Qualitative and quantitative analysis of *Sargassum wightii* and *Kappaphycus alvarezii*

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**Abstract**

During the last four decades, numerous novel compounds have been isolated from marine organisms and many of these substances have been demonstrated to possess interesting biological activities. To date, numerous structurally unusual secondary metabolites, such as flavonoids (which play a role in removal of toxins from skin), alkaloids and phenolic compounds (a powerful free radical scavenging activity agent), sesquiterpenes, diterpenes, phlorotannins have been frequently reported from various species of seaweeds. With this background two seaweeds *Sargassum wightii* (Sw1) and *Kappaphycus alvarezii* (Sw2) were analysed for their active metabolites qualitatively and quantitatively.

**Keywords:** Seaweeds, extracts, secondary metabolites, *Sargassum wightii*, *Kappaphycus alvarezii*

**INTRODUCTION**

Ancient human beings believed that oceans had magical powers to meet any kind of needs of human beings Seaweeds are crucial part of marine ecology and are essential for sustainability of living organisms. There are about 10,000 species, of which 6000 are red algae, 2000 are green algae and 2000 are brown algae [1-4]. The chemical composition of seaweeds varies with species, habitats, maturity and environmental conditions [5]. Importance of Seaweeds lies in its great food value as it is rich in carbohydrates, proteins and lipids (polyunsaturated fatty acid) [6]. Seaweeds possess enormous biologically important and beneficial ingredients like fucoidians, carotenoids [7], fucoxanthin, phlorotannins, flavanoids, algal polyphenols – antivirus, antibacterial, and antioxidative compounds. Enzymic and nonenzymatic antioxidants, vitamins and minerals that aid human health. Marine algae are one of the richest sources of natural antioxidants. The main antioxidant activity of seaweed extracts arises from tocopherols, carotenoids and polyphenols. The seaweeds with high phenolic content have antioxidant properties and are used in prevention off and in treatment of neurodegenerative diseases caused by oxidative stress, and cancer [8-9].

**MATERIALS AND METHODS**

Selection and collection of Seaweed Extracts

Selection of Seaweeds

**Herbal monograph**

<table>
<thead>
<tr>
<th>English name</th>
<th>Seaweeds (Marine Algae)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamil name</td>
<td>Kadal Paasi</td>
</tr>
<tr>
<td>Habitat</td>
<td>Seaweeds have a variety of morphologies, ranging from uni cells and filaments to blades and freshly thalloid forms.</td>
</tr>
<tr>
<td>Parts used</td>
<td>Whole plant</td>
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</table>

The following seaweeds are used for the present study named as Seaweed 1 and Seaweed 2. They are 1. *Sargassum wightii*-(Sw1) 2. *Kappaphycus alvarezii*-(Sw2)

**SEAWEED -1**

**Sargassum wightii** – (SW1)

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<tr>
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</tr>
<tr>
<td>Species</td>
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</tr>
</tbody>
</table>

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**SEAWEED - 2**

**Kappaphycus alvarezii – (SW2)**

- **Genus**: Kappaphycus
- **Kingdom**: Plantae
- **Sub kingdom**: Biliphyta
- **Phylum**: Rhodophyta
- **Sub phylum**: Eurhodophytina
- **Class**: Florideophyceae
- **Subclass**: Rodymeiophycidae
- **Order**: Giartinales
- **Family**: Areschougiaceae
- **Genus**: Kappaphycus
- **Species**: Alvarezii

**COLLECTION OF THE SELECTED SEAWEEDS**

Seaweeds were collected from the Mandabam coastal region, Tamil Nadu and authenticated by SNAP, (Sipcot Natural Alginate Products Private Limited) Ranipet, Tamil Nadu, India.

**METHODS**

**PREPARATION OF EXTRACTS**

The Seaweeds were washed in seawater and freshwater thoroughly to remove the epiphytes and other debris. They were then air dried in shade and coarse powdered. The powder obtained was extracted successively with 3 different solvents - ethanol, methanol and acetone using a soxhlet extractor for 18-20 hrs. The extracts were concentrated at 45ºC under reduced pressure using rotary flash evaporator and stored at 4ºC in refrigerator until further use.

**PHYTOCHEMICAL SCREENING**

**Qualitative analysis**

Qualitative tests were carried out on all the extracts prepared using standard procedures to identify constituents such as alkaloids, carbohydrates, glycosides, proteins and amino acids, phytosterols, tannins, saponins, phenolic compounds, terpenoids, flavanoids.

**a) Detection of Alkaloids**

Solvent free extract of 50mgs of seaweeds was stirred with few ml of dil. HCl and filtered. The filtrate was tested with various alkaloid reagents. Detection of alkaloids was done by four methods.

**Mayer’s test**

To a few ml of filtrate, two drops of Mayer’s reagent was added. A white or creamy precipitate indicated the test as positive.

**Wagner’s test**

To a few ml of filtrate, few drops of Wagner’s reagent was added by the side of the test tube, a reddish brown precipitate confirmed the test as positive.

**Hager’s test**

To a few ml of filtrate, 1 or 2ml of Hager’s reagent was added, a prominent yellow precipitate indicated the test as positive.

**Dragendorff’s test**

To a few ml of filtrate 1 or 2ml of Dragendorff’s reagent was added, a prominent yellow precipitate indicated the test as positive.

**b) Detection of Carbohydrates**

The extract (100mg) was dissolved in 5ml of water, filtered and used for the study. Four tests were performed for the detection of carbohydrates.

**Molisch test**

To 2ml of filtrate two drops of alcoholic solution of napthol was added, the mixture was shaken well and 1ml of Conc. H₂SO₄ was added slowly along the sides of the test tube. A violet ring indicated the presence of Carbohydrates.

**Fehlings test**

1ml of filtrate was boiled on water bath with 1ml of Fehling’s solution A and B. A red precipitate indicated the presence of Carbohydrates.

**Barfoed’s test**

To 0.5ml of filtrate, 0.5ml of Benedict’s reagent was added. The mixture was heated on a boiling water bath for 2mins. A characteristic change indicated presence of sugar.

**Benedicts test**

To 0.5ml of filtrate, 0.5ml of Benedict’s reagent was added, the mixture was heated on a boiling water bath for 2mins. A characteristic change indicated the presence of sugar.

**c) Detection of Saponins**

The extract (50mg) was diluted and made up to 20ml with distilled water. The suspension was shaken in a graduation cylinder for 15mins, formation of a two layer, indicates the presence of Saponins.
d) Detection of glycosides
50mg of extract was hydrolysed with hydrochloric acid for 2hrs on a water bath, filtered and subjected to the following test. Two tests were performed for the detection of glycosides.

Bertrager’s test
To 2ml of filtrate, 3ml of chloroform was added and shaken well. Chloroform layer was separated and 10% ammonia was added to it. Formation of Pink colour indicated the presence of glycosides.

Legal’s test
50 mg of extract was dissolved in pyridine, sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide. Formation of pink colour indicated the presence of glycosides.

e) Detection of proteins and aminoacids
The extract (100mg) was dissolved in 10ml of water and filtered through Whatmann No.1 filtered paper and the filtrate was subjected for analysis of proteins and aminoacids. Three tests were performed for the detection of Proteins and amino acids.

Millon’s test
To 2ml of the filtrate few drops of Millon’s Reagent was added, a white precipitate indicated the presence of proteins.

Biuret test
An aliquot of 2ml of filtrate was treated with one drop of 2% copper sulphate solution. To this 1ml of ethanol (95%) was added followed by excess of potassium hydroxide pellets. Pink colour indicated the presence of proteins.

i) Ninhydrin test
Two drops of Ninhydrin solution was added to two ml of aqueous filtrate. A characteristic purple colour indicated the presence of amino acids.

f) Detection of Flavanoids
A portion of crude powder was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow coloration.

g) Detection of Terpenoids
0.5 g of crude powder was dissolved in 5 ml of methanol. 2 ml of the extract was treated with 1 ml of 2, 4-dinitrophenyl hydrazine dissolved in 100 ml of 2M HCl. A yellow-orange coloration was observed as an indication of terpenoids.

h) Detection of phytosterols
Liberman-Burchcard’s test
The extract (50mg) was dissolved in 2ml of acetic anhydride and to this one or two drops of concentrated sulphuric acid was added slowly along the sides of the test tubes. An array of colour changes indicated the presence of phytosterols.

i) Detection of Phenolic compounds and Tannins
Four tests was performed for the detection of phenolic compounds and tannins.

Ferric chloride test
The extract (50mg) was dissolved in 5ml of water; to this few drops of 5% neutral ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

Gelatin test
The extract (50mg) was dissolved in 5ml of distilled H2O and 2ml of 1% solution of gelatin containing 10% sodium chloride was added to it. Formation of white precipitate indicated the presence of phenolic compounds.

Lead acetate test
The extract (50mg) was dissolved in water and 3ml of 10% lead acetate solution was added. A milky white precipitate indicated the presence of phenolic compounds.

Alkaline reagent test
An aqueous solution of the extract was treated with 10% ammonium hydroxide solution, yellow fluorescence indicated the presence of flavanoids.

Magnesium and hydrochloric acid reduction test
The extract (50mg) was dissolved in 5ml of alcohol, and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) was added. A pink colour to crimson red colour developed which indicated the presence of flavanoids.

QUANTITATIVE ANALYSIS OF BIOCHEMICAL CONSTITUENTS
From the above preliminary test methanol extract of the seaweeds showed a maximum number of secondary metabolites. Therefore the methanol extract was used to pursue further study.

**a) Determination of Total Carbohydrates**

100mg of sample was weighed and hydrolysed in a boiling tube by keeping in a boiling water bath for 3hrs with 5ml of 2.5N HCl and cooled to room temperature, and then it was neutralized with solid sodium carbonate until the effervescence stopped. Then the volume was made upto 100ml with water and centrifuged. The supernatant was collected and 0.5ml and 1ml of aliquots were taken for analysis. Standards were prepared by taking 0.2, 0.4, 0.6, 0.8, and 1.0ml of the working standard and blank was prepared in the same manner as the standards. Volumes of all the tubes were made up to 1ml with water (including the sample). Then 4ml of Anthrone reagent was added and heated for 8mins in boiling water bath, then cooled rapidly. The green colour developed was read at 630nm.

**b) Estimation of Protein**

The working standards were pipette out in a series of test tubes as 0.2, 0.4, 0.6, 0.8 and 1.0 ml respectively and the sample extracts were also pipette out in the volume of 0.1ml and 0.2ml as test. The volume of all the test tubes were made up to 1ml with distilled water. 1ml of the distilled water serves as blank. Then 5ml of alkaline copper reagent was added to all the tubes including blank. After 10 minutes, 0.5ml of Folin-Ciocalteau reagent was added and mixed well and incubated at room temperature in the dark for 30 minutes. The blue colour developed was read at 660nm.

**c) Estimation of total free amino acids**

**Extraction of amino acids**

To 500mg of the powdered sample 5-10ml of 80% methanol was added and centrifuged. The filtrate or supernatant was collected. The extraction was repeated twice with the residues and the supernatants were pooled and used for estimation.

**Technique**

To 0.1ml of extract, 1ml of ninhydrin solution was added and the volume was made up to 2ml with distilled water. All the test tubes were heated in a boiling water bath for 20mins. 5ml of diluents was added and the contents were mixed well, after 15mins the intensity of purple colour against the reagent blank was read colorimetrically at 570nm. The reagent blank was prepared by taking 0.1ml of 80% ethanol. A series of volume from 0.1 - 1ml of the working standards were taken.

**d) Estimation of Proline**

0.5gm of seaweeds was homogenized in 10ml of 3% aqueous sulphosalicylic acid. Filtered the homogenate by using Whatmann No.1 filter paper. To 2ml of filtrate, 2ml of glacial acetic acid and 2ml of acid ninhydrin was added. Then the test tube was boiled in a boiling water bath for 1 hr. Cooled by keeping it in ice bath to terminate the reaction. 4ml of toluene was added to the reaction mixture and stirred well for 20-30 secs. The toluene layer was separated and incubated at room temperature. The intensity of red colour developed was read at 520nm.

**QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS IN METHANOLIC EXTRACT OF SELECTED SEAWEEDS**

**a) Estimation of phenol**

0.5gm to 1.0gm of the seaweeds were exactly weighed and ground with 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 mins. The supernatant was collected. The residue was re-extracted with 5 times the volume of 80% methanol, centrifuged and pooled the supernatant. The supernatant was evaporated to dry. The residue was dissolved in 5ml of water. Pipette out aliquots of standard (0.2 to 2ml) into test tube. Made up the volume of all the tubes to 3ml with distilled water. Then 5ml of Folin-Ciocaltaud reagent was added in each tube. Mixed and placed the tubes in boiling water for exactly 1min. Then the test tubes were cooled and measured the absorbance at 650nm against reagent blank. A standard curve was prepared using different concentration of catechol.

**b) Estimation of β-carotene**

Algal culture was centrifuged at 2000 rpm for 5 mins. To this added 3ml of (2:1) ethanol : hexane mixture was added and 2ml of double distilled water and 4ml of hexane. It was mixed well and centrifuged for 5mins at 2000 rpm. Then the hexane layer was measured at 450nm spectrophotometrically. β- carotene was estimated using the approved AACC (14-50, 1995) method as described below. Eight gram seaweeds was taken in
150 ml glass stoppered Erlenmeyer flask and 40 ml water saturated butanol (WSB) was added. The contents of the flasks were mixed vigorously for 1 minute and kept overnight (16 - 18 hrs) at room temperature under dark for complete extraction of β-carotene. On the following day, the contents were shaken again and filtered completely through Whatman no.1 filter paper into a 100 ml volumetric flask. The optical density of the clear filtrate was measured at 440 nm. Pure WSB was used as blank. The β-carotene content was calculated from calibration curve from known amount of β-carotene as discussed below and expressed as parts per million (ppm). Standard solution of β-carotene was prepared in water saturated butanol (WSB) at the concentration of 5 µg/ml. WSB was prepared by mixing n-butanol with water in 8:2 ratio. Calibration curve was drawn from known amounts of pure β-carotene from 0.25 µg/ml to 1.5 µg/ml which was prepared after suitable dilutions of original stock with WSB in calibrated 10ml volumetric flasks (from 0.5 ml to 3 ml of standard solution in 10 ml). Absorbance of each dilution was measured and a calibration curve was established. β-carotene content of unknown samples was calculated from standard curve.

c) Estimation of Vitamin-C
To 0.5ml of extract, 0.5ml of distilled water and 1.0ml of Tricarboxylic acid (TCA) was added and centrifuged for 20mins. To the supernatant 0.2ml of DTC was added and incubated for 3 hrs at 37°C. Then 1.5ml of H₂SO₄ was added and allowed to stand at room temperature for 30 minutes. The OD was measured at 520nm. The ascorbic acid was measured using the standard curve.

d) Estimation of Vitamin – E
To 1.5ml of seaweed extract, 1.5ml of distilled water was added and centrifuged. To the test added 1.5ml of ethanol and to the blank 1.5 ml of distilled H₂O was added and mixed well. 1.5ml of xylene was added to all the test tubes and centrifuged. The supernatant contained xylene layer. 1.0ml of dipyridy1 reagent was added to all the test tubes. 1.5ml of the reaction mixture was pipetted out and read at 420nm by photochemical colorimeter, then ferric chloride reagent was added to all the tubes and read at 520nm.

e) Estimation of Thiamine
5g of finely powdered sample was weighed in a 250ml of conical flask. Added 100ml of 0.1N H₂SO₄ was added to it without shaking the stopper flask and allowed it stand overnight. On the following morning it was filtered through Whatmann No.1 filter paper, discarded the first 10 to 15ml of filtrate was discarded. 10ml of extract was pipette out in a duplicate into 100ml separating funnels. 10ml of working standard was pipette out in (4-5 replicates). 3ml of 15% NaOH was added into each separating funnel immediately followed by 4 drops (0.2ml) of ferric cyanide solution. It was shaken gently exactly for 30 seconds. Added 15ml of iso butanol was added rapidly from a quick delivery burette or a measuring cylinder. Stoppered immediately and shaken vigorously for 60 seconds and allowed the layer to separate and drained off the bottom layer carefully and discarded it. 1 spatula of sodium sulphate was added directly into separating funnel, stoppered and swirled gently to clarify the extract. Whenever the extract was not clear, a little more Na₂SO₄ was added. Collected the clear extract from the top using a pasteur pipette into a clean dry test tube. A set of sample blank was prepared by pipetting 10ml of the extract and the above procedure was followed and omitted the addition of ferric cyanide. A blank for the standard was prepared separately. Selected suitable primary (360nm) and secondary filters as per the fluorimeter by initially adjusting the standard blank to 0 readings and standard to 100. Then added the sample blank and sample. Since the intensity sometime changed progressively, it was necessary to take 5-6 readings.

f) Estimation of Phlorotannins
The total phlorotannins consist of a complex of different types of individual phlorotannins. Some are soluble in water while some are soluble in solvent. Therefore, RP-HPLC was selected as a suitable tool for quantitative analysis of phlorotannins. The dried algal powder was shaken by a shaker with methanol (2ml) at room temperature for 2hrs. It was filtered through defatted cotton and then added 1.5ml of deionized water and shaken for 5 minutes. The upper non lipid fraction. Was collected and treated the extract twice with 3ml of ethyl ether and taken the upper layer. Ethyl ether was evaporated and the crude phlorotannin was filtered through 0.45 µm cellulose acetate syringe filter and then
g) Estimation of Carrageenans, Fucoidans, Alginate polysaccharides

**Extraction**

Carrageenan containing materials were digested with papain at 70°C in the presence of 1.0 M NaCl. The digest was adjusted to pH 8.0 to 8.5 with NaOH. Celite was added and the mixture filtered over glass wool. Carrageenan in the filtrate was precipitated with cetyl pyridinium chloride (C.P.C.) in the presence of 0.5 to 1.0 M KCl and Celite. The carrageenan-C.P. precipitate was washed with 0.1% C.P.C.-0.05 M KCl and the carbohydrate content determined by the phenol-H$_2$SO$_4$ method.

Mild oxidative treatment is suggested for decolourizing the polysaccharide extracts obtained from brown algal biomass, rendering them suitable for spectrophotometric determination of polysaccharide content. Two reagents were used for the analysis of polysaccharides in brown seaweeds. Sodium chlorite for acidic extracts containing fucoidans and laminarans, and bromine, for alkaline extracts containing alginates. Acidic extracts, after treatment with sodium chlorite and dialysis, were used for the determination of fucoidan by the specific colour reaction of fucose with L-cysteine hydrochloride and sulfuric acid.

Similarly, decolourized alkaline extracts were used for the determination of alginate by specific colour reaction of uronic acids with 3,5-dimethylphenol and sulfuric acid. This reaction was proven to be practically insensitive to changes in monomeric composition of alginates.

h) Estimation of Fucaxanthins (UV-Vis Spectrophotometer)

Pigment extraction from these samples was followed by a method described by Seely et al., with slight modification and Dimethyl Sulforida (DSMO) was used as solvent during maceration. The pigment extracts obtained were then dried using argon gas and collected in a bottle covered with aluminium foil and stored in freezer compartment. The pigment extract was then analysed using TLC to identify the pigment composition based on the spot colour and $R_f$ value using stationary phase Silica gel F-254 and for mobile phase, mix solution of acetonemethanol: JPA: toluene = 5:4:1:90 (v/v). Fucoxanthin content was measured using UV-Vis Spectrophotometer at 450nm.

i) Estimation of Flavanoids

The reaction mixture (3.0ml) comprises of 1.0ml of extract, 0.5ml of aluminium chloride (1.2%) and 0.5ml of potassium acetate (120mm) and incubated at room temperature for 30mins and absorbance was measured at 415nm. Quercetin was used as a positive control.

j) Estimation of Total Ash content

The ash content of the sample was determined by placing a weighed sample in a furnace and heated it to 500°C typically overnight. All the organic material were burnt away leaving just the mineral residue. This residue was weighed and the ash content (g of ash/kg) was calculated.

k) Estimation of Calcium

50 ml of sample was taken in a conical flask. 100 to 200 mg of murexide indicator was added till a pink colour was developed. This was titrated against EDTA solution until the pink colour changes to purple. For better judgement of end point, the purple colour was compared with the water blank and titrated to end point.

l) Estimation of Sodium

This procedure was followed for pre-treatment of the sample, and the concentration of sodium was estimated by flame photometer. The calibration curve was prepared in the range of 0 to 1, 0 to 10 and 0 to 100 mg/L of sodium by using the various standard solutions of sodium. The standard curves
were used for the determination of sodium depending on the initial concentration of sodium in the sample.

m) Estimation of Potassium
Like sodium, potassium can also be determined accurately by flame photometer. The characteristic radiation is 768 nm, the intensity of which can be read on a scale by using a filter for this wavelength by the method of sodium. The same procedure as described for determination of sodium was followed except that to use potassium filter and measured at 768 nm wavelengths instead of sodium filter.

n) Estimation of Iron
In a slightly acid medium, iron (II) ions form an electronegative red-pink complex with 2,2’-dipyridine. Its molar absorption coefficient is 8.7x10^3 M^{-1} cm^{-1}, at wavelength 522 nm. The solution of the complex is stable and Fe (II) bound in the complex with 2,2’-dipyridyl is resistant to oxidation. The reaction of Fe (II) and 2,2’-dipyridine has been used for determination of iron.

RESULT AND DISCUSSION
With potential sources of bioactive compounds of immense pharmaceutical, biochemical and nutraceutical importance [10] numerous structurally unusual secondary metabolites, such as terpenes [2], Phlorotannins [3] and Steroids have been frequently reporting from various species of seaweeds. It is noteworthy that many of the compounds have been demonstrated to have various biological activities, widely ranging from antitumour [3,11], antibacterial [12], antioxidant. These bioactive compounds have provided essential substances for human nutrition and promising drug leads. Thus the present study aims at the quantitative determination of various phytochemicals from two selective seaweeds.

Qualitative Analysis of seaweed extracts
Phytochemical is a natural bioactive compound found in plants, such as vegetables, leaves, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as a defense system against disease or more accurately, to protect against diseases. Phytochemicals are divided into two groups, namely primary and secondary constituents, according to their functions in plant metabolism. The primary constituents comprises of common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds and many more such as flavonoids, tannins and so on. Recent studies have demonstrated that phytochemicals can have complementary and overlapping mechanisms of action, including scavenging of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects. The results of the preliminary screening test using three different solvents like methanol, ethanol and acetone of selected seaweeds are summarized in Table 1 & Table 2.

The phytochemical constituent (or) the secondary metabolites varies from species to species. It reveals that all extracts (methanol, ethanol and acetone) showed the presence of phytochemical constituents. The secondary metabolites identified in the selected seaweed include alkaloids, carbohydrates, saponins, glycosides, proteins, aminoacids, phyto steroids, phenolic compounds, flavonoids, terpenoids and tannins. Most of the phytoconstituents are well known for its medicinal property as well as exhibits various physiological activities.

Of all the three solvent extracts, methanolic extract of the seaweeds showed a maximum number of active components than ethanolic and acetone extracts. Thus for the study only methanolic extracts of seaweeds were used.

Quantitative analysis of phytochemicals in methanolic extract of selected seaweeds
The data of quantitative determination of secondary metabolites are presented in the Table 3, & Table 4 & Fig 1a, 1b, 2a, 2b. Various biochemical and phytoconstituents like carbohydrates, protein, total amino acid, proline, MUFA, PUFA, carrageenan s, fucoidans, alginates, phenol, terepenes, Vitamins and minerals of methanolic extracts of *Sargassum wightii* and *Kappaphycus alvarezii* were quantitatively determined.

With the presence of carbohydrates, proteins and lipids sea weeds serve as good food source. Phenolic compounds polyphenols, phloroglucinol and phlorotannins of the two seaweeds exhibit antioxidant activity and antimicrobial activity is exhibited by the presence of phenolic lipid - Terepenes. The total lipid content; MUFA and PUFA
of the two seaweeds are very important as a nutraceutical for human intake. The presence of polyols and sugars (Glycerol, Mannitol, glucose) non essential aminoacids (Proline) act not only as osmotic effectors, but also as stabilizers for the structure and function of macromolecules and organites. They also protect enzymatic activities and cell membranes. Fucoxanthin, a major carotenoid that is present in both the seaweeds has several physiological activities such as antioxidant, anti-carcinogenic, anti inflammatory, anti-obesity and antidiabetic. The presence of Agar, Fucoidans alginate, Carrageenans exhibits that the two sea weeds has wide application in food industry. Presence of β Carotene in Seaweeds enrich their value as a functional food and also suggests lower incidence of cancer and neuro degenerative diseases. With the presence of antioxidants and antioxidant enzymes like α tocopherol, superoxide dismutase, GSH, Catalase, Ascorbic acid, the two seaweeds exhibit antioxidant property. Presence of vitamins like Thiamine, Vitamin C & E and Minerals like Fe, Ca, Na and K has have enabled us to consider them as an important food source. Seaweeds also provide a higher concentration of salts combined with sodium, potassium, calcium and the concentration is higher than that of terrestrial plants. Sodium is involved in extra cellular and intracellular fluid balance and maintenance the viscosity of the blood. The maximum content of the sodium in the seaweed extract may help to relieve antioxidant stress and control the complication of liver damage. Potassium is the principal element of the intracellular fluid, it is mainly concerned with the homeostasis and influence of the muscle. The presence of potassium may therefore enhance the tissue repair of the liver. The results revealed that both the seaweeds possess quite a number of components with more or less in equal composition, which may be responsible for many of their pharmacological action.
Fig 1a. Biochemical factors of *Sargassum wightii* and *Kappaphycus alvarezii*

Fig 1b. Biochemical factors of *Sargassum wightii* and *Kappaphycus alvarezii*
Fig 2a. Phytochemicals & mineral constituents of *Sargassum wightii* and *Kappaphycus alvarezii*

Fig 2b. Phytochemicals & Mineral constituents of *Sargassum wightii* and *Kappaphycus alvarezii*
Table 1. Qualitative Phytochemical Screening of various extracts of *Sargassum Wightii*

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<td>1</td>
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<tr>
<td>2</td>
<td>Carbohydrates</td>
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<td>Saponins</td>
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<tr>
<td>10</td>
<td>Tannins</td>
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</tbody>
</table>

* + = Present; - = Absent*

Table 2. Qualitative Phytochemical Screening of various extracts of *Kappaphycus alvarezii*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemicals</th>
<th>Ethanol extract</th>
<th>Methanol extract</th>
<th>Acetone Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Proteins and amino acids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Phytosterol</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Terepenoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* + = Present; - = Absent*

Table 3. Biochemical Factors of the Sea weeds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Factors</th>
<th><em>Sargassum wightii</em></th>
<th><em>Kappaphycus alvarezii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates (g/100g)</td>
<td>41.2±0.32</td>
<td>40.8±0.48</td>
</tr>
<tr>
<td>2</td>
<td>Alginites (g/100g)</td>
<td>13.9±0.12</td>
<td>14.4±0.21</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides (g/100g)</td>
<td>1.2±0.09</td>
<td>1.8±0.09</td>
</tr>
<tr>
<td>4</td>
<td>Agar (g/100g)</td>
<td>11.2±0.15</td>
<td>12.9±0.15</td>
</tr>
<tr>
<td>5</td>
<td>Fucoidans (g/100g)</td>
<td>2.1±0.15</td>
<td>1.4±0.09</td>
</tr>
<tr>
<td>6</td>
<td>Carrageenans (g/100g)</td>
<td>2.6±0.09</td>
<td>3.0±0.12</td>
</tr>
<tr>
<td>7</td>
<td>Protein (g/100g)</td>
<td>14.3±0.26</td>
<td>18.4±0.20</td>
</tr>
<tr>
<td>8</td>
<td>Fat (g/100g)</td>
<td>1.7±0.07</td>
<td>1.1±0.12</td>
</tr>
<tr>
<td>9</td>
<td>14:0 % fatty acid</td>
<td>6.0±0.19</td>
<td>4.4±0.18</td>
</tr>
<tr>
<td>10</td>
<td>20:5 % fatty acid</td>
<td>8.1±0.15</td>
<td>6.6±0.12</td>
</tr>
<tr>
<td>11</td>
<td>MUFA</td>
<td>29.3±0.29</td>
<td>22.3±0.15</td>
</tr>
<tr>
<td>12</td>
<td>PUFA</td>
<td>7.9±0.15</td>
<td>6.7±0.19</td>
</tr>
<tr>
<td>13</td>
<td>Total amino acid (g/100g)</td>
<td>15.3±0.27</td>
<td>12.0±0.21</td>
</tr>
<tr>
<td>14</td>
<td>Proline (g/100g)</td>
<td>19.2±0.15</td>
<td>13.1±0.27</td>
</tr>
</tbody>
</table>
Table 4. Phytoconstituents, Vitamins and Minerals Factors of Sea woods

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Factors</th>
<th>Sargassum wightii</th>
<th>Kappaphycus alvarezii</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total Phenolics (mg/g)</td>
<td>35.0±0.40</td>
<td>32.1±0.10</td>
</tr>
<tr>
<td>2.</td>
<td>Crude phlorotannin (%)</td>
<td>0.8±0.06</td>
<td>0.6±0.06</td>
</tr>
<tr>
<td>3.</td>
<td>β- Carotene (µg100g)</td>
<td>32.9±0.15</td>
<td>30.6±0.23</td>
</tr>
<tr>
<td>4.</td>
<td>Ascorbic acid (mg/100g)</td>
<td>446±2.61</td>
<td>356±2.10</td>
</tr>
<tr>
<td>5.</td>
<td>Thiamine (µg100g)</td>
<td>0.3±0.06</td>
<td>0.2±0.04</td>
</tr>
<tr>
<td>6.</td>
<td>α – Tocopherol (mg/g lipid)</td>
<td>1.2±0.03</td>
<td>1.1±0.06</td>
</tr>
<tr>
<td>7.</td>
<td>Fucoxanthin(mg/g)</td>
<td>21.4±0.23</td>
<td>22.2±0.17</td>
</tr>
<tr>
<td>8.</td>
<td>Flavonoids(mg/g)</td>
<td>21.7±0.15</td>
<td>18.3±0.20</td>
</tr>
<tr>
<td>9.</td>
<td>Total Ash g/100g</td>
<td>4.8±0.15</td>
<td>4.6±0.20</td>
</tr>
<tr>
<td>10.</td>
<td>K mg/100g</td>
<td>527.3±1.80</td>
<td>416.3±2.35</td>
</tr>
<tr>
<td>11.</td>
<td>Na mg/100g</td>
<td>453.3±2.83</td>
<td>512.7±3.29</td>
</tr>
<tr>
<td>12.</td>
<td>Fe mg/100g</td>
<td>11.8±0.12</td>
<td>9.7±0.20</td>
</tr>
<tr>
<td>13.</td>
<td>Ca mg/100g</td>
<td>384.7±2.61</td>
<td>442±1.68</td>
</tr>
</tbody>
</table>

CONCLUSION

Conventional drugs used in the treatment of neurological disorders, hepatic disease and hepatic cancer are sometimes inadequate and can have serious adverse effects. It is therefore necessary to search for alternate drugs of doubtful efficacy. Marine organisms are potential source of highly bioactive secondary metabolites such as terpenes, alkaloids, polyphenols, phlorotannins, steroids, carotenes, etc., and thus could exhibit antimicrobial, anti-inflammatory, antioxidant, antitumour and antidiabetic activity. From the present study, it is concluded that the two selected seaweeds (Sargassum wightii and Kappaphycus alvarezii) with potentially productive sources of highly bioactive secondary metabolites, may be recommended for the development of new pharmaceutical agents.

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CONFLICT OF INTEREST

No Conflict of Interest.

REFERENCES


