GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Preliminary phytochemical screening for various secondary metabolites, quantitative and qualitative analysis of Yemeni brown seaweed *Sargassum vulgare*

Diana Faisal al-hashdy [1], Amina Mohammed El-Shaibany [1], Salwa Mohammed Raweh [1], Abdulrahman Abdullah Humaid [2, 3, *] and Mahmoud Mohamed El –Aasser [4]

[1] *Department of Pharmacognosy, Pharmacy College, Sana`a University, Sanaa, Yemen.*
[2] *Department of Biology, Faculty of Science, Sana`a University, Sanaa, Yemen.*
[3] *Department of Pharmacy, Faculty of Medical Science, University of Modern Science, Sana`a, Yemen.*
[4] *The Regional Center for Mycology and Biotechnology, Al-Azhar University, 11787, Nasr City, Cairo, Egypt.*

GSC Biological and Pharmaceutical Sciences, 2022, 20(01), 298–313

Publication history: Received on 11 June 2022; revised on 20 July 2022; accepted on 22 July 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.20.1.0294

Abstract

Sargassum Vulgare was collected from the Red sea coastlines, Al-Hodeida, Yemen, and dried. The dried powder was subjected to consecutive extraction by methanol. The dried methanol extract was subjected to solvent fractionation (n-hexane-chloroform-ethyl acetate and n-butanol solvents). *Sargassum vulgare* polysaccharides (S.R1P, S.R1M, S.R5s, and S.R5ss) were isolated by precipitation with methanol. [19] The methanol extract and n-hexane fraction were evaluated phytochemically. The physicochemical characteristics and FT-IR analysis of *Sargassum vulgare* polysaccharides (S.R1P, S.R1M, S.R5s, and S.R5ss) were investigated, the chemical composition of the *Sargassum vulgare* n-hexane fraction was evaluated by Gas Chromatography-Mass Spectrometry (GC-MS) analysis [8]. Phytochemical investigation revealed that both methanol extract and n-hexane fraction of *Sargassum vulgare* contained alkaloids, phytosterols, terpenoids, fats, and fixed oils. Moreover, *Sargassum vulgare* methanol extract exhibited a high amount of carbohydrates (41.33%) mainly polysaccharides [40][41][42]. The physicochemical characteristics and FT-IR analysis of polysaccharides (S.R1P, S.R1M, S.R5s, and S.R5ss) revealed that all the obtained polysaccharides were alginates with traces of sulfate polysaccharides except S.R1M. [30][34][35]

Keywords: *Sargassum Vulgare*; Secondary Metabolites; Phytochemical Screening; Physical Tests

1. Introduction

Sargassum spp. have a broad geographical distribution from Central America, through Australia, New Zealand, Asia, Europe and Africa [53] spanning the three ocean basins of the Atlantic, Pacific and Indian Oceans, inhabiting temperate, subtropical and tropical habitats starting from the beach up to coral reefs along the littoral and sub-littoral areas Figure 1 [54]. The genus has been considered to be the most species-rich genus of the marine macrophytes with 400 species being identified to date [55]. The bioactive secondary metabolites of Sargassum species have not been completely investigated, while, their major constituents are known and used in folk medicine.). According to the modern Chinese pharmacopeia, „„Hai Zao""can be used to treat goiter scrofula, swelling and pain of testes, edema due to retention of phlegm and morbid fluids. In modern Chinese medical practice, „„Hai Zao""has also been used to treat arteriosclerosis, skin diseases, high blood pressure, hepatosplenomegaly, neurosis, angina pectoris, acute esophagitis, chronic bronchitis. Furthermore, several unique compounds have been isolated as meroterpenoids, phlorotannins and fucoidans from Sargassum species, which may be responsible for their medicinal activities. Other compounds such as phytosterols, sulfoglycolipids, and polyunsaturated fatty acids have been barely reported in this genus [56].

* Corresponding author: Abdulrahman Humaid
Department of Biology, Faculty of Science, Sana`a University, Sanaa, Yemen.

2. Materials and method

2.1. Seaweed collection and Identification

Sargassum vulgare thallus had been collected from Al-Hodeida governorate (Red Sea coast) which represents western coastline of Yemen by handpicking in May 2018. The seaweed was identified by Dr. Abdulsalam Alkory (Head of Marine Science Department, Faculty of Science, and Al-Hodeida University).

2.2. Seaweed preparation

The fresh seaweed (5 kg) was cleaned with seawater to remove associated sand debris. Then, the seaweed transferred to the laboratory using polythene bags and thoroughly washed with sterile tap water to take away excess salt from the surface of the seaweed then washed with sterilized distilled water [37]. Finally, samples were air-dried at room temperature in the darkness, until get completely dried biomass and ground into a fine powder by a grinder (Philips,China) subjected for extraction[20].

2.3. Preparation of *Sargassum vulgare* methanol extract

Sargassum vulgar dried powder (2.42 kg) was macerated in 7L methanol: water (6:1) for 7 days with continuous shaking in a mechanical shaker at room temperature at constant stirring rate at 100 round per minute (Shaker, G F L , Germany). Then, the obtained extract was filter by filter paper (Whattman No1). These two steps were repeated till obtained a pale filtrate. The obtained filtrates were concentration through a rotary evaporator (Buchi, Rotavapor R-215; Switzerland) with temperature not exceed 45 ˚C [43].The dried extracts yields were calculated based on initial dry weights by applying the following formula:

$$\% \text{ of yield} = \frac{\text{Weight of methanol extract}}{\text{Weight of dried sample powder}} \times 100$$

Finally, the dried extracts were stored in airtight glass bottles, at room temperature for subsequent phytochemical screening and biological activity evaluation.

2.4. Solvent fractionation of Sargassum vulgare methanol extract

Fractionation was applied by liquid-liquid partition, methanol extract of *Sargassum vulgare* (342.18 gm) was suspended in methanol: water (2:1) and partitioned with different solvents polarities as n-hexane, chloroform, ethyl acetate, and butanol respectively [16]. Each fraction was evaporated using a rotary evaporator (Buchi, Rota-vapor R-215; Switzerland) then air dried. The final yields of each fraction was calculated by applying the following formula:

$$\% \text{ of yield} = \frac{\text{Weight of dried fraction}}{\text{Weight of methanolic extract}} \times 100$$

Finally, the dried fractions were stored in airtight bottles at room temperature until used. If any crystal was formed, the crystals decanted from the solvent and washed with methanol many times until get no colors in methanol solution and air dried.

2.5. Phytochemical evaluation of Sargassum vulgare methanol extract and n-hexane fraction

Phytochemical analysis of *Sargassum vulgare* methanol extract was carried out according to standard methods [43].The n-hexane fraction was tested for the positive results obtained by the methanol extract.

2.5.1. Polysaccharides Identification Tests

Physical and chemical tests applied to identified the obtained polysaccharides by compared them to fucoidan standard from the literature and the standard alginic acid (pharmaceutical/food grade) according to [4].

Chemical Tests

Chemical tests are applied to identified carbohydrates, sulfates, and polysaccharides.[13]

Qualitative thin layer chromatographic analysis of methanol extract and n-hexane fraction

Active chemical constituents and their retention factor (R_f) values of *Sargassum vulgare* methanol extract and n-hexane fraction were investigated by thin layer chromatography. [9]

Three solvent systems were applied to identify phytochemical components, solvent system I ; benzene: ethyl acetate(1:1) was used for triterpenoid determination. For alkaloids analysis, solvent system II; ethyl acetate: methanol: water (100:13.5:10) was applied. Solvent system III; hexane: ether: acetic acid (80:20:2) was used for the lipids determination [36]. The developed TLC plates were sprayed with suiTablele reagents heated at l00˚C for 5-10 min, then evaluated under UV light (Vilber Lourmat, France)at wavelengths of 254 nm and 365 nm[32] [46][14].

2.6. Quantitative Phytochemical Analysis of methanol extract and n-hexane fraction

2.6.1. Quantitative determinations are applied to determine the relative quantity of the components as carbohydrates alkaloids, and phyto-sterols,

Estimation of Total Carbohydrates Content

The total carbohydrates in a sample can be estimated by anthrone test [13].

2.6.2. Determination of total alkaloids

This method characterized by a simple, sensitive, and rapid spectrophotometric method for total alkaloids quantification without interference of other constituents. [41]

Estimation of sterols by Liberman–Burchard method

Total sterols content was determined by Liberman–Burchard reagent. [40]

Determination of total phenolic contents by Folin-Ciocalteu reagent

Phenolic compounds determination involved the reduction of phenolic compounds by phosphomolybdic / phosphotungstic components in Folin-Ciocalteu reagent. [31].

Fourier transform infrared (FT-IR) spectroscopy

Fourier transform infrared (FT-IR) spectroscopy are used to study the spectra of the obtained residues [4]

3. Results

3.1. Yields of Sargassum vulgare methanol extract

The methanol extracts of *Sargassum vulgare* yields were 389.41 g which have characterized by greenish black color Figure (1). During drying the first methanol extract by a rotary evaporator (Buchi, Rotavapor R-215; Switzerland), greenish white precipitate is formed. The yields of the total methanol extract and the residue obtained from the first methanol extract named (S.R1) are 371.03(14.8%) and 18.38 g (0.74%), respectively.

S.M.E

S.R1

Figure 1 *Sargassum vulgare* methanol extract (S.M.E) and Sargassum vulgare residue 1(S.R1)

3.2. Fractionation Yields

Table (1, 2) showed that the maximum yield is for the aqueous fraction followed by n- hexane fraction then n-butanol fraction. The lowest yields are for chloroform and ethyl-acetate fractions

Methanol extract of *Sargassum vulgare* (342.18g) was fractionation by liquid-liquid Figure (2)

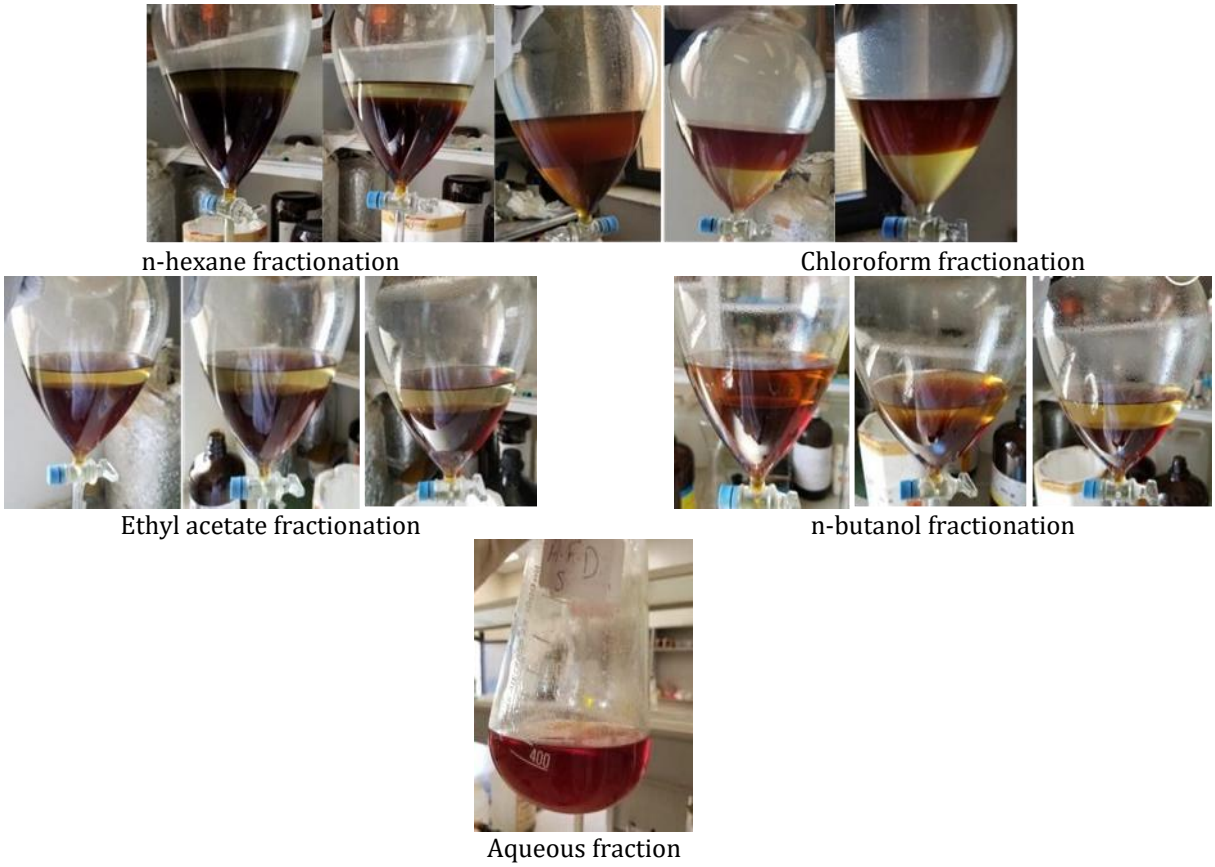

Figure 2 Fractionation of Sargassum vulgare methanol extract

Table 1 Extraction Yields

Sample	Yields (g)	% (w/w)
S.M.E*	371.03	14.8
S.R1*	18.38	0.74
S.R1P*	9.94	54.08 from S.R1
S.R1M*	3.81	20.73 from S.R1

S.M.E*: Sargassum vulgare* methanol extract; $S.R_1$: *Sargassum* residue1; $S.R_{1P}$: *Sargassum* residue 1purified;$S.R_{1M}$: *Sargassum* residue after methanol purification.

Table 2 Fractionation Yields

Fraction	Yield (g)	% (w/w)
Hexane fraction	45.47	13.3
Chloroform fraction	1.67	0.49
Ethyl-acetate fraction	1.41	0.41
Butanol fraction	12.76	3.73
Aqueous fraction	98.77	28.86
S.R5s*	6.31	1.84
S.R5ss*	24.46	7.15

* $S.R_{5s}$: *Sargassum* residue after aqueous fraction; $S.R_{5ss}$: *Sargassum* residue after aqueous fraction supernatant

3.3. Phytochemical evaluation

Thin layer chromatography investigation of methanol extract and n- hexane fraction

Thin layer chromatography (TLC) was applied to investigate the presence of the secondary metabolites in methanol extract and n-hexane fraction through different solvent systems and spray reagents. Figure (3). Table. (3)

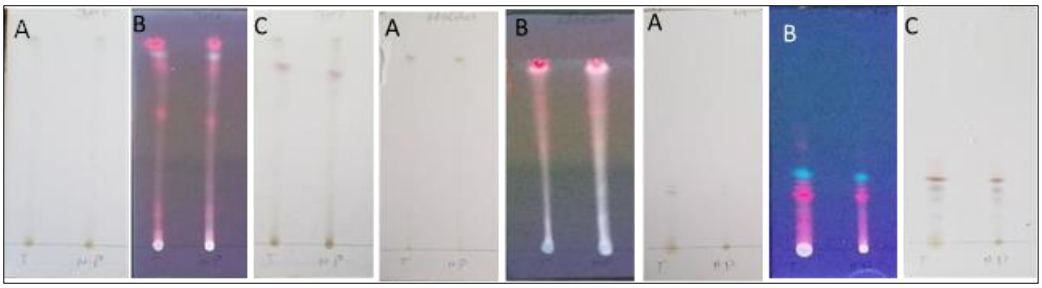

Figure 3 TLC chromatograms of (1) tri-terpenoids, (2) alkaloids and (3) lipids : A=day light, B=under 356 UV, and C= after spraying

Table 3 TLC Results

Phyto-chemicals	Solvent system	No. of Spots	R_f.
Tri-terpenoids	Benzene: EtOAc (1:1)	5	0.89,0.86, 0.75, 0.67, 0.37, 0.33
Alkaloids	EtOAc: MeOH: Water (100:13.5:10)	N.D	N.D
Lipids	n-hexane: ether: A.A (80:20:2)	7	0.34,0.28, 0.27, 0.23,0.22,0.18, 0.11

3.4. Phytochemical screening of *Sargassum vulgare* methanol extract and n-hexane fraction

Table 4 Phytochemical screening of methanol extract and n-hexane fraction

No	Phytochemicals	Tests	S.M.E*	S.H.F*
1.	Alkaloids	Mayer's test	+	+
		Dragendorff's test	+	+
		Wagner's test	+	+
		Hager's test	+	+
2.	Glycosides	Modified Bortrager's test	-	-
		Keller–Killani test	-	-
3.	Phenolic compounds/ Tannins	Shinoda test	-	-
		Ammonia test	+	-
		Lead acetate test	+	+
		Ferric chloride test	-	-
		Gelatin Test	+	-
4.	Protein and amino acids	Xanthoprotein Test	-	-
		Ninhydrin Test	-	-
		Biuret Test	-	-
5.	Phytosterols	Salkowaski test	+	+
		Leibermann Burchard's test	+	+

6.	Carotenoids	Carotenoids test	0-	-
7.	Carbohydrates	Molisch's test	+	-
		Fehling's test	+	-
		Benedict's test	-	-
8.	Saponins	Foam test	-	-
9.	Fats and fixed oil	Spot test	+	+

*S.M.E: *Sargassum vulgare* methanol extract; S.H.F: Sargassum vulgare n-hexane fraction;+ :Positive results; - : Negative result

Phytochemical analysis of *Sargassum vulgare* methanol extract was carried out according to standard methods (43)[47].The n-hexane fraction was tested for the positive results obtained by the methanol extract Figure(4) Table .[4]

Figure 4 1-Extract; 2-Mayer's test; 3- Dragendorrf 's Test; 4-Wagner'test; 5- Hager'stest; 6-Extract; 7- Modified Bortrager's test;8- Cardiac glycosides test: 9- Extract(Stock soln.);10- Lead acetate test; 11-Gelatin test ;12-Extract (High dilution); 13- Lead acetate test;14- Ferric chloride test; 15-Gelatin test16-Extract;17- Shinoda test;18-Alkaline reagent test: 19-Extract; 20-Xanthoprotein test ; 21-Ninhydrine test ; 22-Biuret test: 23-Extract;24- Salkowski's test ; 25-Liebermann Burchard's test: 26- Extract(Stock soln.);27- Carotenoids test; 28:Extract;29:Mayer test;30:Fehling test; 31:Bendict test 32-Extract; 33- Foam test after 30min: 34-Spot Test.

3.5. Physical test

All the obtained residues do not melt till 370˚C except S.R5ss,but they decomposed at 200-350˚C formed dark brown to black at 350˚C .The decomposition temperature of sodium alginate standard is 220.4±4.77 °C. S.R1P decomposes temperature start at 243.8°C and the decomposition ended by dark color (black) at 348.9°C. S.R1M start decompose at 248°C and ended by 299°C. S.R5s decomposition temperature starts at 293.2°C to 343.8°C. While, S.R5ss start melting

at 150.2°C and end by 160.5°C. It was noTable that, all residues are UN melt-able in the tested temperature range compared to alginic acid, sodium salt except S.R5ss

All the residues are freely soluble in distilled water (0.1g in 1mL) and insoluble in methanol. The alginic acid, sodium salt (Sigma- Aldrich, USA) has similar physical properties except its solubility (0.5g suspended in 15mL distill water) a very viscous yellow solution is formed Figure (5), Table 5.

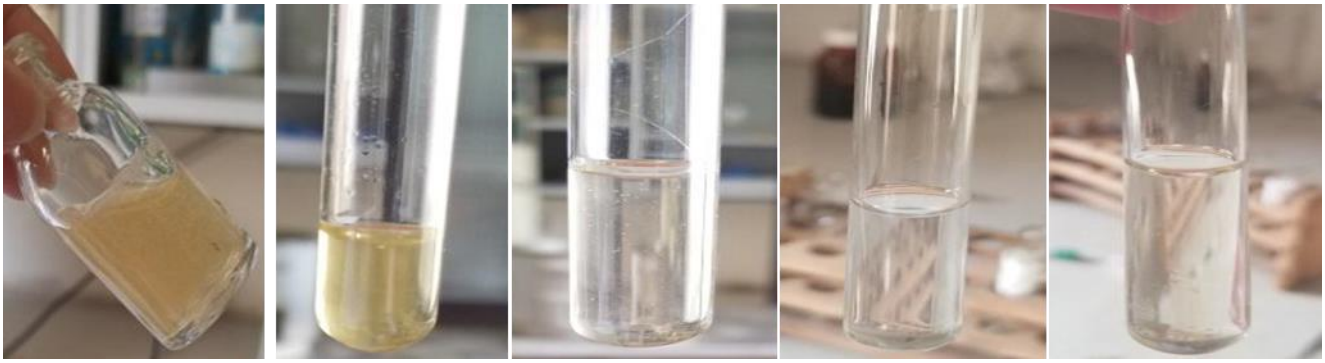

Figure 5 Solubility Test

Table 5 Physical Test Results

R	Color	Odor	Taste	Appearance	Solubility	M.P
$S.R_{1P}$	Greenish white	Fishy odor	Very Salty	Powder	Freely soluble in water; insoluble in methanol	N.M
$S.R_{1M}$	Yellowish white	odorless	Salty	Semi-crystals	Freely soluble in water; insoluble in methanol	N.M
$S.R_{5s}$	White	Fishy odor	Salty	Shiny crystals	Freely soluble in water; insoluble in methanol	N.M
$S.R_{5ss}$	Yellowish white	Fishy odor	Salty	Semi crystals	Freely soluble in water; insoluble in methanol	150.2 160.5°C
Alginic acid	Off white	odorless	Gummy taste	Powder	0.5g to 15mL dis. water	N.M

*R: residues, M.P: melting point; N.M=Non-melt-able; $S.R_{1P}$: purified *Sargassum* residue after methanol extract; $S.R_{1M}$: *Sargassum* residue after methanol wash of $S.R_1$; $S.R_{5s:}$ *Sargassum* residue after methanol wash of aqueous fraction; $S.R_{5ss}$: *Sargassum* residue after methanol wash of aqueous fraction; Freely soluble in water=o.1g in less than1mL distill water

3.6. Quantitative Phytochemical Estimation

Based on the calibration curves of glucose, atropine, cholesterol and gallic acid Figure (6 ,7,8,9) and from the regression equations the total carbohydrates, alkaloids, steroids and phenolic compounds contents are summarized in Table(6,7).

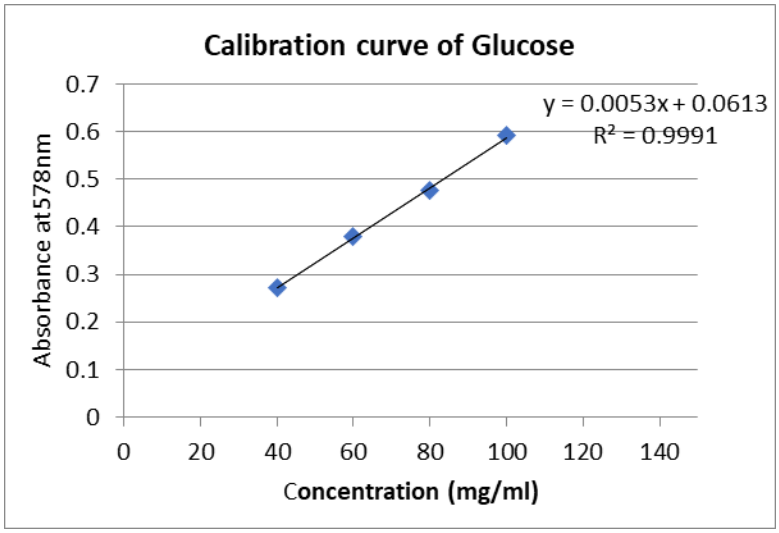

Figure 6 Calibration curve for carbohydrates determination using glucose standard

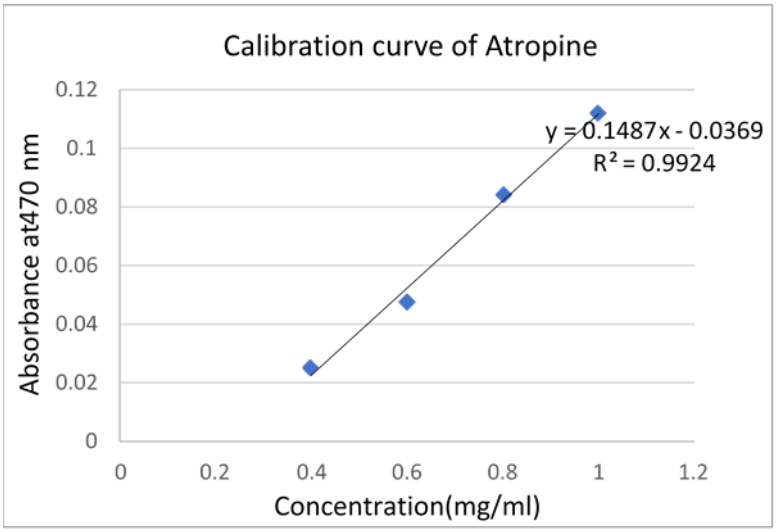

Figure 7 Calibration curve for alkaloids determination using atropine standard

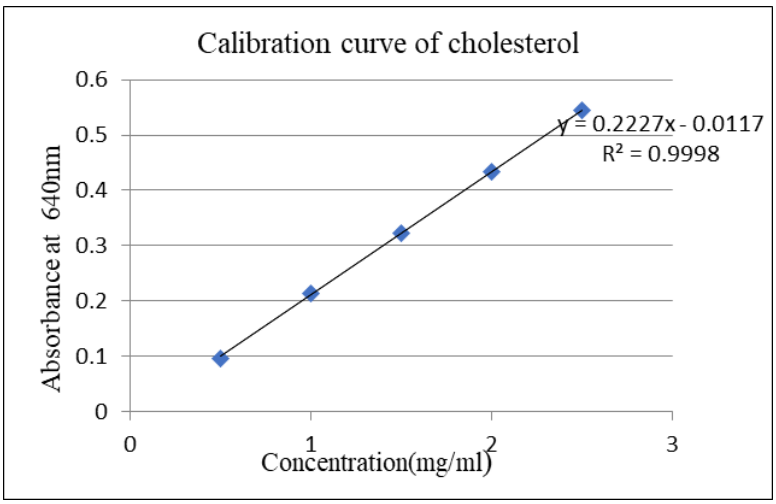

Figure 8 1Calibration curve for sterols determination using cholesterol standard

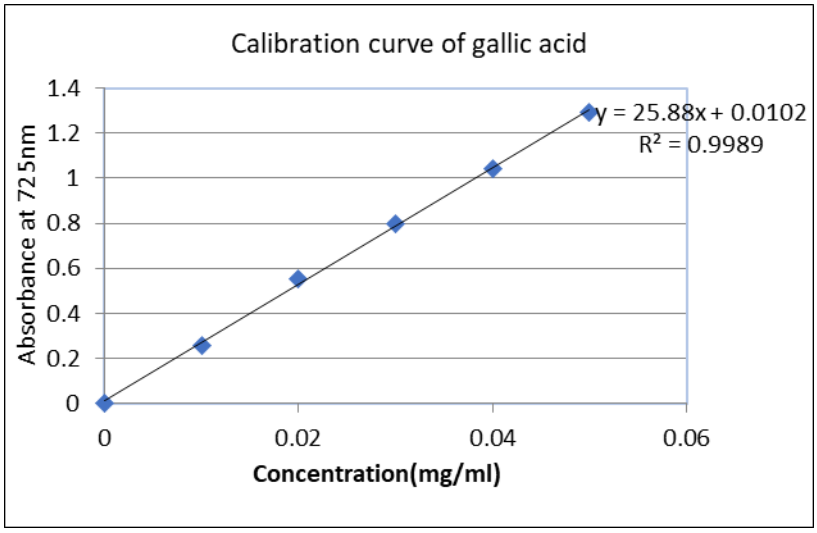

Figure 9 Calibration curve of gallic acid for total phenolics contents determination

Table 6 Chemical Test Results

Residues/Tests	**S.R$_{1P}$**	**S.R$_{1M}$**	**S.R$_{5s}$**	**S.R$_{5ss}$**	**Alginic acid**
Molish's test	++	++	++	++	++
Barium chloride test	-	-	-	-	-
Lugol's test	-	-	-	-	-
Lead acetate (10%)	+	++	++	++	+

*(++): positive results; (+): slightly turbid ;(-): negative results.

Table 7 Quantitative phytochemical estimation

Phytochemical compounds	**S.M.E***	**S.H.F***
Carbohydrates (mg/g glucose equivalent)	41.33±0.011	-
Alkaloids (mg/g atropine equivalent)	2.079±0.0106	1.979±0.0063
Sterols (mg/g cholesterol equivalent)	0.23±0.001	0.78 ±0.005
Phenolic contents (mg/g gallic acid equivalent)	0.00044 ± 0.0034	-

* S.M.E=*Sargassum vulgare* methanol extract; S.H.F= *Sargassum vulgare* hexane fraction; Mean ±Standard deviation

As seen, all the residues obtained from *Sargassum vulgare* are polysaccharides based on the positive results of the molish's test. There are differences in the formed colored after the addition of sulfuric acid between residues and alginic acid sodium salt. All the residues formed purple ring between the two layer and the alginic acid produced brown ring. Negative results are obtained with all the polysaccharides with barium chloride test and lugol's tests that means no sulfate and starch.

3.7. FT-IR spectroscopy

In the present study, the IR spectra of four polysaccharides obtained from Sargassum vulgare are compared to that of alginic acid, sodium salt purchased from Sigma- Aldrich. Their spectra data are summarized in Table [8].

As seen below, S.R1P and S.R5ss polysaccharides demonstrated mainly typical IR absorption bands to the alginic acid, sodium salt (standard). While, S.R1M and S.R5s showed quite differences to the standard.

Moreover, all the polysaccharides shared typical polysaccharide absorption bands Figure (10,11,12,13,15) including a broad band at 3200-3400 cm-1 correspond to hydrogen bonded O-H stretching vibrations and C-H stretching vibrations at 2939- 2924 cm-1 [17].

Table 8 FT-IR spectra data of polysaccharides

N	Sample*	FT-IR (KBr) (cm^{-1})
1	$S.R_{1P}$	3425.58, 2939.52, 2299.15, 1651.07, 1519.91, 1411.89, 1342.46, 1226.73, 1103.28, 1033.85, 925.83, 802.39, 725.23, 570.93.
2	$S.R_{1M}$	3417.86, 2684.91, 2067.69, 1635.64, 1388.75, 1126.43, 617.22,486.06
3	$S.R_{5S}$	3410.15, 3232.70, 3140.11, 1635.64, 1411.69, 1080.14, 1033.85, 717.52.
4	$S.R_{5SS}$	3317.95, 2931.80, 1635.64, 1458.18, 1373.32, 1334.74, 1249.87, 1195.87, 1087.85, 1026.13, 954.41, 933.55, 879.54, 717.52, 624.94.
5	*Alginic acid*	3479.58, 2924.09, 2175.70, 1620.21, 1419.51, 1303.88, 1087.85, 1033.85, 948.98, 887.26, 817.82, 725.23,624.94.

*$S.R_{1P}$: purified *Sargassum* residue after methanol extract; $S.R_{1M}$: *Sargassum* residue after methanol wash of $S.R_1$; $S.R_{5S}$: *Sargassum* residue after methanol wash of aqueous fraction; $S.R_{5SS}$: *Sargassum* residue after methanol wash of aqueous fraction

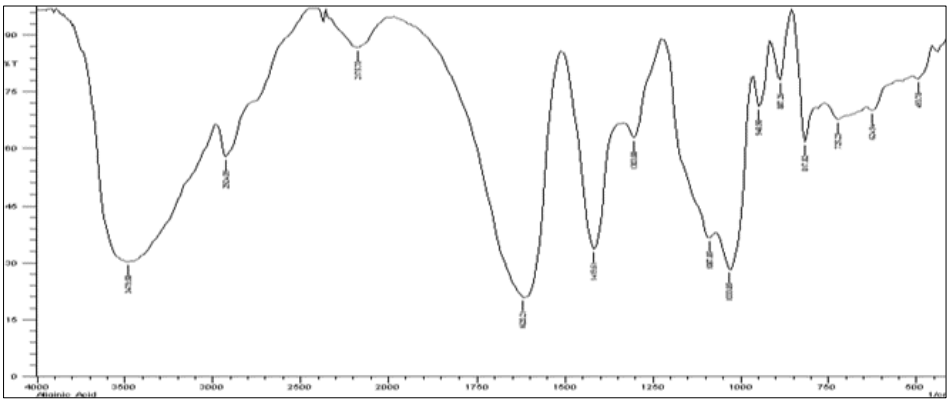

Figure 10 FT-IR spectrum of alginic acid, sodium salt

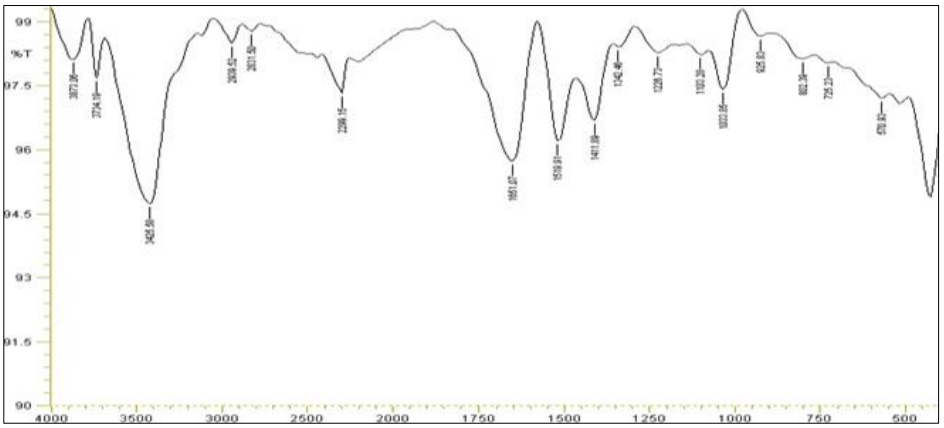

Figure 11 FT-IR spectrum of S.R1P

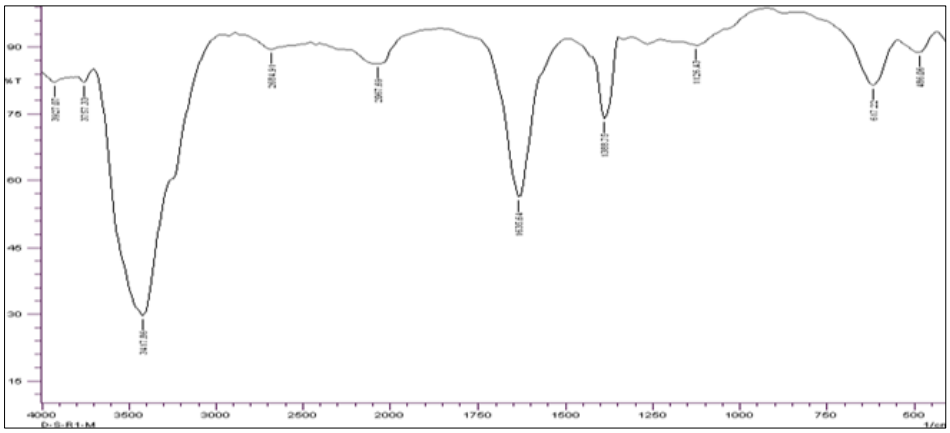

Figure 12 FT-IR spectrum of S.R$_{1M}$

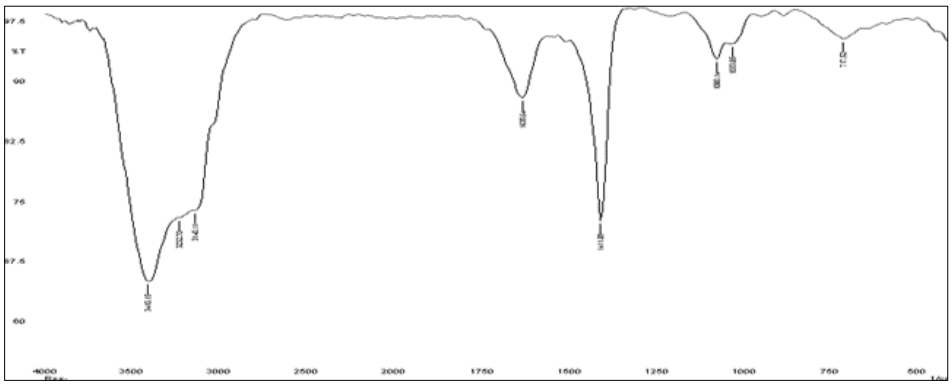

Figure 13 FT-IR spectrum of S.R$_{5S}$

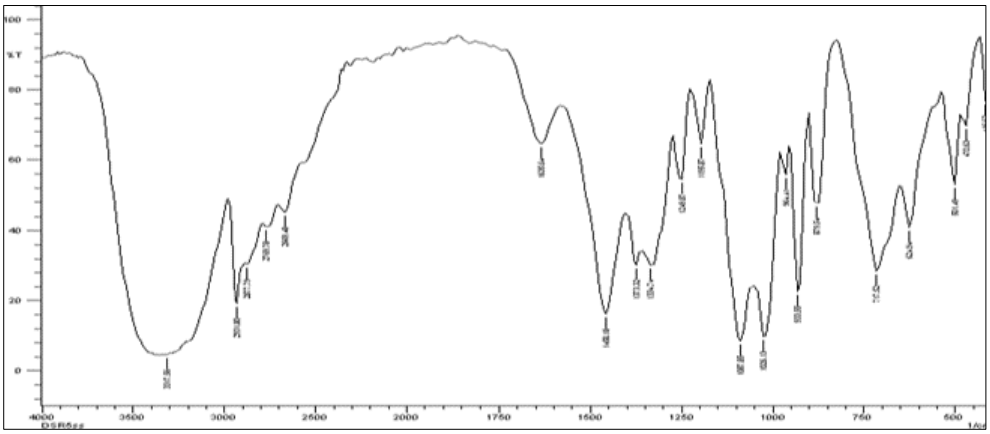

Figure 14 FT-IR spectrum of S.R$_{5SS}$

The bands at 1620.21–1651.07 cm−1 and 1411.51–1458.18 cm−1 were corresponded to carbohydrate O-C=O asymmetric stretching vibrations and C–OH symmetric vibrations of carboxylate groups, respectively which consider as characteristic bands of alginate salt rather than free acid [42];[15];[2] which are presented in all the isolated polysaccharides compared to alginic acid, sodium salt (standard) except for S.R1M

Two IR bands at approximately 1100 and 1025 cm−1, are assigned to mannuronic and the O-H bending of guluronic acid units, respectively of alginates [2]; [42]; [39].

The bands at 1303.3–1342.46 cm−1 (C–C–H, and O–C–H deformation), 1080.42– 1103.85 cm−1(C–O stretching vibrations) and 1026.13- 1033.85 cm−1(C–O and C–C stretching vibrations) of pyranose rings [2].

The fingerprint region (950-750 cm-1) showed three characteristic absorption bands in all polysaccharide compare to standard. The first bands at 948.98, 947.9 and 925.83 cm-1 are assigned to the C-O stretching vibration of uronic acid residues in standard, S.R5SS and S.R1P, respectively. The second bands at approximately 887.26 and 878.1 cm-1 were assigned to the C1-H deformation vibration of β-mannuronic acid residues in S.R5SS and S.R1P, respectively. Finally, the third band at 817.82 cm-1 was characteristic of mannuronic acid residues which present only in alginic acid, sodium salt standard Fig.(4.6) [15]; [10]; [11]; [24]; [2]; [33]; [12];

According to W. Mackie, (1971), and [45][33], two characteristic bands around 808 and787 cm−1, were attributed to mannuronic (M), guluronic (G) acids units, respectively of alginates [44]; [33]. As mentioned in Table (12) all the polysaccharides have two bands around 802.39 and 725.23 cm−1 except S.R5s and S.R1M. Moreover reported that the IR bands at 944-927 and 821-817cm−1 each represented the characteristic absorptions from guluronic acid and mannuronic acid, respectively [6].

4. Discussion

Sargassum vulgare methanol extract was investigated for its phytochemical constituents and it was observed the presence of polysaccharides, alkaloids, phytosterols, terpenoids, fats, and fixed oils

These qualitative phytochemicals results are consistent with the results of some *Sargassum* species as *Sargassum wightii, Sargassum polycystum, Sargassum duplicatum, Sargassum angustifolium, Sargassum oligocystum* and *Sargassum boveanum* and show disagreement in the presence of polyphenolic compounds, tannins, flavonoids, and saponins [7] [20] [21]; [38][32].

Among *Sargassum vulgare* fractions, the aqueous fraction shows the highest yield(28.86%)Table.(1,2)among the five fractions possibly due to high levels of polar compounds (water-soluble components), such as polysaccharides.

The quantitative phytochemical determination of methanol extract revealed that the main phytochemicals were carbohydrates (41.33 ± 0.011 mg/g glucose equivalent Table.(7). This result is consistent with the previous reports [26]; El-c et al., 2021).

The physical properties of *Sargassum vulgare* polysaccharides Table.(5),

All the obtained polysaccharides do not melt-able till 370˚C accept R5ss, but they decomposed at temperature 243.8-293.2˚C formed dark brown to black color compared to alginic acid, sodium salt standard which is decomposed at 220.4˚C. According to Helmiyati and Aprilliza's study, who assumed that pure sodium alginate had a higher decomposition temperature value than isolated sodium alginate due to the crystallinity index. Likewise, R5ss decomposes at 150.2-160.5 ˚C lower than the standard which is consistent with. On the other hand, the remaining polysaccharides show a higher melting point than alginic acid, sodium salt. It might be due to their higher crystallinity index or due to the presence of other impurities [17].

The chemical tests results are mentioned in Table.(7) and Figure(4). The results show that the residues are carbohydrates, with no or trace amount of sulfate. These results are similar to the (4) study. The chemical test results are found to resemble alginic acid, sodium salt standard tests. [23]

alginates salt are the main polysaccharide found in brown seaweed *Sargassum vulgare* samples, rather than alginic acid despite no bands appearing around 1730 cm-1 and appeared around 1600 cm-1 contributed to alginate salt form rather than alginic acid, free acid form [15]

According to the results obtained by physical, chemical tests and FT-IR analysis*, Sargassum vulgare* polysaccharides are mainly alginates compared to the commercial alginic acid, sodium salt except for S.R1M or alginates with traces amounts sulfated polysaccharides. This is in agreement with several studies that reported the co-extraction of fucoidan with alginate [33]; (5).Therefore, these polysaccharides should be further demonstrated by more studies in the future.

FT-IR spectroscopy of *Sargassum vulgare* polysaccharides Table.(8) Figure (11,11,12,13,14)revealed that the prominent peaks are mainly attributed to alginates which are consistent with other isolated alginates [10]; [42]; [15]; [33]; [25]; [17]; [1]; [11]; [12]

Many studies have been reported that the strong band at 1263 cm−1 and the sharp band at 849 cm−1 corresponding to the asymmetric stretching vibrations of S=O and C– O–S, respectively, indicated the presence of sulfate polysaccharides [33];[11]; [39]. In this study, there is a small band around 1250 cm-1 without a sharp peak around 850 cm-1 which may contribute to the presence of a little number of sulfated polysaccharides extracted with alginates. In agreement with the early published works [33]; [11]; [39]. Moreover, the peaks around to 603- 622 and 563-583cm−1 were also attributed to the asymmetric and symmetric O=S=O deformation of sulfates [18] These bands appear as small shoulder in S.R1P Fig.(11)And small intense in S.R5ss Figure(13)spectra indicated the presence of a trace amount of sulfated polysaccharides with alginate which is consistent with (15]; [2].

All obtained polysaccharides have two bands around 802.39 and 725.23 cm−1 except S.R5s Figure(13) and S.R1M Figure(12), which are attributed to mannuronic (M) and guluronic (G) acids units, respectively. These are in agreement with the early published reports [44]; [33]; [6].

Interestingly, the IR spectrum of S.R5s Figure(13) shows the more intense band at 1080.14 cm-1 than a small band at 1033.85 cm-1, indicating the presence of small amounts of mannuronic acid comparable to guluronic acid. This is confirmed by the fingerprint region which shows the only band at 717.52 cm−1, which is assigned to guluronic (G) acids units. Consequently, S.R5s is alginate rich in guluronic (G) acids units which are in agreement with [29] [42]; [2]. Additionally,reported that 780 cm−1 band was characteristic for typical guluronic acids indicating the polysaccharides were not fucoidans

IR spectrum of S.R5ss Figure(14) shows similar intensities bands around 1087.85 and 1026.13 cm-1, which is attributed to a similar quantity of guluronic acid and mannuronic acid units [39]. On contrary, [44]. stated that Sargassum vulgare alginates (high and low viscosity) showed higher values of homopolymeric mannuronic acid blocks [44]

5. Conclusion

Marine environment is an extraordinary reservoir of bioactive natural products, many of them exhibit a novel structural features not found in terrestrial plant natural products. The numbers of novel marine metabolites are increasing every year, indicating that the marine organisms are potentially productive sources of highly bioactive secondary metabolites that may lead to the development of new pharmaceutical agents .The results of the present study confirmed that *Sargassum vulgare* may be rich sources of phytoconstituents which can be isolated and further screened for various biological activities.

Compliance with ethical standards

Acknowledgments

The authors are grateful to The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Disclosure of conflict of interest

The authors declare that they have no competing interests.

References

[1] Ardalan, Y., Jazini, M. and Karimi, K. (2018) 'Sargassum angustifolium brown macroalga as a high potential substrate for alginate and ethanol production with minimal nutrient requirement', *Algal Research*, 36, pp. 29–36. doi:10.1016/j.algal.2018.10.010.

[2] Balboa, E.M., Rivas, S., Moure, A., Domínguez, H., *et al.* (2013) 'Simultaneous extraction and depolymerization of fucoidan from Sargassum muticum in aqueous media', *Marine Drugs*, 11(11), pp. 4612–4627. doi:10.3390/md11114612.

[3] Banu, K.S. and Cathrine, L. (2015) 'General Techniques Involved in Phytochemical Analysis', *International Journal of Advanced Research in Chemical Science (IJARCS)*, 2(4), pp. 25–32.

[4] Bayuran, R.G. (2017) 'Antimitotic and Antiangiogenic Assay of Fucoidan from Sargassum oligocystum', *Asian Society of Teachers for Research*, 1(November), pp. 40–60. Available at: https://aseanresearch.org/downloads/astr/publication/BAYURAN.pdf.

[5] Bedoux, G., Caamal-Fuentes, E., Boulho, R., Marty, C., *et al.* (2017) 'Antiviral and cytotoxic activities of polysaccharides extracted from four tropical seaweed species', *Natural Product Communications*, 12(6), pp. 807–811. doi:10.1177/1934578x1701200602.

[6] Cong, Q., Xiao, F., Liao, W., Dong, Q., *et al.* (2014) 'Structure and biological activities of an alginate from Sargassum fusiforme, and its sulfated derivative', *International Journal of Biological Macromolecules*, pp. 1–8. doi:10.1016/j.ijbiomac.2014.05.056.

[7] Devi, J.A.I., Balan, G.S. and Periyanayagam, K. (2013) 'Pharmacognostical study and phytochemical evaluation of brown seaweed Sargassum wightii', *Journal of Coastal Life Medicine*, 1(3), pp. 199–204. doi:10.12980/jclm.1.2013c959.

[8] EL-Shafay, S.M. (2014) 'Biochemical Composition of Some Seaweed From Hurghada Coastal Along Red Sea Coastal, Egypt', *International Journal of Basic & Applied Sciences*, 14(01), pp. 29–35.

[9] El-sheekh, M.M., Abd, R., Khalek, E., Bases, E.A., *et al.* (2021) 'Comparative assessment of antioxidant activity and biochemical composition of four seaweeds , Rocky Bay of Abu Qir in Alexandria , Egypt', *Food Science and Technolog*, 2061(June), pp. 29–40.

[10] Fenoradosoa, T.A., Ali, G., Delattre, C., Laroche, C., *et al.* (2010) 'Extraction and characterization of an alginate from the brown seaweed Sargassum turbinarioides Grunow', *J Appl Phycol*, 22, pp. 131–137. doi:10.1007/s10811-009-9432-y.

[11] Fernando, Shanura, I.P., Jayawardena, T.U., Sanjeewa, K.K.A., *et al.* (2018) 'Ecotoxicology and Environmental Safety Anti-in fl ammatory potential of alginic acid from Sargassum horneri against urban aerosol-induced in fl ammatory responses in keratinocytes and macrophages', *Ecotoxicology and Environmental Safety*, 160, pp. 24–31. doi:10.1016/j.ecoenv.2018.05.024.

[12] Flórez-Fernández, N., Domínguez, H. and Torres, M.D. (2019) 'A green approach for alginate extraction from Sargassum muticum brown seaweed using ultrasound-assisted technique', *International Journal of Biological Macromolecules*, 124, pp. 451–459. doi:10.1016/j.ijbiomac.2018.11.232.

[13] Gerhardt, P., R.G.E, M., Wood, W.A. and Krieg, N.R. (1994) 'Total Carbohydrates Protocol', in *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology, pp. 518–522.

[14] Ginneken, V.J.T. Van, Helsper, J.P.F.G., Visser, W. De, Keulen, H. Van, *et al.* (2011) 'Polyunsaturated fatty acids in various macroalgal species from north Atlantic and tropical seas', *Lipids in Health and Disease*, 10(1), p. 104. doi:10.1186/1476-511X-10-104.

[15] Gómez-Ordóñez, E. and Rupérez, P. (2011) 'FTIR-ATR spectroscopy as a tool for polysaccharide identi fi cation in edible brown and red seaweeds', *Food hydrocolloids*, 25(6), pp. 1514–1520. doi:10.1016/j.foodhyd.2011.02.009.

[16] Guedes, É.A.C., da Silva, T.G., Aguiar, J.S., de Barros, L.D., *et al.* (2013) 'Cytotoxic activity of marine algae against cancerous cells', *Brazilian Journal of Pharmacognosy*, 23(4), pp. 668–673. doi:10.1590/S0102-695X2013005000060.

[17] Helmiyati and Aprilliza, M. (2017) 'Characterization and properties of sodium alginate from brown algae used as an ecofriendly superabsorbent', *Materials Science and Engineering*, 188, pp. 1–5. doi:10.1088/1742-6596/755/1/011001.

[18] Hifney, A.F., Fawzy, M.A., Abdel-gawad, K.M. and Gomaa, M. (2015) 'Industrial optimization of fucoidan extraction from Sargassum sp. and its potential antioxidant and emulsifying activities', *Food Hydrocolloids*, 9(22), pp. 1–42. doi:10.1016/j.foodhyd.2015.09.022.

[19] Kapuge, K., Sanjeewa, A., Priyan, I., Fernando, S., *et al.* (2017) 'Anti-inflammatory activity of a sulfated polysaccharide isolated from an enzymatic digest of brown seaweed Sargassum horneri in RAW 264 . 7 cells', *Nutrition Research and Practice*, 11(1), pp. 3–10.

[20] Khaled, N., Hiba, M. and Asma, C. (2012) 'Antioxidant and Antifungal activities of Padina Pavonica and Sargassum Vulgare from the Lebanese Mediterranean Coast', *Advances in Environmental Biology*, 6(1), pp. 42–48.

[21] Kok, J.M.L., Jee, J.M., Chew, L.Y. and Wong, C.L. (2016) 'The potential of the brown seaweed Sargassum polycystum against acne vulgaris', *Journal of Applied Phycology*, 28(5), pp. 3127–3133. doi:10.1007/s10811-016-0825-4.

[22] Kolsi, R.B.A., Salah, H. Ben, Jardak, N., Chaaben, R., *et al.* (2017) 'Sulphated polysaccharide isolated from Sargassum vulgare: Characterization and hypolipidemic effects', *Carbohydrate Polymers*, pp. 1–24. doi:10.1016/j.carbpol.2017.04.083.

[23] Kordjazi, M., Etemadian, Y., Shabanpour, B. and Pourashouri, P. (2018) 'Chemical composition antioxidant and antimicrobial activities of fucoidan extracted from two species of brown seaweeds (Sargassum ilicifolium and S . angustifolium) around Qeshm Island', *Iranian Journal of Fisheries Sciencesn*, pp. 1–19. doi:10.22092/IJFS.2018.115491.

[24] Laroche, C. and Michaud, P. (2009) 'A novel alginate from the brown seaweed Sargassum turbinaroides (Sargassae).', *Advances in Fermentation Technology*, pp. 72–92.

[25] Latifi, A.M., Nejad, E.S. and Babavalian, H. (2015) 'Comparison of extraction different methods of sodium alginate from brown alga Sargassum sp. Localized in the Southern of Iran', *J Appl Biotech*, 2(2), pp. 251–255.

[26] Lim, S., Choi, A., Kwon, M., Joung, E., *et al.* (2018) 'Evaluation of antioxidant activities of various solvent extract from Sargassum serratifolium and its major antioxidant components Department of Seafood and Aquaculture Science , Gyeongsang National University ', *Food Chemistry*, 11, pp. 1–30. doi:10.1016/j.foodchem.2018.11.058.

[27] Lim, S.J., Wan Aida, W.M., Maskat, M.Y., Latip, J., *et al.* (2016) 'Characterisation of fucoidan extracted from Malaysian Sargassum binderi', *Food Chemistry*, 209, pp. 267–273. doi:10.1016/j.foodchem.2016.04.058.

[28] Matloub, A.A. and Awad, N.E. (2012) 'Phycochemistry of some Sargassum spp . and their cytotoxic and antimicrobial activities', *Egypt Pharm J*, 11, pp. 99–108. doi:10.7123/01.EPJ.0000419800.62958.79.

[29] Minghou, J., Yujun, W., Zuhong, X. and Yucai, G. (1984) 'Studies on the M : G ratios in alginate', *Hydrobiologia*, 116/117, pp. 554–556.

[30] Mohsen, M., Mohamed, S., Ali, F. and El-Sayed, O. (2007) 'Chemical structure and antiviral activity of water-soluble sulfated polysaccharides from Sargassum latifolium', *J. Appl. Sci. Res*, 3(February 2015), pp. 1178–1185.

[31] Mythili, K., Umamaheswara Reddy, C., Chamundeeswari, D. and Manna, P.K. (2014) 'Determination of Total Phenol, Alkaloid, Flavonoid and Tannin in Different Extracts of Calanthe Triplicata.', *Journal of pharmacognosy and phytochemistry*, 2(4), pp. 40–44.

[32] Narayan, B., Miyashita, K. and Hosakawa, M. (2004) 'Comparative evaluation of fatty acid composition of different Sargassum (Fucales, Phaeophyta) species harvested from temperate and tropical waters', *Journal of Aquatic Food Product Technology*, 13(4), pp. 53–70. doi:10.1300/J030v13n04_05.

[33] Peng, Y., Xie, E., Zheng, K., Fredimoses, M., *et al.* (2013) 'Nutritional and chemical composition and antiviral activity of cultivated seaweed sargassum naozhouense Tseng et Lu', *Marine Drugs*, 11(1), pp. 20–32. doi:10.3390/md11010020.

[34] Pereira, H., Polo, C., Rešek, E. and Engelen, A. (2012) 'Polyunsaturated Fatty Acids of Marine Macroalgae : Potential for Nutritional and Pharmaceutical Applications', *Mar. Drugs*, 10, pp. 1920–1935. doi:10.3390/md10091920.

[35] Pereira, R.C., Barreto-bergter, E. and Starai, V.J. (2014) 'Glycolipids from seaweeds and their potential biotechnological applications', *Frontiers in Cellular and Infection Microbiology*, 4(174), pp. 1–5. doi:10.3389/fcimb.2014.00174.

[36] Pérez, R., MaTableosch, X., Llebaria, A., Balboa, M.A., *et al.* (2006) 'Blockade of arachidonic acid incorporation into phospholipids induces apoptosis in U937 promonocytic cells', *Journal of Lipid Research*, 47(3), pp. 484–491. doi:10.1194/jlr.M500397-JLR200.

[37] Permeh, P., Saeidnia, S., Mashinchian-Moradi, A. and Gohari, A.R. (2012) 'Sterols from Sargassum oligocystum, a brown algae from the Persian Gulf, and their bioactivity', *Natural Product Research*, 26(8), pp. 774–777. doi:10.1080/14786419.2010.548812.

[38] Ramu, S., Murali, A. and Jayaraman, A. (2019) 'Phytochemical screening and toxicological evaluation of sargassum wightii greville in wistar rats', *Turkish Journal of Pharmaceutical Sciences*, 16(4), pp. 466–475.

[39] Rhein-knudsen, N., Tutor, M., Ajalloueian, F. and Meyer, A.S. (2017) 'Characterization of alginates from Ghanaian seaweeds : Sargassum spp. and Padina spp.', *Food* brown *hydrocolloids*, 71, pp. 236–244. doi:10.1016/j.foodhyd.2017.05.016.

[40] Sabir, S.M., Hayat, I. and Ahmed, S.D. (2003) 'Estimation of Sterols in Edible Fats and Oils', *Pakistan Journal of Nutrition*, 2(3), pp. 178–181. doi:10.3923/pjn.2003.178.181.

[41] Shamsa, F., Monsef, H., Ghamooshi, R. and Verdian-rizi, M. (2008) 'Spectrophotometric determination of total alkaloids in some Iranian medicinal plants Abstract ':, *Thai J. Pharm. Sci. 32*, 32, pp. 17–20.

[42] Sinha, S., Astani, A., Ghosh, T., Schnitzler, P., *et al.* (2010) 'Polysaccharides from Sargassum tenerrimum: Structural features, chemical modification and anti-viral activity', *Phytochemistry*, 71(2–3), pp. 235–242. doi:10.1016/j.phytochem.2009.10.014.

[43] Thangaraj, P. (2016) *Pharmacological Assays of Plant- Based Natural Products,Progress in Drug Research 71*. Edited by K.D. Rainsford. Switzerland: Springer International Publishing. doi:DOI 10.1007/978-3-319-26811-8_32.

[44] Torres, M.R., Sousa, A.P.A., Silva Filho, E.A.T., Melo, D.F., *et al.* (2007) 'Extraction and physicochemical characterization of Sargassum vulgare alginate from Brazil', *Carbohydrate Research*, 342, pp. 2067–2074. doi:10.1016/j.carres.2007.05.022.

[45] W.Mackie (1971) 'Semi-quantitative estimation of the composition by infra-red spectroscopy of alginates Astbyy', *Carbohydiute research*, 20, pp. 413–415.

[46] Wagner, H. and Bladt, S. (1996) *Plant drug analysis: a thin layer chromatography atlas.* New York, Springer Science &Business Media.

[47] Wang, H., Ooi, E.V., Ang, P.O. and . (2008) 'Antiviral activities of extracts from Hong Kong seaweeds', *Journal of Zhejiang University: Science B*, 9(12), pp. 969–976. doi:10.1631/jzus.B0820154.

[48] Wang, H.D., Li, X., Lee, D. and Chang, J. (2017) 'Potential biomedical applications of marine algae', *Bioresource Technology*, 244(May), pp. 1407–1415. doi:10.1016/j.biortech.2017.05.198.

[49] Wang, L., Jeon, Y. and Kim, J. (2020) 'In vitro and in vivo anti-inflammatory activities of a sterol-enriched fraction from freshwater green alga , Spirogyra sp .', *Fisheries and Aquatic Sciences*, 9, pp. 23–27.

[50] Wang, P., Xu, G., Bian, L., Zhang, S., *et al.* (2006) 'Study on sterols from brown algae (Sargassum muticum)', *Chinese Science Bulletin*, 51(20), pp. 2520–2528. doi:10.1007/s11434-006-2124-y.

[51] Xiao, X., Yuan, Z. and Li, G. (2013) 'Preparation of phytosterols and phytol from edible marine algae by microwave-assisted extraction and high-speed counter-current chromatography', *Separation and Purification Technology*, 104, pp. 284–289. doi:10.1016/j.seppur.2012.11.032.

[52] Zhen, X.H., Quan, Y.C., Jiang, H.Y., Wen, Z.S., *et al.* (2015) 'Fucosterol, a sterol extracted from Sargassum fusiforme, shows antidepressant and anticonvulsant effects', *European Journal of Pharmacology*, 768, pp. 131–138. doi:10.1016/j.ejphar.2015.10.041

[53] Gloria, N.D.A. and Kweku, A.A. deGraft-J. (2016) 'Preliminary investigation into the chemical composition of the invasive brown seaweed Sargassum along the West Coast of Ghana', *African Journal of Biotechnology*, 15(39), pp. 2184–2191. doi:10.5897/ajb2015.15177.

[54] Yoshida, T. (1989) 'Taxonomy of Sargassum', *The korean Journal of Phycology*, 4(2), pp. 107–110.

[55] Mattio, L. and Payri, C.E. (2011) '190 years of sargassum taxonomy, facing the advent of DNA phylogenies', *Botanical Review*, 77(1), pp. 31–70. doi:10.1007/s12229-010-9060-x.

[56] Liu, L., Heinrich, M., Myers, S. and Dworjanyn, S.A. (2012) 'Towards a better understanding of medicinal uses of the brown seaweed Sargassum in Traditional Chinese Medicine: A phytochemical and pharmacological review', *Journal of Ethnopharmacology*, 142(3), pp. 591–619. doi:10.1016/j.jep.2012.05.046.