IJPSR (2023), Volume 14, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 March 2022; received in revised form 19 April 2023; accepted 22 May 2023; published 01 June 2023

ANTIBACTERIAL ACTIVITY FROM DIFFERENT PARTS OF *MORINGA OLEIFERA* (L.) AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Jitendra Kumar Verma [1], Tabish Qidwai [*1] and Rohit Saluja [2]

Faculty of Biotechnology [1], Institute of Bio-Sciences and Technology, Shri Ramswaroop Memorial University, Lucknow-Deva road, Barabanki - 225003, Uttar Pradesh, India.
Department of Biochemistry [2], All India Institute of Medical Sciences Bibinagar, Hyderabad - 500007, Telangana, India.

Keywords:

In-situ gel, Acyclovir, Anti-viral, HPMC E50 LV, Pluronic F-127

Correspondence to Author:
Dr. Tabish Qidwai

Associate Professor,
Faculty of Biotechnology,
Institute of Bio-Sciences and Technology, Shri Ramswaroop Memorial University, Lucknow-Deva road, Barabanki - 225003, Uttar Pradesh, India.

E-mail: tabish.iet@gmail.com

ABSTRACT: Antibacterial activity of methanolic extracts of different parts (leaves, flowers, green pods and roots) of *Moringa oleifera* plant were evaluated against gram positive bacteria *Bacillus subtilis (Bs), Staphylococcus aureus* (Sa) and gram negative bacteria *Klebsiella pneumonia* (Kp), *Escherichia coli* (Ec), *Pseudomonas aeruginosa* (Pa). The activities were performed by the agar well diffusion and cup plate method, with methanol as the negative and tetracycline as a positive control. Secondary metabolites extracted from all the parts of *M. oleifera* were found active against bacteria. Furthermore, the antimicrobial activity of the mentioned plant extracts was also examined against various gram-positive and gram-negative bacterial strains. It was observed that against *K. pneumonia*, leaves extracts (17.6 mm) of *M. oleifera* possess higher antibacterial activity as compared to others, flower extract (19.2mm) against *B. subtilis*, leaves extracts (22.6 mm) more effective against *E. coli*, pod extracts (22.5 mm) against *S. aureus* and leaf extract (21.9 mm) showed against *P. aeruginosa* as compared to others. The results reveal that *M. oleifera* might be a potential source for the treatment of gram-positive and gram-negative bacteria.

INTRODUCTION: Infectious diseases exert a significant socio-economic burden on countries, with a wide distribution of pathogens. Bacterial, plasmodium, and fungal infections affects people in many countries. The most acute group includes multidrug-resistant bacteria that pose a particular threat in hospitals, nursing homes, and among patients whose care requires devices such as ventilators and blood catheters. They cover *Pseudomonas* and various Enterobacteriaceae (including *Klebsiella*, *E. coli*, *Serratia*, and *Proteus*).

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(6).2894-98

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(6).2894-98

They can cause severe and often lethal infections [14]. In addition, *M. oleifera* seeds shown strong antimicrobial effect against *Enterococcus faecalis* and *Staphylococcus aureus*starins [7-9]. The seed extract and recombinant protein of *M. oleifera* are effective against *Staphylococcus aureus*, *Streptococcus pyrogenes, Streptococcus mitis*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Escherichia coli*, and *Legionella pneumophila* for up to 150 min [12].

Klebsiella pneumoniae are common intestinal bacteria that can cause lethal infections. Resistance in *K. pneumoniae* to last resort treatment (carbapenem antibiotics) has spread to all regions of the world. *K. pneumoniae* is a major cause of hospital-acquired infections such as pneumonia, bloodstream infections, and infections in newborn and intensive-care unit patients. In some countries,

carbapenem antibiotics do not work in more than half of the patients treated for *K. pneumoniae* infections due to resistance (WHO, 2020). The development of drug resistance in pathogenic organisms has created problems in their treatments. Although a lot of research is being done effective treatment options are limited; therefore novel therapeutic compounds are urgently needed to prevent the aforementioned diseases.

Additionally, *M. oleifera* plant and its parts have well-known medicinal properties and have been used as an anti-inflammatory, anti-hypertensive, antimicrobial, anti-oxidant, anti-diabetic, anti-hyperlipidaemic, anti-neoplastic, anti-pyretic, anti-ulcer, cardio protectant and hepatoprotectant [10]. Several important compounds, such as bactericidal and fungicidal, have been found in *M. oleifera* [5-13]. Methanolic extract of *M. oleifera* had a greater antibacterial effect than chlorhexidine (control group) [7]. *M. oleifera* has gained fame as a nutritional immune booster in globally. Its consumption might be helpful in combination with traditional drugs like anti-malarial [11]. In the past, researchers focused only on leaves of *M. oleifera* for antibacterial properties. *C*urrent study aimed to investigate the antibacterial activities of methanolic extract of different parts of *M. oleifera* against disease-causing bacteria by disc agar diffusion method.

MATERIALS AND METHODS:

Collection of Plant Material: The leaves, flowers, green and pods of *M. oleifera* was collected from angelic region of Uttar Pradesh, India. Root collected from 30 days old plant in the nursery. The collected material was washed with water and shade dried, made to coarse powder and then packed in polythene bags for further analysis.

Extraction of Active Principles: The leaves, flowers, green pods, and root of *M. oleifera* has been dipped into polar and nonpolar solvents for the extraction. After 48 hours of incubation, filtration was done with polar & non-polar filtrates. The weighed amount of *M. oleifera* all parts powder was packed in an extraction thimble and placed in an extraction chamber which was suspended above the flask containing the solvent and below a condenser. In the last extraction process, the flask containing the extract was removed and solvent was evaporated using a rotary evaporator. The crude extract was stored in a refrigerator for antibacterial analysis.

Test Organisms: Gram positive bacteria *B. subtilis (Bs), S. aureus* (Sa), and gram negative bacteria *K. pneumonia* (Kp), *E. coli* (Ec), *P. aeruginosa* (Pa) were pure cultures used to test bacterial strains. The agar well diffusion and cup plate method performed the tests, with methanol as negative and tetracycline as a positive control.

Antibacterial Activity Assay: *In-vitro* antibacterial activity of selected plant extracts were tested by disc agar diffusion method [4-15] was applied for antibacterial assay. The extracts were incorporated into appropriate medium and subsequently bacterial spore suspension was inoculated for bacterial susceptibility testing. The preparation was incubated at appropriate temperatures. After incubation, the zone of inhibition (diameter) originating in each disc of the medium was measured to resolve antibacterial activity of different parts of the plant. Each experiment was carried out in triplicate, and the mean diameter of the inhibition zone was measured in millimetre.

Determination of Minimum Inhibitory Concentration (MIC): The MIC for bacterial isolates was carried out using the tube dilution technique as described by Akinyemi *et al.* (2005) [1]. Whereas 0.5 mg of extracts and tetracycline was loaded from 5 mg / mL stock solutions from 100 µl of total volume.

RESULTS: Antibacterial activity of different parts of methanolic extract (leaves, flowers, green pods and root) of *M. oleifera* plant were analyzed against gram positive bacteria *B. subtilis (Bs), S. aureus* (Sa) and gram negative bacteria *K. pneumonia* (Kp), *E. coli* (Ec), *P. aeruginosa* (Pa). The present study suggested that antibacterial activity is shown in plant & their parts. In contrast, 0.5 mg concentration of both extracts and tetracycline was loaded from 5 mg / mL stock solutions from 100 µl of total volume. Secondary metabolites extracted from all four parts of *M. oleifera* were found to be active against bacteria. Furthermore, the antimicrobial activity of the mentioned plant extracts was also examined against various gram-

positive and negative bacterial strains, and the results are summarized in **Table 1**. From the data, it was observed that against gram-negative bacteria *K. pneumonia*, leaves extracts (17.6 mm) of *M. oleifera* possess higher antimicrobial activity as compared to root extracts (16.1 mm), pod extracts (14.7mm) and lowest in flower extract (13.8 mm).

Antimicrobial activity of leaf extract (19.2mm) showed good antimicrobial activity against gram-positive bacteria *B. subtilis* as compared to pod extracts (14.3mm), root extracts (14.0 mm) and lowest in leaves extract (11.3 mm). However, leaves extracts (22.6mm) of the plant were found to be more effective against gram-negative bacteria *E. coli* than the pod extracts (19.6 mm), root extracts (16.9mm), and lowest in flower extracts (14.9 mm). Furthermore, pod extracts (22.5 mm) of the plant were found to be more effective against gram-positive bacteria *S. aureus* than the flower extracts (17.2 mm), root extracts (16.9 mm), and lowest in leaf extracts (14.1 mm).

Additionally, antimicrobial activity of leaf extract (21.9 mm) showed against gram-negative bacteria *P. aeruginosa* as compared to pod extracts (15.33 mm), but root and flower extracts no activity have been shown as described in **Fig. 2, 3** and **Table 1.**

TABLE 1: ANTIBACTERIAL TESTING OF M. *OLEIFERA*, METHANOLIC EXTRACTION OF LEAF, ROOT, AND STEM AGAINST GRAM-POSITIVE BACTERIA *B. SUBTILIS (BS), S. AUREUS* (SA) AND GRAM-NEGATIVE BACTERIA *K. PNEUMONIA* (KP), *E. COLI* (EC), *P. AERUGINOSA* (PA)

Bacteria	Pathogens	Zone of Inhibition (mm)				
		Flower extract (0.5 mg / mL)	Pod extract (0.5 mg / mL)	Root extract (0.5 mg / mL)	Leaves extract (0.5 mg / mL)	Tetracycline (0.5 mg / mL)
Gram-positive	*B. subtilis*	19.2 ± 0.9	14.3 ± 0.6	14 ± 0.1	11.3 ±1.4	21.3 ±1.4
	S. aureus	17.2 ± 1	22.5 ± 1.3	16.9 ± 1.8	14.1 ± 1.2	21.1 ± 1.2
Gram-negative	*K. pneumoniae*	13.8 ± 0.6	14.7 ± 1.1	16.1 ± 1.1	17.6 ± 1.5	22.6 ± 1.5
	E. coli	14.9 ± 0.8	19.6 ± 0.5	16.9 ± 1.8	22.6 ± 1	20.6 ± 1
	P. aeruginosa	0.0	15.33± 1.1	0.0	21.9 ± 1.6	20.9 ± 1.6

Note: Value are presented as mean±S.E. of triplicate experiments.

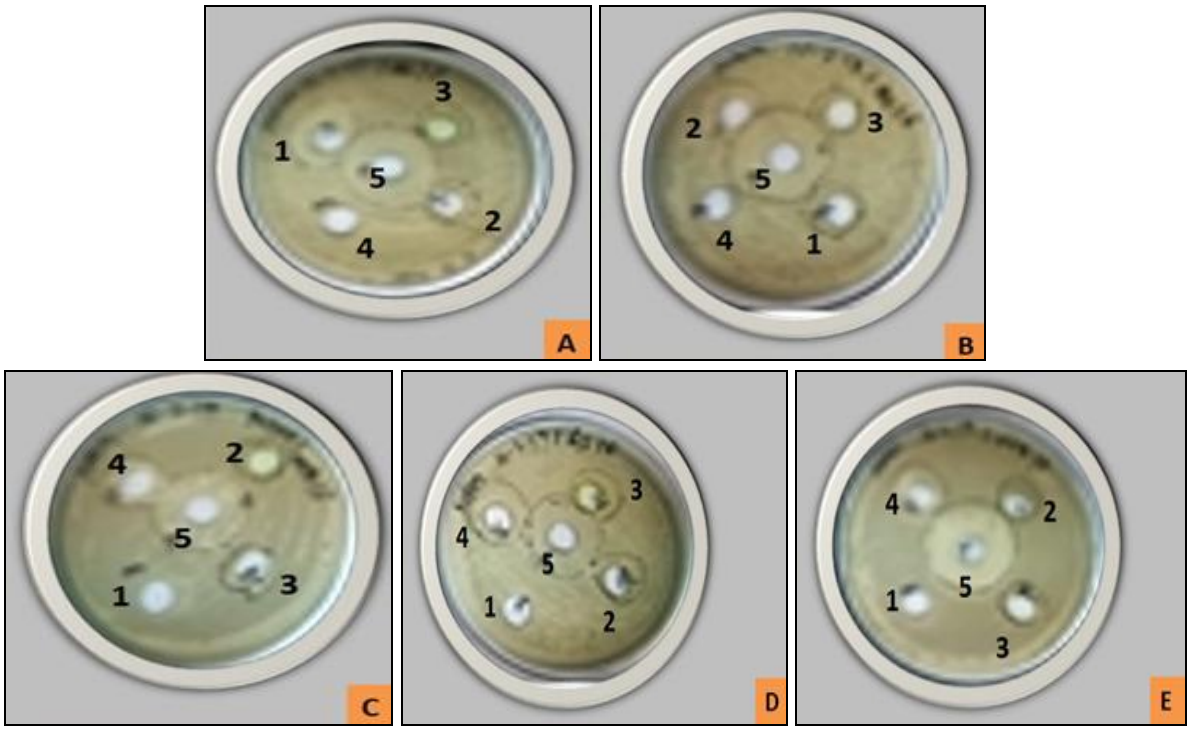

FIG. 1: ANALYSIS OF ANTIBACTERIAL ACTIVITY OF DIFFERENT PARTS OF *MORINGA OLEIFERA* AGAINST GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA: 1-FLOWER EXTRACT, 2-POD EXTRACT, 3-ROOT EXTRACT, 4-LEAVES EXTRACT, 5-TETRACYCLINE AS CONTROL. A- ZONE OF INHIBITION (MM) OF *B. SUBTILIS*, B- ZONE OF INHIBITION (MM) OF *S. AUREUS*, C- ZONE OF INHIBITION (MM) OF *K. PNEUMONIA*, D- ZONE OF INHIBITION (MM) OF *E. COLI* , E-ZONE OF INHIBITION (MM) OF *P. AERUGINOSA*

After performing the antibacterial testing, it was found that extracts other than methanol could not show antibacterial activity as no clear zone of inhibition was obtained after the antibacterial sensitivity test.

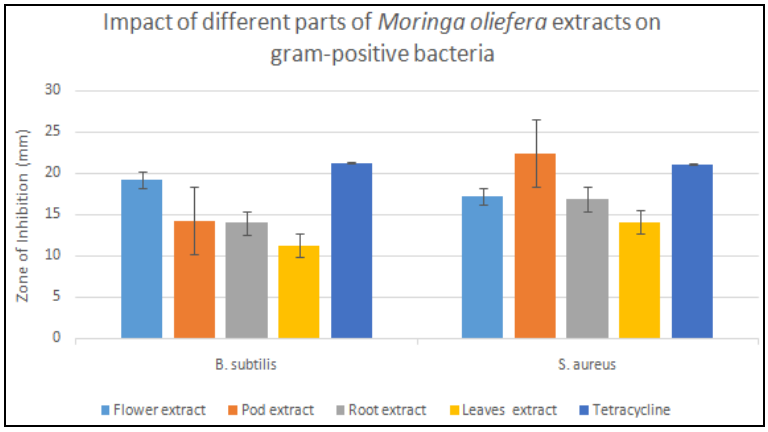

FIG. 2: GRAPHICAL REPRESENTATION OF ANTIBACTERIAL ACTIVITY OF DIFFERENT PARTS OF *M. OLEIFERA* METHANOL EXTRACT AND TETRACYCLINE EFFECT AGAINST GRAM-POSITIVE BACTERIA.

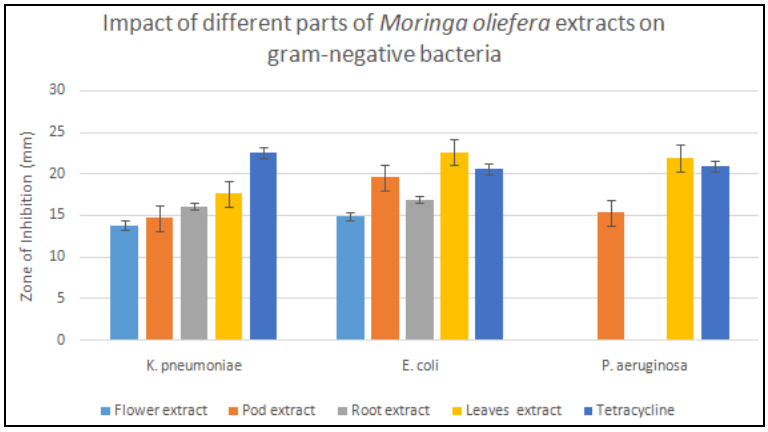

FIG. 3: GRAPHICAL REPRESENTATION OF ANTIBACTERIAL ACTIVITY OF DIFFERENT PARTS OF *M. OLEIFERA* METHANOL EXTRACT AND TETRACYCLINE EFFECT AGAINST GRAM-NEGATIVE BACTERIA.

DISCUSSION: As the prevalence of diseases are being increased throughout the world, in which *M. oleifera* plant has rich in secondary metabolites expend therapeutic potential against a wide range of diseases, therefore, exploitation of *M. oleifera* and its products would be beneficial against the diseases above. In the present study four parts of the plant were used to evaluate for their antibacterial activity against gram positive bacteria *B. subtilis (Bs), S. aureus* (Sa) and gram negative bacteria *K. pneumonia* (Kp), *E. coli* (Ec), *P. aeruginosa* (Pa). The antimicrobial activities of *M. oleiferaleaves*, roots, bark and seeds were investigated *in-vitro* against bacteria, yeast, dermatophytes and helminths pathogenic to man [2]. Due to presence of glucosinolates and isothiocyanates; the stable isothiocyanate 4-[(α-l-rhamnosyloxy) benzyl] isothiocyanate (moringin) was evaluated for their antibacterial activity [6]. Additionally, methanol, chloroform, ethyl acetate and aqueous bark extracts of *Moringa oleifera* were evaluated for their antibacterial activity against bacteria *viz. Staphylococcus aureus*, *Citrobacter freundii*, *Bacillus megaterium* and *Pseudomonas fluorescens* [15]. Based on the results, it has been suggested that *M. oleifera* has antibacterial due to the presence of lipophilic compounds and different metabolites (carboxylic acid, 2, 4-diacetyl phloroglucinol, enzymes, and chitinases) in plant cell walls [8]. Methanolic extract of flower and pod shown adequate activity against gram-positive bacteria *B. subtilis (Bs), S. aureus*(Sa) and leaves against gram-negative bacteria *K. pneumonia* (Kp), *E. coli* (Ec), *P. aeruginosa* (Pa).

CONCLUSION: The result of the present study showed that the methanolic extract of leaves and pod demonstrated better antibacterial activity

against gram-positive and gram-negative bacteria than other plant parts. Leaves and pods can be used for further product development. Furthermore, it is important to evaluate key compounds responsible for the antibacterial activity.

ACKNOWLEDGEMENTS: The author thanks to Dr. Tabish Qidwai for providing innovative suggestions.

CONFLICTS OF INTEREST: The authors declare none conflict of interest statement.

REFERENCES:

1. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC and Fasure KA: Screening of crude extracts of six medicinal plants used in South – West Nigerian unorthodox medicine for antimethicillin resistant *S. aureus* activity. BMC Complimentary Alternative Medicine 2005; 5-6.
2. Armando C, Ofyluz C, Ofelia M, Patricia M and Patricia M: Pharmacological properties of *Moringa oleifera*: Preliminary screening for antimicrobial activity. Journal of Ethnopharmacology 1991; 33: 213-216.
3. Bauer AW, Kirby WM, Sherris JC and Turck M: Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 1966; 45(4): 493 – 496.
4. Cakir A, Kordali S, Zengin H, Izumi S and Hirata T: Composition and antifungal activity of essential oils isolated from Hypericumhussopifolium and H. heterrophyllum. J Flavour Fragrance 2004; 19: 62 – 68.
5. Giacoppo S, Rajan TS, Nicola GRD, Lori R, Rollin P, Bramanti P and Mazzon E: The Isothiocyanate isolated from *Moringa oleiferashows* potent Anti-inflammatory activity in the treatment of Murine Subcute Parkinson's Disease. Rejuvenation Res 2017; 20(1): 50-63.
6. Gigliola B, Luciano DP, Aniello SM, Simona B, Alessandro L, Alberto B, Stefania M and Angela BM: A Stable Isothiocyanate from *Moringa oleifera*, Activates the Somatosensory and Pain Receptor TRPA1 Channel *in-vitro*. Molecules 2020; 25(4): 976.
7. Hijar LA, Luis MAA, Garcia SC, Soto NG and Mendoza JDV: Antibacterial and Cytotoxic Effetcs of *Moringa oleifera* (Moringa) and *Azadirachta indica* (Neem) Methanolic extracts against stains of *Enterococcus faecalis*. International Journal of Dentistry 2018; 10: 1155.
8. Jabeen R, Shahid M, Jamil A and Ashraf M: Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. Pakistan Journal of Botany 2008; 40: 1349–1358.
9. Juliana C, Santiago C, Juan AP, Javier GP and Walter DO: Purification and Characterization of a Novel Thermostable Papain Inhibitor from *Moringa oleifera* with Antimicrobial and Anticoagulant Properties. Pharmaceutics 2021; 13: 512.
10. Kou X, Li B, Olayanju JB, Drake JM and Chen N: Nutraceutical or Pahrmacological Potential of *Moringa oleifera* Lam. Nutrients 2018; 10:343.
11. Olawoye OS, Adeagbo BA and Bolaji OO: *Moringa oleifera* leaf powder alters the pharmacokinetics of amodiaquine in healthy human volunteers. Journal of Clinical Pharmacy and Therapeutics 2018; 10(1111): 12725.
12. Sangeeta C, Raman V, Ramachandran C, Eric BD, Inamul HM, Ghazala S, Momna R, Fazle E, Su JY and D HO: *In-vitro* and *in-silico* Screening and Characterization of Antimicrobial Napin Bioactive Protein in *Brassica juncea* and *Moringa oleifera*. Molecules 2021; 26: 2080.
13. Somsak V, Borkaew P, Klubsri C, Dondee K, Bootprom P and Saiphet B: Antimalarial properties of aqueous crude extracts of *Gymostemma pentaphyllum* and *Moringa oleifera* leaves in combination with Artesunate in Plasmodium berghei infected Mice. Journal of Tropical Medicine 2016; 10(1155): 8031392.
14. WHO: publishes list of bacteria for which new antibiotics are urgently needed, 27 February 2017, News release, GENEVA.
15. Zaffer M, Ahmad S, Sharma R, Mahajan S, Gupta A and Agnihotri RK: Antibacterial activity of bark extracts of *Moringa oleifera* Lam. against some selected bacteria. Pakistan Journal of Pharmaceutical Sciences 2014; 27.6. 1857-1862.

How to cite this article:
Verma JK, Qidwai T and Saluja R: Antibacterial activity from different parts of *Moringa oleifera* (L.) against gram-positive and gram-negative bacteria. Int J Pharm Sci & Res 2023; 14(6): 2894-98. doi: 10.13040/IJPSR.0975-8232.14(6).2894-98.