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Determination of Essential Elements, Total Protein and Evaluation of Antifungal Activity of A Marine Alga *Codium Laevigatum* M Nizamuddin (Chlorophyta) Collected From the Coastal Areas of Pakistan

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Abstract: Dissimilar extract of ethanolic extract of *Codium laevigatum* (Chlorophyta) screened for antifungal activity against 5 fruit spoiling fungi . All extract inhibited fungal growth. Chloroform extracts showed maximum inhibition ratios followed by methanol, ethyl acetate, aqueous and ethanol extracts. Elemental analysis *Codium laevigatum* was carried out for 11 elements *viz.*, Calcium Ca, Cadmium, Cd, Chromium Cr, Copper Cu, Iron Iron Fe, K, Potassium Mn, Magnesium Mg, Nickel Ni, Lead Pb , Zinc Zn , Cr accumulation was the lowest in *Codium laevigatum* , while Mg was present with highest concentration. Total protein content of *Codium laevigatum,* was calculated as 10.3 % .

Keywords: Antifungal Activity, Marine Alga, Chlorophyta, trace metal ions.

1. INTRODUCTION

Seaweeds have been used as medicine for the treatment of different diseases. Chemical analysis of marine algae is a valuable source of lipid, protein and carbohydrates on commercial scale. (Chapman and Chapman 1980).Chemical analysis of Marine algae has implied as the cheapest dietary source for human beings. (Abbot, 1988). The algal proteins has shown beneficial effects on human nutrition in their health (Sultana *et al.*, 1992).The fungal spoilage of post-harvest and losses of fruits has attached attention for the food safety, Mycotoxin contamination of foods has potential health hazards and are cited (Shun-ichi, 2005).As reported post harvest losses of fruits due to pests and diseases in the field, storage, and market usually amount 25% to 50 % total production. (Harvey, 1978) Seaweeds as fungicide protect fruit from different pathogens (Washington *et al.*, 1999). While some trace elements are considered toxic viz., As, Br, Cd, Hg, Pb, Sb, others are essential (Cu, Zn) for growth of human body (Moauro *et al.*, 1993). Marine algae are rich in protein therefore the dietary primary product is economic (Dave and Parekh, 1975). In the view of the above mention importance of seaweeds, the chemical composition protein elements and then fungal inhibitory activity is important.

2. MATERIALS AND METHODS

Collection of fruit spoiling fungi: *Aspergillus flavus* Link ex Gray, *Aspergillus. niger* Van Tieghem, *Aspergillus. ochraceus* K. Wilh, *Penicillium funiculosum* Thom and *Phytophthora cetricola Sawada* were isolated from the rotten and spoiled fruits obtained from vegetable markets and cold storages of Nawabshah and Hyderabad, Pakistan. The infected tissues of fruit surface sterilized with 2% bleach for 10 minutes were inoculated on Potato Dextrose Agar (PDA) (Merck) medium in Petri plates. The fungi were maintained on (PDA) slants at 10-25^{o}C and subculture in the same member was keep on monthly basis throughout this study.

Collection of seaweeds: The seaweed *Codium laevigatum M* Nizamuddin *was* collected during April 2005 from Sandpits, Manora, Buleji, Paradise-point and Hawks Bay costal areas of Karachi, Pakistan. *Codium laevigatum* sample was collected and preserved after mounting on a herbarium sheet and the another sheet was amount by Prof Mustafa Shameel. The samples were washed thoroughly with seawater followed by fresh and distilled water to remove the sand particles and epiphytes. They were shade dried at room temperature for 15 days and used for the extractions.

Extractions: 1 kg of dried plant material was dipped in ethanol for 20 days. The solution was Filtered using No. 42 Filter paper and concentrated under reduced pressure using rotary evaporator and the extract as syrupy liquid. From this residue five different extracts i.e. ethanol, methanol whereas ethyl acetate, chloroform, aqueous extract were shaken vigorously in successive manner by using separating funnel. the extracts were saturated under reduced pressure.

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Bioassay: All test isolates were inoculated onto Potato Dextrose Agar (PDA) plates and incubated at 25 °C for 6-10 days to obtain actively growing cultures consisting of mycelia and conidia. Antifungal activity was carried out by the strip platting method. Two gram of each extract was dissolved in 10 ml of its respective solvent. Mycelial discs, 5 mm diameter, cut from the periphery of the young growing cultures of test fungi, were aseptically transferred to the PDA medium in Petri plates. Four blotting paper strips (each 3X1.24 cm size) were dipped in 1ml extract of known concentration and left for the solvent evaporation in vacuum chamber. The impregnated strips with extract fraction were placed around the mycelial disk of test fungi. Sterilized strips were placed around the test fungi. Colony diameter was recorded after the 72 hours of each test and compared with respective controls. Three replicates were used for each treatment.

Digestion of the seaweeds: The algal material was initially dried under shade at room temperature and later on in an oven at 60-80 ºC for 1 hour. Elemental assay: The samples were investigated for elemental analysis by using atomic absorption spectrophotometer (AAS), Hitachi Ltd. 180-50.S.N5721- at National Center of Excellence for Analytical Chemistry University of Sindh, Jamshoro. Appropriate working standard solution was drawn for each element. The calibration curves were obtained for concentration vs absorbance. The data were statistically analyzed by using manipulation fitting of straight line by least square method. All elements were determined in seaweeds under this investigation procedure. A blank reading was also taken and necessary correction was made during the calculation of percentage concentration of various elements.

Total protein analysis: The protocol of ISI-24-1-e (Amina *et al.,* 2007) was used for the determination of total nitrogen which was calculated using a nitrogen conversion factor of 6.25.

3. RESULTS AND DISCUSSION

Antifungal activity: The maximum inhibition activity was observed against P. funiculosum 81% in chloroform extract .The maximum inhibition activity was observed against test fungi, *A. ochraceus* 80% in ethyl acetate extract: The maximum inhibition activity was observed against test organism *P. funiculosum* 78% and methanol extract: showed the 73% in ethanol extract against the *A. ochraceus* and minimum antifungal activity showed the minimum inhibition against *A nigier* 20% Ethyl acetate extract

As cited in literature, the eighty two macroalgae 18 chlorophyceace, 25 pheophyceace and 39 rhodophyceace indicate the antimicrobial and antifungal activity against 3 gram (+) positive and 2 (–) negative bacteria, one yeast using the agar diffusion *(*Salvador *et al.,* 2007) Forty species of seaweeds were collected from Karachi coast and several biological tastes were conducted on them in order to investigate their antimicrobial, antifungal, phytotoxic and insecticidal activities, Brown seaweed were shown having greater antibacterial activity than the green and red ones out of the screened algal species. *Chaetomorpha antennina, Gracilaria foliifera,Jolyna laminarides* exhibited greatest antifungal activity (Rizvi *et al.,* 2003)

Elemental analysis: *Codium laevigatum* from the different locations of Karachi coast was analyzed for the composition of Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn elements (Table1). The amount of iron was the highest among them Iron (Fe) 2694.6 m/kg Potassium (K) 12007 mg/kg , Calcium (Ca) 19710.3, m/kg Magnesium (Mg) 497.2, mg/kg, Zinc (Zn) 337 mg/kg, Manganese (Mn) 22.67, mg/kg, Copper (Cu) 15.22, Nickel (Ni) 8.8 mg/kg Cadmium (Cd) 5.0 mg/kg Lead (Pb) 2.11, mg/kg Chromium Cr 1.9 mg/kg The amount of Iron was the highest among them. Mg varied according to the collection point of *Codium lavigateum* maximum amount calculated as 2694.6Mg/Kg ,of Iron was present in the samples of seaweed collected from Buleji and minimum amount 15961.0 mg/kg was present in the samples from Manora. The concentration of Fe, K, Ca, Mg, Mn and Cu was higher than other elements and the amount of Cd, Ni, Pb and Cr was minimum from 1 to 8 mg/kg, whereas Cu and Mn was 11 to 15 mg/kg and Zn was 290 to 337 mg/kg in *Codium lavigateum* from the Karachi coast. 2001) if we compare with the pervious reported data then the Ca is present in highest as 80750 ppm and lowest as 6880 ppm (Rizvi and Shameel, 2001), The concentration of Mg, K, Fe, Zn was higher than other elements and the amount of Cd, Ni, Pb and Cr was minimum from 1 to 7 ppm, whereas Cu and Mn was 11 to 20 ppm and Zn was 122 to 411 ppm as cited in *S. robusta* from the Karachi coast.(Amina, *et al.,* 2007) however the present studies showed maximum amount of Iron element in *Codium laevigatum*. The difference in the elemental could be assigned as the ecological and geophysical factors,

4. CONCLUSION

Total protein analysis: Total protein contents of the sample collected from different location on dry weight basis showed 25% (Sandpits), 20% (Manora), 19% (Buleji and Paradise-Point, Costal area Karachi, Pakistan) and 17.35% The highest value of total protein

recorded was 25% (Sandpits), in *Codium laevigatum* from sites of (Sandpits), coast of Karachi comparing this data with total protein contents on dry weight basis shown 32% (Sandpits), 29% (Manora), 25% (Buleji and Paradise-Point) and 29.45% from Hawks Bay sites in *S. robusta* of Karachi coast. (Khanzada, *et al.*, 2007) which is quite comparable with the problem contains results of *Codium laevigatum*.

Table -1. Elementolgy *of Codium laevigatum* Nizamuddin

S. No	Name Of Elements	Symbol	Amount mg/ Kg
1	Calcium	Ca	19710.
2	Cadmium	Cd	5.0±
3	Chromium	Cr	1.9±
4	Copper	Cu	15.22
5	Iron	Fe	2694.6
6	Lead	Pb	2.11
7	Potassium	K	12007
8	Magnesium	Mg	497.2
9	Manganese	Mn	22.67
10	Nickel	Ni	8.8
11	Zinc	Zn	337

REFERENCES:

Abbot, I. A. (1988). Food and products from algae. In: *Algae and Human Affairs.* (Eds.): C. A Lembi and. J. R. Waaland, (2004) Cambridge Univ. Press, Cambridge. 135-147.

Anonymous (1985) The Wealth of India (Raw Materials). Vol. 1A. New Delhi, CSIR, 138–159.

Chapman, V. J. and D. J. Chapman. (1980). *Seaweeds and their uses.* Chapman and Hall, London.3rd Edition. 315- 334.

Dave, M. J. and R. G. Parekh. (1975). Protein content of green seaweeds from Saurashtra coast. *SaltRes. and Ind.*, 11: 41-44.

Eckert, J. W. and J. M. Ogawa. (1985). The chemical control of post harvest diseases: Subtropical and ropical fruits. *Anu. Rev. of Phytopathol*., 23: 421-454.

Harvey, J. M. (1978). Reduction of losses in fresh market fruits and vegetables. *Annu. Rev. Phytopathol.*, 16: 321-341.

Khanzada, A. K., W. Shaikh, T. G. Kazi A. Kabir and S. Kabir. (2007). Antifungal activity, total protein and elemental analysis of seaweed, *Solieria robusta* (Greville) Kylin from the coast of Karachi. *Pak. J. Bot*., 39(3): 931-937

Moauro, A., L. Triolo, P. Avino and L. Ferrandi. (1993). Determination of macro minerals and trace elements in human blood serum. *Dev. Plant. Soil Sci.*, 53: 13-18.

Rizvi, M. A. and M. Shameel. (2003). Biological activity and elementology of benthic algae from Karachi Coast. *Pak. J. Bot.* 35(5): 717-729.

Rizvi, M. A. and M. Shameel. (2001). Distribution of elements in marine algae of Karachi coast. *Pak. J.Bot.*, 33: 357-363.

Sultana, V. S., E. Haque, J. Ara and M. Athar.(2005**)** Comparative efficacy of brown, green and red seaweed in the control of root infecting fungi in okra. International J. Environment. Sci. Tech., 2 (2):129-132.

Shun-ichi, U. (2005) Review Article on Fungal Spoilage of foods and its risk assessment. *Japanese Journal of Medical Mycology*, 46 (1): 1-5.

Washington, W. S., S. Engleitner, G. Boontjes and N. Shanmuganathan. (1999). Effects of fungicides, seaweed extract, tea tree oil and fungal agents on fruit rot and yield in strawberry. *Aust. J. Exp. Agric.*, 39: 487-494.