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ORIGINAL ARTICLE

Evaluation of Antimicrobial Activity of Leaf Extracts of Moringa, Laxmitaru, Mullatha, and Communist Paccha against *Enterococcus faecalis* and *Candida albicans*: An *in vitro* Study

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ABSTRACT

Introduction: General health depends on the oral health. A vast majority of synthetic antimicrobial agents were developed to control oral infections. Side effects and drug resistance of these antimicrobial agents became barrier in successful treatment. Global scenario is now changing toward the use of nontoxic and eco-friendly products. Medicinal plant extracts are emerging as alternative to synthetic drugs.

Aim: This *in vitro* study evaluated antimicrobial activity of leaf extracts of Moringa, Laxmitaru, Mullatha, and Communist paccha against *Enterococcus faecalis* and *Candida albicans*.

Materials and methods: Ethanolic leaf extracts of Moringa, Laxmitaru, Mullatha, and Communist paccha were prepared. *E. faecalis* and *C. albicans* were cultured on agar plates and leaf extracts were added. The plates were incubated at 37°C for 24 hours. Ethanol was used as positive control. Agar well diffusion test was performed and zone of inhibition was calculated in millimeter. Result was analyzed statistically.

Results: Ethanolic extract of Mullatha leaf showed maximum zone of inhibition followed by Moringa, Laxmitaru, and Communist paccha against *E. faecalis* and *C. albicans* respectively

Conclusion: Study suggested the use of leaf extracts of Moringa, Laxmitaru, Mullatha, and Communist paccha as endodontic irrigant and as antifungal agent in oral candidal infections.

Keywords: Antimicrobial activity, *Candida albicans*, Communist paccha, *Enterococcus faecalis*, Laxmitaru, Moringa, Mullatha.

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INTRODUCTION

Oral health is the mirror image of general health and it is related to the quality of life that extends beyond the functions of craniofacial complex. Oral health problems continue to be a major health problem nowadays. A vast majority of synthetic antimicrobial agents were developed to control oral infections in last decades. But the side effects and drug resistance became barrier in successful treatment. Hence, the needs for developing safer antimicrobial agents are continuing. As global scenario is now changing toward the use of nontoxic and eco-friendly products and synthesis of modern drugs from traditional medicinal plants, natural products and their derivatives including synthetic analogs represent over 50% of all drugs in clinical use, along with those derived from higher plants representing the 25% of the total.[1] As per World Health Organization, 80% of world's population depend on traditional plant medicine for their primary health care needs.[2] Medicinal plants are the richest bioresource of drugs with relatively low side effects.[3] So, medicinal plant extracts are emerging as alternative to synthetic drugs.

Endodontic infections are polymicrobial in nature. Among oral pathogens, *E. faecalis* is the most resistant pathogen seen in secondary endodontic infection.[4] Success of endodontic treatment depends on the eradication of *E. faecalis* from reaching periapical area. *Candida albicans* is the most common opportunistic organism in oral cavity and it is also seen in infected root canal.[5] In this dynamic microbial environment, selection of an effective antimicrobial agent to treat infection is critical. Moringa, Laxmitaru, Mullatha, and Communist paccha are medicinal plants which are used to treat various health problems. But dental reflections of these plants are limited and not

explored properly. This study evaluated the antimicrobial activity of leaf extracts of Moringa, Laxmitaru, Mullatha, and Communist paccha against *E. faecalis* and *C. albicans*.

MATERIALS AND METHODS

Mature fresh leaves of Moringa, Laxmitaru, Mullatha, and Communist paccha were collected and taxonomic identification of plants was performed (Table 1). Leaves were washed in sterilized distilled water, shade dried, and powdered; 100 gm of powdered leaf sample was subjected to Soxhlet extraction with aqueous alcohol (70/30). The extract was then evaporated to complete dryness under vacuum.

Agar Diffusion Method

Muller Hinton agar medium (1 L): The medium was prepared by dissolving 33.8 gm of the commercially available Muller Hinton agar medium (HI Media) in 1,000 mL of distilled water.[6] The dissolved medium was autoclaved at 15 lb pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm Petri plates (25–30 mL/plate) while still molten. One liter of nutrient broth was prepared by dissolving 13 gm of commercially available nutrient medium (HI Media) in 1,000 mL distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lb pressure (121°C) for 15 minutes.

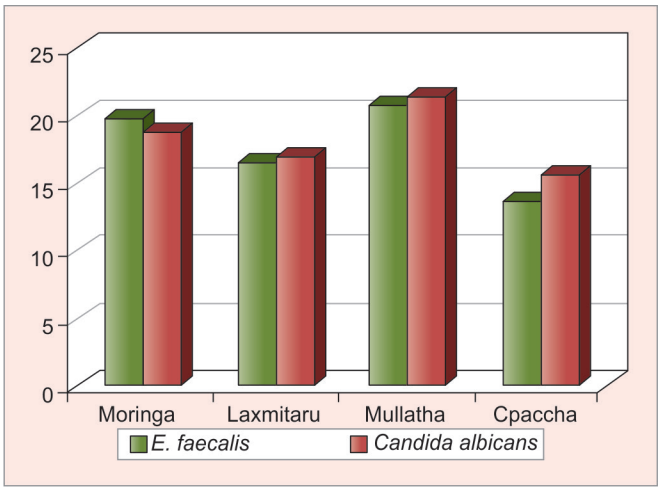
Petri plates containing 20 mL Muller Hinton agar medium were seeded with bacterial culture of *E. faecalis* (growth of culture adjusted according to McFards Standard, 0.5%). For antifungal activity, potato dextrose agar plates were prepared and overnight grown species of fungus, *C. albicans* was swabbed. On these plates, wells of approximately 6 mm diameter and 4 mm depth was bored using a well cutter and 50 µL leaf extracts were added. The plates were then incubated at 37°C for 24 hours. Ethanol was used as positive control. The antimicrobial activity was assayed by measuring the zone inhibition in millimeter. Experiment was carried out three times and mean zone of inhibition was calculated.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance and compared by *post hoc* Tukey test. The level of significance was set to 5% ($p < 0.05$).

Table 1: Medicinal plants used in this study

Common name	*Botanical name*
Moringa	*Moringa oleifera*
Laxmitaru	*Simarouba glauca*
Mullatha	*Annona muricata*
Communist paccha	*Chromolaena odorata*



Graph 1: Graphical representation of zones of inhibition of different herbal extracts

Table 2: Mean inhibitory zone against *E. faecalis* and *C. albicans*

	Zone of inhibition (mm)	
Leaf extract	*Enterococcus faecalis*	*Candida albicans*
Mullatha	20.84 + 0.16[a]	21.43 + 0.29[a]
Moringa	19.82 + 0.60[a]	18.84 + 0.46[a,d]
Laxmitaru	16.50 + 0.28[a,b]	17.00 + 0.76[a,e]
Communist paccha	13.76 + 1.01[a,c]	15.66 + 0.33[a,e]

Data are presented as mean ± standard error; [a]$p < 0.001$ when antimicrobial activity for *E. faecalis* and *C. albicans* was compared between groups; [c]$p < 0.001$ and [b]$p = 0.005$ when intragroup comparison was made for *E. faecalis*; [e]$p = 0.001$ and [d]$p = 0.023$ when intragroup comparison was made for *C. albicans*.

RESULTS

Ethanolic extract of Mullatha leaf showed maximum zone of inhibition followed by Moringa, Laxmitaru, and Communist paccha against *E. faecalis* and *C. albicans* respectively. Ethanol showed no zone of inhibition. Results are shown graphically in Graph 1 and in Table 2.

On statistical comparison, all four leaf extracts showed statistically highly significant antimicrobial activity against *E. faecalis* and *C. albicans* ($p < 0.001$). On intragroup comparison for antimicrobial activity against *E. faecalis*, Mullatha leaf extract showed a highly significant ($p = 0.001$) and significant antimicrobial activity ($p = 0.005$) when compared with Laxmitaru and Communist paccha extracts respectively. Moringa and Mullatha leaf extracts have comparable statistically nonsignificant ($p < 0.668$) antimicrobial activity.

On intragroup comparison for antimicrobial activity against *C. albicans*, Mullatha leaf extract showed a highly significant result ($p = 0.001$) when compared with Laxmitaru and Communist paccha extracts, whereas it showed significant activity (0.023) when compared with Moringa leaf extract.

DISCUSSION

Oral diseases have high impact on quality of life and may lead to systemic and threatening diseases.[7] Due to resistance and side effects, many synthetic chemicals which are effective against microorganisms are not used commonly.[8] As a part of alternative medicine, plant products are introduced into modern medicine, which are cost-effective, easily available, and with less side effects.

In this study, *E. faecalis* and *C. albicans* were included based on the literature and considering its significant role in endodontic infections.[4] *Enterococcus faecalis* is a Gram-positive cocci, which is mainly involved in endodontic infections and asymptomatic chronic peri-radicular lesions. It is also seen in failed endodontic cases and is resistant to calcium hydroxide due to its proton pump.[4]

Enterococcus faecalis can also survive by genetic polymorphism and its ability to bind to dentin, invade dentinal tubules, and survive starvation.[4] *Candida albicans* is the most common oral pathogen in opportunistic infections.[4] It is also found in endodontic infections, immune-deficient conditions, and in patients with broad spectrum antibiotics.[8,9]

Methodology of this study follows the standards established for agar diffusion test and study design is more consistent with other studies testing antimicrobial activity.[10] Moringa or drumstick plant belongs to the family Moringaceae.[11] Studies reported that it is a good source of natural antioxidants like ascorbic acid, carotenoids, flavonoids, and saponins.[12] Antimicrobial activity could be attributed to these constituents. Main action is proven to be through cell membrane perturbations.[13] In this study, a significant zone of inhibition was observed for Moringa against both *E. faecalis* and *C. albicans*. This is in consistent with study of Shailemo et al[14] which exhibited antimicrobial activity of moringa against *E. faecalis*.

Laxmitaru or Paradise tree (*Simarouba glauca*) is famous for its medicinal properties and it belongs to family Simaroubaceae.[15] Its bark, leaf, and fruit extracts are used to treat various diseases. Leaf extract has got analgesic, antimicrobial, antiviral, and astringent properties.[16] Saponins, an alkaloid present in the leaf extract, play an important role in antimicrobial activity. In the present study, significant zone of inhibition was observed against both test microbes. But a study by Mathew et al[17] reported no zone of inhibition against *E. faecalis*. This may be due to difference in extract preparation and *E. faecalis* strain in this study, which is a clinical isolate that may have posed individual characteristics with regard to resistance and tolerance toward various chemical agents.

Annona muricata is a medicinal plant which belongs to family Annonaceae used from traditional time to treat various human pathologies.[18] Phytochemical compounds like tannin, saponins, flavonoids, and alkaloids are seen in these leaf extracts.[19] Flavonoids form complexes with extracellular proteins and bacterial cell wall, thus exhibiting antibacterial effects. Tannins exhibit antimicrobial effect through cause membrane disruption and enzyme inhibition.[20] Alkaloids interfere with deoxyribonucleic acid replication and ribonucleic acid transcription, which are vital for microbial functioning. It has antidiabetic, antitumoral, and antimicrobial activities.[20] Studies reported that methanolic extracts of *A. muricata* leaf extract exhibited inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia*.[21] In this study ethanolic extract showed maximum zone of inhibition against both organisms. Similar result is observed in study by Mathew et al[17] in which zone of inhibition is seen against *E. faecalis*.

Communist paccha or vanapaccha (*Chromolaena odorata*) is a common medicinal plant belonging to family Asteraceae.[22] It is used to treat burns, soft tissue wound, and various skin infections.[23] The leaves are crushed and applied topically on wounds. Leaf extracts contain tannins, saponin, terpenoids, flavonoids, which synergistically act against microbes.[24] In this study, leaf extract exhibits significant activity against both test organs but maximum zone of inhibition is seen against *C. albicans*.

Significant antimicrobial activity of Laxmitaru, Communist paccha, Moringa, Mullatha against *E. faecalis* and *C. albicans* suggests their use as endodontic irrigant and as antifungal agent in oral candida infections. Being natural products, easily available, low cost, and reduced toxicity, these leaf extracts act as better promising antimicrobial agents in dentistry.

CONCLUSION

This study evaluated the antimicrobial activity of Moringa, Laxmitaru, Mullatha, and Communist paccha against *E. faecalis* and *C. albicans*. Mullatha leaf extract showed maximum antimicrobial activity against both test microbes. Extracts of Laxmitaru, Communist paccha, *Moringa oleifera* also showed statistically significant antimicrobial activity. Though this study is done *in vitro*, it gives a broader idea of antimicrobial activity of these herbal, suggesting their use as endodontic irrigant and as antifungal agent in oral candidal infections. Further evaluations have to be carried out to find out minimum inhibitory concentration and clinical trials to check their biocompatibility and safety.

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