ECRONICON OPEN ACCESS

EC NUTRITION
Research Article

Determination of Polyphenol Content, Antioxidant Activity and Potential Human Health Benefits of *Gracilaria tikvahiae*

Cassandra Evans[1]*, Douglas Kalman[1] and Robert Speth[2]

[1]*Nutrition Department, Dr. Kiran C Patel College of Osteopathic Medicine, Nova Southeastern University, Fort Lauderdale, Florida, United States*

[2]*Pharmaceutical Sciences Nova Southeastern University, Davie, Florida, United States*

***Corresponding Author:** Cassandra Evans, Nutrition Department, Dr. Kiran C Patel College of Osteopathic Medicine, Nova Southeastern University, Fort Lauderdale, Florida, United States.

Received: December 03, 2022; **Published:** December 05, 2022

Abstract

The red algae, *Gracilaria*, has a history of use in the food, biotechnological, nutraceutical, and pharmaceutical industries. *Gracilaria* appears to have potential further development use as a nutritional food, due to its unique composition. The purpose of this study was to determine the relative total polyphenol content, characterizing phenolic compounds and measuring antioxidant potential of *G. tikvahiae*. Analysis of extracts were performed using the Folin-Ciocalteu methods, high performance liquid chromatography-mass spectrometry (HPLC-MS) and ferric- reducing antioxidant power (FRAP) to determine polyphenol content and antioxidant activity. Nine phenolic compounds in *G. tikvahiae* were identified. Phenolic compounds in order of abundance were: Quercetin, 3,4-dihydroxybenzoic acid, kaempferol, pungenol, gallic acid, naringenin, apigenin, isorhamnetin, and protocatechuic aldehyde. Antioxidant activity was observed in all *G. tikvahiae* extracts. The results established the presence of a variety of phenolic compounds suggesting that *G. tikvahiae* has potential to serve as a nutritional agent with antioxidant properties. This study highlights the potential of *G. tikvahiae* to be a functional food that warrants further research.

Keywords: *Antioxidants; Polyphenols; Functional Foods*

Introduction

The relationship between diet and optimal health has led to a growing interest in functional foods and their bioactive compounds. In recent years, polyphenol-rich foods, beverages, and dietary supplements have been studied to ascertain their role in reducing oxidative stress while promoting overall health. There is an increasing demand for natural, polyphenol-rich, foods and dietary supplements for use as complementary and integrative health therapies [1,2].

Naturally occurring polyphenols are found within marine algae and seaweeds. A variety of studies have determined the nutrient rich profile and broad range of potential health benefits of marine seaweeds [3-9]. One particular seaweed, *Gracilaria*, contains a diverse array of bioactive compounds and phytochemicals that have made this red seaweed a highly sought-after product for use in many industries [5,7-10]. It is currently harvested for human consumption and for agar production [11]. Traditional uses of *Gracilaria* suggest a broad range of benefits in human health [3,5-9]. Animal studies using *Gracilaria* extracts have validated many of these health benefits [6,10,12,13]. The nutrition profile in *Gracilaria* contains a diverse array of compounds including amino acids, lipids/fatty acids, minerals, vitamins, polysac-

charides and phytochemicals [5,9]. Differences in nutrient composition are observed when comparing different species, which is likely attributed to environment, seasonal variations, and nutrient availability. Common amino acids found in abundance in this red algae include aspartic acid, alanine, glutamic acid, glutamine, proline and serine [4-6,8,10,14-17]. Current literature shows that *Gracilaria* contain small amounts of lipids, 1-3% of dry weight [5,8,14,17]. Typical lipid profiles include palmitic acid, oleic acid, stearic acid, and polyunsaturated fats such as linoleic acid and docosahexaenoic acid (DHA) [5,8,14]. This seaweed contains many minerals including sodium, potassium, manganese, calcium, iron and copper [5,10]. Its vitamin content includes vitamins A, B1, B6, C and E [5,6,8,14,17]. Additionally, *Gracilaria* contain numerous bioactive primary and secondary metabolites such as carotenoids, terpenoids, polyphenols, xanthophylls, chlorophylls, phycobilins, and alkaloids [5,14,17].

The aforementioned marine-based phytonutrients have been of great interest in drug development. Early-stage developmental work has tested these compounds experimentally for treatment of inflammation, cancer, neurodegenerative diseases, etc. [8,17]. It is interesting to also note that *Gracilaria* species have a history of use in folk medicine, treating conditions such as respiratory disease, thyroid disorders and edema [17]. The literature suggests that the polyphenols found in *Gracilaria* exhibit powerful antioxidant properties. There are 160 recorded species of *Gracilaria* yet only 25% of these species have been studied [17]. Certain species, namely *G. edulis, G. corticate, G. tenuistipitata* and *G. cornea,* have been the focus of most of the *Gracilaria* research. This is the first study to characterize polyphenols and antioxidant activity in *Gracilaria tikvahiae.* The specific aims of this study were to (i) measure total polyphenol content in *G. tikvahae*, (ii) determine the individual phenolic compounds found in *G. tikvahae* and (iii) measure antioxidant activity present in *G. tikvahiae*. This study provides new insights on the chemical composition of *G. tikvahiae* and its potential to be a valuable functional food/dietary supplement.

Materials and Methods

Sample preparation

The strain of *Gracilaria* studied were obtained from existing *Gracilaria sp.* currently growing within an open-air wet lab at the Urban Farming Institute, Oakland Park, Florida. All samples were rinsed under cold water and place in a dehydrator at the lowest temperature 35ºC for 12 hours. The dried samples were pulverized and stored in an air-tight container at 4ºC until analysis.

Polyphenols were extracted from *Gracilaria tikvahae* using methods previously described by Francavilla., *et al*. (2013), and Machu., *et al*. (2015), with slight modifications [8,31]. For methanol extractions, 1g of dried algae sample was mixed with 2.5 ml solvent and placed on an oscillator for 24 hrs at room temperature. A total of 7 repeated extractions were performed on the same sample. The resulting supernatant was combined and stored at -80ºC until analysis. For methanol/acetone/water (7:7:6) extractions, 1g of dried algae was mixed with 15 ml solvent and placed on an oscillator for 24 hrs at room temperature. The resulting supernatant was stored at -80ºC until analysis.

Total polyphenol content

The total phenolic content in *Gracilaria tikvahae* supernatant extracts was determined using the Folin-Ciocalteu assay as described Singleton with slight modifications [18]. Ten µL of sample or gallic acid standard solution was added to a well plate. Next, 50 µL of diluted Folin reagent was added to each well. 150 µL of sodium bicarbonate was added to each well to prevent the formation of precipitates. Following a 120 min. incubation period at 20ºC, absorbance was measured at 765 nm using a Synergy HTX Multi-Mode Reader**.** Total phenolic content was determined as milligram of gallic acid equivalent per gram *Gracilaria tikvahae*, using the equation obtained from a standard gallic acid calibration curve. Gallic acid is frequently used as a standard in the Folin-Ciocalteu method due to its solubility and low-cost [18]. Results are expressed as gallic acid equivalents.

Phenolic compounds

High performance liquid chromatography - mass spectrometry was used to identify phenolic compounds in the samples. Specifically, 7.5 mg (dry weight) of sample was weighed accurately into a 1.5-mL microcentrifuge tube followed by adding 10 µL of a mixture of ten isotopic-labeled internal standards (ISTDS) and 250 µL of 80% methanol in water (v/v). Each tube was then vortexed for 30 minutes and spun down for 5 minutes at 14,000 rpm. After that, supernatant from each tube was transferred and filtered through 0.22 µm Acrodisc® syringe filters with the GHP membrane for UHPLC-HRMS analyses.

A Thermo Scientific Vanquish UHPLC system and an Agilent reversed-phase Zorbax Eclipse XDB C18 column (3.0 mm × 100 mm, 3.5 µm particle size, 80 Å pore size) with an ACQUITY UPLC CSH C18 VanGuard Pre-column (2.1 mm × 5 mm, 1.7 µm particle size, 130 Å pore size) were used for all online UHPLC-HRMS analyses with a Thermo Scientific Q-Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer. The controlling software for the sample analysis was Thermo Xcalibur. The UHPLC gradient method is listed in table 1.

Time (min)	Flow (ml/min)	%A	%B	Curve
Run				
0.00	0.50	99.0	1.0	5
0.50	0.50	99.0	1.0	5
3.50	0.50	40.0	60.0	5
4.50	0.50	1.0	99.0	5
5.80	0.50	1.0	99.0	5
5.81	0.50	99.0	1.0	5
8.00	0.50	99.0	1.0	5

Table 1: *UHPLC gradient method.*

[1]Curve 5 represents the gradient curve mode.

The chromatographic separation of polyphenols was achieved using an 8-minute LC gradient method, and the LC column chamber was maintained at 30°C. The flow rate was 500 µL/min, and the sample injection volume was 5 µL. Data acquisition was done using parallel reaction monitoring (PRM) in a negative ionization mode, and analyte peak identification followed by peak integration was done using Trace Finder 4.1 software. The sample sequence was included with quality control samples at three different concentrations (low, mid, and high) and calibration solutions at eight different concentrations. The ratios of the signal intensity of each analyte to its corresponding ISTD were plotted against the known concentrations of the standard mixtures to build the calibration curves.

Antioxidant activity

Ferric reducing anti-oxidant power (FRAP) assay was used to determine antioxidant activity based on methods previously described by Benzie and Sanz-Pintos., *et al* [19]. FRAP reagent was prepared as required by mixing 25 ml acetate buffer (300 mmol/1 acetate buffer, pH 3.6 (3.1g $C_2H_3Na0_2$•3Hp), 2.5 ml TPTZ (10 mmol/1 TPTZ (2,4,6-tripyridyl-s-triazine) solution, and 2.5 ml $FeCl_3$-$6H_2O$ solution ((20 mmol/1 $FeC1_3$•$6H_2O$). 50 µL of Iron (II) standard was added to a well plate. 50 µL diluted sampled (20 µL extract/30 µL H_20) was added to a well plate. 100 µL FRAP reagent was added to each well. Absorbance was measured immediately at 540 nm using a BioTek Synergy HTX Multi-Mode Plate Reader.

Results

Total polyphenol content

Total polyphenol content (TPC) from the different extracts of *G. tikvahae* and *U. lactua* are reported in table 2. TPC values are expressed as gallic acid equivalents (GAE) mg/g of dried algae.

Sample	GAE (mg/g of dry weight)
Methanol extraction	
G. tikvahae	0.357 ± 0.029
U. lactua	0.162
Methanol acetone extraction	
G. tikvahae	0.373
U. lactua	0.407

Table 2: *Total polyphenol content.*

Phenolic compounds

The following phenolic compounds were identified in the *G. tikvihae* samples: Quercetin, 3,4-dihydroxybenzoic acid, kaempferol, pungenol, gallic acid, naringenin, apigenin, isorhamnetin, and protocatechuic aldehyde (Table 3).

Polyphenol	Concentration (mg/kg)
3,4-dihydroxybenzoic acid	0.433 ± 0.003
Apigenin	0.06 ± 0.0004
Gallic Acid	0.121 ± 0.001
Isorhamnetin	0.044 ± 0.0002
Kaempferol	0.318 ± 0.001
Naringenin	0.073 ± 0.0003
Protocatechuic aldehyde	0.034 ± 0.0001
Pungenol	0.267 ± 0.003
Quercetin	0.466 ± 0.001

Table 3: *Phenolic compounds.*

Antioxidant activity

Antioxidant activity from the different extracts of *G. tikvahae* and *U. lactua* are reported in table 4. Results are expressed as FRAP value.

Discussion

The total polyphenol content has been determined in the following species: *G. edulis* (16.26 mg GAE/g), *G. corticata* (4.09 mg GAE/g), *G. salicornia* (3.91 mg GAE/g), *G. debilis* (3.9 mg GAE/g), *G. fergusonii* (2.8 mg GAE/g) *G. dura* (2.5 mg GAE/g), *G. bridiae* (1.13 mg GAE/g), and *G. chilensis* (2.6 mg GAE/g) [8,15,20-25]. See table 5. Contrary to what the authors hypothesized, *G. tikvahiae* had considerably lower amounts of phenolic compounds when compared to other *Gracilaria* species. Similarly, TPC values for *U. lactua* were lower than reported values in other studies [26,27]. Factors such as time of harvest and extraction techniques can influence polyphenol content [4,28,29].

Sample	FRAP Value (μmolTE/mg)
Methanol extraction	
G. tikvahae	129.001
U. lactua	60.681
Methanol acetone extraction	
G. tikvahae	38.367
U. lactua	17.0145

Table 4: *Antioxidant activities.*

Species	TPC Value (mg GAE/g)	Reference
G. gracilis	2.3-31.8	Francavilla, M., et al., The red seaweed *Gracilaria gracilis* as a multi products source. Mar Drugs, 2013. 11(10): p. 3754-76.
G. eludis	16.26	Ganesan P, Kumar CS, Bhaskar N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresour Technol*. 2008;99(8):2717-2723. doi:10.1016/j.biortech.2007.07.005
G. corticata	4.09	Arulkumar, A., et al. (2018). "Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (*Gracilaria corticata* and *Gracilaria edulis*) from Palk Bay, India". Biocatalysis and Agricultural Biotechnology 15: 63-71.
G. salicornia	3.91	Kumar, M., Kumari, P., Trivedi, N. *et al.* Minerals, PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India. *J Appl Phycol* 23, 797–810 (2011). https://doi.org/10.1007/s10811-010-9578-7
G. debilis	3.9	Arulkumar, A., et al. (2018). "Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (Gracilaria corticata and Gracilaria edulis) from Palk Bay, India". Biocatalysis and Agricultural Biotechnology 15: 63-71.
G. fergusonii	2.8	1.0
G. dura	2.5	Arulkumar, A., et al. (2018). "Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (Gracilaria corticata and Gracilaria edulis) from Palk Bay, India". Biocatalysis and Agricultural Biotechnology 15: 63-71.
G. bridiae	1.13	Souza BW, Cerqueira MA, Martins JT, et al. Antioxidant potential of two red seaweeds from the Brazilian coasts. *J Agric Food Chem*. 2011;59(10):5589-5594. doi:10.1021/jf200999n
G. chilensis	2.6 ± 0.6	Ortiz-Viedma, J., et al., Protective Effect of Red Algae *(Rhodophyta)* Extracts on Essential Dietary Components of Heat-Treated Salmon. Antioxidants (Basel), 2021. 10(7)

Table 5: *Previously reported TPC values of Gracilaria sp.*

To our knowledge, this is the first report on specific phenolic compounds in *G. tikvahiae.* To compare to other species, *G. vermiculophylla* was reported to have three polyphenols: gallic, protocatechuic and gentisic acids [28]. *G. texorii* and *G. asiatica* have quercetin and hesperidin [28]. Similar to Capillo., *et al*'s. findings, *G. tikvahiae* was found to contain quercetin and gallic acid [28]. Interestingly, this study found different polyphenols such as 3,4-dihydroxybenzoic acid, kaempferol, and pungenol.

Phenolic compounds in *Gracilaria* can act as free radical scavengers. Antioxidant assays observed high free radical scavenging activity in *G. eludis* extract which correlated with total polyphenol content [30]. This correlation suggests that polyphenols present in *G. eludis* extract are responsible for the antioxidant activity. Similarly, correlations between polyphenol content and radical scavenging and reducing activity were found in *G. changii* extracts [13]. *Gracilaria sp.* possess a greater radical scavenging activity when compared to currently marketed dietary supplements [5,8,31,32]. Polyphenolic compounds in *Gracilaria* can also decrease reactive oxygen species (ROS) production and inhibit Nf-κB activation which is consistent with the action of well-studied polyphenols such as curcumin [6,9,15,20,32,33]. Other compounds present in *Gracilaria sp.*, such as sulfated polysaccharides, are reported to exhibited antioxidant properties [15]. Using a methanol extraction, the ferric reducing ability of *G. changii* was reported as 17.61 **±** 0.33 (**µ**molTE/mg) and *G. chilensis* was 625.25 ± 0.22 (**µ**molTE/mg) [13,30,34]. The reported FRAP values of methanol extracts in this study were greater than *G. changii* but less than *G. chilensis*. Storage time and conditions can affect antioxidant levels [35]. The methanol/acetone extraction was performed after the methanol extractions which could account for the variability in FRAP values. Previous research has reported antioxidant activity to be proportional to total polyphenol content [25]. Conversely, this investigation reported good reducing value (FRAP) but lower levels of total polyphenol content. Other unaccounted bioactive compounds could be responsible for *G. tikvahiae's* antioxidant potential. The reported antioxidant activity of *U. lactua* are consistent with other studies which reported low antioxidant potential [26,27].

Conclusion

The study results established presence of a variety of phenolic compounds. Specifically, 3,4-dihydroxybenzoic acid, Kaempferol, and Pungenol are believed to be unique to *G. tikvahiae.* Despite the presence of phenolic compounds, the TPC reported in this study appeared low compared to other species. Further research is warranted to determine if different extraction or cultivation methods could explain this difference. This study highlights the potential of *G. tikvahiae* to be as a marine-based phytonutrient which has potential uses within a variety of regulated industries (i.e. food, pharmaceutical, dietary supplement, etc.) worthy of future research.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, CE and DK; methodology, CE, DK and RS.; experimental procedures, CE and RS; data analysis, CE and RS; writing-original draft preparation, CE; writing-review and editing, CE, DK and RS; supervision, DK and RS; funding acquisition, CE, DK and RS. All authors have read and agreed to the published version of the manuscript.

Funding Support

This research was funded by Nova Southeastern University, Health Professions Divisions Research Grant, index number 334521.

Consent for Publication

Not applicable.

Availability of Data and Material

Data and material are available upon request.

Code Availability

Not applicable.

Acknowledgements

We thank John Albee, Jeff Manchester, and the Urban Farming Institute for granting access to their wet lab and support of the project. We thank the Dr. Nadine Mikati and Stephanie Petrosky from the Nutrition department, College of Osteopathic Medicine at Nova Southeastern University for their guidance and support.

Bibliography

1. SM., *et al*. "Dietary Supplement Use Among Adults: United States, 2017-2018". *NCHS Data Briefs* 399 (2021).
2. Ganesan AR., *et al*. "Seaweed nutraceuticals and their therapeutic role in disease prevention". *Food Science and Human Wellness* 8.3 (2019): 252-263.
3. Tasneem S., *et al*. "Molecular pharmacology of inflammation: Medicinal plants as anti-inflammatory agents". *Pharmacological Research* 139 (2019): 126-140.
4. Matteo Francavilla MF., *et al*. "The Red Seaweed *Gracilaria gracilis* as a Multi Products Source". *Marine Drugs* 11.10 (2013): 3754-3776.
5. Rosemary T., *et al*. "Biochemical, Micronutrient and Physicochemical Properties of the Dried Red Seaweeds *Gracilaria edulis* and *Gracilaria corticata*". *Molecules* 24.12 (2019): 2225.
6. Yang JI., *et al*. "Aqueous extracts of the edible *Gracilaria tenuistipitata* are protective against H(2)O(2)-induced DNA damage, growth inhibition, and cell cycle arrest". *Molecules* 17.6 (2012): 7241-7254.
7. Nabil-Adam A., *et al*. "Marine Algae of the Genus *Gracilaria* as Multi Products Source for Different Biotechnological and Medical Applications". *Recent Patents on Biotechnology* 14.3 (2020): 203-228.
8. Francavilla M., *et al*. "The red seaweed *Gracilaria gracilis* as a multi products source". *Marine Drugs* 11.10 (2013): 3754-3776.
9. Korivi M., *et al*. "Seaweed Supplementation Enhances Maximal Muscular Strength and Attenuates Resistance Exercise-Induced Oxidative Stress in Rats". *Evidence-Based Complementary and Alternative Medicine* (2019): 3528932.
10. Chakraborty K and T Antony. "First report of spiro-compounds from marine macroalga *Gracilaria salicornia*: prospective natural anti-inflammatory agents attenuate 5-lipoxygenase and cyclooxygenase-2". *Natural Product Research* 35.5 (2021): 770-781.
11. Charles Y., *et al*. "*Gracilaria* Culture Handbook for New England". WrackLines 72 (2012).
12. Sanz-Pintos N., *et al*. "Macromolecular Antioxidants and Dietary Fiber in Edible Seaweeds". *Journal of Food Science* 82.2 (2017): 289-295.
13. Chan PT., *et al*. "Antioxidant activities and polyphenolics of various solvent extracts of red seaweed, *Gracilaria changii*". *Journal of Applied Phycology* 27.6 (2014): 2377-2386.
14. de Almeida CL., *et al*. "Bioactivities from marine algae of the genus *Gracilaria*". *International Journal of Molecular Sciences* 12.7 (2011): 4550-4573.

15. Souza BWS., *et al*. "Chemical characterization and antioxidant activity of sulfated polysaccharide from the red seaweed *Gracilaria birdiae*". *Food Hydrocolloids* 27.2 (2012): 287-292.

16. Makkar F and K Chakraborty. "Previously undescribed antioxidative azocinyl morpholinone alkaloid from red seaweed *Gracilaria opuntia* with anti-cyclooxygenase and lipoxygenase properties". *Natural Product Research* 32.10 (2018): 1150-1160.

17. Torres P., *et al*. "A comprehensive review of traditional uses, bioactivity potential, and chemical diversity of the genus *Gracilaria* (Gracilariales, Rhodophyta)". *Algal Research* 37 (2019): 288-306.

18. Singleton VL., *et al*. "[14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent". *Methods in Enzymology* 299 (1999): 152-178.

19. Benzie IF and JJ Strain. "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay". *Analytical Biochemistry* 239.1 (1996): 70-76.

20. Kumar M., *et al*. "Minerals, PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India". *Journal of Applied Phycology* 23.5 (2010): 797-810.

21. Ganesan P., *et al*. "Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds". *Bioresource Technology* 99.8 (2008): 2717-2723.

22. Souza BW., *et al*. "Antioxidant potential of two red seaweeds from the Brazilian coasts". *Journal of Agricultural and Food Chemistry* 59.10 (2011): 5589-5594.

23. Arulkumar A., *et al*. "Phytochemical composition, *in vitro* antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (*Gracilaria corticata* and *Gracilaria edulis*) from Palk Bay, India". *Biocatalysis and Agricultural Biotechnology* 15 (2018): 63-71.

24. Kumar M., *et al*. "Minerals, PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India". *Journal of Applied Phycology* 23.5 (2011): 797-810.

25. Ortiz-Viedma J., *et al*. "Protective Effect of Red Algae (Rhodophyta) Extracts on Essential Dietary Components of Heat-Treated Salmon". *Antioxidants (Basel)* 10.7 (2021): 1108.

26. Kellogg J and MA Lila. "Chemical and *in vitro* assessment of Alaskan coastal vegetation antioxidant capacity". *Journal of Agricultural and Food Chemistry* 61.46 (2013): 11025-11032.

27. Aslan E., *et al*. "Monitoring the antioxidant activities by extracting the polyphenolic contents of algae collected from the Bosphorus". *Marine Pollution Bulletin* 141 (2019): 313-317.

28. Capillo G., *et al*. "New insights into the culture method and antibacterial potential of *Gracilaria gracilis*". *Marine Drugs* 16.12 (2018): 492.

29. Machu L., *et al*. "Phenolic content and antioxidant capacity in algal food products". *Molecules* 20.1 (2015): 1118-1133.

30. Gunathilaka TL., *et al*. "*In-vitro* antioxidant, hypoglycemic activity, and identification of bioactive compounds in phenol-rich extract from the marine red algae *Gracilaria edulis* (Gmelin) Silva". *Molecules* 24.20 (2019): 3708.

31. KC., *et al*. "Anti-inflammatory activity of aqueous extracts of *Gracilaria*". *International Journal of Current Pharmaceutical Research* (2017): 17-19.

32. Vijayavel K and JA Martinez. "*In vitro* antioxidant and antimicrobial activities of two Hawaiian marine Limu: *Ulva fasciata* (Chlorophyta) and *Gracilaria salicornia* (Rhodophyta)". *Journal of Medicinal Food* 13.6 (2010): 1494-1499.

33. Nour Yahfoufi NA., *et al*. "The immunomodulatory and anti-inflammatory role of polyphenols". *Nutrients* 10.11 (2018): 1618.

34. Narasimhan MK., *et al*. "*In vitro* analysis of antioxidant, antimicrobial and antiproliferative activity of *Enteromorpha antenna, Enteromorpha linza* and *Gracilaria corticata* Extracts". *Jundishapur Journal of Natural Pharmaceutical Products* 8.4 (2013): 151-159.

35. Nekvapil T., *et al*. "Decrease in the antioxidant capacity in beverages containing tea extracts during storage". *Scientific World Journal* (2012): 361698.

Volume 17 Issue 12 December 2022