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Phytochemical Study Of Seaweeds *Sargassum wightii* And Ulva lactuca

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Abstract
The present study aims at assessing the phytochemical and physicochemical nature of two marine seaweeds: Sargassum wightii (a brown alga) and Ulva lactuca (a green alga). A fine powder of samples of these two marine seaweeds was made and stored in airtight containers by the researcher after gathering them from the shore waters of the maritime region near Rameswaram, rinsing with sterilized water to cleanse surface contaminations, and desiccating for a period of seven days in shade for more detailed examination.
As an effective extracting agent, methanol was applied as the reagent for the extraction process of phytochemical constituents. Biologically active metabolites were examined through comprehensive phytochemical evaluations employing both descriptive and measurable analyses of the methanolic fractions. Moreover, principal physicochemical specifications including moisture content, the overall mineral residue and its acid-resistant and water-soluble components were assessed. The qualitative ash assay disclosed that significant mineral elements such as chlorine, phosphorus, and iron were present in both Sargassum wightii and Ulva lactuca, whereas Sulphur was found only in Ulva lactuca.
The characteristic phytocontituents such as terpenoids, steroids, tannins, flavonoids, phenols, proteins, and glycosides were identified in Sargassum wightii through primary phytochemical analysis. In contrast, the results revealed that alkaloids, flavonoids, carbohydrates, phytosterols, phenols, steroids, proteins, and glycosides were found in Ulva lactuca. Quantitative analysis revealed that Sargassum wightii contained phenolic compounds at 2.82 ± 0.38 mg/mL, flavonoids at 2.97 ± 1.29 mg/mL, and tannins at 2.65 ± 0.13 mg/mL, whereas Ulva lactuca exhibited significantly higher levels of phenolics (17.37 ± 1.03 mg/mL), along with flavonoids (2.62 ± 0.108 mg/mL) and alkaloids (68.07 ± 0.39 mg/mL).
Keywords: *Seaweed; Phytochemicals; Physicochemical analysis; Phenol; Extraction; Pigmentation.*

INTRODUCTION

Marine macro-algae, generally known as seaweeds, belong to a distinctive group of photosynthetic organisms classified from simple unicellular forms to complex multicellular thalloid structures. These algae contribute substantially to the littoral biosphere as primary producers with excellent light-harvesting capabilities despite lacking vascular tissues [1]. These seaweeds are widely classified into three main categories according to pigment composition into brown (Phaeophyceae), red (Rhodophyceae), and green (Chlorophyceae) groups which comprise species having distinguished morphological characteristics, chemical compositions, and biological processes.
Apart from their environmental importance, seaweeds are well known for their outstanding medicinal properties and exceptional nutritional values, with rich essential minerals, trace elements, and vitamins. They are also capable of scavenging free radicals owing to their antioxidant constituents. Especially, brown algae have antioxidant and anti-inflammatory effects owing to their rich carotenoid content such as fucoxanthin [2]. Seaweeds find medicinal and nutritional applications as they generate distinctive phytochemical functional ingredients including sulfated polysaccharides, phlorotannins, and halogenated metabolites [3]. Brown algae are nutrient-rich and contain a wide range of antimicrobial, anti-diabetic, and anticancer bioactive compounds among all seaweeds [4]. The genus Sargassum constitutes a large group of brown macro-algae predominantly found across tropical and moderate maritime environments, representing the family Sargassaceae and order Fucales. There are at least 38 seaweed species of this genus in South Asia. *Sargassum wightii* is regarded as one of the most biologically and commercially valuable brown seaweeds in this region. Extensive studies on S. wightii have shown that it possesses therapeutic compounds used in medicines to treat diseases [5].
There are more than 7,000 recognized green seaweeds, constituting a diverse group of green

algae. *Ulva lactuca* is a ubiquitous green alga belonging to the Ulvaceae family, often known as sea lettuce or U. fenestrata due to its foliated morphology or its perforated appearance, respectively. This green alga has gained momentum by contributing immensely to food systems, agriculture, biomedical development, and renewable energy technologies. *U. lactuca* has become an ingredient in nutrient-rich food and fodder owing to its rich content of polysaccharides, proteins, lipids, and micronutrients. It has been integrated into functional foods, animal feed, bio-enhancers, and antimicrobial agents [6, 7]. In addition, this green seaweed finds application in developing biodegradable materials and renewable industrial biopolymers from its extracted polysaccharides.

The present study explores the marine algae *Sargassum wightii* and *Ulva lactuca* through comparative analysis of their phytochemical and physicochemical traits, aiming to identify bioactive constituents with potential applications in pharmaceutical and industrial sectors.

II. MATERIALS AND METHODS

A. Selection of Study Area and Seaweed Samples

The study explores the marine algae *Sargassum wightii* (a brown alga) and *Ulva lactuca* (a green alga), which are abundantly found along the coastal belt adjacent to Rameswaram in Tamil Nadu, India. A large number of marine algae with proven ethno-medicinal and nutritional value are available profusely in this region, well known for its biodiversity. The present study focuses on bioactive compounds and nutraceuticals of these two seaweeds by investigating and comparing their phytochemical and physicochemical properties, owing to their abundance along this coastline.

B. Collection of Seaweed Samples

The sample seaweeds, *Sargassum wightii* (brown alga) and *Ulva lactuca* (green alga), for exploration were freshly collected from the shoreline waters of Rameswaram, situated on the east coast of Tamil Nadu, India. Immediately, the specimen species were cleansed to extract surface contaminants such as sand and epiphytes by thoroughly rinsing them on the spot with natural seawater, and taken in sterilized and disinfected polythene bags to the laboratory under supervised settings for subsequent examination. These two marine algae were taxonomically
classified through assigning authenticated herbarium accession numbers as BSI/SRC/5/24/2022/Tech/MH 178137 for *Sargassum wightii* and LINN 1275.24 for *Ulva lactuca* by the Botanical Survey of India (BSI).

C. Preparation of Dried Seaweed Powder

The algal samples were carefully examined to ensure the complete removal of epiphytes, necrotic tissues, or any other foreign materials. Thereafter, the specimens were purified with laboratory-grade sterile water to help remove leftover particles and microbes. Later, the sample seaweeds were dried under shade, out of sunlight, for a period of one week to keep their biochemical properties intact. Once completely desiccated, the samples were powdered into a fine, homogeneous form by grinding them mechanically under hygienic conditions and stored in airtight containers for subsequent extraction and phytochemical analyses.

D. Preparation of Extract

Prior to extraction, the dried seaweed material was manually cut into small fragments, thoroughly air-dried, and stored in clean, labeled glass containers under controlled conditions to prevent moisture absorption. Two extraction protocols were employed for obtaining the methanolic extracts.

In the first method, 2 to 5 grams of the seaweed powder were taken in a clean vessel with methanol measuring 30 mL and subjected to reflux on a water bath for 30 minutes. This procedure was repeated twice using fresh portions of methanol (2 × 30 mL). The three extracts were blended together and converted into a 100 mL solution with the addition of methanol, followed by filtering through a 0.45 μm membrane.

The second method involved cold maceration: approximately 30 g of sample powder was placed in methanol for 48 hours at ambient temperature. The combination was intermittently agitated to enhance solute diffusion. After maceration, the extract was filtered using a double-layered cotton cloth followed by gravity-based separation and sedimentation.

The filtrate was concentrated under low pressure at normal temperature through a rotary evaporator. The resulting semisolid sample was refined with 25 millilitres of sterile water, dried to remove residual solvent, andstored in light-resistant containers at ambient temperature until further phytochemical nalysis.

E. Qualitative Analysis of Phytochemical Substances

1. Detection of Alkaloids

To identify the alkaloid content in the seaweed extracts, two standard analytical assays were employed:

Wagner's Method for alkaloid identification

Each one millilitre of the extracted algal sample and Wagner's solution of iodine in potassium iodide were mixed together, resulting in the formation of a reddish-brown precipitate, which indicated the presence of alkaloids.

Alkaloid detection: Dragendorff's reagent assay:

Approximately 0.2 g of the extracted sample was warmed lightly with 2% sulfuric acid for two minutes and then filtered. For further examination, bismuth potassium iodide solution was added drop by drop to the filtrate. The experiment asserted the existence of alkaloid compounds by the development of an orange to reddish precipitate.

2. Detection of Flavonoids Zinc-HCl Test:

The existence of flavonoid compounds was experimentally confirmed with the appearance of a red coloration when two millilitres of the sample were reacted with a pinch of zinc dust, along with the meticulous addition of hydrochloric acid.

3. Detection of Terpenoids

To evaluate the presence of terpenoids, 2.5 millilitres of acetylating agent and 2.5 millilitres of chloroform were transferred into a clean test tube containing 20 grams of methanolic extract. Afterwards, a strong acid reagent (H_2SO_4) was carefully added along the inner wall of the tube. The resulting red-violet colouration substantiated the existence of terpenoids in the sample extract.

4. Carbohydrates Identification Benedict's reagent Assay

Each 0.5 millilitre of an alkaline copper sulphate reagent and the methanolic seaweed extract were transferred to a clean test tube and subsequently placed in boiling water for two minutes. The resulting green to brick-red coloured precipitate, formed in proportion to sugar concentration, confirmed the carbohydrate constituents in the extract.

Fehling's Reagent Assay

Alkaline copper reagent solution (A) and alkaline sodium potassium tartrate solution (B) were added in quantities of eight and five drops, respectively, to 1 mL of the extract in a clean test tube, which was kept in a preheated water system at 100 °C for several minutes. The existence of reducing carbohydrates was affirmed by the formation of a brick-red colouration.

5. Saponin Profiling in Extracts

The dried extract (50 mg) was mixed with laboratory-grade sterile water to a final quantity of 20 mL and thoroughly agitated for fifteen minutes. A stable frothing or foaming layer approximately 2 cm thick, persisting at the top of the mixture, indicated a positive test for the existence of saponins.

6. Phytosterol Profiling in the Extract Liebermann–Burchard Test:

The sample extracts weighing about fifty milligrams and two millilitres of acetic anhydride were transferred into a clean, dry test tube. Thereafter, an acid catalyst (concentrated H_2SO_4) was carefully poured down the inner wall of the tube, drop by drop. A sequence of colour changes—from reddish-brown to blue-green tints—substantiated the observation of phytosterols.

7. Evaluation of Phenolic Substances Ferric Chloride Reagent Assay

The formation of a dark colouration validated the existence of phenolic compounds when a minimal volume of five percent neutral ferric chloride ($FeCl_3$) solution was added to fifty milligrams of the extract, blended uniformly in five millilitres of laboratory-grade sterile water.

Gelatin Precipitation Test:

In a separate assay, fifty milligrams of the extract was blended uniformly in five millilitres of laboratory-grade sterile water, along with the subsequent addition of two millilitres of one percent gelatin reagent prepared in 10% sodium chloride. The resulting white precipitate affirmed the existence of phenolic compounds owing to their capability of forming insoluble complexes with proteins.

Lead Acetate Test

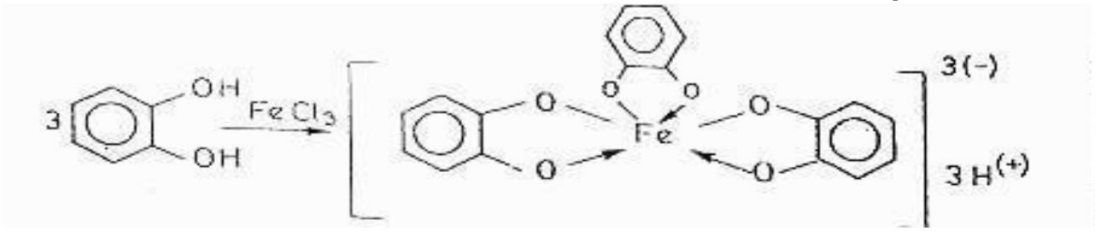
The existence of phenolic compounds was recognized by the formation of a dense white precipitate, resulting from the complexation between phenolic hydroxyl groups and lead ions, when fifty milligrams of the sample extract solution was blended uniformly in laboratory-grade sterile water before adding three millilitres of ten percent aqueous lead acetate solution.

8. Detection of Cardiac Glycosides Legal's Test:

To explore the presence of cardiac glycosides, one millilitre of the extract of the algae sample was dispensed into two millilitres of pyridine in a clean reaction vessel, with the subsequent introduction of a minimal quantity of sodium nitroprusside in droplets. Thereafter, 20 % sodium hydroxide was added little by little to the solution. The change of colour from pink to reddish validated the evidence of cardiac glycosides from chemical interactions between the alkaline reagents and the keto groups.

9. Detection of Tannins

The administration of several microliters of 5 % alcoholic ferric chloride reagent (90% v/v) to a portion



of the filtered sample resulted in the emergence of a dark green or deep blue chromatic shift, substantiating the presence of tannins.

F. Physicochemical Parameters

Adhering to the standard operating procedures presented by the Indian Pharmacopoeia (1966) [8], physicochemical traits— comprising parameters such as moisture loss, total mineral residue, acid-resistant ash, and water-extractable ash —were explored to assess the purity, inorganic content, and stability of the seaweed material. These analytical procedures are consistent with earlier pharmacognostic studies on Ayurvedic drugs [10]. The total ash content was evaluated by incinerating the algae sample at 450 °C in a chamber furnace to achieve complete oxidation of organic matter, leaving inorganic grey ash. Loss on drying, indicating moisture and volatile content, was estimated as the percentage weight loss upon drying at a specified controlled temperature. The cumulative ash content was treated with low-molarity HCl solution to measure the non-acid-soluble ash fraction; the residue resistant to acid digestion indicated silica-rich material. Likewise, the total ash was dissolved in distilled water to estimate water-soluble ash by measuring the undissolved inorganic portion.

Determination of Foreign Matter

To assess the presence of extraneous material, 1 g of the dried seaweed sample was meticulously measured and visually inspected. Any material that differed from the main sample in terms of color, texture, or morphology was considered foreign matter. These unwanted components were manually separated using forceps under proper lighting conditions. The measured quantity of the separated foreign matter was documented, and the percentage was evaluated by subtracting its weight from the original sample mass, thereby estimating the purity of the seaweed material.

Gravimetric Analysis of Moisture

The seaweed powder sample was weighed accurately, placed in a clean, dry vessel, and dried in a laboratory drying oven at a constant temperature of 80 °C for a continuous period of 24 hours to evaluate the moisture level, employing a gravimetric experiment. After this drying process, the sample was stabilized at ambient temperature in a drying chamber and weighed again. The hydration level was assessed through comparison of pre- and post-drying sample weights. The percentage of moisture was then calculated based on the weight loss relative to the original sample mass.

Estimation of Total Ash Value

Approximately 10 g of sample seaweed powder was transferred into the pre-weighed silica vessel and incinerated in a thermostatically regulated furnace at 300 °C for 3 to 4 hours, or until the organic matter was completely oxidized and a uniform grey or white ash was obtained. After cooling the crucible in a desiccator to ambient temperature, the final weight was noted. The complete ash value was estimated

based on the initial sample weight, representing the total amount of inorganic residues present after combustion [11].

Analysis of Water-Extractable Ash Fraction

To estimate the portion of ash soluble in water, 1 g of total ash that had been prepared earlier was blended with 10 mL of laboratory-grade purified water in a suitable container and agitated continuously for 8 hours on a rotary shaker set at 140 rpm to ensure complete dissolution
of soluble components. The resultant solution was filtered using an ashless, laboratory-grade filtration disc. The residue retained on the filter was transferred to a pre-weighed silica vessel and incinerated in a high-temperature combustion chamber for 3 to 4 hours. After reaching ambient conditions, the silica vessel was reweighed. The percentage of water-leachable ash fraction**,** representing the proportion of inorganic salts soluble in water, was calculated based on the difference in ash weight before and after filtration.

Determination of Acid Resistant Ash Content

To estimate the fraction of the total ash insoluble in acid, 1 g of the previously obtained ash was exposed to 10 millilitres of lab-grade H_2SO_4 and incinerated gently to facilitate the dissolution of acid-soluble components. The remaining insoluble residue, transferred into a pre- weighed sintered glass vessel, was thoroughly rinsed with warm, laboratory-grade purified water to remove residual acid, and then dried to achieve an invariable weight. After cooling in a desiccator, the vessel was weighed again. The acid-insoluble ash content, typically consisting of siliceous matter, was measured in relation to the pre-treatment ash mass.

Analysis of Water-Leachable Ash Fraction

A separate 1 gram portion of the cumulative inorganic residue was combined with ten millilitres of laboratory-grade aqueous solvent, blended thoroughly to dissolve the water-soluble components, and filtered using a Whatman ashless filter sheet. The residue retained on the filter was carefully transferred into a pre-weighed silica vessel and incinerated in a regulated calcination furnace for 4 hours at a controlled temperature. After cooling in a desiccator, the silica vessel was weighed again. The proportion of ash insoluble in water was determined by the weight difference, while the amount of ash retained in the aqueous phase was estimated by subtraction.

G. Preliminary Analysis of Ash for Mineral Compounds

To determine the existence of selected inorganic compounds in the seaweed samples, previously prepared ash weighing about 1 gram was diffused in 25 millilitres of 50 % hydrochloric acid and kept for 12 hours at ambient temperature to ensure complete dissolution of the mineral content. Afterwards, the insoluble residue was removed by filtering the solution
using a standard laboratory-grade filter sheet. The clear filtrate was then subjected to qualitative analysis using specific chemical reagents to detect individual elements such as iron (Fe), chloride (Cl), phosphorus (P), and sulphur (S). The appearance of characteristic precipitates or colour changes upon reagent addition served as confirmation of each respective element.

Table-1: Qualitative Detection of Mineral Elements in Ash

SL. NO	PROCEDURE	OBSERVATION	INFERENCE
1.	Following the addition of dilute nitric acid (2 drops), the solution was expanded with 3ml of purified water and reacted with silver nitrate (AgNO) reagent	Development of a pale precipitate	Qualitative conformation of chloride ions (Cl^-)
2.	Test solution was administered with two drops of HCl, gently warmed, and treated with 10 drops of barium chloride ($BaCl_2$).	Formation of white barium sulfate ($BaSO_4$) precipitate	Presence of sulfate ions (SO_4^{2-}), indicating sulfur

3	The sample solution was gently boiled after the addition of two drops of nitric acid. Subsequently four drops of each ammonium molybdate and ammonium hydroxide (NH_4OH) were introduced. Thereafter, the sample solution was held at constant temperature of 60 °C, using thermostatically controlled water bath.	Generation of a yellow sediment	Emergence of phosphate ions (PO_4^{3}), indicating phosphorus
4	Three drops of potassium thiocyanate were added to the sample solution.	Development of reddish brown coloration	Presence of ferric ions (Fe^{3}), indicating iron

Determination of Ash Insoluble in acid

To estimate the acid-insoluble fraction of the total inorganic residue, 1 g of the previously obtained inorganic material was treated with ten millilitres of concentrated sulfuric acid, followed by gentle incineration to ensure complete dissolution of acid-soluble components. The remaining insoluble residue was transferred into a pre-weighed sintered glass vessel, thoroughly rinsed with warm purified water to eliminate residual acid, and then dried to an invariable
weight. The sintered glass vessel was stabilised at ambient temperature in a dry chamber and measured again. The acid-insoluble ash content, typically consisting of siliceous matter, was determined relative to the initial ash mass.

Gravimetric Analysis of Water-Leachable Ash

A separate 1 g portion of the total ash was combined with 10 mL of laboratory-grade purified water and stirred thoroughly to dissolve the water-soluble components. The filtration of the suspension was carried out using an ashless, laboratory-grade Whatman filter sheet. The residue retained on the filter was carefully transferred into a pre-weighed silica vessel and heated in a laboratory incineration chamber for 4 hours at a controlled temperature. After cooling in a desiccator, the weight of the silica vessel was measured again. The change in mass was employed to determine the fraction of ash that remained insoluble in water, while the water- diffusible ash content was deduced by subtracting this value from the original ash weight.

Quantitative Estimation of Phytochemicals

1. Assessment of Total Phenolic Compunds

Employing Folin–Ciocalteu reagent assay, overall phenolic concentration in *Ulva lactuca* and *Sargassum wightii* was estimated in accordance with the protocol outlined by Aparna Buzarbarua [13] (2000), with slight modifications.

One gram of dried and powdered seaweed sample was homogenized in a manual, traditional grinding apparatus with 10 mL of 0.3 % hydrochloric acid in laboratory-grade methyl alcohol. The homogenized sample was exposed to centrifugal force at 10,000 rpm for 20 minutes, followed by meticulous transfer of the supernatant into a pre-cleaned beaker. The separation procedure was executed in two replicates, and the resulting supernatants were merged. The extracted sample fluid was subsequently reduced by gradual volatilization under ambient conditions, and the remaining leftover substance was homogenized in five millilitres of laboratory-grade sterile water.

For spectrophotometric estimation, portions varying between 0.2 millilitre and
2.0 millilitres of the reconstituted extract were dispensed into test tubes and brought up to 3 mL with purified water. Each tube then received half a millilitre of Folin–Ciocalteu's reagent solution, and the contents were mixed thoroughly. After a standing time of 3 minutes, two millilitres of 20% Na_2CO_3 solution was introduced, followed by uniform mixing. The tubes were left undisturbed at ambient temperature for 60 minutes to facilitate chromophore formation. Absorbance of the produced bluish hue was recorded at 630 nm employing a UV–Vis absorbance spectrophotometer, with a reagent blank as reference. The amount of phenolic compounds was quantified and reported in terms of gallic acid equivalents (mg GAE) per gram of dried extract.

2. Assessment of Overall Flavonoid Concentration

Seaweed extracts were analyzed for flavonoid content following a modified colorimetric assay originally

described by Shanmugam et al. [12] (2010). Calibration standards of rutin (0.5– 2.5 mL) were prepared in individual tubes alongside 0.1 mL aliquots of each extract in separate sample tubes. The total volume in each tube, including the control, was brought to 2.5 mL using purified aqueous medium. Thereafter, 75 microliters of a 5% sodium nitrite reagent was introduced, and the contents were left undisturbed to undergo reaction for five minutes under ambient conditions. Following this, 150 microliters of a 10% aluminium chloride reagent was added, and the reaction mixture was kept undisturbed for a further 6 minutes under ambient conditions. Subsequently, 0.5 mL of 1 M sodium hydroxide solution was incorporated, and the contents were mixed thoroughly to develop a pink-colored complex. Absorbance was measured at 510 nm using a UV–Visible spectrophotometer. Flavonoid content was determined by referencing the rutin standard curve and stated as milligrams of rutin equivalents per gram of dry extract (mg RUE/g).

Tannin Measurement

Tannin levels in the seaweed samples were measured by exploiting their ability to form a coloured complex with iron. To set up the calibration, 0.1, 0.2, 0.3, 0.4, and 0.5 mL of tannic acid standard were each diluted to 7 mL with purified water in separate tubes. Each extract aliquot (0.1 mL) was transferred into a clean tube and brought up to a final volume of 7 mL usinga purified aqueous medium. Subsequently, 1 mL of potassium ferricyanide reagent was introduced, followed by the addition of 1 mL of ferric chloride solution. The contents were gently inverted multiple times to promote complete complex formation. Once the chromogenic reaction had progressed sufficiently, the optical density was measured at 700 nm using a UV– Visible spectrophotometer. Tannin levels were quantified based on a standard curve plotted with absorbance values against known concentrations of tannic acid, and results were expressed in terms of catechin reference values per gram of the dried sample material (mg CAE/g).

4. Estimation of Alkaloids

The alkaloid content in the algal extracts was measured with a spectrophotometer, employing 3-Methyl-2-benzothiazolinone hydrazone (MBTH) and theophylline as the reagent and the standard reference compound, respectively, resulting in a coloured chromogenic complex that facilitated quantitative assessment. The different volumes (0.5 to 2.5 mL) of standard theophylline solution were transferred into a series of test tubes to generate a curved calibration graph. For sample analysis, a 1.5 mL portion of seaweed-derived solution was transferred into an individual reaction vessel. Subsequently, 1 mL of 0.01 molar sodium periodate (SPI) and 0.5 mL of 0.1 molar acetic acid were introduced into each vessel, including the reagent control, followed by 2 mL of 0.01 molar MBTH preparations.

Formation of a blue-coloured complex was accomplished by subjecting all reaction tubes to gentle heating for the duration of two minutes. Absorbance for each mixture was assessed at 630 nm using a UV–Visible optical reader after allowing the samples to return to ambient conditions. Alkaloid concentration in the prepared sample was determined by constructing a reference curve through mapping theophylline content along the horizontal axis against corresponding absorbance intensities along the vertical axis, and results were presented in terms of theophylline equivalents (mg THP) per gram of dry material.

Experimental Observations and Analysis Assessment of Plant-Derived Metabolites

Significant levels of secondary metabolites were estimated by subjecting the methanolic extract of *Ulva lactuca* to preliminary phytochemical screening. Data shown in Table 1 demonstrated that the extract, manifesting strong positive reactions for steroids, terpenoids, and flavonoids, indicated their considerable presence in the sample, in addition to moderate existence of phenolic compounds, tannins, alkaloids, and proteins. On the other hand, no noteworthy reaction was observed for saponins and glycosides, indicating either their absence or negligible presence in the tested extract.

Table- 2: Qualitative Phytochemical Screening of *Ulva lactuca*

S.No	Phytochemical Test	Result
1	Alkaloids	+ (Present)
2	Flavonoids	++ (Strongly present)
3	Carbohydrates	+ (Present)
4	Saponins	– (Absent)
5	Phytosterols	+ (Present)

6	Phenols	++ (Strongly present)
7	Tannins	– (Absent)
8	Steroids	++ (Strongly present)
9	Proteins	+ (Present)
10	Glycosides	+ (Present)

Note: (++ = Strongly Present; + = Present; – = Absent)

Interpretation:

It is evident from Table 2 that the seaweed species *Ulva lactuca* was found to be enriched with secondary metabolites. The strong presence of flavonoids, phenols, and steroids, along with antioxidant and structural compounds, was established by the ++ reaction that the extract remarkably exhibited, whereas alkaloids, carbohydrates, phytosterols, proteins, and glycosides were found to be (+) moderately present. The negative results derived from the test indicated the absence of saponins and tannins in the extract. This diverse array of phytochemicals found in *Ulva lactuca*, known as sea lettuce, indicates its valuable source of bioactive ingredients for dietary supplements (nutraceuticals), and therapeutic treatments.

Table-3: Preliminary Phytochemical Analysis for *Sargassum wightii* (Methanol Extract)

S.NO	Phytochemical Test	Result
1	Alkaloids	–(Absent)
2	Terpenoids	+(Present)
3	Steroids	++(Intensely present)
4	Tannins	++(Intensely present)
5	Saponins	–(Absent)
6	Flavonoids	++(Intensely present)
7	Phenols	++(Intensely present)
8	Glycosides	+(Present)
9	Proteins	+(Present)

Legend: ++ = Intensely Present; + = Present; – = Absent

Interpretation:

Table 3 presents that the methanolic fraction derived from *Sargassum wightii* demonstrated potent phytochemical activity with the strong observation of positivity (++ reaction), indicating the significant presence of steroids, tannins, flavonoids, and phenols, possessing antioxidant, anti-inflammatory, and antimicrobial properties. The moderate presence of terpenoids, glycosides, and proteins was identified with the positivity (+) observed. Meanwhile, the absence of alkaloids and saponins in the extract was confirmed with the negative results of the test. These findings ensured that *Sargassum wightii* pre dominantly contained bioactive compounds with remarkable pharmacological value.

Quantitative Analysis of Phytochemical Constituents

The methanolic extracts of both *Sargassum wightii* and *Ulva lactuca* were explored for identifying the proportion of specific phytochemicals such as phenols, flavonoids, tannins, and alkaloids. The results showed that these two seaweeds found a major difference in compound concentration.

In *Sargassum wightii*, the overall phenolic concentration was measured to be 2.82 ± 0.38 milligrams gallic acid equivalent per gram of dry mass, whereas the total flavonoids were estimated to be 2.97 ± 1.29 mg RUE/g dry weight, along with a substantial amount of tannin with a value of 2.65 ± 0.13 mg CAE/g dry weight.

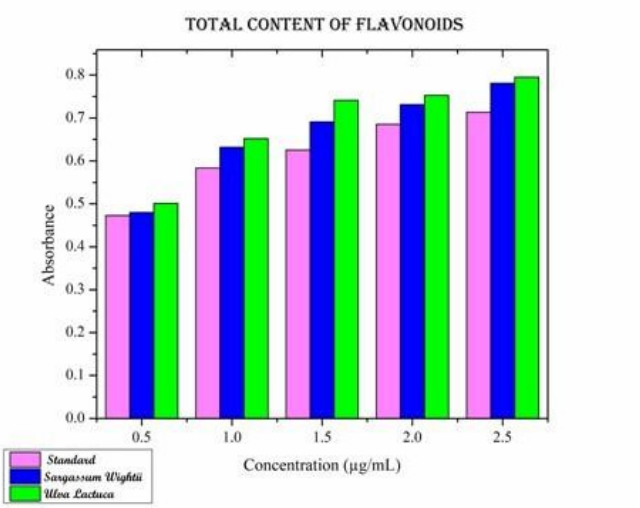
On the other hand, *Ulva lactuca* recorded a predominantly higher phenolic value at 17.37 ± 1.03 mg GAE/g dry weight, expressing its strong antioxidant properties. The flavonoid concentration was estimated to be 2.62 ± 0.108 mg RUE/g dry weight, while the alkaloid concentration

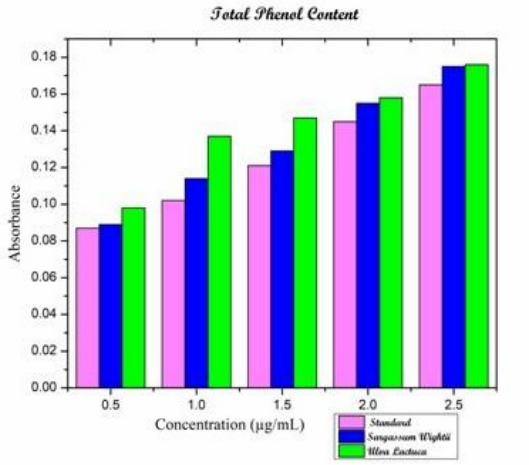
was evaluated at a remarkable 68.07 ± 0.39 mg THP/g dry weight. The presence of highly concentrated phenols and alkaloids in Ulva lactuca reveals its intensified psychopharmacological characteristics.

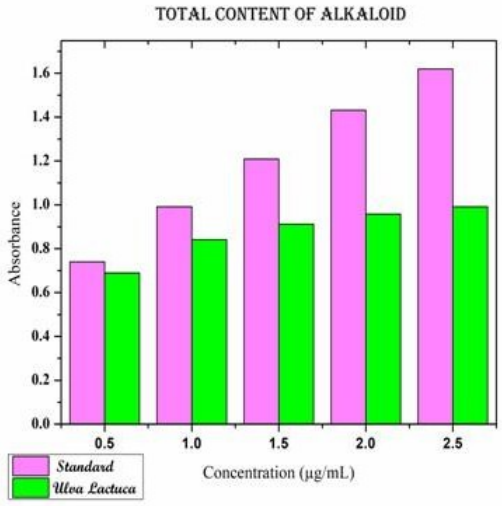
Table -4: Comparative Study of Select Bioactive Compounds in *Sargassum wightii* and *Ulva lactuca*

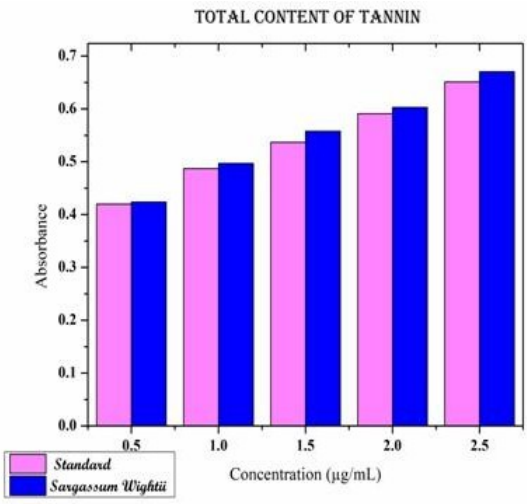
Seaweed	Total Phenol (mg GAE/g dry wt)	Flavonoids (mg RUE/g dry wt)	Tannins (mg CAE/g dry wt)	Alkaloids (mg THP/g dry wt)
Sargassum wightii	2.82 ± 0.38	2.97 ± 1.29	2.65 ± 0.13	– (Not Detected)
Ulva lactuca	17.37 ± 1.03	2.62 ± 0.108	– (Not Detected)	68.07 ± 0.39

Note: Values represent the mean of three independent analyses ± standard deviation (n = 3).
Abbreviations: GAE – Gallic Acid Equivalent; RUE – Rutin Equivalent; CAE – Catechin Equivalent; THP – Theophylline Equivalent; wt – weight.









Interpretation:

Table 4 reveals that the phenolic concentration in *Ulva lactuca* was found higher (17.37 mg GAE/g) than that of *Sargassum wightii* (2.82 mg GAE/g), indicating a strong antioxidant characteristic, whereas the flavonoid level was comparatively a little higher (2.97 mg RUE/g) in *Sargassum wightii* than that of *Ulva lactuca* (2.62 mg RUE/g). At the same time, tannins were remarkably not found at all in *Ulva lactuca*, while there were noticeable tannins (2.65 mg CAE/g) present in *Sargassum wightii*. Similarly, the alkaloid

level in *Ulva lactuca* was notably recorded higher (68.07 mg THP/g), whereas the same was undetected in *Sargassum wightii*. The study presents a clear picture of phytochemical and strong therapeutic properties of these two species.

Pharmacognostic Studies

The standard pharmacognostic parameters such as moisture, ash, pH, and extractive values were assessed to explore the physicochemical characteristics of Sargassum wightii, aiming to ensure the quality, purity, safety, and standardization of the derivatives of medicinal plants employed in pharmaceutical formulations [10].

Table-5: Evaluation of basic physiochemical parameters in *Sargassum wightii*: Moisture, Ash, Extractives, and pH

Parameter	Value
Moisture Content (%)	51.9
Total Ash (%)	36.14
Acid-Soluble Ash (%)	8.3
Water-Soluble Ash (%)	14.5
Water-Soluble Extractive (%)	3.03
Alcohol-Soluble Extractive (%)	4.01
pH Value (5% aqueous solution)	8.62

Interpretation:

The present study reveals that the seaweed *Sargassum wightii* was identified with its hydrophilic characteristics in terms of its high moisture content (51.9 %). It is evident from the study that both organic and inorganic components were present in the marine alga *Sargassum wightii*, from the total ash content found to be 36.14 %, indicating considerable mineral ingredients of a remarkable portion of water-soluble ash (14.5 %) and measurable acid-soluble ash (8.3 %). It is implied that polar and semi-polar contents are effectively extracted employing these solvents, owing to their extractive values in water (3.03 %) and alcohol (4.01 %). The pH value of 8.62 of the 5 % aqueous solution revealed a moderate alkaline nature, generally found in marine macro-algae.

Table-6: Determination of Mineral Constituents in *Sargassum wightii*

S.NO	MINERAL	INFERENCDE
1	Chlorine	Present (+)
2	Sulfur	Absent (–)
3	Phosphorus	Present (+)
4	Iron	Present (+)

Interpretation:

The qualitative mineral assessment in the ash of *Sargassum wightii* disclosed that chlorine, phosphorus, and iron were present, except sulphur, indicating the alga's capability of accumulating elements with nutritional and industrial importance from its acratic environment. The presence of iron supports its contribution to dietary iron intake, while phosphorus plays a vital role in energy metabolism and skeletal health. Chlorine, as an electrolyte, is essential for fluid balance and digestive functions. The ability of Sargassum wightii to bioadsorb and retain such minerals highlights its potential as a functional food ingredient and natural supplement in nutraceutical applications.

Table -7: Quantitative Estimation of Moisture, Ash Content, Extractive Values, and pH of *Ulva lactuca*

Parameter	Value
Moisture Content (%)	47.3
Total Ash (%)	21.1
Acid-Soluble Ash (%)	17.5
Water-Soluble Ash (%)	24.3

Water-Soluble Extractive (%)	27.52
Alcohol-Soluble Extractive (%)	45.3
pH Value (5% aqueous solution)	11.02

Interpretation:
The moisture content of *Ulva lactuca* was found to be 47.3 %, reflecting its naturally high water retention capacity, typical of green macroalgae. The total ash content was 21.1 %, representing the cumulative inorganic residue present after combustion. The acid-soluble ash (17.5 %) indicates a significant portion of the total ash is digestible in acidic media, primarily excluding acid-insoluble materials such as siliceous compounds. The water-soluble ash content (24.3 %) exceeded the total ash percentage, possibly due to the high presence of inorganic salts and water-soluble minerals within the tissue, indicating potent bioavailability of nutrients. Notably, the extractive values were high—27.52 % in water and 45.3 % in alcohol— suggesting that *Ulva lactuca* contains a large proportion of both hydrophilic and semi-polar bioactive constituents. The alkaline pH (11.02) observed in the 5 % aqueous extract is characteristic of many green types of seaweed and may contribute to their antimicrobial or buffering properties. These values collectively highlight the strong phytochemical potential and nutritional richness of *Ulva lactuca*, supporting its application in functional food pharmaceutical, and nutraceutical formulations.

Table-8: Determination of Mineral Constituents in *Ulva lactuca*

S. No	Mineral	Inference
1	Chlorine	Present (+)
2	Sulfur	Present (+)
3	Phosphorus	Present (+)
4	Iron	Present (+)

Interpretation:
The study on the mineral composition of Ulva lactuca asserted that the green macro-alga comprises major mineral components including chlorine, sulphur, phosphorus, and iron,
projecting its mineral-laden nature with strong nutritional and therapeutic applications. Iron is a principal component of haemoglobin and various enzymatic systems in human physiology. For healthy haemoglobin levels, adult males are recommended to intake approximately 15 mg of iron, whereas adult females are advised to intake approximately 10– 12 mg of iron. Therefore, the iron-enriched marine algae Sargassum wightii and Ulva lactuca play a crucial role in addressing malnutrition as mineral supplements.
Furthermore, phosphorus and sulphur present in Ulva lactuca essentially help with metabolic processes, protein synthesis, and cellular detoxification in human physiology as dietary supplements. Significantly, marine algae are well known for their highly effective antioxidant properties. The study asserts that the green seaweed Ulva lactuca is capable of combating oxidative stress that leads to the development of chronic diseases. Previous studies undertaken by many researchers, including Qi et al. (2005) [9], have proclaimed that a sulphated polysaccharide extracted from Ulva species, known as ulvan, shows crucial antioxidant activity, demonstrating the therapeutic properties of this marine alga.

CONCLUSION

The seaweeds, or macro-algae, abundantly found in inshore waters, find applications extensively in both internal and external therapeutic treatments. The present study explored the phytochemical and physicochemical characteristics of methanolic extracts of the seaweeds *Sargassum wightii* (brown alga) and *Ulva lactuca* (green alga), revealing that they are abundant in various therapeutic phytochemicals.
Identification of phytochemical groups of *Sargassum wightii* confirmed the existence of terpenoids, steroids, tannins, flavonoids, phenols, glycosides, and proteins, while *Ulva lactuca* exhibited alkaloids, flavonoids, carbohydrates, phytosterols, phenols, steroids, proteins, and glycosides. The study reaffirms that these marine algae consist of varying secondary metabolites possessing strong medicinal

characteristics, especially owing to their antioxidant, antimicrobial, and therapeutic properties.
These marine algae, *Sargassum wightii* and *Ulva lactuca*, were found to be of high moisture content, 51.9% and 47.3 % respectively, so that they maintained microbial stability and processing due to their capacity for water retention. The seaweeds showed a mineral-rich profile with noticeable total ash content of 36.14 % for *Sargassum wightii* and 21.1% for *Ulva lactuca*.
The study on phytochemical concentration established the critical value of total phenolic, particularly in *Ulva lactuca* (17.37 ± 1.03 mg GAE/g dry weight), while comparing to *Sargassum wightii* (2.82 ± 0.38 mg GAE/g), revealing the fact that the former has high antioxidant activity. It is evident from the study that alkaloids were abundantly present in Ulva lactuca (68.07 ± 0.39 mg THP/g), while tannins were notably found in *Sargassum wightii* (2.65 ± 0.13 mg CAE/g).

The study on mineral composition identified chlorine, phosphorus, and iron in both species, whereas Sulphur was found only in *Ulva lactuca,* highlighting their nutritional and nutraceutical potential on account of the bio-adsorptive and bio-accumulative properties of these seaweeds.

In a nutshell, the study has opened a new avenue for further research on these seaweeds' applications in food, health, and pharmaceutical industries, as *Sargassum wightii* and *Ulva lactuca* demonstrated significant psychopharmacological properties. These findings support the development of eco-sustainable marine -based nutraceuticals, suggesting further exploration of *Ulva lactuca* for pharmaceutical-grade antioxidant compounds.

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Ethical Statement

The authors hereby affirm that the current research titled "Phytochemical Study of Seaweeds *Sargassum wightii* and *Ulva lactuca*" was conducted in accordance with the ethical standards applicable to scientific research. No human participants or animal models were involved at any stage of this study, and therefore ethical clearance from institutional review boards was not required.

All samples of *Sargassum wightii* and *Ulva lactuca* were collected responsibly from the natural coastal environment near Rameswaram, Tamil Nadu, India, with strict adherence to local environmental regulations. The collection process did not involve any endangered or protected marine species, nor did it disrupt the surrounding ecosystem.

The research was carried out solely for academic and scientific advancement, with no commercial interest or conflict of interest involved. The study ensures compliance with the principles of integrity, transparency, and reproducibility in scientific inquiry. All methodologies, analyses, and interpretations were performed with due diligence, following standard laboratory procedures and validated protocols.

The authors declare that the content of this manuscript is original and has not been submitted to or published in any other journal. Appropriate acknowledgments and references have been made to the works of others to avoid any academic misconduct or plagiarism.