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ANTIFUNGAL ACTIVITY, ELEMENTAL ANALYSIS AND DETERMINATION OF TOTAL PROTEIN OF SEAWEED, *SOLIERIA ROBUSTA* (GREVILLE) KYLIN FROM THE COAST OF KARACHI

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Abstract

Various fractions of ethanolic extract of *Solieria robusta* (Greville) Kylin (*Rhodophyta*) were screened for antifungal activity against 5 fruit spoiling fungi isolated from fruits. All fractions were able to inhibit fungal growth. Aqueous fraction showed maximum inhibition ratios followed by methanol, ethyl acetate, chloroform and ethanol. Elemental analysis was carried out for 11 elements *viz.*, Ca, Cd, Cr, Cu, Fe, K, Mn, Mg, Ni, Pb and Zn. Accumulation of Cd was the lowest in *S. robusta*, while Mg was present with highest accumulation. Total protein of *S. robusta* was 25 to 32%.

Introduction

Seaweeds are rich and varied source of bioactive natural products and have been studied as potential biocidal and pharmaceutical agents. They are used in traditional remedies in many parts of the world. Extracted substances from seaweeds have antibacterial actions and other properties include antifungal activities and growth inhibition of plants (Abdussalam, 1990; Scheuer, 1990; Rizvi & Shameel, 2003; Su *et al.*, 1973; Burkholder & Sharma 1969; Chapman, 1980; Arasaki & Arasaki, 1983; Abbott, 1988).

From the second half of the 1990s, an increased attention was given to the fungal spoilage of post-harvest and losses of fruits. It was observed that an international concern about food safety, mycotoxin contamination of foods has gained much global attention in recent times owing to its potential health hazards (Shun-ichi, 2005). Post harvest losses of fruits due to pests and diseases in the field, storage, as well as in transit and commercialization can amount up to 25% of the total production in industrialized countries (Harvey, 1978) and in developing countries damage is often higher exceeding 50%, because of the lack of adequate storage facilities (Eckert & Ogawa, 1985). Seaweeds are also known to aid and stimulate growth of vegetables, fruits and also protect them from different pathogens and physiological hazards either *In vivo* or storage conditions (Washington *et al.*, 1999).

It is an established fact that the sea is full of innumerable wealth viz., minerals, vitamins etc., (Ardissione, 1893; De-Toni, 1895). Most of the trace elements present in the algal biomass are heavy metals and algae have been reported strongly active in heavy metals concentration (Whitton, 1884; Forsberg *et al.*, 1988). While some trace elements are considered toxic viz., As, Br, Cd, Hg, Pb, Sb, others are considered essential (Cu, Zn)

or necessary to human body (Cr, Se) but become health hazardous when their intake values are exceeded (Moauro *et al.,* 1993). Marine algae are rich in protein (Dave & Parekh, 1975). It is, therefore, essential to study their chemical composition considering them as a source of protein or a supplementary food and feed. (Lahaye, 1991; Darcay-Vrillon, 1993; Wahbeh, 1997).

During the present study antifungal activities of red alga *Solieria robusta* (Greville) Kylin from the coast of Karachi were observed against most common fruit spoiling fungi (Table 1). In another investigation the amounts of Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn elements were analyzed in *S. robusta* from Sandpits, Manora, Buleji, Paradise point and Hawks Bay areas of Karachi Coast. Total protein (Dry weight) and its variation according to coastal stations in *S. robusta* was also determined.

Materials and Methods

Collection of fruit spoiling fungi: *Aspergillus flavus* Link ex Gray, *A. niger* Van Tieghem, *A. ochraceus* K. Wilh, *Penicillium funiculosum* Thom and *Phytophthora infestans* de Bary (Table1) were isolated from the rotten and spoiled fruits obtained from vegetable markets and cold storages of district Nawabshah and Hyderabad, Pakistan. The infected tissues of fruit surface sterilized with 2% bleach for 10 minutes were inoculated on PDA (Merck) medium in Petri plates. The fungi were maintained on Potato Dextrose Agar (PDA) slants at 10-25^{o}C and subcultured monthly throughout this study.

Collection of seaweeds: The seaweed *S. robusta* was collected during April 2005 from Sandpits, Manora, Buleji, Paradise-point and Hawks Bay costal areas of Karachi, Pakistan. Seaweed sample was collected and preserved after mounting on a herbarium sheet. 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 100% solvent (1:3 *w/v*) for 20 days. The solution was filtered using Wattsman No. 42 paper and concentrated under reduced pressure using rotary evaporator till the extract become syrupy liquid. From this residue five different fractions i.e. ethanol, ethyl acetate, chloroform, methanol and aqueous fractions were obtained by using separating funnel. Solvents were air dried and the extracts were saturated.

Bioassay: All test isolates were inoculated onto Potato Dextrose Agar (PDA) plates and incubated at 25 ^{0}C for 6-10 days to obtain young, actively growing cultures consisting of mycelia and conidia. Antifungal activity was carried out by the strip platting method. Two gram of each fraction was dissolved in 10ml of its respective solvent and different concentration of 20 mg, 2.0, 0.2 and 0.02 mg per ml^{-1} were obtained by using serial dilution method. 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 fraction 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 in control plates. Colony

diameter was recorded after the 72 hours of each test and compared with respective controls. Three replicates were used for each treatment. Percent inhibition was calculated by the formula as under.

$$PI = \frac{\text{Diameter of control colony} - \text{diameter of test colony}}{\text{Diameter of control colony}} \text{ X } 100$$

Digestion of the seaweeds: The algal material was initially dried under shade at room temperature and later on in an oven at 60-80^{o}C for 1 h. One g of manually crushed sample material was carefully dissolved in 10 ml HNO_3 and the sample was left for 8 hours for the digestion. Acid solution of the sample was then heated gently on hot plate at 100-120^{o}C till the sample was nearly dried. When the sample became cool it was again digested with 8ml HNO_3 and H_2O_2 (2:1) and the samples was dried by keeping on hot plate 100-120^{o}C till 1 ml volume of the sample remained. The sample was cooled and diluted by adding 24ml double distilled water making total volume of 25ml. The colour of diluted sample should be bright yellow; otherwise the solution was filtered through Whatman filter paper No.42.

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 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 (Anon., 1999) was used for the determination of total nitrogen which was calculated using a nitrogen conversion factor of 6.38.

Results and Discussion

Antifungal activity: All five fractions of ethanolic extract of *S. robusta* showed activity against fruit spoiling fungi. The quantum of activity exhibited in the fractions of seaweed varied form mild in ethanol to significant activity in aqueous fractions. Aqueous fraction inhibited 99% growth of *A. niger* with 20 mg/ml concentration. Activity continued to the lower concentration of 0.02 mg/ml. Antifungal activity of aqueous fraction was the highest whereas methanol, ethayl acetate and chlorform fractions showed moderate inhibitions with 20mg/ml concentration. All the test fungi, more or less, were inhibited by all fraction of *S. robusta* extract. It was observed that the aqueous fraction retained the highest inhibition ratios at the lower concentrations. During the bioassays of minimum concentration (0.02mg/ml) a significant ($p<0.05$) inhibition of *A. niger* 89%, *P. infestans* 10%, and *P. funiculosum* 9% were observed in aqueous fraction (Table 2). At the minimal concentration (0.02mg/ml) 18%, 14%, 10% and 6% inhibition of *P. infestans*

was observed in chloroform, ethyl acetate and ethanol fractions, respectively. Where, ethanol fraction was the only fraction it could not inhibit the growth of *P. infestans* while the methanol fraction minutely (1%) inhibited *P. funiculosum*. Inhibition ratios increased at lower concentrations of ethyl acetate fraction against *P. funiculosum* and at the higher concentrations inhibition ratio decreased. Aqueous fraction was the dominant and the most active fraction of the ethanolic extract of seaweed with antifungal activity against all test fungi.

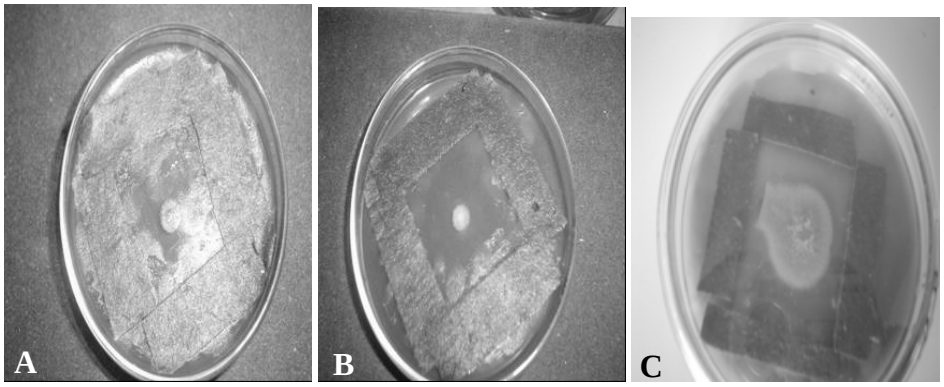

Fig. 1. A: 99% inhibition of *A. niger* in aqueous fraction which is the highest activity amongst all fractions. B: 2nd highest inhibition activity against *P.infestans* by methanol fraction. C: Lowest inhibition activity against *P. funiculosum* by ethylacetate fraction, there are traces of inhibition zones near the proximal areas of impregnated strips. When the concentration lowered the inhibition increased against *P. funiculosum* from 1% to 12%.

Table 1. Collection of fruit spoiling fungi.

S.No.	Host fruit	Collection site	Fungi	Infection %
1.	Banana	Hyderabad vegetable market	*A. flavus*	5
2.	Prunus	Nawabshah vegetable market	*A. niger*	13
3.	Apple	Nawabshah vegetable market	*A. ochraceus*	23
4.	Plum	Dolatpur Safan vegetable market	*P. funiculosum*	1
5.	Mango	Hyderabad cold store	*P. infestans*	2

Solieria robusta (Greville) Kylin, the red alga from the coat of Karachi, Pakistan, inhibited growth of root infecting fungi *Macrophomina phaseolina*, *Rhizoctonia solani* (Sultana *et al.,* 2005) and *Fusarium solani*, human pathogens *Pseudallescheria boydii* and *Trichophyton schoenleinii*, animal pathogenic fungi *Microsporum canis* and *Trichophyton simii* (Rizvi & Shameel, 2003).

Elemental analysis: *S. robusta* from the different locations of Karachi coast was analysed for the compostition of Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn elements (Table 3). The amount of Mg was the highest among them. Mg varied according to the collection point of *S. robusta*, maximum amount 29140.9 ppm of Mg was present in the samples of seaweed collected from Buleji and minimum amount 22961.0 ppm was

present in the samples from Manora. 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 in *S. robusta* from the Karachi coast. Variations of elemental concentrations varied from high in Cr (1.98 - 5.07 ppm) and low in K (17934.0-18695.0 ppm) in *S. robusta* from five stations of Karachi coast. In comparison of previous investigations (Rizvi & Shameel, 2001) where the K was reported in maximum values, present studies showed maximum amount of Mg element in *S. robusta* seaweed. The difference could be ecological, techniques, collection time or as increasing pollution-indicator.

Table 2. *In vitro* antifungal activity of *S. robusta* against fruit spoiling fungi.

Fraction/fungi	Concentrations (mg)			
	20	**2**	**0.2**	**0.02**
		Percent inhibition ± SE		
Ethanol				
A. niger	5 ± 3	0 ± 0	0 ± 0	0 ± 0
A. flavus	12 ± 1.4	0 ± 0	0 ± 0.0	0 ± 0
A. ochraceus	19 ± 3	15 ± 0.8	0 ± 0.6	0 ± 0
P. funiculosum	34 ± 2	5 ± 0	5 ± 0.9	1 ± 0
P. infestans	17 ± 5	4 ± 0.7	2 ± 0.2	0 ± 0
Methanol				
A. niger	18 ± 1	5 ± 1	3 ± 0.5	0 ± 0
A. flavus	2 ± 0.6	1 ± 0.0	0 ± 0	0 ± 0
A. ochraceus	1 ± 0.9	1 ± 1	0 ± 0	0 ± 0
P. funiculosum	19 ± 5	3 ± 6	1 ± 1	0 ± 0
P. infestans	94 ± 3	15 ± 0.9	8 ± 0	6 ± 0.3
Chloroform				
A. niger	60 ± 1	10 ± 0.2	10 ± 0.3	5 ± 0.6
A. flavus	25 ± 0.6	3 ± 0	0 ± 0	0 ± 0
A. ochraceus	15 ± 0.9	8 ± 1	0 ± 0	0 ± 0
P. funiculosum	48 ± 2	12 ± 0.5	0 ± 0.1	0 ± 0
P. infestans	50 ± 2	32 ± 1	18 ± 0.82	18 ± 4
Ethyl acetate				
A. niger	19 ± 5	15 ± 3	0 ± 0	0 ± 0
A. flavus	2 ± 1.2	0 ± 0	0 ± 0	0 ± 0
A. ochraceus	19 ± 3.6	12 ± 8	5 ± 3	0 ± 0
P. funiculosum	1 ± 0.1*	5± 0*	5± 0*	12 ± 7*
P. infestans	89 ± 7	23 ± 5	14 ± 0.23	14 ± 0.3
Aqueous				
A. niger	99 ± 3	90 ± 3	89 ± 3	89 ± 12
A. flavus	30 ± 3	12 ± 3	3± 0.9	1 ± 0.5
A. ochraceus	1 ± 0	1 ± 0.1	0 ± 0	0 ± 0
P. funiculosum	22 ± 7	11 ± 3	9 ± 3	9 ± 1
P. infestans	80 ± 3	45 ± 8	21± 6	10 ± 1.7

*Shows the increase of activity by lowering the concentration of extract.

Table 3. Elemental analysis of *S. robusta* from different areas of Karachi coast (ppm ±SE).

Element	Sandpits	Manora	Buleji	Paradise point	Hawks Bay
Cd	1.59204 ± 0.009	2.1820 ± 0.110	0.97820 ± 0.07	1.68 ± 0.3	1.9812 ± 0.54
Ni	2.24 ± 0.01	2.960 ± 0.081	1.87 ± 0.11	1.85 ± 0.5	2.19 ± 0.75
Pb	4.83 ± 0.2	7.83 ± 0.097	2.68 ± 0.910	3.10 ± 0.6	2.70 ± 1.00
Cr	5.07 ± 0.25	5.18 ± 1.10	1.98 ± 0.87	4.98 ± 0.9	2.37 ± 0.05
Cu	11.43704 ± 0.039	11.9784 ± 0.841	9.77 ± 1.36	10.741 ± 1.2	10.04 ± 0.23
Mn	15.11 ± 0.00085	20.008 ± 1.30	10.25 ± 2.6	13.80 ± 1.65	9.99 ± 0.045
Zn	347 ± 1.87	411.15 ± 10.01	221.0 ± 5.12	122.0 ± 99.3	279.10 ± 0.91
Fe	2036.73 ± 12.33	1822.90 ± 0.01	2551.0 ± 23.18	1996.73 ± 11.01	1987.9 ± 12.59
Ca	2845.94 ± 9.7	2559.990 ± 29.00	3049.0 ± 96.0	2423.00 ± 10.10	2790.30 ± 1.9
K	18165.02 ± 0.9	18021.00± 1101.00	18356.01 ± 211.0	17934.0 ± 29.0	18695.0 ± 0.3
Mg	28774 ± 5	22961.0 ± 2100.00	29140.9 ± 9.0	28941.0 ± 78.0	29101.0 ±25.70

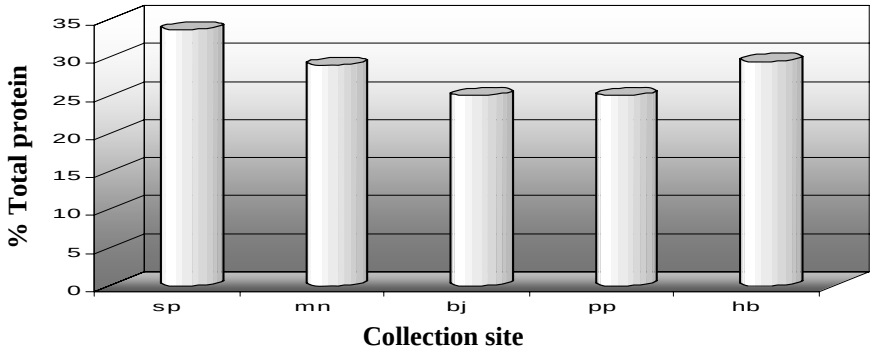

Fig. 2. Total protein (dry weight) analysis of *S. robusta* and variation in the amount of TP from different locations of Karachi Coast. sp=Sandpits, mn=Manora, bj=Buleji, pp=Paradise-Point and hb=Hawks Bay.

Total protein analysis: Total protein contents on dry weight basis showed 32% (Sandpits), 29% (Manora), 25% (Buleji and Paradise-Point) and 29.45% from Hawks Bay sites in *S. robusta* of Karachi coast. The highest value of total protein recorded was 32.62% in *S. robusta*. The highest total protein was reported in *Polysiphonia* sp., and *Ceranium* sp., 25.5%-31%; while the lowest TP in *Gracilaria verrucosa* 0.94% (Dere *et al.*, 2003). The highest value of TP (dry waight) was 35-47% in Division *Rhodophyta* and lowest TP content 5-10% found in Division *Phaeophyta* (Fleurence *et al.*, 1999).

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