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Review Article

A Research on: Development and Assessment of polyherbal Antifungal Ointment for Topical Application

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ABSTRACT

Fungal infections are a significant global health concern, often requiring prolonged treatment with anti-fungal agents that may have adverse effects or lead to resistance. This study focuses on the formulation and evaluation of a poly-herbal anti-fungal ointment incorporating extracts from pomegranate (*Punica granatum*), bael (*Aegle marmelos*), and papaya (*Carica papaya*). These medicinal plants are known for their potent antifungal, antimicrobial, and wound-healing properties. The herbal extracts were obtained using suitable solvent extraction methods and incorporated into an ointment base using standard formulation techniques. The prepared ointment was evaluated for physicochemical properties, including Spreadability, pH, viscosity, homogeneity, and stability. In vitro antifungal activity was assessed against common fungal pathogens such as *Candida albicans* and *Aspergillus niger* using the agar well diffusion method. Results indicated that the formulated polyherbal ointment exhibited significant antifungal activity, comparable to standard antifungal formulations. The formulation was stable, non-irritant, and showed good Spreadability and consistency. This study highlights the potential of combining herbal extracts in topical formulations for safe and effective antifungal therapy.

INTRODUCTION

Indian herbs have long been cherished worldwide for their natural healing properties. With the rising demand for safer and more effective alternatives, herbal plants are gaining popularity in the global market. Unlike synthetic products, herbal remedies typically offer fewer side effects and

better skin compatibility. Herbs possess a wide range of medicinal properties including antioxidant, anti-inflammatory, antiseptic, emollient, antifungal, and antibacterial actions. This makes them particularly valuable in skincare and treatment of common issues like fungal infections. Fungal infections, caused by organisms such as *Candida* and dermatophytes, often result in

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redness, itching, and irritation. While chemical antifungals are available, they can lead to side effects or resistance when used over time. Herbal ointments provide a natural, gentle, and effective alternative without toxic residues or irritation. India's rich traditional systems like Ayurveda, Unani, and even homeopathy have long emphasized herbal treatments. Texts such as the Rigveda and Yajurveda have documented the healing potential of herbs for centuries. Modern science is now catching up, aiming to improve herbal drug delivery methods for better efficacy. Today, herbal products are being developed in both crude and extract forms to offer more targeted relief and overall skin protection. Fungi are mostly multicellular eukaryotic heterotrophs that play a key role in nutrient cycling within ecosystems.

Types of Fungi:

- **Chytridiomycota** - Mostly asexual fungi that produce flagellated spores. They can infect amphibians like frogs by burrowing into their skin.
- **Zygomycota** - Primarily terrestrial fungi, known to grow on various surfaces including food. Example: *Rhizopus stolonifer* (bread mold)
- **Glomeromycota** - Soil-dwelling fungi that form mutualistic relationships with plants. They absorb sugars from plants and help provide minerals from the soil. They reproduce asexually.
- **Ascomycota** - Mentioned in the list but not described; commonly known as sac fungi, includes yeasts and molds.

Fungal infections, or mycoses, can affect both the surface and internal parts of the body.

Types of Fungal infection:

- **Superficial:** Affect skin, hair, nails, and mucous membranes. Includes conditions like ringworm, athlete's foot, oral thrush, and vaginal yeast infections.
- **Deep (systemic):** Involve internal organs such as lungs, heart, or brain, causing serious illnesses like pneumonia, endocarditis, or meningitis. Fungi are found in air, water, plants, and even the human body.



Fig. Fungal Infection

“ointment”-An ointment is a semi-solid, greasy preparation applied to the skin or mucous Membranes for medicinal or protective purposes. It helps in healing, moisturizing, and Delivering active ingredients to treat skin conditions.get it and lower the risk of major side Effects like kidney issues, heart disease, stroke, and nerve damage.

Antifungal ointment: “ointment which is used for destroying fungi or inhibiting their growth.”

Advantages of ointment:

- Absorption
- Long Enhanced -Lasting Effect
- More Potent than Creams
- Creates a Protective Barrier

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Department of Botany

LETTER FOR IDENTIFICATION/ AUTHENTICATION

This is to certify that, the plant material in the form of flowering twig submitted by **Ms. Nakate Pratiksha Kalyan** student of Final Year B. Pharm. of **Samarth Institute of Pharmacy, A/P- Belhe, Tal. Junnar, Dist. Pune** are checked morphologically for identification/ authentication. As far as references used for identification/ authentication, the details of the submitted plants are as follows:

Sr. No.	Common Name	Botanical Name	Part used	Family
1.	Bel	<i>Aegle marmelos</i> (L.) Corrêa	Leaves	Rutaceae
2.	Papaya	<i>Carica papaya</i> L.	Leaves	Caricaceae
3.	Pomegranate	<i>Punica granatum</i> L.	Leaves	Lythraceae


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MATERIAL AND METHOD:

Chemicals: these include beal and papaya leaves extract, pomegranate peel extract, beeswax, shea butter, Lanoline, Sandlewood oil, Honey.

Instrument: Electronic weighing balance, pH meter, Brookfield viscometer, Waterbath, hot air oven, Autoclave, Incubator.

Extraction process for Beal, papaya leaves and pomegranate peel:

Decoction is a method of extraction used primarily in herbal medicine and pharmacognosy to extract water-soluble and heat-stable constituents from

hard plant materials such as roots, bark, seeds, or stems. Unlike infusion, decoction involves boiling the plant material for a prolonged period. Decoction is an extraction method used primarily for tough plant materials like leaves, roots, bark, seeds, and stems to obtain water-soluble and heat-stable active constituents such as alkaloids, glycosides, tannins, and bitter principles. In this process, the plant material is first cleaned, cut, or crushed to increase surface area and may be optionally soaked in water to soften it. The material is then boiled in a specific quantity of water, usually in a ratio of 1 part drug to 16 parts water, for 15 to 60 minutes depending on its hardness. After boiling, the mixture is cooled and

strained to separate the liquid extract from the solid residues. This extract, known as the decoction, can be used immediately or further concentrated if needed. Since decoctions are prone to spoilage, they should be used fresh or preserved appropriately. The efficiency of decoction depends on factors like the type of plant material, duration and intensity of boiling, pH of the solvent, and the nature of the container used. This method is widely used in traditional systems of medicine like Ayurveda and Traditional Chinese Medicine for preparing therapeutic formulations.



Fig. Extraction Process

Content for ointment:

1.Bael: Bael leaves come from the Aegle marmelos tree, also known as Bengal quince or stone apple, native to the Indian subcontinent and Southeast Asia. The tree is considered sacred in Hinduism and Buddhism. Bael has been mentioned in ancient Indian manuscripts from the Vedic era (2000-800 BC). Bael leaves In Hindu mythology, the bael tree is associated with Goddess Parvati and Lord Shiva, symbolizing spiritual growth and prosperity. The trifoliate leaves represent the trinity of Brahma, Vishnu, and Shiva.

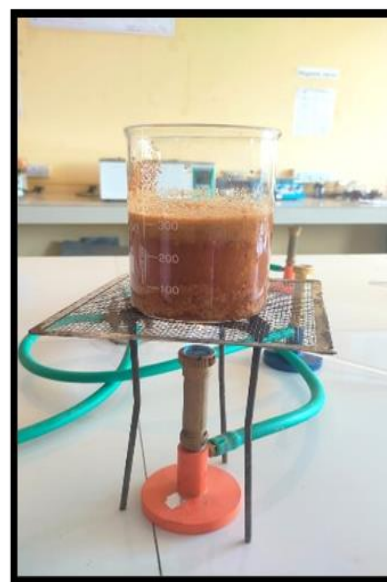


Fig. Aegle Marmelos (Beal)

Scientific Name – Aegle marmelos

Biological Source- It consists of fresh and dried leaves of Aegle marmelos family – Rutaceae

Chemical Constituent- Rutin, Aegeline, Marmeline, Marmesin, Eugenol, Citral, Marmalosin.

Uses – Antifungal, Antibacterial, Anti-Inflammatory, Skin Disorder, Skin Disease

Table no.1 Taxonomical Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliophyta
Order	Sapindales
Family	Rutaceae
Genus	Aegle
Species	Marmelos

2.Papaya: Papaya trees have a rich history dating back to Mesoamerica, specifically southern Mexico and Central America, where they were first domesticated. The papaya plant, scientifically known as *Carica papaya*, is believed to have originated in these regions over 2,000 years ago. Papaya was introduced to the Old World by

Spaniards in the 16th century. It spread rapidly throughout Asia and the South Pacific region, becoming a staple crop in many tropical countries. Today, papaya is grown extensively across the globe, with India being the largest producer, accounting for 38% of the world's supply.



Fig. Papaya Leaves

Scientific Name –Carica papaya

Biological Source- it consist of fresh and dried leaves of Carica papaya Family- Caricaceae

Chemical Constituent- Carpein, Quercetin,psedocarpein,papain,chymopapain,kaempferol

Uses – Inhibit fungal growth,reduce inflammation,wound healing,Emzyatic degradation.

Table No.2 Taxonomical Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliophyta
Order	Brassicales
Family	Caricaceae
Genus	Carica
Species	Carica papaya

3.Pomegranates: Pomegranates have a rich history dating back over 5,000 years, originating from the region of modern-day Iran to northern

India. They're one of the first fruit trees domesticated in the eastern Mediterranean region. Pomegranates were cultivated in the Middle East, India, and the Mediterranean region for several millennia They were introduced to Spanish America in the late 16th century and later to California by Spanish settlers in 1769.Today, pomegranates are grown extensively in West Asia, the Caucasus region, South Asia, Central Asia, and the Mediterranean Basin.

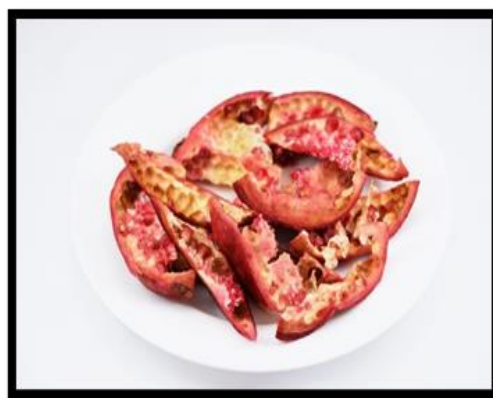


Fig. Pomegranate Peel

Scientific Name –Punica granatum

Biological Source- Pomegranate peel obtained from punica granatum belong to Family-Punicaceae.

Chemical Constituent- punicalalin, Ellagic acid,Gallic acid, ,Ellagitannins,gallotannin

Uses – reduce inflammation,skin repair

Table No.3 Taxonomical Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliophyta
Order	Myrtales
Family	Punicaceae
Genus	Punica
Species	Granatum

Preliminary Phytochemical Investigation: The ethanolic extract underwent qualitative chemical

analysis. Various procedures were employed to detect the presence of different phytochemical compounds in the extract. Key bioactive components in plants include steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, saponins, and glycosides.



Fig. Phytochemical Test for Beal Leaf
Fig. Phytochemical Test for Papaya Leaves



Test:

Test for alkaloid:

- **Mayers test:** 2-3 ml of filtrate, when mixed with small amount of Mayer's reagent, forms a cream coloured precipitate.

- **Dragendorff's reagent:** add dragendorff's reagent into filtrate it gives orange or reddish-brown precipitate indicate alkaloid.

Test for Flavonoid:

- **Lead acetate test:** add lead acetate solution into filtrate it gives a yellow precipitate confirm flavonoid.
- **Shinoda test :** add magnesium turning and conc. HCL to the extract a red colouration indicate flavonoid.

Test for coumarin:

- **NaOH test:** add NaOH to the extract a yellow colouration that disappear with acid confirm coumarin.

Test for phenolic acid:

- **Ferric chloride test:** Add Ferric chloride solution into extract black colouration indicate presence of phenolic acid.

Formulation of polyherbal ointment

• Preparation of polyherbal ointment

1. Selection of excipient: Beal, papaya leaves and pomegranate peel collected from the botanical garden of SIOP, Belhe. The raw material and another chemicals were taken from SIOP, Belhe. All ingredients used are given in table.

2. Method of preparation: In these firstly melt beeswax, shea butter used as ointment base and lanoline at 60–70°C until it gets completely homogeneous. Then add essential oil that is sandalwood oil as flavouring agent. Then prepare aqueous phase heating at 60°C contain honey used as natural preservative. Then slowly add the aqueous phase into oil phase with continuous

stirring using homogenizer. continue stirring until a smooth formation forms. then mixture cool down 40°C, add 2 ml of each extract stir until uniform.

then final step allows the ointment to cool gradually to room temp. while stirring.

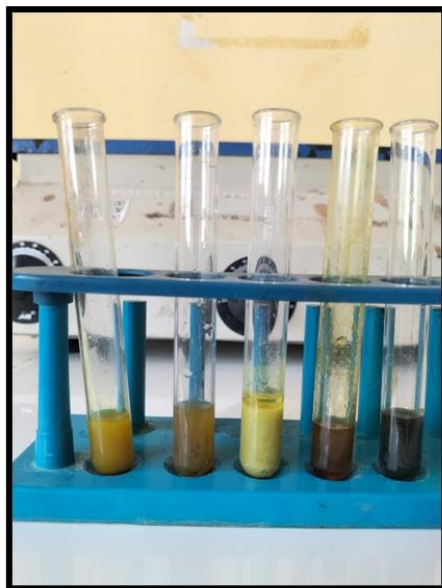


Fig. Phytochemical Test for Pomegranate Peel

Table No.4 Formulation Table

Sr.no	Ingredient	A1	A2	A3	Role
1.	Beal leaves extract	2ml	1.5ml	2.5ml	Antigungal
2.	Papaya leaves extract	1.5ml	2ml	1ml	Skin renewal
3.	Pomegranate peel extract	1ml	1.5ml	2ml	Wound healing
4.	Beeswax	3gm	2.5gm	3.5gm	Emulsifier
5.	Shea butter	4gm	4gm	3gm	Ointment base
6.	Lanoline	2.5gm	3gm	2.5gm	Emolient
7.	Sandlewood oil	1ml	0.8ml	1.2gm	Essential oil
8.	Honey	3.5ml	2.8ml	3gm	Preservative
9.	D.W	1.5ml	1.9ml	1.3gm	Vehicle

Evaluation of Ointment:

1. Physical evaluation: Physical evaluation such as colour, odour, texture, appearance were evaluated.

2. Homogeneity: All formulated ointment underwent homogeneity testing through visual inspection post-container settling, assessing their appearance and the absence of any aggregates.

3. pH: Take 1 gram of each ointment and mix it with 100 ml of water (or a suitable solvent). Then

Let it sit for 2 hours to allow the mixture to settle. After Use a digital pH meter to measure the pH of the mixture. Repeat the measurement three times for each formulation to ensure accuracy. And finally Calculate the average pH value for each formulation. This process helps determine the pH level of each ointment formulation, which is important for ensuring stability, efficacy, and skin compatibility.

4. Spreadability: The Spreadability of the ointment was evaluated using a wooden block

apparatus with a pulley system. About 2 grams of ointment was placed between two glass slides, and a 1 kg weight was applied for 5 minutes to ensure a uniform film. After removing excess ointment, a 50 g weight was attached to the top slide via a string and pulley, and the time taken for the top slide to travel and measured. A shorter time indicated better Spreadability, reflecting the ointment's slip and drag characteristics. This method assessed how easily the ointment spreads, which is crucial for its application and effectiveness.

Spreadability was calculated using the following formula:

$$S = M \times L / T$$

Where, S = Spreadability,

M = Weight in the pan (tied to the upper slide),

L = Length moved by the glass slide and

T = Time (in sec.) taken to separate the slide completely each other.

5.Viscosity: A special device called a Brookfield viscometer was used to measure the ointment's thickness or flowability. this device uses a spindle (a small rotating part) that is inserted into the ointment. spindle was rotated at different speeds (5, 10, 20, 30, and 50 rpm) to test the ointment's viscosity. Before taking each measurement, the ointment was allowed to settle for 2 minutes to ensure accurate results. Each measurement was repeated three times to ensure accuracy.

6.Antifungal activity in vitro techniques:

1.Preparation of PDA :

1. Dissolve PDA powder in distilled water as per manufacturer's instructions (e.g., 39 g in 1 L).

2. Boil to dissolve completely.
3. Sterilize by autoclaving at 121°C for 15 minutes.
4. Pour ~20 mL into each sterile Petri dish under aseptic conditions.
5. Let it solidify at room temperature.

Here's a short version:

2.Inoculating Candida albicans

1. Prepare Candida albicans suspension (0.5 McFarland standard).
2. Dip sterile cotton swab into suspension.
3. Spread evenly on PDA surface (lawn culture).
4. Let it dry for 10-15 minutes.

3.Well Diffusion Assay

1. Create wells (6 mm) in agar plate using sterile cork borer.
2. Fill wells with: 100 µL polyherbal ointment (diluted if needed), positive control, Negative control
3. Avoid overflow, ensure uniform application.
4. Incubate the plate at 28-30°C for 48 Hrs.

Polyherbal Antifungal Ointment: A polyherbal antifungal ointment is a topical formulation composed of extracts from multiple medicinal plants, specifically chosen for their antifungal properties. Polyherbalism is the concept of using more than one herb in a formulation to obtain synergistic therapeutic effects, improve efficacy, reduce toxicity, and target multiple pathways or pathogens simultaneously. It Overcome limitations of synthetic antifungal agents such as



drug resistance, high cost, and skin irritation. An offer a natural and holistic alternative based on traditional medicine systems like Ayurveda and Unani.

Antifungal Activity of *Aegle marmelos*: The leaves of *Aegle marmelos* (Bael) exhibit strong antifungal properties due to their rich content of bioactive compounds like marmelosin, aegeline,

skimmianine, flavonoids, and tannins. Marmelosin and aegeline, in particular, are effective against fungi such as *Candida albicans*. These phytochemicals contribute to the leaves broad-spectrum antimicrobial action. A topical ointment can be formulated by blending Bael leaf extract with a base like Shea butter, along with natural preservatives and skin-friendly ingredient.

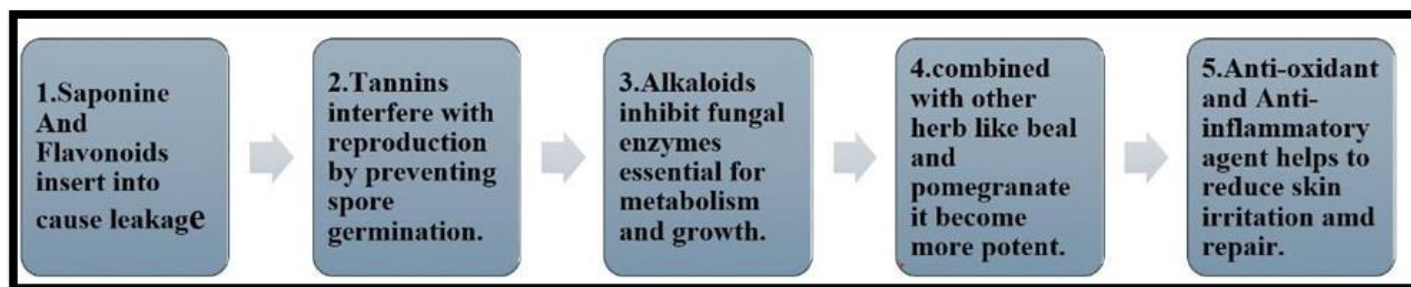


Fig. Antifungal Activity of Aegle marmelos

Antifungal Activity of *Carica papaya* : Papaya (*Carica papaya*) leaves exhibit strong antifungal activity due to their bioactive compounds like flavonoids, alkaloids, tannins, and saponins. These constituents disrupt fungal growth by damaging cell membranes and inhibiting spore germination. When used in polyherbal antifungal ointments alongside herbs like neem or turmeric, their

effectiveness is enhanced. Besides antifungal effects, papaya leaves also provide anti-inflammatory and antioxidant benefits, helping in skin healing. Overall, they offer a natural, cost-effective, and safe alternative to synthetic antifungal treatments.

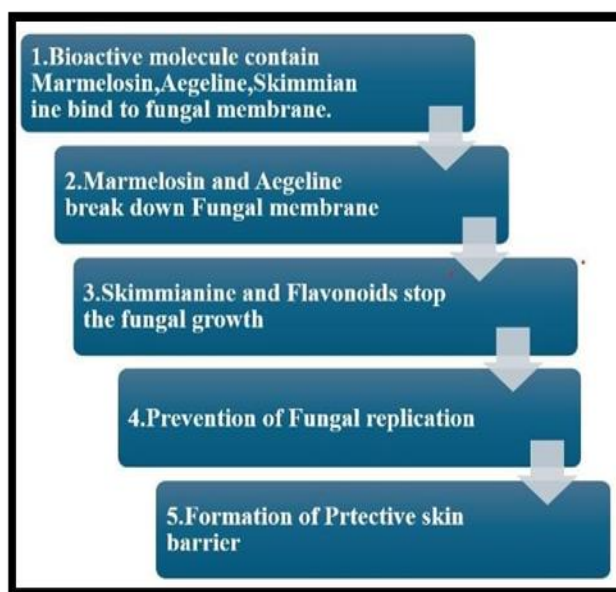


Fig. Antifungal Activity of Carica papaya on skin

Antifungal Activity Of Punica granatum:

Pomegranate peel (*Punica granatum*) exhibits notable antifungal activity on the skin, primarily due to its rich content of bioactive constituents such as ellagic acid, punicalagin, gallic acid, flavonoids, and tannins. These compounds work synergistically to damage fungal cell walls, inhibit enzyme activity, and prevent fungal replication.

Ellagic acid and punicalagin are particularly potent, disrupting the membrane integrity of fungi like *Candida albicans* and *Trichophyton* species. Topical formulations using pomegranate peel extract are valued for being natural, antioxidant-rich, and effective in treating fungal skin infections, making them a promising alternative to synthetic antifungal Ointment

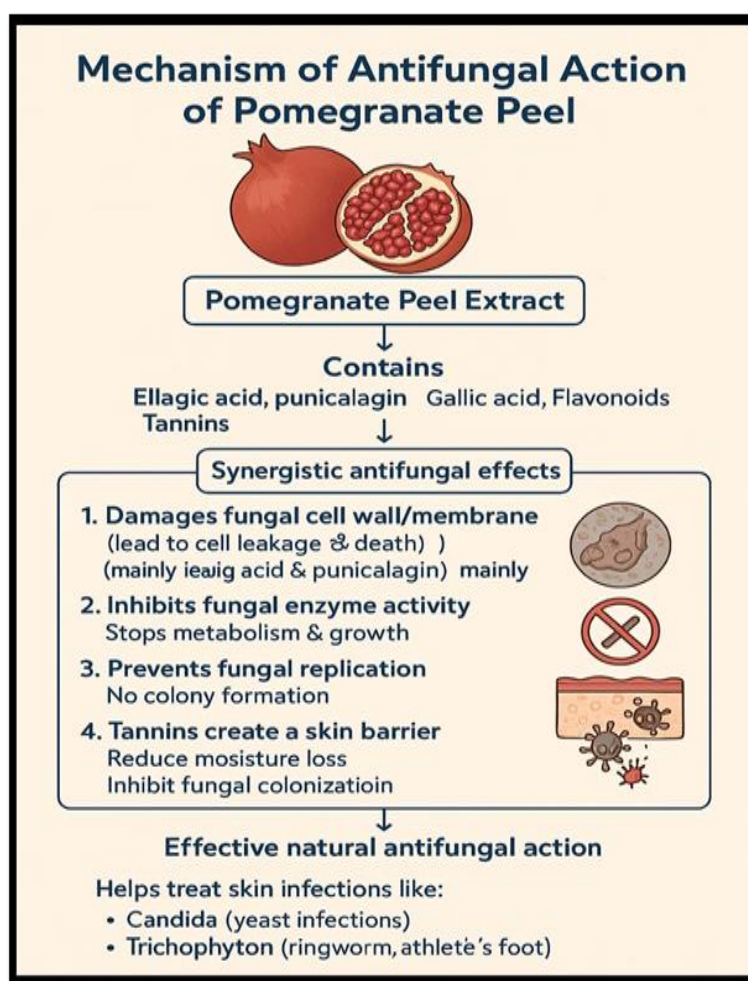


Fig. Antifungal activity of *Punica granatum* on skin.

Mechanism of Action on Polyherbal Antifungal Ointment Skin :

1.Cell Wall Disruption: Multiple constituents target the ergosterol synthesis pathway and cell membrane integrity, leading to fungal cell death.

2.Biofilm Inhibition: Papain and ellagic acid prevent biofilm formation, allowing better penetration of active compounds.

3.Anti-inflammatory Response: Reduces redness, itching, and irritation commonly seen in skin fungal infections.

4.Skin Regeneration: The formulation supports skin healing and barrier repair through antioxidants and enzymatic activity.

5.Prevention of Recurrence: By inhibiting fungal adhesion and maintaining skin integrity, it helps reduce the risk of reinfection

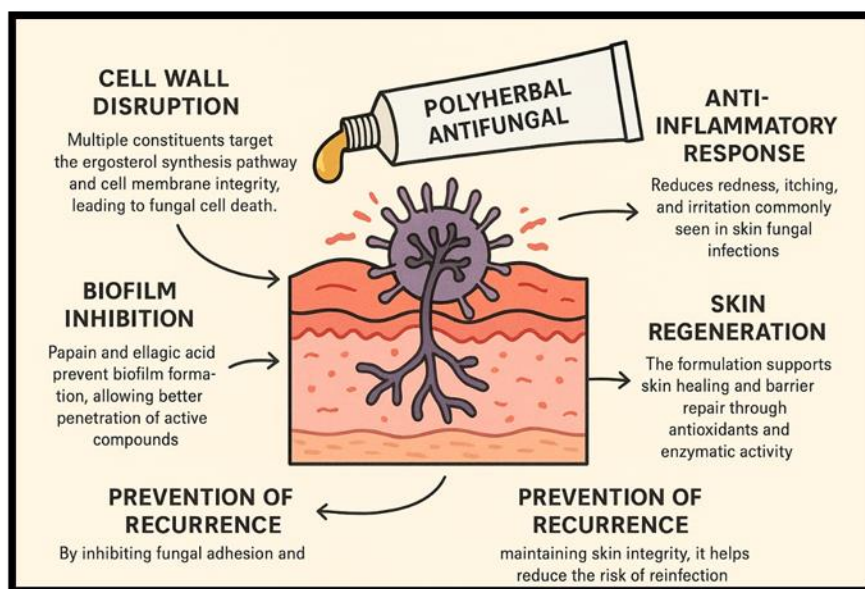


Fig. Machanism of action polyherbal ointment act on skin

RESULT AND DISCUSSION:

Physical evaluation of Ointment:

1.Physical evaluation:

Table. 5 Physical Parameter.

Sr.no	Organoleptic evaluation	Appearance
1.	Colour	Dark Orange
2.	Odour	Slightly aromatic
3.	Texture	Smooth

2.Physicochemical Evaluation:

1) pH determination:

The pH determination of syrup by using Auto pH Meter:

Procedure-

a) **Buffer Preparation:** Prepare 30 mL of buffer solution for each desired pH by mixing

the appropriate volume of stock solutions.

b) **Equilibration:** Allow the prepared buffer solutions to stand for 15 minutes to reach equilibrium.

c) **pH Measurement:** Measure the pH of each solution using an auto pH meter according to standard operating procedures.



Fig 17. Ph Meter.

3.Homogeneity: Formulation were tested for homogeneity by visual inspection after the ointment set in the Container it observed was homogeneous.

Table 6. Homogeneity

Sr.no	Batch	Homogeneity
1.	A1	Homogeneous
2.	A2	Homogeneous
3.	A3	Homogeneous

4.Spreadability: The time in sec require to separate the two slide was taken as measure of Spreadability.

Table 7. Spreadability

Sr.no	Batch	pH	Spreadability
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Table 9 Zone of inhibition.

Test organism	Sample tested	Zone of inhibition	Antifungal activity
Candida albicans	Beal leaves extract	14 mm	Moderate
	Papaya leaves extract	12 mm	Moderate
	Pomegranate peel extract	16 mm	Good
	Polyherbal formulation	21 mm	Excellent
	Std.(Clotrimazole ointment)	23 mm	Excellent

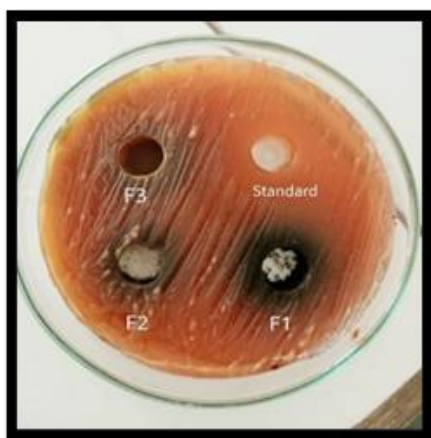


Fig. Zone of inhibition.

CONCLUSION:

1.	A1	6.5 /±0.03	16.11/±0.005
2.	A2	7.07/±0.03	15.50/±0.005
3.	A3	7.2/±0.03	15.38/±0.005

5.Viscosity: Viscosity Of ointment was determined by using Brookfield viscometer at 5,10,15 rpm each reading was taken after equilibrium at sample.

Table 8. Viscosity.

Sr.no	Rpm	Viscosity
1.	5	3608±0.16
2.	10	3712±0.20
3.	15	4018±0.20

6.Zone of inhibition:

The formulated polyherbal antifungal ointment demonstrated significant antifungal activity against common fungal pathogens, validating the traditional use of the selected herbs. The synergistic effect of multiple plant extracts contributed to broad-spectrum efficacy, reduced risk of resistance, and enhanced healing properties. Physicochemical evaluation confirmed the stability, spreadability, and homogeneity of the ointment. Given its natural origin, low toxicity, and therapeutic potential, this polyherbal ointment represents a promising alternative to synthetic antifungal agents for the treatment of superficial fungal infections. Further clinical evaluation is

recommended to substantiate its efficacy and safety in larger populations.

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