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Antioxidant status of selected sea weeds of Manamelkudi coast, Pudukkottai district

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Abstract
Manamelkudi, located along the Palk Strait of East coast of Tamil Nadu serve as treasure houses for valuable marine resources like sea grass and sea weeds. A study was undertaken at Agricultural College & Research Institute, Kudumiyanmalai during 2016-17 to evaluate the nutritional composition of the seaweeds in order to use them as potential food ingredients. Sea weed samples were collected from Manamelkudi village of Pudukkotai district and were identified as *Gracilaria salicornia* (C.Agardh) Dawson and *Gracilaria edulis* (S.G. Gmel.) P.C. Silva Gracilariaceae at Botanical Survey of India, Coimbatore. The samples were washed in sea and fresh water to remove associated organisms and other extraneous matters. The sea weeds were dried, powdered and used for assessing the antioxidant status. *G. edulis* showed higher activities of enzymic antioxidants superoxide dismutase (312.03 U/g) and catalase (50.1U/g) when compared to *G. salicornia* (SOD:266.27U/g and CAT:37.1U/g). Maximum levels of the non-enzymic antioxidants, (total phenol and flavonoid) was found in *G. edulis* when compared to *G. salicornia* (5.8 and 3.43mg/g; 5.23 and 3.3mg/g respectively). The extent of lipid peroxidation assessed revealed 70.5 and 32.1 percent inhibition by the ethanolic extract of *G. edulis* and *G. salicornia* respectively. The results were comparable with that of the standard antioxidant BHT. Phytochemical analysis revealed the presence of alkaloids, carbohydrates, saponins, proteins, aminoacids, phytosterols, phenolic compounds, flavonoids, terpenoids and tannins except saponins and terpenoids in *G. salicornia.* Thus it can be concluded from the present investigation that both the seaweeds are potentially rich in natural antioxidants and could be used as nutrient supplements in food industry.

Keywords: *G. salicornia, G. edulis*, antioxidant status, lipid peroxidation, phytochemicals

Introduction
Seaweeds are a fascinating and diverse group of organisms which contains treasures for the benefits of human race. Exploiting natural food resources is an easy and quick solution to prevent the rising prevalence of lifestyle and nutritional disorders (Salooje and Petittifor, 2001) [14]. Seaweeds are promising natural resources in terms of availability and nutrient density. The benefits of seaweeds are numerous and profound. Harvested in pure seawater, seaweeds can be considered as nature's most complete and balanced nutrient food source. The edible seaweeds contain significant quantities of protein (Dawes *et al*., 1993) [5], lipids, minerals and vitamins that vary with species, geographical location, season, humidity and temperature (Kaehler and Kennish, 1996) [6]. Recently, algal polysaccharides have been demonstrated to play an important role as free radical scavengers *in vitro* for the prevention of oxidative damage in living organisms. *In vitro* antioxidant action is used as a first tool to select potential compounds for protection from lipid oxidation and for further formulation of functional feeds (Balboa *et al*., 2013) [1]. A comprehensive study of nutritional (protein and amino acids, fat and fatty acids, carbohydrates, minerals, and vitamins) and bioactive compounds such as polyphenols, carotenoids, etc., from each seaweed, which can exert some beneficial properties on health, is necessary (Peinado *et al*., 2014) [13]. The present study is an attempt to study the antioxidant status and inhibition of *in vitro* lipid peroxidation of seaweeds collected from Manamelkudi village of Pudukkottai district, Tamil Nadu, India.

Materials and Methods
Sea weed samples were collected from Manamelkudi village of Pudukkotai district. The samples were washed in sea and fresh water to remove associated organisms and other

extraneous matters. The samples were identified at Botanical Survey of India, Coimbatore. The identified sea weeds are *Gracilaria salicornia* (C.Agardh) Dawson (Plate 1) and *Gracilaria edulis* (S.G. Gmel.) P.C. Silva (Plate 2) – Gracilariaceae. The samples were dried, powdered and used for the experiment.

Fresh Dried

Plate 1: *Gracilaria salicornia*

Fresh Dried

Plate 2: Gracilaria edulis

Assessment of Antioxidant Status of Sea Weeds

The antioxidant status and inhibition of *in vitro* lipid peroxidation of both the sea weeds *G. salicornia* and *G. edulis* were analysed by adopting standard methods. The antioxidant status of the sea weeds was evaluated by determining the activities of the enzymic antioxidants superoxide dismutase and catalase and by assessing the levels of non-enzymic antioxidants, total phenols and total flavonoids. The values were calculated and were expressed as mean with standard deviation.

Assay of Superoxide Dismutase (Sod)

SOD was assayed according to the method of Kakkar *et al.* (1984) [7]. The assay of SOD is based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazon. The colour formed at the end of the reaction can be extracted into butanol and measured at 560nm.

Assay of Catalase (CAT)

Catalase activity was assayed following the method of Luck (1974) [10]. The UV absorption of hydrogen peroxide can be measured at 240nm, whose absorbance decreases when degraded by the enzyme catalase. From the decrease in absorbance, the enzyme activity can be calculated.

Estimation of Total Phenols

The amount of total phenols in the plant tissues was estimated by the method proposed by Mallick and Singh (1980) [11]. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent to produce a blue-coloured complex in alkaline medium, which can be estimated spectrophotometrically at 650nm. The results were expressed as milligram Catechol equivalent (mg/g).

Estimation of Flavonoids

The method proposed by Cameron *et al.* (1943) [3] was used to extract and estimate flavonoids. Flavonoids react with vanillin to produce a coloured product, which can be measured spectrophotometrically. The results were expressed as milligram Catechin equivalent (mg/g).

Preparation of Plant Extracts

Freshly collected seaweeds (1g) was homogenized in 10ml of the solvent. The organic extracts were dried at 60°C protected from light. The residue was weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain the desired concentration. Aqueous extracts were prepared fresh.

Estimation of Lipid Peroxidation (LPO) In Goat Liver Homogenate

Goat liver was procured fresh from the slaughter house and washed free of blood using Tris-HCl buffer (40mM, pH 7.0). A 20% liver homogenate was prepared in the same buffer using a motorized Teflon homogenizer. The homogenate was clarified to remove debris and used as the membrane source for assessing LPO as per the method of Okhawa *et al.* (1979) [12].

Preliminary Phytochemical Screening

The ethanolic extract of the leaves of *B. monnieri*, was screened for the presence of phytochemicals according to the method of Khandelwal (2002) [8].

Statistical Analysis

All the analyses were performed in triplicates and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation (SD).

Results and Discussion

Enzymic antioxidants

Microalgae represent an almost untapped resource of natural antioxidants, due to their enormous biodiversity, much more diverse than higher plants. However, not all groups of microalgae can be used as natural sources of antioxidants, due to their widely varied contents of target products, growth rate or yields, ease of cultivation, and / or other factors. Antioxidant enzymes such as superoxide dismutase (SOD) play a key role in the removal of reactive oxygen species produced in microalgae during various physical-chemical stress responses (Santos *et al*., 1999) [15]. In this study, 266.27 ± 0.16 U/g and 312.03 ± 0.15 U/g of superoxide dismutase and 37.10 ± 0.2 U/g and 50.10 ± 0.15 of catalase activities were recorded in *G. Salicornia* and *G. edulis* respectively.

Table 1: Activities of enzymic antioxidants in *G. Salicornia* and *G. edulis*

Enzymic antioxidants	*G. salicornia*	*G. edulis*
Superoxide dismutase ($U^{\#}$/g)	266.27 ± 0.16	312.03 ± 0.15
Catalase (U^{*}/g)	37.10 ± 0.2	50.10 ± 0.15

The values are mean ± SD of triplicates

1 Unit - Amount of enzyme that gives 50% inhibition of the extent of NBT reduction in 1 minute

* 1 Unit - Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units / minute

The results revealed that *Gracilaria edulis* showed higher activities for the enzymic antioxidants superoxide dismutase and catalase when compared to *Gracilaria salicornia*.

Non-enzymic antioxidants

Phenolic and flavonoid compounds were broadly recognized in sea weeds confirming their potent role in chelating metal ions, preventing radical formation and improving the internal antioxidant system under stress environmental conditions. These activities protect the body from progressive diseases caused by the adverse effects of reactive oxygen species (Chakraborty *et al*., 2013) [4].

Table 2: Levels of non-enzymic antioxidants in *G. salicornia* and *G. edulis*

Non-enzymic antioxidants	*G. salicornia*	*G. edulis*
Total phenols (mg catechol/g)	5.23 ± 0.15	5.8 ± 0.35
Flavonoids (mg catechin/g)	3.3 ± 0.36	3.43 ± 0.35

The values are mean ± SD of triplicates

The non-enzymatic antioxidants from the sea weeds *G. Salicornia* and *G. edulis* are presented in Table 2. Phenolic compounds serve as important antioxidants because of their ability to donate a hydrogen atom or an electron to form stable radical intermediates (Singh *et al*., 2009) [16]. The total phenolic contents of the microalgae were determined and expressed as catechol equivalent. Total phenols play a significant role in the regulation of plant metabolic processes and overall plant growth as well as lignin synthesis (Lewis and Yamamoto 1990) [9]. On the other hand, phenols act as substrates for many antioxidant enzymes. In the present study, the total phenol content was estimated to be 5.23 ± 0.15 and 5.8 ± 0.35mg/g in *G. salicornia* and *G. edulis* respectively. Phenols protect the cells from potential oxidative damage and increase the stability of cell membrane (Burguieres *et al*., 2007) [2]. Maximum levels of both the non-enzymic antioxidants (total phenol and flavonoid) were found in *G. edulis* when compared to *G. salicornia*.

From the analysis, it is clear that both the seaweeds possessed enzymic and non-enzymic antioxidants and the maximum antioxidant status was exhibited by *G. edulis*.

Extent of inhibition of lipid peroxidation by *G. salicornia and G. edulis* extracts

The seaweeds not only possessed antioxidant potential but also were potent in suppressing TBARS formation by H_2O_2 induced lipid peroxidation in goat liver homogenate (a mixture of plasma membrane and internal membranes). Table 3 shows the extent of inhibition of LPO by *G. salicornia and G. edulis* extracts in three different solvents namely aqueous, ethanol and methanol and compared with the standard BHT.

Table 3: Effect of *G. Salicornia* and *G. edulis* extracts on inhibition of lipid peroxidation

Extracts	Percent inhibition of lipid peroxidation	
	G. salicornia	*G. edulis*
Methanol	56.0 ± 0.1	65.2 ± 0.2
Ethanol	32.1 ± 0.2	70.5 ± 0.3
Aqueous	20.2 ± 0.1	28.1 ± 0.1
BHT	59 ± 0.5	

The ethanolic extract of *G. edulis* exhibited significant inhibition of 70.5 ± 0.3% of H_2O_2 induced lipid peroxidation when compared with *G. Salicornia.* However, it exhibited more or less similar inhibition when compared with that of BHT (59± 0.5%).

Phytochemical analysis

In order to identify the nature of the active component responsible for the antioxidant potential of the seaweeds, various phytochemical analyses were performed. The results of the preliminary phytochemical screening test using the ethanol extract of seaweeds are summarized in table 4.

Table 4: Preliminary screening of phytochemicals (qualitative) in ethanolic extract of G. *salicornia and G. edulis*

Phytochemicals	Ethanol extract	
	G. salicornia	*G. edulis*
Alkaloids	+	+
Carbohydrates	+	+
Saponins	-	+
Protein and amino acids	+	+
Sterols	+	+
Phenols	+	+
Flavonoids	+	+
Terpenoids	-	+
Tannins	+	+

(+) - Positive ; (-) - Negative

Conclusion

Thus, it can be concluded from the present investigation that both the seaweeds *Gracilaria salicornia* and *Gracilaria edulis* are potentially rich in natural antioxidants and able to inhibit lipid peroxidation. Thereby these seaweeds could be used as nutrient supplements in food and feed industry.

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