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A Review on Bael Fruit Showing Antifungal Activity Against Candida Albicans and Aspergillus Niger

Miss. Shubhangi Krishna Kute and Mrs. Sonal S. Kamble
Yashodeep Institute of Pharmacy (B. Pharm), Pimpalgaon Pandhari, Chhatrapati Sambhaji Nagar, India

Abstract: *The Bael fruit (Aegle Marmelos), a widely recognized medicinal plant in traditional medicine, has gainedattention for its antifungal properties. This review highlights its potential effectiveness against Candida albicans and Aspergillus Niger, two significant fungal pathogens causing infections in humans. Various studies reveal that crude and solvent extracts of Bael fruit exhibit promising antifungal activities. For instance, aqueous and methanolic extracts of Bael fruit demonstrated significant inhibitory effects against C. albicans with minimum fungicidal concentrations (MFC) as low as 31.2 mg/ml and moderate effects against A. Niger at 125 mg/ml. The antifungal activity is attributed to bioactive compounds such as Aegeline, lupeol, and γ-sitosterol, which disrupt fungal growth and survival. Additionally, hexane extracts of Bael leaves have been reported to show effective antifungal potential, further underscoring the therapeutic versatility of the plant. This review synthesizes existing research to underline the importance of Bael fruit as a natural antifungal agent, providing an eco-friendly alternative to synthetic antifungals in combating fungal infections. Further investigations are encouraged to explore its clinical applications and efficacy in combination therapies.*

The Bael fruit (Aegle Marmelos), an essential component of traditional medicinal systems, has demonstrated remarkable potential in combating fungal pathogens such as Candida albicans and Aspergillus Niger. These fungi are major contributors to opportunistic infections in humans, particularly in immunocompromised individuals. This review consolidates findings from various studies that have explored the antifungal efficacy of different extracts and bioactive compounds of the Bael plant. Aqueous, methanolic, and hexane extracts of Bael fruit and leaves have shown significant inhibitory effects against these fungal species. For example, methanolic extracts of the fruit have been reported to inhibit C. albicans with minimum fungicidal concentrations (MFC) as low as 31.2 mg/ml, while aqueous extracts demonstrated moderate activity against A. Niger at 125 mg/ml.

The antifungal activity is attributed to the presence of phytochemicals such as aegelin, lupeol, γ-sitosterol, marmelos, and flavonoids, which act through mechanisms like disrupting fungal cell walls and membranes, inhibiting spore germination, and interfering with fungal metabolic pathway.Hexane extracts of the leaves also demonstrated robust antifungal activity, further emphasizing the plant's versatility.

Keywords: Aegle marmelos, bael fruit, antifungal activity, Candida albicans, Aspergillus niger, phytochemicals, natural antifungal agent, minimum fungicidal concentration (MFC), aegelin, lupeol, γ-sitosterol, marmelosin, flavonoids, fungal infections, synergistic potential, traditional medicine

I. INTRODUCTION

Medicinal plants represent rich source of antimicrobial agents. Medicinal plants have curative properties due to presence of various complex chemical substances found as plant secondary metabolites in one or more parts of them. Plant extracts have been developed and proposed for use as antimicrobial substances .Intrest in large number of traditional natural products has increased . Chemical principles from natural resources have contributed significantly for development of new drugs from medicinal plants Aegle marmelos family Rutaceae, is one of the most important medicinal tree of India, Burma and Ceylon .It prefers dry, sunny and warm parts of the hill slopes with well drained

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loamy soil . Leaves, fruits, stem and roots of this tree at all stages of maturity are used as ethanomedicines against various human ailments.

. Candida albicans is a diploid fungus (a form of yeast) and a causal agent of opportunistic oral and genital infections in humans Under normal circumstances, C. albicans lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis Phanerochaete is a genus of fungi.

The bael fruit (Aegle marmelos), a plant of significant importance in traditional medicinal practices, has drawn attention for its extensive pharmacological activities, including its potent antifungal properties. Native to the Indian subcontinent and Southeast Asia, bael is celebrated not only for its nutritional value but also for its rich reservoir of bioactive compounds such as alkaloids, tannins, phenols, flavonoids, and essential oils. These phytochemicals are believed to contribute to its therapeutic potential, including antimicrobial, antioxidant, and anti-inflammatory activities.

Fungal infections caused by pathogens like Candida albicans and Aspergillus niger are significant global health concerns. C. albicans is a common opportunistic pathogen, causing infections ranging from superficial oral and vaginal candidiasis to life-threatening systemic infections in immunocompromised patients. Similarly, A. niger is a major cause of aspergillosis, which can lead to respiratory issues, allergic reactions, and invasive infections. The growing resistance of these fungi to conventional antifungal drugs has escalated the demand for safer, natural, and cost-effective alternatives.

Preliminary studies have shown that bael fruit extracts, prepared using various solvents such as methanol, aqueous, and hexane, exhibit strong antifungal activity against these pathogens. The mechanism of action involves disruption of fungal cell walls, inhibition of spore germination, and interference with metabolic pathways. Compounds like aegelin, lupeol, γ-sitosterol, and marmelosin have been identified as key contributors to this activity. Additionally, bael extracts have demonstrated potential in enhancing the efficacy of conventional antifungal agents, offering a promising avenue for combination therapies

II. LITERATURE SURVEY

1. Dr. K. Sarvanasingh, P. George Fredrik, and Dr. M. Ramamurthy (2016 March 02)

This study investigated the antifungal and antibacterial activities of Aegle marmelos (Beal fruit) extract against various fungal and bacterial pathogens. The authors used solvent extraction methods to obtain the extract, which was then tested against Candida albicans and Aspergillus niger.

2. Savita, Ajit Pal Singh, and Amar Pal Singh (2021)

This systematic review aims to summarize the existing literature on Aegle marmelos, a medicinal plant traditionally used in various diseases.

3. R.K. Singh et al. (2012)

Phytochemical analysis revealed the presence of flavonoids and phenolic compounds responsible for its medicinal properties. The fruit extract was found to reduce blood sugar levels in diabetic rats.

4. P. Ghosh et al. (2017)

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This research focused on the hepatoprotective activity of Aegle marmelos leaves. Using carbon tetrachloride-induced liver damage in rats, the ethanolic leaf extract demonstrated significant protective effects by reducing liver enzyme levels.

5. S. Kumar et al. (2019)
An investigation into the wound healing properties of Aegle marmelos leaf extract. The study found that its flavonoid content accelerated wound contraction and epithelialization in experimental wound models.

6. M. Sharma and V. Gupta (2018)
This study explored the antimicrobial properties of Aegle marmelos root extract against multidrug-resistant bacterial strains, including MRSA. The results showed promising activity, making it a candidate for alternative antimicrobial therapy.

7. Tiwari et al. (2020)
The study reviewed the use of Aegle marmelos in Ayurvedic medicine. It emphasized its applications in gastrointestinal disorders, particularly its effectiveness in managing diarrhea and constipation

8. R. Mehta and K. Patel (2022)
This study evaluated the cardioprotective effects of Aegle marmelos fruit extract. The results suggested a reduction in LDL cholesterol levels and improvement in heart function in a rat model of hyperlipidemia

III. AIM AND OBJECTIVE

AIM:
To conduct a comprehensive review on the antifungal activity of Beal fruit (Aegle marmelos) extracts, focusing on their efficacy against Candida albicans and Aspergillus niger, and to analyze the potential mechanisms and phytochemical compounds responsible for their antifungal properties.

OBJECTIVE:
1. To compile and review existing research on the antifungal activity of Aegle marmelos fruit extracts.
2. To identify and analyze the phytochemical constituents (e.g., alkaloids, flavonoids, and phenolic compounds) contributing to antifungal activity.
3. To evaluate the effectiveness of Aegle marmelos extracts against Candida albicans and Aspergillus niger through reported studies.
4. To explore the extraction methods and assay techniques used for testing antifungal properties.
5. To provide insights into the potential applications of Aegle marmelos in developing natural antifungal agents for pharmaceutical use

IV. SCOPE AND PLAN OF WORK

SCOPE:
1. Scientific Relevance
Global Fungal Infection Burden: The increasing prevalence of fungal infections caused by Candida albicans and Aspergillus niger poses a significant health threat. This study explores natural alternatives to existing antifungal agents.
Antifungal Resistance: Rising resistance to conventional antifungal drugs like azoles and polyenes makes it crucial to explore plant-derived antifungal compounds like those in Bael fruit.

2. Medicinal and Ethnopharmacological Significance

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Bael Fruit in Traditional Medicine: The review will highlight the traditional uses of Bael fruit (Aegle marmelos) in treating infections, thus integrating ancient knowledge with modern research.
Natural Product Research: Investigates Bael fruit as a sustainable source of antifungal agents, promoting natural alternatives in drug discovery.

3. Research and Development Opportunities
Phytochemical Analysis: Explores active compounds (e.g., alkaloids, flavonoids, coumarins) in Bael fruit that exhibit antifungal properties.
Drug Development: Identifies potential lead molecules for future antifungal drug formulations.
Synergistic Effects: Investigates the potential of Bael fruit extracts to enhance the efficacy of existing antifungal drugs.

4. Pathogen-Specific Insights
Focus on Candida albicans: A critical pathogen responsible for candidiasis, particularly in immunocompromised patients.
Focuson Aspergillusniger: A common cause of aspergillosis, especially in respiratory infections.

5. Applications in Agriculture and Industry
Post-Harvest Pathogen Control: Potential application of Bael fruit extracts to combat fungal contamination in agriculture and food storage.

6. Contribution to Sustainable Medicine
Promotes research on renewable, plant-based antifungal agents, reducing dependency on synthetic drugs and their environmental impact.

7. Academic and Educational Value
Provides a comprehensive review for researchers, academicians, and students in pharmacology, microbiology, and natural product chemistry.
Encourages interdisciplinary collaboration between ethnopharmacologists, mycologists, and pharmaceutical scientists.

PLAN OF WORK
1. Introduction
2. Literature survey
3. Selection of Standard Plant
4. Phytochemial analysis of Bael Fruit
5. Future Scope
6. Summery and Conclusion
7. References

V. METHODOLOGY

MATERIAL AND METHOD
Microorganisms
Antimicrobial activity of plant extracts and synthetic compounds were investigated on seven bacterial species and seven fungal species. The bacterial strains include one Gram positive bacterium Staphylococcus aureus and six Gram negative bacterial strains (Escherichia coli, Pseudomonas Klebsiella aeruginosa, Proteus pneumoniae, vulgarius, Salmonella typhimurium and Vibrio cholerae).The fungal species used in this study are Aspergillus niger, A. fumigatus, A.flavus, Mucor species, Rhizopus species, Penicillium species and Candida albicans.

Cleaning of glassware's

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All glassware's were kept in chromic acid cleaning solution (10%) potassium dichromate in 25% sulphuric acid) for a few hours. The glassware's were washed thoroughly in tap water, followed by detergent solution and finally rinsed with distilled water, and then they were dried in dust- proof cupboard.

Sterilization

Media were sterilized in an autoclave at 15 Lbs pressure for 20 min. The glassware's were sterilized in a hot air oven at 1210c for 3 hrs.

Bacterial medium preparation

Muller-Hinton agar

Eight grams of Muller-Hinton agar was suspended in 1000ml of distilled water and the pH was adjusted to 7.3 and the agar was boiled to dissolve the medium was sterilized by autoclaving at 1210c (15Lbs for 15 minutes and mixed well before pouring ungal medium preparation

Sabouraud dextrose agar and Sabouraud dextrose broth

Choloramphenicol in 2ml ethanol [95%] was added to the hot medium**.**Cycloheximide was dissolved in 2ml acetone and added, while stirring to the hot medium. Streptopenicillin was mixed with the hot medium was yellow and the final PH of the medium was 5.6±0.2.

Plant material

Aegle marmelos

The Aegle marmelos plant leaves were collected from in and around Vandhavasi. The taxonomic identity of the plants was established by the department of Life sciences, Madras University. Voucher specimens were maintained in the herbarium, Department of Botany.

Extract preparation

The plant materials were washed with tap water and then with sterile water. They were then macerated using mortar and pestle using sterile double distilled water at a concentration of one gram of tissue per milli litre on water [1:1 w/v]. The concentrated extracts were weighed and dissolved 5%dimethylsulfoxide [DMSO] individually.

In vitro susceptiblity testing for bacterial species

One gram-positive bacterium [Staphylococcus aureus] and six Gram-negative bacterium [Eschericha coli, Klebsiella penumoniae Psedumonas aeruginosa, Proteus vulgaris, Salmonella typhimurium, Vibrio cholerae] were used for the study.

Disc-Diffusion method [Bauer et al., 1996] Preparation

Whatman No.1, 6mm filtered paper is the disc were prepared and sterilized by autoclaving. These discs were plated and each disc was impregnated with appropriate quantity of stock and dried overnight at 310c.

Tube dilution method

From the plant extract [250mg/ml,] 0.5ml was incorporated into 0.5ml of Muller–Hinton broth to get a concentration of 125mg/ml and serially diluted by double dilution technique to achieve 62.5mg/ml, 31.2mg/ml, 15.6mg/ml, 7.8mg/ml, respectively.

Minimum inhibitory concentration [MIC]

MIC determination 0.5ml of various concentrations of extract [125 to1.95mg/ml] and synthetic compounds [50 to 0.78ul] of bacterial strains inoculum was transferred on to each tube. The last tube of Muller-Hinton broth with 50 µl of inoculum served as positive control. The whole set up in triplicate was incubated at 370 c for 24 hrs. The MIC was the lowest concentration of the extract that did not permit any visible growth after 24 hrs incubation.

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Minimum bactericidal concentration [MBC]

The MBC was determined by sub culturing the above [MIC] serial dilutions after 24 hrs, in Muller-Hinton agar plates using 0.01 µl loop and incubating at 370 c for 24 hrs. MBC was regarded

VI. PHYTOCHEMICAL PROFILE OF BAEL FRUIT

Botanical Information

Scientific Name: *Aegle marmelos*

Family: Rutaceae

Common Names: Bael, Bengal quince, Wood apple, Stone apple

Plant Description

Morphology:

1.It has a thick, soft, and spiny bark, with a strong aromatic smell when crushed.

2. Leaves are trifoliate (three leaflets), ovate to lanceolate in shape.

3. Flowers are greenish-white, fragrant, and borne in clusters.

4. Fruit is a hard-shelled berry, globose, 5–15 cm in diameter, with a woody outer layer. Inside, it contains aromatic, orange-yellow, mucilaginous pulp with numerous seeds embedded in it.

Habitat: Thrives in arid, semi-arid, and subtropical climates. It is drought-tolerant and grows in a variety of soil types, including loamy, sandy, and clay soils.

Phytochemical Composition

The medicinal properties of Aegle marmelos are attributed to its rich phytochemical profile, which includes:

1. Alkaloids:

Aegeline, marmeline, skimmianine.

These have antimicrobial and anti-inflammatory properties.

2. Flavonoids:

Rutin, quercetin, and kaempferol.

Act as antioxidants and anti-inflammatory agents.

3. Phenolic Compounds:

Gallic acid, ferulic acid, and tannins.

Provide antioxidant and antimicrobial activity

4. Terpenoids:

Limonene, β-caryophyllene.

Exhibit anti-inflammatory and hepatoprotective effects.

5. Coumarins:

Marmelosin, psoralen.

Show hepatoprotective and anticancer potential.

6. Carbohydrates:

Includes dietary fiber and pectin, aiding in digestion.

7. Vitamins and Minerals:

Rich in vitamin C, calcium, potassium, and iron.

8. Essential Oils:

Contains compounds like eugenol and citral with antimicrobial properties.

Pharmacological Actions

1. Antidiarrheal:

Tannins and pectin in the pulp provide relief from diarrhea and dysentery.

2. Antimicrobial:

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Effective against bacterial, viral, and fungal infections due to alkaloids and flavonoids.

3. Antioxidant:

Flavonoids and phenolics neutralize free radicals and protect against oxidative stress.

4. Antidiabetic:

Regulates blood glucose levels by enhancing insulin sensitivity and reducing glucose absorption

5. Hepatoprotective:

Protects the liver from damage by reducing oxidative stress and improving detoxification.

6. Cardioprotective:

Lowers cholesterol, prevents lipid peroxidation, and improves heart health.

7. Anti-inflammatory:

Reduces inflammation by inhibiting prostaglandins and cytokines.

8. Immunomodulatory:

Enhances immune system function due to its bioactive components.

9. Gastroprotective:

Alleviates gastric ulcers and improves digestion.

10. Anti-cancer:

Coumarins like marmelosin show potential in inhibiting cancer cell growth.

Traditional Uses

1.Treats gastrointestinal disorders (diarrhea, dysentery, constipation).
2.Manages diabetes in Ayurvedic and Unani medicine.
3.Relieves respiratory ailments (asthma, bronchitis).
4.Used as a general health tonic for immunity and vitality.
5.Provides relief in inflammatory conditions like arthritis.

Dosage Forms

1. Raw Fruit: Consumed fresh or dried.
2. Powder: Dried fruit pulp or leaf powder.
3. Juice: Extracted from ripe fruit pulp.
4. Extracts: Alcoholic and aqueous extracts for capsules/tablets.
5. Topical Applications: For wounds and burns

Dosage (General Recommendations)

Fresh Juice: 30–50 ml/day (adults).
Powder: 3–6 grams/day.
Decoction: 50–100 ml/day.
Dosage may vary based on specific formulations and health conditions.

Toxicity and Safety

Safety: Generally considered safe when consumed in traditional doses.
Adverse Effects: Overconsumption may lead to constipation due to high tannin content.
Caution: Use during pregnancy, lactation, or in children under medical supervision.

Drug Interactions

May enhance the effect of antidiabetic drugs, requiring blood glucose monitoring.
Could interact with anticoagulants and antihypertensive medications

VII. DRUG PROFILE

1.Ageline

Molecular Formula: C10H11NO2
Molecular Weight: 177.2 g/mol
Chemical Structure: Contains a phenyl ring with a methoxy and hydroxy substitution and an amine group.

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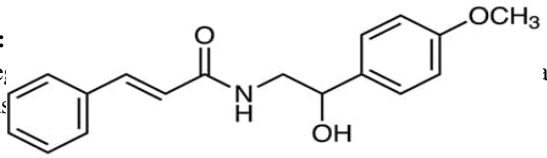
Synonyms: N-methyl-3-(3-hydroxy-4-methoxyphenyl)propan-1-amine

Source
Natural Origin: Found in the leaves of Aegle marmelos (commonly known as Bael tree), a medicinal plant native to India and Southeast Asia.
Structure:

Mechanism of Action of Aegeline:
Aegeline, a compound found in Ae 

ın help fight infections caused by Candida albicans and Aspergillus

1. Disrupting the Fungal Cell Membrane:
Aegeline targets the cell membrane of the fungi, which is made up of a substance called ergosterol. It binds to ergosterol and disrupts the membrane's structure, making it leaky and causing the fungus to lose important substances inside its cells

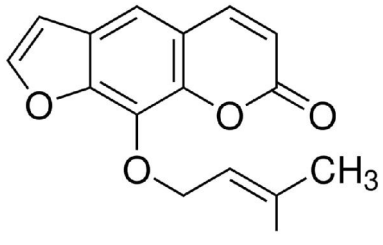
2. Blocking Ergosterol Production:
Aegeline also interferes with the process that produces ergosterol, a crucial component for the cell membrane. Without it, the fungal cell membrane becomes weak, making the fungi more vulnerable.

3.Creating Oxidative Stress:
Aegeline causes the fungi to produce harmful molecules called reactive oxygen species (ROS). These molecules damage the fungus's cell components, such as its DNA and proteins, preventing it from growing and multiplying.

4.Triggering Cell Death:
Aegeline activates pathways in the fungal cells that lead to their programmed death (apoptosis). This helps in eliminating the fungal infection.

2. Marmeline
Molecular Formula: C11H10N2O
Molecular Weight: 186.21 g/mol
Structure: Marmeline is an alkaloid compound featuring an indole moiety.
Sources:
Natural Source: Marmeline is predominantly found in the seeds and leaves of the Murrayakoenigii plant, commonly known as curry leaves.
Plant Family: Rutaceae

Structure:



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Mechanism of Action of Marmeline:
Marmeline, another compound from Aegle marmelos, also has antifungal effects, working against Candida albicans and Aspergillus niger in several ways:

1. Preventing Hyphal Growth:
In Candida albicans, marmeline stops the fungus from changing into a more invasive form called hyphal growth. By preventing this transition, it stops the fungus from invading tissues and forming harmful clusters called biofilms.

2. Stopping Biofilm Formation:
Marmeline also prevents the fungus from forming biofilms—thick, protective layers that fungi create to survive in harsh environments. Without biofilms, fungi are more easily attacked by the immune system or treatments.

3. Disrupting DNA and Protein Production:
Marmeline interferes with the fungus's ability to make DNA and proteins, which are necessary for growth and reproduction. This blocks the fungus from multiplying and spreading.

4. Affecting Mitochondria:
Marmeline damages the mitochondria (the powerhouse of the cell), which reduces the fungus's ability to produce energy. This leads to a shortage of energy in the fungal cell, causing it to die.

5. Weakening the Cell Wall:
Marmeline inhibits enzymes that fungi need to build and maintain their cell walls. Without a strong cell wall, the fungus becomes fragile and more easily destroyed

VIII. APPLICATIONS AND LIMITATION

Applications
1. Medicine Development:
Bael fruit shows potential as a natural source for creating antifungal medications, especially for infections caused by Candida albicans (like oral thrush) and Aspergillus niger (which can cause lung infections).

2. Natural Alternatives:
Its antifungal properties make it a promising alternative to synthetic drugs, especially for tackling drug-resistant fungal infections.

3. Food Preservation:
Bael fruit extracts can act as natural preservatives, preventing fungal growth in stored food.

4. Skin Care:
It could be used in creams or powders to treat or prevent fungal infections on the skin.

5. Crop Protection:
Farmers can use it as an eco-friendly way to protect crops from fungal diseases.

6. Traditional Medicine Support:

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Reinforces the role of natural remedies in modern healthcare

Limitation

1. Lack of Human Trials:
Most research is done in labs, so its effectiveness in humans isn't fully proven yet.

2. Inconsistent Results:
The effectiveness of Bael fruit can vary because its chemical makeup depends on where and how it's grown.

3.No Standard Methods:
There isn't a fixed way to extract or test the antifungal compounds, making it hard to ensure consistent results.

4. Unclear Dosage:
The right amount to use safely and effectively for humans isn't established yet.

5. Unknown Mechanism:
We don't fully understand how it works to kill fungi.

6. Risk of Resistance:
Over time, fungi might become resistant to Bael fruit's antifungal properties, just like with synthetic drugs.

7. Regulatory Challenges:
Approving natural antifungals for medical use can be complicated due to strict regulations.

8. Limited Reviews:
There aren't many comprehensive studies combining lab results, clinical data, and practical uses of Bael fruit for fungal infections

IX. SUMMARY AND CONCLUSION

Summary

1. Antifungal Activity:
Aegle marmelos (bael) exhibits significant antifungal properties, particularly against Candida albicans and Aspergillus niger.
Leaf extracts, particularly aqueous and crude forms, inhibit fungal growth effectively, with clear zones of inhibition observed in laboratory studies.
Crude extracts demonstrate antifungal effects with specific MIC (Minimum Inhibitory Concentration) and MFC (Minimum Fungicidal Concentration) values:
Candida albicans: MIC - 15.6 mg/ml, MFC - 31.2 mg/ml.
Aspergillus niger: MIC - 62.5 mg/ml, MFC - 125 mg/ml.
Unsaponifiable matter from bael seeds also shows activity against Aspergillus niger, further supporting its antifungal potential.

2. Phytochemical Constituents:
Active compounds include alkaloids (e.g., marmeline), terpenoids, phenolic compounds, and flavonoids.
These constituents contribute to the plant's antimicrobial efficacy by disrupting fungal cell structures and inhibiting growth.

3. Traditional and Scientific Relevance:
The antifungal properties align with the traditional use of bael in treating infections.

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X. CONCLUSION

Aegle marmelos is a promising natural antifungal agent due to its rich phytochemical profile and broad-spectrum activity.

The plant's extracts show potential for combating fungal pathogens like Candida albicans and Aspergillus niger, which are responsible for significant human infections.

Its antimicrobial efficacy justifies its traditional use and highlights its potential for development into antifungal therapeutics.

Further research, including in vivo studies and clinical trials, is necessary to confirm its efficacy, safety, and applicability in pharmaceutical formulations

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