IJPSR (2011), Vol. 2, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 March, 2011; received in revised form 06 July, 2011; accepted 23 July, 2011

ANTIBACTERIAL ACTIVITY OF THE LEAVES, BARK, SEED AND FLESH OF *MORINGA OLEIFERA*

V. Priya, P. Abiramasundari, S. Gayathri Devi* and G.P. Jeyanthi

Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Deemed University for Women, Coimbatore- 641 043, Tamil Nadu, India

Keywords:

Moringa Oleifera,

Antibacterial activity.

Correspondence to Author:

Dr. S. Gayathri Devi,

Assistant Professor (SS), Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Deemed University for Women, Coimbatore- 641 043, Tamil Nadu, India

ABSTRACT

Moringa oleifera belongs to an onogeneric family of shrubs and tree, *Moringaceae*. In the Dravidian language there are many local names for *Moringa oleifera* but all are derived from the generic root "Morunga". It has anticancer, anti-inflammatory activity and thyroid status regulator efficacies. An experiment was carried out to study the antibacterial activity of different solvent extracts of *Moringa oleifera* plant by agar well diffusion method against *Bacillus subtilis, Escherichia coli, Klebsiella pnemoniae, Shigella dysentriae* and *Staphylococcus aureus* that frequently cause enteric infections in humans. The benzene, methanol and aqueous extracts of *Moringa oleifera* leaves, bark, seed and flesh have shown strong antibacterial activity against all the organisms tested such as *B. subtilis, E. coli, K. pneumoniae. S. dysentriae* and *S. aureus*. The zone of inhibition ranged from 7-23mm. The plant *Moringa oleifera* can be used as a source of oral drugs to fight infections caused by susceptible bacteria.

INTRODUCTION: Infections due to pathogenic bacteria and fungi represent a critical problem to human health [1]. Despite the extensive use of antibiotics and vaccines programs, infectious diseases continue to be a leading cause of morbidity and mortality worldwide [2]. Microbial infections have been reported to be the major cause of inflammation [3].

Medicinal plants also represent a rich source from which antimicrobial agents can be obtained [4]. Extracts from the leaves of *Alchornea cordifolia* have been reported to inhibit the growth of bacteria such as *Staphylococcus aureus, S. albus, Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa* [5].

Moringa oleifera, an edible tree found worldwide in the dry tropics, is increasingly being used for nutritional supplementation [6]. The tree is valued mainly for the tender pods, which are esteemed as a vegetable. Flowers and young leaves are also eaten as vegetables. The leaves are a rich source of essential aminoacids such as methionine, cysteine, tryptophan and lysine. Decoctions and extracts made from the leaves are also variously employed in native medicine. In Southern India, village people use the fresh leaves to prepare cow and buffalo ghee from butter fat. It has been found that there is a significant increase in shelflife of ghee and that *Moringa* leaves can be a good source of natural antioxidants [7]. The present study was designed to investigate the influence of herbal medicines on the "Antibacterial activity of the leaves, bark, seed and flesh of the *Moringa oleifera*".

MATERIALS AND METHODS

Collection of the plant samples and Preparation of the plant extracts: *Moringa oleifera* plants were procured from local markets in Coimbatore and duly

authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Coimbatore. The leaves, bark, seed and flesh of the plant were separated out and they were washed, shade dried, powdered to coarse size and stored at room temperature. Ten gram of each powder sample was extracted with 100ml of various solvents namely petroleum ether, benzene, chloroform, methanol and distilled water. The organic extracts were obtained by using Soxhlet's apparatus. Period of extraction was 24 hours. The aqueous extract was prepared by dissolving 10g of sample in 100ml of distilled water and boiled at 100°C for 5 hours. Each extract was concentrated to dryness under pressure. They were then dissolved in dimethyl sulfoxide (DMSO) and stored in the refrigerator until use. 0.001g of extracts per 20µl was taken for the assay.

Determination of the Antibacterial Activity:

Selection of the Microorganisms: The bacterial strains used for this study were *Bacillus subtilis, Stapylococcus aureus, Shigella dysentriae, Klebsiella pneumonia* and *Escherichia coli.* All the bacterial strains were grown and maintained in nutrient agar.

Well Diffusion Method: A loopful of the strain was inoculated in 15ml of nutrient broth in a test tube and incubated on a rotary shaker for 24 hours to activate them. Muller Hinton Agar (MHA) was prepared evenly on to the surface of MHA plates using sterile cotton buds, and the well (6mm in diameter) was punctured in the agar medium using sterile stainless cork borer. Each plant extract (20µl) and control chloramphenicol (15µl) was introduced into the wells. Plates were incubated overnight at 37°C. The antibacterial activity was interpreted from the size of the diameter of inhibition zone around each well. It was measured and recorded [8].

Minimum Inhibitory Concentration (MIC): MIC is defined as the lowest concentration of the sample that did not show any growth of the tested microorganisms. In the present study, 100µl of nutrient broth was taken in the wells of a 96 well plate. To this 100µl of sample was mixed with 100µl of sample diluents in the well making two fold dilutions. 100µl of this dilution was transferred and mixed with 100µl of the diluents in the second row making a 4:1 dilution. This proceeds consecutively down the plate making two fold dilutions in each well. After the dilution of the sample is completed, aliquot of test organisms was added to all wells and the microtitre plates were incubated overnight. If the sample concentration was sufficient to kill the organisms, r growth appeared and the wells will be clear. At this point there was an insufficient sample to kill the organism and the well will be cloudy, indicating growth that is the minimum inhibitory concentration of the sample against that specific organism[9].

Minimum Bactericidal Concentration (MBC): MBC was determined for each set of wells from MIC determination, a loopful of broth was collected from those wells, which did not show any growth and inoculated on sterile nutrient agar. Plates inoculated with bacteria were then incubated at 37°C for 24 hours. After incubation, the concentration with no visible growth was noted as minimum bactericidal concentration [10].

RESULTS AND DISCUSSION: The various extracts of *Moringa oleifera* namely petroleum ether, benzene, chloroform, acetone, methanol and aqueous extracts of its leaves, bark, seed and flesh were tested against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Shigella dysentriae* and *Staphylococcus aureus* for their antibacterial activity. The results are presented in **Tables 1-4**.

TABLE 1: ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* LEAF EXTRACT BY WELL DIFFUSION METHOD

BACTERIAL STRAINS	SOLVENT EXTRACTS						Control
	Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Aqueous	
Bacillus subtilis	7	8	10	13	18	15	20
Escherichia coli	14	10	-	8	17	20	22
Klebsiella pneumoniae	9	11	15	14	18	16	18
Shigella dysentriae	18	12	-	10	21	22	23
Staphylococcus aureus	12	14	19	17	20	21	22

(Diameter of zone in mm)

TABLE 2: ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* BARK EXTRACT BY WELL DIFFUSION METHOD

BACTERIAL STRAINS	SOLVENT EXTRACTS						Control
	Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Aqueous	
Bacillus subtilis	-	17	20	17	22	19	22
Escherichia coli	7	10	16	13	18	14	19
Klebsiella pneumoniae	18	15	-	-	20	17	22
Shigella dysentriae	-	13	16	12	20	17	20
Staphylococcus aureus	19	16	10	12	14	18	20

(Diameter of zone in mm)

TABLE 3: ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* SEED EXTRACT BY WELL DIFFUSION METHOD

BACTERIAL STRAINS	SOLVENT EXTRACTS						Control
	Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Aqueous	
Bacillus subtilis	17	22	9	13	18	20	24
Escherichia coli	18	18	15	12	12	17	19
Klebsiella pneumoniae	20	19	15	14	21	22	24
Shigella dysentriae	17	18	16	13	10	17	18
Staphylococcus aureus	18	13	-	-	21	12	23

(Diameter of zone in mm)

TABLE 4: ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* FLESH EXTRACT BY WELL DIFFUSION METHOD

BACTERIAL STRAINS	SOLVENT EXTRACTS						Control
	Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Aqueous	
Bacillus subtilis	10	13	14	10	14	15	24
Escherichia coli	18	15	10	7	13	20	24
Klebsiella pneumoniae	16	17	15	13	14	18	19
Shigella dysentriae	18	21	9	12	15	20	22
Staphylococcus aureus	17	18	15	11	19	8	19

(Diameter of zone in mm)

The zone of inhibition of benzene, methanol and aqueous extracts of *Moringa oleifera* leaves was found to be effective against all the test organisms. The zone of inhibition ranged between 8 and 22mm. The petroleum ether and acetone extracts showed moderate inhibition against *Bacillus subtilis, Escherichia coli* and *Shigella dysentriae*. Chloroform extract was sensitive against *Escherichia coli* and *Shigella dysentriae*.

The zone of inhibition of the extracts of benzene, methanol and aqueous extracts of *Moringa oleifera* bark was found to be effective against all the test organisms. Zone ranged from 10 to 20mm. Chloroform and acetone extracts showed moderate activity against *Bacillus subtilis, Escherichia coli, Shigella dysentriae* and *Staphylococcus aureus* but was sensitive against *Klebsiella pneumoniae.* Petroleum ether did not show inhibition for *Bacillus subtilis* and *Shigella dysentriae.*

The zone of inhibition of petroleum ether, benzene, methanol and aqueous extracts of *Moringa oleifera* seed was found to be effective against the entire organism tested. The zone of inhibition ranged from 10 to 22mm. Chloroform and acetone extracts showed inhibition against the entire organism tested except *Staphylococcus aureus*.

The zone of inhibition of the extracts of *Moringa oleifera* flesh was found to be effective against the entire microorganism tested. The zone of inhibition ranged from 7 to 21mm. Benzene and aqueous extracts showed good activity against *Shigella dysentriae* and *Escherichia coli.* Acetone extract showed moderate inhibition against *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus*. The zone of inhibition produced by the sample extract was

compared with inhibition zone produced by chloramphenicol, which was used as a control.

Minimum Inhibitory Concentration (MIC): Minimum inhibitory concentrations (MIC) were done for selected organisms using benzene, methanol and aqueous extracts which gave maximum zone in well diffusion method. The results were presented in **Tables 5-8**.

TABLE 5: ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* LEAF EXTRACT AGAINST *ESCHERICHIA COLI, SHIGELLA DYSENTRIAE* AND *STAPHYLOCOCCUS AUREUS* BY MINIMUM INHIBITORY CONCENTRATION (MIC)

EXTRACTS	*Escherichia coli* (mg/100ml)					*Shigella dysentriae* (mg/100ml)					*Staphylococcus aureus* (mg/100ml)				
	0	4	2	1	0.5	0	4	2	1	0.5	0	4	2	1	0.5
Benzene	-	+	+	-	-	-	+	+	+	-	-	+	+	+	-
Methanol	-	+	+	+	-	-	+	+	+	-	-	+	+	+	+
Aqueous	-	+	+	-	-	-		+	+	-	-	+	+	+	-

+ indicates positive; – indicates negative

TABLE 6: ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* BARK EXTRACT AGAINST *BACILLUS SUBTILIS, KLEBSIELLA PNEUMONIAE* AND *SHIGELLA DYSENTRIAE* BY MINIMUM INHIBITORY CONCENTRATION (MIC)

EXTRACTS	*Bacillus subtilis* (mg/100ml)					*Klebsiella pneumoniae* (mg/100ml)					*Shigella dysentriae* (mg/100ml)				
	0	4	2	1	0.5	0	4	2	1	0.5	0	4	2	1	0.5
Benzene	-	+	+	+	+	-	+	+	-	-	-	+	+	+	-
Methanol	-	+	+	+	-	-	+	+	-	-	-	+	+	+	+
Aqueous	-	+	+	+	-	-		+	-	-	-	+	+	+	-

+ indicates positive; – indicates negative

TABLE 7: ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* SEED EXTRACT AGAINST *BACILLUS SUBTILIS* AND *KLEBSIELLA PNEUMONIAE* BY MINIMUM INHIBITORY CONCENTRATION (MIC)

EXTRACTS	*Bacillus subtilis* (mg/100ml)					*Klebsiella pneumoniae* (mg/100ml)				
	0	4	2	1	0.5	0	4	2	1	0.5
Benzene	-	+	+	+	-	-	+	+	-	-
Methanol	-	+	+	+	+	-	+	+	+	-
Aqueous	-	+	+	+	-	-	+	+	-	-

+ indicates positive; – indicates negative

TABLE 8: ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* FLESH EXTRACT AGAINST *ESCHERICHIA COLI, KLEBSIELLA PNEUMONIAE* AND *SHIGELLA DYSENTRIAE* BY MINIMUM INHIBITORY CONCENTRATION (MIC)

EXTRACTS	*Escherichia coli* (mg/100ml)					*Klebsiella pneumoniae* (mg/100ml)					*Shigella dysentriae* (mg/100ml)				
	0	4	2	1	0.5	0	4	2	1	0.5	0	4	2	1	0.5
Benzene	-	+	+	-	-	-	+	+	-	-	-	+	+	+	+
Methanol	-	+	+	-	-	-	+	+	-	-	-	+	+	+	+
Aqueous	-	+	+	-	-	-	+	+	-	-	-	+	+	+	-

+ indicates positive; – indicates negative

100µl of the extract of *Moringa oleifera* in the concentration ranges of 4.0 to 0.5 mg were inoculated against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Shigella dysentriae* and *Staphylococcus aureus*.

The bacterial strains *Bacillus subtilis, Shigella dysentriae* and *Staphylococcus aureus* were found to be sensitive against benezene, methanol and aqueous extracts of *Moringa oleifera* at the concentration of 4-1mg/100µl. *Escherichia coli* and *Klebsiella pnemoniae* were found to be sensitive at the concentration of 4-2mg/100µl.

Minimum Bactericidal Concentration (MBC): Based on the MIC results the minimum bactericidal concentration (MBC) was performed against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Shigella dysentriae* and *Staphylococcus aureus* and no visible growth of any of these microorganisms were found.

Since, the *Moringa oleifera* was found to have significant antibacterial activity against microorganisms tested, the results of the present study support the traditional usage of the *Moringa oleifera* and it can be recommended for use as antimicrobial agent in new

drugs for the therapy of infectious disease caused by pathogens.

REFERENCES

1. Eswarappa SM: Location of pathogenic bacteria during persistent infections: Insight from an analysis using game theory. PLoS ONE, 2009; 4(4): e5383.
2. Bloom BR: Microbial infections and immune defence. Nature, 2000; 406: 759.
3. Du-shieng C, Yi-wen C, Wu-Chang C and Ming-Chung L: Naphthalenedione compounds for treating inflammation related disorders and microbial infection. Free patients Online, 2005.
4. Kubmarawa D, Ajoku GA, Enwerem NM and Okorie DA: Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr. J. Biotechnol., 2007; 6(14): 1690-1696.
5. Adeshina GO, Onaolapo JA, Ehinmidu JO and Odama LE: Phytochemical and antimicrobial studies of the ethyl acetate extract of *Alchornea cordifolia* leaf found in Abuja, Nigeria. J. Med. Plant Res., 2010; 4(8): 648-658.
6. Thurber MD and Fahey JW: Adoption of *Moringa oleifera* to content under-nutrition viewed through the lens of the "Diffusion of Innovations" theory. Ecol. Food Nutr. 2009; 48(3): 212-225.
7. Pari L, Karamac M, Kosinska A, Rybarizyk A and Amarowicz R: Antioxidant activity of the crude extracts of drumstick tree (*Moringa oleifera* Lam.) and sweet broomweed (*Scorpia dulcis* L.) leaves. Pol. J. Food Nutr. Sci., 2007; 57(2): 203-208.
8. Rios JL, Recio MC and Villar A: Screening methods for natural products with antibacterial activity, a review of literature. J. Ethnopharmacol., 1988; 23: 127-149.
9. Eloff JN: A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med., 1998; 64: 711-713.
10. NCCLS: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard Fifth edition, NCCLS dominant M1745; NCCLS, Wayne, PA. USA. 2000.

****