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IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF *MORINGA OLEIFERA* AND *MOMORDICA CHARANTIA* SEEDS

F. G. Shoba*, V.A. Babu, M. Parimala and J. Sathya

P.G. & Research Department of Zoology, Voorhees College, Vellore – 632001, Tamilnadu, India

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Correspondence to Author:

Dr. F. Gricilda Shoba

Associate Professor
P.G. & Research Department of
Zoology, Voorhees College, Vellore –
632 001. Tamilnadu, India.

Email: gricildashoba@gmail.com

ABSTRACT: The aqueous and methanol extracts of the seeds of *Moringa oleifera* (Family: Moringaceae) and the seeds of *Momordica charantia* (Family: Cucurbitaceae) were screened for their antimicrobial activity against three human pathogenic bacterial strains and three fungal strains by agar-well diffusion assay. The pattern of inhibition varied with the solvent used for extraction and the microorganisms tested. Among these extracts, methanol extract showed significant antimicrobial activity against all the six tested microbes. The most susceptible microorganism was *Staphylococcus aureus* which showed 8 mm and 7 mm zone of inhibition with methanol extract of *M.oleifera* and *M.charantia* respectively. *M.oleifera* methanol extract was significantly effective against the fungi *C.albicans* as comparable to the standard flucanazole. Preliminary phytochemical analysis of different extracts revealed the presence of flavonoids, terpenoids and tannins in *M.oleifera* methanol extract and the presence of alkaloids, glycosides and tannins in *M.charantia* methanol extract. These compounds may have contributed to their antimicrobial action.

INTRODUCTION: Since time immemorial, our traditional system of medicine and folklore medicine claim that medicinal plant as a whole or their parts are being used in all types of infectious diseases successfully.

Nowadays, Multiple Drug Resistance (MDR) has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such infectious diseases. Adding to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic response¹.

Owing to the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently started paying attention to medicinal plants and biologically active compounds isolated from plant species used in herbal medicine. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world². However, it is necessary to evaluate on a scientific base, the potential use of herbal medicine for the treatment of infectious disease caused by common pathogens.

Moringa oleifera belongs to the family Moringaceae. It is a highly valued plant, distributed in many countries. It has an impressive range of medicinal uses with high nutritional value. Various parts of the plant such as the leaves, roots, seeds, bark, fruits, flowers and immature pods act as cardiac and circulatory stimulants, possess

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antitumour, antipyretic, antiepileptic, anti-inflammatory, antiulcer property³. The seeds of this plant are used for their antibiotic and anti-inflammatory properties to treat arthritis, rheumatism, gout, cramp, sexually transmitted diseases and as a relaxant for epilepsy.

Momordica charantia belonging to the family Cucurbitaceae, is found in tropical and subtropical regions of the world such as India, Asia, South America and widely used as food and medicine. It is said to possess innumerable biological activities like anthelmintic, antibacterial, antidiabetic, anti-inflammatory, antimutagenic, antioxidant, anti-tumour and antiulcer⁴. Traditionally it is said to possess astringent, aphrodisiac, carminative, cytostatic, cytotoxic, depressive, hypocholesterolemic, hypotriglyceridemic, hypoglycaemic, insecticidal, laxative, purgative, stomachic, tonic and vermifuge properties.

The present study was undertaken to evaluate the antimicrobial activity of the methanol and aqueous extracts of the seeds of *Moringa oleifera* and *Momordica charantia*. This study assesses the *in vitro* antimicrobial activity of the above plants against few common pathogenic microorganisms that cause the most common type of infectious disease in rural India.

MATERIALS AND METHODS:

Plant collection: Fresh vegetables of *Moringa oleifera* and *Momordica charantia* were collected from Vellore, Tamilnadu, India and their seeds were removed. The taxonomic identities of these plants were made by the expertise of the Department of Botany, Voorhees College, Vellore. The procured seeds were then washed with 70% alcohol and then rinsed with distilled water. It was shade-dried for two weeks at room temperature and stored in air-tight containers until further use.

Plant extraction: Collected, dried plant samples (seeds) in small pieces were crushed to powder form. For aqueous extract, a lot of 5 g of the sample was soaked in 25 ml of sterile distilled water and kept in a rotary shaker for 12 hours at 30°C. Thereafter, it was filtered with Whatmann

No.1 filter paper. The filtrate was concentrated in a rotary evaporator till a sticky mass was obtained. The resultant *M. oleifera* aqueous extract (MOAE) and *M. charantia* aqueous extract (MCAE) were then weighed and stored at 4°C. For methanol extract, a lot of 5 g of the sample was soaked in 25 ml of methanol and kept in a rotary shaker for 12 hours at 30°C. Thereafter it was filtered with Whatmann No.1 filter paper. The filtrate was concentrated in a rotary evaporator till a sticky mass was obtained. The resultant *M.oleifera* methanol extract (MOME) and *M.charantia* methanol extract (MCME) were then weighed and stored at 4°C.

Test organisms: The bacterial strains used for the test were *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The fungal strains used for the test were *Aspergillus niger*, *Candida albicans* and *Candida kefyr*. All the stock cultures were obtained from Microlabs, Institute of Research and Technology, Vellore, Tamilnadu.

Preliminary phytochemical screening: The extracts obtained were subjected to preliminary phytochemical analysis following standard methods⁵. The extracts were screened to identify the presence or absence of various active principles like alkaloids, carbohydrates, saponins, glycosides, proteins, sterols, phenols, flavonoids, terpenoids and tannins.

Antibacterial activity: The extracts were screened for their antibacterial activity in comparison with the standard antibiotic ciprofloxacin (100 µg/ml) *in vitro* by agar-well diffusion method⁶. Bacterial lawn was prepared using the test organisms on Mueller Hinton Agar (MHA). Wells were punched for 8 mm deep in 30 min old bacterial lawn and each well was based by 50 µl molten MHA. In each step of well cutting, the well cutter was thoroughly wiped with alcohol using sterile micropipettes. Further, wells were filled with 100 µl aliquots of 30 mg/ml solvent extract of the plants. Their respective solvents were used as a negative control. The plates were incubated at 37°C for 24 hours. Antibacterial activities were evaluated by measuring the diameter values of zones of inhibition.

Antifungal activity: The extracts were also screened for their antifungal activity in comparison with the standard antibiotic fluconazole (10 mg/ml) *in vitro* by agar-well diffusion method ⁶. Lawn culture was prepared using the fungal strains on Sabouraud's Dextrose Agar (SDA). The inoculated plates were kept for few minutes for solidification. After solidifying, using well cutter, wells were made at a depth of 8 mm. 100 µl aliquots of the selected extracts were added into the well at a concentration of 30 mg/ml. The plates were incubated at room temperature for 48 hours. The activity of the extracts was determined by measuring the diameter of zones of inhibition. Pure solvents were used as a negative control.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *M.OLEIFERA* AND *M.CHARANTIA*.

Phytochemicals	<i>Moringa oleifera</i>		<i>Momordica charantia</i>	
	Aqueous	Methanol	Aqueous	Methanol
Alkaloids	+	+	-	+
Carbohydrates	+	+	+	+
Saponins	-	-	+	+
Glycosides	+	+	-	+
Proteins	+	+	-	-
Sterols	-	-	+	-
Phenols	+	+	-	+
Flavonoids	+	+	+	+
Terpenoids	+	+	-	-
Tannins	+	+	-	+

The data in the table gives the phytochemicals that have been identified from the aqueous and methanol extracts of *M.oleifera* and *M.charantia* seeds. '+' indicates presence and '-' indicates absence.

Antibacterial activity: The antibacterial activity of the plant extracts were variable according to various organisms and the solvent used. The inhibition zones ranged from 5 mm to 8 mm diameter. The results obtained in the present study revealed that *M.oleifera* methanol extract possessed potential antibacterial activity. When tested, the zone of inhibition was maximum for *S.aureus* (8 mm) followed by *E.coli* (7 mm) and *P.aeruginosa* (5 mm), while the aqueous extract proved to have no effect on the test organisms. Similarly the methanol extract of *M.charantia* exhibited potential bactericidal property showing maximum zone of inhibition for *S.aureus* (7 mm) followed by *E.coli* (5 mm) and *P.aeruginosa* (5 mm). Even here, the aqueous extract was not effective in controlling the growth of the bacteria. The inhibition of *S.aureus*

RESULTS:

Qualitative phytochemical analysis: Qualitative phytochemical analysis of *M.oleifera* seeds showed the presence of alkaloids, glycosides, flavanoids, terpenoids and tannins in both aqueous and methanol extract. *M.charantia* seeds differed in their phytoconstituents in methanol and aqueous extracts. While the aqueous extract contained saponins, flavonoids and sterols, the methanol extract contained saponins, flavonoids, alkaloids, glycosides and tannins. The results are tabulated in **Table 1**.

by MOMME and MCME was comparable to the standard antibiotic ciprofloxacin very closely. The results are represented in **Figure 1**.

Antifungal activity: Antifungal activity of the seed extracts showed moderate activity when compared with the standard antibiotic fluconazole. Among the four extracts, methanol extract of both *M.oleifera* and *M.charantia* showed a very good antifungal activity against all the three tested fungi as depicted in **Figure 2**. However the aqueous extract of *M.oleifera* was effective against *C.kefyr* (8 mm) and *C.albicans* (6 mm). But the aqueous extract of *M.charantia* possessed activity against *A.niger* strain (5 mm) only. Interestingly, MOMME significantly inhibited *C.albicans* similar to the standard fluconazole.

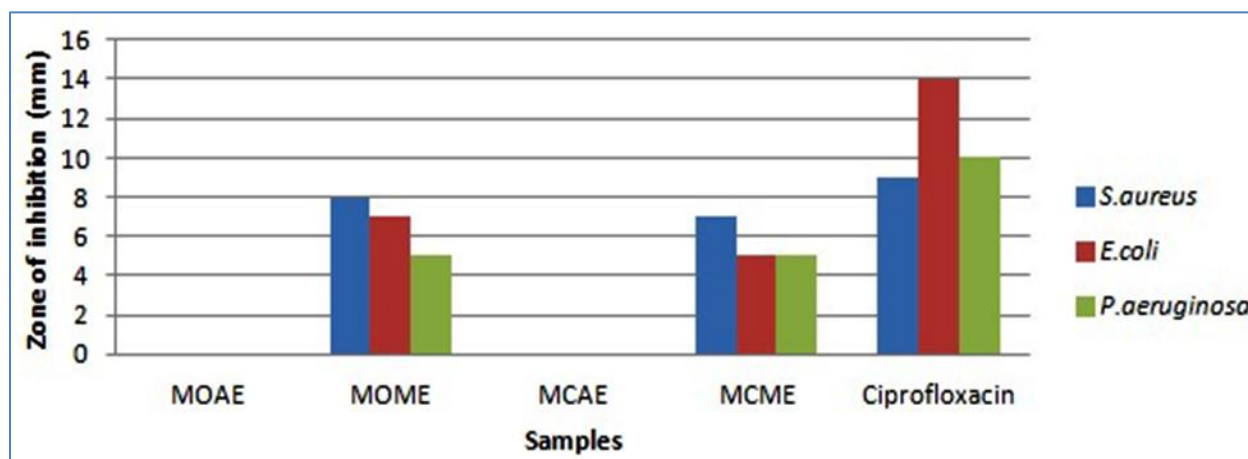


FIGURE 1: ANTIBACTERIAL ACTIVITY OF *M.OLEIFERA* AND *M.CHARANTIA*. Each data point represents the zone of inhibition formed by the extracts against three bacterial strains in comparison with the standard ciprofloxacin. The methanol extracts showed significant activity against the tested organisms.

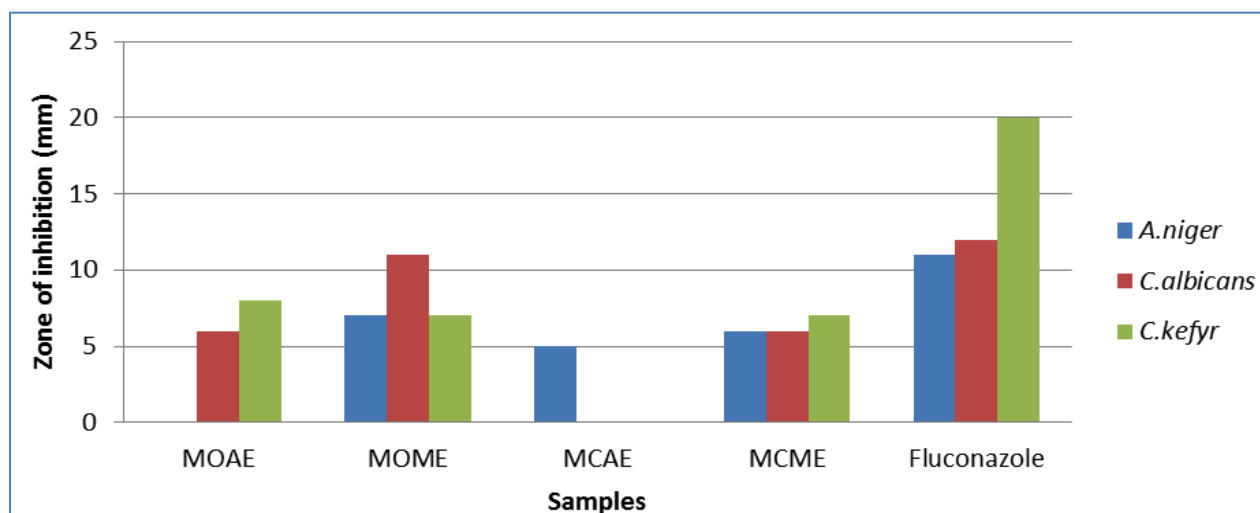


FIGURE 2: ANTIFUNGAL ACTIVITY OF *M.OLEIFERA* AND *M.CHARANTIA*. Each column represents the zone of inhibition formed by the extracts against three fungal strains. The methanol extract possessed significant activity against all the three organisms, when compared to the aqueous extract.

DISCUSSION: It has been reported that higher plants show a promising potential source of new antimicrobial agents⁷. Their activity is directly attributed to the presence of certain phytochemicals in such plants which contribute to its microbicidal property and inhibition of microbial growth by various mechanisms⁸. These botanical compounds extracted depend upon the type of solvent used in the extraction procedure.

In this study, methanol extract of the plants *M.oleifera* and *M.charantia* provided more consistent antimicrobial activity at 30 mg/ml concentration. However, aqueous extract failed to show similar result. It is clear that the methanol

extract contain active phytoconstituents that gives the plant its antimicrobial property.

MOMe had the broadest spectrum of activity on the tested bacterial and fungal strains. This may be due to the presence of flavonoids, terpenoids and tannins. The activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes⁹.

The oil containing terpenoids has exerted a marked inhibition against multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* and

vancomycin-resistant enterococci, *Enterococcus faecalis*¹⁰. Terpenes and phenols from methanolic extract have been reported by different workers as effective antimicrobial compounds^{11, 12}. Possession of alkaloids, tannins and flavonoids has also been shown to enhance antimicrobial property of a plant¹³.

MCME revealed the presence of alkaloids, glycosides and tannins which are absent in the corresponding MCAE. Studies have shown that the bark of *Terminalia arjuna* contains glycosides and tannins which are antibacterial compounds¹⁴. Hence their presence may have added to its activity. Another previous study proved that the aqueous extract of leaves of *M.charantia* was found to exhibit milder antimicrobial activity compared to methanolic extract which apparently indicates that the methanolic extract contains higher concentration of active antimicrobial phyto-compounds such as alkaloids and glycosides^{15, 16}.

Both MOME and MCME were found to contain tannins. Tannins are a group of compounds that has received a great deal of attention in the recent years. One of their molecular actions is to complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation^{17, 18}. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins. They also complex with polysaccharides¹⁹. Among the microorganisms tested, *S. aureus* and *C. albicans* are among the most commonly encountered pathogens in clinical practice and there has been an alarming increase in staphylococcal infections by strains with MDR^{20, 21, 22}.

Here, both MOME and MCME have shown intrinsic bioactivity against *S. aureus*. Therefore the result reconfirms that the methanolic extract of the plants have power over the microorganisms especially against *S. aureus*. By further isolation and characterization of the methanol extracts of *M. oleifera* and *M. charantia* seeds, the exact active fraction and phytoconstituent responsible for their efficiency can be identified.

CONCLUSION: To conclude, this study evaluated the antimicrobial efficiency of the aqueous and methanol extracts of *M.oleifera* and *M.charantia* seeds and the methanol extract was found to possess significant antimicrobial activity comparable to the standard antibiotics. This may be due to the presence of flavonoids, terpenoids and tannins in *M.oleifera* seeds and the presence of alkaloids, glycosides and tannins in *M.charantia* seeds. These can be used to develop new drugs for the therapy of infectious diseases with particular reference to those caused by *S. aureus* and *C. albicans*. However, further studies should be undertaken to elucidate the exact mechanism of action by which the extracts exert their antimicrobial effect.

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