ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation and comparison of antibacterial activity of leaves, seeds and fruits extract of *momordica charantia*

Manju Makhija*, Dharmendra Ahuja, Bankim Chand Nandy, Shipra Gautam, Kalpana Tiwari, Ankita Awasthi, Priya Saxena

*Department of Pharmaceutical Sciences, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India.

ABSTRACT

Momordica charantia is a well known plant in Asia including India which posses wide range of pharmacological activities. These drug have been used in India as folk remedy in the form of decoctions and infusions to treat bacterial infections and also claimed to be an effective against variety of skin conditions like psoriasis, acne, wounds etc. The present investigation is carried out to study and compare antibacterial activity of different parts of *Momordica charantia* extracts with different solvents on four microorganisms by disk diffusion method. Methanolic, ethanolic, petroleum ether and aqueous extract of leaves, seeds and fruits of the plant were evaluated for antibacterial activity using the disk diffusion method on four microorganisms (*Staphylococcus aures, Pseudomonas aeruginosa*, *Escherichia coli, and Salmonella typhi*). Zone of inhibition was calculated. Results indicate that the different concentrations of various extracts under study exhibit antibacterial activity and among the various extracts, fruit extracts have shown better activity as compared to other parts of extracts. Among the various extracts, aqueous extracts have shown better antibacterial activity.

Keywords: *Momordica charantia*, Disc Diffusion Method, Antibacterial activity, Zone of inhibition.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

In recent years, several diseases and microbial infections such as respiratory infections, bacterial meningitis, sexually transmitted as well as hospital acquired infections, particularly those caused by the members of the family Enterobacteriaceae have shown considerable resistance to a number of antibacterial agents, such as penicillin, ampicillin, and flouroquinolones among many others [1-4]. *Momordica Charantia* or Bitter Melon, also known as balsam pear or Karela, is a tropical vegetable, is a common food in Indian cuisine and has been used extensively in folk medicine as a remedy for diabetes. It is a very common herb having various medicinal properties for the treatment of different kind of disease, viz. antifungal, wound healing and antidiabetic agents [5, 6].

Popularity of *Momordica charantia* in various systems of traditional medicine for several ailments (antidiabetic, abortifacient, anthelmintic, contraceptive, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies) focused the investigator's attention on this plant [7].

Most recent researches on the plant show that it has ability to inhibit the enzyme guanylate cyclase that is thought to be associated with psoriasis, leukemia and tumor pathogenesis [8, 9]. Therefore, drastic measures should be adopted to control the use of antibacterial agents, to understand the genetic mechanisms of bacterial resistance, and to continue studies to develop new drugs. Ultimately, this may greatly contribute to provision of more appropriate and efficient antibacterial agents to the patient.

However, a few previous studies have had evaluated the antibacterial activity, and very variable results had been reported. In present study antibacterial study of different parts of plant, extracted with different solvents is compared.

MATERIALS AND METHODS

Collection and authentication of plant materials

The leaves, seeds and fruits of *Momordica charantia* were collected from garden of B.R. Nahata college of Pharmacy, Mandsaur MP. The plant material was identified by comparing with voucher specimen no. BRNCP/C/006/2008 deposited in the herbarium of Department of Pharmacognosy, B. R. Nahata college of Pharmacy, Mandsaur, MP and the specimens voucher were deposited in the department herbarium for further reference. The all parts of plants were separately shade dried, cut in to small pieces, air-dried and pulverized in to course powder by using a dry grinder and passed through the sieve before being stored in closed vessel for further use.

ISSN: 0975-8585

Microorganisms used

Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa and Escherichia coli were procured from B.R. Nahata college of Pharmacy, biotech lab. Mandsaur MP.
Gentamycin (500 mg) was dissolved in 1000 ml distilled water was used as positive controls.

Drug and chemicals

The drug Gentamycin (Loba chemical, Mumbai) purchased from commercial sources. Nutrient agar (Hi media), Nutrient Agar Media was prepared by using S.D. Fine Laboratories Chemicals.
All other chemicals were analytical grade and used as such without further testing.

Extraction

Shade dried leaves, seeds, fruits of *Momordica charantia* were powdered and weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature for 48 hours, using pet ether, methanol, ethanol and water [10]. Alcoholic extract was concentrated under the vacuum in rotary flash evaporator and successively in hot air oven till solid to semisolid mass. Extracts were stored in an airtight container in refrigerator below 10°C.
The different extracts were used in different concentrations (0.2 mg μl^{-1}, 0.4 mg μl^{-1}, 0.6 mg μl^{-1},0.8 mg μl^{-1}, 1 mg μl^{-1}) to evaluate the antibacterial properties using Paper Disc Diffusion Method.

Preliminary photochemical investigations

The extract of all parts of *Momordica charantia* were subjected to qualitative tests for the identification of various active constituents viz. glycosides, alkaloids, flavonoids, proteins, saponins, carbohydrates, amino acids and tannins, free reducing sugar, saponins using standard test procedures [11-12] (Table I).

Table 1 Phytoconstituents present in different leaf, seed, and fruit extract of *Momordica charantia*

Phytoconstituents	Leaf extract				Seed extract				Fruit extract			
	M	E	A	PE	M	E	A	PE	M	E	A	PE
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	-	-	+	+	-	-	+	+	+	+
Carbohydrates	+		+	+	+	+	+	+	+	+	+	+
Saponins	-	-	+	+	-	-	+	+	-	-	+	+
Amino acids	-	-	-	-	-	-	-	-	-	-	-	-
Flavanoids	-	-	-	+	-	-	-	+	+	-	-	+
Proteins	+	+	+	-	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+

M- Methanolic extract, E-Ethanolic extract, A-Aqueous extract, PE-Petroleum ether extract

ISSN: 0975-8585

Antibacterial activity testing

A loopful of each assayed strain was inoculated into 15 ml of Nutrient broth and incubated at 37 ºC for 24 hours to activate the strains. One milliliter of microbial suspension was pipetted onto the surface of solidified agar plate, and gently rotated by hand so as to cover the entire surface with the microbial suspension. The plates were allowed to dry for at least 15 minutes prior introduction of the shortly prepared extract impregnated paper disks onto the agar by means of sterile forceps. The control antibiotic disks were also incorporated at the centre; subsequently the agar-plates were incubated overnight at 37ºC. Pure solvents (petroleum ether, methanol ethanol and water) were used as negative controls. Diameters of zones of inhibition (ZI) were measured and recorded in millimeters.

RESULTS & DISCUSSION

Phytoconstituents present in different leaf, seed, and fruit extracts of *Momordica charantia* are shown in table 1. Zone of Inhibition in different concentration of Leaf, seed, fruit extracts are shown in table 2, 3 & 4 and figure 1, 2, 3 respectively.

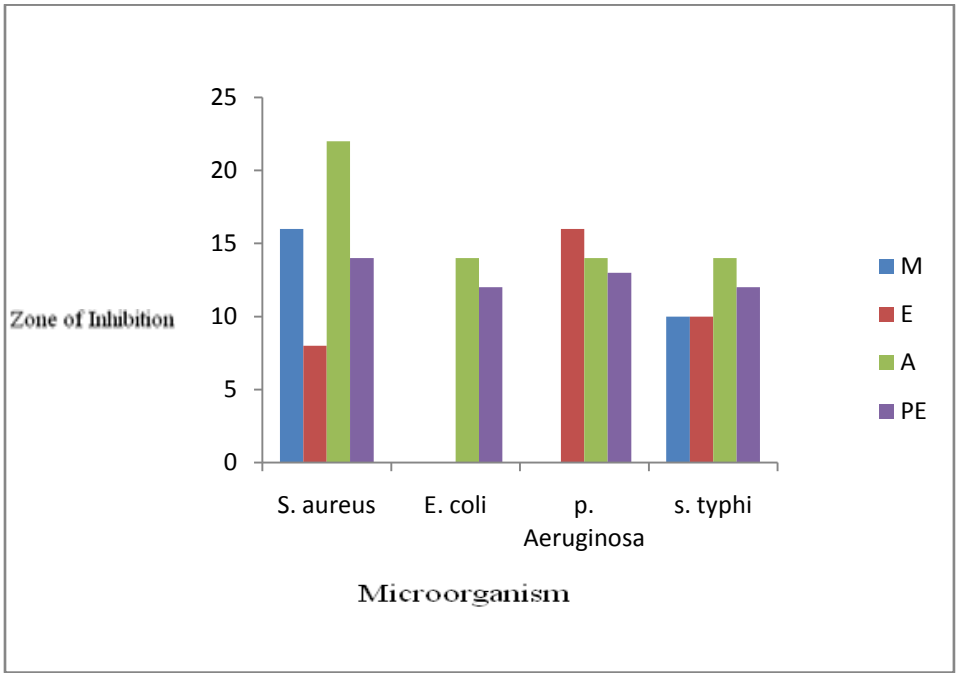

Figure 1: Zone of Inhibition in different concentrations (mg µl $^{-1}$) of leaf Extract

ISSN: 0975-8585

TABLE 2: Zone of Inhibition in different concentrations (mg µl $^{-1}$) of Leaf Extract

Zone of inhibition (mm)*						
Microorganism		**Concentration (mg µl $^{-1}$)**				
		0.2	**0.4**	**0.6**	**0.8**	**1**
S. aureus	**M**	-	13±0.52	16±0.45	16±0.6	16±0.58
	E	-	-	-	-	08± 0.87
	A	-	14±0.34	18±0.43	18±0.84	22±0.4
	PE	-	8±0.73	12±0.11	12±0.51	14±0.34
E. coli	**M**	-	-	-	-	-
	E	-	-	-	-	-
	A	-	10±0.56	10±0.51	14	14±0.69
	PE	-	8±0.64	10±0.51	10	12±0.67
Pseudomonas Aeruginosa	**M**	-	-	-	-	-
	E	-		14±0.23	14±0.31	16±0.89
	A	-	12±0.45	12±0.34	14	14±0.34
	PE	-	8±0.21	11±0.23	11±0.48	13±0.45
Salmonella typhi	**M**	-	8±0.13	8±0.45	10±0.87	10±0.16
	E	-	8±0.18	10±0.89	12±0.65	10±0.81
	A	-	10±0.34	10±0.9	12±0.76	14±0.93
	PE	-	08±0.18	10±034	12±0.89	12±0.89

M- Methanolic extract, E-Ethanolic extract, A-Aqueous extract, PE-Petroleum ether extract
While (-) stands for negative result or no antibacterial activity.
*Values are in terms of Mean ± SEM of results done in triplicate.

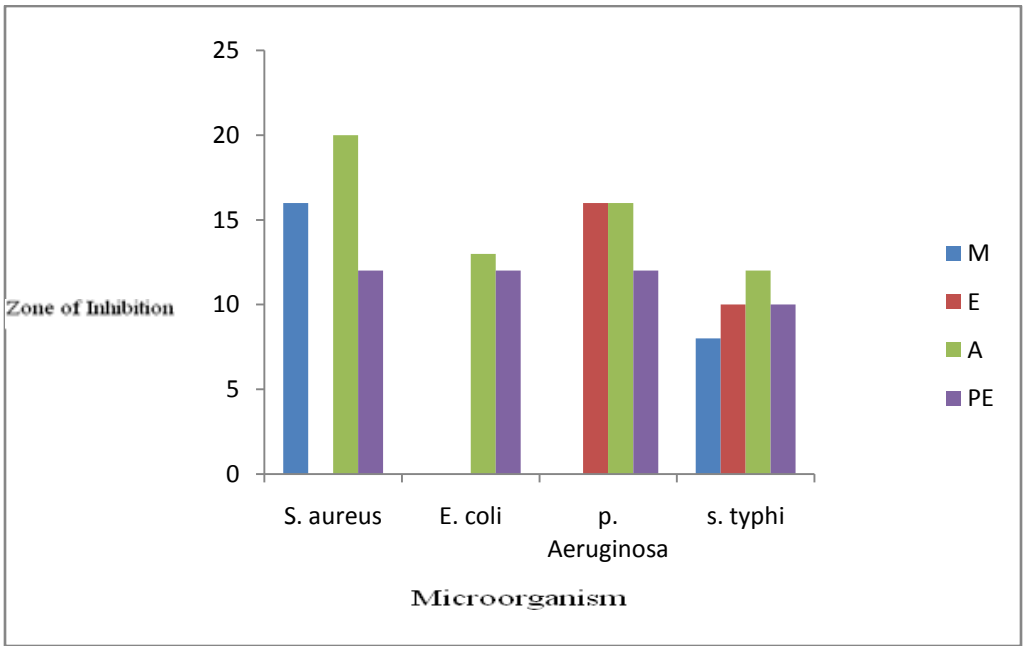

Figure 2: Zone of Inhibition in different concentrations (mg µl $^{-1}$) of seed Extract

ISSN: 0975-8585

TABLE 3: Zone of Inhibition in different concentration (mg µl $^{-1}$) of seed Extract

Zone of inhibition (mm)*						
Microorganism		Concentration (mg µl $^{-1}$)				
		0.2	0.4	0.6	0.8	1
S. aureus	M	-	13±0.52	16±0.45	16±0.6	16±0.58
	E	-	-	-	-	-
	A	-	18±0.51	20 ±0.51	20 ±0.51	20 ±0.51
	PE	-	08±0.51	10±0.51	12±0.51	12±0.51
E. coli	M	-	-	-	-	-
	E	-	-	-	-	-
	A	-	10±0.51	10 ±0.51	13±0.51	13±0.51
	PE	-	8±0.51	8±0.51	12±0.51	12
Pseudomonas Aeruginosa	M	-	-	-	-	-
	E	-		8±0.51	10±0.51	16±0.51
	A	-	12±0.51	12±0.51	16±0.51	16±0.51
	PE	-	8±0.51	10±0.51	11±0.51	12±0.51
Salmonella typhi	M	-	-	6±0.15	6±0.37	8±0.96
	E	-	6±0.18	10±0.89	10±0.65	10±0.85
	A	-	08±0.34	10±0.9	10±0.56	12±0.63
	PE	-	06±0.18	06±084	08±0.8	10±0.23

M- Methanolic extract, E-Ethanolic extract, A-Aqueous extract, PE-Petroleum ether extract
While (-) stands for negative result or no antibacterial activity.
*Values are in terms of Mean ± SEM of results done in triplicate.

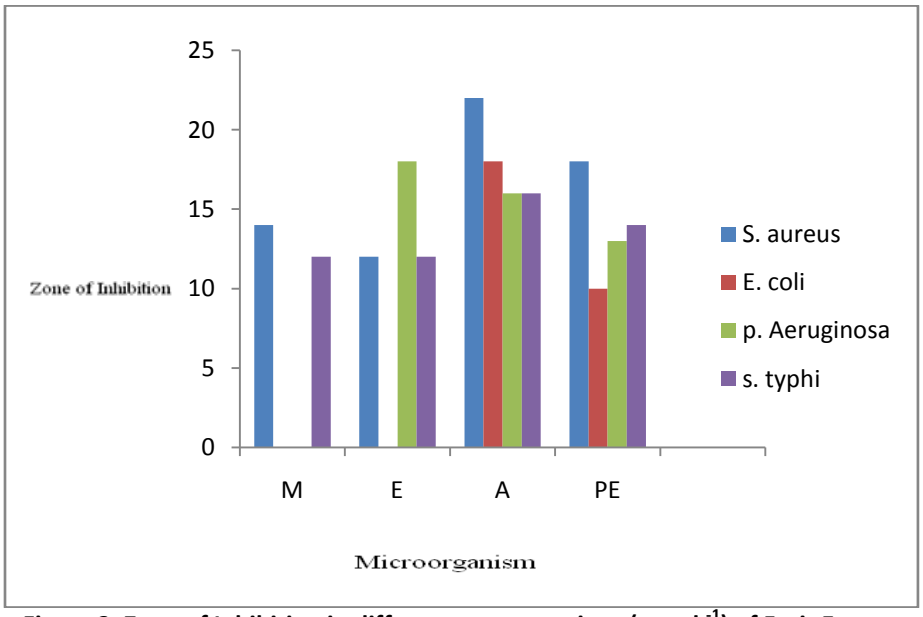

Figure 3: **Zone of Inhibition in different concentrations (mg µl $^{-1}$) of Fruit Extract**

ISSN: 0975-8585

TABLE 4 : Zone of Inhibition in different concentration (mg µl $^{-1}$) of Fruit Extract

Zone of inhibition (mm)*						
Microorganism		**Concentration (mg µl $^{-1}$)**				
		0.2	**0.4**	**0.6**	**0.8**	**1.0**
S. aureus	**M**	-	10±0.31	12±0.85	12±0.59	14±0.42
	E	-	8±0.43	10±0.97	10±1.2	12±0.971
	A	-	20±0.5	22±0.64	22±0.73	22±0.82
	PE	-	14±0.22	14±0.49	18±0.61	18±0.32
E. coli	**M**	-	-	-	-	-
	E	-	-	-	-	-
	A	-	14±0.49	14±0.51	18±0.11	18±0.21
	PE	-	8±0.73	8±0.51	10±0.83	10±0.34
Pseudomonas aeruginosa	**M**	-	-	-	-	-
	E	-	10±0.89	16±0.78	16±0.54	18±0.11
	A	-	12±0.49	14±0.51	16±0.51	16±0.42
	PE	-	10±0.78	12±0.73	12±0.64	13±0.45
Salmonella typhi	**M**	-	8±0.16	8±0.78	10±0.56	12±0.34
	E	-	8±0.55	08±0.21	10±0.18	12±0.54
	A	-	10±0.45	12±0.97	14±0.98	16±0.75
	PE	-	08	12±0.64	12±0.23	14±0.44

M- Methanolic extract, E-Ethanolic extract, A-Aqueous extract, PE-Petroleum ether extract
While (-) stands for negative result or no antibacterial activity.
*Values are in terms of Mean ± SEM of results done in triplicate.

Table 5. Zone of Inhibition of control for different microorganism.

Microorganism	*S. aureus*	*E. coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*
Positive control	24	16	25	17

Zone of Inhibition of control for different microorganism is shown in table 5. Petroleum ether as well as aqueous extracts of leaves, seeds and fruits exhibited higher antibacterial activity. Petroleum ether extracts showed milder antibacterial activity compared to the aqueous extracts, which indicates that aqueous extracts contain higher concentration of active antibacterial agents. But also this could be attributable to other constituents like alkaloids, glycosides, volatile oils or tannins, which are all found in more abundant amount in fruits of *Momordica charantia* as fruit extract exhibited higher antibacterial activity of all the other extracts [7]. *Staphylococcus aureus* was sensitive to these extracts. This justifies the traditional use of this plant in treatment of skin infections [13, 14].

ISSN: 0975-8585

CONCLUSION

From the above results it can be concluded that aqueous and petroleum ether crude extracts of leaves, seeds and fruits of *Momordica charantia* have adequate antibacterial activity. Fruit extracts possess relatively higher antibacterial activity compared to other extracts. Further studies are recommended that will involve various strains of microorganisms, purification of the most active antibacterial components. Toxicity studies should also be done to determine their safety.

REFERENCES

[1] WHO-Global Principles for the Containment of Antibacterial Resistance in Animals Intended for Food. Last update January 2008; Accessible at: www.who.int/ emc/diseases/zoo/who_global_principles.html.
[2] Okeke IN, Aboderin OA, Byarugaba DK, Ojo KK and Opintan JA. Emerg Infect Dis. Perspective 2007; 13 (11):393-96.
[3] Centre for diseases control (CDC). National Nosocomial Infections Surveillance System.
[4] Accessible at: www.cdc.gov/ncidod/dhqp/nnis.html.
[5] Denyer SP, Hodges NA, Gorman SP. Bacterial resistance to antibiotics and Clinical uses of antibacterial drugs. In Hugo & Russell's Pharmaceutical Microbiology. 5th Ed. Blackwell Publishing, 2004. Chapt. (13-14): 220-250.
[6] Nadkarni KM. 1982. The Indian Materia Medica. 3rd ed. Popular parakashan, Bombay 238.
[7] Christopher W. PhcogMag 2005;1(1):7-8.
[8] Grover JK, Yadav SP. J Ethnopharmacol. 2004;93(1):123-32.
[9] Nagasawa H, Watanabe K, Inatomi H. Am J Clin Med 2002; 30(2–3): 195–205.
[10] Takemoto DJ. Prep Biochem 1983; 13(4): 371–93.
[11] Roopashree T S, Raman Dang , Shobha Rani RH, Narendra C. Int J Applied Research in Natural Products 2008; 1(3):20-28.
[12] Kokate. CK, Purohit AP, Gokhale SB. Pharmacognosy. 39th ed.Pune: Nirali prakashan. 2007. p108-109.
[13] Khandelwal KR. Practical Pharmacognosy. 12th ed. Pune: Nirali prakashan 2004: p149-160.
[14] Khan MR., Omoloso AD. Khan, MR. Korean J Pharmacol 1998; 29(3): 155–58.
[15] Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN. Indian J Exp Biol 1988; 26(11): 883–904.