

## Short Communication

# Antimicrobial Activity of Crude Extract Obtained from the Root of *Plumbago zeylanica*

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Ethanol extract of *Plumbago zeylanica* root was investigated for its antimicrobial activities against 11 human pathogenic bacteria and 6 phytopathogenic fungi using disc diffusion method and poisoned food technique respectively. The extract exhibited good antibacterial and antifungal activities against the test organisms. Among the test bacteria, *Vibrio cholerae* was found to be the most sensitive to the extract showing the highest diameter of zone of inhibition and lowest minimum inhibitory concentration (MIC) value (200 µg/ml). The extract was also very effective against *Escherichia coli* and *Pseudomonas aeruginosa* showing MIC value of 250 µg/ml. Among the phytopathogenic fungi tested, *Curvularia lunata* exhibited the highest sensitivity to the extract with an MIC value of 150 µg/ml, which was followed next by *Colletotrichum corchori* and *Fusarium equiseti* (MIC value 250 µg/ml). The root extract from *P. zeylanica* seems promising since it showed both antibacterial and antifungal activities.

**Keywords:** Antimicrobial activity, *Plumbago zeylanica*, Ethanol extract, Minimum inhibitory concentration (MIC)

Plants are still widely used in ethnomedicine around the world<sup>1</sup>. Plant metabolites are proved to be the most important group of compounds that showed wide range of antimicrobial activity<sup>2-4</sup>. Much of the work has been done in India<sup>5</sup> and other countries<sup>3-4,6</sup>. However, a relatively little work has been done in this field in Bangladesh<sup>2,7</sup>.

It has been reported that the shrubby perennial herb, *Plumbago zeylanica* (family Plumbaginaceae), possesses antimicrobial activities<sup>5</sup>. It grows in shady places of wastelands and graveyards all over Bangladesh. Roots and aerial parts principally contain an orange yellow pigment, plumbagin, a naphthoquinone and a fatty alcohol<sup>5</sup>. Its other constituents in roots are chitranone, zeylanone, dihydrosterone, 2-methyl naphthaquin, plumbazeylanone and terpenoids, lupeol and teraxesterol. The plant also contains alkaloids, glycosides, tannin, saponins and steroids<sup>5</sup>. The plant has been used both in Ayurvedic and Unani systems for its different activities such as in curing laryngitis, ringworm infection and other skin disease. Paste is applied externally in leprosy<sup>5</sup>. Other species of *Plumbago* are also reported by several workers for their biological activities such as antiparasitism<sup>8</sup>, insect antifeedant<sup>9</sup> and antitumoral<sup>6</sup>. In this study, antimicrobial activity of *P. zeylanica* was evaluated against some human pathogenic bacteria and plant pathogenic fungi.

Root of *P. zeylanica* was collected in fresh condition in December 2004 from a garden owned by the Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Chittagong,

Bangladesh, where a voucher specimen was maintained. The root was cleaned, cut into small pieces (1-2 cm), dried in oven at reduced temperature (45-50°C) and ground to fine powder. The dried powder (250 g) was kept steeped twice overnight in 95% ethanol. They were combined, filtered and centrifuged at 5,000 rpm for 20 min. The extract was then concentrated to a gummy material under reduced pressure at 50°C by using a rotary vacuum evaporator.

*In vitro* antibacterial and antifungal activities of the crude extract of the plant were determined by disc diffusion method<sup>10</sup> and poisoned food technique<sup>11</sup> respectively. Mueller-Hinton medium (agar and broth) was used for culture of bacteria and Sabouraud medium (agar and broth) was used for culture of fungi. Ethanol solution (5%) of the crude extract was used as the test antimicrobial agent against 11 human pathogenic bacteria and 6 phytopathogenic fungi. The results were compared with the standard antibacterial antibiotic ampicillin (20 µg/disc, Beximco Pharma Bangladesh Ltd., Dhaka) and antifungal antibiotic nystatin (100 µg/ml medium, Beximco Pharma Bangladesh Ltd., Dhaka). Minimum inhibitory concentration (MIC) of the crude extract was determined by macro-dilution broth technique<sup>12</sup>.

Antimicrobial activity of the crude extract from *P. zeylanica* against the pathogenic bacteria is summarized in Table 1. Using the disc diffusion method, the extract exhibited zone of inhibitions ranged from 8 to 18 mm in diameter with 250 µg/disc and 16-30 mm in diameter with 500 µg/disc concentration against the test bacteria. The highest zone of inhibition (30 mm) was recorded

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against *Vibrio cholerae* El Tor. The extract at a concentration 500 µg/disc showed larger zone of inhibition as compared to that formed by the standard ampicillin disk. The MIC values of the crude extract as determined by macro-dilution broth technique against the test bacterial isolates varied between 200 µg/ml in case of *V. cholerae* El Tor and 500 µg/ml in case of *Staphylococcus aureus*. It was apparent that the crude extract was equally active against Gram-positive and Gram-negative bacteria, or enteric and non-enteric pathogens. Similar antibacterial activity of other plant extracts has been reported by other investigators<sup>7,13-14</sup>.

**Table 1.** Antibacterial activity of crude extract from *Plumbago zeylanica*

Bacterium	Diameter of inhibition zone (mm) <sup>a</sup>		Ampicillin (20 µg/disc)	MIC <sup>b</sup> (µg/ml)
	Crude extract (µg/disc)			
	500	250		
<i>Bacillus subtilis</i>	21	13	19	350
<i>Bacillus cereus</i>	23	14	18	300
<i>Bacillus megaterium</i>	22	14	16	350
<i>Staphylococcus aureus</i>	19	12	22	500
<i>Escherichia coli</i>	26	15	10	250
<i>Vibrio cholerae</i> El Tor	30	18	15	200
<i>Shigella dysenteriae</i>	20	12	22	400
<i>Shigella sonnei</i>	16	10	20	500
<i>Salmonella typhi</i>	17	8	20	500
<i>Salmonella paratyphi</i>	20	13	17	450
<i>Pseudomonas mutabilis</i>	24	15	12	250

<sup>a</sup>Measured by disc diffusion method; <sup>b</sup>Measured by macro-dilution broth technique; MIC = Minimum inhibitory concentration

Antifungal activity of the crude extract against 6 phytopathogenic fungi was studied and the results were compared to that of standard antifungal antibiotic nystatin. The results of the inhibition of fungal radial mycelial growth and MIC values are summarized in Table 2. It appeared that the crude extract of *P. zeylanica* inhibited radial mycelial growth of all the test fungi at a concentration of 100 µg/ml medium to varying degrees that ranged from about 25% in case of *Macrophomina phaseolina* and 76% in case of *Curvularia lunata*. The MIC values of the crude extract ranged between 150 µg/ml against *Curvularia lunata* and 500 mg/ml against *Macrophomina phaseolina*. This indicates that root extract of *P. zeylanica* could be used as an eco-friendly antifungal agent in the control of fungal diseases. The extract could also be used by farmers for the control of seed-borne fungal pathogens. Antifungal activities of other plants have also been reported by other investigators<sup>15-16</sup>.

**Table 2.** Antifungal activity of crude extract from *Plumbago zeylanica*

Fungus	Percentage inhibition of fungal mycelial growth <sup>a</sup>		MIC <sup>b</sup> (µg/ml)
	Crude extract (100 µg/ml)	Nystatin (100 µg/ml)	
	<i>Alternaria alternate</i>	34	
<i>Botryodiplodia theobromae</i>	39	70	350
<i>Curvularia lunata</i>	76	75	150
<i>Colletotrichum corchori</i>	56	41	250
<i>Fusarium equiseti</i>	55	45	250
<i>Macrophomina phaseolina</i>	25	72	500

<sup>a</sup>Measured by poisoned food technique; <sup>b</sup>Measured by macro-dilution broth technique; MIC = Minimum inhibitory concentration

Research on natural resources has been encouraged by the World Health Organization<sup>17</sup>. Although natural products from plants are known to control some infectious diseases, the use of plant secondary metabolites for treatment of bacterial and fungal disease has received less attention. The finding of the active compound may be interesting in the search for new efficacious and safe antimicrobial agent against a variety of pathogenic bacteria and fungi. Our results could stimulate further pharmacological studies seeking new antimicrobial agents from the plant resources. The present investigation confirms that there are antibacterial and antifungal properties in the crude extract of *P. zeylanica* root. Further study is necessary to purify the active compound(s) that could be tested for antimicrobial activity.

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