ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 11, Issue 4, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICAL SCREENING AND HEAVY METAL ANALYSIS OF *ULVA RETICULATA*

GOMATHI K, ANNA SHEBA L*

Department of Biochemistry, School of Life Sciences, Vels University, Pallavaram, Chennai, Tamil Nadu, India.
Email: lawrencesheeba@gmail.com

Received: 10 October 2017, Revised and Accepted: 15 December 2017

ABSTRACT

Objective: *Ulva reticulata* is a marine edible green seaweed widely distributed along the coastal lines of India. The present study was designed to screen the phytochemicals and evaluate heavy metals content of *U. reticulata* collected from Rameshwaram, Tamil Nadu, India, in the perspective of assessing their therapeutic value and/or safety in relation to its uses.

Method: The seaweed sample was subjected to extraction with solvents of different polarities (methanol, ethanol, acetone, chloroform, and petroleum ether) and screened for phytochemicals according to standard methods. Heavy metal analysis was also performed with the dried sample powder using inductively coupled plasma optical emission spectrophotometer (ICP-OES).

Result: Preliminary phytochemical analysis revealed the presence of reducing sugar, proteins, flavonoids, glycosides, alkaloids, and anthraquinones. ICP-OES indicated the seaweed to contain relative amount of heavy metals in the order of iron (Fe) > chromium (Cr) > Zinc (Zn) > nickel (Ni) > copper (Cu) > lead (Pb) - cadmium (Cd) and is within the permissible limits set by the WHO/FAO, except Fe and Cr.

Conclusion: The different solvent extracts of *U. reticulata* showed the presence of the number of phytochemicals. Furthermore, the present study has revealed the presence of heavy metals in *U. reticulata* which can be a representative picture of the dissolved metals in the aquatic phase.

Keywords: *Ulva reticulata*, Phytochemicals, Inductively coupled plasma optical emission spectrophotometer, Heavy metals.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i4.23012

INTRODUCTION

Marine organisms serve as the prolific source for natural products with therapeutic and nutritive properties. Many of these products do not exhibit structural and chemical features of the terrestrial natural products. They are structurally unique compounds of biomedical importance [1]. Among the marine organisms, seaweed or macroalgae have attracted the biologist as it produces a vast number of bioactive compounds.

Edible seaweeds have been found to have high nutritional value as they are the rich sources of carbohydrates, minerals, proteins, pigments, and vitamins [2]. Since ancient times, these plants have been exploited as a source of food, feed, cosmetics, fertilizer, and source of traditional medicine in many countries, in particular, it has been a staple food in Southeast Asian countries [3,4]. Seaweeds as a whole or its constituents are incorporated into food for consumption, markedly as a whole food, and it is not considered as a component of western diet [5]. However, seaweed consumption has been increasing all over the world due to their natural composition. Its health-promoting effects and nutritive value are attributed to their phytochemicals, polysaccharides, vitamins, fiber, low-fat content, low calorific value, and minerals. Seaweeds are generally rich in phytochemicals with antiviral [6], antioxidant [7], antifungal [8], antibacterial [9], antitumor [10], antihypertensive [11], antihyperlipidemic [12], and antiproliferative activities [13]. Commonly, the phytochemicals in seaweeds belong to the chemical classes including brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulfur-nitrogen heterocyclics, sterols, terpenoids, polysaccharides, peptides, proteins, halogenated ketone, alkanes, and cyclic polysulfides [14]. Besides their use as food, feed, and medicine, seaweed also has the capacity to bioaccumulation of heavy metals at a rapid rate. Although some of the heavy metals at low concentrations are necessary for the biological process such as the growth of the plants, at threshold concentration these become toxic, these heavy metals are introduced into the aquatic system due to anthropogenic activities [15]. It has been reported that, among the heavy metals arsenic, cadmium, chromium, lead, and magnesium rank the five to affect public health [16].

Ulva sp. (Phylum Chlorophyta, Class *Ulvophyceae*, Order *Ulvales*, Family *Ulvaceae*), a group of green algae, is widely distributed along the coasts of the world's ocean [17,18]. So far, few species of *Ulva* have been studied extensively. It has been used as food and nutritional supplement in Southeast Asian countries and North and South America. *Ulva reticulata* is one of the edible seaweeds of the *Ulva* genera [19]. This species is light to dark green in color and is found on rocks in a low intertidal zone along shorelines with moderate water movement [20].

As per the data from algaebase, *U. reticulata* is found to be widely distributed in Indo-west Pacific region, Southeast Asia, Southwest Asia, Northern Pacific Ocean, and Eastern and Western Indian Ocean. In South India, coastal lines of Tamil Nadu, particularly Gulf of Mannar, Rameshwaram to Kanyakumari, are highly concentrated with *U. reticulata* [20].

Although *macroalgae* exhibited a broad spectrum of nutritional and therapeutic values, its bioaccumulating property emphasizes the need to determine its heavy metal composition to ensure safety consumption. In this context, this study was aimed to screen the phytochemicals and evaluate the heavy metal content of *U. reticulata* collected from Rameshwaram, Tamil Nadu.

METHODS

Collection of seaweed

The seaweed *U. reticulata* was collected from Rameshwaram, Tamil Nadu, India (Latitude: 9° 16' 48" N; Longitude: 79° 18' 0" E). The study area is laid by Southeast of Bay of Bengal and Indian Ocean.

After collection, the seaweed was thoroughly washed with sea water and subsequently with tap water and distilled water to remove the epiphytes, sand, and mud particles. The collected seaweed was authenticated by Dr. P. Jayaraman, Plant Anatomy Research Center, Tambaram, Chennai. The collected seaweed was allowed to shadow dry and was finely powdered using mixer grinder.

Heavy metal analysis

1 g of homogenized sample was taken in a cleaned microwave digestion vessel. An exothermic reaction during digestion process was avoided by adding 1 ml of hydrogen peroxide. 8 ml of suprapure HNO3 was added into the vessel. 8 ml of suprapure HNO3 was added into an empty flask as well, which served as blank. The vessels were capped after 10 min and then kept in microwave hot block unit. The digestion cycles were carried out according to ramping method. After completion, the digested sample was allowed to cool to room temperature by keeping it in hume hood. The digested sample was filtered using No 1 Whatman filter paper and diluted accurately with 25 ml double ionized water and then subsequently transferred into 50 ml Tarson tube for spectrophotometric analysis. The samples were analyzed quantitatively for Cu, Cr, Ni, Fe, Pb, Cd, and Zn using inductively coupled plasma optical emission spectrophotometer (ICP OES-720ES-Agilent). The analysis of all of the minerals was carried out in triplicates. All the chemicals and solvents used for experiments were of analytical grade.

Preparation of extract

Dried and coarsely powdered *U. reticulata* was separately macerated with solvents such as methanol, ethanol, acetone, chloroform, and petroleum ether in the ratio 1:5 (g/ml) and kept soaked for 4 d with mechanical shaking at regular intervals. The solvents were filtered, distilled under vacuum, and dried in a vacuum desiccator to obtain the methanol, ethanol, petroleum ether, chloroform, and acetone extract of *U. reticulata*. The different extracts obtained were subjected to preliminary phytochemical screening following standard methods [21,22].

Phytochemical analysis

Test for carbohydrates

Molisch test

Few drops of Molisch reagent was added to 2 ml of the extract and mixed well. 2 ml of concentrated sulfuric acid was added to this solution. Formation of the red-violet ring at the junction of the solution indicates the presence of carbohydrates.

Fehling's test

1 ml of Fehling's A and 1 ml of Fehling's B solutions were added to 1 ml of the extract in a test tube and heated in a water bath for 10 min. Formation of red precipitate indicates the presence of reducing sugar.

Test for phenolic compounds

Ferric chloride test

A little extract was dissolved in 2 ml of distilled water. This was followed by addition of few drops of 10% ferric chloride. Formation of blue/green color indicates the presence of phenols.

Test for alkaloids

Extracts were dissolved individually in dilute HCl and filtered.

Dragendroff's test

Dragendroff's reagent was added to the filtrate, and the formation of orange precipitate indicates the presence of alkaloids.

Mayer's test

To 1 ml of the filtrate, 2 ml of the reagent was added. Formation of white or pale precipitate shows the presence of alkaloids.

Test for flavonoids

Alkaline reagent test

The extract was stirred with a few drops of sodium hydroxide. Formation of intense yellow color, which becomes colorless on the addition of few drops of dilute acid, indicates the presence of flavonoids.

Test for tannins

To 2 ml of the extract, 2 ml of distilled water and few drops of ferric chloride solution were added. Formation of green precipitate was an indication of the presence of tannins.

Test for saponin

5 ml of extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam indicates the presence of saponin.

Test for quinone

1 ml of the extract was treated with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

Test for anthraquinone

1 ml of the extract was macerated with ether, and after filtration, aqueous ammonia was added. The formation of red, pink, or violet coloration in the aqueous layer after shaking indicates the presence of anthraquinones.

Test for glycosides

Picric acid was added to the extract and made alkaline. A stable orange color was formed in the presence of glycosides.

Test for proteins

To 1 ml of the extract, few drops of Biuret reagent were added. The formation of the blue color indicates the presence of protein.

RESULTS AND DISCUSSIONS

Although Indian coastal regions are rich in *Ulva* sp., they are underutilized and yet to be popularized. According to literature, the chemical composition of seaweed contributes to its efficacy as nutraceutical and traditional medicine. On the other side, seaweeds of genus *Fucus*, *Enteromorpha*, *Laminaria,* and *Ulva* are tagged as bioaccumulation of heavy metals [23]. The WHO in several of its recommendations has emphasized the need to ensure the safety of edible plants and plant products in terms of their heavy metal composition. It was in this standpoint, and our study was attempted to carry out preliminary screening and heavy metal analysis in *U. reticulata*.

Phytochemical analysis

In the present study, the preliminary phytochemical analysis was performed with methanol, ethanol, acetone, chloroform, and petroleum ether extracts. The results were depicted in Table 1.

A good solvent depends on its property of optimal extraction and its capacity in conserving the stability of the chemical structure of desired compounds [24]. Among the five different extracts, methanol, chloroform, and acetone extracts showed the presence of a maximum number of phytochemical compounds, whereas the ethanol extract showed the presence of a minimum number of compounds. It has been certainly understood that factors such as species, subspecies, geographical location, harvest, and method of extraction affect the chemical composition of the extract obtained from the seaweed [25]. The present investigation illustrates the presence of reducing sugar, flavonoids, glycosides, alkaloids, anthraquinones, phenolic compounds, and proteins and absence of saponins, quinones, and tannins.

Reducing sugars and proteins which are the basic nutrients showed its presence in all the extracts. Marine algae are the most important source of carbohydrates. The marine carbohydrates demonstrate a wide spectrum of industrial, biomedical, and biological applications [26,27]. Many algal soluble polysaccharides are found to be related to hypercholesterolemia and hypoglycemic activities [26]. *Ulva* sp. is a potent source of ulvan, a water-soluble sulfated polysaccharide possessing antioxidant, anticoagulant,

Table 1: Preliminary phytochemical screening of different solvent extracts of *U. reticulata*

Phytoconstituents	Methanol	Ethanol	Acetone	Chloroform	Petroleum Ether
Phenolic compounds	-	-	-	+	-
Carbohydrates	+	+	+	+	+
Flavonoids	-	-	+	-	-
Glycosides	+	-	+	+	+
Saponins	-	-	-	-	-
Alkaloids	+	-	+	+	+
Anthraquinones	+	-	-	-	-
Quinones	-	-	-	-	-
Proteins	+	+	+	+	+
Tannins	-	-	-	-	-

U. reticulata: Ulva reticulate, +: Present, - : Absent

immunostimulatory, antilipidemic, antibacterial, antiprotozoal, and antifungal activities [28].

Green seaweeds contain high amounts of polyphenols such as catechin, epicatechin, epigallocatechin gallate, and gallic acid [29]. Phenolic compounds were noticed only in the chloroform extract. They are well known for its antioxidant property combating free radical-mediated diseases. Phenolic compounds are extensively used as anti-inflammatory, antimicrobial, antiviral, and anticancer agents [29].

Similar to phenolics, flavonoids were noticed only in the acetone extract. Flavonoids are found to possess significant pharmacological activities such as antioxidant, anticancer, antiviral, antibacterial, antiallergic, and antiosteoporotic[30].

Alkaloids are extensively used as drugs due to its pharmacological activities including antihypertensive effect, antianalgesic effect, antiarrhythmic effect, antimalarial activity, and anticancer actions. Antibiotic activities are common for alkaloids and they are used as antiseptics [31]. Referring to our study, alkaloids were found in all the extracts except ethanol. This result was in contrast with the study conducted in *Urophora fasciata* in which alkaloids did not show their presence in any of the four extracts tested [32]. This may be due to change in chemical composition of seaweeds with respect to species.

Methanol, chloroform, acetone, and petroleum ether extracts have been reported to contain glycosides. Glycosides were also found to be present in other species of *Ulva* such as *U. fasciata*, *Ulva lactuca,* and *Ulva intestinalis* [33]. Glycosides include glycosides of vitamins, polyphenolic glycosides, alkaloid glycosides, glycosides in the group of antibiotics, glycopeptides, cardiac glycosides, and steroid and terpenoid glycosides. These glycosides exhibit many biological activities including antibiotic drugs, schizophrenia treatment, immunomodulatory, and hypolipemic activities [34].

Anthraquinones are a class of aromatic compound possessing a broad spectrum of bioactivities, such as cathartic, anticancer, anti-inflammatory, antimicrobial, diuretic, vasorelaxing, and phytoestrogen activities [35]. In the present study, methanol extract was reported to contain anthraquinone. Anthraquinones were also found to be present in green seaweeds such as *Caulerpa racemosa*, *Caulerpa peltata*, *Caulerpa taxifolia*, *Codium fragile,* and *Chlorodesmis fastigiata* collected from Maharashtra and Goa, India [36]. However, other species of *Ulva* genera, *U. fasciata* Delile, and *U. lactuca* Linn. collected from the southern coast of Tamil Nadu did not show the presence of anthraquinones [37].

Heavy metal analysis

The data pertaining to heavy metal analysis in *U. reticulata* were presented in Table 2.

The relative abundance of the studied heavy metals was in the order of Fe>Cr>Zn>Ni>Cu. Pb and Cd are at below detection limits. The order of heavy metals in the selected seaweed is the representative picture of the dissolved heavy metals in the study area. Apart from the study area, there are several other factors that affect the concentration of heavy metals and it includes algal bioaccumulation capacity, season, pH, geographic factors, time of harvesting, and salinity. The bioaccumulation capacity of the seaweed varies between species. For instance, *U. lactuca* collected from Cockburn sound of Western Australia has been found to be a good indicator of Cd, Fe, Mn, and Pb [38]. Similarly, *Ulva rigida C. Agardh* and *Enteromorpha linza* (Linnaeus) from Thermaikos Gulf, Greece, exhibited the seasonal and local variation of Cr, Ni, and Co concentrations [39]. In a study conducted in Olaikuda, Rameshwaram, Southeast coast of India, the reproductive part of two species of *Turbinaria* such as *Turbinaria decurrens* and *Turbinaria ornata* is found to have highest accumulation capacity of As, Pd, Cd, and Cr [40].

Table 2: Heavy metal composition of *U. reticulata*

Heavy metals	Quantity (ppm)
Chromium (Cr)	19.3
Copper (Cu)	12.2
Zinc (Zn)	15.8
Nickel (Ni)	15.3
Iron (Fe)	141.5
Cadmium (Cd)	BDL (0.1)
Lead (Pb)	BDL (0.1)

BDL: Below Detection Limit. *U. reticulata: Ulva reticulate*

In the present work, among the heavy metals studied, Fe was found to be abundant and its level exceeded the permissible limit set by the FAO/WHO (1984) for a medicinal plant (20 ppm) [41]. Such a high content of Fe is seen in *Sargassum* sp. grown in Rameshwaram coast. The presence of high Fe content in marine plants may be due to its role in metabolic functions of the plant. Further, increased photosynthesis and respiration in marine plants enables them to uptake more Fe. In humans, Fe plays an important role in red blood cell production and is used against anemia, tuberculosis, and growth disorder [42]. While its wide importance, its higher concentration becomes a matter of great concern.

According to the WHO, the permissible level of Pb, Cd, Cr, Cu, Ni, and Zn in medicinal plant and food is 10, 1, 1.5, 10, 15, and 50 ppm, respectively [41]. Further, CEVA 2014 has set 0.5 and 5 mg/kg as the tolerable limit of Cd and Pb, respectively, in dried seaweed for human consumption [43,44]. In line with the aforementioned permissible levels suggested by the WHO and CEVA, Zn, Cu, Pb, and Cd in the studied seaweed were found to be within the permissible limits, whereas Cr exceeds the limit and Ni content was at the borderline.

Although human requires chromium in very small amount to regulate many processes in the human body including homeostasis, chromium toxicity leads to nephrotoxicity, nasal and lung ulcers, skin ulcers, spinal/joint degeneration, depressed immune system, and lymphatic swelling [45,46].

Nickel is not a cumulative toxicant; however, chronic exposure causes chronic dermatitis, kidney disorder, chronic bronchitis, lung cancer, pulmonary fibrosis, and acute respiratory distress syndrome [47].

Cd and Pb were not detected in our seaweed. Perhaps, Cd and Pb are non-essential trace element and have a detrimental effect on the human body. Pb poisoning causes convulsions, chronic hepatitis, CNS disorder, anemia, and kidney damage and reduces fertility and delayed puberty, whereas Cd causes respiratory distress, liver and kidney damage, anemia, and cardiovascular disorders [48].

Zn and Cu are the essential elements in our body. Zn is the second most abundant transition metal in organisms after iron. Its role in nerve function, male fertility, formation of red and white blood cells, heart function, and normal growth is well known [49]. Cu helps in absorption of iron. It is also used for cellular defense, protecting mucus membrane and preventing anemia, osteoporosis, delayed wound healing and the development of aortic aneurysms, and loss of hair color [49]. This study has highlighted that Cu and Zn are present within permissible limits and the concentration of Zn is more compared to Cu.

CONCLUSION

The present work revealed the presence of phytochemicals and heavy metals in *U. reticulata*. Of all the heavy metals studied, Fe and Cr exceed the permissible limits which alarm to have more insights about their health implications. Further investigation should be carried out based on their area and season of cultivation and physicochemical parameters that affect the bioavailability of metals.

AUTHORS CONTRIBUTIONS

All authors have equal contribution in bringing out this article.

CONFLICT OF INTEREST

None.

REFERENCES

1. Carte BK. Biomedical potential of marine natural products: Marine organisms are yielding novel molecules for use in basic research and medical applications. Biosci 1996;46:271-86.
2. Arasaki S, Arasaki T. Low Calories, High Nutrition: Vegetables from the Sea to Help you Look and Feel Better. Tokyo: Japan Publications; 1983.
3. Dhargalkar VK, Pereira N. Seaweed: Promising plant of the Millennium. Sci Cult 2005;71:60-6.
4. Patia MP, Sharma SD, Nayaka L, Panda CR. Uses of seaweed and its application to Human welfare: A Review. Int J Pharm Pharm Sci 2016;8:12-20.
5. MacArtain P, Gill CI, Brooks M, Campbell R, Rowland IR. Nutritional value of edible seaweeds. Nutr Rev 2007;65:535-43.
6. Wang H, Ooi EV, Ang PO Jr. Antiviral activities of extracts from Hong Kong seaweeds. J Zhejiang Univ Sci B 2008;9:969-76.
7. Indu H, Seenivasan R. *In vitro* antioxidant activity of selected seaweeds from Southeast coast of India. Int J Pharm Pharm Sci 2013;5:474-84.
8. Santhanam S, Aseer M, Sugathan S, Joseph S, George SK, Kalimuthusamy NS. Antimicrobial activity of seaweeds extracts against multiresistant pathogens. Anna Microbiol 2008;58:535-41.
9. Rao P, Parekh K. Antibacterial activity of Indian seaweed extracts. Bot Mar 2009;24:577-82.
10. Noda H, Amano H, Arashima K. Antitumor activity of marine algae. Hydrobiologia 1990;204-205:577-84.
11. Faezah S, Khoo KS, Hoe SZ, Lam SK. Antihypertensive effects of edible brown seaweeds in rats. Int J Adv Appl Sci 2016;3:103-9.
12. Yoon NY, Kim HR, Chung HY, Choi JS. Anti-hyperlipidemic effect of an edible brown algae, *Ecklonia stolonifera*, and its constituents on poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. Arch Pharm Res 2008;31:1564-71.
13. Afef D, Syrine L, Valerie LM, Jacques R, Abderrahman B. Antiproliferative activity and phenolics of the Mediterranean seaweed *Laurencia obusta*. Ind Crops Prod 2013;47:252-5.
14. Bhakuni DS, Rawat DS. Bioactive Marine Natural Products. 1st ed. India: Anamya Publishers; 2005.
15. Ansari TM, Marr IL, Tariq N. Heavy Metals in Marine Pollution Perspective-A Mini Review. J Appl Sci 2004;4:1-20.
16. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. In: Luch A, editros. Molecular, Clinical and Environmental Toxicology. Experientia Supplementum. 1st ed. Basel: Springer; 2012. p. 165-217.
17. Wolf MA, Sciuto K, Andreoli C, Moro I. *Ulva* (*Chlorophyta, Ulvales*) biodiversity in the North Adriatic sea (Mediterranean, Italy): Cryptic species and new introductions. J Phycol 2012;48:1510-21.
18. Kong F, Mao Y, Cui F, Zhang X, Gao Z. Morphology and molecular identification of *Ulva* forming green tides in Qingdao China. J Ocean Univ China 2011;10:73-9.
19. CABI. *Ulva reticulata*. In: Invasive Species Compendium. Wallingford, UK: CAB International; 2017. Available from: http://www.cabi.org/isc.
20. Guiry MD, Guiry GM. Algae Base. Galway: World-Wide Electronic Publication, National University of Ireland; 2017.
21. Savithramma N, Rao ML, Rukmini K, Devi PS. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. Int J Chemtech Res 2011;3:1394-402.
22. Harborne JB. Phytochemical Methods. 2nd ed. London: Chapman and Hall; 1984.
23. Markert BA, Breure AM, Zechmeister HG editors. Bioindicators and Biomonitors: Principles, Concepts, and Applications. UK: Elseiver; 2003.
24. Harborne JB. Phytochemical methods a guide to modern techniques of plant analysis. Plant Pathol J 1999;48:146.
25. Renuka B, Sanjeev B, Ranganathan D. Evaluation of phytoconstituents of *Caralluma nilagiriana* by FTIR and UV-VIS spectroscopic analysis. J Pharmacogn Phytochem 2016;5:105-8.
26. Ismail GA. Biochemical composition of some Egyptian seaweeds with potent nutritive and antioxidant properties. Food Sci Technol 2017;37:294-302.
27. Sudha PN, Aisverya S, Nithya R, Vijayalakshmi K. Industrial applications of marine carbohydrates. Adv Food Nutr Res 2014;73:145-81.
28. Patel S. Therapeutic importance of sulfated polysaccharides from seaweeds: Updating the recent findings. 3 Biotech 2012;2:171-85.
29. Vinayak RC, Sudha SA, Chatterji A. Bio-screening of a few green seaweeds from India for their cytotoxic and antioxidant potential. J Sci Food Agric 2011;91:2471-6.
30. Elsherif KM, Najah Z, Kawan E. Phytochemical screening and heavy metals contents of *Nicotiana glauca* plant. Int J Pharm Pharam Res Human 2015;4:82-91.
31. Roberts MF, Wink M. Alkaloids Biochemistry, Ecology, and Medicinal Application. New York and London: Plenum Press; 1998.
32. Raj GA, Chandrasekaran M, Jegan S, Venkatesalu V. Phytochemical analysis and antifungal activity of *Ulva* Species from the Kanniyakumari Gulf of Mannar, South Coast India. Nat Prod Ind J 2016;12:104.
33. Abdel-Khaliq A, Hassan HM, Rateb ME, Hammouda O. Antimicrobial activity of three *Ulva* species collected from some Egyptian Mediterranean seashores. Int J Eng Res Gen Sci 2014;2:648-69.
34. Kren V, Martinkova L. Glycosides in medicine: The role of glycosidic residue in biological activity. Curr Med Chem 2001;8:1303-28.
35. Chien SC, Wu YC, Chen ZW, Yang WC. Naturally occurring anthraquinones: Chemistry and therapeutic potential in autoimmune diabetes. Evid Based Complement Alternat Med 2015;2015:357357.
36. Kotnala S, Garg A, Chatterji A. Screening for the presence of antimicrobial activity in few Indian seaweeds. Pertanika J Trop Agric Sci 2009;32:69-75.
37. Babu A, Johnson M, Patric RD. Chemical profile of selected green seaweeds of Southern coast of Tamil Nadu, India. Int J Res Engg Biosci 2014;2:103-13.
38. Talbot V, Chegwidden A. Cadmium and other heavy metal concentrations in selected biota from Cockburn Sound, Western Australia. Aust J Mar Freshw Res 1982;33:779-88.
39. Haritonidis S, Malea P. Seasonal and local variation of Cr, Ni and Co concentrations in *Ulva rigida* C. Agardh and *Enteromorpha linza* (Linnaeus) from Thermaikos Gulf, Greece. Environ Pollut 1995;89:319-27.
40. Roy S, Anantharaman P. Heavy metals accumulation of different parts of *Turbinaria* spp. along the olaikuda Coast, Rameshwaram, Tamil Nadu, India. Int Adv Res J Sci Eng Tech 2017;4:99-102.
41. Evaluation of certain food additives and contaminants. Thirty-third report of the joint FAO/WHO expert committee on food additives. World Health Organ Tech Rep Ser 1989;776:1-64.
42. Claude B, Paule S. The Manual of Natural Living. 1st ed. Guildford Surrey: Biddles Limited; 1979.
43. CEVA. Edible Seaweed and French Regulation - Synthesis Made by CEVA. France: CEVA; 2014.
44. Llanos NL, Dalawampu SM. Heavy metals in edible seaweeds from coastal areas of Manila Bay and Roxas city, Philippines. Int J Adv Res

2017;5:1429-34.
45. Naithani V, Pathak N, Chaudhary M. Evaluation of heavy metals in two major ingredients of ampucare. Int J Pharm Sci Drug Res 2010;2:137-41.
46. Chandaka M, Murali K, Ramanji R, Mohammed O. Comparative studies on phytochemical screening and metal analysis of hydro alcoholic extracts of *Beta Vulgaris*, *Carica Papaya,* and *Vitis vinifera*. Juniper Online J Public Health 2017;2:1429-34.
47. Das K, Das S, Dhundasi S. Nickel, its adverse health effects and oxidative stress. Indian J Med Res 2008;128:412-25.
48. Ayaz M, Junaid M, Subhan F, Ullah F, Sadiq A, Ahmad S, *et al*. Heavy metals analysis, phytochemical, phytotoxic and anthelmintic investigations of crude methanolic extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. BMC Complement Altern Med 2014;14:465.
49. Rajendran K, Sampathkumar P, Govindasamy C, Ganesan M, Kannan R, Kannan L. Levels of trace metals (Mn, Fe, Cu and Zn) in some Indian seaweeds. Mar Pollut Bull 1993;26:283-5.