Evaluation of Antioxidant and Free Radical Scavenging Potential of *Halimeda tuna*

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Abstract

Seaweeds are regarded as a prospective source of bioactive compound. In the current study, the algae *Halimeda tuna* have been extracted using hexane, chloroform and ethanol were tested for phenolic, tannin, flavonoid content. Also, *in vitro* antioxidant activity interms of DPPH, Hydroxyl, Superoxide, Nitric oxide radical scavenging assay and FRAP assay. The presence of higher point of phenolic, tannin and flavonoid content were identified from ethanolic extract. Also, a result of a greater rate of DPPH and superoxide radical scavenging activity was observed through chloroform extract. The hexane and ethanolic ectracts exhibited a good result for hydroxyl and nitric oxide scavenging activity. The test results implied *Halimeda tuna* to possess good antioxidant potential.

Key Words: Seaweeds, *Halimeda tuna*, Extracts, Antioxidant, Radical Scavenging Activity

1. Introduction

The powerful role of antioxidants in inhibiting free radicals provides a great secure against various infectious and degenerative diseases. The presence of antioxidant properties were reported in variety of marine algae (Yasantha *et al.*, 2006). The chemical compound free radical is an unpaired electron swing around the chief layer of the nucleus. In order to attain stability, these free radicals extract the electrons which causes damage molecular (Evans and Halliwell, 1999). The further increment of theses free radicals leads to cell death and oxidative stress creation (Wiseman and Halliwell, 1996). A variety of synthetic antioxidants such as butylated hydroxyanisol (BHA), bytylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) are commercially available. The restrictions of these synthetic alternatives were arised due to their side effects (Huang, 2004). Hence herbal or algal extracts possessing antioxidant must be researched to identity the actual content to acquire its beneficial effects so as to reduce the usage of synthetic antioxidants.

In this paper, antioxidant of the algae *Halimeda tuna* was reported with the measurement of total phenolic, tannin and flavonoid content. The DPPH, Hydroxyl, Superoxide, Nitric oxide radical scavenging activity and FRAP assay were assessed and reported.

2. Materials and Methods

The collected samples were cleaned, shade dried and extracted in soxhlet extractor with hexane, chloroform and ethanol. The percentage yield was calculated. The extracts were subjected to the following *in vitro* assays such as

2.1 Total phenolics and tannin determination

The determinations of total phenolics were done using method described by Siddhuraju and Becker (2003). The analysis was performed in triplicate and the results were expressed as gallic acid equivalents. The tannins were also estimated using the same extracts having treated it with Polyvinyl Polypyrrolidone (PVPP)

(Siddhuraju and Manian, 2007). The supernatant of the centrifuged sample contains only simple phenolics other than tannins. Precipitation of tannin would have taken place along with the PVPP. The tannin was the calculated using

Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%)

2.2 Determination of Total Flavonoid Content

The modified calorimetry method earlierly reported by Zhisten *et al.,* (1999) was used to determined the flavonoid content. Absorbance of the mixture was determined at 510 nm versus water blank. The results were expressed as rutin equivalent.

2.3 Free Radical Scavenging Activity

With the basis of hydrogen donating or radical scavenging ability, the determination of antioxidant activity of various solvent extracts could be made effectively. The standard method of Blois (1958) was used for DPPH radical scavenging activity. According to the method expressed by Klein *et al.,* (1991), Sreejayan and Rao (1997) and Beauchamp and Fridovich (1971), the hydroxyl radical, nitric oxide radical and superoxide radical scavenging activity were executed respectively. The absorbance of the sample was measured at 517 nm, 412 nm, 546 nm and 590 nm for DPPH, hydroxyl, nitric oxide and superoxide radical scavenging activity respectively. The percentage inhibition of free radicals could be calculated using the formula

% radical scavenging activity = (control OD-sample OD / control OD) x 100

The ferric reducing antioxidant assay was carried out using the method Benzie and Strain 1996. At 593 nm the absorbance was measured.

3. Results and Discussion

The total phenolic, tannin and flavonoid content of *Halimeda tuna* were compared among the three solvent extracts (hexane, chloroform and ethanol). Higher amount of antioxidants were present in ethanol and lower level of antioxidants were present in hexane.

Phytochemicals such as phenolics, saponins, steroids and terpenoids were reasons for good antioxidant activities (Chejara *et al.,* 2014). Flavonoids are a group of polyphenolic compounds which influence the radical scavenging inhibition of hydrolytic and oxidative enzymes and also act as an anti-inflammatory agent (Okwu, 2001a and Okwu, 2001b).). Antioxidant activity has also been reported in the following algal species such as *Sargassum pallidum* (Ye *et al.,* 2009), *Halimeda incrassata* (Nonoa *et al.,* 2011). The ability of the phenolic substances including flavonoids, phenolic acids, tannins and lignins to act as potential antioxidants has been extensively investigated (Rice-Evan *et al.,* 1996; Suja *et al.,* 2005).

In the report of free radical scavenging activity obtained, the percentage inhibition of the selected free radicals by three different sample extracts were compared below. DPPH radicals were inhibited to a greater extent of 43.16% by chloroform. Hexane and ethanolic extract showed almost similar level of DPPH scavenging activity. Devi *et al.,* 2014 reported a positive correlation between phenolic compounds and DPPH hydroxyl radical scavenging activity in *Halimeda tuna* and *Gracilaria foliifera*. The hydroxyl ions were inhibited to a considerable level of 72.73% through hexane extract followed by ethanolic extract and

chloroform extract. The most reactive hydroxyl free radical can be formed from superoxide anion and hydroxyl peroxide. The free radical has an extremely short half-life but is capable of causing a great damage to living organisms (Cheesman and Slater, 1993; Lee *et al.,* 2005). The superoxide ions were scavenged appreciably by chloroform extract (36.78%) of *Halimeda tuna* compared to that of hexane and ethanolic extract.

Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated, after the oxygen is taken into living cells (AI-Mamun *et al.,* 2007). The nitric oxide scavenging activity was greater by ethanolic extract (46.52%) compared to that of hexane and chloroform. The ability to reduce ferric ions indicates that the antioxidant compounds are electron donors and could reduce the oxidized intermediate of lipid peroxidation processes, thus acting as primary and secondary antioxidants (Yen and Chen, 1995; Matanjun *et al.,* 2008). Finally antioxidant power was at a highly creditable and distinguishable range through ethanolic extract (4158.15 mmol Fe (II)/g) compared to that of hexane and chloroform.

4. Conclusion

Halimeda tuna, a macroalgae has been taken under present study. It has been extracted using hexane, chloroform and ethanol. The presence of total phenolics, tannin, flavonoid content were determined. The percentage of inhibition of free radicals using three different solvent extracts were also tested and reported.

Table 1. Levels of Total Phenol, Tannin and Flavonoid in *Halimeda tuna*

S.No	Sample	Phenol (mg TAE/g)	Tannin (mg TAE/g)	Flavonoid (mg RE/g)
1	Hexane	16.36 ± 1.27	6.93 ± 1.27	0.30 ± 0.02
2	Chloroform	62.40 ± 3.33	45.76 ± 3.33	0.94 ± 0.05
3	Ethanol	108.15 ± 3.33	87.63 ± 1.27	3.76 ± 0.07

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

Table 2. Free Radical Scavenging Activity of *Halimeda tuna*

S.No	Sample	DPPH (%)	Hydroxyl (%)	Superoxide (%)	Nitric Oxide (%)	FRAP (mmol (Fe(II)/g)
1	Hexane	25.06 ± 0.14	72.73 ± 0.35	25.64 ± 0.39	27.11 ± 0.34	1862.53 ± 7.60
2	Chlorofom	43.16 ± 0.09	48.43 ± 0.39	36.78 ± 0.34	35.65 ± 0.28	1396.59 ± 16.46
3	Ethanol	24.26 ± 0.21	69.15 ± 0.34	25.40 ± 0.26	46.52 ± 0.28	4158.15 ± 5.57

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