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A Review on Role of Chemical and Biological Markers in Standardization of Herbal Product Pippermint

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ABSTRACT:- In the study of pippermint oil found that which chemical constituent are present and what is there medicinal uses. The term "herbal drug" refers to a plant or part of a plant that has been converted into phytopharmaceuticals through simple processes such as collection or harvesting, drying, and storage. Herbal medicines are extremely important in today's world. Standardisation of herbal takes place to avoid adulteration and substitution. The quality and purity of herbal drugs were confirmed by standardisation. HPLC, HPTLC, TLC, GC-MS, LC-MS, and other analytical techniques are used to standardise herbal drugs. The condition of a drug is determined by its collection, storage, and processing. Identity, purity, and active ingredient content are three important pharmacopoeia definitions. The WHO publishes guidelines for the evaluation of herbal drugs. 1) Evaluation of quality control 2) evaluation of safety 3) evaluation of toxicity 4) Evaluation of stability. There are various herbal drug evaluation parameters. A) Physical Analysis B) Chemical Analysis C) Morphological Analysis D) Analytical Evaluation, and so on. Zandu Balm, Chyawanprash, herbal shampoo, skin care products, ointment, oil, liquid extract, and other herbal medicine preparations are available in the market. Herbal medicine is used by approximately 80% of the population today. "The important thing about herbal drugs is that they always produce harmless benefits rather than harmful effects." On the basis of use it becomes highly used herb product which are use for therapeutic effect and we also found that how much essential standardization of herbal product to improve the quality of the herbal product in these article also mention that which regulatory are control its quality and which test are perform during and after finished product.

Keywords: Standardization, Herbal drug, WHO, Evaluation method , Quality control, PEO,IBS.

I. INTRODUCTION: -

Herbs are used from long time period as a medicinal purpose. So it's known as traditional medicine due to their medicinal properties. The constantly increase the demand of herbal medicine in globally and the rapid expansion of the global market for herbal product required to check there safety and quality of herbal material and there finished herbal products has become a major used for health authorities, pharmaceutical industries and the public. The safety and efficacy of herbal medicine are depend on their quality and there dose quantity. The identification, quality, and purity of herbal medicine medications are confirmed through standardization.

Standardization of herbal formulation is essential in order to assess of quality drugs based on the concentration of their active principles, physical, chemical, phyto-chemical, standardization and in-vitro, in-vivo parameters. Now days to maintain the standardization and the quality evaluation of herbal product by using the Organolaptic properties, Botanical properties, Physical properties, Chemical properties, Biological properties of the herbal drugs and product.

- **Organolaptic Properties:-** Organolatic properties refers to evaluation by using the sensory organs like skin, eye, tongue, nose, and ear.
- **Botanical Properties:-** On the basis of their taxonomy plant are categorized such as Class, Order, Family, Genus, and Species and the

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nature of plant such as flavonoid contain or alkaloids.

- **Physical Properties:-** Physical parameters also include the color, odor, and the appearance of the product, clarity, viscosity, moisture, pH, disintegration time, friability, hardness, flow ability, ash value, and others physical value are consider.
- **Chemical Properties:-** The chemical parameter are generally perform to know about the quality and the quantity of the active pharmaceutical ingredient and separate out them. It include qualitative, quantitative and chromatography analytical part. Chromatography analysis is done by using TLC, HPLC, HPTLC, GC, UV, GC-MS and fluorimetry technique.
- **Biological Properties:-** In biological parameter we discuss about the pharmacological effect, therapeutic properties, toxicological effect, microbial contamination, antagonistic, and the other effect such as systems of biological approach.

Standardization is a not easy process to perform in it considers numerous factors which effect the bio efficacy and reproducible therapeutic effect. To achieve high quality oriented herbal product we should taken right form of plant with proper identification, season and area of collection and their extraction and purification process and rationalizing the combination in the case of poly herbal drugs.

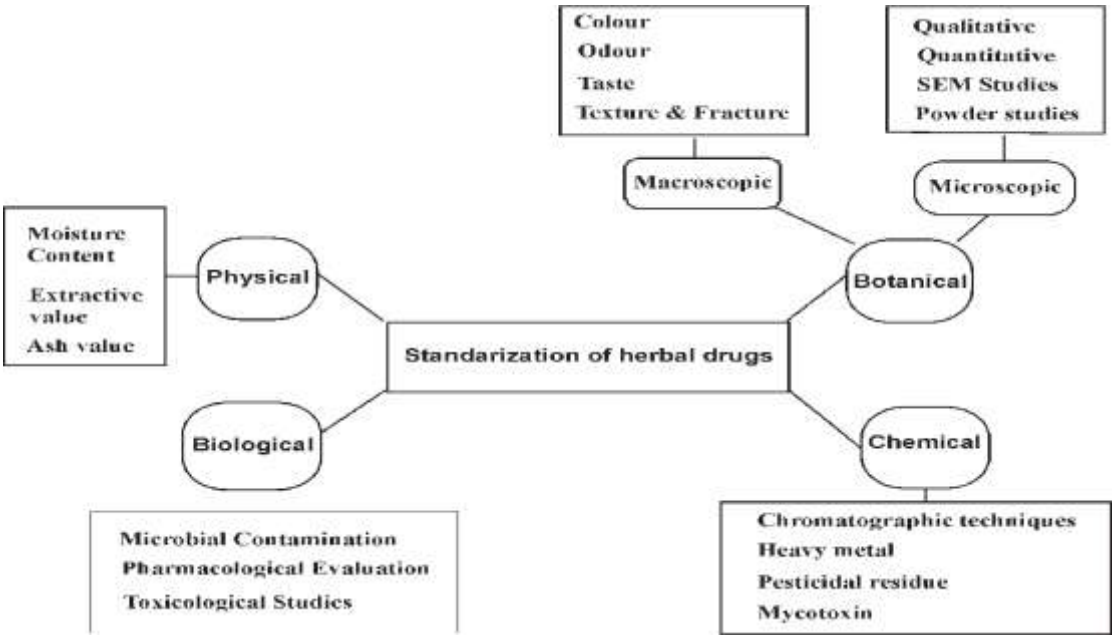

Figure 1: A schematic representation of herbal drug standardization

The standardization of crude drug materials includes many steps "each and every steps should be authenticated" to improve the quality and avoid to loss of materials.

a) Stage of collection.
b) Parts of the collected plant.
c) Regional status.
d) Botanical identity like phyto morphology, microscopical and histological analysis.

Various histological parameter studies are:-

i. Leaf constant: - Palisade ratio, Vein islet number, Vein termination, Stomatal number and Stomatal index.
ii. Trichomes.
iii. Stomata.
iv. Quantitative microscopy.
v. Taxonomical identity.
vi. Foreign matter.
vii. Organoleptic evaluation.
viii. Ash values and extractive values.
ix. Moisture content determination.
x. Chromatographic and spectroscopic evaluation.
xi. Heavy metal determination.
xii. Pesticide residue.
xiii. Microbial contamination.

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xiv. Radioactive contamination.

In general, the herbal formulation can be standardized schematically in order to formulate the medicament using raw materials collected from different localities and the comparative chemical efficacy of different formulation batches will be observed. The preparations with the highest clinical efficacy will be chosen. After checking all of the routine physical, chemical, and pharmacological parameters for all batches in order to select the final finished product and validate the entire manufacturing process.

Guidelines for Herbal Drug Standardization:-

According to WHO (1996a and b, 1992), standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects such as selection and handling of crude material, safety, efficacy, and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumers, and product promotion.

The WHO guidelines can be summarised as follows:

i. Reference to the drug's identity. Botanical evaluation includes sensory characteristics, foreign organic matter, microscopy, histological and histochemical evaluation, quantitative measurements, and so on.

ii. Refers to the drug's physicochemical properties. Chromatographic fingerprints, ash values, extractive values, moisture content, volatile oil and alkaloidal assays, quantitative estimation protocols, and so on.

iii. Information on pharmacological parameters, biological activity profiles, bitterness values, hemolytic index, astringency, swelling factor, foaming index, and so on.

iv. Toxicology information- pesticide residues, heavy metals, microbial contamination such as total viable count, pathogens such as E. coli, Salmonella, P. aeruginosa, S. aureus, Enterobacteria, and so on.

v. Contamination by microorganisms.

vi. Contamination with radioactive materials.

Who Guidelines for Monograph Title:-

Botanical: - Sensory evaluation, Foreign matter, Microscopy measurement.

Physicochemical TLC: - Ash, Extractable matter, Water content and volatile matter, Volatile oils.

Pharmacological: - Bitterness value, Haemolytic activity, Astringency, Sterling index, Foaming index.

Toxicological: - Pesticide residue, Arsenic, Metals.

Microbial contamination: - Total viable count, Pathogens, Aflatoxins, Radioactive contamination.

Standardization of Herbal Drug/Products:- Due to increased demand for medicinal plants, commercial production of herbal medicines and their trade is the fastest growing sector of industry today; the supply line is negatively impacted, resulting in adulteration and substitution for genuine drugs.

- **Fluorescence quenching**: When a plant extract is spotted on a fluorescent silica gel layer and exposed to UV light, it appears as a spot on a fluorescent background, causing quenching, and the intensity of the quenching is directly proportional to the concentration of the extract. For fluorescence quenching, a silica gel GF plate was used as an adsorbent. Hexane, toluene, ether, ethyl acetate, butanol, methanol, and water were used as solvents.
- **Use of fingerprinting and marker compounds for identification and standardization of botanical drugs**: Chemical and chromatographic techniques can be used to help identify a herbal material or extract. For fingerprinting, chromatographic techniques such as HPLC, TLC, GC, and capillary electrophoresis, as well as spectroscopic methods such as IR, NMR, and UV, can be used. DNA fingerprinting has been widely used in many species, for example, DNA fingerprinting of **Piperita** species and their adulterants. Marker compounds can be used to assist in the identification of herbal materials, to set specifications for raw materials, to standardize botanical preparations during all aspects of manufacturing processes, and to obtain stability profiles.
- **Thin layer chromatographic determination of Menthol in an herbal medicinal product containing MENTHA PIPERITA extract**: When the retention factor (Rf = 0.25) of the standard solution spot was compared to the retention factors of the samples, it was discovered that all samples contained menthol, with M1 and M4 containing the most. Menthyl acetate was identified in all samples, with M4 and M5 having the highest concentrations. The chromatographic profiles of the five samples were compared, and M1 and M2 were nearly

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identical in terms of the number of spots, color, and size. M3 and M4 samples showed a spot just below Rf = 0.5, while M5 showed a yellowish orange, not intense, spot just below the blue-violet spot located at Rf 0.79, indicating that this sample contains a compound not found in other studied oils.

Need of Standardization :

- The need for standardization of herbal medicine is important to achieve the potential quality and stability of the product.
- If the quality and stability of the product is assured, the best quality of active ingredient has been used. But, the principle of standardization is not stated in the pharmacopoeia.
- Absence of quality standards has admitted an adverse effect which may lead to death.
- To evaluate the parameters for standardization, specific devices are required as per GMP acquisition. Several factors like bio efficacy, reproducible therapeutic effects influence the standardization of herbal medicine.
- The main factor is adulteration of herbal ingredients which can be done intentionally or unintentionally such as lack of storage, mixing of one ingredient with another, same name of herb or substitution with excipient material.
- In the manufacture of allopathy products, herbal extracts are used as excipients, standardization is an essential procedure to be followed to test their bioactivity.

➢ **Advantages of herbal medicine:**

- Low/minimal cost
- Potency and efficiency
- Increased resistance
- More protection
- Fewer side effects
- Full accessibility
- Recyclable

➢ **Disadvantages of herbal medicine :**

- Cannot cure Quick Sickness and Accidents
- Risks of self-medication
- Complexity of standardization.

MARKERS IN HERBAL PRODUCT: - Markers are reference materials that are chemically determined elements of an herbal item. They might or might not participate in the therapy process. Nevertheless, even when they support the healing activity, there may not be proof that they are primarily to blame for the clinical effectiveness. Markers are categorized in to two classes;

- **Chemical markers:-** Chemical markers are defined by the European Medicines Agency (EMEA) as chemically defined constituents or groups of constituents of a herbal medicinal product that are of interest for quality control purposes regardless of whether they have any therapeutic activity. Chemical markers are biochemical constituents that include primary and secondary metabolites as well as other macromolecules such as nucleic acids. The concentration of a chemical marker can indicate the quality of a herbal medicine. Many research areas benefit from the study of chemical markers, including the authentication of genuine species, the search for new resources or substitutes for raw materials, the optimisation of extraction and purification methods, structure elucidation, and purity determination. Systematic research using chemical markers may lead to drug discovery and development. The EMEA divides chemical markers into two categories: analytical markers and active markers.

i. Analytical markers- are the constituents or groups of constituents that serve solely for analytical purposes.
ii. Active markers- are the constituents or groups of constituents that contribute to therapeutic activities.

Applications of chemical markers:-

- Adulterant identification- An adulterant of gamboges was distinguished from the authentic sample using an HPLC-UV method that employed eight caged xanthones as chemical markers.
- Differentiation of herbal medicines from a variety of sources.
- Determination of the best harvesting season.
- Confirmation of collection locations
- Evaluation of processing method.
- Herb quality assessment
- Identification and quantitative determination of proprietary products • Stability test of proprietary products- The stability test is used to evaluate product quality over time and determine recommended shelf life.
- Diagnosis of herbal intoxication- Toxic components may be used as chemical markers in screening methods, such as rapid diagnosis of acute hidden aconite poisoning in urine samples by HPLC-MS.

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- Lead compounds for new drug discovery- The components responsible for the therapeutic effects may be investigated as lead compounds for new drug discovery.

- **DNA Markers / Biological Markers: -** DNA markers are trustworthy for revealing polymorphisms since each species' genetic makeup is distinct and unaffected by ageing, physiological changes, or environmental variables. As DNA may be extracted from both fresh and dried organic plant tissue, detection is not limited by the sample's physical shape.

A biomarker, also known as a biological marker, is a quantifiable indicator of a biological state or condition. Biomarkers are frequently measured and evaluated in blood, urine, or soft tissues in order to investigate normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers are used in a wide range of scientific disciplines.

Variety of biomarkers

Nowadays, a wide variety of biomarkers are used. Each biological system (such as the cardiovascular, metabolic, or immune systems) has its own set of biomarkers. Many of these biomarkers are relatively simple to measure and are included in routine medical exams.

A general health check, for example, may include measurements of blood pressure, heart rate, cholesterol, triglycerides, and fasting glucose levels. Body measurements such as weight, BMI, and waist-to-hip ratio are commonly used to diagnose conditions such as obesity and metabolic disorders.

Characteristics of an ideal biomarker :-

A perfect biomarker has certain characteristics that make it suitable for testing a specific disease condition. An ideal marker should have the following characteristics:

- ✓ Measurement is simple and safe.
- ✓ It is less expensive to follow up.
- ✓ Modifiable through treatment.
- ✓ The same across gender and ethnic groups.

Biomarkers as health and disease predictors

Biomarkers are used to predict serious illnesses such as diabetes and cardiovascular disease. Each biomarker indicates whether a disease or health state exists and can be combined to provide a detailed picture of how healthy a person is and whether or not a diagnosis is required.

Steps involve in processing of herbal drugs: - First we have need to identify the things we have required and which chemical need to cure and prevent the disease according there nature they are categories and flowing steps are involve in herbal extraction.

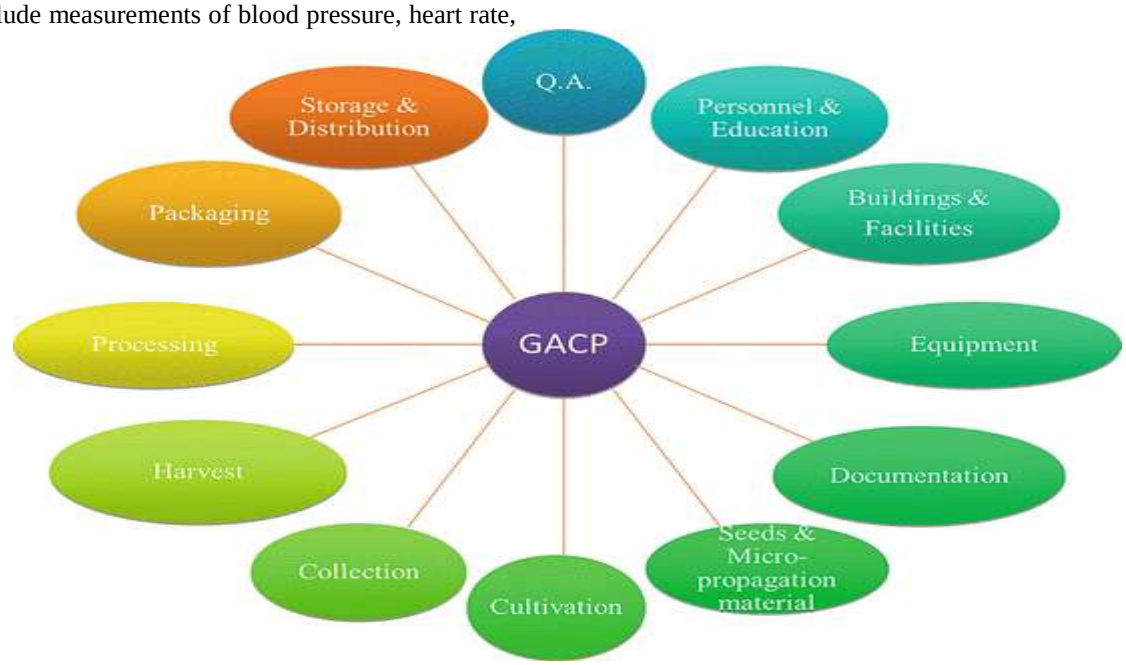

Figure-02: Content of GACP

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a) **Selection of herb: -** The spices of botanical variety selected for cultivation should be the same as the specify in the official pharmacopoeia or National document. In case of new variety of medicinal plant should be identified and documented before the cultivation.

b) **Identification of herbal material:-** Identification is perform of the basis of Botanical identification, Specimen and Seeds and other propagations material.

- **Botanical Identification:-** The species , sub-species , genus, variety and other part of the plant form cultivations should be verified from a qualified botanist institute and the record of herbariums.
- **Specimen:-** In case of new plant with medical properties and identify is unknown a specimen of the plant should be submitted to the National Harberium for identification and documentation.
- **Seed and other propagation material:-** The supplier of seeds and other propagation material should be specified information relating to identifying quality as well as breeding history. The seeds and propagation material should be free from contamination and disease in order to promote healthy plant growth.

c) **Cultivation of medicinal herbs plant:-** Cultivation of medicinal plant required intensive care and management as various factor such as environment, soil, irrigation, pest, etc. play a important role. Good Agriculture Practices In Cultivation (GACP), Conservation Agriculture (CA). This means to improve conservation and make use effective of natural resources.

d) **Collection of herbs:-** For the collection of herbal medicinal plant a proper time should be selected herbs are selected for the collection at a stage when they yield maximum amount of chemical constituent, skilled labours should be engaged as they are trained to identified and select the herbs at a proper stage.

e) **Processing of herbal raw material:-** Processing of herbal raw material involves various step from which the crude drug under goes after the harvesting.

Figure-03: 5 Primary Steps in Herbal Processing

- **Primary processing :-** It include simple processing by which the herbs are prepared like sorting of different part, Garbling, cleaning, Drying etc.

i. **Garbling :-** This process help in insuring the purity and clean of the harvest material dirt like soil dust, impurities like insect deal tissue and residual non medicinal

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plant and separated from raw material. The process depend on the part of plant to be prepared the process may involved procedure such as removing of dirt, foreign substance, discarding, damage part, peeling of the bark, sieving, treaming, Removal of hair from root etc..

ii. **Washing:-** After garbling the herbal raw material should be clean well to remove the trash of reaming soil, dirt and other impurities from surface. The root rhizome and tubers are washes with clean water during the washing process scraping and brushing may be necessary.

iii. **Parboiling:-** After washing herbal raw material need to under goes parboiling process in which they are put in boiling water in short time period. This may helps in improving the storage like of the raw material and preventing contamination.

iv. **Leaching:-** some impurities can be removed by subjective the plant material under running water called leaching it used to remove impurities from the surface of the herb part.

v. **Drying:-** Drying process occurs naturally and artificial in which herbal plant and there part get dried. Naturally drying process we used sun light and the shade of sun light in the presence of air. In the artificial drying process we used the different types of the equipment such as Tray dryer and vacuum dryer.

- **Secondary processing:-** It included technique such as removal of foreign substance, prevention of microbial, enhancing efficacy of drugs, reducing the toxicity, extraction using suitable solvent, concentration and drying of extract.

1. **Cutting / Sectioning and communication:-** The herbal material are process by cutting and sectioning in to smaller size which are in convenient for storage as well as extraction. Various size can be obtain depending on the part of herbs and extraction method use it may be small particles cores powder and fine powder.
2. **Aging / Sweating:-** Aging refer to storing the raw material are processed by specific time after harvesting. It is generally done under sun or shade for up to a years. During the process of aging excessive water is evaporated and enzymatic reaction may occurs to alter the chemical composition of herbal material.
3. **Baking / Roasting:-** It is the process of drugs heating where the herbal materials heated in the oven. The temperature of heating and duration of baking or roasting vary from one herbal material to another until the drug developed the specific color.
4. **Boiling/ Streaming:-**In the boiling process that drugs is cooked in water or any other liquid solvent such as milk, wine or animal urine.
5. **Stir/ Frying:-** In this process the herbal are put in spot of frying pan and continuously stirring for a specific period under heat until the external color change, carbonized. To facilitate uniform heating the drug materials can be add mixed withstand talc clay.
6. **Fumigation:-** Sometimes the harvesting raw materials are subjects to fumes. Fumigation with sulpher dioxide is commonly employed for some medicinal herbs for the purpose of preserving, color, improved appearance, bleaching and preventing the growth of insect and mould.
7. **Extraction of herbal material:-** Extraction is the process of separation in which the chemical constituent present in plant and tissue are removed by using selective solvent which is known as "Menestrum".

- **Steps of herbal extraction:-**

1. **Infusion:-** It is liquid preparation obtained by extracting herbal maerial with either cold or hot water without boiling.
2. **Decoction:-** It is also liquid preparation obtain by boiling the herbal material with water.
3. **Fluid extraction:-** It is liquid preparation obtained maceration and percolation of herbal material the ratio will be one part liquid contain one part of herb.
4. **Tincture:-** It is a dilute alcoholic extract of herbal material with 5-10 part of solvent.
5. **Powder extract: -** It is a form of herbal preparation which is processed into dry granulated or powder material.

Advantages of DNA markers over chemical markers:-

❖ DNA marker analysis is more reliable than chemical marker analysis.

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- They provide an efficient and accurate method of testing the authenticity of hundreds of samples at the same time, whereas traditional chemical-based methods typically take several days to verify.
- Any compound that acts as a chemical marker must be unique to that particular species.
- Not all plants have a distinct chemical compound, and the same chemical marker is used to identify two or more plants.
- Furthermore, the concentration of secondary metabolites and other biochemical markers may change due to environmental factors, making correct botanical identification difficult, whereas genetic markers are unique and are unaffected by age, physiological conditions, or environmental factors.

Selection of herbal plant:- For the study of the biological and chemical markers of herbal plant and maintain there standardization in the herbal product we are chosen the peppermint which are commonly use in the Indian kitchen as the spices.

Introduction:- Peppermint, also known as mentha piperta, is a common herb grown in Europe and North America. Since time immemorial, peppermint oil has been used to treat headaches, common colds, neuralgia, and other ailments. This review focuses on peppermint oil's antispasmodic properties. Peppermint oil has a fresh, menthol aroma, is clear to pale yellow in colour, and has a watery viscosity. India is the world's largest mint oil producer and exporter. Mint oil, as well as its constituents and derivatives, is used in the food, pharmaceutical, perfumery, and flavouring industries. Its main constituent, menthol, is used in the production of lozenges, toothpastes, pain relievers, cold compresses, Dabur Pudin Hara, and other products. Mint oil is made from the leaves of the plant Mentha. The oil is used to treat stomach disorders such as indigestion, gas, acidity, and so on. It is the main ingredient of ayurvedic medicines like Dabur's 'Pudin Hara'. The oil is a natural source of menthol, which is found in cough drops and ointments such as Vicks Vaporub. The capsules were found to be beneficial in terms of decreasing total procedure time, reducing colonic spasm, increasing endoscopist satisfaction, and reducing pain in patients during colonoscopy. Peppermint is consumed as a tea, tincture, oil, or extract and applied topically as a rub or liniment. It is frequently used in paediatric patients to treat abdominal pain, irritable bowel syndrome, nausea, and the symptoms of coughs and colds. Historical and Popular Applications Mentha piperita, the Latin name for peppermint, is derived from the Greek Mintha, the name of a mythical nymph thought to have metamorphosed into the plant, and the Latin piper, which means pepper. It is one of the world's oldest medicinal herbs, with applications in both Eastern and Western cultures. The herb was used in cooking and medicine by the Greek, Roman, and Egyptian cultures. Peppermint is now one of the most economically important aromatic and medicinal crops grown in the United States. The global production of peppermint oil is approximately 8000 tonnes per year.

Figure-04: Benefit of Peppermint

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Peppermint leaf and oil are used in folk medicine, flavouring agents, cosmetics, and pharmaceutical products all over the world. Peppermint oil is the most commonly used of all volatile oils. Peppermint is regarded by herbalists as an astringent, antiseptic, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mildly bitter, analgesic, anticatarrhal, antimicrobial, rubefacient, stimulant, and emmenagogue. It is also considered to be antiseptic. . Inhaling peppermint oil vapour relieved respiratory congestion. Tea infused with peppermint oil was used to treat coughs, bronchitis, and other respiratory ailments. Inflammation of the mouth and throat. This method is still used. It has traditionally been used to treat colic in infants, flatulence, diarrhoea, indigestion, nausea and vomiting, morning sickness and anorexia, as well as as a spasmolytic to reduce gas and cramping. Peppermint oil is currently used to treat IBS, Crohn's disease, ulcerative colitis, gallbladder and biliary tract disorders, and liver problems. Menstrual cramps can be relieved with peppermint oil. Externally, peppermint oil is used to treat neuralgia, myalgia, headaches, migraines, and chicken pox.

Extraction and isolation:- PEO is a complex mixture of biologically active secondary metabolites such as menthol, menthone, neomenthol, iso-menthone, and so on. Because the phytochemical property of PEO is quite specific and is associated with the biological activities, pharmacological effects, and applications of PEO, extraction of Mentha and isolation of PEO are important.

- **Extraction of Mentha:-** Mentha is grown from rhizomes and harvested at the start of flowering for maximum yields of high-quality essential oils. Extraction of bioactive components from plants is always a challenge; several different methods can be used to extract essential oils with specific qualities of active compounds from plant materials. Steam distillation, hydrogenation distillation, microwave-assisted extraction, supercritical fluid extraction, ultrasonic-assisted extraction, and countercurrent extraction are some methods for extracting active compounds from essential oils. The rate of recovery and chemical composition of essential oil components in plants, particularly medicinal plants, are determined by the original plant raw materials and the extraction technology used. Mentha is harvested, sun-dried, and ground in a blender. Following sieving, the average particle size and particle size range were determined, and the moisture content in the plant material was determined gravimetrically by drying at 105 °C until constant weight was achieved. By reducing the average particle size of Mentha to 0.224 mm and increasing the moisture content to 8.77%, mass transfer limitations can be reduced and extraction improved. The byproducts are then collected in barrels for steam distillation. Essential oil components are typically extracted using hydro- distillation and ethanol solid-liquid extraction, and further rectification with fractional distillation technology can improve the aromatic odour of the oil. Mentha's main chemical components are menthol, menthone, neomenthol, and iso-menthone. Hydro-distillation, steam distillation, and microwave distillation are three common extraction methods for essential oil components. The more traditional methods for extracting essential oils from Mentha include standard Soxhlet and supercritical fluid extraction, microwave-assisted extraction, and ultrasonic-assisted extraction. PEO yield can be increased by adjusting different power settings. A possible alternative method is conventional hydrodistillation, a traditional method proven to separate the essential oil components of Mentha. Microwave-assisted hydrodistillation can also be used to separate PEO. Although hydrodistillation and steam distillation are superior in terms of extraction yield and quantification of major compounds, microwave-assisted distillation can reduce extraction time from 180 minutes to 30 minutes.
- **Isolation of PEO components:-** PEO contains twenty-six different compounds. Menthol, menthone, neomenthol, and iso-menthone are the chemical components that are currently thought to be important and effective in PEO. The purification of these chemical constituents is crucial for pharmacological research. The range of different stationary phases, the thickness of different films, and the length of the column all influence component isolation in gas chromatography (GC). Mass spectrometry (MS) improves the ability to distinguish between compounds, and overlapping components may necessitate spectroscopy to aid in MS characterization. It is self-evident in all cases that complete separation of the components simplifies the downstream identification process. The search for improved separation capability continues, particularly in

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the field of multidimensional gas chromatography.

- **Isolation of menthol:-** Menthol (also known as menthol camphor) is a cyclic monoterpene alcohol that is found in large quantities in Mentha. Menthol, in conjunction with menthone, iso-menthone, and other compounds, imparts a cool minty scent to plants. Menthol is biosynthesized in plants via an 8-step secondary metabolism pathway. PEO contains high concentrations of menthol and menthone, and the former is typically obtained by steam distilling Mentha sylvestris. The essential oil components were separated on a silica gel column using gradient elution with ethane followed by ethane/AcOEt chromatography, menthol components were identified using high-performance thin-layer chromatography, and menthol was separated using nuclear magnetic resonance (NMR) analysis. After the esterification reaction, menthol can also be isolated by vacuum distillation using a micro distillation apparatus.
- **Isolation of menthone:-** By injecting PEO into the precap-GC system, a mixture containing menthone can be obtained. Menthone can be isolated by repeating the preceding procedure and eluting with chloroform. Furthermore, after separating and purifying PEO with the Varian 3300 Gas Chromatograph, menthone can be isolated using an HP-InnoWax capillary column or an HP-5 capillary column in a GC-MS system.
- **Isolation of neomenthol:-** Neomenthol, a cyclic monoterpene, is a stereoisomer of menthol. Menthone can be broken down into epimer alcohols such as iso-menthol and neomenthol. In flowering Mentha leaf discs, some menthol was converted to menthyl acetate, while the majority of menthol and neomenthol were converted to neomenthyl -D-glucoside. The major monoterpene component 1-menthone is converted to iso-menthol in flowering Mentha. Menthone is converted to menthol and menthyl acetate in Mentha leaf discs, and a significant portion of 1-menthol can be converted to d-neomenthyl--D glucoside, according to a study of menthone metabolism in Mentha leaf discs.
- **Isolation of iso-menthone:-** The main compounds in Mentha leaves are menthone and iso-menthone, which are highly synthesised during the filling of the epidermal oil glands in the rapidly growing young Mentha. A small amount of neomenthyl acetate and menthol glycosides are produced concurrently, resulting in the distinct metabolic profile of the epimeric reduction products in PEO. The iso-menthone component can be obtained by drying on anhydrous magnesium sulphate and concentrating after 2 hours of steam micro-distillation and simultaneous cyclohexane extraction. According to reports, iso-menthone can be obtained directly through water distillation.
- **Chemical Constituents of Peppermint:-** Menthol is the main component of peppermint essential oil and is primarily responsible for the agent's anti-spasmodic properties. Peppermint oil contains limonene (1.0-5.0%), cineole (3.5-14.0%), menthone (14.0-32.0%), menthofuran (1.0-9.0%), isomenthone (1.5-10.0%), menthyl acetate (2.8-10.0%), isopulegol (0.2%), menthol (55.0%), pulegone (4.0%), and carvone (max. 1.0%).

Biological activities of PEO:- PEO is primarily metabolised in the body through its reaction with glucuronic acid and excreted in urine or faeces. PEO has anti-inflammatory, antibacterial, antiviral, scolicidal, immunomodulatory, antitumor, neuroprotective, antifatigue, and antioxidant properties, according to a growing body of evidence.

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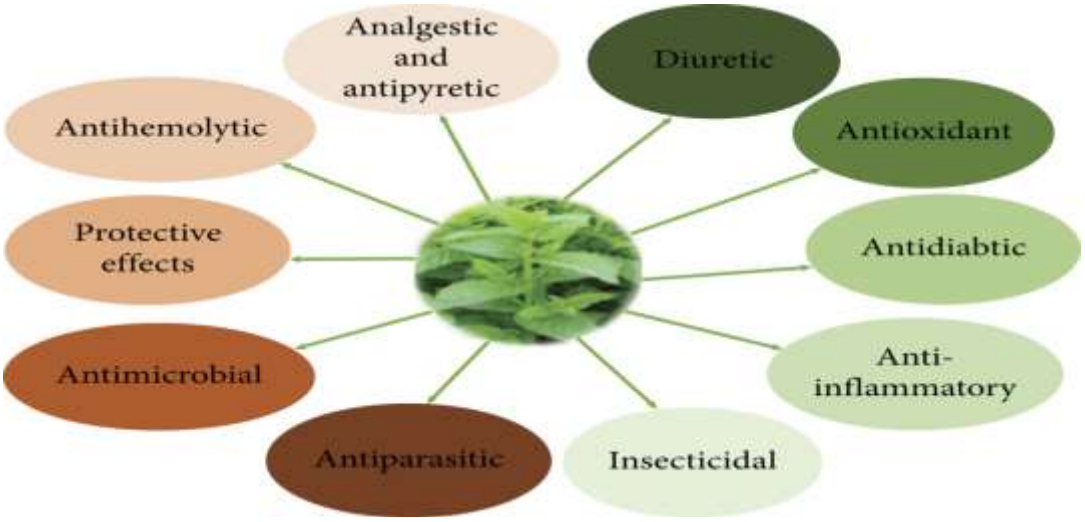

Figure 05: Biological and pharmacological properties of Mentha spicata.

- **Anti-inflammatory activity:-** Menthol is a TRPM8 (transient receptor potential melastatin 8) channel agonist. Menthol can activate the TRPM8 channel in irritable bowel syndrome (IBS), inhibiting the chemical and mechanosensory responses of nociceptive TRP channels and decreasing the release of proinflammatory mediators from nerve endings. PEO can control IBS by suppressing the expression of pro-inflammatory cytokines while increasing the levels of anti-inflammatory cytokines. PEO can prevent xylene-induced intestinal inflammation in mice and acetic acid-induced colitis in rats when taken orally. Menthol's gastroprotective effect is primarily due to its anti-inflammatory activity, which is linked to prostaglandin E2 (PGE2) production, K+-ATP channel activation, and antisecretory effect. PEO has been shown to effectively reduce excessive inflammation and subsequent atopic dermatitis-like lesions by inhibiting the ERK-NF-B pathway Menthol can also help to reduce inflammation and oxidative stress. PEO inhibits carbachol-induced muscle contraction involving the autonomic ganglia and has anti-inflammatory and analgesic properties, particularly in respiratory disease. PEO has also been shown to have anti-gout and analgesic properties, as well as strong anti-inflammatory activity in croton oil-induced mouse ear edoema by inhibiting NO and PGE2 production.
- **Antibacterial activity:-** PEO has powerful antibacterial activity, according to mounting evidence. Staphylococcus aureus is a superbug of the Staphylococcus genus that has become a troublesome bacterial strain in modern invasive medication. PEO inhibits the growth of human pathogenic bacteria such as Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis, Escherichia coli, Neisseria gonorrhoeae, and Pseudomonas aeruginosa. PEO has a strong antibacterial effect on Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, and Escherichia coli as determined by broth microdilution and disc diffusion methods. PEO has been shown in studies to suppress Streptococcus pneumonia, Salmonella enteritidis, and Salmonella typhi, as well as various skin moulds and Candida albicans. PEO can also stop the growth of fungal strains like Aspergillus niger and Vibrio. Furthermore, PEO can inhibit worm movement and kill adult Salmonella mansoni and schistosomes [34]. The menthol in PEO has been shown to reduce Gram-negative pathogens' quorum sensing activity. In several ways, PEO can be effective against Chlamydia trachomatis. For starters, PEO inhibits Chlamydia trachomatis and prevents it from entering host cells. Second, PEO inhibits Chlamydia trachomatis replication, reducing infectivity and thus infection progression. The combination of PEO and erythromycin can reduce Chlamydia trachomatis replication and reduce antibiotic dose. PEO has the ability to reduce the number of Clostridium nucleatum, plankton, and biofilm-embedded cells, indicating that it is an

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effective therapeutic agent for oral disease. Furthermore, by modulating the fungal biota, PEO can alleviate abdominal pain in IBS-like rats. PEO can synergize with other agents to produce synergistic antibacterial effects against Gram-positive and Gram-negative microorganisms, according to research. When PEO is combined with gentamicin, the amount of gentamicin needed to kill bacterial strains is significantly reduced. PEO has been shown to have a strong bactericidal effect on Escherichia coli and to destroy the integrity of the bacterial cell membrane. Furthermore, PEO can inhibit the formation of bacterial biofilm and reduce biofilm activity and bacterial adhesion.

- **Antiviral and scolicidal activity:-** Virus particle fusion experiments revealed that after the particles were treated with PEO, the entry of HIV-1 into cells was significantly reduced and the virus's replication efficiency could be inhibited at an early stage of virus infection. PEO has been shown in studies to rapidly reduce the infectivity of HIV-1 virions at non-cytotoxic concentrations. The human respiratory syncytial virus (RSV) is a syncytial virus that infects the lungs. It has been demonstrated that PEO has a high level of activity against RSV. The free virus is extremely sensitive to PEO. PEO appears to inhibit herpes simplex virus (HSV) entry into host cells. PEO reduces viral infectivity, possibly through direct interactions with the viral envelope and glycoprotein. Furthermore, PEO may disrupt the later stages of the HSV-1 life cycle, resulting in viral replication suppression. It has been reported that Iranian Mentha species have potent scolicidal activity against hydatid cyst protoscolices. Mentha aquatica 200 mg/ml methanol extract has the highest scolicidal activity (99.54%) after 30 minutes of exposure, implying that antiparasitic agents may be developed from Mentha.
- **Immunomodulatory activity:-** Phagocytes, particularly macrophages, are the first-line innate immune system effectors, eliminating pathogenic microorganisms that invade the host. The recognition of pathogen-associated molecular patterns (PAMP) is associated with macrophage activation. PEO was discovered to modulate immune activity via phagocytosis in an in vitro study. PEO can also inhibit airway epithelial hyperplasia, collagen deposition, and goblet cell activation in asthmatic mice by decreasing IL-6 levels through regulation of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) phosphorylation.
- **Antitumor activity:-** Cell renewal and proliferation are balanced by cell death, such as apoptosis, under normal conditions. During tumour development, this balance is disrupted, allowing cancer cells to proliferate. PEO has weak to moderate antiproliferative properties and may inhibit neuroblastoma growth by down-regulating the expression of some oncogenes, including epidermal growth factor receptor. PEO significantly inhibits the proliferation of colorectal cancer cells SW480 by inducing apoptosis and arresting the cell cycle at the G1/G0 and G2/M stages. PEO's inhibitory effect on cancer cells was greatly enhanced when combined with sage (Salvia officinalis L.). According to another study, PEO may inhibit gene expression in cancer cells by suppressing the activity of Topoisomerase I.
- **Neuroprotective effect:-** Menthol in PEO increases phasic and tonic -aminobutyric acid (GABA) A receptor-mediated currents in neurons throughout the periaqueductal grey (PAG). Menthol appears to inhibit tonic current via extrasynaptic GABAA receptors lacking the delta subunit. Menthol-induced enhancement of GABAergic inhibition within the PAG has the potential to modulate this brain region's analgesic and anxiolytic functions. Menthol increases the decay time of spontaneous inhibitory postsynaptic currents (IPSC) in PAG neurons while having no effect on IPSC dynamics in hippocampal CA1 pyramidal neurons. Furthermore, menthol has concentration-dependent GABAA and nicotinic receptor binding properties as well as significant inhibition of acetylcholinesterase, potentially increasing acetylcholine synaptic availability. PEO can increase Ca^{2+} concentration and prolong the depolarization response, providing neuroprotection in CAD cells under oxidative stress. Menthol also has a significant effect on sensory ganglion neuron subpopulations. Menthol increases the sensation of cooling and improves the response of isolated sensory neurons to temperature cooling. The results indicate that menthol can suppress cold-sensing trigeminal neurons while having little effect on oral trigeminal thermal sensors. Because PEO acts as a

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smooth muscle relaxant that reverses acetylcholine-induced contraction through calcium channel blockade and antagonises serotonin-induced contraction, it may affect the enteric nervous system and interfere with gastrointestinal neuromotor function. by inhibiting contractility directly and causing circular smooth muscle relaxation in the colon. Topical stimulation of PEO, which can be applied to the skin over the inflamed muscle, inhibits nociceptive neurons in the skin and alleviates muscle pain by activating skin nociceptors in a rat model of inflammatory myalgia. The nasal-brain pathway is a potential route for drug delivery because it bypasses the blood-brain barrier, and nasal administration of PEO can increase bioavailability for the treatment of neurodegenerative disease.Furthermore, PEO can relieve bronchospasm by increasing nitric oxide production and regulating K+ channel opening.

- **Antifatigue activity:-** Physical fatigue is defined as the inability to maintain voluntary activities and is linked to physical deterioration. Exercising causes an abnormal accumulation of blood lactate and blood urea nitrogen, which can cause metabolic disruption and, eventually, fatigue. PEO peripheral injection promotes mouse walking behaviour. Menthol can stimulate the adrenal cortex to increase energy and lower blood lactate levels, which may increase cellular energy metabolism by stimulating the central nervous system. . In addition, PEO may increase lung capacity in healthy subjects, allowing more oxygen to reach the brain and effectively eliminating fatigue. Furthermore, PEO improves mental refreshment and increases body alertness. PEO can modulate the brain's olfactory pathway, alleviate anxiety, reduce pain and impulse, and improve sleep quality, all of which contribute to its antifatigue activity.
- **Antioxidant activity:-** ROS, such as superoxide, hydroxyl, and peroxy radicals, are important in the pathogenesis of many diseases, including neurodegenerative disease, cancer, cardiovascular disease, and inflammatory afflictions. Mentha species and PEO have antioxidant and free radical-scavenging properties. Neutrophil infiltration, free radical formation, and increased oxidative stress have all been identified as pathogenic factors in inflammatory bowel disease (IBD). Menthol in PEO reduces oxidative stress in colon tissue and lowers malondialdehyde levels and the end product of lipid peroxidation. Furthermore, due to its antioxidant activity, PEO reduces melanin synthesis in B16-F10 cells by decreasing the expression of microphthalmia-associated transcription factor (MITF), tyrosinase-related protein (TRP) 1, TRP-2, and tyrosinase.

Pharmacology and clinical action of Peppermint:- Menthol, menthone, cineol, and several other volatile oils are active constituents in peppermint oil, which is made by distilling the ground parts of the peppermint plant. In vitro studies show that peppermint oil can relax GI smooth muscle, possibly through an antagonistic effect on calcium channels in the gut. Peppermint oil has also been shown to relax the lower esophageal sphincter, resulting in gastroesophageal reflux. This discovery has resulted in the popularity of enteric-coated peppermint formulations, which bypass the upper GI tract unmetabolized, facilitating its effect in the lower GI tract while having no effect in the upper tract.

- **Irritable bowel syndrome: -** Peppermint oil has received the most attention in the treatment of IBS. Combinations of peppermint oil and other botanical medicines have also been investigated as potential treatments for non-ulcer dyspepsia. When used topically, the oil has also been used to treat tension headaches. Because peppermint oil has the potential to relax the lower esophageal sphincter, resulting in heartburn symptoms, most trials have used enteric-coated preparations. Although the results of studies on the use of this herb for the treatment of IBS symptoms have been mixed4,5, there appears to be a trend indicating mild effectiveness in the reduction of some IBS symptoms, particularly flatulence and abdominal pain and distension. A metaanalysis of five trials involving 175 patients found that peppermint oil had a statistically significant benefit over placebo in the treatment of IBS symptoms.
- **Reduction of colonic spasm during GI procedures:-** Because of its relaxing properties on smooth muscle, peppermint oil administered via enema has been studied in two trials as a way to reduce symptoms of gastrointestinal spasm during barium enema administration and possibly during colonoscopy.10,11 In a randomised controlled

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trial (RCT) of 383 patients undergoing barium enemas, 37 to 41 percent of those who received peppermint oil experienced a non-spasm examination, compared to 13.4 percent of those who received placebo (P.001).10 An RCT of 141 patients undergoing barium enemas found no residual spasm in 60% of the treated group versus 35% of the control group (P.001).

- **Non-ulcer dyspepsia:-** Several clinical trials have shown that a combination of enteric-coated peppermint oil and caraway oil can reduce symptoms of non-ulcer dyspepsia (e.g., fullness, bloating, gastrointestinal spasm),12,13 but the specific preparation used in these trials is not commercially available in the United States. A meta-analysis of several trials of Iberogast, a preparation containing peppermint and caraway oils as well as other herbal extracts, found it effective in the treatment of functional dyspepsia.14 This advantage may be due to the preparation's relaxing effect on the lower esophageal sphincter, which results in pressure equalisation between the stomach and the oesophagus as well as a reduced sensation of bloating and abdominal pressure. However, in patients who are predisposed to gastroesophageal reflux, this effect could theoretically result in reflux symptoms. Because multiple herbs were used in these trials, drawing definitive conclusions about the specific effects of peppermint in this condition is difficult.
- **Tension headache:-** Two studies have found that applying peppermint oil topically can help relieve tension headache symptoms.15,16 In one randomised controlled trial, 32 patients were tested using a variety of topical herbal preparations.15 Patients who used a peppermint and ethanol preparation had a significant analgesic effect when compared to those who received a placebo. A second randomised controlled trial comparing the efficacy of topical peppermint oil and acetaminophen on 164 headaches in 41 patients discovered that a 10% peppermint oil preparation significantly reduced headache intensity after 15 minutes.16 There was no statistically significant difference in efficacy between peppermint oil and paracetamol, and no side effects were reported.
- **Respiratory protection:-** Exposure to fine particulate matter (PM) is one of the risk factors for aggravated airway inflammation and pulmonary destruction in asthma. PEO nebulization was used to treat asthmatic mice caused by PM10. PEO reduces respiratory epithelial hyperplasia, collagen deposition, and goblet cell activation in asthmatic mice, with lower levels of IL-6 and pro-inflammatory T helper 2-specific cytokines, as well as down-regulation of JAK2 and STAT3 phosphorylation, implying that PEO relieves asthma by inhibiting IL-6/JAK2/STAT3 axis activity. PEO, a relaxant, may act as an antispasmodic agent, as PEO and its component pulegone are both effective against acetylcholine- and KCle-induced contraction of rat tracheal smooth muscle, without influencing the rate of contraction.The function of the ganglia and NO. Furthermore, PEO inhibits CaCl2-evoked contraction in depolarized rat trachea, indicating that extracellular calcium entry is hampered. Menthol has been shown to suppress the respiratory sensory irritation response to various cigarette smoke irritants, as evidenced by the fact that menthol, an agonist of the TRPM8 ion channel in cold-sensitive sensory neurons, can abolish the mouse irritation response to acrolein, an agonist of the transient receptor potential ankyrin 1. Furthermore, menthol can reduce sensitivity to acetic acid, cyclohexanone, and transient receptor potential cation channel subfamily V member 1 (TRPV1). These findings suggest that menthol is efficiently absorbed in the respiratory tract, resulting in a local concentration high enough to activate sensory TRP channels.
- **Hypoglycemic and hypolipidemic effects:-** Diabetes mellitus is a metabolic disorder that causes hyperglycemia, polyuria, polydipsia, and polyphagia, as well as potential complications such as retinopathy, nephropathy, neuropathy, ketoacidosis, and so on. PEO has been shown to lower blood glucose levels, increase insulin and C-peptide levels, and improve pancreatic beta cell structure, implying that PEO could be used as a hypoglycemic agent for diabetes mellitus and its complications. Furthermore, PEO can effectively resist hyperlipidemia by improving lipid metabolism, lowering serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol while increasing high-density lipoprotein cholesterol levels and decreasing atherosclerosis index in

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hyperlipidemic rats. PEO can also increase hepatic glutathione levels, improve liver function and antioxidant activity, and thus increase blood glucose uptake and utilisation, which may explain PEO's hypolipidemic and hypoglycemic effects.

- **Skin protection:-** PEO is commonly used to treat wounds, skin infections, inflammation, eczema, hives, psoriasis, scabies, and insect bites [80]. The terpenes in PEO may act as solubilizers across the epicuticle barrier, increasing drug distribution in the stratum corneum. PEO alters skin permeability and promotes hair growth by enhancing vascular formation in the dermal papilla of hair, which may aid in the early stages of hair growth, indicating a promising approach for the treatment of hair loss with PEO. PEO is said to have a very high sun protection factor (SPF) value because it can form an even and long-lasting sunscreen on skin and protect it from sun and wind drying. Chronic pruritus is defined as itching that lasts longer than 6 weeks and is severe enough to interfere with daily activities. Itching can be caused by a variety of skin conditions as well as non-dermatological conditions. Topical PEO has been shown to relieve itching sensations by activating A-delta fibres and k-opioid receptors, making it effective in the treatment of chronic pruritus. Furthermore, PEO can reduce pregnancy pruritus (PG) caused by hormonal changes. It is well understood that topical administration of PEO produces a long-lasting cooling effect on the skin against facial neuralgia, owing to spatial changes in cold receptor calcium channels and relaxation of pericranial muscle, resulting in cutaneous blood flow to the forehead.
- **Brain and nervous system protection:-** PEO is said to be beneficial to the brain and nervous system. PEO contains neuroactive menthol and menthone. These compounds can inhibit cholinesterase and bind to nicotine and GABAA receptors, increasing neuronal activity. The continuous administration of PEO greatly aids in the elimination of mental fatigue.

Analysis Method of Peppermint Oil:-Various techniques have been used by different researchers to analyse peppermint oil. Peppermint oil is chemically analysed to determine its constituents using solid-phase microextraction coupled with gas chromatography/mass spectrometry (SPME-GC/MS). More efficient methods for standardisation and quantification of biological active constituents are required to improve the efficacy, safety, and quality of commercially available herbal drugs. Because mint oil is the most common ingredient in pharmaceutical and cosmeceutical formulations, its quality must be ensured for the formulations. The current study entails the use of official quality control tests to authenticate commercial mint oil samples, as well as physicochemical tests and TLC fingerprint profiles to identify the active constituents.

II. MATERIALS AND METHODS:-

- **Collection and Identification of Samples:-** Sample Collection and Identification At the same time, three samples of peppermint oil were collected from different essential oil vendors in the Karachi market. For the test procedure, the samples were coded as P-1, P-2, and P-3. Sigma-Aldrich Company Ltd provided the genuine reference standard for peppermint oil.
- **Determination of Physicochemical Characteristics:-** The various physicochemical properties of peppermint oil samples, such as relative density, refractive index, and optical rotation, were determined using British Pharmacopoeia methods and the results were compared to the official specified ranges.
- **Identification of Menthol in Different Samples of Peppermint Oil:-**

Test solution: 0.1 g of the substance to be examined was dissolved in toluene and diluted to 10 ml with the same solvent.
Reference solution: 50 mg of menthol was dissolved in toluene and diluted to 10 ml with the same solvent.
Plate: TLC silica gel F254 plate (5-40 µ)
Mobile phase: Ethyl acetate and toluene (5:95, v/v).
Application: 10 ul of the reference solution and 20 µl of the test solution were applied as bands of 10 mm on TLC plate. **Development:** Over a path of 15 cm.
Drying: Dried in air.
Detection: Examined in ultraviolet light at 254 nm (Camag, Muttenz, Switzerland).
Results: The zones of menthol present in the chromatograms were compared with that of with the reference standard.

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- **Identification of Menthol in Different Samples of Peppermint Oil by TLC:-** TLC analysis of peppermint oil samples revealed the presence of menthol (Fig. 1). Table 2 shows the R values of menthol in peppermint oil samples, as well as the Rr values of menthyl acetate. All of the samples had R values that corresponded to the standard spots of menthol (Rr=0.91) and menthyl acetate (R=0.82). The results showed that menthol and menthyl acetate were present in all of the samples, as the R values were found to be very close to that of the reference standard, confirming their presence in the samples.

Table 1. Physicochemical studies of Mentha piperita samples.

No.	Sample	Relative Density	Optical Rotation	Refractive Index
1.	P1	0.958	-15.8	1.460
2.	P2	1.065	-14.2	1.467
3	P3	0.884	-15.2	1.533

Table 2. R_f value of menthol in Mentha piperita samples.

No.	Sample	R_f value of Menthol	R_f value of Menthyl Acetate
1.	Standard	0.91	0.82
2.	P1	0.91	0.82
3.	P2	0.90	0.80
4.	P3	0.91	0.82

- **Determination of Acid Value:-** Table 3 shows the acid values of the peppermint oil samples. According to British Pharmacopoeia13, the acid value should not be greater than 1.4. All peppermint oil samples were found to have an acid value in the range of 1.299-1.396, meeting the pharmacopoeial specification.

Table 3. Acid value of Mentha piperita samples.

No.	Sample	Acid Value
1.	P-1	1.396
2.	P-2	1.342
3.	P-3	1.299

III. CONCLUSION:-

Mentha piperita have various types of chemical constituent which have large scale use in industry. Due to its high demand in pharmaceutical industry it is mandatory to maintain its quality and its standard by it provide its maximum therapeutic and pharmacological effect on human body. Herbal medicine quality control aims to ensure their quality, safety, and efficacy. Chemical manufacturers play an important role in our current

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quality control operations. Fluorescence quenching, combining chromatography and spectroscopy, biological assays, the use of biomarkers in fingerprinting, and more recent techniques for the standardisation of herbal drugs are all available. To maintain its quality and standard there is some regulatory bodies which are maintain the parameter of herbal product standards. In industry maintain the and check the quality of standard herbal product generally we perform the Gas Chromatography technique.

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