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Chemical composition and antioxidant activities of Jeddah corniche algae, Saudi Arabia

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KEYWORDS

Seaweeds;
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Abstract The increased use of natural product in the pharmaceutical industry has led to an increase in demand for screening for bioactive compounds in marine algae. An important economic algae, through chemical composition analysis and their antioxidant activities were investigated in this study. Chemical composition analysis of three algal samples from the Chlorophyta *Ulva lactuca* (U), Phaeophyta *Sargassum crassifolia* (S) and Rhodophyta *Digenea simplex* (D) was tested. Main components were sugars (57.40–185.13 mg/g dry weight), uronic acids (29.3–45.26 mg/g dry weight), sulfate (94.7–181.2 mg/g dry weight), amino acids (7.6–16.7 mg/g dry weight) and small amounts of betaines (2.38–8.47 mg/g dry weight). Hydrolyzed chemical composition analysis fractions of algal extract was shown a great proportion of sugars plus sulfate (as polysaccharide composed) ranges between 332 and 538.2 mg/g dry weight with trace amounts of uronic acids ($\leq 9\%$). All three algal extract showed antioxidant activities on lipoxygenase, DPPH and on Ames test. Two of aqueous extracts (U and D) inhibited lipoxygenase activity by less than 50%, where as the methanolic extract (S) caused 76% inhibition of the control. In all cases, the methanolic extract were more inhibitory than the aqueous extract. The (S) showed the highest antioxidant activity with DPPH (69%) in aqueous extract and in methanol extract with Ames test (85%). Both U and D showed antioxidant activity with DPPH in hexane by less of 25% where as in both aqueous and methanolic extracts by less than 50% of the control. Aqueous and methanolic extracts of U and D showed high inhibition by Ames test which caused 70% and 75% respectively. IR spectra of algal

Abbreviations: (U), *Ulva lactuca*; (S), *Sargassum crassifolia*; (D), *Digenea simplex*; DPPH, α -diphenyl- β -picrylhydrazyl; HPLC, high performance liquid chromatographic

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extracts (U; D and S) range from 1450 to 750 cm^{-1} were very similar absorption band at 1430, 1370, 1250, 1130, 1110, 1050 and 1020 cm^{-1} . Absorption bands were due to uronic acids, glucosides and sulfate. The presence of sulfated polysaccharide material in the fractions UF2, DF2 and SF2 were found as cell wall storage of marine algae, confirmed by ^{13}C NMR spectroscopy. It is concluded that the algal species probably have a different components and can be used in the activities of antioxidant enzymes as reduced the risks of enzymes. But the correlation between the chemical composition and antioxidant activities of algal extracts needs further investigation.

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1. Introduction

During the last years, many studies have been made on biological activities of the seaweed (Ehresmann et al., 1977), and could be potential rich sources of natural antioxidants (Matanjun et al., 2008). Traditionally, seaweeds have been used in the treatment of various infectious diseases (Hoppe, 1999), and reports of many active compounds have been isolated and their structure determined (Vairappan et al., 2001; Mundt et al., 2003). Among the features of marine algae and their substance, several extracts were screened on an antioxidant capability (Latham, 2008), and their inhibitory activity on lipoxygenase enzyme (Mori et al., 2003), as well as by radical scavenging activity, using a stable free radical (Matsukawa et al., 1997). Until now, however, no screening for antioxidant activities has been done with Jeddah corniche algae, even though the abundance and diversity of algae in the coastal waters of the Jeddah corniche are very high (Mutawie, 2006). We therefore decided to incorporate these studies into our test protocols to antioxidant activities of selected algae.

2. Materials and methods

2.1. Algal materials

Algal samples were collected along Jeddah corniche, about 22 km through the longitudinal direction of N–W. Samples were randomly selected at depths of about 20–100 cm and three different samples belong to different classes of algae were collected. Samples of algae were cleaned of barnacle, gastropod and other contaminants at the site then immediately transported to the laboratory in polyethylene container. Part of that they were air-dried at room temperature and other part were stored polyethylene plastic bags as fresh material in freezer until used.

2.2. Extract preparation

Dry algal material (100 g) was extracted with methanol (absolute) in a Soxhlet apparatus for 8 h according to the method of Blunden et al. (1981). The extract was concentrated under reduced pressure at 60 °C using rotary evaporator then filtered, washed with about 25 ml distilled water and stored in the dark at 4 °C according to the method of Nascimento et al. (1993).

2.3. Composition analysis

Specimens of extract samples were used for composition analysis, total sugar content was determined by modified method described by Hellebust and Craigie (1978). Hydrolyzed sugar

at 105 °C for 2.5 h were also determined with the same technique described by Adams (1965). Uronic acids assays were carried out using the method described by Blumenkrantz and Asboe-Hansen (1973). Amino acid assays were carried out using the method described by Matoh et al. (1980). Betaines estimation were carried out using HPLC assay according to the method of Gorham (1984). Sulfate content was determined according to the method of David and James (1979). Sulfate hydrolyzed with 1 M HCl was calculated and expressed as sulfate equivalents according to the method of Ruperez et al. (2002).

2.4. Infrared spectra

Extract samples were centrifuged at 3000 (rpm) for 30 min and after lyophilization, characterized by infrared spectra (FT-IR/NICOLET-ESPN670).

2.5. Fractionation

Fractionation of extracts by centrifugations yielded two fractions (F1 and F2). Fractions were extracted with MeOH-CHCl_3 and soluble fraction (F1) as a major containing fraction. The process was repeated twice and the combined supernatants were used. The residue was then sequentially extracted with a successive MeOH-CHCl_3 and the final volume was measured and noted as fraction (F2). Samples of each fractions from the three algal samples (F1 and F2) were tested for hydrolyzed chemical composition. Fraction (F2) was run on silica gel column chromatographed using chloroform and methanol as described by Vairappan et al. (2001), and after lyophilization, characterized by ^{13}H NMR according to the method of Pengzhan et al. (2003).

2.6. Lipoxygenase test

The assay for enzyme (lipoxygenase) activity was carried out as described by Matsukawa et al. (1997). The reaction mixture contained 0.2 M borate buffer pH 9.0, Tween 20, linolic acid, an enzyme solution (0.1 U/ml dissolved in ice cold borate buffer). The enzyme reaction was carried out in the cuvette and monitored at 234 nm using UV-visible recording spectrophotometer (160A-Shimadzu, Japan), The percentage inhibition was defined by the presence and absence of fraction material.

2.7. DPPH test

Radical scavenging activity of the fraction material was determined by the use of a stable free radical DPPH (α -diphenyl- β -picrylhydrazyl). The oxidation and decolorization of DPPH

was followed in absorbance at 540 nm. according to Matsukawa et al. (1997).

2.8. Ames test

Plate test consist of 100 µl of the fraction, 100 µl of bacterial strain (TA/104) and 100 µl H₂O₂ incubated for 30 min. Enzyme (0.5 ml) was added to the top agar (0.5 mM histidine/biotin solution). Mixture was transformed to the glucose plate and incubated at 37 °C for 48 h. Colonies were counted and the percentage inhibition was defined as described by Maron and Ames (1983).

3. Statistical analysis

The experimental data were analyzed for statistical significance between control and mean values of treated groups, using Origin 5.0 software. Values were presented as means (±) Standard Error (SE). Data were analyzed by Student's *t*-test. Differences with *P* < 0.05 were considered significant.

4. Results and discussion

4.1. Chemical analysis

The chemical compositions analysis of algal extract of three chosen species *Ulva lactuca* (U), *Digenea simplex* (D) and *Sargassum crassifolia* (S) from green, red and brown algae respectively were shown (Table 1). Total recovery (21.1–42.4%) of the algal dry weight corresponded to nondialyzable compounds, as free minerals and low-molecular-weight substances were removed during extraction and centrifugation process. The result was consistent with infrared analysis. Main and highest components were sugars (57.40–185.13 mg/g dry weight), uronic acids (29.3–45.26 mg/g dry weight), sulfate (94.7–181.2 mg/g dry weight), whereas amino acids had very low (7.6–16.7 mg/g dry weight) and small amounts of betaines (2.38–8.47 mg/g dry weight), as lowest content components in all algal extracts. Studies on chemical compositions from brown algae, showed their relatively high sulfate content (Haroun-Bouhedja et al., 2000).

4.2. Antioxidant activities

Antioxidant activities of algal extracts (Table 2) was estimated from their ability to inhibit lipoxygenase activity or to oxidized and decolorized the DPPH and to determine the inhibition of bacterial colonies as by Ames test (Maron and Ames, 1983). Two of aqueous (U and D) extracts and one of methanol (S)

extract inhibited lipoxygenase activity by less than 50% of the control. The most potent methanol extract was that of (S), which caused 76% inhibition. In all cases, the methanol extract (hexane not determined) were more inhibitory than the aqueous extract. The sequence of antioxidant activity as assayed by lipoxygenase inhibition by the methanol extract was as follows: (S) > (D) > (U). Of the seaweed extracts tested, from (S) showed the highest antioxidant activity with DPPH (69%) in aqueous extract and in methanol extract with Ames (85%) test (hexane not determined). The current literature reports that many different in vitro methods are being used to evaluate antioxidants of interest in many biological systems (Frankel and Meyer, 2000). In some of these protocols, samples were extracted with organic solvents (Yan et al., 1998) and in aqueous (Matsukawa et al., 1997); however, on these conditions one single test being used to evaluate inhibition A good efficiency in the vitro inhibition of LDL oxidation was reported by Jimenez-Escrig et al. (2001). Extracts (U and D) showed antioxidant activity with DPPH in hexane by less of 25% where as in both aqueous and methanol extracts by less than 50% of the control. Both aqueous and methanol extracts (U and D) were also examined by Ames test and showed high inhibition (70–75%). The antioxidant activity of brown was attributed to their phloroglucinol content (Ruperez et al., 2002). Our results are in agreement with Matsukawa et al. (1997), who found that the antioxidant activity of brown algae was superior to that of red or green groups. Antioxidant potential of sulfated polysaccharides from the brown algae was higher than that of agar-like sulfated galactans from the red algae (Ruperez, 2001). Fujimoto and Kaneda (1980) reported the chloroform-soluble phospholipids fraction of *Eisenia bicyclis* showed high antioxidant activity.

4.3. Fractions analysis

Fractions (F1 and F2) contained a high proportion of sulfate, sugars and quite low content of uronic acids in tested hydrolyzed fractions of the three algae. Hydrolyzed chemical composition analysis fractions of algal extract were shown in Table 3. F1 and F2 contained sugars, uronic acids and sulfate as the main constituent of all algae. The low level of soluble sugar (SF1) in (S) could be related to the high values of insoluble (SF2 – 123.8 mg/g dry weight) associated to lignin or dietary fiber of alga. A great proportion of three algae could be sugars plus sulfate (as polysaccharide composed) ranges (332–538.2 mg/g dry weight) with trace amounts of uronic acids (≤9%). The amounts and type of compounds separated after centrifugation procedure into different fractions would, of course, vary from algal yield. Fraction values obtained to be (14.93 g) in (SF1), (13.41 g) in (DF1) and (12.28 g) in (UF1).

Table 1 Chemical composition analysis of algal extract (mg/g dry weight).

Algal sample	Yield (%)	Sugar	Uronic acids	Sulfate	Betaines	Amino acid	Total recovery (%)
<i>Ulva lactuca</i> (U)	21.79 ± 0.6	68.40 ± 0.06*	29.3 ± 0.04*	94.7 ± 0.21*	8.47 ± 0.62	7.6 ± 0.1	21.1
<i>Digenea simplex</i> (D)	23.52 ± 0.8	185.13 ± 0.12*	45.26 ± 0.2*	173.1 ± 0.42	6.35 ± 0.32	14.30 ± 0.24	42.4
<i>Sargassum crassifolia</i> (S)	25.11 ± 1.2	57.40 ± 0.22	36.06 ± 0.04	181.2 ± 0.41*	2.38 ± 0.1	16.7 ± 0.22	29.4

Data are mean value of triplicate determinations (±) SD = standard deviation.

* Significant at *P* < 0.05.

Table 2 Antioxidant activities of algal extract.

Algal extract	(% Inhibition)								
	Aqueous			Methanol ^a			Hexane ^b		
	Lt	At	Dt	Lt	At	Dt	Lt	At	Dt
<i>Ulva lactuca</i> (U)	24	n.d.	34.9	39	70	30.2	n.d.	n.d.	18.7
<i>Digenea simplex</i> (D)	48	n.d.	42.3*	52	75	41.0	n.d.	n.d.	21.5
<i>Sargassum crassifolia</i> (S)	65*	n.d.	69.3*	76	85	49.7*	n.d.	n.d.	24.9*

Lt = lipoxigenase test; At = Ames test; Dt = DPPH test; n.d. = not determined.

^a Correlation between soluble extract in aqueous and methanol (% inhibition), $r = 0.802$, $P < 0.05$; and between aqueous and hexane.

^b $r = 0.70$, $P < 0.05$.

* Significant at $P < 0.05$.

Table 3 Hydrolyzed chemical composition analysis in fractions (F1 & F2) of algal extract (mg/g dry weight).

Fraction	Yield (g)	Sugar	Uronic acids	Sulfate
UF1	12.28	27.5 ± 0.26*	14.3 ± 0.13	39.4 ± 0.02
UF2	4.78	136.69 ± 0.04	14.8 ± 0.01	127.9 ± 0.01
DF1	13.41	75.32 ± 0.16	17.03 ± 0.2	58.02 ± 0.55
DF2	5.16	212.21 ± 0.01	24.43 ± 0.2	192.6 ± 0.26
SF1	14.93	21.7 ± 0.22	15.09 ± 0.21	45.07 ± 0.22
SF2	6.96	123.8 ± 0.03	20.4 ± 0.19	215.7 ± 0.31

Data are mean value of triplicate determinations (±) SD = standard deviation.

* Significant at $P < 0.05$. UF1 & UF2 = *Ulva lactuca*; DF1 & DF2 = *Digenea simplex*; SF1 & SF2 = *Sargassum crassifolia*.

This would mainly contain the majority of quaternary alkaloids and N-oxides (Blunden et al., 1982). On the other hand, fraction yield was found (6.96 g) in (SF2), (5.16 g) in (DF2) and (4.78 g) in (UF2). These fractions (F2) would mainly presented the polysaccharide as insoluble residue which contained may be small amounts of free sugar from cellulose (Pengzhan et al., 2003) with slight amount of alkaloids (Dragendorff-positive compounds) such as betaines (Blunden et al., 1982).

4.4. Infrared spectrum

IR spectra of algal extract from the three algal species (U; D and S) are shown in Fig. 1. Wavenumbers range from 1450 to 750 cm^{-1} and the extracts (U, D and S) were very similar absorption band (at 1430, 1370, 1250, 1130, 1110, 1050 and 1020 cm^{-1}). A large absorption band at 1430 cm^{-1} in with a small shoulder at 1450 cm^{-1} was due to uronic acids, in agreement with a higher uronic acids content (Ruperez et al., 2002). Two important bands were assigned at 1370 and 1050 cm^{-1} corresponding respectively to the stretching of C=O of uronic acids and the vibration of the C–O–C bridge of glucosides (Pengzhan et al., 2003). The absorption at 1230–1020 cm^{-1} was due to sulfate and attributed to stretching of C–O–S (Pantakar et al., 1993; Ruperez et al., 2002). The band close to 1250 cm^{-1} was quite similar to that previously used for calculating total ester sulfate content of carrageenan and agar (Melo et al., 2002).

4.5. NMR analysis

The ^{13}C NMR spectrum of (UF2, DF2 and SF2) from *Ulva lactuca*, *Digenea simplex* and *Sargassum crassifolia* respec-

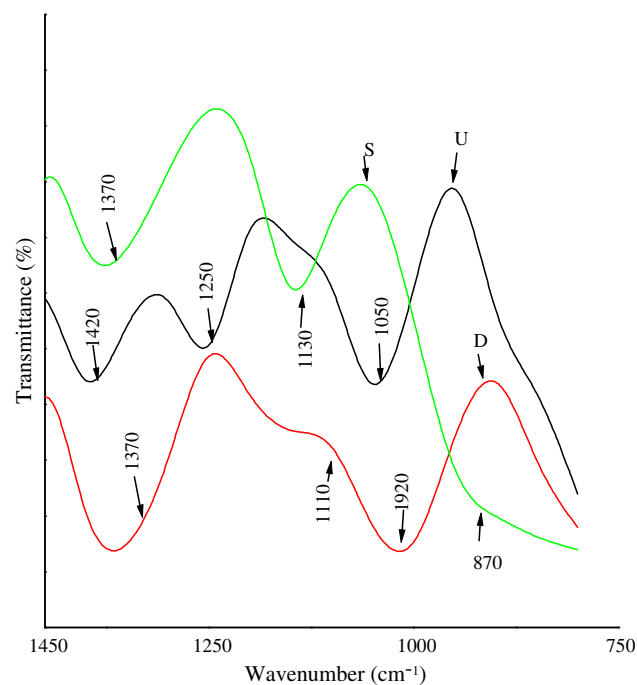


Figure 1 Infrared absorption spectrum of algal extracts from; *Ulva lactuca* (U); *Digenea simplex* (D); *Sargassum crassifolia* (S). Wavenumbers range from 1450 to 750 cm^{-1} .

tively are shown (Table 4). The signals assignment were done by comparison with the previously published data (Lahaye et al., 1998; Melo et al., 2002). The major signals type

Table 4 Chemical shift assignment for ^{13}C NMR spectra of main units from algal extract.

Residue unit	^{13}C Carbon chemical shift						References
	C1	C2	C3	C4	C5	C6	
G	102.4	70.2	82.2	68.8	75.3	61.4	Lahaye et al. (1998)
A	98.3	69.9	80.1	77.4	75.7	69.4	
G'	103.7	69.8	81.2	69.1	75.9	61.8	
L6S	101.3	69.3	71.1	79.1	70.3	67.9	Melo et al. (2002)
G	102.0	69.8	81.8	68.4	75.0	61.0	
A	98.0	69.4	79.7	77.0	75.2	69.3	
G6M-4S	102.2	70.1	80.2	71.4	70.1	68.6	
G'	103.1	69.9	81.8	69.0	75.5	61.3	
L6S	100.8	69.5	71.4	79.9	70.4	67.2	
*Fraction							*Observed spectra
UF2	100.1	72.5	81.1	71.4	71.1	70.3	
DF2	99.7	70.5	80.3	71.2	72.8	62.2	
SF2	99.8	71.3	79.1	78.4	74.3	69.3	

G = (103) linked β -D-galactose; A = (1-4) linked 3,6 anhydrogalactose; G6M-4S = 6-O-methyl-D-galactose-4 sulfate; G'-L6S = (1-3) β -D-galactose (1-4)- α -L-galactose-6-sulfate.

* UF2 = *Ulva lactuca*, DF2 = *Digenea simplex*, SF2 = *Sargassum crassifolia*.

G6M-4S (6-O-methyl-D-galactose-4 sulfate) was prominently observed in the spectrum from (UF2); G'-L6S (1-3) β -D-galactose (1-4)- α -L-galactose-6-sulfate was prominently observed in the spectrum from (DF2) and A as basic repeating structures of (1-4) linked 3,6 anhydrogalactose was prominently observed in the spectrum from (SF2). The major signals for algal fractions (UF2, DF2 and SF2) were observed in the spectrum corresponding to polysaccharides materials as expected, their cell wall storage of marine algae sulfated polysaccharides, as the same as those of other (Pengzhan et al., 2003). In summary, polysaccharides which are associated as expected with sulfate from algal extracts and especially (F2) which exhibited antioxidant potential. Nevertheless, at present, the mechanisms by which sulfated polysaccharides from the marine algae exert their antioxidant power are still unknown.

5. Conclusion

Marine algae exert their antioxidant power are still unknown. In this sense, it is of great interest to have available highly purified and well characterized sulfated polysaccharides with which to elucidate their mode of action. Algal species probably have a different components and can be used in the activities of antioxidant enzymes as reduced the risks of enzymes. But the correlation between the chemical composition and antioxidant activities of algal extracts needs further investigation.

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Chemical composition and antioxidant activities of Jeddah corniche algae, Saudi Arabia

المدخر الكيماوي وأنشطة مضادات الأكسدة في طحالب كورنيش جدة، المملكة العربية السعودية

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قسم الأحياء، كلية العلوم التطبيقية، جامعة أم القرى، مكة المكرمة، المملكة العربية السعودية¹
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ملخص البحث: مع ازدياد استخدام المنتجات الطبيعية في الصناعات الدوائية، فقد أصبح البحث ضرورة في الكشف عن مركبات ذات نشاط وفعالية في الطحالب البحرية. ويظهر الجانب الاقتصادي في الطحالب من خلال الكشف للمحتوى الكيميائي، إضافة إلى وجود ما يعزز ذلك، من خلال فاعليتها كمضادات للأكسدة. ووفقاً للظروف التجريبية المهنية للقياس، أظهرت الدراسة تباين في التحليل الكمي الكيميائي للعينات المقدر والممتلئة لطحلب الأولفا من (الطحالب الخضراء) والسرجاسم من (الطحالب البنية) والديجينا من (الطحالب الحمراء)، حيث احتلت السكريات الكلية ما بين (57.40 - 185.13 مليجرام/جم وزن جاف) وحمض اليورونيك (29.3-45.26 مليجرام/جم وزن جاف) والكبريتات (94.7- 181.2 مليجرام/جم وزن جاف) والأحماض الأمينية (7.6-16.7 مليجرام /جم وزن جاف)، إلى جانب ذلك تمثل قدراً يسيراً من البيتين تراوح ما بين (2.38 – 8.47 مليجرام /جم وزن جاف)، في جميع عينات الطحالب المختارة في الدراسة. كما أظهرت نتائج التحليل الكمي الكيميائي للمركبات لما بعد عملية التحلل، حيث شكل كل من السكريات الكبريتات قدراً توافيقاً في اتجاه الزيادة في المحتوى وبدرجة أكبر عن محتوى ما قبل التحلل تراوح ما بين (332 - 538.2 مليجرام /جم وزن جاف)، في حين احتل حمض اليورونيك أدنى نسبة تواجد ($\geq 9\%$). كما بينت جميع المستخلصات المعدة أنشطة إيجابية كمضادات للأكسدة على مجموعة الإختبارات الكاشفة ومنها: الليوأوكسجنيز و الـ دي بي بي اتش وأختبار أميس. إلى جانب ذلك أوضح مؤشر الفعالية للمسخلص المائي لكل من طحلب الأولفا والديجينا نشاطاً لا يقل عن مقدار (50 %)، بينما أظهر المستخلص الكحولي لطحلب السرجاسم نشاطاً مماثلاً لا يقل عن مقدار (76%) عن مستوى الضابط القياسي. في حين تبين من نتائج أختبارات المسخلص المائي والكحولي لطحلب السرجاسم قدرته الإيجابية العالية لإختزال الـ دي بي بي اتش (69%) وأختبار أميس (85%). كما أظهر كل من مستخلص محلول الهكسان لطحلب الأولفا والديجينا إيجابية لإختزال الـ دي بي بي اتش بمقدار أقل من (25%) بالنسبة للمجموعة القياسية، بينما أظهرت المستخلصات المائية والكحولية لنفس الطحلبين مقدار أقل من (50%) بالنسبة للمجموعة القياسية. ومن خلال حساب معدل التثبيط على أختبار أميس بفاعلية المستخلص المائي والكحولي لطحلب الأولفا والديجينا تبين بلوغ النسبة ما بين 70 – 75% على التوالي. كما أظهرت الإختبارات الأولية بإستخدام أطياف الأشعة فوق الحمراء قدرتها على الإمتصاص في المناطق الطيفية الواقعة (1450 – 750) لكل من الطحالب المختارة في الدراسة. وقد أكدت النتائج عن وجود تقارب في أطوال الأمتصاص (1430، 1370، 1250، 1050، 1110، 1130، 1020 سم⁻¹). وفي السياق التكميلي لمجريات التحاليل الكاشفة أظهرت النتائج إحتماية وجود وبصورة عامة في مجمل التقديرات، حمض اليورونيك والجليكوسيدات والكبريتات في المواقع الممتصة. كما يعزز التوافق الكبير للمسح الرنيني المغناطيسي، التأكيد بجلاء في غالب الأمر وجود كمياً من عديدات السكريات الكبريتية وبشكل عام في معظم المستخلصات المعزولة كمخزون مدخر في جدر الطحالب البحرية. وخلاصة القول أنه وبالرغم من تعددية أجناس الطحالب إلا أنها تحتوي على مركبات متفاوتة وتؤدي نفس الفاعلية في تضاد إنزيمات الأكسدة أو ربما تقلل من معدل الخطورة، في حين قد نجد أن معدل العلاقة التوافقية بين المدخر الكيميائي وكنشاط مضاد للأكسدة لازال يحتاج إلى دراسات مستفيضة.