JOURNAL OF ALGAL BIOMASS UTILIZATION
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J. Algal Biomass Utln. 2010, **1** (2): 33-41

Preliminary Phycochemical Analysis and *In Vitro* Antibacterial Screening of *Pithophora Oedogonia* (Mont.) Wittrock- A Freshwater Green Alga Forming Mats in the Water Bodies

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ABSTRACT

Antibacterial activity of the methanolic extract of dried up filamentous green alga *Pithophora oedogonia* (Mont.) Wittrock was assayed against two gram +ve (*Bacillus subtilis* and *Staphyllococcus areus*) and three gram –ve (*Escherichia coli*, *Helicobacter pylori* and *Salmonella typhae*) bacteria under culture conditions, using the agar disc diffusion technique. Incubation of the Mullar–Hinton agar plates for 24h at 30^0C, supplemented with the five test bacteria along with 25µl methanolic extract revealed strong inhibitory effect, showing highest inhibition zone (18.1 mm) in case of gram +ve bacteria. Preliminary phycochemical analysis was also performed on the powdered algal sample employing chemical methods and TLC to assay the bioactive compounds, which revealed the presence of six principal bioactive compounds- phenolic, saponin, tannin, amino acid, steroid and flavonoid.

Key Words: *Pithophora oedogonia*, Algal extract, Phycochemicals, Antibacterial activity

INTRODUCTION

The aquatic plants are rich source of structurally novel and biologically active metabolites. The metabolites (primary and /or secondary) produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry (Kusumoto *et. al.*, 1995). The aquatic plants are known to produce certain bioactive molecules which interact with other organism in the environment, inhibiting bacterial or fungal

growth (antibiotic activity) or modulating the development of other organisms which are found in the vicinity of that plant (allelopathic activity). In the field of research involving bioactive substances of plant origin, a greater interest has now arisen in algae. The first investigation on antibiotic activity of alga was carried out by (Pratt *et. al.,* 1944). Several products of algal origin such as alginate, carrragenean and agar as phycocolliods have been used for decades in medicine and pharmacy. Since algae have been used in traditional medicine for a long time (Fitton, 2006), and also some algal substances have bacteriostatic and bactericidal activity, they have been extensively studied by several researchers (Burkholder *et. al.,* 1960; Ehresmann *et. al*., 1977; Moreau *et. al*., 1984; Reichelt and Borowitzka, 1984; Hornsey and Hide, 1985; Vlachos *et. al*., 1999; Gonzalez del Val *et. al*., 2001; Nora *et. al*., 2003; Freile-Pelegrin and Morales, 2004; Salvador *et. al*., 2007). Many authors had found antibacterial activities of microalgae due to fatty acids (Cooper *et. al*., 1983; Findlay and Patil, 1984; Viso *et. al*., 1987). Among several such algae, *Pithophora oedogonia* a green filamentous fresh water alga which forms mat and creates nuisance in the water bodies has been shown to have antibacterial activity in *in vitro* condition against gram-positive and gram-negative bacteria. This study aimed at investigations of the phycochemicals and antibacterial properties of the methanolic extract from fresh water green alga *Pithophora oedogonia* against five bacterial isolates in order to validate it as an antimicrobial remedy. This study will also hopefully expose new frontiers on the current applications of the plant extract. Selection of crude plant extracts for screening programs has the potential of being more successful in the initial steps than the screening of pure compounds isolated from natural products.

MATERIAL AND METHODS

Collection of plant material

The material was collected from aquatic bodies situated inside the Banaras Hindu University campus and was identified as *Pithophora oedogonia* (Mont.) Wittrock using research papers and monographs/ books.

Preparation of algal extract

The algal material collected from nature was washed under running tap water to remove epiphytes and associated debris and dried at 40^0 C in an oven for 4-5 days. Crushed the dried up algal material with the help of pestle and mortar and kept it in 90% methanol for 7-8 days. At the end of extraction, extract was filtered through Whatman no.1 filter paper to remove all unextractable matter, including cellular materials and other constituents that

are insoluble in the extraction solvent. The filtrate was concentrated under reduced pressure by using a rotatory evaporator. The methanolic extract was transferred to a hot air oven where it was dried to a constant weight at 45^0C. Portion of the residue was used for phyco-constituents' analysis, while the rest was used for the bacterial susceptibility test.

Phycochemical analysis

Phycochemcial analysis of the extract was carried out using chemical methods and confirmation was done by TLC according to the methodology proposed by Indian Pharmacopeia (1985) and Harborne (1998). By this analysis, the presence of several phycochemicals like amino acids, phenolics, alkaloids, flavonoids, tannins, saponins, steroids, sugars, glycosides etc. were tested. The results are presented in Table 1.

Test organisms

The extract was tested on the two Gram positive (*Bacillus subtilis* and *Staphyllococcus areus*) and three Gram negative bacteria (*Escherichia coli*, *Helicobacter pylori* and *Salmonella typhae*). All the strains were procured from the Laboratory of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Bacterial Media (Muller Hinton Media)

35g of Muller Hinton Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15 lb pressure for 15 minutes. The sterilized media were poured into petri dishes.

Bacterial susceptibility testing

The sensitivity testing of the methanolic extract was determined using agar disc diffusion method (Bauer *et. al*., 1966). The strains of bacteria obtained were inoculated in conical flasks containing 100 ml of nutrient broth. These conical flasks were incubated at 30^0 C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar, poured in Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs had been impregnated with 25 µl of *Pithophora* extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight under the conditions mentioned above. After incubation, the clear zones around the discs were measured and expressed in millimeter as a measure of their antibacterial activity.

RESULTS AND DISCUSSION

The preliminary phycochemical analysis of the *Pithophora oedogonia* (Mont.) Wittrock extract revealed the presence of amino acids, sugar, protein, phenolics,

steroids, saponins, and tannins presented in Table 1.

Table 1. Preliminary phytochemical analysis of methanolic extract the green alga *Pithophora oedogonia* (Mont.) Wittrock

S. No.	Constituents	Methanol extract
1	**Alkaloids**	
	(i) Dragendorff's test	-
	(ii) Meyer's test	-
2	**Carbohydrates**	
	(i) Mohch's test	+ +
	(ii) Fehling (reducing sugar) test	+
	(iii) Fehling (combined reducing sugar) test	+ +
3	**Cardiac glycosides**	
	(i) Killer-killanis test	-
	Flavonoids	
4	(i) Shinoda's test	+
	(ii) $FeCl_3$ test	-
5	**Saponins**	
	(i)Frothing test	+ +
6	**Terpenes and steroids**	
	(i) Salkowski test	+ +

	(ii) Libarman-Burchard's test	+
7	**Phenolics /Tannins**	
	(i) $FeCl_3$ test	+ +
	(ii) Lead acetate test	+
8	**Amino acids**	
	(i) Ninhydrine test	+ +
9	**Protein**	
	(i) Biurette test	+ +

Key: - = Negative (absent); + = Positive (slightly present); ++ = Positive (moderately present)

The test for alkaloid and glycosides, however, showed negative result. Determination of disc diffusion assay showed that the methanolic extract of *Pithophora oedogonia* tested exhibited an antibacterial effect against pathogenic as well as non-pathogenic test bacteria. Significant effect on growth inhibition of Gram positive and Gram negative bacteria was also noticed. It was noted that among all the tested organisms, the Gram-positive bacterial strain, *Bacillus subtilis,* registered maximum susceptibility to the *Pithophora* extract, showing 18.1 mm inhibition zone and the Gram-negative strain, *Helicobacter pylori* proved least susceptible as indicated by 12.64 mm inhibition zone. These differences may be attributed to the fact that while the cell wall in Gram-positive bacteria consists of a single layer that of Gram-negative is a multi-layered and quite complex structure (Yao and Moellering, 1995). Of the information available on antimicrobial activity of algae all world over, marine algae seem to dominate the scene for their antimicrobial activity. Reichelt and Borowitzka, (1984) and Salvador *et. al*. (2007) screened the antimicrobial activity of 82 marine algae as fresh and lyophilized forms. Hornsey and Hide (1974) tested 151 species of British marine algae and reported that although antibacterial activity was more evident in some taxonomic groups, it also varied seasonally. It has also been indicated that the antibacterial activity is due to different chemical agents present in the

extract, including flavonoids and triterpenoids and other compounds of phenolic nature or free hydroxyl group, classified as active antimicrobial compounds (Rojas *et. al*., 1992). The antibacterial activity of both pathogenic and non-pathogenic representing Gram +ve and Gram –ve nature of the test bacteria as assayed in the present study in terms of zone of inhibition as a measure of efficiency is presented in Table 2.

Table 2. Antibacterial activity of methanolic extract (25µl) of *Pithophora oedogonia* (Mont.) Wittrock on five bacterial strain of varied nature

Sl. No.	**Bacterial strains used**	**Zone of Inhibition (mm)**	**Remark**
1.	*Bacillus subtilis*	18.1±0.44	Gram +ve
2.	*Staphylococcus areus*	17.10±0.54	Gram +ve
3.	*Escherichia coli*	15.80±0.53	Gram -ve
4.	*Salmonella typhimurium*	12.64±0.42	Gram -ve
5.	*Helicobacter pylori*	15.67±0.52	Gram -ve

*All the values are mean standard deviation of three determinations.

CONCLUSIONS

Herbal drugs are gaining momentum at a very fast pace all world over for the cure of various human ailments due to ready availability of the plant resources, their cheapness and most importantly side effectlessness of these remedies.

Antibiotics provide the main basis for the therapy of bacterial infections from which humans often suffer. High genetic variability of bacteria, however, enables them to rapidly evade the action of antibiotics by developing antibiotic resistance in them. Thus, there is a need of continuing search for new and more potent antibiotics to cope the demonds. According to World Health Report of Infectious Diseases (2000), overcoming antibiotic resistance is the major issue for the next millennium.

In view of the above, exploitation of *Pithophora oedogonia* found in the monoalgal form as mats in natural freshwater habitats, while on one hand will help mitigating nuisance and pollution load in the concerned water body, by virtue of good antibacterial activity shown by this alga it can also be profitably used to discover bioactive principales that may serve as leads for the development of new pharmaceuticals addressing the novel therapeutic needs of the mankind. Such screening of various natural organic compounds and identifying active agents is the need of the hour. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly for their practical uses.

Based on the present and past studies, it is concluded that the neglected lower plants, algae represent a new source of antimicrobial formulation with stable and biologically active compounds and a scientific data base needs to be established for their use in medicine as herbal remedies. The knowledge about the botanical preparations from aquatic algal forms, likewise, require future intensive investigations and trials from view points of ethnobotany, phytochemistry and pharmacology. Human beings being the most important species of biosphere safety of the herbal remedies to life must be ensured before they are prescribed and marketed. Identification of promising plant resources and their scientific exploitation for drug development, on sustained basis, can turn out to be a million doller projects for the Indian sub-continent which has enormous plant wealth.

ACKNOWLEDGEMENTS

The authors express sincere thanks to the UGC, New Delhi for providing financial assistance in the form of UGC Research Fellowship to APS and to the head of the Department of Botany, for providing necessary facilities.

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