www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Antibacterial screening and qualitative phytochemical estimation of selected aquatic plants

Minal Jani*[1], Saumiya Shah[1] and Sujit Prajapati[2]

[1]Anand Mercantile College of Science, Management and Computer Technology, Anand- 388120, Gujarat, India
[2]B. R. Doshi School of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

ABSTRACT

Ignoring little economic importance aquatic plants are considered as menace. Present investigation has been carried out on five different aquatic plants *Hygrophila auriculata, Polygonum glabrum, Lemna gibba, Ludwigia adscendens* and *Najas marina* for their probable potent antibacterial activity as well as active phytochemical components. Plants were collected from Pariyej and Kanewal lakes located surrounding Anand district, Gujarat, India. Crude extracts of all five plants and their different parts were prepared in water, hexane, ethyl acetate and methanol respectively. These extracts were assayed for the presence of antibacterial activity by agar well diffusion method against three Gram positive (*Bacillus subtilis, Bacillus cereus* and *Staphylococcus aureus*) and one Gram negative (*Proteus vulgaris*) bacterial strains. Furthermore the minimum inhibitory concentration (MIC) and qualitative phytochemical testing has been done for actively reported plants. Five crude extracts prepared in methanol showed best response. Out of 36 different crude extracts of above five plants and their parts in different solvents 15 extracts showed positive antibacterial activity. The phytochemical evaluation revealed that *Hygrophila auriculata* stem and leaf showed presence of alkaloids where as *polygonum glabrum* leaves showed presence of phenolic compounds, tannins and alkaloids.

Keywords: Aquatic plants; antimicrobial; tannins; phenolic compounds; alkaloids

INTRODUCTION

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. Ethnobotanical studies pertains to the aquatic macrophytes of Gujarat state reveals that a total of 112 species under 90 generas, belonging to 47 families are used for the medicinal purpose by the aboriginals (Patel, 2009). Thus it is strongly require to put efforts towards unearthing the knowledge in this regards.

Apart from this, aquaculture is also a rapidly expanding sector of the global economy with an average growth rate of 8.8 percent per annum since 1970 (Arthur et al., 2009). Due to intensive fish farming, medicines are needed to maintain animal health. Commercial antibiotics such as oxilinic acid and tertacycline were applied in fish fed against a variety of bacterrial pathogens of fish. This antibiotics spread pollution to the environment. As an alternative, aquatic plants can become the potent source for the allelopathic studies (Ghobrial et al., 2007).

In the present study five different aquatic plants and their parts were used for the screening of their antimicrobial activities against three Gram positive and one Gram negative bacterial strains. Plants showed positive activities were further qualitatively estimated for their phytochemical constituents i.e. tannins, alkaloids, saponins, cardiac glycosides, flavonoides, terpenoides and phenolics.

MATERIAL AND METHODS

Plants collection

Five plant species; *Ludwigia adscendens* and *Polygonum glabrum* from Mahisagar River, Vasad; *Najas marina* from Valli Kanewal Lake near Tarapur, and *Hygrophila auriculata* and *Lemna gibba* from roadside, Anand-Tarapur highway were collected during January 2011. The healthy and disease free collected plants were tested for the antibacterial activity.

Preparation of plant extracts

Plants and plant parts were thoroughly washed with running tap water, blotted, air dried and powdered by grinder (Maharaja Mixer Ltd). Ten grams of each plant powdered material was soaked in 50 ml of distilled water for 24 hours at room temperature under shaking condition (130-140 rpm). The extracts were filtered with the help of eight layers of muslin cloth. Each plant

* Corresponding Author
Email: nidhuminu@yahoo.co.in
Contact: +91-7567213060
Received on: 18-09-2011
Revised on: 30-09-2011
Accepted on: 03-10-2011

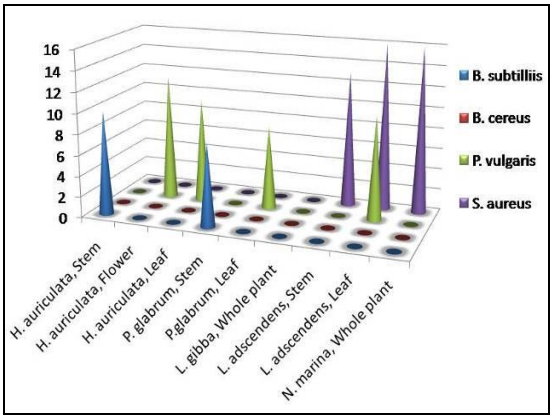

Figure 1: Antibacterial activity of methanol extracts

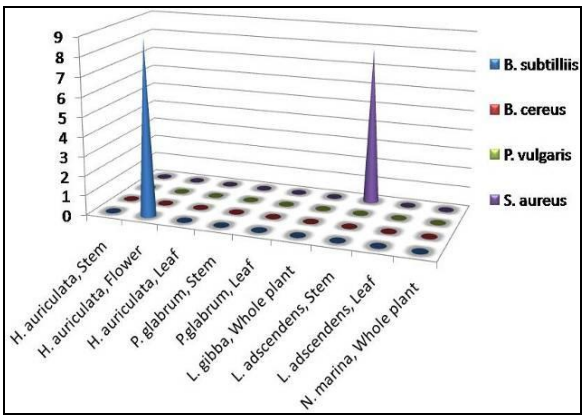

Figure 2: Antibacterial activity of hexane extracts

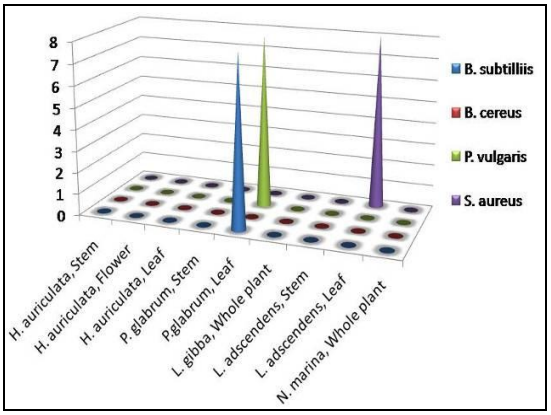

Figure 3: Antibacterial activity of ethyl acetate extracts

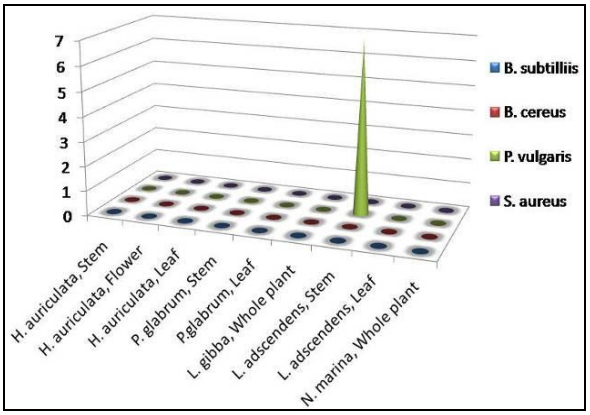

Figure 4: Antibacterial activity of Aqueous extracts

filtrate was dried at room temperature and transferred to eppendorf tubes. Each residual material from the funnel was dried again and resuspended in 50 ml hexane and the above steps were repeated. Similarly, the residual materials were re-extracted with same volumes (50 ml) of ethyl acetate and methanol respectively.

Antimicrobial activity testing of plant extracts

In the present study, 36 extracts of the examined different plant parts were used. Their antibacterial activity was studied by the agar well diffusion method (Perez et al., 1990). From the stock, 100 mg of each plant extract were suspended in one milliliter of Dimethyl sulfoxide (DMSO). Three Gram positive (B*acillus subtilis, Bacillus cereus* and *Staphylococcus aureus*) and one Gram negative bacteria (*Proteus vulgaris*) were selected for the screening. A fresh bacterial culture of each of the tested pathogens (100 µl having 10^8 CFU/ml) was added to 10ml of soft agar and poured on agar plates and allowed to solidify. A well of 8mm diameter punched off with sterile cup borer and then it was filled with 100 µl of the respective plant parts extract. Plates were placed for 30 minutes in refrigerator for diffusion of extracts and then incubated at 37°C for 24 hours. Zone of inhibition (excluding well diameter) appeared was measured as a property of antibacterial activity.

Antibiotics; ampicillin, streptomycin and chloramphenicol at a concentration of 100 µg/ml and 100% DMSO were used as positive and negative controls, respectively. The assay was performed in duplicate and repeated twice for better results.

Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration was evaluated by the two fold serial broth dilution method. Plant extracts showing inhibition zone were selected for MIC. Selective broth medium was used for dilutions as well as preparing inoculums. The bacterial cell density was maintained uniformly throughout the experimentation at $1X10^8$ CFU/ml by comparing with 0.5 Mc Farland turbidity standards. DMSO and the used selective medium were included as controls and each assay was repeated thrice.

Phytochemical analysis

Qualitative phytochemical analysis of plant extracts was carried out for tannins, alkaloids, saponins, cardiac glycosides, flavonoides, terpenoides and phenolic compounds as described by Raaman, 2006.

RESULTS

Results illustrated that the aqueous extracts of the tested five plants didn't display antibacterial activity except that of the stem of *L. adscendens* which showed 7 mm zone of inhibition against *P. vulgaris* (Fig. 4).

Table 1: Values of minimum inhibitory concentration (MIC) of plant extracts against *B. subtilis*

Plant name	Plant part used	Extract	MIC value (mg/ml)
H. auriculata	Stem	Methanol	0.5
	Flower	Hexane	0.5
P. glabrum	Stem	Methanol	1
	Leaf	Ethyl acetate	1

Table 2: Values of minimum inhibitory concentration (MIC) of plant extracts against *P. vulgaris*

Plant name	Plant part used	Extract	MIC value (mg/ml)
H. auriculata	Flower	Methanol	0.031
	Leaf	Methanol	0.25
P. glabrum	Leaf	Ethyl acetate	1
		Methanol	0.5
L. adscendens	Stem	Aqueous	2
	Leaf	Methanol	0.5

Table 3: Values of minimum inhibitory concentration (MIC) of plant extracts against *S. aureus*

Plant name	Plant part used	Extract	MIC value (mg/ml)
L. adscendens	Stem	Hexane	0.5
		Methanol	0.031
	Leaf	Ethyl acetate	1
		Methanol	0.031
N. marina	Whole plant	Methanol	0.031

Table 4: Phytochemical constituents of crude solvent extracts of selected plants

Plant Name	Part used	Extract	Tannins	Saponins	Cardiac Glycosides	flavonoides	Terpenoids	Phenolic Compounds	Alkaloids
H. auriculata	Stem	Methanol	-	-	-	-	-	-	+
	Flower	Ethyl acetate	-	-	-	-	-	-	+
P. glabrum	Leaf	Aqueous	+	-	-	-	-	+	+

Absent = (-) Present = (+)

Hexane extract of *H. auriculata* flower and *L. adscendens* stem showed inhibition against *B. subtilliis* (8) and *S. aureus* (8) respectively (Fig. 2). Ethyl acetate extracts of *P. glabrum* and *L. adscendens* leaves showed 8 mm zone of inhibition against *B. subtilliis* and & *P. vulgaris* and *S. aureus*, respectively (Fig. 3). Methanol extract of *H. auriculata* and *P. glabrum* stems showed 10 and 8mm zone of inhibition against *B. subtilliis.* Methanol extract of *hygrophilla auriculata* flower and leaf*, polygonum glabrum* leaf and *Ludwigia adscendens* leaf showed 12, 10, 8 and 10 mm zone of inhibition *against P. vulgaris,* respectively. Also, methanol extract of stem and leaf of *Ludwigia adscendens* showed 13 and 16 mm zone of inhibiton while *Najas marina* whole plants showed 16 mm zone of inhibition against *S. aureus* (Fig. 1). No extract of any plant showed inhibition against *Bacillus cereus.* Out of the four crude extracts of five studied plants, methanol extract showed the best response. The plant *L. gibba* didn't show any activity against all the tested bacteria in all four extract.

The minimum inhibitory concentration has been calculated for the positively responded plants and results showed that methanol extracts of *Ludwigia adscendens* and *Najas marina* had the lowest MIC values against *S. aureus* (0.031 mg/ml). MICs of other plant extracts are shown in (Tables 1-3).

Qualitative phytochemical estimations were conducted for 15 extracts that showed positive response. Out of those 15 only 3 plant extracts showed (Table 4) positive responses to alkaloids, Tannins and phenolic compounds.

DISCUSSION

Generally, plant extracts are usually more active against Gram positive bacteria than Gram negative bacteria (Basri and Fan, 2005). It was also found in the present investigation, where, out of 15 positive responded extracts only 3 extract showed positive response against *P. vulgaris,* gram negative bacteria.

Many studies revealed that plants methanol extracts inhibited the growth of tested bacteria more than plants aqueous extracts. *Juniperus oxycedrus* aqueous extract showed no effect to 56 species of bacteria; however methanol extracts of *J. oxycedrus* inhibited the growth of 24 out of 56 bacteria (Karaman et al., 2003). The study of Abu-Shanab et al., 2005 also concluded that the methanol extract of the plants shows best results. The present investigation also supports the above observations.

Nine out of 15 positively responded extracts were of methanol. Ahmed et al., 2005 reported that the methanolic extract of *L. adscendens* showed a broad spectrum of antibacterial activity against all the bacteria tested except *Staphylococcus aureus*. Irrespective to this conclusion, our results displayed that methanolic extract of *L. adscendens* showed a good response against *Staphylococcus aureus* i.e. 0.031 MIC value.

Little work has been done on the antibacterial activity of *N. marina,* however allelopathic activity has been reported by Gross et al., 2003 and Hilta and Grossb, 2008. Here it was shown that N. marina had active ingredients and showed activity against *S. aureus*.

Similarly little work has been done on the *P. glabrum* although phytochemical analysis of *P. glabrum* has revealed four sesquiterpenes (Jacobsson and Muddathir, 1992). Methanolic extracts of *P. glabrum* stem and leaf showed activity against *B. subtilis* and *P. vulgaris*, respectively and phytochemical estimation of *P. glabrum* in present work revealed the presence of tannins, phenolic compounds and alkaloids.

Boily and vampuyvelde, 1986 and Valientic et al., 1995 examined the antimicrobial activity of ethanolic extracts of the leaves, stem, fruits and root of *H. auriculata* against bacterial and fungal growth and reported that the leaves exhibited active anti-microbial activity against *Staphylococcus aureus*, *Candida albicans*, *Mycobacterium canis* and *Trichophyton mentagraphytes*, while the stem exhibited activity against *Candida albicans*, *Mycobacterium canis* and *Trichophyton mentagraphytes.* It was reported that the chloroform and alcoholic extracts of *Hygrophila spinosa* exhibited significant antibacterial activity, whereas the aqueous extract of the same has moderate activity and the petroleum ether extract had the weakest activity against *Escherchia coli, Staphylococcus aureus, Bacilus subtilis* and *Pseudomonas aeruginosa* (Patra et al., 2008). In the present conducted study *H. auriculata* stem, leaf & flower methanol extracts showed positive activity against *B. subtilis* and *P. vulgaris*, respectively.

CONCLUSION

Out of 5 different plants *Hygrophila auriculata*, *Polygonum glabrum* and *Lemna gibba* showed good antimicrobial activity against all four bacteria. Methanolic and ethyl acetate extract of *Hygrophila auriculata* stem and flower, respectively showed presence of alkaloid. Aqueous extract of *Polygonum glabrum* leaf showed presence of tannins, phenolic compound and alkaloids.

ACKNOWLEDGEMENT

Authors are thankful to management of AMCOST for providing lab facility for the present study.

REFERENCES

Abu-Shanab B, Adwan G, Abu Safiya D, Adwan K and Abu-Shanab M: Antibacterial activity of Rhus coriaria L. extracts growing in Palestine, J. Islam. Univ. Gaza, Vol. 13, No. 2, 2005, pp. 147-153.

Ahmed F., Selim M.S.T. and Shilpi J.A.: Antibacterial activity of Ludwigia adscendens, Fitoterapia, Vol. 76, No. 5, 2005, pp. 473-475

Arthur, J.R., Bondad-Reantaso, M.G., Campbell, M.L., Hewitt, C.L., Phillips, M.J. and Subasinghe, R.P. Understanding and applying risk analysis in aquaculture: a manual for decision-makers. FAO Fisheries and Aquaculture Technical Paper; No. 519/1. Rome, FAO, 2009

Basri, D.F. and Fan, S.H.: The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents, Indian J. Pharmacol., Vol. 37, No. 1, 2005, pp. 26-29.

Boily Y and Vampuyvelde L.: Screening of medicinal Plant of Rawanda for Antimicrobial activity, J. Ethanopharmacol, Vol.16, No. 1, 1986, pp. 1-13.

Ghobrial, M.G., S. Omar and T. Adnan: Potential for finding new bioactive agents from selected aquatic plant extracts against enteric bacteria, Egyptian journal of Natural Toxins, Vol. 4, No. 2, 2007, pp. 12-25.

Gross E. M., D. Erhard and E. Ivanyi: Allelopathic activity of Ceratophyllum demersum L. and Najas marina ssp. Intermedia (Wolfgang) Casper, Hydrobiologia, 2003, 506–509: 583–589.

Hilta S. and E. M. Grossb: Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes?, Basic and Applied Ecology, Vol. 9, No. 4, 2008, pp. 422-432

Jacobsson, U. and A. Muddathir: Four biologically active sesquiterpenes of the drimane type isolated from Polygonum glabrum, Phytochemistry., Vol. 31, 1992, 4207-4211.

Karaman I, F Sahin, M Gulluce, H Ogutcu, M Sengul and A Adiguzel: Antimicribial activity of aqueous and methanol extracts of Juniperus oxycedrus, L.J. Ethnopharmacol., Vol. 83, 2003, 231-235.

Patel, A., Aquatic angiosperms of Gujarat, M.Sc. dissertation thesis, Sardar Patel Universiry, Vallabh Vidyanagar, India, 2009

Patra A., S. Jha, N. Murthy and A. Vaibhav: Anthelmintic and antibacterial activities of Hygrophila spinosa

T. Anders, Research J. Pharm. and Tech., Vol. 1, No. 4, 2008, 531-533

Perez, C., M., Paul, and P., Bazerque: An Antibiotic assay by the agar well diffusion method, Acta. Bio. Med. Exp., Vol. 15, 1990, 113-115

Raaman, N. Phytochemical Techniques, New Delhi: 2006, New India Pub Agency

Valientic, A. J., L. vanhoof, J. Totte, A. Lasure, B. Vanden, D. Rwangaboo and P.C. Mvukiyuwami: Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties, J. Ethanopharmacol., Vol. 46, No. 1, 1995, 31-47.