

Egyptian Journal of Botany http://ejbo.journals.ekb.eg/



Physicochemical Characterization of *Sargassum latifolium* at Ras Sudr Shores - Red Sea Coast of Egypt

Seham E. Abu Ahmed^{(1)#}, Mohamed A. Deyab⁽¹⁾, Nagwa I. Hassan⁽¹⁾, Mostafa M. El-Sheekh⁽²⁾

⁽¹⁾Botany and Microbiology Department, Faculty of Science, Damietta University, Damietta El-Gededa, P.O. 34517, Egypt; ⁽²⁾Botany Department, Faculty of Science, Tanta University, Tanta, Egypt.

S*ARGASSUM latifolium* is an edible brown alga collected from the red seashore in Egypt during autumn. This research aimed to investigate the physicochemical properties and biogenic molecules of *S. latifolium*. The optimal physicochemical properties of red seawater were a moderate temperature (24°C), salinity (40.12%), hydrogen ion concentration (7.8), dissolved oxygen (7.37mg/L), a slightly alkaline pH, a slightly elevated biochemical oxygen demand (1.8mg/L), and an electrical conductivity of 53.67mmhos/cm. *S. latifolium*'s biochemical composition is rich in carbohydrates (45.52mg/g dry weight) and low in protein (6.38mg/g dry weight). *S. latifolium* contained 89.23% and 23.37% moisture and ash, respectively. The bioactive compounds in an aqueous crude extract of *S. latifolium* revealed numerous functional groups, including -OH, -NH, -CH, -COOH, CO, and C–C. The resulting aqueous extract of *S. latifolium* contained steroids, terpenoids, flavonoids, phenols, coumarins, and quinones.

Keywords: Fourier Transformed Infrared, Natural products, Sargassum latifolium, UV.

Introduction

Due to its physical location and partial isolation from the open oceans, the Red Sea is unique among basins and occupies an ideal position (Halim, 1984). The richness and beauty of the Red Sea's natural surroundings are a major factor in its national and international appeal (Hoegh-Guldberg, 1999). Large macroalgae that grow attached to rocks and along the shoreline are referred to as seaweeds, and they can be found in a variety of aquatic environments (Ismail et al., 2019). They can produce numerous secondary metabolites (Val et al., 2001; Smit, 2004) with a wide range of biological activities, such as antiviral, antibacterial (Chakraborty et al., 2010; El-Shafay et al., 2016; Farghl et al., 2021), antifungal, and antitumor (TÜney et al., 2006) properties. In comparison to those found in the leaves of higher plants, the variety of secondary metabolites in seaweed has always

piqued the interest of biochemists. Isoprenoids (terpenes, carotenoids, and steroids), polyketides (phlorotannins), amino-acid-derived natural products (alkaloids), and shikimates (flavonoids) are the primary and secondary metabolites found in algae (Mendis & Kim, 2011; El-Sheekh et al., 2020). The phylum Phaeophyta contains a variety of macrophytes. More than 1500 Phaeophyta species have been identified (Davis et al., 2003). The brown color of Phaeophyta is caused by the carotenoid fucoxanthin, which covers other pigments (Hashim & Chu, 2004). Over 400 species of the brown seaweed genus Sargassum (Phaeophyceae) belong to the family Sargassaceae (Mattio & Payri, 2011). This organism is found in all oceans and is used as both medicine and food by numerous cultures. In addition, it produced bioactive compounds, including meroterpenoids, phlorotannins, fucoidans, sterols, and glycolipids (Iqbal et al., 2008).

*Corresponding author email: dr_seham2016@yahoo.com
Received 23/02/2022; Accepted 01/06/2022
DOI: 10.21608/ejbo.2022.123635.1920
Edited by: Prof. Dr. Salama A. Ouf, Faculty of Science, Cairo University, Giza 12613, Egypt.
©2022 National Information and Documentation Center (NIDOC)

This paper focused on the study of seaweeds vegetation the collection of *Sargassum latifolium* and its associated macroalgae from Ras Sudr Red Sea coast of Egypt during autumn 2020 to determine the predominance of macroalgal species, physicochemical analysis of the most dominant seaweed (*S. latifolium*), and qualitative and quantitative phytochemical composition of *Sargassum latifolium* aqueous extract.

Materials and Methods

Study area

Ras Sudr, located on the Red Sea coast of Egypt (Fig. 1), is one of Egypt's most important algal growth regions (intertidal zone). Ras Sudr is located on the Red Sea coast in the Gulf of Suez at a longitude of 32° 43' East and latitude 29° 35' North. It is part of the South Sinai Governorate. Ras Sudr is located on the western side of the Sinai Peninsula, 200km from Cairo and approximately 60km from the Ahmed Hamdi tunnel crossing in Suez. It is also almost directly opposite the resort of Ayn Elsokhna on the opposite red seashore. The coastline of Ras Sudr is 95km long.

Physicochemical analysis of water

The water's temperature was measured with a Celsius Thermometer, and the water's hydrogen ion concentration (pH) was measured with a Horizon Ecology Co pH meter 5995. Using a Y.S.I. Model 33 (yellow springs instrument) S-C-T Meter % MHOS, salinity (%), conductivity (EC), and total dissolved salts (TDS) were measured. According to Kopp & McKee (1983) dissolved oxygen (DO) was measured. According to APHA (1992),

biological oxygen demands (BOD), reactive (ortho) phosphate, and calcium hardness as CaCO, (EDTA Titrimetric Method) were measured. Total alkalinity (T. ALK) and Phenolphthalein alkalinity (phph ALK) were measured using the method of Kumar & Ravindranath (1998). In addition, Ramteke & Moghe (1988) identified the presence of chlorides. The Golterman et al. (1978) method was used to determine ammonianitrogen. Nitrite was measured based on Dean Adams' (1990) research while the concentration of nitrates was measured based on Strickland's (1972) research. Total nitrogen was measured according to Patton & Kryskalla (2003), while total phosphorus was determined according to USEPA (1983) guidelines (1983). According to Dean Adams (1990), total hardness as CaCO₂ (EDTA Titrimetric Method) was determined. Magnesium hardness was determined according to Hawk et al. (1947) and Moore (1986). Heavy metals were carried out according to Table 1.

Description, collection, and preparation of seaweeds

Sargassum latifolium: Thallus up to 25cm long, erect, attached to the substratum by a small perennial discoid holdfast, with a short and perennial stipe giving rise to a 3mm broad, distinctly zigzag-shaped main axis. Phylloids are alternate, lanceolate, 1.5–2.5cm long, and 1–3mm broad, with serrate but more prominently toothed margins up to 5mm in diameter, spherical to subspherical, pedicellate, and smooth vesicles. The receptacles were cylindrical, furcated, and up to 6mm long.



Fig. 1. Map of the collection sites of Sargassum latifolium

Egypt. J. Bot. 62, No. 3 (2022)

In order to evaluate the percentage and coverage of each species, quadrates $(1m \times 1m)$ were used to collect samples of macroalgae from the shoreline to a depth of 2m in the area under study. At low tide, algae samples were collected manually with a sharp, rectangular shovel. At the collection site, samples were washed with seawater to remove adherent sediments and contaminants, placed in plastic bags, and transported to a laboratory for further processing (Fig. 2). According to Asharf and Mohamed (2013), the relative abundance of each individual was calculated according to the following equation:

Abundance % = No of individuals of a given species \times 100 \div Total no. of all



Fig. 2. Sargassum latifolium

Sample preparation

Sargassum latifolium was washed multiple times with tap water and distilled water to remove all traces of salt from its surface. The remaining water was drained, and the seaweed was spread on blotting paper and dried at room temperature in the shade before being ground into powder. The ground-dried algal material is stored in dry plastic bags until use.

Preparation of Sargassum latifolium aqueous extract

Sargassum latifolium was made by combining 1g of algal powder with 100mL of distilled water and shaking continuously with a glass stirring rod at 60°C for 15min. Using Whatman No. 1 filter paper, the resultant extract was filtered and used to initiate further study.

Characterization of Sargassum latifolium

Biochemical characterization of aqueous extract of Sargassum latifolium

Dubois et al. (1956) phenol sulfuric acid method was used to determine carbohydrate content. According to Lowry et al. (1951), protein content was determined.

Physical characterization of Sargassum latifolium

Water content: The moisture content of *Sargassum latifolium* was determined using the method of Omar et al. (1988).

Ash content: Three sets of dry samples (5g each) were placed in crucibles and dried at 105°C for 30min. For determining the amount of ash in the dry samples, the following equation was used:

Ash content (percentage) = (Weight of ash/weight of dry algae) \times 100

UV-vis scan analysis of Sargassum latifolium

ATI Unicam-UV Visible Vision Software V3.20 was used to determine the UV absorbance spectrum of *S. latifolium* aqueous extract between 190 and 750nm (John Peter Paul & Shri Dev, 2013).

Fourier transform infrared spectrometry (FT-IR)

The Mattson 5000 FT-IR spectrometer was used to record the FT-IR spectrum. The dry weight of *Sargassum latifolium* was crushed with KBr powder and pressed into pellets for 400–4000cm⁻¹ FT-IR spectroscopy (Wang et al., 2004).

Qualitative analysis of natural products in S. latifolium aqueous extract

The phytochemical analysis of aqueous algal extract was detected using Savithramma et al. (2011) standard method. It was conducted to determine the presence or absence of the major naturally occurring chemical compounds, including alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones, and glycosides.

Quantitative analysis of phytochemical substances in S. latifolium aqueous extract

Estimation of total phenolic content (TPC) The Folin–Ciocaltaeu test described by Tambe & Bhambar (2014) was used to determine the TPC of the extract. Next, 1mL of the sample (1mg/mL) was combined with 1mL of Folin–Ciocalteu's phenol reagent. After 5min, 10mL sodium carbonate solution (7%) and 13mL deionized distilled water were added to the mixture and thoroughly combined. Before measuring the absorbance at 760nm, the solution was kept at 23°C in the dark for 90min prior to absorbance measurement. The total phenolic concentration was determined using extrapolation of the calibration curve generated by creating a gallic acid solution. In triplicate, the phenolic chemicals were measured. The TPC was calculated as gallic acid equivalents (GAE) per gram of dry material.

Estimation of total tannin content

Total tannin content was determined using the Folin-Ciocalteu method as described by CI & Indira (2016). A 10mL volumetric flask was filled with 0.1mL of algal extract, 7.5mL of distilled water, 0.5mL of Folin-Ciocalteu phenol reagent, and 1mL of a 35% sodium carbonate solution, then diluted to 10mL with distilled water. The liquid was thoroughly mixed before being stored for 30min at 25°C. Furthermore, a set of tannic acid reference standard solutions (20, 40, 60, 80, and 100µg/mL) were prepared using the same method. At 700nm, the absorbance of test and standard solutions were measured using a UV-visible spectrophotometer. The tannin content was determined three times. Tannin concentration was measured in milligrams of equivalent tannic acid per gram of dried plant material.

Estimation of Total flavonoid content

In addition, we utilized Chang et al. (2002) AlCl₃ colorimetric method to calculate the total flavonoid content. The standard solution was made by dissolving 10mg of rutin in 10mL of methanol to obtain a 1000µg/mL solution. In various tubes, aliquots ranging from 0.01 to 0.08mL of the above stock solution were placed. Each test tube contained 1.5mL of methanol, 0.1mL of 10% AlCl,, 0.1mL of 1M CH,COOK, and 2.8mL of distilled water. The reaction mixture was maintained at room temperature for 30min. The absorbance of the resulting solutions was compared to the absorbance of the reagent blank at 415nm. The calibration curve obtained by plotting absorbance versus concentration was determined to be linear over this concentration range. 10mg of extracts were dissolved in 10mL of methanol to produce 1mg/mL solutions. Subsequently, the calibration curve was used to determine the total flavonoid concentration in the test sample. The rutin equivalent (mg RE/g extract) was used to determine the total flavonoid concentration of the extract.

Results and Discussion

Physicochemical analysis of water

The physicochemical analysis of water in the study area is presented in Table 1. The spatial and temporal variability of environmental characteristics in coastal waters is substantial (Bosak et al., 2012). Due to their location at the interface between terrestrial and marine ecosystems, these habitats are referred to as "critical transition zones" and are subject to increased anthropogenic influences, primarily as a result of the increasing human population density in coastal regions (Levin et al., 2001).

TABLE 1. Physicochemical analysis of seawater

Element	During autumn
Temperature	$24^{\circ}C \pm 0.5$
Hydrogen ion concentration (pH)	7.8 ± 0.1
Salinity (%)	40.12 ± 0.02
Conductivity (EC)	53.67 mmhos/cm ± 0.2
Total dissolved salts (T.D.S)	$34.16 \text{g/L} \pm 0.06$
Dissolved oxygen (DO)	$7.37 mg/L \pm 0.2$
Biochemical oxygen demands (BOD)	$1.8 mg/L\ \pm 0.02$
Total alkalinity (T. ALK)	$2.68 meq/L \pm 0.2$
Chlorides	25.78 g/L \pm .25
Free CO ₂	$18.95 \text{mg/L} \pm 0.3$
CO ₃	$2.3 mg/L \pm 0.09$
HCO ₃	$0.74 \text{mg/L} \pm 0.02$
Total hardness	$3.88 \text{g/L} \pm 0.02$
Calcium hardness	$1.42g/L\pm0.03$
Magnesium hardness	$2.46g/L\pm0.02$
Total phosphorous	$2.82 \text{mg/L} \pm 0.03$
Phosphates (PO ₄)	$0.03 \text{mg/L} \pm 0.02$
Total nitrogen	$1.95 \text{mg/L} \pm 0.03$
Nitrate	$0.58 \text{mg/L} \pm 0.01$
K, Ca, Na, Mg, Fe, Mn, Zn and Cu (mg/L)	472, 412, 12051, 2.69, 1.70, 1.90, 0.56 and 1.68 respectively

Water temperature is the most influential environmental factor on organisms, chemical reactions, and biological reactions. Air temperature, seasonal variation, winds, water depth, waves, and heat input or loss in shallow areas along the shore all influence the water temperature of natural bodies of water. In contrast, it affects the growth and metabolic rates of marine organisms, specifically photosynthesis and respiration processes in macroalgae (Zou & Gao, 2014). Recent research indicates that Sargassum spp. can survive between 8°C and 36°C, which could explain the intact rhizoid at 34°C (Iver et al., 2004; Raikar et al., 2001). Physiologically, low temperatures can halt biochemical activity in the thallus body of macroalgae, whereas high temperatures will damage the enzyme and destroy the biochemical mechanisms in the thallus (Luning, 1990).

Water temperature values (24° C in autumn) were consistent with the findings of Madkour (2013); 20.7°C–28.2°C and Dorgham et al. (2012); 22.8°C–30.5°C in various sites. In addition, Nassar et al. (2015) determined that the temperature of the northern portion of the Suez Gulf was 17°C during the winter, 31.50°C during the summer, and the annual mean value of 24.35°C. According to the seasonal variations, seawater in the northern Red Sea, Egypt showed the highest temperature values (29.25°C–32.00°C) during summer and the lowest values (17.96°C–21.97°C) during winter (Abdelmongy & El-Moselhy, 2015).

In autumn, the salinity of the water was 40.12%. The elevated salinity along the Red Sea coast results from increased evaporation in enclosed (Madkour, 2013; Abdelmongy & El-Moselhy, 2015). The pH of the water is an important environmental factor for the survival, development, and physiology of aquatic organisms. In the area of interest, the pH was 7.8. Another study found that the Red Sea's shoreline salinity ranged between 40.23 and 41.43g/L throughout the year, while the pH ranged between 7.78 and 8.07 (Abdelmongy & El-Moselhy, 2015).

Due to the relatively high salinity of the water, the microelements total P (2.82mg/L), PO₄ (0.26mg/L), Total N (1.95mg/L), and nitrate (0.58mg/L) are abundant. Consistent with the findings of Nassar et al. (2015), who stated that the dissolved nitrate along the eastern coast of the Suez Gulf of Egypt ranged between the maximum value of 0.078mg/L and the minimum value of

0.01 mg/L and that PO_4 was very low (0.002 mg/L-0.016 mg/L) throughout the year. Our measurements were in agreement with their findings. In addition, Abdelmongy & El-Moselhy (2015) discovered that the dissolved nitrates along the northern Egyptian coast of the Red Sea ranged from 0.009 to 0.3 mg/Land that the dissolved PO_4 ranged from 0.002 to 0.05 mg/L throughout the year.

The conductivity of an electrolytic solution is determined by the ions present and their concentrations. The conductivity readings indicate the TDS concentration in wastewater (Emara et al., 2015). According to our research, the Red Sea's conductivity is 53.67mmhos/cm. Conversely, Emara et al. (2015) discovered that the surface seawater conductivity in the northwestern Suez gulf varied between 49 and 51mmhos/cm.

TDS (34.16g/L) comprise inorganic salts such as calcium (412mg/L), magnesium (2.69mg/L), potassium (472mg/L), sodium (12051mg/L), bicarbonates (0.74mg/L), chlorides (25.78g/L), and sulfates are dissolved in water along with a small amount of organic matter. According to Emara et al. (2015), the TDS of surface seawater in the northwestern Suez gulf varied between 34 and 36g/L.

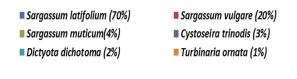
Monitoring dissolved oxygen in coastal areas will serve as an indicator of water quality and a tool for assessing ecosystem health (Australia, 2002). Unless harmful substances are present, dissolved oxygen is the best indicator of the impact of pollution on an aquatic ecosystem (Lester, 1975). The majority of aquatic organisms require dissolved oxygen for respiration. It influences the solubility and availability of nutrients, and low dissolved oxygen levels promote the release of nutrients from sediments, thereby reducing the production of aquatic ecosystems. In this study, the DO concentration was 7.37mg/L. Coastal waters require a minimum of 4.0mg/L of oxygen, but 5.0mg/L is preferred for optimal ecosystem function and carrying capacity (Gilbrich et al., 1978). According to the findings, during autumn, DO concentrations frequently exceeded the threshold amount (4mg/L). During these seasons, the greatest phytoplankton bloom was associated with a slight increase in dissolved oxygen and good water column mixing (Girgis, 1980).

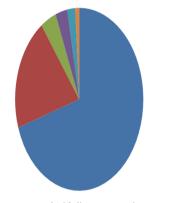
Biological oxygen demand (BOD) is a significant component. Streams with high

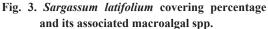
concentrations of organic pollutants, such as sewage, fishing vessel waste, and industrial waste, can have a significantly elevated BOD. A 5-day BOD of less than 2mg/L is indicative of extremely healthy streams, while contaminated streams may approach 10mg/L. A BOD of 1mg/L was also cited as an indicator of nearly pure water (Association et al., 1912). According to these studies, our research area was not significantly affected by human activities. Due to the effect of warm temperature on the account and activity of microorganisms' water content, BOD levels were low (1.8mg/L) at our research site during the fall.

Seaweeds abundance and algal sampling

Sargassum latifolium was selected for this study as it was the predominant macroalgal species (70%) in our study area, followed by Sargassum Vulgare (20%), Sargassum muticum (4%), Cystoseira trinodis (3%), Dictyota dichotoma (2%), and Turbinaria ornata 1% (Fig. 3).







The growth and distribution of seaweeds are varied according to the physicochemical characteristics of seawater and the substrate of the habitat, as well as the growth and distribution of seaweeds vary (Kadi, 2005). Generally, *Sargassum* is associated with the presence of water nutrients such as nitrate and phosphate (Noorjahan et al., 2019), as well as micronutrients, including Fe, I, Mn, and Mg (Circuncisao et al., 2018). *Sargassum* thrives in protected or turbulent waters and rocky environments (Pakidi & Suwoyo, 2017). According to our findings, the physicochemical characteristics of seawater in our study area are optimal for the growth of *Sargassum*, as are the substrate characteristics (rocky and sandy). Abu Ahmed et al. (2021) discovered that *Sargassum vulgare* was the predominant macroalgal species (53%) along the coast of Hurghada by the Red Sea, followed by *S. muticum* and *S. crispum*.

Characterization of Sargassum latifolium

Biochemical characterization of S. latifolium The results obtained regarding the chemical composition of S. latifolium, including its total protein and carbohydrate content, indicated that the total protein content was 6.38mg/g dry weight, and the carbohydrate content was 45.52mg/g dry weight. Studies on the chemical composition of seaweed have revealed that they are rich in proteins, lipids, carbohydrates, and trace minerals. Protein content in brown seaweeds ranges between 5 and 15% of the dry weight (Burtin, 2003; Chakraborty & Santra, 2008; Manivannan et al., 2008; Rohani-Ghadikolae et al., 2012). Due to varying seasonal and environmental growth conditions (Dawczynski et al., 2007), the protein content of Sargassum latifolium was low in the present study.

Carbohydrates are an essential component of metabolism because they provide the energy necessary for respiration and other vital functions. According to the study done by Marinho-Soriano et al. (2006), the most common carbohydrates in brown seaweeds include fucoidan, laminarin, cellulose, and alginates (Dawczynski et al., 2007). Light intensities and temperature appeared to increase carbohydrate synthesis while decreasing protein and lipid content. Compared to other species of Sargassum, the carbohydrate content of S. latifolium in our study (Table 2) was high (45.52mg/g dry weight) in comparison to other species of Sargassum (Chennubhovla et al., 1987; Goecke et al., 2012; Murugaiyan et al., 2012; Parthiban et al., 2013; Rameshkumar et al., 2013).

TABLE 2. Chemical composition of Sargassum latifolium

Total carbohydrate (mg/g dry weight)			45.52 ± 0.632		
Total protein (r	ng/g dry weight)	6	0.38 ± 0.541		
Physical	characterization	of	Sargassum		

Water and ash contents: S. latifolium contained 89.23% and 23.37% moisture and ash, respectively. Fouda et al. (2019) reported that the moisture content of six seaweeds collected along the coast of the Red Sea ranged from 88.08% to 94.09%, and the ash content ranged from 19.60% to 45.48%. According to Rupérez (2002), brown seaweeds have higher ash content (21.37%– 45.04%) than green, red, and most terrestrial plants. The ash content varies based on climatic, geographical, and physiological factors (Sanchez-Machado et al., 2002; Mendis & Kim, 2011). A high proportion of variable ash indicates a significant amount of diverse mineral components (El-shafay, 2014).

UV-vis spectral analysis of Sargassum latifolium

Figure 4 displayed the ultraviolet scan spectrum analysis of *S. latifolium* aqueous extract. The diagram depicted the separation of aqueous extract compounds between 200 and 400nm. Osman et al. (1993) postulated that peaks in the range of 200 to 400nm identify carboxylate and proteinaceous components. Maximum absorption at 294nm is attributable to flavonoids, phenolics, and their derivatives (Rajeswari & Jeyaprakash, 2019).

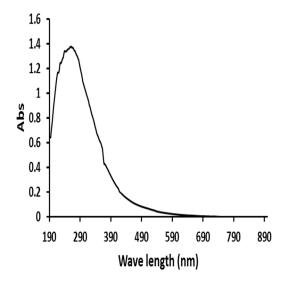


Fig. 4. UV absorbance spectrum of the aqueous extract of *Sargassum latifolium*

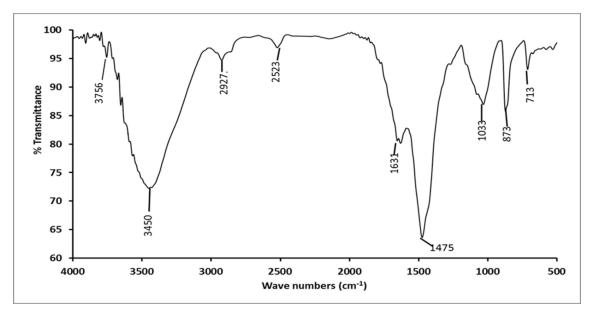
FT-IR characterization of Sargassum latifolium

The functional groups of any bioorganic substance could influence its biological activity. Furthermore, the structure of *S. latifolium* compounds was deduced using FT-IR analysis to identify the fundamental groups present. After transferring the crude powder of *Sargassum latifolium* into the FT-IR, the primary functional group was separated based on the peak ratio of the peaks. Figure 5 depicts the FT-IR spectrum results and peak values associated with the bioactive component's functional groups. The FT-IR spectrum of S. latifolium revealed functional groups with characteristic peaks at 3450, 2927, 2523, 1631, 1475, 1033, 873, 713, 579, and 531cm⁻¹. The broad and powerful absorption bands at 3756 and 3450cm⁻¹ are caused by the free O-H and N-H stretching vibrations of amino acids (Rao, 1963). Alcohol and acids may be assigned to spectral peaks in the range 4000cm⁻¹-3400cm⁻¹ (Flórez-Fernández et al., 2019). The -CH chemical group is responsible for the spectral band at 2927cm⁻¹ (Stojanovic et al., 2012). The weak absorption band observed at 2523cm⁻¹ corresponds to the characteristic peaks of the carboxylic group, the C-O stretching (Thirunavukkarasu et al., 2014). Peaks around 1628 and 1428cm⁻¹ indicate symmetric and asymmetric carboxylate anion (COO⁻) stretching vibrations, respectively (Flórez-Fernández et al., 2019).

The absorption bands located at 1030cm⁻¹ correspond to the C–H bending or C–O or C–C stretching vibrations of carbohydrates (Li et al., 2004) and polysaccharides (Nakamoto, 2009) found in *S. latifolium*. The band observed around 873cm⁻¹ is a result of aromatic molecules. C–H indicated the presence of an aromatic ring pigment compound (Figueira et al., 1999). The weak absorption band centered at 713cm⁻¹ confirms the presence of carbohydrates (Anand & Suresh, 2015) due to the C–H bending vibration (Anand & Suresh, 2015).

Qualitative characterization of aqueous extract

The preliminary phytochemical analysis of aqueous seaweed extract revealed the presence of terpenoids, steroids, flavonoids, phenols, coumarins, and quinones (Table 3). However, tannins, saponins, glycosides, and alkaloids were absent. Previous research has demonstrated that seaweeds are a rich source of bioactive compounds such as sterols, carotenoids, fatty acids, pigments, tannins, bromophenols, flavonoids, polysaccharides, and phenolic acids, which have been investigated for their biological properties (Zainuddin et al., 2020) Consistent with our findings, phytoconstituents of seaweed, such as phenols and flavonoids, have been found to inhibit free radicals (Verma et al., 2015; El-Sheekh et al., 2020).



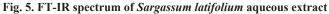


TABLE	3.	Phytochemical	analysis	of	Sargassum
latifolium aqueous extract					

Phytochemical	Sargassum latifolium
parameters	aqueous extract
Alkaloids	absent
Terpenoids	present
Steroids	present
Tannins	absent
Saponins	absent
Flavenoids	present
Phenols	present
Coumarins	present
Quinones	present
Glycosides	absent

Quantitative analysis of the aqueous extract of Sargassum latifolium

S. latifolium's Phenols (0.652mg gallic acid/g dry wt), flavonoids (0.182mg RUE /g dry wt), and tannins (0.0001mg C.A.E./g dry wt) are detailed in Table 4. Marine organisms are a rich source of new and physiologically active metabolites, providing the pharmaceutical industry with bioactive compounds of interest. It is believed that phenol compounds are the primary bioactive components of algal cells, and depending on their composition and concentration, they can either stimulate or inhibit microbial growth (Moubayed et al., 2017). According to the quantitative analysis, the total phenol content of *S. latifolium* was greater than the total flavonoid content, which was followed

by the tannin content. Rout et al. (2022) found that the total phenolic and flavonoid contents of *S. wightii* varied quantitatively according to the solvent used for extraction. The crude methanol extract of *Sargassum wightii* contained significantly more total phenolics (2.74mg GAE/g dry wt) and flavonoids (2.62mg RUE/g dry wt) than the aqueous extract (1.14GAE/g dry wt and 1.21mg RUE/g).

TABLE 4. Quantitative analysis of *Sargassum latifolium* aqueous extract

Total phenolics	0.652mg gallic acid/ g dry wt
Total flavenoids	0.182mg RUE / g dry wt
Total tannins	0.0001mg C.A.E. / g dry wt

Conclusion

According to the findings of this study, the brown seaweed *Sargassum latifolium* was the dominant macroalgal species along the shores of Ras Sudr, Red Sea Coast, Egypt. The optimal physicochemical properties of Red Sea water were a temperature of 27°C, a salinity of 40.12%, pH of 7.8, dissolved oxygen concentration of 7.37mg/L, and biological oxygen demand concentration of 1.8mg/L. According to its biochemical composition *Sargassum latifolium* is rich in carbohydrates (45.52mg/g dry wt) and low in protein (6.38mg/g dry wt). *S. latifolium* aqueous extract qualitative analysis revealed the presence of terpenoids, steroids, flavonoids,

phenols, coumarins, and quinones. In contrast, tannins, saponins, glycosides, and alkaloids are absent. UV scans of an aqueous extract of *S. latifolium* revealed peaks at 294nm that were attributed to flavonoids and phenolic derivatives. The FT-IR analysis of *S. latifolium* revealed the presence of multiple functional groups, including -OH, -NH, -CH, -COOH, CO, and C–C.

Competing interests: The authors report no conflicts of interest regarding this work.

Authors' contributions: SEA, MAD, and MME proposed the idea of this study. SEA and NIH designed the experimental work and made the measurements. SEA, MAD, NHI and MME analyzed and interpreted the data and wrote the manuscript. SEA and NHI performed the calculations and statistical analysis, participated with MAD and MME in the analysis and interpretation of the data, revised the manuscript, MME checked the manuscript for plagiarism, SEA acted as the corresponding author. All authors participated in the drafting of the manuscript and have read and approved the final draft.

Ethics approval: Not applicable.

References

- Abdelmongy, A.S., El-Moselhy K.M. (2015) Seasonal variations of the physical and chemical properties of seawater at the Northern Red Sea, Egypt. *Open Journal of the Ocean and Coastal Sciences*, **2**(1), 1-17.
- Abu Ahmed, S.E., Deyab, M.A., El-Ashry, F.S., El-Adl, M.F. (2021) Qualitative and quantitative phytochemical composition of *Sargassum vulgare* at Hurghada Red Sea Coast- Egypt. *Scientific Journal for Damietta Faculty of Science*, **11**(1), 10-19.
- Anand, M., Suresh, S. (2015) Marine seaweed Sargassum wightii extract as a low-cost sensitizer for ZnO photoanode based dye-sensitized solar cell. Advances in Natural Sciences: Nanoscience and Nanotechnology, 6(3), 035008.
- APHA, A. (1992) WPCF (American Public Health Association, American waterworks association, water pollution control federation) standard methods for the examination of water and wastewater. Stand. Methods Exam. Water Wastewater 17.

- Association, A.P.H., Association, A.W.W.W., Federation, P.C., Federation, W.E. (1912) Standard Methods for the Examination of Water and Wastewater, American Public Health Association.
- Australia, N.W. (2002) National Land and Water Resources Audit, Australian Catchment River and Estuary Assessment, Vol. 1. NLWRA Common Wealth of Australia.
- Bosak, S., Šilović, T., Ljubešić, Z., Kušpilić, G., Pestorić, B., Krivokapić, S., Viličić, D. (2012) Phytoplankton size structure and species composition as an indicator of trophic status in transitional ecosystems: the case study of a Mediterranean fjord-like karstic bay. *Oceanologia*, 54(2), 255-286.
- Burtin, P. (2003) Nutritional value of seaweeds. Electronic Journal of Environmental, Agricultural and Food Chemistry, 2(4), 498-503.
- Chakraborty, S., Santra, S.C. (2008) Biochemical composition of eight benthic algae collected from Sunderban. *Indian Journal of Marine Science*, 37(3), 329 – 332.
- Chakraborty, K., Lipton, A., Raj, R.P., Vijayan, K. (2010) Antibacterial labdane diterpenoids of Ulva fasciata Delile from southwestern coast of the Indian Peninsula. *Food Chemistry*, **119**(4), 1399-1408.
- Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, **10**(3), 178–182.
- Chennubhovla, V., Najmuedin, M., Ramalingam, J., Kaliaperumal, N. (1987) Biochemical composition of some marine algae of Mandapam cost (South India). Symposium on Research and Development in Marine Fisheries. Mandapam camp.
- CI, K.C., Indira, G. (2016) Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of Strobilanthes Kunthiana (Neelakurinji). *Journal of Medicinal Plants*, 4, 282-286.
- Circuncisao, A.R., Catarino, M.D., Silva, A.M.S. (2018) Minerals from macroalgae origin: Health benefits and risks for consumers. *Marine Drugs*,

16(11), 400.

- Davis, T.A., Volesky, B., Mucci, A. (2003) A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research*, 37(18), 4311-4330.
- Dawczynski, C., Schubert, R., Jahreis G. (2007) Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry*, **103**(3), 891-899.
- Dean Adams, V. (1990) "Water and Wastewater Examination Manual", Lewis Publishers, Chelsea, USA.
- Dorgham, M., El-Sherbiny, M., Hanafi, M. (2012) Environmental properties of the southern Gulf of Aqaba, Red Sea, Egypt. *Mediterranean Marine Science*, 13(2), 179-186.
- Dubois, M., Gilles, K.A., Hamilton, J. K., Rebers, P., Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356.
- El-shafay, S.M. (2014) Biochemical composition of some seaweed from Hurghada coastal along Red Sea coastal, Egypt. *International Journal of Basic* and Applied Sciences, 14(1), 29-35.
- El Shafay, S.M., Ali, S.S., El-Sheekh, M.M. (2016) Antimicrobial activity of some Seaweed species from Red Sea, against multidrug resistant bacteria. *Egyptian Journal of Aquatic Research*, **42**(1), 65-74.
- El-Sheekh MM., El Shafey Sh., El-Shenody R, Bases E. (2020) Comparative assessment of antioxidant activity and biochemical composition of four seaweeds, Rocky Bay of Abu Qir in Alexandria, Egypt. *Food Science and Technology*, **41**(1), 29-40.
- Emara, M., Farid, N., El-Sabagh, E., Ahmed, O., Kama, E. (2015) Physico-chemical study of surface seawater in the northwestern Gulf of Suez. *Egyptian Journal of Chemistry*, 56(5), 345-365.
- Farghl A., Al-Hasawi, El-Sheekh M.M. (2021) Assessment of antioxidant capacity and phytochemical composition of brown and red seaweeds collected from Red Sea Coast. *Applied Sciences*, **11**, 11079.
- Figueira, M., Volesky, B., Mathieu, H. (1999) Instrumental analysis study of iron species

biosorption by Sargassum biomass. *Environmental Science & Technology*, **33**(11), 1840-1846.

- Flórez-Fernández, N., Domínguez, H., Torres, M. (2019) A green approach for alginate extraction from Sargassum muticum brown seaweed using ultrasound-assisted technique. *International journal of biological macromolecules*, **124**, 451-459.
- Fouda, W.A., Ibrahim, W.M., Ellamie, A.M., Ramadan, G. (2019) Biochemical and mineral compositions of six brown seaweeds collected from red sea at Hurghada coast. *Indian Journal of Jeo Marin Sciences*, 48(04), 481-491.
- Gilbrich, W., Helmer, R., Gale, R., Siddiqi, R., Organization, W.H. (1978) Water quality surveys; a guide for the collection and interpretation of water quality data-Prepared by the IHD/WHO Working Group on Quality of Water.
- Girgis, A. (1980) Investigation of level and effects of pollutants on saline lakes and littoral marine environment. Part II: Hydrography of Lake Qarun, *Scientific Report*, Academy of Scientific Research and Technology. Institute of Oceanography and Fisheries, Cairo.
- Goecke, F., Escobar, M., Collantes, G. (2012) Chemical composition of *Padina fernandeziana* (Phaeophyceae, Dictyotales) from Juan Fernandez Archipelago, Chile. *Revista Latinoamericana de Biotecnologia Ambiental Y Algal*, 3(2), 95-104.
- Golterman, H., Clymo, R., Ohstand, M. (1978) "Methods for Physical and Chemical Analysis of Freshwater". Blackwell Scientific Publication, London, 214p.
- Halim, Y. (1984) Plankton of the Red Sea and the Arabian Gulf. Deep-Sea Research Part A. Oceanographic Research Papers, 31(6-8), 969-982.
- Hashim, M., Chu, K. (2004) Biosorption of cadmium by brown, green, and red seaweeds. *Chemical Engineering Journal*, 97(2-3), 249-255.
- Hawk, F., Oser, L., Summerson, S. (1947) A convenient titrimetric ultramicromethod for the estimation of urea and Kjeldahl. *Journal of Biological Chemistry*, **156**, 281.

Egypt. J. Bot. 62, No. 3 (2022)

- Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, **50**(8), 839-866.
- Iqbal, S., Haleem, S., Akhtar, M., Zia-ul-Haq, M., Akbar, J. (2008) Efficiency of pomegranate peel extracts in stabilization of sunflower oil under accelerated conditions. *Food Research International*, **41**(2), 194-200.
- Ismail, G.A., Gheda, S.F., Abo-shady, A.M., Abdelkarim, O.H. (2019) *In vitro* potential activity of some seaweeds as antioxidants and inhibitors of diabetic enzymes. *Food Science and Technology*, 40, 681-691.
- Iyer, R., Clerc, O.D., Bolton, J., Coyne, V. (2004) Morphological and taxonomic studies of *Gracillaria* and *Gracilariopsis* species (Gracilariales, Rhodophyta) from South Africa. *South African Journal of Botany*, **70**(4), 521-539.
- John Peter Paul, J., Shri Devi, S.D.K. (2013) Phytochemical screening of *Padina tetrastromatica* Hauck. *American Journal of PharmTech Research*, **3**(5), 214-222.
- Kadi, A. (2005) Beberapa catatan Kchadiran marga Sargassum di perairan Indonesia (Several notes of Sargassum clan attendance waters of Indonesia). Oseana, 30, 19-29.
- Kopp, J., McKee, G. (1983) Methods for Chemical Analysis of Water and Wastes. US Environ. Prot. Agency Rep(600/4), 79-020.
- Kumar, S., Ravindranath, S. (1998) Water Studies– Methods for monitoring water quality. Center for Environment Education (CEE), Banglore, Karnataka, India 191p.
- Lester, W. (1975) Polluted river: River Trent, England. *River Ecology*, **2**, 489-513.
- Levin, L.A., Boesch, D.F., Covich, A., Dahm, C., Erséus, C., Ewel, K.C., Kneib, R.T., Moldenke, A., Palmer, M.A., Snelgrove, P. (2001) The function of marine critical transition zones and the importance of sediment biodiversity. *Ecosystems*, 4(5), 430-451.
- Li, Y.M., Sun, S.Q., Zhou, Q., Qin, Z.J. Tao, X. Wang, J., Fang, X. (2004) Identification of American ginseng from different regions using FT-IR and

two-dimensional correlation IR spectroscopy. *Vibrational Spectroscopy*, **36**(2), 227-232.

- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265-275.
- Luning, K. (1990) "Seaweeds: Their Environment, Biogeography, and Ecophysiology", pp. 287-293. Wiley Interscience Publication New York.
- Madkour, H.A. (2013) Impacts of human activities and natural inputs on heavy metal contents of many coral reef environments along the Egyptian Red Sea coast. *Arabian Journal of Geosciences*, **6**(6), 1739-1752.
- Manivannan, K., Thirumaran, G., Devi, G.K., Hemalatha, A., Anantharaman, P. (2008) Biochemical composition of seaweeds from Mandapam coastal regions along Southeast Coast of India. *American-Eurasian Journal of Botany*, 1(2), 32-37.
- Marinho-Soriano, E., Fonseca, P., Carneiro, M., Moreira, W. (2006) Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresource Technology*, 97(18), 2402-2406.
- Mattio, L., Payri, C.E. (2011) 190 years of *Sargassum* taxonomy, facing the advent of DNA phylogenies. *The Botanical Review*, **77**(1), 31-70.
- Mendis, E., Kim, S.K. (2011) Present and future prospects of seaweeds in developing functional foods. *Advances in Food and Nutrition Research*, 64, 1-15.
- Moore, P.D. (1986) Methods in plant ecology. Weed Biology and Managment, 8(4), 250-259.
- Moubayed, N.M., Al Houri, H.J., Al Khulaifi, M.M., Al Farraj, D.A. (2017) Antimicrobial, antioxidant properties and chemical composition of seaweeds collected from Saudi Arabia (Red Sea and Arabian Gulf). *Saudi journal of biological sciences*, 24(1), 162-169.
- Murugaiyan, K., Narasimman, S., Anatharaman, P. (2012) Proximate composition of marine macroalgae from Seeniappa Dhaka, Gulf of Mannar region, Tamil Nadu. *International Journal* of Research in Marine Sciences, 1(1), 1-3.

- Nakamoto, K. (2009) "Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part B: Applications in Coordination, Organometallic, and Bioinorganic Chemistry", John Wiley & Sons.
- Nassar, M.Z., El-Din, N.G.S., Gharib, S.M. (2015) Phytoplankton variability in relation to some environmental factors in the eastern coast of Suez Gulf, Egypt. *Environmental Monitoring and Assessment*, **187**(10), 1-18.
- Noorjahan, A., Aiyamperumal, B., Anantharaman, P. (2019) Isolation and characterization of seaweed endophytic fungi as an efficient phosphate solubilizers. *Biosciences Biotechnology Research Asia*, 16, 33-39.
- Omar, S., Ahmad, N., Ahmad, F. (1988) Composition of alginate from brown seaweeds, *Sargassum* and *Padina* spp. *Pertanika (Malaysia)*, **11**, 79-85.
- Osman, M.E., Menzies, A.R., Williams, P.A., Phillips, G.O., Baldwin, T.C. (1993) The molecular characterisation of the polysaccharide gum from *Acacia senegal. Carbohydrate Research*, 246(1), 303-318.
- Pakidi, C.S., Suwoyo, H.S. (2017) Potensi dan pemanfaatan bahan aktif alga cokelar *Sargassum* sp. (Potential and use of active ingredients of brown algae *Sargassum* sp.) *Octopus. Journal of Fisheries Science*, 6, 551-532.
- Parthiban, C., Saranya, C., Girija, K., Hemalatha, A., Suresh M., Anantharaman, P. (2013) Biochemical composition of some selected seaweeds from Tuticorin coast. *Advances in Applied Science Research*, 4(3), 362-366.
- Patton, C.J., Kryskalla, J.R. (2003) Methods of analysis by the US Geological Survey National Water Quality Laboratory: Evaluation of alkaline persulfate digestion as an alternative to Kjeldahl digestion for determination of total and dissolved nitrogen and phosphorus in water, US Department of the Interior, US Geological Survey.
- Raikar, S.V., Lima, M., Fujita, Y. (2001) Effect of temperature, salinity and light intensity on the growth of Gracilaria spp. (Gracilariales, Rhodophyta) from Japan, Malaysia and India. *Indian Journal of Marine Sciences*, **30**, 98-104.

Rajeswari, R., Jeyaprakash, K. (2019) Bioactive

Egypt. J. Bot. **62,** No. 3 (2022)

potential analysis of brown seaweed Sargassum wightii using UV-VIS and FT-IR. *Journal of Drug Delivery and Therapeutics*, **9**(1), 150-153.

- Rameshkumar, S., Ramakritinan C., Yokeshbabu, M. (2013) Proximate composition of some selected seaweeds from Palk Bay and Gulf of Mannar, Tamilnadu, India. *Asian Journal of Biomedical and Pharmaceutical Sciences*, **3**(16), 1.
- Ramteke, D., Moghe, C. (1988) A Course Manual on Water and Waste Water Analysis. National Environmental Engineering Research Institute, Nagpur, India.
- Rao, C.N.R. (1963) Chemical applications of infrared spectroscopy. "Chemical Applications of Infrared Spectroscopy", pp. 681-681.
- Rohani-Ghadikolaei, K., Abdulalian, E., Ng, W.K. (2012) Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. *Journal of food science and technology*, **49**(6), 774-780.
- Rout, S., Rath, B., Bhattamisra, S.K., Rath, I., Kumar, A. (2022) Antioxidant and anti-inflammatory activities of methanol and aqueous extracts of *Sargassum wightii. Journal of Herbmed Pharmacology*, **11**(1), 75-82.
- Rupérez, P. (2002) Mineral content of edible marine seaweeds. *Food Chemistry*, **79**(1), 23-26.
- Sanchez-Machado, D., Lopez-Hernandez, J., Paseiro-Losada, P. (2002) High-performance liquid chromatographic determination of α-tocopherol in macroalgae. *Journal of Chromatography* A, **976**(1-2), 277-284.
- Savithramma, N., Rao, M.L., Rukmini, K., Devi, P.S. (2011) Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. *International Journal of ChemTech Research*, 3(3), 1394-1402.
- Smit, A.J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. Journal of Applied Phycology, 16(4), 245-262.
- Stojanovic, R., Cvitanovic, B.A., Manojlovic, V., Komes, D., Nedovic, V., Bugarski, B. (2012) Encapsulation of thyme (Thymus serpyllum L.) aqueous extract in calcium alginate beads. *Journal*

of the Science of Food and Agriculture **92**(3), 685-696.

- Strickland, J. (1972) A Practical Handbook of Seawater Analysis 167. Bull. Fish. Res. Board Can., Ottawa.
- Tambe, V.D., Bhambar, R.S. (2014) Estimation of total phenol, tannin, alkaloid and flavonoid in Hibiscus tiliaceus Linn. wood extracts. *Journal of Pharmacognosy and Phytochemistry*, 2(4), 41-47.
- Thirunavukkarasu, R., Pandiyan, P., Subaramaniyan, K., Balaraman, D., Manikkam, S., Sadaiyappan, B., Jothi, G.E.G. (2014) Screening of marine seaweeds for bioactive compound against fish pathogenic bacteria and active fraction analysed by gas chromatography-mass spectrometry. *Journal of Coastal Life Medicine*, 2(5), 367-375.
- TÜney, İ., Cadirci, B.H., Ünal, D., Sukatar, A. (2006) Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). *Turkish Journal of Biology*, **30**(3), 171-175.
- USEPA (1983) Methods for Chemical Analysis of Water and Wastes, Environmental Monitoring and Support Laboratory Cincinnati, Ohio.
- Val, A., Platas, G., Basilio, A., Cabello, A., Gorrochategui, J., Suay, I., Vicente, F., Portillo,

E., Río, M., Reina, G. (2001) Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *International Microbiology*, **4**(1), 35-40.

- Verma, K., Shrivastava, D., Kumar, G. (2015) Antioxidant activity and DNA damage inhibition in vitro by a methanolic extract of *Carissa carandas* (Apocynaceae) leaves. *Journal of Taibah University for Science*, 9(1), 34-40.
- Wang, Y., Zhang, M., Ruan, D., Shashkov, A.S., Kilcoyne, M., Savage, A.V., Zhang, L. (2004) Chemical components and molecular mass of six polysaccharides isolated from the sclerotium of Poria cocos. *Carbohydrate Research*, **339**(2), 327-334.
- Zainuddin, E., Tassakka, A., Manggau, M., Syamsuddin, R. (2020) Preliminary study of cultivated algae from South Sulawesi as antibacterial agent against fish pathogenic bacteria. *IOP Conference Series: Earth and Environmental Science*, IOP Publishing.
- Zou, D.H., Gao, K.S. (2014) The photosynthetic and respiratory response to temperature and nitrogen supply in the marine green macroalga *Ulva conglobata* (Chlorophyta). *Phycology*, **53**, 86-94.

الخصائص الكيميانيه والفيزيانيه للسرجاسم لاتيفوليوم من شاطىء راس سدر، البحر الأحمر، مصر

سهام السيد أبو أحمد (1)، محمد على دياب (1)، نجوى ابر اهيم حسن (1)، مصطفى محمد الشيخ (2)

⁽¹⁾قسم النبات و الميكروبيولوجي - كلية العلوم - جامعة دمياط - دمياط الجديدة- مصر، ⁽²⁾قسم النبات – كلية العلوم – جامعة طنطا- طنطا- مصر

يحتل البحر الأحمر موقعا مثاليا بسبب عزلته الجزئيه عن المحيطات المفتوحه وموقعه الجغرافي المتميز لذلك تم تجميع طحالب بحريه كبيره من منطقه رأس سدر على شاطى البحر الأحمر ودراسة التوزيع الخضرى لهذه الطحالب باستخدام اطارخشبي (متر مربع) من خلال الوزن الطازج والجاف ونسبه التوزيع. تبين أن طحلب السرجاسم لاتيفوليوم هو الأكثر سياده والأعلى نمو وتم دراسه الخواص الفيزيائيه والكيميائيه للماء وتبين انها معتدله الحراره والملوحه والأس الهيدروجينى وغنيه بالعناصر الغذائيه اللازمه للنمو. تم تجفيف الطحلب لعمل مستخلص مائى ودراسه الخواص الكيميائيه والفيزيائيه له وقد تبين أن الطحلب من الكربوهيدرات ونسبه ضئيله من البروتين كما يحتوى المستخلص على مركبات طبيعيه مثل التربينات والاسترودات والفلافينويد والكومارين والكينون.