

## Research Article

# Alginate level, its physical properties and biochemical composition of three selected brown seaweeds (*Colpomenia sinuosa*, *Dictyota dichotoma* and *Iyengaria stellata*) of Karachi coast, Arabian Sea

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### Abstract

The global population increasing day by day means that food production needs to increase by 70% by 2050, placing pressure on the quality of food standards. If we see our earth which is 70% covered by water all types of water have algae both micro or macro. Macro algae or Seaweed are available in large quantities; they appear to be the most suitable raw material for commercial exploitation. The key objective of the present research study was to assess the brown seaweed species potential as a source of alginate and its physical properties (relative density and viscosity) and biochemical content (carbohydrate, ash) in species of brown seaweeds *Colpomenia sinuosa*, *Dictyota dichotoma* and *Iyengaria stellata* collected from the different shores of Karachi coast (Hawks Bay, Buleji and Manora). Standard methods were used for all parameters. The yield of alginate was recorded in *Colpomenia sinuosa* 8.74-13.09 %, *Dictyota dichotoma* 9.11-10.99 %, and *Iyengaria stellata* 8.40-10.51 %. The range of relative density of the alginate extracted from *Colpomenia sinuosa* 0.8-0.945, *Dictyota dichotoma* 0.75-0.889 and *Iyengaria stellata* 0.81-0.85 whereas viscosity was in the range of 77.12-98.95 in *Colpomenia sinuosa*, 84.4-93.32 in *Dictyota dichotoma* and 79.11-93.66 in *Iyengaria stellata*. The maximum carbohydrate was recorded in *Iyengaria stellata* (29.16-39.21%), ash content in *Dictyota dichotoma* (24.8-30.66%), and the moisture content in *Dictyota dichotoma* whereas biomass was high in *Colpomenia sinuosa* (314.17-487.16). The study of alginate and their physical properties, biochemical composition, and biomass reveals their suitability to be a good source for human consumption. Further studies are necessary for the isolation and characterization of the active molecules, which can be used to treat various oxidative stress-related diseases. Alginate polysaccharides not only possess health benefits but can also act as a means to strengthen the economy of a country through exports and imports.

**Keyword:** Alginate; Extraction; Karachi coast; Pheophytes; Polysaccharides

### Introduction

Approximately 15000 species of macroalgae or seaweeds are identified still and broadly

classified into three large groups based on their pigmentation: green (Chlorophytes), brown (Phaeophytes), and red (Rhodophytes)

algae [1]. Seaweed has been used for centuries for botanical, industrial and pharmaceutical due to its high nutritive content [2]. Brown seaweeds have been extensively used for their traditional, ethnobotanical, and medicinal properties like the other two groups (green and red). Traditionally Chinese use hot water extract from certain seaweeds in the treatment of cancer. In recent years, a family of fucose-containing sulfated polysaccharides from brown seaweeds has attracted attention due to their high bioactive properties and found their application in cosmetics, functional foods, and dietary supplements [3]. In our daily lives, we use seaweed polysaccharides that are used in the manufacturing of toothpaste, soaps, shampoos cosmetics, milk, ice cream, processed food, air fresheners, and a host of other items [2].

The use of enzymes for seaweed biorefinery is a promising biotechnological application that has been widely used to improve the extraction efficiency of bioactive components [4]. Alginic acid or alginates extracted from brown seaweeds are used in frozen desserts, pudding (both in standard cooked), pie and pastry filling, dessert gel, fabricated foods, salad dressing, meat and flavor sauces [2]. Nowadays, the field of marine natural products becomes more sophisticated. Seaweeds produce a variety of biologically active components with different structures and interesting functional properties [5]. Brown seaweed, as a raw material for alginate extraction, can be found in Indonesian waters. Two types of brown seaweeds that grow predominantly in Indonesia are *Sargassum* sp. and *Turbinaria* sp. However, not like *Eucheuma cottonii* which has been cultured successfully in many areas in Indonesia, *Turbinaria* sp. and *Sargassum* sp. have not been cultured intensively [6]. There had been

several research reports on alginate extraction using acid followed by the addition of formalin solution [7].

Biochemical constituents of seaweeds such as carbohydrates, ash, protein, amino acid, and lipids, etc. have been extensively investigated in various parts of the world, to mention a few: *Dictyota ceylinica* from Sunderban, India [8], *Laminaria*, *Sargassum* and *Fucus* sp. From Saint Martin's Island of Bangladesh [9], *Laminaria* sp., *Undaria pinnatifida* and *Hizikia fusiforme* from China, Japan, and Korea [10] *Sargassum horneri* from Japan sea of Toyama prefecture, Japan [11]. Engelen *et al.* [12] Studied the biomass of *Sargassum polyceratum* around the Island of Curacao, Netherlands Antilles. The people of Eastern countries take seaweeds as part of their diet for a long time and in the west, these marine plants are utilized industrially as a source of polysaccharides [13, 14]. Today seaweed and its products are commonly marketed in Europe.

Nowadays the people of Pakistan are facing two problems one is increasing population and the other is increasing prices of food and other needs items. Every man is looking for alternatives for each item of his/her need. To solve this problem, it is necessary to search out an alternate. Seaweeds are one of the richest and most promising sources, utilized as food, hydrocolloids (agar, carrageen, and alginic acid), medicines, cosmetics, and fertilizer. Seaweeds can be used as an alternate source of food for solving these two problems. The seaweeds of the Arabian Gulf are the least studied and unexploited resources. In the present study three brown algae *Colpomenia sinuosa*, *Dictyota dichotoma*, and *Iyengaria stellate* (Fig. 1) were selected for extraction of alginic acid analyzing their biochemical composition and biomass.



**Figure 1.** General view of *Colpomenia sinuosa*, *Iyengaria stellata* and *Dictyota dichotoma*

### Materials and Methods

Three different species of brown seaweeds *Colpomenia sinuosa*, *Iyengaria stellata*, and *Dictyota dichotoma* were collected from three different shores of Karachi coast viz, Hawks Bay, Buleji, and Manora. Collected seaweeds were, washed with seawater followed by fresh water to remove adhering impurities and epiphytes, shade-dried and grounded into fine homogenized powder, and stored in plastic bottles at 4°C until further analysis. Standard methods were used for the alginic acid extraction and its viscosity and relative density [15, 16] biochemical composition, and biomass [17, 18].

#### Extraction of alginic acid

The sample of seaweeds (10 grams) and 1% aqueous calcium chloride (300 ml) were taken in a 1L beaker and kept on a hot plate with stirred contents continuously by high torque electric stirrer at 60 °C for 15 minutes. The mixture was then centrifuged at 2000 rpm for 5 minutes. Discarded the supernatant and returned the seaweed residue to the 1L beaker for re-extraction with a further portion of hot aqueous calcium chloride. Combined the residues and washed with water and diluted acid HCl (0.05M). Filled the centrifuge bottles with distilled water up to ¾

point and then mixed thoroughly before centrifugation for 5 minutes. Discarded supernatant and fill the bottles with HCl (0.05 M) up to ¾ point. Again the contents were mixed and centrifuged. Washed residual seaweed twice more with hydrochloric acid and supernatant each time discarded.

Returned the residue of the above sample and treated with 300 ml aqueous sodium carbonate (0.5%) in 1 L beaker. Stirred contents continuously and heated at 45°C for 30 minutes. The mixture was centrifuged two times and saved the supernatant. Pour the alginate solution slowly stirring by glass rod into the 100 ml aqueous calcium chloride (25%) in a 2L beaker and continue stirring for an additional 10 minutes. Centrifuged precipitated calcium alginate and discarded supernatants. After that the calcium alginate for filtration in preweighed filter paper under vacuum. Washed the calcium alginate first with 50% 200 ml ethanol and then 300 ml cold hydrochloric acid (1M). The alginic acid was washed on the filter paper, with (50%) aqueous ethanol until completely pure from chloride. Tested the filtrate with one drop of silver nitrate solution until no formation of chloride precipitate is evident. Washed with 95 % 200 ml ethanol, acetone and 100 ml

diethyl ether for solvent exchange, and dried in vacuo in desiccators for 15 minutes. Finally weighed and calculated the

percentage yield of partially purified alginic acid. The alginic acid yield (in weight percent) is defined as:

$$\text{Yield (\%)} = \frac{\text{Dried mass of extracted final alginic acid product} \times 100}{\text{Dried algal mass before acid extraction}}$$

### Assessment of purity

25ml standardized aqueous sodium hydroxide (0.1M) added in 200 mg dry alginic acid powder in to a 100 ml beaker and kept at hotplate stirrer at 30<sup>0</sup>C until total dissolution of acid. Back-titrated excess alkali with standardized hydrochloric acid (0.1M) and used phenolphthalein as an indicator (red to colorless). Finally, the calculated percentage of alginic acid purity that 1 ml sodium hydroxide is equivalent to 0.0194g alginic acid in the hydrated form in which it exists even after solvent exchange.

### Viscosity and relative density

The viscosity and relative density of alginic acid were determined by the method of Haug [15] and Whyte [16]. The viscosity of 1 % solution was determined at 20<sup>0</sup>C by using Ubbelohde type Viscometer manufactured by Techino Nominal whereas the relative density of 1 % solution was determined simply by using the R.D. bottles of 10 ml.

### Carbohydrates

The carbohydrate was estimated by using the phenol sulphuric acid method [17]. 50mg of dried sample of seaweed was homogenized and diluted with distilled water up to 25 ml. 1 ml diluted sample was taken in triplicate test tubes, then 1 ml phenol reagent and 5 ml Sulphuric acid (conc.) were added in each test tube. For blank 1 ml distilled water, 1ml Phenol reagent and 5 ml of H<sub>2</sub>SO<sub>4</sub> were taken in a test tube. All tubes were allowed to stand at room temperature for 10 minutes and then kept in a water bath at 30<sup>0</sup>C for 20 minutes. The color intensity was noted at 490 nm against the reagent blank on a spectrophotometer.

### ASH

Ash or total inorganic contents were determined by the standard method of A.O.A.C [18]. One gram of dried sample was taken in a pre-weighed crucible and ignited in a muffle furnace at 550<sup>0</sup>C for eight hours. Ash was cooled in a desiccator at room temperature and weighed. The difference of weights before and after ignition gave the ash content.

### Moisture

The moisture content of brown seaweed was calculated by the standard method of A.O.A.C [18]. One gram of sample powder was dried in an oven at 110<sup>0</sup>C for 24 hours, cooled, and weighed. The process was repeated until a constant weight was obtained. The difference in initial and final weight gave the moisture content.

### Results and Discussion

The data reveals high variability in the content of alginic acid and its physical properties in between seaweed species, sampling shores, and collection time. The concentration of alginic acid in *Colpomenia sinuosawas* was found to vary from 6.46-14.85 % with the mean value of 12.12±2.05 % at Hawks Bay, 8.74±3.85 % at Buleji and 13.09±2.48 % at Manora (Table 1). The relative density of the alginic extracted from *Colpomenia sinuosa* was found to vary from 0.72-0.99 unit with the mean value of 0.94±0.053 unit at Hawks Bay, 0.80±0.11 unit at Buleji and 0.94±0.49 unit at Manora. Another physical property of alginic acid the range of viscosity was 66.54-124.7 centipoise (cp) with the mean value of 98.95±13.62 cp at Hawks Bay, 77.12±8.79 cp at Buleji and 90.05±16.33 cp at Manora (Table 1).

The concentration of alginic acid in *Dictyota dichotoma* was found to vary from 6.93-14.61 % with the mean value of 10.99±0.98 % at Hawks Bay, 9.19±2.09 % at Buleji and 9.11±1.75 % at Manora (Table 1). The relative density of the alginic acid extracted from *Dictyota dichotoma* was found to vary from 0.76-0.97 units with the mean value of 0.75±0.30 units at Hawks Bay, 0.89±0.04 units at Buleji and 0.88±0.02 units at Manora island Karachi. The range of viscosity was 69.6-99.7 ep cp with the mean value of 93.32±7.18 cp at Hawks Bay, 84.4±11.32 cp at Buleji, 85.1±5.25 ep at Manora (Table 1).

In *Iyengaria stellata* the concentration of alginic acid was found to vary from 6.58-12.61% with the mean value of 10.51±1.79 % at Hawks Bay, 8.40±1.29 % at Buleji and 9.54±1.87 % at Manora (Table 1). The relative density of the alginic acid extracted from *Iyengaria stellata* was found to vary from 0.56-0.96 units with the mean value of 0.85±0.82unit at Hawks Bay, 0.81±0.12 unit at Buleji and 0.84 ± 0.44 unit at Manora (Table 1). The viscosity ranged from 56.8-100.2 cp with the mean value of 93.66±6.46 cp at Hawks Bay, 79.11±12.37 cp at Buleji site, and 92.06±6.04 cp at Manora (Table 1).

**Table 1. Alginic acid (%) and its physical properties (relative density and viscosity) in brown seaweeds of Karachi coast**

S. No.	Name of species	Shores	Alginic acid	Relative density	Viscosity
1	<i>Colpomenia sinuosa</i>	Hawks Bay	12.12±2.05	0.945±0.05	98.95±13.62
		Buleji	8.74±3.85	0.80±0.11	77.12±8.79
		Manora	13.09±2.48	0.94±0.49	90.05±16.33
2	<i>Dictyota dichotoma</i>	Hawks Bay	10.99±0.98	0.75±0.30	93.32±7.18
		Buleji	9.19±2.09	0.89±0.04	84.4±11.32
		Manora	9.11±1.75	0.88±0.02	85.1±5.25
3	<i>Iyengeriastellata</i>	Hawks Bay	10.51±1.79	0.85±0.08	93.66±6.46
		Buleji	8.40±1.29	0.81±0.12	79.11±12.37
		Manora	9.54±1.87	0.84±0.04	92.06±6.04

The concentrations of carbohydrate in *Colpomenia sinuosa* were found to vary from 29.76-40.37 % with the mean value of 32.51±1.71 % at Hawks Bay, 36.62±2.74 % at Buleji and 31.20±1.91 % at Manora (Table 2). The ash concentrations of *Colpomenia sinuosa* were found to vary from 18.69-32.41 % with the mean value of 30.93±1.36 % at Hawks Bay, 29.98±1.18 % at Buleji and 19.04±0.50% at Manora (Table 2) whereas the range of moisture was 1.67-11.2 % with the mean value of 6.89±0.44 % at Hawks Bay 5.50±3.44 % at Buleji and 10.85±0.49% at Manora (Table 1). The biomass of *Colpomenia sinuosa* from all shores was 215-680 gm<sup>-2</sup> with a mean value of 314.17±59.19 gm<sup>-2</sup> at Hawks Bay, 487.16±205.48 gm<sup>-2</sup> at Buleji and 455±35.35gm<sup>-2</sup> at Manora (Table 2).

In *Dictyota dichotoma* the concentrations of carbohydrates were found to vary from 23.64-34.29 % with the mean value of 30.47±1.82 % at Hawks Bay, 22.52±2.54 % at Buleji and 30.93±1.62 % at Manora (Table 2). The ash concentrations were found to vary from 7.42-29.34 % with the mean value of 26.53±2.02 % at Hawks Bay, 26.29±1.75% at Buleji, and 14.12±7.50 % at Manora (Table 2). The range of moisture was 6.27-11.29 % with a mean value of 7.70±1.00 % at Hawks Bay, 7.93±0.93 % at Buleji, and 8.80±1.58 % at Manora (Table 2). The biomass of *Dictyota dichotoma* from all shores was 190-390 gm<sup>-2</sup> with the mean value of 288.3±42.97 gm<sup>-2</sup> at Hawks Bay, 280±86.09 gm<sup>-2</sup> at Buleji, 273±54.49 gm<sup>-2</sup> at Manora (Table 2).

The concentrations of carbohydrates in *Iyengaria stellata* were found to vary from

19.67-46.85 % with the mean value of  $29.34 \pm 2.04\%$  at Hawks Bay,  $29.16 \pm 3.04\%$  at Buleji and  $39.21 \pm 4.48\%$  at Manora (Table 2). The ash concentrations of *Iyengaria stellata* were found to vary from 21.15-34.46 % with the mean value of  $30.66 \pm 2.76\%$  at Hawks Bay,  $24.80 \pm 2.75\%$  at Buleji and  $25.61 \pm 0.65\%$  at Manora (Table 2). The range of moisture was 3.23-6.89 % with the mean

value of  $5.84 \pm 0.57\%$  at Hawks Bay,  $4.67 \pm 0.95\%$  at Buleji, and  $5.26 \pm 0.65\%$  at Manora (Table 2). The biomass of *Iyengaria stellata* ranged from 210-920  $\text{gm}^{-2}$  at all shores with the mean value of  $376.88 \pm 61.48 \text{ gm}^{-2}$  at Hawks Bay,  $373.3 \pm 148.74 \text{ gm}^{-2}$  at Buleji and  $381.87 \pm 221.71 \text{ gm}^{-2}$  at Manora (Table 2).

**Table 2. Biochemical composition (%) and biomass ( $\text{gm}^{-2}$ ) in brown seaweeds of Karachi coast**

S. No.	Name of species	Sites	Carbohydrate	Ash	Moisture	Biomass
1	<i>Colpomenia sinuosa</i>	HawksBay	$32.51 \pm 1.71$	$30.63 \pm 1.36$	$6.89 \pm 0.44$	$314.17 \pm 59.19$
		Buleji	$36.62 \pm 2.74$	$29.98 \pm 1.18$	$5.50 \pm 3.44$	$487.16 \pm 205.48$
		Manora	$31.20 \pm 1.91$	$19.04 \pm 0.50$	$10.85 \pm 0.49$	$455 \pm 35.35$
2	<i>Dictyota dichotoma</i>	HawksBay	$30.47 \pm 1.82$	$26.53 \pm 2.02$	$7.70 \pm 1.00$	$288.3 \pm 42.97$
		Buleji	$22.52 \pm 2.54$	$26.29 \pm 1.75$	$7.93 \pm 0.93$	$280 \pm 86.09$
		Manora	$30.93 \pm 1.62$	$14.12 \pm 7.50$	$8.80 \pm 1.58$	$273 \pm 54.49$
3	<i>Iyengaria stellata</i>	HawksBay	$29.34 \pm 2.04$	$30.66 \pm 2.76$	$5.84 \pm 0.57$	$376.88 \pm 61.58$
		Buleji	$29.16 \pm 3.04$	$24.80 \pm 2.75$	$4.67 \pm 0.95$	$373.3 \pm 148.74$
		Manora	$39.21 \pm 4.48$	$25.61 \pm 0.65$	$5.26 \pm 0.65$	$381.87 \pm 221.71$

A wide variation was found in carbohydrates, ash and alginic acid extracted from all three studied attached seaweed (*Colpomenia sinuosa*, *Dictyota dichotoma* and *Iyengaria stellata*) species collected from different shores (Fig. 2). In the present work, the color of alginic acid was dark brown i.e., the commercial standard was (1-4). no significant correlations were found between alginic acid, carbohydrates, and ash. The relative density and viscosity also show variation between species and shores. The viscosity of alginic acid from different shores of the Karachi coast is variable and depends strongly upon the collection time and drying conditions of the seaweed. To avoid the destruction of alginic acid the algae should be collected, transported, and dried as quickly as possible due to polymer chain of alginic acid is sensitive to the high isolation temperature [20].

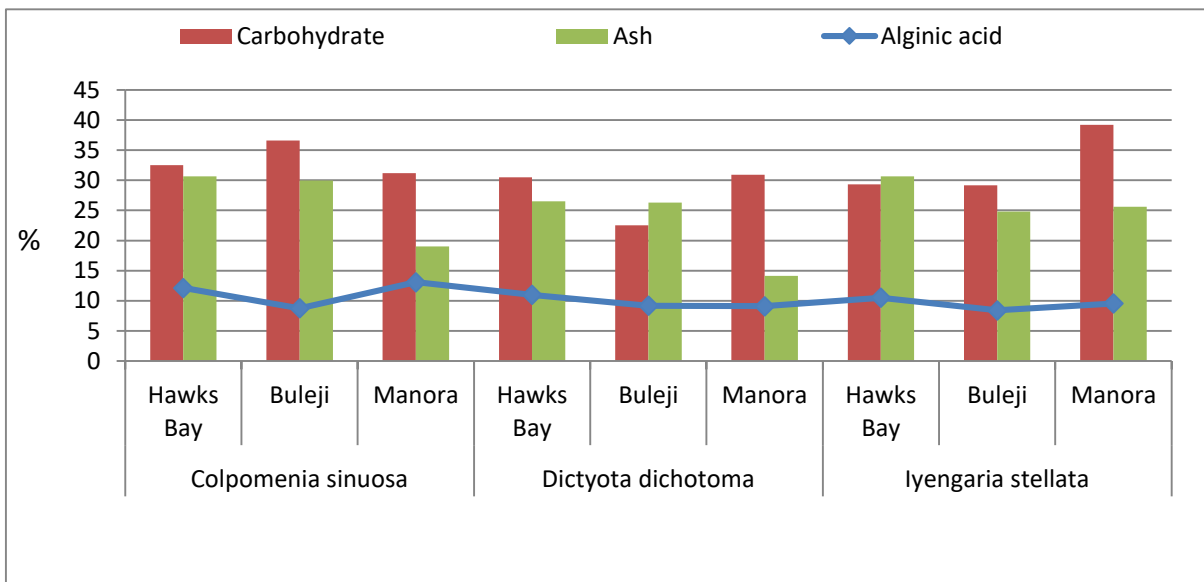
This study showed that the results for yield of alginic acid extracted from species of brown

seaweeds obtained during the study were similar to the values reported by Arvizu *et al.* [21] from Baja California Sur, Mexico, Chandia *et al.* [22] from South of Chile and Miller [23] from New Zealand. Recently Jothisarawathi *et al.* [24] have also given the same results of alginic acid content in brown seaweeds of the Gulf of Mannar, India when compared with the present study. The viscosity of alginic acid was also similar to the values reported by Jothisarawathi *et al.* [25].

The results of carbohydrate were recorded in the present study have much resemblance with the results of Chakraborty and Santra [8] for the *Dictyota dichotoma* species that collected from Sunderban India. The present results also agree with the results of Arvizu *et al.* [21] given for the brown seaweed *Eisenia arborea* from Baja California Sur, Mexico. Hossain *et al.* [11] Results for moisture agree well with the result of the present study. Marine plants especially seaweeds have 80-

90 % water and are considered biologically important for metabolism because it participate in the chemical reactions of metabolism as a source of hydrogen ions

[11]. Azad *et al.* [9] described in their study that high concentrations of carbohydrates and moisture were due to the immature or young stage of the plant.



**Figure 2. Variation in carbohydrates, ash and alginic acid in brown seaweeds of Karachi coast**

### Conclusion

From the present results, it is clear that the seaweed species, that were analyzed in the present study, are edible and can be used for human food because all studied species of seaweed have high quantities of alginic acid content and biomass with considerable amounts of biochemical constituents (carbohydrate, ash and moisture) that make them valuable as commercial species. So it is concluded that brown seaweed (*Colpomenia sinuosa*, *Dictyota dichotoma* and *Iyengaria stellata*) found on the coast of Karachi can be used as a source of alginic acid in different kinds of food, medicines, textiles, paper, cosmetics and fertilizer industries for daily consumption.

### Authors' contributions

Conceived and designed the experiments: R Qari, Performed the experiments: FK Siyal & R Qari, Analyzed the data: FK Siyal, Contributed reagents / materials / analysis

tools: R Qari, Wrote the paper: T Jatt & R Qari.

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