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Antioxidant properties of marine algae: an overview

Reem Mohammed Alharbi*

Biology Department, College of Science, University of Hafer Al Baten, Hafer Al-Baten, **Saudi Arabia**

***Correspondence:** rmalharbi@uhb.edu.sa **Accepted: 0**1Feb.2019 **Published online:** 12Apr. 2019

Oxidative stress and reactive oxygen species have been linked with the commencement of a variety of chronic human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer. The exigency of components with antioxidant efficacy is growing as it is recognized. The antioxidant effects of these components are mainly assigned to scavenging efficacy against hydroxyl and superoxide radicals, quenching singlet, triplet oxygen, chelating ability, and reducing power. There are various artificial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Propylgallate (PG) and butylated hydroxyquinone (TBHQ) are commercially obtainable and actually applied. However, the use of these artificial antioxidants for medicine or food components has been bounded by the toxicity and safety that can develop to the problems of the potential health in human. Therefore, many investigators have owing interest to many types of innate antioxidants that can be used without toxicity in human. Marine algae are known to be a wealthy source of these components. Antioxidant efficiency has been announced in a great kind of marine algae, including green, brown and red algae. Natural antioxidants from algae are known to play a significant function against various diseases. The discovered antioxidant components in algae from taxa related to these groups have effort anti-inflammatory, anti-aging, dietary, antimicrobial, cytotoxic, antimalarial, anti-proliferative, and anticancer properties. Therefore, the present overview was summarized in the recent research on the antioxidant efficacy of marine algae, methods, and components.

Keywords: Marine algae; brown algae; red algae; green algae; free radicals; antioxidants.

INTRODUCTION

Reactive oxygen species (ROS), such as the superoxide radical (O_2^-), the hydroxyl radical (OH), the peroxide radical (RO) and the nitric oxide radical (NO) in the organisms are produced by non-enzymatic and enzymatic reactions (Eseyin et al., 2015). It may attack biological molecules including lipids, proteins, enzymes, DNA or RNA and by doing so, provoke tissue lesions resulting in ageing, atherosclerosis and carcinogenesis (Mellouk et al., 2017).Nearly, all living organisms have a protection antioxidant device but unable to prevent oxidative damage completely (Wang et al., 2008). Moreover, the body's device technicality would play a role in the form of antioxidants and would try to minimize the injury by acclimating itself to the stressful conditions. Antioxidant components act as free radical scavengers to keep living organisms from the systemic production of (ROS), lipid peroxidation, protein damage and DNA breaking (Kokilam and Vasuki, 2014;Shu et al., 2016; Shao-Chi, 2017).

Lifestyle diseases such as anemia, arthritis, asthma, atherosclerosis, cancer, aging, cardiovascular diseases, diabetes, hypertension, inflammation, myocardial infarction, atherosclerosis, Alzheimer's, cancer and Neurodegenerative diseases are advertise to be occasion by chronic oxidative stress (Li et al., 2014). Also, previous investigations have specified that the hyperlipidemia was

accompanied with the ultra-production of reactive oxygen species (ROS) (Heitzer et al., 2001; Snehal et al., 2009). This oxidative stress has been inspecting by living organisms, utilizing enzymatic and non-enzymatic antioxidant. Therefore, exogenous and endogenous factors affect the antioxidant activity which leads to oxidative stress. Oxidant stress is a phenomenon affecting individuals of all ages and social conditions throughout the world. However, older individuals are, in this respect, more vulnerable due to their weakening antioxidant profile. Indeed, it has been determined that the occurrence of oxidative stress is inversely proportional to the antioxidant capacity of the organism (Sanchez-Roman et al., 2013).

Artificial components with antioxidant efficacy e.g. butylated hydroxyanisole, butylated hydroxytoluene, and tertiary butyl hydroquinone are commonly ancient in processed food that may cause poisonous belongings to the human. Hence, there is an urgency to improve alternative natural antioxidant to clobber these issues.

Recently, many natural antioxidant components have been proved to have prospect antihyperlipidemic efficacy (Adigun et al., 2016). This was interpreted as one of the influential ways to get hypolipidemic medicine. The concept of the antioxidant-rich extract was pilot to the functional food and nutraceuticals sector to fortify and add nutritional value to existing conventional foods such as bread, beverages, and eggs. It would be a cost and time effective strategy in this industry as it does not require stringent isolation of pure components as do medicine and pharmaceutical industries. Actually, it is beneficial to extract a group of effective materials rather than single matter because interaction in a combinational group would report protagonist and synergistic effects that contribute to exalted antioxidant properties (Chan et al., 2013;Thoo et al., 2013).

The earlier decisions confirmed that the natural products from higher plants might support to minimize the effects of oxidative stress (Chan et al., 2013). Several researchers are focusing on these issues to identify potent natural components without any side effects. On the other hand, round seventy percent of our planet is covered by oceans, which contain a wide diversity of marine organisms. These organisms exhibited highly percent of natural products (Wijesekara et al., 2011). Among these marine organisms, marine macroalgae which generally recognized as seaweeds have a group of bioactive substances such as vitamins, fatty acids polysaccharide, polyphenols, and peptides, with different chemical structures and functional properties, supports much health benefits to the living organisms (Marudhupandi et al., 2014).

Marine macroalgae are superabundant along all sea coastlines and the details of their chemical constituents and physicochemical features are desired in order to perfect the growing demand for marine macroalgae products. Marine algae have an extremely numerous of species in the world and been able to be divided into several developmental systems. Broadly, three types of marine algae can be defined according to their color: brown algae, green algae, and red algae. Actually, interest in less-known marine algal species is increasing not only because of their potential active properties due to specific functional components but mostly for their nutritious value (Iba˜nez and Cifuentes, 2013).

In the marine environment, algae are exposed to extreme conditions (ultraviolet light, salinity, temperature and high oxygen concentrations), leading to the formation of free radicals and other oxidizing agents. However, they still developed well without any serious structural and photodynamic damage during metabolism. From this fact, it can be suggested that they possessed some protective mechanisms and/or produced several secondary metabolites which helped them to exist in these harsh conditions (Jiménez-Escrig et al., 2001). On the other hand, the role of the natural antioxidants from algae has been increasing in food or medicinal materials because the synthetic antioxidants (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and alpha-tocopherol) used in reducing oxidative damages need to replace due to side effects such as liver damage, carcinogenesis (Munir et al., 2013). Marine algae have been increasingly attracted in the search for bioactive compounds to develop new drugs and healthy foods (Mohamed et al., 2012). Particularly, brown algae (Phaeophyta) are of great interest due to their potential ability to produce a variety of secondary metabolites such as fucoxanthin, phenolic compounds, sulphated polysaccharide, terpenoids, bromophenols which can benefit human health (Balboa et al., 2013). These components have higher antioxidant potential and found to exhibit anticancer activity against several types of cancers *in vitro* (Dellai et al., 2013).

In brown algae, phenolic compounds have a high ratio compared to the others and are mainly responsible for antioxidant activity of the extracts (Farvinand Jacobsen, 2013). The structure of

these compounds varies from the simple molecules, such as phenolic acid, to more complex compounds constructed by several units of the phloroglucinol monomer (1, 3, 5-trihydroxybenzene) called phlorotannins (Gupta and Abu-Ghannam, 2011). Besides, pigment such as fucoxanthin is one of the most abundant carotenoids of brown algae and estimates around 10% of total carotenoids found in nature (Rajauria et al., 2016). The structure of fucoxanthin includes a usual allenic bond and 5, 6-monoepoxide in its molecule, and almost all of them were *trans*-fucoxanthin in brown algae (Jaswir et al., 2013). Fucoxanthin was also responsible for antioxidant activity of algal extracts reported by several researchers (Foo et al., 2015).

Considerable marine macroalgae species confirm natural antioxidant ability that can safeguard the human body from free radicals and disturb the progress of many diseases such as heart diseases, diabetes, and hypertension (Kolanjinathan et al., 2014; Collins et al., 2016). Flavonoid and Phenolic components were widely established in marine macroalgae emphasize their effective role in forbidding the radical formation, chelating metal ions, and improving the internal antioxidant system under stressful environmental conditions. These activities protect the human body from advanced diseases caused by the adverse effects of reactive oxygen species (Chakraborty et al., 2013).

Thousands of years ago, mankind used marine algae as food and medicine. Marine algae extracts are gaining increasing attention due to their unique composition and the potential for widespread use in industry. A variety of novel (green) extraction techniques have been devised for converting marine algae biomass into marine algae extracts, such as enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and ultrasound-assisted extraction (UAE), which are capable of extracting seaweeds' biologically active compounds without causing degradation (Shao-Chi, 2017).

Antioxidant substances in marine algae contribute to the endogenous defense mechanism against external stressful conditions (Ranjala et al., 2013). Antioxidant properties of some red, brown and green algae extracts have shown that they vary proportion to the content of antioxidative compounds (Zubia et al., 2007; Yuan et al., 2005). In fact, the antioxidant activity in algae acts via several processes and compounds such as lipophilic scavengers (carotenoids), enzymatic scavengers (catalase, superoxide dismutase and peroxidase), and polyphenols (Mittler, 2002). Many studies indicated a close relationship between anticancer activity of algae and their contents of antioxidant compounds such as polyphenols and flavonoids. Marine algae extracts contain substantial amounts of polyphenols such as catechin, epicatechin, epigallocatechingallate, and gallic acid, as reported in *Halimeda* sp. (Chlorophyceae) (Yoshie et al., 2002).

Chunying et al., (2000) described that twenty-seven species of Chinese marine algae were tested for antioxidant activity. Among them, 15 marine algae had significant antioxidant activity in at least one of the organic solvent extracts. The antioxidant activity was high in seaweed species namely *Gloiosiphonia capillaries*. (Cox et al., 2010) investigate the antioxidant properties of edible marine algae; *Himanthalia elongata, Palmaria palmata, Laminaria digitata, L. saccharina, Chondrus crispus* and *Enteromorpha* sp. They found that the total phenolic contents of dried methanolic extracts were significantly different among the different marine investigated macroalgae. They also reported the *H. elongata* had the pivotal phenolic content and also had the definitive DPPH scavenging efficacy with a 50 percent inhibition level at 0.125µg/ml of extract.

Some workers evaluated the total phenolic content and antioxidant efficacy of 50% aqueous methanol extracts of the marine algae: *Kappaphycus alvarezzi*, *Padina antillarum*, and *Caulerpa racemosa*. It was found that, *Padina antillarum* have the capability total phenolic content, 2430 and 208 mg gallic acid equivalents /100 g dried sample and ascorbic acid equivalent antioxidant capacity of 1140 and 85 mg AA/100 g (Chew et al., 2008).

Two main types of antioxidant are responsible for the prevention of the free radicals generation (Cotgreave et al., 1988). Epidemiological data obtained in rodents showed the protective effect of red and green algae against intestinal, skin and breast cancer. Kumar et al., (2008) reported the antioxidant activity of three selected Indian brown marine algae namely *Sargassumm arginatum, Padina tetrastomatica*, and *Turbinariaconoides*. It was found that ethyl acetate fractions of the marine brown alga *Sargassumm arginatum* have the highest total antioxidant activity of ascorbic acid equivalent/g extract. While, petroleum ether fraction of *Turbinaria conoides* recorded lower deoxyribose efficacy. *In-vitro* investigation, the antioxidant efficacy of three Indian red marine

algae, namely *Euchema kappaphycus, Gracilaria edulis,* and *Acanthophora spicifera*was studied. It was found that the ethanol fractions of *Acanthophora spicifera* were recorded the higher total antioxidant activity among all the studied fractions. But *Gracilaria edulis* was reported higher antioxidant efficacy of petroleum ether fraction (Kumaret al., 2008).

Godard et al., (2009) investigated the effects of *Ulva* dietary treatments on hypercholesterolemic of three groups of hamsters, each one with four animals. Hamsters were fed with a high cholesterol diet for 84 days and the high cholesterol diet was replaced for an equivalent fiber weight of the green alga *Ulva*. Plasma cholesterol, non-HDL cholesterol and triglycerides were reduced in the studied hamsters with *Ulva* dietary treatments and increased the liver glutathione peroxidase efficacy and showed thiobarbituric acid reactive substances compared with the control which was fed with cellulose. Plasma antioxidant capacity increased with the treatment and aortic fatty streak area was noticed to be decreased by 70% in *Ulva* dietary rats

Antioxidant activity of ulvan (sulfated polysaccharide) extracted from the marine green alga *Ulva pertusa* was studied by (Qi et al., 2010) . Results reported a significant inhibitory effect of elvan on superoxide and radicals. It isindicated that *Ulva pertusa* has a stronger antioxidant activity. Other work was conducted by Yuan and Walsh (2006) on *Laminaria* and *Porphyra* species. The potential of these algae extracts havethe ability to reduce the risk of intestinal or mammary cancer in the studied animal. Also, evaluation the effect of *Palmaria palmata, Laminaria setchellii, Macrocystis integrifolia,* and *Nereocystis leutkeana* extracts on the human cervical adenocarcinoma cell line (Hela cells) were studied (Yuan and Walsh, 2006). The antiproliferative activity exhibited by the marine algae extracts were positively correlated with the total polyphenol contents, suggesting a link related to the content of polyphenols and phlorotannins including mycosporine like amino acids and phenolic acids present in the studied marine algal species. In similar studies, the antioxidative activity of enzymatic extracts from seven species of brown marine algae was conducted by (Heo et al., 2005). They reported a prominent effects in hydrogen peroxide scavenging activity (approximately 90 percent) higher than that of the commercial antioxidants.

In previous studies, Sachindra et al., (2007) studied the ability of some brown and red marine algae extracts isolated from Indian origin to scavenge different radicals and quench singlet oxygen. It was found that, the methanol extract from brown algal species exhibited higher radical scavenging activity in contract to the red algal species and the activity is correlated to the high polyphenol content in the studied marine algae.

Kolsi et al.,(2017) evaluate the composition and the antioxidant activity of hexane, ethyl acetate and methanol extracts of the green algae (*Codium fragile*) collected from Sfax (Tunisia) during spring 2013. The antioxidant activity of the algal extracts was determined using the procedures of total antioxidant activity, free radical scavenging (DPPH-decolorization method) and ABTS radical-scavenging activity. According to the results, it was noted that this green alga has important carbohydrate content, followed by lipids and proteins with a higher content in polyphenol, flavonoid and low levels of tannins in the three extracts of the seaweed with an interesting antioxidant *in vitro* capacity showed at the methanol extract. The tested extract showed excellent interfacial concentration-dependent properties. Overall, the results suggested that *Codium fragile* is promising source of natural antioxidants and ACE inhibitor agents and could, therefore, be used as alternative additives pharmaceutical preparations.

Hlila et al., (2017) examined the antioxidant and the antimicrobial activities of the marine algae *Padinapa vonica* (*P. pavonica*) and *Enteromorpha* sp. from the Tunisian Mediterranean coast.It was found the highest amount of phenolic compound was recorded in the *P. pavonica* acetone extract (90.61 ± 0.11) mg catechin equivalent/g extract]. This brown algae sample demonstrated greater DPPH and ABTS radical scavenging ability potential in comparison to other green seaweed, *Enteromorpha* sp. They recommended the possible use of *Padina pavonica*as source of antioxidant and antimicrobial compounds.

The potential of two marine green macroalgae (*Cladophora rupestris* and *Codium fragile*) as a source of bioactive phenolic compounds was explored by Gwladys et al., (2017). The antioxidant, mineralogenic, and osteogenic activities also were evaluated. Their results demonstrated a positive correlation between phenolic fractions and biological activity, suggesting that phenolic compounds extracted from marine green macroalgae may represent promising molecules toward therapeutic applications in the field of bone biology

Taheri et al.,(2017) studied the anti-cancer

and anti-oxidant activities of organic and water extracts of brown algae (*Cystoseira indica*) collected from the shores of Chabahar, Iran.The chloroform extract showed the best reducing power compared to the other infusions, with an average of 0.36±0.02 μg/μL. Also, chloroform extract showed the best metal chelating activity with an average of 62.18±0.86 μg/μL (P<0.05). The best free radical scavenging activity observed in the ethanol and methanol extracts with concentrations of 15.83 and 33.21 μg/μL, respectively; the inhibitory activity of methanol extracts was better than ethanol extract (P<0.05). In conclusion they foundthe extract of the brown algae (*Cystoseira indica*) can be proposed as an antioxidant and anticancer compound for preclinical and clinical studies.

Mellouk et al., (2017) evaluated the total phenolic and lipid content, fatty acids profiles and *in vitro* antioxidant activities of aqueous and solvent extracts of the red alga *Asparagopsis taxiformis*, through six different investigations. the results of the antioxidant screening performed at 1.0 mg/ml, revealed that aqueous and methanolic extracts exhibited higher inhibition against superoxide and nitric oxide radicals and excellent radical scavenging activity [with half maximal inhibitory concentration (IC_{50}) values 5.1 and 15.0 μg/ml, respectively], demonstrating improved antioxidant behavior when compared with standard ascorbic acid (which has an IC_{50} value of 3.7 μg/ml). Scavenging activity of the aqueous and methanolic extracts exhibited a strong peroxidation inhibition against linoleic acid emulsion system at a concentration of 300 μg/ml in comparison to the butylatedhydroxyltoluene. Although all the studied extracts exhibited ferric reducing power, the aqueous and methanolic extracts had greater hydrogen donating ability. The antioxidant activity of potent studied marine alga *Asparagopsis taxiformis*in this work stimulated the authers to recommended the using of *Asparagopsis taxiformis* as well as being used as a functional food, may be developed as novel pharmaceutical compounds and may be used as anti-ageing agents. Infurther studies, antioxidant activity of *Laurencia papillosa* dichloromethane, dichloromethane: methanol (1:1,v/v), methanol extracts were investigated by Omar et al., (2018).

Among the three algal extracts, dichloromethane:

Methanol (1:1 v/v) extract exhibited the greatest antioxidant activity with a very low IC50 (110.8 μg mL^{-1}) followed by water extract (225.7 μg mL^{-1}) and finally dichloromethane extract (310.2 μg mL^{-1}).

In parallel studies, Maheswari et al., (2018) investigate the antioxidant, antibacterial activity and GC-MS analysis of active compounds present in the methanol extract of the brown alga *Padina tetrastromatica.* For antioxidant activity, DPPH radical assay, ABTS radical cation scavenging assay, phosphomolybdenum reduction assay and Fe3+ reducing power assay were carried out. This study indicates that the methanol extract of *Padina tetrastromatica* have significant inhibitory activity on clinical pathogens. The phytoconstituents of the algae possess good antioxidant potential and other significant biogenic activities. The presence of 2-Phenyl-4H-1-benzopyran-4-one could be one of the reasons for the antioxidant property of the extract.

In other studies, Dang et al.,(2018) investigate six brown algae (*Sargassum vestitum, Sargassum linearifolium, Phyllospora comosa, Padina sp., Hormosira banksii* and *Sargassum podocanthum*) for the chemical profile and antioxidant activity. The results showed that the extracts *H. banksii, S. vestitum* and *Padina sp.* indicated the significantly higher total phenolic compound (TPC) and antioxidant activities (ABTS, DPPH and FRAP) compared to the other species (P < 0.05) and comparable to positive controls: butylated hydroxytoluene, ascorbic acid and alpha-tocopherol at the concentrations (0.06 mg mL^{-1}). Fucoxanthin was also found in six species and isolated for evaluating antioxidant activity. In addition, the phenolic compounds were mainly responsible for antioxidant activity of the extracts, while fucoxanthin showed quite high antioxidant activity. It is suggested that *S. vestitum, H. banksii* and *Padina sp.* have the potent for extracting bioactive components and further applications in food and pharmaceutical industries.

Also, Chibi et al., (2018) research the antioxidant activity of the red alga *Halopitys incurvus* harvested from El Jadida coast (Morocco). After having set up an adapted protocol of extraction by different solvent polarities (Chloroform/Methanol (2:1, V: V), Chloroform, and Isopropanol/water (7:3, V: V), the antioxidant activity was evaluated by two complementary techniques: on thin layer chromatography (TLC) plate and spectrophotometry using the free radical DPPH (2, 2-diphenyl-1-picrylhydrayl). The tests were validated by comparison with reference antioxidant substances (ascorbic acid and δ-tocopherol).The preliminary screening of the

extracts on TLC plate allowed to target the scavenger activity of the DPPH radical in the various prepared extracts. The evaluation of the scavenging power of the extracts against DPPH by spectrophotometry confirmed the results of the preliminary screening and shows that the extracts resulting from H. incurvus have a real antioxidant activity in the crude extract (Chloroform/ Methanol), and in the Chloroformic extract and in the Isopropanolic extract compared to the EC50 value of δ-tocopherol.

In similar studies Miranda-Delgado et al., (2018) evaluate the content of polyphenols, flavonoids and anthraquinones from sequential extracts from four algae species from along the Chilean coastline: *Desmarestia ligulata, Dictyota kunthii, Laurencia chilensis* and *Chondracanthus chamissoi*. The greatest antioxidant activity was detected in the ethyl acetate and dichloromethane extracts from L. chilensis in its TRAP potential, ethyl acetate of D. kunthii in its FRAP potential, and finally D. ligulata in its DPPH radical scavenging activity.

In addition studies, six brown algae, *Sargassum vestitum, Sargassum linearifolium, Phyllospora comosa, Padina sp., Hormosira banksii* and *Sargassum podocanthum*, were investigated by Dang et al., (2018) for the chemical profile and antioxidant activity. The results showed that the extracts *H. banksii, S. vestitum* and *Padina sp*. indicated the significantly higher total phenolic compound (TPC) and antioxidant activities (ABTS, DPPH and FRAP) comparable to positive controls: butylated hydroxytoluene, ascorbic acid and alpha-tocopherol at the concentrations (0.06 mg mL^{-1}). Fucoxanthin was also found in six species and isolated for evaluating antioxidant activity. They recommended that, six algal species had a significant difference in terms of TPC, TFC, tannins and fucoxanthin as well as antioxidant activities (ABTS, DPPH and FRAP).

Antioxidant procedures

Chemicals and reagents:

DPPH (1,1–diphenyl – 1,2 – picrylhydrazyl), Gallic acid, Rutin , potassium ferricyanide, trichloroacetic acid (TCA), aluminum chloride, Ferric chloride, sodium nitroprusside, sulphanilamide, napthylethylenediamine dihydrochloride, ammonium molybdate, ammonium persulphate, ascorbic acid, sodium nitrite, thiobarbituric acid (TBA), Folin and Ciocalteu's phenol reagent and all solvents used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Collection of marine algal samples

Collection of marine algal samples at a depth of 1–2.5 m. Fresh marine algae thoroughly washed with sea-water and placed in plastic bags. When arrived at the laboratory, the marine algae thalli washed again with tap water then with distilled water and dried in shadow (25 ± 2◦C) for 20 days away from sunlight and heat such as to preserve as much as possible the quality of the initial material. Dried marine algae thalli will milled in a mechanical grinder, to obtain a fine and homogeneous powder (0.2 mm mesh size), they could be stored in well- sealed amber glass bottles at 4◦C until further use. The collected marine algae will thoroughly wash with freshwater and then with distilled water and dried in shade at room temperature. Next, the dried marine algae will blended, sieved and the powder was packed in an airtight container.

Preparation of the algal extracts

The algal extracts could be freeze-dried for 48 h using a freeze dryer and the dried algae will ground to give ≤0.6 mm particle size. The algae will extracted with the ethanol (70%) using ultrasonic bath (Soniclean, 220 V, 50 Hz and 250W; Soniclean Pty Ltd, Stepney, SA, Australia) set at temperature of 30 °C, time of 60 min and power of 150W as described by Dang et al.,(2017). The process could be repeated (n = 3) till the samples became colourless. The extracts will filter and the filtrates will concentrate using a rotary evaporator (Buchi Rotavapor B-480, Noble Park, Vic., Australia). These extracts will freeze-dry to obtain the crude powders and then stored at −20 °C until analysis.

Phycochemical screening

A freshly prepared methanolic extracts of the investigated algae could be qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract will performed using the following reagents and chemicals: Alkaloids with Dragendorff's and Mayer's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions, and saponins with the ability to produce suds. These tests were identified by characteristic color changes using standard procedures (Trease and Evans, 2002).

Determination of polyphenols, flavonoids and condensed tannin content

The total phenolic compounds in marine algae could be determined using the Folin- Ciocalteu reagent according to the method of Marinova et al., (2005). Two hundred µl of the sample (0.1 g/ml) in triplicate will incubated with 1 ml of diluted Folin-Ciocalteu reagent (1:2 with water) for 5 min. 1 ml of 7% Na_2CO_3will added to the reaction mixture which will incubated again for 90 min. thereafter, the absorbance will read at 750 nm using Jenway UV-VIS 6305 spectrophotometer. The total phenolic content is expressed as gallic acid equivalent (GAE) in milligrams per gram of dry sample. Total flavonoid content will determined using assay described by Zhishen et al.,(1999) with slight modifications. Briefly, 1 ml of algal extract (0.1g/ml) will dilute with 4 mL of water and 0.3 mL of NaNO2 (5% w/v) will add. After 5 min, 0.3 mL of $AlCl_3$ (10% w/v) will added followed by the addition of 2 mL of NaOH (1 M) six min later. The volume will increase to 10 mL by adding 2.4 mL distilled water and the sample incubated at RT for 15 min. The absorbance will take at 510 nm. The assay will performed in triplicate, and the flavonoids content will determined by interpolating the absorbance of the samples against a calibration curve constructed with rutin standard (1.0–5.0 mg/mL) and expressed as milligrams of rutin equivalent per gram of extract (mg Rutin/g).

Total condensed tannin content could be determined according to the method of Julkunen-Titto (1985). Briefly, a 50 µl aliquot of each extract was mixed with 1.5 ml of 4% vanillin (prepared with methanol) and then 750 µl of concentrated HClwill added. The solution will shake vigorously and left to stand at room temperature for 20 min in darkness. The absorbance against blank will read at 500 nm. Tannic acid will used to prepare the standard curve and results will express as mg tannic acid equivalents (TE)/g extract.

***In-vitro* antioxidant evaluation**

Reducing power

The reducing capacity of marine algae extracts could be investigated according to the method of Oyaizu (1986). Various concentrations of algal extracts (6.25-50 mg /ml will mixed with 2.5 ml of phosphate buffer (2 M, *p*H 6.6) and 2.5 ml of potassium ferric cyanide (1%), and the mixture will incubated at 50°C for 20 min. After which, then the mixture will incubated at 50 0C for 20 min. 1.5 ml of 10% TCA will added to the reaction mixture which will centrifuged at 1000×g for 10 min. The supernatant (0.5 ml) will mixed with distilled water (1 ml), FeCl3 (0.5 ml, 0.1%) and the absorbance will measured at 665 nm. The higher the absorbance of the reaction mixture the greater is the reducing power.

Evaluation of total antioxidant capacity (TAC) by phosphomolybdenum method:

The total antioxidant capacity could be evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al., (1999). A 0.3 ml of extract will mixed with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After that, the absorbance of the green phosphate/Mo complex will measured at 695 nm. The higher absorbance value indicated higher antioxidant activity.

DPPH free radical-scavenging activity

The free radical scavenging activity of marine algae extracts will measured by DPPH according to Wong, et al., (2006) method. Briefly, 40µl of methanolic extract of algae (25 mg/m) will added to 3 ml of DPPH (0.1 mM) in methanol solution, shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance will measured at 517 nm using a UV- visible spectrophotometer (UV-VIS 6305 model, Jenway, Germany).The percent of DPPH scavenging effect will calculated as follows:

$\% DPPH = ([A_C – A_S]/A_C) \times 100$

Where $A_C$is the absorbance of the control reaction and $A_S$is the absorbance in the presence of the sample.

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity could be measured spectrophotometrically according to Garrat, (1964) method. One ml of sodium nitroprusside (10 mM) in phosphate buffer will added to 0.5 ml of sample (1.25 mg/ml) and incubated at 25 ºC for 150 minutes. Thereafter, 0.5 ml of the reaction mixture containing nitrite ions will removed and added 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid), shaken and allowed to stand for 5 min. then 1 ml of naphthylethylene diamine dihydrochloride (0.1%) will added, mixed and allowed to stand for 30 min. The absorbance of the mixture will measured at 540 nm against the corresponding blank solutions at 546 nm. The percentage of scavenging activity will measure with reference to ascorbic acid as standard.

Deoxyribose radical scavenging activity

An assay mixture containing EDTA (1 mM), FeCl3 (10 mM), H2O2 (10 mM) and deoxyribose (10 mM) will added to the marine algae extracts (12.5mg/mL) dissolved in distilled water with ascorbic acid (1 mM) in 50 mM phosphate buffer. The mixture will incubated at 37˚C for 1 hour and 1.0 mL of the incubated mixture will mixed with 1 mL of 10% TCA and 1 mL of 0.4% TBA (in glacial acetic acid, pH adjusted by NaOH) to develop the pink chromagen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is expressed as percentage inhibition of deoxyribose degradation and will calculated as previously reported (Heo et al., 2005).

Thiobarbituric acid reactive assay (TBARS)

The assay will performed as described by Halliwell and Gutteridge,(1999). Four mgs of the algal extracts will take in a test tube and will evaporated to dryness at 80°C .1 ml of 0.15M potassium chloride will added to the tubes and followed by 0.5ml of linoleic acid (1mg) with 0.15 M KCl. Peroxidation will initiated by the addition of 100 µl of 2mM ferric chloride. After incubating the tubes for 30min at 37°C, the peroxidation reaction will stopped by adding 2ml of ice cold HCL (0.25N) containing 15% TCA & 0.38% TBA. The samples will kept at 80°C for 1 hr, cooled and centrifuged at 7500rpm. The absorbance of the supernatant, containing TBA-MDA complex will read at 532nm. The anti-lipid peroxidation activity (ALP%) will calculated using the formula :

$ALP\% = ([A_C - A_S]/A_C) \times 100$

Where A_C is the absorbance of the control reaction and A_S is the absorbance in the presence of the sample.

CONCLUSION

In conclusion, there are indeed a great variety of antioxidant compounds from marine algae. The priority for the next decades should be focused in the development of new alternative compounds and/or the recovery of natural molecules that would allow the consistent and proper control of many Reactive oxygen species (ROS) related diseases. Complacency and delay will have major detrimental effects on future public health. Marine algae may be an answer to unsolved and growing global problems, a novel untapped source to combat various disorders. The mentioned algae exhibited various antioxidant activities against ROS and it would be an excellent candidate as a natural antioxidant source which can be applied in food and pharmaceutical industry. Detailed information and data on these activities need to be undertaken with individual species. There is a number of challenges ahead like the isolation of the antioxidant components present in the algae also *In vivo* testing on Human beings and further in large-scale controlled studies.

CONFLICT OF INTEREST

The author declared that the present study was performed in the absence of any conflict of interest.

ACKNOWLEGEMENT

No acknowledgment for anybody.

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