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Phycochemistry and Anti Bacterial activity of Freshwater Green Algae *Nitella tenuissima* (Desv.) Kiitz

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Abstract

Antibacterial activity of stonewort green macro alga, *Nitella tenuissima* (Desv.) Kiitz was studied in five different solvents *viz*., methanol, ethanol, ethyl acetate, chloroform and distilled water against four human pathogenic bacteria *Staphylococcus aureus, Bacillus cereus, Streptococcus mutans, Citrobacter koseri* by two different methods *viz*., agar well diffusion method and paper disc method. A preliminary phycochemical screening for protein, carbohydrate, total chlorophyll and total phenol content estimation were also studied in all the algal extract. The results showed that the methanolic extract of *Nitella tenuissima* (Desv.) Kiitz recorded appreciable anti bacterial activity. The zone of inhibition in methanol extract of disc method was 20 mm (against *Staphylococcus aureus* and *Bacillus cereus*) and 20 & 28 mm in methanol & ethyl acetate of well diffusion method respectively (against *Staphylococcus aureus*). Among the algal extracts studied, the methanol extracts registered high zone of inhibition against all the bacterial strain tested in both the methods.

Keywords: *Nitella tenuissima* (Desv.) Kiitz, Phycochemical, Antibacterial activity, Freshwater, Macro algae, Methanoilic extract.

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1. Introduction

Algae are the very important and diverse group of plant kingdom, being many bioactive compounds algae becoming good area for research (Kamble and Chavan, 2010). Need for the new therapeutic compounds had been expanding steadily because of the evolving resistance of microorganisms to existing antibiotic. The emergence of resistant bacteria has created a major concern and urgent need for a new anti-bacterial agent (Rajasulochana *et al*., 2009). Many of the algae are known for the production of bioactive substances (Spratt, 1994). Several works have been carried out on the extracts from algae to know their anti microbial activities. It is well known that calcified algae are very significant as far as their ecological and economic importance is concerned (Davis, 1994). A four-gene phylogenetic analysis was conducted to investigate relationship between the group charophytes and land plants. This analysis supports the hypothesis that the land plants are placed phylogenetically within the Charophyta identifies the Charales (stoneworts) as the closest living relatives of plants (Wray and Tsuda,1977). As Charales is closest living of higher plants this species is expected to have a high proportionate of bioactive compounds and antibacterial properties. Furthermore, microorganisms are causative agents in both food poisoning and food deterioration and so antimicrobial agents from plants are of interest as natural preservatives (Yukokuroglu *et al*., 2001). Phycochemistry is the study of natural products and chemical constituents occurring within algal thallus from a biological point of view (Shameel, 1990). The excessive amount of chlorophyll a and chlorophyll b is present in freshwater algae (e.g. members of the phyla Volvophycota, Chlorophycota and Charophycota). Several studies have been carried out to with various algal species to see their antibacterial activity. However, the present study objective is to find out the antibacterial and phycochemical properties of fresh water green macro algae, *Nitella tenuissima* (Desv.) Kiitz, an indicator of fresh water algal flora which was not done previously.

2. Materials and Methods

2.1 Algal material

Nitella tenuissima (Desv.) Kiitz were collected from Aarivakkam Lake, Kattangolathur, Kancheepuram District, Tamilnadu, India during October to December 2011(Wood, 1964).

The collected sample were cleaned with distilled water to remove all the extraneous matter such as epiphytes, sand particles, pebbles, shells etc., The sample were then thoroughly washed with freshwater, blotted and spread out in shade under room temperature for drying.

2.2 Preparation of extract

The shade dried sample was ground to a fine powder using pestle and mortar. The algal sample was mixed with various solvents such as methanol, ethanol, ethyl acetate, chloroform and distilled water. Extraction was done by soaking one part of algal sample into ten parts of solvent (1:10) ratio *viz*., methanol, ethanol, ethyl acetate, chloroform and distilled water. The extract was filtered through cheese cloth followed by filtration using Whatman No.1 filter paper. The extraction was repeated until the powder was free of extractable substances. The pooled extracts were concentrated under rotary evaporator at 40°C and then under room temperature. Finally the residues of each extracts were collected in separate vials and used for experiment.

2.3 Antibacterial Activity

2.3.1 Bacterial strains used for assay

Staphylococcus aureus (*S.aureus*), *Bacillus cereus* (*B. cereus*), *Streptococcus mutans* (*S. mutans*) and *Citrobacter koseri (C. koseri)* were obtained from the Centre of Advanced Studies in Botany(CAS), University of Madras. The bacterial stock cultures were maintained at 4^{O}C in a refrigerator.

2.3.1.1 Inhibitory effect by disc diffusion method

In vitro antibacterial activity was carried out by using Mueller Hinton Agar (MHA) following the method described (Karol *et al*., 2001). The MHA

plates were prepared by pouring 15mL of media into each sterile petri plates and allowed to cool. Swabs were prepared from various stock cultures of bacteria and were spreaded over agar surface to make a lawn. The residual extract of sample was mixed with respective solvent in the ratio of 1:10 and 2µl of sample was loaded uniformly on sterile discs (5mm in dia) *i.e.*, 200µg/ disc. Similarly, respective solvent used without sample was also loaded on sterile filter paper discs of 5mm in diameter as solvent control and labelled. Then soaked discs were air dried aseptically to ensure evaporation of solvents. Dried disc with impregnated algal and solvent extracts were carefully placed at uniform distances over agar surface with the help of sterile forceps and ensured for correct implantation by applying gentle press over disc. Then the plates were allowed to dry for 30 minutes. Then the petriplates were incubated at 35°C for 24 hours. After the incubation time, plates were observed for inhibitory zone formation (in mm) around the disc of different extracts on microbial lawns in agar surface and recorded.

2.3.1.1 Inhibitory effect by the agar well diffusion method

In vitro antibacterial activity was screened by using MHA following the method described (Attaie *et al*., 1987). The MHA plates were prepared by pouring 15mL of media into each sterile petri plates and allowed to cool. Swabs were prepared from various stock cultures of bacteria and were spreaded over agar surface to make a lawn. Five wells were made with sterile cork borer (6mm) in each plate. The residual extract of sample was mixed with respective solvent in the ratio of 1:10 and 2µl of sample was loaded uniformly in wells. *i.e.*,200µg/well. Similarly, respective solvent used without sample was also loaded in wells as solvent control and labelled. The inoculated plates were incubated at 37°C for 24 hours. After incubation period, the diameter of the clear zone around the well was measured (in mm) and recorded.

2.4 Phycochemical Analysis

The methanol extract of *Nitella tenuissima* (Desv.) Kiitz were also studied for their phycochemical constituents.

2.4.1 Estimation of chlorophyll

The shade dried sample was weighed and homogenized with 100% acetone and centrifuged at 5000 rpm for 10 min. The pellet obtained was again added with 100 % acetone, covered with aluminium foil and kept overnight at 4°C. Then the sample was centrifuged at 5000 rpm for 10 min. The supernatant was separated and the absorbancies was read at 470 nm, 644.8 nm and 661.6 nm. The amounts of Chl a, ChI b and total chlorophyll were calculated as per the formula mentioned (Lichtenthaler and Wellburn, 1985; Lichtenthaler, 1987)

2.4.2 Estimation of carotenoid

The sample was prepares as same of chlorophyll estimation and the absorbencies was read at 470 nm and total carotenoids were calculated as per the formula mentioned (Lichtenthaler and Wellburn, 1985; Lichtenthaler, 1987).

2.4.3 Estimation of total protein:

Total protein content was estimated by following (Bradford, 1976). Five gm of the fresh sample was homogenized in 5 mL of 0.1 M sodium phosphate buffer at pH 7 and then centrifuged at 5000 rpm for 10 minutes. The supernatant was taken for the estimation of total protein. 0.2 mL of sample was added to 5 mL of protein reagent (Coomassie Brilliant Blue G-250) and mixed thoroughly by vortexing and the solution was allowed to stand for 5 minutes. The absorbance was read at 595 nm against a reagent blank using a spectrophotemeter. The amount of protein was calculated by using a standard graph prepared with Bovine Serum Albumin (BSA) ranging from 10-100 ug/ml.

2.4.4 Estimation of total carbohydrate

Total carbohydrate content was estimated by following the method described by (Dubois, et al.,1956).5gms of the dried sample was homogenized with 5 mL of 0.1M sodium phosphate buffer at pH 6.8 and centrifuged at 5000 rpm for 10 minutes. To 1 mL of sample, 1

mL of 5 % phenol and 5 mL of H_2S0_4 were added. The mixture was thoroughly mixed by vortexing and the solution was allowed to stand at room temperature for 30 minutes. The OD was read at 490 nm using spectrophotometer and standard graph was prepared with different concentrations of D-glucose ranging from 10-100 ug/mL to calculate the amount of carbohydrate.

2.4.5 Determination of total polyphenol content

The total polyphenol of the extracts was determined by spectrophotometric method (Dewanto *et al*.,2002). Three hundred micro litre of the methanolic extract prepared above was introduced into test tubes followed by the addition of 1.5 mL of a Folin-Ciocalteau's reagent (10X dilutions) and 1.2 mL of sodium carbonate (7.5% w/v) and vortexed for 15 sec and incubated for 30 minutes at 40°C for color development. The absorbance was measured at 760nm against the blank without sample using spectrophotometer. Total polyphenolic content was expressed as mg tannic acid equivalent per gram of dry weight (mg TAE/g dry weight) through the standard graph prepared with tannic acid, ranging from 10 to 100 ug/ml.

3. Results and Discussion

The results of the experiment revealed that, appreciable results were obtained in methanol extract in both the methods tested. In disc diffusion method of methanol extract, the zone of inhibition was noticed for the majority of the pathogens tested and the zone of inhibition ranged from 5mm to 20mm. When the extracts were tested in well diffusion method of methanol extract, maximum zone of inhibition (28 mm and 20 mm) obtained in ethyl acetate and methanol against *S. aureus* and the zone of inhibition ranged from 20mm to 2mm.

Table -1: Antibacterial activity of green macro algae *Nitella tenuissima* (Desv.) Kiitz

S. No	Bacteria	Zone of inhibition in mm by different solvent extracts									
		Methanol		Ethanol		Distilled water		Ethyl acetate		Chloroform	
		*	#	*	#	*	#	*	#	*	#
1	*Staphylococcus aureus*	20.0	20.0	5.0	3.0	-	-	7.0	28.0	-	-
2	*Bacillus cereus*	20.0	15.0	2.0	5.0	-	-	8.0	15.0	12.0	16.0
3	*Streptococcus mutans*	10.0	6.0	8.0	8.0	-	-	6.0	15.0	7.0	8.0
4	*Citrobacter koseri*	5.0	2.0	2.0	15.0	-	-	7.0	17.0	-	-

*-Paper Disc method; #- Well Diffusion method; - = No activity.

3.1 Disc diffusion method

The zone of inhibition of the extract (200µg/disc) was observed as follows in methanol extract, 20 mm against *S.aureus* and *B. cereus*, 10 mm against *S. mutans* and 5mm against *C. koseri*. In chloroform extract, 12mm and 8mm against *B. cereus* and *S. mutans* and no zone was formed around *S. aureus* and *C. koseri*. In ethyl acetate extract, 8mm against *B. cereus*, 7mm against *S.aureus* and *C. koseri* & 6mm against *S. mutans*. In ethanol extract, 8mm against *S. mutans*, 5mm against *S.aureus*, 2mm against *B. cereus* and *C. koseri*. In distilled water extract, inhibition zone was not formed. In this method, the zone of inhibition ranged from 20 mm to 2 mm. (Table -1)

3.2 Agar well-diffusion method

The zone of inhibition of the extract (200µg/well) was observed as follows. In methanol extract, 20 mm against *S.aureus*, 15mm against *B. cereus*, 6mm against *S. mutans* and 2mm against *C. koseri*. In chloroform extract, 16mm against *B. cereus*

and 8 mm against *S. mutans* and no zone was formed around *S. aureus* and *C. koseri*. In ethyl acetate extract, 28mm against *S.aureus*, 17mm against and *C. koseri* and 15 mm against *B. cereus* and *S. mutans*. In ethanol extract, 15mm against *C. koseri S.aureus*, 8mm against *S. mutans*, 5mm against *B. cereus*, and 3 mm against *S.aureus*. In distilled water extract, inhibition zone was not formed. In this method, the zone of inhibition ranged from 28mm to 2mm (**Table -1**).

Table -2: Phycochemistry of green macro algae *Nitella tenuissima* (Desv.) Kiitz

S.no.	Parameters	Result
1	Protein*	0.8µg/mg
2	Total Carbohydrate#	4.4 g/mg
3	Chlorophyll a#	1.14mg/l
4	Chlorophyll b#	0.97mg/l
5	Total chlorophyll#	0.28 ug /1.5mg
6	Total carotenoid#	1.40mg/l
7	Total polyphenol#	13.0 mg TAE/g dry weight

* Fresh algal sample; # dried algal sample.

Among the four extracts used in the present study, the activity was in the order methanol >ethyl acetate>ethanol >chloroform> distilled water (Table -1) agreeing with observations of (Vijaya Parthasarathy *et al*.,2004) that methanol is a better solvent for algal extraction and separation of variety of phycochemicals that produce maximum inhibitory effect on both gram positive and gram negative bacteria. The present study reveals the poor activity of water extract in the algal species, agreeing with earlier reports (Hodgson, 1984) that the use of organic solvents is always better for extraction compared with water extraction. Solvent controls recorded no inhibition zone in both the method and was not given in table.

Over the last decades there has been a lot of work in the area of elucidating the active principle of herbal medicines and synthesizing the active constituent for medical use. Research has shown that a number of potent herbs don't show activity or show reduced activity after separation and synthesis of the active principles (Cowan.,1999). An earlier study reported the Anti-bacterial activity of methanol extract of freshwater microalgae including *Chara contraria and Nitella flexilis* (Omulokoli *et al*.,1997). Which inhibited the growth of *Bacillus cereus a*nd *Corynebacterium diphtheria w*hich belongs to phylum charophyta also tue species of this study belongs to. Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria (Omulokoli *et al*.,1997). As the macro algae used in this study possess antibacterial activity, we can elucidate active alkaloid composition of this species.

3.3 Phycochemical analysis

The results of the phycochemical study revealed that protein, carbohydrate, Chlorophyll a, Chlorophyll b, total chlorophyll, carotenoid and total polyphenol content was 0.8µg/mg, 4.4 g/mg, 1.14mg/l, 0.97mg/l, 0.28ug /1.5mg, 1.40mg/l and 13.0 mg TAE/g dry weight, respectively. (Table -2) The presence of phycochemicals can be attributed to the complex mixture of phycochemicals which possess antioxidant, antimicrobial, anticancer and antiviral activity. The compounds responsible for these activities include phenolic compounds, sulphated polysaccharides and organic acids (Liu, 2003; Podsedek,2007).

4.0 Conclusions

It is concluded from the present investigation that, methanolic extract of fresh water macroalgae *Nitella tenuissima* (Desv.) Kiitz extract as antibacterial agents would be useful in controlling various pathogenic bacteria instead of synthetic products which causes hazardous problems. As the methanolic extract of *Nitella tenuissima* (Desv.) Kiitz had shown appreciable anti-microbial activity, we can identify active alkaloid contents, a step forward towards pharmacological application in future studies. The results also showed the

presence of protein, carbohydrate, chlorophyll, carotenoid, polyphenol content. As polyphenol content is present, antioxidants may also be present, Further studies on antioxidant activity are needed to confirm it.

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6.0 References

Kamble, S. M. and Chavan A.M.2010. Antibacterial activity of some fresh water algae, *Journal of Experimental Sciences*, 1(2):05-06.

Rajasulochana, P., Dhamotharan, R., Krishnamoorthy, P. and Murugesan, S. 2009. Antibacterial activity of the extracts of marine red and brown algae. *Journal of American Science*, 5: 20-25.

Spratt, B.G. 1994. Resistance to antibiotics mediated by target alterations. *Science*, 264: 388-393.

Davis, R. D. 1994. The gift of dyslexia, Berley Publications, New York.

Wray, F.O. and Tsuda R.T. 1977. Bibliography of marine benthic algae in Micronesia. *Micronesica* 13: 85-120.

Buyukokuroglu, M.E., Gulein, I., Oktay, M. and Kufrevioghu, O.I. 2001. *In vitro* antioxidant properties of dantrolene sodium. *Pharmacol.Res*., 44:491-494.

Shameel, M. 1990. Phycochemical studies on fatty acids from certain seaweeds. *Bot. Mar.*,33:429-432.

Wood, R.D. 1964. A synopsis of the Characeae, *Bulletin of the Torrey Botanical Club* 91(1):35-46.

Karol, M.C., Court, R.M., Climino, M.T., and Delwiche.C.F. 2001. The closest living relatives of land plants. *Science*, 294:235(1-3).

Attaie, R., Whalen, P.J., Shahani, K.M. and Amer, M.A. 1987. Inhibition of growth of staphylococcus aureus during production of acidophilus yogurt. *J. Food Prot*., 50(3):224-228.

Lichtenthaler, H. and Wellburn, A. 1985. Determination of total carotenoids and chlorophylls A and B of leaf in different solvents. *Biochem. Soc. Trans.*, 11: 591-592.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids, the pigments of photosynthetic biomembranes. In: Douce, R., Packer, L. (eds) *Methods Enzymol.*, 148. Academic Press Inc, New York, pp. 350-382.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., 72: 248-254.

Dubois, M.., Gills, K.A., Hamilton, K., Rober P.A and Smith, F. 1956. Colorimetric method of determination of sugars and related substances. *Anal chem*., 28:1-350.

Dewanto, V., Wu, X., Adom, K.K., Liu, R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food.chem.*, 50: 3010-3014.

Vijaya Parthasarathy, M. D., Prema, M. and Krishnamurthy, A. 2004. Preliminary report on the antibacterial activity of extracts from spirogyra sp. Zygnematacea - Chlorophyceae. *Indian Hydrobiology*, 7: 229-233.

Hodgson, L.M. 1984. Antimicrobial and antineoplastic activity in some South florida Seaweeds. *Botanica Marina*. 28: 387-390.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*., 12: 564-582.

Omulokoli, E., Khan, B. and Chhabra, S. C 1997. Antiplasmodial activity of four Kenyan Medicinal plants. *J. Ethno pharmacol*., 56:133-137.

Liu, R. H. 2003. Health Benefits of Fruit and Vegetables are from Additive and Synergistic Combinations of Phytochemicals. *American Journal of Clinical Nutrition*, 78 (3): 517S-520S.

Podsedek, A. 2007. Natural Antioxidants and Antioxidant Capacity of *Brassica* Vegetables: A Review. Lwt-*Food Science and Technology*, 40 (1): 1-11.