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Short communication

Antibacterial activity of *Plumbago indica*

Dibyajyoti SAHA*, Swati PAUL

BGC Trust University, Department of Pharmacy, Chittagong, BANGLADESH

The antibacterial activity of the methanol extract of *Plumbago indica* (Plumbaginaceae) was evaluated against eleven pathogenic bacteria by the disc diffusion method using ciprofloxacin as a standard. In the screening, the methanol extract of the plant showed varying degrees of antibacterial activity with zone of inhibition ranging from 7.0-25.0 mm. The highest antibacterial activity was seen against with *Staphylococcus aureus*, *Salmonella typhi* and *Salmonella paratyphi*. The Minimum Inhibitory Concentrations (MIC) of the methanol extract of *P. indica* were found to be 31.25-125 µg/mL for bacteria species used in the screening.

Key words: *Plumbago indica*, Plumbaginaceae, Antibacterial activity, Methanol extract, Ciprofloxacin.

Plumbago indica'nın Antibakteriyel Aktivitesi

Plumbago indica'nın (Plumbaginaceae) metanol ekstresinin antibakteriyel aktivitesi disk difüzyonu yöntemi ile siprofloksasin standart olarak kullanılarak 11 patojenik bakteriye karşı değerlendirilmiştir. Tarama çalışmasında, bitkinin metanol ekstresinin 7.0-25.0 mm arasında değişen inhibisyon zonu ile farklı derecelerde antibakteriyel aktiviteye sahip olduğu gösterilmiştir. En yüksek antibakteriyel aktivite *Staphylococcus aureus, Salmonella typhi* ve *Salmonella paratyphi*'ye karşı tespit edilmiştir. Taramada *P. indica* bitkisinin methanol ekstresine ait Minimum İnhibitör Konsantrasyon (MİK) değerleri 31,25-125 µg/mL olarak bulunmuştur.

Anahtar kelimeler: *Plumbago indica*, Plumbaginaceae, Antibakteriyel aktivite, Metanol ekstresi, Siprofloksasin.

***Correspondence:** E-mail: saha.dibyajyoti@gmail.com, Tel: +88 - 01752560434

INTRODUCTION

In Bangladesh, *Plumbago indica* (Plumbaginaceae) named "agnichita" belonging to the family Plumbaginaceae. The family is sometimes referred to as the leadwort family or the plumbago family. Most species in this family are perennial herbaceous plants, but a few grow as shrubs. The plants have perfect flowers and are pollinated by insects. They are found in many different climatic regions, from arctic to tropical conditions, but are particularly associated with salt-rich steppes, marshes, and sea coasts. *Plumbago* is popularly known as "chittiramulam", in Tamil and "white leadwort" in English. *Plumbaginaceae* is distributed as a weed throughout the tropical and subtropical countries of the world. The family *Plumbaginaceae* is composed of 10 genera and 280 species. The genus *Plumbago* includes 4 species, namely *P. indica* L., *P. rosea* L., *P. capensis* L. and *P. zeylanica* L., which are distributed in several parts of India (1). *P. indica* root promotes appetite and has long been marked as a powerful antiseptic. A liniment made from bruised root mixed with a few amount of bland oil is used in treating rheumatism, paralysis, leucoderma, enlarged glands and buboes and scorpion-sting (2). Scraped root is inserted into the mouth of the womb to procure illegal abortion, a tincture of the root is used in secondary syphilis, leprosy, dyspepsia, hemorrhage, piles, flatulence, loss

of appetite and the milky juice of the plant is used in ophthalmia, scabies and as an antiseptic agent (3). In order to establish the above assertion about their validity, these medicinal plants must be subjected to extensive study in different research works. For this reason, *P. indica* was selected and subjected for chemical, biological and pharmacological investigations to explore the antimicrobial, cytotoxicity, antidiarrhoeal, anti-motility, analgesic activity of methanol extracts of the above mentioned plant species.

MATERIALS AND METHODS

Collection of plant material

Plumbago indica was collected in rainy season (25th June 2012) from Naramuk, Rajsthali of Rangamati district. After collection, suitable herbarium sheet for each plant with some general information were prepared and send to Bangladesh Council of Scientific and Industrial Research (BCSIR), Baluchara, Chittagong for identification with herbarium / Accession No. 36085.

Extraction

The collected plants (leaves and stems) was separated from undesirable materials or plants or plant parts and was shed-dried (35-50°C). The plant was ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until extraction commenced. About 75 gm of powdered plant material of *Plumbago indica* was taken in a clean, flat bottomed amber glass container and soaked in 350 ml of methanol. The container with its contents was sealed and kept for a period of 10 days accompanied by continuous shaking. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton materials. Then they were filtered by using Whatman filter paper number 1 and the solvent was made to evaporate under the room temperature. The residues were stored in a refrigerator until further studies.

Bacterial cultures

To investigate the antibacterial activity both the gram positive and gram negative species were selected. For the evaluation, the gram positive and gram negative of clinical isolates: *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysentariae, Vibrio cholera, Shigella sonnei* and *Salmonella paratyphi* were selected respectively. All the clinical species were supplied by the Faculty of Microbiology, Chittagong University, Chittagong, Bangladesh. All the test strains were maintained on nutrient agar slopes and were sub-cultured. These bacteria served as test pathogens for antibacterial activity assay using disc diffusion method.

Sterilization

Antibacterial screening was done in laminar hood maintaining all precautions required to avoid any contamination derives the test. UV light was kept switch on before half an hour working in laminar hood to avoid any accidental contamination. Petridishes and other glass wares sterilized by autoclaving at a temperature of 121°C and a pressure of 15 lbs/sq. inch for 15 min. Blank discs were kept in a covered petridish and subjected to dry heat sterilization at 180°C for 1 h. Then, they were kept in a laminar hood under UV light for 30 min.

Culture media preparation

The nutrient agar medium (HiMedia Laboratories Pvt. Ltd. India) is used to demonstrate the antibacterial activity and to make subculture of the test organism

Drugs and chemicals used for antibacterial assay

Ciprofloxacin (500 µg/disc) and dimethyl sulphoxide (DMSO) as solvent.

Antibacterial activity

Disc diffusion method (4) was used to test the antibacterial activity of the methanolic extract of plant against eleven bacteria. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amount of the test substances dissolved in methanol (40 µg/mL) using micropipette and the residual solvents were completely evaporated. Discs containing the test material (250 µg/disc & 500 µg/disc) were placed on

nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of Ciprofloxacin (500µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4^0C) for 24 hours to allow maximum diffusion of test samples. The plates were then incubated at 37 ^{0}C for 24 h to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter (5). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Table 1. Composition of nutrient agar medium

Ingredients	Amounts
Beef extract	1.50 g
Peptic digest of animal tissue	5.0 g
Sodium chloride	5.0 g
Yeast extract	1.50 g
Agar	15.0 g
Distilled water	q.s to 1000 ml
28.0 g is recommended for 1000mL distilled water	

Minimum Inhibitory Concentration

Lowest dose of an antibiotic that dose the inhibition of the growth of a particular no (s) organism (s) upon which it acts is called the 'Minimal Inhibitory Concentration' (MIC). The present study protocol was designed and performed due to the fact that the MEAC are found as a positive antimicrobial agent and the extract acts preciously on a number eleven of gram positive and Gram negative bacteria species are made to test. For MIC test the 'Serial tube dilution technique (6) is used.

Clinical pathogenic species

The above mentioned 11 species of bacteria were screened for minimum inhibitory concentration of the crude methanolic extract of *Plumbago indica*

Sterilization

Test tubes containing Nutrient broth (for bacteria), media and other glass wares were sterilized by autoclaving at a temperature of 121°C and a pressure of 15 lbs/sq inch for 15 minutes. Then, they were kept in a laminar hood under UV light for 30 minutes.

Culture media preparation

The nutrient broth medium (HiMedia Laboratories Pvt. Ltd. India) is used to demonstrate the antibacterial activity and to make subculture of the test organism.

Table 2. Composition of nutrient broth medium

Ingredients	Amounts
Beef extract	5.0 g
Peptic digest of animal tissue	5.0 g
Sodium chloride	1.50 g
Yeast extract	1.50 g
Distilled water	q.s to 1000 ml
13.0 g is recommended for 1000ml distilled water	

Assay procedure

Ten screw cap test tubes were taken and serially marked 1, 2, 3, 4, 5, 6 & 7 for sample solutions and the rest three T_M for medium, T_{MI} for medium & inoculum and T_{MS} for medium & solvent respectively. 1 mL of nutrient broth medium were taken in all test tubes and sterilized in an autoclave at a temperature of 121°c and a pressure of 15 Ibs/sq. inch for 30minutes. After 30 minutes, 1 mL of 1000 µg sample was added to the no-1 marked tube and the tube was shaken gently for proper mixing of the content. 1 ml of the content form the 1st tube was added to the no 2 marked tube that action was performed upto the no.7 marked tubes, after proper mixing 1 mL content form the 7 marked tube was discarded. 10µL of specified bacterial suspension of the clinical pathogen were added to the 1 to 7 and T_{MI} marked tubes by a suitable micropipette (10-100µl). For T_{MS} only 1mL ethanol was added, after shaking 1mL of the mixture was discarded from the tube. T_M contain only 1 mL medium. All the test tubes were subjected for incubation at 37 °C for 18 h.

RESULTS AND DISCUSSION

Determination of antibacterial activity

The antibacterial activity of the crude extracts were evaluated by the disc diffusion method against 4 gram positive and 7 gram negative pathogenic bacteria using ciprofloxacin as standards. In the screening, the methanol extract of *Plumbago indica* showed varying degrees of antimicrobial activities with zone of inhibition ranging from 7.0-25.0 mm, while the highest antibacterial activity was seen against with *Staphylococcus aureus, Salmonella typhi* and *Salmonella paratyphi.*

Table 3. Antibacterial activity of the crude extract of MEPI, standard and blank

Tested bacteria	Zone of inhibition (mm)			
	MEPI		S	C
	A	*B*	*500 µg/disc*	
Gram Positive Species				
Bacillus subtilis	10	18	24	-
Bacillus megaterium	9	17	25	-
Bacillus cereus	14	25	28	-
Staphylococcus aureus	11.5	24	27.5	-
Gram Negative Species				
Pseudomonas aeruginosa	10	21	31	-
Escherichia coli	12	23	28	-
Shigella dysentariae	9.5	21	32	-
Shigella sonnei	7	18	27	-
Salmonella typhi	13	20	23.5	-
Vibrio cholera	7.5	19	24	-
Salmonella paratyphi	13.5	22	26	-

MEPI= Methanol extract of *Plumbago indica* L. A = 250µg/disc, B = 500µg/disc, S = Standard (ciprofloxacin) & C = Control

Determination of Minimum Inhibitory Concentration (MIC)

From the table 4, it was depicted that methanol extract of *Plumbago indica* inhibited the growth of *Bacillus cereus, Salmonella typhi* significantly at the dose of 31.25 µg/mL then followed by *Staphylococcus aureus, Shigella dysentariae, Salmonella paratyphi* by 62.50 µg/mL, *Bacillus subtilis, Bacillus megaterium, Pseudomonas aeruginosa, Escherichia coli, Shigella sonnei,* and *Vibrio cholerae* by 125 µg/mL.

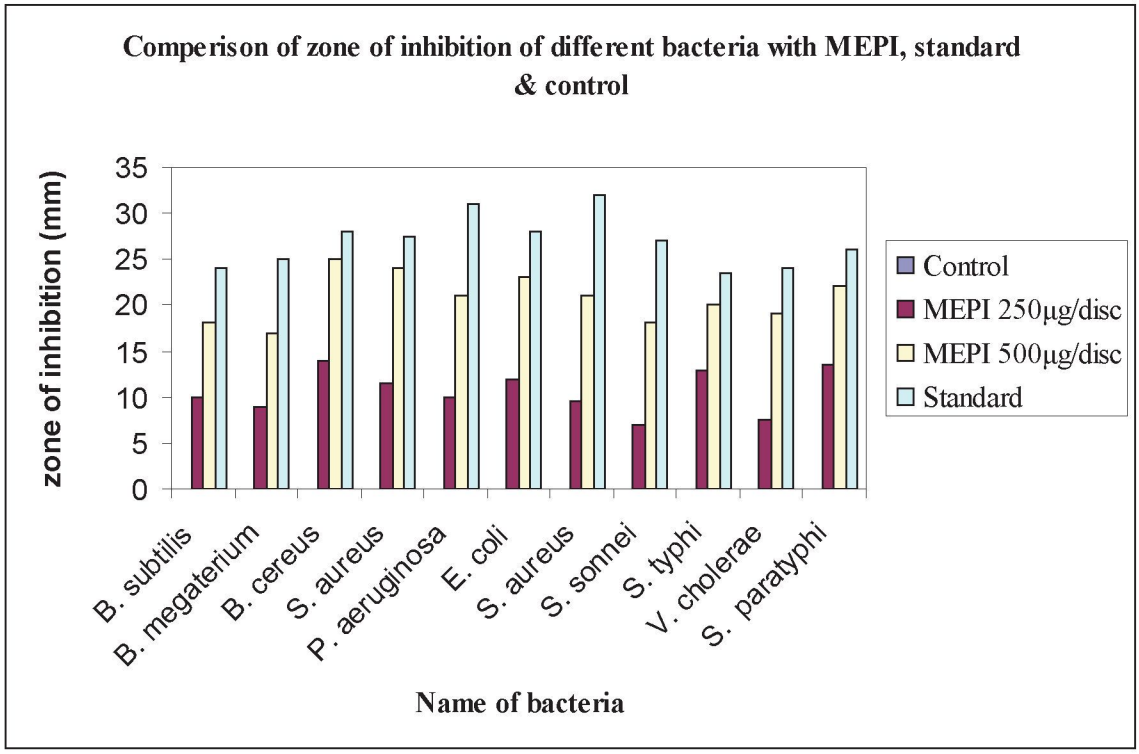

Figure 1. Graphical representation of antibacterial activity of methanol extract of *Plumbago indica* compared with standard antibiotic and control

Table 4. Minimum inhibitory concentration of the MEPI against the clinical pathogenic bacteria

Marked test tubes	Medium added (mL)	Sample solution (µg/mL)	Inoculums added (µL)	Growth of clinical pathogens										
				Gram Positive Species				*Gram Negative Species*						
				Bacillus subtilis	*Bacillus megaterium*	*Bacillus cereus*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Shigella dysentariae*	*Shigella sonnei*	*Salmonella typhi*	*Vibrio cholerae*	*Salmonella paratyphi*
1	1	500	10	-	-	-	-	-	-	-	-	-	-	-
2	1	250	10	-	-	-	-	-	-	-	-	-	-	-
3	1	125	10	-	-	-	-	-	-	-	-	-	-	-
4	1	62.5	10	+	+	-	-	+	+	-	+	-	+	-
5	1	31.25	10	+	+	-	+	+	+	+	+	-	+	+
6	1	15.625	10	+	+	+	+	+	+	+	+	+	+	+
7	1	7.8125	10	+	+	+	+	+	+	+	+	+	+	+
T_{MI}	1	0	10	+	+	+	+	+	+	+	+	+	+	+
T_{MS}	1	0	10	-	-	-	-	-	-	-	-	-	-	-
T_M	1	0	0	-	-	-	-	-	-	-	-	-	-	-

MIC = Minimal inhibitory concentration, MEPI = Methanol extract of *Plumbago indica*, T_{MI} = Test tube containing medium & inoculum, T_{MS} = Test tube containing medium & solvent, T_M = Test tube containing medium, (+) = Growth and (-) = No growth

CONCLUSION

In the screening, the methanol extract of *Plumbago indica* showed varying degrees of antibacterial activity with zone of inhibition ranging from 7.0-25.0 mm, while the highest antibacterial activity was seen against with *Staphylococcus aureus, Salmonella typhi* and *Salmonella paratyphi.* The Minimum Inhibitory Concentrations of the methanol extracts was found to be 31.25-125 μg/mL for bacteria used in this study.

REFERENCES

1. Vijver VDL, Distribution of plumbagin in the Plumbaginaceae, Phytochemistry, 11(5), 3247-3248, 1974.
2. Rathinasabapathi B, Walid M. Fouad WM, Sigua AC, β-Alanine betaine synthesis in the Plumbaginaceae, purification and characterization of a trifunctional, *S*-adenosyl-l-methionine-dependent *N*-methyltransferase from *Limonium latifolium* leaves, Plant Physiol, 126 (3), 1241-1249, 2001.
3. Ghani A, Medicinal plants of Bangladesh with chemical constituents and uses, J Asiatic Soc Bangladesh, 2(3), 4-19, 1998.
4. Bauer A.W, Kibry WMM, Sheries JC, Turek M, Antibiotic succeptibility testing by a standard single disc method, Am J Sci, 1, 103-195, 1951.
5. Roland R, Antibiotics: An Introduction, F Hoffmann La Roache & Co. Basle, Switzerland, 2nd ed, 70-71, 1982.
6. Andrews JM, Determination of minimum inhibitory concentrations, J Antimic Chemother, 48, 5-16 , 2001.

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