



## EVALUATION OF MICROALGAE FROM HIMALAYAN REGION FOR NUTRACEUTICAL ACTIVITIES

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### ABSTRACT

Microalgae serve as a predominant biological source for nutraceutical compounds along with the lipids and dyes. In the present study, microalgal species from the Himalayan region, India have been evaluated for nutraceutical activities such as carbohydrate, protein, phenolic, flavonoid contents and DPPH activities. The evaluation showcased that *Scenedesmus dimorphus* was found to be the highest producer of protein and carbohydrate than the *Scenedesmus quadricauda* and *Chlorella* sp. Among the microalgal species, *Chlorella* sp. was found to have a more antioxidant, phenolics, flavonoid, and DPPH activities over *Scenedesmus* sp. y. Overall, the present study showcase the unexplored potential of microalgae for various nutraceutical activities. This unexplored algal source may serve as an untapped resource for nutritional and food-biotech communities to do research for its incorporation as a palatable feed material and also as an aquatic, and animal feed.

**KEYWORDS:** Micro algae; *Scenedesmus* sp.; *Chlorella* sp.; Nutraceutical; Anti-oxidant



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## INTRODUCTION

Nutraceutical sector is one of the rapidly growing global sectors with a market of \$142.1 billion and is expected to touch \$204.8 billion in 2017 with a growth rate 6.3% for annum<sup>1</sup>. Nowadays, people are paying more attention towards the functional foods due to their combined nutraceutical, biological and physiological effects in treating different health ailments. Ideally, the functional foods have to be rich in proteins, carbohydrates, vitamins, minerals along with the anti-oxidant, phenolic and scavenging activities. The nutraceutical abilities of microalgae have acknowledged by many researchers for the protein, vitamins and mineral contents<sup>2</sup>. Many algal species such as *Spirulina* and *Chlorella* Sp. considered as GRAS (Generally Regarded As Safe) for human and animal consumption and can be utilized as a nutraceutical compound for its intervention in human or animal health problems. Moreover, the characteristics of faster growth rates, immense biodiversity, simple media requirements, easy adaptability to various environments makes microalgae as a profound source than animal-, plant- and microbial- sources for different nutraceutical compounds. Usually, Microalgal biomass is considered as an unusual protein source due to the presence of high protein content and essential amino acids<sup>3</sup>. The overall digestibility of carbohydrates from dry microalgal biomass is more compared with other sources. The biomass of *Scenedesmus* sp. has been reported for its nutraceutical properties towards malnutrition problem<sup>4</sup>. Antioxidants and flavonoids play a major role in therapeutic treatments as an inhibitor of Reactive Oxygen Species (ROS) with scavenging effect on OH·, HOCl·, singlet oxygen and lipid peroxyl radicals). Microalgae also have been reported for antioxidants and flavonoids activities<sup>5,6</sup>. To combat the existing prevalent malnutrition problems in developing countries, exploration of nutraceutical compounds from untapped sources is utmost important. To harness the full potential of microalgae from untapped regions such as Himalayas, India, the locally isolated strains have to be explored for nutraceutical activities. Having the high productivities with *Chlorella* sp. and *Dunaliella* sp. with other industrial commodities, the present research work has been intended to evaluate the nutraceutical activities of dried lyophilized biomasses of *Scenedesmus dimorphus*, *Scenedesmus quadricauda* and *Chlorella* sp.

## MATERIALS AND METHODS

### Chemical reagents and solvents

The chemicals and reagents used in this study were of reagent and analytical grade and procured from Merck, India and SRL, Mumbai. Standards of 2,2'-diphenyl-1-picrylhydrazyl (DPPH), Folin Ciocalteu reagent were procured from Sigma, USA.

### Biomass Production

Three species of microalgae viz. *Scenedesmus dimorphus*, *Scenedesmus quadricauda* and *Chlorella* sp. previously isolated and maintained in our laboratory were used in this study<sup>7</sup>. Each species was grown using the BG11 medium<sup>8</sup> in two-liter conical flasks and cultures were kept in a growth chamber and at 18-25° C, and provided 16:8 h light and dark cycles. The cultures were

aerated by an air pump with 0.22 µm sterilized air filter to avoid settling and sticking to the surface of the flask and grown for 25 days to achieve the full growth.

### Preparation of algal powder and algal extract

After 25 days, the biomasses were centrifuged at 7000 rpm for 15 min at 4°C. The respective pellets of algal biomass were freeze-dried at -80°C for 2 h in a lyophilizer. The respective algal powders were stored at -80°C for further studies. A precisely weighed (50 mg) dried, lyophilized algal powder was extracted with different solvents namely, methanol, chloroform, hexane and aqueous water (100 ml) and kept in an orbital shaker for overnight. The obtained extracts were filtered and the respective filtrates were collected and used for further nutraceutical evaluations.

### Estimation of nutraceutical activities

The total phenolic content of the microalgal extracts were estimated by the Folin-Ciocalteu method by measuring the absorbance at 720 nm using microplate reader<sup>9</sup> and the results were expressed in terms of µg/g dry weight of algal biomass. The total flavonoid content (µg/grams dry weight of algae) of the extracts was estimated using the Aluminium chloride colorimetric method<sup>10</sup> by monitoring the absorbance at 510 nm. DPPH scavenging activity of the microalgal extracts was estimated by measuring the absorbance at 517 nm<sup>11</sup> and the respective antioxidant activities were reported in terms of % of inhibition. The total protein content (µg/g dry weight of algal biomass) of the extracts was measured using the Lowry method using BSA as a standard<sup>12</sup>. The total carbohydrate content of the extracts was estimated using the Anthrone method was utilized to measure the carbohydrate content (µg/grams dry weight of algae) of algal extracts by measuring the absorbance at 620 nm<sup>13</sup>.

## RESULTS AND DISCUSSION

The inclusion of microalgal stuff in food and feed material is one of the most promising resources of nutritional value enhancement by maintaining the well-balanced chemical composition of the food and feed material. Utilization of microalgal biomass as a nutraceutical material is an interesting and innovative approach to the development of healthier food and feed products.

### Estimation of total phenolic content

Phenolic compounds act as antioxidants due to their ability to donate a hydrogen atom or an electron to form stable intermediates radicals. Moreover, the phenolic compounds have a profound role in treating different humans and animal health ailments<sup>14</sup>. The total phenolic content of *Chlorella* sp., *S. dimorphus*, and *S. quadricauda* extracted with methanol, chloroform, hexane and aqueous water has been presented in Fig. 1 (a). It was found that the total phenolic content of the freeze-dried algal biomass varies with the extracting solvent. Among the different solvents, the methanolic extracts showed the higher total phenolic content whereas the water extracts showed the lower phenolic content. The methanolic extract of *Chlorella* sp. exhibited the highest phenolic content (13000 µg / gDW of microalgae) which followed by the methanolic extracts

of *S. quadricauda* (10,000 µg) and *S. dimorphus* (8800 µg). The fact of higher phenolic contents associated with the methanolic extracts was attributed to the more affinity of phenolic compounds towards polar solvents such as methanol/hexane<sup>15,16</sup>. The extracted polyphenols likely to be polar compounds due to the more polarity of methanol over the other utilized organic solvents such as hexane and chloroform.

#### Estimation of total flavonoid content

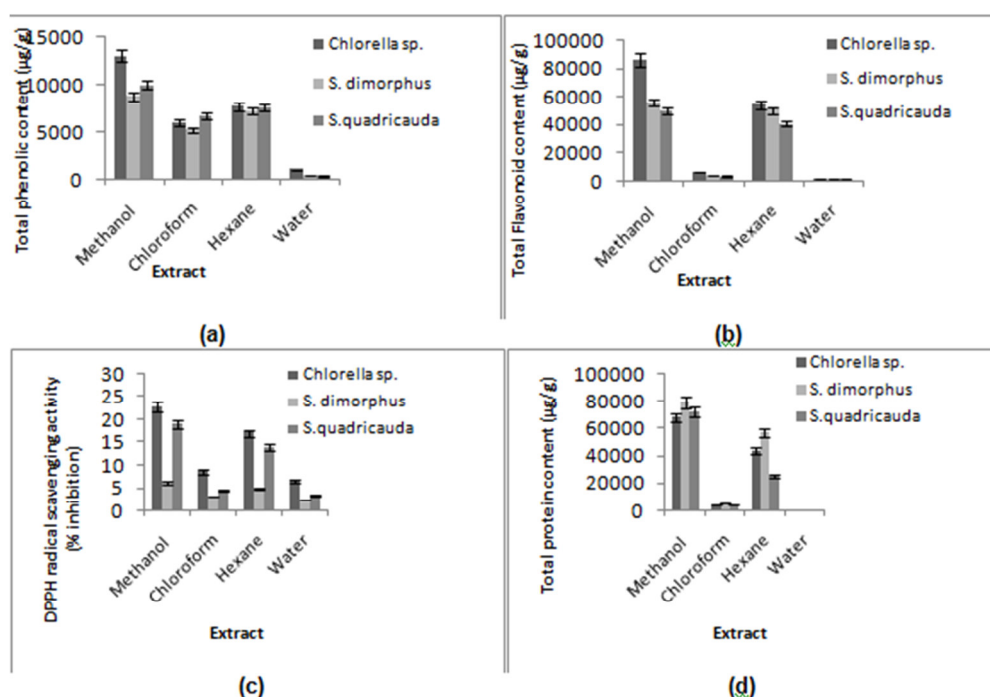
The hydroxyl group of flavonoids is the main contributor towards their antioxidant activity, which has been reported for anti-viral, anti-tumor, anti-allergic, anti-platelet, anti-inflammatory and anti-oxidant properties<sup>11</sup>. The highest flavonoid content has been found in the methanolic extract of *Chlorella sp.* (86000 µg/g DW of microalgae) followed by *S. dimorphus* (56000 µg) and *S. quadricauda* (50000 µg) (Fig. 1b) followed by the hexane, chloroform and aqueous extracts of free-dried algal biomasses. Several researchers also reported high flavonoid content associated with the methanolic extracts<sup>15,16</sup>.

#### Estimation of antioxidant activity (DPPH)

The antioxidant activities of freeze-dried microalgal extracts were evaluated for the scavenging effect against the free radical DPPH through micro-plate assay. In this case also, the methanolic extract of *Chlorella sp.* shown strong radical scavenging activity (50 µg/ml, with a percentage decrease of 22.7%) which followed by methanolic extracts of *S. quadricauda* (with a percentage decrease of 19 %) and *S. dimorphus* (free radical scavenging activity of 6.2%) (Fig. 1c). The scavenging effects of different extracts on the DPPH radical decreased in the order of methanol>hexane>chloroform>water at a concentration of 50 µg/ml. It is evident from these results that the methanol extracts have a remarkable effect on scavenging of free radicals. The study revealed the efficiency of methanolic extracts of *Chlorella sp.* and *S. quadricauda* extracts in the prevention of reactive radicals from damaging various biomolecules such as lipoproteins, DNA, amino-acids, sugar, proteins and PUFA in biological and food systems<sup>17</sup>.

Figure 1

The (a) total phenolic content, (b) flavonoid content, (c) DPPH scavenging activity (d) total protein content of methanol, chloroform, hexane and water extracts of *Chlorella sp.*, *S. dimorphus* and *S. quadricauda*. All values are represented as  $\pm$ sd of three replications.



#### Estimation of total protein content

The highest protein content was exhibited by methanolic extract of *S. dimorphus* (79000 µg/g DW of microalgae) followed by *S. quadricauda* (72000 µg) and *Chlorella sp.* (68000 µg) (Fig. 1d). Amongst all the solvents, the maximum proteins were extracted in the methanolic extract. Thus, suggesting that these microalgae contain more polar amino acids such as glutamine, asparagine, histidine, serine, threonine, tyrosine, cysteine, methionine and tryptophan. The polar amino acids participate mainly in hydrogen bonding<sup>18</sup>.

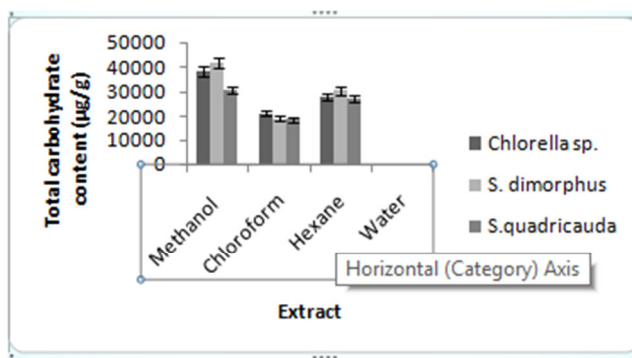
#### Estimation of total carbohydrate content

Carbohydrates are an important energy source for the

mammals which provide energy for respiration and other important biological processes. In the present study, higher carbohydrate content has been observed with the methanolic extract of *S. dimorphus* (42000 µg/g DW of microalgae) followed by methanolic extracts of *Chlorella sp.* (38400 µg) and *S. quadricauda* (30800 µg) (Fig. 2). In this case also, polarity plays an important role in carbohydrate release by the tested solvents. The lower carbohydrate contents have been observed with the chloroform and aqueous water extracts. Hence, it was concluded that the carbohydrate content of microalgae is not only species specific but also polarity particular of the extracted solvent<sup>18</sup>.

Figure 2

The total carbohydrate content in methanol, chloroform, hexane and water extracts of *Chlorella sp.*, *S. dimorphus* and *S. quadricauda*. All values are represented as  $\pm$  sd of three replications.



## CONCLUSION

The present study put forth the three microalgal species from the Himalayan region, India having nutraceutical activities. Among all the extracts, methanolic extracts of microalgae exhibited higher activities than the other solvent extracts because of its strong polarity. Phenolic and flavonoid content, as well as DPPH scavenging activity, were found to be maximum in *Chlorella sp.* whereas, the protein and carbohydrate content were found to be maximum in *S. dimorphus*, which also showed the comparable anti-oxidant activity. Thus, the *Chlorella sp.* and *S. dimorphus* could use in functional foods and dietary supplements owing to their anti-oxidant and nutritional properties. This study gave an insight into the nutritional value of indigenously isolated

microalgae for its possible inclusion into the food and feeds supplements. Further research has to be performed to enhance the reported nutraceutical yields through media- and process- engineering.

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## CONFLICTS OF INTEREST

Conflict of interest declared none.

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