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Biological Screening of *Eichornia crassipes* against Different Pathogenic Microbes: An In Vitro Study

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Abstract

The present research is a biological screening of *Eichornia crassipes* (Pontederiaceae). Dichloromethane and methanol extracts of the whole plant were investigated for their antibacterial, antifungal, phytotoxic, and cytotoxic activities. The antibacterial activity was evaluated using agar well-diffusion method against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Shigella flexneri, Pseudomonas aeruginosa,* and *Salmonella typhi*. The antifungal activity was evaluated using the agar tube–dilution method against *Candida albicans*, *Candida glabrata*, *Aspergillus flavus, Microsporum canis,* and *Fusarium solani*. The phytotoxicity activity was determined using Lemna bioassay against *Lemna minor*. Brine shrimp–cytotoxicity assay was determined against brine-shrimp larvae. Dichloromethane extract exhibited significant phytotoxicity (100% growth regulation) at 1,000 μg/ml concentration against *Lemna minor* whereas methanolic extracts showed moderate (75% growth regulation) phytotoxicity at the same concentration. Methanolic extract showed cytotoxicity at the highest level of dose whereas dichloromethane extract showed no activity having Etoposide as standard drug. Both of the extracts have nonsignificant antifungal and antibacterial activity.

Keywords: *Eichornia crassipes*; Biological screening; Cytotoxicity; Phytotoxicity.

1. INTRODUCTION

Medicinal plants have a promising future because there are about half million plants around the world, and the medical activities of most of them have not been investigated yet, and their medical activities could be decisive in the treatment of present or future illnesses [1]. The search for new drugs derived from plants has accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are working on phytochemicals that lead to the development of new bioactive compounds in treating infectious diseases [2]. The recent failure of antibiotics against certain pathogens may result in a strong urge to find an alternative treatment or to identify new antimicrobial agents from medicinal plants [3]. *Eichornia crassipes* is one of the traditional natural sources. Flowers are traditionally used for treating the skin problems of horses [4]. The species is also used as a tonic [5].

2. MATERIALS AND METHODS

2.1. Plant Material

The whole plant of *Eichornia crassipes* was collected from the surroundings of Lower Bari Doab Canal district, Sahiwal, Pakistan. The plant materials were identified by Prof. Dr. Altaf Ahmad Dasti, Institute of Pure and Applied Biological Studies, Bahauddin Zakariya University, Multan. A voucher specimen is deposited in the Herbarium of the Institute.

2.2. Extraction

The air-dried plant material was grounded and extracted successively with dichloromethane and methanol (thrice with each solvent) at room temperature with occasional shaking for 24 h. The extracts were concentrated by Rotavapor-R20 at 35°C.

2.3. Antibacterial Bioassay: Agar Well Diffusion Method

The petri plates are prepared with an inoculated media. Three wells of 8 mm diameter are cut on one plate with a borer and sealed with a drop of inoculated sterile media. All the solutions, i.e, the extract, solvent and reference standard (imipenum 10 μg/disc.) were poured into their respective well by means of a sterilized pipette. The petri dishes were incubated at 37°C for 24-48 h. The zones of inhibition were measured with a vernier caliper [6].

2.4. Antifungal Bioassay

Test fungi such as *Trichphyton longifusus, Candia albicans, Aspergillus flavus, Microsproum canis, Fusarium solani, and Candia glabrata* were employed for preliminary screening. Extracts were dissolved in sterile Dimethyl Sulphoxide (DMSO) to serve as a

stock solution. Sabouraud dextrose agar was prepared by mixing Sabouraud 4% glucose agar and agar in distilled water. Test tubes containing media were autoclaved at 121°C for 15 min. The tubes were allowed to cool to 50°C, and the desired concentration of extract was added into nonsolidified media. The tubes were allowed to solidify at room temperature. Each tube was inoculated with a 4 mm diameter piece of inoculum removed from a seven-day-old culture of fungi. All culture-containing tubes were inoculated at an optimum temperature of 28-30°C for growth for 7-10 days. The culture was examined at least twice a week during the incubation. The results were expressed in μg [6].

The selection of bacteria and fungi was based upon their parasitic activity and their availability; for example, *Trichphyton longifusus* is a common cause of athlete's foot, whereas virulent *E. coli* can cause urinary tract infections.

2.5. Phytotoxic Bioassay

The prepared inorganic medium of 5.5-6.0 pH was attained with KOH pellets. 8 sets of 20 vials each for 500, 50, 5ppm and control were prepared for testing. 15 mg of each of crude methanol and dichloromethane extracts of plant (*Eichornia crassipes*) were dissolved in 15 ml of related solvents. 1000 μl, 100 μl and 10 μl solutions of extract were added to 500 ppm, 50 ppm and 5 ppm vials respectively. The solvent was placed for overnight to evaporate. 2 ml of E-medium and a single plant of Lemna minor L. having a rosette of three fronds was added to each testing vial. These vials were placed in a glass dish that was filled with water up to 2 cm and it was sealed with stopcock grease and glass plate. The glass dish was placed in a growth chamber for seven days at temperature of 25ºC under fluorescence and incandescent light. Number of fronds of each test vial were counted and recorded on 3rd and 7th day [6].

2.6. Brine Shrimp–Lethality Bioassay

Brine shrimp–cytotoxicity assay was accomplished according to the standard procedure described by McLaughlin (1991). Three concentrations (1,000, 100, and 10 ppm) of the plant extracts were used in this assay. Brine-shrimp larvae were hatched in a small partitioned tank in artificial seawater. Illumination was provided on one side to attract newly hatched larvae. Brine-shrimp larvae with the second instar stage were used in this assay. Plant extracts of respective concentrations were added to dram vials. To each dram vial, ten brine-shrimp larvae were added. The negative control was prepared by evaporating 0.5 ml of methanol in dram vials and then by adding sea-salt solution to it. Following 24 h of incubation, the survivors were counted by using a magnifying glass. The experiment was repeated three times. The mortality data was transformed by probit analysis in a finny computer program to estimate the ED_{50} value. The percentage of mortality was also calculated at all concentrations [7].

3. RESULTS AND DISCUSSION

Dichloromethane and methanol extracts of the whole plant of *Eichornia crassipes* were studied for their antibacterial, antifungal, and phytotoxic properties and for their brine shrimp–lethality bioassay.

The antibacterial activity of the extracts was tested against *Eschericha coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureu, Pseudomonas aeruginosa and Salmonella typhi.* Both the extracts exhibited no activity at the concentration of 0.3 mg/ml (Table 1).

Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani and Candida glabrata were employed for the fungitoxic effect of the extracts. Dichloromethane extract showed 25% inhibition against *Aspergillus flavus,* at the concentration

Table 1: Results of in vitro antibacterial bioassay of *Eichornia crassipes*.

Extract	Name of bacteria	Zone of inhibition of sample (mm)	Zone of inhibition of standard drug (mm)
MeOH extract	*Eschericha coli*	–	25
	Bacillus subtilis	–	50
	Shigella flexinari	–	28
	Staphylococcus aureus	–	48
	Pseudomonas aeruginosa	–	23
	Salmonella typhi	–	28
DCM extract	*Eschericha coli*	–	25
	Bacillus subtilis	–	50
	Shigella flexinari	–	28
	Staphylococcus aureus	–	48
	Pseudomonas aeruginosa	–	23
	Salmonella typhi	–	28

Concentration of sample: 0.3 mg/ml of DMSO; size of well: 6 mm (diameter) standard; standard drug: imipenum, 10 μg/disc.

Table 2: Results of in vitro antifungal bioassay of *Eichornia crassipes*.

Extract	Name of fungus	Linear growth (mm) Sample	Control	% Inhibition	Standard drug	MIC (μg/ml)
MeOH	*Candida albicans*	100	100	0	Miconazole	110.8
	Aspergillus flavus	100	100	0	Amphotericin B	20.20
	Microsporum canis	100	100	0	Miconazole	98.4
	Fusarium solani	100	100	0	Miconazole	73.25
	Candida glabrata	100	100	0	Miconazole	110.8
DCM	*Candida albicans*	100	100	0	Miconazole	110.8
	Aspergillus flavus	75	100	25	Amphotericin B	20.20
	Microsporum canis	100	100	0	Miconazole	98.4
	Fusarium solani	100	100	0	Miconazole	73.25
	Candida glabrata	100	100	0	Miconazole	110.8

Concentration of sample: 400 μg/ml of DMSO, minimum inhibitory concentration (MIC), incubation time: 27 (28 ± 1C°), incubation period: 7 (7-10 days).

Table 3: Results of in vitro phytotoxic bioassay of *Eichornia crassipes*.

Extract	Plant name	Concentration of compound (μg/ml)	No. of Fronds Sample	Control	% Growth regulation	Concentration of standard drug (μg/ml)
MeOH	*Lemna minor*	1,000	5	20	75	0.015
		100	12		40	
		10	13		35	
DCM		1,000	0	20	100	
		100	19		5	
		10	20		0	

Standard drug: Paraquat.

Table 4: Results of in vitro cytotoxic bioassay of *Eichornia crassipes*.

Extract	Dose (μg/ml)	No. of shrimps	No. of survivors	LD_{50} (μg/ml)	STD drug	LD_{50} (μg/ml)
MeOH	1,000	30	12	366.521	Etoposide	7.4625
	100	30	18			
	10	30	25			
DCM	1,000	30	23	21,778.36	Etoposide	
	100	30	25			
	10	30	29			

of 400 μg/ml for an incubation period of seven days at 27°C with reference to amphotericin B as standard, while methanol extract was found to be inactive (Table 2).

Dichloromethane extracts of the whole plant of *Eichornia crassipes* showed significant phytotoxicity at concentrations of 1,000, 100 and 10 μg/ml against *Lemna minor*, whereas methanolic extracts of the whole plant of *Eichornia crassipes* showed moderate phytotoxicity at the same concentration.

The methanolic extract of *Eichornia crassipes* showed cytotoxicity at the highest level of dose with LD_{50} 366.521 μg/ml, whereas the dichloromethane extract showed no activity having etoposide as the standard drug.

4. CONCLUSION

The results showed that *Eichornia crassipes* possesses strong phytotoxic and cytotoxic activities using different solvents. Moreover, it may be a source of herbal pesticides because of strong phytotoxic properties. There is a need for further investigation on the isolation of allelochemicals that were involved in its phytotoxic activity. The methanolic extract possesses strong in vitro cytotoxic activity. Further investigation is required to find its specific potential on different types of cancer cell lines.

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None.

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