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# Biochemical and Antibacterial Studies on Green Algae of Visakhapatnam Coast

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**Biochemical and antibacterial activities were investigated with the crude extracts of five green algal species viz., *Ulva fasciata*, *Enteromorpha compressa*, *Caulerpa racemosa*, *Caulerpa taxifolia* and *Cladophora patentiramea* to evaluate the total carbohydrate, protein and lipid content and their antibacterial activity against gram positive and gram negative bacteria. Carbohydrate content was found to be high in *U. fasciata and C. racemosa*, whereas protein and lipid contents were high in *Caulerpa* species. Chloroform – methanol, n-butanol and diethyl ether extracts of all five green algal species showed different levels of antibacterial activity against *Escheriachia coli, Klebsiella pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis*.**

**Key words:** Green algae, Antibacterial activity, Biochemical studies, Visakhapatnam seacoast.

Visakhapatnam coast (Lat. 17$^0$41' N Lon. 83$^0$ 17' E.) supports the luxuriant growth of more than 100 different species of macro algae.[1-3] As a consequence of an increasing demand for biodiversity in the screening of natural products for nutrients and antibiotics, there is a greater interest in marine organisms. Seaweeds especially green algae are low in fats, rich in proteins, carbohydrates, vitamins and natural antioxidants, which are not found in land plants.[4-6] Most of the green algal species are lithophytes and grow in the littoral and sub littoral zones of Visakhapatnam coast. Macro algae are important source of primary and secondary metabolites and they are potential source of many bioactive compounds.[7-11] The anti-bacterial activity in organic solvent extracts of six species of marine algae against multi-antibiotic resistant bacteria was reported by Marasneh[12], Sastry *et al.,*[13] and Bushra Begum *et al.,*[14] also reported the antibacterial activity in the organic solvent extracts of seaweeds against Gram-positive and Gram-negative pathogenic bacteria. Keeping this in mind, five green algal species viz., *Ulva fasciata*, *Enteromorpha compressa*, *Caulerpa racemosa*, *Caulerpa taxifolia* and *Cladophora patentiramea* were collected from different

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stations of the Visakhapatnam seacoast and their total carbohydrate, protein, lipid contents and antibacterial activities were determined.

## MATERIAL AND METHODS

### Work stations

Work stations were same as in our earlier studies.[15]

### Algal samples

*Ulva fasciata*, *Enteromorpha compressa*, *Caulerpa racemosa*, *Caulerpa taxifolia*, *Cladophora patentiramea* were collected during low tide from four different stations of Visakhapatnam coast in sterile polyethylene zip lock bags and immediately transferred to laboratory. The algal samples were washed thoroughly with sterilized seawater followed by double distilled water to remove any adherent material and then samples were shade dried at room temperature and used for the preparation of aqueous and organic solvent extracts.

### Bacterial strains

Antibacterial activity of crude extracts of algal species were tested against the Gram-negative strains of *Escherichia coli* (ATCC – 11775), *Klebsiella pneumoniae* (ATCC – 13883), and Gram-positive strains of *Bacillus subtilis* (ATCC – 6051), *Staphylococcus aureus* (ATCC – 12600).

### Preparation of algal extracts

Shade dried samples were ground into fine powder. These powdered samples were used in the preparations of 10% (w/v) aqueous and organic extracts. Aqueous extract was prepared with double distilled water and organic extract were prepared with diethyl ether, chloroform-methanol mixture (2:1) and n–butanol. The crude extracts were evaporated to dryness under reduced pressure at 40$^0$C. The dry residue samples were

**Table 1.** Antibacterial activity of Green algal species of Visakhapatnam seacoast

| Seaweed species | Formulations | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | *E. coli* | *K. pneumoniae* | *B. subtilis* | *S. aureus* |
| *Enteromorpha compressa* | Aqueous | - | - | - | - |
| | Diethylether | 10 | - | - | - |
| | Chloroform-methanol | 12 | + | 12 | + |
| | n-butanol | + | - | + | 14 |
| *Ulva fasciata* | Aqueous | - | - | - | - |
| | Diethylether | 10 | - | - | 14 |
| | Chloroform-methanol | 14 | - | + | 10 |
| | n-butanol | - | - | - | - |
| *Caulerpa racemosa* | Aqueous | - | - | - | - |
| | Diethylether | 11 | + | - | - |
| | Chloroform-methanol | 12 | + | - | + |
| | n-butanol | - | - | 14 | + |
| *Caulerpa taxifolia* | Aqueous | - | - | 10 | 11 |
| | Diethylether | 12 | - | - | - |
| | Chloroform-methanol | 13 | 10 | 17 | 13 |
| | n-butanol | + | + | - | - |
| *Cladophora patentiramea* | Aqueous | - | - | - | - |
| | Diethylether | 11 | - | - | - |
| | Chloroform-methanol | 12 | 10 | 10 | + |
| | n-butanol | - | - | - | - |

-No activity; +Inhibition zone < 10mm

separately weighed, dissolved and diluted in respective solvents to obtain a final concentration of 1 mg/ml.

**Estimation of total carbohydrates**

Total carbohydrate content of green algal species was estimated by phenol–sulphuric acid method[16] using glucose as standard.

**Estimation of total lipid content**

Total lipid content of green algal species was estimated by Bligh and Dyer method.[17]

**Estimation of total Protein**

Total protein content of green algal species was estimated by method[18] using bovine serum albumin (BSA) as standard.

**Antibacterial activity**

Antibacterial activities of green algal species were determined by agar well diffusion method.[19] Nutrient agar plates were prepared by pour plate method. To the molten sterile nutrient agar medium (40°C - 45°C) 1.0 ml growth culture of concerned test organism ($1\times10^8$ cells) was mixed thoroughly and poured into sterile flat bottomed petridish (6.0 cm diameter) and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and 50 µl of seaweed extract (1.0 mg /ml) was added to each well aseptically and plates were incubated at 37°C for 24 hours and the zone of inhibition was determined. Respective pure solvents were used as controls.

## RESULTS AND DISCUSSION

The total carbohydrate, lipid and protein contents of *U. fasciata*, *E. compressa*, *C. racemosa*, *C. taxifolia* and *C. patentiramea* are presented in Fig. 1-3 respectively. Out of the five green algal species studied *U. fasciate* and *C. racemosa* was found to contain higher content of carbohydrate whereas *C. taxifolia* and *C. racemosa* were found to contain high content of lipid and protein respectively. Samples collected from stations I & II have been found to contain relatively low amount of carbohydrate, lipid and protein content compared to the samples from

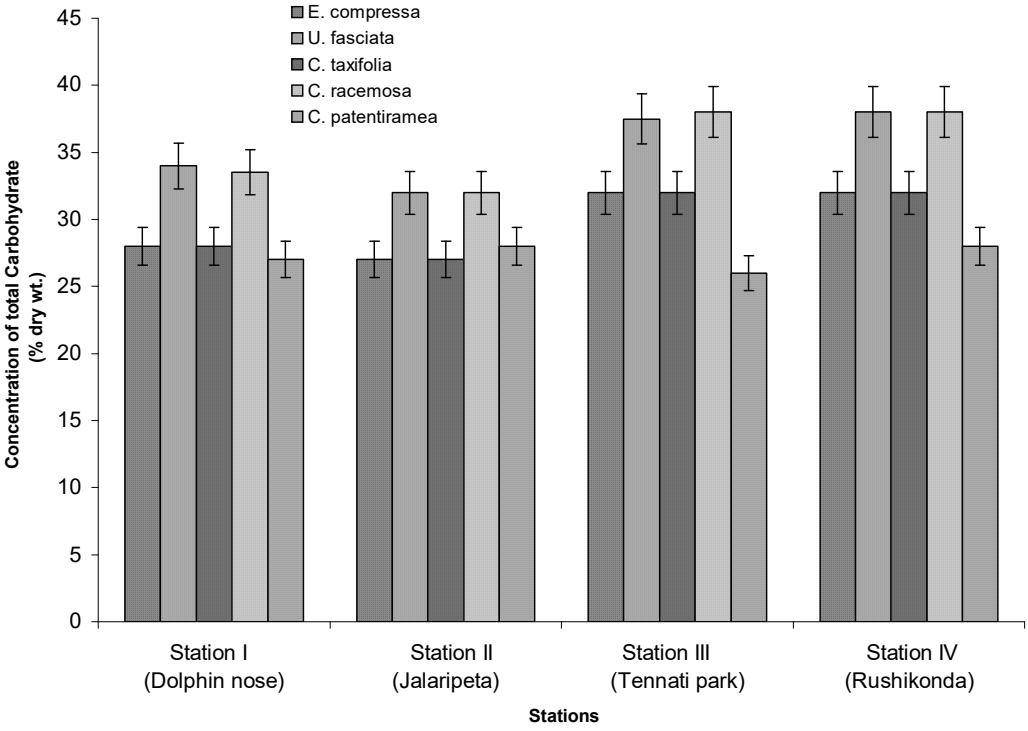


**Fig. 1.** Total carbohydrate content of green algal species from four different stations of Visakhapatnam seacoast

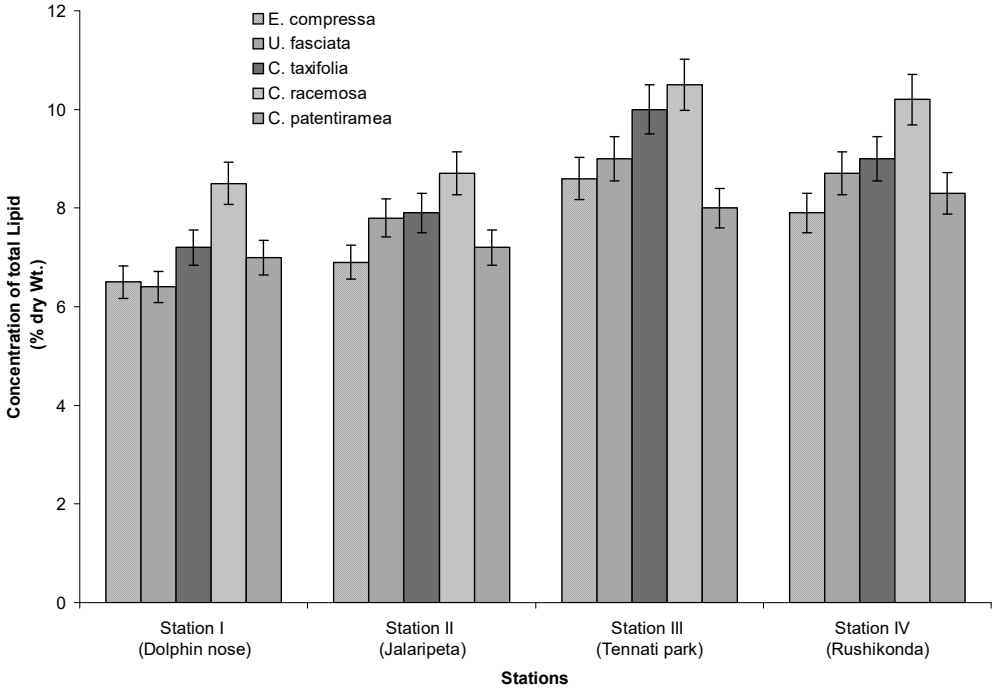


**Fig. 2.** Total content of green algal species from four different stations of Visakhapatnam coast

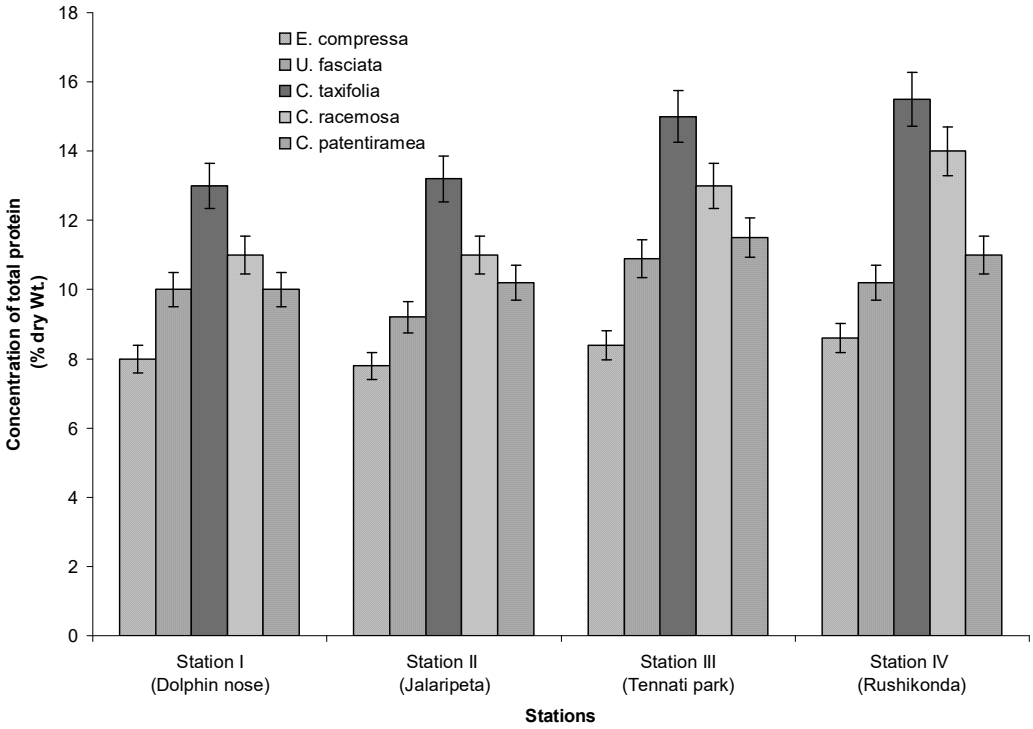


**Fig. 3.** Total protein content green algal species from different stations of Visakhapatnam seacoast

stations III and IV. This may be due to the effect of industrial effluent and sewage pollutants on carbohydrate, lipid and protein metabolism[20-21].

Antibacterial activity of the green algal extracts is presented in Table 1. Out of five green algal species studied the aqueous extract of *C. taxifolia* exhibited moderate activity against gram positive bacteria. Whereas, organic solvent extracts of all five green algal species found to showed different degrees of antibacterial activity. Chloroform-methanol extract of all species exhibited activity against gram positive and negative bacteria tested. Most significant antibacterial activity was observed with chloroform-methanol extract of *C. taxifolia* against all four bacterial strains tested. Whereas, Diethyl ether extract of all five green algal species exhibited activity against *E. coli* and and n-butanol extract of *E. compressa* and *C. recemosa* exhibited antibacterial activity against *S.aureus* and *B. subtilis* respectively. Henry [22] and McGaw [23] showed that both saturated and unsaturated fatty acids of plant source exhibited antibacterial activity against different bacterial strains.

In the present study the significant antibacterial activity of chloroform-methanol extract of green algal species may be due to presence of fatty acid or other hydrophobic compounds. This work provides scope for ethno-medicinal investigations on green algal species of Visakhapatnam coast. Further work is needed to identify the active principle(s) present in organic solvent extracts.

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## REFERENCES

1. Umamaheswara Rao M and Sreeramulu T. An annotated list of the marine algae of Visakhapatnam (India). *Bot J Linn Soc.,* 1970a; **63**: 23-45.
2. Umamaheswara Rao M and Sreeramulu T. The fruiting behaviors of some marine algae of Visakhapatnam (India). *Bot Mar.,* 1970b; **13**: 47–49.
3. Umamaheswara Rao M Additions to algal flora of Visakhapatnam coast. *Phykos*., 1999; **38**: 113–126.
4. Dangeard, L. Nutritional properties of microalgae potentials and constraints. In: Hand book of microalgal mass culture. Richmond. A (ed), CRC Press, Florida, USA. 1940; 339-358.
5. Chapman, V.J., and Chapman, D.J. Seaweeds and their uses. Chapman and Hall, London, 1980; 66.
6. Lahaye M and Kaffer B. Seaweed dietary fibers structure physicochemical and biological properties relevant to intestinal physiology. *Sci Ailments.,* 1997; **17**: 563-564.
7. Faulkner, D.J. Marine natural products. *Nat Prod Rep.,* 1986; **3**: 2-33.
8. Bhakuni,D.S., B.N, Dhawan., H.S, Gray., A.K., Goe., B.N., Mehrota., R.C., Srimal and M.N., Srivastava, Bioactivity of marine organisms: Part VI – Screening of some marine flora from Indian coast. *Indian J. Exp. Biol.,* 1992; **30**: 512-517.
9. Jose Vittor M, Lima–Filho, Ana FFU Carvalho, Sissi M Freitas and Vanta MM Melo. Antibacterial activity of extracts of six macro algae from the north eastern brazilian coast. *Braz J Microbiology*., 2002; **33**: 1-3.
10. Blunt JW, Copp BR, Munro MH, Northcote PT and Prinsep MR. Marine natural products. *Nat Prod Rep* 2003; **20**: 1-48.
11. Venkata Raman B, Rao DN and Radhakrishnan TM. *Enteromorpha compressa (L) greville* an edible green alga as a source of anti-allergic principle(s) *Ind J Clin Biochem* 2004; **19**: 105-109.
12. Marasneh I, Jamal M, Kashasneh M and Zibdeh M. Antibiotic activity of marine algae against multi-antibiotic resistant bacteria. *Microb*., 1995; **83**: 23-26.
13. Sastry V.M.V.S and Rao G.R.K. Antibacterial substances from marine algae- successive extraction using benzene, chloroform and methanol. *Bot Marina*., 1994; **37**: 357-360.
14. Bushra Beegum,N.R and T, Ganga Devi. Antibacterial activity of selected seaweeds from Kovalam southwest coast of India. *Asian J. Microbiol. Biotech. Envi. Sci.*, 2003; **5**: 319-322.
15. Rajagopal, S.V., Radhakrishnan, T.M and Venkata Raman, B. Studies on biochemical and antibacterial activities of red algae of Visakhapatnam seacoast. *Asian J. Microbiol, Biotech and Envi Sci*., 2006; **8**(1): 115-118.
16. Krishnaveni, S., T. Balasubramanian and S.

Sadasivam. *Food Chem*., 1984; **15**: 229.

17. Bligh and Dyer method of total lipid estimation In: Jayaraman, J. Laboratory Manual in Biochemistry, Wiley Eastern Limited, New Delhi. 1996; pp. 96 – 97.
18. Lowry,O.H, N.J. Rose Brough., A.F. Farr and R.J. Randall. *J Biol Chem.,* 1951; **193**: 256-275.
19. Perez, C., M. Pauli and Bazereque, P. An antibiotic assay by the agar well diffusion method. *Acta Biologiac et Medicine Experimentalis*., 1990; **15**: 113-115.
20. Umamaheswara Rao, M., S.B.K. Murthy and V. Mohanchand. *Indian J. Bot.,* 1984; **7**: 50.
21. Umamaheswara Rao, M., V. Mohanchand and S.B.K. Murthy. In marine plants; their biology, chemistry and utilization, edited by V. Krishnamurthy & A.G. Untawale, Seaweed Res. Util. Assoc, Madras. 1985; 109.
22. Henry, G.E., R.A. Momin., M.G. Nair and D.L Dewitt. Antioxidant and cyclooxygenase activities of fatty acids found in food. *J Agri Food Chem*., 2002; **50**: 2231-2234.
23. McGaw, L.J., A.K. Jager., J and Van Staden. Isolation of antibacterial fatty acids from *Schotia brachypetala*. *Fitoterapia.,* 2002; **73**: 431-433.