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ANTIBACTERIAL ACTIVITIES AND ELEMENTAL ANALYSIS OF SOME SEAWEEDS COLLECTED FROM KARACHI COAST

Kashif Ahmed*, Tahira Ashraf and Shamsa Naz

Department of Chemistry, N.E.D. University of Engineering and Technology, Karachi, Pakistan.
*Corresponding Author's Email: kashif25473@yahoo.com

ABSTRACT

The present work shows antibacterial activities and mineral contents (K, Na, Ca, Mn, Fe and Zn) in some seaweed samples which were collected from Karachi Coastal area. All extracts of *Sargassum muticum* showed the highest antibacterial activities (water 3.5±0.5 mm, ethanol 4.9±0.1 mm, methanol 12.4±0.2 mm) while only methanol extract of *Sarconema fucellatum* (7.6±0.1 mm), *Solieria robusta* (9.5±0.1 mm) and water extract (3.2±0.5 mm) of *Campia compressa* showed inhibition zone. Ethanol and methanol extracts of *Sargassum ilicifolium* (3.5±0.1 mm and 8.6±0.1 mm, respectively) and *Sargassum swartzii* (4.6±0.1 mm and 10.1±0.2 mm respectively) also showed inhibition zones. The study showed the potential of *Sargassum muticum* as the natural source of antibacterial compounds.
The differences in the concentrations of mineral contents were observed between the different species of seaweeds. The brown seaweed had the highest K (*Sargassum ilicifolium*, 47.7±5g/kg) and Ca (*Stoechospermum marginatum*, 15.7±1.4 g/kg) mean concentrations while the green seaweed had the highest mean Zn (*Ulva fasciatalatum*, 30.8±28 mg/kg) and red algae has the highest Na (*Osmundea pinnatifida*, 38.8±1.1 g/kg), Fe (*Osmundea pinnatifida*, 956±26 mg/kg) and Mn concentrations (*Sarconema fucellatum*, 262±14). Outcome of the study is the information about the potential to be used for curing bacterial and fungal infections and mineral contents for humans use and other utilizations.

Keyword: Antibacterial activity, *Staphylococcus aureus*, elemental analysis, mineral contents

INTRODUCTION

Seaweeds have been consumed as food for centuries in various parts of world because of their taste, rich source of minerals, vitamins, antioxidants and nutritional elements (Jimenez-Escrig and Cambrodon, 1999; Manivannan, *et. al.* 2011). They are often utilized as animal feed, medicine and bio fertilizers (Robledo and Freile-Pelegrin, 1997).

Antibiotics are compounds which can damage the growth or metabolism of pathogenic microorganisms. Nevertheless, these have been connected with negative aspects such as allergic reactions, some level of toxicity, side effects, hypersensitivity and high cost (Thomashow and Weller, 1995;; Schinor, *et. al*., 2007). Consequently, scientists are now exploring alternate antimicrobial compounds especially from seaweed. Even though, extracts of seaweed contain compounds which can act as antibiotic, antioxidants, anti-inflammatory and anticoagulant were explored by marine researchers yet they there is a need of further survey for using degradable natural derivatives of biochemical origin (Alam and Qasim, 1993; Karaman *et. al.*, 2003)

The flavor of the seaweeds is associated with the many useful micronutrients that they contain. They contain higher concentrations of minerals than land-dwelling vegetables (Rohani-Ghadiko-laei *et. al*., 2012) therefore; they are wonderful alternate of dietary sources of minerals elements (Bocanegra *et. al*., 2009). Now a day there is an increasing trend of utilizing dried seaweeds as a food supplements. On internet many websites of sea vegetable are busy in promoting seaweeds as good iron source despite the lack of bioavailability studies (Sawada *et. al.*, 2014). Survey of seaweeds for high mineral contents and encouraging their intake would be an economical way to help reduce the mineral deficiency (Zimmermann and Hurrell, 2007; Sawada *et. al.*, 2014).

In line with the current trend, the objective of this study was to determine concentrations of several minerals (K, Na, Ca, Mn, Fe and Zn) and to scrutinize the antibacterial activities of water, ethanol and methanol extracts of seaweed samples of *Sargassum swartzii*, *Stoechospermum marginatum*, *Sargassum muticum*, *Sargassum ilicifolium*, *Sarconema fucel*, *Codium tomentosum*, *Campia compressa*, *Caulerpa racemosa*, *Solieria robusta*, *Ulva fasciata latum*, *Codium iyengarii* and *Osmundea pinnatifida* collected from Karachi Coastal area.

MATERIALS AND METHODS

Sample collection

Twelve seaweed samples were collected from Karachi Coastal area in the month of January for analysis including four green [*Codium iyengarii* (Bϕrg), *Codium tomentosum* (Stackhouse), *Ulva fasciata* (Delile) and

Caulerpa racemosa (Forssk) J. Ag.], four brown algae [*Stoechospermum marginatum* (C. Ag.) Kŭtz, *Sargassum swartzii* (Turner) C. Ag., *Sargassum muticum* (Fensholt), *Sargassum ilicifolium* (Turner) C. Ag.] and four red algae [*Sarconema fucellatum* (Zanard), *Solieria robusta* (Grev.) Kylin, *Campia compressa* (Harv.) and *Osmundea pinnatifida* (Huds) Stack]. The seaweed identification was based on the morphological characteristics.

Extract preparation

Each species of seaweeds were washed by deionized water and then 500 g of each species was kept for shade-drying up to four days and then oven-drying at 70° C to obtain a constant weight and crushed in the grinder. Maceration method (with the help of water, ethanol and methanol) was used for obtaining extracts.

Determination of percentage yield of soluble mass

Percentage yield soluble mass in extract of various samples of seaweeds were determined by the following equation Percentage Yield = (mass of extract containing soluble mass / sum of powdered sample and solvent) x 100

Antibacterial activity

A pathogenic bacterium *Staphylococcus aureus* was used in the study and its pathogenicity was established by Koch's postulates (Holland *et al*., 2014). The antibacterial activity was done by the method of Murugan and Santhana (2003). The control which was used in the study was streptomycin. After creating the lawn culture of *Staphylococcus aureus*, the extract loaded disc was placed on the lawn culture of the test organism. The plates were incubated for 48 h at 37 °C for circular clear area (inhibition zone) around the discs. Zone of inhibition was measured.

Sample analysis for minerals

Each species of seaweeds were washed by deionized water and then 1.5 kg of each species was kept for shade-drying up to four days and then crushed in the grinder. The seaweed samples were digested and determined according to the procedure described by Herna´ndez Sua´ rez *et al*. (2007). Briefly one gram of dried seaweed was weighed in digestion tubes and 8 mL of Nitric acid were added. The mixture was heated in a water bath till complete digestion. After cooling at room temperature 1 mL of hydrochloric acid was added and heated to 160° C for 5 min. Then, this solution was quantitatively transferred and adjusted to 10 mL with Milli-Q deionized water. The determination of the minerals (K, Na, Ca, Mn, Fe and Zn) was performed using atomic absorption spectrometry. The analysis on dried material was done in triplicate. The values were expressed as dry weight of the marine algae. The samples were grouped as green, brown and red seaweeds for data analysis.

RESULTS AND DISCUSSION

In the present work total twelve seaweed species were checked for their percentage yields and antibacterial activities. The percentage yield of extracts isolated from seaweed using various solvents are mentioned in Table 1. It can be observed that percentage yields are different from sovent to solvent. The yields of all extracts in methanol were highest followed by ethanolic extract and then water extracts from the same seaweed species. Some species of seaweeds (*Ulva fasciata*, *Stoechospermum marginatum*, *Codium tomentosum*, *Codium iyengarii*, *Caulerpa racemosa* and *Osmundea pinnatifida*) do not showed inhibition zone which indicated that water, ethanol and methanol are not suitable solvents for extraction of antibacterial compounds in these species (Zheng, *et. al*., 2001; Zubia, *et. al.*, 2008; Serap, *et. al.*, 2009). Leela and Satirapipathkul (2011) reported that methanol is the best choice as a solvent to extract antibacterial substances from living system. It is due to the ability of methanol to dissolve both (polar and non-polar) types of compounds (Serap, *et. al.*, 2009).

Antibacterial activities of various seaweed extract (Table 2) obtained using different solvents were examined by measuring the inhibition zone created in bacterial culture (*Staphylococcus aureus*). Inhibition zone is the distance between the ends of the filter paper disc (containing extract) and the area where the bacterium growth appears. All extracts of *Sargassum muticum* showed the highest antibacterial activities (water 3.5±0.5 mm, ethanol 4.9±0.1 mm, methanol 12.4±0.2 mm) while only methanol extract of *Sarconema fucellatum* (7.6±0.1 mm), *Solieria robusta* (9.5±0.1 mm) and water extract (3.2±0.5 mm) of *Campia compressa* showed inhibition zone. Ethanol and methanol extracts of *Sargassum ilicifolium* (3.5±0.1 mm and 8.6±0.1 mm respectively) and *Sargassum swartzii* (4.6±0.1 mm and 10.1±0.2 mm respectively) also showed inhibition zones. In overall, methanolic extract perform best compared to other solvents.

Extracts obtained from different seaweed species shows different inhibited zones against bacteria possibly due to method of extraction, maturity, effect of environment and different in their biochemical composition (Zubia, *et.*

al.,, 2008). A variety of solvents have been used for selection seaweeds for antibacterial activity and the most suitable solvent for the most effective extraction of seaweeds is still ambiguous (Zheng, *et. al.*, 2001). Cox, Abu-Ghannam, and Gupta (2010) reported that extraction of antimicrobials from different species of seaweeds were solvent dependent, he observed that methanol was a better solvent for antimicrobials extraction from brown seaweeds which is full agreement with our results. Gupta and Abu-Ghannam (2011) mentioned that bioactive compounds having antimicrobial activities are found on the cell wall of seaweed and have natural tendency of preventing cells from microbial attack.

Table 1. Percentage yield of soluble mass of seaweed samples in various solvents.

Seaweeds	Solvents		
	Water	**Ethanol**	**Methanol**
Sargassum swartzii	1.6±0.1	3.8±0.1	4.9±0.2
Sargassum muticum	2.1±0.1	2.7±0.1	6.8±0.2
Sargassum ilicifolium	1.2±0.2	2.5±0.1	4.1±0.1
Ulva fasciata	0.6±0.1	1.6±0.1	3.2±0.1
Stoechospermum marginatum	0.7±0.1	1.8±0.2	3.8±0.2
Codium tomentosum	0.8±0.2	1.2±0.2	2.3±0.2
Codium iyengarii	0.5±0.3	1.9±0.2	3.4±0.3
Caulerpa racemosa	0.6±0.2	1.3±0.1	2.7±0.3
Solieria robusta	0.3±0.1	2.3±0.1	3.9±0.2
Sarconema fucellatum	0.8±0.2	2.1±0.1	3.6±0.1
Campia compressa	0.6±0.1	1.4±0.2	2.1±0.1
Osmundea pinnatifida	0.8±0.2	1.6±0.1	1.9±0.1

Table 2. Inhibition zone of solvent extracts obtained from seaweed against *Staphylococcus aureus*.

Seaweeds	Inhibition zone extracts in solvents (mm)			Inhibition zone of Streptomycin
	Water	**Ethanol**	**Methanol**	
Sargassum swartzii	--	4.6±0.1	10.1±0.2	21.7±0.5 mm
Sargassum muticum	3.5±0.5	4.9±0.1	12.4±0.2	
Sargassum ilicifolium	--	3.5±0.1	8.6±0.1	
Ulva fasciata	--	--	--	
Stoechospermum marginatum	--	--	--	
Codium tomentosum	--	--	--	
Codium iyengarii	--	--	--	
Caulerpa racemosa	--	--	--	
Solieria robusta		--	9.5±0.1	
Sarconema fucellatum	--	--	7.6±0.1	
Campia compressa	3.2±0.5	--	--	
Osmundea pinnatifida	--	--	--	

The values of minerals concentration are presented (Table 3). According to these results the highest concentration of K was present in *Sargassum ilicifolium* **(**47.7±5g/kg**)** while lowest in *Sargassum swartzii* (5.2±5g/kg); *Osmundea pinnatifida* contained highest Na (38.8±1.1g/kg) and *Sarconema fucellatum* contained lowest (3.08±0.1 g/kg); maximum quantity of Ca in *Stoechospermum marginatum*, (15.7±1.4 g/kg) and minimum in *Osmundea pinnatifida* (1.80±0.3 g/kg); *Sarconema fucellatum* (262± 14 mg/kg) contained highest Mn while *Sargassum ilicifolium* contained lowest (6.41±2.4 mg/kg); iron content was maximum in *Osmundea pinnatifida* (956±26 mg/kg) while minimum in *Codium tomentosum* (121±6 mg/kg); *Ulva fasciatalatum* contained maximum concentration of Zn (30.8± 28 mg/kg) while *Osmundea pinnatifida* contained minimum concentration (1.38±0.8 mg/kg) among all samples of seaweed. When variations in mineral concentrations on the basis of genera were noted then it was observed that the brown seaweed had the highest K (*Sargassum ilicifolium*, 47.7±5 g/kg) and Ca (*Stoechospermum marginatum*, 15.7±1.4 g/kg) mean concentrations while the green seaweed had the highest mean Zn (*Ulva fasciatalatum*, 30.8±28 mg/kg) and red algae has the highest Na (*Osmundea pinnatifida*, 38.8±1.1 g/kg),

Fe (*Osmundea pinnatifida*, 956±26 mg/kg) and Mn concentrations (*Sarconema fucellatum*, 262±14). Our data on mineral concentration were similar to the data published by some authors (Larrea-Marı´n, *et. al*., 2010; Romarı´s-Hortas, *et. al*., 2010; Kumar, *et. al*., 2011; El Din and El-Sherif, 2012).

Establishing the reference values for minerals in seaweed is difficult because there are many factors which influence the mineral concentrations. Environmental features of each region such as the salinity, temperature and pH of the seawater (Lodeiro, *et. al*., 2005; Marinho-Soriano, *et. al*., 2006), sampling seasonality (Vasconcelos and Leal, 2001) and age of the fronds influence the uptake and accumulation of the minerals. Moreover minerals concentrations can vary enormously among the different families, genera and species of seaweed, even under similar environmental conditions, geographical origin and harvesting time (Riget, *et. al*., 1997; Marinho-Soriano, *et. al*., 2006; Kumar, *et. al*., 2011). This could be explained due to differences in the bio sorption of minerals as a consequence of differences in the amount and composition of polysaccharides in their cell walls (Davis, *et. al*., 2003; Bocanegra, *et. al*., 2009).

Table 3. Mineral Concentrations (SD±) per kg of dry mass of various species of seaweed from Karachi Coastal area.

Seaweed Sample	Mineral concentrations					
	K (g/kg)	Na (g/kg)	Ca (g/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
Sargassum swartzii	5.2±5	18.0±1.7	8.03±2.5	12.5±6.8	399±10	12.5± 12
Sargassum muticum	28.2±1	15.5± 1.8	11.4±1.7	29.6± 3	426±18	29.6± 30
Sargassum ilicifolium	47.7±5	32.8± 7.1	9.68±0.7	6.41±2.4	285±7	6.41±2.4
Ulva fasciata latum	19.3±9	19.5± 1.4	9.08±8.8	34.5±2.6	198±7	30.8± 28
Stoechospermum marginatum	25.3±2	17.4± 4.7	15.7±1.4	21.7±2.1	311±13	2.38±07
Codium tomentosum	9.82±6	11.1± 6.5	3.97±1.4	67.1±21	121±6	16.2± 11
Codium iyengarii	37.8±4	7.81±1.6	6.45±1.6	27.2± 7	155±5	7.48±4.1
Caulerpa racemosa	6.45±4	21.4± 2.6	4.26±0.9	86.0±11	137±3	10.1±0.4
Solieria robusta	19.7±7	22.4± 1.4	4.58±3.0	171±8.2	515±23	7.08±5.0
Sarconema fucellatum	23.5±3	3.08±0.1	10.4±0.8	262± 14	342±14	8.52±0.3
Campia compressa	13.8±5	6.98±0.1	3.87±0.6	133± 13	453±17	8.51±0.9
Osmundea pinnatifida	5.49±2	38.8±1.1	1.80±0.3	2.47±0.9	956±26	1.38±0.8

CONCLUSION

It can be concluded that *Sargassum muticum* showed the highest antibacterial activity (inhibition zone of water extract 3.5±0.5 mm, ethanol extract 4.9±0.1 mm and methanol extract 12.4±0.2 mm) and its methanolic extract has a potential to be used as natural antibiotics. In future further work should be done for purification and determination of the structure of active antibacterial compounds.

A clear variation in the concentration of minerals was found between the seaweed species. The brown seaweed had the highest K (*Sargassum ilicifolium*, 47.7±5g/kg) and Ca (*Stoechospermum marginatum*, 15.7±1.4 g/kg) mean concentrations while the green seaweed had the highest mean Zn (*Ulva fasciatalatum*, 30.8±28 mg/kg) and red algae has the highest Na (*Osmundea pinnatifida*, 38.8±1.1 g/kg), Fe (*Osmundea pinnatifida*, 956±26 mg/kg) and Mn

concentrations (*Sarconema fucellatum*, 262±14). It was concluded that seaweeds are good source minerals as evident from values and can be used as mineral supplement for human consumption and other purposes.

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