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Study of Antimicrobial and Antifungal Properties of the Formulation of Moringa Concanensis

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ABSTRACT

Bacterial and fungal skin infections pose a significant and increasing global burden, as evidenced by recent statistics and trends. Skin and Soft Tissue Infections (SSTIs) are notably prevalent, surpassing urinary tract infections and pneumonia combined in incidence. Factors contributing to the rise of SSTIs include an aging population, higher numbers of immunocompromised individuals, and the emergence of multi-resistant pathogens. In the United States, the economic impact of SSTIs, predominantly from hospitalizations, was estimated at $13.8 billion in 2012[1-2].

Fungal skin infections also present a substantial global health issue, ranking as the most prevalent skin disease globally in 2017. The burden varies regionally, with sub-Saharan Africa showing particularly high rates. For instance, Mali had the highest DALY rate for fungal skin diseases in 2017. These infections contribute significantly to the overall disease burden worldwide [3].

Emerging challenges such as antimicrobial resistance further complicate treatment efforts across bacterial, fungal, and viral infections. This has spurred intensified efforts in antimicrobial discovery and production. Meanwhile, advancements in diagnostic technologies have enhanced the precision of identifying causative agents, potentially reducing the need for empirical treatments and improving patient outcomes.

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INTRODUCTION

Antibacterial agents are pivotal in antimicrobial therapy, often used in combination with other treatments to ensure efficacy and reduce antibiotic resistance risks [6-7]. They also serve in disinfection and sterilization processes, eliminating harmful bacteria from surfaces, though their overuse can contribute to the development of resistant strains. The widespread use of antibacterial agents has led to the emergence of antibiotic-resistant pathogens, prompting the development of new antibacterial agents and the exploration of strategies like increased environmental sampling and metagenomics.

Significant examples include penicillin, discovered in 1928, which targets bacterial cell walls and marked the advent of the antibiotic era [6]. Another example is Dettol, a mixture of chloroxylenol and terpineol, commonly used as an antiseptic for wounds. Aldehydes, such as glutaraldehyde, formaldehyde, and ortho-phthalaldehyde (OPA), are highly effective against bacteria, fungi, and viruses, and are used in the sterilization of surgical instruments. Despite their crucial role in managing bacterial infections and preventing disease spread, the misuse and overuse of antibacterial agents necessitate careful and judicious use to mitigate the development of antibiotic resistance and ensure sustainable treatment options [8].

Overusing antibacterial agents: the overuse of antibacterial agents can have serious consequences, including the promotion of antibiotic resistance, disruption of the microbiome, adverse health effects, environmental contamination, and potential links to increased allergies. Prudent and targeted use of these agents is recommended The key risks of overusing antibacterial agents include:

1. **Disruption of the Microbiome:** Antibacterial agents can disrupt the natural balance of beneficial bacteria in the body's microbiome. This

can lead to increased susceptibility to infections, such as Clostridioides difficile (C. diff) infections.[9]

2. **Increased Antibiotic Resistance:** Overuse of antibacterial agents can contribute to the development of antibiotic-resistant bacteria, as the agents can select bacteria that are resistant to them. This can lead to infections that are difficult to treat.[10]
3. **Adverse Health Effects:** Antibacterial agents can cause side effects like diarrhea, gastrointestinal disturbances, and even life-threatening allergic reactions in some people.
4. **Environmental Contamination:** Overuse of antibacterial agents can lead to their release into the environment, potentially contributing to the spread of antibiotic resistance in the broader ecosystem.[9]
5. **Increased Medicalization of Self-Limiting Conditions:** Overuse of antibacterial agents for viral or non-bacterial infections that would resolve on their own can lead to unnecessary medicalization and increased healthcare costs.[10]
6. **Potential Link to Allergies:** Some research suggests that excessive use of antibacterial agents in healthy households may interfere with the normal maturation of the immune system, potentially increasing the risk of allergies in children.[11]

Table 1.1. Antibacterial agents and antifungal agents' difference:

Characteristic	Antibacterial Agents	Antifungal Agents
Target Microorganisms	Primarily target bacteria	Primarily target fungi
Mechanisms of Action	Disrupt bacterial cell walls, inhibit protein synthesis, or interfere with bacterial replication	Disrupt fungal cell membrane, inhibit fungal cell wall synthesis, or interfere with fungal replication
Chemical Composition	Can be derived from natural sources or synthesized in a laboratory	Can be derived from natural sources or synthesized in a laboratory
Side Effects	Can cause allergic reactions, gastrointestinal disturbances, and kidney damage	Can cause allergic reactions, gastrointestinal disturbances, and liver damage
Examples	Penicillin, tetracycline	Clotrimazole, fluconazole
Use	Used to treat bacterial infections	Used to treat fungal infections
Target Structures	Target bacterial cell wall, protein synthesis, or replication	Target fungal cell membrane, cell wall synthesis, or replication
Antimicrobial Activity	Inhibit bacterial growth or kill bacteria	Inhibit fungal growth or kill fungi
Classification	Antibiotics, antiseptics, disinfectants	Antifungals, antifungal creams, oral antifungals
Examples of Infections Treated	Pneumonia, tuberculosis, urinary tract infections	Athlete's foot, ringworm, candidiasis

These differences reflect the unique structural and metabolic features of bacterial and fungal cells, allowing antimicrobial agents to target and inhibit or kill these microorganisms effectively while minimizing harm to human cells.

Moringa concanensis

Moringa concanensis Nimmo, commonly known as the Drumstick family, is a species of flowering plant native to Pakistan and western India. It is primarily found in seasonally dry tropical biomes and is known for its medicinal properties, which are used in traditional systems of medicine such as Ayurveda, Siddha, and Sowa-Rigpa [26].

The plant is a small tree with thick bark, hairless except for younger parts and inflorescence. Its leaves are bipinnate, measuring 45 cm long, with 4-6 pairs of leaflets that are broadly elliptic to round, 2.5-3.8 cm long, and 1.25-2.5 cm broad. The flowers are borne in lax velvety panicles up to 45 cm long and are small, yellowish, with red or pink veins. They have 5 minutely velvety sepals and oblong or oblong-spoon-shaped petals. The fruit is a linear pod, 30-45 cm long, and sharply 3-angled [27].

Moringa concanensis is used in traditional medicine to treat various diseases. The leaves, flowers, and seeds are used to prepare various remedies, including those for fever, rheumatism, and skin conditions. Phytochemical analysis of the plant's leaves, flowers,

and seeds has revealed the presence of various compounds such as alkaloids, flavonoids, terpenoids, carbohydrates, proteins, and amino acids. Phenol and saponin were found in methanol extracts of leaves and flowers, while steroids, anthraquinone, tannin, oils, and resins were absent.

Fig No 1: leaves and Flower of Moringa concanensis

Taxonomical Classification: Moringa concanensis Nimmo, commonly known as Konkan Moringa, is a species of the genus Moringa within the family Moringaceae. Here is its detailed taxonomical classification:

- **Kingdom:** Plantae
- **Phylum:** Tracheophyta
- **Class:** Magnoliopsida
- **Order:** Brassicales
- **Family:** Moringaceae
- **Genus:** Moringa
- **Species:** Moringa concanensis Nimmo [29-30]

Material & Method

Collection and authentication of the plant

The plant materials used in this experiment were collected from local markets and identified through comparison with standard herbarium specimens available at the Botanical Survey of India, Dehradun. This comparison, facilitated by the transaction reference Bharat Kosh transaction ref. no. 2506240007890, dated 25/06/2024, confirmed the plant species as Moringa concanensis Herb. of the Moringaceae family. The collected plant materials were subjected to pharmacognostic studies to ensure the authenticity and quality of the plant materials used for the evaluation of antibacterial and antifungal activities.

- The collected plant materials were thoroughly cleaned, dried, and pulverized for further processing in the study. The various parts of Moringa concanensis, including leaves, bark, and seeds, were crushed in a mixer and then passed through a sieve to obtain a fine powder. This powder was then used for phytochemical screening, antimicrobial assays, and subsequent formulation processes. The drying and pulverization of the plant materials are essential to prepare them for extraction and ensure the consistency and reliability of the experimental results

Preparation of Plant Extracts

- The collected plant materials of Moringa concanensis were first thoroughly washed with distilled water to remove any dust and impurities. After cleaning, the materials were air-dried in the shade at room temperature for 7-10 days until they were completely dehydrated. Once dried, the plant part (leaves) was separately ground into a fine powder using a mechanical grinder. The powdered plant materials were then stored in airtight containers to prevent moisture absorption and contamination.

Extraction Process

- **Methanol Extract** The solvents used for solvent extraction included 50 % ethanol, and distilled water, chosen for their varying polarities to ensure a broad spectrum of phytochemicals is extracted.75 grams of the dried plant powder were placed in a Soxhlet apparatus and extracted with 500 mL of 50 % ethanol for 6-8 hours. The extraction was carried out until the solvent in the siphon tube became colorless, indicating complete extraction. The extracts were then filtered through Whatman No. 1 filter paper and concentrated under a water bath. The concentrated extracts were dried in a desiccator and stored at 4°C until further use.
- **Aqueous Extract**: 75 grams of the dried plant powder were mixed with 500 mL of distilled water and heated at 60°C for 3 hours. The mixture was allowed to cool and then filtered through Whatman No. 1 filter paper. The extracts were then filtered through Whatman No. 1 filter paper and concentrated under a water bath which was stored at 4°C until further analysis.
- **Storage of Extracts** The dried extracts were weighed and their yields were calculated. They were then stored in labeled, airtight containers at 4°C to maintain their stability and prevent degradation until used for further phytochemical screening, and antibacterial, and antifungal assays.

SUMMARY AND CONCLUSION

- The findings from the study highlight the significant potential of Moringa extracts and seed oil in pharmacological applications due to their diverse bioactive properties. The ethanolic and

aqueous extracts demonstrated different yields, with the ethanolic extract yielding 9.2% and the aqueous extract yielding 7.2%. Phytochemical screening revealed the presence of flavonoids and steroids in both extracts, with additional terpenoids present in the aqueous extract, indicating the presence of various bioactive compounds essential for therapeutic efficacy.

- Physicochemical analysis of Moringa leaves showed consistent moisture content, ash value, and pH levels, ensuring the stability and quality of the extracts. The titratable acidity of the 50% alcoholic extract was determined, indicating its acidic content, which is crucial for evaluating the stability and shelf-life of the extracts.
- UV-Vis spectrophotometric analysis identified the absorption maximum (λmax) for usnic acid at 234.5 nm, facilitating further quantitative estimations. The total phenolic and flavonoid contents of the pumpkin pulp extract were quantified, with the ethanolic extract showing higher phenolic (7.78 mg/g) and flavonoid (3.155 mg/g) content compared to the aqueous extract.
- Antibacterial activity assessment using the agar well diffusion method demonstrated that both extracts exhibited significant antibacterial effects against various pathogenic bacteria, with the ethanolic extract showing a higher zone of inhibition. Similarly, antifungal testing revealed that Moringa seed oil exhibited strong antifungal activity against Malassezia furfur at higher concentrations, comparable to the standard antifungal treatment ketoconazole.
- The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) tests indicated that Moringa seed oil concentrations above 5% effectively inhibited the growth of Malassezia furfur. This concentration-dependent antifungal activity emphasizes the potential of Moringa seed oil as an effective natural antifungal agent.
- In conclusion, the study underscores the promising pharmacological applications of Moringa extracts and seed oil, demonstrating significant antibacterial and antifungal activities. The physicochemical stability, bioactive compound presence, and effective inhibition of pathogenic microorganisms position Moringa as a valuable natural source for developing therapeutic agents. Future research should focus on further elucidating the mechanisms of action, optimizing extraction processes, and exploring clinical applications to fully harness the therapeutic potential of Moringa extracts.

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