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Impact of Various Solvents on Yield and Activity of Phenolics and Flavonoides of *Ulva lactuca* (Chlorophyta) Algae

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ALA, EKB and SSM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MHA and SSM managed the analyses of the study, the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Context: The degenerative diseases, due to generation of reactive oxygen species are one of the main human health hazards in the densly populated cities.
Objective: This study aims at providinga scientific support related to richness of antioxidants in the

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marine *Ulva lactuca* (Chlorophyta) algae and their high scavenging activity against various free radicals.

Materials and Methods: Three different solvents were applied on *Ulva lactuca* (Chlorophyta) algae to compare the recovery of phenolics and flavonoids. The reducing power and scavenging activity of the extracts, against hydroxyl and nitrate radicals, were assessed.

Results: The ethyl acetate extract (EAE) was superior to that of the ethanol and chloroform in obtaining the highest significant recovery of phenolics and flavonoids from *Ulva lactuca* (Chlorophyta) algae ($P < 0.05$). In addition, the EAE had the highest significant reducing power and scavenging activity against three radicals ($P < 0.05$) compared to other two solvents.

Conclusion: The EAE helped in uncovering the richness in antioxidants of *Ulva lactuca* (Chlorophyta) algae, associated with significant scavenging activity against various radicals. This scientific evidence could be as a prophylactic against degenerative diseases, pending the proof of its safety and efficacy in experimental *in vivo* trials.

Keywords: Ulva lactuca (Chlorophyta) algae; flavonoids; phenolics; solvent; yield.

1. INTRODUCTION

Free radicals are molecular species that contain one or more unpaired electron, capable of independent existence and associated with high activity [1]. Free radicals are generated during metabolism as reactive oxygen species (ROS) or reactive nitrogen species (RNS). The ROS might be generated as a result of toxicity of different chemicals, pesticides, radiation, pollutant and smoke. These radicals can target the cellular proteins, nucleic acids, producing degenerative diseases [2]. The excessive production of ROS, associated with a decrease in the antioxidant capacity of the human or animal body, leads to oxidative stress. Antioxidant agents act by inhibiting the initial step of oxidation of lipid, resulting in a slow rate of oxidation. These agents reduce the complications of chronic ailments including diabetes, cancer and vascular diseases [3]. The synthetic antioxidant agents, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), exhibit potent free radical scavenging properties, with documented side effects [4].

The search for discovering effective natural antioxidant compounds, with high potency in scavenging free radicals, is becoming the objective of many investigators [5].

Natural products or their derivative compounds can act as antioxidants, that can be used in integrative and complementary medicine, aiming at protection against pathogenesis and complications resulting of many chronic diseases. Medicinal plants are recently used in pharmaceutical industry, due to their richness in active ingredients that could be incorporated in future veterinary or human drugs [6]. Among the known various active ingredients of naturally-occurring herbs on our planet are phenols, epicatichin and flavonoides compounds.

Marine algae considered as promising pharmaceutical sources and are according to their components and pigments. It considered as a source of biological active components and utilised as free radical scavenger and inhibition of many bacterial species [7].

Nowadays there is a new vision on nanoparticles and delivery system to increase its bioavailability and increasing biodiversity in exploring novel therapeutic agents from natural products, the marine algae organisms are with promising interest, since they are possess a wide range of biological activities such as [7].

Ulva lactuca (Chlorophyta) algae is known to contain a wide variety of compounds, with sporadic paucity of documentations related to its biological and pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial and cytotoxic activities. In addition, there is a paucity of data related to the impact of various solvent extracts on yield and quantitative free radical scavenging activities in algae of [8].

Solvents are widely used for extraction of plant bioactive compounds. However, the yield of extraction is dependent on the presence of a variety of active compounds with different properties and polarities that may affect their solubility in the solvent. Polar solvents (acetone, ethanol, ethyl acetate and methanol) are used for extraction of polyphenols from a plant matrix [9]. The ethanol and methanol have been extensively used to extract antioxidant compounds from

plants, while phenolic compounds were efficiently extracted by mixtures of acetone and ethanol.

The objective of this research is to study the impact of various solvent extracts on the yield and free radical scavenging properties of phenolics and flavonoides of *Ulva lactuca* (Chlorophyta) algae.

2. MATERIALS AND METHODS

2.1 Preparation of Algae Extract

Ulva lactuca (Chlorophyta) algae samples were collected in clean sterile jar from Red sea shore Jeddah, Saudi Arabia and subjected to taxonomy scheme of identification by a specialised botanist at the Botany Department, Faculty of Science, KAU, Saudi Arabia. The samples were washed, and suspended for 24 hours in different solvents namely, ethylacatate (EAE), ethanol (EE), or chloroform (CE) (100g/1000 ml), by each of the three solvents, was centrifuged for 10 min. at 8000*x*g, followed by deproteinization with isopropanol (1:1), and concentration by rotary evaporisation at 4ºC. All the reagents used during this study were of high analytical grade [10,11].

2.2 Assay of Total Phenolic Content

The total phenolic content in each of the three extracts of *Ulva lactuca (Chlorophyta) algae* was quantified according to a previously described method [10]. Briefly, a volume of 0.2mlof each extract is added to2.5ml of 10% Folin ciocalteau's reagent, followed by addition of 2ml of 10% Na_2CO_3, an incubation for 1 hour at 37°C and reading the absorbance of the developed color at 760nm. A standard curve was established using different concentrations of Gallic acid. The determined total phenols is reported in Milligram Equivalence to Gallic acid per One Gram of the Extract.

2.3 Assay of Total Flavonoid Content

The total flavonoid content in each of the three extracts of *Ulva lactuca (Chlorophyta) algae* was quantified according to a previous documented method [11]. Briefly, a volume of 0.5 ml of each extract was added to 2ml of 10% $AlCl_3$ and to 0.5 ml of potassium acetate (120 mM), followed by anincubation for a period of 30 min at 30°C, and reading the absorbance of the developed color at 415 nm. The standard curve was established by using different concentrations of Quercetin. The reporting of the mean of total flavonoid was on the basis of milligram of Quercetin per gram of extract.

2.4 Reducing Power Capacity

The reducing power capacity of the three extracts was determined by measuring their ability to reduce ferric chloride solution [12]. Briefly, aone ml of each extract was mixed with one ml of 200 mM sodium phosphate buffer (pH 6.6) and 1ml of 1% potassium ferric cyanide, incubated at 40°C for 15 min., followed by the addition of one ml of 10 % TCA, and a centrifugation at 2000x*g* for 5 minutes. A volume of two ml of the supernatant was collected and mixed with 1 ml of 0.1% ferric chloride, followed by reading the absorbance of the developed color at 700 nm. The mean % reduction power of the extract is reported by its equivalence to 100µg ascorbate /ml.

2.5 Scavenging Capacity

The mean of free radical scavenging capacity of the extracts against 1, 1- diphenyl-2-picryhydrazyl (DPPH) was determined according toa previously documented method .The formulae used in its calculation was:

Percent Scavenging Activity = $A_{0Control}$- $A_{0sample}$ abs./ $A_{0Control}$ x 100

The A_0 is the absorbance using Gallic acid as a Standard Control (Serial dilution of gallic acid used as standard (20- 100µg /ml).

2.6 Scavenging Power against Hydroxyl Radical

The mean of scavenging power of different extracts on hydroxyl radical was determined using the method of Kuda et al. [12]. Ascorbic acid was used as standard, and the mean of scavenging power is reported in percentage.

2.7 Scavenging Capacity against Nitric Oxide

The mean of scavenging activity against nitric oxide was determined by the main reaction with sodium nitroprusside-Griess reagent [13]. The mean of percentage of inhibition was compared to that obtained with Trolox.

2.8 Statistical Analysis

The data were analysed by using the SPSS software. The student's *t*-test was used to examine the statistical significance of differences among the means of the different treatments within this Complete Randomized Design. The significant differences are reported at P value of < 0.05.

3. RESULTS

Data of Table 1 show the impact of different solvents on the recovery of phenolics and flavonoids from *Ulva lactuca* (Chlorophyta) algae. The phenolic content ranged between a minimum mean of 38.9 GAE/g, recovered by chloroform, to a maximum mean of 77.3 GAE/g, recovered by ethyl acetate solvent ($p<0.05$); however, the extracted flavonoid range was from 31.2 to 60 mgQE/g. The Ethyl acetate extract showed the highest yield of phenolics and flavonoids compared to the other two solvents ($p<0,05$).

Table 2 shows the comparison of means of % ferric-reducing power properties of *Ulva lactuca* (Chlorophyta) algae at different weights of extracts, resulting from three solvents, in comparison to the reducing power of control-Vitamin C. The means of the reducing power against ferric ions of different concentrations of the control Vitamin C and EAE were significantly higher than those obtained by similar concentrations of EE and CE ($P<0,05$). It is worth noting that the control-Vitamin C included at around 95 µg /ml was able to reach to the highest % reducing power of 93%. The mean % scavenging activity against 1-diphenyl-2-picrylhydrazyl radical of the control-Gallic acid and EAE were significantly greater than that obtained by EE and CE at scavengers concentrations between 20-80 mcg/ml ($P<0,05$) (Table 2). The scavenging activity of all extracts and the control increased with an increasing concentration of the extract between 20 to 80 mcg/ml.

A comparison of the % scavenging activity, by the three different extracts of *Ulva lactuca* (Chlorophyta) algae and the control-Vitamin C, against hydroxyl radicals is shown in Table 2.

The mean % scavenging activity against hydroxyl radical of the control-Vitamin C and EAE were significantly greater than that obtained by EE and

Table 1. The impact of different solvents on the recovery of phenolics and flavonoids from *Ulva lactuca (Chlorophyta) algae* algae (Mean ±S.D)

Extracts	Mean of phenolics (mg GAE/g extract)	Mean of flavonoids (mg QE/g extract)
Ethyl acetate	77.3±5.9[a]	60 ±7.0 [a]
Ethanol	45.5±3.2[b]	34.5±4.8[b]
Chloroform	38.9±2.8[b]	31.2±3.3[b]

[a-b]Means in a column followed by different alphabet superscripts are significantly different at P< 0.05

Table 2. Reducing power capacity, scavenging capacity, scavenging power against hydroxyradical, scavenging power against nitric oxide of different algae extracts (Mean ± SD)

Animal groups Parameters	Ethylacetate extract (EAE)	Ethanol extract (EE)	Chloroform extract (CE)	Vitamin C
Reducing power capacity Mean± SD	92.1±7.3	87 ±3.4	72.7±5.9	95.4±8.8
P_1 value	N.S	<0.05	0.01	--------
Scavenging capacity antioxidants (%) Mean± SD	19± 1.7	42.0± 2.5	34.1± 2.5	29.9±2.3
P_1 value			N.S	<0.001
Scavenging power against hydroxyradical (%) Mean ±SD	21± 1.4	49.4± 4.5	38.4± 2.27	31.3± 3.32
P_1 value		<0.001	<0.001	<0.001
P_2 value				
P_3value				
Scavenging power against nitric oxide (%) Mean± SD	21± 1.4	49.4± 4.5	38.4± 2.27	31.3± 3.32
P_1 value	---	<0.001	<0.001	<0.001

P_1 versus vitamin C; P_2: EE versus EAE; P_3: CE versus EAE

CE at scavengers concentrations between 20-60 mcg/ml (P<0.05). It is worth noting that the Ethanol Extract (EE) was the only one that kept a homogeneous dose-dependent capacity at higher weights between 60 to 100 µg /ml. The comparison of the impact of different solvents on the mean scavenging activity of *Ulva lactuca (Chlorophyta) algae* extracts against nitric oxide, compared to control-Trolox, is shown in Table 2. The mean % scavenging activity against nitric oxide radical of the control-Trolox and EAE were significantly greater than that obtained by EE and CE at scavengers concentrations between 20-60 mcg/ml (P<0,05).

4. DISCUSSION

In the present study, the concentrations of flavonoid and phenolics, and the scavenging capacity of different dilutions of three solvent extracts of *Ulva lactuca (Chlorophyta) algae* algae against different radicals, were determined. The ethyl acetate extract (EAE) has proved its highest significant efficiency in extracting the phenolics and flavonoids from the algae of *Balanites aegyptiacea,* compared to the ethanol extract (EE) and chloroform extract (CE) (Table 1). This is in agreement with a paucity of previous researches related to extraction of phenolics [14] and flavonoids [15] from algae. The main reason for this high efficiency in extraction is most likely the chemical nature of phenolics, constituted of a hydroxyl group bonded to an aromatic hydrocarbon, and the nature of flavonoids, composed of 15-carbon skeleton of two phenyl rings and a third heterocyclic ring, that made these chemicals highly soluble in the ethyl acetate solvent, which is an ester of ethanol and acetic acid [16].

The percent reducing power capacity of the three different extracts, applied at a range of concentrations between 20 to 100 mcg/ml, proved the presence of a highest significant capacity by the EAE compared to the EE and CE. This expected trend is likely due to the highest efficiency in recovering wider spectrum of members of phenolics and flavonoids (Table 2). It is previously documented that the reducing power capacity is correlated positively to the nature of the members of phenolics [17] and flavonoids in the extract.

The EAE showed its highest scavenging capacity, specifically on DPPH (Table 2). This could be due also to the nature of members of phenolics and flavonoids recovered by the EAE. Previous works documented the correlation between members of recovered phenolics and flavonoids to scavenging capacity against DPPH [18]. It is worth noting that the decay in scavenging activity of DPPH in the two curves of Gallic acid and EAE at higher weights of 100 mcg could be due to unavailability of enough amounts of DPPH used in the test protocol.

The apparent dose-dependent scavenging capacity against hydroxyl radicals by the three extracts and the control-vitamin C allowed for comparisons, showing the highest capacity by EAE (Table 2). This is another indication of the reason behindthis difference, which is most likely the chemical nature of the recovered scavengers. Previous works on scavenging capacity against hydroxyl radicals showed the correlation between the nature of phenolics and flvonoids [19] on the scavenging capacity against hydroxyl radicals.

The EAE showed also its highest scavenging capacity against nitric oxide compared to the two other extracts (Table 2). Previously documented researches correlated positively the scavenging activity against nitric oxide to the chemical nature of flavonoids [20] and phenolics [21] in the extracts.

The phenolic, flavonoids, vitamin C, phytosterol and carotenoids are well established as antioxidant bioproducts. The reducing power capacity of flavonoids mediates their antioxidant effects by removal of free radicals [22]. Phenolic compounds of different chemical nature exhibit different levels of antioxidant properties through the chelating of metal ions. Previous works showed that extracts of *Ulva lactuca (Chlorophyta) algae* result in strong inhibition of lipid peroxidation [23,24]. The reduction capabilities of *Ulva lactuca (Chlorophyta) algae* extract was compared to ascorbic acid or Trolox. The reducing power of ethyl acetate extract of *Ulva lactuca (Chlorophyta) algae* was remarkable, and the reducing power of the extract rose as the concentration of the extract gradually increased.

5. CONCLUSION

In conclusion, the presence of bioactive compounds in *Ulva lactuca (Chlorophyta) algae* extracts could make it a good source of antioxidant compounds that can be used in treating degenerative diseases caused by free

radicals, as well as to justify the basis of using this plant's extract in folkloric remedies.

It is recommended in the future research to identify and determine the concentration of the different recovered members of phenolics and flavonoid by the three solvents, that will help in the interpretation of the differences obtained in their free radical-scavenging capabilities.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lordan S, Ross RP, Stanton C. Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. Mar Drugs. 2011;9:1056-100.
2. De Almeida CL, Falcão H de S, Lima GR, Montenegro C de A, Lira NS, de Athayde-Filho PF. Bioactivities from marine algae of the genus *Gracilaria*. Int J Mol Sci. 2011;12:4550-73.
3. Ganesan P, Kumar CS, Bhaskar N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Bioresour Tech. 2008;99:2717-23.
4. Dhargalkar VK, Neelam P. Seaweed: Promising plant of the millennium. Science and Culture. 2005;71:60-6.
5. Ilhami G, Metin TU, Munir O. Evaluation of antioxidant and antimicrobial activities ofClary Sage. Turk J Agric. 2003;28:25-33.
6. Ismet B, Cumhur A, Hilal K. Antimicrobial and antioxidant activities of *Cystoseira crinita* Duby and *Ulva intestinalis* Linnaeus from the coastal region of Sinop, Turkey. Journal of Coastal Life Medicine. 2015;3: 441-5.
7. Pietra F. Secondary metabolites from marine microorganisms; bacteria, protozoa, algae and fungi: Achievements and perspective. Natural Product Reports. 1997;14:453-64.
8. Kelecom A. Secondary metabolites from marine microorganism. Annals of Brazilian Academy of Sciences. 2002;74:151-70.
9. Parekh J, Chanda SV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol. 2007;31:53-8.
10. Nwinyi OC, Chinedu NS, Ajani OO, Ikpo CO, Ogunniran KO. Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. Afr J Food Sci. 2009;3:77-81.
11. Md. Monir H, Md. Nur A, Nizam U, Mohammad BU, Sk. Ferozuddin AC. *In vitro* antioxidant, antimicrobial and *in vivo* peripheral analgesic activities of methanol and petroleum ether extracts of whole plant of *Uraria lagopoides* DC. British Journal of Pharmaceutical Research. 2015;8:1-14.
12. Kuda T, Tsunekawa M, Goto H. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. Journal of Food Composition and Analysis. 2005;18:625-33.
13. Finkel T, Holbrook NJ. Oxidants, oxidativestress and the biology of ageing. Nature. 2000;408:239-47.
14. Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 2007;102:771-6.
15. Foon TS, Ai LA, Kuppusamy P, Yusoff MM, Govindan N. Studies on in vitro antioxidant activity of marine edible seaweeds from the east coastal region of Peninsular Malaysia using different extraction methods. J Coast Life Med. 2013;1:193-8.
16. Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a redalga, *Polysiphonia urceolata*. Food Chem. 2006;95:37-43.
17. Kelman D, Posner EK, McDermid KJ, Tabandera NK, Wright PR, Wright AD. Antioxidant activity of Hawaiian

marinealgae. Mar Drugs. 2012;10:403-16. Rosaline SD, Sakthivelkumar S, Rajendran K, Janarthanan S. Screening of selected marine algae from the coastal Tamil Nadu, South India for antibacterial activity. Asian Pac. J. Trop. Biomed. 2012;2:S140–S46.

18. Harborne JB. Phytochemical methods: Aguide to modern techniques of plant analysis. 3rd edition, New York, Chapmanand Hall. 1998;1-150.
19. Ranganathan A. Phytochemical analysis of *Carallum anilagiriana* using GC-MS. J. Pharmacog and Phytochem. 2014;3:155-9.
20. Rajesh PM, Natvar PJ. *In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radicals cavenging methods. Journal of Advanced Pharmacy Education and Research. 2011;1:52-68.
21. Premalatha M. Phytochemical characterization and antimicrobial efficiency of seaweed samples, *Ulva fasciata* and *Chaetomorpha antennina.* International Journal of Pharma and Bio Sciences. 2011;2:288-93.
22. Sundaram R, Lawrance A, Balakrishnan M. Antibacterial activity of *Ulva reticulata* from southwest coast of Kanyakumari, India. Journal of Coastal Life Medicine. 2016;4:246-7.
23. Seenivasan R, Indu H, Archana G, Geetha S. The antibacterial activity of some marine algae from South East coast of India. Am. Eurasian J. Agric. Environ. Sci. 2010;9(5): 480–489.
24. Shimizu Y. Microalgal metabolites: A new perspective. Annu. Rev. Microbiol. 1996; 50:431–465.

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