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Original Article

Antimicrobial Efficacy and Phytochemical Analysis of Three Aquatic Plant Species in Bangladesh

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Aquatic plants are generally considered as breeding grounds for mosquitoes and other harmful vectors of diseases. However, in recent years, some research has been carried out to test their significance as sources of antimicrobial lead molecules. The aim of this research was to study the phytochemical composition of local aquatic plant species and test their antimicrobial effect against selected bacterial strains. Three different aquatic plant samples were collected from a large water body near Dhaka. Ethanol and ethyl acetate extracts of the plant species: *Eichhornia crassipes*, *Pistia stratiotes*, and *Spirodela polyrrhiza* were tested against *Staphylococcus aureus*, *Salmonella typhi*, and *Lactobacillus* spp. Out of the eight different extracts, only the ethyl acetate extracts prepared from *Eichhornia crassipes* showed significant anti-microbial activity against *Staphylococcus aureus* and *Salmonella typhi*. In disk diffusion tests, zone of inhibitions of ethyl acetate extracts of *Eichhornia crassipes* leaves and stems were 8.00 ± 0.5 mm and 7.83 ± .29 mm respectively. In well diffusion tests, zone of inhibitions of ethyl acetate extracts of *Eichhornia crassipes* leaves and stems were 18.00 mm and 20.00 mm respectively. Zones of inhibition of ethyl acetate extracts of *Eichhornia crassipes* stems against *Staphylococcus aureus* were 7.67 ± 0.29 mm and 12.00 mm respectively in disk and well diffusion tests. Zone of inhibition of ethyl acetate extracts of *Spirodela polyrrhiza* was 8.17 ± 0.29 mm against *Staphylococcus aureus* in disk diffusion tests. No extracts showed any antimicrobial potential against *Lactobacillus*. Phytochemical composition analysis showed the presence of alkaloids, flavonoids, steroids, phenolics, tannins, glycosides, and cardiac glycosides in the different ethanol and ethyl acetate extracts. Tannins were absent in all extracts and saponins were absent in all ethyl acetate extracts.

Keywords: Aquatic plants, Anti-microbial, Phytochemical composition

Introduction

Plants have been the basis of sophisticated traditional medicine systems for thousands of years which have led to the isolation of a great number of chemical compounds with potentials for various uses[1]. The recent interest in plant derived drugs is mainly due to the current widespread belief that they are safe, clinically effective, better tolerated by patients, less expensive and globally competitive[2,3].

Aquatic plants, covering up surfaces of water bodies, can be algae, bryophyte, petridophyte and angiosperm. They grow entirely or partly in water bodies and mostly have adverse effects on aquatic ecosystem resulting in economic losses. However, studies on the phytochemical components of aquatic plants have shown that these plants are rich in flavonoids which can confer potent antimicrobial effects[4,5]. Hence, aquatic plant species can be promising sources of potential anti-microbial lead molecules.

Bangladesh is a country crisscrossed by a large number of rivers and smaller water bodies. A wide range of aquatic plant species thrive in these aquatic reservoirs. In Bangladesh about 130 angiospermic, 6 pteridophytic, 3 bryophytic and several hundred algae species have been identified as aquatic plants. Some amphibious plants are also available. True aquatic plants are identified as planktonic, floating, benthic, submerged or emergent types. Other than algae about 35 species are submerged, 20 are surface immersed, and 17 are free floating[6].

This research focused on three aquatic plant species widely distributed throughout Bangladesh:

1) Water lettuce (*Pistia stratiotes*)

2) Water Hyacinth (*Eichhornia crassipes*)

3) Common Duckweed (*Spirodela polyrrhiza*)

The antimicrobial effect of these species has been widely tested out in countries like India and China[7-14]. However, very little research work on them in Bangladesh was found through extensive literature review[15]. Some information of aquatic weed species used for traditional medicinal practices in rural areas of Bangladesh was found from ethnomedicinal survey reports[16, 17]. As aquatic weeds differ widely in their chemical composition depending upon species, season and location, an insight into their chemical composition is essential for their proper utilization[13]. One of the objectives of this research was to identify the phytochemical constituents of these species collected from water

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bodies in Bangladesh. As these species grow in great abundance throughout water bodies here, attempts should be made to find some use out of them. This work focused on finding out the anti-microbial effects of the mentioned plant extracts against microorganisms that were not tested in previous studies in Bangladesh. Another significant observation about one of the species, *Eichornia crassipes*, is that in rural Bangladesh, the large leaves of this plant are used to cover up containers of milk. It is assumed that the leaves prevent the milk from spoiling. This study aimed to find out whether the plant leaves confer any anti-microbial activity against the microorganisms involved in milk spoilage.

Materials and Methods

- Plant materials: The selected plant species, Water lettuce (*Pistia stratiotes*), Water Hyacinth (*Eichhornia crassipes*), and Common Duckweed (*Spirodela polyrrhiza*) were collected from two different water bodies situated in Tikitpur village under Rohitpur thana, Munshigonj district in July, 2017.
- Sample preparation: Roots of the selected plants were separated, washed with tap water followed by distilled water to remove all debris and unwanted associated parts. The samples were then dried to constant weight. The dried specimens were then ground with a blender to fine powder[5].
- Extract preparation: *E. crassipes* samples were divided into leaves and stems. For the other two species, the stems were too small to be separated from the whole plant and as such, the extracts were prepared from the whole plant. Extracts from the dried leaves and stems of the samples were prepared in 95% ethanol and 95% ethyl acetate (20 g of each in 200 mL solvent)[18]. The extracts were finally concentrated using rotary evaporator at 42ºC. All steps are illustrated in figure 1.
- Stock solution preparation: Ethanol and Ethyl acetate extracts were dissolved in ethanol and DMSO respectively, at a concentration of 100 mg/mL. Extracts were diluted to 50mg/mL concentration. So, stock solutions with two different concentrations (100 mg/mL and 50 mg/mL) from each of the extracts were prepared. These were stored at -10ºC for further analysis.
- Phytochemical assays: Standard phytochemical assays (Wagner's test for alkaloids, Salkowaski's test for steroidal compounds, froth test for saponins, lead acetate test for tannins, test for flavonoids and phenolic compounds, Killer-Killani test for glycosides) were being carried out to determine the composition of the various extracts[19-21].
- Antimicrobial assay: Antimicrobial effect of the extracts were tested against *Staphylococcus aureus*, *Salmonella typhi* and *Lactobacillus* spp and compared with standard antibiotics (Imipenem, Chloramphenicol, Doxycycline and Ciprofloxacin). Here two types of tests- disk diffusion and well diffusion tests were done to confirm the results. The inhibitory effects of all ethanol and ethyl acetate extracts were calculated and compared by measuring the activity index. By using the following formula, the activity index was calculated:

Activity index (AI) = Zone of inhibition of extract/ Zone of inhibition of standard antibiotic.

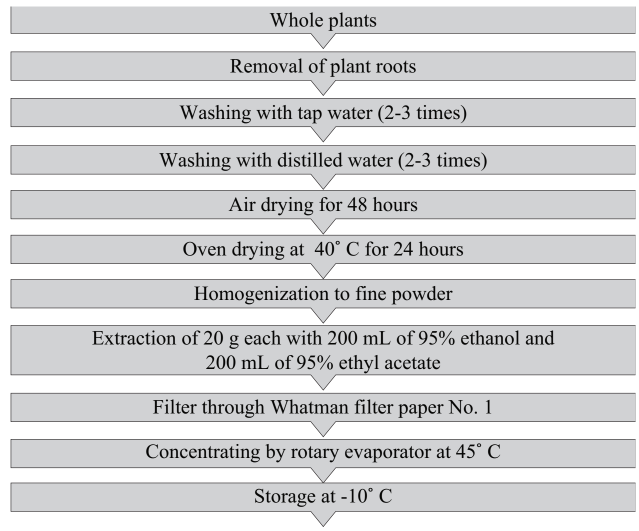

Figure 1. *Plant sample preparation and extraction*

Results

The weight of the final dried, powdered samples are listed below. The powdered samples were stored at 4ºC while the ethanol and ethyl acetate extracts were stored at -10ºC for further use.

Table 1. *Dry weight of samples obtained*

Plant species	Initial weight	Final dry weight
Water lettuce (*Pistia stratiotes*)	3.7 kg	152.48g
Water Hyacinth (*Eichhornia crassipes*)	3.5 kg (leaves), 7.5 kg (stems)	125 g (leaves), 137 g (stems)
Common Duckweed (*Spirodela polyrrhiza*)	2 kg	134 g

Phytochemicals assay results

The extracts were tested for various phytochemicals found which are usually found in plants. The crude extracts were tested for the presence or absence of alkaloids, steroidal compounds, phenolic compound, flavonoids, saponins, tannins, glycosides and cardiac glycosides. In the following table, summary of phytochemical test results are given. Here ethanol and DMSO worked as negative control as ethanol and ethyl acetate extracts were dissolved in ethanol and DMSO respectively.

Table 2. *Summary of phytochemical analysis results for the extracts*

Extract	Solvent	Alkaloids	Saponins	Steroidal Compounds	Flavonoids	Tannins	Phenolic Compounds	Glycosides	Cardiac Glycosides
ECL	EtOH	+	+	+	+	-	+	+	+
	Etac	+	-	+	+	-	+	+	+
ECS	EtOH	+	+	+	+	-	+	+	+
	Etac	-	-	+	+	-	+	+	+
PS	EtOH	+	+	+	-	-	+	+	+
	Etac	+	-	+	+	-	+	+	+
SP	EtOH	+	+	+	+	-	+	+	+
	Etac	+	-	+	+	-	+	+	+

Note: (+) = Presence of Phytochemicals; (-) = Absence of Phytochemicals; ECL = *Eichhornia crassipes* leaves; ECS = *Eichhornia crassipes* stems; PS = *Pistia stratiotes*; SP = *Spirodela polyrrhiza*; EtOH = Ethanol; DMSO = Dimethyl Sulfoxide;

Anti-microbial assay results

Anti-microbial efficacy of the eight extracts was tested against *Staphylococcus aureus*, *Salmonella typhi* and *Lactobacillus* spp. The results of the disk diffusion and well diffusion tests are summarized in the following table.

Table 3. *Summary of anti-microbial assay results*

Extract	**Solvent**	**Content**	**Disk Diffusion**						**Well Diffusion**						
			Zone of Inhibition (ZOI) (mm)			**Activity Index (AI)**			**Content**	**Zone of Inhibition (ZOI) (mm)**			**Activity Index (AI)**		
			Salmonella typhi	*Staphylococcus aureus*	*Lactobacillus*	*Salmonella typhi*	*Staphylococcus aureus*	*Lactobacillus*		*Salmonella typhi*	*Staphylococcus aureus*	*Lactobacillus*	*Salmonella typhi*	*Staphylococcus aureus*	*Lactobacillus*
ECL	EtOH	2mg	-	-	-	-	-	-	100 µl	-	-	-	-	-	-
		1mg	-	-	-	-	-	-							
	Etac	2mg	8.00 ± 0.5	-	-	0.24 ± 0.01	-	-	100 µl	18.00	-	-	0.65 ± 0.24	-	-
		1mg	-	-	-	-	-	-							
ECS	EtOH	2mg	-	-	-	-	-	-	100 µl	-	-	-	-	-	-
		1mg	-	-	-	-	-	-							
	Etac	2mg	7.83 ± .29	7.67 ± 0.29	-	0.23 ± 0.004	0.21 ± 0.004	-	100 µl	20.00	12.00	-	0.72 ± 0.27	0.37 ± 0.03	-
		1mg	-	-	-	-	-	-							
PS	EtOH	2mg	-	-	-	-	-	-	100 µl	-	-	-	-	-	-
		1mg	-	-	-	-	-	-							
	Etac	2mg	-	-	-	-	-	-	100 µl	-	-	-	-	-	-
		1mg	-	-	-	-	-	-							
SP	EtOH	2mg	-	-	-	-	-	-	100 µl	-	-	-	-	-	-
		1mg	-	-	-	-	-	-							
	Etac	2mg	-	8.17 ± 0.29	-	-	0.23 ± 0.004	-	100 µl	-	-	-	-	-	-
		1mg	-	-	-	-	-	-							

Note: (-) = No zone of inhibition; ECL = *Eichhornia crassipes* leaves; ECS = *Eichhornia crassipes* stems; PS = *Pistia stratiotes*; SP = *Spirodela polyrrhiza*; EtOH = Ethanol; Etac = Ethyl Acetate

Discussion

Phytochemical analysis showed the presence of alkaloids, flavonoids, steroids but no tannins in ethanol extracts of *Eichhornia crassipes* which is consistent with previous findings [19,22]. Similarly, alkaloids, flavonoids, steroids and glycosides but no tannins were found in ethanol extracts of *Spirodela polyrrhiza* which confirms previous findings[23]. Tannins were also absent from *Pistia stratiotes* extracts[24].

As for the anti-microbial efficacy test results, only the ethyl acetate extracts of *Eichornia crassipes* leaves and stems showed minor activity against *Salmonella typhi* (zones of inhibition in disk diffusion method, 8.00 ± 0.5 mm and 7.83 ± .29 mm respectively and in well diffusion method, 18.00 mm and 20.00 mm respectively). Ethyl acetate extracts of *Eichhornia crassipes* stems showed anti-microbial activity against *Staphylococcus aureus* (zone of inhibitions were 7.67±0.29 mm and 12.00 mm respectively in disk and well diffusion tests). Ethyl acetate extracts of *Spirodela polyrrhiza* showed antimicrobial activity against *Staphylococcus aureus* (zone of inhibition in disk diffusion method 8.17±0.29 mm).

Literature review shows that the extracts of the three aquatic plant species has anti-microbial activity against several species.

Methanolic extracts of *Eichornia crassipes* were shown to inhibit *Bacillus subtilis*, *Streptococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*[25]. Aqueous extracts of *E. crassipes* leaves showed antimicrobial activity against *Proteus vulgaris*, *Salmonella typhi*, and *Bordetella bronchiseptica*[26]. Ethanol and chloroform extracts of this plant showed significant activity against *Monascus ruber* and aqueous and ethanolextracts showed significant activity against *Aspergillus fumigates*[22]. Another study showed significant anti-microbial effect of the ethanolic, methanolic and aqueous extracts of *E. crassipes* leaves and roots extracts against *E. coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella cholerasuis*, *Shigella* sp., *Klebsiella pneumoniae*, *Serratia liquefaciens*, *Brenneria nitgrifluens*, *Bacillus subtilis*, *Bacillus cereus*, *Erwinia carotovora*, *Staphylococcus aureus*, *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium italicum*[27]. However, the ethanolic extract of *E. crassipes* did not show significant anti-microbial effect against *Staphylococcus aureus* in this study.

The ethanol extract of *S. polyrrhiza* showed broad spectrum antimicrobial activity against gram-negative strains of *Aeromonas hydrophila*, *Psedomonas putida*, *P. aeruginosa*, *P. fluorescens*, *Vibrio parahaemolyticus*, *V. alginolyticus*, *E. coli* and fungal pathogens, *Candida albicans* and *Saprolegnia parasitica*[23].

The ethanol and hot water fractions of *P. stratiotes* was reported to exert anti-microbial action on a few pathogenic bacteria while chloroform fraction of the same plant possess both anti-fungal and antibacterial activities on some pathogens[28,12,14].

In this study, only the ethyl acetate extracts showed anti-microbial potential. Further research is needed to identify the specific compounds responsible for anti-microbial activity in these extracts. None of the extracts showed any significant activity against *Lactobacillus* spp. Future works could include testing out the efficacy of these extracts against other pathogens and also testing their anti-oxidant properties.

Conclusion

Aquatic plant species are mostly considered as useless weeds. These species are collected and discarded which is a loss of resources. However, they can be of great use if useful phytochemicals were isolated from them. Research focusing on the native aquatic plants in Bangladesh will help in understanding their chemical constituents and potential future applications. The future objective of this work is to continue exploring the aquatic plant species of Bangladesh as sources of anti-microbial lead molecules.

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