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SCREENING OF ANTI-OXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *HYPNEA VALENTIAE* (TURNER) MONTAGNE (RED ALGAE) COLLECTED FROM RASTHAKADU, KANYAKUMARI DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

The present study was undertaken to assess the anti-oxidant activity of ethanolic extract of *Hypnea valentiae* (Turner) Montagne collected from Rasthakadu coast, Kanyakumari District in the south east coast of Tamil Nadu, India. Free radical scavenging activity was evaluated using 1,1-diphenyl2-picryl hydrazyl (DPPH) free radical by the method of Mensor *et al.* The percentage of scavenging activity of DPPH by ethanolic extracts at 100µg, 200µg, 300µg, 400µg and 500µg were 28.22, 35.18, 48.75, 57.19 and 66.56%, respectively. These results showed that the DPPH assay in which 500µg showed the highest total anti-oxidant capacity, followed by 400µg, 300µg and 200µg, and lastly 100µg. The results showed that DPPH scavenging activity was increased, when the concentration of ethanolic extracts was also increased. Vitamin C, a strong anti-oxidant was used as control, and the anti-oxidant potential was compared to all tested samples.

KEYWORDS: Algae, *Hypnea valentiae*, Anti-oxidant, Ethanolic, DPPH

1. INTRODUCTION

It was proved that reactive oxygen species (ROS) generated in the human body can cause oxidative damages associated with many degenerative diseases such as atherosclerosis, coronary heart diseases, aging and cancer.[1] Reactive oxygen species (ROS) such as superoxide radical ($O_{2.}^-$), hydroxyl radical (OH^-), peroxyl radical (ROO^-) and nitric oxide radical (NO^-) can outbreak the biological molecules namely lipids, proteins, enzymes, DNA and RNA which leads to cell or tissue injury. ROS induce peroxidation of lipids (polyunsaturated fatty acids) generating secondary oxidants like heptanol and hexanal which contributes to oxidative rancidity, deteriorating the flavor of the food. These not only cause a loss in food quality but are also believed to be associated with carcinogenesis, mutagenesis, arthritis, diabetes, inflammation, cancer and genotoxicity.[2] To overcome the complications a variety of synthetic antioxidants namely Butylated Hydroxy Toluene (BHT), Butylated Hydroxy Anisole (BHA), Propyl Gallate (PG) etc. have been used as food preservatives. However, these synthetic antioxidants have many side effects such as liver damage and are suspected to be mutagenic and neurotoxic. Hence, most consumers prefer additive-free foods or a safer approach like the utilization of more effective antioxidants of natural origin.[3,4]

Recently, various phytochemicals like polyphenols, which are widely distributed in plants, have been reported to act as free radical scavengers.[5] Marine plants like Algae contain high amount of polyphenols. Marine macro algae constitute an important renewable resource in the marine ecosystem. Some of the commercially utilized marine macro algae can be used as fertilizers, food additives and animal feed.[6] Although Marine algae possess a wide range of application in food and pharmaceutical industry, the anti-oxidant activities of many types of algae in the South Indian coastal area are still unexplored. The main objective of the present study is to evaluate the anti-oxidant activity of *Hypnea valentiae* (Turner) Montagne, a marine Red algae.

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

The collection of *Hypnea valentiae* (Turner) Montagne (Marine Red Algae) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking from Rasthakadu, Kanyakumari district located in the south east coast of Tamil Nadu, India. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. The samples were packed separately in polythene bags in

wet conditions and brought to the laboratory, followed by, thoroughly washed in tap water and distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution.[7]

2.2. Preparation of ethanolic extract

For the preparation of ethanolic extract of *Hypnea valentiae* (Turner) Montagne, the collected plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered sample was packed in Soxhlet apparatus and extracted with ethanol for 8h. The excess amount of ethanol was evaporated and fine ethanolic crude powder was prepared and stored in the refrigerator for the anti-oxidant activity.[8]

2.3. Antioxidant activity

DPPH Free Radical Scavenging Assay

Ethanolic extracts from *Hypnea valentiae* (Turner) Montagne were analyzed for the anti-oxidant activity based on the scavenging activity of the 1,1-diphenyl2-picryl hydrazyl (DPPH) free radical using the method of Mensor et al.[9] Ethanolic extracts were prepared in triplicates at different concentrations (100-500µg/ml) and transferred into 1ml of 0.3mM ethanolic DPPH solution (Sigma Aldrich). Samples were left to stand for 30 minutes in the light and the absorbance was measured at 517nm, zeroing the spectrophotometer with ethanol as a blank. The DPPH radical had a dark violet colour solution, and once neutralized, became pale yellow allowing visual monitoring of the radical reaction. Vitamin-C was used as a positive control. The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition Percentage} = 1 - \frac{\text{Absorbance of Sample} - \text{Absorbance of Blank}}{\text{Absorbance of Control}} \times 100$$

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity

DPPH Free Radical Scavenging Assay

Crude ethanolic extracts of *Hypnea valentiae* (Turner) Montagne at various concentrations (100-500µg) were tested for anti-oxidant activity via the DPPH assay. The experimental results are presented in Table, where ethanolic extracts were established to possess anti-oxidant activity. Vitamin C was used as a positive control for the DPPH assay. Anti-oxidant activity was determined by assaying the reduction of DPPH radicals. The inhibition

percentage of all tested samples showed a concentration dependent pattern as shown in Table-1. The percentages of anti-oxidant property of the ethanolic extracts at concentrations ranging from 100-500µg however, were lower than vitamin C. Vitamin C had over 90% scavenging activity at a concentration of 100µg, whereas the tested ethanolic extracts required a concentration of 500µg to reach below 70%. The percentage of scavenging activity of DPPH by ethanolic extracts from *Hypnea valentiae* (Turner) Montagne at 100µg, 200µg, 300µg, 400µg and 500µg were 28.22, 35.18, 48.75, 57.19 and 66.56%, respectively. Among the various concentration of ethanolic extracts used, 500µg ethanolic extract of *Hypnea valentiae* (Turner) Montagne had the strongest scavenging ability while 100µg ethanolic extract of *Hypnea valentiae* (Turner) Montagne had the lowest. The results showed that the scavenging activity was increased when the concentration of ethanolic extract was also increased. Vitamin C, a strong anti-oxidant was also used as control, and the anti-oxidant potential was compared to all tested samples.

Table 1: Scavenging effects on DPPH free radical by various concentrations of Ethanolic extracts of *Hypnea valentiae* (Turner) Montagne and Vitamin C.

Concentration	Percentage of anti-oxidant effect on DPPH	
	Vitamin C	Ethanolic Extract
100µg	90.69±2.11	28.22±1.14
200µg	93.24±1.43	35.18±1.98
300µg	99.57±2.99	48.75±1.32
400µg	99.92±1.76	57.19±1.66
500µg	99.98±2.34	66.56±1.02

The anti-oxidant activity of several naturally occurring compounds have been known for decades. Recently, many types of marine macro algae have been considered as source of reactive oxygen species inhibitors. They can be used as food additives and can also provide protection against tissue oxidation.[10] The present investigation has also proved that the ethanolic extract of *Hypnea valentiae* (Turner) Montagne possess anti-oxidant activity to scavenge free radicals. Dietary natural antioxidants are reported to help in preventing aging and other diseases. There are some evidences that marine algae contain compounds with a relatively high anti-oxidant and anti-proliferative activity. Marine macro algae are low in fat but contain vitamins and bioactive compounds like terpenoids, sulfated polysaccharides and polyphenolic compounds, the latter being a potential natural antioxidant not found in land plants.[11]

Anti-oxidant compounds scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus reduce the level of oxidative stress and slow or prevent the development of complications associated with oxidative stress related diseases.[12] Many synthetic antioxidants have shown toxic and mutagenic effects, which have shifted attention towards naturally occurring anti-oxidants. A great number of naturally occurring substances like marine algae have been recognized to have anti-oxidant abilities.[13] The present investigation has shown that ethanolic extracts of *Hypnea valentiae* (Turner) Montagne which exhibited significantly higher DPPH scavenging activity. Since the effects of anti-oxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability.

4. CONCLUSION

On the basis of results in the present study, it can be concluded that the ethanolic extracts of *Hypnea valentiae* (Turner) Montagne is capable of scavenging a wide range of synthetic and naturally occurring free radicals. It is evident from the present study that the ethanolic extracts of *Hypnea valentiae* (Turner) Montagne could be utilized as a good natural source of anti-oxidants and a possible food supplement or as an anti-microbial agent in pharmaceutical industry. However the active components responsible for the anti-microbial activities need to be evaluated. The data may contribute to a rational basis for the use of anti-oxidant rich marine algal extracts in the therapy of diseases related to oxidative stress. The finding of the current report appear useful for further research aiming to isolate, identify and characterize the specific phenolic compounds in *Hypnea valentiae* (Turner) Montagne for its industrial and pharmaceutical applications.

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