مجلة القُلزم العلمية

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موجهات النشر

تعريف المجلة:

مجلة (القُلزم) للدراسات العلمية مجلة علمية محُكمة تصدر عن مركز بحوث ودراسات دول حوض البحر الأحمر - السودان، بالشراكة مع أكاديمية المنهل للعلوم - السودان. تهتم المجلة بالبحوث والدراسات العلمية والمواضيع ذات الصلة بدول حوض البحر الأحمر.

موجهات المجلة:

1. يجب أن يتسم البحث بالجودة والأصالة، وألا يكون قد سبق نشره قبل ذلك.
2. على الباحث أن يقدم بحثه من نسختين. وأن يكون بخط (Traditional Arabic) بحجم 14 على أن تكون الجداول مرقمة وفي نهاية البحث وقبل المراجع على أن يشار إلى رقم الجدول بين قوسين دائريين ().
3. يجب ترقيم جميع الصفحات تسلسلياً وبالأرقام العربية بما في ذلك الجداول والأشكال التي تلحق بالبحث.
4. المصادر والمراجع الحديثة يستخدم أسم المؤلف، اسم الكتاب، رقم الطبعة، مكان الطبع، تاريخ الطبع، رقم الصفحة.
5. المصادر الأجنبية يستخدم اسم العائلة (Hill, R).
6. يجب ألا يزيد البحث عن 30 صفحة، وبالإمكان كتابته باللغة العربية أو الإنجليزية.
7. يجب أن يكون هناك مستخلص لكل بحث باللغتين العربية والإنجليزية على ألا يزيد على 200 كلمة بالنسبة للغة الإنجليزية. أما بالنسبة للغة العربية فيجب أن يكون المستخلص وافياً للبحث بما في ذلك طريقة البحث والنتائج والاستنتاجات، مما يساعد القارئ العربي على استيعاب موضوع البحث وبما لا يزيد عن 300 كلمة.
8. لا تلزم هيئة تحرير المجلة بإعادة الأوراق التي لم يتم قبولها للنشر.
9. على الباحث إرفاق عنوانه كاملاً مع الورقة المقدمة (الاسم رباعي، مكان العمل، الهاتف، البريد الإلكتروني).

نأمل قراءة شروط النشر قبل الشروع في إعداد الورقة العلمية.

بسم الله الرحمن الرحيم

كلمة التحرير

الحمد لله رب العالمين، والصلاة والسلام على سيدنا محمد وعلى آله وصحبه أجمعين

وبعد:

القارئ الكريم ،،،

السلام عليك ورحمة الله وبركاته.. نطل على حضراتكم من نافذة جديدة من نوافذ النشر العلمي وهي مجلة القلزم العلمية، ونحن في غاية السعادة والمجلة تصل عددها الثامن والثلاثون بفضل الله تعالى ومنته.

القارئ الكريم:

هذه المجلة تصدر بالشراكة مع أكاديمية المنهل للعلوم وهي إحدى الأكاديميات السودانية الفنية التي وضعت بصمات مميزة في مسيرة البحث العلمي، وهذا العدد هو الثامن والثلاثون في إطار هذه الشراكة العلمية التي تأتي في إطار استراتيجية مركز بحوث ودراسات دول حوض البحر الأحمر في تفعيل الحراك العلمي والبحث داخل السودان وخارجه..

القارئ الكريم:

هذا العدد يشتمل على عدد من البحوث والدراسات المهمة ذات البعد النظري والتطبيقي ولضمان نجاح واستمرارية هذه المجلة بإذن الله تعالى نأمل أن يرفدنا الباحثون بمزيد من اسهاماتهم العلمية المميزة مع خالص الشكر والتقدير للجميع..

أسرة التحرير

المحتويات

Characterization of Phytochemical Constituents and Evaluation of Biological Activities of Enteromorpha intestinalis from Red Sea, Sudan

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Abstract:

Seaweeds and their extracts have attracted great interest in the pharmaceutical industry as a source of bioactive compounds. Studies have demonstrated the cytotoxic activity of macroalgae towards different types of cancer cell models, and their consumption has been suggested as a chemo-preventive agent against several cancers, such as breast, cervix, and colon cancers. The main objectives of this investigation are characterized some phytochemical constituents and evaluated some biological activities of *Enteromorpha Intestinalis* from the Red Sea, Eastern Sudan. The results of the phytochemical screening of E. *Intestinalis* revealed the presence of alkaloids, saponins, tannins, cardiac glycosides, phytosterols, terpenoids, flavonoids, and bitter principles. The main identified constituents in the pet. ether extract, analyzed by GC/MS, was the diterpene phytol (29.16%), followed by palmitic acid methyl ester (25.45%) and fucosterol (18.42%). *E. Intestinalis* was investigated to evaluate its antibacterial activity against two strains of gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two strains of gram-

negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and one fungal strain (*Candida albicans*) using the cup-plate method. *E. Intestinalis* revealed considerable activity towards both gram-positive, gram-negative bacteria or tested concentrations up to 40 µg/ml. It also exhibited a pronounced activity, against the *C. albicans*. The antioxidant activity which was detected using the DPPH radical scavenging method was found to be low (20±0.01). the detection of minerals content of E. *intestinalis* by using inductively coupled plasma emission spectrometry (ICPES) revealed the presence of microelements like K, Na, and S, as well as one trace element, Si. Proximate analysis of *E. Intestinalis* indicated that it contained moisture (7.731%), protein (16.625%), lipids/fats (0.864%), ash (31.072%), and crude fiber (5.710%).

توصيف المكونات الكيميائية النباتية وتقييم الأنشطة البيولوجية لطحلب الانترومورفا اينتستينالاس في البحر الأحمر، السودان

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المستخلص:

جذبت الأعشاب البحرية مكوناتها اهتماما كبيرا في مجال الأدوية الصناعة كمصدر للمركبات النشطة بيولوجيا. وقد أظهرت هذه الدراسة النشاط السام للخلايا للطحالبية تجاه أنواع مختلفة من نماذج

الخلايا السرطانية، وتم تجربته كعامل وقائي كيميائي ضد العديد من أنواع السرطان مثل سرطان الثدي وعنق الرحم والقولون. الأهداف الاساسية لهذة الدراسة في التعرف علي بعض المكونات الكيميائية النباتية وتقييم بعض الأنشطة البيولوجية لطحلب الانترومورفا اينتستينالاس من البحر الأحمر، شرق السودان. كشفت نتائج التحليل الكيميائي لطحلب الانترومورفا عن وجود قلويدات، وصابونين ، وعفص ، وجليكوسيدات قلبية ، وفيتوستيرول ، وتيربينويدات ، وفلافونويد ، ومرير. وتم استخراج الأثير، وتحليلها بستخدام طريقة GC/MS ، واظهرت النتائج ان فيتول ديتيربين (29.16٪) ، يليها حمض بالميتيك ميثيل استر (25.45٪) ثم فوكوستيرول (18.42٪). تم اجراء تحليل وفحص طحلب الانترومورفا لتقييم نشاطها المضاد للبكتيريا ضد سلالتين من البكتيريا موجبة الجرام (المكورات العنقودية الذهبية والعصية الرقيقة) وسلالتين من البكتيريا سالبة الجرام (الإشريكية القولونية والزائفة الزنجارية) وسلالة فطرية واحدة (المبيضات) باستخدام طريقة لوحة الكأس. كشف طحلب الانترومورفا عن نشاط كبير تجاه كل من البكتيريا موجبة الجرام أو سلبية الجرام أو التركيزات المختبرة بتركيز يصل إلى 40 جم/مل. كما أظهر نشاطا واضحا ضد C. albicans. تم العثور على النشاط المضاد للأكسدة الذي تم الكشف عنه باستخدام طريقة DPPH لتكون منخفضة (-0.01 0.02). تم تحليل محتوى المعادن فى طحلب الانترومورفا باستخدام جهاز طيف الانبعاثات البلازمي كشفت عن وجود عناصر مثل الكالسيوم، البوتاسيوم، والكبريت، والسيليكا. تحليل تقريبي لطحلب الانترومورفا أشارت إلى أنها تحتوي على رطوبة (7.731٪)، بروتين (16.625٪) ، دهون/دهون (0.864٪) ، رماد (31.072٪) وألياف خام (5.710٪).

Introduction:

Algae are mostly aquatic and grow in various types of waters. Seaweed polyphenols, known as phlorotannin's, possess antioxidant activity, and polyphenols extracted from brown and red seaweeds have shown antioxidant activity (Abdul et al., 2016). Seaweeds in general are used as vermifuges and antiscorbutic, besides curing cough, stomach, chest, bladder, and kidney ailments (Boney, 1965).

Red Sea seaweeds possess algal proteins called phycobiliproteins that have antioxidant properties, which could be beneficial in the prevention or treatment of neurodegenerative diseases caused by oxidative stress (Alzheimer's and Parkinson's) as well as in the treatment of gastric ulcers and cancers. They also contain polyunsaturated fatty acids (Omega 3 and Omega 6 fatty acids), which are played important role in the prevention of cardiovascular diseases,

osteoarthritis, and diabetes. Besides, seaweeds are an excellent source of known vitamins such as A, B, especially B_{12}, C, D, E, and Vitamin K, as well as essential amino acids (Drum, 2021). Seaweed polyphenols, known as phlorotannin's, possess antioxidant activity, and polyphenols extracted from brown and red seaweeds have shown antioxidant activity (Abdul et al., 2016).

Entromorpha intestinalis is a green alga in the family Ulvaceae, known by the common names sea lettuce, gutweed and grass kelp (Guiry, 2007). *E. intestinalis* is a conspicuous bright grass-green seaweed (Amsler andSearles, 1980). *E. intestinalis* occurs in a wide range of habitats on all levels of the shore, where suitable support is available; it will grow on rocks, mud, sand, and in rock pools. It is abundant in brackish water areas, where there is appreciable freshwater run-off, and in wet areas of the splash zone. It is a common epiphyte on other algae and shells. The seaweed may become detached from the substratum and buoyed up by gas, rise to the surface, where it continues to grow in floating masses (Blomster et al., 2002).

This investigation are important to shown characterize phytochemical constituents and the evaluate biological activities of *Enteromorpha intestinalis* from the Red Sea, Eastern Sudan.

Materials and Methods:

3.1 Materials:

3.1.1 Plant material:

The sample of marine algae *Enteromorpha intestinalis* was collected and provided by the Department of Biological Oceanography, Faculty of Marine Sciences, Red Sea University, and authenticated by Dr. Awatif Sugga, Department of Biological Oceanography.

3.1.1.1 Sample preparation:

The foreign matter was removed from the sample, and the dried sample was powdered using a mortar and pestle.

3.1.2 Test microbial strains:

The microbial strain was provided by the National Research Centre, Khartoum. They are: *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans.*

3.2 Methods:

3.2.1 Extract preparation:

25 grams of the powdered *E. intestinalis* were extracted successively with light petroleum ether (b. p. 40–60 °C) and 70 % ethanol, respectively, by using a Soxhlet extraction apparatus in a water bath, for six hours for each solvent, then each solvent was evaporated. The extract yield for each solvent was calculated based on the original sample weight.

3.2.2 Phytochemical screening:

3.2.2.1 Extract preparation:

Five grams of the powdered material were extracted by boiling under reflux with 50 ml of 70% ethanol in a water bath for 30 minutes and filtered. The solvent was then evaporated.

3.2.2.2 Phytochemical screening tests:

Phytochemical screenings were conducted to detect the presence of phytochemicals in the mangrove gum ethanolic extract according to the methods described by (Roy, 2016).

3.2.3 GC-MS analysis method of analysis:

3.2.3.1 Sample Preparation:

2 ml of the sample was thoroughly mixed with 7 ml of alcoholic sodium hydroxide (Noah) that was prepared by dissolving 2 g in 100 ml of methanol. 7 ml of alcoholic sulfuric acid (1 ml H_2SO_4 to 100 ml methanol) was then added. The mixture was shaken for 5 minutes. The contents of the test tube were left to stand overnight. 1 ml of supersaturated sodium chloride (NaCl) was then added, and the contents were shaken. 2 ml of normal hexane was added, and the contents were shaken thoroughly for three minutes. Then the n-hexane layer (the upper layer of the test tube) was taken using a disposable syringe. 5 µl of the n-hexane extract was diluted with 5 ml of diethyl ether. Then the mixture was filtered through a 0.45 µm syringe filter and dried with 1 g of anhydrous sodium sulfate as a drying agent, and 1 µl of the diluted sample was injected into GC.MS instrument.

3.2.3.2 GC/MS Conditions:

The qualitative and quantitative analysis of the sample was carried out using GM/MS technique model (GC/MS-QP2010-Ultra) from Japan's 'Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was injected using split mode, with helium as the carrier gas and a flow rate of 1.61 ml/min. The temperature program was started from 60 °C with a rate of 10 °C/min to 300 °C as the final temperature degree, with a 5-minute hold time. The injection port temperature was 300 °C, the ion source temperature was 200 °C and the interface temperature was 250 °C. The sample was analyzed by using scan mode in the range of m/z 40–500 charges to ratio and the total run time was 29 minutes. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST), results were recorded.

3.2.4 Proximate analysis:

Proximate analysis procedures were determined by the association of official analytical chemists methods (Horwitz andInternational, 2002).

1. Moisture content determination:

The aluminum dish was placed in a drying oven at 105°C for 2 hours. After that, the crucible was placed in the desiccators to allow cooling. The aluminum dish was weighed, and 2 g of the powder was placed in the aluminum dish. The sample was dried in a drying oven for 3 h at 105°C, and then weighed to determine the percentage of dry weight and the percentage of moisture content.

2. Ash content determination:

Two grams of sample were placed in a crucible, the weight recorded, and placed in a muffle oven at 550°C for 8 h.

3. Fat content determination:

The fat content was determined by directly extracting the sample with petroleum ether in a Soxhlet extractor for 4 h. The residue in the round bottom flask after solvent removal represents the fat content of the sample.

4. The crude protein content determination:

The crude protein content of the samples was estimated by the macro-Kjeldahl method, in which the sample was digested with a known quantity of acid. The digested material was distilled after the addition of alkali. The released ammonia was collected in 4% boric acid. The resultant boric acid, which now contained the ammonia released was then titrated against 0.1 N, HCl. The percentages of nitrogen were converted to protein by multiplying by 6.25.

5. Crude fiber content determination:

Two grams of sample were put into a 250mL conical flask, and 1.25% sulfuric acid solution was added. The sample was heated for about 30 min, filtered, and washed until traces of acid could not be detected using pH paper. The Whatman paper 5B with a 125micrometer pore size was placed in the Buchner flask. The acid extracted was transferred into a 250 mL conical flask, and 1.25% NaOH solution was added subsequently. The sample was heated again for 30 min, filtered using a vacuum filter, and washed with water until the base was undetected. The whole material was transferred into a crucible and dried for 12 h at 120°C. After that, the crucible was placed in a muffle oven at 550°C for 12 h and the weight of crucible was recorded.

3.2.5 Mineral content determination:

Mineral content was determined by analyzing the ash obtained from 1g of the sample using inductively coupled plasma emission spectroscopy (ICPES).

3.2.6 Antimicrobial Activity Test:

3.2.4.1 Preparation of the test organisms:

3.2.4.1.1 Preparation of bacterial suspensions:

One ml aliquots of a 24-hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37 ºC for 24-hours. The bacterial growth was harvested and washed off with 100 ml of sterile normal saline to produce a suspension containing about 10^8-10^9 C.F.U/ml. The suspension was stored in the refrigerator at 4 °C until used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Bajpai et al., 2008). Serial dilutions of the stock suspension were made in sterile normal saline solution, and 0.02 ml volumes of the appropriate dilution were

transferred by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry, and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colonies forming units per ml suspension.

Each time, a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

3.2.4.1.2 Preparation of fungal suspension:

The fungal cultures were maintained on sabouraud dextrose agar (SDA), incubated at 25 °C for 4 days. The fungal growth was harvested, and washed with sterile normal saline, and finally suspended in 100ml of sterile normal saline. The suspension was stored in the refrigerator until used.

3.2.4.2 Testing of antibacterial susceptibility (Disc diffusion method):

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts, and performed using a mueller hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (Kiehlbauch et al., 2000). Bacterial suspension was diluted with sterile physiological solution to 10^8 cfu/ml (turbidity= McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on the surface of MHA, and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No. 1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extract. The inoculated plates were

incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured (Kiehlbauch et al., 2000).

1- The antibacterial activity results were expressed in terms of the diameter of the zone of inhibition: <9mm was considered inactive, 9–12 mm as partially active, 13–18 mm as active, and >18mm as very active (Amsler andSearles, 1980).

2- The results were expressed in terms of the diameter of the inhibition zone: < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active (Alves et al., 2000).

3.2.7 Antioxidant activity (DPPH radical scavenging assay):

The DPPH radical scavenging was determined according to the method of (Shimada et al., 1992). with some modification. In 96-well plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C°C. The concentration of DPPH was kept at (300µ μm). The test samples were dissolved in DMSO, while DPPH was prepared in ethanol. After incubation, the decrease in absorbance was measured at 517nm using a multiplate reader spectrophotometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated control group. All tests and analyses were run in triplicate (Shimada et al., 1992).

4.Results and Discussion:

4.1 Extractable matter content:

Upon successive extraction of the *E. intestinalis* with petroleum ether and ethanol, the yield was found to be (0.812%) and (43.6%) for the solvents, respectively.

4.2 Phytochemical screening:

Phytochemical screening of the *E. intestinalis* crude ethanolic extract to detect the presence of the different secondary metabolites is shown in Table 1.

Table 1. Phytochemical screening of the E. intestinalis ethanolic extract

Test	Observation	Result
Test for alkaloids Mayer's reagent Dragendoroff's reagent Wagner's reagent	Precipitate formation	Presence of alkaloids
Test for saponin Foam test	Persistent foam Development	Presence of Saponins
Test for phenols (tannins) Ferric chloride test	Blackish-green color formation	Presence of phenolic compounds
Test for anthraquinone (Borntrager's test)	No red or pink color formed	Anthraquinones were not present
Test for flavanoids Potassium hydroxide test	Yellow color development	Flavanoids were not present
Test of cardiac glycoside Killer killiane test	Reddish brown ring formed at the interphase	Presences of cardiac glycosides
Test for steroids Liebermann's test	Red violet ring formed at the interphase	Presences of Steroids
Test for bitter principles Potassium hydroxide test	Red color development	Presence of bitter principles

The phytochemical screening results of *E. intestinalis* from the Red Sea in Eastern Sudan resemble those obtained from screening *E. intestinalis* from India in the presence of flavonoids, alkaloids, steroids, saponins, and phenols (Shankhadarwar, 2015). Also, this result is in agreement with the (Roy, 2016) who found that the phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, glycosides, saponins, and cardiac glycosides.

4.3 GC-MS analysis of the pet. ether extract:

The gas chromatogram of the pet. ether extract of *E. intestinalis* showed the detection of 15 compounds (Figure 2). Their identification is based on their mass spectra. The identified compounds with their percentages are shown in Table 2.

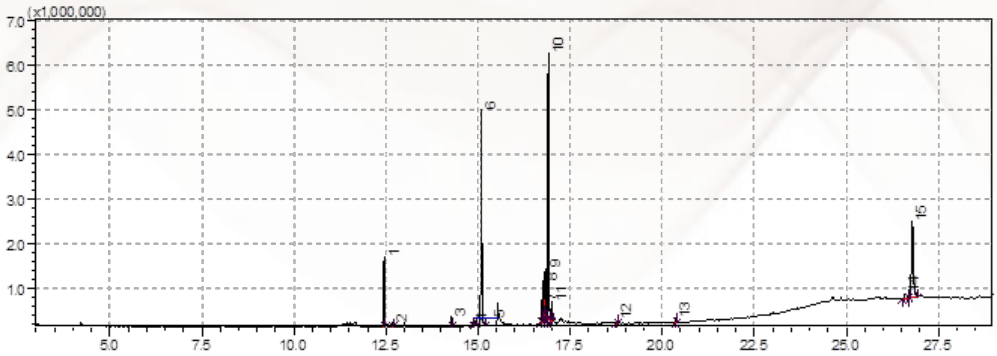


Figure 2. The gas chromatogram of pet. ether extract

Table 2. Petroleum ether extract composition Shahdara of E. intestinalis

ID#	Name	Ret. Time	Area	Area%
1	8-Heptadecene	12.450	2496989	7.15
2	Heptadecane	12.666	171452	0.49
3	Hexahydrofarnesyl acetone	14.277	384754	1.10
4	7-Hexadecenoic acid, methyl ester, (Z)-	14.849	153459	0.44
5	Palmitoleic acid (9-Hexadecenoic acid) methyl ester	14.893	332192	0.95
6	Palmitic acid (Hexadecanoic acid), methyl ester	15.086	8885046	25.45
7	Linoleic acid (9,12-octadecadienoic acid (Z, Z), methyl ester)	16.732	769761	2.20
8	10-Octadecenoic acid (Z)-methyl ester	16.775	1268042	3.63
9	11-Octadecenoic acid, methyl ester	16.825	2116398	6.06
10	Phytol	16.906	10179889	29.16
11	Methyl stearate . Stearic acid, methyl ester	16.998	820938	2.35

12	Arachidic acid (Eicosanoic acid), methyl ester	18.755	135889	0.39
13	Behenic acid (Docosanoic acid), methyl ester	20.371	227743	0.65
14	γ-Sitosterol (Stigmast-5-en-3-ol, (3α)-)	26.592	545206	1.56
15	Fucosterol (Stigmasta-5,24(28)-dien-3α-ol, (E)-)	26.791	6430284	18.42

The main identified constituents in the pet. ether extracts were the diterpene phytol (29.16%), followed by palmitic acid methyl ester (25.45%) and fucosterol (18.42%).

In medicinal fields, phytol has shown antioxidant activity (Santos *et al*., 3013) as well as anti-inflammatory and antiallergic effects (Ryu et a*l*., 2011). Recent studies have revealed that phytol is an excellent immunostimulant, superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity (Lim et al., 2006).

Fucosterol, a phytosterol in marine algae, was found to increase in the anti-oxidant enzymes such as hepatic cytosolic superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-px) activities of 33.89, 21.56, and 39.24%, respectively, in CCI4-intoxicated rats. These results suggest that fucosterol possesses not only anti-oxidant, but also hepatoprotective activities in rats (Lee et al.,2003).

Palmitic acid (16:0) is the most common saturated fatty acid found in the human body and can be provided in the diet or synthesized endogenously from other fatty acids, carbohydrates, and amino acids. Palmitic acid represents 20–30% of total fatty acids in membrane phospholipids, and adipose triacylglycerols (Carta et al., 2015).

Palmitic acid is a major component of palm oil (44% of total fats), but significant amounts of PA can also be found in meat and dairy products (50–60% of total fats), as well as cocoa butter (26%) and olive oil (8–20%) (Innis, 2016).

Other saturated fatty acids detected were stearic acid (18:0) (2.35%), arachidic acid (20:0) (0.39%), and behenic acid (22:0) (0.65%).

Linoleic acid (18:2 n-6) which is the parent compound of the omega-6 family among the polyunsaturated fatty acids, was detected in the amount of 2.20%.

On the other hand, linoleic acid, a surfactant agent, has become popular in the beauty products industry because of its beneficial properties for the skin. It has anti-inflammatory, acne-reductive, and moisture-retentive properties when applied topically to skin. (Letawe et al., 1998). Another unsaturated fatty acid detected in trace amounts is palmitoleic acid (16:1 n-9) (0.95%). Pet. ether extract also showed the presence of alkane hydrocarbons in heptadecane isomers (7.54%).

4.4 Proximate analysis:

Proximate analysis of *E. intestinalis* is shown in Table 3.

Table 3. *P*roximate Composition of *E. intestinalis*

Chemical Composition	Percentage composition
Moisture	7.731%
Ash	31.072%
Protein	16.625%
Fat	0.864%
Fiber	5.710%
Carbohydrates	37.998%
Total:	100%

The nutritional composition of E. intestinalis in the present study, from the red sea, resembles those in E. intestinalis from Southern Thailand and Mexico in the content of protein, ash, and differs in crude fiber content, which is

lower in E. intestinalis from the Red Sea in Sudan than those from Thailand and Mexico, in which protein (14.6–19.5% DW), lipid (2.1–8.7% DW), ash (25.9–28.6% DW), soluble fiber (25.3–39.6% DW), insoluble fiber (21.8–33.5% DW), and total dietary fiber (51.3–62.2% DW) (Aguilera-Morales et al., 2005) (Benjama andPayap, 2011).

4.5 Mineral content:

The detection of minerals content of *E. intestinalis* by (ICPES) revealed the presence of macroelements like K, Na, and S. Table 4.

Table 4. Elemental composition of *E. Intestinalis*

Elements detected	Concentration
Rb	ppm 7.8
K	4.8ppb
Na	160ppb
S	100ppb
Si	159ppb

E. intestinalis has macroelements like ***K, Na,*** and ***S***, also has one trace element, ***Si,*** as shown in the table above. This result is in agreement to those of (Benjama andPayap, 2011) who found that *U. intestinalis* was rich in Mg, K, Cl, Na, and Ca.

4.6 Antimicrobial activity screening:

The crude alcoholic extract of *E. intestinalis* was investigated to evaluate its antibacterial activity against two strains of gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two strains of gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*) and one fungal strain (*Candida albicans*) using the cup-plate method. The evaluation

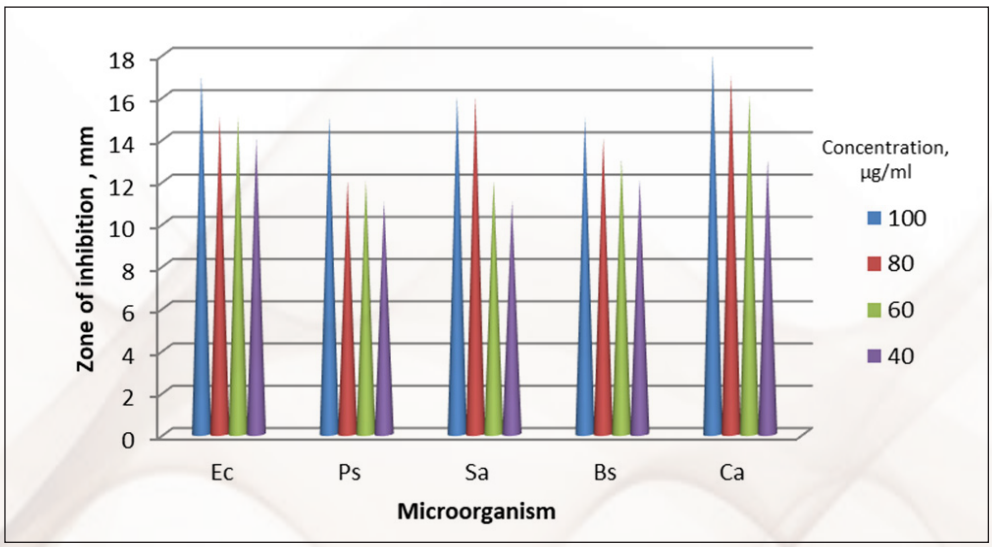
of the activity of the extract against the different strains is recorded in Table 5. and illustrated in Figure 3.

Table 5. Antimicrobial activity of E. intestinalis ethanolic extract

Concentration, µg/ml	Zone of inhibition, mm				
	E. coli	P. aeruginosa	S. aureus	B. subtilis,	C. albicans
100	17	15	16	15	18
80	15	12	16	14	17
60	15	12	12	13	16
40	14	11	11	12	13

The antibacterial activity (NCCLS, 1990):

- <9mm zone is considered inactive.
- 9–12 mm is considered partially active.
- 13–18 mm is considered active.
- >18mm is considered very active.



From the histogram in Figure 3, it could be noticed that, *E. intestinalis* revealed considerable activity towards both gram-positive, gram-negative bacteria or tested concentrations. It also exhibited a pronounced activity against *C. albicans*.

The activity of the ethanolic extract may be due to the presence of certain secondary metabolites, e.g.: phenolic compounds in the extract (both tannins and flavonoids), saponins, and fatty acids.

Phenolic compounds are the main antimicrobial agents in plants (Tiwari et al., 2009). Even though the exact antimicrobial mechanism of phenolic compounds is not clear, phenolic compounds are commonly known for their antimicrobial effects (Tiwari et al., 2009) (Ciocan and Ioan, 2009). The ability of phenolic compounds to alter microbial cell permeability, thereby permitting the loss of macromolecules from the cell interior, could help explain some of the antimicrobial activity. Another explanation might be that phenolic compounds interfere with membrane function and interact with membrane proteins, causing deformation in structure and functionality (Bajpai *et al*., 2008). A combination of phenolic compounds can provide synergistic antimicrobial effects and can contribute to a better antimicrobial reaction as compared to the reaction of an individual compound (Tafesh *et al*., 2011). In addition, the effect of phenolic compounds can be concentrations dependent; at low concentration, phenols affect enzyme activity, while at high concentrations they cause protein denaturation (Delaquis and Mazza, 1995). Also, the antimicrobial activity of saponins has been proven (Bahraminejad *et al*., 2007). Therefore, the saponin content may contribute to the antimicrobial activity of the ethanolic extract.

On the other hand, the antimicrobial activity of the extract may be due to the fatty acid composition of its lipid fraction. This could be proved by the findings obtained by Roy *et al*. (2016) in which the n-Hexane extract of the seaweed *E. intestinalis* showed antibacterial activity against *E. coli, Bacillus*

sp., *Staphylococcus aureus, Pseudomonas* sp, as well as antifungal activity against *C. Albicans*.

It is known that; the antimicrobial activity of each fatty acid can be influenced by the fatty acids structure, and shape including the length of carbon chain and number of double bonds. Fatty acids with longer carbon chains usually exhibit a stronger inhibitory effect than those with shorter chains. Unsaturated fatty acids also tend to have a higher inhibitory effect than saturated ones. (Desbois and Smith, 2010). Even though the antimicrobial effect of fatty acids is well studied, the mechanism by which fatty acids inhibit the bacterial growth is not well-defined (Desbois and Smith, 2010; Kankaanpa *et al*., 2001). In general, lipids inactivate microorganisms mainly by disruption of the bacterial cell wall or membrane, inhibition of intracellular replication, or inhibition of an intracellular target (Driessen *et al*., 1995).

4.7 Antioxidant activity screening:

The antioxidant activity of *E. intestinalis* ethanolic extract was investigated using a DPPH radical scavenging assay (Table 6).

Table (6): Antioxidant activity of *E. intestinalis* ethanolic extract

Sample	%RDS±SD (DPPH)
E. intestinalis extract	20±0.01
Standard (propyl gallate)	90±0.01

It could be noticed that the *E. intestinalis* ethanolic extract exhibited moderate reduction capability and a free radical scavenging effect. The antioxidant activity of the ethanolic extract may be due to the content of the phytosterol, fucosterol, which is known for its antioxidant activity (Tiwari *et al*., 2009; Ciocan and Ioan, 2009). Also, the activity may be due to its content of the phytosterol, fucosterol, which is known for its antioxidanta ctivity (Lee *et*

al.,2003) or the diterpene phytol, which is also known for its antioxidant activity (Ryu *et al*., 2011).

2.2 Chemical Composition of E. intestinalis:

2.2.1 Secondary metabolites:

The phytochemical screening of *E. intestinalis* from India revealed the presence of flavonoids, tannins, alkaloids, glycosides, saponins, and cardiac glycosides (Satarupa *et al*., 2016; Shankhadarwar, 2015). detected presence of flavonoids, alkaloids, steroids, sapnonins and phenols in *E. intestinalis*.

2.2.2 Mineral composition:

Its composition reflects what is present in the water, and it is known to accumulate heavy metals, such as Cu, Cd, Zn, etc. (Chakraborty et al., 2014). *E. intestinalis* from southern Thailand was found to be rich in Mg, K, Cl, Na, and Ca (Benjama and Masniyom 2011).

2.2.3 Nutritional composition:

Chemical analysis indicated that *Enteromorpha spp*. (from Mexico) has 9–14% protein (digestibility 98%), 2–3.6% ether extract, 32–36% ash, and n-3 and n-6 fatty acids (10.4 and 10.9 g/100g) of total fatty acid, respectively (Aguilera-Moralesa *et al*.,2003).

Metin and Baygar (2018) determined the nutritional composition of green macroalgae (*E. intestinalis*) collected from Muğla during different seasons and concluded that *E. intestinalis* can be utilized in a variety of ways depending on its intended field of usage, due to its higher content of crude protein in summer, ash and vitamin C in spring and polyunsaturated fatty acids in autumn.

E. intestinalis, collected from the Pattani Bay in southern Thailand in the rainy and summer seasons of 2007– 2008, were investigated for its nutritional

value. It was found that the green seaweed contained high level of protein (14.6–19.5% DW), lipid (2.1–8.7% DW), ash (25.9–28.6% DW), soluble fiber (25.3–39.6% DW), insoluble fiber (21.8–33.5% DW), and total dietary fiber (51.3–62.2% DW) (Benjama and Masniyom 2011).

2.3 Biological Activities:

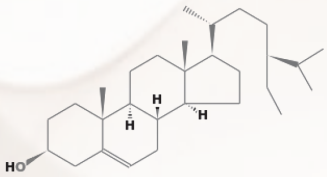
The *E. intestinlas* have biological activities such as antioxidant, antimicrobial, and cytotoxic potential (Abbott and Hollenberg, 1992).

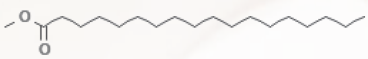
2.3.1 Antimicrobial activity:

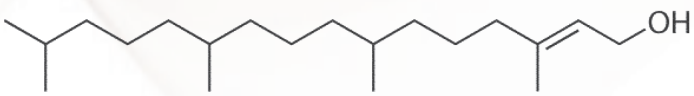
Srikonga *et al*. (2017) studied the antibacterial activities of crude extracts with different solvents (methanol, ethanol, dichloromethane, and hexane) from *E. intestinalis* from southern coast of Thailand. They concluded that only the hexane extract had significant antibacterial activities for gram-positive bacteria but not gram-negative bacteria.

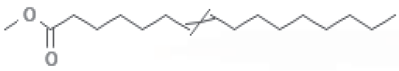
2.3.2 Antioxidant activity:

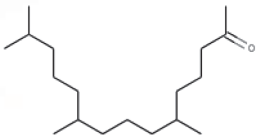
Among different solvent extracts (methanol, ethanol, dichloromethane, and hexane) the highest inhibition radical scavenging effects on (DPPH), and (ABTS) activities were observed in the dichloromethane extract with IC50 = 0.92 mg/ml and 1.50 mg/ml, respectively (Srikonga *et al*. 2017).

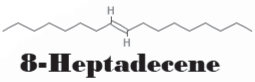












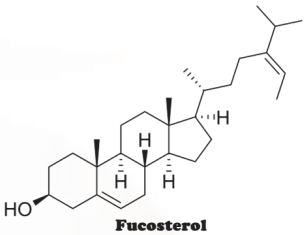


Figure (**27**) Structural of Major Compounds found in Extract of *Enteromorpha intestinalis*

**9,12-Octadecadienoic acid (Z,Z)-,
methyl ester**

Appendix:

Antimicrobial Activities:

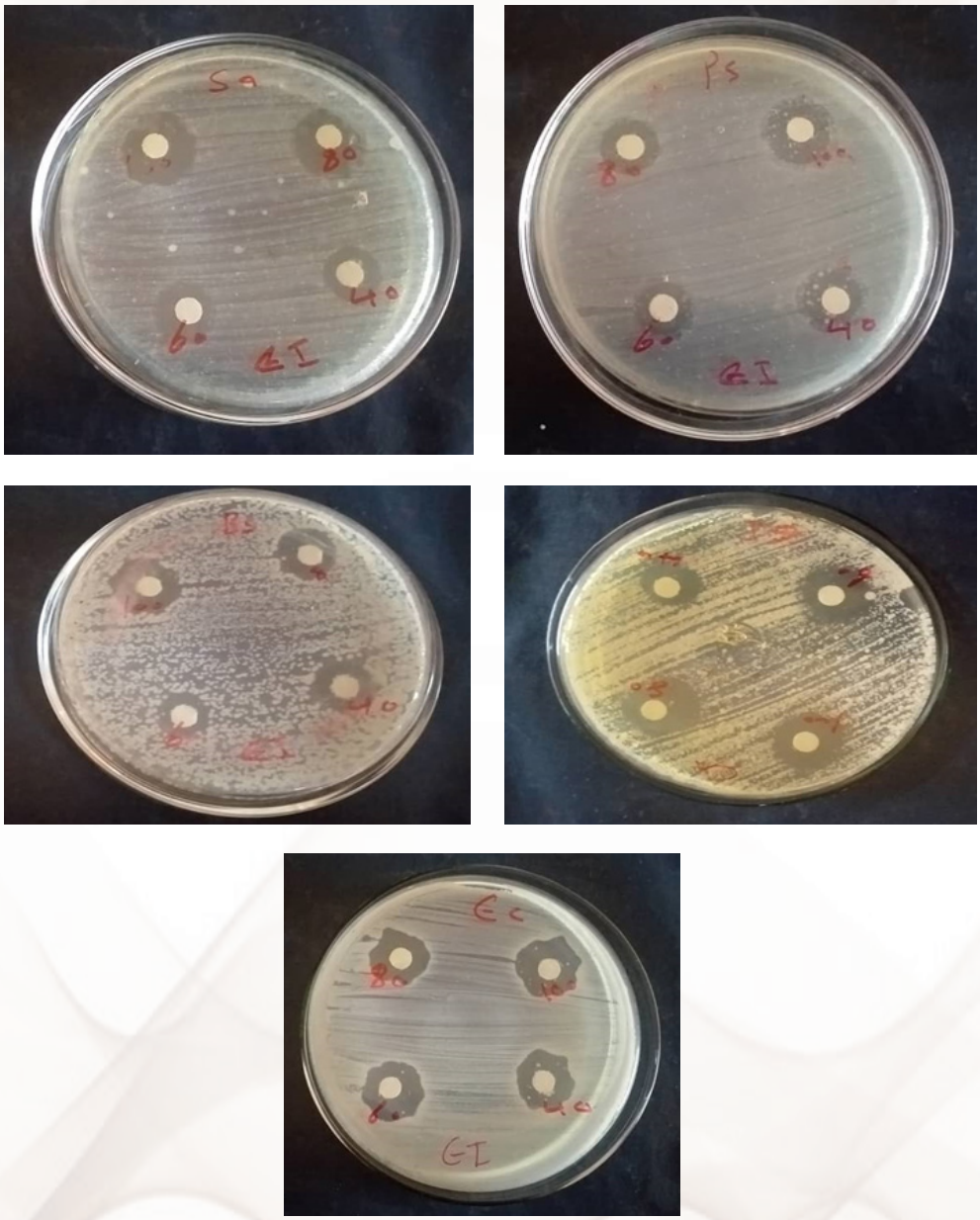


Figure (28) Antimicrobial Activities

5. Conclusion and Recommendations:

5.1 Conclusion:

In conclusion, the results of this study indicates that *E. intestinalis* contained important primary and secondary metabolites which enable it to be used as nutraceutical or food supplement. A among these constituents were omega-3 and omega-6 fatty acids, the diterpene phytol and the phytosterol fucosterol, as well phenolic compounds. It also contained macro elements like *K, Na,* and S. On the other hand, *E. intestinalis* was found to be active against both Gram-positive and Gram-negative bacteria as well as *C. albicas.*

5.2 Recommendation:

1. Further studies are needed on the biological and pharmacological activities of seaweeds and their highly bioactive secondary metabolites because of their potential in the development of new pharmaceutical agents.
2. It is recommended to isolate of fucosterol which exhibits various biological therapeutics, including anticancer, antidiabetic, antioxidant, hepatoprotective from *E. intestinalis* during its abundance season in the Red Sea to be used in the medical field.

Reference

(1) Abdul, Q. A., Choi, R. J., Jung, H. A., and Choi, J. S. (2016). Health benefit of fucosterol from marine algae: a review. J Sci Food Agric, 96(6), 1856-1866. doi:10.1002/jsfa.7489

(2) Aguilera-Morales, M., Valdez, C., Carrillo, S., González, B., and F.P, P.-G. (2005). Chemical composition and microbiological assays of marine algae Enteromorpha spp. as a potential food source. Journal of Food Composition and Analysis, 18, 79-88. doi:10.1016/j.jfca.2003.12.012

(3) Alves, T. M., Silva, A. F., Brandao, M., Grandi, T. S., Smania, E., Smania Junior, A., and Zani, C. L. (2000). Biological screening of Brazilian medicinal plants. Mem Inst Oswaldo Cruz, 95(3), 367-373. doi:10.1590/s0074-02762000000300012

(4) Amsler, C. D., and Searles, R. B. (1980). VERTICAL DISTRIBUTION OF SEAWEED SPORES IN A WATER COLUMN OFFSHORE OF NORTH CAROLINA1. Journal of Phycology, 16(4), 617-619. doi:https://doi.org/10.1111/j.1529-8817.1980.tb03080.x

(5) Bajpai, V. K., Rahman, A., Dung, N. T., Huh, M. K., and Kang, S. C. (2008). In vitro inhibition of food spoilage and foodborne pathogenic bacteria by essential oil and leaf extracts of Magnolia liliflora Desr. J Food Sci, 73(6), M314-320. doi:10.1111/j.1750-3841.2008.00841.x

(6) Benjama, O., and Payap, M. (2011). Nutritional composition and physicochemical properties of two green seaweeds (Ulva pertusa and U. intestinalis) from the Pattani Bay in Southern Thailand. Songklanakarin Journal of Science and Technology, 33, 575-583.

(7) Blomster, J., Maggs, C., and Stanhope, M. (2002). Molecular and morphological analysis of Enteromorpha intestinalis and E. compressa

(Chlorophyta) in the British Isles. Journal of Phycology, 34, 319-340. doi:10.1046/j.1529-8817.1998.340319.x

(8) Boney, A. D. (1965). Aspects of the Biology of the Seaweeds of Economic Importance. Advances in Marine Biology, 3, 105-253.

(9) Drum, R. (2021). SEA VEGETABLES FOR FOOD AND MEDICINE *Island Herbs* (pp. 98297-90025). USA: Waldron.

(10) Guiry, M. D. J., D.M.; Rindi, F.; McCarthy, T.K. (Eds). (2007). *New Survey of Clare Island. Vol. 6: The Freshwater and Terrestrial Algae* (Vol. 6). Dublin: Royal Irish Academy.

(11) Horwitz, W., and International, A. (2002). *Official methods of analysis of AOAC International* (17. ed., current through revision ed.). Gaithersburg, Md.: AOAC International Gaithersburg, Md.

(12) Kiehlbauch, J. A., Hannett, G. E., Salfinger, M., Archinal, W., Monserrat, C., and Carlyn, C. (2000). Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York state laboratories. J Clin Microbiol, 38(9), 3341-3348. doi:10.1128/jcm.38.9.3341-3348.2000

(13) Letawe, Boone, and PiÉRard. (1998). Digital image analysis of the effect of topically applied linoleic acid on acne microcomedones. Clinical and Experimental Dermatology, 23(2), 56-58. doi:10.1046/j.1365-2230.1998.00315.x

(14) Roy, M. (2016). Antimicrobial and Phytochemical Screening of Seaweeds: Enteromorpha intestinalis and Ulva lactuca Collected from Indian Sunderbans Delta Region.

(15) Shankhadarwar, S. (2015). Phytochemical screening of marine algae Ulva lacuta (Linn.) and Enteromorpha intestinalis (Linn.). 7, 419-423.

(16) Shimada, K., Fujikawa, K., Yahara, K., and Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry, 40(6), 945-948. doi:10.1021/jf00018a005

(17) Tiwari, B. K., Valdramidis, V. P., O'Donnell, C. P., Muthukumarappan, K., Bourke, P., and Cullen, P. J. (2009). Application of natural antimicrobials for food preservation. J Agric Food Chem, 57(14), 5987-6000. doi:10.1021/jf900668n

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