Chemical Composition, Nutritional Value, Bioactive Compounds Content and Biological Activities of the Brown Alga (*Sargassum Subrepandum*) Collected from the Mediterranean Sea, Egypt

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ABSTRACT

The present study aims to determine the chemical composition, nutritional value, bioactive compounds content and biological activities of the brown alga (*Sargassum subrepandum*) collected from the Mediterranean Sea coasts, Egypt. Data of proximate chemical composition of *Sargassum subrepandum* powder indicated that carbohydrates were the largest compound (59.68 ± 3.23%) followed by ash (16.23 ± 1.14 %), crude fiber (9.18 ± 2.02 %), total protein (4.98 ±0.61%) and crude fat (0.91 ± 0.12%.). Also, the total energy Kcal/100g, G.D.R. (g) for protein (63 g) and G.D.R. (g) for energy (2900 Kcal) for the same samples were 266.83± 4.73, 1265.06±20.67g and 1086.83±15.48, respectively. Furthermore, bioactive compounds content of *Sargassum subrepandum* powder indicated that polysaccharides were the largest compound (152.45± 19.32 mg starch equivalent. g^{-1}) followed by phenolics (122.67 ± 20.34 mg gallic acid equivalent. g^{-1}), tannins (34.15 ± 6.90 mg catechin equivalent. g^{-1}), carotenoids (30.78± 6.41 mg. g^{-1}), flavonoids (29.31 ± 5.67 mg catechin equivalent. g^{-1}) and anthocyanin's (3.65± 0.44 mg Cyanidin 3-glucoside, CCy3G equivalent.$100g^{-1}$). The samples also recorded several very high biological activities which include inhibition of low-density lipoprotein (LDL) oxidation, scavenging of free radicals, and antibacterial and antifungal. Such important biological effects could play important roles in strategies to combat/treat many diseases, especially those for which oxidative stress is one of the mechanisms for its occurrence i.e. diabetes, cancer, atherosclerosis, etc. Therefore, the present study recommended that brown alga (*Sargassum subrepandum*) powder and/or extracts to be included in our daily diets, drinks, food supplementation and pharmacological formulae.

Keywords: *Sargassum subrepandum*, inhibition of LDL oxidation, antioxidant activities, free radicals scavenging activity, antibacterial, antifungal.

INTRODUCTION

With the exacerbation of the crisis of chemically synthesized treatments and drugs, and the resulting health complications and dangerous side effects, the world's attention is directed to searching for bioactive compounds from natural sources. On top of these sources are marine plant organisms such as brown algae, due to their rapid growth and reproduction in various climatic conditions, their lack of need for special care treatments, and their richness in various groups of bioactive compounds. All these factors and others make it an affordable and accessible source of food ingredients, as well as a promising source of pharmaceutical compounds.

Brown algae belong to Family, *Phaeophyceae* are a large group of mostly marine multicellular algae, including many seaweeds located in different countries around the world including Egypt. They are a diverse group of aquatic organisms, which are described as having the ability to perform photosynthesis. Some types of brown algae are also familiar to most people as food and as habitats. Despite this, there is a vast and diverse world of algae including BA that not only help us with life, but are essential to our existence in it (Guiry, 2001). BA are members of the Heterokontophyta category of eukaryotic creatures, which are identified by their chloroplasts being surrounded by four membranes, implying that they evolved through a symbiotic interaction between a basic eukaryote and another eukaryotic cell. Most brown algae contain the pigment fucoxanthin, which gives them their name and gives them their unique greenish-brown hue. They are the only heterokonts that mature into multicellular forms with distinct tissues, but they reproduce using flagellated spores and gametes that look very similar to heterokont cells. Their nearest cousins, according to genetic analyses, are yellow-green algae (Mann and Martin, 2002).

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Despite the presence of herbivorous predators, brown algae are usually seen as the dominant flora in shallow water tropical and subtropical settings in Egypt. As a result, there appears to be a strong link between secondary metabolite synthesis and predator avoidance in this family (Gerwick *et al.,* 1981). BA is currently the most dominating group in the Egyptian coast's littoral zone. There are around 1500–2000 BA species worldwide. Some species, such as those belonging to the genus Sargassum, produce unique floating seaweed mats in the tropical and subtropical seas of the Red and Mediterranean Seas, which serve as habitats for a variety of species (Rushdi *et al.,* 2020). They are also important for the ecology since they fix carbon (Mann and Martin, 2002). They're crucial in commercial applications since they've become the focus of their study (El-Gamal, 2020; Elhassaneen et al., 2020 and Fayez, 2021). For example, members of *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 Rushdi *et al.,* 2020). Polysaccharides, which include alginates, cellulose, and sulfated polysaccharides like fucoidans and laminarins, are also important components. Proteins, free mannitol, minerals (inorganic and organic) such as iodine and arsenic, polyphenols, peptides, fatty substances, and different colors are among the other components (Chapman and Chapman, 1980, Helen, 2003 and El-Gamal, 2020 and Rushdi *et al.,* 2020). Alginates, the most frequently used algal extract, are block copolymers of mannuronic and guluronic acid sugars that have been employed as thickening agents in the food business and as binders, gelling agents, and wound absorbents in the pharmaceutical industry (Helen, 2003).

Brown algae are used dried in condiments and soup bases, or fresh in salads, rolls, and stews, or with rice, from a nutritional and therapeutic standpoint. The overall content of certain traditional Asian diets is suggested to play a role in the low incidence of cancer, particularly breast cancer (Kanke *et al.,* 1998; Funahashi *et al.,* 2001and Lawson, 2001). In addition, Adami *et al.* (1998) discovered that the Japanese population has a nine-fold lower incidence of breast cancer than the Western population, while the Korean population has an even lower incidence. Furthermore, Yamori *et al.,* (2001) showed that dietary algae have been linked to the relative longevity and health of Okinawan Japanese communities in research. In these investigations, Okinawan descendants living in Brazil were compared to Okinawans. The former is more likely to develop cardiovascular and other problems. For 10-week dietary intervention research, immigrants at high risk for disease in Brazil were given 3g of decosahexaenoic acid, 5g of seaweed including BA (wakame) powder, and 50 mg of isoflavonoids from soybean. This combination decreased blood pressure and cholesterol levels, inhibited bone resorption signs in the urine, and reduced the risk of diabetes. Many researchers have discovered that consuming up to 4% of one's diet in BA (*Sargassum subrepandum* L.) powder was useful in preventing obesity-related issues such as oxidative stress, immunological parameters, and bone abnormalities (El-Gamal, 2020 and Elhassaneen *et al.,* 2020).

Although all the previous studies and others dealt with many of the biological effects of brown algae, there is still a need to conduct more and more research to explore other roles that this important food source can play. Therefore, the present study aims to determine the chemical composition, nutritional value and bioactive compounds content in brown alga (*Sargassum subrepandum*) collected from Mediterranean Sea coasts in Egypt. Also, the different biological activities including antioxidant activities and antimicrobial effects of such algae will be in the scope of this study.

MATERIAL AND METHODS

Ethical approval

The biological model, rats, used in the experimental design of the study was ethically approved by the Scientific Research Ethics Committee (Animal Care and Use), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval no. 05-SREC- 01-2020).

Materials

Brown alga samples

Brown alga *(Sargassum subrepandum*) samples were collected from the coasts of the Mediterranean Sea, Alexandria, Alexandria Governorate, Egypt. The samples were drained from water and verified by the staff in the Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Chemicals

Bioactive compounds standard [gallic acid (GA), catechin (CA), α-tocopherol and Butylated hydroxytoluene (BHT)], DDPH (2,2-diphenyl-1-picrylhydrazyl), AAPH [2,2′-Azobis(2-methylpropionamidine) dihydrochloride], $CuSO_4$ and dimethyle sulfoxide (DMSO)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.

Biological model

Adult male albino rats (130±10 g per each) were obtained by special arrangement from the Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Methods

Preparation of brown alga (*Sargassum.subrepandum*) powder

Brown alga (*Sargassum subrepandum*) samples were 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%. In a high-speed mixer, the dried samples were crushed into a fine powder (Moulinex Egypt, El Araby Co., Benha, Egypt). The material that went through an 80 mesh filter was retained, sealed in polyethylene bags, and stored at 40°C in the refrigerator for future studies.

Preparation of brown alga (*Sargassum subrepandum*) extracts

Brown alga (*Sargassum subrepandum*) powder was used for their different types of extracts as follow: 20 g dried BA plus 180 ml water were homogenized and transferred to a beaker, where they were agitated at 200 rpm for 1 hour at room temperature in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany). Filtration via Whatman No. 1 filter paper separated the extract from the residue. The remaining residue was extracted twice more, and the two extracts were blended together. The residual solvent was extracted at 55°C under reduced pressure using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The identical methodology was performed as before, with the exception that the extraction medium was changed to Methanol (80%, v/v) and Ethanol (80%, v/v). A rotary evaporator was used to extract the residual solvent at reduced pressure at 45°C.

Chemical analysis of brown alga (*Sargassum subrepandum*) powder

Brown alga 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)

Determination of nutritional value of brown alga (*Sargassum subrepandum*) powder

Total energy value

Total energy (Kcal/100 g) of brown alga (*Sargassum subrepandum*) powder samples was calculated according to Insel *et al*, (2002) using the following equation: Total energy value (Kcal/100 g) = 4 (Protein % + carbohydrates %) + 9 (Fat %)

Satisfaction of the daily needs of adult man (25-50 years old) in protein

Grams consumed (G.D.R., g) of food (wet weight basis) to cover the daily requirements of the adult man (63 g) in protein was calculated using the RDA (1989) values. Percent satisfaction of the daily requirement of adult man in protein (P.S., %) when consuming the possibly commonly used portions in Egypt i.e. one bag (100 g weight), was also calculated.

Satisfaction of the daily requirements of adult man (25-50 years old) in energy

Grams consumed of food (wet weight basis) to cover the daily requirements of energy man (G.D.R., g) were calculated using the RDA (Recommended dietary allowances) which are 2900 Kcal /day for man as given by RDA (1989). The recent satisfaction (P.S., %) of the daily needs of an adult man (25 -50 years old, 79 kg weight and 176 cm height) in energy upon consumption the commonly used portion at homes in Egypt, i.e. i.e. one bag (100 g), was also calculated.

Bioactive compounds determination

Total phenolics and carotenoids

Total phenolics in brown alga (*Sargassum subrepandum*) extracts were determined using Folin-Ciocalteu reagent according to Singleton and Rossi, (1965) and Wolfe *et al.*, (2003). 200 milligrams of the sample were extracted for 2 hours at room temperature with 2 mL of 80 percent MeOH containing 1% hydrochloric acid on an orbital shaker set to 200 rpm. After centrifugation at 1000g for 15 minutes, the supernatant was decanted into 4 mL vials. The pellets were combined and analyzed for total phenolics. 100 microliters of extract were mixed with 0.75 mL Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand for 5 minutes at 22 ^{0}C; after 90 minutes at 22 ^{0}C, 0.75 mL sodium bicarbonate (60 g/L) solution was added to the mixture, and absorbance was measured at 725 nm. Gallic acid and equivalents are used to express the results (GAE). The total carotenoids in 80% acetone extract were determined by using the method reported by Litchenthaler, (1987).

Total flavonoids

Total flavonoids contents in brown alga (*Sargassum subrepandum*) extracts were estimated using the colorimetric assay described by Zhisen *et al.*, (1999). To aliquot (0.05 mL) of the extract/standard (Catechin, CA), 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 Extracts were tested at final concentrations of 0.1 and 0.15 mg/mL. Total flavonoid content was measured in mg of catechin equivalent (CAE) per gram of dry extract (standard curve equation: y=0.0003x- 0.0117, r^2 = 0.9827).

Total anthocyanins

Brown alga (*Sargassum subrepandum*) samples were extracted with a mixture of MeOH /H_2O/ 0.01% HCl, (50: 50: 1, v/v/w) as described by Lee *et al.*, (2011). The extract was evaporated to dryness in a vacuum rotary evaporator in a water bath at 40 ^{0}C. The resultant yield was 12% (w/w) and then the residue was diluted in distilled water of pH 5.6 and the total content of anthocyanins. in the sample was measured spectrophotometrically using the molar extinction coefficient of cyanidin-3,5-diglucoside (26 300 $M^{-1}cm^1$).

Total polysaccharides

Total polysaccharides were determined in brown alga (*Sargassum subrepandum*) samples 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 in brown alga (*Sargassum subrepandum*) samples by the method of Van-Burden and Robinson (1981) and expressed as mg catechin per g of DW.

Antioxidant activity determination

DPPH radical scavenging assay

The ability of brown alga (*Sargassum subrepandum*) extracts to scavenge free radicals was determined using the DPPH radical scavenging assay developed 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 brown alga (*Sargassum subrepandum*) extract at different concentrations (12.5–150 µg/mL). The reaction mixture was completely vortexed and kept at room temperature for 30 minutes. The mixture's absorbance was measured spectrophotometrically at 517 nm (UV-160A; Shimadzu Corporation, Kyoto, Japan). BHT was utilized as a benchmark. Percentage DPPH radical scavenging activity was calculated by the following equation: DPPH radical scavenging activity (%) = [(A0− A1)/A0] ×100. where A_0, the absorbance of the control, and A_1, the absorbance of the BA/BHT. Then inhibition (%) was plotted against concentration, and IC_{50} was calculated from the graph.

Peroxyl radical (ROO^-)-scavenging activity

An improved oxygen radical absorbance capacity (ORAC) assay, similar to that described by Ou *et al.*, (2001) was used to determine peroxyl radical-scavenging activity in brown alga (Sargassum subrepandum). In a test tube, the sample (200 µl), phosphate buffer (3.5 ml, 75 mM, pH 7.4), and FL (100 µl, 35 nM) were combined and incubated at 37 ^{0}C for 5 minutes before the initial fluorescence intensity was measured. 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) (200 mL, 75 g/L) was added to start the reaction. Fluorescence readings were taken every three minutes until the fluorescence intensity was zero. Excitation and emission wavelengths were 493 and 515 nm, respectively. Trolox was also used as a calibration standard to confirm the accuracy of the final results.

Inhibition of low-density lipoprotein (LDL) oxidation

The inhibition of LDL oxidation in brown alga (*Sargassum subrepandum*) was measured using the Princen *et al.* (1992) method. Serum was obtained from an adult male white albino rat of the Sprague Dawley strain and diluted with phosphate buffer (50 mM, pH 7.4) to a concentration of 0.6 percent. 10 µl DMSO or 10 µl DMSO with varying quantities of all examined algal extracts were combined with 5.0 ml diluted serum. To start the reaction, 20 µl of $CuSO_4$ solution (2.5 mM) was added, and the absorbance at 234 nm was measured at room temperature every 20 minutes for 140 minutes. The net area under the curve was used to get the final result.

Antibacterial and antifungal tests

Staphylococcus aureus, *Escherichia coli* and *Candida albicans* (from the collection of the Microbiology Department, Faculty of Agriculture, Damietta University, Damietta, Egypt) were used as test microorganisms. The agar cup methods described by Spooner and Sykes (1979) were used to determine the antibacterial and antifungal properties of brown alga (*Sargassum subrepandum*) extracts.

Statistical Analysis

All tests/measurements were performed in triplicate and the results were presented as mean standard deviations (SD). The Student t-test and the MINITAB 12 computer program were used for statistical analysis (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

Proximate composition of brown alga *(Sargassum subrepandum*) powder

Data in Table (1) shows the proximate chemical composition of *Sargassum subrepandum* powder. From such data it could be noticed that carbohydrates were the largest compound (59.68±3.23%) followed by ash (16.23±1.14 %), crude fiber (9.18 ± 2.02 9.18 ± 2.02 %), total protein (4.98 ±0.61%) and crude fat (0.91±0.12%). Such data are in accordance with that reviewed by many authors which mentioned that the greater portion of *Sargassum subrepandum* is carbohydrates (Percival, 1979; Mabeau and Fleurence, 1993; Holdt and Kraan, 2011; Fayez, 2021). Although brown algae are representing low-calorie foods however their carbohydrate content is generally high. It can represent up to 75% of dry weight (Lahaye, 1991). While Holdt and Kraan, (2011) reported that brown algae genus *Sargassum* 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. In several cases, brown algae including *Sargassum subrepandum* carbohydrates possessing a fiber level greater than those recorded for many vegetables or fruits (Darcy-Vrillon, 1993; MacArtain *et al.,* 2007). Hydrocolloids such as alginate, agar, carrageenans, fucoidan, and laminarin are abundant among the fibers in algae. Also, Erniati *et al*., (2016) found that the brown algae genus *Sargassum* contain carbohydrates, proteins, ash, vitamins (vitamins B1, B2, B6, B16, C and niacin), and macro and microminerals comprising K, Na, Mg, P, I, and Fe. Furthermore, Erbabley and Junianto, (2020) reported that chemical composition of brown algae *(S. filipendulla)* comprised 21.61%, 24.79%, 0.19%, 2.31%, and 52.20% of water, ash, fat, protein, and carbohydrate content respectively. The mineral level consisted of 21.52, 0.50, 21.53 and 27.60 mg/g of Mg, Fe, Na, and K, respectively. Brown algae contain fat/lipid between 0.9% and 4% of dry weight (Dawczynski *et al.,* 2007). Whereas Antonopoulou *et al.,* (2005) reported that fats contain up to 1-3% of dry weight. Also, Khotimchenko, (2005) and Mendis and Kim, (2011) found that the brown algae genus *Sargassum* 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 chemical composition of brown algae including *Sargassum* genus varies with one or a combination of the following factors, species, season, geographical location, light intensity and duration, water and air temperature, water depth, nutrients in media, water pH, water salinity and residents' activities (Antonopoulou *et al.,* 2005; Guschina and Harwood, 2006; El-Gamal, 2020).

Nutritional evaluation of brown alga (*Sargassum subrepandum*) powder

The nutritional evaluation of the brown alga (*Sargassum subrepandum*) powder is shown in Table (2). From such date it could be noticed that the total energy was ranged 266.83± 4.73 Kcal/100g, G.D.R. (g) for protein (63 g) was 471.84-1265.06±20.67g, G.D.R. (g) for energy (2900 Kcal) was 1086.83±15.48, P.S./ 100 g for protein (63g) was 7.90±0.80% and P.S./100 g for energy (2900 Kcal) was 9.20±0.62%. The nutritional evaluation reported was partially in accordance with that observed by Fayez, (2021). Although brown algae genus *Sargassum* represents low-calorie foods i.e. consumption of 100 g powder cover only 9.20% of the daily requirement of the adult person for energy (2900 Kcal).

Table 1. Proximate chemical composition of brown alga (*Sargassum subrepandum*) powder

Component (g/100g)	Content
Moisture	9.02±0.98
Total protein	4.98 ±0.61
Crude fat	0.91 ± 0.12
Ash	16.23 ± 1.14
Crude Fiber	9.18 ± 2.02
Carbohydrate	59.68 ± 3.23

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

Table 2. Nutritional value of brown alga (Sargassum subrepandum) powder

Parameter	Value
Energy (Kcal/100g)	266.83± 4.73
G.D.R. (g) for protein (63 g)	1265.06±20.67
G.D.R. (g) for energy (2900 Kcal)	1086.83±15.48
P.S./100 g (%) for protein (63g)	7.90±0.80
P.S./100 g (%) For energy (2900 Kcal)	9.20±0.62

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

This is due to their fat content, the high calories component, is generally low. In a similar study, Dawczynski *et al.,* (2007) found that Brown algae contain fat/lipid between 0.9% and 4% of dry weight while, Antonopoulou *et al.,* (2005) and Rodrigues *et al.,* (2015) reported that fats contain up to 1-3% of dry

weight. Such data confirm the possibility of successfully using the brown algae genus *Sargassum* in nutritional applications for obese and overweight patients.

Bioactive compounds and dietary fiber content in brown alga (*Sargassum subrepandum*) powder

Bioactive compounds in brown alga (*Sargassum subrepandum*) were represented in Table (3). It is possible to deduce from such data that polysaccharides were the largest compound (152.45±19.32 mg starch equivalent. g^{-1}) followed by phenolics (122.67 ± 20.34 mg gallic acid equivalent. g^{-1}), tannins (34.15 ± 6.90 mg catechin equivalent. g^{-1}), carotenoids (30.78± 6.41 mg. g^{-1}), flavonoids (29.31 ± 5.67 mg catechin equivalent. g^{-1}) and anthocyanin's (3.65± 0.44 mg Cyanidin 3-glucoside, CCy3G equivalent.$100g^{-1}$). Also, brown alga (*Sargassum subrepandum*) were riched in dietary fiber (48.78±3.98 g/100g). In a similar study, El-Gamal, (2020) found that the total carotenoids and total phenolics content in ethanol extract were 358-511mg.100 g^{-1} and 593-4278 mg EGA.100 g^{-1}, respectively. 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. Many of those 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). Alginates, the most frequently used of the brown algae extracts, are block copolymers of mannuronic and guluronic acid sugars that have been utilized as thickening agents in the food business and as binders, gelling agents, and wound absorbents in the pharmaceutical industry (Helen, 2003).

From a nutritional point of view, several biological activities for polysaccharides include anticoagulant, antithrombotic, anti-inflammatory, anti-obese, antiviral, immune system-boosting properties and anti-osteoporosis (Nagaoka *et al.,* 2000; El-Gamal, 2020; Elhassaneen *et al.,* 2020). Also, 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 (Ito and Tsuchida, 1972; 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*).* Other bioactive compounds i.e. Phenolics, flavonoids, carotenoids and Anthocyanin's which were determined in the brown algae genus *Sargassum* 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, 2020; El-Gamal, 2020). Such previous effects of these compounds are due mainly to their magical biological/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 (2004) and El-Gamal, (2020) reported many different types of carotenoids were found in brown algae species. Both carotenoids and chlorophylls must be attached to peptides in order to create pigment-protein complexes in the membrane during photosynthesis (Macpherson and Hiller, 2003; Neilson and Durnford, 2010).

Table 3. Total content of bioactive compounds and dietary fiber in brown alga (*Sargassum subrepandum*)

Component	Mean±SD
Dietary fiber (g/100g)	48.78± 3.98
Phenolics (mg gallic acid equivalent. g^{-1})	122.67 ± 20.34
Flavonoids (mg catechin equivalent. g^{-1})	29.31 ± 5.67
Carotenoids (mg .g^{-1})	30.78± 6.41
Tannins (mg catechin equivalent. g^{-1})	34.15 ± 6.90
Anthocyanin's (mg Cyanidin 3-glucoside, CCy3G equivalent.$100g^{-1}$).	3.65± 0.44
Polysaccharides (mg starch. g^{-1})	152.45± 19.32

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

Finally, algae samples were riched in dietary fiber. Carbohydrates are available as polysaccharide, which is not taken up by the human body and are regarded as dietary fibers. 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, brown algae carbohydrates possess a fiber level greater than those recorded for many vegetables or fruits (Darcy-Vrillon, 1993; MacArtain *et al.,* 2007). Hydrocolloids such as alginate, agar, carrageenans, fucoidan, and *laminarin* are abundant among the fibers found in algae. The value of consuming reasonable levels of dietary fiber for

human health has been addressed by several authors (Forsythe *et al.,* 1976 and Ballesteros *et al.,* 2001). Fibers are primarily insoluble and can bind bile acids, according to Camire *et al.* (1993) and Elbasouny *et al.* (2019). One of the processes through which certain forms of dietary fiber lower plasma cholesterol is thought to be bile acid binding. Furthermore, El-Sadany (2001) investigated the hypocholesterolemic effect of dietary fiber and discovered that rats fed potato peels for four weeks had a 40% reduction in plasma cholesterol content and a 30% reduction in hepatic fat cholesterol levels when compared to animals fed only cellulose supplemented diet. Furthermore, a high dietary fiber consumption has a positive impact on blood glucose profiles and related health issues in both healthy and diabetic people of both types. Dietary fiber can affect the absorption of other simple sugars by affecting the stomach emptying time. Many other writers have also proven the effect of dietary fiber on blood glucose and insulin responsiveness (Chandalia *et al.,* 2000 and reviewed in Al-Weshahy and Rao, 2012).

Antioxidant activities of brown alga (*Sargassum subrepandum*) extracts

DPPH radical scavenging activity

The free radical scavenging activity (%) of brown alga (*Sargassum subrepandum*) different extracts and standard (BHT) are shown in Figure 1 and Table 4. It was clear from the statistics that the ethanol extract had the highest activity of the extracts. The radical scavenging activity of water, ethanol, and methanol extracts were 85.00, 92.83, and 88.96 percent, respectively, at a concentration of 100 g/mL, whereas the standard BHT was 96.62 percent. For the IC_{50}, the water, ethanol and methanol extracts were recorded 18.81±0.84, 10.03±0.93 and 12.14±0.77 µg/mL, respectively. The IC_{50} of BHT (standard) was 8.01±0.31 µg/mL. The free radical scavenging activity of different tested extracts and standard was in the following order: standard (BHT)> ethanol extract> methanol extract> water extract**.** The theory of the DPPH radicals scavenging activity test is based on measurement of the *diene conjugation* ( ) by absorption at 234 nm in the presence of DDPH (2,2-diphenyl-1-picrylhydrazyl) substrate is commonly used for determining the oxidative stability of the brown alga (*Sargassum subrepandum*) sample (Antolovich *et al.,*2002). Several studies indicated that The DPPH approach has been successfully utilized to assess the antioxidant activity and oxidative stability of various parts, such as fruits, vegetables, algae, plant by-products, etc. (Kahkonen *et al.,*1999; El-Gamal, 2020; Fayez, 2021). Also, Lien *et al*., (2008) and Elhassaneen *et al.,* (2020) proved that Free radical scavenging activity is critical for preventing the harmful effects of free radicals in diseases such as obesity, diabetes, cancer, cardiovascular, neurological, pulmonary, nephropathy, and bone diseases. The results of this study suggest that all the brown alga (*Sargassum subrepandum*) extracts showed free radical scavenging activity due to their high content of different categories of bioactive compounds (antioxidants) including flavonoids, carotenoids, anthocyanin's, tannins, polysaccharides, phenolics etc.

Inhibition of low density lipoprotein (LDL) oxidation

Dose-dependent inhibition of CuSO4-induced LDL oxidation *in vitro* by brown alga (*Sargassum subrepandum*) extracts is shown in Figure (2). The data indicated that the inhibitive action of the all brown alga (*Sargassum subrepandum*) extracts against CuSO4-induced LDL oxidation, as evidenced by decreased conjugated dienes ( ) production in a dose-dependent fashion. A comparative study between brown algae extracts clarified that the water, ethanol and methanol extracts acted more dramatically in protecting LDL against oxidation. The protecting LDL against oxidation activity of different tested extracts was in the following order: ethanol extract> methanol extract> water extract.

The present data with the others proved that such effect could be attributed to the different bioactive compounds as antioxidants (flavonoids, carotenoids, anthocyanin's, tannins, polysaccharides, phenolics, vitamins, volatile components etc.) contained in such brown algae extracts (Plaza *et al.,* 2010; Shinichi, 2011; El-Gamal, 2020; Fayez, 2021). In a similar study, Aly *et al.,* (2018) found that some plant parts extracts contented the same bioactive compounds including onion skin, tomato pomace and eggplant peels protect LDL against oxidation *in vitro*. Also, Aviram *et al.,* (2000) and

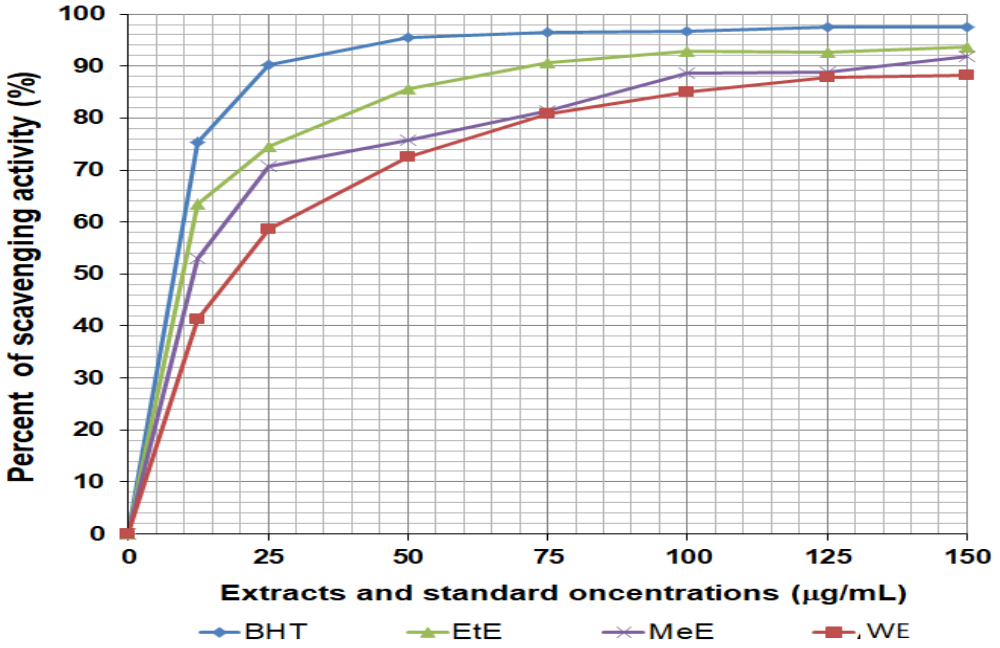

Fig. 1. DPPH radical scavenging activity (%) of brown alga (*Sargassum subrepandum*) extracts and standard (BHT)*

* Each value represents the mean value of three replicates. BHT, Butylated hydroxytoluene, EtE, Ethanol extract, MeE, Methanol extract, WE, Water extract.

Table 4. IC50 (DPPH) of brown alga (*Sargassum subrepandum*) extracts and BHT (Standard)

Name of sample	BHT	WE	EtE	MeE
IC_{50} (µg/mL)	8.01 ± 0.31 ^{c}	18.81 ± 0.84^{a}	10.03±0.93^{b}	12.14 ± 0.77 ^{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≤0.05. BHT, Butylated hydroxytoluene, EtE, Ethanol extract, MeE, Methanol extract, WE, Water extract

Li *et al.*, (2006) have the same effect when applied with pomegranate juice, which was attributed to the high levels of phenolic compounds and ascorbic acid contained in the juice (such as found in brown algae extracts). They also found that the pomegranate peel extract acted more efficiency as compared to the pulp extract, in protecting LDL against oxidation due to its higher content of phenolic compounds. Such mechanisms of actions, protecting LDL against oxidation by phenolic compounds, could be included increasing the levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in the liver and lungs as well as an increase in inhibition of NADPH-dependent lipid peroxidation (Majid *et al.*, 1991; Elbasouny *et al.*, 2019; Elgamal, 2020 and Elhassaneen *et al.*, 2020).

Furthermore, phenolic compounds had a complicated relationship with peroxyl radicals (ROO-) and inhibited LDL oxidation (Laranjinha *et al.*, 1994 and Sadeek *et al.*, 2019). "Oxidative modification of lipoproteins" theory posits that LDL oxidation plays a crucial role in early atherosclerosis, according to various research (Aviram *et al.*, 2000 and Chisolm and Steinberg, 2000). Due to its cytotoxic effects on artery cells, oxidized LDL is atherogenic and promotes monocytes to adhere to the endothelium, resulting in the formation of atheromatous plaques (Hong and Cam, 2015). Our present data with the others proved that the brown alga (*Sargassum subrepandum*) extracts could be used successfully as a promising tool in the prevention of atherosclerosis through inhibiting the LDL oxidation process.

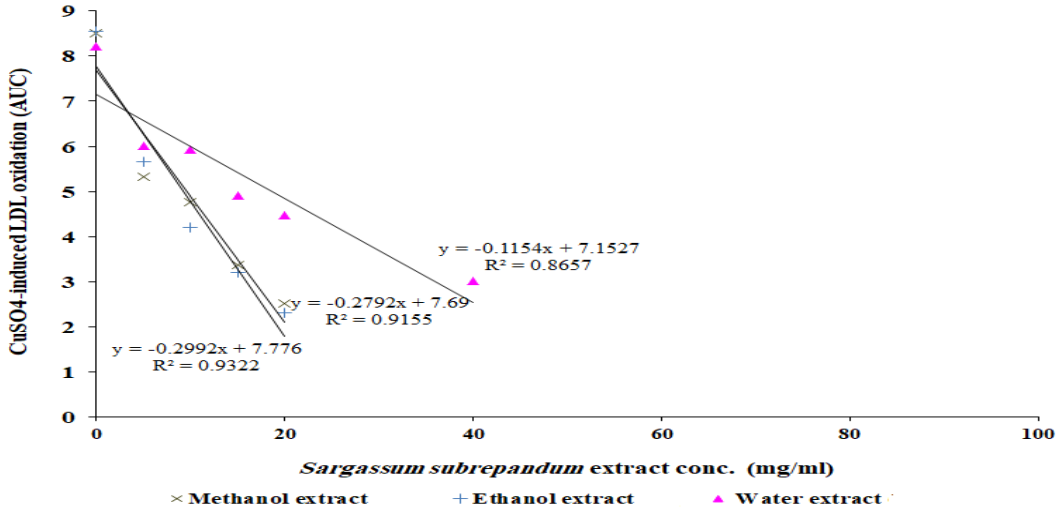

Fig. 2. Dose-dependent inhibition of CuSO4-induced LDL oxidation *in vitro* by brown alga (*Sargassum subrepandum*) extracts. The *conjugated diene* ( ) formation was measured as the absorbance at 234 nm and the result is expressed as the area under the curve (AUC)

Peroxyl radical (ROO-)-scavenging activity

In the present study, the peroxyl radicals ($ROO^{\cdot}$) prevention capacity assay was used to compare the preventive capacity of brown alga (*Sargassum subrepandum*) extracts against $ROO^{\cdot}$ (Li *et al.*, 2006). The process is based on the antioxidants' metal-chelating properties, and the so-called preventive capacity against ROO is linked to the extract's metal-chelating ability. In this approach, ROO- is generated using AAPH [2,2′-Azobis(2-methylpropionamidine) dihydrochloride] and fluorescein is used as a sensitive probe for free radical attack. The results revealed that, as compared to the brown alga (*Sargassum subrepandum*) extracts, the water, ethanol and methanol extract acted more dramatically in preventive capacity against $ROO^{\cdot}$ formation/ scavenging activity (Figure 3).

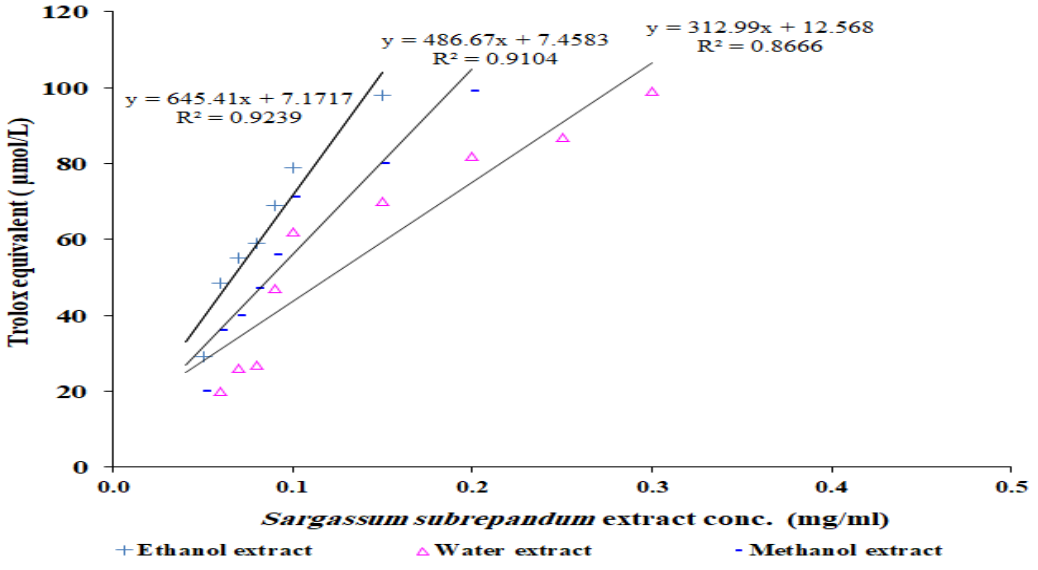

Fig. 3. Dose-dependent $ROO^{\cdot}$ scavenging capacity of brown alga (*Sargassum subrepandum*) extracts as determined by improved oxygen radical absorbance capacity assay

In terms of scavenging ROO-, the ethanol extract appeared to be more effective than the other extracts. Many investigations have shown that ROO- occurs during lipid oxidation in live cells under oxidative stress (Thomas,1999 and Aly, 2017). Free radicals, such as ROO-, can travel a long distance and react with sulfhydryl groups, (-SH), which are found in many biomolecules that make up the body's antioxidant defense system, such as glutathione (GSH) fractions and related enzymes. (Aly, 2017; Elbasouny *et al.,* 2019; EL-Gamal, 2020). Numerous studies have shown oxidative stress plays an important role in the mechanisms of occurrence of many diseases such as diabetes, anemia, cancer, obesity, cardiovascular diseases, bone diseases, aging, pulmonary diseases, etc. (Dhalla *et al.,* 2000; Aly, 2017; Elbasouny *et al.,* 2019; Sajal *et al.,* 2019; Elhassaneen *et al.,* 2020, Sayed Ahmed, 2016; Mehram *et al.,* 2021 $_{a-b}$). Thus, the results of the current study indicated that brown algae (*Sargassum subrepandum*) extracts are a promising tool in the prevention strategy of those diseases through scavenging some of the free radicals formed during oxidation of lipids in oxidative stress.

Antibacterial and antifungal activities of brown alga (*Sargassum subrepandum*) extracts

Antibacterial and antifungal activities of brown alga (*Sargassum subrepandum*) extracts were investigated and shown in Table 5. The highest activity (inhibition zones) against the gram-positive bacteria *Staphylococcus aureus* (17.4±1.3 mm) was recorded for EtE followed by MeE and WE (16.7±0.8 and 14.4±1.5, respectively).

Table 5. Antibacterial and antifungal activities of brown alga (*Sargassum subrepandum*) extracts

Extract	Bacteria		Fungi
	Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
EtE	5.5±0.7*	17.4±1.3[a]	15.8±09 [a]
MeE	5.4±0.2*	16.7±0.8 [a]	15.7±1.2 [a]
WE	4.2±0.5*	14.4±1.5 [b]	11.1±1.4 [b]

BHT, Butylated hydroxytoluene, EtE, Ethanol extract, MeE, Methanol extract, WE, Water extract**.** *Diameter of the inhibition zone less than 10 mm means absence of activity. Means with different superscript letters in the same raw are significantly done differently at p≤0.05.

respectively). The same behavior was observed for the antifungal activity against *Candida albicans.* Also, no activity against Gram-negative bacteria *Escherichia coli*

was detected by all tested extracts. Such variations recorded in the amount of the antibacterial and antifungal activities measured for the *Sargassum subrepandum* extracts are mainly due to the difference in polarities of extracting solvents. The ethanol and methanol solvent are higher in improving the recovery of the bioactive compounds in *Sargassum subrepandum* than water, subsequently increasing their efficiency in inhibiting bacteria and fungi. In a similar study, Kamenarska *et al.* (2002) discovered that only gram-positive bacteria were resistant to toluene (non-polar chemicals, including volatile compounds) and methanol: chloroform (1:1) extracts (compounds with average polarity). The ethanol extract (which contains more polar chemicals) has modest antibacterial and antifungal action in addition to gram-positive bacteria. Also, Omar *et al.,* (2012) reported that the growth inhibitions of bacteria by S*argassum* genus extracts were affected by seasonality. They discovered that such growth inhibitions were more common in autumn samples than in summer samples. The current study's findings, along with those of others, verified brown algae's potential as an antibacterial and antifungal agent against gram-positive bacteria.

In conclusion, the data of the study supported our hypothesis that brown alga (*Sargassum subrepandum*) contains several categories of phytochemicals include phenolics, flavonoids, carotenoids, anthocyanin's, tannins, polysaccharides etc., with other compounds that are responsible for different biological activities. The biological activities studied here including antioxidant activities (DPPH radical scavenging activity, inhibition of low-density lipoprotein (LDL) oxidation and Peroxyl radical (ROO-)-scavenging activity), and antibacterial and antifungal activities. Such important biological effects could play important roles in strategies to combat/treat many diseases, especially those for which oxidative stress is one of the mechanisms for its occurrence. Therefore, we recommended that brown alga (*Sargassum subrepandum*) powder and/or extracts be included in our daily diets, drinks, food supplementation and pharmacological formulae.

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الملخص العربي

التركيب الكيميائي والقيمة الغذائية والمركبات النشطة حيويا والأنشطة البيولوجية للطحلب البنى والتى تم الحصول عليه من البحر الأبيض المتوسط *Sargassum subrepandum* بجمهورية مصر العربية

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تهدف الدراسة الحالية إلى تقدير التركيب الكيميائي والقيمة الغذائية والمركبات النشطة حيويا والأنشطة البيولوجية للطحلب البنى *Sargassum subrepandum* التي تم جمعها من سواحل البحر الأبيض المتوسط بجمهورية مصر العربية. أشارت نتائج التركيب الكيميائي لمسحوق الطحلب البنى *Sargassum subrepandum* الى أن الكربوهيدرات (59.68 ± 3.23٪) كانت أكبر نسبة بين المركبات المقدرة يليها الرماد (16.23 ± 1.14٪)، الألياف الخام (9.18 ± 2.02٪)، البروتين الكلي (4.98 ± 0.61٪)، والدهون الخام (0.91 ± 0.12٪). أيضا ، كان إجمالي الطاقة الكلية (Kcal / 100g) ، الـ G.D.R. بالجرام للبروتين (على اساس 63 جم) ، والـ G.D.R. بالجرام للطاقة (على اساس2900 كيلو كالوري) لنفس العينات 266.83 ± 4.73 ، 1265.06 ± 20.67 جم ، 1086.83 ± 15.48 جم على التوالي. اضافة الى ذلك ، أشار تحليل المركبات النشطة حيويا فى مسحوق الطحلب البنى *Sargassum subrepandum* إلى أن السكريات العديدة (152.45 ± 19.32 مجم مكافئ النشا/جم) كانت تمثل أكبر نسبة ، يليها الفينولات (122.67 ± 20.34 مجم مكافئ حمض الجاليك /جم) ، التانينات (34.15 ± 6.90 مجم كاتشين مكافئ /جم)، الكاروتينات (30.78 ± 6.41 مجم. /جم) ، الفلافونويدات (29.31 ± 5.67 مجم مكافئ كاتشين/جم) والأنثوسيانين (3.65 ± 0.44 مجم مكافئ Cyanidin 3−glucoside, CCy3G / جرام). كما سجلت العينات أيضًا العديد من الأنشطة البيولوجية الهامة بدرجة كبيرة والتي تشمل تثبيط أكسدة البروتين الدهني منخفض الكثافة (LDL) ، وإزالة/كسح الجذور الحرة ، والنشاط المضاد للبكتيريا والفطريات. لذلك يمكن أن تلعب هذه الأنشطة البيولوجية الهامة أدوارًا مهمة في استراتيجيات مكافحة / علاج العديد من الأمراض ، خاصة تلك التي يكون الإجهاد التأكسدي أحد آليات حدوثها مثل مرض السكري والسرطان وتصلب الشرايين وما إلى ذلك. لذا توصى الدراسة الحالية بأن يتم تضمين مسحوق الطحلب البنى *Sargassum subrepandum*وكذلك مستخلصاته في وجباتنا الغذائية اليومية والمشروبات والمكملات الغذائية والمستحضرات الدوائية.

الكلمات المفتاحية: الطحلب البنى *Sargassum subrepandum* ، تثبيط أكسدة البروتينات الدهنية منخفضة الكثافة، الأنشطة المضادة للأكسدة ، النشاط الكاسح للجذور الحرة ، النشاط المضاد للبكتيريا والفطريات.