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EVALUATION OF BIOCHEMICAL COMPONENT AND ANTIMICROBIAL ACTIVITY OF SOME SEAWEEEDS OCCURRING AT KARACHI COAST

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Abstract

Nutritionally valuable seaweeds are being used as fresh or dried vegetables or as ingredients in wide variety of prepared foods. Interest in seaweeds has increased markedly through out the world due to their value in nutrition and in medicine. In this study 16 seaweed species collected from Karachi coast were examined for the estimation of ascorbic acid (vitamin C), calcium and 9 for protein, carbohydrate and lipid contents. Lipid content was found less as compared to carbohydrate and protein in most of the brown and red seaweeds. All the seaweeds showed the presence of calcium at varying concentrations, while ascorbic acid was found in fourteen seaweeds in considerable amount. Intake of edible seaweeds having ascorbic acid and calcium may protect the human from the diseases related to deficiency of calcium and vitamin C. Microbes and microbial infection remains a threat to human life. In this study, ethanol extract of 5 seaweeds were screened for antibacterial activity, while 9 tested against root rotting fungi. Most of the brown species had shown potent antimicrobial activity followed by red algal species. The highest antibacterial activity was found in ethanol extract of brown seaweed species *Dictyota dichotoma* var *intricata* and *D.indica* against *Salmonella typhimurium* followed by *D. indica* and *Halimeda tuna* against *Bacillus subtilis* with a zone of inhibition of 15, 15, 14, and 14 mm respectively. *Dictyota dichotoma* var. *intricata, D.indica, Sargassum lanceolatum* and red *Melanothamnus afaqhusainii* produced a zone of inhibition of 11, 10, 9, 11mm respectively against gram negative bacteria *Salmonella typhimurium* even at lowest concentration of 2mg/disc. The ethanol extract of most of the species was found less active against tested fungi (*Rhizoctonia solani, Macrophomina phaseolina, Fusarium solani* and *F. oxysporum*). Only two brown seaweeds *Sargassum ilicifolium* and *Cystoseira indica* had shown antifungal activity to some extent.

Introduction

Seaweeds are traditionally used as sea vegetables in Asia but in western countries they have been used as gelling or thickening agents. From nutritional point of view they are low calorie food, with high concentration of minerals (Mg, Ca, P, K and I), vitamins, proteins, indigestible carbohydrates and low concentration of lipids (Jimenez Escrig & Cambrodon, 1999; Chapman & Chapman 1980, Qasim, 1991). Seaweed mineral content is higher than that of land plants and animals (Ito & Hori, 1989, Ortega-Calvo *et al*., 1993). The mineral fraction of some seaweeds accounts for upto 36% of dry weight. Among all the minerals calcium plays a vital role in maintaining structure of the body, calcium may ranges from 60-226mg/ 100 g of seaweeds (Fajarado *et al.*, 1998; Risso *et al.*, 2003). Whereas quality of protein and lipids in seaweeds is acceptable compared with other vegetables, mainly due to their high content in essential amino acids and their relative high levels of unsaturated fatty acids (Jimenez-Escrig & Cambrodon, 1999). The protein content of brown seaweeds is generally less, where as higher protein contents are recorded in green and red seaweeds (Burton, 2003). Lipids represent low percentage of dry algal material and show interesting polyunsaturated fatty acid composition (Burton, 2003). Human body requires vitamin C for normal physiological functions. It helps in the metabolism of tyrosine, folic acid and typtophan, lowers the blood cholesterol and contributes to the synthesis of the amino acids and is needed for tissue growth and wound healing (Iqbal *et al.*, 2004). Ascorbic acid, the accepted name for vitamin C is found in citrus fruits, peppers, strawberries, tomatoes, broccoli, turnip and other leafy vegetables and in small amounts in milk and fish (Iqbal *et al.*, 2004; Platt *et al*., 1963). Similarly calcium is most abundant mineral in human body and it is important for formation of bones, teeth, coagulation of blood (Springhouse, 2003). Calcium deficiency causes diseases like arthritis, high blood pressure and osteoporosis, calcium deficit causes nerve fiber irritability and repetitive muscle spasms (Springhouse, 2005).

Seaweeds also contain various biologically active compounds and secondary metabolites. Many compounds possessing cytotoxic, antiinflammatory, antibacterial, antifungal activities (Lindequiest & Schweder, 2001; Newman *et al*., 2003). Vallinayagam *et al*., (2009) investigated antibacterial activities of four seaweeds (*Gracilaria edulis, Ulva lactuca, Sargassum wightii* and *Padina gymnospora*) and found them active against seven bacterial species. Another study reveals that *Haligra* sp. was potent against *Staphyloccocus aureus* which is responsible for the food borne diseases in the canned foods (Devi *et al*., 2008). Karabay-Yavasoglu *et al.* (2007), identified 40 different volatile compounds from red seaweed *Jania rubens*, they reported the more potent antimicrobial activity of methanol and chloroform extract of seaweed than hexane, dichloromethane extracts and volatile oil. Karachi coastal area inhabitant of a number of seaweed species belonging to Rhodophycota, Phaeophycota and Chlorophycota and have a broad spectrum biological activities (Ara *et al.*, 1998; 1999; 2002ab; 2005; Ayesha *et al.*, 2010; Sultana *et al*., 2011.). Antimicrobial activity of seaweeds occurring at Karachi coast has been reported (Ara *et al.*, 2002a; Usmanghani & Shameel, 1986; Rizvi & Shameel, 2005). However, diverse reports are made from different parts of the world

about antimicrobial activity of different seaweed species (Taskin *et al.*, 2007; Vairappan, 2003). The present report describes the quantification of ascorbic acid (vitamin C), calcium, protein carbohydrate and lipids in some seaweeds occurring at Karachi coast. It also summarizes the antimicrobial effects of some seaweeds which were previously not reported.

Material and Methods

Collection and identification of seaweeds: Seaweeds belonging to Phaeophycota [*Dictyota dichotoma* var. *intricata, D. indica* Sond.ex Küts., *Dictyota divaricata, Iyengaria Stellata* (Børg.) Børg., *Jolyna laminarioides Guimaraes, Sargassum tenerrimum* J. Ag., *S. Swartzii* (Turn.) C. Ag., *S. lanceolatum, S. ilicifolium* (Turn.) C. Ag., *S. virgatum*, *Spatoglossum asperum* J, Ag., *Cystoseira indica* (Thivy et Doshi) Mairh, *Stoechospermum marginatum* (C. Ag.) Kütz.], Chlorophycota [*Codium iyengarii* Børg., *Halimeda tuna* and *Ulva fasciata* Delile., *U. lactuca* L., *Rhizoclonium implexum* (Dillw.) Kütz.] and Rhodophycota [*Melanothamnus afaqhusainii Shameel, Solieria robusta* (Greville) Kylin] were collected from Buleji beach of Karachi coast in different seasons at low tide. Seaweed species exposed on sand and rocks were collected in plastic bags and brought to the Laboratory. The voucher specimens and herbarium sheets were prepared. Each species of seaweed was washed thoroughly with freshwater to remove salt and epiphytes, dried under shade. Dried seaweeds were powdered in an electric grinder and stored in polyethylene bags at room temperature until used.

Preparation of ethanol extract: Dry powder of seaweed (500 g) was extracted with ethanol (4 vol.) for 1 week. Extracts were pooled, filtered through cotton wool/ Whatman No. 1 filter paper and concentrated to dryness under reduced pressure on rotary vacuum evaporator at 40°C and weighed.

Preparation of water extracts: Water extracts were prepared by homogenizing one gram of seaweed in 100ml of water. They were filtered by Whatman No. 1 filter paper and stored in refrigerator at 4^{o}C for the estimation of carbohydrate, lipid and protein. Whereas for the estimation of calcium and ascorbic acid water extracts were lyophilized on a Freeze Dryer.

Estimation of carbohydrates: Carbohydrates were estimated by Dubois method (Dubois *et al.*, 1956). One ml of homogenate was taken in triplicates; 1mL of 5% phenol was added to all tubes followed by 5mL of concentrated sulphuric acid. Mixture was incubated at room temperature and read against reagent blank at 490 nm.

Estimation of proteins: Proteins were detected by Lowry method (Lowry *et al.*, 1951). 0.1mL of homogenate was taken in triplicates with addition of 5mL alkaline reagent and mixture was incubated for 15 minutes. 0.5 mL of Folin phenol reagent was added and read against blank at 750 nm.

Estimation of lipids: To evaluate the total lipids in seaweed samples, lipids were extracted by chloroform methanol method. Tubes were pre-weighed and 0.25g of ground seaweed was added to the tubes, Seven ml of chloroform methanol mixture was added to the tubes and were shaken, Samples were filtered and solution was allowed to evaporate. Test tubes were re-weighed after evaporation (Erikson, 1993).

Estimation of calcium and ascorbic acid: Lyophilized extracts of seaweeds were used for the detection of calcium and ascorbic acid (vitamin C) in seaweeds. A concentration of 1, 10, 20 and 50 mg per mL were prepared in distilled water. Calcium and ascorbic acid were estimated using RQflex 10 (Merck), kit method.

Antimicrobial screening: The ethanol extract of seaweed species was tested for antibacterial and antifungal activity using Agar disc diffusion method (Ara *et al*., 2002a), where 200 mg/mL of the crude extract of seaweeds prepared in ethanol. The sterilized thick filter paper discs (5 mm) were loaded with 2, 4 and 6mg/disc of ethanol extract, only ethanol served as a negative control and streptomycin for gram negative bacteria/penicillin for gram positive bacteria and Topsin-M for fungi (10µg/disc) served as the positive control. The discs were dried in order to evaporate organic solvent. Petri plates were poured with specific media, (Nutrient ager for antibacterial activity and Czepak's Dox agar for antifungal activity). Bacterial lawn (*Bacillus subtilis, Styphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*) were prepared on the nutrient agar media containing plates and discs loaded with seaweed's extract were placed at different peripheral positions of Petri dishes. Each treatment was replicated three times and plates were inoculated at 28°C. For antifungal activity a 5 mm disc of actively growing culture of test fungi (*Rhizoctonia solani, Macrophomina phaseolina, Fusarium solani* and *F. oxysporum*) was inoculated in the center of the Petri dishes after placing the discs loaded with different concentration of seaweed extracts. Zone of inhibition was recorded after 48 hours for antibacterial activity, and after 5 days for antifungal activity.

Results and Discussion

More than 200 seaweeds are utilized commercially world wide of which 65 % are used as human food (Zemek-White & Ohno, 1999). In Asia, seaweeds have been used in human nutrition since ancient times. The most common seaweeds used as food include brown algae such as *Laminaria*, *Undaria* and Hijiki *(Sargassum fusiformis)* and a species of red algae Nori (*Porphyra)*. In this study, biochemical composition revealed that percentage of lipids in seaweeds was less than protein and carbohydrate. The amount of protein in red seaweeds was in range of 4-7 g% of dry matter, while carbohydrate contents were high i.e., 40-45g%. The protein contents in green seaweeds were in the range of 2-8 g % of dry weight (Table 1). The highest protein content was found in *Ulva lactuca*. There are reports that, the amount of protein portion of brown seaweeds is low (3-15g % of the dry weight), compared with that of green or red seaweeds, (10-47 g %) of the dry weight (Askari & Askari, 1983).

Table 1. Concentration of protein, carbohydrates and lipids in some seaweeds of Karachi coast.

Seaweeds	Protein g %	Carbohydrates g %	Lipids g %
Codium iyengarii	2.01	7.0	4.0
Halimeda tuna	2.0	10.0	4.0
Rhizoclonium implexum	5.6	14.4	8.0
Ulva lactuca	8.15	8.8	1.2
Melanothamnus afaqhusainii	4.3	40.0	1.4
Dictyota indica	7.41	11.0	2.0
Solieria robusta	4.5	45	2.8
Sargassum variegatum	3.0	36.0	1.0
Dictyota dichotoma var. *intricata*	5.0	12.8	6.8

In this study, sixteen seaweed species were examined for ascorbic acid and calcium content. Out of them fourteen showed presence of ascorbic acid (vitamin C) in appreciable amount (Table 2). There are report that vitamin C strengthens the immune system activities, intestinal absorption of iron, control the formation of conjunctive tissues and matrix of bony tissues and also act as antioxidant (Burton, 2003). Vitamin C protects small blood vessels from damage, this may prevent excessive menstrual blood loss (Cohen & Rubin, 1960). Presence of vitamin C in some seaweeds of Karachi coast has been reported by Qasim & Barkati (1985).

In this study that all the sixteen seaweed species examined were found to contain calcium at varying concentration with highest in *Dictyota indica* (Table 2). Calcium is one of the most important elements in the diet because it is structural component of bones, teeth, soft tissues and is essential in many of the body's metabolic process and account for 1 to 2 percent of adult body weight and of which 99 percent is stored in bones (Springhouse, 2003).

Seaweed farming is highly developed in Japanese coastal areas and main species grown there are *Porphyra* (Nori), *Laminaria* (Kombu) and *Undaria* (Wakame) having business of more than 100 million dollars. Due to rapidly growing population of the world and emerging food crises, seaweed resources are seemed as alternate source of food which can also protect the consumers from several diseases. However, fluctuation in nutritional composition in seaweeds must be taken into account to consider the commercial collection to use it in human nutrition (Fajardo *et al.*, 1998).

Table 2. Concentrations of calcium and ascorbic acid in some seaweeds of Karachi coast.

Seaweeds	Calcium (ppm)	Ascorbic acid (ppm)
Codium iyengarii	9	Low
Spatoglossum asperum	8	38
Stoechospermum marginatum	8	61
Cystoseira indica	37	34
Sargassum variegatum	8	51
Sargassum swartzii	13	52
Sargassum tenerrimum	35	33
Sargassum ilicifolium	15	32
Iyengaria stellata	6	Low
Solieria robusta	7	27
Halimeda tuna	15	33
Rhizoclonium implexum	14	33
Dictyota dichotoma var. *intricate*	10	52
Dictyota indica	80	32
Melanothamnus afaqhusainii	53	34
Jolyna laminarioides	7	27

In this study, the ethanol extract of 5 seaweeds, 3 brown *Dictyota dichotoma* var. *intricata, D. indica, Sargassum lanceolatum* and a red *Melanothamnus afaqhusainii*, were found active against different bacterial strains even at lowest concentration of 2 mg/disc (Table 3). Antibacterial activity of five species of seaweeds revealed that red and brown seaweeds had greater antibacterial activity than the green algae, which is in agreement with findings of Rao *et al*. (1991). Similarly, Karabay-Yavasoglu (2007) have identified 40 volatile compounds consisted of n-docosan, n-eicosan and tetratriacontane from a red alga *Jania rubens* and found that methanol and chloroform extracts (4 mg/disc) showed more potent antimicrobial activity. Another researcher found that halogenated compound Elatol from red algae *Laurencia* spp., inhibited six species of bacteria. Its antibacterial potency was as good as or better than the six commercially available antibiotics (Vairappan, 2003). In our study red species *Melanothamnus afaqhusainii* had shown good antibacterial activity against all pathogenic bacteria except *P. aeruginosa*. Our results also showed that the highest activity at concentration of 6mg/disc was measured in ethanolic extract of seaweed *Dictyota dichotoma* var. *intricata* and *D. indica* against *S. typhimurium* followed by *Halimeda tuna* and *D. indica*, against *B. subtilis* with a zone of 15, 15, 14 and 14 mm respectively. *D. dichotoma* var. *intricata* was potently active against all bacteria at all concentrations with zone of inhibition of 9, 10, 12 mm against *B. subtilis*, 9, 10, 13 mm against *S. aureus*, 8, 8, 10mm against *E. coli*, 11, 12, 15 mm against *S. typhimurium* and 9, 11, 12 mm against *P. aeruginosa*.

Table 3. Antibacterial activity of seaweed species.

Seaweed species	*B. subtilis*			*S. aureus*			*E. coli*			*S. typhimurium*			*P. aeruginosa*		
	2 mg	4 mg	6 mg	2 mg	4 mg	6 mg	2 mg	4 mg	6 mg	2 mg	4 mg	6 mg	2 mg	4 mg	6 mg
Halimeda tuna	7	10	14	0	7*	9	9	9	10	8	9	10	7*	8*	*10
Dictyota dichotoma var *intricata*	9	10	12	9	10	13	8	8	10	11	12	15	9*	*11	12*
Dictyota indica	8	11	14	0	0	9	9	9	10	10	12	15	0	7*	8*
Melanothamnus afaqhusainii	9	9	10	8	9	10	8	9	11	10	11	12	0	8*	8*
Sargassum lanceolatum	8	10	12	8	8	9	8	9	11	9	11	13	0	8*	8*

*Produced weak zone and later bacteria grown

Table 4. Antifungal activity of seaweed species.

Seaweed species	*Fusarium oxysporum*			*Fusarium solani*			*Macrophamina phaseolina*			*Rhizoctonia solani*		
	2 mg	4 mg	6 mg	2 mg	4 mg	6 mg	2 mg	4 mg	6 mg	2 mg	4 mg	6 mg
Halimeda tuna	0	0	0	0	0	0	0	0	0	0	0	0
Ulva fasciata	0	0	0	0	0	0	0	0	0	0	0	0
Jolyna laminarioides	0	0	0	0	0	0	0	0	0	0	0	0
Melanothamnous afaqhusainii	0	0	0	0	0	0	0	0	0	0	0	0
Sargassum ilicifolium	0	**8**	**10**	0	0	0	0	0	**10**	0	0	0
Sargassum lanceolatum	0	0	0	0	0	0	0	0	0	0	0	0
Sargassum swartzii	0	0	0	0	0	0	0	0	0	0	0	0
Sargassum tenerrimum	0	0	0	0	0	0	0	0	0	0	0	0
Cystoseira indica	0	0	**6**	0	0	0	0	0	**9**	0	0	0

The ethanol extract of seaweed species (*H. tuna, J. laminarioides, M. afaqhusainii, C. indica, S. ilicifolium, S. lanceolatum, S. tenerrimum, S. swartzii* and *U. fasciata*) were tested for antifungal activity against root infecting fungi. *S. ilicifolium* was effective at a concentration of 4 and 6 mg/disc with inhibition zone of 8 and 10 mm respectively against *F. oxysporum*. The same seaweed was effective against *M. phaseolina*, produced an inhibition zone of 10 mm (Table 4). Other researchers also reported that *U. fasciata* and *S. ilicifolium* were active against some fungal species including *F. solani*, with % inhibition of 55.71 and 44.28 (Rizvi & Shameel, 2005). The ^{1}H-NMR analyses revealed the presence of polyunsaturated esters in *S.wightii* (Vallinayagam *et al.*, 2009), so these polyunsaturated esters may be the compound responsible for antimicrobial activity in different *Sargassum* species.

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