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The Therapeutic Promise of Citrus limon: A Review of its In-Vitro Antioxidant and Anti-Inflammatory Effects

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ABSTRACT: Citrus plants, members of the Family Rutaceae have long been utilized in Traditional Indian medicine for the treatment of scurvy, Rheumatism , stomach ailments, diarrhea ,and liver disorders. In addition to their therapeutic applications, Citrus plants are well known for their potent antioxidant properties, underscoring their significance in both Ethnomedicine and modern pharmacological research. Citrus essential oils are fascinating because they sit at the intersection of botany, chemistry and human health. the Rutaceae family, with its rich diversity has given us plants whose oils are aromatic and also pharmacologically active. What makes citrus oils particularly valuables is their complex Phytochemical composition-mainly Terpenes {like limonene, Aldehydes ,Alcohols, Esters, Flavonoids } these mixture of compounds are known for both the Organoleptic properties { Flavour, fragrance} and the bioactivities like Antibacterial, anticancer, anti-inflammatory, antioxidant properties. The study has an objective to investigate the composition of phytochemicals in citrus limon leaf powder and evaluate their potential pharmacological activities majorly antioxidant and anti-inflammatory activity by evaluating their chemical constituents and carrying out testing procedures in various applications, the review highlights the importance of citrus limon leaves as a sustainable source of natural bioactive compounds. Citrus Genus are the most cultivated fruit crops ,rich in carotenoids, Flavonoids , and many other bioactive compounds of nutritional and nutraceutical values.

KEYWORDS: Antioxidant activity, Anti-inflammatory activity, Antidiabetic, Flavonoids, lemon leaf- Extract.

INTRODUCTION

The genus **Citrus** is part of the family Rutaceae, commonly used as a Flavoring and acidifying component in food. Numerous Citrus species can be found globally, including **Citrus limon** (lemon), **Citrus medica** (citron), **Citrus aurantium** (sour orange), **Citrus paradisi** (grapefruit), **Citrus reticulata** (mandarin, tangerine), **Citrus clementina** (clementine), and **Citrus sinensis** (sweet orange). In addition to this essential oil and its elements, the fruits of Citrus limon provide flavonoid compounds with various biological effects. Numerous illnesses have emerged in recent years, most of which are caused by **"oxidative stress**," which is the outcome of an imbalance between the production of pro-oxidants Citrus is one of most significant fruit crops in the world because of its Global availability, variety diversity, and appeal, all of which enhance human nutrition and health**.** Reactive oxygen species (ROS) are appreciated for its role in numerous cellular functions, like cellular communication and immune mechanisms. Cellular ROS may interact with different elements, like **carbohydrates, fats, proteins**, and **nucleic acids**. High levels of ROS lead to cellular harm and numerous long-term illnesses affecting humans. The equilibrium between ROS and antioxidants is crucial to uphold

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wellness and decrease the likelihood of long-term illnesses. Our body instinctively depend regarding antioxidants to sustain acceptable levels of ROS, which encompasses, internal antioxidants like **albumin, bilirubin, glutathione** (GSH), and uric acid; **antioxidant enzymes, involving superoxide dismutase (SOD), catalase, glutathione peroxidase, hemeoxygenase and NADPH; and dietary an**tioxidants, including vitamins C and E carotenoids and several polyphenolic substances**.**

Fig : 1

TAXONOMICAL CLASSIFICATION

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Sapindales

Family : Rutaceae
Subfamily : Aurantioideae
Genus : Citrus
Species : limon

PHYTOCHEMICAL CONSTITUENTS

S.NO	PHYTOCHEMICALS	CONFIRMATORY TESTS
1.	Alkaloids	Mayers test and Dragendroff Test
2.	Flavonoids	Aluminium Chloride or Zhishen
3.	Tannins	Gelatintest,lead acetate Test
4.	Carbohydrates	Molisch's Test
5.	Saponins	Foam Test
6.	Fixed Oils	Spot Test
7.	Phytosterols	Libermann-Burchard Test
8.	Phenols	FC Method
9.	Steroids	Ring Test
10.	Proteins	Biuret Test
11.	Cardiac Glycosides	Ring Test
12.	Reducing Sugars	Fehling's Test
13.	AminoacidS & proteins	Millions Test Ninhydrin Test

Table : I

IDENTIFICATION TESTS

Alkaloids: Examples for Alkaloids in citrus limon leaves: **Caffeine, Theobromine, Theophylline.**
Four milliliters of 1% diluted hydrochloric acid were mixed with 0.5 grams of aqueous extract. After boiling, it was filtered.
Mayers Test: A few drops of Mayer's reagent were added to one milliliter of the filtrate for the Mayer's test. Alkaloids were detected by **turbidity or precipitation**.
Dragendorff's test: A few drops of Dragendorff's reagent were added to one milliliter of the filtrate. Alkaloids were detected by an **orange-brown precipitate**.

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Flavonoids: Examples for Flavonoids in citrus limon leaves: **Hesperidin, Eriocitrin ,Narirutin and Diosmin.**
Aluminium chloride Test: A total amount 0.5 ml of the solution of sample is mixed with 2 ml of distilled water and add 0.15 ml of 5% $NaNO_2$ solution. After a duration of 6 minutes, 0.15 ml of a 10% $AlCl_3$ solution was introduced and allowed to sit for another 6 minutes, after which quantity containing 2 ml of a 4% NaOH solution is mixed into the combination. Addition of water to achieve a final volume of 5 ml, then mixture is stirred thoroughly and allow to stand for other 15 minutes. The development of a **pink hue**confirms the presence of flavonoids.
Tannins: Examples for Tannins in Citrus limon leaves: **Tannic acid, Gallic acid, Ellagic acid, Catechin, Epicatechin.**
Gelatin Test: A sample of 3 grams of extract in aqueous form was mixed with 6 milliliters of distilled water. The solution was filtered, Few amount of 10% ferric chloride solution is added. The appearance of **bluish green color** confirms the tannins presence in sample.
Lead Acetate Test: In the lead acetate test, a few drops of lead acetate solution of 10% concentration were added to 5milliliters of aqueous extract. White precipitate formation results due to the presence of Tannins.
Carbohydrates: Examples for Carbohydrates in citrus limon leaves: **Glucose, Fructose, Sucrose.**
Molisch's Test: Two milliliters of aqueous extract are mixed with Molisch's Reagent. After adding a tiny quantity of concentrated sulfuric acid to the mixture, a distinct layer was left to form. After giving the mixture a good shake, it was allowed to stand for a few more minutes. The solution was then diluted by adding five milliliters of distilled water. The presence of carbohydrates was shown by the formation of a purple precipitate ring.
Saponins: Examples for Saponins in citrus limonleaves**: streroids and Triterpenoids.**
Foam Test: 0.5 Grams of Ethanolic extract was boiled and the resulting Mixture was filtered in a test tube 2.5 ml of the Filtrate was taken and 10 ml of distilled water was added. The mixture was shaken thoroughly for a few minutes and then left to stand for a sometime The appearance of Frothing along with the Formation of a Honeycomb structure indicated the presence of Saponins**.**
Fixed Oils: Examples for fixed oils in citrus limon**: Limonene, beta-pinene, Geranial.**
Spot Test: A small amount of extract was squeezed in two batches. Cover with filter paper and let dry. Oil stains are noticeable, If observed under direct sunlight, oil stains will adhere to the filter paper. It shows the **non volatile oil** presence.
Phytosterols: Examples for phyto-sterols in Citrus limon **Beta-Sytosterol, Stigmasterol, Campestrerol**.
Liberman-Burchard Test: The small amount of extract was solubilised in 5 ml of chloroform. Especially 2 to 3 drops of this chloroform solution which is added to treat the Concentrated sulfuric acid. To do this, add a tiny drops of dilute acetic acid.3ml of acetic anhydride was added.**bluish green ,which shows the presence of Phytosterols.**
Phenols: Examples for phenols in citrus limon leaves: **Eriocitrin, quercitin-3-rutinoside,catechol, quercitin, caffeoyl-N-tryptophan].**
Fc-Method: An aliquot extract of sample is added to 5 ml of Folin-Ciocalteu reagent and mix thoroughly sodium carbonate in 4 ml. Vortex the tube for 15 seconds to mix leave at 40 degrees for 30 minutes to develop color the appearance of **blue color results in the occurrence of Phenol.**
Proteins and amino acids: Examples for proteins and amino acids in citrus limon leaves: **Glutamic acid, arginine, aspartic acid, leucin, glycine, proline, serine, alanine, valine and threonine.**
Biuret Test: Aqueous extract of 0.5 ml is treated with an equal volume of 1% sodium hydroxide, to which a few drops of copper sulfate solution has been carefully added. The solution turns **purple Indicating presence of proteins.**
Ninhydrin test: Add a few drops of ninhydrin reagent to the sample extract. After mixing carefully, bring the solution to a boil Soak in water for 2-3 minutes. **Bluish black color is observed due to the** proteins in sample and gently heated. **reddish brown Color precipitate that shows the tyrosine residues**; It is found in most proteins. sample Extract.

Cardiac Glycosides:
Ring Test: Acetic anhydride of 2 ml is added to 0.5 g of extract. Then alone a drop of 1% ferric chloride along with a small amount of concentrated added sulfuric acid**. Appearance of a brown ring in interphase**. indicated the presence of deoxy sugars, which showed the cardiac glycoside in sample extract.
Reducing Sugars Test: Examples for reducing sugars in citrus limon leaves: **Glucose, Fructose, Galactose, Ribose, Xylose.**

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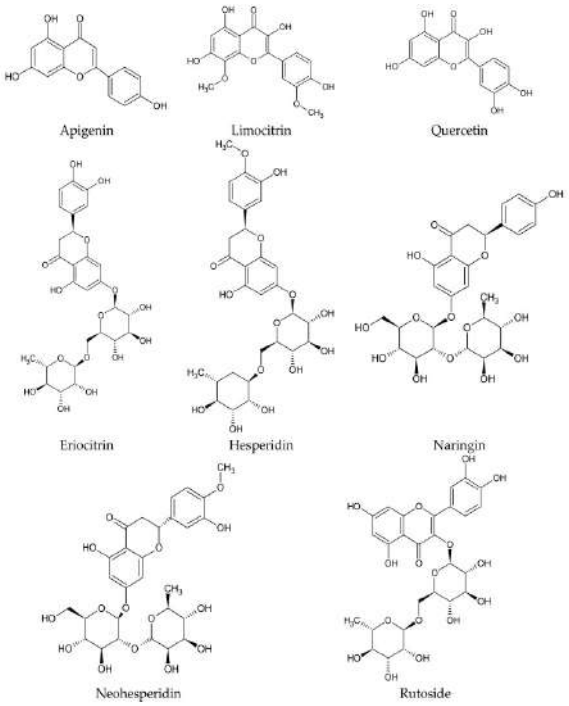
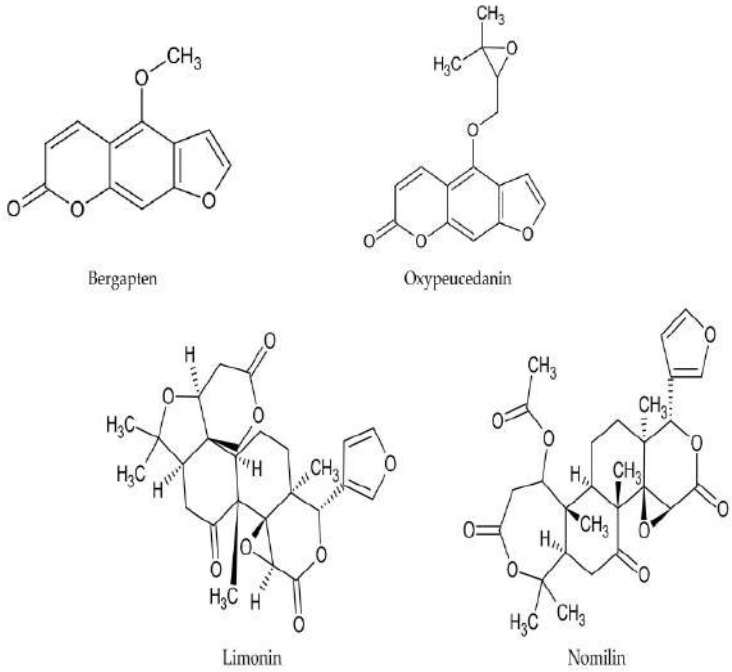


Fehling's Test: A small amount of Fehling's reagent was added to the aqueous solution. Extracted and boiled the mixture for 2 minutes. The occurrence of Reducing sugars is observed due to **Brick Red** color.

STRUCTURAL REPRESENTATION OF PHYTOCHEMICAL CONSTITUENTS



EXTRACTION METHODS

In the pharmaceutical industry Extraction refers to the process of using selective solvents in typical extraction techniques to separate the medicinally active chemicals of plant or animal tissues from the inactive or inert components. These plant based products are comparatively impure liquids, semisolids or powders that are solely meant to used externally or orally in the form of tinctures and fluid extracts Conventional Extraction Techniques like **Maceration, Percolation and Soxhlet extraction** often use organic solvents and require a significant volume of solvents and a long extraction period.

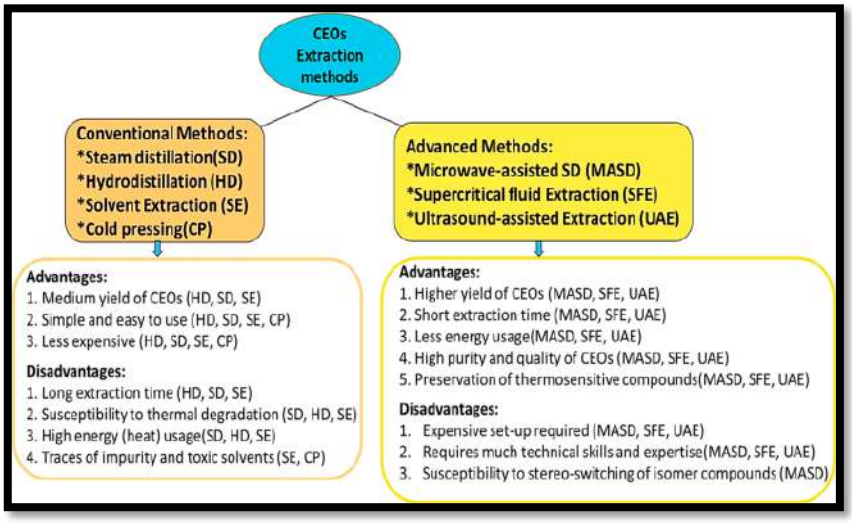


Fig :II

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CONVENTIONAL EXTRACTION TECHNIQUES

Maceration: This technique includes agitating the powdered substance using an orbital shaker incubator at 250 rpm overnight with a solvent, then proceeding to filtration and solvent evaporation. A measured combination of lemon peel and leaf powder (50 grams each) is mixed with 500 ml of solvent. The prepared solution was stored in an orbital shaking incubator at 37°C and then filtered and evaporated to dryness with a water bath. Ultimately, a solidified mass of the extract was achieved.

Percolation: This is the method most commonly employed to obtain active components in the creation of tinctures and liquid extracts. The dry components are dampened with a suitable volume of the solvent and permitted to rest for around 4 hours in a tightly sealed container, after which the mass is compressed and the lid of the percolator is secured. Extra solvent is introduced to create a thin layer on top. the mixture is allowed to steep in the mass, and the sealed percolator for 24 hours. The discharge point of the percolator then opened and the liquid inside is permitted to drip gradually. More solvent is introduced as necessary, until the percolate accounts for approximately three-fourths of the needed amount of the completed product. The extract is subsequently compressed and the fluid is introduced to the percolate. Adequate solvent is incorporated to achieve the necessary volume, and the mixed liquid is made clear through filtration or by allowing it to settle afterward. through decantation. The procedure is retrieved until a drop of the solvent from the percolator when it evaporate do not remain a residue.

Decoction: It is an appropriate technique for extracting the components. able to dissolve in water and which cannot be annihilated by the influence of temperature . Decoction is a preparation uses water to remove active ingredients from herbal plant substances. During this procedure, the liquid formulation is created by boiling the vegetation combined with water. Decoction is a technique of selection when dealing with challenging and fibrous plants, barks and roots and with vegetation that contains water-soluble compounds.

The plant material is basically fragmented or pulverized. Clay-coated copper containers. Water is added and the pot is warmed over a flame. If the substance is soft, four times. Water is utilized for every 1 part of the drug; if the drug is somewhat hard. Water is utilized eight times, and if the medication is particularly tough, it's used sixteen times water is advised. The combination is subsequently heated to a boil on low heat until it decreases to one-quarter of initial volume in the event of soft drugs, and one-eighth in aid of moderate or extremely potent narcotics. The extract is subsequently cooled and filtered**.**

Soxhlet Extraction: Soxhlet device is a dedicated glass refluxing system primarily utilized for organic Solvent extraction through Soxhlet is a common method and a proven method, which exceeds in efficacy compared to traditional extraction techniques aside from for, within restricted areas of use, the retrieval of compounds that a re-sensitive to heat. The solid material in powdered form is situated in a thimble constructed from filter paper and is situated within the Soxhlet setup. The device is equipped with a RB flask with the solvent included and to a reflux condenser. The liquid in the RB flask is heated to boiling softly, the steam rises through the lateral tube, liquefied the condenser and descends into the thimble holding the substance and gradually fills the Soxhlet. When the dissolving agent reaches the summit of the connected tube it flows over into the flask, therefore eliminates the segment of the material, which it has drawn out.

NON-CONVENTIONAL TECHNIQUES

The new ways to get things out of plants are really good at doing their job. They include using microwaves to help with steam, which is called microwave-assisted steam distillation and also using microwaves with water and gravity which is called Microwave hydro-diffusion and Gravity. There is also a way that uses waves to help get things out which is called **ultrasound-assisted extraction** and a way that uses special fluids to get things out. These new ways are called "**green extraction methods**" because they do not make much carbon dioxide and they do not use as much energy so they are good for the earth.

Ultrasound assisted Extraction:

This procedure uses ultrasonic waves to break down the cell walls of plants and herbs, which catalyzes the release of bioactive substances. Because the waves generate cavitation, which causes the micro-bubbles in the solvent to collapse, the extraction efficiency for the UAE method is higher. Using the UAE technique has several benefits, such as lower solvent use, higher extraction yields, and faster extraction timeframes.

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Microwave assisted Extraction:

The microwave technique is characterized by using electromagnetic waves at a frequency of 2.45 GHz, but according to the literature it ranges from 0.3 GHz to 300 GHz. In microwave assisted extraction (MAE), the waves interact with polar molecular compounds such as water, causing damage to citrus residues due to the increase in heat and pressure inside the cell walls. The porosity caused favouring the transfer of waste molecules and as a consequence, an improvement in the yield of the extracts without affecting the compounds. Low extraction time and energy consumption are the main advantages**.**

PHARMACOLOGICAL ACTIONS

Antioxidant Activity

Previous studies assess the antioxidant activity of Citrus limon essential oils. Citrus essential oils are significant in decreasing nitrite content and levels of Lipid Peroxidase, beside improving glutathione reduced concentration (GSH) levels, superoxide dismutase, catalase, and GPx activities in the mouse hippocampal tissue. Citrus essential oils' antioxidant properties may be linked to the phenolic compounds present in their leaves**.[21]** citrus limon essential oil exhibits antioxidant properties through the prevention of lipoperoxidation, offering significant from oxidative stress. Moreover, it helps reduce the risk of lifestyle-associated skin disorders by regulating the equilibrium of oxidative stress. C.limon demonstrates strong antioxidant characteristics, mainly because of flavonoids, limonoids, and phenolic substances. These substances capture free radicals, diminish oxidative stress and safeguard cells from oxidative harm. The activity of antioxidant in C. limon play a role to its capability in preventing or controlling different chronic illnesses, such as heart conditions, neurodegenerative illnesses, and oncology. C. limon, often referred to as lemon, is famous for its strong antioxidant properties, that are necessary for its medicinal possibility. The antioxidant features of C. limon are linked to the existence of diverse bioactive compounds, such as flavonoids, limonoids, coumarins, phenolic compounds, and ascorbic acid. These antioxidants assist in counteracting damaging free radicals, lower oxidative tension, and protect cells from oxidative stress. Review indicates that the antioxidant activity of flavonoids in Citrus limon, especially hesperidin and hesperetin, extend beyond merely scavenging free radicals. These substances also boost the body's antioxidant defenses by the stimulation of the ERK/Nrf2 signaling pathway.

Anti-Inflammatory Activity

Anti-inflammatory activity of C. limon in detail flavonoids, such as diosmetin, inflammatory response. By modulating these inflammatory pathways, limonoids contribute to the anti-inflammatory activity of C. limon. It contains coumarin derivatives, including umbelliferone, bergapten, and osthol, which possess anti-inflammatory papigenin, quercetin, and kaempferol, which exhibit potent anti-inflammatory effects. Flavonoids inhibit the activity of pro-inflammatory enzymes, such as cyclooxygenase and lipoxygenase thereby reducing the synthesis of inflammatory mediators like prostaglandins and leukotrienes. Additionally, flavonoids modulate signalling pathways involved in inflammation, such as nuclear factor-kappa B and mitogen-activated protein kinases leading to decreased expression of inflammatory genes. Limonoids in C. limon, such as limonin and tomlin, demonstrate anti-inflammatory activity. They inhibit the release of pro-inflammatory cytokines releasing from immune cells, which are tumor necrosis factor-alpha and interleukin-6 Limonoids also suppress the activation of NF-κB, the transcription factor endulged in the properties of Coumarins inhibiting the activity of pro-inflammatory enzymes and reduce the production of inflammatory mediators, such as prostaglandins and leukotrienes. Furthermore, they inhibit the activation of immune cells involved in inflammation, such as macrophages and neutrophils. Phenolic compounds present in C. limon, including hydroxycinnamic acids, contribute to its anti-inflammatory activity. These compounds inhibit the release of pro-inflammatory cytokines and chemokines from immune cells..

OTHER ACTIVITIES OF CITRUS LIMON

Antidiabetic Activity

C. limon, is a proven bio-remedy for diabetes mellitus because it has antidiabetic properties. The bioactive substances found in C. limon, Examples like flavonoids, limonoids, and compounds of Phenols, are significant for the plant's antidiabetic qualities.. It has been illustrated that C. limon improves glucose metabolism, that shows better glycemic control. The bioactive substances in C. limon controls blood glucose levels by escalating insulin sensitivity and promoting cell uptake of glucose. Type 2 diabetes people who have insulin resistance profited mostly from this effect. Metabolism is the process by which our body uses the energy from digested food for growth and function. The consumed food is transformed into a type of blood sugar, with glucose being the

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fundamental element of blood sugar. The Major source of fuel to the body is glucose. Without insulin, glucose is unable to enter our cells. A category of organic compounds called polyphenols is found in lemons, with a considerable concentration in lemon peel. Review indicates that polyphenols significantly reduced fat accumulation, elevated blood glucose levels and insulin resistance, the onset of hyperlipidemia, and weight gain, all of which are various facets of obesity .Antidiabetic effects improve insulin sensitive Antidiabetic effects: Extracts improve insulin sensitivity and inhibit essential enzymes that degrade the carbohydrates, which are α-amylase and α-glucosidase, which helps improve glycemic control**.**

Anticancer Activity

limon, widely called as Lemon, shows promising anticancer activity, rendering it a focal point in cancer research The bioactive components of C. limon are responsible for its anticancer effects. Compound such as limonoids, flavonoids, and phenolics substances, that play a role in its healing properties.. It includes Limonoids, limonin and Tomlin, demonstrate potential antiproliferative properties. Limonoids restrict the development and expansion of cancer cells by triggering cell cycle halt, enhancing apoptosis (programmed cell demise), and inhibiting tumor cell infiltration and spread. They also display antioxidant characteristics, minimizing oxidative stress and cellular harm linked to cancer progression. It is abundant in flavonoids, including hesperidin and diosmetin, showcasing cancer-fighting potential. Flavonoids demonstrate their anticancer properties. The anticancer activity will show by citrus limon with the Stimulation of Apoptosis Process of colorectal cancer cells**.**

Hepatoprotective Activity

C. limon has hepatoprotective properties and has been known for its effectiveness in liver health. It helps protect the liver against toxic substances, oxidative stress, and inflammation. C. limon exerts its hepatoprotective effects by enhancing antioxidant defences, reducing lipid peroxidation, and modulating liver enzymes involved in detoxification Citrus limon essential oil may aid liver detoxification by stimulating cytochrome P450 and boosting liver enzymes like glutathione S-transferase, especially in situations of prolonged liver toxicity.

Cardioprotective Activity

Citrus limon leaves having significant cardioprotective Activity because of Presence of bioactive Compounds which includes Flavonoids like Hesperidin, elicitin and diosmin, compounds of phenol, vitamin-C, limonoids and Essential oils. These Phytoconstituents shows Strong Antioxidant and Anti-inflammatory properties safeguarding the heart against inflammation and oxidative Stress which are significant in Contributing in cardiovascular Diseases. They cause Reduction in Lipid Peroxidation ,increases the Endogenous activity of Antioxidant enzymes which consists of Superoxide dismutase, Catalase and Glutathione and enhances Endothelial function causing improved blood Circulation. it can also helpful for the lowering of Blood Pressure and reduces the Oxidation LDL Extract Cholesterol. Citrus it will show decrease in Myocardial injury and maintain the Cardiac Function. It can acts as a Natural Cardioprotective agent and it has Proven Safety and therapeutic efficacy in Humans**.**

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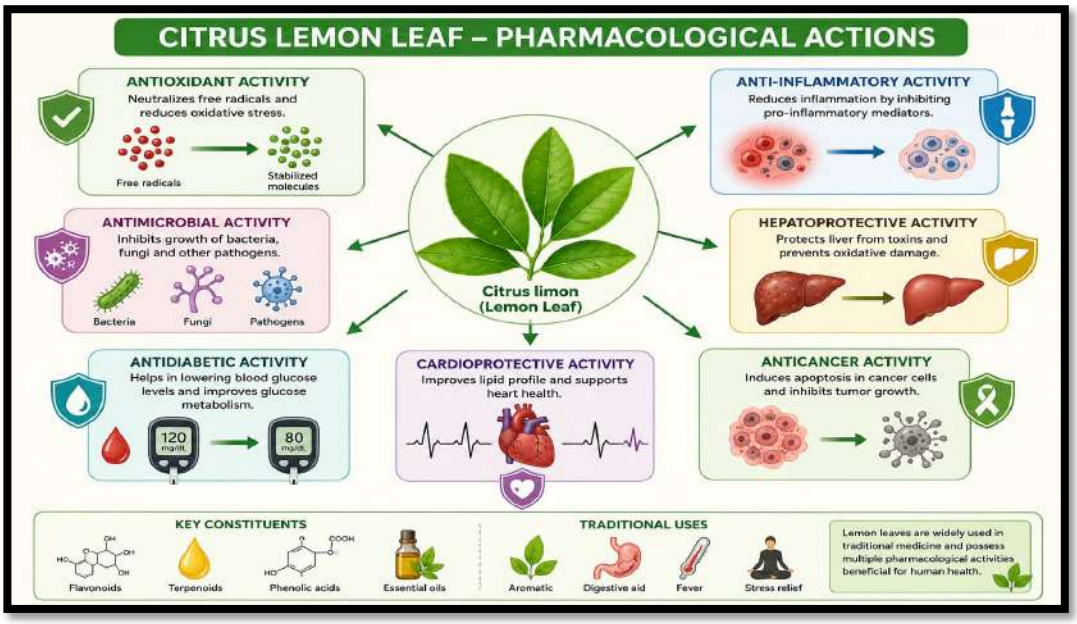

Fig :III

THERAPEUTIC USES

Health conditions	Active Constituents	Mechanism of Action
Skin Care	Vitamin-c, Flavonoids	Antimicrobial, Antioxidant, Cell Renewal
Eye Health	Antioxidants	Prevents Macular degeneration
Gout	Vitamin-c, Flavonoids	Reduces uric acid, Detoxifies
Gum Diseases	Vitamin-c, Potassium	Promotes Healing, Inhibits Bacteria
Peptic Ulcers	Limonoids, Flavonoids	Antioxidant, Antimicrobial, Mucosal Repair
Scurvy	Vitamin-c	Collagen Synthesis
Digestive Disorders	Flavonoids, citric acid	Stimulates bile& Enzyme secretion
Hemorrhoids	Fibre, Antioxidants	Relieves Constipation and
Hypertension	Eriocitrin, Hesperidin, Vitamin-C	Vasodilation Cholestrol Reduction

Table : II

Cardiovascular Wellness: Lemon provides significant advantages for cardiovascular wellness.
Blood Pressure Control: The elevated potassium levels and flavonoids may reduce blood pressure by aiding in the relaxation of blood vessels.
Cholesterol Control: The soluble fiber (pectin) and flavonoids found in lemons might assist in reducing LDL (bad) cholesterol levels.

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Vascular Health: Due to its antioxidant and anti-inflammatory properties, it enhances vascular endothelial function and lowers the likelihood of atherosclerosis

Preventing Kidney Stones: Due to citric acid content in high ratio, lemon juice raises citrate levels in urine . Citrate lowers the likelihood of kidney stone development by inhibiting calcium crystallization in the urine. It is particularly effective in treating calcium oxalate stones.

Fig : IV

INVITRO MODELS OF ANTI-OXIDANT ACTIVITY OF CITRUS LIMON

DPPH Assay

Assessment of 2, 2-diphenyl-1-picrylhydrazyl (DPPH)activity of scavenging radicals. The antioxidant activity of the essential oil can be assessed by using the scavenging of the DPPH radical (Sigma-Aldrich, Germany)examination. In summary, 50 µl portions of different concentrations of the EO will combine with 5 µl of DPPH solution (0.004% in(methanol). The combination was stirred and leave to sit at ambient temperature in the dark for about 30 minutes. The absorbance of the solution was subsequently measured at 517 nm. DPPH The solution will act as a negative control, ascorbic acid act as a positive control, and highest free radical scavenging activity was determined by reduced absorbance .of the reaction mixture. EO solution.

$$\%Inhibition = \frac{(A - A_1)}{A} * 100$$

A = Control absorbance
A_1= Sample absorbance

Scavenging of hydrogen Peroxide

The scavenging activity of EOC against H_2O_2 can be done using the technique by Kavoosi and Rowshan.11 In summary, 1 mL of H2O2 (50 mM in 100 mL phosphate buffer pH 7.4) will be combined with 100 µL of essential oils (0–0.3 mg/mL in DMSO). Subsequently, the mixture was agitated vigorously and allowed to incubate for 60 minutes at 37°C. Continuing this, the absorbance of Reaction mixture was calculated at 230 nm in comparison with the blank solution which consists phosphate buffer without Hydrogen peroxide. The scavenging activity of H2O2 was measured using the equation:

H_2O_2 scavenging activity (%):

[Absorbance of control – Absorbance of sample/Absorbance of control] × 100.

IC50 was determined from the graph that plotted percentage inhibition versus various concentrations of essential oil.

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INVITRO MODELS OF ANTI - INFLAMMATORY ACTIVITY OF CITRUS LIMON

Analysis by Gas Chromatography-Mass Spectrometry

The chemical makeup of the extracted essential oils will be examined with a Trace 1310/ISQ-LT gas chromatograph (GC) that has a flame ionization detector and a VF-5MS capillary column (60 m × 0.25 mm × 0.25 µm). The injector temperature was established at 250 °C, and the carrier gas flow of helium rate was 1.0 mL/min. The oven was kept at 50 °C for 5 minutes and then raised to 65 °C at a rate of 10 °C/min, followed by an increase to 210 °C (10 minutes) at 5 °C/min, and finally adjusted to 325 °C (10 minutes) at 20 °C/min. The parameters for mass spectroscopy (MS) were: electron ionization mode: 70 eV; temperature of ion source: 270 °C; and mass spectra range: 35–550 amu. The substances of the oil are recognized by checking similarity with their spectra of Mass with those listed in the NIST library. Identification of compounds relied on correlating the relative retention times with that of an n-alkane mixture (C8–C30, Sigma-Aldrich, St. Louis, MI, USA).

In vitro Inhibition of albumin denaturation Assay

The complete 5 ml reaction mixture includes egg albumin of 0.2 ml and 2.8 ml of a solution comprising phosphate-buffered saline (PBS, pH 6. 4), along with 2 ml of various concentrations of Citrus macroptera Montr. EO. Consequently, the ultimate concentration results (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml) were synthesised based on established protocol [8-12]. An identical amount of distilled water was utilized as a control. The prepared mixtures are subsequently placed in an incubator at 37±2°C for 15 minutes, followed by heating for 5 minutes at 70°C. After allowed to cool cooling, the absorbance was taken at 660 nm using blank vehicles. The control drug utilized in this experiment was diclofenac sodium, administered at a end concentration of 100–500 µg/ml, and it was handled in the same manner for absorbance measurement. The protein denaturation percentage of inhibition was computed using the formula:

$$\% \text{ inhibition} = 100 \times [\text{abst/absc} - 1]$$

Where abst represents the test sample absorbance and absc denotes the control absorbance**.**

CONCLUSION

This Review demonstrates that Citrus limon leaves is a highly appealing subject for various Scientific Investigations It emphasizes the therapeutic benefits of citrus limon, or lemon, demonstrating a close relationship between traditional medicine and contemporary science. Lemons have long been used in medicine because of their many health advantages, which include antioxidant, anti-inflammatory, antibacterial, and antidiabetic properties. Additionally, it has the potential to enhance mood, memory, and metabolic health. All things considered, lemon leaves offer a useful natural cure and support healthcare integrity and the research endorses the incorporation of Citrus limon into contemporary nutraceutical products, functional dietary options, and preventive health care approaches. Phytochemicals and nutrients found in citrus plants display various health advantages and medicinal qualities. Lemons leaves, abundant in bioactive e substances like flavonoids, coumarins, and essential oils, along with vitamin C, which improves immune system performance, aids skin wellness, and aids in collagen production. Citrus plants exhibit diverse pharmacological tasks, such as antioxidant, anti-inflammatory, antitumor, and anti-diabetic characteristics.. The existence of substances such as D-limonene and polyphenolic compounds found in lemon aids in their cancer-fighting and anti-diabetic characteristics, while their antioxidant properties, linked to vitamin C and phenolic substances, Improves overall well-being.

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