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Antimicrobial and Phytochemical Analysis of *Pithophora varia*

Maruthanayagam Alaguprathana[1*] and Mani Poonkothai[1]

[1]Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, 641 043, Coimbatore, Tamilnadu, India.

Authors' contributions

This entire work was done by author MA. Author MP gave an idea for this work. Both authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

The present study is aimed to investigate the antimicrobial as well as phytochemical constituents of fresh water green algae *Pithophora varia.* The algae was extracted with acetone, benzene, chloroform and ethanol separately. The extracts were screened for antibacterial activity against two Gram positive (*Staphylococcus aureus, Bacillus subtilis*) and five Gram negative (*Klebsilla pneumoniae, Proteus vulgarius, Pseudomonas aeroginosa, Salmonella typhi* and *E. coli)* bacteria and the antifungal activity was assessed against *Aspergillus niger, Trichodrma sp., Aspergillus flavus* and *Aspergillus fumigatus* using agar well diffusion assay. The ethanol extract of *P.varia* was showed significant antibacterial as well as antifungal activity against *Bacillus subtilis, E. coli* and *Trichodrma sp, Aspergillus niger* separately. The zone of inhibition range from 18-20 mm respectively. The phytochemicals present in algal extracts are alkaloids, phenols, amino acids, saponins, tannins, steroids, flavonoids and Quinones. Each active compound shows different activities against different types of diseases like cancer, liver disorders, diabetes, arthrosclerosis and inflammatory diseases. The results showed that the *P. varia* extract have great potential as antimicrobial and phytochemical compounds against microorganism and they can be used in the treatment of diseases caused by resistant microorganisms.

**Corresponding author: E-mail: alaguprathanaphd.adu.ac.in@gmail.com;*

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1. INTRODUCTION

Aquatic organisms have a rich source of structurally different and biologically active compounds. Potential bioactive composites namely Secondary or primary metabolites are generated by aquatic organisms which play a vital role in the pharmaceutical industry. Microorganisms produce a wide range of chemicals such as alkaloids, indoles, macrolides, peptides, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons. A few of these chemicals may inhibit the growth of pathogenic microorganisms [1,2].

Microbial infections are one of the prominent causes of health problems, physical disabilities and mortalities around the world. The prolonged use of synthetic drugs develops resistance against pathogens. Hence the uses of green medicines are healthier and safer than synthetic medicines because of their limited side effects. Algae are an important source of biologically active secondary compounds, which is used for the biological control of pathogens. Algae have a significant attraction as the natural source of bioactive molecules with a broad range of biological activities, including antimicrobial, antiviral, antioxidant and anti-inflammatory effects [3]. Special attention for antiviral, antibacterial and antifungal activities against human pathogens has been reported by [4-6]. The ability of algae to produce antimicrobial compounds could be used as a defense agent against pathogens and also as used as a pharmaceutical bioactive natural compound [7]. More than 150,000 diversities of algae are found in fresh water are very few have been identified for antimicrobial activity [8].

1.1 *Pithophora varia* Distribution, Habitat and Description

P. varia is found state wide but found most commonly from the piedmont eastward throughout the coastal plain. It thrives in shallow ponds with low flow and is often associated with ponds receiving nitrogen and phosphorous enrichment. It may form extensive surface mats during the summer months. This dense and prolific growth often interferes or prevents fishing, irrigation or other utilization of the pond. *P. varia* is recognized as one of the most difficult and persistent species of aquatic vegetation to control.

P. varia belongs to the family of filamentous green algae. It may be found growing on the bottom or in dense mats on the surface. This algae is often described as resembling a tangled mass of cotton or wool like growth which is very course to the touch. Under magnification it was composed of irregularly branched filaments usually with numerous swollen spore-like reproductive cells known an akinetes. It may range in colour from lime green to a dark greenish brown. The surface mats generally form in warmer weather when gas bubbles, produced by the plant, are trapped within the dense algal growth, causing them to become buoyant. Disturbance of these mats by high wind or heavy rain events may cause them to temporarily sink to the bottom. This often gives a false impression that the growth has disappeared, only to have it return to the surface within several days.

Due to this justification, the present work aims to evaluate the antimicrobial and photochemical activities of *Pithophora varia* using different solvents.

2. MATERIALS AND METHODS

2.1 Collection of Algae

Pithophora varia was collected from a fresh water pond near Perur, Coimbatore, Tamilnadu. Algal mats were thoroughly washed with distilled water to remove dirt's, epiphytes and debris. The algal mats were dried for a week under shade and the dried samples were cut into small pieces and then ground with a mortar and pestle and made into powder.

2.2 Preparation of Algal Extracts

The algal extracts were prepared using the organic solvents such as acetone, benzene, chloroform and ethanol. Ten gram of algal powder was homogenized with 100ml of respective solvents. The extract preparation was left three days in an orbital shaker incubator at 30°C and filtered. The supernatants were then transferred to a pre-weighed beaker and evaporated in room temperature. The dried extracts was weighed and dissolved in 2 ml of DMSO (dimethylsulphoxide) are considered as a stock. The stocks were used for antimicrobial and phytochemical analysis.

2.3 Selection of Microorganisms for Antimicrobial Activity

2.3.1 Collection of bacterial strains

Totally seven human pathogenic bacterial strains were selected for the present investigation. The bacterial strains are *Staphylococcus aureus, Bacillus subtilis* (Gram-positive) and *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeroginosa, Salmonella typhi* and *E.coli* (Gram-negative) were obtained from Spire labs, Coimbatore, Tamil Nadu. The collected bacterial strains were inoculated onto the nutrient broth and incubated at 35°C for 24 hr.

2.3.2 Isolation of fungi

One gram of soil was taken from sewage/ contaminated area and mixed with 10 ml of distilled water followed by serial dilution up to 10^{-8}. The diluted water samples were inoculated in sterilized Petri plates containing Rosebengal chloramphenicol agar (RCA) medium. The plates were incubated at room temperature for five days. After the incubation period, the fungal colonies were identified in all the dilutions 10^{-1}-10^{-8} using lacto phenol cotton blue staining method. The identified fungal isolates are *Aspergillus niger, Trichoderma sp., Aspergillus flavus* and *Aspergillus fumigatus.* The fungal strains were inoculated onto Rosebengal chloramphenicol broth and incubated at room temperature for three days.

2.4 Antimicrobial Activity Assay

The antimicrobial activity of algal extracts was determined using agar well diffusion method done by Bauer et al. [9]. The culture media was prepared and autoclaved at 120°C for 15 min. The sterile Petri dishes were poured with nutrient agar (NA) medium for bacteria and Rosebengal chloramphenicol agar (RCA) medium for fungus. Six wells of 8.0 mm diameter were made on the culture medium. Afterwards, the bacteria and fungus were swabbed on the NA and RCA plate respectively using a sterile cotton swab, the cultures were uniformly spread over the surface of plates. Each well was filled with 20 µl of algal extracts along with chloramphenicol (negative control) and nystatin (positive control). Then the plates were incubated at 35°C for 24 hr (for bacteria) and room temperature for 72 hr (for fungi) and observed for a zone of inhibition around the well. The diameter of inhibition was measured.

2.5 Phytochemical Studies

The phytochemical analysis of the various organic solvent extract was carried out using standard methods. The presence and absence of phytoconstituents such as alkaloids, phenols, amino acids, saponins, tannins, steroids, flavonoids and quinones were tested based on that of Harborne [10] method.

3. RESULTS

The acetone, benzene, chloroform and ethanolic extracts were evaluated for their potential bioactivity against seven human bacterial pathogens and four fungi.

Table 1 revealed that the antibacterial activity of algal extracts showed their potency against bacterial strains. Among all the extracts, the ethanol extract was found to be effective against *Bacillus subtilis, E. coli* respectively (Fig. 1(a)). The zone of inhibition ranges from 18 to 19 mm alone. The inhibition zones produced by the ethanolic extract compared with zones produced by chloramphenicol and nystatin was used as a control.

The antifungal activity of algal extracts was determined against fungal isolates were shown in Table 2. All the extracts of *P. varia* have some considerable inhibitory effect on the growth of fungal species. Among these, the ethanol extract has a significant inhibitory effect against *Trichoderma. sp* and *Aspergillus niger* (18 mm and 20 mm diameter inhibitory zones respectively) (Fig. 1(b)). Similar activities were carried out by Goud et al. [11] and Prashant et al. [12] who investigated that ethanolic extract of blue-green alga and green algae have shown good antifungal activity.

Phytoconstituents were extracted by using different solvents of increasing polarity like acetone, benzene, chloroform and ethanol. The results obtained in the present investigation, the acetone, benzene, chloroform and ethanol extracts of *P. varia* showed the presence of alkaloids, phenols, amino acids, saponins, tannins, steroids, flavonoids and Quinones. Among the various extracts, the ethanolic extract showed more phytochemical compounds followed by acetone extract.

Table 1. Antibacterial activity of algal extracts

S. no.	Bacterial strains	Inhibition zone diameters (mm)					
		Acetone	Benzene	Chloroform	Ethanol	Chloramphenicol (negative control)	Nystatin (positive control)
1	*Staphylococcus aureus*	12	9	8	14	-	20
2	*Klebsilla pneumoniae*	7	6	11	10	-	22
3	*Proteus vulgarius*	10	8	9	12	-	23
4	*Pseudomonas aeroginosa*	11	8	13	13	-	20
5	*Salmonella typhi*	12	10	14	14	-	23
6	*E. coli*	15	11	9	18	-	21
7	*Bacillus subtilis*	16	12	11	19	-	22

Table 2. Antifungal activity of algal extracts

S. no.	Fungal strains	Inhibition zone diameters (mm)					
		Acetone	Benzene	Chloroform	Ethanol	Chloramphenicol (negative control)	Nystatin (positive control)
1	*Aspergillus niger*	16	13	12	18	-	22
2	*Trichodrma sp.*	19	14	11	20	-	20
3	*Aspergillus flavus*	12	9	6	15	-	18
4	*Aspergillus fumigatus*	14	11	13	17	-	22

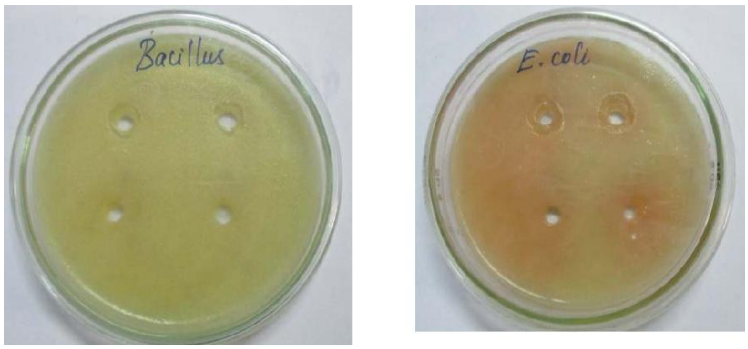

Fig. 1(a). Antibacterial activity with *Basillus subtilus* and *E. coli*

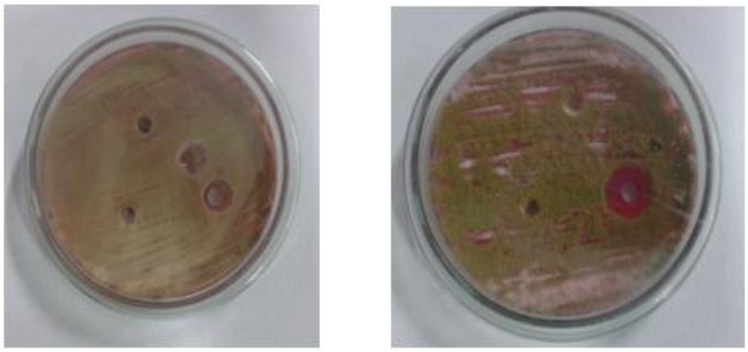

Fig. 1(b). Antifungal activity with and *Aspergillus sp.*

Table 3. Phytochemical analysis of algal extract: (+) presence, (-) absence

S. no.	Constituents	Acetone	Benzene	Chloroform	Ethanol
1	**Alkaloids**				
	Mayer's reagent	-	-	-	-
	Dragendroff's reagent	+	-	-	+
	Wagner's reagent	-	+	-	+
	Hager's test	+	-	+	-
2	**Phenol**				
	Ferric chloride	-	-	-	+
	Lead acetate test	-	+	-	+
3	**Amino acid**				
	Ninhydrin test	-	-	+	+
4	**Flavonoids**				
	Schinoda's test	+	-	+	-
	Ammonia test	-	+	-	+
5	**Saponins**				
	Froth test	+	-	-	+
	Sodium Bicarbonate	+	-	-	+
6	**Tannins**				
	Breamer's test	+	-	-	+
7	**Quinones**				
	Brontrager's test	+	-	-	+

4. DISCUSSION

The results reported in this study are in accordance with Chowdhury et al. (2015) who reported that the ethanolic extract of *Oscillatoria sancta* showed maximum antimicrobial activity against *E. coli* (16.1 mm). This result supports the findings of many authors [13,14] they screened the most active compounds in macroalgae, biochemical analysis is being undertaken to determine the structure and nature of compounds responsible of the bioactivity of the extracts with high antibacterial activity. Not only the presence of a particular compound which makes these organisms, interesting but also their huge diversity and the possibility of not only harvesting them but also of growing them at different conditions, leading to an enrichment of some bioactive compounds [15,16].

Our results highlighted the in vitro antifungal activity of the tested algal extracts, although in recent years, most of the compounds were reported as antibacterial in human medicine. It is expected that the antifungal activity found by us to be done in the presence of bioactive molecules, as phenolic compounds (phlorotannins, terpenes, alkaloids), polysaccharides or fatty acids, many of these structures being identified as antimicrobials [17]. Using organic solvents which are able to extract a lot of lipophilic compounds (glycolipid, phenolic-terpenoids, unsaturated fatty acids and hydroxylated acids), the higher antifungal activity found in ethanol extracts, compared to water extracts could be explained by Hanaa et al. [18] (2008) and Mundt et al. [19]. The main reasons for using the algal extract as antifungal agents is their natural origin and low chance of pathogens developing resistance and less environmental hazards [20].

Flavonoids, as antioxidants may prevent the progressive impairment of pancreatic bête cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes [21] who also stated that the flavonoids are probably the most important natural phenolic due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties. Flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation, and also as potential therapeutic agents against a wide variety of diseases.

Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss [22]. Saponins are also having some strong antitumor effects and found beneficial in targeting and inhibiting the tumour angiogenesis by suppressing its inducer in the endothelial cells in blood vessels and preventing and adhering, invasion of metastasis of tumour cells [23].

Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria. Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties [24].

5. CONCLUSION

The present study indicates that the antimicrobial property of the *P.varia* against the selected bacteria strains and fungi isolates was varying depending upon the solvent medium used for extraction. Among the tested extracts the methanolic extract showed as a promising and potential solvent for the extraction of antimicrobial compounds. The phytochemical screening of algal extract showed the presence of alkaloids, phenols, amino acids, saponins, tannins, steroids, flavonoids and Quinones which may be attributed to its antimicrobial activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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