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*Research Article*

# PHYTOCHEMICAL CHARACTERIZATION OF *SARGASSUM WIGHTII, KAPPAPHYCUS ALVAREZII* AND *GRACILARIA CORTICATA* AFTER PHYCOCOLLOID EXTRACTION

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**ABSTRACT**

Seaweeds are a fascinating and diverse group of organisms categorized under macro algae living in the earth's oceans. Seaweeds are classified into three major groups; the green algae (Chlorophyta), the brown algae (Phaeophyta) and the red algae (Rhodophyta). Seaweeds are placed into one of these groups based on their pigments and coloration. Among a huge array of seaweeds, the three selected seaweeds are *Sargassum wightii*, *Gracilaria corticata* and *Kappaphycus alvarezii* found in the coastal areas of Gulf of Mannar, Tamil Nadu on the basis of their valuable commercial products alginate, agar and carrageenan respectively. The samples were investigated for the presence of phytochemicals like tannins and polyphenols using fodder collected after extraction of respective valuable products by employing different extraction methods. Retention of several phytochemicals to a greater extent gives a hope of using the waste from seaweed industry for obtaining value added compounds from seaweeds. The results of different tests in this study showed that these extracts and their bioactive components can be used as strong modulators on oxidative stress hence can be used for drug preparations while their phycocolloids for drug formulations. The selected biomass could be recommended for use in dietaries to combat protein energy for malnutrition or micronutrient deficiencies due to proven nutritional elements and enlight upon intergrated aquaculture to emphasize on seaweed biofiltration in modern mariculture.

**Keywords**: *Sargassum wightii*, *Gracilaria corticata*, *Kappaphycus alvarezii*, Phycocolloids.

## INTRODUCTION

India has coastline of about 7,500 km, a sizable exclusive economic zone and a large shelf area. This biogeographically important area harvests variety of natural resources, flora & fauna widely used for fuel, food and feed in human welfare. The Southern Coast of India bears luxuriant growth of seaweeds, globally used for phycocolloids. Many of the seaweeds possess bioactive components which contain low calories, and they are rich in vitamins, minerals, proteins, polysaccharides, steroids and dietary fibers (Govindasamy *et al*., 2012). Seaweeds have recently received significant attention for their potential as natural antioxidants and determination of protein content of algae can provide important information on the chemical characteristics of algal biomass.The three seaweeds selected based on their commercial importance found in the coastal areas of Gulf of Mannar, Tamil Nadu are *Sargassum wightii*, *Gracilaria corticata* and *Kappaphycus alvarezii*.

Phycocolloids refer to those polysaccharides extracted from both fresh water and marine algae. Polysaccharides derived from marine red and brown algae such as agar, carrageenan and algin are economically important and have commercial significance, since these polysaccharides exhibit high molecular weight, high viscosity, stabilizing and emulsifying properties (Ji Minghou, 1990 and Armisen R. and F. Galatas 1987). These colloids have many applications in food, pharmaceutical, cosmetic, biotechnological industries etc as gelling agents, thickeners or stabilizing and emulsifying agents (Kaladharan et al., 1999). The basic structure of agar is a regularly alternating sequence of 3 -

linked-β D galactopyranose and 4 - linked 3 -6 anhydro -α- L- galactopyranose.

Carrageenan which is also derived from different genera of Rhodophyta is a linear polysaccharide with a repeating structure of alternating 1, 3-linked- β- D galactopyranose and 1-4 linked α- D- galactopyranose units. The 3 linked units occur as the 2-and 4-sulphate, while the 4-linked units occur as the 2-sulphate, 2,6- disulphate, the 3,6 anhydride and the 3,6 anhydride 2- sulphate. Algin extracted from genera of Phaeophyta is a linear polymer based on two monomeric units, β- D mannuronic acid and α- L guluronic acid (Istinii et al., 1994).

Many scientists reported that chlorophyll present in algae is the highest known source of chlorophyll. This green pigment is reported to be vital for rapid assimilation of amino acids. Red and green seaweeds contain carotenoids such as beta carotene, lutein, violaxanthin and fucoxanthin in brown seaweeds (Aranzazu Bocanegra *et al*., 2009). Main carotenoids present in the red algae are beta-carotene, alpha carotene and their dihydroxylated derivatives such as zeaxanthin and lutein (Yan *et al*., 1999). Phloratannin ranges from five to 15 % of dry weight in seaweeds. It plays an essential role in preventing disease linked to oxidative stress.

## MATERIALS AND METHODS

### Collection of Seaweeds

The seaweeds *Kappaphycus alvarezii, Sargassum wightii* and *Gracilaria corticata* were collected from Gulf of Mannar, near Rameswaram coastal region, Tamil Nadu. Algal samples were cleaned from epiphytes, extraneous matter and necrotic were removed. Samples were collected in sterilized polyethylene bags, and transported to the laboratory. Samples were washed thoroughly with sea water then sterile distilled water, air dried, cut into small pieces and then ground until a fine powder is obtained.

### Preparation of seaweed extract

The 10 g powdered seaweed was soaked in 100 mL of ethanol and incubated for 3 hours to homogenize the sample. Then it was filtered through Whatman No.1 filter paper. To the filtrate again 50 mL of solvent was added and incubated for 1hour and then filtered again. Filtrate was collected and stored in the refrigerator for phytochemical analysis.

### Extraction of Phycocolloids

**Carrageenan extraction -** *Kappaphycus alvarezii*

50 g of dried seaweed sample was washed with tap water and then incubated in 2 L of 6 % KOH solution in an 80˚C water bath for 3 hours. The samples were washed overnight in tap water. The samples were then stirred lightly in 1 L of distilled water and boiled for at least 1 hour until the algae disintegrated and the carrageenan extract was filtered with muslin cloth. The fodder or sap was stored for screening of different immunostimulatory properties (Dinesh Kumar Mishra et al., 2007).

**Alginate extraction -** *Sargassum wightii*

50 g of sample was treated with 500 mL of 0.2 N sulphuric acid in a slow shaker overnight at room temperature, in order to remove the acid soluble salt. The mixture was filtered through nylon and washed with 50-100 mL distilled water and filtered. The residue was extracted with 500 mL of 1% sodium carbonate solution and kept in a shaker at room temperature overnight and filtered again with muslin cloth. The sap was stored for screening of different immunostimulatory properties (Umamaheswara Rao, 1969).

**Agar extraction -** *Gracilaria corticata*

50 g of the samples were incubated in l L of 5% NaOH solution at 70˚C in a water bath for 2 hours and washed in tap water for 30 min. The algae were then stirred lightly in 1 L of 1.5% sulphuric acid solution at room temperature for 2 hours and further washed in tap water overnight to completely eliminate the acid. Samples were boiled for 90 minutes in 1 L of distilled water and the agar extract was filtered through a muslin cloth and fodder was stored (Chennubhotla *et al.,* 1977).

### Phytochemical Characterization of Seaweeds

The dried, powdered samples and fodders after extraction of phycocolloids were subjected to qualitative tests for the identification of phytochemical constituents for comparison.

**Test for Saponins** (Froth formation Test)

2 mL seaweed extract was taken in a test tube to which 1 mL of water was added and shaken well till froth is formed.

**Test for Steroids** (Salkowski test)

5 mL of seaweed extract was taken in a test tube to which few drops of concentrated sulphuric acid was added.

**Test for Tannins** (Ferric chloride Test)

2 mL seaweed extract was taken in a test tube to which 2 mL of 1% ferric chloride solution was added.

**Test for Quinones**
1 mL seaweed extract was taken in a test tube to which 2 mL of sodium hydroxide solution was added.

**Test for Anthraquinones**
3 mL of the seaweed extract was added to a 1 mL of ether and filtered to which a little amount of caustic soda was added.

**Test for Ketones**
2 mL of the seaweed extract was added to few crystals of resorcinol and equal volume of hydrochloric acid was added followed by heating.

**Test for Reducing sugars**
1 mL of the extract was added with few drops of Fehling's solution I and II and observed for colour change.

**Test for Flavones**
1 mL seaweed extract was taken in a test tube to which 1 mL of 10% sodium hydroxide solution or ammonia was added.

**Estimation of Protein**
**Precipitation of protein**
Protein precipitation was followed Berges *et al* (1993). Two proportions of 25% trichloroacetic acid (TCA) added to the extracts were tested: 2.5:1 and 3.0:1 (TCA: homogenate, v/v). Tubes containing TCA and homogenate were kept in an ice bath for 30 minutes and then centrifuged for 20 minutes at 4˚C. Supernatants were discarded; pellets were washed with cold 10% TCA and centrifuged again. Pellets formed after the second centrifugation were suspended in 5% TCA at room temperature, in a proportion of 5:1 (5% TCA: precipitate, v/v) and centrifuged at 21˚C for 20 minutes. Supernatants were discarded and pellets were kept in the tubes until quantification of protein was done a few minutes later. When the protein analysis was not performed immediately, pellets were stored at −20˚C until further analysis. Precipitated protein was suspended in 0.5 mL 1.0 N NaOH and 2.0 mL 0.1 N NaOH for Lowry assay.

**Protein analysis (Lowry method)**
Solution A: 1% Copper sulphate in 10 mL distilled water.
Solution B: 2% sodium potassium tartrate in 100 mL of distilled water.
Solution C: 1 g sodium hydroxide and 5 g sodium carbonate dissolved in 250 mL of distilled water.
50 mL of solution C was mixed with 0.5 mL (each) of solution A and B. 1 mL of seaweed extract was taken in a test tube to which 5 mL of the above mixture was added following which 0.5 mL of Folin–Ciocalteu reagent was added. The tubes were incubated for about 30 minutes and the optical density was measured in a ELICO SL 159 UV-Vis Spectrophotometer at 650 nm. The protocol for extraction and precipitation of macroalgal protein was followed to get more accurate results (Elisabete Barbarino *et al*., 2005).

**Estimation of Lipid**
10 mg of dried powder sample taken in a test tube, 5 mL of chloroform: methanol (2:1) mixture was added. The mixture was incubated at 37˚C for 24 hours after closing the mouth of the test tube with aluminum foil. After the incubation, the mixture was filtered using a filter paper. The filtrate was collected in a 10 mL preweighed beaker, which was kept on a hot plate. The chloroform: methanol mixture was evaporated leaving a residue at the bottom of the beaker. The beaker with the residue and the weight of the empty beaker was calculated.

**Estimation of Total sugar**
10 mg of the sample was taken with 5 mL of 80% ethanol and was centrifuged at 4000 rpm. To the supernatant, 0.5 mL was taken and 5 mL of anthrone reagent was added. The tubes were kept in a boiling water bath for 15 minutes followed by incubation in dark room for another 15 minutes. The colour intensity developed was read in a ELICO SL 159 UV-Vis Spectrophotometer at 490 nm. Total flavonoid content was expressed as mg glucose equivalents (GE)/100g dry mass samples were analyzed in duplicates.

**Estimation of Flavonoids**
Total flavonoid content was measured by the Aluminum chloride calorimetric assay. 10 mg of the sample was taken with 5 mL 80% ethanol and centrifuged at 4000 rpm. The supernatant was added to 10 mL test tube containing 4 mL of double distilled water to which was added 0.3 mL of 5% sodium nitrate. After 5 minutes 0.3 mL 10% aluminum chloride was added. At sixth minute, 2 mL of 1M sodium hydroxide solution was added and the total volume was made up to 10 mL with double distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg catechin equivalents (CE)/100g dry mass samples were analyzed in duplicates.

## RESULTS AND DISCUSSION

### Extraction of Phycocolloids

The hydrocolloids extracted were poured in Petri plates and were found to solidify at a faster rate and the gel strength was higher compared to the same lab scale products. All the three hydrocolloids were water soluble and translucent.

### Phytochemical Characterization of Seaweeds

**Test for Saponins** (Froth formation Test)
Stable froth was formed in all three seaweeds indicating presence of saponins.

**Test for Steroids** (Salkowski test)
The formation of yellow coloured indicates presence of steroids and the quantity is almost same in all the three seaweeds.

**Test for Tannins** (Ferric chloride Test)
The extracts were turned into blue colour indicating the presence of tannins in which *Gracilaria corticata* showed the least.

**Test for Quinones**
All three extracts were turned into blue colour indicating the presence of quinones but *Kappaphycus alvarezii* showed invariantly a very high amount of quinones than the other two seaweed extracts.

**Test for Anthraquinones**
The seaweed extract was turned into red colour indicating the presence of anthraquinones except that of *S.wightii*.

**Test for Ketones**
All three extracts were turned into pink indicating the presence of ketones in which *Sargassum wightii* showed the least amount of ketones.

**Test for Reducing sugars**
The seaweed extract was turned into red to reddish brown colour indicating the presence of reducing sugars.

**Test for Flavones**
The extracts were turned into yellow colour indicating the presence of flavones.
Thus, the phytochemical screening of seaweeds showed the presence of saponins, steroids, tannins, quinones, ketones and reducing sugars in all the three seaweed samples tested even though there may be a difference quantitatively.

### Estimation of Protein

Among the three seaweeds *Gracilaria corticata* was found to have highest amount of protein followed by *Kappaphycus alvarezii* and *Sargassum wightii* which would support their role in a nutraceutical.

### Estimation of Lipid

The extract from *Sargassum wightii* was found to have higher lipid content than *Kappaphycus alvarezii* and *Gracilaria corticata* but the amount of lipid compared to other compounds are fairly low (at levels that would augment a balanced diet if consumed regularly).

### Estimation of Flavonoids

The extract from *S. wightii* was found to have higher flavonoid content than *K. alvarezii* and *G. corticata*. The flavonoids being a group of polyphenols are one of the compounds mainly responsible for anti-oxidant and anti-inflammatory activity. Also few specific flavonoids are responsible of odour-masking capacity.

### Estimation of Total Sugar

The extract from *Gracilaria corticata* was found to have higher total sugar content than *Sargassum wightii* while the *Kappaphycus alvarezii* showed the least. Good amount of carbohydrate in seaweeds proves it to be a rich source of energy that also helps in controlling body weight, improved digestion and dietary fibers prevents the accumulation of cholesterol in arteries.

## CONCLUSION

Phycocolloids agar, alginate and carrageenan from the three selected seaweeds were easily extracted and of high quality with good physiological properties like viscosity, gelling capacity and odorless. Phytochemical screening of constituents and characterization studies from the fodder collected after phycocolloid extraction proved the presence of appreciable amount of compounds like proteins, carbohydrates, lipids and flavonoids specially recommended for nutraceuticals which is the evident that the waste from such industries can be used for manufacturing variety of compounds and it does act as a step towards solid waste management. Detailed investigation on the compositions of each component involved is absolutely necessary to establish appropriate applications which may open new frontiers for human consumption of this seaweed world-wide.

**Table 1: Preliminary Phytochemical Analysis of Seaweeds**

| S.No | Compound Name | *S.wightii* | *G.corticata* | *K.alvarezii* |
|---|---|---|---|---|
| 1 | Saponins | + | ++ | ++ |
| 2 | Steroids | + | ++ | ++ |
| 3 | Tannins | +++ | + | ++ |
| 4 | Quinones | ++ | + | +++ |
| 5 | Anthraquinones | - | ++ | ++ |
| 6 | Ketones | + | ++ | +++ |
| 7 | Reducing sugars | +++ | ++ | + |
| 8 | Flavones | ++ | + | +++ |

**Table 2: Estimation of Protein**

| Seaweed Extract | Absorbance (650nm) | Amount of Protein (µg/mL) |
|---|---|---|
| *G. corticata* | 0.68 | 136 |
| *S. wightii* | 0.36 | 72 |
| *K. alvarezii* | 0.45 | 90 |

**Table 3: Estimation of Lipid**

| Seaweed extracts | Weight of beaker (g) | Amount of Lipid (g) |
|---|---|---|
| *G. corticata* | 45.77 | 0.02 |
| *S. wightii* | 45.82 | 0.07 |
| *K. alvarezii* | 45.78 | 0.03 |
| Empty beaker | 45.75 | - |

**Table 4: Flavonoid Estimation**

| Seaweed extracts | Absorbance | Amount of Flavonoids (mg (CE)/100g seaweed) |
|---|---|---|
| *G. corticata* | 0.07 | 0.18 |
| *S. wightii* | 0.31 | 0.92 |
| *K. alvarezii* | 0.16 | 0.60 |

**Table 5: Estimation of Total Sugar**

| Seaweed extracts | Absorbance (490 nm) | Amount of Flavonoids (mg (GE) /100g seaweed) |
|---|---|---|
| *G. corticata* | 0.23 | 0.3 |
| *S. wightii* | 0.11 | 1.02 |
| *K. alvarezii* | 0.09 | 0.85 |


**Fig. 1: Seaweeds (a) *Kappaphycus alvarezii, (b) Sargassum wightii* and (c) *Gracilaria corticata***

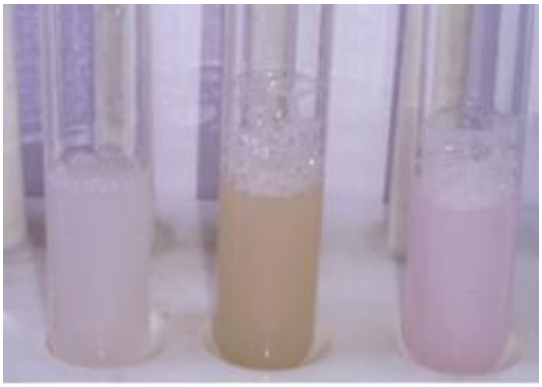


**Fig. 2: Froth formation Test**

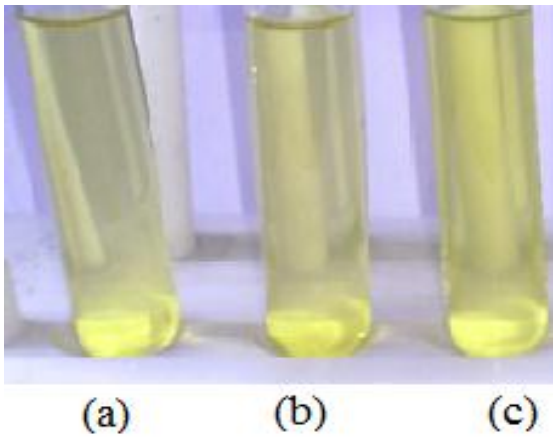


**Fig. 3: Salkowski test**

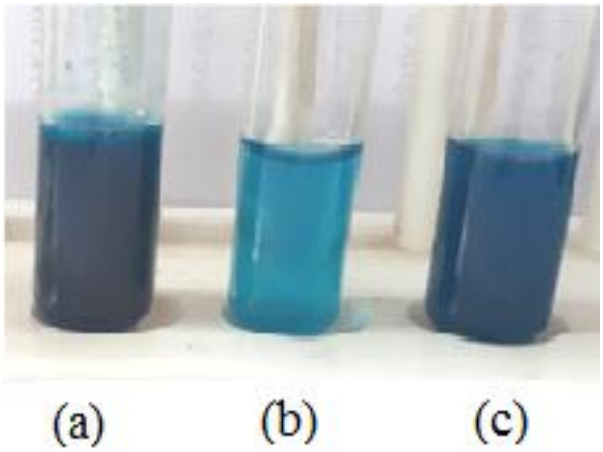


**Fig. 4: Ferric chloride Test**

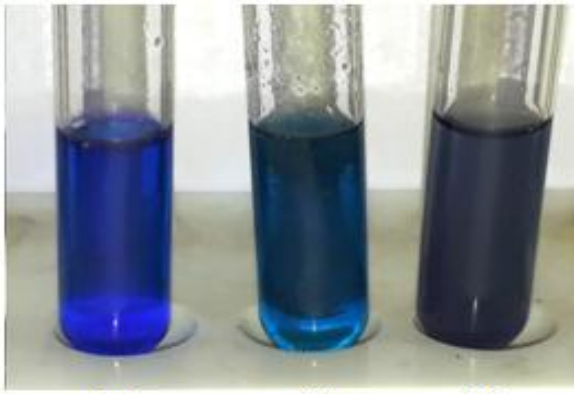


**Fig. 5: Test for Quinones**

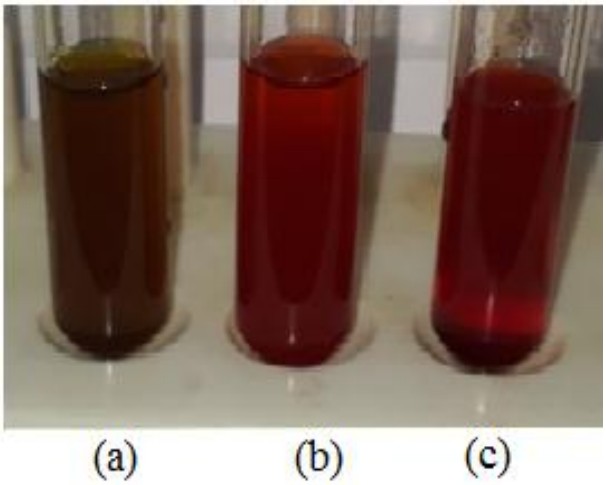


**Fig. 6: Test for Anthraquinones**

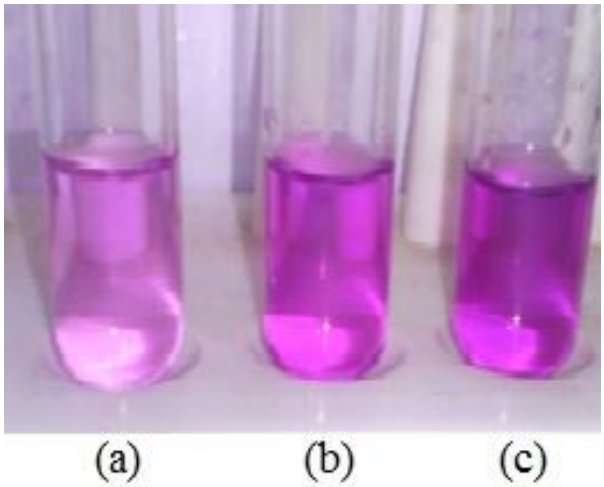


**Fig. 7: Test for Ketones**

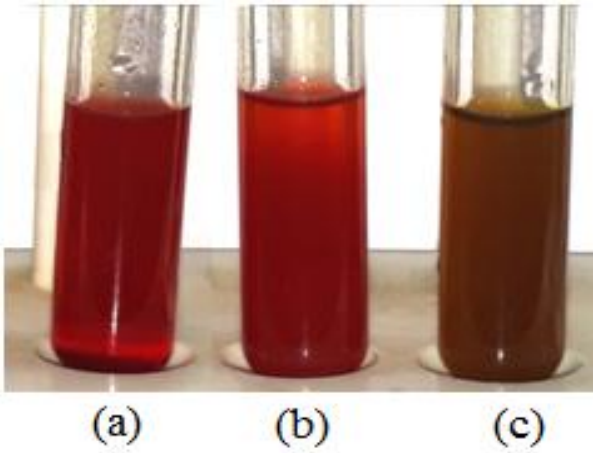


**Fig. 8: Test for Reducing sugars**

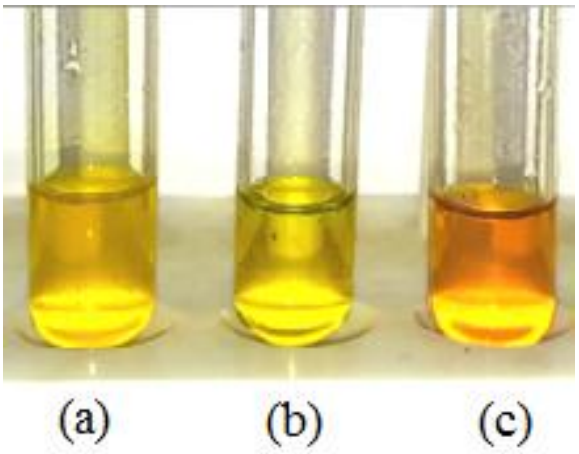


**Fig. 9: Test for Flavones**

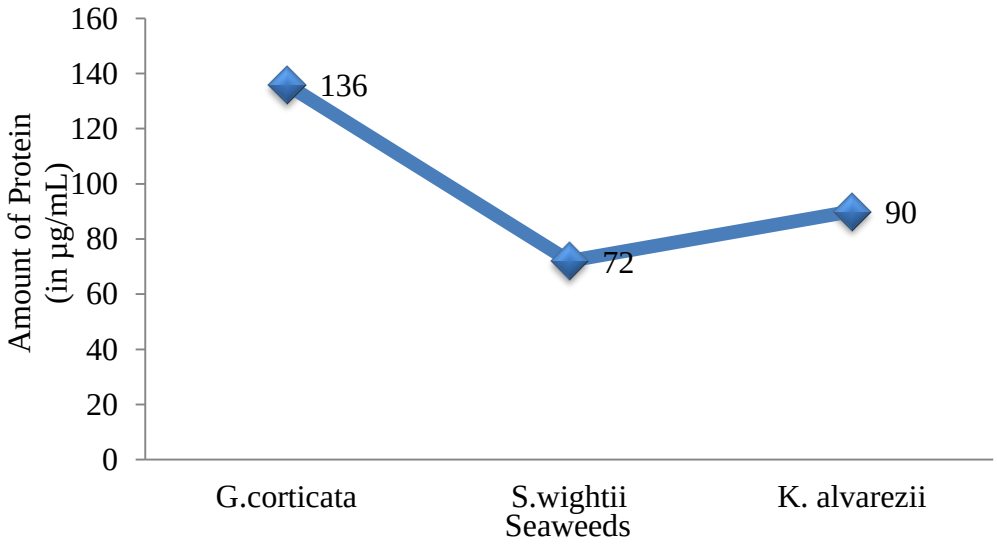


**Fig. 10: Estimation of Protein (Lowry method)**

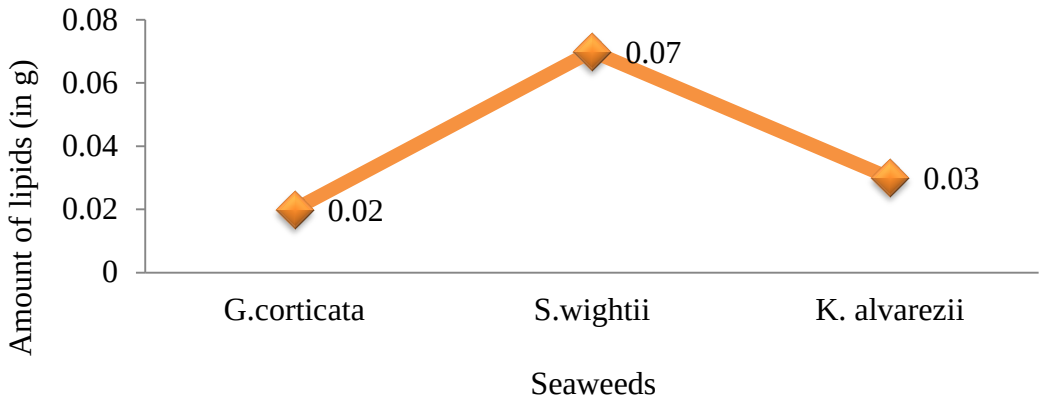


**Fig. 11: Estimation of Lipid**

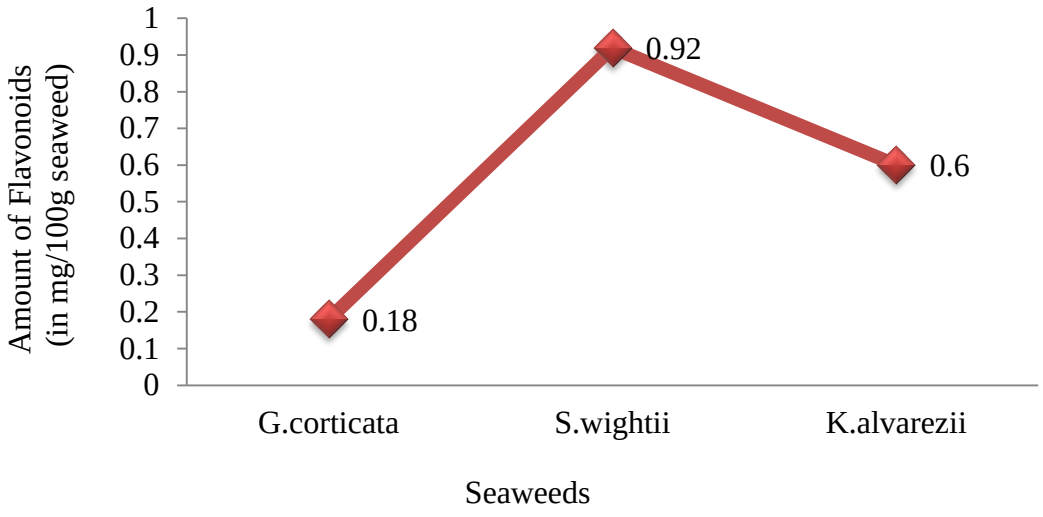


**Fig. 12: Flavonoid estimation**

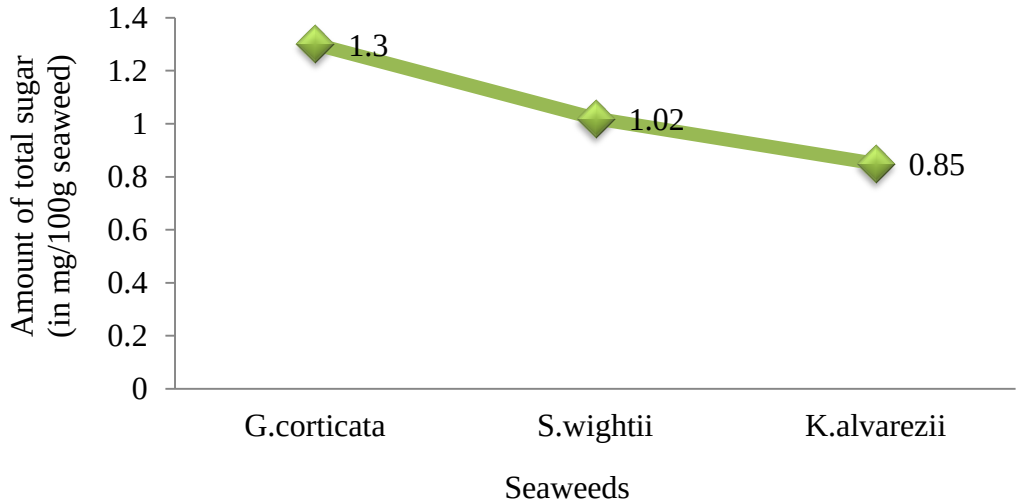


**Fig. 13: Estimation of Total Sugar**

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