Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(4):479-483

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Studies on the potential therapeutic effects on the aquatic macrophytes namely *Cabomba aquatica, Ceratophyllum demersum* and *Hygrophila corymbosa*

Malathy R. and Shaleesha A. Stanley*

Department of Biotechnology, Jeppiaar Engineering College, Chennai, Tamil Nadu, India

---

ABSTRACT

Aquatic plants maintain water clarity by preventing the re-suspension of bottom sediments. Certain water plants can absorb and break down polluting chemicals. The current study on three different aquatic plants Cabomba aquatica, Ceratophyllum demersum and Hygrophila corymbosa was proved to have antimicrobial activity. Crude extracts of plants and were prepared in acetone, butanol and methanol. The presence of antimicrobial activity by agar well diffusion method against one gram positive (Staphylococcus aureus), one gram negative (Escherichia coli) bacterial strain and one fungi (Aspergillus niger) was studied and qualitative phytochemical screening was performed. 100% butanol extracts of Hygrophila corymbosa showed highest antibacterial activity (12mm) against E.coli and 50% methanol extracts of Cabomba aquatica showed high antifungal activity (18mm) against A.niger. The phytochemical evaluation revealed that all three plant leaves showed presence of secondary metabolites like tannins, steroids, glycosides, flavonoids, phenolic compounds and alkaloids.

Key words: Aquatic plants, Antimicrobial activity, Phytochemical evaluation.

INTRODUCTION

The discovery of therapeutic medicinal products has historically been largely accidental, through trial and error involving observation of the therapeutic effects of “naturally” produced compounds such as penicillin from the mold *Penicillium notatum*.

Aquatic plants form one of the most productive ecosystems of the world and essential life supporting systems, providing a wide array of benefits to human kind. Due to intensive fish farming, medicines are needed to maintain animal health. Commercial antibiotics such as oxolinic acid and tertacycline were applied in fish feed against variety of bacterial pathogens of fish. This antibiotics spread pollution to the environment. As an alternative, aquatic plants can become the potent source for the allelopathic studies [4].

Seaweeds have been traditionally used in human and animal nutrition. Seaweeds are rich source of bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals. Important polysaccharides such as agar, alginates and carrageenans obtained from seaweeds are used in pharmaceutical as well as in the food industries [1]. The inhibitory substances biosynthesized by the seaweeds were noted as early as in 1917 [6]. The first observation regarding antibiotic activities of seaweeds was reported by Pratt et al., 1944. Recent

findings evidenced that seaweeds contained antibacterial [19], antiviral [3, 12], antifungal, cytotoxic [16] and larvicidal potentials [18].

Moreover, Su [14, 15] investigated the antimicrobial activity of the extracts of 24 macrophytes against several bacteria and fungi. The antimicrobial compounds were identified as tannic acid, gallic acid and ethyl gallate. Secondary metabolites in rooted floating leaved species are alkaloids, whereas submerged taxa contain both simple phenols and flavonoids [5]. Other investigations have implicated organic extracts and isolated hydrocarbons from free floating-leafed and submerged macrophyte species, which induce suppression of potential microbial colonizers [2].

As effective strategy of the present study, various organic solvents have been used to extract the possible active ingredients from aquatic plants, aiming at investigating the antimicrobial activity that the selected aquatic plants extracts may exert against the bacteria and fungi.

EXPERIMENTAL SECTION

Sample collection

Three aquatic plant species (*Cabomba aquatica, Ceratophyllum demersum, Hygrophila corymbosa)* were collected from Rettari lake, Tamil nadu, India. Collected samples were washed with seawater to remove epiphytes and other marine organisms. The plants were transported to the laboratory in sterile polythene bags.

Extraction of macrophytes

In the laboratory, samples were rinsed with tap water and were shade dried, cut into small pieces and powdered in a mixer grinder. Organic solvents (acetone, butanol and methanol) were used for extraction. Each powdered sample (1g) was soaked in about 50 ml of the solvent for 24hrs at room temperature in shaking condition . The filtrate dried at room temperature and transferred to eppendorf tubes [8].

Phytochemical analysis

The three (acetone, butanol and methanol) extracts of following plants was subjected to different chemical tests for the detection of different phytoconstituents using standard procedure [7, 13 and 17].

Test organisms

The antibacterial activity was carried out by using a gram negative bacteria *(Escherichia coli)* and gram positive bacteria *(Staphylococcus aureus).*The antifungal activity was carried out by *Aspergillus niger.* The pure bacterial and fungal cultures were inoculated in nutrient broth and incubated at 37º C for 2-8 h till the turbidity developed.

Determination of Antimicrobial assay

The antimicrobial activity was carried out by using agar well diffusion method. The solvents like acetone, butanol and methanol were used to collect the plant extracts and were tested against the microbes with 50% and 100% of extracts [11].

Antibacterial assay

Overnight grown bacterial culture was transferred to sterile Petri plate with Mueller Hinton agar medium and was spread with sterile spreader to create a lawn. About 3 wells of 6mm diameter were made in each plate with the help of a sterile cork borer. Among the three, two wells were placed with the 25µL of 50% and 100% of the extracts using sterile pipettes and remaining one well was mentioned as control. The Petri dishes were prepared and incubated for 18-24hrs at 37°C and the zone of inhibition around the well was measured in nearest millimeter. Each experimental result was determined by the average of triplicates.

Antifungal assay

Young fungal cultures were incubated for 2-3 days at room temperature and seeded on Sabouraud Dextrose Agar plates (SDA) for bioassay by agar well diffusion method. Wells (6mm diameter) were punched using sterile cork borer. 25µL of each extract inserted into the wells and incubated at 37°C for 3 days the plates were observed for the presence of inhibition zones. Gentamycin (25µL) and 1% Dimethyl sulfoxide (DMSO) was used as positive control and negative control respectively. Bioassay was performed twice for better result.

RESULTS AND DISCUSSION

The present study carried out on the aquatic plant sample revealed the presence of medicinally active constituents. The phytochemical constituents of the selected plants investigated are summarized in (Table 1). The plant extracts have been analyzed and reported the presence of flavonoids, glycosides, phenols, saponins, and tannins of selected plants, which could be responsible for the observed antimicrobial property.

Table 1. Phytochemical constituents present in different extracts of *C.aquatica, C.demersum* and *H.corymbosa*

Aquatic plants	***Cabomba aquatica***			***Ceratophyllum demersum***			***Hygrophila corymbosa***		
Extracts	**Acetone**	**Butanol**	**Methanol**	**Acetone**	**Butanol**	**Methanol**	**Acetone**	**Butanol**	**Methanol**
Alkaloids	+	+	+	+	_	+	+	_	+
Cardiac glycosides	+	+	+	+	+	+	_	+	_
Flavanoids	_	_	_	_	+	_	_	_	_
Tannins	+	+	+	_	_	_	_	_	+
Phenols	_	_	_	_	_	_	_	_	+
Proteins	_	_	_	_	_	_	_	_	_
Saponins	_	_	_	_	_	_	_	_	_
Terpenoids	+	_	+	_	_	_	+	_	+
Quinone	_	_	_	_	_	_	_	+	+
Steroids	_	+	_	_	_	_	+	+	+
Glycosides	_	_	+	+	+	+	_	_	_

The antimicrobial activity of the selected Macrophytes (*Cabomba aquatica, Ceratophyllum demersum, Hygrophila corymbosa)* were extracted with three different solvents like acetone, butanol and methanol and determined the activity against two pathogenic organisms both Gram positive *(Staphylococcus aureus)* and Gram negative *(Escherichia coli)* bacteria and fungi *(Aspergillus niger)* by agar well diffusion method were tabulated in the Table 2.

Table 2. Inhibition zones of the tested microbes caused by the different aquatic plant extracts

Aquatic plants	**Extractant**	**Bacteria**		**Fungi**
		E.coli	***S.aureus***	***A.niger***
Negative control	DMSO	-	-	-
Cabomba aquatica	Acetone 50%	-	-	-
	Acetone 100%	-	-	22mm
	Butanol 50%	-	-	-
	Butanol 100%	-	-	-
	Methanol 50%	-	-	28mm
	Methanol 100%	-	-	-
	Gentamycin	22mm	22mm	
	Penicillin			32mm
Ceratophyllum demersum	Acetone 50%	-	-	18mm
	Acetone 100%	-	-	-
	Butanol 50%	-	-	-
	Butanol 100%	-	-	-
	Methanol 50%	-	-	-
	Methanol 100%	-	-	-
	Gentamycin	22mm	22mm	
	Penicillin			32mm
Hygrophila corymbosa	Acetone 50%	-	-	-
	Acetone 100%	-	-	-
	Butanol 50%	10mm	-	-
	Butanol 100%	12mm	-	18mm
	Methanol 50%	-	-	-
	Methanol 100%	-	-	-
	Gentamycin	22mm	22mm	
	Penicillin			32mm

The highest activity was seen in 100% butanol extract (12mm) of *H.corymbosa* against *Escherichia coli*. but the same time there was no activity recorded for all three plant extracts against *Staphylococcus aureus* and antifungal property was found in all three plants i.e., 50% methanol extract (28mm) and 100% acetone extract(22mm) of

C.aquatica, 50% acetone extract (18mm) of *C.demersum* and 100% butanol extract (18mm) of *H.corymbosa* against *Aspergillus niger.*

Today seaweeds are the raw materials for many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries [9]. The common major compounds like alkaloids, steroids, glycosides and cardiac glycosides can be extracted using polar solvents such as acetone, butanol and methanol during Phytochemical process. These results were compared with the recently reported qualitative phytochemical tests which were used to detect the presence of alkaloids, tannins, saponins, flavonoids, glycosides and phenols from the aquatic plants.

Marine genus synthesizes active constituents which are used in traditional and complementary medicine. Different varieties of marine algae were reported to contain active ingredients that can cure diseases. Nowadays, higher percentage of population prefer to use remedies of natural origin for curing illness as these claimed to produce less side effects [20]. The present study was focused on three different extract of *C.aquatica, C.demersum* and *H.corymbosa* for the presence of phytochemical substances and antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi.

Alkaloids are commonly found to have antimicrobial properties [10]. The preliminary Phytochemical screening of three macrophytes indicates the presence of chemical constituents playing an indispensable role in antibacterial activity. The findings of this study also pave the way for further research to identify the specific active compounds that are responsible for its claimed antibacterial activity.

CONCLUSION

The study confirms that all three plant extracts used in this investigation possess antibacterial and antifungal activity against the used organisms. In addition, the efficiency of the extracts varied with solvent used in the extraction. Comparing the effects of plant extracts on three different organisms, it is evident that all three plants showed response against *A.niger* followed by *E.coli* and *S.aureus*. The nature and number of these active components are not clear, however it still worthwhile to take into account the use of these bioactive materials as antimicrobial agents. Hence these compounds should be investigated for natural antimicrobial agents and more studies should be carried out to purify and identify these compounds.

Acknowledgement
The authors gratefully acknowledge Jeppiaar Engineering College, Chennai for providing with the laboratory infrastructure to carry out the study. We are also immensely thankful to our friends and family for their limitless support.

REFERENCES

[1] A Bocanegra; Sara Bastida; Juana Benedi; Sofia Rodenas; J Francisco Sanchez–Muniz. *J.Med food*., **2009,** 12 (2): 236-258.
[2] SD Elakovich; JW Wooten. *J. Aquat. Plant Manage.*, **1989** 27: 78-84.
[3] HS Garg; T Sharma; DS Bhakuni; BN Pramanik; AK Bose. *Tetrahedron Letters*., **1992,** 33: 1641-1644.
[4] MG Ghobrial; S Omar; T Adnan. *Egyptian Journal of Natural Toxins*., **2007,** 4(2): 12-25.
[5] B Gopal; U Goel. *Bot. Rev.*, **1993,** 59: 155–210.
[6] JB Harborne. Phytochemical Methods. *Chapman and Hall Ltd., London.*, **1973,** 49-188.
[7] R Harder; A Oppermann. *Arch. Microbiol.*, **1953,** 19: 98-401.
[8] B Manju; L Shrine Deepthi; A Shaleesha Stanley. *Adv Bio Tech.*, **2012,** 12(06):30-32.
[9] VK Mishra; F Temelli; PF Ooraikul Shacklock; JS Craigie. *Botanica Marina.*, **1993,** 36(2): 169-174.
[10] E Omulokoli; B Khan; SC Chobra. *Ethnopharmacology*., **1997,** 56: 133-137.
[11] R Seenivasan; M Rekha; H Indu; S Geetha. *J. of Applied Pharmaceutical Science* **2012,** 2 (10): 159-169.
[12] J Serkedjieva. *Phytotherapy Research*., **2004,** 18: 480-483.
[13] A Sofowara. Medicinal plants and Traditional medicine in Africa. *Spectrum Books Ltd, Ibadan, Nigeria*., **1993,** 191-289.
[14] KL Su; Y Abul-Hajj; EJ Staba. *Lloydia.*, **1973,** 36: 80-87.

[15] KL Su; Y Abul-Hajj; EJ Staba. *Lloydia.*, **1973,** 35: 72-79.
[16] HF Tang; YH Yi; XS Yao; QZ Xu; SY Zhang; HW Lin. *J. of Asian Natural Product Research*., **2002,** 4: 95-105.
[17] GE Trease; WC Evans. *Pharmacognosy*. Bailliere Tindall, London, Edn 11, **1989,** 45-50.
[18] TS Thangam; K Kathiresan. *Bot. Mar.*, **1991,** 34: 537-539.
[19] I Tuney; BH Cadirci; D Unal; A Sukatar. *Turkish J. of Biol*., **2006,** 30: 171-175.
[20] N Tyagi; A Bohra . *Biochem. Cell. Arch*., **2002,** 2: 21-24.