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المركبات النشطة بيولوجيا والأنشطة المضادة للأكسدة للطحالب البنية التي تم جمعها من شواطئ البحار المصرية

Bioactive compounds and antioxidant activities of brown algae collected from the shores of the Egyptian seas

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المستخلص:

تنتمى الطحالب البنية إلى مجموعة Heterokontophyta، وهي مجموعة كبيرة من الكائنات حقيقية النواة التى تتميز بوجود بلاستيدات خضراء محاطة بأربعة أغشية، وتنتشر الطحالب البنية في مصر بشكل طبيعي على مساحات كبيرة من شواطئها البحرية. لذا تهدف الدراسة الحالية إلى تحديد المركبات النشطة حيويا والأنشطة المضادة للأكسدة الموجوده بالطحالب البنيه التي تم جمعها من الشواطئ المصرية. وقد أظهرت النتائج إلى أن الكربوهيدرات كانت أكبر مركب بنسبه (٦٠.٥٩٪) يليها الألياف الغذائية (٤٨.٨٧%)،الرماد (١٩.٤٥٪) والألياف الخام (٧.١٤٪) والبروتين الكلي (٤.٠٢٪) والدهون الخام (٠.٨٢٪)، كذلك وجد ان السكريات العديدة كانت أكبر المركبات النشطة حيويا تليها الفينولات ثم التانينات والكاروتينات وأقلها الفلافونيدات ،كما أشار اختبار بيتا كاروتين إلى أن مستخلص الإيثانول للطحالب البنية سجل أعلى نشاط مضاد للأكسدة يليه مستخلص الميثانول ثم اخيرا مستخلص الماء. كما وجد ان المستخلص المائي يمتلك أعلى نشاط في إزالة الجذور الحرة. اما بالنسبة لمقياس IC_{50} فقد سجل كل من المستخلص المائي ومستخلص الايثانول ويليهم المستخلص الميثانولي القيم التاليه ١٩,٩٢، ١٠.٤١، ١٢.٥٢ ميكروجرام / مل على التوالي، في حين ان مقياس IC50 لـ BHT (المركب القياسى) كان بنسبه ٧.٧٨ ميكروجرام / مل. كما اظهرت كل من اختبارات البيتا كاروتين وازاله الجذور الحره لكل من المستخلصات المختبرة والمركبات القياسية (المرجعية) المضادة للأكسدة الترتيب التالى من حيث درجة النشاط: المركبات القياسية (BHT/ α-tocopherol) > المستخلص الايثانولي > المستخلص الميثانولي >المستخلص المائي. وخلص النتائج إلى ان الطحالب البنية ومستخلصاتها تحتوي على عدة مجموعات من المركبات النشطة حيويا مع مركبات/مكونات أخرى مسؤولة عن الأنشطة البيولوجية المختلفة بما في ذلك الأنشطة المضادة للأكسدة وإزالة الجذور الحرة. لذلك، توصى الدراسة بإدراج الطحالب البنية ومستخلصاتها في وجباتنا اليومية والمشروبات والمكملات الغذائية والتركيبات الدوائية.

الكلمات المفتاحية : مستخلصات الطحالب البنية، التركيب الكيميائي ، اختبار بيتا –كاروتين ، نشاط إزالة الجذور الحرة.

Bioactive compounds and antioxidant activities of brown algae collected from the shores of the Egyptian seas

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Abstract:

Brown algae (BA) belongs to the group Heterokontophyta, a large group of eukaryotic organisms distinguished most prominently by having chloroplasts surrounded by four membranes. In Egypt, BA is naturally spread over large areas of marine beaches. The present study aims to determine the chemical composition and bioactive compounds in BA samples collected from the shores of the Egyptian Seas. Also, biological activities of the same BA samples extracts will be studied. Results indicated that carbohydrates were the largest compound (60.59%) followed by Dietary fiber (48.78 %), ash (19.45%), crude fiber (7.14%), total protein (4.02%) and crude fat (0.82%). Also, polysaccharides were the largest compound followed by phenolics, tannins, carotenoids and flavonoids. β-carotene assay (BCA) indicated that ethanol extract (EtE) recorded the highest antioxidant activity followed by methanol extract (MeE) and water extract (AqE). The values of EtE extract are coming well i.e. closing to the line of 50 mg α-tocopherol followed by MeE and AqE, respectively. Also, AqE possessed the highest free radical scavenging activity (FRSA). For Half-maximal inhibitory concentration (IC_{50}), the AqE, EtE and MeE recorded 19.92, 10.41 and 12.52 µg/mL, respectively. The IC_{50} of BHT (standard) was 7.78 µg/mL. The BCA and FRSA of different tested extracts and standards were in the following order: standards (BHT/ α-tocopherol) >EtE >MeE >AqE**.** In conclusion, Brown algae extracts (BAE) contain several classes of phytochemicals with other compounds that are responsible for different biological activities including antioxidant and radical scavenging activities. Therefore, we recommended brown algae powder and/or extracts to be included in daily diets, drinks, food supplementation and pharmacological formulae.

Keywords: Brown algae extracts, chemical composition, β-carotene assay, free radical scavenging activity.

Introduction

Algae are a diverse group of aquatic organisms, which are described as having the ability to perform photosynthesis. Some types of algae are also familiar to most people. For example, seaweed, pond scum, or algae blooms in lakes. Despite this, there is a vast and diverse world of algae that not only help us with life but are essential to our existence in it. In general, Marine algae are classified as brown, red, or green algae (**Guiry and Nic, 2001**). Brown algae (BA) belongs to Family, *Phaeophyceae* are a large group of mostly marine multicellular algae, including many seaweeds located in colder Northern Hemisphere waters. They play an important role in marine environments, both as food and as habitats. For example, *Sargassum* creates unique floating mats of seaweed in the tropical waters of the Sargasso Sea that serve as the habitats for many species. Many brown algae, such as members of the order Fucale's , commonly grow along rocky seashores. Some members of the class, such as kelp, are used as food for humans. Worldwide, over 1500–2000 species of brown algae are known. Some species, such as *Ascophyllum nodosum*, are important in commercial use because they have become subjects of extensive research in their own right. They have environmental importance too through Carbon fixation (**Mann and Martin, 2002**). BA belongs to the group Heterokontophyta, a large group of eukaryotic organisms distinguished most prominently by having chloroplasts surrounded by four membranes, suggesting an origin from a symbiotic relationship between a basal eukaryote and another eukaryotic organism. Most BA contains the pigment fucoxanthin, which is responsible for the distinctive greenish-brown color that gives them their name. They are unique among heterokonts in developing into multicellular forms with differentiated tissues, but they reproduce through flagellated spores and gametes that closely resemble cells of other heterokonts. Genetic studies show their closest relatives to be the yellow-green algae.

In Egypt, BA is also frequently encountered as the major vegetation in shallow water tropical and subtropical habitats, even though herbivorous predators are plentiful. Hence, the correlation between secondary metabolite synthesis within this family and predator avoidance seems to be pronounced (**Gerwick *et al.*, 1981**). In the littoral zone of the Egyptian coast, BA is currently the most dominant group. Members of the *Sargassum* genus represent valuable sources of several compounds including proteins, lipids, minerals, essential fatty and amino acids, and bioactive compounds (**Hossain *et al.*, 2003, El-Gamal, 2020 and Elhassaneen *et al.*, 2020**). Also, BA consists mainly of water (90 %) in the native state. Polysaccharides are major

components and comprise alginates, cellulose, and sulfated polysaccharides such as fucoidans and laminarins. Other components include proteins, free mannitol, minerals such as iodine and arsenic (inorganic and organic), polyphenols, peptides, fatty compounds, and various pigments (**Chapman and Chapman, 1980, Helen, 2003 and El-Gamal, 2020**). Alginates, probably the most widely used of the algal extracts, are composed of block copolymers of mannuronic and guluronic acid sugars and have been adopted by the food industry as thickening agents and by the pharmaceutical industry as binders, gelling agents, and wound absorbents (**Helen, 2003**).

From a nutritional and therapeutic point of view, BA is used dried in condiment and soup bases or eaten fresh in salads, rolls, or stews, or with rice. It is thought that the overall content of certain traditional Asian diets contributes to the low incidence of cancer, particularly breast cancer (**Kanke *et al.*, 1998; and Lawson *et al.*, 2001**). The unique levels of seaweed intake contribute to the variance in the levels of breast cancer (**Funahashi *et al.*, 2001**). There is a nine-fold lower incidence of breast cancer in the Japanese population and an even lower incidence in the Korean population compared to the incidence in the West (**Adami *et al.*, 1998**). The relative longevity and health of Okinawan Japanese populations have been attributed in part to dietary algae in studies (**Yamori *et al.*, 2001**). These studies compared Okinawan descendants who were living in Brazil with Okinawans. The former has a higher risk of developing cardiovascular and other diseases. For a dietary intervention study, 3g of docosahexaenoic acid, 5g of seaweed (wakame) powder, and 50 mg of Isoflavonoids from soybean (*Glycine soja*) were given daily to immigrants, at high risk for developing diseases, in Brazil for 10 weeks. This combination reduced blood pressure and cholesterol levels, suppressed the urinary markers of bone resorption, and attenuated a tendency toward diabetes. Also, consumption of BA powder up to 4% of the diet was effective in protecting against obese complications including oxidative stress status, immunological parameters and bone disorders.

All of these previous therapeutic effects require conducting more studies to identify the bioactive compounds in brown algae and their different biological effects. Therefore, the present study aims to determine the bioactive compounds in brown algae collected from the shores of the Egyptian seas. Also, the biological activities i.e. antioxidant activities of such plant part ethanolic extract will be in the scope of this investigation.

Materials and Methods

Materials

BA samples

Dried BA *(Sargassum subrepandum*) were collected from the coasts of the Mediterranean Sea, Alexandria, Alexandria Governorate, Egypt. The BA samples were verified by the staff in the Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Chemicals

Bioactive compounds standard (gallic acid, catechine, α-tocopherol and Butylhydroxy toluene) were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals (Except as otherwise stated), reagents and solvents were of analytical grade were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt.

Methods

Preparation of BA powder

BA was cleaned and sorted manually and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 55 ^{0}C until arriving by the moisture in the final product to about 10%. The dried samples were ground into a fine powder in high mixer speed (Moulinex Egypt, ElAraby Co., Benha, Egypt). The material that passed through an 80 mesh sieve was retained for use.

Preparation of BA extracts

BA powder was used for their different types of extracts as follow: A 20 g from dried BA plus 180 ml water were homogenized and transferred to a beaker and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent was removed under reduced pressure at 55°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The same protocol was followed as before with changing the extraction medium with Methanol (80%, v/v) and Ethanol (80%, v/v) respectively. The residual solvent was removed under reduced pressure at 45°C using a rotary evaporator. All extracts (Aqueous, Methanolic and Ethanolic) could be ready for the antioxidant activity studies.

Chemical analysis of BA

BA samples were analyzed for proximate chemical composition including moisture, protein (T.N. × 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy), fat (soxhelt miautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber and dietary fiber contents were determined using the methods described in the **AOAC, (1995)**. Carbohydrates calculated by differences: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber). Total energy (Kcal/100 g) was calculated according to **Insel *et al,* (2002)** using the following equation: Total energy value (Kcal/100 g) = 4 (Protein % + carbohydrates %) + 9 (Fat %).

Determination of bioactive compounds in BA

Total phenolics and carotenoids

Total phenolics in BA extracts were determined using Folin-Ciocalteu reagent according to **Singleton and Rossi, (1965)** and **Wolfe *et al.*, (2003)**. Two hundred milligrams of the sample were extracted for 2 h with 2 mL of 80% MeOH containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 min and the supernatant decanted into 4 mL vials. The pellets were combined and used for the total phenolic assay. One hundred microliters of extract were mixed with 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 ^{0}C for 5 min; 0.75 ml of sodium bicarbonate (60g/L) solution was added to the mixture after 90 min at 22 0C, absorbance was measured at 725 nm. Results are expressed as gallic acid and equivalents (GAE). The total carotenoids in 80% acetone extract were determined by using the method reported by (**Litchenthaler,1987**).

Total flavonoids

Total flavonoids contents in BA extracts were estimated using the colorimetric assay described by **Zhisen *et al.*, (1999)**. To aliquot (0.05 mL) of the extract /standard (catechin CAE), 150 µL of sodium nitrate (5 %) and 2.5 mL of distilled water were added. After 5 min, 0.3 mL of aluminum chloride (10 %) was added. At 6 min, 1 mL of NaOH (0.001 M) and 0.55 mL distilled water was added to the mixture and left at room temperature for 15 min. The absorbance of the mixtures was measured at 510 nm spectrophotometer (UV-160A; Shimadzu Corporation, Kyoto, Japan). Samples of extract were evaluated at a final concentration of 0.1 and 0.15 mg/mL. Total flavonoid contents were expressed as catechin equivalent, CAE (standard curve equation: $y = 0.0003x - 0.0117$, $R^2 = 0.9827$), mg of CA/g of dry extract.

Total polysaccharides

Total polysaccharides were determined by spectroscopic analysis technique using a UV- visible-light spectrophotometer. Samples were extracted and measured according to the method of **Vazirian *et al.*, (2014).** Starch was used as a standard and the results were expressed as mg of starch equivalents per g of dw.

Tannins

Tannins were determined by the method of **Van-Burden and Robinson (1981)** and expressed as mg catechine per g of dw.

Antioxidant activity

β-carotene bleaching (BCB) assay

Antioxidant activity (AA) of plant extracts and standards (α-tocopherol and BHT) was determined according to the BCB assay following a modification of the procedure described by **Marco, (1968)**. For a typical assay, 1mL of *β*-carotene solution, 0.2 mg/mL in chloroform, was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid and 0.2 mL of Tween 20. Each mixture was then dosed with 0.2 mL of 80% MeOH (as control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50 ml) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal auto-oxidation at 50 ^{0}***C for 2 h. The absorbance of the solution at 470 nm was monitored on a*** spectrophotometer (beckman DU-50) by taking measurements at 10 min intervals, and the rate of bleaching of *β*-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Various concentrations of BHT and α-tocopherol in 80% methanol were used as the control. Antioxidant activity (AA) was all calculated as percent inhibition relative to control using **Al-Saikhan *et al.*, (1995)** equation as follow: AA= ($R_{control}$ - R_{sample}) / $R_{control}$ x 100, Where: $R_{control}$ and R_{sample} were the bleaching rates of *β*-carotene in reactant mixture without antioxidant and with plant extract, respectively.

DPPH radical scavenging assay

The free radical scavenging ability of BA extracts was tested by DPPH radical scavenging assay as described by (**Desmarchelier *et al.*,1997**). A solution was prepared, and 2.4 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM in methanol) was mixed with 1.6 mL of *G. lucidum* extract at different concentrations (12.5–150 μg/mL). The reaction mixture was

vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm (UV-160A; Shimadzu Corporation, Kyoto, Japan). BHT was used as a reference. Percentage DPPH radical scavenging activity was calculated by the following equation: DPPH radical scavenging activity (%) = [(A0− A1)/A0] ×100 where A0, the absorbance of the control, and A1, the absorbance of the BA / BHT. Then inhibition (%) was plotted against concentration, and IC50 was calculated from the graph.

Statistical Analysis

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

Results and Discussion

Proximate composition, dietary fiber and total energy of BA

Data in Table (1) shows the proximate composition, dietary fiber and total energy of BA. From such data, it could be noticed that carbohydrates were the largest compound (60.59%) followed by Dietary fiber (48.78 %), ash (19.45%), crude fiber (7.14%), total protein (4.02%) and crude fat (0.82%.). Such data are following that reviewed by many authors, almost all of them mentioned that the greater portion of BA carbohydrates is available as polysaccharide, which is not taken up by the human body and is regarded as dietary fibers (**Percival, 1979; Holdt and Kraan, 2011**). Dietary fibers are good for human health as they make an excellent intestinal environment by favoring the growth of intestinal microflora, including probiotic species so they can be considered as prebiotic (**Tosh and Yada, 2010**). In several cases, BA carbohydrates possess a fiber level greater than those recorded for many vegetables or fruits (**MacArtain *et al.*, 2007**). Among the fibers, hydrocolloids such as alginate, agar, carrageenans, fucoidan, and laminaran are present in large proportions in algae. Many of these polysaccharides play significant roles in food processing and human nutrition. For example, they are used in foods as thickeners, gelling agents, and emulsion stabilizers (**Bixler and Porse, 2011**). Other studies have also reported numerous biological activities including anticoagulant, antithrombotic, anti-inflammatory, ant obese, antiviral, immune system-boosting properties and anti-osteoporosis (**Elhassaneen *et al.*, 2020**). Although BA are representing low-calorie foods however their carbohydrate content is generally high. It can represent up to 75% of dry weight. While **Holdt and Kraan, (2011)** reported that BA contains a large amount of carbohydrate as structural, storage, and functional polysaccharides, and the total carbohydrate content may range

from 20% to 76% of dry weight depending on the species. BA contains fat/lipid between 0.9% and 4% of dry weight (**Dawczynski *et al.*, 2007**) reported that fats contain up to 1−3% of dry weight. Also, (**Khotimchenko, 2005; Mendis and Kim, 2011**) found that BA has a very little lipid content, ranging from 1% to 5% of dry matter (**Rodrigues *et al.*, 2015**). From all the above studies and others, it could be concluded that the proximate chemical composition of BA varies with species, geographical location, season, water temperature, depth, light intensity, nutrients, pH, salinity or a combination of these factors (**Guschina and Harwood, 2006**).

Table 1. Proximate composition, dietary fiber and total energy of BA

Component	Content
Moisture	7.98±1.03
Dry matter	92.02±1.76
Total protein (g/100g)	4.02 ±0.57
Crude fat (g/100g)	0.82 ± 0.21
Ash (g/100g)	19.45 ± 0.28
Crude Fiber (g/100g)	7.14 ± 3.91
Carbohydrate (g/100g)	60.59 ± 4.06
Dietary fiber (g/100g)	48.78± 3.98
Total energy (Kcal/100g)	265.82±3.04

*Each value represents the mean of three replicates ±SD.

Bioactive compounds in BA

Bioactive compounds in BA were shown in Table (2). From such data it could be noticed that Polysaccharides were the largest compound (152.45± 19.32 mg starch. g^{-1}) followed by phenolics (122.67 mg gallic acid. g^{-1}), tannins (34.15 ± 6.90 mg catechine. g^{-1}), carotenoids (30.78± 6. 41mg.g^{-1}) and flavonoids (29.31 ± 5.67 mg catechin. g^{-1}). Such data are partially following that reported by **El-Gamal, (2020)** the total carotenoid content in ethanol extract was 358 - 511mg.100g^{-1} and total phenolics was 593 -4278 mg EGA.100 g^{-1}. Also, **Chapman and Chapman, (1980)** and **Helen, (2003)** found that polysaccharides are major components and comprise alginates, cellulose, and sulfated polysaccharides such as fucoidans and laminarins Also, alginates, probably the most widely used of the algal extracts, are composed of block copolymers of mannuronic and guluronic acid sugars and have been adopted by the food industry as thickening agents and by the pharmaceutical industry as binders, gelling agents, and wound absorbents (**Helen, 2003**).

Table 2. Total content of bioactive compounds in BA

Component	Range	Mean±SD
Phenolics (mg gallic acid. g^{-1})	89.56-141.43	122.67 ± 20.34
Flavonoids (mg catechin. g^{-1})	23.67-33.86	29.31 ± 5.67
Polysaccharides (mg starch. g^{-1})	123.65-162.51	152.45± 19.32
Carotenoids (mg .g^{-1})	28.19-38.8	30.78± 6.41
Tannins (mg catechine. g^{-1})	24.65- 41.15	34.15 ± 6.90

*Each value represents the mean of three replicates ±SD.

Many previous studies, along with others, have proven that bioactive compounds (Phenolics, flavonoids and carotenoids) which was determined in this study in BA play important biological roles in preventing and/or treating many diseases such as diabetes, atherosclerosis, cancer, obesity, bone, anemia and aging (**Elhassaneen *et al.*, 2016, 2019-a, 2020, 2021; El-Gamal, 2020**).

Such previous effects of these compounds are due mainly to their magical antioxidant activities. The phenolics are secondary metabolites defined as aromatic benzene ring compounds possessing one or more hydroxyl groups bonded directly to an aromatic ring, including their functional derivatives. These phytochemicals display a wide variety of structures, from simple moieties to polymers with high molecular weight and biogenetically they arise from two main primary synthetic pathways; the shikimate pathway and the acetate pathway (**Dai and Mumper, 2010**). Regarding the carotenoids, **Britton, *et al.*,2004** reported many different kinds of carotenoids were found from the algal species studied. For photosynthesis, both carotenoids and chlorophylls are necessarily bound to peptides to form pigment-protein complexes in the thylakoid membrane. Five main kinds of the complexes described below are isolated from some algae, and the pigment compositions are investigated (**Durnford, 2003; Neilson and Durnford, 2010**). Exceptionally in cyanobacteria, myxol glycosides and some carotenoids are located in the cytoplasmic membrane for protection from high-light (**Masamoto *et al.*, 1999**). Another bioactive compound in BA, polysaccharides, help protect against potential carcinogens and they clear the digestive system and protect surface membranes of the stomach and intestine. They absorb substances like cholesterol, which is then eliminated from the digestive system i.e. hypocholesterolemic and hypolipidemic responses (**Burtin, 2003**). This is often coupled with an increase in the fecal cholesterol content and a hypoglycaemic response (**Dumelod *et al.*, 1999**). On the other side, the polysaccharide-mediated potentiation of immune function is thought to be the major mechanism of antitumor action by algae

*(***Liu, 1999 and Wasser, 2002***).* In conclusion, formulating nutraceuticals from BA has shown to present interesting health-promoting benefits.

Antioxidant activities of BA extracts

***β* -Carotene Bleaching (BCB)**

The decrease in absorbance of *β*-carotene in the presence of different brown algae extracts (and well-known antioxidants used as standards) with the oxidation of β-carotene and linoleic acid is shown in Table (3) and Figure (1). Such data indicated that ethanol extract (EtE) recorded the highest concentration followed by methanol extract (MeE) and water extract (AqE). The values of EtE extract absorbances through 120 min are coming well i.e. closing to the line of 50 mg α-tocopherol MeE and AqE, respectively. Also, such data proved the high stability of all algae extracts when compared with the most common antioxidant standards α-tocopherol. The present data are following that obtained by **El-Gamal, (2020) and Elhassaneen *et al.*, (2020)** who found that different extracts of BA have highly significant ($p \leq 0.05$) differences in antioxidant activity (AA= 30.00 - 84.41%) when it was calculated by the different methods. The highest AA was recorded for the ethanol extract (EE) while the lowest one was for the Hexane extract (HE). Also, such attention was recorded by **Elhassaneen and Abd Elhady, (2014), Sayed-Ahmed, (2016) and Aly *et al.*, (2018)** who studied the antioxidant/BCB stability of many plant parts extracts by different media/solvents commonly distributed in the Egyptian local markets.

Table 3. Antioxidant activity of brown algae extracts assayed by the β-carotene bleaching method (BHT α–tocopherol at 50 mg/L concentration was used as a reference)

Extracts	Time (min)												
	0	10	20	30	40	50	60	70	80	90	100	110	120
AqE	**0.898**	**0.865**	**0.824**	**0.808**	**0.796**	**0.713**	**0.698**	**0.694**	**0.676**	**0.660**	**0.614**	**0.600**	**0.591** [b]
MeE	**0.898**	**0.879**	**0.871**	**0.870**	**0.863**	**0.810**	**0.806**	**0.778**	**0.774**	**0.765**	**0.735**	**0.731**	**0.728** [a]
EtE	**0.898**	**0.898**	**0.890**	**0.888**	**0.877**	**0.852**	**0.825**	**0.798**	**0.789**	**0.775**	**0.762**	**0.761**	**0.761** [a]
Control	**0.898**	**0.730**	**0.601**	**0.536**	**0.503**	**0.456**	**0.432**	**0.396**	**0.334**	**0.262**	**0.232**	**0.191**	**0.164** [c]
a-Toc, 50 mg/L	**0.898**	**0.899**	**0.898**	**0.889**	**0.887**	**0.857**	**0.845**	**0.849**	**0.832**	**0.814**	**0.811**	**0.803**	**0.798**[a]

* Values with different superscript letters in the same raw are significantly did different at $p \leq 0.05$.

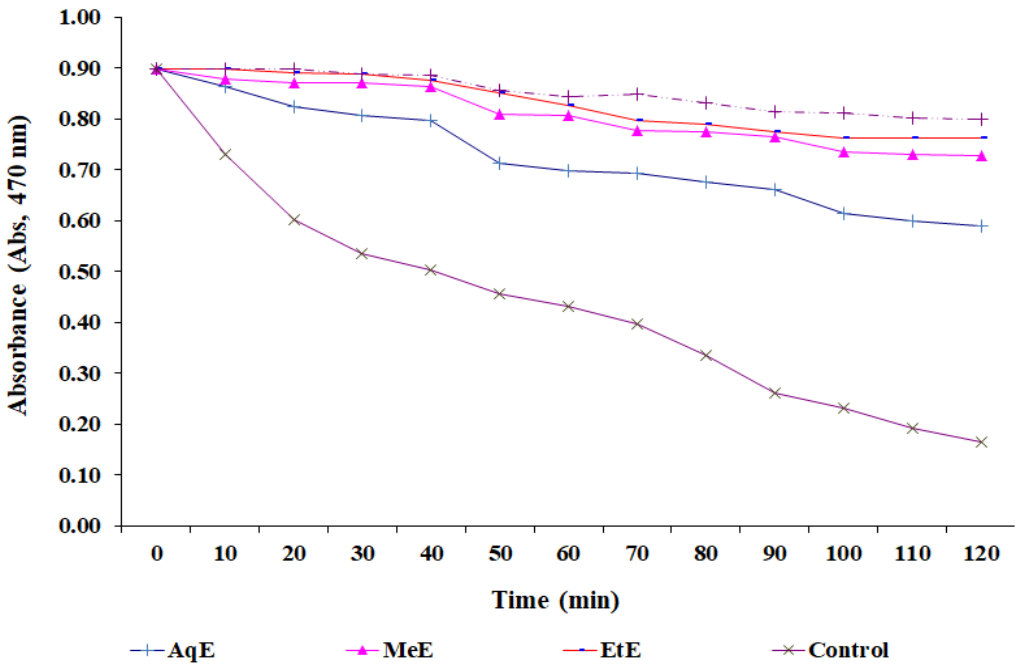

Figure 1. Antioxidant activity of different extracts of *G. lucidum* assayed by the β-carotene bleaching method (BHT and α–tocopherol at 50 mg/L concentration was used as a reference).

* AqE, aqueous extract, MeE, methanol extract, EtE, ethanol extract, α-Toc, alpha-tocopherol, BHT, Butylhydroxy toluene

In general, *β* -Carotene Bleaching (BCB) assay is based on measuring the ability of an antioxidant to inhibit lipid peroxidation. In this method, a model system made of *β*-carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant. The free linoleic acid radical formed upon the abstraction of a hydrogen atom (H^+) from one of its methylene groups (-HC=CH-) attacked the β-carotene molecules, which lost the double bonds and therefore, its characteristic orange color. The absorbance of the solution at 470 nm was monitored on a spectrophotometer by taking measurements at 10 min intervals, and the rate of bleaching of *β*-carotene was calculated by fitting linear regression to data over time according to **Marco (1968)**. For several decades, such methods used efficiency technique for estimation/evaluation the antioxidant activity of huge plant parts extracts (**Elhassaneen and Abd Elhady, 2014, Shalaby, 2015; Elhassaneen *et al.*, 2016; Sayed-Ahmed, 2016, Badawy, 2017, Aly *et al.*, 2018 and Elhassaneen *et al.*, (2019 a-b).** Our results revealed that ethanol and methanol extracts of BA had almost similar free radical scavenging

activity when compared with standards tocopherols. Therefore, those results suggest that all the extracts from BA showed radical scavenging activity by their hydrogen donating ability.

DPPH radical scavenging activity

The free radical scavenging activity (FRSA) of the different extracts of BA and standard BHT are shown in Figure 2 and Table 4. From such data, it could be noticed that among the extracts, AqE possessed the highest activity. At a concentration of 100 µg/mL, the scavenging activity of AqE, EtE and MeE was 82.52, 87.82 and 91.23%, respectively, whereas at the same concentration, the standard BHT was 96.62%. For the IC50, the AqE, EtE and MeE recorded 19.92, 10.41 and 12.52 µg/mL, respectively. The IC50 of BHT (standard) was 7.78 µg/mL. The FRSA of different tested extracts and the standard was in the following order: BHT > EtE > MeE > AqE**.**

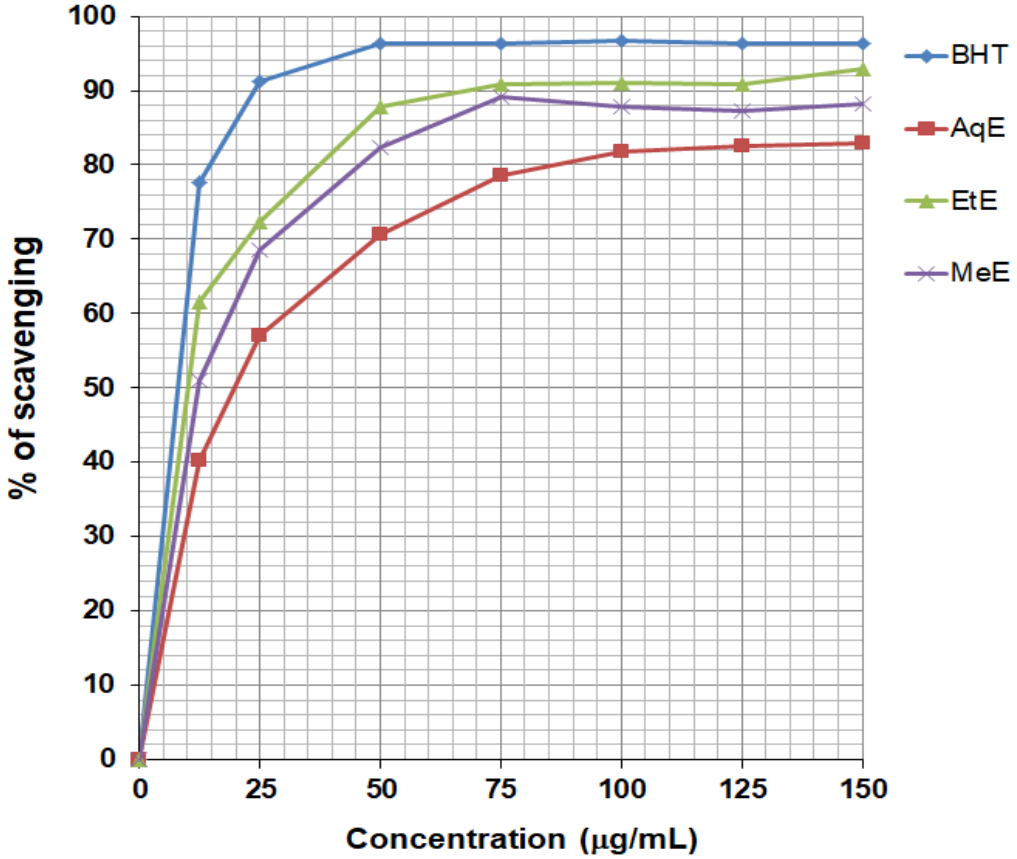

Figure 2. DPPH radical scavenging activity of BA extracts
*Each value represents the mean value of three replicates

Table 4. IC_{50} (DPPH) of BA extracts

Name of sample	BHT	AqE	EtE	MeE
IC_{50} (µg/mL)	7.78 ± 0.51^{c}	19.92 ± 0.99^{a}	10.41 ± 1.02^{b}	12.52 ± 0.87^{b}

* Each value represents the mean value of three replicates ±SD. Values with different superscript letters in the same raw are significantly done differently at $p \leq 0.05$.

The theory of the DPPH radical scavenging activity test is based on the measurement of diene conjugation by absorption at 234 nm is commonly used for determining the oxidative stability of a sample. The usual substrate for the determination of conjugated dienes is DDPH (2,2-diphenyl-1-picrylhydrazyl). DPPH methodology has been used successfully to evaluate the antioxidant activity of different parts including fruits, vegetables, algae, plant by-products, etc. (**Kahkonen *et al.*,1999**). Several studies proved that FRSA is very important to prevent the adverse role of free radicals in different diseases such as obesity, diabetes, cancer, cardiovascular, neurological, pulmonary, nephropathy diseases. The results obtained in this study suggest that all the extracts from BA showed FRSA due to their rich content of different categories of bioactive compounds including phenolics, flavonoids, carotenoids, etc.

In general, antioxidants may have a positive effect on human health as they can protect the human body against damage by reactive oxygen species (ROS) and nitrogen oxygen species (NOS), which attack macromolecules including membrane lipids, proteins and DNA, leading to many health disorders including cancers, diabetes, heart vascular diseases, aging, inflammatory diseases, obesity, anemia, etc. (**Halliwell and Aruoma, 1991; Yang *et al.*, 2001, Salman, 2016; Mahran *et al.*, 2018 and Aly *et al.*, 2018; Elhassaneen *et al.*, 2019-b and 2021; and Mehram *et al.*, 2021a-b**). Additionally, deterioration of some foods has been identified due to oxidation of lipids or rancidity and the formation of lipid peroxidation products causes a decrease in the nutritional value of lipid foods, and affects their safety and appearance. Recently, there is a considerable interest in the development of antioxidants from natural sources, such as marine algae (**Isuru *et al.*, 2011; El-Gamal, 2020 and Elhassaneen *et al.*, 2020**). They also reported that extracts from BA have induced immune activity and also induced nitrite (NO) production.

In conclusion, the data of the study supported our hypothesis that brown algae powder and extracts contain several classes of phytochemicals with other compounds that are responsible for different biological activities including antioxidant and radical scavenging activities. Therefore, we recommended brown powder and/or extracts to be included in our daily diets, drinks, food supplementation and pharmacological formulae.

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