

Formulation And Evaluation Of Anti-Inflammatory Activity Of Citrus Lemon Peel

GIRAM V. B.^{1*}, SANAP J. B.², GHUBE D. D.³, TATHE P R^{4*}

¹Research Scholar, Department of Pharmacology, Samarth College of Pharmacy, Deulgaon Raja, Dist. Buldhana – 443204, Maharashtra, India
Orcid Id: 0009-0004-6704-8559

²Associate Professor, Department of Pharmacology, Samarth College of Pharmacy, Deulgaon Raja, Dist. Buldhana – 443204, Maharashtra, India
Orcid Id: 0000-0001-8999-5128

³Head of the Department, Department of Pharmacology, Samarth College of Pharmacy, Deulgaon Raja, Dist. Buldhana – 443204, Maharashtra, India
Orcid Id: 0009-0001-8012-0736

⁴Principal, Samarth College of Pharmacy, Deulgaon Raja, Dist. Buldhana – 443204, Maharashtra, India
Orcid Id: 0000-0003-2163-2974

*Corresponding Author: Giram V. B. & Tathe P. R.
vaishnavigiram6@gmail.com & tathepr@gmail.com

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ABSTRACT

The present study was focused on the formulation and assessment of citrus limon peel extract for topical anti-inflammatory properties. Lemon peel, which is generally considered an agricultural waste material, contains several bioactive phytoconstituents such as flavonoids, limonene, hesperidin, vitamin C, and polyphenolic substances with strong anti-inflammatory and antioxidant qualities. The objective of this research was to prepare a suitable topical formulation using lemon peel extract and to evaluate its physicochemical characteristics and anti-inflammatory potential.

Citrus limon peels were gathered, shade dried, ground into a powder, and then Soxhlet extracted using ethanol as a solvent. The obtained extract was concentrated and evaluated for various phytochemical constituents. Different formulations of gel were prepared using suitable polymers and excipients in varying concentrations to obtain stable and effective topical preparations. The prepared formulations were assessed for stability, washability, spread ability, homogeneity, pH, viscosity, and physical appearance. Compatibility studies and preliminary phytochemical screening were also carried out to confirm the presence of active constituents responsible for anti-inflammatory activity.

Keywords: Citrus limon peel extract; Lemon peel; Anti-inflammatory activity; Topical gel formulation; Phytochemical screening; Ethanolic extract; Natural bioactive compounds; Flavonoids; Antioxidant activity; Herbal formulation; Soxhlet extraction; Physicochemical evaluation; Topical drug delivery; Stability studies; Polyphenols.

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INTRODUCTION

When the body recognizes dangerous stimuli like viruses, damaged cells, or irritants, it triggers inflammation, a defensive immunological response. It is an essential part of the healing process, allowing the immune system to remove threats and repair damaged tissue [1]

Citrus limon (lemon) peel extract contains various phytochemicals, including phenols, flavonoids, and terpenes. Phytochemicals are non-nutritive plant substances that have the ability to prevent disease. Although they are not necessary for the human body to survive, they protect against illnesses [2]

Types of Inflammation

1. Acute Inflammation:

This is the body's first line of defense against an infection or unexpected tissue injury. Its objective is to eliminate the damaging stimuli and initiate the healing process.

2. Chronic Inflammation:

In contrast, chronic inflammation persists for months or even years. It can occur when the initial cause of inflammation is not resolved or when the immune system mistakenly attacks healthy

tissue. Chronic inflammation is associated with various diseases, including autoimmune disorders, cardiovascular diseases, and some cancers. It often results in ongoing tissue damage and can significantly impact health and quality of life [3].

An essential physiological process, inflammation is a complicated biological reaction to damaging stimuli like irritants, viruses, or damaged cells. It is a protective process designed to get rid of necrotic cells and tissues, eliminate the underlying cause of cell damage, and start the healing process. Acute inflammation serves as a vital defence mechanism, but chronic inflammation can cause many pathological conditions, such as diabetes, arthritis, cardiovascular disease, neurodegenerative diseases, and some types of cancer [4].

In response to a wide range of harmful agents (such as bacterial infection, physical trauma, chemicals, or any other phenomena), inflammation is a crucial physiological reaction that ultimately aims to perform the dual functions of limiting damage and fostering tissue healing. Inflammatory pathways are essential for immune monitoring, optimal healing, and post-injury regeneration [5].

*Author for Correspondence: Giram V. B. & Tathe P. R.

Citrus fruit, i.e., lemon (*Citrus limon*), has been identified as an excellent source of bioactive phytochemicals that are visualized to exhibit anti-inflammatory activity. Citrus crops have been found to contain rich chemical diversity in their makeup, such as flavonoids, limonoids, phenolic acids, volatile oils, and vitamins, for the antioxidant, antimicrobial, and anti-inflammatory activities [6].

Lemon peel, usually wasted as residue from juice and food processing industries, contains higher bioactive compound content levels compared to pulp. This provides lemon peel a potential agent for creating natural anti-inflammatory products, while simultaneously making it possible to enhance industrial waste sustainably. This reveals lemon peel as a possibility for the development of natural anti-inflammatory products, as well as opening a window of opportunity for industrial waste valorization in a sustainable scenario. In addition, recent studies have revealed that lemon peel extracts may exhibit synergistic activity when combined with other bioactive compounds, thereby enhancing their therapeutic efficacy [7].

By generating cells and mediators that battle foreign materials and prevent infection, the inflammation process protects our body against disease. The Rutaceae family, citrus is the herb most widely used in traditional medicine. Citrus is the indigenous genus in the tropical and subtropical regions of Asia [8]. Citrus fruits are the principal winter fruits, and they are a mainstay of Mediterranean cuisine. Citrus fruits provide therapeutic properties and are highly nutrient-dense. They are commonly cultivated in the tropics, where they are indigenous [9].

Mechanism of Inflammation

The inflammatory response involves several key processes:

- Vasodilation: Increased blood flow to the afflicted area causes blood vessels to enlarge, resulting in warmth and redness.
- Increased Permeability: When blood arteries become more permeable, white blood cells, proteins, and fluids can leave the bloodstream and enter the tissues, resulting in edema.
- Cellular Response: White blood cells, such as macrophages and neutrophils, move to the site of infection or injury. These cells play a vital role in engulfing pathogens and debris, secreting signalling molecules (cytokines) that further amplify the inflammatory response [10].

Numerous *in vitro* studies have demonstrated the anti-inflammatory properties of lemon peel. Ethanolic and aqueous extracts of lemon peel have been found to suppress protein denaturation, stabilize erythrocyte membranes, and suppress lipoxygenase activity, which are all established models for the evaluation of anti-inflammatory activity *in vitro* [11].

Citrus lemon peels, which are otherwise wasted, are an excellent source of bioactive substances with a variety of possible applications and research prospects. Flavonoids, pectin, and vitamin C are some such compounds that possess anti-inflammatory activities [12].

In vivo experiments also enhance the medicinal prospects of lemon peel. Oral administration of aqueous, ethanolic, or methanolic extracts of lemon peel significantly lowered paw edema caused by carrageenan and inflammation caused by formalin in albino Wistar rats, effects similar to those seen with reference NSAIDs diclofenac and indomethacin.

The aroma and health advantages of lemon peel come from its abundance of bioactive components [13].

- D-limonene
- Vitamin C
- Flavonoids
- Pectin
- Minerals

Citrus fruits are processed industrially to yield huge volumes of peel as waste, which, if not harnessed, adds to environmental pollution. Valorisation of lemon peel into anti-inflammatory products presents a green and cost-effective strategy that encompasses principles of green chemistry and circular economy. By transforming low-value byproducts into high-value therapeutics, these strategies offer double advantages of health promotion and environmental conservation [14].

In spite of the increasing evidence for the anti-inflammatory activities of lemon peel, there is still a lack of systematic investigations that combine phytochemical profiling, formulation development, and overall biological activity assessment. The majority of prior investigations have been limited to *in vitro* tests or *in vivo* models alone. There is little work available on the formulation development, including gels or nano-formulations, that would optimize bioavailability and therapeutic effectiveness [15]. In addition, precise mechanistic investigations revealing the molecular mechanisms regulated by lemon peel phytochemicals are limited. Closing such gaps has the potential to unlock the discovery of safe, efficacious, and sustainable anti-inflammatory therapies from natural sources [16].

An important physiological response to many dangerous substances, including chemicals, physical trauma, bacterial infections, and other phenomena, is inflammation. ultimately aiming to achieve the dual goals of promoting tissue healing and preventing damage. Immune surveillance, optimum healing, and post-injury regeneration all depend on inflammatory mechanism [16].

The inflammatory process defends our body by producing cells and mediators that fight foreign substances and prevent infection. However, conditions including psoriasis, inflammatory bowel disease, and rheumatoid arthritis can be brought on by persistent or severe inflammation. It plays a key role in many infectious and non-infectious diseases such as diabetes, cancer, cardiovascular disease, rheumatoid arthritis, Alzheimer's, and arteriosclerosis, also contributing to autoimmune disease damage. Severe inflammation may release mediators into the bloodstream, causing fever [17].

Citrus peels are rich in minerals and phytochemicals, including glycosides, volatile oils, and β and γ -sitosterol. Ascorbic acid, flavones, polyethoxylated compounds, and phenolic compounds display several essential activities that are very unusual in other plants. Citrus peels have long been used as a traditional remedy for scurvy, digestive and respiratory conditions, peptic ulcers, eye infections, gum disease, gout, skin care, piles, urinary issues, and weight loss. It also serves as a sterilizer and disinfectant [18].

Introduction to Citrus limon and Ethnomedicinal Relevance

Citrus limon (L.) Burm. f., commonly known as lemon, is one of the most widely cultivated and medicinally valuable fruit-bearing plants of the Rutaceae family. Citrus limon is an evergreen small tree or shrub with glossy green leaves, white fragrant flowers, and bright yellow oval fruits with characteristic acidic pulp and aromatic peel. Originally from Asia's subtropical regions, citrus limon is now widely grown in tropical and subtropical areas [19]. Citrus limon has become extremely valuable as a medical plant resource in addition to being a significant food fruit. Over the years, the plant parts, including fruit juice, peel, seeds, leaves, and essential oil, have been used in medicine for their medicinal properties. Recent advances in phytopharmaceuticals have added greater value to Citrus limon due to the discovery of numerous bioactive phytochemicals responsible for the medicinal properties [20].

From a botanical perspective, Citrus limon is a member of the Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida, Order Sapindales, Family Rutaceae, Genus Citrus, and Species

Citrus limon. It is a medium-sized thorny shrub or tree, reaching a height of about 3–6 meters. The leaves are simple, alternate, elliptic or ovate, with an abundance of volatile oil glands, giving it a characteristic citrus odor [21]. The fruit contains an outer colored epicarp or flavedo with an abundance of essential oil glands, an inner white mesocarp or albedo, and a juicy segmented endocarp. Of all the components, the peel is of utmost pharmaceutical value, having a rich reservoir of flavonoids, limonoids, coumarins, phenolic acids, pectin, and volatile monoterpenes [22].

The ethnomedicinal significance of Citrus limon is highly correlated with traditional medicine systems practiced in Asia, Mediterranean countries, the Middle East, and Africa. In Ayurvedic medicine and traditional Indian home medicine, Citrus limon is utilized for treating digestive disorders, sore throats, nausea, cleansing the skin, washing wounds, reducing fever, and treating minor inflammatory conditions. The peel of Citrus limon is specifically utilized as a rubefacient agent, deodorizer, insect repellent, and soothing agent in traditional medicine systems [23]. Folk medicine practitioners often apply dried peel powder for treating swellings, insect bites, minor pains, and skin irritations. These traditional practices clearly indicate its potential for inclusion in topical anti-inflammatory formulations. Citrus fruits, including Citrus limon, have been considered traditional Persian and Unani medicine for treating various conditions. These medicinal plants have been utilized for treating conditions like digestive inflammations, headaches, skin eruptions, and muscular discomforts through preparations made from peel infusions and essential oils [24]. In Mediterranean folk medicine, Citrus limon peel infusions and essential oils have been utilized for treating joint pains after massage, inflammatory swellings, and post-traumatic bruising. These traditional practices have historical importance, especially considering the long empirical evidence supporting its topical application for treating various conditions [25].

One of the main reasons for the continued popularity of Citrus limon in traditional medicine is its high phytochemical content. Citrus limon peel is rich in flavonoids like hesperidin, eriocitrin, diosmin, quercetin, naringin, and limocitrin, they have all been shown to have anti-inflammatory and antioxidant qualities. In addition, Citrus limon peel contains a high percentage of D-limonene, beta-pinene, gamma-terpinene, citral, linalool, and alpha-terpineol, all of which have been reported to have membrane-stabilizing activity, free radical scavenging activity, and anti-edematous activity [26]. The traditional usage of citrus limon peel in the treatment of inflammatory diseases is supported scientifically by the presence of all these bioactive components. Citrus limon peel is an underutilized part of this medicinal plant, and it is rich in bioactive compounds. While Citrus limon juice is consumed for its health benefits, Citrus limon peel is thrown away like any other vegetable or household waste. In recent times, natural product scientists have realized the importance of Citrus limon peel and other fruit peels in terms of their high medicinal potential. The concept of utilizing Citrus limon peel for medicinal purposes can be related to the concept of converting waste to wealth, where all the agro-industrial waste is utilized for producing high-value drugs [27].

Another significant aspect of its ethnomedicinal value is its use in skin and cosmetic therapy. The traditional formulations containing lemon peel have been widely used for skin cleansing, reducing oiliness, inflammation, and erythema; soothing insect bites; and reducing mild inflammatory forms of acne. Such applications may be related to anti-inflammatory, antimicrobial, and antioxidant properties, which are highly relevant to the development of topical semisolid dosage forms. The natural fragrance of lemon peel oil is also an added advantage, making it highly acceptable to

consumers. It is therefore a highly promising ingredient for the development of herbal gel formulations [28].

The popularity and widespread use of lemons worldwide, especially for culinary, cosmetic, and aromatherapy purposes, have also contributed to its medicinal value. In aromatherapy, lemon peel oil is often used for massage to relieve inflamed muscles, fatigue, mild sprains, and stress-related headaches. Such applications may be related to circulation-enhancing and anti-inflammatory properties, possibly through the action of volatile monoterpenes and the natural cooling effect [29]. From a research point of view, Citrus limon can be seen to be an optimal choice for ethnomedicine-based phytopharmaceutical development. Its historical safety profile for human consumption, its ready availability, its pleasant odor and taste, and its chemical profile all make it an optimal choice for topical anti-inflammatory formulations. Ethnomedicinal relevance can be seen to offer traditional justification for Citrus limon, while scientific pharmacological data offer mechanistic justification for its choice for this study. From an Indian perspective, where Citrus limon is readily available, culturally acceptable, and economically inexpensive, its use as a phytopharmaceutical raw material can be seen to have significant public health implications [30]. In this respect, the formulation of a standardized anti-inflammatory gel based on Citrus limon peel can be seen to offer an economically viable, patient-friendly, and culturally acceptable alternative to synthetic topical NSAIDs and steroids.

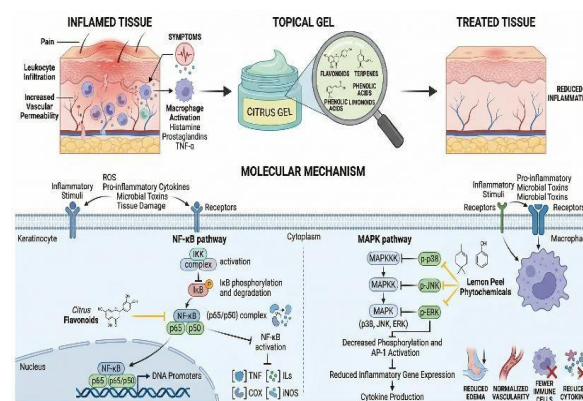


Fig. Inflammation Pathway

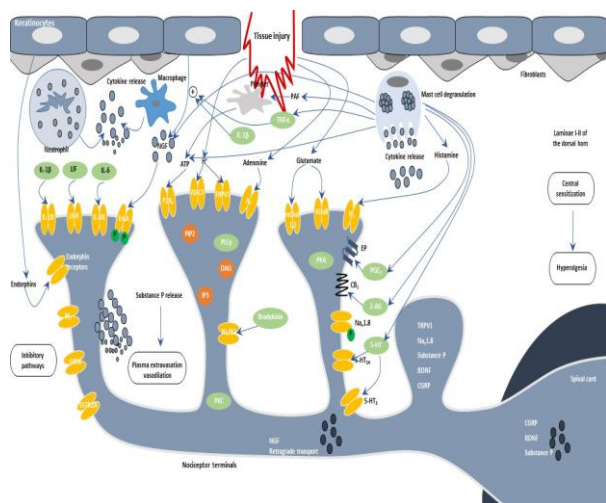
Molecular Mechanisms of Anti-inflammatory Action

The underlying mechanisms of the anti-inflammatory property at the molecular level form the primary scientific foundation for the therapeutic utility using phytochemicals taken from Citrus limon peels to create a gel system using evidence-based medicine principles [31]. Inflammation is neither an abrupt nor an isolated phenomenon but a very organized sequence of events involving intracellular signal transduction, transcription factors, inflammatory enzymes, cytokines, oxidative reactions, and immune cell functions. This therapeutic effect of Citrus limon peel extract is attributed to its multiple targets, especially when working in synergy with other molecules including flavonoids, terpenes, phenolic acids, and limonoids

Fig. Molecular Mechanism of Anti-inflammatory action

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway is a major role among the several pathways that cause inflammatory reactions [32]. It is considered to be the master switch regulating inflammation through the transcription of various pro-inflammatory genes. In a resting state, NF-κB stays inactive inside the cell as it binds to inhibitory proteins, namely the IκB complex. However, once an inflammatory stimulus (such as tissue damage, oxidative stress, microbial toxins, cytokines, or reactive oxygen species) activates the NF-κB complex, phosphorylation and the ensuing breakdown of the IκB complex take place [33]. TNF-α, IL-1β, IL-6, COX-2, iNOS, chemokines, and adhesion molecules are among the pro-inflammatory genes whose transcription is regulated by NF-κB when it enters the nucleus. Flavonoid compounds found in citrus fruits can suppress the action of NF-κB through inhibition of its activation, thus lowering the production of inflammatory mediators [34]. The second major pathway involves mitogen-activated protein kinases (MAPK), with several kinases including ERK, JNK, and p38 kinase as its members. MAPK kinases are activated when the

skin is exposed to oxidative stress, cytokines, lipopolysaccharides, and physical injury. Once activated and phosphorylated, these proteins act through transcriptional regulation and enhance the production of inflammatory mediators and cytokines. It has been demonstrated that citrus flavonoids and citrus peel extracts considerably reduce the phosphorylation of MAPK family members, resulting in decreased inflammatory gene expression



and thus preventing macrophages' activation, development of edemas, and infiltration of leukocytes. Therefore, this cascade is very important in localized dermal inflammation [35].

Another important mechanism of inhibitory effects is the inhibition of cyclooxygenase (COX) and lipoxygenase (LOX). Cyclooxygenase and lipoxygenase enzymes are used in inflammation to convert free fatty acids, particularly arachidonic acid, that are produced from membrane phospholipids. These enzymes convert arachidonic acid to prostaglandins, thromboxane, and leukotrienes, which induce vasodilation, vascular permeability, pain sensitivity, and chemotactic activity in white blood cells. Citrus limon peel substances have been proven to block the arachidonic acid metabolism pathway, leading to decreased formation of prostaglandins and leukotrienes. Two important compounds are d-limonene and α-terpineol [36].

PLANT PROFILE

Fig: Citrus lemon Taxonomical Classification

Kingdom	plantae
Division	Tracheophyta[37]
Class	Magnoliopsida
Order	Sapindales (Dicotyledas)
Family	Rutaceae[38]
Sub Family	Aurantioideae
Genus	Citrus
Species	Citrus X Limon[39]
Clade	Tracheophytes
Clade	Eudicots
Clade	Rosids[40]
Trib	Citreae
SubTrib	Citreae[41]
Indian Name	Limbu, Nimbu (Hindi), Dodda nimbe (kannada), Elumicchai (Tamil)[42]
Botanical Name	Citrus Limon

Table: Taxonomical Classification

Useful Parts:Fruit:

The Citrus limon fruits are highly appreciated for their usage in nutrition, medicine, and industries. They are full of citric

acid and vitamin C and are very helpful for human health. They are widely employed in drinks, preserving foods, pharmaceuticals, and cosmetics owing to their refreshing taste and antioxidative effect

The flesh of the fruits is generally incorporated into different types of foods, including jams and nutraceuticals. The peel, which is regarded to be the most valuable pharmaceutical component of the fruit, consists of several bioactive components like flavonoids (hesperidin and eriocitrin), as well as limonene. The peel has considerable anti-inflammatory, antioxidant, antimicrobial, and possible anti-Alzheimer effects [43].

Seeds:

Seeds are sources of fixed oils and limonoids that make them biologically active. The antimicrobial effects of these seeds and their insecticidal nature make them valuable. Furthermore, because of their therapeutic properties, lemon seeds have long been utilized in herbal medicine [44].

Leaves:

The leaves contain volatile oils and are often used for different reasons. Some common applications include using them in tea to get the freshness from the leaves, using them in aromatherapy to gain the soothing effect, and also for their medicinal value[45]

Flowers:

flowers provide a valuable source of essential oils that are extracted using techniques such as steam distillation. The flowers have fragrant compounds that give them the pleasing scent and healing properties. Essential oil that is extracted from the flowers of lemons has bioactive compounds that have relaxing, refreshing, and mood-lifting qualities[46].

Bark:

Bark from the lemon plants are known to contain bioactive substances like tannins and alkaloids, which have been found to possess significant pharmacological activity. An alkaloid is a compound synthesized naturally, and most of them display therapeutic action within the body. On the other hand, tannins are compounds classified as polyphenols and have been observed to have astringent, antibacterial, and antioxidant qualities [47].

Root:

Historically, the root of the lemon tree has been used in folk medicine owing to its healing potential, especially in treating inflammatory conditions and digestive disorders. It is thought that the roots contain bioactive substances that make them valuable from a medicinal perspective.

As part of inflammation treatment, the lemon root is applied because of its possible anti-inflammatory properties that can be useful for relieving inflammation and irritation and eliminating pain in the body. The roots are incorporated into various natural remedies designed to deal with inflammation. Lemon roots are used for treating digestive issues as well. They are thought to assist in digestion and alleviate symptoms such as indigestion. Overall, the roots play an important role in maintaining normal functioning of the gastrointestinal tract [48].

Wood:

Although not one of the important woods used as a source of timber, the wood from the lemon tree still finds useful applications, particularly in the rural areas. In particular, it is an ideal wood for use as fuel because it is easily combustible and serves to generate sufficient amounts of heat energy when burnt. Because of its reasonable hardness and availability, it can be used to manufacture simple wooden tools. Such tools include handles and simple household goods. This is besides the main functions of the lemon tree as a source of lemons and medicinal plants[49]

Medicinal properties and uses:

Medicinal properties:

Antioxidant

For example, lemon belongs to the citrus family that has flavonoids and vitamin C are antioxidants that stop oxidative harm from happening. by eliminating the free radicals. This ensures that there is no cell damage, thus preventing chronic disease conditions like heart diseases [50].

Anti-inflammatory

The anti-inflammatory properties of lemon help in reducing swelling. Its components are responsible for the inhibition of inflammatory substances; hence, it reduces pain and other symptoms caused by inflammation [51].

Antimicrobial

Lemon acts effectively as an antimicrobial against both fungi and bacteria. Lemon's essential oils and citric acid give it its antibacterial qualities [52].

Antidiabetic

The use of lemon assists in better glucose metabolism, which can regulate blood glucose levels. It lowers insulin resistance and can help with diabetes treatment [53].

Immunomodulatory

The immunomodulatory action of lemons comes from its ability to strengthen immune responses. Lemon's vitamin C component contributes to an improved immune response [54].

Medicinal Uses:

Fruit Juice

- Lemon juice acts as a powerful immunity booster due to its high vitamin C content, which fortifies the body's anti-infection defences.
- It is also antiscorbutic, indicating that it assists in the prevention and treatment of scurvy, a condition brought on by a lack of vitamin C, by maintaining healthy skin, gums, and blood vessels [55].

Peel

- The lemon peel contains flavonoids and essential oils that provide anti-inflammatory effects, helping reduce swelling and pain.
- It has strong antioxidant properties, protecting cells from oxidative damage.

- It also shows antimicrobial activity, assisting in preventing the development of dangerous microbes [56].

Essential Oil

- Lemon essential oil has antiseptic properties, making it useful for preventing infections and cleansing wounds.
- It is widely used for stress relief in aromatherapy, as its refreshing aroma helps in relaxation and improving mood [57].

Leaves

- The leaves of lemon can be used as a febrifuge as they help lower body temperature.
- Moreover, the leaves aid in proper digestion, thus they can also be used as digestive aids [58].

Chemicals and reagents:

Category	Chemical/Reagent	Purpose/Use
Solvents for Extraction	Ethanol (Analytical Grade)	Used for extraction of phytoconstituents from lemon peel [61]
Gel-Forming Agents	Carbopol 934	Polymer used for gel formation and viscosity increase
Neutralizing Agent	Triethanolamine (TEA)	Adjusts pH and neutralizes Carbopol to produce gel structure[62]
Preservatives	Methyl Paraben	Prevents microbiological development and enhances shelf life[63]
	Propyl Paraben	Acts as antibacterial preservative
Humectants	Glycerin	Moisturizing chemical that increases skin hydration
	Propylene Glycol	Enhances moisture retention and medication penetration[64]
Buffer Solutions	Phosphate Buffer (pH 6.8–7.4)	preserves physiological pH throughout preparation and assessment
Standard Drug	Diclofenac Sodium	Used as standard anti-inflammatory medication for comparison
	Ibuprofen	Alternative reference standard for anti-inflammatory action[65]

Table: Chemicals and reagents

Collection and authentication

Fresh and healthy lemons are picked for the experiment to ensure the quality of plant samples. The fruits are cleaned using distilled water to eliminate the presence of dust, dirt, and microbial contaminants on their surface. The peels are then isolated from the fruits using appropriate methods without

Seeds

- The seeds of lemon have anti-microbial properties which prevent bacteria from flourishing.
- Additionally, the seeds of lemon also possess insecticidal properties that deter or kill insects [59].

MATERIALS AND METHODS

plant materials:

Citrus limon’s fresh peels serve as the source material used in the experiment. The selection of lemons takes place very cautiously; only those which are healthy, free of disease, and have reached full maturity are selected to ensure good quality peels. Once obtained, these peels are stripped off the fruit by hand and washed thoroughly using distilled water [60].

causing any harm to their tissues. Proper authentication of the plant samples is performed to confirm their botanical identity. Fresh fruit of citrus limon (L) were collected from the garden of Samarth college of Agriculture, Deulgaon Raja. Maharashtra India, during the months of (Jan-2026) The fruits were selected with care to guarantee that they remained undamaged. fungal infection, or insect infestation.

Extraction of Lemon Peel Method: Soxhlet Extraction

1. Preparation of dried peel powder

The collected lemon peels are first shade-dried at room temperature to remove moisture while preventing degradation of heat-sensitive phytoconstituents. After complete drying, the peels are pulverized using a mechanical grinder to obtain a coarse powder. The plant material's surface area is increased via powdering, which enhances efficient solvent penetration and improves extraction of active constituents [66].

2. Placing powder in Soxhlet extractor

A certain amount of the powdered peel is carefully weighed using weighing balance and placed inside a thimble of filter paper/cellulose material. The powder-filled thimble is then positioned inside the Soxhlet extractor main body. The thimble should be tightly packed but with enough space for circulation of the solvent through siphon action.

3. Introduction of ethanol as an extraction solvent

The choice of ethanol as an extraction solvent is informed by its capability of dissolving both polar and semi-nonpolar phytochemicals found in lemon peel, including flavonoids, phenolic compounds, and essential oil components. A volume of ethanol is introduced into the round bottomed flask attached to the Soxhlet extraction apparatus.

4. Continuous Heating and Extraction (6 – 8 Hours)

The round bottom flask is heated by placing it in a heating mantle. As the heat is applied, the ethanol gets vaporized and moves up through the distillation arm where it condenses in the condenser. The solvent that condenses keeps washing the herbal matter in the extraction chamber until the chamber fills up to a certain extent. As soon as the chamber reaches its maximum level, the solvent with dissolved matter is siphoned back to the flask.

5. Collecting and Filtration of Extract

When the extraction process ends, the flask containing the ethanolic extract is cooled down. Then, the filtrate is filtered through Whatman paper in order to isolate any suspended matter and debris from the plant.

6. Concentration through rotary evaporator

After filtering out the extract, A rotary evaporator is then used to concentrate it under low pressure. The ethanol is removed from the mixture at low temperatures to prevent degradation of heat-sensitive substances [67].

7. Semi-solid crude extract isolation

Once the total amount of the solvent is evaporated from the reaction mixture, the semi-solid crude extract from Citrus limon peel can be isolated. After determining the weight, the isolated extract is packed in a sterile vial and kept in a refrigerated environment for subsequent processing

Extraction Objective

The primary objective of the extraction procedure is to extract biologically active compounds present in the lemon peel, which contribute to their medicinal and anti-inflammatory activities. This step enables the separation of the active phytochemicals from the plant matrix so that they can be put into use for further applications.

Isolation of Flavonoids (Hesperidin and Eriocitrin)

Flavonoids are essential polyphenols that occur naturally in lemon peel. Hesperidin and eriocitrin are two flavonoids with strong anti-inflammatory and antioxidant properties. The flavonoids neutralize free radicals, protect from oxidative damage, and inhibit the inflammatory factors causing swelling and cell damage. Through isolation, the concentration of these active phytochemicals can be increased, thus facilitating their use as ingredients of gels [68].

Isolation of Limonene

Limonene is an important bioactive molecule that is the main constituent of citrus fruits' essential oils like lemon peels. Limonene possesses anti-inflammatory, anti-microbial, and protective effects on the skin. In addition, limonene plays a key role in enhancing penetration, allowing the active components to penetrate deeper into the skin tissues [69].

Extraction of Phenolic Substances

The phenolic substances that are extracted from lemon rind have been known to possess various properties that make them antioxidants as well as having protective qualities. This is because they prevent lipid peroxidation as well as minimizing cellular damage while promoting tissue repair through regulating inflammatory reactions. The extraction of the phenolic substances makes the crude extract medicinally valuable and is very essential in improving the anti-inflammatory gel formulation [70].

Generally, the extraction of phenolic substances is done in order to get a crude extract containing flavonoids, limonene, and phenols [71][72]

Table: Composition of Citrus lemon Peel Gel Formulations

Ingredients (% w/w)	Function	F1	F2	F3
Citrus lemon Peel Extract	Active anti-inflammatory agent	2.0	3.0	4.0
Carbopol 934	Gelling agent	1.0	1.0	1.0
Glycerin	Humectant/Moisturizer	5.0	5.0	5.0
Methyl Paraben	Preservative	0.20	0.20	0.20
Propyl Paraben	Preservative	0.05	0.05	0.05
Triethanolamine	pH Adjuster	q.s.	q.s.	q.s.

Distilled Water	Vehicle	q.s. to 100	q.s. to 100	q.s. to 100
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Anti-inflammatory activity evaluation In-vivo methods (Animal Studies) [73], [74]

Carrageenan-Induced Paw Edema

The carrageenan-induced paw edema test is among the most widely used in-vivo experimental procedures that have been utilized in studying the anti-inflammatory effects of herbal products such as the gel extracted from the peel of Citrus limon. The procedure determines the capability of the herbal product in inhibiting induced inflammation.

Procedure:

1. Selection of Experimental Animals

- Healthy rats will be selected for conducting the experiment to guarantee accurate results.
- The selection of rats having identical weights and ages ensures reduction in biological variability that may affect the inflammation process
- The use of uniform rats is necessary for making correct comparison among control, standard, and test groups.

2. Acclimatization of Animals

- Before the experiment, the chosen rats are subjected to acclimatization to the laboratory environment for a few days.
- The animal is kept in normal environmental conditions like:
 - Correct temperature and humidity
 - Proper lighting
 - Sufficient ventilation
 - Ready access to adequate food and water
- Acclimatization is essential to reduce stress in the animal and provide it the opportunity to adjust to the experiment environment.

3. Experimental Grouping

- Once acclimatized, the rats are grouped randomly to compare the efficacy of anti-inflammatory activity.
- Rats in each group have the same number of individuals in order to be accurate with experiments.

4. Control Group

- No substance is administered to this group, except the base without the actual Citrus limon peel extract.
- It helps to determine how the inflammation reaction will take place normally in absence of treatment.

- The control group serves as a baseline for comparison with treated groups.

5. Standard Group (Diclofenac)

- The animals under this group will be administered Diclofenac, which is a common anti-inflammatory drug.
- Diclofenac serves as a standard to assess the efficacy of the herbal gel formula.
- The output from this group verifies that the test model is valid.

6. Test Group (Lemon Peel Extract)

This is the test group that will be administered with the gel that contains the extract from citrus limon peels. This test group's objective is to evaluate the herbal product's ability to reduce inflammation. The decrease in paw edema is an indication of the efficacy of the lemon peel extract.

7. Importance in Research Study

- Scientific validity and reproducibility are assured by correct choice and groupings.
- The correct comparison between untreated, standardly treated, and test treated animals is assured.
- Aids in establishing the anti-inflammatory effects of the formulation.

RESULT AND DISCUSSION

Plant materials:

Fresh fruit of citrus limon (L) were collected from the garden of Samarth college of Agriculture, Deulgaon Raja. Maharashtra India



Fig. Citrus Lemon

Organoleptic Evaluation of Citrus Lemon peel

Table: Organoleptic Evaluation of Citrus Lemon peel

Evaluation parameter	Results
Color	Yellow to yellowish-green
Odor	Strong, characteristic citrus aroma

Taste	Slightly bitter and aromatic
Shape	Irregular curved pieces of peel
Texture	Rough outer surface and spongy inner surface
Surface	Presence of oil gland on outer peel

Table: Organoleptic Evaluation of Citrus Lemon peel

Phytochemical screening test:

Sr.no	Phytochemical constituents	Name of test	observation	Result
1	Alkaloid	Hager test	The test solution mixed with the sodium hydroxide solution. The Formation of yellow color precipitate which confirms the presence of alkaloids.	+
2	Steroids	Salkowski test	The test solution was mixed with 1ml of chloroform. Then added carefully 1ml of concentrated sulphuric acid and shaken gently. A reddish -brown color in the chloroform layer and green fluorescence in the acid layer confirm the presence of the steroidal ring.	+
3	Flavonoids	Alkaline reagent test	Test solution mixed with 20% sodium hydroxide solution. Then added dilute HCL, a yellow color forms and disappears on the addition of HCL which indicate the presence of flavonoids.	+
4	Saponin	Foam test	The test solution was mixed with 5mL of distilled water in the test tube and shaken vigorously. The formation of stable foam indicates presence of saponin.	-
5	Glycosides	Keller-killiani test	One ml of the plant extract was treated with 2mL glacial acetic acid containing a indicates the confirmation of keller-kiliani test.	+
6	Tannins	Ferric chloride test	The test solution was mixed with 2mL of ferric chloride. Formation of a dark brown color indicates the presence of tannins.	+




				
7	proteins	Xanthoproteic test	The test solution was mixed with 1ml of concentrated nitric acid and boiled. A yellow color precipitate was formed. After cooling, sodium hydroxide solution was added. Formation of orange color confirms proteins	+ 
8	Phenolic compounds	Ferric chloride test	The test solution was mixed with 2ml of ferric chloride. Formation of blue or green color indicates the presence of phenolic compounds.	+ 

Table: Phytochemical screening test

- Lemon peel extract

Determination of ash value Procedure

A dry, clean silica crucible was precisely weighed and designated as W1. The crucible was filled with two to three grams of air-dried citrus limon peel powder, and the weight was once more noted as W2. After that, the crucible was heated in a muffle furnace at 500–600°C until the sample was completely incinerated and white or gray ash was obtained, indicating the removal of organic matter. After incineration, the crucible was carefully removed and cooled in a desiccator for about 30 minutes. Finally, after weighing the ash-filled crucible, the final weight was noted as W3.

Formula for Total Ash Value

$$\text{Total Ash Value (\% w/w)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where:

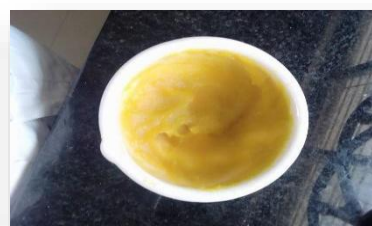
- W1/W1 = Weight of empty crucible
- W2/W2 = Weight of crucible + sample before ignition
- W3/W3 = Weight of crucible + ash

Fig. Ash Value

Thin Layer Chromatography (TLC)

Requirements

- TLC plate (Silica gel 60 F254)
- Capillary tubes
- Developing chamber
- Solvent system (mobile phase)



- UV chamber or iodine chamber

Procedure

To create a transparent solution, a tiny amount of ethanolic Citrus limon peel extract was dissolved in either ethanol or methanol and thoroughly mixed. The TLC developing chamber was filled with the mobile phase solvent, sealed, and given 15 to 20 minutes to saturate. Using a capillary tube, a tiny amount of the extract was applied to a pencil line that was drawn about 1 cm above the TLC plate's lower edge. The line was subsequently allowed to dry. The solvent was allowed to climb upward by capillary action after the TLC plate was carefully positioned inside the chamber with the spot above the solvent level. The plate was removed, the solvent front was promptly noted with a pencil, and the plate was dried when the solvent front approached the top of the plate. The produced spots were then seen using an iodine chamber or UV light at 254 or 366 nm, where various chemicals showed up as distinct spots.

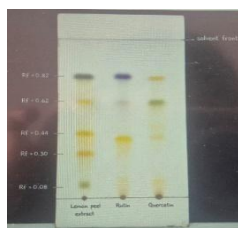


Fig. TLC plate

Formulation of Gel

The gel containing *Citrus limon* peel extract was prepared using Carbopol as a gelling agent. The gel base was created by dispersing Carbopol in distilled water and allowing it swell completely. With constant stirring, the lemon peel extract was separately dissolved and added to the hydrated gel.

Preservatives and glycerin were added to improve stability and moisture retention. Finally, triethanolamine was added dropwise to adjust the pH and convert the dispersion into a smooth, homogeneous gel. The prepared gel was packed in airtight containers for further evaluation of anti-inflammatory



activity.

Fig. Formulation of Gel Physical Evaluation of Gel

Parameter	Observation
Colour	Light yellow
Odour	Characteristic lemon odour
Appearance	Smooth
Homogeneity	Good

Table: Physical Evaluation of Gel pH of Gel

Formulation	pH
Lemon peel gel	5.0 – 7.0

Table No: 08 pH of Gel Viscosity Result

Formulation	Viscosity	Observation
F1	18,500 ± 120 cP	Moderate viscosity, good spreadability
F2	22,300 ± 150 cP	Optimum viscosity, smooth and stable gel
F3	26,700 ± 140 cP	High viscosity, slightly thick consistency

Table: Viscosity Result Drug Content Uniformity

Formulation	Drug content
Lemon gel	95–99 %

Table: Drug Content Uniformity

Anti-Inflammatory Activity Result

Paw Volume Observation

Anti-Inflammatory Activity [Paw Volume Result Table (Using Vernier Caliper)] Observation Time Intervals

- 0 hr — Before carrageenan injection

- 1 hr — Initial inflammation phase
- 2 hr — Development phase
- 3 hr — Peak inflammation
- 4 hr — Late phase inflammation

Paw Thickness Result Table (Measured by Vernier Caliper)

Group	Treatment	0 hr (mm)	1 hr (mm)	2 hr (mm)	3 hr (mm)	4 hr (mm)
Control	Carrageenan only	3.20	4.85	5.60	6.10	5.95
Standard	Diclofenac gel	3.18	4.10	4.45	4.20	3.84
F1	Lemon peel gel	3.21	4.35	4.90	4.60	4.25

F2	Lemon peel gel	3.19	4.15	4.55	4.30	3.95
F3	Lemon peel gel	3.20	4.50	5.05	4.75	4.40

**Table No: 12 Paw Thickness Result Table
Anti-Inflammatory Activity [Paw Volume Result Table
(Measured by Mercury Plethysmograph)]**

Observation Time Intervals

- 0 hr – Before carrageenan injection (normal paw volume)

- 1 hr – Early phase inflammation
- 2 hr – Progressive edema
- 3 hr – Peak inflammatory response
- 4 hr – Late phase inflammation

Paw Volume Result Table (Mercury Plethysmograph Method)

Group	Treatment	0 hr (ml)	1 hr (ml)	2 hr (ml)	3 hr (ml)	4 hr (ml)
Control	Carrageenan only	0.52	0.88	1.18	1.35	1.30
Standard	Diclofenac gel	0.51	0.70	0.82	0.78	0.65
F1	Lemon peel gel	0.50	0.76	0.96	0.91	0.84
F2	Lemon peel gel	0.51	0.72	0.85	0.80	0.71
F3	Lemon peel gel	0.52	0.79	0.99	0.94	0.86

Table: Paw Volume Result Table

DISCUSSION

The aim of the current study was to create, assess, and look into the anti-inflammatory properties of a herbal gel that contained extract from citrus lemon peel. The findings of the study indicate that lemon peel possesses significant pharmacological potential due to the existence of several bioactive phytoconstituents, including flavonoids, limonene, hesperidin, eriocitrin, phenolic compounds, and vitamin C. These components are widely known for their anti-inflammatory and antioxidant properties, which may help to lessen tissue swelling and inflammation.

The extraction of lemon peel was successfully performed using the Soxhlet extraction method with ethanol as a solvent. Ethanol was selected because of its ability to extract several polar and semi-polar phytoconstituents efficiently. The obtained extract showed the presence of important secondary metabolites during phytochemical screening. It was discovered that the main active ingredients with anti-inflammatory properties were flavonoids and phenolic compounds. These compounds may inhibit the synthesis and reduction of inflammation by the release of inflammatory mediators like prostaglandins, histamine, serotonin, and cytokines.

CONCLUSION

The present study on the “Formulation and Evaluation of Anti-Inflammatory Activity of Citrus Lemon Peel” successfully demonstrated the pharmaceutical and therapeutic potential of lemon peel as a organic and natural source of anti-inflammatory agents. The research was carried out with the objective of preparing a topical gel formulation containing extract of *Citrus limon* peel and evaluating its anti-inflammatory activity along with different physicochemical parameters. The study's conclusions validate the custom of using citrus peel to treat skin conditions and irritation..

In this research work, fresh peels of *Citrus limon* were gathered, dried, ground into a powder, and extracted using ethanol as the solvent in the Soxhlet extraction process. The extraction process efficiently isolated various bioactive phytoconstituents present in the lemon peel. Important secondary metabolites like flavonoids,

phenolic compounds, tannins, alkaloids, and terpenoids have been identified by phytochemical screening. These phytoconstituents are well known for their antioxidant and anti-inflammatory properties. By preventing the release of inflammatory mediators, flavonoids and phenolic compounds are important in reducing inflammatory responses.

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