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ORIGINAL RESEARCH ARTICLE

Phytoconstituent Analysis and Antibacterial Activity of *Nitella flagelliformis* A. Braun Collected from a Pond of Khamargachi of Hooghly District, West Bengal, India

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ABSTRACT

The present paper was communicated with the screening of some phytoconstituents present in crude extract of *Nitella flagelliformis* A. Braun and *in vitro* antibacterial activity study of four organic solvent extracts **(**benzene, chloroform, acetone and methanol**)** of the same alga, collected from a pond of Khamargachi of Hooghly district, West Bengal. The tested alga showed positive results of having bio-active compounds and specific inhibitory effect on three Gram positive and five Gram negative tested pathogenic bacteria. Acetone extract showed the presence of higher phenolic content of 27.03 mg/g of dry material whereas chloroform extract exhibited the occurrence of both flavonoid and flavonol content in higher amount (527.30 and 186.69 mg/g of dry material). Antibacterial activity revealed that benzene and acetone extracts were best active against most of the bacterial strains whereas chloroform and methanolic crude extracts were comparatively less active against the same bacterial strains.

Key words: Antibacterial activity, Hooghly district, *Nitella* Agardh, Pathogenic bacteria, Phytoconstituent analysis, West Bengal.

INTRODUCTION

India is an important country in the world where ancient systems of medicine such as siddha, ayurveda and unani have been in practice for many years (Prashantkumar *et al.,* 2006). Microalgae are the natural sources of bioactive molecules, as they are found to be potent to produce bioactive compounds in culture (Goud *et al.,* 2007). The macroalgae have a significant attraction as natural source of bioactive molecules with a broad range of biological activities, such as antibacterial, antiviral, antitumorals, antioxidant and anti-inflammatory (Sreenivasa rao 1995; Vineela and Elizabeth, 2005; Patra *et al.,* 2008; Patra *et al.,* 2009). Algae are the source of amino acids, terpenoids, steroids, phenolic compounds, phlorotannins, halogenated ketones and alkanes and cyclic polysulphides (Taskin *et al.,* 2007). Bioactive molecules are accumulated either in algal cells or excreted into the outer environment. *Nitella flagelliformis* A. Braun is a fresh water charophycean alga belonging to the order charales of the family Characeae which is grown on sandy mud near shore as submerged condition. It is grayish green and characterized by position of sex organs whose male sex organ globule lies above the female sex organ nucule.

The antimicrobial bioactive molecules could be used against pathogens and to manufacture pharmaceuticals. Although few works have been done on phytochemical and antibacterial activities of some algae genera, no works regarding phytochemical evaluation and antibacterial activities have not been done till now with this alga *N. flagelliformis.* The objective of the study is to correlate phytoconstituents with biological activity of the said alga. Hence in the present investigation, phytochemical screening was performed to detect active components of the tested algal extract and antibacterial activity to evaluate inhibitory effect against eight pathogenic bacterial strains.

MATERIALS AND METHODS

Collection of algal sample:

Algal sample was collected in sterilized plastic packets and sterilized glass containers from a pond of Khamargachi (23.83^{o}N, 88.20^{o}E) of Hooghly district, West Bengal. Detailed study was

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made by examining specimens under Olympus microscope (Model-CH20i) for identification of species. Identification of taxa was accomplished with the help of authentic literatures (Imahori, 1954; Pal *et al.,* 1962; Wood and Imahori, 1964, Wood and Imahori, 1965).

Preparation of extracts:

Before extraction, the algal material was washed under running tap water to remove adhering soil particles, epiphytes and associated debris. It was then shade-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, chloroform, 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 and 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 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 7 before assay of antibacterial activity.

Qualitative measurement of phytochemicals:

The algal samples were subjected to phytochemical screening for the detective of phenol, flavonoids and flavnol using the method of Harborne (Sinha, 2012).

Quantitative measurement of phytochemicals:

Estimation of total phenol content: The quantity of total phenolic content of crude extract was determined according to Folin-Ciocalteu procedure (Singleton and Rossi, 1965). 20-100 µl of the tested samples were introduced into test tubes in which 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 presented as gallic acid equivalents (GAE) in milligram per gram (mg g^{-1}) of extract.

Determination of total flavonoids: Total flavonoids were determined with the method of Ordonez *et al.,* (2006). 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of sample and kept at room temperature for one hour. The absorbance was measured at 420 nm (UV-visible spectrophotometer Hitachi U 2000 Japan). The presence of flavonoids was indicated by the formation of yellow color. Total flavonoid contents were expressed 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 of the algal extracts were 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 taken 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: $y = 0.0049x + 0.0047$, $R^2 = 0.9984$, where y was the absorbance and x was the quercetin equivalent (mg/g).

Bacterial strains:

Gram positive bacteria: *Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus*.

Gram negative bacteria: *Vibrio cholera, Pseudomonas aeruginosa, Shigella flexneri, Escherichia coli*. *Shigella dysenteriae*.

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.

Antibacterial activity test:

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^oC 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, screening of phytoconstituents and *in vitro* antibacterial activity study using eight strains of bacteria were carried out from the macroscopic green alga *Nitella flagelliformis* A. Braun, collected from Khamargachi pond of Hooghly district, West Bengal. The

phytoconstituents screening of benzene, chloroform, acetone and methanol crude extract showed the presence of different types of active components (**Table 1**). It was found that there was a wide variation in the amount of total phenolic content ranging from 4.39±0.04 to 27.03±0.11mg GAE/g of dry material (**Table 2**). Similarly the flavonoid and flavonol content of the said extracts in terms of rutin equivalent and quercetin equivalent ranged between 123.09±1.01 to 527.30±0.04 mg/g and 93.97±6.17 to 186.69±1.39 mg/g of dry material, respectively (**Table 2**). However, acetone extract showed the presence of higher phenolic content 27.03±0.11 mg/g while least amount was observed in the methanol extract (4.39±0.04 mg/g) and intermediate in benzene and chloroform extract (**Table 2**). Chloroform extract showed the presence of both flavonoid 527.30±0.04 mg/g and flavonol 186.69±1.39 mg/g content in higher amount (**Table 2**).

(Table 3) showed the antibacterial activities of organic solvent extracts of *Nitella flagelliformis* against 3 Gram positive and 5 Gram negative bacterial strains. The extracts exhibited antibacterial activities against all the tested pathogenic bacteria. Benzene and acetone extracts showed somewhat better antibacterial activity than that of other extracts. Acetone extract and benzene extract exhibited highest inhibitory effect of 24 mm and 22 mm against *Shigella flexneri* and *Staphylococcus aureus* respectively. Chloroform extract was found as the most sensitive against *Shigella flexneri* and *Pseudomonas aeruginosa* by forming inhibition zone measured as 16mm and 15mm.In contrast, somewhat lower antibacterial activity was observed in case of methanol extract of *Nitella flagelliformis*. Here, highest antibacterial activity (18mm) was noticed against *Shigella flexneri*. The aqueous extract did not show any remarkable activity against the pathogenic bacteria except *Shigella flexneri* and *Bacillus subtilis*. The study revealed that benzene and acetone extracts showed maximum inhibition zones than other extracts. This variation of antibacterial activity of the organic solvent extracts of *Nitella flagelliformis* might be due to the presence of different antibacterial substances within the algal plant cells. Similar type of observations were encountered by Patra *et al*., (2009) while working on evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum* sp.) extract from Chilka lake, Orissa.

Table 1: Qualitative estimation of phytoconstituents of *Nitella flagelliformis*

Photochemical	Water	Benzene	Chloroform	Acetone	Methanol
Phenol	+	+	+	+	+
Flavonoid	+	+	+	+	+
Flavonol	+	+	+	+	+

+ indicates presence or positive reactions

Table 2: Quantitative estimation of phytoconstituents of *Nitella flagelliformis*

Photochemical	Benzene	Chloroform	Acetone	Methanol
Phenol	25.38±0.22	14.10±0.13	27.03±0.11	4.39±0.04
Flavonoid	141.26±1.56	527.30±0.04	123.09±1.01	150.62±0.03
Flavonol	132.27±1.79	186.69±1.39	93.97±6.17	98.94±1.11

Table 3: Antibacterial activity of different solvent extracts of *Nitella flagelliformis*

Solvent Used	Inhibition zone diameter (mm)							
	Bs	***Ml***	***Sa***	***Ec***	***Sd***	***Pa***	***Vc***	***Sf***
Benzene	18	21	22	20	19	21	19	22
Chloroform	10	12	14	12	11	15	12	16
Acetone	20	23	24	22	21	23	20	24
Methanol	13	13	12	13	12	12	11	18
Water	10	9	9	10	8	8	7	10

Ec = Escherichia coli, Sf = *Shigella flexneri, Pa = Pseudomonas aeruginosa, Sd = Shigella dysenteriae, Vc = Vibrio cholerae, Bs = Bacillus subtilis, Ml = Micrococcus luteus, Sa = Staphylococcus aureus.*

CONCLUSIONS

This experimental result reflected the presence of bioactive metabolites in the of algal plant cells which were soluble in these solvents. It was clear that extraction by organic solvents provided a higher efficiency for antimicrobial activities as compared to aqueous extracts. Among all the solvents benzene and acetone extracts exhibited maximum antibacterial activity which indicated the exclusion of most of the bioactive metabolites in these solvents.

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