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EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITY OF *CITRUS LIMON* LEAF EXTRACT

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Keywords:

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ABSTRACT: *Citrus limon* is the commonly available medicinal plant in India. Which is utilized for the various pharmacological and food activities. During this year, commercial production of lemons was approximately 4.3 million tons that harvested from 25734 hectares in Iran. Nowadays, the interest in essential oils and their application in food preservation have been amplified as the natural additives to improve the shelf-life and the safety of food products, due to the risk in using synthetic preservatives. Also, food-borne diseases are a growing public health problem worldwide, calling for more impressive preservation tactics. Lemon has many bioactive components such as citric acid, Ascorbic acid, minerals, flavonoids and essential oils. In present work *Citrus limon* leaves subjected for extraction and followed by phytochemical Investigation and antimicrobial activity. The result shows that *Citrus limon* leaves ethanolic extract showing significant antimicrobial activity. The antimicrobial activity of the plant extract will be beneficial for the treatment of various microbial diseases such as fungal infection, skin rashes, ringworm and other skin related problems.

INTRODUCTION: Lemon [*Citrus limon* (L.)] is a prominent member of the citrus family. The Rutaceae family contains roughly 130 genera divided into seven subfamilies that are used for a variety of purposes around the world. Lemon is a tiny evergreen tree that grows best in hotter regions, with global production exceeding 160 million tons in 2014. It has also been adapted to drier climates, such as those found in Iran.

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Lemons were collected from 25734 hectares in Iran this year, resulting in a commercial yield of almost 4.3 million tones 1, 2. Due to the risk of employing synthetic preservatives, the interest in essential oils and their application in food preservation has grown. Furthermore, food-borne infections are an increasing public health concern around the world, necessitating more effective preservation techniques. Citric acid, ascorbic acid, minerals, flavonoids, and essential oils are just a few of the bioactive components found in lemons [1, 2].

Citrus essential oils are a complex mixture of over 400 elements with 85-99 percent volatile and 1-15 % non-volatile components that are generally regarded as safe (GRAS). Monoterpene, sesquiterpene, and their oxygenated derivatives

make up the volatile components, whereas non-volatile chemicals include hydrocarbons, flavonoids, sterols, fatty acids, coumarins, waxes, carotenoids, and psoralens [3-4].

In addition lemon juice production, essential oils which acquired by cold pressing of the peel or distillation of leaves are broadly applied as an aroma enhancer in beverages, bakery and food products, also serves as a flavoring agent to mask the unpleasant taste of drugs in pharmaceutical, and as fragrance in perfumery and cosmetic industries Numerous studies have been performed on the chemical composition, antimicrobial, antifungal, antioxidant and radical scavenging abilities of the essential oils of peel and leaf of various species and/or cultivars of lemon around the world, where limonene has been always the prominent component found in all peel essential oils, whereas the leaf essential oil was generally rich in limonene, in some case other compounds were the major constituents. The primary component of Japanese lemon leaf oil was geranial, which was followed by limonene and neral.

Caryophyllene was found to be the most abundant component in Egyptian lemon leaf oil, followed by linalool, nerol, and limonene. While limonene was present in Italian, Turkish, and Chinese lemon leaf oils, it was followed by -pinene and geranial. The primary constituents of the Benin lemon leaf oils were limonene, -pinene, and citronellal. Although it is well known that limonene is one of the most common ingredients in lemon leaf oils, the composition of essential oils varies depending on where they are produced. Different agents, such as genetic, environmental, and experimental factors, influence the quality and quantity of essential oils [5].

To the best of our knowledge, no research has been done on the composition, antioxidant, or antibacterial properties of lemon leaf essential oils from Iran. The purpose of this work is to use GC-MS to identify the volatile constituents of essential oils from lemon leaves grown in the south of Iran and to assess their antioxidant activity using the DPPH radical scavenging test. Furthermore, the antibacterial activities of essential oils will be studied against six Gram positive and Gram negative microorganisms. Citrus essential oils are a complex mixture of over 400 elements with 85-99 percent volatile and 1-15 percent non-volatile components that are generally regarded as safe (GRAS). Monoterpene, sesquiterpene, and their oxygenated derivatives make up the volatile components, whereas non-volatile chemicals include hydrocarbons, flavonoids, sterols, fatty acids, coumarins, waxes, carotenoids, and psoralens [3-4].

Essential oils obtained by cold pressing the peel or distillation of the leaves are widely used as an aroma enhancer in beverages, bread, and culinary products, as well as a flavoring agent to disguise the bad taste of medications in pharmaceutical, and as fragrance in perfumery and cosmetic sectors. Many studies have been conducted around the world on the chemical composition, antimicrobial, antifungal, antioxidant, and radical scavenging abilities of the essential oils of the peel and leaf of various species and/or cultivars of lemon, where limonene has always been the most prominent component found in all peel essential oils, whereas the leaf essential oil was generally rich in limonene, in some cases other compounds were the major C.

The primary component of Japanese lemon leaf oil was geranial, which was followed by limonene and neral. Caryophyllene was found to be the most abundant component in Egyptian lemon leaf oil, followed by linalool, nerol, and limonene [3-4]. While limonene was present in Italian, Turkish, and Chinese lemon leaf oils, it was followed by pinene and geranial. The primary constituents of the Benin lemon leaf oils were limonene, pinene, and citronellal. Lemon leaf essential oil has also been shown to have antibacterial and antioxidant properties in the past.

Although it is well known that limonene is one of the most common ingredients in lemon leaf oils, the composition of essential oils varies depending on where they are produced. Different agents, such as genetic, environmental, and experimental factors, influence the quality and quantity of essential oils. Furthermore, the antibacterial activities of essential oils will be studied against six Gram positive and Gram negative microorganisms. India is the world's most important and affordable source of medical plants and plant products. These medicinal herbs have been used extensively in Ayurvedic medicine for millennia.

Many of these plants have recently gained popularity as a result of their distinctive ingredients and diverse applicability in a variety of emerging disciplines of research and development. Nanoscience is centred on manipulating individual atoms and molecules to create materials for use at sub-microscopic scales. Physical, chemical, and biological knowledge at sizes ranging from single atoms and molecules below the nanometer to 100nm are included. During the last two decades, the production of nanoparticles has brought nanotechnology, which has resulted in innovative chemicals being used in a variety of industries. Diagnostics, antimicrobial agents, drug delivery, textiles (clothing), electronics, bio-sensing, food industry, paints, cosmetics, medical devices, and the treatment of several acute and chronic diseases such as malaria, hepatitis, cancer, and AIDS are all examples of pure metals in nanoparticle form [5-6]. Lemon leaves are ovate, oblong, and taper to a point on the non-stem end and are small to medium in size. Green leaves with fine-toothed margins and a little rippling grow alternately along the branches. A prominent central stem with some tiny veins running throughout the leaf is also seen [7].

Kingdom: Plantae

Order: Pates

Family: Rutaceae

Genus: Cymbopogon

Species: *Cymbopogon citrates*

FIG. 1: LEMON AND ITS LEAF

MATERIALS AND METHODS:

Plant Material: Lemon levees were harvested in April from a nearby location in the Ahmednagar area and dried in the shade. It's important to choose organs that are typical and healthy.

Pharmacogenetic Study: For morphological and histological analyses, fresh leaves were obtained. Microscopical features, physicochemical parameters, and phytochemical inquiry were all investigated using coarse powder. Transverse sections of leaves were produced and stained as per normal procedure for microscopical examinations. The powder microscopy was carried out [8-9].

Physicochemical Investigation: Analysis The percentage of ash values and extractive values were determined using a well-established approved method and procedure. The conventional approach was used for preliminary screening [10-13].

Ash Value: Ash values, especially in powder form, are useful in establishing the quality and purity of crude pharmaceuticals. The goal of ashing vegetable medications is to get rid of any organic debris that might interfere with an analytical assessment. When crude pharmaceuticals are burned, they frequently produce an ash that contains carbonates, phosphates, and silicates of sodium, potassium, calcium, and magnesium [13-15].

Extraction of Lemon Levees Extract

FIG. 2: SOXHLET'S EXTRACTION ASSEMBLY

Apparatus: Ethanol, weighing balance, two necked round bottom flask, heating mantle, thermometer, 500 ml healer, measuring cylinder, screening apparatus [14].

Procedure:

- ❖ First assembled the setup for Soxhlet extractor.
- ❖ Then next weight 40 gm. Lemongrass on weighing balance.
- ❖ Make thimble from filter paper add the lemongrass into the Thimble.
- ❖ Place the thimble in the Soxhlet chamber.
- ❖ Add 150 ml ethanol into the bottom flask.
- ❖ Place the flask in the heating mantle.
- ❖ Set the temperature at 90 degrees Celsius.
- ❖ Adjust the temperature of the heating mantle.
- ❖ Set the time of process for 1 hour then 1.20 min then 1.40 min. And take the runs according to the parameters.

Determination of Ash Values:

Total Ash: Take around 2 or 3 g of the ground drug, carefully weighed, and place it in a tarred platinum or silica dish that has been previously torched and weighed.

Place the ground drug in a fine, equal layer on the dish's bottom. Incarnated by progressively increasing the heat not surpassing dull red heat until carbon is removed, then cooled and weighed [15].

Acid- insoluble Ash: Boil the whole ash with 25 mL dilute hydrochloric acid for five minutes, collect the insoluble matter in a Gooch crucible or on ash-free filter paper, wash with hot water, ignite, and weigh. Calculate the percentage of acid-insoluble ash using the air-dried medication as a reference [16].

RESULTS AND DISCUSSION:

Phytochemical Test: After performing phytochemical test these components are present i.e. carbohydrate, Pentose sugar, Hexose sugar, Proteins, Amino acid, Flavonoid, Alkaloid, Steroids

TABLE 1: PHYTOCHEMICAL TESTS

Sr. no.	Ingredients	Conclusion
1.	Carbohydrate	+
2.	Pentose sugar	+
3.	Monosaccharides	-
4.	Hexose sugar	+
5.	Non reducing polysaccharides	-
6.	Proteins	+
7.	Amino acid	+
8.	Fat and oil	-
9.	Volatile oil	-
10.	Glycosides	-
11.	Steroids	+
12.	Alkaloid	+
13.	Flavonoid	+

Physicochemical Analysis:

TABLE 2: PHYSICOCHEMICAL ANALYSIS OF POWDERED DRUG

Name of test	Acid insoluble ash value	Sulphated ash value
Weight of empty dish	59.01 gm	59.01 gm
Weight of drug taken	2 gm	0.85 gm
Weight of ash + dish	60.96 gm	59.86 gm
Percentage of ash	95%	51%

Antimicrobial Activity: The agar well diffusion method was used to test the antibacterial activity of lemon extracts against Gram positive and negative isolates. The microbial susceptibility was summed up in the water extracts of all the materials tested, which revealed a variety of inhibitory effects.

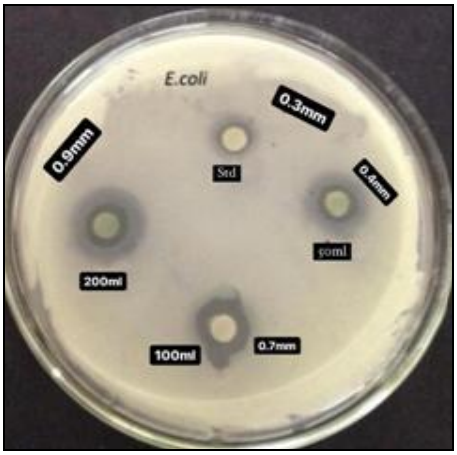

FIG. 3: ANTIMICROBIAL ACTIVITY OF *E. COLI*

TABLE 3: ANTIMICROBIAL TEST OF *E. COLI*

Concentration (µg/ml)	Zone of Inhibition (mm)
50	0.3
100	0.5
200	0.8
Ciprofloxacin (STD)	0.9

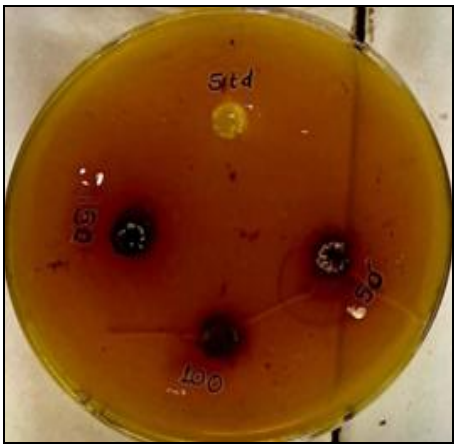

FIG. 4: ANTIMICROBIAL ACTIVITY OF *S. AUREUS*

TABLE 4: ANTIMICROBIAL ACTIVITY OF *S. AUREUS*

Concentration (µg/ml)	Zone of Inhibition (mm)
50	0.3
100	0.5
200	0.7
Ciprofloxacin (STD)	0.8

CONCLUSION: *Citrus limon* is a popular plant in India that has a variety of medicinal characteristics. *Citrus limon* leaves were extracted and then tested for phytochemicals and antibacterial activity in the current study. The results reveal that the ethanolic extract of *Citrus limon* leaves has strong antibacterial action. The plant extract's antimicrobial action will be effective in the treatment of a variety of microbial disorders, including fungal infection, skin rashes, ringworm, and other skin-related issues.

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CONFLICT OF INTEREST: Nil

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