RESEARCH PAPER

Antimicrobial efficacy of *Moringa oleifera* against caries causing pathogens: An in-vitro study

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ABSTRACT
Moringa oleifera is a traditional plant with medicinal properties, found in the tropical region of North India. This study was conducted to investigate the antimicrobial activities of the ethanolic extract of the leaves of the *Moringa oleifera* plant. *Moringa oleifera* leaves were dried, powdered, and an extract was prepared using a cold extraction method. The method employed for antibacterial assays of plant extracts was the agar-well diffusion method. Strains of caries-causing pathogens, *Lactobacillus, S. mutans,* and *S. pyogenes* were used for monitoring the anti-bacterial activity of the moringa extract. Subsequently, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated. The ethanolic leaf extracts of *M. oleifera* plant showed no antibacterial activity at the concentration of 10 mg/ml against all the 3 test bacteria. At 25 and 50mg/ml, the extract exhibited moderate antibacterial activity, and at 75mg/ml, it exhibited a strong antibacterial activity against *Lactobacillus* species. At 50 and 75 mg/ml, the extract exhibited moderate antibacterial activity against *Streptococcus mutans*. The extracts were ineffective against the *Streptococcus pyogenes* strain. The MIC values were 6.25 at 25mg/ml for *Lactobacillus* and at 50mg/ml for mutans species. The MBC values were 25 at 25mg/ml for *Lactobacillus* and at 50mg/ml for mutans species. Antimicrobial activity of the ethanolic leaf extract of *M. oleifera* was observed against *Lactobacillus* and *Streptococcus mutans*.

Keywords: *Moringa oleifera*, antimicrobial, *Streptococcus mutans*, ethanolic leaf extract
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INTRODUCTION

The *Moringa oleifera* (M. oleifera / Moringingaceae, English: drumstick tree) since ages, has been an important constituent of the Indian diet. The drumstick tree cultivation profoundly covers most parts of the country. Owing to its medicinal properties, nearly all the parts of the plant, including leaves and fruits, have been used as vegetables and also considered as a part of traditional medicine. Studies have reported that the leaves of the plant also possess properties like "antitumor, hypotensive, cardioprotective, wound healing activities, and use for eye diseases".[1]

M. oleifera leaves are a good source of natural antioxidants and are rich in beta-carotene, vitamin C, vitamin E, and polyphenols. In addition to that, it is an outstanding indigenous source of vitamins, proteins, and minerals.[2] The therapeutic value of the plant was observed regarding anti-diabetes, anti-atherosclerosis, anti-infertility, anti-rheumatoid arthritis, pain relief, anti-depression, and diuretic and thyroid regulation in various studies.[2-4] Inhibition of the growth of breast, pancreatic, and colorectal cancer cells was reported to be seen in *M. oleifera* leaf and bark extracts.[5,6] Significant reduction in glucose to normal levels has been found in *M. oleifera* without any obvious cytotoxicity.

Antimicrobial properties are possessed by various parts of Moringa roots, flowers, bark, and stem, including seeds.[7,8] Promising anti-bacterial properties were reported in the

aqueous and ethanolic extracts of the leaves of *M. oleifera*, with strong inhibitory potentials against "Gram-positive species (*Staphylococcus aureus and Enterococcus faecalis*) over Gram-negative species (*Escherichia coli, Salmonella, Pseudomonas aeruginosa, Vibrio parahaemolyticus, and Aeromonas caviae*)".[9] The aforementioned indicates that the bioactivity of *M. oleifera* has garnered incredible attention over the last few decades, making its underlying mechanisms and pharmacological functions more appreciable.

Subsequently, it led to the rising exploration of its usage. With respect to the dental aspect, it was found that *Moringa* had a protective effect on enamel and dentin remineralization similar/or better than fluoridated toothpastes.[10] It was also formulated as a novel dental remedy in the form of toothpaste and mouthwash, and higher mean inhibition was observed with respect to toothpaste against *S.aureus* and *S.mutans*, in comparison to mouthwash.

Commercial antibiotics meant for prevention and cure are becoming resistant to bacteria at a rate that brings about a lot of concern. Considering the fact that a new source of antimicrobial agents is observed in natural products of higher plants, the focus of many research groups has now shifted towards medicinal plant research.

Our literature search revealed that there is a lack of evidence about the antimicrobial activity of herbal extracts like *Moringa oleifera* against dental caries-causing microorganisms. Hence, the present study was meant to evaluate the in vitro antibacterial properties and to determine the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of various extracts of *Moringa oleifera* against common caries pathogens.

MATERIALS AND METHODS

Preparation of the plant extract

The freshly collected *Moringa oleifera* leaves from the Chandaka bioreserve forests, Bhubaneswar, Odisha, were brought to the Department of Pharmacy, Siksha O Anusandhan University. A sample plant from the collected lot was submitted to the Regional Plant Resource Center, Bhubaneswar, for final identification by the in-house taxonomists, and a herbarium was submitted with the reference number RPRC 11237. According to the process of maceration, the extraction method was undergone. The *M.oleifera* leaves were dried out in an open surface at room temperature (37˚C) for a few days, till they were completely dry. After that, the dried moringa leaves were powdered.

About 20 grams of the *M.oleifera* plant samples were powdered, followed by percolation at room temperature with 300 mL 99.9% ethanol in a 400 mL beaker. Using foil paper, the beaker was covered, shaken up, and left undisturbed for two weeks, followed by regular shaking at intervals. The suspensions were filtered, and the filtrates were concentrated after two weeks, by means of a rotary evaporating machine at 40°C. For further analysis, marking of the extracts was done, and subsequent storage in the refrigerator.

Agar-well diffusion method for antibacterial assays of plant extracts

An antibacterial activity of the *Moringa oleifera* leaf extract was determined by the agar-well diffusion method. To examine the antibacterial activities of plant extract, strains of *Streptococcus mutans*, *Streptococcus pyogenes*, and *Lactobacillus* species were used. Preparation of bacterial lawns was done with fully punched 6 mm thick agar. Similarly, when a lawn was 30 minutes old, 6-8 wells were prepared. The base of each well is composed of 50 µl molten Blood agar and Muller-Hinton agar, in duplicates. Furthermore, the wells were filled with 100 µl aliquots of 30 mg/ml solvent-extracts of the plant. Dilution of the solvent extracts from the original stock of plant extracts was done earlier by 10% DMSO (Dimethyl Sulphoxide) solution. Incubation of plates was done at 37°C for 18-24 hours. Evaluation of the antibacterial activities was done by assessing the diameter of zones of inhibition. The experiment was carried out thrice. Results were presented from the third repeated experiment. An aliquot of 100 µl of Chlorhexidine (2%) with an average diameter of zone of inhibition of 21 mm was used along with 10% DMSO solution, considered as the reference control (Perez et al. 1990).[11] The 10% DMSO solution exhibited the absence of any antibacterial activity (Rath et al 2013).[12]

Determination of MIC and MBC values of the plant extracts

The MIC test reflects the smallest level of antimicrobial agent responsible for inhibition of growth. On the contrary, MBC reflects the smallest level of antimicrobial agent, leading to microbial death. MIC and MBC of active plant extracts were evaluated by appropriate dilutions from original stock solutions of each plant extract for the following concentrations: 0**,** 1.562, 3.125, 6.25, 12.5, 25, 50, and 100 mg plant extract per ml in aliquots of 10% DMSO solution. For each solvent-extract, separate experiments were conducted. On a 96-well (12×8) microliter plate, an aliquot of 80 µl of each dilution of a solvent-extract was released. Further, an aliquot of 100 µl MH broth (HI Media), an aliquot of 20 µl bacterial inoculum (10^9CFU/ml), including a 5 µl-aliquot of 0.5% Triphenyl tetrazolium chloride (TTC- colorless dye) were also released. The micro-titre plate incubation was done at 37°C for 18 hours, after pouring all the above materials to a well. Growth inhibition was considered after the occurrence of pink colouration due to TTC, and also the absence of colouration. The first well of the micro-titre plate, devoid of plant extract, was the control (Eloff et al. 1998).[13] In wells where pink colour was not evident, the MIC value was noted. Further, a sub-culture of bacteria

was done on nutrient agar, from each well of the micro-titre plate. MBC value was noted as the level of dilution, if bacterial growth was absent on the nutrient agar. Results from the second repeated experiment were presented (Rath et al 2013).[12]

RESULTS

The ethanolic leaf extracts of *M. oleifera* plant exhibited no antibacterial activity at a concentration of 10 mg/ml against all the 3 test bacteria. At 25 and 50 mg/ml, the extract exhibited moderate antibacterial activity against *Lactobacillus* species, having a zone of inhibition of 15 and 17mm, whereas at a concentration of 75mg/ml, the extract exhibited good antibacterial activity against *Lactobacillus* species, having a zone of inhibition of 21 mm. Similarly, at 50 and 75 mg/ml, the extract exhibited moderate antibacterial activity against *Streptococcus mutans* having zone of inhibition 16 and 18 mm, respectively. The extracts were ineffective against *Streptococcus pyogenes* strain [Figure-1,2].

Figure 1. Antimicrobial assay, as zone of inhibition by the agar-well diffusion method of *Moringa oleifera* against *Lactobacillus sp*

Figure 2. Antimicrobial assay, as zone of inhibition by the agar-well diffusion method of *Moringa oleifera* against *Streptococcus mutans*

The ethanolic leaf extracts of *M. oleifera* plant exhibited a MIC value of 6.25, 3.125 mg/ml; 1.56 mg/ml at a concentration of 25, 50 and 75 mg/ml against *Lactobacillus* species, whereas the MBC values of 25, 12.5 mg/ml; 6.25 mg/ml at the same concentrations against *Lactobacillus* species Similarly, at 50 and 75 mg/ml the extract exhibited at MIC value 6.25 mg/ml; 3.125 mg/ml against *Streptococcus mutans* with a MBC value of 25 and 12.5 mg/ml, at same concentration respectively. The extracts were ineffective against *Streptococcus pyogenes* strain [Table-1,2].

Table 1. Antimicrobial assay, as zone of inhibition by the agar-well diffusion method of *Moringa oleifera*

Bacterial Strains	Zone of inhibition			
	10mg/ml	25 mg/ml	50 mg/ml	75 mg/ml
Lactobacillus sp.	0	15	17	21
Streptococcus mutans	0	0	16	18
Streptococcus pyogenes	0	0	0	0

Table 2. MIC and MBC values extracts of *Moringa oleifera*

Bacterial Strains	Plant Extracts							
	10mg/ml		25mg/ml		50mg/ml		75mg/ml	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Lactobacillus sp.	0	0	5.00	25	2.50	10.00	1.25	5.00
Streptococcus mutans	0	0	0	0	5.00	25	2.50	10.00
Streptococcus pyogenes	0	0	0	0	0	0	0	0

DISCUSSION

The *Moringa oleifera* leaf extract showed antibacterial activity against the major caries-causing pathogens like *Lactobacillus* and *Streptococcus mutans*, but it did not show any antibacterial activity against *Streptococcus pyogenes*. Studies have shown that the highest action was demonstrated by the ethanolic extracts of *M. oleifera*, while the least action was demonstrated by the aqueous extracts against the causative agent of typhoid fever, *S. typhi*.[14] Ethanol extracts have shown the highest significant mean inhibition values among all other modes of extracts, like acetone or ethyl acetate.[15] This makes us believe in the fact that ethanolic extracts of plant extracts work better than aqueous or any other means of extraction. Evidence from literature reviews also supports that little or no antimicrobial activity is exhibited by the aqueous extracts of plants.[16,17]

Consideration was made for leaf extract in our study based on the greatest antimicrobial efficacy of the leaves part of *M. oleifera* in comparison to other plant parts. These results were in agreement with some studies, which indicate that the leaves of *Moringa oleifera* contain bioactive substances responsible for antibacterial properties against a broad array of microorganisms. Certain phytochemical compounds, such as "flavonoids, saponins, tannins and other phenolic compounds", constitute to provide antimicrobial activities to the leaves of *M. oleifera* plant.[18,19] The presence of such compounds may be the reason for the antimicrobial activities reflected in this study.

The antimicrobial activities of *Moringa oleifera* leaves, flowers, and seeds were tested in vitro against "fungus, gram-negative and gram-positive bacteria".[20] It was found effective against *E. coli, S. typhi, P. aeruginosa, S. aureus, Streptococcus-B-haemolytica, B. sterothermophilus, S. pyogenes*, etc. In the study by Isitua et al, *M.oleifera* ethanolic leaf extract was found to exhibit antimicrobial activity against *Streptococcus pyogenes* at 100ug/ml.[21] On the contrary, our study showed no antimicrobial activity against pyogenes at 25, 50, or 75mg/ml concentration. This difference could be owing to the different concentrations of the extract used.

The mean zone of inhibition of ethanolic leaves extract of *M. oleifera* against *Streptococcus mutans* was 13.00^{aA} in a study by Elgamily et al.[15] A 6mm zone of inhibition was noted by Amabye et al.[22] In our study, the minimum zone of inhibition for the same was 16mm at a concentration of 50mg/ml. The slight variation may be due to different concentrations of leaf extracts used in our study.

The World Health Organization estimates that more than 80% of the world's population deals with their primary health care needs by relying upon traditional medicines.[23] Formulation of new dental products could be done by using plant extracts, owing to their antimicrobial potentials, in order to control oral pathogens.

CONCLUSION

The present investigation confirmed the antimicrobial potential of the *Moringa oleifera* leaves, making it a possible reason for promising applications of medicinal plants for making novel dental remedies (toothpaste,

mouthwash, etc.) to control caries pathogens. The ethanol extract showed comparable zones of inhibition, too. Thus, the use of this plant will be of great potential in terms of cost, affordability, availability, and accessibility. Further research to determine the toxicity of the leaf extract could be done.

DECLARATIONS

Ethical Approval:

This study did not involve human participants or animals. Ethical approval was not required

Informed Consent:

Not applicable (no human participants)

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