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Evaluating the Efficacy of Azadirachta Indica, Mentha Spicata, Rosmarinus Officinalis, Aegle Marmelos in Combating Skin Infection Caused By Bacteria

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ABSTRACT
The rising occurrence of antibiotic-resistant bacteria has spurred the search for alternative antimicrobial substances derived from natural materials. This research examines the antibacterial properties of plant extracts from mint (Mentha spicata), neem (Azadirachta indica), rosemary (Rosmarinus officinalis), and Indian bael (Aegle marmelos) against bacteria that cause skin infections, such as Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa. The extracts were created using ethanol, methanol, and water as solvents and were assessed for their antibacterial activity employing agar well diffusion and minimum inhibitory concentration (MIC) methods. The results indicated that all four plant extracts displayed notable antimicrobial properties, with the ethanol extracts exhibiting the strongest effects. Particularly, extracts from rosemary and Indian bael produced significant inhibition zones against Staphylococcus aureus and Escherichia coli. Phytochemical analysis revealed the presence of bioactive compounds, including flavonoids, tannins, and terpenoids, which are likely responsible for the demonstrated antimicrobial effects. These findings imply that extracts from mint, neem, rosemary, and Indian bael could serve as natural antimicrobial agents for managing skin infections, offering a feasible alternative to traditional antibiotics. Additional research is needed to isolate the active compounds and assess their clinical effectiveness and safety.

Keywords: Bacterial skin infection, Azadirachta indica, Rosmarinus officinalis, Mentha spicata, Aegle marmelos.

I. INTRODUCTION

Skin infections are a common health issue that affects people of all ages, resulting from a variety of pathogens such as bacteria, viruses, fungi, and parasites. the body's largest organ, the skin acts the primary barrier against these harmful agents. Nevertheless, when the skin's surface is damaged, it becomes vulnerable to infections. Maintaining good hygiene, receiving timely treatment, and sometimes seeking medical help are essential for effectively managing skin infections.

Bacterial infections, including impetigo, cellulitis, and folliculitis, are widespread and can vary in severity. Major bacteria responsible for these infections include Staphylococcus aureus and Streptococcus pyogenes, which are linked to conditions like impetigo, cellulitis, and folliculitis. Impetigo mainly affects children and typically presents as red sores that burst open and form a honey-colored crust. Cellulitis is a deeper infection marked by redness, swelling, warmth, and pain, while folliculitis leads to red, swollen bumps that can be itchy or painful. Additionally, other noteworthy bacteria such as Pseudomonas aeruginosa can lead to infections in damp conditions, including hot tub folliculitis and serious infections from burns or wounds.

Neem (Azadirachta indica) is a renowned plant for its potent antimicrobial properties, effective against a broad spectrum of microorganisms. The plant's antimicrobial compounds, including azadirachtin, nimbin, nimbidin, gedunin, and salannin, exhibit bactericidal, fungicidal, and virucidal activities. Neem has been found to be effective against various pathogens, such as Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus subtilis, Candida albicans, Aspergillus niger, and Trichophyton mentagrophytes, as well as HIV, HSV, and influenza viruses.

Mentha spicata, commonly known as spearmint, is a versatile perennial herb renowned

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for its culinary, medicinal, and aromatic uses. Mentha spicata have broad range of applications, from culinary delights to therapeutic benefits, underscores its status as a valuable and multifunctional herb. Its ongoing scientific exploration continues to reveal new potential uses, further solidifying its place in both traditional and modern contexts.

Rosmarinus officinalis, commonly known as rosemary, is a woody, perennial herb native to the Mediterranean region, renowned for its aromatic, needle-like leaves and distinctive, pine-like scent. Belonging to the Lamiaceae family, which also includes mint, sage, and lavender, rosemary has been cherished since ancient times for its culinary, medicinal, and ornamental value. Rosemary's robust flavor and aroma make it a favorite ingredient in Mediterranean cuisine, where it is used to season meats, potatoes, and vegetables. Its essential oil, derived from the leaves, is used in cooking, baking, and as a flavoring agent in various foods and beverages. Rosemary pairs well with a variety of foods, including meats like lamb, chicken, and pork, as well as roasted vegetables and potatoes.Rosemary essential oil contains compounds such as rosmarinic acid, cineole, and camphor, which have demonstrated antimicrobial activity against various bacteria and fungi. This makes rosemary oil effective in inhibiting the growth of microorganisms that cause skin infections, such as acne, athlete's foot, and fungal infections.

Aegle marmelos, commonly known as bael or bael fruit, is a tropical fruitbearing tree native to the Indian subcontinent and parts of Southeast Asia. The tree, which belongs to the Rutaceae family, is distinguished by its aromatic, greenish-brown fruit, which has a hard, woody rind and a sweet, aromatic pulp inside. Bael is renowned for its nutritional and medicinal benefit. It is particularly noted for its high content of vitamin C and its potential to support digestive health. Traditional medicine systems, such as Ayurveda, use various parts of the bael tree, including the fruit, leaves, and bark, for their therapeutic properties.

II. MATERIALS AND METHODS

Collection of Plant Samples: Fresh, healthy leaves of Azadirachta indica (Neem), Mentha spicata (Spearmint), Rosmarinus officinalis (Rosemary), and Aegle marmelos (Bael) were carefully hand-picked from the Garden fields. The selection of plants was based on their botanical authenticity, vigor, and absence of any visible signs of disease or pests. The leaves were chosen at the optimal stage of maturity to ensure maximum bioactive compound content. The collected plant material was promptly transported to the laboratory in sterile, sealed containers to prevent contamination and maintain freshness. The containers were labeled with the botanical name, date, and time of collection to ensure accurate tracking and documentation.

Preparation of plant sample: To facilitate the extraction process, the freshly harvested leaves of Azadirachta indica, Mentha spicata, Rosmarinus officinalis, and Aegle marmelos were cut into small pieces to increase their surface area. This step ensured efficient drying and extraction of bioactive compounds. The cut leaves were then dried using a hot air oven at varying temperatures to remove excess moisture. The temperature and duration of drying were optimized for each plant species to prevent degradation of bioactive compounds: Azadirachta indica: 40°C for 6 hours, Mentha spicata: 45°C for 4 hours, Rosmarinus officinalis: 50°C for 5 hours, Aegle marmelos: 55°C for 7 hours. The dried leaf material was then pulverized into a fine powder using a high-speed mixture blender. This step ensured uniform particle size and increased the surface area for efficient extraction. The resulting powder was stored in airtight containers, such as glass jars or polypropylene bags, at a moisture-free location to preserve its integrity and prevent degradation. The containers were labeled with the botanical name, date, and time of processing to ensure accurate tracking and documentation.

Extraction of plant sample: A suitable solvent was carefully selected for the extraction process. The selected solvent was a mixture of ethanol and water (70:30 v/v), which is a commonly used solvent for plant extraction due to its Polar nature, allowing for effective extraction of polar bioactive compounds, Low toxicity and safety for food and pharmaceutical applications, Ability to dissolve a wide range of bioactive compounds. Approximately 10 grams of each leaf powder (Azadirachta indica, Mentha spicata, Rosmarinus officinalis, and Aegle marmelos) was accurately weighed using a digital balance and placed in a Soxhlet extraction apparatus. Continuous circulation of the solvent through the plant material. Controlled temperature (78°C) to optimize extraction efficiency. The extraction process was carried out for 6 hours, ensuring complete extraction of bioactive compounds. The extracted solution was then collected and subjected

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to further processing, such as concentration and purification, to obtain the final extract.

Isolation of bacterial strains: Pure cultures of the test organisms, namely Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus), were obtained from Proteo Genie, PSG College of Technology, Coimbatore. These microbial strains were selected for the antimicrobial study due to their clinical significance and representation of gram-negative and gram-positive bacteria, respectively. The test organisms were cultured on nutrient agar slants to promote optimal growth and maintenance. The cultures were periodically sub-cultured to ensure their viability and purity. The sub-culturing process involved transferring a small inoculum from the original culture to a fresh nutrient agar slant, allowing the microorganisms to grow and multiply. The cultures were preserved at a refrigerated temperature of 4°C to slow down microbial growth and maintain their stability prior to use in the antimicrobial study. This preservation method allowed for the maintenance of the cultures' viability and ensured their availability for future use.

Antimicrobial activity: Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similar to the procedure used in diskdiffusion method, the agar plate surface is inoculated by spreading a volume (500ul) of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile 1000µl tip, and sample volume (100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated at 37°C under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and zone of inhibition checked after incubation period. Here 1mg/ml concentration of Gentamycin is used as an antibiotic control for Bacterial culture.

III. RESULT AND DISCUSSION

The antibacterial activity of four plant extracts - Azadirachta indica, Mentha spicata, Rosmarinus officinalis, and Aegle marmelos - was evaluated against two types of bacteria: gram-positive Staphylococcus aureus (S. aureus) and gram-negative Escherichia coli (E. coli). The evaluation was conducted using cultured nutrient agar plates.The leaf extracts of Rosmarinus officinalis and Aegle marmelos demonstrated significant antibacterial activity against S. aureus, as evidenced by a pronounced inhibition zone. In contrast, E. coli exhibited reduced susceptibility to these extracts, resulting in a smaller inhibition zone. The inhibition zone is a clear area around the extract where bacterial growth is inhibited, indicating the extract's antibacterial effectiveness.

S. No	Sample	ZONE OF INHIBITION FOR MICROORGANISM (In mm)	
		E. coli	S. aureus
1	Mint	Slightly clearance	13 mm
2	Vilvam	13 mm	16 mm
3	Rosemery	Slightly clearance	24 mm
4	Neem	No Zone	16 mm
5	Gentamycin	31 mm	29 mm

Table.3.1. Antimicrobial activity – Zone of inhibition

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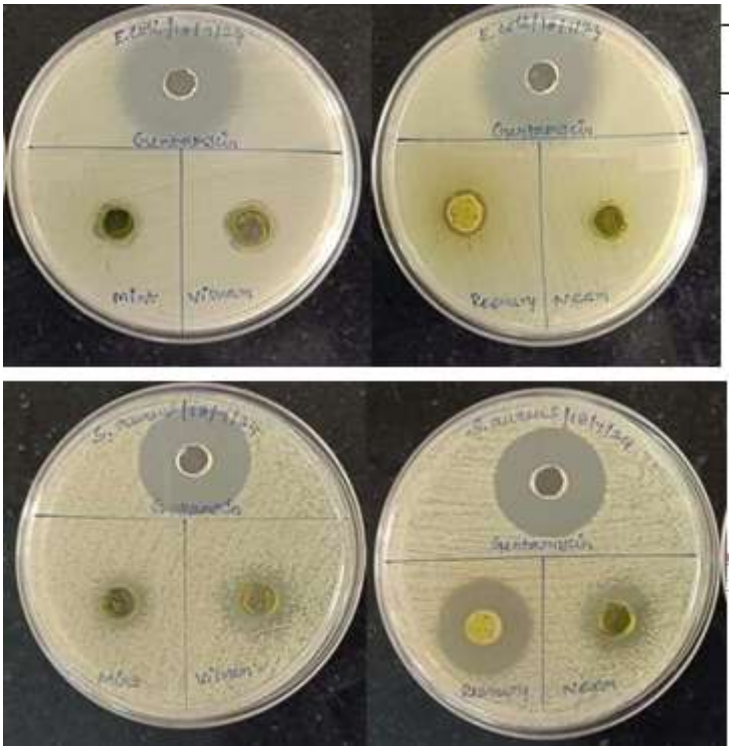

Fig.3.1. Antimicrobial activity

IV. CONCLUSION

The results suggest that Rosmarinus officinalis and Aegle marmelos leaf extracts possess selective antibacterial properties, with a greater effect on gram-positive bacteria (S. aureus) than gram-negative bacteria (E. coli). This selective activity could be attributed to the unique composition of bioactive compounds in each extract, which may target specific bacterial cell wall components or metabolic pathways.

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