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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF *HYPNEA VALENTIAE* (RED ALGAE), GULF OF MANNER, RAMESWARAM, TAMIL NADU

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ABSTRACT

The present study was to confirm whether different methods show the same sensitivity or capacity to discriminate the antioxidant activity of the different crude extracts of *Hypnea valentiae*. The crude extracts were analyzed using the following methods; 2, 2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging, Hydroxyl radical scavenging, Superoxide radical scavenging, ABTS radical scavenging and reducing power assay. The crude extracts of *H. valentiae* were found to have strong antioxidant properties. The antioxidant activity values quantified through superoxide and ABTS were higher than the ones obtained by DPPH, hydroxyl and reducing power and these values varied among extracts. The results indicated that the methanol extract is the most potent antioxidant. This holds great promise for the use of *Hypnea valentiae* as a source of strong antioxidant compounds.

Keywords: Red algae, ABTS, DPPH, Reducing power, Free radical activity.

1. INTRODUCTION

Free radicals may cause diseases as diverse as cancer, diabetes, Alzheimer and Parkinson through inducing oxidative stresses, cell damage and death [1]. Reactive oxygen species (ROS) are produced endogenously from metabolic activity in the human body or exogenously from smoking, air pollutants, radiation, zone and industrial chemicals. Research into the natural products chemistry and chemical defenses of algae over the past 40 years has resulted in the isolation of over 15,000 novel compounds, many of which have shown to have bioactive properties [2]. Marine algae are one of the largest producers of biomass in the marine environment. Recent reports have exhibited that marine algae are rich in bioactive compounds including polysaccharides, poly unsaturated fatty acids, poly-phenolic compounds, antioxidants, peptides, essential vitamins and minerals [3]. The marine algae are considered an important source of secondary metabolites such as antiviral, antioxidant, anticancer, antimicrobial, anti-inflammatory, anticoagulant and antibiotic agents. Among marine organisms, red algae have been identified as an under-exploited plant resource, although they have long been recognized as valuable sources of structurally diverse secondary metabolites. All orders of red algae contain a large number of species that actively concentrate chlorine and bromine ions and incorporate them into halogenated organic molecules.

Hypnea valentiae are dark red-brown, 100-300 mm tall, with definite upright branches bearing, radial side branches, gradually shorter. It also Short spine-like branches, few on main branches, point upward. They contain higher amounts of protein, fiber and minerals, thus considered an essential nutritional food. They also help improve the immune system, stimulating the functions of leukocytes. It is believed that this type of algae is a great adjuvant in the treatment of urinary tract infections, asthma, stomach disorders, skin diseases, boils, obesity, and high cholesterol levels. They also proved to be effective in the treatment of ulcers and tumors. Seaweed extracts possess potent bacterial activity against the bacterial strain, thus supporting their folkloric usage, promising a future scope for the use of these marine seaweeds against microbial populations [4]. Therefore, the present research was conducted to quantify the antioxidant activity of crude extracts from marine red algae *Hypnea valentiae* using five different in-vitro assays.

2. MATERIAL AND METHODS

2.1. Collection of sample

Fresh algae samples were collected from the coastal region of Gulf of Manner, Rameswaram region, Tamil Nadu. The collected samples were washed with sea water and brought to the laboratory in a plastic bag containing sea water to prevent evaporation.

2.2. Preparation of extracts

Five g of the fine powder of *H. valentiae* was extracted successively with 100 ml of alcoholic and organic solvents (Petroleum ether, Benzene, Ethyl acetate, Methanol and Ethanol) in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No. 41 filter paper separately and all the extracts were concentrated in a rotary evaporator. All the concentrated extracts were subjected for in vitro antioxidant activity.

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging activity

The DPPH is a constant free radical and is extensively used to measure the radical scavenging activity of antioxidant component. This process is based on the reduction of DPPH in methanol solution in the company of a hydrogen donating antioxidant due to the arrangement of the non-radical form DPPH. Using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) the free radical scavenging action of all the extracts was assessed as per the previously reported process. DPPH of 0.1mM solution in methanol was prepared. 1 ml of this solution was poured into 3 ml of the solution at different concentrations (50, 100, 200, 400 and 800µg/ml). The mixtures were shaken dynamically and allowed to stand at room temperature for 30 minutes [5]. After that the absorbance was measured at 517nm using a UV-VIS spectrophotometer (Genesys 10 UV: Thermoelectron corporation). Ascorbic acid was employed as the standard. The lesser absorbance values of reaction mixture identify higher free radical scavenging action. Using the subsequent formula the ability to scavenge the DPPH radical was computed.

DPPH scavenging activity (% inhibition) = (A0 - A1)/ A0 X 100

Where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were carried out in triplicates and the outcomes were averaged.

2.3.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging properties was analyzed according to the method described by Halliwell *et al*. [6]. Stock answers of $FeCl_3$ (10mM), Ascorbic Acid (1mM), EDTA (1mM), H_2O_2 (10mM) and Deoxyribose (10mM) were put in distilled deionized water. The assay was executed by adding 0.1 ml EDTA, 0.1 ml H_2O_2, 0.01 ml of $FeCl_3$, 0.36 ml of deoxyribose, 1.0 ml of the extract of diverse concentration (50, 100, 200, 400 & 800µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 hour. 1.0 ml of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging achievement of the extract is accounted as % inhibition of deoxyribose. The degradation is figured by using the succeeding equation

Hydroxyl radical scavenging activity = (A0 - A1) / A0 X 100

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were carried out in triplicates and the results were averaged.

2.3.3. Superoxide radical scavenging activity

The superoxide anion scavenging action was calculated as elucidated by Srinivasan *et al*. [7]. The superoxide anion radicals were made in 3.0 ml of Tris--HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract of different concentrations (50, 100, 200, 400 & 800 µg/ml) and 0.5 ml Tris--HCl buffer (16 mM, pH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25°C for 5 min and the absorbance was estimated at 560nm against a blank sample, ascorbic acid. The percentage inhibition was determined by using the following equation. Superoxide radical scavenging activity = (A0 - A1) / A0 X 100

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were achieved in triplicates and the results were averaged.

2.3.4. Antioxidant Activity by Radical Cation (ABTS+)

ABTS assay was supported on the slightly modified technique of Huang *et al*. [8]. By reacting 7 mM ABTS solution with 2.45 mM potassium persulphate, ABTS radical cation ($ABTS^+$) was prepared. This mixture is

permitted to be in the dark at room temperature for 12-16 hrs previous to use. With ethanol to an absorbance of 0.7±0.02 at 734 nm the $ABTS^+$ solution was added. Following this trolox standard to 3.9 ml of diluted $ABTS^+$ solution or addition of 100µL of sample, absorbance was calculated at 734 nm by Genesys 10S UV-VIS (Thermo scientific) accurately after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = (A0 - A1) / A0 X 100

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were repeated thrice and the end results were averaged.

2.3.5. Reducing Power

The reducing power of the extract was established by the method of Kumar and Hemalatha [9]. 1.0 ml of solution containing 50, 100, 200, 400 & 800 µg/ml of extract was mixed up with sodium phosphate buffer (5.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 ml, 1.0%). The mixture was incubated at 50°C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°C) in a refrigerated centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700nm. The experiment was performed thrice and results were averaged.

3. RESULTS AND DISCUSSION

Marine sources are emerging as good alternate source for bioactive substances. Nowadays red algae are used as dietary supplements in daily life to animals and human and are easily metabolized. Macroalgae dietary fibres perform varied range of functions such as antioxidant, antimicrobial, antimutagenic, anticoagulant, antitumor etc. The antioxidant activity of Hypnea valentiae have been quantified by the DPPH, ABTS, Superoxide radical, Hydroxyl radical and reducing ability. The antioxidant activity of plant extracts can be quantified by different methods of measurement; in fact, it is recommended to use at least two different methods [10] and the results of a test system can be used to establish a ranking [11]. In our study, the five methods used to quantify the antioxidant activity of the selected red algal species showed the antioxidant capacity of these.

Antioxidant potential of the crude algae extracts was evaluated by DPPH radical scavenging activity. The DPPH radical scavenging activity was found to be increasing as dose increases. The results are expressed as % inhibition of DPPH and reported in fig.1.With respect to the antioxidant activity quantified by DPPH in each of the crude extracts, the lowest value was observed in benzene followed by petroleum ether. Significant activity was recorded in Ethyl acetate extract. The concentration of *Hypnea valentiae* ethyl acetate extract needed for 50% inhibition (IC_{50}) was 33.86 mg/ml, while ascorbic acid needed 30.45 mg/ml (table 1). The DPPH method was evidently introduced nearly 50 years ago by Blois [5] and it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. DPPH scavenging activity is influenced by the polarity of the reaction medium, chemical structure of the radical scavenger and the pH of the reaction mixture, sample concentration and reaction time. In this study, free radical scavenging activities of the crude extracts of *Hypnea valentiae* and standard ascorbic acid were determined by using DPPH method.

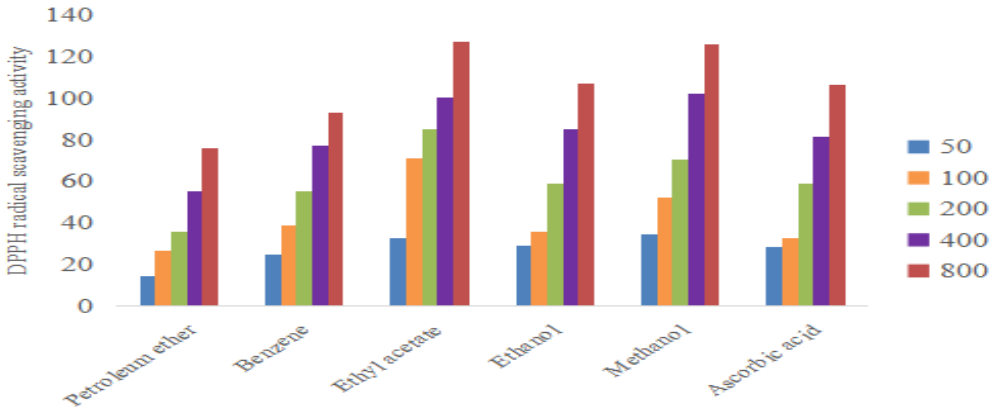

Fig. 1: Effect of different solvent extracts of *H. valentiae* on DPPH assay

Regarding the antioxidant activity quantified by hydroxyl radical, the crude extracts with the highest values were ethanol and ethyl acetate. The other crude extracts with the lowest to moderate antioxidant activity. The concentration of *Hypnea valentiae* ethanol extract needed for 50% inhibition (IC_{50}) was found to be 32.99 mg/ml, whereas 32.47 mg/ml (table 1) needed for ascorbic acid. The result obtained in the study showed that the ethanol extract revealed significant OH radical scavenging activity compared to standard Ascorbic acid (Fig. 2). The hydroxyl radical is a highly reactive oxygen-centered radical that is formed from the reaction of various hydroperoxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acid in membranes, and most biological molecules and is known to be capable of abstracting hydrogen atoms from membrane lipids and can bring about the peroxidic reaction of lipids [12]. *Hypnea valentiae* exhibited concentration-dependent scavenging activity against hydroxyl radicals generated in a Fenton reaction system.

Superoxide radical scavenging capacities of the crude extracts tested varied from 22.18 to 142.84% which represents a variation of standard ascorbic acid (fig.3). Methanol extract exhibited the significant antioxidant activity (142.84%) followed by ethanol (132.39%) and ethyl acetate (109.12%). In this assay, benzene (98.15%) revealed the lowest antioxidant potential (Fig. 3). The IC_{50} value of methanol extract of *Hypnea valentiae* on superoxide radical was found to be 33.66 mg/ml and 29.45 mg/ml for ascorbic acid, respectively (Table 1). Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents, e.g., hydroxyl radicals. The superoxide anion radical scavenging activity of the ethyl acetate extract may be due to the existence of phenolic compounds. The increase in superoxide anion radical scavenging is due to the increase in a number of phenolic hydroxyl groups in the molecule.

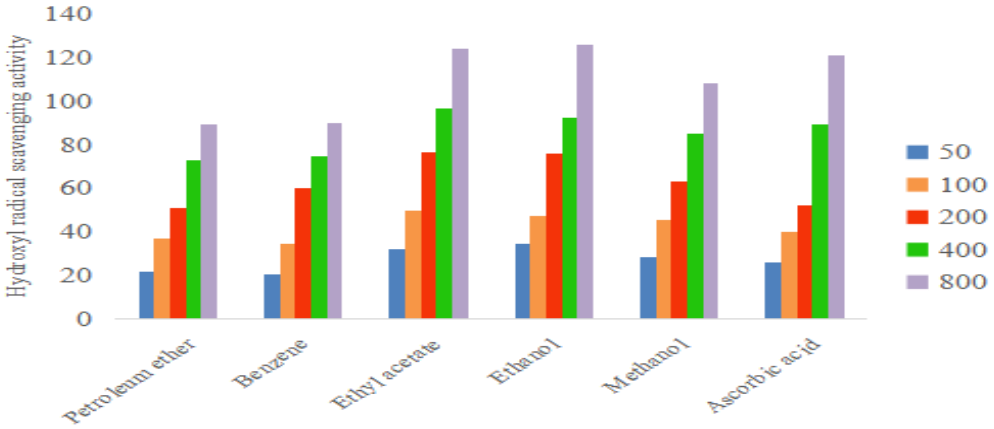

Fig. 2: Effect of different solvent extract of *H. valentiae* on Hydroxyl assay

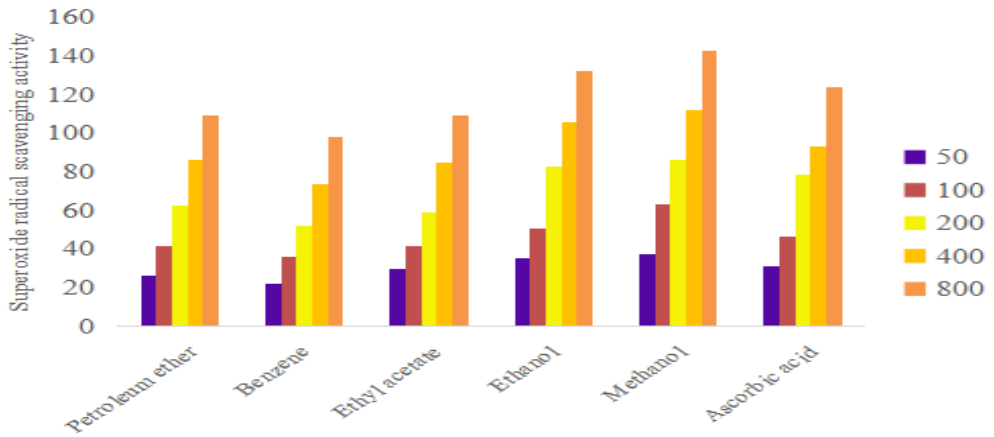

Fig. 3: Effect of different solvent extract of *H. valentiae* on Superoxide assay

ABTS assay is an excellent tool for evaluating the hydrogen-donating antioxidants and chain-breaking antioxidants [13]. The decolorization of the $ABTS^{+}$ was observed, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734nm. The crude extract from *H. valentiae* was fast and effective scavengers of $ABTS^{+}$ radical (fig.4). This activity is comparable with that of trolox, the standard antioxidant used in this study. The percentage inhibition was 129.25%, 127.16%, 111.37%, 110.12%, 98.24% and 112.45% in ethyl acetate, ethanol, ethanol, benzene, petroleum ether and trolox respectively at 800µg/ml, the highest concentrations tested. The quantity of *H. valentiae* ethyl acetate extract required to produce 50% inhibition of ABTS radical 28.47 mg/ml whereas 26.14 mg/ml (Table 1) needed for trolox. The scavenging of ABTS (129.25 %) by the extracts was found to be higher than that of DPPH (127.35 %). Several factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals [14]. Similarly Wang *et al*. [15], found that some compounds that have ABTS+ scavenging activity could not scavenge DPPH.

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [16]. The ferric reducing assay quantifies the ability of an antioxidant capacity to reduce a reactive oxygen species against that species oxidative power. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [17]. The results are showed in fig.5. As seen from the graph, the methanol extract exhibited a reducing power almost equal to that of Ascorbic Acid. The methanol extract showed a very good reducing power at lower concentrations. However, at higher concentrations, its reducing power was lesser than that of the ethanol extract.

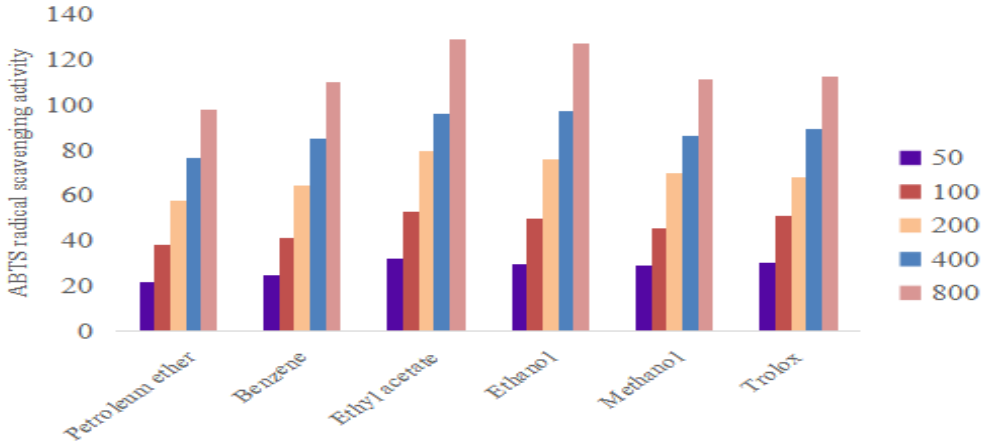

Fig. 4: Effect of different solvent extract of *Hypnea valentiae* on ABTS assay

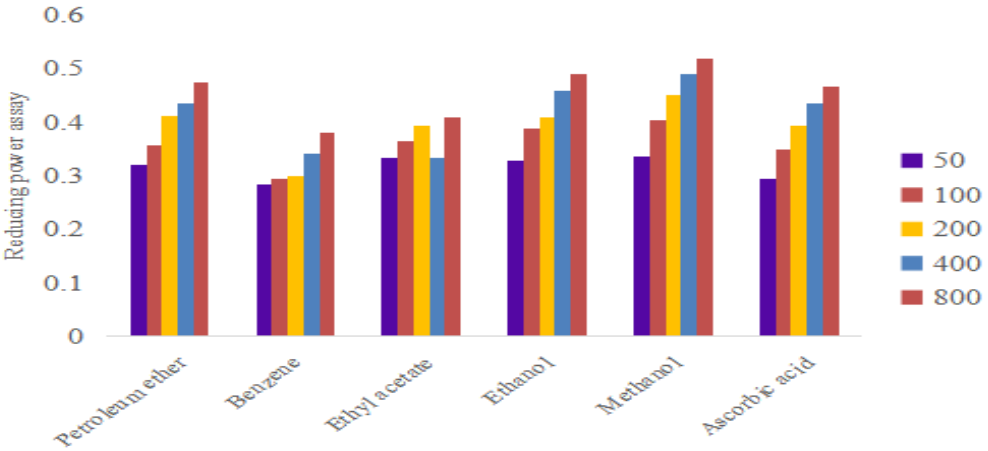

Fig. 5: Effect of crude extracts of *H. valentiae* on reducing power assay

Table 1: IC_{50} values of crude extracts

Tests	IC_{50} values					
	Petroleum Ether	Benzene	Ethyl Acetate	Ethanol	Methanol	Ascorbic acid
DPPH assay	23.66	27.48	33.86	30.16	33.35	30.45
Hydroxyl assay	28.92	29.85	32.80	32.99	31.72	32.47
Superoxide assay	28.88	26.47	28.30	32.18	33.66	29.45
ABTS assay	24.87	26.27	28.47	28.65	25.96	26.14

4. CONCLUSION

This work represents the ample screening of antioxidant activities in red macroalgae from the Gulf of Manner, Rameswaram. The results clearly showed that crude extracts of the selected red algae tested possess antioxidant activity to varying degrees. This screening emphasized the great antioxidant potential of *Hypnea valentiae*, which was found to be equivalent to the antioxidant activity of commercial antioxidant molecules assessed in the same study. Supplementary to these findings, it would be worth while carrying out additional experiments on the isolation, identification and characterization of the bioactive compounds of the most active extracts, especially from *H. valentiae*, and on the understanding of their mechanisms of action.

Conflict of interest

None declared

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