Global Journal of Pharmacology, 3 (2): 59-62, 2009
ISSN 1992-0075

Total Flavanoid and *in vitro* Antioxidant Activity of Two Seaweeds of Rameshwaram Coast

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Abstract: This study aims to evaluate the total flavonoid and invitro antioxidant activity of two seaweeds such as *Ulva lactuca* and *Sargassum wightii.* The total flavanoid content and antioxidant activity of methanolic extract was higher in 2.02±0.07 mg GAE/g and 1.16±0.11 mg GAE/g respectively in *S. wightii*. The FTIR analysis reveals presence of the polyphenolic signals at different ranges. The moderate antioxidant activities in flavanoids were estimated was compared with the gallic acid standard.

Key words: Seaweed, Flavonoids, Antioxidant activity, FT-IR analysis

INTRODUCTION

Flavonoids are one of non-nutritive chemicals in seaweeds exhibit several biological effects such as anti-inflammatory, anti hepatotoxic and anti- ulcer actions. Flavonoids may help in protection of diseases (anti-inflammatory, anti hepatotoxic and anti- ulcer actions) by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body. Flavonoids have potent, anti-allergic, anti-viral and have free radical scavenging abilities and also provide protection against cardiovascular mortality. They also exhibited anti-coagulant, anti-hyperlipidase, anti-nephritic, vasodilative effects and human immuno deficiency virus (HIV) type 1 integrase inhibition.

A free radical is a molecule with one or more unpaired electrons in the outer orbital. Many of these free radicals are in the form of reactive oxygen and nitrogen species, these can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free-radical formation [1]. Oxidative stress has been linked to cancer, aging, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's). Reactive oxygen species(ROS) such as superoxide radical (O2•–), hydroxyl radical(OH•), peroxyl radical (ROO•) and nitric oxide radical(NO•), attack biological molecules such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis carcinogenesis [2] and may lead to the development of chronic diseases related to the cardio and cerebrovascular systems [3]. The most commonly used synthetic antioxidants presently used are butylatedhydoxyanisole (BHA), butylatedhydoxytoluene (BHT) Propylgallate (PG) and test butylatedhydroquinone. However, these synthetic antioxidants have side effects such as liver damage and carcinogenesis [4]. Free-radical scavengers are antioxidants which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and subsequent lipid peroxidation, protein damage and DNA strand breaking [5]. Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicines to replace synthetic antioxidant. Seaweeds have received special attention as a source of natural antioxidants [6]. Seaweeds are known source of pharmacological and food additives with potential health effects like antioxidative and anticarcinogenic [7,8]. Based on the above facts, we now report the effect of the methanol extract of two seaweeds such as *Sargassum wightii* and *Ulva lactuca* for its potential antioxidant property and also to evaluate the flavanoid content.

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MATERIALS AND METHODS

Preparation of Methanol Extract of Seaweed: Fresh plants of *U. lactuca* Linn (Chlorophyceae) and *S. wightii* Greville (Phaeophytceae) were collected from the intertidal region of the Rameshwaram, southeast coast of India. Then the plants were washed thoroughly with sea water to remove extraneous materials and brought to the laboratory in plastic bags containing water to prevent evaporation. Samples were then shade dried till constant weight obtained and ground in an electric mixer. The powdered samples were than stored in refrigerator.

Extraction of Flavonoids from Seaweeds: Total flavonoids were extracted according to the method of Hertog [9]. Briefly each 5g of sample was homogenized with 40ml of 75% methanol with t-butyl hydroquinone (w/v) using a pestle and mortar. 10ml of 6N hydrochloric acid was added carefully mixed and refluxed at 90°C for 2hr. After cooling, the supernatant was filtered through Watmann No.1 filter paper and transferred in a volumetric flask with methanol. After replacing air with nitrogen gas to inhibit decomposition of flavonoids, the extracts were kept at -80° C for further analysis.

Estimation of Total Flavonoids The determination of flavonoids can be made indirectly by estimating the Total Phenolic Contents (TPC). The determination of total phenolic content, measured as Gallic acid equivalent by using Folin and Ciacalteau reagents [10,11]. 1ml of sample (diluted to 50 to 25% of original concentration with methanol) 0.5ml of Folin Ciacalteau's reagent (2 N) and 3ml of Na_2CO_3 (200mg/ml) were taken and mixed in the given order. The mixture was vortexed and the reaction was allowed to 15min at room temperature and absorbance was measured at 725nm in spectrophotometer (HITACHI 220S). Gallic acid was used as standard and the equivalents (w/w) were determined from a standard concentration curve.

Determination of Total Antioxidant Activity: Total antioxidant activity of seaweed extracts was determined according to the method of Prieto *et al.* [12]. Briefly 2ml of sample was taken at different concentrations (50, 10, 250, 500 and 1000 µg) and mixed with 1ml of standard reagent 0.6 M Sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate. Then Reaction mixture was incubated at 95° C for 90min. Absorbance of all the sample was measured at 635nm.

Ft-ir Spectrophotometry (Fourier Transform-infra Red Spectrum Analysis): The solid samples of *S.wightii* and *U.lactuca* (10 mg) was mixed with 100mg of dried Potassium bromide (Kbr) and compressed to prepared as a salt disc. The disc was then read spectrophotometrically (Bio-Rad FTIR-40-model, USA). The frequencies of different components present in each sample were analyzed. The same procedure was followed for the standard.

RESULTS AND DISCUSSION

The total flavanoid content of methanolic extract was 1.35±0.04 mg GAE/g and 2.02±0.07 mg GAE/g respectively in *U. lactuca and S. wightii* and the total antioxidant activity of methanolic extract was also higher in *S. wightii* (1.16±0.11 mg GAE/g) whereas *U. lactuca* showed (0.91±0.09 mg GAE/g) (Table.1). All these results indicate flavanoids extracted from *S. wightii* and *U. lactuca* could be an important source of antioxidant molecules. The capacity of flavanoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavanoids are important for their antioxidant and free radical scavenging activities. Plant Phenolic in general is effective free radical scavengers and antioxidants. Phenolic compounds are commonly found in the edible brown, green and red seaweeds in which the antioxidative property has been correlated to their phenolic content [13,14]. There are few reports on the antioxidant capacity of seaweeds and the mechanism of seaweeds as antioxidative agents is still not fully understood. Hence further research is underway to analyze and isolate the active compounds responsible for the antioxidant and antimicrobial activity from both the seaweeds. Some authors claim that there is no correlation between the total phenolic content and the radical scavenging capacity [15], so it was important to examine the correlation between the total phenolic contents and total antioxidant capacity of the studied seaweeds. The results of the present study reveal that there is a strong correlation between antioxidant activity and phenolic content.

Table 1: Total flavonoid content and total antioxidant activity of seaweeds

Seaweeds	Total flavonoids (mg GAE/g)	Total antioxidant activity mg GAE/g
Ulva lactuca	1.35±0.04	0.91±0.09
Sargassum wightii	2.02±0.07	1.16±0.11

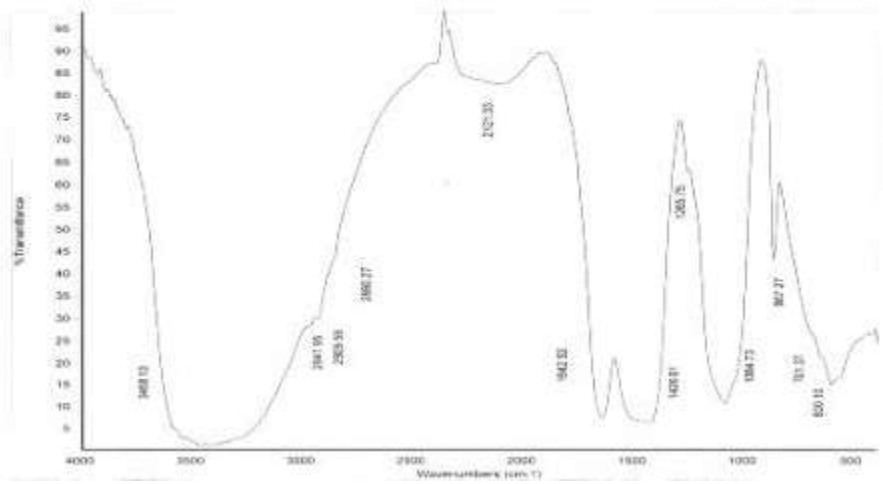

Fig. 1: FT-IR spectrum of *S. wightii*

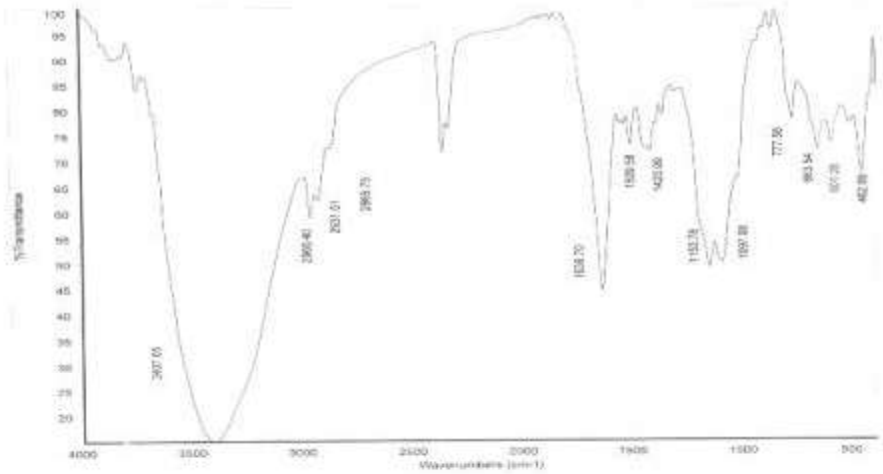

Fig. 2: FT-IR spectrum of *U. lactuca*

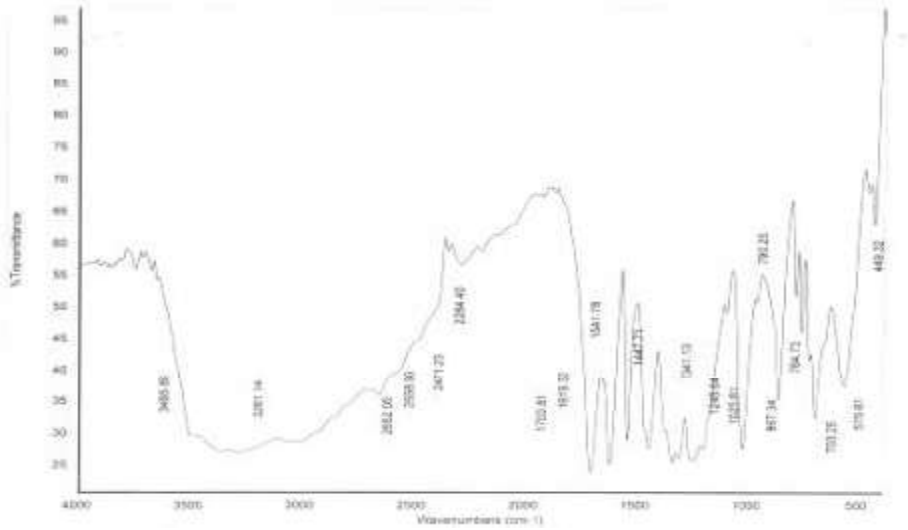

Fig. 3: FT-IR spectrum of Gallic acid standard

It is possible that the antioxidant of both the seaweed extract *S. wightii* and *U.lactuca* can be the result of their high concentration of phenolic compounds. The total phenolic content expressed as gallic acid equivalents was higher for the *S. wightii* and *U.lactuca* extracts.

Ft-ir Spectral Analysis: The FT-IR analysis of the samples was done and the functional groups associated were determined (Fig. 1-3). The FT-IR spectrum of both the sample was obtained and the effective peaks were compared with that of the standard gallic acid. The FT-IR spectrum of the standard gallic acid contains thirteen major peaks at the range of 3407.05, 2960.40, 2931.51, 2865.75, 1639.70, 1509.58, 1420.99, 1153.78, 1097.08, 777.58, 663.54, 601.28, 462.89cm-1:whereas the FT-IR spectrum of the *S.wightii* and *U.lactuca* samples also recorded the same number of peaks lying between 449.32 cm^{-1} and 3495.89 cm^{-1} and 462.89 cm^{-1} and 3407.05 cm^{-1} respectively. This finding helps in further research in the investigation of other seaweeds with different solvent fraction for their antioxidant activity and it also useful to utilize of these plants as a source food and medicine.

ACKNOWLEDGEMENT

The authors are grateful to Prof. S.T. Balasubramanian, Director, Centre of Advanced Study in Marine Biology, Annamalai University for providing the necessary facilities.

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