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ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA*

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ABSTRACT

Moringa oleifera, belongs to the Moringaceae family, is an indigenous plant and native to the North India region, Also known as the miracle tree, is a rich source of essential nutrients and bioactive compounds with medicinal potential. This study employed Soxhlet extraction using 90% ethanol to obtain crude extracts from dried powdered *Moringa* leaves. The extraction yielded a high amount of phytochemicals, including phenolics and flavonoids. The antibacterial activity of the extract was evaluated against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria using the Kirby-Bauer well diffusion method. The extract demonstrated concentration-dependent antibacterial effects, with the highest inhibition observed at 100 µl, showing greater sensitivity in *S. aureus* compared to the Gram-negative strains. This difference is attributed to structural variations in bacterial cell walls influencing phytochemical permeability. The findings highlight *Moringa oleifera* as a promising natural antibacterial agent, particularly effective against Gram-positive bacteria. Further studies are recommended to isolate active compounds and elucidate their mechanisms of action.

KEYWORDS: *Moringa oleifera*; Anti-Bacterial Activity; Ethanolic extract; Phytochemical constituents.

INTRODUCTION

MORINGA OLEIFERA

Moringa oleifera is universally referred to as the miracle plant or the tree of life. *Moringa oleifera* is one of the vegetables of the Brassica order and belongs to the family Moringaceae. The Moringaceae is a single genus family with 13 known species.[1] The Moringa plant derives this name based on its uses, particularly with regard to medicine and nutrition. It is a plant native to the sub Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan[2] Almost all the parts of this miracle tree have been found to be very useful. Leaves are used as forage, tree trunk for making gums, power nectar in honey and powdered seeds for water purification.[3]

M. oleifera has in recent times been advocated as an outstanding indigenous source of highly digestible protein, calcium, iron, vitamin C and carotenoids suitable for utilization in many of the developing regions of the world where undernourishment is a major concern.[4] Moringa leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas.[5]

Drumstick is one of the promising plant which could contribute to increased intake of some essential nutrients and health promising phytochemicals. It is very significant for its medicinal value, numerous parts of the plant such as roots , seeds, bark, leaves, fruits and immature pods, flowers act as a cardiac and circulatory drugs, anti pyretic, antiulcer, anti-inflammatory and antiepileptic.[6]

MATERIALS AND METHOD

PRE-EXTRACTION PREPERATION FOR PLANT SAMPLE

The integrity of the biomolecules in the plant leaves requires sample preparation prior to extraction. *Moringa oleifera* leaves were cleaned and dried under the shade. The dried sample was ground and sieved (20 mesh) to become powder. The powdered form is kept in a sealed container such as desiccators to prevent moisture trapped in the samples until it is used for the extraction. The presence of moisture could promote the growth of unwanted fungal.

AUTHENTIFICATION OF PLANT

The plant material was identified by the Botanist Dr. Rajasamarsen K Modi, Associate Professor & Head, department of Studies & Research in Botany, Government First Grade College, Naubad Bidar.

SOXHLET EXTRACTION

Soxhlet extraction is a solvent extraction method that is routinely used for the separation of bioactive compounds from plant sources.[7] Soxhlet extraction was employed in the present work to powdered and dried Moringa oleifera leaves to yield a crude extract to be used for determining antibacterial activity.[8]

The dried powdered Moringa leaves was packed into a cellulose thimble and introduced into the main chamber of a Soxhlet extractor. The solvent, 90% ethanol (v/v), was used in extracting at a solvent-to-sample ratio of 4:1 (v/w).[9] Extraction was kept steady at the temperature of 60–70°C on the heating mantle, where the vapours of ethanol got condensed and recirculated through the plant material with the result that the bioactive compounds dissolved.[7]

After the solvent siphon arm became almost colorless, showing total extraction, the extraction was stopped.[8]

Soxhlet extraction provided a high yield and would be likely to preserve a wide range of phytochemicals, e.g., flavonoids and phenolics, with proven antimicrobial activity.[9]

ANTIBACTERIAL METHODOLOGY

The Antibacterial activity of metal oxide was performed by Kirby-Baur well diffusion method.[9] 24 hours freshly grown (0.5 McFarland turbidity Standard) cultures of S. *aureus* (Gram positive), *E. Coli* (Gram negative), *Klebsiella pneumoniae* (Gram negative) in nutrient broth medium were inoculated by lawn culture technique on sterile solidified Muller Hinton Agar (HIMEDIA-M173) plates with the help of sterile cotton swabs.

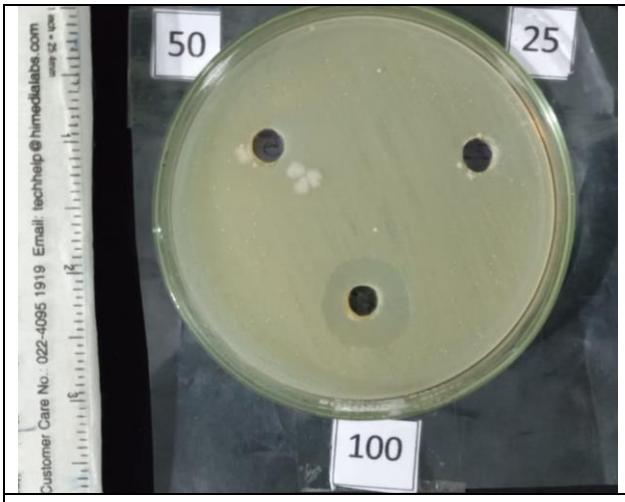
The stock solutions of concentration 1 mg/ml were prepared in DMSO solvent. Three wells were bored on the MHA media plates with the help of sterile cork borer (8mm). Each well loaded with solution following concentration 25µl, 50µl, 100µl respectively. Then allowed to diffuse at room temperature for 2-3 hours. The plates were incubated in the upright position at 37 $^{\circ}$C for 24 hours. Then the zones of inhibition were observed.

RESULT

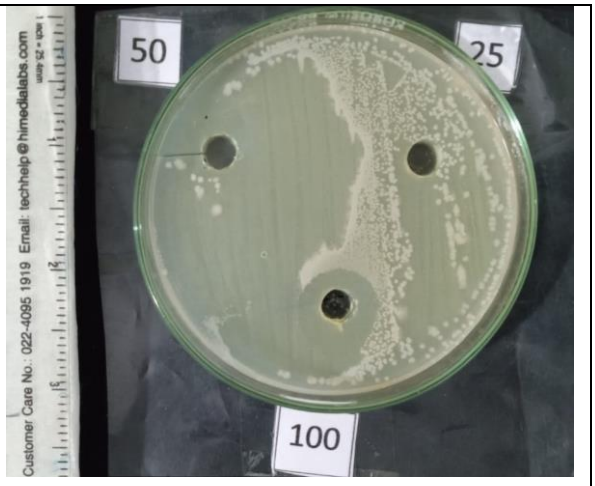
Table 1: Antibacterial Activity of Moringa Oleifera Leaves Extract.

Antibacterial activity			
Zone of Inhibition in mm			
Organisms	25 µl	50 µl	100 µl
EC01-MO	NI	NI	18
SA03-MO	NI	NI	22
KP04-MO	NI	15	19
Note :- Strain EC01- *Escherichia coli*, SA03- *Staphylococcus aureus*, KP04- *Klebsiella pneumoniae*, NI- No Inhibition			

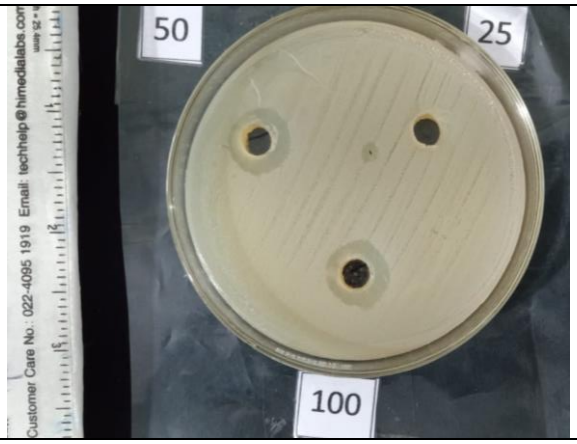
Figure 1: Plates showing Antibacterial Activity of Moringa Oleifera Leaves Extract.



EC01-MO



SA03-MO



KP04-MO

DISCUSSION

Antibacterial efficacy of the ethanol extract of Moringa oleifera against three bacterial isolates, namely Escherichia coli (EC01) and Klebsiella pneumoniae (KP04) Gram-negative bacteria, and Staphylococcus aureus (SA03) a Gram-positive bacterium, was tested. The extract showed EC-50 concentration-related inhibition where the lowest volume (25 µl) was inactive against all the isolates.

The extract showed moderate inhibition at 50 µl against Klebsiella pneumoniae (15 mm) and E. aureus was not affected, implying partial susceptibility by the Gram-negative KP04 strain even at intermediate levels of the extract. At 100 µl (the highest tested volume), there were strong antibacterial activities against all test organisms. Worthy of note was Staphylococcus aureus (Gram-positive) with the largest zone of inhibition (22 mm), an indication of greater sensitivity to the Moringa extract than that of the Gram-negative strains, E. coli (18 mm) and Klebsiella pneumoniae (19 mm).

This variation in susceptibility between Gram-positive and Gram-negative bacteria is due to structural differences in cell walls. Gram-positive bacteria have a thicker layer of peptidoglycan but do not have an outer membrane like in Gram-negative bacteria, and therefore are generally more permeable to phytochemicals. The outer membrane of the Gram-negative bacteria would provide most of the antimicrobial agents a hindrance, possibly accounting for the comparatively smaller inhibition zones recorded in E. coli and Klebsiella pneumoniae at lower concentrations of the extract.

The antibacterial activity reported here is probably attributed to the flavonoids and phenolic compounds found in the extract, which have been shown to disrupt microbial membrane structures and block enzyme activities necessary for bacterial growth. The enhanced activity against S. aureus indicates the use of Moringa oleifera extracts as natural antimicrobial agents, especially against Gram-positive bacteria.

CONCLUSION

The research on *Moringa Oleifera* is needed to gain more importance in India. It contains essential nutrients and can be exploited for a variety of purposes. Sohxlet extracted ethanolic crude leaves of M. oleifera also showed promising antibacterial activity, especially at high concentrations, against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli and Klebsiella pneumoniae) bacteria. Activity against the Gram-positive strain was greater, as per known structural differences in bacterial cell walls and susceptibility to phytochemicals. These observations validate the potential of Moringa oleifera as a natural antibacterial source of molecules, particularly against Gram-positive bacterial infections. Isolation of the active molecules and determination of their mode of action are suggested in future studies.

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