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Formulation and Evolution of Antifungal Cream from Aegles Marmelos Leaves

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ABSTRACT
This project focuses on the formulation and evaluation of an antifungal herbal cream containing Aegles marmelos extracts as the active ingredient to combat Candida albicans, a common fungal pathogen responsible for various infections, including oral and vaginal candidiasis. Aegles marmelos, a plant known for its medicinal properties, contains alkaloids such as atropine, scopolamine, and hyoscyamine, which exhibit antimicrobial and antifungal effects. The project aims to extract these bioactive compounds from the plant's leaves and incorporate them into a cream formulation. The creams effectiveness will be evaluated based on its ability to inhibit the growth of Candida albicans in vitro, through methods like Minimum Inhibitory Concentration (MIC) determination, and antifungal activity assays. The cream is intended to provide a topical, easy-to-apply alternative for treating fungal infections, leveraging the natural properties of Aegles marmelos for its potential therapeutic application. The study also assesses the cream's stability, pH, and skin irritation potential to ensure its safety and efficacy as a topical antifungal treatment. The overall aim of the project is to offer a sustainable, plant-based treatment option with reduced side effects compared to conventional antifungal drugs

keywords: Aegles marmelos (Beal), Antifungal activity, MIC (minimum inhibitory concentration), cream,

1. INTRODUCTION

Medicinal plants are vital in modern healthcare as they provide bioactive compounds that inspire new drug discoveries. They play an integral role in traditional medicine, pharmaceuticals, and even health supplements. Remarkably, over 80% of the world's population depends on plants for primary healthcare, as noted by the WHO. Their relevance has grown in response to the rising resistance to synthetic drugs. Take *Aegle marmelos* (Bael tree), for instance—celebrated in Indian tradition for its healing properties. Its fruit and leaves address issues like diarrhoea and asthma, while its bark juice is linked to boosting fertility. Medicinal plants are eco-friendly, effective, and offer fewer side effects, making them a reliable choice in healthcare.[1]

Superficial fungal infections of the skin, hair, and nails are becoming increasingly common, affecting over 40 million people worldwide, especially in developing countries. Dermatophytes, responsible for conditions like tinea and onychomycosis, and *Candida* species are the primary culprits. For individuals

with weakened immune systems, *Candida* can cause severe complications by invading deeper tissues, leading to systemic candidiasis.

Topical antifungal treatments are favored for their targeted application, fewer systemic side effects, improved efficacy, and patient convenience. Common agents like polyenes, azoles, allylamine/benzylamines, and ciclopirox are available in forms such as creams, gels, and sprays. Effective treatments rely on the drug's ability to penetrate the outer skin layer to reach the deeper epidermis.
Recent advancements focus on innovative delivery systems—like nanoparticles, vesicular carriers, and colloidal systems—to enhance skin penetration and maximize treatment effectiveness. These approaches are designed to overcome limitations of conventional methods, such as skin irritation and low active ingredient levels, ensuring better outcomes and addressing the growing global challenge of fungal infections. These innovations hold promise for advancing patient care and managing fungal diseases more effectively.

Types of Fungi:

- Chytridiomycota
- Zygomycota
- Glomeromycota
- Ascomycota

Yeasts and dermatophytes are frequently the source of fungal skin infections. They reside on the outermost layer of the epidermis and are more prevalent in diabetics and obese people with large skinfolds. Dermatophytids, which are allergic reactions to the fungus and do not arise from touch with the diseased area, are rashes that are caused by fungal infections and can appear on other parts of the body.

Cream

Definition- An cream is a topical preparation. An antifungal cream is a cream that is used to destroy fungi or inhibit their growth.[2][3] "Cream is a semisolid preparation of a medication for topical use (on the skin) that contains a water base. Essentially, it is a preparation of oil (often lanolin or petrolatum) in water."

Advantages of Cream:

1) Able to calm inflammation
2) Promote skin tone
3) Easy to formulate
4) Wide Availability (over the counter for mild infections) [4]
5) Easily water washable and easy to wipe away.

Plants are important to humans because they contain active compounds that serve as the basis for medications and have been utilized in Indian traditional medicine for centuries. Aegle marmelos (L.) Correa, commonly referred to as Bael, is a valuable source of bioactive compounds and ingredients that are used in natural remedies for a variety of ailments. Bael is rich in phytochemicals, alkaloids, tannins, essential oils, gums, resins, coumarin, and polysaccharides, which make it effective against numerous diseases while being environmentally sustainable.[5][6]

2. OBJECTIVE OF RESERCH

1) **Sustainability and Cost-Effectiveness of antifungal cream.**
2) **Good Consumer Acceptability.**
3) **Antifungal creams aim to fully eliminate infections, preventing fungal regrowth or spread, reducing recurrence risk, and ensuring lasting skin health.**
4) **Developing a stable and effective herbal cream involves crafting formulations with plant-based extracts while maintaining optimal texture, balanced pH levels, and long-lasting shelf stability.**

DRUG PROFILE OF *AEGLES MARMELOS*:

Biological source:

Fig No.01 AEGLE MARMELOS LEAF

Aegle marmelos, often called bael, Bengal quince, golden apple, or wood apple, is a remarkable tree from the Rutaceae family.[7] It is native to the Indian subcontinent and Southeast Asia. This deciduous beauty can reach up to 13 meters tall and holds a special place in both traditional medicine and cultural practices. Known for its therapeutic benefits, the bael tree is cherished not only for its health-giving properties but also for the meaning it carries in various cultural rituals. Truly a gift of nature

Synonyms:

Hindi (Bel, Bael, Sripal); Sanskrit (Bilva, sriphal, shivadruma, Shiv apala); Telugu (Maledu); Bengali Khmer (Phneou or pnoi); Vietnamese (Bhavnau); Malay (M (Modjo). (Bel)); Gujarati (Bil); Kannada (Bil Patra, kumbala, malura); Tamil (Kuva Lum); Thai (Matum and mapin).[9]

Morphological features:

The leaves of the bael tree are quite fascinating. They are trifoliate, meaning they grow in clusters of three.[8] Each leaf has a rounded base and ends in a pointed tip. As they mature, their colour changes; young leaves start off pale green or pinkish with a delicate, hairy texture, while adult leaves transform into a deep, smooth green.

The leaflets vary in size, typically ranging from about 5–14 cm in length and 2–6 cm in width. Their shape is oval, tapering toward the tip, with edges that are either smooth or gently toothed. A striking feature is the network of 4–12 pairs of veins running through each leaflet, which connect along the edges.

Ecology:

The bael tree, native to India, has spread its roots far and wide. You'll find it thriving in countries like Bangladesh, Egypt, Malaysia, Myanmar, Pakistan, Sri Lanka, and Thailand. In India, it often grows naturally in dry woodlands across the slopes and fields of central and southern regions. Similarly, in places like Burma, Pakistan, and Bangladesh, it can be spotted in mixed deciduous forests and arid landscapes. This resilient tree is well-suited to diverse environments, making it a true survivor in nature's tapestry.[10]

Botanical Description:

The bael tree grows at a gentle pace and typically remains medium to small in size, reaching about 25 to 30 feet in height when mature. Its stem is soft yet dense, giving it a sturdy appearance. You'll also notice a few spiky branches scattered across its structure, adding a rugged touch to its overall charm. Nature took its time to design this resilient tree! As for the full botanical description, that can be explored in detail, showcasing the intricacies of this incredible plant.[11][12]

Taxonomy:

- Kingdom: Plantae
- Order: Sapindales
- Family: Rutaceae
- Subfamily: Aurantioideae
- Genus: Aegle
- Species: *A. marmelos* [13]

Chemical constituents:

Leaf	Skimmianine, Aeglin, Rutin, -sitosterol, -sitosterol, Flavone, Lupeol, Cineol, Citral, Glycoside, O-isopentenyl, Hallo diol, Mameline, Citronellal, Cuuminaldehyde phenylethyl Cinna amides, Euginol,

Uses:

1.Anti- inflammatory;
2. Anti-fungal;
3. Anti-acne
4. Anti-oxidant activity;
5. Anti-ulcer activity;
6. Anti-diabetic activity;
7. Anti-malarial activity;

8. Anti-cancer activity;
9. Anti-bacterial activity;
10. Anti-microbial activity;
11. Anti-viral activity;

3. PREFORMULATION STUDY:

Preformulation testing is crucial for developing stable, effective, and safe drug dosage forms.[14] It involves studying the phytochemical properties of a compound to optimize drug delivery and ensure performance. These studies define substance properties and guide drug-excipient combinations, confirming the identity and suitability of plant extracts for formulation development.

Collection of leaf of Aegles marmelos:

- **Collection:** Collect the leaves during September and October.
- **Washing:** Wash the leaves.
- **Drying:** Dry the leaves in a hot air oven at 55°C.
- **Grinding:** Grind the dry leaves separately.
- **Sieving:** Pass the ground leaves through a 60-mesh sieve.[15][16]

Fig. No.02 Powder form of *Aegle Marmelos*.

Extraction method:

Extraction method of Beal leaf powder using Soxhlet

Apparatus:

The preparation of the ethanolic extract of *Aegle marmelos* leaves involved the use of a Soxhlet apparatus. Extraction continued until the extract passing through the syphon tube turned colorless. Upon completion of the extraction cycle, the alcohol-based extract was collected and concentrated, yielding a greenish-black residue. The Bael leaf extract was then stored in a dark, cool environment within a tightly sealed, airtight container. [17]

Formulation of antifungal cream:

Table No. I Formula 0f Antifungal Cream

Sr. No	Ingredient	Formulation (f1)	Formulation (f2)
1	Bael Leaves Extract	1.5 g	1.5g
Oil phase			
2	Steric Acid	3.3 g	3.3g
3	Cetyl Alcohol	1.2g	1.2g
4	Liquid Paraffin	1.2 g	1.2g
Water phase			
5	Water	22.05ml	21.04ml
6	Glycerin	1.5ml	2.0ml
7	Triethanolamine	0.45ml	0.45ml
8	Methyl Paraben	0.3	0.4ml

Formulation Preparation:

The antifungal cream preparation involves heating the oil and water phases separately to 70°C. The oil phase is then gradually incorporated into the water phase under constant stirring, forming an oil-in-water emulsion. The mixture is stirred until the cream achieves a smooth, opaque consistency. Ethanolic extracts of Bael leaves are added, along with Methylparaben, serving as a preservative, is also blended into the formulation.[18]

METHOD OF PREPARATION

Fig.no 04. weighing of ingredients

Fig. no 05. Soxhlet extraction

Fig no. 06. Rota evaporator

Fig no.07 Extract Sample of Aegle Marmelos

Fig no.09 Excipient used during formulation

Fig. no.09 Final product

4. EVALUTION OF HERBAL ANTIFUNGAL CREAM

Phytochemical screening-

- **Test for Alkaloids:**
  Wagner's Test: Few drops of Wagner's reagent were added into 2 to 3 ml extract. Formation of reddish brown precipitate indicates the presence of alkaloids
- **Test for Flavonoids**:
  Pew's Tests: Zinc powder was added into 2-3 ml. extract, followed by drop wise addition of con. HCl. No formation of purple red or cherry color indicates the absence of flavonoids.
- **Test for Glycosides:**
  Molisch's Test: 2 drops of Molisch's regent were added into 1 ml of extract, and 2 ml of concentrate H2SO4 was added carefully into above solution. Formation of violet ring at the junction indicates the presence of glycosides.
- **Test for Phenols**:
  Phenol Tests: 0.5 ml of FeCl3 (w/v) solution was added into 2 ml of test solution, formation of an intense color indicates the presence of phenols.
- **Test for Saponins**:
  Foam Test: The extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 minutes. Not formation of foam layer, indicates the absence of saponins.
- **Test for Tannins:**
  Lead acetate test: Few drops of 10% lead acetate solution were added into 5 ml of extract. Formation of yellow or red precipitate indicates the presence of tannins**.**

Evalution of cream:

To assess the developed cream, a physical evaluation was conducted. This involved carefully observing its color, fragrance, texture, and overall condition. These characteristics are essential in determining the quality and appeal of the cream.[19]

Irritancy:

A small area measuring 1 cm² was marked on the left side of the back for testing. The cream was applied to the marked spot, and the application time was noted. Over the next 24 hours, the skin was monitored for any signs of irritation, redness (erythema), or swelling (edema). All observations were carefully recorded to assess the cream's effect on the skin. A detailed yet straightforward way to ensure its safety and effectiveness!

Wash Ability:

Apply a small amount of cream to your hands and rinse with tap water.

pH:

Disperse 0.5 g of cream in 50 ml of distilled water and measure with a digital pH meter.

Spreadability:

Spreadability was measured by timing the separation of two microscope slides compressed with cream under a set weight, calculating diffusion capacity as m×l/tm \times l / t for effectiveness. The spread ability of cream is 8.5 g.cm/sec.

Greasiness:

The cream was tested by applying a thin layer, or smear, onto the skin's surface. This was done to check whether the smear felt oily or greasy. Additionally, the cream's physical properties were closely examined, including its color, fragrance, and overall appearance. These observations help evaluate the cream's quality and texture for practical use.

Physical Property.

Sr. No	Tests	Observation
1	Color	Pale green
2	Odour	Characteristic
3	Appearance	Semi-solid

Stability studies:

Test	After One Month
Physical Appearance	Semi-Solid
Texture	Smooth and Creamy
Color	Pale Green
Odour	Aromatic
pH value	6.4
Thermal Stability	Stable
Degradation of Product	No

5. Result

Phytochemical screening:

SR.NO	TESTS	RESULT
1	Alkaloids	+ve
2	Glycosides	+ve
3	Flavonoids	+ve
4	Phenol	+ve
5	Saponin	+ve
6	Tannins	+ve

Table No. II

Physical evaluation:

Sr. No	Parameters	Formulation 1(F1)	Formulation 2(F2)
A) Physical evaluation			
1	Color	Pale green	Pale green
2	Oduor	Characteristics	Characteristics
3	Homogeneity	Fairly good	Good
B) Chemical evaluation			
1	pH	6.4	6.7
2	Phase Separation	Slightly phase separation	No phase separation
3	Viscosity	0.0389 n Pas	-
4	Spreadability	Good	Good

5	Washability	Good	Good
6	Irritancy	No Irritancy	No Irritancy

Table No III

Stability test:

Formulation	**Temperature**	**1 Month stability study**	**Parameter**	**Result**
F1	25^0 C to 30^0C (± 2°C)	7 days, 14 Days,30 days	color	Pale green
			Slightly phase separation	No
			Homogeneity	Fairly Good
F2	31^0C to 35^0C (± 2°C)	7 days, 14 Days,30 days	Color	Pale green
			Phase separation	No
			Homogeneity	Good

Table no IV

Minimum inhibitory concentration -

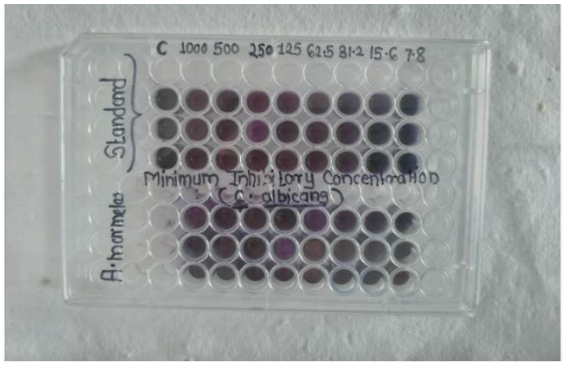

Fig No 1.9

Discussion of MIC results after a certain incubation period. The test samples of A. marmelos showed minimum inhibitory concentration of 15.6 µg/ml respectively against C. albicans compared to the standard.

SR.NO	Sample code	Concentration Ug/ml	Absorbance at 600nm			Mean	%growth of inhibition
			Test 1	Test 2	Test 3		
1	Control		1.603	1.603	1.603	**1.603**	-
2	Standard	7.8	-	-	-	-	-
	(Metronidazole)	15.6	1.538	1.538	1.538	**1.538**	**4.05%**
		31.2	1.421	1.421	1.421	**1.421**	**11.35%**
		62.5	1.397	1.397	1.397	**1.397**	**12.85%**
		125	0.834	0.834	0.834	**0.834**	**47.97%**
		250	0.611	0.611	0.611	**0.611**	**61.88%**
		500	0.531	0.531	0.531	**0.531**	**66.87%**
		1000	0.422	0.422	0.422	**0.422**	**73.67%**
	Aegles marmelos	7.8	-	-	-	-	-
		15.6	1.480	1.480	1.480	**1.480**	**7.67%**
		31.2	1.415	1.415	1.415	**1.415**	**11.72%**
		62.5	1.210	1.210	1.210	**1.210**	**24.51%**
		125	1.134	1.134	1.134	**1.134**	**29.51%**
		250	0.915	0.915	0.915	**0.915**	**42.91%**
		500	0.710	0.710	0.710	**0.710**	**55.70%**
		1000	0.640	0.640	0.640	**0.640**	**60.07%**

Table No. VI Effects of compound against C.albicans

6. Summary

This paper investigates the antifungal properties of an herbal cream derived from Aegles marmelos species, with emphasis on its effectiveness against various fungal pathogens. The study begins with a comprehensive review of the traditional medicinal uses of Aegles marmelos, focusing on its phytochemical constituents known for their antifungal activity. The research method includes the extraction of biological activity compounds from Marmelos Aegles, monitoring the development of cream preparations. The in vitro antifungal test is implemented to evaluate the effectiveness of cream on common fungal strains, and the results show a significant antifungal activity. The results of the study show that Aegles marmelos creams not only offer a natural alternative to synthetic antifungals, but also represent a promising treatment option for fungal infections. The thesis concludes with recommendations for further research into the stability of the cream formula and its potential use in clinical settings, highlighting the importance of studying herbal remedies in modern medicine.

7. CONCLUSION

In recent years, it has been observed that the demand for herbal medicines and cosmetics is increasing due to the increasing number of side effects caused by synthetic chemicals used in the products under development. The primary goal of this study was to create a cream that is both stable and effective, all while avoiding the use of synthetic chemicals. The focus was on ensuring functionality and quality through natural ingredients, promoting a safer and more sustainable formulation. The chemical substances used in the prepared preparations are replaced with the material of natural origin. I created an antifungal cream using AEGLES Marmelos. AEGLES Marmelos leaves antimicrobials, antibacterial, antioxidant, and anti -inflammatory characteristics. The composition has a higher concentration of Aegles Marmelos Extract, indicating excellent antifungal activity, and is suitable for the skin. The evaluation test shows that the composition is tolerated, with high permit PH, propagation ability, non -GOD, transparency, and less than ADR.

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