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Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *APHANOTHECE PALLIDA* (KÜTZ.) RABENGH. COLLECTED FROM DIARA POND, HOOGHLY, WEST BENGAL

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ABSTRACT
The present paper was dealt with the qualitative and quantitative screening of some phytoconstituents of a blue green alga *Aphanothece pallida* **(**Kütz.**)** Rabengh. and *in vitro* antibacterial activity study of four various organic solvent extracts with different polarities **(**benzene, chloroform, acetone and methanol**)** of the same alga, collected from a pond of Diara of Hooghly district, West Bengal. The tested alga showed positive results of having active organic compounds and specific inhibitory effects on three gram positive and one gram negative tested pathogenic bacterial strains. The qualitative screening results revealed that *Aphanothece pallida* contained steroid, terpenoid, phenol, flavonoid and flavonol in all the solvent extracts studied. Quantitative estimation of chloroform and benzene extracts showed the presence of higher and lower phenolic contents 5.38±0.14 mg/g and 2.99±023 mg/g respectively. Acetone extract showed the highest flavonoid content 10.86±0.24 mg/g whereas, methanol extract possessed high amount of flavonol content 27.06±0.47 mg/g and low amount of flavonoid content as 5.88±0.20 mg/g. Antibacterial activity revealed that *Bacillus subtilis* and *Staphylococcus aureus* were more susceptible whereas *Micrococcus luteus* and *Escherichia coli* were comparatively less susceptible against all the four solvent extracts.

Keywords: *Aphanothece Pallida, Phytoconstituents Analysis, Antibacterial Activity, Hooghly, West Bengal*

INTRODUCTION
Cyanobacteria are known to produce metabolites with diverse biological activities such as antibacterial, antifungal, antiviral, anticancer, anti-plasmodial, algaecide, anti-platelet aggregation and immuno-suppressive activities (Priyadharshini *et al*., 2013). Cyanobacteria are alternative source of a variety of bioactive compounds, lipids or fatty acids, proteins, enzymes, pigments and compounds of pharmaceuticals and neutraceutical values (Ordog *et al*., 2004). A number of cyanobacteria and micro algae produce various biologically active compounds. These include antibiotics which in laboratory tests inhibited bacteria and fungi of human diseases (Kulik, 1995). Previously, Singh *et al*., (2005) extracted some bioactive compounds from cyanobacteria and microalgae in India.
Rania and Hala (2008) investigated antibacterial and antifungal activity of cyanobacteria and green micro algae from Egypt. Patil *et al*., (2009) assessed antibacterial potentiality of some indigenous cyanobacterial species. Khairy and El-Kassas (2010) extracted active substances from some blue green algal species which were used as antimicrobial agents. *Aphanothece pallida* **(**Kütz.**)** Rabenh. is a mucilagenous, colonial, free floating blue green alga, under the order chroococcales of class cyanophyceae. It is a very common algal form and widely distributed throughout the district. There is paucity of information regarding phytochemical screening and antibacterial activity of this species. Hence the present study had been taken from this state.

MATERIALS AND METHODS
Collection of Algae
Algal sample was collected in plastic and glass containers from Diara pond (22°.79'N, 88°.28'E) of Hooghly district (20°30'32″-23°1'20″N and 87°30'20″-80°30' 15″E), West Bengal very carefully because of their softness nature in aquatic habitat. Detail study was made by examining specimens under Olympus

microscope (Model-CH20i) for determination of species. Identification of the taxa accomplished with the help of authentic literatures (Geitler, 1932; Desikachary, 1959).

Preparation of Extracts

Before extraction, the selected algal material was washed under running tap water to remove adhering soil particles, epiphytes and associated debris and dried up at room temperature.

Solvent Extracts

The dried up algal samples were powdered with mortar and pestle for preparation of solvent extracts. Crushed samples were kept in contact with solvents **(**Benzene, chloroforn, acetone and methanol**)** for 8-10 days at room temperature followed by shaking for 14 h in rotary shaker. At the end of extraction, extracts were filtered through a bacterial filter (Whatman no. 1 filter paper) and then the filtrate was concentrated under reduced pressure by using a rotary evaporator to such a volume that 1 ml of extract would correspond to 5 gram of gross weight. Solvent extracts were transferred to a hot air oven for dry up to a constant weight at 45^0C. Then the filtrate residue was used for the bacterial susceptibility test. However, when the pH was out of range it was adjusted to pH 7 before assay of antibacterial activity.

Phytochemical Screening

Qualitative Tests for Phytochemicals

All the extracts were subjected to preliminary phytochemical screening as described by Trease and Evans (1989) and Harborne (1998).

Quantitative Estimation of Total Phenolic Content

The amount of total phenolic content of crude extract was determined according to Folin-Ciocalteu procedure (Singleton and Rossi, 1965). 20-100 µl of the tested sample was introduced into test tubes; 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were allowed to stand for 30 min. Absorption at 765 nm was measured (UV-visible spectrophotometer Hitachi U 2000, Japan). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram (mg g^{-1}) of extract.

Determination of Total Flavonoids

Total flavonoids were estimated using the method of Ordonez *et al*., (2006). In 0.5 ml algal sample, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After one hour at room temperature, the absorbance was measured at 420 nm (UV-visible spectrophotometer Hitachi U 2000 Japan). Appearance of yellow color indicated the presence of flavonoids. Total flavonoid contents were calculated as rutin (mg/g) using the following equation based on the calibration curve: $y = 0.0182x - 0.0222$, $R^2 = 0.9981$, where y was the absorbance and x was the rutin equivalent (mg/g).

Determination of Total Flavonols

Total flavonols in the algal extract was estimated using the method of Kumaran and Karunakaran (2006). To 2.0 ml of sample (standard), 2.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm (UV-visible spectrophotometer Hitachi U 2000, Japan) was read after 2.5 h at 20°C. Total flavonol content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y = 0.0049 x + 0.0047$, $R^2 = 0.9984$, where y was the absorbance and x was the quercetin equivalent (mg/g).

Bacterial Strains

Gram Positive Bacteria

Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus. All the bacterial cultures were produced from ID and BG Hospital, Kolkata. These bacterial stains were maintained on nutrient agar slant at 4°C and subcultured for 24 h before use.

Gram Negative Bacteria

Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Shigella flexneri, Vibrio cholera.

Antibacterial Activity

Antibacterial activity tests were performed using agar well diffusion method (Bauer *et al*., 1966) to evaluate antibacterial activities of various solvent extracts of the selected algal species. The strains of bacteria were inoculated at 30^0C for 24h. Media were prepared using Muller Hinton agar, then poured

into Petri dishes and inoculated with the test organism from the seeded broth. 25µl algal extract was introduced onto the upper layer of the agar plate. The plates were incubated over night. After incubation, the clear inhibition zones on the agar plates were visible. Each test was performed 3 times and antibacterial activities were expressed as the mean of diameter of inhibition zones (measured in mm.)

RESULTS AND DISCUSSION

For the first time, the qualitative and quantitative screening of phytoconstituents and *in vitro* antibacterial activity using eight strains of bacteria were carried out of a microscopic blue green alga *Aphanothece pallida* **(**Kütz.**)** Rabengh. from West Bengal, India. The qualitative phytochemical screening of different organic solvents of various polarities viz. benzene, chloroform, acetone and methanol extract exhibited the presence of different types of active components (Table 1). The qualitative screening results revealed that *Aphanothece pallida* contained steroid, terpenoid, phenol, flavonoid and flavonol in all the solvent extracts studied.

Table 1: Qualitative estimation of phytoconstituents of *Aphanothece pallida* collected from Diara pond of Hooghly district, West Bengal

Name of the algal species	Collected from	Phytochemicals present in Benzene, chloroform, acetone and methanol solvent extracts								
		Alkaloids	Tannin	Steroid	Saponin	Glycoside	Terpenoid	Phenol	Flavonoid	Flavonol
Aphanothece pallida	Pond at Diara, Hooghly, W. B	-	-	+	-	-	+	+	+	+

+ indicated presence or positive reactions and – indicated absence or negative reactions

Table 2: Quantitative estimation of some phytoconstituents in different solvents of *Aphanothece pallida* collected from Diara pond of Hooghly district, West Bengal

Phytochemicals types	Amounts of selected phytochemicals in different Solvents			
	Benzene(mg/g)	Chloroform(mg/g)	Acetone(mg/g)	Methanol(mg/g)
Phenolic Content	2.99±0.23	5.38±0.14	3.07±0.22	4.87±0.14
Flavonoid Content	7.97±0.22	8.46±0.10	10.86±0.24	5.88±0.20
Flavonol Content	11.08±0.78	17.89±0.78	8.12±0.89	27.06±0.47

Table 3: Antibacterial activity of different solvent extracts of *Aphanothece pallida* collected from Diara pond of Hooghly district, West Bengal

Solvents Used	Name of bacteria and inhibition zones (mm) with (Mean ± SE)							
	Bs	***Ml***	***Sa***	***Ec***	***Sd***	***Pa***	***Vc***	***Sf***
Benzene	10±0.05	9±0.04	8±0.03	8±0.04	-	-	-	-
Chloroform	16±0.05	14±0.06	15±0.06	12±0.05	-	-	-	-
Acetone	12±0.06	11±0.05	10±0.04	9±0.04	-	-	-	-
Methanol	14±0.06	12±0.05	13±0.05	10±0.04	-	-	-	-
Water	7±0.04	-	8±0.03	-	-	-	-	-

Ec= Escherichia coli, Sf= Shigella flexneri, Pa= Pseudomonas aeruginosa, Sd= Shigella dysenteriae, Vc= Vibrio cholerae, Bs= Bacillus subtilis, Ml= Micrococcus luteus, Sa= Staphylococcus aureus; - indicated not detected.

The quantitative estimation of different organic solvents of various polarities viz. benzene, chloroform, acetone and methanol extracts exhibited the presence of different types of active components that were

responsible for antioxidant and antibacterial potentialities. Analyses of results showed that total phenolic content was ranged from 2.99±023 to 5.38±0.14 GAE mg /g of dry material (Table 2). Similarly, the flavonoid and flavonol content of the said extract in terms of rutin equivalent and quercetin equivalent were determined between 5.88±0.20 to 10.86±0.24 mg/g and 8.12±0.89 to 27.06±0.47 mg/g of dry material, respectively (Table 2). However, chloroform extract showed the presence of higher phenolic content 5.38±0.14 mg/g while least amount was observed in the benzene extract 2.99±023 mg/g and intermediate condition was seen in acetone and methanol extracts. Acetone extract showed the highest flavonoid content 10.86±0.24 mg/g, whereas, methanol extract confirmed high amount of flavonol content 27.06±0.47 mg/g and low amount of flavonoid content as 5.88±0.20 mg/g.

Results in table 3, showed the antibacterial activities of various organic solvent extracts of *Aphanothece pallida* **(**Kütz.**)** Rabengh. against three Gram positive and five Gram negative bacterial strains. The extracts showed antibacterial activities against only four tested pathogenic bacteria out of eight. Chloroform and methanol extracts exhibited somewhat better antibacterial activities than that of other two organic solvent extracts. *Bacillus subtilis* and *Staphylococcus aureus* were more susceptible whereas *Micrococcus luteus* and *Escherichia coli* were comparatively less susceptible against all the four solvent extracts. Chloroform and methanol extracts both of them revealed the highest inhibition zones of 16 mm and 14 mm against the same bacteria *Bacillus subtilis*. Comparatively somewhat lower antibacterial activities were recorded by benzene and acetone extracts of *Aphanothece pallida*. The aqueous extract did not showed any remarkable activities against the pathogenic tested bacteria. This variation of antibacterial activities of the organic solvent extracts of this alga might be due to their presence of different antibacterial substances within the algal plant cells. Richard *et al*., (1980) while working on cyanobacteria and eukaryotic algae also found antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. Same findings were observed in this present study. Vijaya (2004) made observations that methanol was a better solvent for extraction and separation of variety of phytochemicals that produced inhibitory effect against Gram positive and Gram negative bacteria. Similar type of trend was noticed in this study. Goud *et al*., (2007) while testing antibacterial activity and screened biomolecular composition of certain fresh water micro-algae from river Godavari (India) made a conclusion that Gram positive bacteria were more susceptible than Gram negative bacteria. Same observations were encountered in the present work.

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