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EVALUATION OF ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC CONTENT OF SELECTED MICROALGAE OF KUMAUN HIMALAYAN REGION

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ABSTRACT

In order to identify new sources of safe and inexpensive antioxidants, the antioxidant capacity and total phenolic content of 5 microalgae isolated from fresh water lakes of Kumaun Himalayan Region were evaluated, using DPPH-Radical scavenging activity assay and the Folin-Ciocalteu method, respectively. The microalgae were extracted using methanol by a three step sequential extraction procedure. Most of these microalgae were evaluated for the first time for their antioxidant activities. It was found that microalgae *Chlorella vulgaris* and *Mougetia parvula* possessed the higher antioxidant activities and thus could be a potential rich source of natural antioxidants. In addition, the correlation coefficients between the antioxidant capacities and the phenolic content were very high (R^2 = 0.9882). It is clear from the present investigation that phenolic compounds played key role in regulating antioxidant capacities of these microalgae.

KEYWORDS: Antioxidant capacities, Microalgae, Kumaun Himalayan Region, Phenolic content

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1.INTRODUCTION

The use of antioxidants to prolong the shelf life of foodstuffs is ubiquitous. Epidemiological studies have demonstrated an inverse association between intake of fruits and vegetables and mortality from age related diseases, such as coronary heart disease and cancer, which may be attributed to their antioxidant activity[1,2]. The most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG). However, there has been growing concern over their safety and toxicity. Therefore, development and utilization of more effective antioxidant from natural resources are desired for use in foods or medicinal stuff to replace the synthetic antioxidants. Algal biomass and algae-derived compounds have a very wide range of potential applications, from animal feed and aquaculture to human nutrition and health products. Some algae are considered as rich sources of natural antioxidants[3,4]. Although macroalgae have received much more attention as potential natural antioxidants[5,6], there has been very limited information on antioxidant activity of microalgae[7,8]. Microalgae may serve as a continuous and reliable source of natural products, including antioxidants, because they can be cultivated in bioreactors on a large scale[9]. Furthermore, the qualities of the microalgal cells can be controlled in making them free from herbicides, pesticides and other toxic compounds by using clean nutrient media for their growth[10]. The value of microalgae as a source of natural antioxidants is further enhanced by the relative ease of purification of target compounds[11].

In terrestrial plants, an important class of antioxidants are phenolic compounds, more specifically the relatively complex flavonoids, which show several antioxidant mechanisms. Flavonoids can inhibit lipid oxidation[12] by directly scavenging ^{0}OH, HOCl, singlet oxygen and lipid peroxyl radicals, by metal chelation and by inhibiting lipoxygenase. Little information is however available about the presence of phenolic compounds in microalgae. Recent research[13] showed that several classes of flavonoids, such as isoflavones, flavanones, flavonols and dihydrochalcones can be found in microalgae and cyanobacteria. This indicates that, although microalgae are evolutionary more primitive than terrestrial plants, or even belong to completely different evolutionary lineages, they are capable of producing relatively complex polyphenols.

Microalgae represent an almost untapped resource of natural antioxidants, due to their enormous biodiversity, much more diverse than higher plants. However, not all groups of microalgae can be used as natural sources of antioxidants, due to their widely varied contents of target products, growth rate or yields, ease of cultivation, and/or other factors. Reports on the antioxidant activity of microalgae are limited, especially concerning the relationship between their phenolic content and antioxidant capacity. Therefore, it was desirable to identify some rich sources of antioxidants from a large group of microalgae and to evaluate the relationship between these two parameters.

Algae are exceedingly conspicuous in fresh water habitats as lakes, ponds, pools, streams and reservoirs of temporary nature in Kumaun region. Their growth is dependent upon the factors as light, oxygen, carbon-di-oxide, temperature, water and suitable mineral salts. Although a good deal of work has been done on the systematics of fresh-water algae by different workers[14, 15, 16] but our knowledge concerning the occurrence and distribution of algae in Kumaun region is inadequate. This renewed interest in algae as a source of antioxidant, has unfortunately, not been appreciated in our region. The aim of this study was, therefore, to identify new sources of safe and inexpensive antioxidants from microalgae species of lakes of Kumaun Himalayan region using DPPH radical scavenging activity and total phenolic contents and to investigate the relationship between antioxidant capacity and phenolic content.

2. MATERIALS AND METHODS

Algae were collected in bulk from the surface, subsurface and sides of Nainital, Bhimtal and

Sattal lake of Kumaun Himalayan region. Algal biomass was handpicked from fresh water bodies and immediately after collection; samples were brought to the laboratory, air dried for two days later on dried at 40^0C in an oven for 2-3 days till the dry weight was constant. A part of fresh algae samples were observed for identification and preserved in Lugol's solution. To ensure complete sedimentation the samples were allowed to stand for 24 hours. The supernatant was discarded and temporary and permanent mounts were prepared. The identification of algal material was done following Smith[17] (1950) and Prescott[18] (1969) system of classification. The algal material is deposited in department of biotechnology, Bhimtal Campus, Kumaun University for future reference. The algal samples collected and analyzed were identified as *Microcystis aruginosa*, a blue green alga and rest were green algae *Hydrodictyon reticulum, Spirogyra orientalis, Chlorella vulgaris, Cosmarium nitudulum, Mougetia parvula*. Similar dry weights (100g) of all samples were taken for further experiments and analysis.

2.1 Preparation of the extract

Crude extracts of algal samples were prepared using the modified method from Lim et al. (2002)[19]. 10 g of dried samples were extracted with methanol (100 ml) for 30 minutes at 40° C followed by centrifugation (4500 × g, 10 min) to extract the supernatant. The extraction was repeated thrice and further transferred to conical flask and washed with chloroform to remove pigments. The extract was evaporated under reduced pressure (72 mbar) with a rotary evaporator until all dried extract was collected in air tight container and stored at 4° C for further use.

2.2 Estimation of total phenolic content

Phenolic content was estimated by the Folin-Ciocalteu procedure according to the slightly modified procedure used by Hajimahmoodi et al. (2010)[20]. For this, 200 µL extract was mixed with 1.5 mL of Folin–Ciocalteu reagent (previously diluted tenfold with distilled water) and allowed to stand at room temperature for 5 min. Next, 1.5 mL sodium bicarbonate solution (60 gL^{-1}) was added to the mixture. After incubation for 90 min at room temperature, the absorbance was measured at 750 nm. Total phenolics were calibrated against gallic acid standard solutions (25-150 mg L^{-1} in 50 % methanol) and are expressed as mg gallic acid equivalent (G.A.E.) g^{-1} biomass. Each experiment was performed in triplicates at each concentration.

2.3 DPPH radical scavenging activity

The free Radical Scavenging Activity (RSA) was determined according to the method of Chandini et al. (2008)[21] with slight modification. One millilitre of algal extracts in methanol with concentration of 0.45 mg mL^{-1} was mixed with 2 mL of 0.08 mM methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl). The mixtures were then vortexed, left for 30 min at room temperature in the dark and the absorbances were measured at 517 nm. BHT was used as the positive control. 1.0 millilitre of methanol mixed was taken as blank and mixed with 2 mL of 0.08 mM methanolic solution of DPPH. Radical scavenging activity was calculated using the equation:

Radical Scavenging Activity (%) = [1 - (A sample/A blank)´100%]

2.4 Statistical Analysis

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

3. RESULTS AND DISCUSSION

3.1 Total phenolic contents of the microalgae

Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities, such as anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities. These activities may be related to their antioxidant activity[22]. Thus, the total phenolic content of 5 microalgal species collected from different lakes of Kumaun region was also evaluated, using the Folin–Ciocalteu method. The total phenolic content of different microalgal species were shown in Table 1. The phenolic content of various microalgal species

varied from 2.40 to 11.15 mg GAE g^{-1}DW. Except *Microcystis aruginosa* (2.40 mg GAE g^{-1}DW) and *Hydrodictyon* reticulum (2.79 mg GAE g^{-1}DW) all other microalgae showed relatively high phenolic content (>3 mg GAE g^{-1}DW) belongs to *Mougetia parvula* (7.66 mg GAE g^{-1}DW), *Cosmarium nitudulum* (8.40 mg GAE g^{-1}DW) and *Spirogyra orientalis* (8.60 mg GAE g^{-1}DW). *Chlorella vulgaris* was found to have highest phenolic content (11.15 mg GAE g^{-1}DW). Phenolic content as measured in this study was comparable to the previous studies[23, 20, 24], although Cha et al. (2010)[25] detected higher levels in extracts from *C. vulgaris* obtained by pressurised liquid extraction at elevated temperatures using 90% ethanol as extractant. Polyphenols act as antioxidants through single electron transfer and through hydrogen atom transfer. It is also well known that the antioxidant activity of polyphenols is related to the degree and pattern of hydroxylation and extent of conjugation in polyphenols. At present, little is known about the polyphenolic components that are present in microalgae. Recent work by Klejdus et al. (2010)[13] and Kovácik et al. (2010)[26] indicate that a variety of phenolic classes are present in microalgae, but further identification of phenolic components is needed to better understand the differences in the contribution of phenolics to the antioxidant activity of microalgae.

3.2 Free Radical Scavenging Activity

The parameter used to measure the free radical scavenging activity of the extract was % radical scavenging activity of DPPH (% DPPH-RSA). The presented data in Fig 1 indicated that RSA in extract concentration of 0.45 mg mL-1 were in the range of 20 - 75% from algal extract. These extracts had lower antioxidant activity than the commercial antioxidant BHT (100 ppm, 89.83%). The study used DPPH method to test the ability of the antioxidative compounds functioning as proton radical scavengers or hydrogen donors[27, 28]. DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger. The highest % RSA was found at *Chlorella vulgaris* (74%). The extreme condition at Kumaun Himalayan Region encouraged the algae to form a defence system against photodestruction by UV radiation and exhibit radical scavenging properties.

3.3 Correlation between antioxidant capacity and phenolic content

The correlation coefficient (R^2) between the antioxidant capacity and the phenolic content of the 5 microalgae species selected in Kumaun Himalayan region was determined (Fig 2). The correlation coefficient between the antioxidant capacity and the phenolic content was found to be 0.9882 which suggest significant positive correlation between antioxidant capacity of microalgae species and their total phenolic content. Several studies have compared antioxidant activity and phenolic content in fractionated extracts of biomass from one or a few species of microalgae. These studies found that fractions that were rich in phenolic compounds also had a high antioxidant capacity[29, 23, 30], while another study found the opposite[30]. A more recent study used two antioxidant tests (DPPH-radical scavenging and Ferric Reducing Antioxidant Potential, FRAP) and a significant relation between antioxidant activities and phenolic content was noticed[20]. Sharatchandra and Rajashekhar (2013)[34] showed in four cyanobacteria species isolated from a sulpfur spring in the western ghats of Karnataka have high phenolic content and exhibited antioxidant activity. The photoprotective effect of phenols or phlorotannins depends not only from their accumulation, but also from their high antioxidant and radical-scavenging capacities, which may be involved in other cytoprotective roles[31, 19, 32]. In fact, microalgae could produce a wide range of antioxidant compounds, including for example, carotenoids, polyunsaturated fatty acids and polysaccharides[9, 33]

Table1.Total phenolic content of methanol extract of various microalgae species isolated from various Lake of Kumaun Himalayan Region. Data is given in mean ± SD (n = 3) and expressed as Gallic acid equivalence (GAE) in mg

Algal species	Total phenol content (mg GAE g^{-1} DW)
Microcystis aruginosa	2.40±0.12
Hydrodictyon reticulum	2.79±0.16
Spirogyra orientalis	8.60±0.26
Chlorella vulgaris	11.15±1.12
Cosmarium nitudulum	8.40±0.24
Mougetia parvula	7.66±0.27

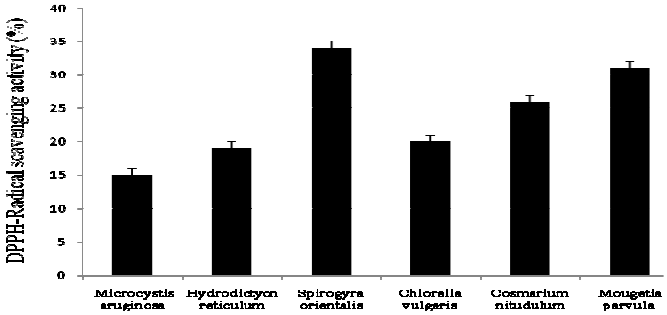

Figure1

DPPH-Radical scavenging activity of methanol extract of various microalgae species isolated from fresh water lakes of Kumaun Himalayan Region. Data is given in mean ± SD (n = 3).

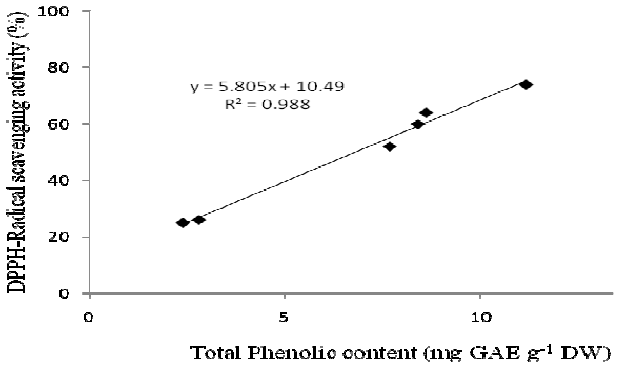

Figure 2

Correlation between the antioxidant capacity and phenolic content of various microalgae species isolated from fresh water lakes of Kumaun Himalayan Region. GAE: gallic acid equivalents

4. CONCLUSION

The study illustrated for the first time the antioxidant capacity and phenolic content of five microalgal species selected from the fresh water lakes of Kumaun Himalayan Region. The correlation coefficient between the antioxidant capacities and the phenolic contents was significant, and phenolic compounds were a major contributor to the antioxidant capacities of these algae. *Chlorella vulgaris* and *Mougetia parvula* were found to have higher antioxidant capacities and thus could be potential rich source of natural antioxidants. Further

identification of phenolic substances from these microalgae is required to evaluate whether microalgae may contain novel phenolic compounds that are not known from terrestrial plants. The fact that the phenolic content differed almost threefold between different samples of the same species suggests that the phenolic content of selected microalgae may be optimised by selecting the appropriate cultivation and processing conditions.

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