A DISSERTATION ON

FINGERPRINTING OF ESSENTIAL OILS

SUBMITTED TO THE DEPARTMENT OF BIOSCIENCE

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILLMENT

FOR THE

DEGREE OF MASTER OF SCIENCE IN BIOCHEMISTRY

BY

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UNDER THE SUPERVISION OF

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Mr. Himanshu Kumar Mishra** during the months Feb-June, 2022 reported in the present thesis was under my Co- supervision. The results reported by his are genuine and script of the thesis has been written by the candidate himself. The thesis entitled is **"The Fingerprinting of Essential Oils"** is therefore, being forwarded for the acceptance in partial fulfillment of the requirements for the award of the degree of M. Sc Biochemistry, Department of Biosciences, Integral University, Lucknow, (U.P).

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To whom it may concern

This is to certify that **Mr. Himanshu Kumar Mishra**, a student of M.Sc. Biochemistry (2nd year/4th semester), Integral University Lucknow, has completed his four-month dissertation work entitled **"Fingerprinting of Essential Oils "** successfully**.** He has completed his work at CSIR-CIMAP (Central Institute of Medicinal and Aromatic Plants), Lucknow, under the guidance of **Dr. Ratnasekhar CH.** The dissertation was a compulsory part of his M.Sc. degree.

I wish his good luck and a bright future.

Dr. Snober S. Mir

Head of department of Bioscience

DECLARATION

I, **HIMANSHU KUMAR MISHRA**, a student of the "M.Sc. BIOCHEMISTRY" session 2020-2022, Department of Bioscience, Integral University Lucknow, declare that I am solely responsible for all of the work presented in this thesis entitled **"FINGERPRINTING OF ESSENTIAL OILS".** Which is being submitted to Integral University, Lucknow, Uttar Pradesh, India for partial fulfillment for the award of the degree of Master of Science in Biochemistry (2022), has been carried out by me under the supervision of **Dr. RATNASEKHAR CH,** Scientist, Phytochemistry Division, CSIR-CIMAP, Lucknow, U.P., India.

I further declare that; I take responsibility for the accuracy of this dissertation report.

Place: Himanshu Kumar Mishra

Date:

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Before I present my work, I would like to grateful acknowledge the contribution ofall those people who have helped me in the work described in this dissertation. I'lltry nonetheless, and if your name isn't there, please know that my thanks is just as great as it is for those who are.

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HIMANSHU KUMAR MISHRA

ABBREVEATIONS AND SYMBOLS

%	Percent
mg	Milli gram
nm	Nanometer
cm	Centimeter
α	Alpha
β	Beta
δ	Delta
ml	Milli liter
µl	Micro liter
˚C	Degree Celsius
EO	Essential oil
MIR	Mid-infrared
NIR	Near-infrared
NMR	Nuclear magnetic resonance
GC-MS	Gas chromatography mass-spectrometry
FT-NIR	Fourier transform-near infrared
IRMS	Isotope-ratio mass spectrometry
FID	Flame ionization detector
DSC	Differential scanning calorimetry
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
PCA	Principal Component Analysis

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1. INTRODUCTION

Secondary metabolites, which help plants repel predators and draw pollinators, are abundant in nature. Plant oils and extracts have been utilized for a variety of uses for thousands of years. Essential oils are a complex blend of volatile substances generated from the secondary metabolism of aromatic and other plant species. Steam or hydro distillation is commonly used to isolate volatile metabolites from plant material; the fragrant mixture of compounds obtained is known as an essential oil (EO). EOs are primarily composed of volatile terpenes and hydrocarbons.

According to EQ de Lima et al., the antimicrobial properties of plant extracts and essential oils derived from medicinal plants have been empirically known for hundreds of years but have only recently been scientifically confirmed.

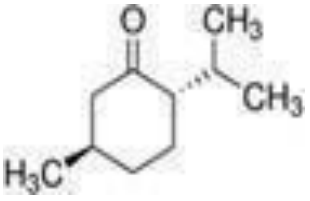
Essential oils are classified based on their extraction methods, chemical components present in oil, aroma, and so on. Extraction methods include steam distillation, cold pressing, and solvent extraction, while chemical composition includes terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids, and aroma includes Citrus, Herbaceous, Medicinal, Floral, Resinous oils, and Woody, Earthy, Minty, and Spicy oils.

Figure 1: Essential oils

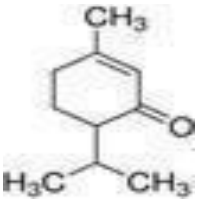
To make peppermint oil, the fresh leaves and stems of *Mentha piperita*, a member of the family Lamiaceae, are often hydrodistilated, steam distilled, or solvent extracted. It is usually gone through the process of rectification and fractionation before use. Peppermint oil is an essential oil that contains various chemical compounds, including menthol, which gives peppermint to its refreshing properties. It is a popular essential oil all over the world [Sachan et.al. 2013].

Peppermint oil contains 4.5-10% w/w esters in the form of Methyl acetate, less than 44 percent w/w free alcohols in the form of Menthol, and 15-32 percent w/w ketones in the form of Menthone [Miloua et al. 2022].

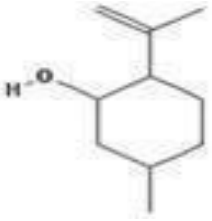
The following figure includes the structures of the main components present in peppermint-



Menthone



Piperitone



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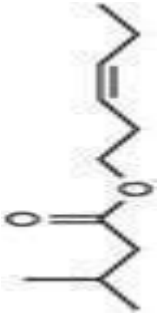
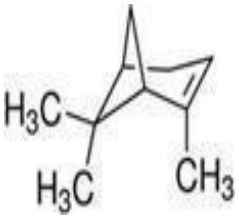
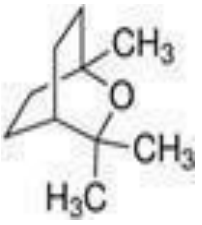


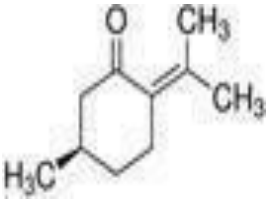
Cis-3-Hexenyl isovalerate



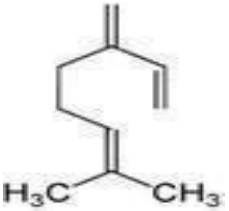
α-Pinene



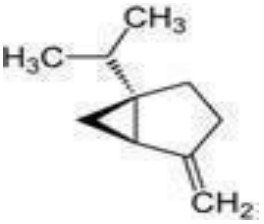
Eucaliptol



Pulegon



β-Myrcene



Sabinene

According to statistics, global essential oil output in 2017 was expected to be more than 150 thousand tonne, worth nearly $6 billion USD, three times increase in amount since 1990 (45 thousand tonne), with 50% of it occurring since 2007. Several economic analyses predict that growth will continue, with the industry expected to reach 370 thousand tonne per year and be worth more than $10 billion by the 2020s [Barbieri et al. 2018].

Essential oils, in addition to their applications in the improvement of health issues, cure the most common health issues such as migraines and nausea. It is also used in the food industry due to its antibacterial, antimicrobial, and antifungal properties, as well as its preservative potential in comparison to foodborne pathogens. Aromatherapy is becoming more popular as a complementary therapy due to its unique properties, which include coping with some cancer side effects and promoting wound healing [Irsad et al. 2022].

Essential oils require quality control to ensure their safety and to distinguish adulteration and fraud. Unfortunately, adulteration of essential oils is common along supply chains, causing concern. Essential oils require quality control to ensure their safety and to detect adulteration and fraud. Adulteration of essential oils is unfortunately common along supply chains, raising concerns in the essential oil industry. The addition of cheaper essential oil (e.g., sweet orange added to bitter orange, *Mentha arvensis* in *Mentha piperita*, or lavandin added to lavender) orsynthetic materials (e.g., synthetic linalool and linalyl acetate added to bergamot essential oil) taints essential oil frequently [Baser et al. 2009]. The normalized percentage areas of selected markers can easily detect this type of adulteration. Furthermore, because plant biosynthesis is guided by stereochemistry and terpenes/terpenoids are generally chiral compounds with a specific enantiomeric composition [Cagliero et al. 2016; Dewick et al. 2002], chiral marker compounds can be used to detect essential oil adulteration caused by the addition of synthetic volatile compounds. To recover quality control and detect fraud and adulteration caused by the addition of cheap synthetic materials or volatiles from other sources to EO, enantiomeric recognition is required.

Dilution with vegetable oils is another type of EO adulteration that results in scent loss. Vegetable oils were chosen because they are inexpensive and have a

density and texture similar to essential oils [Baser et al. 2009]. This type of adulteration is deceptive because it does not change the EO's qualitative composition or the relative percentage abundance of the marker compounds. Diluting the final product, on the other hand, interferes with the sensory and biological properties of the EO while also committing commercial fraud. In this case, the normalized percentage area is no longer diagnostic, and a true quantitative analysis is required, assuming that acceptable reference quantitative data is available.

The following table lists the most common adulterants found in essential oils:

Pure oil	**Adulterants**
Cedarwood oil Virginia	Cedarwood oil Chinese
Cinnamon bark oil	Cinnamon leaf oil / Cinnamic aldehyde
Lemon oil	Orange terpenes / Lemon terpenes
Peppermint oil	Corn mint oil
Geranium Bourbon	Geranium oil from China
Rosemary oil	Eucalyptus globulus oil
Bitter Almond oil	Benzaldehyde
Lavender oil	White camphor oil, Spanish sage oil
Lemon grass oil	Citral
Spearmint oil	L- carvone
Wintergreen oil	Methyl salicylate
Cardamom oil	Triphenyl acetate / linalyl acetate
Siberian Fir	Chinese Fir
Anise oil	Anethole
Cypress oil	α-pinene, δ-3-carene and Myrcene

Table 1: Common adulterants used in essential oils

Gas chromatography (GC), ideally in conjunction with mass spectrometry, is currently regarded as the gold standard for essential oil quality control (MS). Methods based on this technique can detect volatile compounds present in essential oils, as well as confirm the identity of such compounds. Isotope-ratio mass spectrometry and chiral GC analysis have proven to be extremely effective in combating adulteration caused by the addition of synthetic versions of

compounds found in essential oils [Schipilliti et al. 2012; Paraschos et al. 2016]. When non-volatile substance adulteration is suspected, liquid chromatography (i.e., high performance liquid chromatography, HPLC) may be useful. [Fan et al.2015].

However, this technique is not widely used in industry for quality control, and GC- based methods continue to predominate, with their attendant limitations.

Our present study involves use of sophisticated instruments to identify the chemical composition in the essential oil called **"Fingerprinting of essential oils"** and also to detect the amounts of adulterants that are added in that essential oil and its analysis is done by FT-NIR spectroscopy under proper guidance at CSIR-CIMAP, Lucknow.

2.Review of literature

Humans have been using essential oils for thousands of years. The extraction of essential oils from plants has been documented for over 5000 years. Aromatic substances were also used in antiquity, including Rome, Greece, the Middle East, andthe Far East. In Europe, the therapeutic and repellent properties of plants that produce aromatic compounds were recognized [Mucha et al. 2021]. Term "essential oil" is derived from the drug Quinta essential, which was named by a Swiss physician, Paracelsus von Hohenheim, in the sixteenth century. Numerous authors have attempted to define essential oils. "The essential oil is the product obtained from a vegetable raw material, either by steam distillation or mechanical process from the epicarp of Citrus, or dry distillation," according to The French Agency for Normalization. The essential oil is then physically separated from the aqueous component. Products obtained from vegetable raw materials using various methods, such as non-aqueous solvent or cold absorption, according to this definition.

The Lamiaceae (syn. Labiatae) family of plants contains a number of medicinal and aromatic plants. There are over 232 genera and approximately 7200 species in the family [Harley et al. 2004]. Secondary metabolites such as terpenes/essential oilsand other components are found in the epidermal glands of the majority of Lamiaceae plants' leaves, stems, and reproductive structures. With 25–30 species found worldwide, particularly in South Africa, Australia, and temperate Eurasia, the genus Mentha is an important taxon in the Lamiaceae family [Dorman et al. 2003]. The taxon is important for commercial as well as medicinal purposes. Indeed, various plant parts of this genus, such as leaves, flowers, and stems, are frequently used to provide aroma and flavour in herbal medicine, teas, or as additives in spice mixtures for various foods [McKay et al. 2006].

Mentha spp. has been used as a folk remedy for aliment ulcerative, anorexia, nausea, flatulence, bronchitis, liver complaints, and colitis due to its stimulant, antiemetic, diaphoretic, carminative, anti-inflammatory, analgesic, emmenagogue, antispasmodic, and ant catarrhal properties [Iscan et al. 2002].

Essential oils are soluble in alcohol, ether, and fixed oils but insoluble in water due to their non-polar nature. At room temperature, essential oils are generally colorless or yellowish liquids. With the exception of a few oils, they have a distinct odor and density less than unity (cinnamon, sassafras, and vetiver). They have a high refractive index and optical activity [Dhifi et al. 2016].

Essential oils are primarily composed of volatile and aromatic compounds and are extracted from various parts of plants, primarily the leaves and flowers. They are secreted from plants via the hydrolysis of some glycosides or from the protoplasm via the degradation of cell membrane and resin materials. Terpenes, sesquiterpenes, peroxides, esters, phenols, aldehydes, and ethers are the most abundant in essential oils.

2.1 Essential oils classification:

Essential oils are classified based on their extraction methods, notes, chemical composition and aroma, and so on.

2.1.1 Classification based on extraction methods:

Various methods are currently used for essential oil extraction, but the most common and prevalent methods are steam distillation, cold pressing, and solvent extraction. Essential oils are classified into the following categories based on these methods:

- Steam-Distilled Oils: It is the most traditional method of essential oil extraction. The plant material was placed in a container while steam passed through it in this method. Heat from steam causes pockets of aromatic molecules and oils in plants to open. Following their release, these molecules rise with steam and pass through a closed system. The aromatic steam is now cooled and distilled with cold water, allowing the essential oils to condense and transform into liquid form. The liquid mixture is separated once more, this time into two or more essential oils and hydrosol or aromatic water. Many factors influence oil quality and purity, including the pressure of steam passed through plant material, the coolant used, and the temperature of the closed system during oil production.
- Cold-pressed or Expressed oils: This method is used to extract oils from citrus trees (where oils are produced from the rind of fruits like tangerines,

grapefruits, lemons, oranges and others). Because of their high therapeutic value, they are classified as essential oils and are known as expressed oils. Mechanical pressure is used in this method to force oils out of the fruits in juice form. Because oils in their juicy form contain a lot of water, a separation process is used to separate the oils from the water. Cold-pressed oils spoil faster than other oils, which is one disadvantage of this method.

- Solvent Extracted Oils: Some plant materials are heat (steam) or cold-pressing sensitive. When they are subjected to any of these methods, the oil producedmay be contaminated or of poor quality. Some plants, such as Jasmine, Rose, Orange, Blossom, Tuberose, and Oak, are extracted using solvents to avoid this impurity. Ether, alcohols, hexane, and petroleum are used as solvents. The plant material is passed through hydrocarbon solvents in this method. The essential oils are separated when the solvent mixture is filtered and distilled under low pressure. One disadvantage of this method is that solvent residues can sometimes remain in the oils, causing allergic reactions in some people.

2.1.2 Classification Based on Chemical Composition:

- Every essential oil contains more than a hundred components, but the number of components varies between oils. Terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids are the two most important active compounds in essential oils. These two groups are derived from distinct primary metabolic precursors and are synthesized via distinct metabolic pathways. Essential oils, like all organic compounds, are made up of hydrocarbon molecules and can be further classified such as-
- Terpenoids: Terpenes and terpenoids are the primary components of many plant and flower essential oils. Terpenoids are the most important components of most plant essential oils and are found in the monoterpenoid and sesquiterpenoid families.
- Monoterpene / Monoterpenoid: Monoterpenes are found in nearly all essential oils and have a carbon structure of 10 with at least one unsaturated bond. Examples of monoterpenes and monoterpenoids include geraniol, terpineol (found in lilacs), limonene (found in citrus fruits), myrcene (found in hops), linalool (found in lavender), and pinene (present in pine tree). Because

they are highly reactive to air and heat sources and easily oxidized, oils containing monoterpenes do not last long.

- Sesquiterpenes and Oxygenated Compounds: Sesquiterpenes are 15-carbon atom compounds with the molecular formula C15H24 and complex pharmacological actions, such as chamazulene, which is found in German chamomile.

- Esters: Esters are the condensation product of acid and alcohol (the process is known as esterification) and are found in a wide variety of essential oils. Linalyl acetate, a well-known ester found in bergamot, clary sage, lavender, and petit grain, as well as geraniol acetate found in sweet marjoram, are two beneficial compounds found in essential oils.
- Ketones: Ketones are organic compounds with a carbonyl group (>CO) bonded to two carbons that are commonly found in oils used in the upper respiratory system. Ketone-containing essential oils include Clary sage, Hyssop, Idaho, Tansy, Rosemary, and Western red cedar.

2.1.3 Classification Based on Aroma:

Essential oils can also be classified based on their aroma/smell. Citrus, Herbaceous, Medicinal, Floral, Resinous oils, and Woody, Earthy, Minty, and Spicy oils are included in this classification.

- Citrus Oils: This category includes essential oils with a distinct citrus flavor. Citrus oils are produced by plants such as bergamot, grapefruit, lemon, lime, orange, and tangerine.
- Herbaceous Oils: Oils extracted from plants that are otherwise very useful herbs. Plants such as Basil, Chamomile, Melissa, Clary Sage, Hyssop, Marjoram, Peppermint, and Rosemary can be used to extract these oils.
- Camphoraceous Oils: These are essential oils that have a specific healing property. Some of these essential oils come from Cajeput, Tea Tree, borneol- like, earthy and mug wort-like, and rosemary-like plants, with a fruity, dried plum- like background.
- Floral Oils: This category includes oils made from floral parts or that contain the floral essence of plants. Plants that produce these oils include Geranium, Jasmine, Lavender, Rose, Neroli, Chamomile, and Ylang-Ylang.

- Woody Oils: Essential oils with woody aromas or extracted from plant barks and other woody parts. Such oils are produced by plants such as cedar, cinnamon, cypress, juniper berry, pine, and sandalwood, among others.
- Earthy Oils: Essential oils with an earthy aroma or that are extracted from plant roots and other earthy parts. Some of these oils are produced by Angelica, Patchouli, Vetiver, and Valerian.
- Spicy Oils: Spice or spicy plant oils such as thyme, cloves, aniseed, black pepper, cardamom, cinnamon, coriander, cumin, ginger, and nutmeg.

Estimating global essential oil output and trade is difficult [Verlet et al. 1992]. Many countries do not keep domestic production or export figures for some of the highest- volume oils, while the rest are buried in codes that cover a wide range of items. Worldwide essential oil output was expected to exceed 150,000 tons in 2017, valued at nearly $6 billion USD, representing a threefold increase in volume since 1990 (45,000 tone), with half of it occurring since 2007. Several economic analyses predict that growth will continue, with the industry expected to reach 370,000 tons per year and be worth more than $10 billion USD (current dollars) by the 2020s [Barbieri et al. 2016].

Essential oils contain varying amounts of alcohols, aldehydes, ketones, phenols, esters, ethers, and terpenes. The world's largest producers of essential oils are China and India, followed by Indonesia, Sri Lanka, and Vietnam. Morocco, Tunisia, Egypt, and Algeria are major African oil producers; the Ivory Coast, South Africa, Ghana, Kenya, Tanzania, Uganda, and Ethiopia are minor players [Lawrence et al. 2009]. Essential oils are also produced in large quantities in North America. Natural aromatic plant materials are abundant in the United States (US), Canada, and Mexico, and Argentina, Paraguay, Uruguay, Guatemala, and the island of Haiti all contribute significantly to sector production. Aside from the major oil-producing countries mentioned above, many others of lesser importance exist, including France, Germany, Taiwan, Japan, Jamaica, and the Philippines. Many producers also come from low-cost peasant-type operations and developing-country economies (65 percent of world production). Orange derivatives from Brazil and China, corn mint from India and China, lemon from Argentina and Spain, Eucalyptus from China and India, peppermint from the United States and India, citronella from China and

Indonesia, sassafras from China, lime from Mexico, lavandin from France and Spain, and patchouli from Indonesia are just a few examples. The sector employs approximately 1 million farmers and accounts for 0.06 percent of all farms globally (1,600 million). More than two-thirds of total essential oil crop production is accounted for by the top three essential oil crops (orange, mint, and lemon), which total approximately 100,000 tones. Patchouli, Citronella, Eucalyptus globulus, Clover leaf (production range: 1,000–10,000 tones), and Vetiver, Geranium, Nutmeg, and Lavender (production range: 50–400 tones) are all grown on small farms or harvested from forests. Small farmers continue to dominate essential oil production, contributing significantly to the local incomes of developing-country rural populations.

Essential oils were exported for $4.38 billion USD and imported for $4.54 billion USD in 2016 [Barbieri et al. 2016]. The top exporters were the US ($47 billion USD), Germany ($28 billion USD), the UK ($26 billion USD), and France ($22 billion USD), in that order [Comrade et al. 2018], while the top importers were France ($65 billion USD), the US ($47 billion USD), Germany ($41 billion USD), and Ireland ($35 billion USD). The EU, led by France, Germany, and the United Kingdom, is the world's largest importer of essential oils. Europe dominates the essential oil market geographically, accounting for roughly 40% of global exports in 2016. Due to rising demand for natural cosmetics, increased awareness, and increased supply, the region is expected to maintain its dominance throughout the analysis period.

Essential oils are plant extract-derived products that have been used in industrial and home-made products on a large scale. Essential oils are commonly used in pest control, cleaning, and anti-inflammatory actions, among other products and personal care products. Wound healing, rejuvenation, and relaxation can all be aided by essential oils. Essential oils, in addition to their applications in the improvement of health issues, cure the most common health issues such as migraines and nausea. Itis also used in the food industry due to its antibacterial, antimicrobial, and antifungal properties, as well as its preservative potential in comparison to foodborne pathogens. Aromatherapy is becoming more popular as a complementary therapy due to its unique properties, which include coping with some cancer side effects and promoting wound healing [Irsad et al. 2020].

2.2 Applications of essential oils:

2.2.1 Application of essential oils in the food industry: The US Food and Drug Administration has classified the use of essential oils as GRAS (Generally Recognized as Safe) antimicrobial additives in food, and they are rich sources of biologically active compounds with known antimicrobial and antioxidant properties, attracting interest as food additives [Llana et al. 2015; Manso et al. 2014; Wrona et al. 2015; Atarés et al. 2016].

They have recently gained popularity as a food additive due to their potent antimicrobial and antioxidant properties [Calo et al. 2015]. The fundamental approach to ensuring food safety is to use active packaging to reduce the initial microbiological load and/or to inhibit the growth of residual microorganisms during post-process applications such as production and storage [Herman et al. 2017]. Because of their numerous applications, cinnamon essential oils have been described as the most important essential oils used in the food and cosmetic industries, particularly as an antimicrobial agent [Nollet et al. 2017]. Cinnamon oil encapsulated in cyclodextrin nano sponges, according to Semiauto, could be used for antimicrobial food packaging [Simionato et al. 2019]. Furthermore, using yoghurt as a food model, garlic essential oil demonstrated its potential as a natural food preservative by exhibiting appropriate physicochemical properties, particularly in acidic food products [Clemente et al.2016].

2.2.2 Pharmacological applications:

Essential oils are said to have a wide range of pharmacological properties. Individuals and businesses have recently developed methods to modify the effects of pharmaceutical products. These herbs and extracts have been used as cancer treatments, antioxidants, antimicrobials, and anti-inflammatory agents [Nieto et al. 2017]. Luo et al. demonstrated the anti-inflammatory activities of six essential oils in a 12-O- tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation model, and their results showed that these six essential oils inhibited inflammation to some extent in a dose-dependent manner and markedly relieved ear edema [Luo et al. 2019]. According to Chen et al., essential oils of *Scutellaria baicalensis* are anti- tumorigenic and inhibit the growth of HeLa and A549 cells [Chen et al. 2016]. Some essential oils and their

constituents may act as natural antioxidants [Sun et al 2017]. Bacterial pathogens are becoming resistant to multidrug antibiotics, resulting in disease severity increasing. They can form biofilms, which are associated with drug tolerance, and a lack of immunity in host cells increases the number of potentially fatal bacterial infections in the human body [Raut et al. 2014]. Essential oils extracted from medicinal aromatic plants like peppermint (*Mentha piperita*), thyme (*Thymus vulgaris*), and fennel (*Foeniculum vulgare*) have also been shown to be effective against Gram-negative and Gram-positive bacteria, viruses, fungi, and yeast. Eos are thought to aid higher plant defense mechanisms [Reichling et al. 2010]. *Copaifera officinalis* essential oil, according to Bisht, contains -cadinene, germacrene D, -humulene, -copaene, germacrene B, caryophyllene, and bisabolene, which inhibit *Escherichia coli* and *Staphlococcus aureus* [Bist et al. 2014].

2.2.3 Effects of Essential Oils on Pathogens: Pharmacologic and antimicrobial activity tests have been performed on essential oils. Various essential oils' antimicrobial activities have been tested in vitro. The most common methods are agar diffusion tests, serial broth or agar dilution tests, and vapor phase tests [31]. These oils are thought to play a role in the defence mechanisms of phytopathogenic microorganisms [Camele et al. 2010; Mancini et al. 2014; Elshafie et al. 2015].

2.2.4 Application as antimicrobial agent:

Selected essential oils appear to have the advantage of inhibiting the growth of potential pathogens while having only a minor effect on beneficial intestinal microbiota members. *Clostridium perfringens* strains were discovered to be sensitive to carvacrol, cinnamaldehyde, citral, limonene, and thymol, especially at higher concentrations tested (500 mg/l), as well as oregano oil, rosemary oil, and thyme oil [Ouwehand et al. 2010]. Clove oil, an essential oil extracted from the clove plant, *Syzygium aromaticum*, has been reported to act as a bioactive substance in apples, particularly against *Botrytis cinerea*, *Monilinia fructigena* Hone, *Penicillium expansum*, and *Phlyctema vagabunda* Furthermore, essential oils from basil (*Ocimum basilicum*), fennel (*Foeniculum sativum*), lavender (*Lavandula officinalis*), marjoram (*Ocimum majorana*), oregano (Ocimum vulgare L), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*),

sage (*Salvia officinalis*),savory(*Satureja montana).*

2.2.5. Applications in Aroma therapy:

Aromatherapy is defined as "the treatment of anxiety or minor medical conditions by rubbing pleasant smelling natural oils into the skin or inhaling their aroma." It is the use of aromatic essential oils to improve the emotional and physical health and beauty of the body. Our sense of smell is important to our overall health, according to science. When inhaled, essential oils travel directly to the limbic system of the brain via the olfactory system. True, our emotions and chemical balance are affected by a specific scent. Essential oils are also absorbed through the skin and circulated throughout the body, allowing them to reach all internal organs. By carefully selecting one or more oils, you can experience beneficial effects promoting overall health - and even specific targets. The advantages are determined by the unique nature of each individual's response to an aromatic stimulus.

2.3 Adulteration of essential oils:

In order to increase profits or meet some established ISO requirement, essential oils are typically adulterated by the addition of synthetic and natural compounds, both related and unrelated to the oil's composition. Keeping this in mind, the root cause of adulteration is economic motivation.

Adulteration is a widespread problem in the food and nutraceutical industries. Becauseessential oils are an expensive commodity, it is estimated that more than 80% of thosemarketed as pure and natural essential oils are adulterated in some way.

Factors that encourage adulteration include:

Price: One of the most important drivers is a desire to purchase cheaper oils: some oils must be produced using expensive raw materials, labor, and investments, so there will undoubtedly be some price margin. If the buyer wants to buy at a low price, the supplier must definitely adulterate in order to keep their business running. If you see a very low price for one of the most expensive rose essential oils, it is an indication of adulterated or inferior rose oils. Rose oils do not have unusual chemistry, so its components can be obtained from cheaper oils such as palma rosa, geranium, lemongrass, and so on. The price difference between adulterated and pure oils can be more than 1000

percent, but the profit of adulteration also depends on the type of adulteration.

Aroma: Adulteration does not have to be done in large quantities and it can also be done in small amounts to improve the smell. To create an authentic aroma, several synthetic fragrances are added in trace amounts. Many essential oils are scented with ethyl vanillin, cyclamen aldehyde, galaxolide, and other compounds.

Natural Variability: Chemical composition variation always motivates suppliers to meet certain specifications such as ISO or other standards. Chemicals are more widely available and less expensive than producing pure and natural EOs. As a result, many EO suppliers are primarily focused on finding marker-free aroma chemicals rather than farming or increasing production capacity.

2.3.1 Types of adulteration include:

- **Dilution by non-volatile components or cooking oils:** Heavier components of other oils or cooking oils (such as castor, sunflower, maize, canola, nut oils, and so on) are less expensive, odorless, and undetectable by standard GCMS. The addition of heavier components raises the concentration of essential oils.

- **Synthetic**: Synthetic compounds can be made from two types of precursors: natural and petrochemical-based. Petrochemical-derived precursors fail isotopic testing (C14 or deuterium), whereas natural-derived precursor-derived synthetic compounds pass.
- **Natural Isolates**: Many essential oil molecules are derived from similar but less expensive plant products. Linalool is commonly obtained through fractional distillation from Ho-Wood or leaf. Another well-known molecule obtained from Eucalyptus EO via fractional distillation is 1,8 cineole. Similarly, limonene can be extracted from orange oil. The sophisticated addition of those natural isolates is frequently undetectable by the conventional method of EO analysis.
- **Adding similar oils:** Lavandin in lavender, corn mint in peppermint, E. citriodora in citronella, and orange in bergamot/grapefruit/lemon oils are all difficult. As a result, this falls under the category of sophisticated adulteration.

2.4. Analytical techniques that are commonly used to detect adulteration in essential oils:

2.4.1 Gas chromatography- Mass spectrometry (GC-MS): To analyze volatile organic compounds, gas chromatography (GC) is used in conjunction with one of

several detection systems. Gas chromatography is the first step in essential oil analysis because it separates the mixture into individual components. A 'column' is used to achieve this separation. A small amount of essential oil is injected at the beginning of the column, and a gas pushes the mixture through the column to the other side, where each of the separated components meets a detector, usually a mass detector (MS) or a flame ionization detector (FID). When a mass spectrometer is connected to a gas chromatograph, the process is known as gas chromatography- mass spectrometry (GC-MS).

The column is slowly heated from a low starting temperature, typically in the 40- 60 °C range, and then raised at a rate of 3-5 °C per minute until it reaches a maximum

temperature in the 280–300 °C range. The column is filled with an inert gas, such as nitrogen or helium, and essential oil components travel in the direction of gas flow as vapors. The term "gas chromatography" derives from the use of nitrogen or helium gas and does not describe the physical state of the essential oil components as they pass through the column. Because essential oil components exist in the column as liquids/ vapors, they usually elute before reaching their boiling point at atmospheric pressure, which is 297°C. The boiling point of spatulous in a pressurized column is much higher according to thermodynamic theory, but it usually elutes between 150 and 200 °C. Furthermore, while limonene has a boiling point of 176 degrees Celsius, it usually elutes between 105 and 115 degrees Celsius. Much larger components, such as volatile diterpenes and coumarins, have boiling points that are much higher than the operating conditions of the machine; for example, incensole acetate has a boiling point of 420 °C, but it is still eluted in gas chromatography like other components, albeit with a longer retention time.

The inlet gas pressure (helium or nitrogen), intermolecular interactions with the stationary phase (the column), temperature, and the concentration and vapor pressure of each individual molecule all influence the physical state of essential oil components (and volatile derivatives) as they travel through the column [Hively et al. 1968]. When essential oil components pass through the column, they are in a vapor phase; however, once stationary, they are adsorbed into the column matrix as a liquid.

The packing inside the column (stationary phase), which can be interactive (polar or chiral) or non-interactive, can also influence essential oil component separation in chromatography (non-polar). The column is typically a thin hollow tube 30 meters long that is coiled into a circle for convenience before being placed in an 'oven,' which is a temperature-controlled cabinet. The hollow space in the column is filled with a polar or non-polar stationary phase, such as polyethylene glycol (wax) or phenyl/methyl polysiloxane base.

Two primary diagnostic methods are used to identify essential oil components. The first is the mass spectrometer's 'fingerprint' or fragment pattern.

In gas chromatography, the mass spectrometer uses an electron beam to ionize the molecules, a technique known as electron impact ionization. As a result, the compound is bombarded with a precisely measured load of electrons, typically 70 millivolts, and the fragment masses are determined.

2.4.2 Isotope-ratio mass spectrometry (IRMS): Isotope-ratio analysis using isotope- ratio MS (IRMS) or stable-isotope-ratio analysis is used to certify the naturalness of one or more components of an essential oil (SIRA). Plants are distinguished by their metabolic assimilation of atmospheric CO2, specifically by reaction intermediates derived from CO2 incorporation (molecules with three or four carbon atoms). The isotopic fractionation of metabolites in C3 and C4 plants differs, and this difference allows plants to be distinguished by their isotopic ratio [O'Leary et al. 1981; Ghashghaie et al.2003]. Isotopic variations in natural compounds are measured using the principle that most chemical elements have different stable isotopes, which result in different molecular weights [Rundel et al. 2012]. The stable-isotope ratios of carbon, hydrogen, oxygen, or nitrogen within metabolites can be used to detect the accidental or intentional addition of a synthetic product (mostly of fossil origin), or even to differentiate between different geographical or botanical origins. The analysis of isotopic data has become the gold standard for determining the origin and naturalness of flavors and fragrances.

IRMS is frequently combined with combustion/pyrolysis (C/P-IRMS) for adulteration control. This technique can reliably distinguish natural from synthetic mandarin essential oil based on C-isotope-ratio measurements for terpinen-4-ol,

-terpinene, - terpineol, and terpinolene. Carvacrol and thymol H-isotope ratio measurements [Schipilliti et al. 2010; Nhu-Trang et al. 2006; Hör et al. 2001] can be used to determine the authenticity of thyme and oregano essential oils. Furthermore, IRMS detects synthetic benzaldehyde in bitter almond oil.

IRMS is a very effective technique, but it requires a significant financial investment as well as an experienced operator. Its use also necessitates the creation of databases, which takes time.

2.4.3. Nuclear Magnetic Resonance spectroscopy: NMR spectroscopy provides information for the control of authenticity by determining stable-isotope ratios, allowing for the measurement of isotopic patterns within natural and synthetic molecules for the purposes of differentiation. The investigation of site-specific natural-isotope fractionation (SNIF-NMR), which is based on measuring deuterium/hydrogen (D/H) ratios at specific positions of a molecule, has enabled the identification of plant precursors [Cordella et al. 2002; Martin et al. 1993].

Deuterium quantification NMR detected significant differences in deuterium-isotope distribution depending on molecule origin, and it has the potential to characterise the enantiomeric purity of compounds like -pinene or methyl salicylate. The presence of synthetic linalool in essential oils is also detected using SNIF-NMR, as is the addition of (-) bisabolol extracted from Vanillosmopsis plants to chamomile oil.

Although NMR is a powerful technique, it necessitates compound isolation, databases, an experienced operator, and a significant investment.

2.4.4 Chiral Gas Chromatography Analysis: It is a very powerful technique for essential oil verification that is becoming increasingly important in adulterant detection. Many plant metabolites are chiral molecules, with enantiomers differing between species within the same genus. Despite having the same physicochemical properties, their optical activity differs. For example, (R)-limonene contributes to the smell of oranges, whereas (S)-limonene contributes to the smell of lemon. The main odorant in caraway essential oil is (S)-carvone (it smells like cumin), and (R)-carvone is found in spearmint essential oil (smell of spearmint).

In other cases, one or more stereoisomers may be less active or even

odorless, such as (R)-linalool, which has a strong floral note but is weaker than (S)-linalool, or (2S,4R)-cis-rose oxide, which has a strong rosy scent but is odorless [Mosandl et al. 1995]. Chiral analysis can detect adulteration of natural products with synthetic substitutes, usually in the racemic form, or bulking oils from other crops by using values of enantiomeric purity and enantiomeric excess. These values are composed of a measured ratio of detected enantiomers expressed in percentages and a relative difference of separated enantiomers expressed in percentages [Mosandl et al. 1995; Thao et al. 2010; Tournier et al. 2007; Barba et al. 2012].

As a result, chiral analysis is critical in the analysis of essential oils and has emerged as one of the most important analytical techniques in recent years. It is a low-cost, sensitive technique, but method development takes time, particularly because no universal chiral stationary phase exists [Rubiolo et al. 2010]. During the processing or storage of essential oils, some non-enzymatic reactions or racemization can occur, resulting in false-positive chiral analysis results

2.4.5 High- Performance Thin Layer Chromatography: Recent advances in this technique have primarily been observed in the quality of stationary phases and the efficiency of detection techniques. It was possible to create high-performance stationary phases with smaller particle sizes and narrow size distributions. This improved the resolution and reproducibility of TLC analysis. New stationary phases have also been developed and are now commercially available, such as chiral phases. (HP)TLC quickly became the preferred method for analysis and control (e.g., in the adulteration of ylang-ylang essential oil by sunflower oil).

(HP)TLC is an automated technique that allows for the rapid analysis of multiple samples (more or less complex) at the same time. It is also considered a greener technique because it reduces waste material (including volatile organic compounds) and energy costs.

Despite these benefits, (HP)TLC has some drawbacks as an off-line technique and requires an initial investment for equipment acquisition.

Other techniques' applications have shifted toward adulteration control. Differential scanning calorimetry (DSC), for example, is defined as "the

measurement of the change in the difference in the heat flow rate to the sample and to a reference sample while they are subjected to a controlled temperature programmed" [Prager et al. 1981]. It is based on assessing the effects of temperature-programmed scans, which can result in structural changes or decompositions [Cordella et al. 2002]. Its use in assessing sample purity has changed its use and has been tested in quality control. Orange, lemongrass, and basil essential oils have all been used in this manner. Because of the presence of dominant substances in their composition, they have distinct DSC profiles (approximately 90 percent limonene, 66 percent citral, and 84 percent methyl chavicol). In such cases, DSC can generate fingerprints with high precision [Martins et al. 2012].

Thermal diffusivity, like photoacoustic spectroscopy (PS), which is primarily used for gas analysis, can also be used for authentication. Since the introduction of more efficient lasers, its application areas have expanded. In a study on concentrated citrus oils, for example, PS was used to measure thermal diffusivity in essential oils to differentiate between different extraction processes [López Muñoz et al. 2012].

3. OBJECTIVE

The study was targeted to analyze the Fingerprinting of Essential oils by experimentation of:

- Collection of essential oils and adulterants
- Detection of adulterants in pure essential oils by FT-NIR

4. Materials and methods

4.1 Instrumentation:

Fourier transform near-infrared spectroscopy (FT-NIR)

FT-NIR based on the principle that, when a sample is exposed to NIR light, it absorbs it and activates the corresponding molecular vibrations. The result is an NIR spectrum with absorption bands of various molecular groups (most notably N-H, C-H, and O-H bonds) at typical wavelengths.

Figure 2: Fourier transform near- Infrared spectroscopy (FT-NIR)

Because it allows for simple, quick, and non-destructive measurements of chemical and physical components, FT-NIR spectroscopy is appealing to the food industry. It allows for remote measurements and easy analysis of various types and forms of samples. For direct and simultaneous measurements of multiple constituents in food matrices, NIR instruments can be easily deployed in the field or on process lines. Its non-destructive nature, as well as the requirement for little or no sample preparation prior to measurement, have all contributed to the increased interest in FT-NIR over FT-MIR.

NIR spectroscopy is the study of compound absorption in the NIR range (10000–4000 cm^{-1}) of the electromagnetic spectrum.

FT-NIR technology has been quickly adopted by the food and chemical industries. It is commonly used in the food industry for composition analysis, such as rapid measurement of fat, protein, moisture, sugars, and so on. A few specific applications of FT-NIR in food analysis include cholesterol determination in dairy products and analysis of edible oils and fats.

One common and critical application for food safety is the rapid differentiation of bacterial species. FT-NIR spectra represent the ratio of various chemical groups present in the sample, allowing for the detection of subtle differences. SIMCA and other multivariate classification methods have enabled sample clustering based on biochemical differences while reducing random noise. SIMCA generates a three- dimensional (3D) model of the samples based on the first three principal components that account for most of the differences between the samples.

4.2 Collection of samples:

Pure *Mentha spicata* (Spearmint), *Mentha piperita* (Peppermint), *Mentha citrata* (Orange mint) and *Mentha arvensis* (Corn mint) were obtained from the supermarket. Coconut oil and Soyabean refined oil was bought from local market.

Essential oils and preparation of spiked samples:

We take 5ml Borosile culture vials which is sterilized and free from moisture and other impurities. Then make 80 samples (20 samples of each ie- *Mentha pipperita, Mentha arvensis, Mentha spicata and, Mentha citrata*) of pure oils by adding about 500µl in each vial.

For spiked samples, we make 40 samples (20 samples of Coconut adulterated and 20 samples of Soyabean refined oil adulterated in *Mentha pipperita*) of 1% and 5% (v/v) respectively. To create calibration curves for each analyte, we used two concentration levels. In the scope of this study, 80 spiked samples were prepared. Samples were kept at 4ºC in dark place until spectral analysis.

4.3 Methodology:

The near-infrared (NIR) spectra were collected using a Thermo Scientific Antaris-II FT-NIR Spectrophotometer in the transflectance mode. NIR spectra were collected with resolution of 4 cm^{-1} and 64 scans spanning the 4,000–10,000 cm^{-1} spectral range.
Sample containing vials are put on the trans flection plate and cover the vials with opaque beaker.

5. RESULTS AND DISCUSSIONS

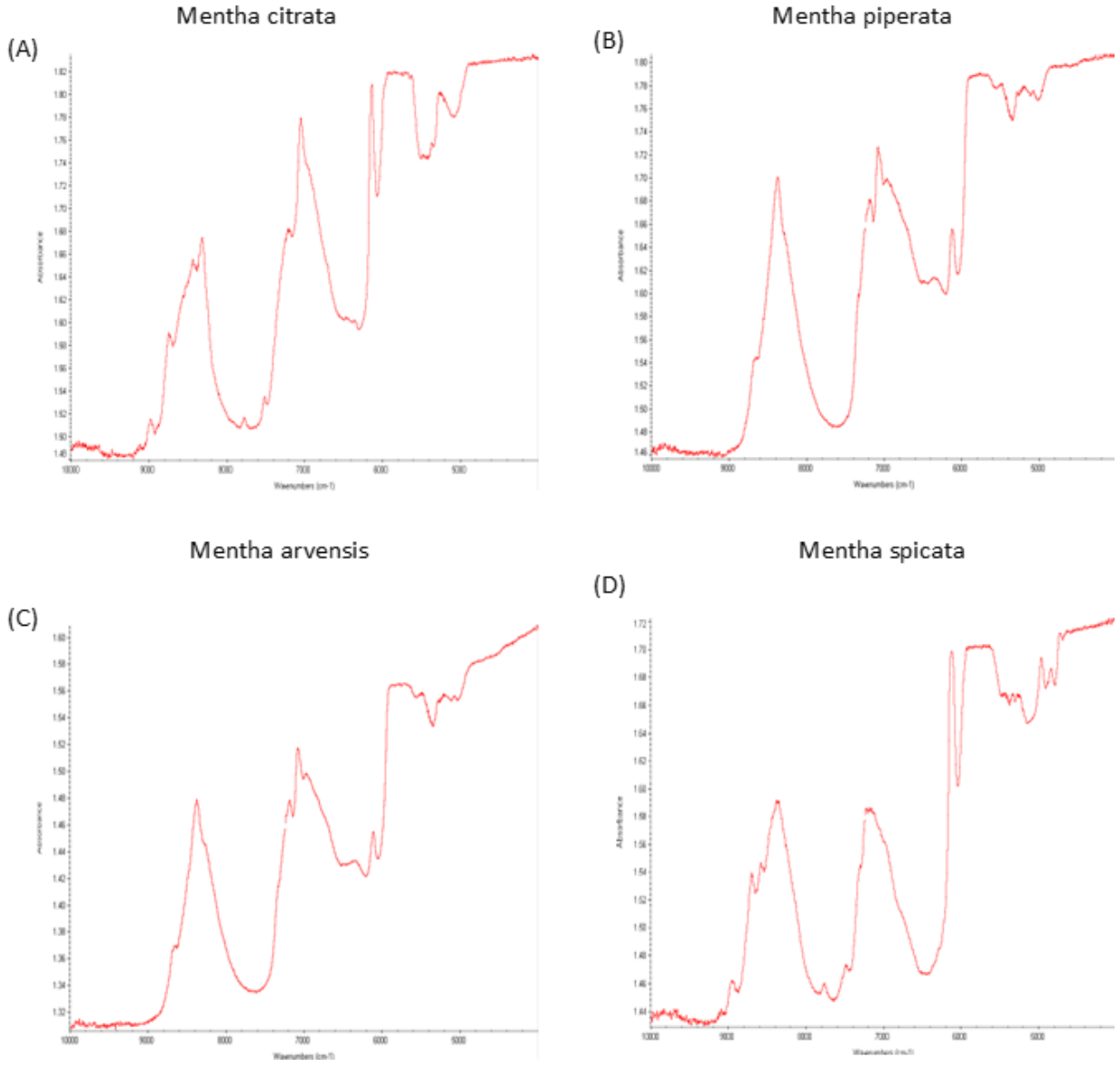


Figure 3: Absorption spectra of pure Mentha oil: (A) spectra of *Mentha citrata*; (B) spectra of *Mentha piperata*; (C) spectra of *Mentha arvensis*; (D) spectra of *Mentha spicata*

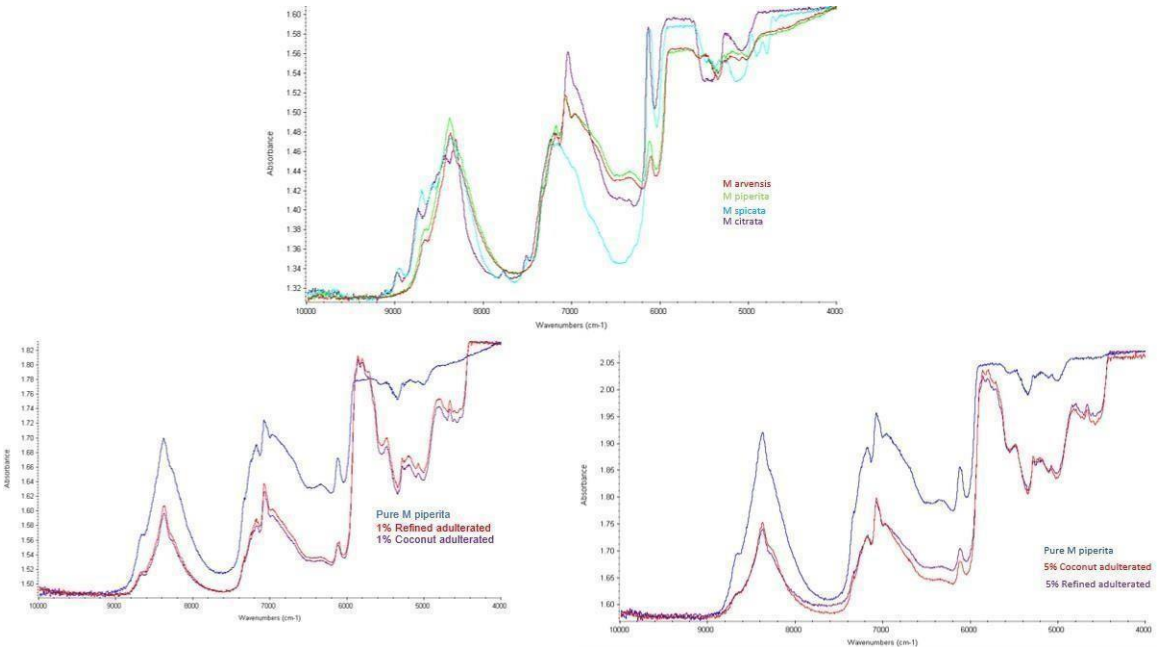


Figure 4: Absorption spectra of different Mentha oil: (A) spectra of pure *Mentha citrata*, *Mentha piperata*, *Mentha arvensis*, *Mentha spicata*; (B) plot of spectra of adulterated 1% *Mentha piperata;* (C) plot spectra of adulterated 5% *Mentha piperata*.

We tried to validate the method using FT-NIR technique and clear observations can be found. From the above shown spectral plots, it can be observed that the major difference in the spectral lines of adulterated and pure *Mentha piperata* oil can be found in the NIR spectral wavelength ranging from 4500-6000 cm^{-1} (wavenumber). This difference in the spectral pattern of this particular spectrum is due to the variation in the functional groups and fatty acid components of *Mentha piperata* oil and adulterant oils.

From the above spectra (B), it is seen that NIR is able to differentiate between the adulterated and pure *Mentha piperata* oil even at a minute concentration of 1% adulteration. The adulteration was performed by mixing 1% coconut oil and 1% refined oil. It has also been observed that the NIR Spectral peaks of adulterated oil using coconut oil and using refined oils are different having different amplitude however we cannot rely on this method for identification of adulteration.

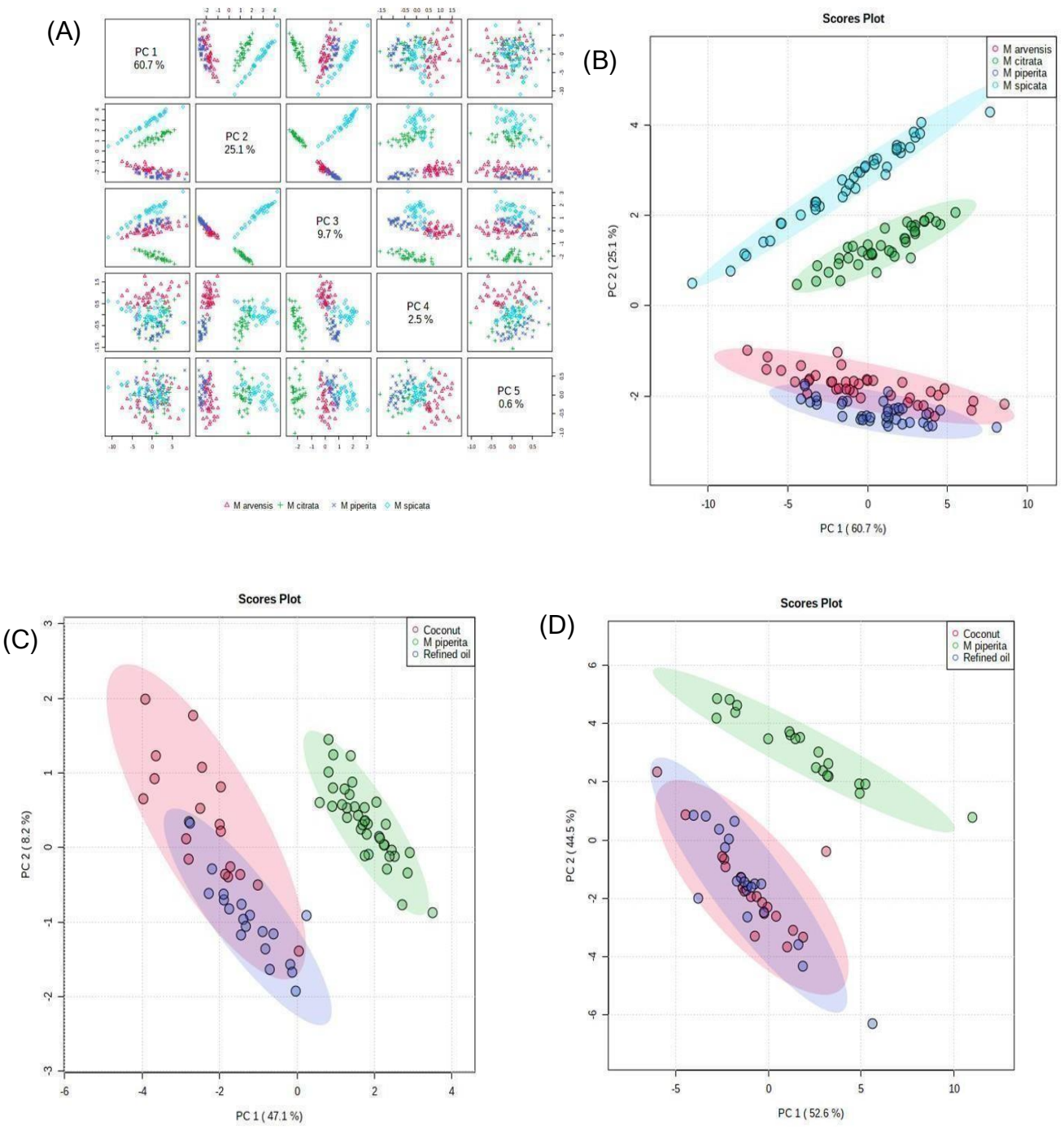


Figure 5: (A) Principal component analysis score plot of different types of Mentha oil. (B) principal component analysis of (C) plot of principal component of *Mentha piperata* adulterated with 5% coconut and refined oil; (D) plot of principal componentof *Mentha piperata* adulterated with 1%coconut and refined oil.

5.1 Principal component analysis (PCA):

Principle component analysis is a method for reducing the dimensionality of a large data set by transforming a large set of variables into a small set that retains the majority of the information in the large set. PCA is a traditional feature extraction and data representation technique that is widely used in pattern recognition and computer vision. In PCA-based pattern recognition, 2D (two-dimensional) matrices must first be transformed into 1D. (1-dimensional). Principle component analysis is a method for reducing the dimensionality of a large data set by transforming a large set of variables into a small set that retains the majority of the information in the large set. PCA is a traditional feature extraction and data representation technique that is widely used in pattern recognition. There is a multivariate analysis of principal components was performed. Principal component analysis (PCA) was performed in order to have a better visualization of all the information contained in the data set. The first five principal components conjugate more variation of species presented here. Out of first five the two principal component explains PC1 (60.7%), PC2 (25.1%) 85.8 % out of100% explains principal component variation (as shown in Figure 5 A).

6. CONCLUSION

Essential oils smell great, reduce stress, treat fungal infections, and help you sleep. They are concentrated extractions from plants. A process called distillation turns the “essence” of a plant into a liquefied form for many medicinal and recreational uses. There is a wide variety of essential oils available. An adulterant is a chemical which acts as a contaminant when combined with other substances. Adulterants are added to pure substances to extend the quantity while reducing the quality. From the NIR spectra of oils, PCA models were built which were able to predict some components despite of activate method like GC-MS, GC-IRMS for identification of adulteration.

So there the exactly adulterations happens but however, the NIR representation using a present approach could be able to identify the adulteration. So, this can be successfully used for the fields. This would be a very rapid and non-destructive technique for the identification of essential oil through FT-NIR.

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