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Formulation and Evaluation of Herbal Anti-Inflammatory Cream from Aegle Marmelos

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ABSTRACT

Herbal-based topical formulations are increasingly recognized as safer and more biocompatible alternatives to synthetic anti-inflammatory agents. In line with this growing interest, the present study focuses on the development of a naturally derived anti-inflammatory cream utilizing the extract of Aegle marmelos, a medicinal plant widely valued in traditional systems for its therapeutic potential. This research highlights the importance of incorporating phytochemical-rich herbal ingredients into modern topical delivery systems to achieve effective, stable, and skin-friendly formulations.In this study, Aegle marmelos leaves were collected, processed, and subjected to Soxhlet extraction using eth.anol as the solvent. Phytochemical screening of the extract confirmed the presence of key bioactive compounds such as flavonoids, phenolics, tannins, alkaloids, saponins, glycosides, and terpenoids—constituents known to exhibit potent anti-inflammatory and antioxidant properties. A herbal cream formulation (F1) was successfully prepared as an oil-in-water (O/W) emulsion using optimized conditions and a combination of pharmaceutical excipients including stearic acid, cetostearyl alcohol, beeswax, coconut oil, glycerin, and triethanolamine.The prepared formulation was subjected to comprehensive evaluation, covering organoleptic characteristics, physicochemical parameters, physical stability, skin safety, and therapeutic efficacy. The cream displayed desirable properties such as smooth consistency, homogeneous appearance, pleasant herbal odor, and a skin-compatible pH of 6.7. It demonstrated excellent spreadability, easy washability, and no signs of phase separation. Stability studies conducted over 60 days further confirmed the robustness and shelf-life potential of the formulation, showing no significant change in pH, color, consistency, or texture. Skin irritation studies verified that the cream was non-irritant and safe for topical application.The in vitro anti-inflammatory activity, assessed using the protein denaturation method, revealed significant inhibition, thereby confirming the therapeutic effectiveness of the Aegle marmelos extract.Overall, the findings establish that the developed herbal anti-inflammatory cream is safe, stable, non-irritant, and pharmaceutically acceptable. The study strongly validates the use of Aegle marmelos as an effective natural alternative for topical management of inflammation and provides a promising foundation for future herbal-based dermatological formulations.

Keywords: Aegle marmelos, herbal cream, anti-inflammatory, topical formulation, phytochemicals, Soxhlet extraction, oil-in-water emulsion, stability, skin safety, protein denaturation assay.

INTRODUCTION

Overview of Anti-inflammatory Creams: -

An anti-inflammatory cream is a topical semisolid dosage form designed to deliver active pharmaceutical ingredients directly to the site of inflammation. These preparations are applied externally to the skin to reduce swelling, redness, and pain, thereby providing local relief without significant systemic exposure. Anti-inflammatory creams are among the most widely prescribed dermatological and musculoskeletal formulations due to their convenience, effectiveness, and minimal side effects. Such creams may contain synthetic agents like corticosteroids and non-steroidal anti- inflammatory drugs (NSAIDs), or natural plant-based extracts possessing anti- inflammatory and antioxidant properties. The topical route bypasses the gastrointestinal tract and hepatic metabolism, ensuring that the drug acts locally where it is needed most. Additionally, anti-inflammatory creams enhance patient compliance because of their ease of application, pleasant texture, and fast onset of action.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

A typical anti-inflammatory cream comprises:

- Active ingredient: Provides therapeutic effect (e.g., hydrocortisone, diclofenac, or herbal extracts).
- Base: A suitable emulsion system (O/W or W/O) to facilitate spreading and absorption.
- Emulsifier: Ensures physical stability and consistency.
- Humectants and emollients: Maintain skin hydration and smoothness.
- Preservatives and antioxidants: Prevent microbial growth and degradation.
- Fragrance and coloring agents: Improve aesthetic and patient acceptability.

The formulation goal is to develop a stable, non-irritating, cosmetically elegant product that provides effective relief with minimal adverse reactions.

Inflammation and Anti-inflammatory Agents

Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants. It is a protective mechanism that eliminates the initial cause of injury, removes necrotic cells, and initiates tissue repair. However, when this process becomes chronic, it leads to conditions such as arthritis, dermatitis, and psoriasis.

The inflammatory response typically involves five cardinal signs:

1. **Rubor (Redness)** – due to vasodilation and increased blood flow.
2. **Calor (Heat)** – resulting from enhanced metabolic activity and perfusion.
3. **Tumor (Swelling)** – caused by plasma exudation and cellular infiltration.
4. **Dolor (Pain)** – due to prostaglandin and bradykinin release.
5. **Functio laesa (Loss of function)** – impairment due to tissue damage and swelling. Anti-inflammatory agents work by interfering with the biochemical mediators responsible for these processes. These agents are broadly classified as:

- **Corticosteroids:** Suppress multiple inflammatory pathways by inhibiting phospholipase A2 and reducing prostaglandin and leukotriene synthesis.
- **NSAIDs:** Inhibit cyclooxygenase (COX) enzymes, reducing prostaglandin production and thereby alleviating pain and swelling.
- **Herbal Agents:** Contain phytochemicals such as flavonoids, tannins, and alkaloids that exhibit anti-inflammatory, antioxidant, and wound-healing properties.

In the modern era, herbal anti-inflammatory preparations are preferred due to their safety, biocompatibility, and lower incidence of adverse reactions compared to synthetic agents.

Cream as a Dosage Form

A cream is a smooth, viscous, semisolid emulsion containing both an aqueous and an oily phase. It serves as a vehicle for topical application of therapeutic agents, providing hydration and protection to the skin. Creams are classified mainly into two types:

1. Oil-in-Water (O/W) Creams: The oil phase is dispersed in water, producing non- greasy, easily washable formulations ideal for cosmetic and medicinal purposes.
2. Water-in-Oil (W/O) Creams: The water phase is dispersed in oil, producing greasy and occlusive preparations that enhance hydration of dry skin.

Advantages of creams as dosage forms include:

- Enhanced patient comfort and acceptability due to smooth texture.
- Localized drug delivery directly to the affected area.

- Reduced systemic side effects compared to oral therapy.
- Ability to incorporate a wide range of drugs, including hydrophobic and hydrophilic compounds.
- Ease of removal from the skin after application.

Creams are ideal for dermal and transdermal delivery because they promote controlled release of drugs, improve bioavailability, and allow for formulation flexibility.

Advantages of Anti-inflammatory Creams

Anti-inflammatory creams offer several benefits over systemic therapy:

- Localized action: Targets inflammation directly at the site, reducing systemic exposure.
- Rapid relief: Fast onset due to direct absorption through the epidermis.
- Reduced side effects: Avoids gastrointestinal irritation and hepatic first- pass metabolism.
- Enhanced patient compliance: Non-invasive, easy to apply, and cosmetically acceptable.
- Versatility: Can be used for both acute and chronic inflammatory conditions.
- Protective and soothing: Cream bases often contain emollients that prevent dryness and protect damaged skin.

These advantages make topical anti-inflammatory creams the preferred dosage form for treating localized inflammatory disorders.

Advantages of Herbal Creams

Herbal creams have gained immense attention as they combine traditional medicinal wisdom with modern formulation science. Their growing popularity is attributed to:

1. Safety and biocompatibility: Natural plant extracts are less likely to cause allergic or irritant reactions.
2. Multiple pharmacological actions: Herbs often exhibit anti-inflammatory, antioxidant, antimicrobial, and wound-healing activities simultaneously.
3. Cost-effectiveness: Raw materials are inexpensive and widely available.
4. Sustainability: Derivedfrom renewable plant sources,supporting eco- friendly production.
5. Minimal side effects: Suitable for long-term or repeated use without systemic toxicity.

Thus, herbal anti-inflammatory creams represent a safer and greener alternative to synthetic products.

Ideal Properties of a Cream: -

An ideal topical cream should:

- Possess effective anti-inflammatory and analgesic properties.
- Exhibit smooth, homogeneous, and non-gritty texture.
- Spread easily without excessive greasiness or stickiness.
- Show good stability under various storage conditions.
- Have pH compatible with skin (approximately 5.5–7.0).
- Be non-irritating, non-sensitizing, and hypoallergenic.
- Permit satisfactory drug release and skin penetration.
- Retain aesthetic appeal, with pleasant color and odor.



Fig.No.1: Anti-inflammatory Creams

History and Evolution of Anti-inflammatory Creams

The evolution of anti-inflammatory topical formulations can be traced through four major phases:

a. **Ancient and Traditional Period**: Ancient civilizations such as the Egyptians, Indians, and Chinese used herbal pastes, oils, and poultices for treating wounds and inflammation. Ingredients like turmeric, sandalwood, aloe vera, and camphor were widely used for their soothing and healing properties.

b. **19th Century:** Scientific isolation of active constituents such as salicin from willow bark in 1828 (precursor of aspirin) laid the foundation for modern anti-inflammatory therapy. The invention of petroleum jelly (Vaseline) in 1872 revolutionized semisolid formulations by providing a stable, inert base.

c. **20th Century:**

- 1950s: Introduction of topical corticosteroids such as hydrocortisone revolutionized dermatological therapy.
- 1970s–1980s: Development of NSAID-based gels and creams such as diclofenac and ketoprofen improved patient compliance.
- Late 20th century: Emergence of emulsion and liposomal drug delivery systems for enhanced penetration and sustained release.

d. 21st Century: Focus has shifted to herbal, nanoemulsion, and transdermal systems that combine traditional extracts with modern delivery technologies. Emphasis is now placed on biocompatibility, sustainability, and consumer safety, leading to renewed interest in plant-derived anti-inflammatory agents.

1.7 Selection of Aegle marmelos :-

Aegle marmelos (Family: Rutaceae), commonly known as Bael, is a medicinal plant extensively used in Ayurveda. All parts of the plant—leaves, fruit, bark, and roots— possess therapeutic properties, but the leaves are particularly known for their anti-inflammatory, antioxidant, and wound-healing activities.

The leaves of *Aegle marmelos* are rich in a variety of **bioactive phytoconstituents** that contribute to its diverse therapeutic properties. The major constituents include **alkaloids**, such as aegeline, which are known for their potent anti-inflammatory and antioxidant effects. The plant also contains abundant **flavonoids**, notably rutin and quercetin, which play a crucial role in scavenging free radicals, reducing oxidative stress, and enhancing tissue repair. Additionally, the presence of **tannins and coumarins** provides astringent and antimicrobial properties that aid in wound contraction and protection against microbial invasion. **Saponins and glycosides** further contribute to the plant's pharmacological profile by exhibiting anti-inflammatory, analgesic, and immunomodulatory effects.

Due to this rich phytochemical composition, *Aegle marmelos* exhibits multiple **pharmacological activities**, including **anti-inflammatory, antimicrobial, antioxidant, analgesic, hepatoprotective,** and **wound-healing properties**. These combined actions make it a promising candidate for use in the formulation of topical anti-inflammatory preparations, where it can promote healing, reduce inflammation, and protect the skin from oxidative damage.

Reasons for selection:

1. Scientifically validated anti-inflammatory potential in various in vivo models.
2. Traditional use in treating wounds, swelling, and skin infections.
3. Availability, low cost, and minimal toxicity.
4. Rich source of bioactive compounds suitable for topical delivery.

Hence, *Aegle marmelos* leaf extract represents an effective, natural alternative for developing a stable and efficient anti-inflammatory cream.

Structure and Function of Skin

The skin is the largest organ of the human body, accounting for about 16% of total body weight and covering an area of approximately 1.8 square meters in an average adult. It serves as a multifunctional protective barrier between the internal organs and the external environment. The skin provides mechanical protection, prevents water loss, and acts as the first line of defense against microbial invasion, chemical substances, and ultraviolet radiation. In addition to protection, it also performs important roles in temperature regulation, sensory perception, vitamin D synthesis, and excretion of certain waste products through perspiration. Structurally, the skin is composed of three principal layers—the epidermis, dermis, and hypodermis (subcutaneous tissue)—each with distinct histological features and physiological functions. The harmonious interaction among these layers ensures the maintenance of homeostasis and protection of the body from external insults.

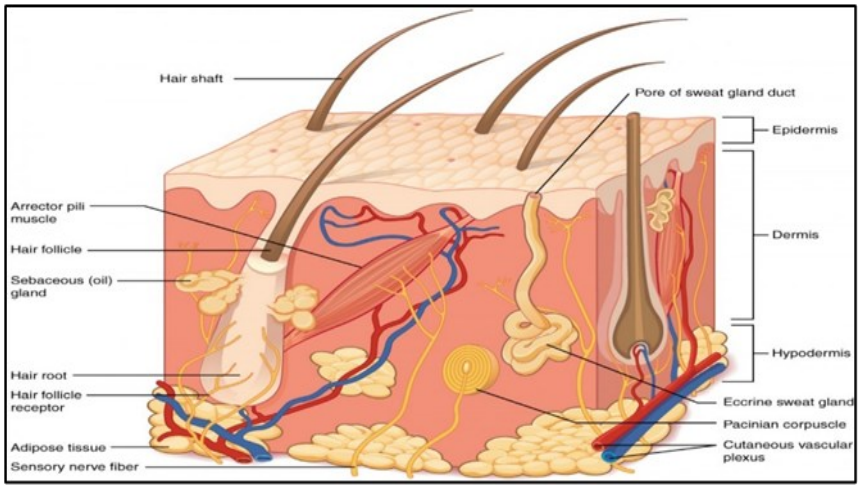


Fig.No.2: ANATOMY OF SKIN

Epidermis

The epidermis is the outermost and thinnest layer of the skin, composed mainly of stratified squamous epithelial cells. Its thickness varies depending on body site—being thicker on the palms and soles (about 1.5 mm) and thinner on the eyelids and lips (about 0.05 mm). As an avascular layer, it obtains nutrients by diffusion from the dermis below. The main cell type is the keratinocyte (≈90%), with other specialized cells including melanocytes (produce melanin for UV protection), Langerhans cells (immune defense), and Merkel cells (sensory function). The epidermis forms a physical, chemical, and biological barrier that prevents fluid loss and protects against pathogens. It undergoes continuous renewal, with cells migrating from the base to the surface over about 28 days.

LAYERS OF THE EPIDERMIS

The epidermis has five layers from the deepest to the outermost:

1. Stratum Basale (Basal Layer): Single layer of dividing cells that generate new keratinocytes. It also contains melanocytes and Merkel cells,

playing a vital role in regeneration and wound healing.

2. Stratum Spinosum (Prickle Cell Layer): Several layers of keratinocytes joined by desmosomes, providing strength and flexibility. Langerhans cells here offer immune protection.

3. Stratum Granulosum (Granular Layer): Flattened cells containing keratohyalin granules and lipid bodies that form a waterproof barrier and reduce transepidermal water loss (TEWL).

4. Stratum Lucidum (Clear Layer): Found only in thick, hairless skin (palms and soles), this translucent layer enhances protection against friction.

5. Stratum Corneum (Horny Layer): The outermost layer, made of dead, keratinized cells embedded in lipids, forming the main protective barrier. Its "brick-and-mortar" structure regulates hydration and controls drug permeability, making it the key layer in transdermal absorption.

Dermis

The dermis lies beneath the epidermis and provides strength, elasticity, and nourishment. It is much thicker, containing collagen and elastin fibers, blood vessels, lymphatics, nerve endings, hair follicles, and glands.

It consists of two regions:

- **Papillary layer:** Thin upper layer with loose connective tissue and capillaries that nourish the epidermis.
- **Reticular layer:** Dense lower layer with collagen and elastin providing tensile strength and resilience.

The dermis plays an important role in temperature regulation and in the absorption and diffusion of drugs once they cross the epidermis.

Hypodermis (Subcutaneous Tissue)

The hypodermis, or subcutaneous layer, connects the skin to underlying muscles and bones. It is composed mainly of adipose tissue and loose connective tissue, along with larger blood vessels and nerves.

Its major functions include:

- Thermal insulation and temperature regulation
- Cushioning against mechanical shocks
- Energy storage in fat cells
- Anchoring of skin to deeper tissues

Although not directly involved in drug absorption, the hypodermis influences drug distribution and systemic delivery for formulations intended for deeper penetration.

DRUG PROFILE:



Fig.No.3.1: Aegle marmelos Plant

Biological Source:

The fresh or dried, mature leaf of *Aegle marmelos* is its biological source. The scientific name of *Aegle marmelos* is Aegle marmelos (L.) Corradi belongs to the family *Rutaceae*. The leaves are trifoliate (three leaflets per leaf), aromatic, and are usually harvested when fully mature. They may be shade-dried for use in herbal formulations. It is also referred to as Bael, Bilva (in Sanskrit), Bel Patra (in Hindi), or Wood Apple (in English).

Taxonomical Classification of Aegle Marmelos:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade:Rosids

Order: - Sapindales

Family: - *Rutaceae*

Genus: - Aegle.

Species: Aegle marmelos.

Morphological Features:

The Aegle marmelos plant is a medium-sized deciduous tree that can grow up to 12–15 meters in height. The leaves are compound and trifoliate, consisting of three distinct leaflets attached to a common petiole. The leaflets are alternate and lanceolate to ovate in shape, with an entire margin and a pointed (acute) apex. The surface of the leaves is smooth (glabrous) and leathery in texture, with a distinct aromatic smell when crushed due to the presence of essential oils. Each leaflet is 5–10 cm long and 2–5 cm wide. The central leaflet is larger than the lateral ones. Leaves are attached to the stem by a long petiole (2–4 cm), and stipules are absent. The plant produces fragrant white or greenish- white flowers, usually found in axillary panicles or cymes. The flowers are bisexual, with five petals and a small, cup-shaped calyx. The corolla is tubular, and the stamens are numerous. The fruit is a large, round to oval berry, hard-shelled, initially green, and turns yellow upon ripening. It contains a mucilaginous pulp with numerous seeds.

Organoleptic (Sensory) Characteristics:

1. **Color**: Fresh Leaves: Bright green to dark green
2. **Dried Leaves**: Pale green to yellowish-green
3. **Oduor**: Strongly aromatic when crushed, due to the presence of volatile oils like limonene and eugenol
4. **Taste:** Bitter and astringent (t titer and astringent (typical of many medicinal leaves)

Macroscopic (Visible) Morphology:

Type of Leaf:

a) **Category:** Compound leaf

b) **Leaf Type:** Trifoliate (each leaf consists of three leaflets)

c) **Phyllotaxy (Arrangement**) : Alternate on stem

d) **Leaf Attachment:** Petiolate (with a distinct petiole)

e) **Venation:** Reticulate (net-like vein arrangement)

f) **Stipules:** Absent

Leaflet Morphology:

a) **Number of Leaflets:** 3 per leaf (trifoliate)

b) **Shape**: Ovate to lanceolate (broadest below the middle, tapering at both ends)

c) **Apex:** Acute to obtuse (pointed to rounded tip)

d) **Base**: Cuneate (wedge-shaped)

e) **Margin**: Entire (smooth, not serrated)

f) **Surface**: Glabrous (smooth, non-hairy) on both adaxial (upper) and abaxial (lower) sides

g) **Texture**: Leathery (coriaceous), thick and firm to touch

h) **Color Gradient**: Young Leaves: Lighter green, soft and tender Mature Leaves: Darker green, firm and tough.

CHEMICAL CONSTITUENTS OF AEGEL MARMELOS:

Aegle marmelos, commonly known as bael, contains a diverse array of chemical constituents, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, and coumarins. Notably, marmelosin, a coumarin derivative, is a key bioactive compound with antioxidant, antidiabetic, and hepatoprotective properties. Other coumarins like imperatorin and psoralen have shown antimicrobial and anti-

inflammatory effects. The fruit also contains various vitamins (A, B, C), minerals (calcium, phosphorus, potassium, iron), and essential oils. The pulp of the bael fruit is rich in bioactive compounds like carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids, and terpenoids. Specific alkaloids include aegeline, aegelenine, and fragine. The leaves contain tannins, including skimmianine. The fruit also contains essential oils, with γ-Cadinene, δ-carene, and α-pinene exhibiting antifungal activity. Furthermore, bael fruit is a source of vitamins such as riboflavin, vitamin B1, vitamin A, vitamin B2, ascorbic acid, vitamin C and thiamine.

1. BESSWAX:



Fig.No-3.2: BESSWAX

Table No.1: Properties of Bees Wax

IUPAC Name	Hexadecenoicacidtriacontylester
Molecure Weight	600–1000g/mol
Appearance	Yellow to brown solid(refined is white)
Odour	Mild honey like
Melting Point	62– 64^{0}C
Density	0.95– 0.98 g/cm^3
AcidValue	17 -24
Solubility	Insoluble in water, soluble in oils, ether

USES:

1. **Cosmetics**: Emulsifier, emollient, and fragrance in soap sad perfumes.

2. **Pharmaceuticals**: Tablet coating, and ingredient in ointments.

3. **Food Industry**: Food additive (E901) to provide shine, film for wrapping cheese, stiffening agent.

4. **Industrial**: Thickener, emulsifier, and stiffen in gagent in cosmetics, polish ingagent, waterproofing, and lubricant.

5. **Traditional Uses**: Batik fabrics, lost-wax casting, water proofing wood and leather,

Streng thening threads, candle-making.

2.Steraic Acid



Fig.No-3.3: Steraic Acid

Table No.2: Properties of Steraic Acid

IUPAC Name	Octadecanoic acid
Molecure Weight	284.484g/mol
Appearance	White solid (flakes o waxy substance)
Odour	Odorless or mild fatty odor
Melting Point	69.3 °C (156.7 °F)
Density	0.95– 0.98 g/cm^3
Acid Value	194 to 212 mg KOH/g
Solubility	Insoluble in water, soluble in oils, ether
Bioling Point	361 °C

Uses:

1 Stearic Acid for Skin: Stearic acid is a popular ingredient in skincare products due to its moisturizing and emollient properties. It helps strengthen the skin's barrier, lock in moisture, and reduce inflammation. Many creams, lotions, and soaps contain stearic acid to improve texture and stability.

2 Stearic Acid in Cosmetics: In cosmetics, stearic acid acts as a thickening agent and emulsifier. It is used in products like foundations, lipsticks,

and mascaras to provide a smooth, creamy consistency.

3 Stearic Acid in Food: As a food additive (E570), stearic acid is used in baked goods, frozen dairy products, and candies. It acts as a stabilizer, emulsifier, and anti-caking agent.

4 Stearic Acid in Soap Making: Stearic acid is a key ingredient in soap production. It helps create a hard, long-lasting bar of soap with a rich lather. Many soap recipes use stearic acid flakes or powder for consistent results.

5 Stearic Acid for Candle Making: In candle production, stearic acid is added to wax to improve hardness, opacity, and burn time. It is often used in combination with paraffin or beeswax.

6 Industrial Applications: Stearic acid is used in the production of rubber, plastics, and metalworking fluids. It acts as a lubricant, release agent, and softening agent.

7 Stearic Acid in Pharmaceuticals In the pharmaceutical industry, stearic acid is used as an excipient in tablets and capsules. It helps bind ingredients together and ensures proper drug release. Magnesium stearate, a derivative of stearic acid, is commonly used as a lubricant in pill manufacturing.

3. Cetostearyl Alcohol: -



Fig.No-3.4: Cetostearyl Alcohol

Table No.3: Properties of Cetostearyl Alcohol

IUPAC Name	**hexadecan-1-ol; octadecan-1-ol**
Molecure Weight	284.484g/mol
Appearance	White or off-white waxy solid (flakes, pellets, or granules)
Odour	Mild, characteristic waxy or oil-like odor, can be odorless
Melting Point	48°C to 56°C(approx.118°F to133°F)
Density	~0.82 g/cm³ at 20°C
Acid Value	0.2 to 1.0 mg KOH/g
Solubility	insoluble in water but is soluble in oils and organic solvents like ethanol
Boiling Point	249°C

Uses:

1.Cosmetics and Personal Care: It primarily functions as an emulsifier and thickener to stabilize and texturize creams, lotions, and conditioners.

2.Cosmetics and Personal Care: It acts as an emollient, softening and smoothing the skin and hair by preventing moisture loss.

3.Pharmaceutical Industry: It serves as a necessary excipient base for formulating topical medications like ointments, creams, and gels.

4.Pharmaceutical Industry: It provides structural stability to drug delivery systems and ensures the consistent dispersal of active ingredients.

5.Food Industry: Its components (like cetyl alcohol) are sometimes used as a food additive or in the creation of flavorings and decorations on candies.

6.Industrial Chemistry: It is utilized as a chemical intermediate in the synthesis of various types of surfactants and detergents.

7.Industrial Chemistry: It can be employed as a component in certain lubricants, waxes, and industrial polishes.

4.Coconut Oil: -



Fig.No-3.5: Coconut oil

Table No.4: Properties of Coconut oil

IUPAC Name	**(1-decanoyloxy-3-octanoyloxypropan-2-yl) dodecanoate**
Molecure Weight	Between 640 and 680 g/mol.
Appearance	Clear to milky white to slightly yellow
Odour	Odorless or faint, characteristic coconut aroma/taste
Melting Point	20-28 °C (68-82 °F)
Density	0.925g/cm³ (or 925kg/m³)
AcidValue	0.2 to 1.0 mg KOH/g
Solubility	Insoluble in water, soluble in organic solvents (alcohol, ether, chloroform)
Bioling Point	232 °C(>450 °F) or~225 °C

Uses:-

1.Food Science: Coconut oil is used for its excellent cooking stability.

2.Cosmetology (Beauty/Personal Care): It functions as a natural moisturizer and hair conditioner.

3.Medicine/Nutrition: It acts as an antimicrobial agent due to its richness in lauric acid.

4.Chemical Industry: It serves as a source for detergents and surfactants.

5.Boosting energy: Contains medium-chain triglycerides (MCTs)

6.Antimicrobial properties: Lauric acid may help fight some microorganisms.

7.Skin protection: Helps reduce moisture loss.

5.Propyl Paraben:



Fig.No-3.6: Propyl Paraben

Table No.4: Properties of Propylparaben

IUPACName	Propyl 4-hydroxybenzoate
Molecure Weight	180.20g/mol
Appearance	white, crystalline powder that is odorless or has a faint odor.
Odour	Either no odor or a faint, pleasant, aromatic odor
Melting Point	Between 95°C and 99°C
Density	1.06g/cm
AcidValue	
Solubility	very slightly soluble in water, but freely soluble in alcohols like ethanol and acetone
Bioling Point	113 °C

USES: -

1. Cosmetics: Used as a preservative in creams, lotions, shampoos, and makeup products to prevent microbial growth.
2. Pharmaceuticals: Added to medicines, ointments, and injectable solutions to extend shelf life by inhibiting bacteria and fungi.
3. Food Industry: Used as a preservative in processed foods and beverages to prevent spoilage and extend shelf stability.
4. Personal Care Products: Commonly used in deodorants, shaving products, and toothpaste for antimicrobial protection.
5. Other: Used in industrial products like adhesives and oils as a preservative to prevent microbial contamination.

Glycerin



Fig.No-3.7: Glycerin

Table No.4: Properties of Propylparaben

Chemical formula	C3H5(OH)3
Molecular mass	92.09382 g mol-1
Density	1.261 g cm-3
VISCO Sity	1.5 Pa.s

Melting point	18.2°C
Boiling point	290°C
Food energy	1 4.32 kcal g-¹
Flash POIN	160°C(closed cup)
Surface tension	64mN * m ^ - 1

MATERIALS AND METHODOLOGY:

Materials:

The drug and equipment used in the project research given in the following table. All the materials used were of analytical grade.

Table No 08. Instrument/Equipment

Sr. No.	Equipment/Instrument name	Model/ Company
1.	Soxhlet apparatus	IC-122 / Borosilicate glass
2.	Hot air oven	LSC-358 Labline
3.	Water bath	ASI instrument for scientific water bath active scientific industries. Dombivali-421202.
4.	Stirrer	Elektrocraft PVT. LTD. Mumbai
5.	Round bottom flask	Borosilicate glass
6.	Auto digital pH meter	LT-111 Labline

Table No 09. List of Drug / Materials

Sr. No.	Materials	Manufacturer
1.	*Aegle Marmelos* Extract	Local field of Belgaum, Karnataka
2.	Stearic Acid	MWIT Turkewadi
3.	Cetostearyl Alcohol	MWIT Turkewadi
4.	Coconut Oil	MWIT Turkewadi
5.	Beeswax	MWIT Turkewadi
6.	Glycerine	MWIT Turkewadi
7.	Triethanolamine	MWIT Turkewadi
8.	Distilled Water	MWIT Turkewadi

METHODOLOGY

PREFORMULATION STUDY:

Preformulation testing represents the initial and crucial phase in the systematic development of dosage forms for a drug substance. It involves the evaluation of the physicochemical properties of the active compound to optimize drug delivery, stability, and therapeutic efficacy. The objective of a preformulation study is to gather essential information that characterizes the drug substance and establishes a scientific foundation for combining it with suitable pharmaceutical excipients in the formulation process. In the present study, a preformulation evaluation was carried out on the selected herbal extracts to assess their physicochemical characteristics and compatibility with the excipients used in the formulation of an anti-inflammatory cream. This step ensured the development of a stable, effective, and safe topical dosage form.

Collection of *Aegle Marmelos*:

The *Aegle Marmelos* plants powder was collected from local field of Belgaum Karnataka, India.



Fig. No. 4.1: *Aegle Marmelos*

Preparation of Plant Extract of *Aegle Marmelos*The dried leaves of ***Aegle*** *Marmelos* were coarsely powdered, and 25 g of the powdered material was accurately weighed and placed in a thimble holder. Extraction was carried out using a Soxhlet apparatus with ethanol as the solvent. About 250 mL of ethanol was added to the distillation flask, and the thimble was plugged with cotton to prevent the transfer of plant particles into the solvent flask. The extraction process was continued for about 3 hours until the solvent in the siphon tube became colourless, indicating complete extraction.

USES: -

1. Cosmetics & Personal Care: Used in creams, lotions, soaps, and toothpaste as a humectant to retain moisture.

2. Pharmaceuticals: Used in cough syrups,ointments, suppositories, and as a solvent or sweetening agent.

3. Food Industry: Used as a sweetener,preservative, and humectant in baked goods, candies, and beverages.

4. Industrial Applications: Used in antifreeze lubricants, plastics, and as a softening or smoothing agent.

5. Other: Used in herbal extracts, printing inks, and as a humectant in tobacco

7.Triethanolamine



Fig.No-3.7: Glycerin

Table No.4: Properties of Propylparaben

Acidity/alkalinity	pH = 10.5(0.1 Nsolution solution)
Bolling point	335°C
Freezing point	21.6 °C
Flashz point	208°C
Hygroscopicity	very hygroscopic.
Melting point	20-21°C
Moisture content	0.09%
Solubility	Triethanolamine may turn brown on exposure to air and light.

USES: -

1. Cosmetics & Personal Care: Used in creams, lotions, soaps, and toothpaste as a humectant to retain moisture.

2. Pharmaceuticals: Used in cough syrups, ointments, suppositories, and as a solvent or sweetening agent.

3. Food Industry: Used as a sweetener, preservative, and humectant in baked goods, candies, and beverages.

4. Industrial Applications: Used in antifreeze, lubricants, plastics, and as a softening or smoothing agent.

5. Other: Used in herbal extracts, printing inks, and as a humectant in tobacco 12 PM

Methyl paraben

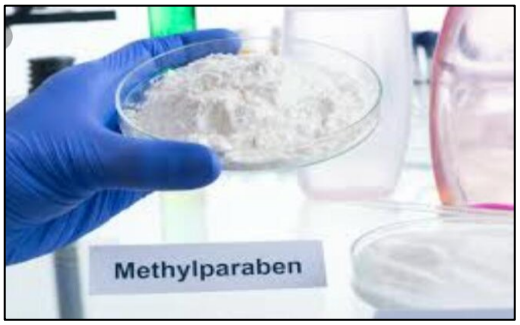


Fig.No-3.8: Methylparaben



Fig.No.4.2: Soxhlet of *Aegle Marmelos*

The obtained ethanolic extract was then filtered and concentrated under reduced pressure to remove excess solvent. The concentrated extract was dried to yield a thick, semisolid ethanolic extract of ***Aegle Marmelos***, which was stored in an airtight container for further studies.



Fig.No.4.3: Extracted Residue

Table No 10. List of Ingredients and Its Use

Sr. No.	Materials	Uses
1.	*Aegle Marmelos* Extract	Anti-inflammatory
2.	Stearic Acid	Emulsifier
3.	Cetostearyl Alcohol	Emollient
4.	Coconut Oil	Moisturizer
5.	Beeswax	Thickener
6.	Glycerine	Humectant
7.	Triethanolamine	Stabilizer
8.	Distilled Water	Solvent
9.	Methyl paraben	Preservative against bacteria
10.	Propyl paraben	Antioxidant Stabilizer

Table No 11. Formulation Table for Anti-Inflammatory Cream

Sr. No	Ingredients	F1
1.	*Aegle Marmelos* Extract	3gm
2.	Stearic Acid	3.9gm
3.	Cetostearyl Alcohol	2.1gm
4.	Coconut Oil	5.1ml
5.	Beeswax	2.1gm
6.	Glycerine	2.1ml
7.	Triethanolamine	0.6ml
8.	Methyl paraben	0.051gm
9	Distilled Water	14.1ml
10	Propyl paraben	0.021gm
	Total	**30 gm**

METHOD OF PREAPARATION OF ANTI-INFLAMMATORY CREAM:

The method for the preparation of the 30 g Aegle marmelos anti-inflammatory cream begins by accurately weighing all components and heating the separate phases: the Oil Phase (3.9 g Stearic acid, 2.1 g Cetostearyl alcohol, 2.1 g Beeswax, 5.1 mL Coconut oil, and 0.021 g Propyl paraben) and the Aqueous Phase (14.1 mL Distilled water, 2.1 mL Glycerin, 0.6 mL Triethanolamine, and 0.051 g Methyl paraben) are both simultaneously heated to a critical temperature of 72°C until all solids are dissolved. Following temperature equalization, the Aqueous Phase is slowly introduced into the Oil Phase under high-shear agitation to form a stable O/W emulsion stabilized by the in situ formation of Triethanolamine Stearate; stirring continues during cooling until the temperature drops to 42 °C , at which point the heat-sensitive 3 g Aegle marmelos leaf extract is thoroughly incorporated, followed by the fragrance below 30 °C .The finished cream is then transferred to its final container, and a sample is reserved for initial quality control tests including pH determination, homogeneity assessment, and stability testing.

Method of Preparation of anti-inflammatory cream:



Fig.No.4.4: Aegle marmelos leaves Powder



Fig.No.4.5: Soxhlet extraction

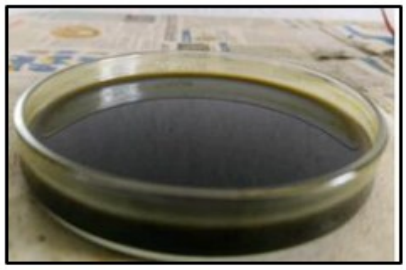


Fig.No.4.6 : Extracted residue



Fig.No.4.7 : Excepeints used during formulation



Fig.No.4.8: Heating of oil or water phase



Fig.No.4.9: Mixing of two-Phase



Fig.No.4.10: Final Product

Phytochemical Screening

Phytochemical screening is carried out to identify and verify the presence of bioactive constituents in herbal extracts, helping in the detection of species-specific compounds and characteristics. The ethanolic extract of ***Aegle*** *Marmelos* was subjected to preliminary phytochemical analysis, which revealed the presence of alkaloids, tannins, saponins, flavonoids, steroids, and terpenoids.

- **Test for Alkaloids:**

(a) Dragendorff's' test: To 1 mi of the extract add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer's test: To 1 ml of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution), Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

- **Test for saponins:**

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponin.

- **Test for Glycosides**

(a) Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

- **Test for carbohydrates and sugars:**

(a) Fehling's test: To 1 mi of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

- **Test for tannins and phenolic compounds:**

(a) To 1ml of the extract, add femic chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

- **Test for flavonoids:**

(a) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, addition of a and indicates the presence of disappears flavonoid.

- **Test for steroids:**

(a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3m of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

- **Test for triterpenoids**

Noller's test: Dissolve two or three granules or tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids



Fig No. 4.11: Phytochemical Screening of Leaves of *Aegle Marmelos*

EVALUATION TESTS FOR HERBAL GEL

Following evaluation parameters were preferred to ensure super priority of prepared anti-inflammatory cream

ORGANOLEPTIC PROPERTIES:

The anti-inflammatory cream was evaluated for color, odor, appearance and texture to ensure uniformity and quality.

1. **Color:** The color of the cream was observed visually under natural light. A small quantity of the formulation was placed in a clean, transparent container, and the uniformity of color was noted. Any signs of discoloration, fading, or non-uniformity were recorded.

2. Odor: The odor of the cream was assessed by carefully smelling a small portion of the formulation. The characteristic fragrance of the cream was noted, and attention was paid to detect any unusual or unpleasant odors, which could indicate microbial contamination or chemical degradation. Odor evaluation is crucial for patient acceptability and can provide early indication of formulation instability.

3. Texture: The texture of the cream was evaluated manually by gently rubbing a small amount between the fingers. The smoothness, consistency, and homogeneity of the cream were checked, and the presence of any grittiness, lumps, or phase separation was noted. Texture assessment ensures that the cream has a uniform and acceptable consistency, which is important for ease of application and therapeutic performance.



Fig.No.4.12: Herbal Cream

A. PHYSICOCHEMICAL PROPERTIES:

1. Determination of pH: The pH of the formulated herbal cream was determined using a digital pH meter. One gram of the cream was accurately weighed and dispersed in 10 mL of distilled water, then allowed to stand for two hours to reach equilibrium. The electrodes of th pH meter were immersed in the dispersion, and the pH value was recorded. Before measurement, the pH meter was calibrated using standard buffer solutions of pH 4.0 and 7.0 to ensure accuracy.



Fig No.4.13: Digital pH Meter

2. Spredability: A sufficient quantity of the sample is placed between two glass slides, and a weight of 1 grams is exerted on the slides for 5 minutes. The spreadability can be represented by the formula, S= ml/t, where m denotes the weight applied to the upper slide, I signifies the distance moved on the glass slide, and represents the duration taken. The following formula is used to calculate the spreadability,

$$\textbf{Spreadability (S)} = \frac{\textbf{ML}}{\text{T}}$$

Were,

M =Weight tied to upper slid

L =Length of glass slides

T =Time taken to separate the slides



Fig.No.4.14: Spreadability

3. Phase Separation: To evaluate phase separation, a small quantity of the anti-inflammatory cream was placed in a clean, transparent glass container and

observed at room temperature. The sample was then subjected to stress conditions, including heating at 40°C and 45°C and centrifugation at 3000rpm for 30 minutes. After each condition, the Cream was inspected for any signs of separation, such as an oily layer, sedimentation, or liquid release. No phase separation was observed, indicating that the formulation was physically stable under the tested condition.



Fig.No.4.15: Phase Separation

4. Homogeneity: The uniformity of the herbal cream was evaluated based on the visual appearance and feel.



Fig.No.4.16: Homogeneity

5.Skin Irritation Test: Mark the designated area of 1 cm^2 on the left dorsal surface. Then apply the cream to that marked area and record the time. Afterward, evaluate for any signs of irritation, erythema, and edema at intervals up to 24 hours, and document the findings.



Fig.No.4.17: Skin Irritation

6.Washability Test: A small quantity (approximately 0.5 g) of the formulated Cream was applied evenly on a marked area of the skin. The ease and extent of removal were then evaluated by gently washing the area with tap water. The observation was noted based on how easily the cream was removed without leaving any residue.



Fig.No.4.18: Washability Test

1. **Stability Test:** The prepared formulation (F1) was subjected to stability testing to evaluate its physical and chemical stability. The formulation was stored in tightly closed containers at room temperature (25 ± 2 °C and 60% RH) for a period of 30 days. Samples were withdrawn at predetermined intervals of 0, 15 and 30 days and evaluated for various parameters such as appearance, color, odor, pH and spreadability. The pH was determined using a calibrated digital pH meter. The physical parameters such as appearance, color and odor were observed visually and spreadability was determined by

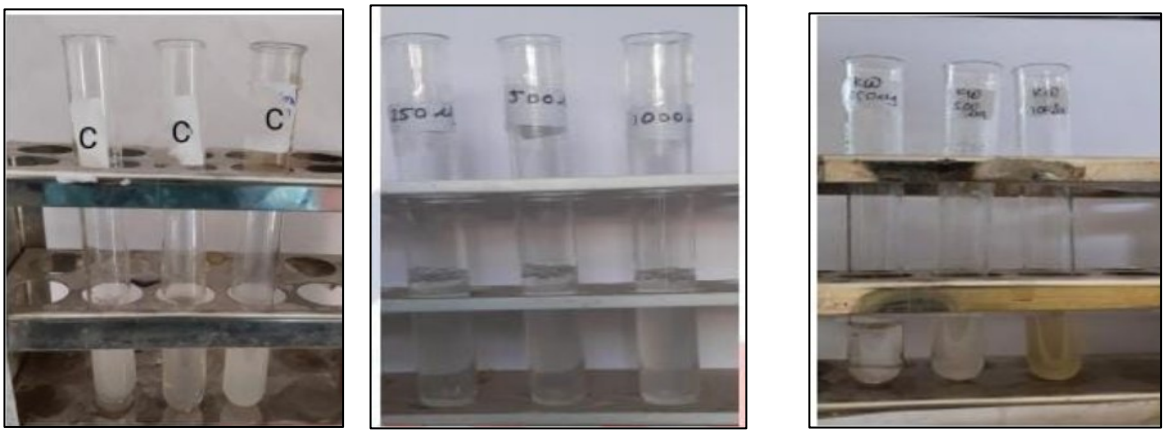
the slip and drag method. The obtained results were recorded and compared with the initial values to assess any significant changes during the storage period.

2. Anti-inflammatory Test:

In vitro anti-inflammatory activity by Protein denaturation method

The reaction mixture (10 mL) consisted of 0.4 mL. of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 4 mL. of Sample (1000µg/ml). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37°c ±2) in a incubator for 15 min and then heated at 70°c for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at concentration 1000 µg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$



Control sample **Standard Diclofenac sodium** **Sample – F2**

Fig No.4.19: Anti-inflammatory activity

RESUIT AND DISUSSION:

Qualitative Phytochemical Test: The phytochemical tests were performed on various extracts of Aegle marmelos (Bael), and the results are shown in Table 12. The extractstested positive for flavonoids, saponins, tannins, phenolic compounds, carbohydrates, alkaloids, glycosides, and proteins, while , steroids, and were noted to be present in low concentration or absent in some extracts. These results indicate the presence of bioactive compounds, particularly phenolics and flavonoids, which are well-known to be responsible for the plant's analgesic, anti-inflammatory, and antioxidant properties, thereby strongly supporting its use in an herbal anti-inflammatory cream formulation.

Table No. 12: Qualitative Chemical Examination of Various Extracts of Aegle marmelos

Test	Extracts (Result)
flavonoids,	+
saponins,	+
phenolics	+
glycosides	+
tannins	+
carbohydrates	+
alkaloids	+
steroids	-
terpenoids	+

Organoleptic Evaluation:

The organoleptic properties of the Anti-inflammatory were evaluated, and the results are presented in Table No. 13.

Table No.13: Organoleptic Evaluation of Anti-inflammatory

Sr.no	Organoleptic Properties	Observation
1.	Color	Green
2.	Odor	Slight plant like smell
3.	Appearance	Smooth, homogeneous

DISCUSSION:

The organoleptic evaluation of the formulated anti-inflammatory cream showed that it possessed a green color, a slight plant-like odor, and a smooth, homogeneous appearance. These characteristics indicate proper incorporation of herbal ingredients, good aesthetic appeal, and uniformity of the formulation. The mild odor and smooth texture suggest good patient acceptability, while the consistent color and homogeneity reflect the physical stability and quality of the cream. Overall, the organoleptic properties support the suitability of the formulation for further evaluation.

pH determination:

Table No.14: pH of Anti-inflammatory Cream

Sr.no	Parameter	Result
1.	pH	6.7

Discussion:

The pH value of 6.7 is slightly above the average acidic pH of the skin (4.5-5.8), bringing it close to a neutral pH of 7.0. This range is generally considered acceptable for topical application. The primary discussion point is dermal safety: a pH near the skin's natura state ensures the formulation will not cause irritation, dryness, or disrupt the skin's acid mantle. Furthermore, maintaining this pH is essential for the chemical stability of the active ingredients (the Aegle marmelos extract) and the physical integrity of the cream base over the product's shelf-life.

Spreadability of Anti-inflammatory cream

Table No.15: Spreadability of Anti-inflammatory Cream

Sr.no	Parameter	Result
1.	Spreadability	5.5gm

Discussion:

The smooth, semi-solid texture and the spreadability value of 5.5 gm indicate excellent flow properties. This allows the cream to be easily dispensed and spread efficiently over the affected area, ensuring uniform coverage and potentially enhancing drug absorption.

Phase separation of Anti-inflammatory cream

Table No.16: Phase separation of Anti-inflammatory Cream

Sr.no	Parameter	Result
1.	Phase separation	No

CONCLUSION

The present research successfully achieved its primary objective by formulating, optimizing, and evaluating a stable and effective herbal anti-inflammatory cream using *Aegle marmelos* extract. Comprehensive phytochemical screening confirmed the presence of potent bioactive constituents including flavonoids, phenolics, tannins, alkaloids, and saponins which strongly justify the plant's traditional medicinal applications and validate its selection as the principal therapeutic agent in the formulation. The developed cream displayed excellent physical, chemical, and therapeutic characteristics, including a smooth semi-solid texture, green color, and mild herbal odor. Physical evaluation parameters such as **pH (6.7)**, **spreadability (5.5 g)**, **washability**, and **homogeneity** showed that the

formulation is **skin-friendly, non-greasy, easily spreadable, and patient-compliant**. Importantly, no **skin irritation** was observed, indicating good dermatological safety. Its smooth consistency, uniform green coloration, appropriate pH, good spreadability, easy washability, and complete absence of phase separation indicate a well-designed and stable emulsion system. Dermatological safety was confirmed through skin irritation studies, which showed no adverse reactions, while stability studies demonstrated that the formulation reliably maintains its quality throughout the testing period. The anti-inflammatory evaluation further revealed significant inhibition of protein denaturation, supporting the strong therapeutic potential of the *Aegle marmelos* extract. Overall, the study concludes that the formulated herbal anti-inflammatory cream is **safe, stable, natural, eco-friendly, cost-effective, and therapeutically effective** for topical use. Given the increasing global preference for herbal and chemical-free skincare products, this formulation represents a promising alternative to synthetic anti-inflammatory agents, offering fewer side effects and greater consumer acceptability. Additionally, the use of readily available plant material makes the formulation economically feasible and environmentally sustainable, aligning with the growing interest in green pharmaceutical technologies. The results of this research also highlight the broader potential of *Aegle marmelos* as a valuable ingredient in modern phytopharmaceutical and cosmeceutical applications. The phytochemical richness of the plant suggests further opportunities for developing formulations targeting wound healing, antioxidant protection, and antimicrobial activity.

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