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In vitro antioxidant activity of freshwater green macroalgae, *Nitella tenuissima* (Desv.) Kiitz

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Abstract: The present study was conducted to find the antioxidant value of fresh water macro algae - *Nitella tenuissima (*Desv.) Kiitz. Antioxidants scavenges free radicals thereby preventing oxidative damage and can be used in cardiovascular and anti-inflammatory diseases. Methanolic extract of sample have been screened for the total phenols by Folin-Ciocalteau's method and radical scavenging activity by 1, 1 - diphenyl-z-picrylhydrazyl (DPPH) method. The methanolic extract showed phenol content of 13.0 mg TAE/g dry weight and 62% inhibition on radical scavenging activity at the maximum concentration tested, *viz.,*1.0 mg/mL with the IC_{50} value of 0.72mg/mL. Hence the scavenging activity increases with the concentration indicates the *Nitella tenuissima* (Desv.) Kiitz. has higher antioxidant activity, so proves as the good source of antioxidants and can be used in pharmacological purpose.

Key words: *Nitella tenuissima* (Desv.) Kiitz., DPPH assay, Total phenol content, antioxidant activity.

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1. Introduction

The necessity of compounds with antioxidant activity is increasing as it is realized that the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been linked in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer (Collier *et al*., 1990, Boynes *et al*., 1991) Free radicals are implicated for more than 80 diseases including Diabetes mellitus, arthritis, cancer, ageing etc. In the treatment of these diseases, antioxidant therapy has gained an utmost importance. (Anbarasan *et al*., 2011) Several studies have investigated the antioxidant activity of natural products in marine and fresh water algae (Fujimoto *et al*, 1984; Matsukawa *et al*.,1997; Lim *et al.*, 2002).

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Several compounds of fresh water algae showed pharmacological activities and discovery of compounds primarily for deadly diseases like cancer, Acquired Immuno Deficiency Syndrome (AIDS), Arthritis etc., while other compounds have been developed for analysis or to treat inflammation. In recent years there has been an upsurge of interest in herbs, spices and vegetables that contain natural substances with health promoting or pharmaceutical properties. Free radicals, especially reactive oxygen and nitrogen species have been implicated as mediators of chronic deteriorative inflammatory and auto immune diseases, including rheumatoid arthritis, cancer, diabetes and cardiovascular disease etc (Tsao and Deng, 2004). Antioxidant compounds are widely used compounds to counter the free radicals mediate the oxidative stress in the cell (Blokhina, 2003). These antioxidants can be derived from plants and algae (Bhaskara rao *et al.*, 2011) Keeping the above view in mind, the *in vitro* screening of antioxidant potential in algae was conducted.

2. Materials and Methods

2.1. Algal material

Nitella tenuissima (Desv.) Kiitz were collected from Aarivakkam Lake, Kattangolathur, Kanchipuram district, Tamilnadu, India during October to December 2010. The collected sample were cleaned with distilled water to remove all the extraneous matter such as epiphytes, sand particles, pebbles, shells etc., The sample were then thoroughly washed with freshwater, blotted and spread out in shade under room temperature for drying.

2.2. Preparation of extract

The shade dried sample was ground to a fine powder using pestle and mortar. Extraction was done by soaking 25g of algal sample into 100 mL of methanol solvent (1:4) ratio. The extract was filtered through cheese cloth followed by filtration using Whatman No.1 filter paper. The extraction was repeated until the powder was free of extractable substances. The pooled extracts were concentrated under rotary evaporator at 40°C and then under room temperature. The concentrated extract was redissolved in methanol and used for the estimation of total polyphenol content and for the antioxidant activity using DPPH method.

2.2. Antioxidant Activity

2.2.1. Determination of Total polyphenol content

The total polyphenol of the extract was determined by spectrophotometric method as described by Dewanto et al (2002). Three hundred micro litre of the methanolic extract prepared above was introduced into test tubes followed by the addition of 1.5 mL of a Folin-Ciocalteau's reagent (10X dilutions) and 1.2 mL of sodium carbonate (7.5% w/v) and vortexed for 15 sec and incubated for 30 minutes at 40°C for color development. The absorbance was measured at 760nm against the blank (without sample) using spectrophotometer. Total polyphenolic content was expressed as mg tannic acid equivalent per gram of dry weight (mg TAE/g dry weight) through the standard graph prepared with tannic acid, ranging from 10 to 100 ug/ml.

2.2.2. DPPH assay

The scavenging activity of the stable 1, 1 - diphenyl-z-picrylhydrazyl (DPPH) free radical was determined by the method as described by Marwah et al (2007). Briefly, the reaction medium contained 2 mL of 0.135 mM DPPH violet solution in methanol and 2 mL of algal extract (methanol for the control). Similarly, 2mL of methanolic extract of ascorbic acid (0.2mg/mL) was mixed with 2 mL of 0.135 mM DPPH. The methanol with DPPH served as control. The reaction mixture was incubated in the dark for 15 minutes and the absorbance was recorded at 517 nm using UV-VIS spectrophotometer. The experiments was conducted in triplicates and the scavenging activity was calculated. The decrease in absorbance on the addition of test sample was used to calculate the scavenging activity and expressed by the inhibition percentage (% IP) of DPPH radical, following the equation:

Inhibition percentage (% IP) = $[(A_c - A_s) / Ac] \times 100$.

Where A_c, and A_s are the absorbancies of the control and of the test sample after 15 minutes, respectively. The sample effective concentration 50% of DPPH (EC_{50}) was determined.

3. Results and Discussion

The beneficial effects derived from phenolic have been attributed to their antioxidant activity (Heim, 2002). The methanol extract of *Nitella tenuissima* (Desv.) Kiitz were studied for their total phenol content and its antioxidant radical scavenging activity. The phenolic

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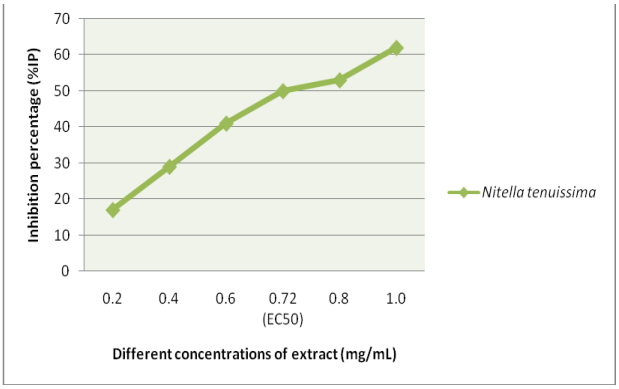
contents estimated in the methanol extract of *Nitella tenuissima* (Desv.) Kiitz. was 13.0 mg/g of dry weight in tannic acid equivalent, respectively. The dose-response curve of DPPH radical scavenging activity of the methanol extract of *Nitella tenuissima* (Desv.) Kiitz. was compared with ascorbic acid. At a concentration of 0.2 mg/mL, the scavenging ability on DPPH radical of *Nitella tenuissima* (Desv.) Kiitz. was 17%. However, at 1.0 mg/mL, the scavenging activities of the extract of sample showed 62%. The scavenging activity was concentration dependent (Graph-1). It was recorded as 17, 29, 41, 53, and 62 at the concentrations 0.2, 0.4, 0.6, 0.8 and 1.0 (mg/ml) respectively. The scavenging activity of Ascorbic acid 0.2mg/mL was 57%. The EC_{50} values of the scavenging activity of *Nitella tenuissima* (Desv.) Kiitz. extract showed 0.72 mg/mL on DPPH radicals (Table -1) Decreasing in the absorbance value of DPPH solution indicates an increase of the DPPH radical scavenging activity.



Graph-1 Showing Inhibition percentage (IP) of methanolic extract of *Nitella tenuissima* (Desv.) Kiitz.

Natural antioxidants are not limited to terrestrial sources and reports have revealed seaweeds to be rich sources of natural antioxidant compounds (Lim *et al*., 2002; Duan *et al*., 2006; Kuda *et al*., 2007). Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties (Duan *et al*., 2006; Kuda *et al*., 2007; Wang *et al*., 2009).

Table 1: *In vitro* antioxidant activity of fresh water green macro algae, *Nitella tenuissima* by DPPH assay

S.No	Concentrations (mg/mL)	Inhibition percentage (%IP)
1	0.2	17
2	0.4	29
3	0.6	41
4	0.8	53
5	1.0	62
6	Ascorbic acid 200µg/mL	57

The methanolic extract of *Nitella tenuissima* (Desv.) Kiitz. was screened for totol polyphenol content by Folin-Ciocalteau's method and its antioxidant activity by DPPH Assay. Screening the antioxidant activity by free radical scavenging showed that the scavenging activity was increased with increasing extract concentrations. Higher extract concentrations increased the scavenging activity especially for methanol extract of *Nitella tenuissima* (Desv.) Kiitz.

4. Conclusion

From the above result, a strong correlation has been observed between the phenols and antioxidant activity which has been previously reported by (Velioglu et al., 1998, Javanmardi et al., 2003, Kahkonen, et al., 1999). The presence of phenol in the methanolic extract of algae, *Nitella tenuissima* (Desv.) Kiitz. clearly demonstrates that the methanolic extract of algae possess good antioxidants and was comparable with that of standard ascorbic acid.

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