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Potential Antimicrobial, Antioxidant and Anityrosenase Activities achieved by Selected Species of Marine Macroalgae

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Marine macroalgae are very rich source of phenolic compounds endowed with antioxidant and antimicrobial properties. The aim of this study was to evaluate the bioactivity composition of three selected green, brown and red algae – viz., *Halimeda opuntia*, *Padina pavonica* and *Halymenia* sp., respectively from Jeddah coast in Saudi Arabia. The antimicrobial properties against pathogenic Grame positive, Grame negative bacteria and fungi were tested. *In vitro* antioxidant activities including 1, 1-Diphenyl -2-picrylhydrazyl (DPPH) radical scavenging, antityrosenase activity and lipid peroxidation activity were studied. The highest amount of phenolic compound was found in the extract obtained from *Padina pavonica* this extract also showed good antioxidant activity expressed by its capacity to scavenge superoxide anion and to inhibit lipid peroxidation. *Halimeda opuntia*, *Padina pavonica* exhibited good antioxidant activity when compared to *Halymenia* sp. The maximum antimicrobial activity was shown by the extract of *Halimeda opuntia*, *Padina pavonica* against *Klebsiella pneumoniae* ATCC 27736 (32mm). The results showed that marine macroalgae exhibited varying degrees of antioxidant and antimicrobial properties.

Key words: Marine macroalgae, antimicrobial, antityrosenase, antioxidant, total phenolic.

Oxidation is essential for living organisms. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent[1]. Reactive oxygen species (ROS) are produced during oxidation[2]. Excessive accumulation of ROS will result in cellular injuries, including lipid peroxidation, protein oxidation, and DNA damage, which are involved in development of a variety of diseases including cellular aging, mutagenesis, carcinogenesis, hepatopathies, diabetes, and neurodegeneration[3]. Damages to the functioning cells later on will cause chronic diseases such as cancers, coronary heart disease, Alzheimer and Parkinson.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Almost all organisms possess antioxidant defense systems including endogenous and exogenous antioxidants. Endogenous antioxidants that naturally present in the body are divided into two; enzymatic antioxidants, including glutathione peroxidases, superoxide dismutase and catalase[4] and also nonenzymatic antioxidant, including α-tocopherol (vitamin E), β-carotene, ascorbate (vitamin C), and glutathione[5]. Incomplete protection of endogenous antioxidants is covered

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by the antioxidants from the outside source, which is known as exogenous antioxidants; including vitamin A, C, E, polyphenols and carotenoids that are obtained through daily food consumption[6]. However, these systems are insufficient to prevent the damage entirely in some cases[7].

The antioxidant is needed to counter act the oxidation process by slowing down or completely terminate the process. Epidemiological and clinical studies on plant-based foods such as vegetables, fruits and medicinal plants have proved that terrestrial plants are rich with antioxidants compounds[8-9]. Macroalgae are the most important source of natural antioxidants[10-11]. They are exposed to very high ultra violet (UV) radiation, high salinity and intensive photosynthetic activities that will lead to the formation of oxidative stress. The presence of carotenoids, photosynthesis pigments and other antioxidative agents is the adaptation mechanism to protect them from the oxidation damages. It has been demonstrated that natural products from marine algae such as phycoerythrobilin, chlorophyll *a*, chlorophyll *b* and fucoxanthin which are accessory pigments have established antioxidant activities[12]. Algae have a broad spectrum of chemical such as polyphenolic compounds[13] and biological activities including antioxidant and free radical scavenging properties[14-16]. Epidemiological studies have indicated that regular consumption of foods rich in phenolic compounds is associated with reduced risk of cardiovascular diseases, neurodegenerative diseases, and certain cancers[17-18].

Marine macroalgae are considered as source of bioactive compounds which have broad spectrum of biological activities. Compounds have been detected in green, brown and red algae with antiviral, antifungal and antibacterial activities[19-21]. Many marine Macroalgae were screened for their antimicrobial activity. Extracted substances from marine algae have antibacterial and antifungal activities[22-24]. Synthetic antimicrobials and antioxidant compounds are commonly used in food industry for preserving food and its quality but those have been suspected of toxic and exerting carcinogenic effect. So the aim of the present study was to estimate the biochemical activities of marine microalgae collected from Jeddah coast, Saudi Arabia, and find the correlations between the antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Chemicals and reagents

Dimethyl sulfoxide (DMSO), sodium carbonate, potassium dihydrogen phosphate, di-potassium hydrogen phosphate and iron (III) chloride 6-hydrate were purchased from Fisher Scientific (Louhgborough, UK). Trichloroacetic acid, 1,1-diphenyl-2-picrylhydrazyl, iron (II) sulfate 7-hydrate, b-carotene (Type 1: synthetic), 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Folin–Ciocalteau's reagent, thiobarbituric acid and linoleic acid were obtained from Fluka (Buchs, Switzerland). Potassium ferricyanide was obtained from Unilab (Mandaluyong City, The Philippines) sodium phosphate buffer, pH 7, methanol and L-(+)-ascorbic acid were purchased from Merck (Darmstadt, Germany). Muller Hinton Agar (MHA), nutrient Agar medium (Difco).

Microorganisms

The organisms used were: Gram positive bacteria namely *Bacillus megaterium* ATCC 25848, *Bacillus subtilus* NRRL B-543, *Sarcina lutea* ATCC27853 and *Staphylococcus aureus*; NRRL B-313, Gram negative bacteria *Escherichia coli*; NRRL B-210, *Pseudomonas aeruginosa* NRRL B23 27853, *Klebsiella pneumoniae* ATCC 27736 and *proteus vulgaris* NRRL B-123. The pathogenic yeast was *Candida albicans* NRRL Y-477 and fungi *Aspergillus niger* NRRL-3. These microorganisms were obtained from Natural Research center, Department of Chemistry of Natural and Microbial product, Cairo, Egypt. The Gram positive and negative bacteria were grown and maintained in nutrient agar media, also the pathogenic yeast and fungi were grown and maintained in MHA medium[21].

Seaweed Collection and Processing

Three marine macroalgae samples, *Halimeda opuntia*, *Padina pavonica* and *Halymenia* sp., were collected from Jeddah coast 2013. The algal sample was handpicked and washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes and then washed thoroughly using tap water. They were shade dried. The dried algae were finally pulverized in the commercial grinder and the powdered algae samples were stored at 4°C and used for further analysis.

Solvent Extraction

Extraction of the algae was carried out with methanol. The solvent was evaporated using rotary vacuum evaporator. Stock solutions of the crude extract was prepared by dissolving the extract using DMSO[25].

Biochemical Analysis

The three algae species were tested for their *in vitro* antioxidant activity by using different assays. Different concentration of the solvent extracted test samples were used *viz*, 0.2, 0.4 0.6, 0.8 and 1.0 mg/ml were used for the assay. Standards were also taken in their respective concentrations.

Estimation of Total Phenolics (TPC)

Total phenolic content (TPC) of algae extracts was determined using the Folin–Ciocalteau assay[26]. The reaction mixture contained 1ml of algae extracts, 0.5ml of the Folin–Ciocalteu reagent, 0.75ml of 20g/100ml sodium carbonate and 3ml of pure water. The mixture was heated in a water bath at 40°C for 20 min and then cooled. The absorbance at 765nm was measured spectrophotometer (Hitachi U-2001, model 121-0032) and used to calculate the phenolic contents using gallic acid as a standard. The total phenolic contents were then expressed as gallic acid equivalent (GAE), in mg/g dry sample. All experiments were performed in duplicate.

DPPH radical-scavenging activity

One milliliter of Algae extracts with different dilutions was added to 2 ml of DPPH (5.9 mg/100 ml methanol) against control[27]. All tubes were incubated for 30min and the absorbance measured at 517 nm. The DPPH scavenging ability was calculated as

$$I(\%) = (1- AS/ AC) \times 100 \quad ...(1)$$

Where AC is the absorbance of the control reaction (containing all reagents except the tested compound) and AS is the absorbance of the tested compound. The % of inhibition was determined from a graph plotting percentage inhibition against extract concentration. All experiments were performed in duplicate.

Lipid peroxidation inhibition (LPI) activity

The antioxidant activity of algae extracts was determined by measuring the % inhibition of peroxidation in a linoleic acid system as described by[28]. The dried extracts (5 mg) for each treatment was added to a solution containing linoleic acid (0.13 ml), 99.8% ethanol (10 ml) and 0.2 M sodium phosphate buffer, pH 7, (10.0 ml). The resulting mixture was then diluted to 25.0 ml with distilled water. To 2.0 ml of the sample solution, was added 1.0 ml of 20% aq. trichloroacetic acid and 2.0 ml of aq. thiobarbituric acid (TBA) solution. The final sample concentration was 0.02% w/v. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on absorbance on the final day. In both methods, antioxidant activity is described by percent inhibition. After stirring (3 min), the absorption was measured at 530 nm. A control was performed with linoleic acid but without extracts. ascorbic acid (200 ppm) was used as a positive control. The maximum peroxidation level observed as 168 h (7 days) in the sample that contained no antioxidant component was used as a test point. Percent inhibition of linoleic acid peroxidation was calculated to express antioxidant activity.

Percent inhibition of linoleic acid peroxidation:

$$= [(\text{Absorbance of control - Absorbance of sample})/ \text{Absorbance of control})] \times 100 \quad ...(2)$$

Tyrosinase inhibition

Tyrosinase inhibitory activity was determined by a spectrophotometric method, as described by[29] using a modified dopachrome method with L-DOPA as the substrate. A 5 mg aliquot of the extract was weighed and dissolved in 2 ml of 50% DMSO. Then, 40 ll of sample was added to 80 ll of 0.1 M phosphate buffer (pH 6.8), 40 ll of 0.02 mg/ml tyrosinase and 40 ll of L-DOPA (2.5 mM) in a well of a 96-well microtitre plate. The samples were incubated for 30 min at 37 °C. Each sample was accompanied by a blank that contains all components except L-DOPA. Absorbance was measured at 475 nm, using 700 nm as a reference. The extract of *Hibiscus tiliaceus* was used as the positive control. Results were compared with a control and a blank containing 50% DMSO in place of the sample solution. Kojic acid and quercetin were used as the positive controls. The percentage of tyrosinase inhibition was calculated as:

$$\%I = [(A \text{ control} - A \text{ sample})/ A \text{ control})] \times 100$$

Where %I inhibition percentage and A is the absorbance.

Antimicrobial Assay

Screening of antimicrobial activity was performed by well diffusion technique[30]. The nutrient agar ((NA) for pathogenic bacteria and Muller Hinton Agar for fungi. The plates were seeded with 0.1 ml of the standardized inoculums of each test organism. The inoculums were spread evenly over plates with glass spreader. The seeded plates were allowed to dry in the incubator at 37° C for 20 minutes. A standard cork borer of 8 mm was used to cut uniform wells on the surface of media and 100 µl of each extract was introduced in the wells. The inoculated plates were incubated at 30-37° C for 24-96 hours and zone of inhibition was measured to the nearest millimeter (mm). The zone of inhibition produced by the algae extracts was compared with DMS as negative control [31].

Statistical Analysis

All experiments were conducted in triplicate (n=3). The mean of parameter phenol and antioxidant actifity were examined for significant by analysis of variance (ANOVA) using statgraphic Centuion XVI. Significant differences between the mean of parameters were determined by using the LSD test (P<0.05)

RESULTS AND DISCUSSIONS

In the present study the green *Halimeda opuntia* extract showed a good radical scavenging activity followed by red *Halymenia* sp., while the brown *Padina pavonica* showed the lowest mean antioxidant activity (Fig 1). Likewise earlier report demonstrated that the brown algae showed maximum antioxidant activity exhibited higher phenolic content[32]. A good DPPH scavenging activity of the macroalgae extracted from the brown seaweed *Padina pavonica* was also studied[33]. The DPPH free radicals scavenging activity in different sp., brown macroalge methanol extracts exceeded 54%, the most active seaweed species being green *Halimeda opuntia* (61%), followed by red *Halymenia* sp (54%) and brown *Padina pavonica* (36%)[34]. The brown algae *L. variegata* showed maximum inhibition in the DPPH radical scavenging assay[35]. In another studies the brown algae *F. vesiculosus* exhibited maximum DPPH radical scavenging activity compared to the red algae[36]. A comparison study on the antioxidant activity of red and brown algae showed that the scavenging activity of all samples on the DPPH radical was found to be strong. In general, the scavenging effects on the DPPH radical increased sharply with increasing concentration of all the samples and standards to a certain extent and then slowly increased. The difference in the DPPH radical

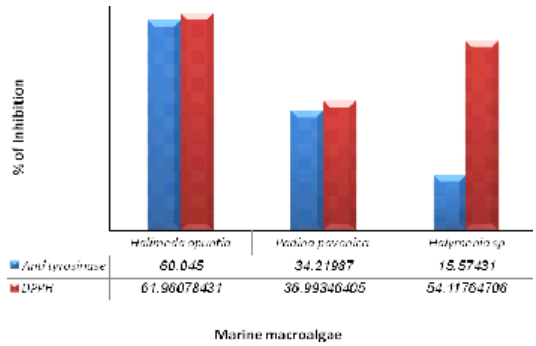

Fig. 1. (DPPH) radical-scavenging activity and *Tyrosinase* inhibition activity of *Marine macralgae*

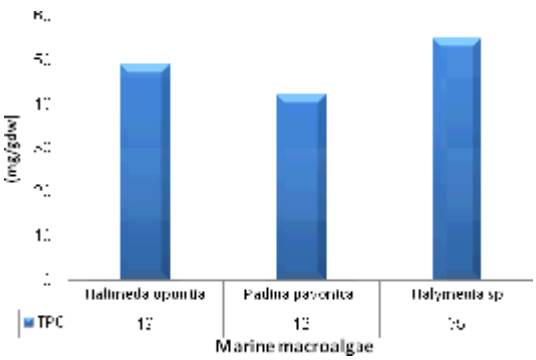

Fig. 2. Total phenolic content (TPC) of *Halimeda opuntia*, *Padina pavonica* and *Halymenia* sp

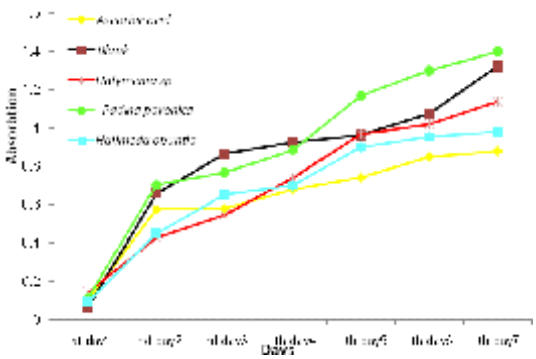

Fig. 3. Inhibition of linoleic acid oxidation by marine macroalgae samples, *Halimeda opuntia*, *Padina pavonica* and *Halymenia* sp.

scavenging activity of each extract in different extracts included that the extracting solvent used would affect the radical scavenging potency. This may be due to the different polarities of each antioxidant compound group present in the seaweeds[37].

The antityrosinase activity also showed in Fig (1) tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions, such as food browning and melanisation of human skin. Therefore, these agents have good commercial potential in both food processing and cosmetic industries. The methanolic extract of 3 macroalgae were screened. *Halimeda opuntia* showed the highest anti-tyrosinase activity and *Halymenia* sp 60% and 34% of inhibition respectively, but slightly lower inhibition apper with *Padina pavonica 15%*. The results obtained were similar to those of [38-39] who did mass screening on several algae species in samples with an inhibition percentage above 50% and were described to have high tyrosinase inhibition activity. Tyrosinase inhibition activity was comparable to *Etlingera* sp. (fulgens, elatior), which fell in the range of 49–55% [40].

The Phenolic compound are commonly found in algae and have been reported to have several biological activity including antioxidant properties. Early reports revealed that marine sea weeds extracts, especially their polyphenols, have antioxidant activity[41]. Total phenolic content (TPC) of marine macroalgae of methanolic *Halimeda opuntia*, *Padina pavonica* and *Halymenia* sp., were reported in (Fig 2). The data showed that, the highest value of total phenolic content were appeared in brown and green algae 55 and 49 mg/gdw respectively, while the red algae recorded the lowest total phenolic content 42mg/gdw.

The existence of phenolic compounds in green, brown and red macroalgae is understood to be associated with their protective mechanisms during certain adverse conditions. Higher amount of phenolic compounds is produced during the hot climate and during the early stage of the growth in order to prevent the photooxidative damage and sea grazers, respectively[41]. On the other hand there are a positive correlation between the DPPH-scavenging activity and total phenolic content of the extracts suggests that the presence of phenolic contents within the algae might be the major contributors to the antioxidant activity of *H. discoidea*. Many studies have examined the role of phenolic contents in relation to the antioxidant activity[42].

The compounds belong to phenolic, flavonoid, tanin, and alkaloid groups, and the compounds with many sulfide groups. In addition, a series of polyphenolic compounds and related phenolic compounds such as epigallocathecin, cathechol, caffeic acid, myricetin and hespedirin have been isolated from the same genus *Halimeda macroloba*[43]. Proton donation causes the violet colored-DPPH radical turn to colorless non-radical compounds. Thus, the radical capture activity could be counted from DPPH radical scavenging. The remaining DPPH radical content was

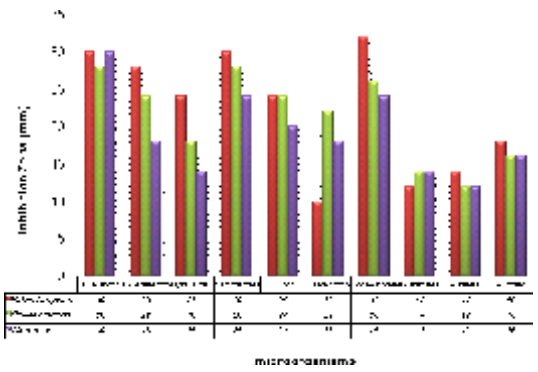

Fig. 4. The diameter of inhibition zone (mm) surrounding *Marine macroalgae* impregnated wells in presence of various microorganisms

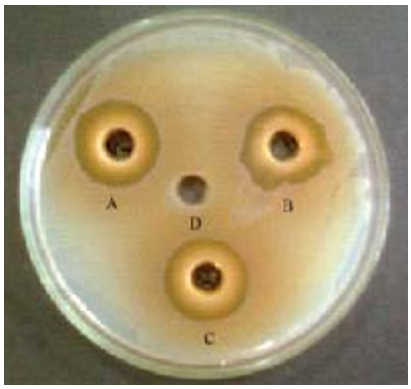

Fig. 5. Agar plates containing zones of inhibition among the Gram negative bacteria *Klebsiella pneumonia, where* (A) *Halimeda opuntia*, (B) *Padina pavonica* (C) *Halymenia* sp and (D) control

spectrophotometrically measured at ≈ 517 nm[44]. The inhibitory ability against the free radicals is affected by the extent of extract concentration. The DPPH test was extensively used in natural product studies for antioxidant isolation and extract and pure compound ability to absorb the radicals.

The results showed in Fig (2) revealed that phenolic compounds existed in the algae extract had significantly contributed to the antioxidant property. However, negative correlation between total phenolic contents and antioxidant capacity did exist in some studies[41]. The mechanism of phenolic compound as an antioxidant relies on the structure of aromatic rings that attached to the hydroxyl groups[45]. The hydrogen atom of the hydroxyl group will be donated to the unstable free radicals and thus terminating the oxidative activity. However, negative correlation between total phenolic contents and antioxidant capacity did exist in some studies[46].

The inhibition of lipid peroxidation of seaweed extracts is shown in Fig. 3. Autooxidation of linoleic acid without the addition of algal extracts or commercial antioxidants was accompanied by a rapid increased in absorbance that reached 1.340 in 7 days (control). The inhibitory effect of green algae at 0.5 mg mL^{-1} is equivalent to BHT and significantly higher than the effects of commercial antioxidants tested. Red algae also showed inhibitory effect of lipid peroxidation, significantly higher than BHA and α- tocopherol. In this assay, the antioxidant activity of brown algae showed the lowest antioxidant activity; nevertheless, its inhibitory effect is equivalent to that of α-tocopherol. These data are in accordance with previous studies that have demonstrated the inhibition of lipid peroxidation by extracts from Rhodophyta[47], Phaeophyta[48] and Chlorophyta[49].

Moreover, these results suggest that antioxidants from *C. baileyana* and *A. longicaulis* are more effective as chain breaking molecules rather than reductors, whereas those of *L. variegata* have very good reductive capacity, but low chain breaking capacity[50]. In the future, identification of these molecules will be helpful to understand the different antioxidant mechanisms observed in this study.

The antimicrobial effects of crude methanol extracts of three species of marine algae (green, red, and brown) on the growth of various Gram positive, negative bacteria and fungi using agar diffusion method are shown in Fig (4). The extracts showed a significant antibacterial activity against Gram positive as well as Gram negative bacteria that confirms previous findings[51-52]. The algal methanolic extracts displayed different degrees of antimicrobial activities against different microorganism, whereas green algae have high active against all tested bacteria *Halimeda opuntia* (green algae) which was in agreement with other reports[53], while brown *Padina pavonica* showed moderated activity against tested strains the *Halymenia* sp. found to be more resistant. The *Bacillus subtilus, Staphylococcus aureus* and *Sarcina lutea* were found to be more sensitive (widest zones of inhibition) among the Gram negative bacteria (*Klebsiella pneumonia Escherichia coli* and *P. aeruginosa*), *Candida albicans* and *Aspergillus niger* was found to be more resistant.

On the other hand, most of tested algae showed inhibitory activity against the tested bacteria (*Klebsiella pneumoniae B. subtilis, S. aureus* and *Bacillus subtilus*) fig (5). In general, the Gram negative bacteria were more resistant (without zones of inhibition), than the Gram positive bacteria.

Overall, antibacterial activity of methanolic extracts in (Fig4) showed algae profoundly distinct antibacterial activity by having observable inhibition with diameters ranging from12 to 32 mm on tested bacteria. In this study, the brown and green algae extracts were found more active than red algae extracts, however green algal extracts yield higher antibacterial activity than brown algae extracts which was in parallel with earlier investigation[54]. Most of the active compounds of marine algae show antibacterial activities. Many metabolites isolated from marine algae have been shown to possess bioactive effects[55]. However, Antimicrobial activity depends on algal species and on extraction efficiency of their active compounds as well as location, seasons of the year and temperature of the water. In contrast, our results showed that the methanol extract of *U. lactuca* inhibited all the test organisms. This difference may be attributed to location or seasonal variations[56].

CONCLUSION

In conclusion, Marine algal natural products are rich sources of antioxidants. In fact, some marine algae are edible. Our results indicate that the antioxidant properties of three kinds of algae have been investigated the algal species collected in the current study from Jeddah coast represents rich source of valuable medicine compounds and their extracts exhibit a significant capacity of antibacterial activities especially green algae. In the future, therefore screening their natural products will be of great interest and further studies should be undertaken to characterize the active compounds residing in these types of algae as well as to evaluate the effects of each individual compound on microorganisms. Moreover, toxicological studies are need to be performed for drug discovery

REFERENCES

1. Sies H., Oxidative stress: Oxidants and antioxidants". *Experimental Physiology*. 1997; **82**(2): 291–5.
2. Hu L, Yu W, Li Y, Prasad N and Tang Z., Antioxidant activity of extract and its major constituents from okra seed on rat hepatocytes injured by carbon tetrachloride. *BioMed Research International*. 2014: 1-9.
3. Supardya NA, Ibrahima D, Sulaimanb SF and Zakariaa NA., Free radical scavenging activity, total phenolic content and toxicity level of *Halimeda discoidea* (decaisne) extracts (Malaysia's green macroalgae). *International Journal of Pharmacy and Pharmaceutical Sciences.* 2011; **3**(5): 397-402.
4. Langseth L., The data support a role for antioxidants in reducing cancer risk. *Oxidants, Antioxidants and Disease Prevention* International Life Sciences Institute (ILSI Europe) Brussels, Belgium 1995.
5. Droge W., Free radicals in the physiological control of cell function. *Physiol. Rev.* 2002; **82**: 47-96.
6. Pietta PG., Flavonoids as antioxidants. *J. Nat. Prod.* 2000; **63**:1035-1042.
7. Simic MG., Mechanisms of inhibition of free-radical processes in mutagenesis and carcinogenesis. *Mutation Research.* 1988; **202**(2): 377–386.
8. Matsukawa R, Dubinsky Z, Kishimoto E, Masaki K, Masuda Y, Takeuchi T, Chihara M, Yamamoto Y, Niki E, Karube IA., Comparison of screening methods or antioxidant activity in seaweeds. *J. Appl. Phycol*. 1997; **9**: 29-35.
9. Aguilera J, Bischof K, Kartsen U, Hanelt D, Wiencke C., Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defense systems against high light stress. *Mar. Biol.* 2002; **140**: 1087-1095.
10. Prasad KN, Xie HH, Hao J., Antioxidant and anticancer activities of 8-hydroxypsoralen isolated from wampee [*Clausena lansium* (Lour.) Skeels] peel. *Food Chemistry*. 2010; **118**(1): 62–66.
11. Farasata M, Khavari-Nejada RA, Nabavib SMB and Namjooyanc F., Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweed from Northern Coasts of the Persian Gulf. *Iranian Journal of Pharmaceutical*. 2014; **13**(1): 163-170.
12. Pangestuti R and Kim SK., Biological activities and health benefit effects of natural pigments derived from marine algae. *J. Funct. Foods.* 2011; **3**(4): 255-66.
13. Ramah S, Etwarysing L, Auckloo N, Gopeechund A, Bhagooli R, Bahorun T., Prophylactic antioxidants and phenolics of seagrass and seaweed species: A seasonal variation study in a Southern Indian Ocean Island, Mauritius. *Internet Journal of Medical Update*. 2014; **9**(1): 27-37.
14. Zhang WW, Duan XJ, Huang HL, Zhang, Y, Wang BG., Evaluation of 28 marine algae from the Qingdao coast for antioxidative capacity and determination of antioxidant efficiency and total phenolic content of fractions and subfractions derived from *Symphyocladia latiuscula* (Rhodomelaceae). *J. Appl. Phycol.* 2007; **19**: 97-108.
15. Murugan K and Iyer VV, Antioxidant and antiproliferative activities of extracts of selected red and brown seaweeds from the Mandapam Coast of Tamil Nadu. *Journal of Food Biochemistry.* 2014; **38**: 92–101.
16. Kokilam G and Vasuki S., Biochemical and phytochemical analysis on *Ulva fasciata* and *Caulerpa taxifolia. International Journal of Pharmacy and Pharmaceutical Science Research.* 2014; **4**(1): 7-11.
17. Soory M., Nutritional antioxidants and their applications in cardiometabolic diseases. *Infectious Disorders.* 2012; **12**(5): 388-401.
18. Aboul-Enein HY, Berczynski P and Kruk I, Phenolic compounds: the role of redox regulation in neurodegenerative disease and cancer. *Mini-Reviews in Medicinal Chemistry.* 2013; **13**(3):

385-398.
19. Hellio GB, Brrener AM, Pons G, Cotlenceau Y and Le Gal Borgongman., Antibacterial and antifungal activities of extracts of marine algae from Breittany. France. Use as antifouling agents. *Applied Microbiology and Biotechnology*. 2002; **54**: 543–549.
20. Newman DJ, Cragg GM and Snader KM., Natural products as source of new drugs over the period 1981-2002. *Journal of Natural Products* 2003; **66**: 1022-1037.
21. Tuney I, Cadirci B.H, Unal D. and Sukatar A., Antibacterial activities of the Extracts of marine algae from the coast of Urla (Izmir, Turkey). *Turkish Journal of Biology*. 2006; **30**: 171-175.
22. Rizvi MA and Shameel M., Pharmaceutical Biology of seaweeds from the Karachi Coast of Pakistan.*Pharmaceut. Biol*. 2005; **43**(20): 97-107.
23. Asnad and Abbass T., Screening of potential seaweeds against *Fusarium* species isolated from fruits and vegetables in Baluchistan, Pakistan. *International Journal of Biosciences*. 2014; **4**(3):131-138.
24. Arunachalam P, Uthandakalai R, Rajsmail R., Evaluation of antibacterial activity of some selected green seaweed extracts from Muttam coastal areas, Kanyakumari, Tamil Nadu, India. *Journal of Coastal Life Medicine*. 2014; **2**(2): 112-115.
25. Sanger G , Widjanark SB, Kusnadi J, Berhimpon S., Antioxidant Activity of Metanol Extract of Sea Weeds Obtained from North Sulawesi *Food Science and Quality Management*. 2013; **19**: 63-70
26. Zhou K., and Yu L., Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. Lebensmittel-Wissenschaft und- Techn., 2006; **39**: 1155–1162.
27. Kaur R, Geetanjali R and Adarsh V., Evaluation of antifungal and antioxidative potential of hydrolytic products of glucosinolates from some members of Brassicaceae family. *J.P.B.C.S.*, 2011; **3**(10): 218-228.
28. Ozsoy N, Can A, Yanardag R, Akev N., Antioxidant activity of Smilax excelsa leaf extracts. *Food Chem*., 2008; **110**: 571-583.
29. Chan E CW, Lim Y Y, Wong L F, Lianto F S, Wong S K and Lim K K., Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chemistry*, 2008; **109**: 477–483.
30. Kivanc M and Kunduhoglu B., Antimicrobial activity of fresh plant juice on the growth of bacteria and yeast. *J. Qafqaz Univ*., 1997; **1**: 26-53.
31. Ishrat R, Shaista A and Hidatullah A., Antimicrobial potential of seeds extract of Raphanus sativus, *Pak. J. Bot.*, 2008; **40**(4): 1793-1798.
32. Kumaran A and Karunakaran RJ., In vitro antioxidant properties of methanol extracts of five Phillanthus species from India. LWT, 2007; **40**: 344–352.
33. Hong Ye, Keqi Wang, Chunhong Zhou, Jun Liu, Xiaoxiong Zeng. Purification, antitumor and antioxidant activities in vitro of polysaccharides from the brown seaweed Sargassum pallidum, *Food Chem* 2008; **111**: 428–432.
34. Yan XJ, Chuda Y Suzuki M and Nagata T Fucoxanthin as the major antioxidant in Hijikia fusiformis, a common edible seaweed. *Biosci Biotechnol Biochem*, 1999; **63**: 605–607
35. Wei Y, Li Z, Hu Y and Xu Z., Inhibition of mouse liver lipid peroxidation by high molecular weight phlorotannins from Sargassum kjellmanianum. *J Appl Phycol*, 2003; **15**: 507–511.
36. Ismail A, Hong TS., Antioxidant activity of selected commercial seaweeds. *Mal J Nutr*, 2002; **8**: 167–177.
37. Marinova EM, Yanishlieva NV., Antioxidative activity of extracts from selected species of the family Lamiaceae in sunflower oil. *Food Chem*, 1997; **58**: 245-248.
38. Baurin N, Arnoult E, Scior T, Do Q T and Bernard P., Preliminary screening of some tropical plants for anti-tyrosinase activity. *Journal of Ethnopharmacology*, 2002; **82**: 155–158
39. Lee K T, Kim B J and Kim J H., Biological screening of 100 plant extracts for cosmetic use (1): Inhibitory activities of tyrosinase and DOPA auto-oxidation. *International Journal of Cosmetic Science*, 1997; **19**: 291-298.
40- Chan E C W, Lim Y Y, Wong L F, Lianto F S, Wong S K and Lim K K., Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chemistry*, 2008; **109**: 477-483.
41. Del Val AG, Platas G, Basilio A, Cabello A, Gorro-chateui J, Suay I, Vicente F, Portillo E, DeRio MJ, Reina GG and Pelaez F., Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *International Microbiology*, 2001; **4**: 35-40.
42. Lavanya R. and Veerappan N., Antibacterial potential of six seaweeds collected from gulf of mannar of southeast coast of India. *Advances in Biological Research*, 2011; **5**: 38-44.
43. Vairappan CS, Daitoh M, Suzuki M, Abe T and

Ma suda M., Antibacterial halogenated metabites from the Malysianlaurencia species. *Phytochemistry*, 2001; **58**: 291- 297.

44. Kumar, K. S., K. Ganesan, and P.V. Subba Rao., Antioxidant Potensial of Solvent Extract of Kappaphycus alvarezii (Doty) DotyAn edible seaweed. *J. Food Chemistry* 2008; **107**: 289 – 295.
45. Venkateswarlu S, Panchagnula GK, Gottumukkala AL and Subbaraju GV 2007 Synthesis, structural revision, and biological activities of 4'-chloroaurone, a metabolite of marine brown alga Spatoglossum variabile. Tetrahe-dron, 2008; **63**: 6909-6914.
46. Oh KB, Lee J, Chung S, Shin J, Shin HJ, Kim HK and Lee HS., Antimicrobial activities of the bromophenols from the red alga Odonthalia corymbifera and some synthetic derivatives. *Bioorganic and Medicinal Chemistry Letters*, 2008; **18**: 104-108.
47. Yuan YV, Bone DE and Carrington MF., Antioxidant activity of dulse (Palmaria palmata) extract evaluated in vitro. *Food Chem* 2005; **91**: 485-494.
48. Lim SN, Cheung PCK, Ooi VEC and Ang PO., Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum*. *J Agric Food Chem* 2002 ; **50**: 3862-3866.
49. Cavas L, Yurdakoc K., A comparative study: assessment of the antioxidant system in the invasive green alga Caulerpa racemosa and some macrophytes from the Mediterranean. *J Exp Mar Biol Ecol* 2005 ; **321**: 35-41
50. Zhang P and Omaye ST., Antioxidant and prooxidant roles for β- carotene, α-tocopherol and ascorbic acid in human lung cells. *Toxicol In Vitro* 2001; **15**: 13-24.
51. Tuney I, Cadirci BH, Unal D and Sukatar A., Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). *Turkish Journal of Biology*, 2006; **30**: 171-175.
52. Patra JK, Rath SK, Jena K, Rathod VK and Thatoi H., Evaluation of antioxidant and antimicrobial ac-tivity of seaweed (Sargassum spp.). Extract: A study on inhibition of glutathione-S-transferase activity. *Turkish Journal of Biology*, 2008; **32**: 119-125.
53. Del Va AG, Platas G, Basilio A, Cabello A, Gorro-chateui J, Suay I, Vicente F, Portillo E, DeRio MJ, Reina GG and Pelaez F., Screening of antimicro-bial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *International Mi- crobiology*, 2001; **4**: 35-40.
54. Lavanya, R and Veerappan N., Antibacterial potential of six seaweeds collected from gulf of mannar of southeast coast of India. *Advances in Biological Research*, 2011; **5**: 38-44.
55. Vairappan CS, Daitoh M, Suzuki M, Abe T and Ma- suda M., Antibacterial halogenated metabites from the Malysianlaurencia species. *Phytochemistry*, 2001; **58**: 291- 297.
56. Perez RM, Avila JG and Perez G., Antimicrobial activity of some American algae. *Journal of Ethno- pharmacology*, 1990; **29**: 111-118.