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Research Article

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF TWO SELECTED SEAWEEDS FROM INDIA

Surbhi Agarwal[1]., Komal Chauhan[2] and Aman[3]

[1,2]Department of Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management
[3]Department of Basic and Applied Science, National Institute of Food Technology Entrepreneurship and Management

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ABSTRACT

The present study investigates the presence of phytochemicals and antioxidant activity of two selected seaweeds (SW) from Tamil Nadu, India. The SW studied were brown seaweed *Sargassum fusiforme* (SF) and green seaweed *Ulva lactuca* (UL). The phytochemical screening and antioxidant activity of SWs were carried out using standard methods in eight extracts made in four different solvents for each SW namely, water, acetone, ethanol and methanol. All the results were analysed using statistical software. All eight extracts showed the presence of carbohydrates tannins and phenols while alkaloids and cardiac glycosides were absent in all extracts. Total phenol and antioxidant study shows that maximum amount of total phenols and antioxidants were present in methanol extracts of SF followed by UL except for FRAP assay in which case methanol extract of UL has higher FRAP activity than methanol extract of SF although the difference was not significant. Results shows that seaweeds are rich source of bioactive compounds and antioxidants thus can be used as therapeutic products.

INTRODUCTION

In today's world, the deaths rising from non communicable diseases are on rise. According to WHO (Lindmeier Christian, 2015) report about 16 million people die prematurely before age of 70 due to chronic diseases such as lung diseases, stroke, cancer, and diabetes with most being preventable. About 26.2% of premature deaths have occurred in India between age group of 30 and 70 due to cardiovascular disease, cancer, diabetes or chronic respiratory diseases.

Many chronic degenerative diseases such as cancer, atherosclerosis, chronic fatigue syndrome, rheumatoid arthritis, and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and Huntington's disease have been associated with oxidative stress resulting from formation of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the body through various mechanisms (Rahman, Hosen, Islam, & Shekhar, 2012). For example, oxidative stress generated by free radicals and their products plays an important role in development of atherosclerosis in addition to other factors by oxidizing LDL–c which in turn regulates expression of cellular adhesion molecules leading to plaque formation (Steinberg, n.d.) (Devasagayam *et al*., 2004). In obesity, excessive adipocytes and preadipocytes act as a source of inflammatory cytokines which stimulates production of ROS and RNS (Coppack, 2001). Oxidative stress may lead by neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease by causing oxidative damage to neurons leading to death or dysfunction of neurons (Rahman *et al*., 2012). Formation of ROS may also be stimulated by hyperglycemia in diabetes resulting in hyperglycemia induced trigger of diabetic complications (Dismutases & Fridovich, 1997) (Wei, Lu, Wei, Ma, & Lee, 2001) (Beckman & Ames, 1999) (Henry, 1962) (Finkel & Holbrook, 2000). Oxidants may also lead to inflammation as a result of activation of different kinases and redox transcription factors such as AP-1 and NH-kappa B resulting in resulting in chronic obstructive pulmonary disease (Hoshino & Mishima, 2008) (MacNee, 2001). However, this damage can be prevented to great extent by antioxidants.

Antioxidants, either generated in body or consumed in diet, plays a major role in counteracting the oxidative stress by neutralizing the free radicals. This prevents cells against their toxic effects thus resulting in disease prevention (Pham-Huy,

Corresponding author:* **Surbhi Agarwal
Department of Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management

He, & Pham-Huy, 2008). Also, antioxidants have shown to increase shelf life of food products (Schwarz *et al*., 2001). Thus, now-a-days more of research is being focussed on increasing consumption of antioxidnats because of their health promoting properties. Many higher plants, fruits and vegetables have been found to be a good source of antioxidants (Kumaran and Kumakaran, 2007) with seaweeds being a rich source of antioxidants among marine sources.

Seaweeds are macrophytic algae, a primitive type of plants lacking true roots, stems and leaves (Khan & Satam, 2003). They are found in the intertidal zone attached to rocks, floating on ocean or on the beach. Based on pigmentation, seaweeds are categorized as brown seaweeds, red seaweeds and green seaweeds (Subba Rao & Mantri, 2006). SWs are generally used to produce phycocolloids such as agar, alginates and carrageenan which are used as gelling, thickenin and stabilising agents in many industries such as food, confectionary, textiles, pharmaceuticals, etc (Kaliaperfumal, 1998). Seaweeds are also known to have many health beneficial components such as tannins, antioxidants, phytochemicals, polyunsaturated fatty acids, minerals, vitamins etc. (Khotimchenko *et al*, 2005). Thus, they exhibit many medicinal uses i.e., can be used against oxidative stress, diabetes, inflammation, obesity, hypertension, allergy, cancer etc (Mohamed, Hashim, & Rahman, 2012). Thus, the study was aimed to explore the bioactive potential of two seaweeds namely, *Sargassum fusiforme* (SF) and *Ulva lactuca* (UL) from India as a natural source of antioxidants.

MATERIALS AND METHODS

Sample Collection and Storage

For this study brown seaweed *Sargssum fusiforme* and green seaweed *Ulva lactuca*, were supplied from Prasmo Agri, Kumbakonam, Tamil Nadu. After procurement, seaweeds were washed thoroughly under running tap water to remove any dust, dirt or foreign material adhering to it followed by distilled water. Washed seaweeds were freeze dried for 72 hours, grinded to fine powder, vacuum packed and stored in dry and dark place at 4°C until further analysis. All the chemicals used were of analytical grade.

Extraction

1 gram of seaweed powder was extracted with 30 ml of water, acetone, methanol, and ethanol each for both varieties of seaweeds. Extraction was carried out in dark for 24 hours at room temperature with intermediate shaking for better extraction. After extraction it was filtered and kept in dark at 4°C until further analysis.

Preliminary Phytochemical Screening

All the extracts prepared i.e., extract of SF in water (SF_W), extract of SF in acetone (SF_A), extract of SF in methanol (SF_M), extract of SF in ethanol (SF_E), extract of UL in water (UL_W), extract of UL in acetone (UL_A), extract of UL in methanol (UL_M), and extract of UL in ethanol (UL_E) were analysed for presence of phytochemicals such as tannins, flavonoids, steroids, alkaloids, cardiac glycosides, saponins etc. Phytochemicals were analyzed using standard procedures (Aziz, 2015) (Bhandary, Kumari, Bhat, & Prasad Bekal, 2012)(Devmurari, 2010)(Mamta & Jyoti, 2012).

Total phenolic Content

Total phenolic content (TPC) was measured using method given by (Singleton and Rossi, 1965) using Folin Ciocalteu (FC) reagent. In this, 1ml of sample was reacted with 5ml of FC reagent followed by incubation at room temperature. Then 20% of sodium carbonate was added and volume was made upto 100ml and again incubated for 2 hours at room temperature in dark. The absorbance of colour developed was measure at 765nm. Gallic acid was taken as standard and results were expressed as mg GAE/100g of dried SW.

Antioxidant Activity

Total Antioxidant Activity

Sample was reacted with a solution containing 0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate at 95°C for 90 minutes and then absorbance was measured at 695nm (Prieto, Pineda, & Aguilar, 1999). Ascorbic acid (AA) and Butylated hydroxytoluene (BHT) were used as standard for water soluble and fat soluble antioxidants respectively. Results were expressed in terms of mg AAE/100g dried SW and mg BHT/100g dried SW.

FRAP assay

FRAP reagent was added to sample, incubated for 4 minutes at room temperature. Absorbance was measured at 593nm (Benzie & Strain, 1996). Trolox was used as standard and results were expressed as mg TE/100g dried SW.

Statistical Analysis

All readings were taken in duplicate and results were expressed as mean ± standard deviation (SD). All the results are presented as dry weight of seaweeds. Analysis of results was performed using analysis of variance (ANOVA) and statistical difference between means was calculated using Duncan's multiple range test. Differences were considered significant at a level of $p<0.05$. All statistical analysis was performed using SPSS version 21.0.

RESULTS AND DISCUSSION

Phytochemical Screening

In this study water, acetone, methanol and ethanol extracts of SF and UL were analysed for phytochemical properties. Results are shown in Table 1. Result shows that all eight extracts tested has carbohydrates, tannins and phenolics whereas protein, alkaloids and cardiac glycosides were absent in all extracts. Flavonoids were present in aqueous, methanolic and ethanolic extract of both the seaweeds however, inter-tests has shown variation with respect to flavonoids. Steroids were detected only in methanol and ethanol extracts of UL while being completely absent in SF. However, saponins were present in alcoholic and aqueous extracts of both the seaweeds but were not detected in acetone extracts.

In contrast to these results, (Janarthanan & Kumar, 2013) has shown presence of alkaloid, terpinoids and steroids in acetone and methanol extracts of *Sargassum wightii*. Also, (Feldmann, Hamel, Agardh, & Vahl, 2014) has shown presence of alkaloids in methanol, ethanol and acetone extracts of UL and result for presence of protein in all the extracts was also positive.

Table 1 Preliminary Phytochemical screening of Seaweeds

S.No.	Phytochemicals	Test name	Sragssum fusiforme				Ulva lactuca			
			SF_W	SF_A	SF_M	SF_E	UL_W	UL_A	UL_M	UL_E
1.	Carbohydrates	Molisch's test	+	+	+	+	+	+	+	+
2.	Reducing Sugar	Benedict's test	+	+	+	+	+	+	+	+
		Fehling's test	+	+	+	+	+	+	+	+
3.	Protein	Ninhydrin test	-	-	-	-	-	-	-	-
4.	Tannins + Phenols	Lead Acetate test	+	+	+	+	+	+	+	+
5.	Tannins	Potassium dichromate test	+	+	+	+	+	+	+	+
6.	Alkaloids	Mayer's reagent test	-	-	-	-	-	-	-	-
		Wagner's reagent test	-	-	-	-	-	-	-	-
7.	Cardiac Glycosides	Keller-Killiani's test	-	-	-	-	-	-	-	-
8.	Flavonoids	Lead acetate test	+	-	+	+	+	-	+	+
		Sodium hydroxide test	-	-	-	-	+	+	-	+
9.	Steroids/ Triterpenoids	Salkowski test	-	-	-	-	-	-	+	+
10.	Saponins	Lead acetate test	+	-	+	+	+	-	-	+

These differences may arise due to different extraction procedures used or because of difference in climatic conditions, season of harvest and area from where seaweeds have been procured.

Total Phenolic Content

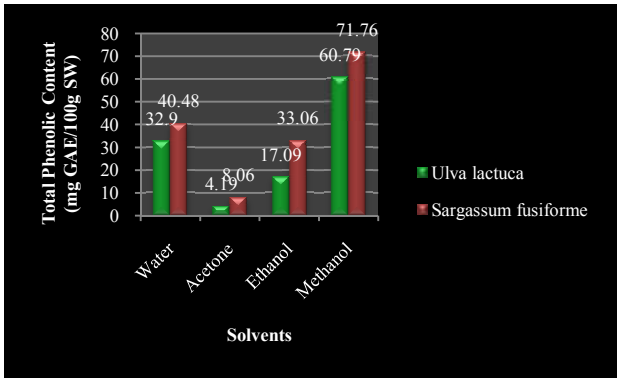
(Flodin *et al*, 1999) and their location in the sea (Connan, Deslandes, & Gall, 2007). (Feldmann *et al*., 2014) has reported higher phenolic content (1.05±0.005mg phenolics/g of UL) in UL where in 2.60±0.002mg phenols/g of SW was present in *Sargassum cinereum* and *Sargassum ilicifolium* which is also

Results of quantitative analysis of antioxidant activity are shown in **Table 2**

Units		*Sargassum fusiforme*				*Ulva lactuca*			
		Water	Acetone	Ethanol	Methanol	Water	Acetone	Ethanol	Methanol
TPC	(mg GAE/100g dried SW)	$40.48\pm12.54^{c,d}$	$8.07\pm7.76^{a,b}$	$33.06\pm18.02^{b,c}$	71.76 ± 3.87^{e}	$32.89\pm15.51^{b,c}$	4.19 ± 0.91^{a}	$17.09\pm1.36^{a,b,c}$	$60.79\pm5.70^{d,e}$
TAA	(mg AAE/100g dried SW)	163.59 ± 20.53^{c}	64.32 ± 3.28^{a}	127.01 ± 3.28^{e}	169.96 ± 8.21^{c}	81.73 ± 1.64^{a}	79.41 ± 3.28^{a}	121.79 ± 12.32^{b}	166.48 ± 19.71^{c}
TAA	(mg BHT/100g dried SW)	$99.76\pm20.41^{a,b}$	82.12 ± 13.61^{a}	258.55 ± 4.53^{c}	479.89 ± 49.90^{d}	70.89 ± 6.81^{a}	75.70 ± 31.76^{a}	$200.81\pm40.83^{b,c}$	271.38 ± 99.81^{c}
FRAP	(mg TE/100g dried SW)	$1.31\pm0.05^{b,c}$	$0.73\pm0.05^{a,b}$	0.59 ± 0.01^{a}	2.68 ± 0.21^{d}	1.46 ± 0.06^{c}	$0.75\pm0.13^{a,b}$	$0.80\pm0.01^{a,b,c}$	2.81 ± 0.74^{d}

*Mean ± SD followed by same letters in the same row are not significantly different ($p \leq 0.05$).

Result shows that among all the four extract, total phenolic content of SF was significantly higher than UL. This could be because of presence of phlorotannins in only brown seaweeds (Ragan and Glombitza, 1986). However, methanol extract of SF has maximum amount of total phenols (71.76±3.87mg GAE/100g of dried SW) among all the extracts followed by the methanol extract of UL with 60.79±5.70mg GAE/100g of dried SW with acetone extract of UL having the lowest amount of total phenolics (4.19±0.91mg GAE/100g of dried SW). Ethanol extracts of both seaweeds has higher phenol content as compared to acetone extracts with ethanol extract of SF having significantly higher content as compared to acetone extract of SF.



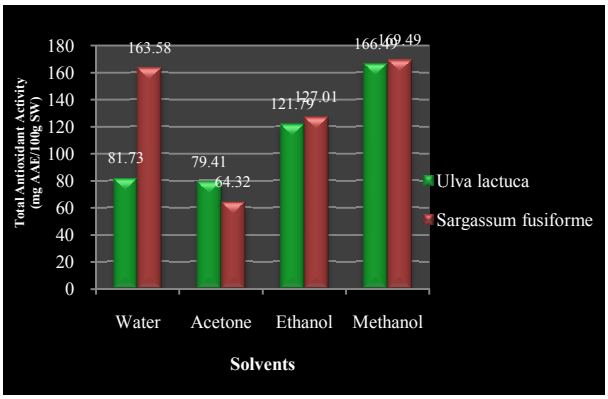
Graph 1 Total Phenolic Content of Seaweeds

(Wang, Jónsdóttir, & Ólafsdóttir, 2009) has shown that water extract less amount of polyphenols as compared to polar organic solvents. However, researchers shows that phenolic content of seaweeds varies according to the climatic conditions

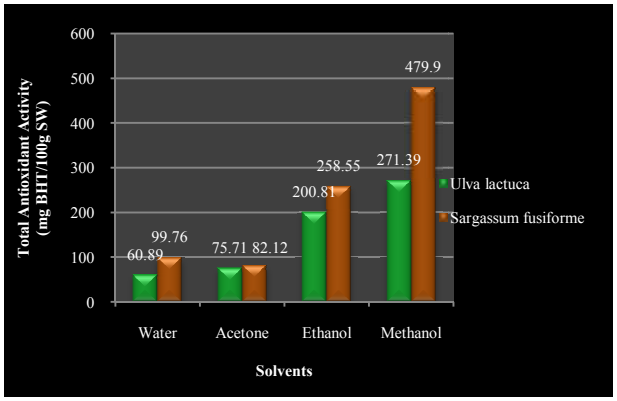
higher as compared to the present study. Whereas phenolic content of *Sargassum marginatum* was found to be 11.00±0.10mg GAE/g of methanol + chloroform extract by (Chandini, Ganesan, & Bhaskar, 2008). *Sargassum plagiophyllum* conatins 7.48±0.02mg GAE/g of extract of phenols (Chakraborty, Madacherry, & Makkar, 2016). Among green seaweeds *Enteromorpha prolifera* also found to have higher amount of phenols (47.9±0.2mg GAE/g of sample) (Cho, Lee, Kang, Won, & You, 2011).

Total Antioxidant Activity

The total antioxidant activity (TAA) of SF and UL are shown in graph 2. When estimating in terms of ascorbic acid equivalents, results shows that methanol extracts of both the seaweeds has higher amount of total antioxidant activity with SF having the highest with 169.97±8.21mg AAE/100 g of dried SW as compared to 166.48±19.71mg AAE/100 g of dried SW for UL. Water extract of SF also has appreciable amount of TAA and is not significantly different from methanol extract of both seaweeds. However, acetone extracts of both seaweeds have shown to have lower amount of TAA among all the four extracts followed by ethanol extract. Acetone extract of SF exhibit the lowest amount of TAA of 64.32±3.28mg AAE/100g of dried SW. Also, TAA of ethanol extracts of both the seaweeds is significant different from other extracts. This could be because of the fact that antioxidant activity depends strongly on the type of the solvent used as compounds with different polarity exhibits solvent specific solubility rate. Also it is dependent on the climatic condition in which it is grown (Kumar, Sucheta, Deepa, Selvamani, & Latha, 2008).



a



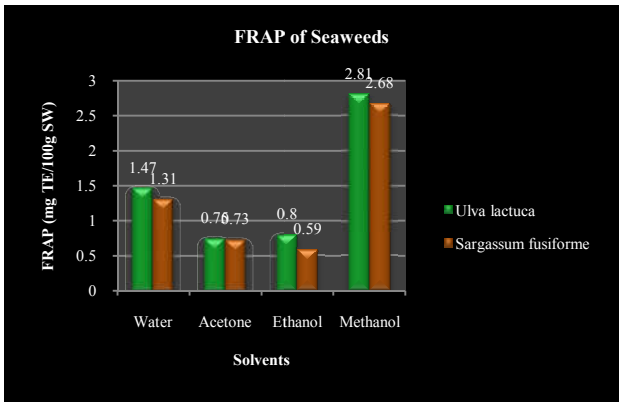
b

Graph 2 Total Antioxidant Activity of Seaweeds (a) using ascorbic acid as standard (b) using BHT as standard

With respect to the TAA in terms of BHT equivalence, water extract of UL has the lowest activity of 70.89±6.81mg BHT/100g of dried SW followed by the acetone extract of UL. From the results it can be seen that although TAA of water and acetone extract of both the seaweeds are not significantly different from each other but it is significantly different from ethanol and methanol extracts of both the seaweeds. Methanol extract of SF has shown to have maximum amount of TAA of 479±49.90mg BHT/100g of dried SW when estimated in terms of BHT equivalent. Also among all the extracts, extracts of SF has more TAA than UL which could be because of presence of bioactive compounds such as fucoxanthin and phlorotannins in brown seaweeds (Xiaojun, Xiancui, Chengxu, & Xiao, 1996) (Yan, Chuda, Suzuki, & Nagata, 1999).

FRAP assay

FRAP assay gives information regarding ability of a compound to reduce reactive species (Benzie & Strain, 1996). From graph 3 it can be seen that the methanol extract of UL has maximum FRAP activity followed by methanol extract of SF i.e., 2.81mg TE/100g of dried SW and 2.68±0.21mg TE/100g of dried SW respectively. Lowest FRAP activity was found in ethanol extract of SF with 0.59±0.03mg TE/100g of dried SW. In contrast to TPC and TAA, FRAP activity of all extracts of SF is lower than respective extracts of UL. Brown seaweeds such as Fucus serratus and Fucus vesiculosus have found to have 113.4±18.5 µM ascorbic acid equivalent/g of dried SW and 109.8±17.7 µM ascorbic acid equivalent/g of dried SW respectively (O'Sullivan *et al*., 2011).



Graph 3 FRAP of Seaweeds

This shows, that seaweeds are rich source of bioactive compounds such as phenols and antioxidants, however results have shown greater variation with respect to content of specific antioxidants mentioned in literature. This difference could be due to differences in extraction procedures or solvents used, difference in species of seaweeds and geographical location from where they are procured.

CONCLUSION

This study was conducted to estimate the antioxidant activity of two Indian seaweeds i.e, *Sargassum fusiforme* and *Ulva lactuca* from Tamil Nadu. The results clearly show that both seaweeds are rich source of antioxidants and contain bioactive constituents as can be seen from phytochemical screening of the extracts. However, further studies are needed to understand the structure and geometry of these photochemical and antioxidant compounds. Also, animal trials are required for better understanding of their mechanism of action as free radical scavengers and as therapeutic agents. These antioxidant and phenolic compounds need to be assessed for any toxic or harmful effects. Thus, these phytochemical and antioxidant constituents from seaweeds may be used in pharmaceutical, nutraceutical, cosmetics or food industry in future.

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