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Cultivation, Extraction, Identification and Various Pharmacological Activity of Citrus Oil

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Abstract: *Citrus Limon, commonly known as lemon, is a fruit rich in essential oils with a wide range of potential health benefits. This review paper explores the chemical composition, extraction methods, and biological activities of Citrus limon essential oil (CLEO). The major constituents of CLEO include limonene, β-pinene, γ-terpinene, and citral. These compounds have been shown to possess antimicrobial, antioxidant, anti-inflammatory, and anticancer properties. The potential applications of CLEO in various fields, including food preservation, cosmetics, and pharmaceuticals, are also discussed. Further research is needed to fully understand the mechanisms of action of CLEO and to explore its potential as a safe and effective natural remedy.This review paper explores various extraction techniques for obtaining essential oil from Citrus limon, including traditional methods like hydrodistillation and cold pressing, as well as modern techniques such as microwave-assisted extraction and ultrasound-assisted extraction. The chemical composition of the extracted essential oil, primarily consisting of limonene and citral, is discussed, along with its potential health benefits and applications. The paper also highlights the importance of sustainable extraction practices and the potential for valorizing citrus by-products to obtain valuable essential oils.*

Keywords: citrus oil, cultivation, identification, antioxidant property, Anticancer, antioxidant, antiulcer, antidiabetic, antimicrobial

I. INTRODUCTION

Lemon is an important medicinal plant that belongs to Rutaceae family. Citrus fruits such as orange, lemon, and lime, have been widely cultured and processed into juice.3 During the manufacture of citrus juice, very large amounts of byproduct wastes, such as peels are formed every year. 4 Citrus peels exhibit a broad spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities.[Sheila John. Et al.2017]. Citrus limon belongs to Rutaceae family, common name is lemon and this originated from South East Asia, probably in India or Southern China. Lemon is a pale yellow, elliptically shaped berry fruit. Citrus fruit in general contain sugar, polysaccharide, organic-acid, lipids, carotenoids, vitamins, minerals, flavonoids, bitter lemonoids and volatile compounds. Lemon is a good source of potassium, calcium & vitamin C. Limon or lime juice have been reported to exhibit antimicrobial activity against Vibrio cholera.[Amit Pandey .et al.2011].

The majority of the citrus essential oil is found in the flavado part of the peel, with smaller amounts found in the leaves, flowers, fruits, and seeds. Terpene hydrocarbons, oxygenated compounds, and non-volatile compounds are three fractions that can be roughly split into the more than a hundred different chemicals that comprise essential oils. The terpene fraction can make up 50 to 95% of the oil. Germicidal, antioxidant, and antibacterial properties, and anticarcinogenic activities are all present in the essential oil. [HemlataKarne. et al2023].

India along with Mexico, Argentina and Brazil accounts for almost half the lemon production in the world. Lemon is one of the most commonly utilized fruits in the world due to its sour taste and nutritional values. A sample of Lemon fruits. is Because of the huge consumption of Lemon juice throughout the world, a large amount of wet solid waste is produced. This waste mainly includes Lemon peels. [D.C. Sikdar.et al2017].

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Fig.1& 2 . lemon fruit.

The major constituent of citrus essential oil is limonene, a highly lipophilic cyclic monoterpene that accounts for 68-98% of the oil's weight and up to 4% of the weight of citruspeel waste. Limonene is a commonly used food preservation agent and is designated as a generally recognised as safe additive in the Code of Federal Regulations due to its antioxidant properties and fragrance. In actuality, limonene is essential to the global flavor and fragrance industry. Numerous sectors have taken an interest in the existence of limonene, a substantial component of the essential oils present in orange peel that has antibacterial, antioxidant, biological, and herbal fragrance. [HemlataKarne. et al2023].


Fig. 3&4. Essential oil of citrus oil.

Utilization of peel in several possible ways helps reducing solid-waste handling along with adding value to these peel waste.9,10 Currently zest, a food ingredient obtained by scraping or cutting the outer skin of citrus fruits such as lemon and orange is commonly used as a flavoring agent in biscuits, puddings, candy, chocolates, pies, cakes and in sour condiments.[Sheila John. Et al.2017].

TAXONOMICAL CLASSIFICATION:

kingdom	Plantea
subkingdom	Angiosperms
phylum	Eudicots
class	Rosids

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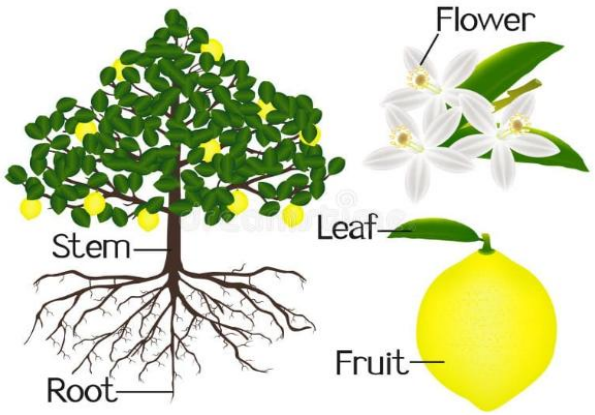
order	Sapindales
family	Rutaceae
genus	Citrus
species	c. limon

Fig.5. parts of lemon tree.



CULTIVATION:

Local cultivars of lime and lemon were still predominant in the country. The exiting lime and lemon orchards were mostly of seedling origin. About 14% of the lime and 13% lemon were propagated vegetatively. Although seedling trees produced good quality fruits, and were readily accepted in the market, there were some problems associated with these planting materials; the trees propagated from seeds were tall in growth habit with longer juvenile phase with prominent thorns. They were reported to be more susceptible to Phytopthora root rot disease. About 9 percent of lime varieties were improved whereas no improved cultivar of lemon was reported. Eureka lemon was found most dominant among improved cultivars. Local varicties were known by different names with the change in localities as Sunkagati, ThuloKagati, Chasme Kagan, Bahramase for lime, and Local Amilo, Nibuwa, LamchoNibuwafor lemon. Improved verities were mostly distributed.[D.D.Dhakal et al.2002].

EXTRACTION:

MATERIAL AND METHOD

Collection of plant material:

Fresh lemons were purchased from a local market or from kitchen garden of house

washed thoroughly under tap water.

The peels were removed using a sterile knife and shade dried for 4-5 days at room temperature.

The dried peels were pulverized using an electric blender and stored in airtight containers for further use.[Sheila John. Et al.2017].

Preparation of the Sample:

Orange peels were cut into intermediate sized pieces and heated in an oven at a temperature of 100^{o} C for 20 min, 30 min and 40 min respectively.

The oven was not preheated.lemon peels were grated to obtain the zest of fresh skin.

Zest of fresh skin was ultimately used for the experimentation. [HemlataKarne. et al2023].

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Preparation of Lemon Peels Sample:

The collected sample of Lemon peels is cleaned and pith is manually separated from the outer coloured part of the peels.

That is because of the reason that the majority of the oil in oil sac present in them.

This is then preheated at a temperature of 45 0C and kept for two hours. [D.C. Sikdar.et al2017].

Preparation of peel extracts:

Two different solvents namely methanol and acetone were used for extraction.

5grams of lemon peel powder was soaked in 100 mL of the respective solvents for 72 hours by maceration technique.

The supernatant was filtered using Whatmann filter paper 1 and Buchner funnel and concentrated using rotary evaporator and dry residue was preserved at 5°C until further use. [Sheila John. Et al.2017].

Material/Instruments Used: The materials/instruments used for this work were round bottom flask, Basket heater, distillation unit, thermometer, measuring cylinder, conical flask, separating funnel, soxhletapperatus. [D.C. Sikdar.et al2017].

Extraction of Oil by Steam Distillation:

100g of pre-heated orange and lemon peels sample is taken in a round bottom flask, and then 200ml of water is added to the preheated peel.

Heat is supplied by temperature controlled basket heater.

At the initial stage, experiment is carried out at a temperature of 80˚C for 60 min.

The distillate is collected in a conical flask.

This is then separated using a separating funnel. The less dense upper layer is the citrus oil. This oil is then stored in a vials.[K Sathya et al.2018].

Extraction of oil by soxhletapperatus:

Essential oil extraction from orange peels was done using the Soxhlet method. The orange peels were pureed using a blender. A round bottom flask was washed, oven dried, and cooled in a desiccator. To carry out this procedure, the ground peels were sieved using a standard 0.6 mm particle size sieve. A dried mass of 10 g of the orange peel powder was weighed, and the weight recorded. The weighed sample was dropped in the Soxhletextractorapparatus as shown inFigure-4. The extraction was carried out using normal hexane, methanol, and petroleum as the extraction solvent. In the Soxhlet apparatus, the solvent in the round bottom flask was heated from the heating mantle to become evaporated and got condensed down through the sample where it was able to extract the oil along, thereby, giving a mixture of oil and solvent, which was later separated [Essential oil extraction from orange peels was done using the Soxhlet method. The orange peels were pureed using a blender. A round bottom flask was washed, oven dried, and cooled in a desiccator. To carry out this procedure, the ground peels were sieved using a standard 0.6 mm particle size sieve. A dried mass of 10 g of the orange peel powder was weighed, and the weight recorded. The weighed sample was dropped in the Soxhletextractorapparatus as shown inFigure-4. The extraction was carried out using normal hexane, methanol, and petroleum as the extraction solvent. In the Soxhlet apparatus, the solvent in the round bottom flask was heated from the heating mantle to become evaporated and got condensed down through the sample where it was able to extract the oil along, thereby, giving a mixture of oil and solvent, which was later separated [Essential oil extraction from orange peels was done using the Soxhlet method. The orange peels were pureed using a blender. A round bottom flask was washed, oven dried, and cooled in a desiccator. To carry out this procedure, the ground peels were sieved using a standard 0.6 mm particle size sieve. A dried mass of 10 g of the orange peel powder was weighed, and the weight recorded. The weighed sample was dropped in the Soxhletextractoapparatus.The extraction was carried out using normal hexane, methanol, and petroleum as the extraction solvent. In the Soxhlet apparatus, the solvent in the round bottom flask was heated from the heating mantle to become evaporated and got condensed down through the sample where it was able to extract the oil along, thereby, giving a mixture of oil and solvent, which was later separated.

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IDENTIFICATION TEST:

Tannins:

Take 5ml of lemon peel filtrate and add 5ml distilled water.
heat at 80-100 0C for 10 min in water bath, then filter it after that add 1% Ferric chloride (5-6 drops).
Dark green color indicates the presence of tannins.

Glycosides:

Extract was hydrolyzed with HCl solution and neutralized with NaOH solution.
Few drops of fehling's solution A & B were added.
red precipitate showed the presence of glycosides.

Reducing sugar:

Extract was shaken with distilled water and filtered.
Filtrate was boiled with fehling's solution A&B for 10 min.
Orange & red precipitate indicates the presence of reducing sugar.

Saponins:

Take 0.2ml sample, add 4.8 ml distilled water.
heated to boil frothing showed the presence of saponins.

Flavonoids:

0.2 ml of sample was added in 0.2ml of NaOH.
add 1-2 drops of HCl.
yellow to colorless showed the positive result.

Phlobatannins:

Take 10 ml of sample and add 0.2ml HCl, then boiled in water bath for 10 min.
Red precipitate indicates positive result.
[Amit Pandeyet al.2011].

PHARMACOLOGICAL ACTIVITY:

Anticancer:

An irregular growth of cells or tissues of body is called cancer. If abnormal cells divide without control and invade nearby tissues is called malignant tumor and also known as malignancy. If the abnormal cell growth does not invade nearby tissues is called benign tumor or non-cancerous growth. A number of chemo protective activities and chemicals are found in plants in which some of these are taking as clinical experiments. A novel procedure for cancer therapy is Inhibition of angiogenesis. The selected plants may be carefully used as anti-angiogenic therapy and in cancer controlling. Citrus limonene contain vitamin C, folate, dietary fibre and other bioactive components, such as carotenoids and flavonoids, which are suggested to be responsible for the prevention of cancer and degenerative disease. Citrus liminoids shows anticancer property in aqueous extract of fruit , containing those compounds which shield the cells from damage which is the cause of cancer.

Antioxidant activity:

The important sources of antioxidants are citrus juices and fruits and these antioxidants are ascorbic acid, flavonoids and phenolic compounds. The source of glycosides and phenolic compounds are citrus peels. The bioactive compounds are phenolic acids which are responsible for the antioxidant and many other biological activities. The highest anti-oxidant activity can be found in citrus fruits. Ascorbic acid (vitamin C) interpretations for a great proportion in some fruits can act as antioxidant. Moreover, other phytoconstituents like carotenoids, fl avonoids, glutathione, and various enzyme systems may also contribute to the antioxidant activity.

Anti-ulcer activity:

The pathophysiology of peptic ulcer dieses involves an imbalance between offensive (acid, pepsin, and H. pylori) as well as suspicious aspects. Indian Medicinal plants and their derivatives have been a valuable source of therapeutic agents to treat various disorders including Antiulcer diseases. Fruit aqueous excerpt also shows the antiulcer activity against gastric ulcer.

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Anti-diabetic activity:

A metabolic disorder is called Diabetes. A system, through which our body utilizes the digested food energy and growth, is known as metabolism. The eaten food is converted into of blood sugar and the basic component of blood sugar is called glucose. The main source of fuel for ourbody is glucose. In absence of insulin, glucose cannot enter our cells. A class of organic substance known as polyphenols which is present in lemon and a high amount in lemon peel. It is investigated that polyphenols considerably inhibited the fat accumulation, high blood glucose levels and insulin resistance, development of hyper lipidemia, weight gain which are different aspects of obesity. A range of essential oils is present in citrus peel. The pathogenic bacteria are killed or their growth is inhibited by suing these essential oils. The lowering of diabetes and anti-per oxidative effects are possessed by citrus peel due to the high content of total polyphenols.

Antimicrobial activity:

The antibacterial activity of the organic acid-rich extract of fresh red chicory was verified against predontopathic bacteria like actionmycies and streptococcus mutant. Oxalic acid and succinic acid were recognized from active extract .entire organic acid with variant level of efficiency were present to reducing biofi lm generation and adhesion of bacteria to the cell. The antibacterial activity was analyzed by well diffusion method. 25 mL of Muller Hinton agar was prepared according to the standard procedure and poured into the plates and then it is allowed to get hard. The inoculums suspension are taken as standard which streaked over the surface of the media by using sterile cotton pad to make sure the confluent development of the organism and 5 minutes are allowed to dry the plates. The different concentrations (50, 75 and 100 g/mL) of the extract were poured into the wells after drying. A standard (1 g/mL) which is used called Tetracycline. Finally, the bacteria are incubated at 37ºC for 24 hours in the inoculated plates.[SaniaRafique et al.2020].

II. CONCLUSION

This review paper has comprehensively explored the cultivation, extraction, identification, and authentication of citrus oils. We have delved into various aspects of citrus oil production, from the optimal growing conditions and harvesting techniques to the diverse extraction methods available. The chemical composition and identification of key compounds within citrus oils have been discussed, highlighting the importance of chromatographic and spectroscopic techniques for their accurate analysis. Additionally, the review has emphasized the significance of authentication methods to ensure the quality, purity, and traceability of citrus oils in the market.Through this review, we have gained valuable insights into the complex and multifaceted world of citrus oil production. Future research directions include further optimizing cultivation practices, developing sustainable and efficient extraction methods, and advancing analytical techniques for comprehensive characterization and authentication of citrus oils. By addressing these areas, we can contribute to the sustainable production of high-quality citrus oils, meeting the growing global demand for natural and authentic products.

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