaceae EOs tested with respect to the locations was observed ($p < 0.05$).

Some essential oils obtained from the plain region showed relatively better scavenging power than those from the hilly region. The variation may be associated with changes in the quantity of eugenol, carvone, linalool, and perilla ketone. Eugenol may be linked to the better radical-scavenging power of *O. tenuiflorum* essential oil. Though there are no detailed studies from Nepal, some previous findings from outside the country have revealed the better antioxidant capacity of *Ocimum* species EOs (Salles Trevisan *et al*., 2006; Zheljazkov *et al*., 2008).

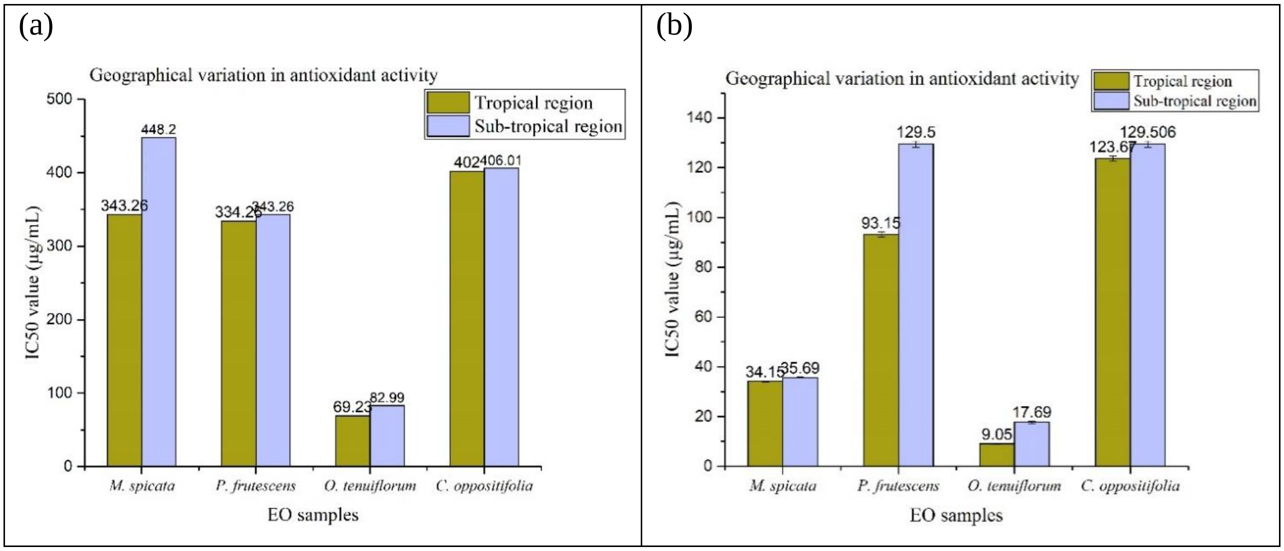


Figure 4. 15 Geographical variation in antioxidant activity of Lamiaceae essential oils (IC_{50} values) in (a) DPPH assay and (b) ABTS assay

4.1.9 Cytotoxicity of Essential Oils

The effects of Lamiaceae essential oils on the viability of different cell lines, like MCF-7 and NIH-3T3, were determined to explore their cytotoxicity and antiproloferative capacity. The cytotoxicity was expressed as the IC_{50} values, which represent the concentration of EOs preventing 50% of cell viability and are shown in Table 4.43. The viability of cell lines after incubation with different concentrations of Lamiaceae essential oils was observed to have prominent impacts. The results displayed that the incubation with variation in concentrations of essential oil influenced the viability of NIH-3T3 and MCF-7 cell lines, as given by a plot of cell viability (%) versus logarithm concentrations (µg/mL) of the essential oil samples (Figures 4.16 and 4.18). The essential oil samples showed an inhibitory effect on the cell lines, depending on the variations in their concentrations. The cytotoxicity was expressed as IC_{50} values, which indicate the concentrations at which 50% of cells were killed in DMEM. The photographs of morphological changes of the 3T3 cell line when treated with *P. frutescens* essential oil and the standard as a function of concentrations (µg/mL) are shown in Figure 4.17. Similarly, photographs of the morphological changes of MCF-7 cells when treated with *P. frutescens* essential oil and the standard treated with the MCF-7 cell line are shown in Figure 4.19. Actually, these morphological changes have been observed with the variation in the concentrations of essential oil samples.

Similarly, some of the essential oils could also enhance cell proliferation at lower concentrations. This capacity might be expressed in terms of cell index percentage, and some essential oils can kill the cells at very high concentrations (100 µg/mL) but activate cell growth at low concentrations. Therefore, their application as anticancer agents would be insignificant. The cell index (%) for these samples is represented by a graph of cell index (%) versus the concentration (µM) for cell proliferation, as shown in Figure 4.20. Essential oils from *O. majorana* (S-14), *M. spicata* (S-16), *M. longifolia* (S-19) and *O. tenuiflorum* (S-22) have a weak proliferation effect at low concentrations against the MCF-7 cell line.

Among the Lamiaceae essential oils employed, *P. frutescens, M. longifolia*, *C. umbrosum*, and *E. strobilifera* showed higher cytotoxic potential against the cell lines tested, with IC_{50} values varying from 7.41 to 23.76 µg/mL. Essential oils from *M. pulegium, M. spicta, P. glabar,* and *O. basilicum* were shown to be the least toxic, with IC_{50} values varying from 99.64 to 90.56 µg/mL. *P. frutescens* (IC_{50} 7.41 and 8.14 µg/mL), *C. umbrosum* (IC_{50} 21.70 and 12.53 µg/mL), *M.*

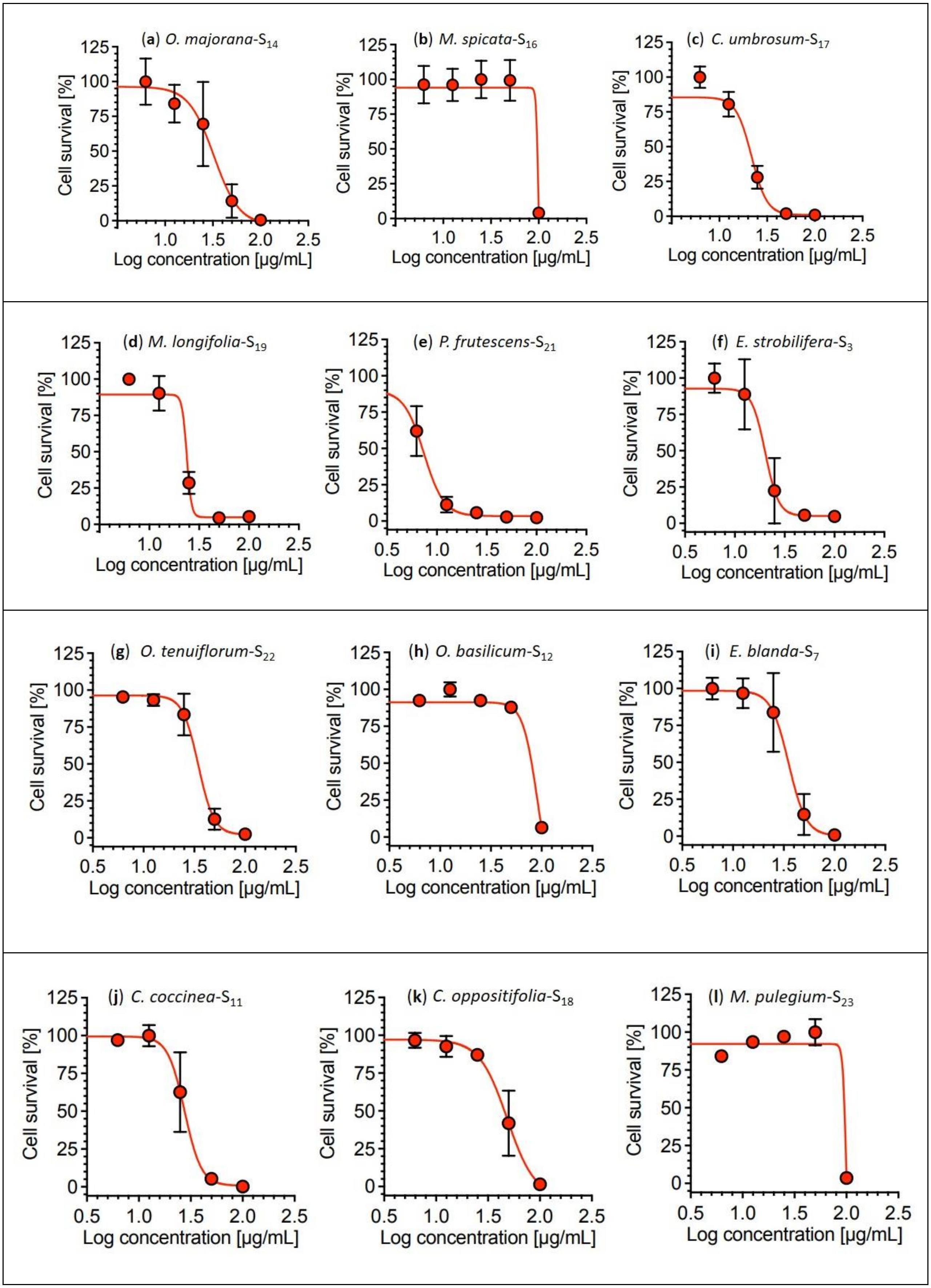
longifolia (IC_{50} 23.76 and 12.12 µg/mL), and *E. strobilifera* (20.17 µg/mL). Essential oils were highly cytotoxic as compared to other species against NIH-3T3 and MCF-7 cell lines, respectively, among which *P. frutescens* essential oil had the most inhibitory effects on cell viability for both cell lines. *M. pulegium* essential oil showed less toxicity (IC_{50} 99.64 µg/mL) against the NIH-3T3 cell line. Similarly, *O. basilicum* essential oils exhibited comparatively less effectiveness in inhibiting cell growth of both cell lines, i.e., less toxicity against the MCF-7 cell line (IC_{50} 92.88 µg/mL) and the NIH-3T3 cell line (IC_{50} 90.56 µg/mL). The IC_{50} values of *C. coccinea* essential oils were 27.58 µg/mL and 24.04 µg/mL on the cell proliferation of NIH-3T3 and MCF-7, respectively. The essential oils derived from *O. tenuiflorum* exhibited the most interesting cytotoxic activity against NIH-3T3 (IC_{50} 34.62 µg/mL) and MCF-7 (IC_{50} 23.43 µg/mL). *E. blanda* essential oil offered moderate cytotoxicity against the cell lines tested. The IC_{50} values of *E. blanda* EO were 35.31 µg/mL and 33.13 µg/mL on the cell proliferation of NIH-3T3 and MCF-7, respectively. *C. oppositifolia* essential oil was considerably less toxic against. NIH-3T3 with an IC_{50} of 48.40 µg/mL, whereas it had considerable cytotoxic potential against MCF-7 with an IC_{50} of 22.37 µg/mL. Similarly, *O. americanum* had comparatively mild cytotoxic activity against NIH-3T3 (49.45 µg/mL) and MCF-7 cell lines (57.42 µg/mL), respectively. In the case of *O. majorana*, the essential oil was found to have considerable cytotoxicity against NIH-3T3 with IC_{50} values of (32.44 µg/mL) and was weaker against MCF-7 (53.51 µg/mL). *P. glaber* essential oil was comparatively less toxic against NIH-3T3 and MCF-7 cell lines, with IC_{50} values of (97.72 µg/mL) and (51.18 µg/mL), respectively. The viability of cells significantly decreased at high concentrations of the essential oils. In accordance with the present investigations, the order of cytotoxic activity of Lamiaceae essential oil, which were comparatively less cytotoxic against NIH-3T3 and MCF-7 cell lines, was as follows: *O. basilicum* (92.88 and 90.56) > *P. glaber* (97.72 and 51.18 µg/mL) > *M. spicata* (98.93 µg/mL) > *M. pulegium* (99.64 and 58.47 µg/mL). However, essential oil samples exhibited weaker cytotoxic potential than that of positive control gemcitabine, with IC_{50} values of 0.4977 µg/mL and 0.5175 µg/mL for MCF-7 and 3T3 cell lines, respectively. Actually, the cytotoxic effects evaluated for the present essential oils are comparatively found to be stronger as .compared to those of other Lamiaceae plants (Perez-Gonzalez *et al.*, 2019). Lamiaceae essential oils displayed the most remarkable cytotoxic activity against the MCF-7 cancer cell line. *P. frutescens* could be a good agent to inhibit breast cancer cell proliferation in comparison to other Lamiaceae plants with lower IC_{50} values. This result is in agreement with some of the previous findings (de Sousa *et al*., 2010; Perez-Gonzalez *et al*., 2019). This cytotoxic activity of *P. frutescens* may be associated with dominat components like perilla ketone, followed by isoegomaketone, or some kind of synergistic effect of minor constituents present in the essential oil sample.

Commonly, it is considered that the vital bioactive constituents of EOs regulate their biological activities. Regarding the published guidelines (Gad, 2009), IC_{50} values of 10-100 µg/mL represent promising inhibitory capacity against cancer cells. This indicates the potent cytotoxic activities of Lamiaceae essential oils. Actually, it has also been described that sesquiterpenes are primarily responsible for the cytotoxicity of these EOs (Sylvestre *et al*., 2005). The cytotoxicity of these oils may involve some type of synergism of minor constituents with the other active or major compounds (Yu *et al*., 2004). There are very limited findings reported in the literature in .the context to the cytotoxicity of Lamiaceae essential oils (de Sousa *et al*., 2010), and therefore, there are apparently no previous results.on the cytotoxicity of many Lamiaceae EOs.

Table 4. 43 Cytotoxicity of essential oils in terms.of IC_{50} values, µg/mL

S.N.	**Lamiaceae EOs and standard**	**Cell lines**	
		NIH-3T3 (IC_{50}, µg/mL)	**MCF-7 (IC_{50}, µg/mL)**
1.	*O. majorana* (14)	32.44	53.51
2.	*M.spicta* (16)	98.93	-
3.	*C. umbrosum(17)*	21.70	12.53
4.	*M. longifolia*(19)	23.76	12.12
5.	*P. frutescence*(21)	7.41	8.14
6.	*E. strobilifera*(3)	20.17	50.08
7.	*O. tenuiflorum* (22)	34.62	23.43
8.	*O. basilicum* (12)	90.56	92.88
9.	*E. blanda* (7)	35.31	33. 13
10.	*C. coccinea* (11)	27.58	24.04
11.	*C. oppositifolia (18)*	48.40	22.37
12.	*M. pulegium* (23)	99.64	58.47
13.	*O. americanum* (15)	49.45	57.42
14.	*P. glabar* (8)	97.72	51.18
15	Gemcitabine	0.517	0.4966

Note: IC_{50} is median inhibitory concentration, '-'= Not determined.



(a) O. majorana-S_{14}
(b) M. spicata-S_{16}
(c) C. umbrosum-S_{17}
(d) M. longifolia-S_{19}
(e) P. frutescens-S_{21}
(f) E. strobilifera-S_{3}
(g) O. tenuiflorum-S_{22}
(h) O. basilicum-S_{12}
(i) E. blanda-S_{7}
(j) C. coccinea-S_{11}
(k) C. oppositifolia-S_{18}
(l) M. pulegium-S_{23}
Cell survival [%]
0
25
50
75
100
125
Log concentration [µg/mL]
0.5
1.0
1.5
2.0
2.5

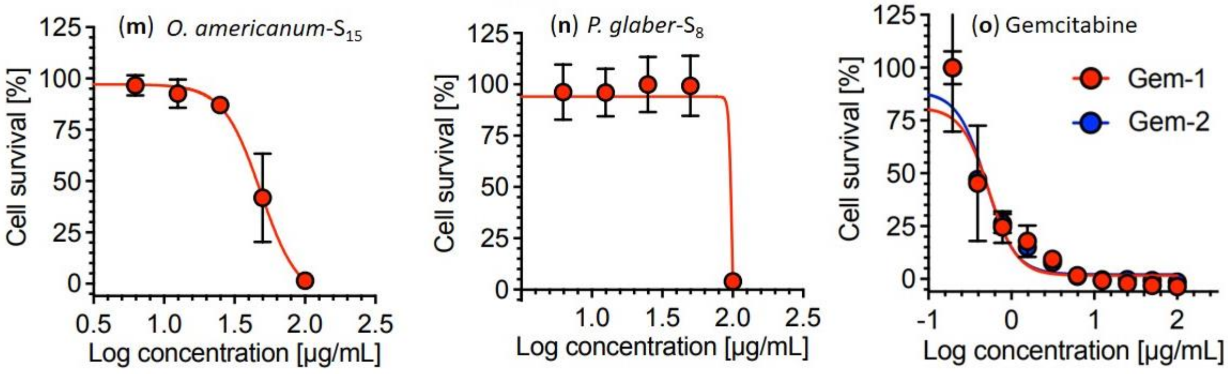


Figure 4. 16 Graph representing the percentage cell survival versus logarithm .of the concentrations (µg/mL) for cytotoxicity of Lamiaceae essential oils and standard against NIH-3T3 cell line (a-o).

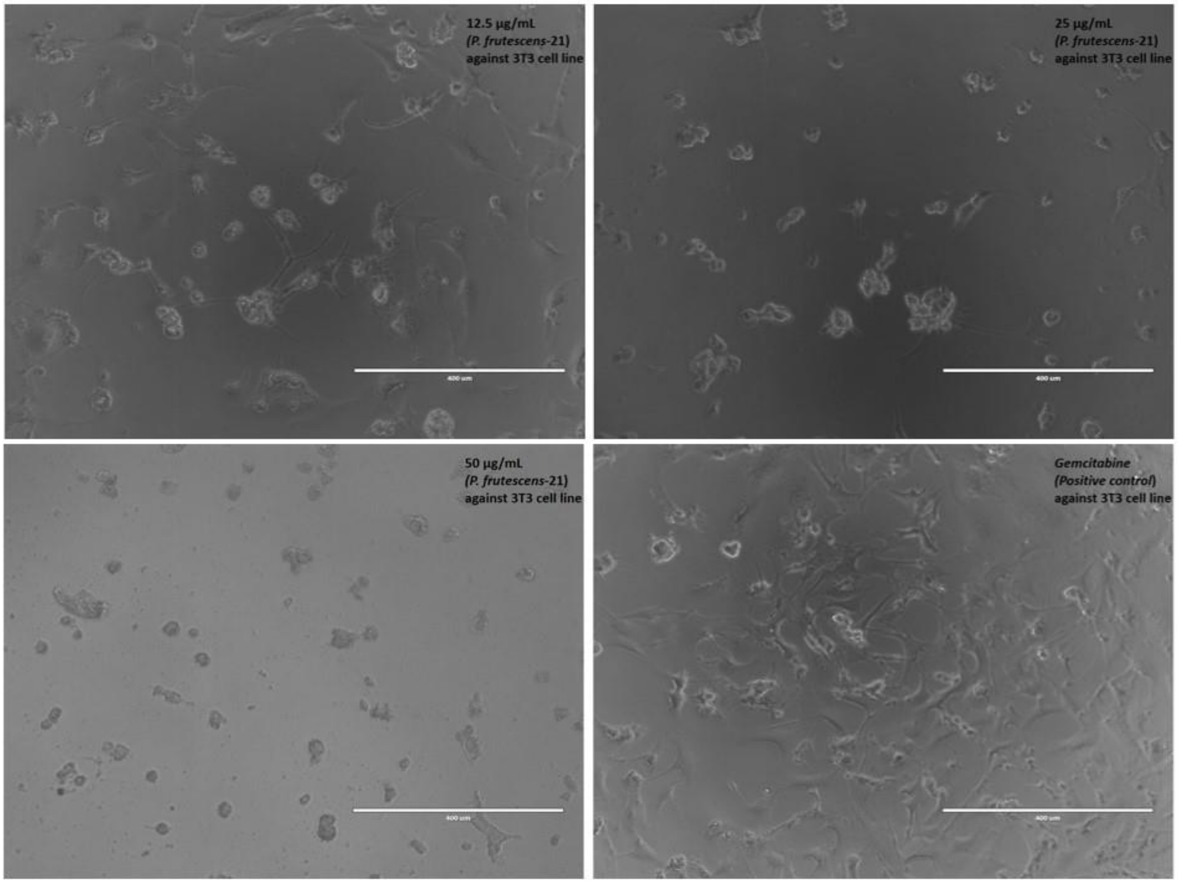
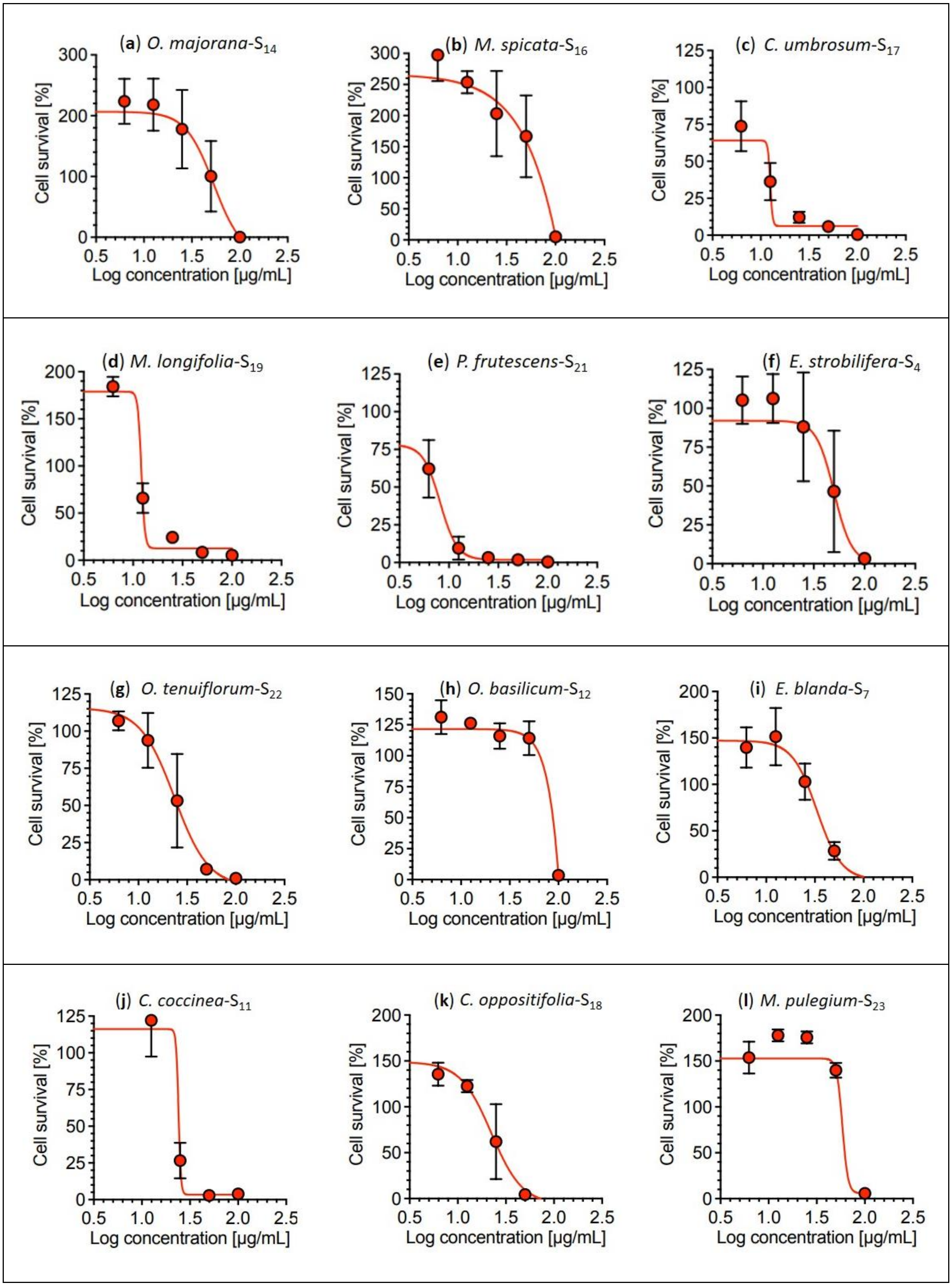


Figure 4. 17 Photographs of morphological changes of 3T3 cell line when treated with *P. frutescens* (S-21) essential oil and standard


(a) O. majorana-S14
(b) M. spicata-S16
(c) C. umbrosum-S17
(d) M. longifolia-S19
(e) P. frutescens-S21
(f) E. strobilifera-S4
(g) O. tenuiflorum-S22
(h) O. basilicum-S12
(i) E. blanda-S7
(j) C. coccinea-S11
(k) C. oppositifolia-S18
(l) M. pulegium-S23
Cell survival [%]
Log concentration [µg/mL]
0.5
1.0
1.5
2.0
2.5

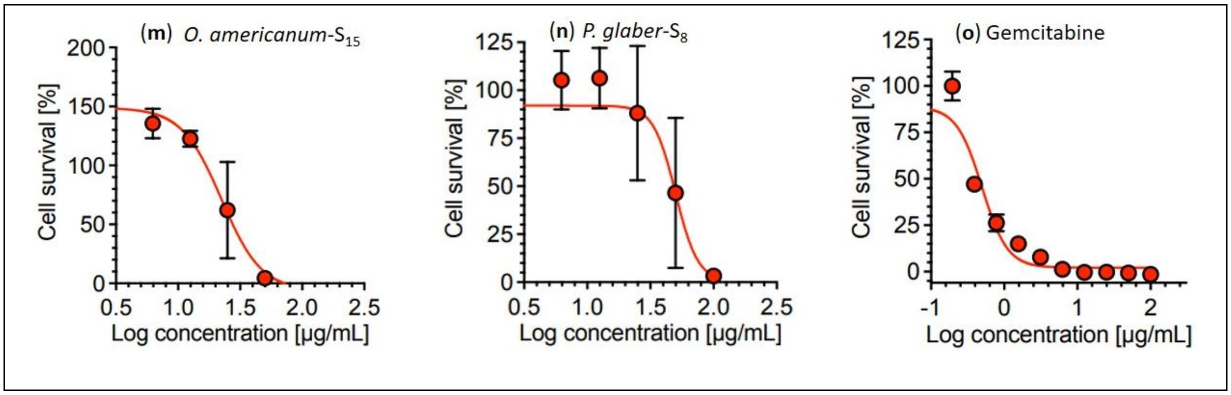
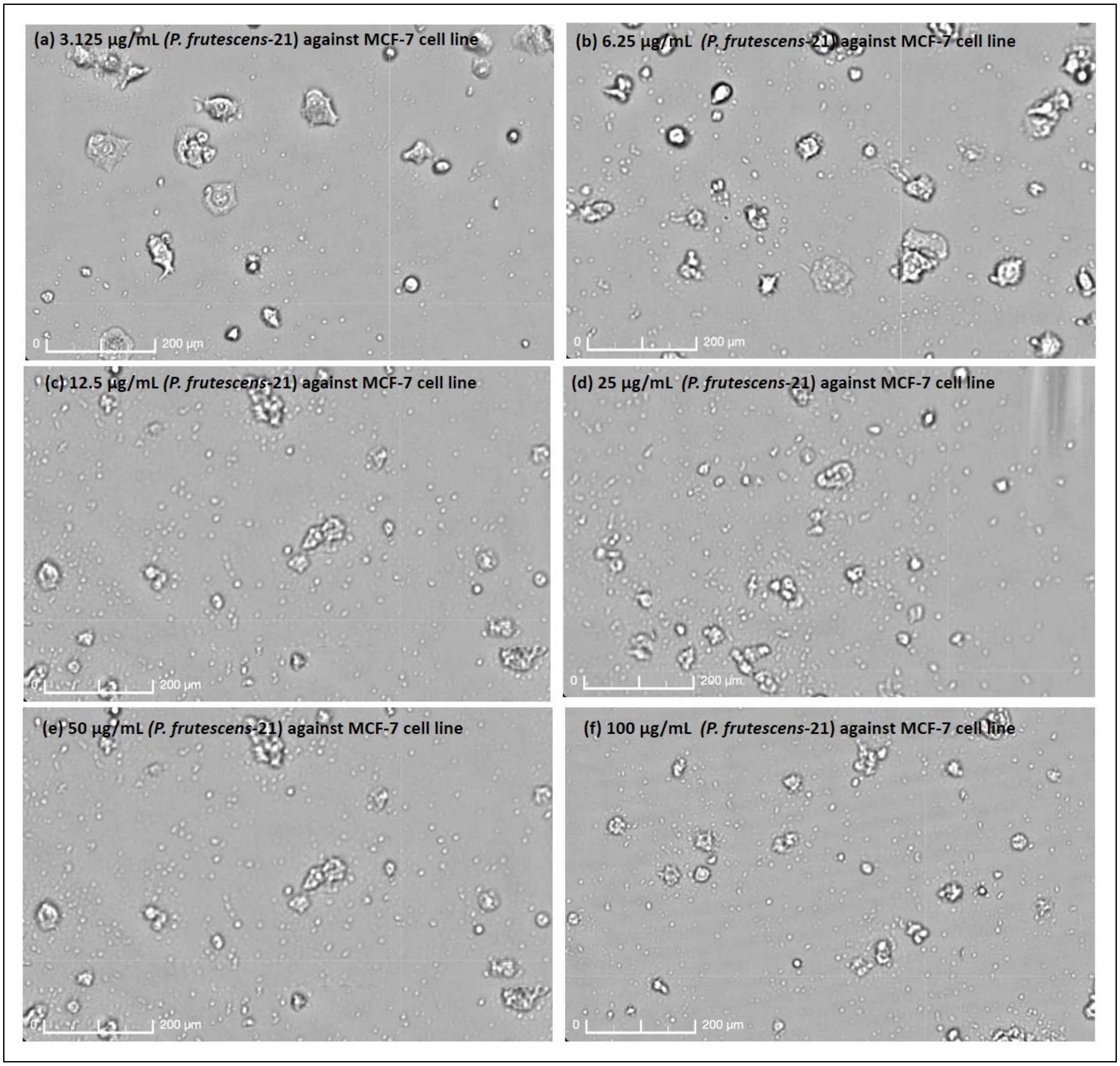


Figure 4. 18 Graph representing the percentage cell survival versus logarithm of the concentrations (µg/mL) for Lamiaceae essential oils and standard against MCF-7 (a-o).



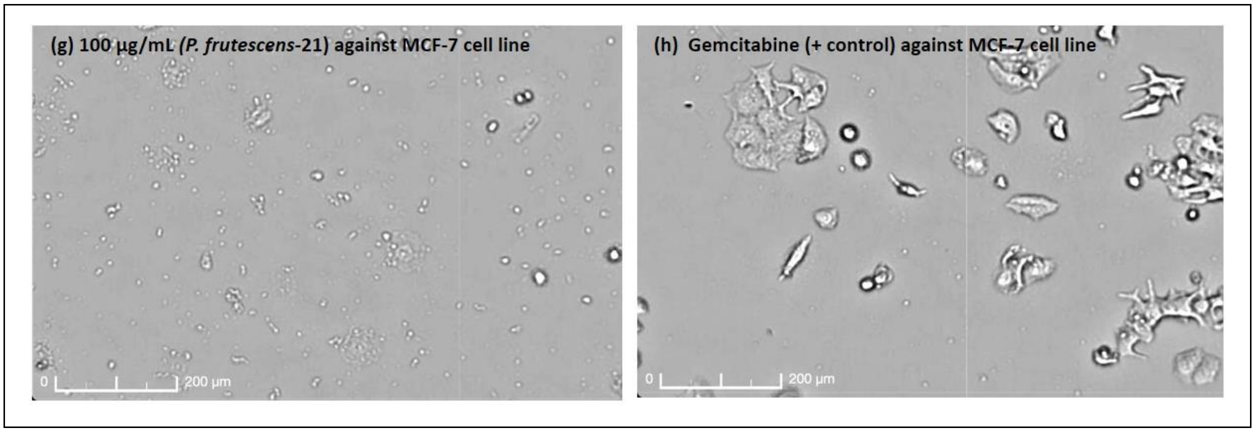
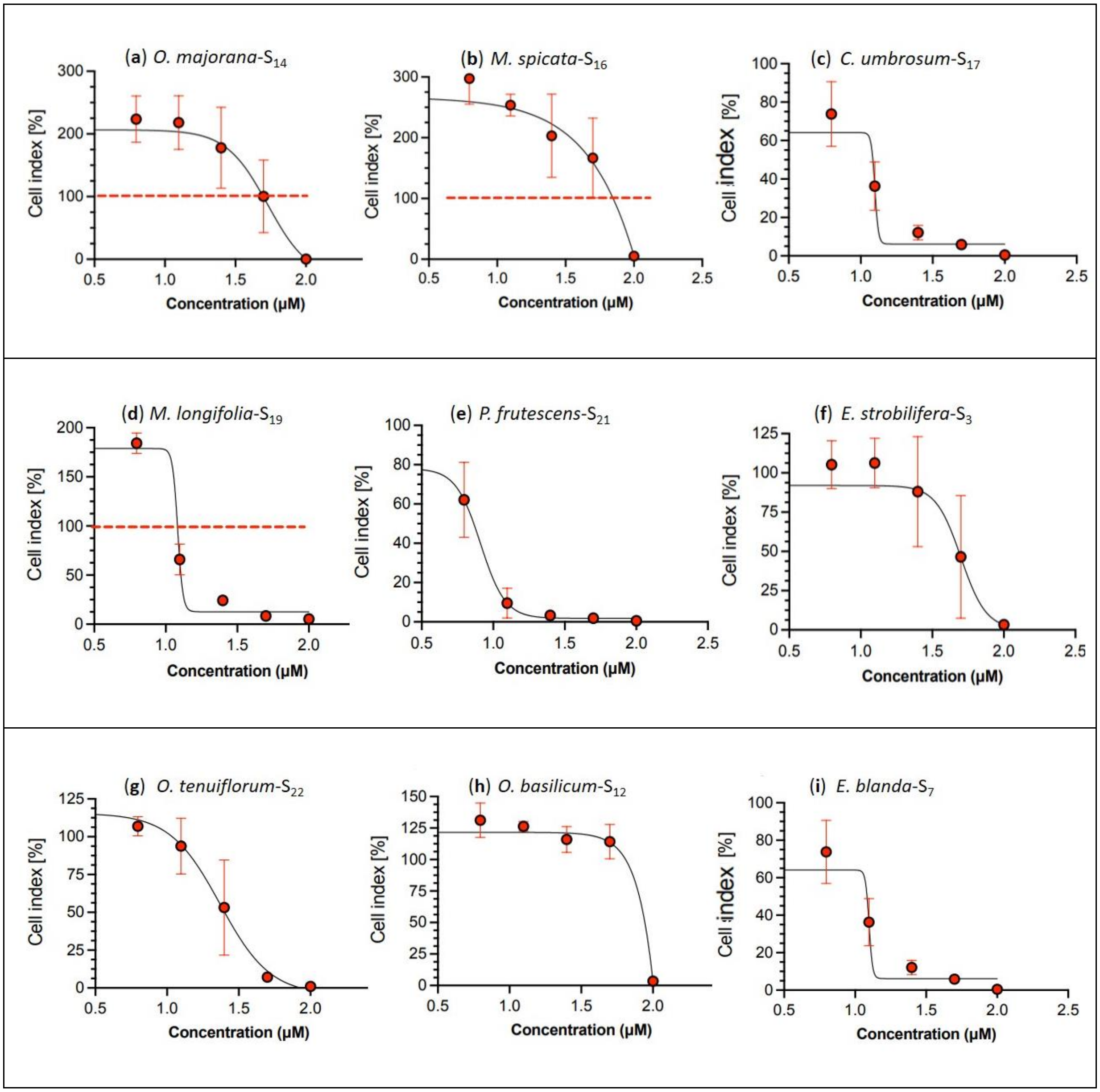


Figure 4. 19 Photographs of morphological changes of MCF-7 cells when treated with *P. frutisens* (S-21) essential oil and standard (a-h).



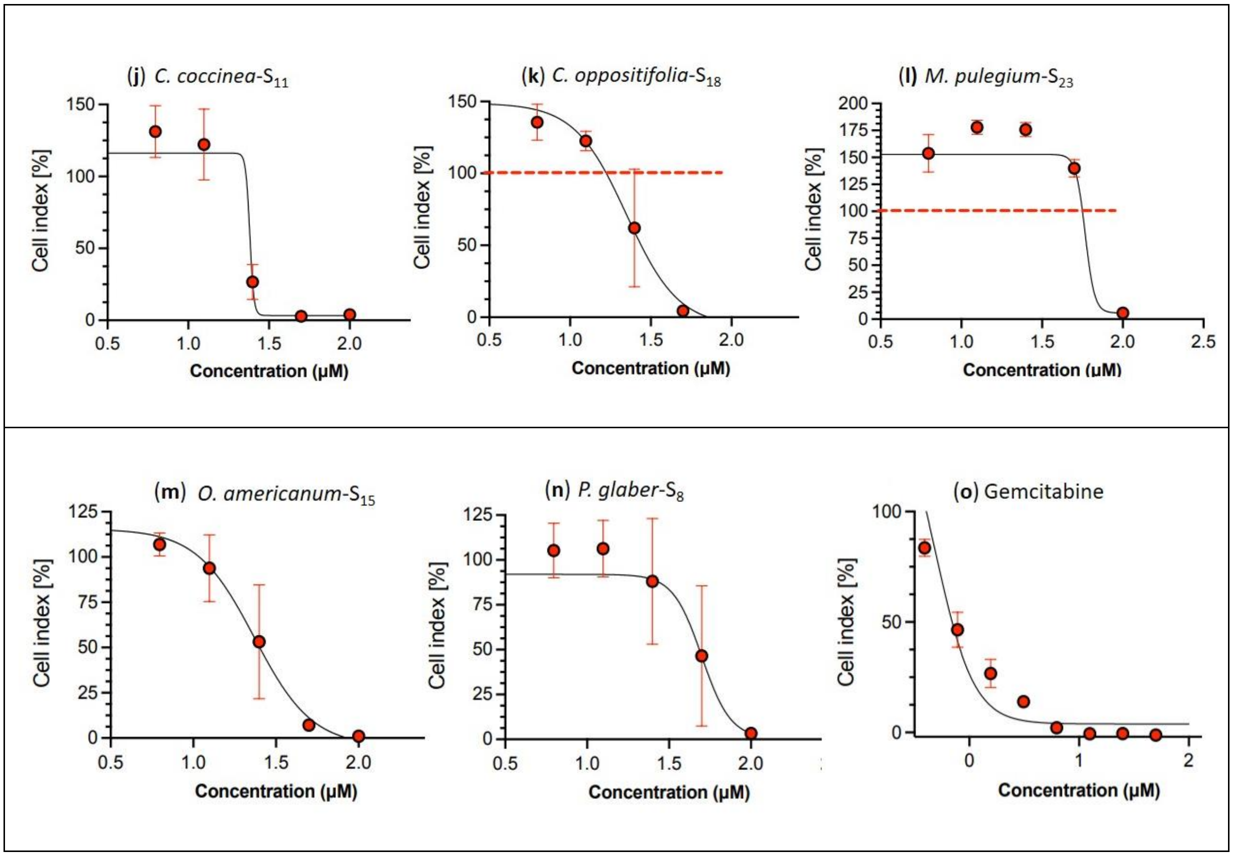


Figure 4. 20 Graph representing cell index (%) versus the concentrations (µM) for cell proliferation of Lamiaceae essential oils and standard against cancer cell line, MCF-7 (a-o).

4.1.10 Formulation of Topical Cream: By Applying Essential Oils

The concentrations of different components were optimized during the formulation period at different trials following the standard protocol based on the handbook of pharmaceutical excipients which is presented in Table 3.3. Here, essential oils with the best antioxidant activity and a pleasant aroma were taken as the best combinations. Then, the cream was employed for various tests.

4.1.10.1 Physical compatibility test

The base cream loaded with essential oils (Figure 4.21-a) showed no characteristic change (NCC) in color, preserving homogeneity, viscosity, and texture when loaded with the cream base for months in separate vials, showing a good compatibility test between essential oil and the base cream. Physical compatibility testing of excipients is required because it is important in product development and commercialization, allowing for the selection of the best excipients and maximizing shelf life (Tan *et al*., 2022).

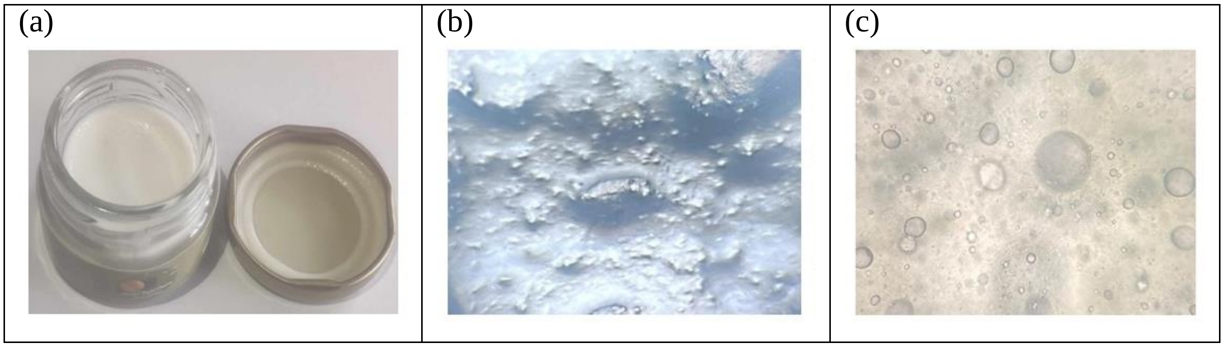


Figure 4. 21 Cream formulation (a) formulated cream enriched with essential oils, (b). homogeneous distribution of cream base and (c) visible cream base (microscopic profile: 40 ×)

4.1.10.2 Organoleptic characteristics

The cream had a uniform texture that was smooth and calming, with no greasiness at all. It had a slightly yellowish color and a fresh aroma (loaded with essential oils). At 180º inversion, the cream did not flow, an optimal requirement for topical application (Salehi *et al*., 2022).

4.1.10.3 Cream type determination

When 1 g of cream was washed with running water and removed with no greasiness left, it meant good oil/water (o/w) cream (visually observed) (Gyawali *et al*., 2020). Figure 4.21 (b, c) shows the microscopic profile (40 ×) representing the homogeneous distribution of cream base only and visible cream base with essential oil droplets seen.

4.1.10.4 Cream spreadability, pH, and centrifugation study

The spreadability of cream lies between 5-7 cm (6.27±0.42 to 5.70±0.17) either at real or accelerated (Table 4.44). This showed that the cream can be easily distributed with little effort on the site of application and shows uniform particle distribution (Tan *et al*., 2022). The pH of cream lies between 6 and 8, ranging from 6.50±0.32 to 6.250±0.04 at either real or accelerated conditions (Table 4.44). An effective cream has a skin physiological pH range of 6–8 (Pinto *et al*., 2021; Tan *et al*., 2022). The formulated cream centrifugation demonstrated a maximum % of 19.67±2.08 to 11.33±0.58 at either real or accelerated (Table 4.44). The cream showed desirable stability in both conditions. An ideal unstable cream emulsion has 100% emulsion instability (Restu *et al*., 2015; Tan *et al*., 2022).

Table 4. 44 Cream spreadability, pH and centrifugation under both normal and accelerated time conditions

	Room temperature			**Freeze-thaw cycling**		
Duration (months)	**Spreadability** (cm) (mean±SD)	**pH** (mean±SD)	**Centrifugation** (%) (mean±SD)	**Spreadability** (cm) (mean±SD)	**pH** (mean±SD)	**Centrifugation** (%) (mean±SD)
0	6.267±0.416	6.350±0.135	11.333±0.577	5.700±0.173	6.280±0.060	15.000±2.646
1	6.133±0.115	6.420±0.080	12.333±1.528	5.967±0.208	6.250±0.040	19.000±1.000
2	6.033±0.231	6.390±0.030	15.000±3.606	5.900±0.458	6.327±0.095	19.667±2.082
3	6.267±0.416	6.267±0.115	17.000±1.000	5.900±0.100	6.500±0.318	19.333±0.577

Where, n = 3 for independent performed experiments

4.1.10.5 Rheological study

The cream has demonstrated a rheology profile whereby the viscosity (Pa. s) decreased but not linearly with increasing shear rate (sec^{-1}) (Table 4.45). The initial apparent viscosity was highest, 152.00±2.65 Pa. s at shear rate of 1.7 sec^{-1}, at lower shear stress initially (51.57±2.65 pa), but apparent viscosity decreased with increasing shear rate (sec^{-1}) in Figure 4.22. This illustrates the thixotropic profile of cream (Korhonen *et al*., 2001). It was established that cream can spread well on the skin surface when rubbed gently, as rubbing decreases the cream's viscosity (Korhonen *et al*., 2001; Pinto *et al*., 2021).

Table 4. 45 Rheological study with changing RPM

RPM	Shear rate (s^{-1})	Viscosity (mean)	SD	Shear stress (mean)	SD
1	1.7	152.000	2.646	51.567	2.646
10	17	72.000	2.646	33.000	6.028
20	34	43.667	3.512	32.556	3.464
30	51	30.103	0.170	37.034	5.508
40	68	20.667	1.155	42.889	4.041
50	85	10.333	0.577	48.444	9.074
60	102	7.000	1.000	56.333	5.774

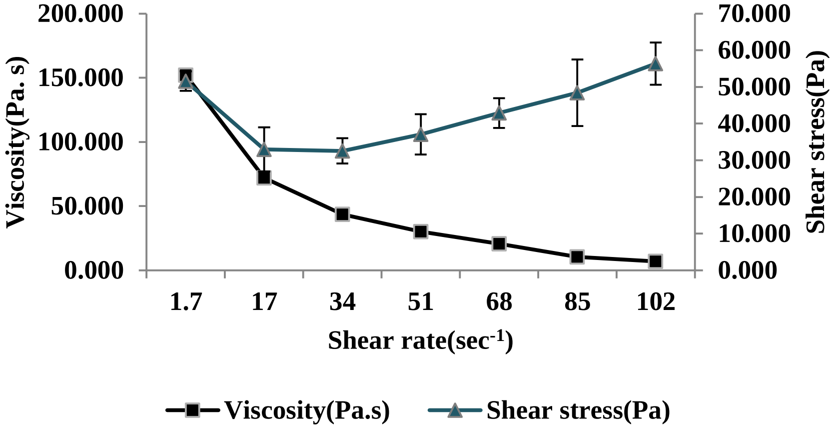


Figure 4. 22 A plot of shear rate vs. viscosity and shear rate vs shear stress

4.1.10.6 Antioxidant assay of cream

The antioxidant activities of ascorbic acid and formulated cream are shown in Table 4.46. Similarly, Annex 11 illustrates the radical-scavenging activity with a concentration-wise trend for standard and formulated creams. In the DPPH assay, the IC_{50} value of cream loaded with essential oils was 75.82±0.06 µg/mL, whereas the ascorbic acid showed 7.44±0.015 µg/mL. This indicates that this cream has good antioxidant activity and skin-beneficial properties. Also, EC_{50} values for cream and ascorbic acid in the FRAP assay were 487.21±2.94 µg/mL and 201.287±1.55 µg/mL, respectively. The FRAP assay also indicated that the cream was rich in antioxidant properties. In the ABTS assay, the IC_{50} values of cream and standard were 65.69±2.666 µg/mL and 7.467±0.849 µg/mL, respectively, indicating the effective antioxidant activity of cream. The antioxidant activities of cream in both real-time and accelerated time exhibit the stability of cream (Table 4.47). Based on the antioxidant activities of essential oil-loaded cream, it had some enriched antioxidant chemicals, such as polyphenol compounds or flavonoids, or some synergistic effects. This property is essential for any cosmetic cream used on the skin because the antioxidant property maintains skin stress and turnover rate by interfering with any ROS in the body. A study performed by Jadoon et al. reported that loading with these oils showed a synergistic antioxidant effect in the formulation. Also, formulated creams have a pleasing, calm, and soothing fragrance, fascinating the product's consumers (Jadoon *et al.*, 2015).

Table 4. 46 Antioxidant activity of formulated creams using different methods

Samples	**DPPH Radical Scavenging**	**FRAP**	**ABTS Radical Scavenging**
	IC_{50} value (µg/mL)	**EC_{50} value (µg/mL)**	**IC_{50} value (µg/mL)**
Ascorbic acid	7.438±0.015	201.287±1.551	7.467±0.849
Formulated Cream	75.816±0.065	487.214±2.937	65.687±2.666

Table 4. 47 The antioxidant activities of cream in both real time and accelerated time

Duration (months)	**Room temperature**			**Freeze-thaw cycling**		
	DPPH activity (IC_{50}, µg/mL) (mean±SD)	**FRAP assay** (EC_{50}, µg/mL) (mean±SD)	**ABTS activity** (IC_{50}, µg/mL) (mean±SD)	**DPPH activity** (IC_{50}, µg/mL) (mean±SD)	**FRAP assay** (EC_{50}, µg/mL) (mean±SD)	**ABTS** activity (IC_{50}, µg/mL) (mean±SD)
0	75.816±0.065	487.214±2.937	65.687±2.666	72.111±1.254	485.587±0.036	62.877±1.025
1	75.480±0.289	485.222±2.010	64.258±1.541	71.444±0.211	483.254±0.784	60.457±0.412
2	72.251±0.694	485.241±1.547	60.124±0.298	70.894±0.087	482.548±0.255	59.214±0.269
3	72.146±0.487	483.62±2.013	61.245±0.471	68.458±0.124	480.217±0.364	58.241±0.244

Where n = 3 for independent performed experiments

4.1.10.7 Stability study

The results of cream spreadability, pH, and centrifugation tests for three months (0, 1, 2, and 3) under both normal and accelerated time conditions are presented in Table 4.44. The antioxidant activities of cream in both real-time and accelerated time were not much influenced. This indicates that all loaded active components remained stable during the analysis period without noticeably degrading (Tan *et al.*, 2022). This shows that the formulation of phytocosmetic cream was good in terms of stability for long-term storage, which is a necessary quality for stability.

4.1.10.8 Permeability study

The permeability study (DPPH activity) for EOs-loaded cream at 0-, 1-, 2-, 3-, 4-, 5-, 6-, 7-, and 24-h intervals is shown in Table 4.48 and Annex 4. As suggested by Table 4.48, the percentage of cream release (DPPH activity) had been gradually increased on every time interval, showing effective DPPH activity (antioxidant activity) after a 4 hour interval with a cream release (DPPH activity) of 61.234 and getting saturated after 6 hours (6 hours-83.648, 7 hours-85.978, and 24 hours-88.369). This followed zero-order kinetics rather than first-order (since $R^2 = 0.983$ of zero-order is greater than first-order). Figure 4.23 shows the zero-order and its relation to skin application (Gyawali *et al.*, 2020; Kirk *et al.*, 2022). Therefore, this formulated cream with EOs can be taken as a good product, retaining various efficacies to improve skin care.

Table 4. 48 Permeability study (DPPH activity) for essential oils loaded cream at different intervals

Time (hrs)	Cumulative % cream released	% cream remaining
0	0	100
1	23.654	76.346
2	32.455	67.545
3	50.287	49.713
4	61.234	38.766
5	71.289	28.711
6	83.648	16.352
7	85.978	14.022
24	88.369	11.631

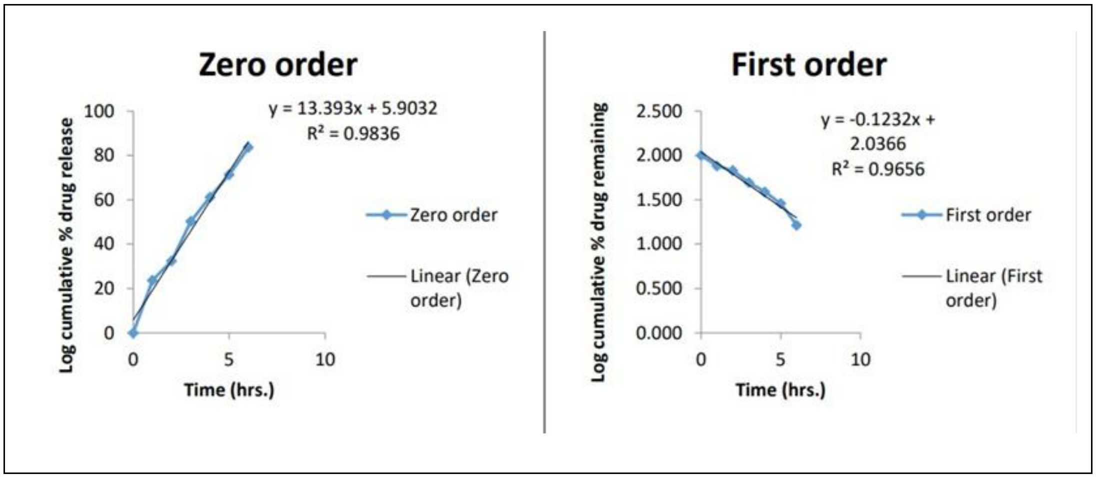


Figure 4. 23 A plot of log cumulative % cream release vs. time showing zero order kinetics and log cumulative % cream remaining vs. time showing 1st order kinetics

CHAPTER 5

5.1 CONCLUSION AND RECOMMENDATIONS

5.1.1 Conclusion

The present research work conducted in this dissertation has generated scientific baseline information on the volatile composition, chiral terpenoids, antimicrobial efficacy, cytotoxicity, antioxidant capacity, and formulation of essential oils of Lamiaceae plants from Nepal. A total of 15 plant species from 10 genera of the Lamiaceae family were collected from different locations in Nepal. The experimental research work was performed to determine the yield, chemical characterization, antioxidant, cytotoxic, and antimicrobial potentials of the essential oils. This study also explored the impacts of seasonal and geographical variations on the yield, composition, biological properties, and chemometrics of Lamiaceae essential oils and finally developed the product by utilizing them.

The results revealed that the variation in the yield of essential oils from Lamiaceae plants relates to harvesting season and geographical region, with an average yield of 0.76%. Among the Lamiaceae species, the plant *Ocimum tenuiflorum* from Kathmandu was found to have the highest essential oil yield of 1.68±0.12%, while *Leucosceptrum canum* had the lowest yield among the samples with 0.20±0.05%. From the GC-MS analysis of essential oils, it was confirmed that there were several bioactive compounds, mainly dominated by oxygenated monoterpenes with an average concentration above 49%. *Mentha pulegium* comprises the highest proportion of oxygenated monoterpenes with 91.63%, followed by *Mentha spicata* (85.3%*)* and *Perellia frutescens* (83.05%), respectively. The Lamiaceae family has been demonstrated to be full of chemotaxonomic markers at the genus level. The most interesting chemotaxonomic markers are germacrene D, linalool, β-caryophyllene, (*Z*)-β-ocimene, (*E*)-β-ocimene, eugenol, α-pinene, β-pinene, etc., which are very common. However, it may not be sufficient to individuate any chemotaxonomic markers. The most leading compounds in the Lamiaceae species were terpinen-4-ol, carvone, eugenol, perilla ketone, methyl chavicol, camphor, piperitenone oxide, β-caryophyllene, β-pinene, and isocaryophyllene, which were found not to be much influenced by seasonal variation in winter and summer. Also, the present study revealed two major groups of volatile classes: the first group with the dominance of oxygenated monoterpene (57.5-91.63%), including carvone, terpinen-4-ol, pinocarvone, camphor, perilla ketone, etc. as major components, and the second group of sesquiterpene hydrocarbons (0.13-18.54%), including β-caryophyllene, α-humulene, *trans*-β-elemene, δ-elemene, germacrene D, etc. as major components in the Lamiaceae essential oils. Additionally, the Lamiaceae essential oils also contained significant amounts of several minor chemical compounds, which are present in lower concentrations. The chiral GC-MS detected several chiral terpenoids (~20 common) in all samples of Lamiaceae species, which is a chemotaxonomic indicator of the Nepalese Lamiaceae family, and also showed the variation in the enantiomeric distribution in the samples. The major chiral terpenoids detected in the essential oils of Lamiaceae plants were α-pinene, camphene, sabinene, β-pinene, limonene, linalool, *cis*-sabinene hydrate, terpinen-4-ol, linalyl acetate, bornyl acetate, α-terpineol, and β-caryophyllene, which is the first extensive report ever regarding Lamiaceae species.

The Lamiaceae essential oils were also found to have very good antioxidant and radical-scavenging potentials. In the DPPH assay for the determination of antioxidant capacity, the IC_{50} values of essential oils ranged from 69.23 µg/mL to 646.58 µg/mL. The ABTS inhibition assay for the evaluation of antioxidant activity showed IC_{50} values varying from 5.88 µg/mL to 146.30 µg/mL. The essential oils from *Ocimum tenuiflorum* were found to be the best antioxidants, whereas the least effective were from *M. pulegium* among all the tested samples. Lamiaceae species from the summer season had better antioxidant activity than those from the winter.

Among the samples studied, *Origanum majorana*, *Mentha pulegium*, and *Ocimum tenuiflorum* were more effective against *Candida albicans*, and *Aspergillus niger*. These essential oils were able to show very low minimum inhibitory concentrations (MICs) (78.1 µg/mL). It was observed that plants growing at the middle range of altitude, i.e., the subtropical region, had better antifungal activity, while those growing at tropical altitude had better antibacterial activity. Since the antimicrobial efficacy may be directly connected to the specific composition of essential oil, the activity could also vary. The Lamiaceae essential oils used for the analysis explored relatively good antibacterial power against Gram-positive bacteria than Gram-negative bacteria.

The essential oils from *Perilla frutescens*, *Mentha longifolia*, *Clinopodium umbrosum*, and *Elsholtzia strobilifera* showed very strong cytotoxic potential against breast cancer (MCF-7) and fibroblast (NIH-3T3) cell lines, with IC_{50} values varying from 7.41 to 23.76 µg/mL. While the least cytotoxicity was observed for *M. pulegium* and *O. basilicum* essential oils, respectively.

Furthermore, the study suggests that the cream made with natural fragrance from essential oils like *O. majorana, O. tenuiflorum,* and *O. basilicum* found superior cosmetic grade since the physical and chemical characteristics of the cream demonstrated acceptance results on both real and accelerated time zone studies, thereby establishing a first-step natural remedy for skin protection. Finally, it could be established that plants of the Lamiaceae family are major sources of essential oils and bioactive components, and they are of great value from commercial perspectives, belonging to tropical regions collected during the summer season.

5.1.2 Recommendations

The present research work highlights the commercial and scientific information on the Lamiaceae family and the future ambition to assimilate it into the product formulation. These are some future perspectives on our research work.

- The present finding provides baseline information for future chemotaxonomic studies of Lamiaceae species.
- Genetic analysis can be added to assist with geographical variation in phytochemicals, which guides researchers' efforts toward the identification of high-metabolite-yielding germplasm.
- A wide range of pharmacological potential, particularly *in vivo* anticancer studies of essential oils, needs to be assessed that can contribute to potential drug discovery.
- Phytochemical variations among the intra-species and inter-species of several other Lamiaceae members can be evaluated.
- The effects of environmental variations on specific phytochemical biosynthesis can be assessed among the members of the Lamiaceae family.
- A single bioactive metabolite present in the essential oil can be isolated for drug discovery as well as for functional food and nutraceutical uses.
- This research work also explores a new model for the development of cosmetic and consumer products by incorporating several essential oils and emphasizes the further study of the formulation of different other products by using essential oil as a natural ingredient.

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ANNEXES

Annex 1: Percentage yields and organoleptic properties of EOs of different Lamiaceae plants

S.N.	EO Samples	EO Yields (%) (Mean±SD)	Appearance	Color	Aroma
1	*O. majorana* (S-1)	0.50±0.05	Slightly viscous liquid	Pale yellow	Strong sweet, spicy odor
2	*M. pulegium* (S-2)	0.63±0.04	Slightly thick liquid	Deep pale yellow	Strong and pleasant odor
3	*E. strobilifera* (S-3)	0.90±0.20	Transparent liquid	Pale yellow	Cool and pleasant odor
4	*O. tenuiflorum* (S-4)	0.50±0.05	Transparent liquid	Pale yellow to yellowish	Strong odor
5	*M. spicata* (S-5)	0.66±0.02	Slightly thick liquid	Pale yellow	Plesant and minty odor
6	*P. frutescens* (S-6)	0.78±0.13	Transparent liquid	Pale yellow	Plesant odor
7	*E. blanda* (S-7)	0.88±0.02	Transparent liquid	Light yellow	Strong odor
8	*P. glaber* (S-8)	0.45±0.02	Transparent viscous liquid	Pale yellow	Plesant odor
9	*O. americanum* (S-9)	0.35±0.02	Viscous liquid	Colorless to pale yellow	Plesant odor
10	*C. oppositifolia* (S-10)	0.25±0.06	Transparent viscous liquid	Dark yellow	Plesant odor
11	*C. coccinea* (S-11)	0.23±0.05	Transparent liquid	Pale yellow	Strong odor
12	*O. basilicum* (S-12)	0.66±0.09	Transparent liquid	Colorless	Cool and pleasant odor
13	*L. canum* (S-13)	0.34±0.02	Transparent liquid	Pale yellow	Strong odor
14	*O. majorana* (S-14)	0.90±0.28	Transparent and slightly viscous liquid	Pale yellow to colorless	Plesant odor
15	*O. americanum* (S-15)	0.42±0.03	Viscous liquid	Colorless to pale yellow	Sweet odor
16	*M. spicata* (S-16)	1.59±0.10	Transparent viscous liquid	Pale yellow	Plesant odor
17	*C. umbrosum* (S-17)	0.20±0.03	Transparent liquid	Pale yellow	Strong odor
18	*C. oppositifolia* (S-18)	0.34±0.02	Transparent liquid	Light yellow	Plesant odor
19	*M. longifolia* (S-19)	1.55±0.10	Transparent liquid	Deep pale yellow	Cool, plesant, minty odor
20	*L. canum* (S-20)	0.15±0.06	Transparent liquid	Pale yellow	Pungent odor
21	*P. frutescens* (S-21)	0.87±0.07	Transparent liquid	Pale yellow	Plesant odor
22	*O. tenuiflorum* (S-22)	1.67±0.13	Transparent liquid	Deep pale yellow	Sweet and pungent
23	*M. Pulegium* (S-23)	0.99±0.05	Transparent liquid	Pale yellow	Strong odor
24	*O. majorana* (S-24)	1.16±0.05	Transparent liquid	Pale yellow to colorless	Plesant and spicy odor
25	*M. spicata* (S-25)	1.62±0.04	Transparent liquid	Deep yellow	Plesant and sweetspicy

26	*O. tenuiflorum* (S-26)	1.68±0.13	Transparent liquid	Pale yellow	Strong odor
27	*O. basilicum* (S-27)	0.88±0.09	Transparent liquid	Colorless	Sweet and pleasant odor
28	*P. frutescens* (S-28)	0.80±0.02	Transparent liquid	Pale yellow	Sweet odor
29	*M. pulegium* (S-29)	0.67±0.07	Transparent liquid	Colorless	Strong, fresh, minty odor
30	*C. coccinea* (S-30)	0.23±0.02	Viscous liquid	Dark yellow	Strong odor
Average % yield in Lamiaceae species		**0.76%**			

Note: Yield values are mean ± standard deviation of three samples of each *Origanum* species, analyzed individually in triplicate

Annex 2: Compound classes used in the multivariate statistical analyses of Lamiaceae essential oil samples.

Name of EO samples	Classes of Terpene					
	Code No.	MH	OM	SH	OS	OT
O. majorana	S-1	27.6	69.2	2.9	0.1	0.25
O. majorana	S-14	9.34	77.25	0.13	2.71	1.68
O. majorana	S-24	21.19	67.57	1.78	0.45	7.51
E. strobilifera	S-3	20.84	57.5	12.37	3.93	3.57
E. blanda	S-7	4.95	74.13	7.98	2.15	5.71
M. spicta	S-5	13.47	79.04	5.05	0.39	1.34
M. spicta	S-16	14.39	74.99	9.31	0.2	0.69
M. spicata	S-25	6.31	85.3	3.21	0.17	1.24
M. longifolia	S-19	10.98	76.32	3.8	0.15	2.6
M. pulegium	S-2	0.89	85.69	3.88	0.66	2.93
M. pulegium	S-23	1.04	91.63	0.31	1.58	2.52
M. pulegium	S-29	3.3	87.65	5.55	1.3	2.15
O. tenuiflorum	S-4	0.2	1.15	57.11	7.28	33.53
O. tenuiflorum	S-22	0.31	0.99	62.54	1.15	35.49
O. tenuiflorum	S-26	0.33	1.16	53.17	7.87	31,20
O. americanum	S-9	11.35	68.57	18.54	0.57	0.96
O. americanum	S-15	6.64	82.38	6.75	2.58	1.49
O. basilicum	S-12	0.3	28.09	4.09	1.06	64.9
O. basilicum	S-27	0.71	28.65	5.6	0.44	62.49
P. frutescens	S-6	0.51	69.46	15.42	10.02	2.46
P. frutescens	S-21	0.09	76.59	13.39	1.09	8.51
P. frutescens	S-28	0.32	83.05	7.41	1.2	8.37
P. glaber	S-8	0.35	2.19	43.64	37.52	0.11
C. oppositifolia	S-10	0.31	1.92	26.67	15.92	42.98
C. oppositifolia	S-18	1.22	0.89	47.51	4.15	33.68
C. coccinea	S-11	0.21	2.64	79.91	5.48	10.24

C. coccinea	S-30	0.24	2.48	76.88	6.01	10.99
C. umbrosum	S-17	4.31	77.66	8.31	4.51	3.17
L. canum	S-13	56.04	1.73	31.09	4.99	3.56
L. canum	S-20	23.12	7.11	60.08	3.56	4.92

Annex 3: Antioxidant activities of Lamiaceae essential oil in terms of IC_{50} value from DPPH and ABTS assays

S.N.	EO Samples	DPPH (Mean±SD)	ABTS (Mean±SD)
		IC_{50}(μg/mL)	IC_{50}(μg/mL)
1	*O. majorana* (1-Spring)	468.7±0.24	49.03±0.20
2	*O. majorana* (14-Summer)	225.61±0.05	36.74±0.44
3	*O. majorana* (24-Summer)	187.44±1.33	34.74±1.00
4	*M. spicata* (5-Winter)	536.89±0.40	80.89±0.80
5	*M. spicata* (16-Summer)	448.2±0.09	35.69±0.20
6	*M. spicata* (25-Summer)	343.26±0.09	34.15±0.21
7	*P. frutescens* (6-Winter)	359.17±0.11	140.1±1.23
8	*P. frutescens* (21-Summer)	343.263±0.09	129.5±1.21
9	*P. frutescens* (28-Summer)	334.26±0.20	93.15±1.04
10	*O. americanum* (9-Winter)	359.17±0.10	129.51±1.21
11	*O. americanum* (15-Summer)	452.793±0.90	145.67±0.20
12	*C. oppositifolia* (10-Summer)	406.01±0.04	129.51±1.21
13	*C. oppositifolia* (18-Winter)	402±0.08	123.67±1.07
14	*C. coccinea* (11-Winter)	182.42±0.11	52.05±0.85
15	*C. coccinea* (30-Winter)	174.7±0.10	38.13±0.73
16	*O. tenuiflorum* (4-Winter)	78.96±0.1	5.88±0.80
17	*O. tenuiflorum* (22-Autumn)	69.23±0.10	9.05±0.20
18	*O. tenuiflorum* (26-Autumn)	82.99±0.12	17.69±0.48
19	*M. pulegium* (2-Autumn)	236.14±0.09	61.44±0.26
20	*M. Pulegium* (23-Summer)	646.58±0.19	145.35±1.00
21	*M. pulegium* (29-Autumn)	375.23±0.04	125.36±1.01
22	*O. basilicum* (12-Summer)	448.21±0.09	61.4±0.26
23	*O. basilicum* (27-Winter)	236.14±0.09	44.39±0.81
24	*L. canum* (13-Winter)	524.37±0.40	71.97±0.31
25	*L. canum* (20-Summer)	380.67±0.09	34.74±1.00
26	*E. strobilifera*(3-Autumn)	188.77±0.72	34.74±1.01
27	*E. blanda* (7-Winter)	452.79±0.90	101.14±0.29
28	*P. glaber* (8-Winter)	621.73±0.17	146.3±1.00
29	*C. umbrosum* (17-Summer)	554.8±0.95	87.2±0.80
30	*M. longifolia* (19-Summer)	501.43±0.48	53.49±0.28
	Ascorbic Acid	6.4±0.34	2.0±1.20
	BHT	12.5±0.05	-
	Quercetin	-	7.8±0.65

Annex 4: Permeability study

Time (hrs)	Cumulative % drug released	% drug remaining	Square root time	Log Cumu. % drug remainining	Log time	Log Cumu. % drug released	% Drug released	Cube Root of % drug remaining (Wt)	Wo-Wt
0	0	100	0.000	2.000	0.000	0.000	100	4.642	0.000
1	23.654	76.346	1.000	1.883	0.000	1.374	23.654	4.242	0.400
2	32.455	67.545	1.414	1.830	0.301	1.511	8.801	4.073	0.569
3	50.287	49.713	1.732	1.696	0.477	1.701	17.832	3.677	0.965
4	61.234	38.766	2.000	1.588	0.602	1.787	10.947	3.384	1.258
5	71.289	28.711	2.236	1.458	0.699	1.853	10.055	3.062	1.580
6	83.648	16.352	2.449	1.214	0.778	1.922	12.359	2.538	2.104
7	85.978	14.022	2.646	1.147	0.845	1.934	2.33	2.411	2.231
24	88.369	11.631	4.899	1.066	1.380	1.946	2.391	2.266	2.376

Annex 5. Seasonal and geographical variation in the yieds of Lamiaceae essential oils

	Seasonal variation			Geographical variation	
EO samples	Summer (%) (Mean±SD)	Winter (%) (Mean±SD)	**EO samples**	Tropical region (%) (Mean±SD)	Sub-tropical region (%) (Mean±SD)
M. spicata	1.59±0.1	0.66±0.02	*M. spicata*	1.62±0.04	1.59±0.1
P. frutescencs	0.87±0.07	0.78±0.13	*P. frutescens*	0.8±0.02	0.87±0.07
O. americanum	0.42±0.03	0.35±0.02	*O. tenuiflorum*	1.67±0.13	1.68±0.13
O. basilicum	0.66±0.09	0.88±0.09	*C. oppositifolia*	0.34±0.02	0.25±0.06
L. canum	0.15±0.06	0.34±0.02			

Annex 6: Seasonal and geographical variation in the antioxidant activities of Lamiaceae essential oils in terms of IC_{50} values (µg/mL) (DPPH Assay)

	Seasonal variation			**Geographical variation**	
EO samples	Summer (IC_{50}) (Mean±SD)	Winter (IC_{50}) (Mean±SD)	**EO samples**	Tropical region (IC_{50}) (Mean±SD)	Sub-tropical region (IC_{50}) (Mean±SD)
M. spicata	448.2±0.09	536.89±0.4	*M. spicata*	343.26±0.09	448.2±0.9
P. frutescencs	343.26±0.19	359.17±0.11	*P. frutescens*	334.26±0.2	343.26±0.09
O. americanum	452.793±0.91	359.17±0.10	*O. tenuiflorum*	69.23±0.1	82.99±0.12
O. basilicum	448.21±0.19	236.14±0.09	*C. oppositifolia*	402±0.08	406.01±0.04
L. canum	380.67±0.09	524.37±0.04			

Annex 7: Seasonal and geographical variation in the antioxidant activities of Lamiaceae essential oils in terms of IC_{50} values (µg/mL) (ABTS Assay)

	Seasonal variation			**Geographical variation**	
EO samples	Summer (IC_{50}) (Mean±SD)	Winter (IC_{50}) (Mean±SD)	**EO samples**	Tropical region (IC_{50}) (Mean±SD)	Sub-tropical region (IC_{50}) (Mean±SD)
M. spicata	35.69±0.2	80.89±0.8	*M. spicata*	34.15±0.21	35.69±0.2
P. frutescencs	129.5±1.21	140.1±1.23	*P. frutescens*	93.15±1.04	129.5±1.21
O. americanum	145.67±0.2	129.51±1.21	*O. tenuiflorum*	9.05±0.2	17.69±0.48
O. basilicum	61.4±0.26	44.385±0.81	*C. oppositifolia*	123.67±1.07	129.51±1.21
L. canum	34.74±1.0	71.97±0.31			

Annex 8: Applications of major compounds of essential oils

Major compounds	**Applications / Uses**
1. Terpinen-4-ol (*Origanum majorana*)	• Terpinen-4-ol is well-known for its strong antimicrobial and antiseptic properties (Sharifi-Rad et al., 2017). • It a valuable ingredient in products like antiseptic creams, wound ointments, skincare products and as immune support. (Do Nascimento et al., 2020)
2, Carvone (*Mentha spicata*)	• Commonly used in flavoring and fragrance applications. • It is used as a flavoring agent in food and beverages. • Its minty or spicy notes make it a popular choice in products like chewing gum, mints, and confectioneries. • Due to its pleasant aroma, carvone essential oil is used in aromatherapy to promote relaxation and reduce stress. (Pina et al., 2022); (Bakrim et al., 2022).
3. Piperitenone oxide (*M. longifolia* / *C. umbrosum*)	• It is used in perfumery, flavoring, and aromatherapy. • It is also used in oral care products and as a flavoring agent in foods and beverages. • It is also used in oral care products and as a flavoring agent in foods and beverages (Bozovic et al., 2015).

4. Menthone (*M. pulegium*)	• It is widely used in the flavor and fragrance industry to impart a minty scent and taste to products. • Menthone's minty flavor makes it a popular ingredient in oral care products like toothpaste, mouthwash, and chewing gum, as it provides a fresh and cooling sensation (Zhao et al., 2022).
5. Pinocarvone (*E. strobilifera*)	• It is used in perfumery for its piney and herbaceous scent. • Additionally, pinocarvone is used in household cleaning products, air fresheners, and personal care items due to its refreshing and invigorating aroma (Do Nascimento et al., 2020); (Zhang et al., 2021).
6. Dihydrotagetone (*E. blanda*)	• Good antimicrobial activity • Used as good chemical pesticides
7. Eugenol (*O. tenuiflorum*)	• It is well recognized as pharmacological properties. • It is iused n various fields: pharmaceutical, food, flavor, cosmetic industry. • It is commonly used in perfumery, as well as in flavoring agents due to its distinct flavor profile. • It has antimicrobial and analgesic properties, which make it suitable for dental care products and topical preparations (Ulanowska & Olas, 2021).
8. Camphor (*O. americanum*)	• It is commonly used for its soothing and cooling properties. • Camphor is often used topically in creams, ointments, and balms to relieve minor aches and pains. • It is also used in aromatherapy for its invigorating and refreshing scent. (Zhang et al., 2021); (Lee et al., 2022).
9. Methyl chavicol, estragole (*O. basilicum*)	• It is used in perfumery and as a flavoring agent in food and beverages. • It is also believed to have potential antimicrobial and antioxidant properties. . (Do Nascimento et al., 2020); (Oriola & Oyedeji, 2022).
10. Perilla ketone (*P. frutescens*)	• Unique fragrance, • Used in perfumery, and aromatherapy for its calming and soothing scents. • It is also utilized in traditional medicine in some cultures for its potential medicinal properties (Wang et al., 2022).
11. Germacrene D (*P. glaber*)	• It is used in perfumery to add depth and complexity to fragrances. • Some germacrene variants might also have potential medicinal properties, but research is ongoing in this area (Alexandre Carvalho et al., 2023), (Saab et al., 2018)..
12. β-caryophyllene (*C. oppositifolia*)	• This compound is notable for its interaction with cannabinoid receptors, specifically CB2 receptors, which has led to research into its potential anti-inflammatory and analgesic effects (Fidyt et al., 2016). • It is also used in perfumery and as a flavoring agent (Bhatia et al., 2008).
13. Isocaryophyllene (*C. coccinea*)	• Isocaryophyllene inhibits the production of picolinic acid, a biochemical that is involved in the inflammatory response. • Isocaryophyllene also has antiinflammatory activity due to its ability to inhibit prostaglandin synthesis. There are no known physiological effects from this compound (Oriola & Oyedeji, 2022).
14. β-pinene (*L. canum*)	• It's used in the fragrance industry to create pine, woodsy, and earthy scents. • Beta-pinene is also being investigated for its potential anti-inflammatory and analgesic properties (Salehi et al., 2019; (Sagorin et al., 2021).

Annex 9: Description of Lamiaceae plant materials used in the present research study

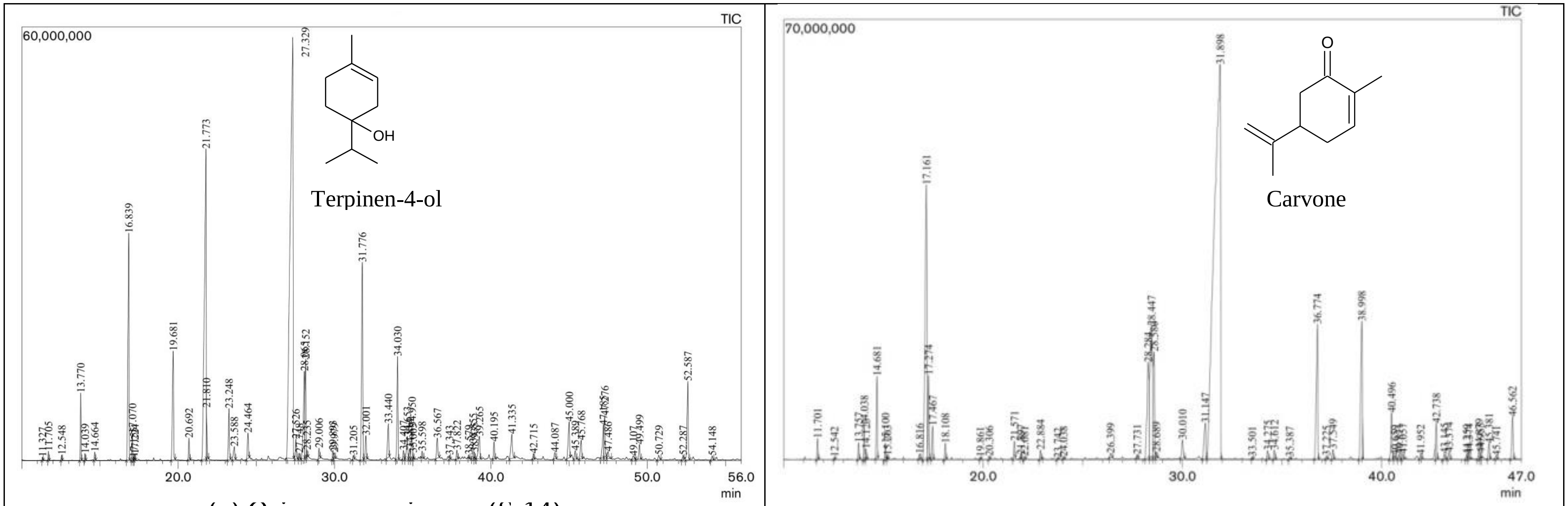
Plant species selected	Location /District	Coordinates	Elevation /Altitude	Parts used	Harvesting month	Traditional Uses
1. *Origanum*						
O. majorana L. Maruwa phool, Raam Tulsi (Nep.), Sweet marjoram (Eng.) [**S. No. 1**]	Nagarjun, Kathmandu	27°43'58.8"N; 85°15'26.5"E	1537 m	Aerial parts	2019 /05/10 (**May-2019**) Spring	Used for external treatment, sprains, and bruises. It is livertonic, expectorant, carminative, diuretic, and antispasmodic, digestive. Leaves are astringent and is aremedy of colic. Essential oils from leaves are used for hot fomentation in acute diarrhea (Baral & Kurmi, 2006; Joshi, 2000).
O. majorana L. [**S. No. 14**]	Sanothimi, Bhaktapur	27°40'48.8"N; 85°22'42.3"E	1336 m	Aerial parts	2019 /05/20 (**Jun-2019**) Summer	
O. majorana L. [**S. No. 24**]	Bafal, Kathmandu	27°41'57.9" N; 85°17'8.4" E	1290 m	Aerial parts	2022/06/15 **(June-2022)** Summer	
2. *Mentha*						
M. pulegium L. [**S. No. 2**]	Gaunkharka, Nuwakot	27° 56' 9.4" N; 85° 28' 57.9" E	2528 m	Aerial parts,	2018/11/15 (**Nov-2018**) Autumn	The dry parts of this plant and its EO are used in traditional medicine (liver and gallbladder ailments, digestive, gout, amenorrhea, increased micturition, colds, skin disorders, and as abortifacient), gastronomy (aromatizing culinary herb), cosmetics,and aromatherapy (Teixeira et al., 2012).
M. pulegium L. [**S. No. 23**]	Gaunkharka, Nuwakot	27° 56' 9.4" N; 85° 28' 57.9" E	2528 m	Aerial parts,	2022/8/31 (**Aug-2022**) Summer	
M. pulegium L. [**S. No. 29**]	Sindhupalchok, Kutumsang	27° 55' 41.5" N 85° 29' 8.4" E	2465 m	Aerial parts,	2022/10/18 (**Oct-2022**) Autumn	
M. spicata L. Pahaadi Pudina (Nep.), Spearmint (Eng.) [**S. No. 5**]	Dhulikhel, Kavre	27° 37' 17.3" N; 85° 32' 25.3" E	1493 m	Leaves	2021/12/10 (**Dec-2021**) Winter	It is antiseptic, carminative, antispasmodic, stimulant and stomachic. It is used to allay nausea, flatulencesickness, vomiting. Leaves given in fever and bronchitis, decoction is applied externally in aphthae (Joshi & Joshi, 2001).
M. spicata L. [**S. No. 16**]	Dhulikhel, Kavre	27° 37' 18.3" N; 85° 32' 25.4" E	1497 m	Leaves	2022/06/20 **(Jun-2022)** Summer	
M. spicata L. [**S. No. 25**]	Bansgadhi, Bardiya	28°15' 14.3" N; 81° 30' 7.0" E	159 m	Leaves	2022/07/15 **(Jul-2022)** Summer	

M. longifolia L. *Tulsi paate* (Nep.) [**S. No. 19**]	Bansgadhi, Bardiya	28° 14' 32.9" N; 81° 31' 16.0' E	159 m	Leaves	2022/07/22 (**Jul-2022)** Summer	Used in anthelmintic, astringent to bowels, used in diseases of heart, bronchitis, loss of appetite, diarrohea and dysentery, leaf juice, antiseptic applied on cuts and wounds (Bhattarai, 1992).
3. *Elsholtzia*						
E. strobilifera (Benth.) Benth Ban bawari, Dhan Tulsi(Nep.) [**S. No. 3**]	Mamche, Barpak, Gorkha	28°12’37.3”N; 84°44’31.2”E	1808 m	Aerial parts	2021/10/26 (**Oct-2021**) Autumn	Plant pase applied to forehead in case of headache. Powdered seeds used for flavouring foodstuff. (Manandhar, 2002) .
E. blanda (Benth.) Benth, Ban Silaam (Nep.), Wild basil(Eng.) [**S. No. 7**]	Machchhegaun, Kathmandu	27°39' 16.13" N; 85°14' 53.2" E	1662 m	Aerial shoot+ ves	2021/12/18 (**Dec-2021**) Winter	EO used for aromatherapy, asa decongestant of nose and throat during cough & cold. Juice used to treat headache. Also applied to cut and wounds (Manandhar, 2002).
4. *Ocimum*						
O. tenuiflorum L. Holy basil, Tulasi (Nep.), [**S. No. 4**]	Bansgadhi, Bardiya	28°15' 14.3" N; 81° 30' 7.0" E	159 m	Aerial parts	2021/12/2 (**Dec-2021**) Winter	Used for treatment viral encephalitis and pulmonary eosionphilia in children, Used in cardiopathy, asthma, leucoderma, haemopathy, vomiting, hiccough, opthalmia, gastropathy, ringworms, skin disease, snake bite and scorpion sting. Aromaic oil from leaves has antibacterial and insecticidal properties (Bhattacharji, 2001; DPR-3, 1997; Joshi, 2000).
O. tenuiflorum L. [**S. No. 22**]	Bansgadhi, Bardiya	28°14' 32.9" N; 81°31' 15.9" E	159 m	Aerial parts	2022/10/10 (**Oct-2022**) Autumn	
O. tenuiflorum L. [**S. No. 26**]	Kirtipur, Kathmandu	27°40’46.8”N; 85°16’43.5”E	1351 m	Aerial parts	2022/10/13 (**Oct-2022**) Autumn	
O. americanum L, Baabari, Tulasi (Nep.), Hoary bash, scred basil (Eng.)[**S. No. 9**]	Thankot, Kathmandu	27° 41' 37.7" N; 85°13' 44.4" E	1383 m	Aerial parts,	2021/12/18 (**Dec-2021**) Winter	Leaves aromatic, acrid, appetizing, digestive, carminative, expectorant, anthelmintic and cardiotonic. Leaf decoction taken for cough, dysentery, mouth wash, leaf paste applied over parasitic skin diseases (Joshi, 2000).
O. americanum [**S. No. 15**]	Thankot, Kathmandu	27° 41' 46.7" N; 85° 13' 45.7" E	1380 m	Aerial parts,	2022/06/12 (**Jun-2022**) Summer	

O. basilicum L. Raam tulsi, Baawari phool(Nep.), sweet basil [**S. No. 12**]	Gajehada, Kapilvastu	27°40’7.9”N; 83°10’12.9”E	133 m	Aerial parts,	2021/12/25 (**Dec-2021**) Winter	Used to cure dyspepsia, cogh, constipation, and intermittent fevers. Also useful in otalgia, cephalalgia, rthralgia, dyspepsia, colic helminthiasic, cardiac debility, ringworm and other skin diseases. Flowers diuretic, carminative, stimulant and demulcent (DPR-3, 1997; Joshi, 2000)
O. basilicum L. [**S. No. 27**]	Gajehada, Kapilvastu	27°40’7.9”N; 83°10’12.9”E	133 m	Aerial parts,	2022/8/25 (**Aug-2022**) Summer	
5. ***Perilla***						
P. frutescens (L.) Britton,Silaam (Nep.), Acute common perilla(Eng.) [**S. No. 6**]	Dhulikhel, Kavre	27° 36' 59.4" N; 85° 32' 13.5" E	1351 m	Leaves	2021/12/13 (**Dec-2021**) Winter	Stems are useful in feeling of oppression in chest, abdominal distention, morning sickness, fetal distress. Leaves are used in cold, headache, cough, nausea, vomiting, food poisoning from fish and crabs. Fruits are given in productive cough and wheezing.(Joshi & Joshi, 2001)
P. frutescens (L.) [**S. No. 21**]	Dhulikhel, Kavre	27° 36' 59.4" N; 85° 32' 13.5" E	1474 m	Leaves	2022/08/27 (**Aug-2022**) Summer	
P.frutescens (L.) [**S. No. 28**]	Bansgadhi, Bardiya	28°14' 32.9" N; 81°31' 15.9" E	159 m	Leaves	2022/08/24 (**Aug-2022**) Summer	
6. ***Pogostemon***						
P. glaber Benth**.** Rudhilo, Naam paani (Np) [**S. No. 8**]	Machchhegaun, Kathmandu	27°39'32.3"N ; 85°14'34.6"E	1647 m	Leaves	2021/12/18 (**Dec-2021**) Winter	Root used for the treatment of gastric trouble(Gewali, 2008), Plant juice is applied on forehead to treat headache, root juice is effective against indigestion and fever. Leaf decoction is given to cure asthma of children (Manandhar, 2002; Rajbhandari, 2001)
7. ***Colebrookea***						
C. oppositifolia Sm. Dhusure, Dhursil (Nep.) [**S. No. 10**]	Machchhe Narayan Temple, Kathmandu	27° 39' 33.3" N; 85° 14' 39.6" E	1657 m	Leaves	2021/12/15 (**Dec-2021**) Winter	Roots used in epilepsy. Leaves are applied in wounds and bruises. Leaf juice used in a veterinary, anthelmintic, in dysentery, opthlamic problems (DPR-3, 1997; Rajbhandari, 2001)
*C. oppositifolia S*m. [**S. No. 18**]	Bhakunde Besi, Kavre	27° 33' 13.7" N; 85° 38' 53.4" E	1136 m	Leaves	2022/7/17 (**Jul-2022**) Summer	

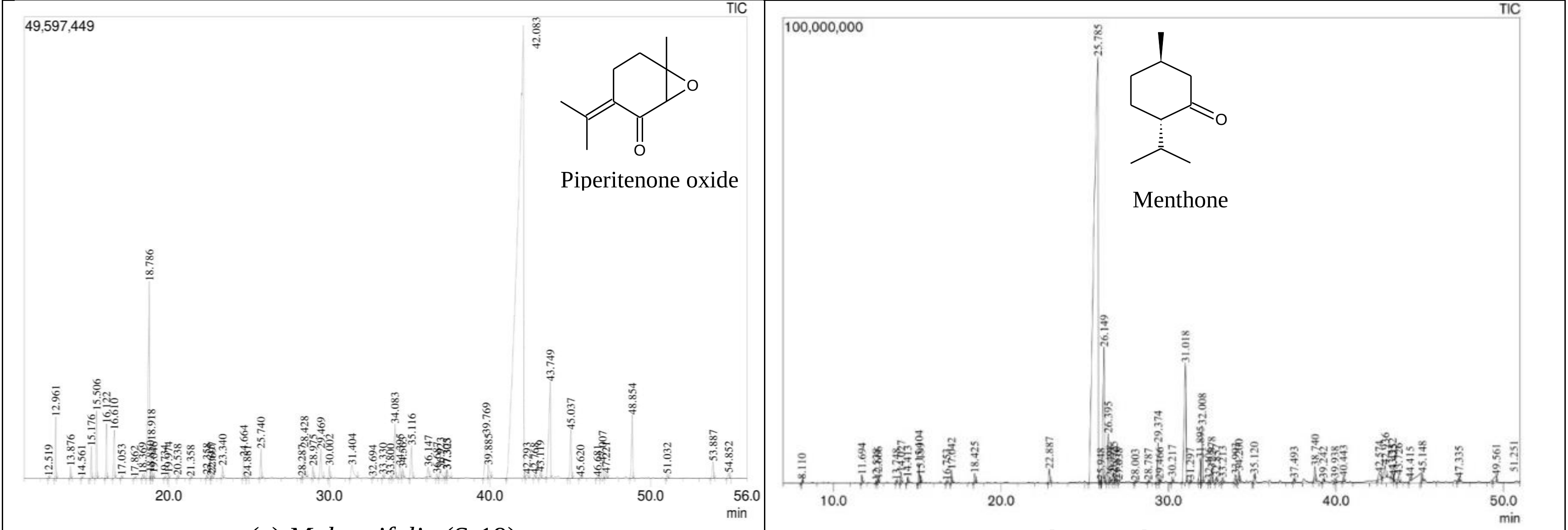
8. *Colquhounia*						
C. coccinea Wall. (Nep.) [**S. No. 11**]	Bethanchowk, Kavre	27° 30’ 0.8’’N; 85° 26’ 28.8’’E	1760m	Shoot Leaves	2022/1/16 (**Jan-2022**) Winter	Used as culinary herbs such as basil, mint, sage, savory, lavender and perilla. (Bhatt et al., 2009)
C. coccinea Wall. [**S. No. 30**]	Gosaikunda, Rasuwa	28° 13' 0’ N ; 85° 34' 60’ E.	4360 m	Shoot Leaves	2022/1/25 (**Jan-2022**) Winter	
9. *Leucosceptrum*						
L. canum Sm. Bhusure, Ghurmiso [**S. No.13**]	National Botanical Garden, Lalitpur	27°35’48.9”N; 85°22’48.5”E	1514 m	Leaves	2022/2/8 (**Fab-2022**) Winter	Used in headache, fever, stomach pain, bleeding from foreign wounds, closed fractures, yellow pus-filled sores (roots, leaves).
L. canum Sm. [**S. No.20**]	NBG, Lalitpur	27°35’48.9”N; 85°22’48.5”E	1514 m	Leaves	2022/8/1 (**Aug-2022**) Summer	
10. *Clinopodium*						
C. umbrosum (M. Bieth.) C. Koch Suparnasaa (Nep.), Wild basil, Calamint (Eng.) **[S. No. 17**]	Bakhundole, Kavre	27° 37' 4.8" N; 85° 32' 18.2" E	1499 m	Aerial parts	2022/6/17 (**Jun-2022**) Summer	Leaf juiceis applied in cuts, wounds (Manandhar, 2002).

Annex 10: GC-MS chromatograms showing separation of different volatile compounds of Lamiaceae family essential oil samples



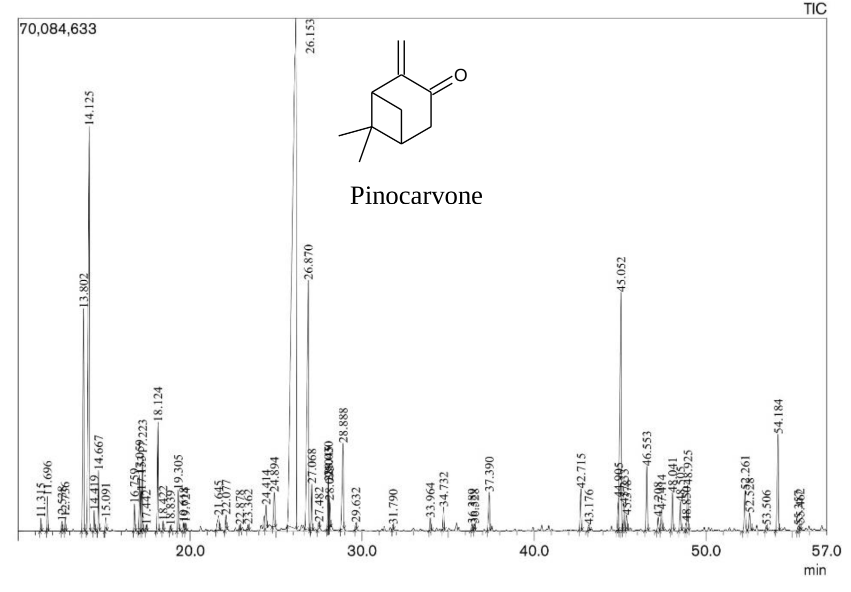
(a) *Origanum majorana* (S-14)

(b) *Mentha spicata* (S-5)

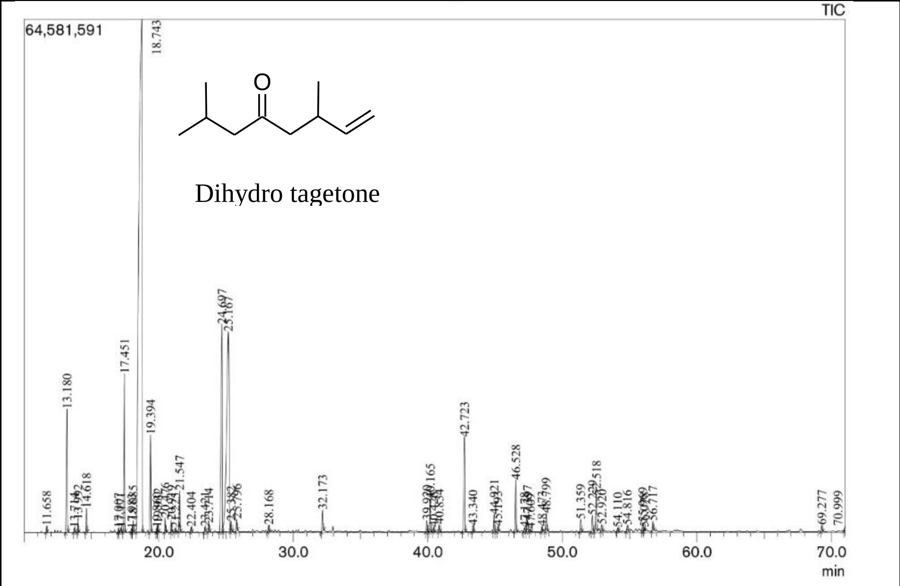


(c) *M. longifolia* (S-19)

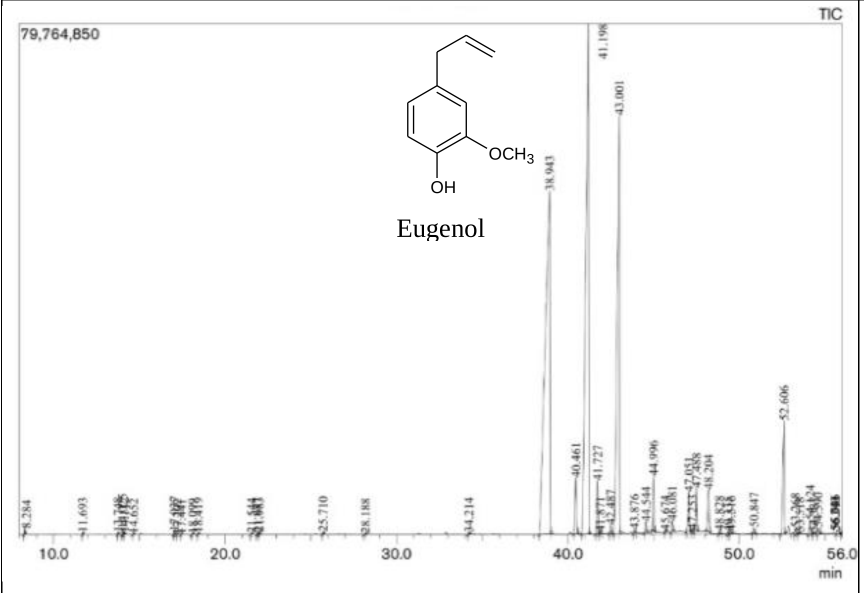
(d) *M. pulegium* (S-23)



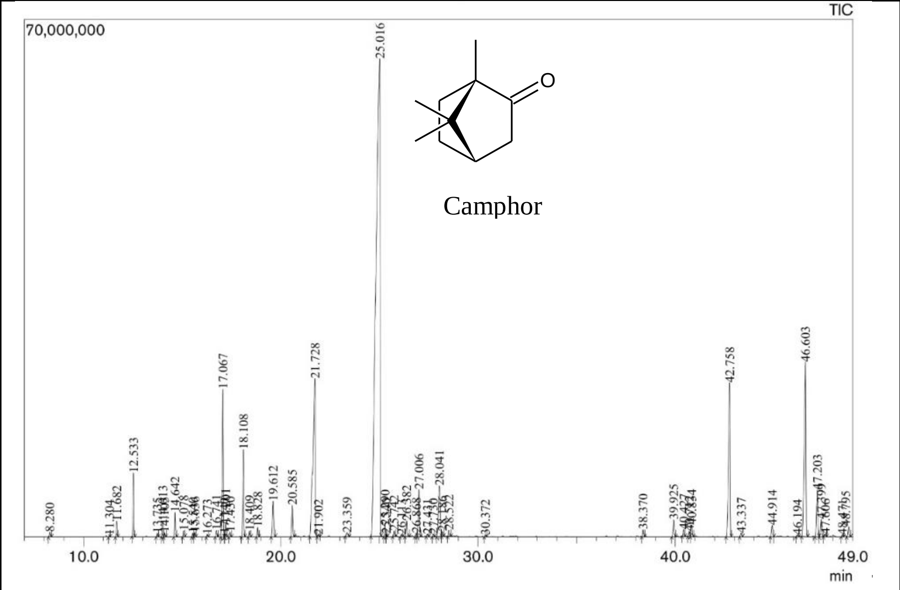
(e) *E. strobilifera* (S-3)



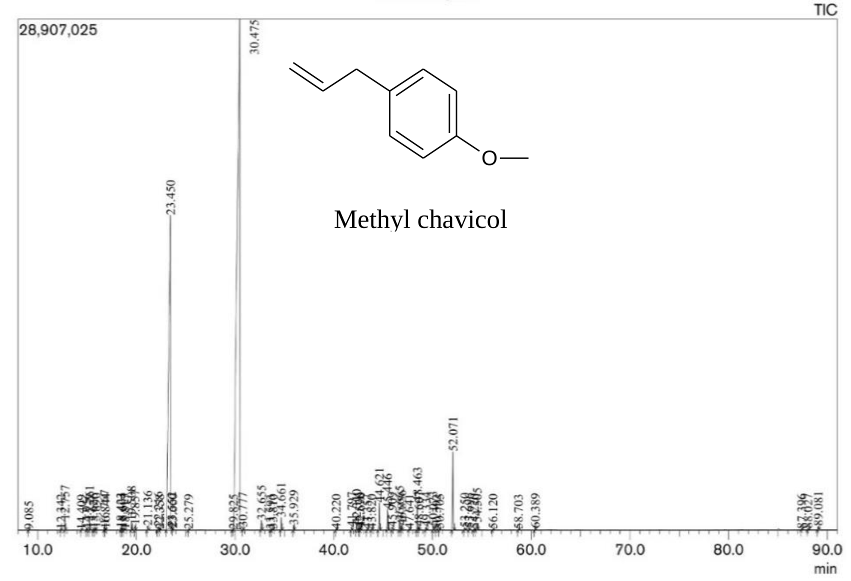
(f) *E. blanda* (S-7)



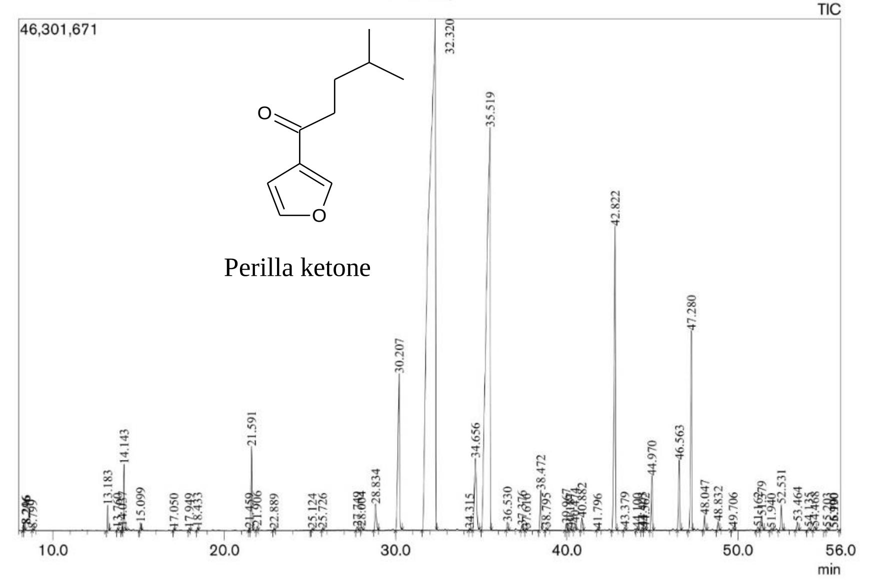
(g) *O. tenuiflorum* (S-4)



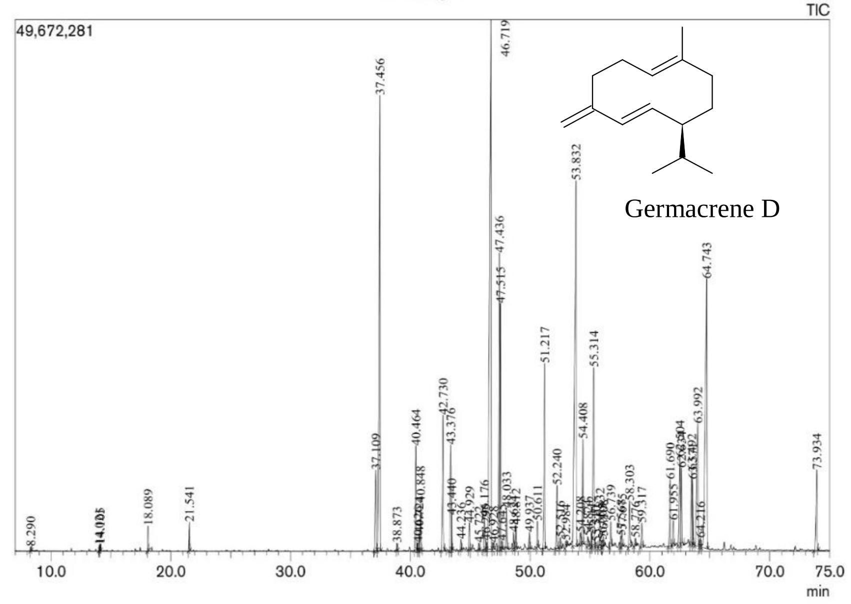
(h) *O. americanum* (S-9)



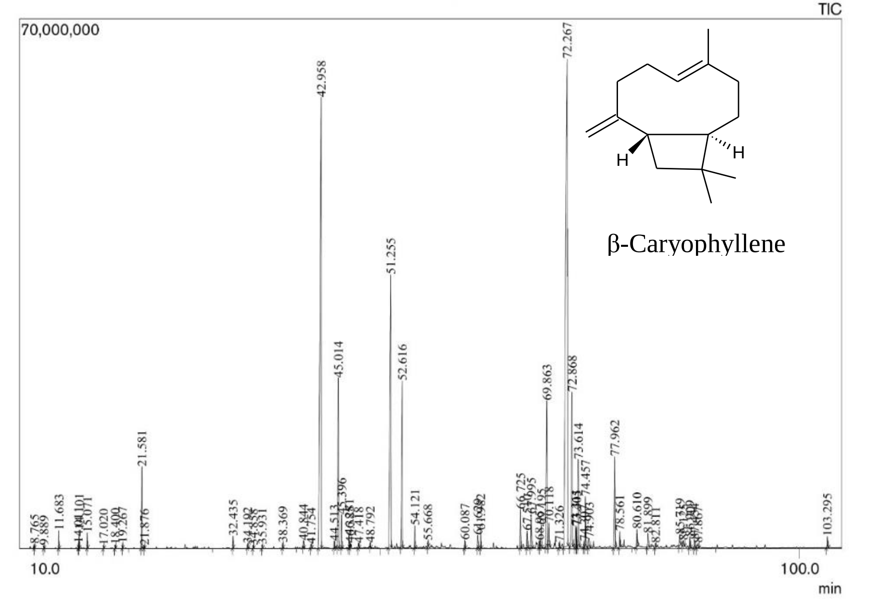
(i) *O. basilicum* (S-12)



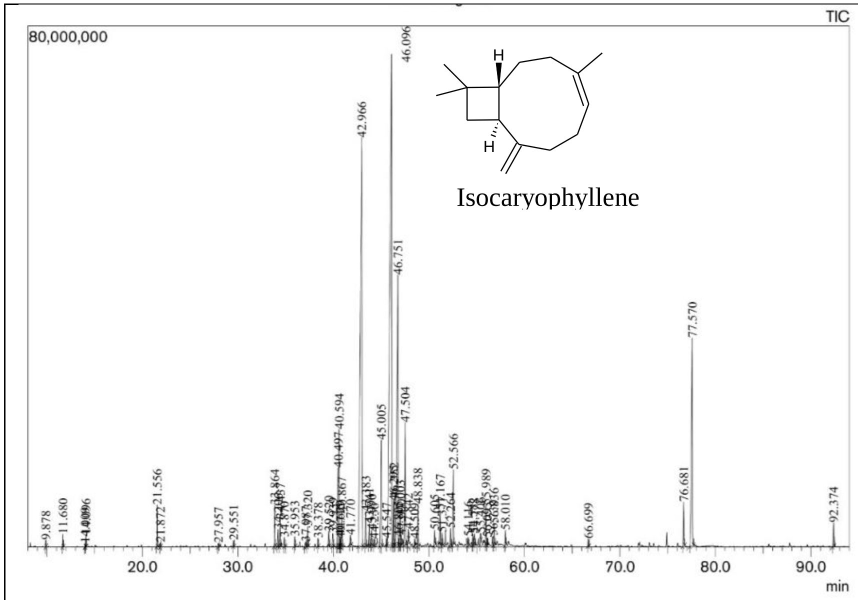
(j) *P. frutescens* (S-21)



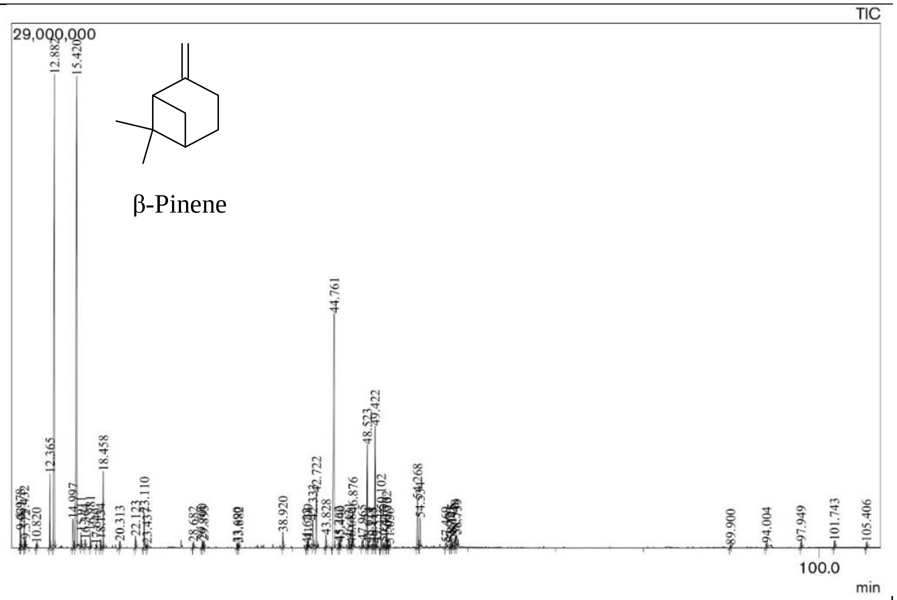
(k) *P. glaber* (S-8)



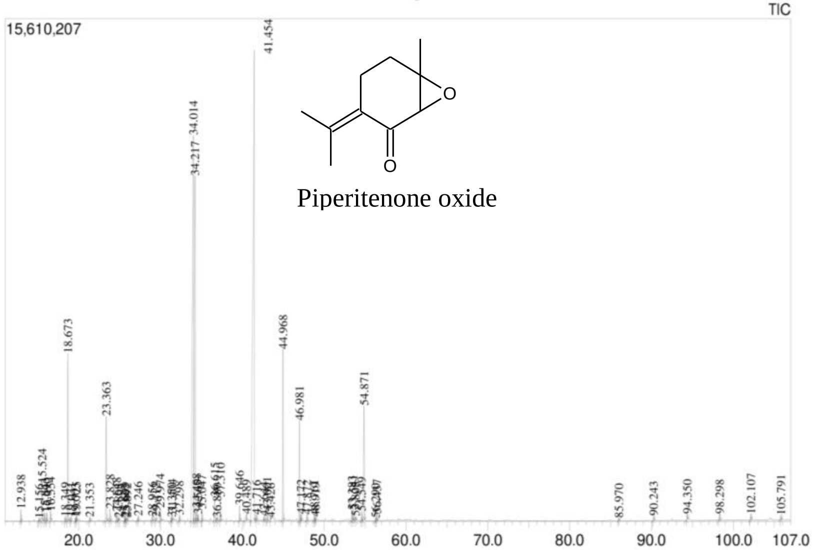
(l) *C. oppositifolia* (S-10)



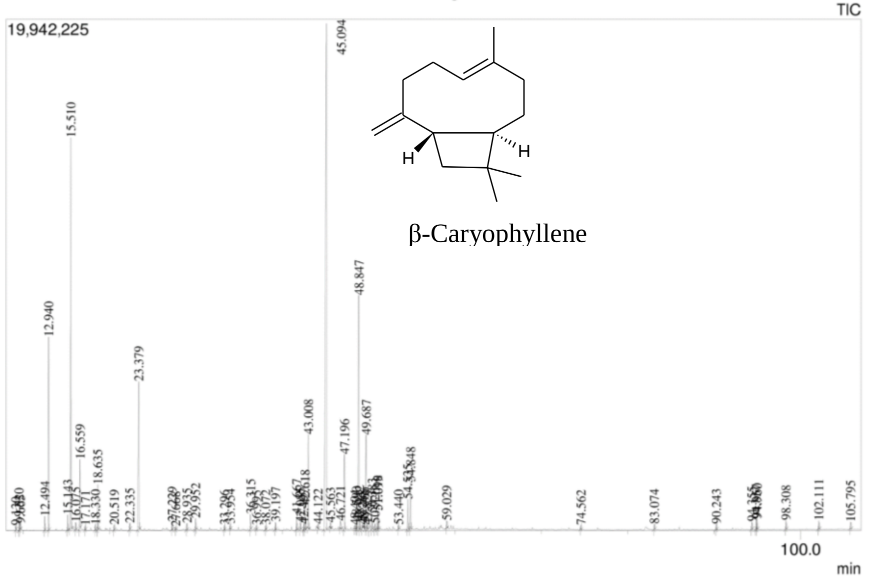
(m) *C. coccinea* (S-11)



(n) *L. canum* (S-13)

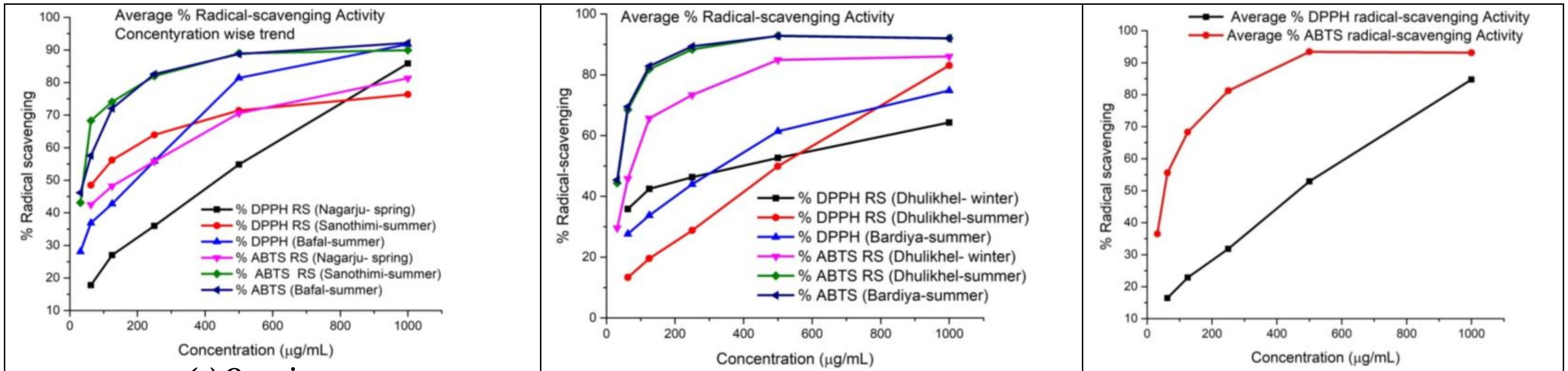


(o) *C. umbrosum* (S-17)

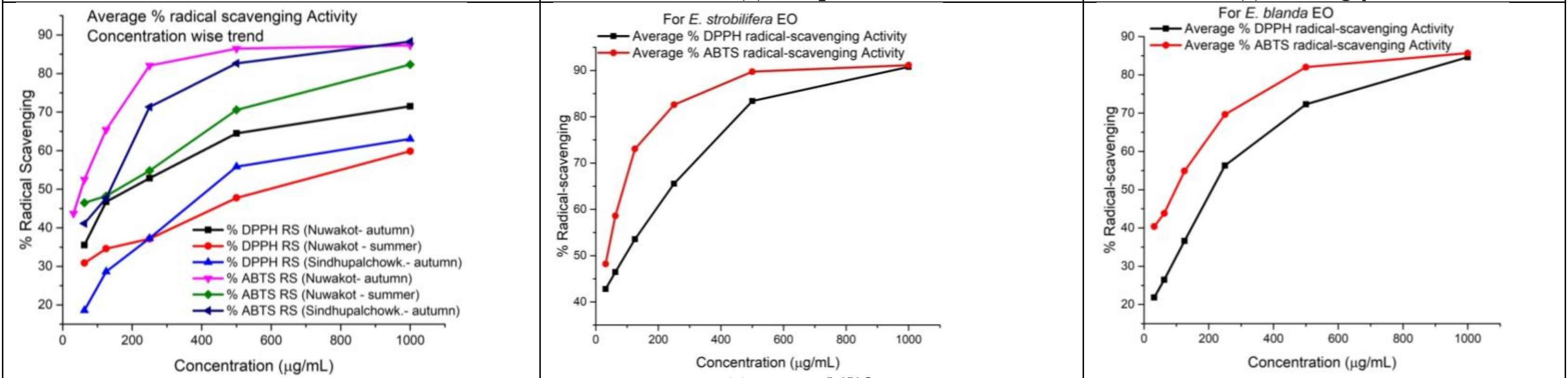


(p) *L. canum* (S-20)

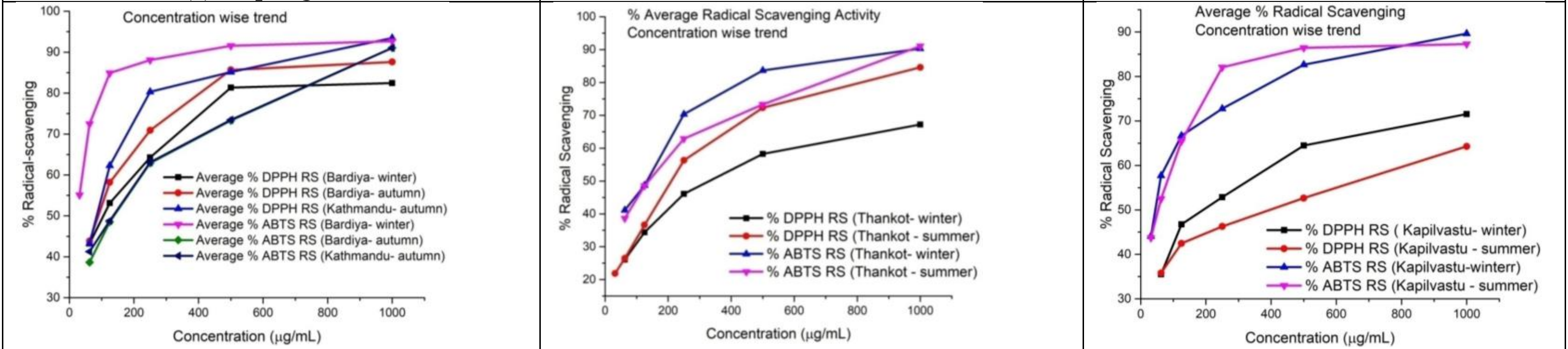
Annex 11: Radical-scavenging activity different Lamiaceae EOs based on concentration wise trend for DPPH and ABTS assays



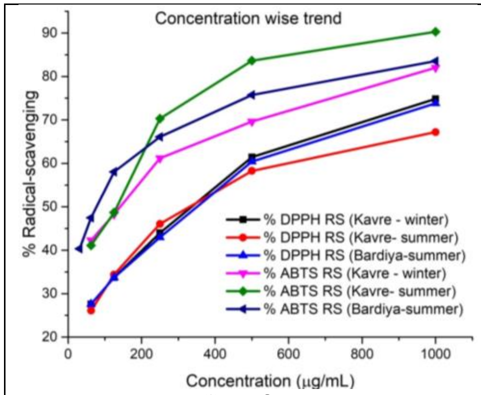
(a) O. majorana *(b) M. spicata* *(c) M. longifolia*



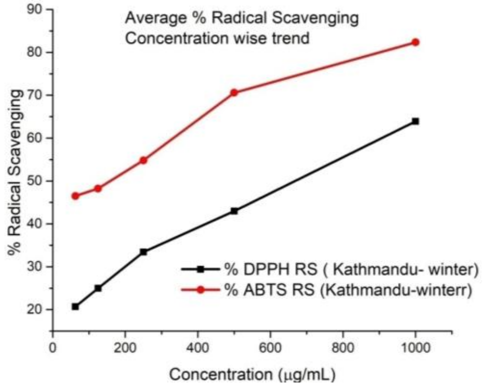
(d) M. pulegium *(e) E. strobilifera* *(f) E. blanda*



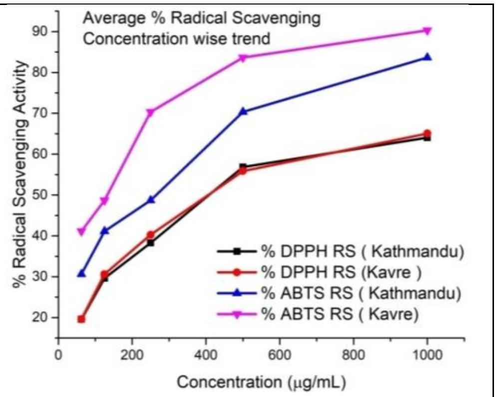
(g) O. tenuiflorum *(h) O. americanum* *(i) O. basilicum*



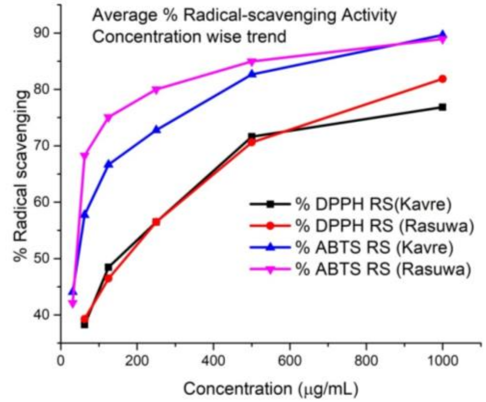
(j) P. frutescens



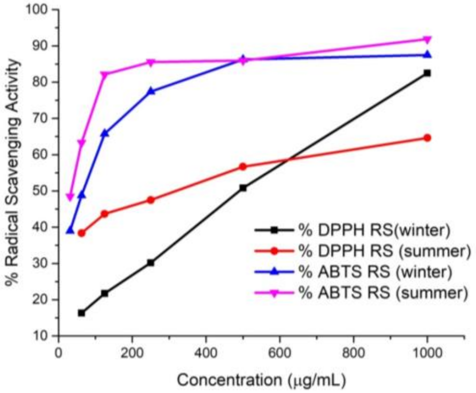
(k) P. glaber



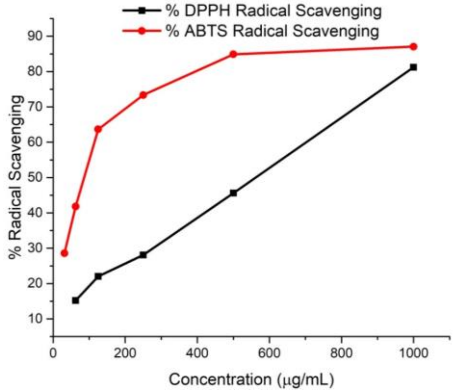
(l) C. oppositifolia



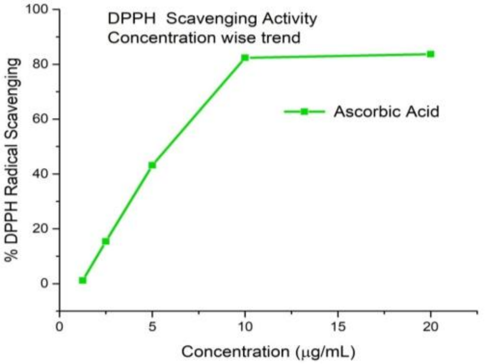
(m) C. coccinea



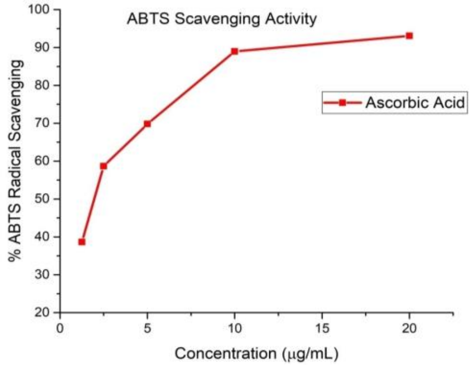
(n) L. canum



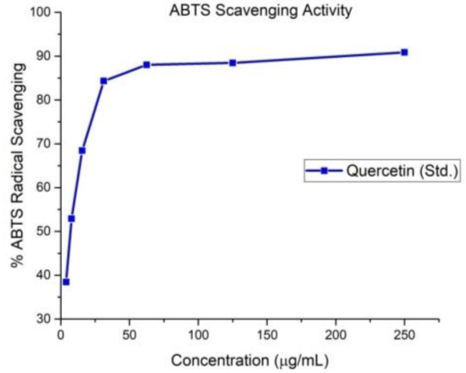
(o) C. umbrosum



(p) Ascorbic acid (standard)-DPPH

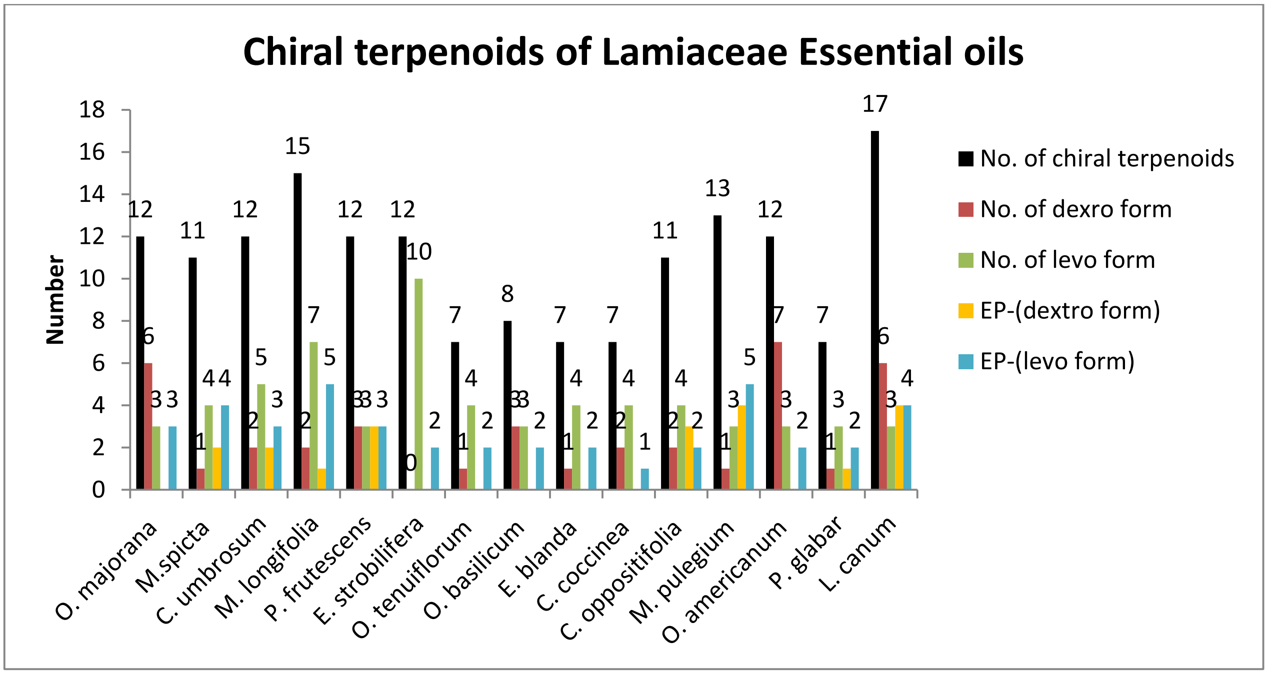


(q) Ascorbic acid (standard)-ABTS



(r) Quercetin (standard)-ABTS

Annex 12: Enantiomeric distribution of chiral compounds in Lamiaceae essential oils



Total chiral compounds No. = 163; Total dextro form=38 (37.62%); levo form = 63 (62.37%); enantiomerically pure dextro from = 20 (32.26%) and enantiomerically pure dextro from = (67.74%).The common chiral compounds in all lamiaceae essential are: α-thujene, α-pinene, sabinene, β-pinene, camphene, limonene,1-octen-3-ol, linalool,α-terpineol,cis- sabinene hydrate,menthone, β-caryophyllene, germacrene D,terpinen-4-ol, borneol, β- bisabolene, δ-cadinene, δ-cadinene , trans-β- ionone, trans-nerolidol

Annex 13: Letters issued by the National Herbarium and Plant Laboratories, Godawari, Lalitpur, Nepal for identification of plants

नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वनस्पति विभाग
राष्ट्रिय हर्वेरियम तथा वनस्पति प्रयोगशाला

गोदावरी, ललितपुर

पत्र संख्या: 078/079
च.नं. 968

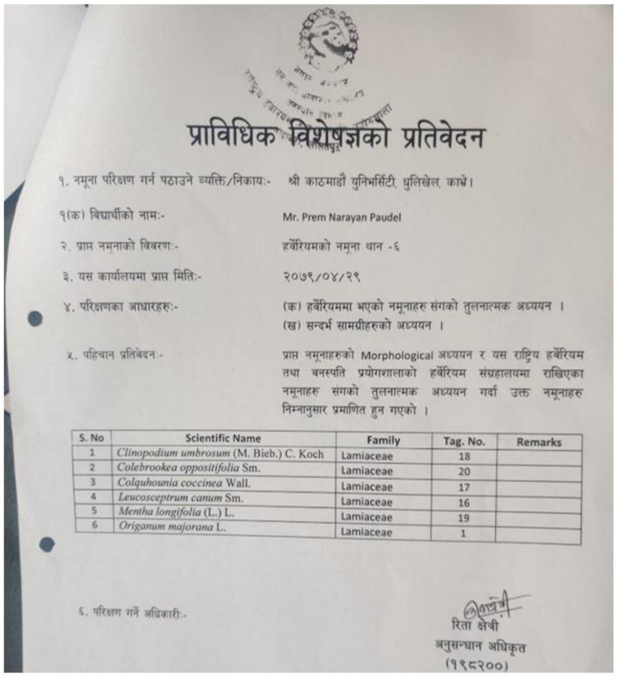
मिति २०७८/१०/२३

विषयः नमूनाहरु पहिचान सम्वन्धमा।

श्री Kathmandu University
School of Health Science
धुलिखेल, काठमाडौं।

प्रस्तुत विषयमा तहाँ कार्यालयको सन् २०२१/१२/१४ को पत्र साथ वनस्पतिका नमूनाहरु प्राप्त भई व्यहोरा अवगत भयो। पत्र मार्फत ल्याइएका नमूनाहरुको पहिचान गरी प्राविधिक विशेषज्ञको प्रतिवेदन (पाना १) यसै पत्रसाथ संलग्न गरी पठाइएको व्यहोरा अनुरोध छ।

78/10/23
सुभाष खत्री
वरिष्ठ अनुसन्धान अधिकृत
(१६३६८१)
कार्यालय प्रमुख



प्राविधिक विशेषज्ञको प्रतिवेदन

१. नमूना परिक्षण गर्न पठाउने व्यक्ति/निकाय:- श्री काठमाडौं युनिभर्सिटी, धुलिखेल, काभ्रे।

१(क) विद्यार्थीको नाम:- Mr. Prem Narayan Paudel

२. प्राप्त नमूनाको विवरण:- हर्बेरियमको नमूना थान -६

३. यस कार्यालयमा प्राप्त मिति:- २०७९/०४/२९

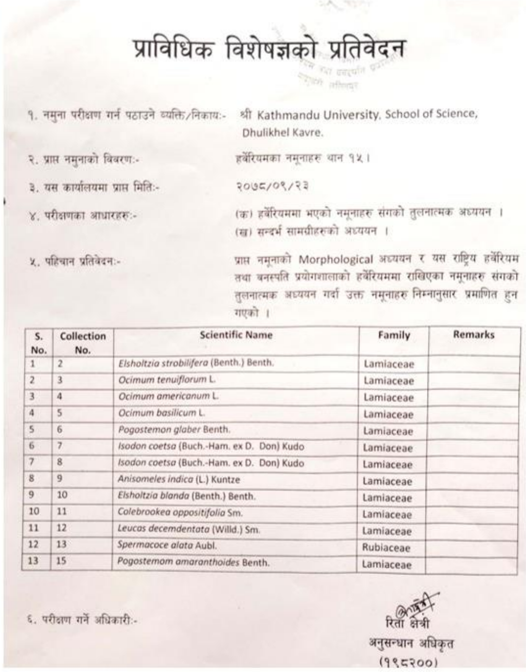
४. परिक्षणका आधारहरु:- (क) हर्बेरियममा भएको नमूनाहरु संगको तुलनात्मक अध्ययन ।
(ख) सन्दर्भ सामग्रीहरुको अध्ययन ।

५. पहिचान प्रतिवेदन:- प्राप्त नमूनाहरुको Morphological अध्ययन र यस राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशालाको हर्बेरियम संग्रहालयमा राखिएका नमूनाहरु संगको तुलनात्मक अध्ययन गर्दा उक्त नमूनाहरु निम्नानुसार प्रमाणित हुन गएको ।

S. No	Scientific Name	Family	Tag. No.	Remarks
1	*Clinopodium umbrosum* (M. Bieb.) C. Koch	Lamiaceae	18	
2	*Colebrookea oppositifolia* Sm.	Lamiaceae	20	
3	*Colquhounia coccinea* Wall.	Lamiaceae	17	
4	*Leucosceptrum canum* Sm.	Lamiaceae	16	
5	*Mentha longifolia* (L.) L.	Lamiaceae	19	
6	*Origanum majorana* L.	Lamiaceae	1	

६. परिक्षण गर्ने अधिकारी:-

रिता क्षेत्री
अनुसन्धान अधिकृत
(१९८२००)



प्राविधिक विशेषज्ञको प्रतिवेदन

१. नमुना परीक्षण गर्न पठाउने व्यक्ति/निकाय:- श्री Kathmandu University, School of Science, Dhulikhel Kavre.

२. प्राप्त नमुनाको विवरण:- हर्बेरियमका नमूनाहरु थान १५।

३. यस कार्यालयमा प्राप्त मिति:- २०७८/०९/२३

४. परीक्षणका आधारहरु:- (क) हर्बेरियममा भएको नमूनाहरु संगको तुलनात्मक अध्ययन ।
(ख) सन्दर्भ सामग्रीहरुको अध्ययन ।

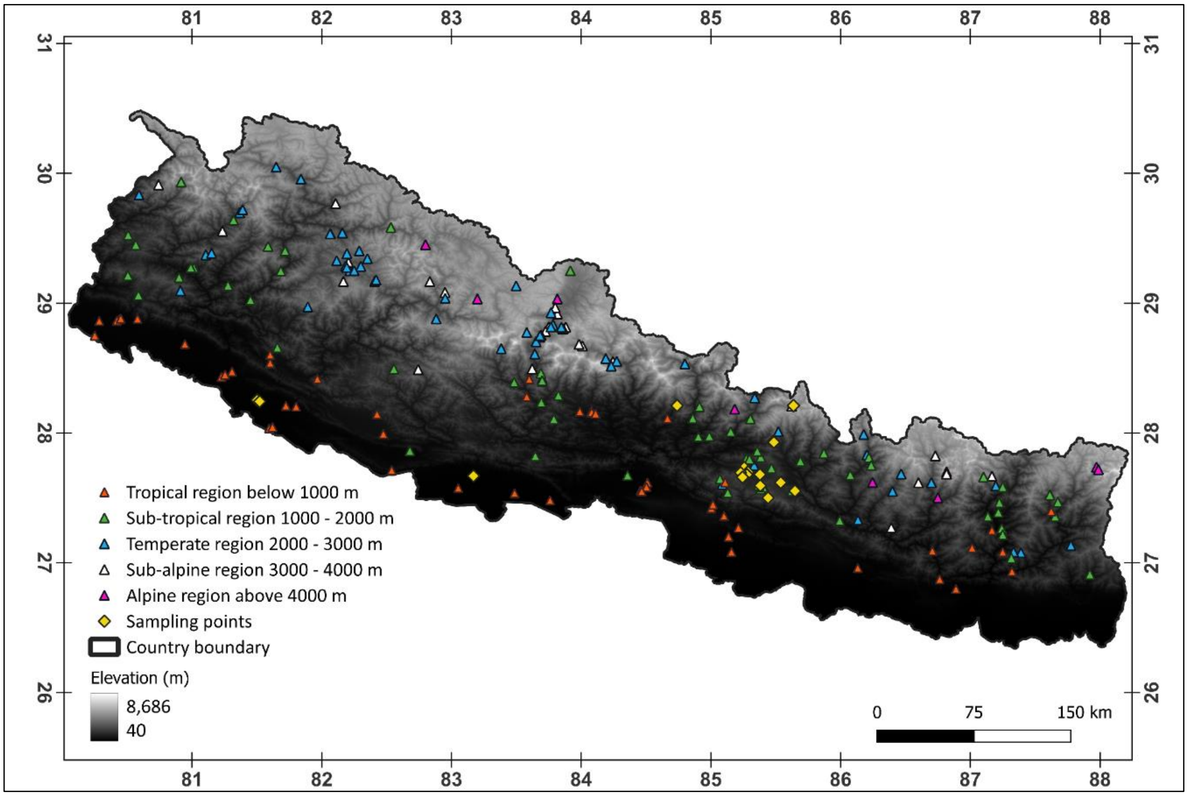
५. पहिचान प्रतिवेदन:- प्राप्त नमूनाको Morphological अध्ययन र यस राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशालाको हर्बेरियममा राखिएका नमूनाहरु संगको तुलनात्मक अध्ययन गर्दा उक्त नमूनाहरु निम्नानुसार प्रमाणित हुन गएको ।

S. No.	Collection No.	Scientific Name	Family	Remarks
1	2	*Elsholtzia strobilifera* (Benth.) Benth.	Lamiaceae	
2	3	*Ocimum tenuiflorum* L.	Lamiaceae	
3	4	*Ocimum americanum* L.	Lamiaceae	
4	5	*Ocimum basilicum* L.	Lamiaceae	
5	6	*Pogostemon glaber* Benth.	Lamiaceae	
6	7	*Isodon coetsa* (Buch.-Ham. ex D. Don) Kudo	Lamiaceae	
7	8	*Isodon coetsa* (Buch.-Ham. ex D. Don) Kudo	Lamiaceae	
8	9	*Anisomeles indica* (L.) Kuntze	Lamiaceae	
9	10	*Elsholtzia blanda* (Benth.) Benth.	Lamiaceae	
10	11	*Colebrookea oppositifolia* Sm.	Lamiaceae	
11	12	*Leucas decemdentata* (Willd.) Sm.	Lamiaceae	
12	13	*Spermacoce alata* Aubl.	Rubiaceae	
13	15	*Pogostemom amaranthoides* Benth.	Lamiaceae	

६. परीक्षण गर्ने अधिकारी:-

रिता क्षेत्री
अनुसन्धान अधिकृत
(१९८२००)

Annex 14: Distribution of Lamiaceae plants across different geographical locations of Nepal



Annex 15: Photographs of selected Lamiaceae plants taken during sample collection


Colquhounia coccinea Walll.


Ocimum basilicum L.


Elsholtzia strobilifera (Benth.)


Leucosceptrum canum


Ocimum americanum

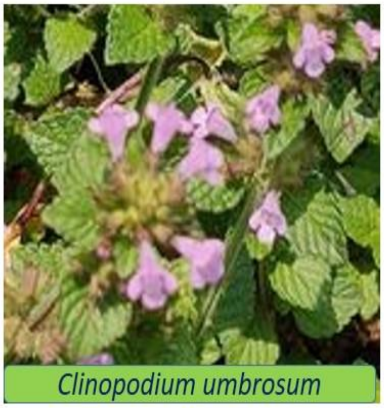

Elsholtzia blanda


Colebrookea oppositifolia Sm


Origanum majorana L


Mentha spicata L.


Perilla frutescens


Clinopodium umbrosum


Mentha spicata L.

Mentha pulegium

Mentha longifolia

Ocimun tenuiflorum L.

Pogostemon glaber Benth.

Origanum majorana L.

Ocimun tenuiflorum L.

Annex 16: Some glimpses during the plant sample collection at different parts of Nepal

Annex 17: Some glimpses during the laboratory works