Original Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

EVALUATION OF MICROALGAE FROM HIMALAYAN REGION FOR NUTRACEUTICAL ACTIVITIES

AKANSHA BAJPAI[1#], VIJAY KUMAR GARLAPATI[1#], RAKESH SINGH GOUR[1] AND ANIL KANT[1]

[1]Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, HP-173234, India
[#]Equal contribution was made for this research.

ABSTRACT

Microalgae serve as a predominant biological source for nutraceutical compounds along with the lipids and dyes.In the presentstudy, 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 *Scendesmus dimorphus* was found to be the highest producer of protein andcarbohydrate than the *Scendusmus quadricauda* and Chlorella sp. Among the microalgal species, *Chlorella sp.* was found to have a more antioxidant, phenolics, flavonoid,and DPPH activities over *Scendusmus* sp. y. Overall, the present study showcase the unexplored potential of microalgae for various nutraceutical activities. This unexplored algal source may serve as an untappedresource 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.; Cholrella sp.;* Nutraceutical*;* Anti-oxidant

ANIL KANT

Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, HP-173234, India

***corresponding Author**

Received on: 19-01-2017
Revised and Accepted on: 15-02-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.b174-178

INTRODUCTION

Nutraceutical sector is one of the rapidly growing global sectors with a market of $142.1 billion and isexpected to touch $204.8 billion in 2017 with a growth rate 6.3% for annum[1].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 mineralcontents[2]. 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, immensebiodiversity, simple media requirements, easy adaptability to various environmentsmakes microalgae as a profound source than animal-, plant- and microbial- sources for differentnutraceutical compounds.Usually, Microalgal biomass is considered as an unusual protein source due to the presence of high protein content and essential amino acids[3]. The overall digestibility of carbohydrates from drymicroalgal biomass is more compared with other sources. The biomass of *Scenedesmus sp.* has been reported for its nutraceutical properties towardsmalnutrition problem[4]. Antioxidants and flavonoids play a major role in therapeutic treatments as an inhibitorof Reactive Oxygen Species (ROS)with scavenging effect on OH-l, HoCl-, singlet oxygen and lipid peroxyl radicals). Microalgae also have been reported for antioxidantsand flavonoidsactivities[5,6].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 Chlorellasp.

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 Ciocalteau reagent were procured from Sigma, USA.

Biomass Production

Three species of microalgae viz. *Scenedesmus dimorphus*, *Scenedesmusquadricauda* and *Chlorella sp*. previously isolated and maintained in our laboratory were used in this study[7]. Each species was grown using the BG11 medium[8]in two- liter conical flasksand 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 ofalgal powder and algal extract

After 25 days, the biomasses werecentrifuged at 7000 rpmfor 15 min at 4^{o}C. The respective pellets of algal biomass were freeze-dried at -80^{o}C for 2 hin a lyophilizer. The respective algal powders were stored at -80^{o}C for further studies.A precisely weighed (50 mg) dried, lyophilized algal powderswere extracted withdifferent 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-Ciocalteau method by measuring the absorbance at 720 nm using microplate reader[9]and the results were expressed in terms ofµg/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[10]by monitoring the absorbance at 510 nm. DPPH scavenging activity of the microalgal extracts was estimatedby measuring the absorbanceat 517nm[11] and the respective antioxidant activities werereported in termsof % 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[12].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[13].

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[14]. 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 biomassvaries with the extracting solvent. Among the different solvents, the methanolic extracts showed the higher total phenolic contentwhereas 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 wasattributed to the more affinity of phenolic compounds towards polar solvents such as methanol/hexane[15,16].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[11].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 researchersalso reported high flavonoid content associated with the methanolic extracts[15,16].

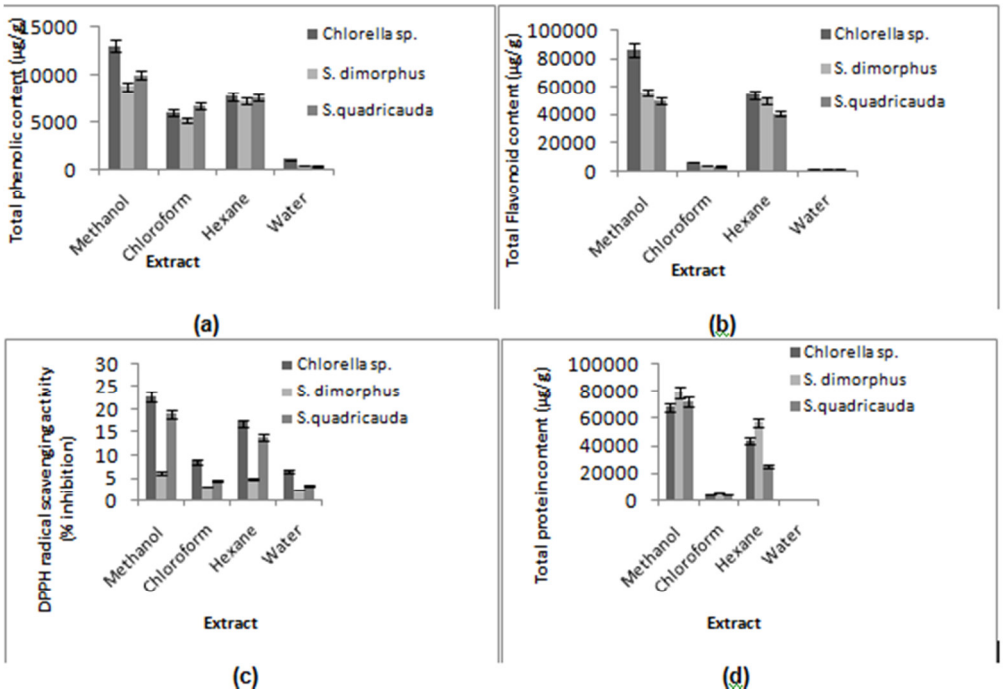
.

Estimation of antioxidant activity (DPPH)

The antioxidant activities of freeze-dried microalgal extracts were evaluated for the scavengingeffect against the free radical DPPHthrough 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 50µg/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* extractsin the prevention of reactive radicals from damaging various biomolecules such as lipoproteins, DNA,amino-acids,sugar, proteins and PUFA in biological and food systems[17]

Figure 1

The (a) total phenolic content, (b) flavoniod 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 ±sd of three replications.



Estimation of total protein content

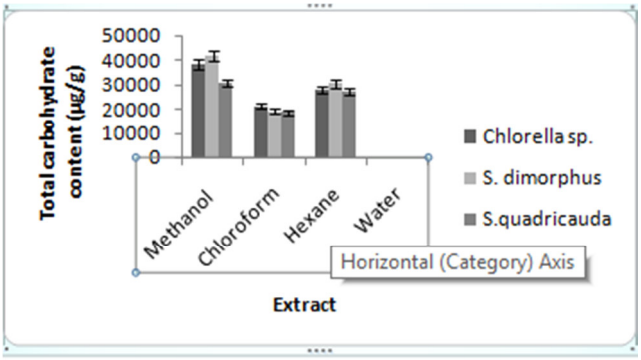
The highest protein content was exhibited by methanolic extract of *S. dimorphus* (79000 µg/g DW of microalgae) followed by *S. quadricauda* (72000 µg) and *Chlorella sp.* (68000 µg) (Fig.1d). Amongst all the solvents, the maximum proteins were extracted in the methanolic extract. Thus, suggesting that 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[18].

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 particularof the extracted solvent[18]

Figure 2

The total carbohydrate content in methanol, chloroform, hexane and water extracts of Chlorella sp., S. dimorphusand S.quadricauda. All values are represented as ± 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 exhibitedhigher 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 usein 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 feedsupplements. Further research has to be performed to enhance the reported nutraceutical yields through media- and process- engineering.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Jaypee University of Information Technology, Waknaghat, HP, India for providing research facilities for execution of the present study.

CONFLICTS OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Nutraceuticals world. Global Nutraceuticals Industry Analysis till 2017 – Emerging Markets in Asia-Pacific and Latin America to Drive Growth; 2013. Available from: http://www.nutraceuticalsworld.com/issues/2013-09/view_industry-news/nutraceuticals-market-to-reach-2048-billion-by-2017#sthash.daayPQ4F.dpuf
2. Halim R, Webley PA, Martin GJO. The CIDES process: Fractionation of concentrated microalgal paste for co-production of biofuel, nutraceuticals, and high-grade protein feed. Algal Res. 2015 Nov 1; 19: 299-06.
3. López CVZ, García MCC, Fernández FGA, Bustos CS, Chisti Y, Sevilla JMF. Protein measurements of microalgal and cyanobacterial biomass.Biores. Technol. 2010 May 21; 101: 7587-91.
4. Gouveia L, Batista AP, Sousa I, Raymundo A, Bandarra NM. Microalgae in novel food products. In: Konstantinos N, Papadopoulos PP, editors. Food Chemistry Research Developments.New York: Nova Science Publishers, Inc.; 2008. P.75-11.
5. da Silva BV, Barreira JCM, Oliveira MBPP. Natural phytochemicals and probiotics as bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery technologies.Trend. Food Sci. Technol. 2016 April 1; 50: 144-58.
6. Pietta PG. Flavonoids as antioxidants. J.Natural Product. 2008 July 1;63 (7): 1035-42.
7. Gour R, Kant A, Chauhan R. Screening of micro algae for Growth and lipid accumulation properties. J. Algal Biomass Utiliz. 2014 Jan 5; 5(1):38-41.
8. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 1979 March 1; 111: 1-61.
9. Taga MS, Mille EE, Pratt DF. Chia seeds as a source of natural lipid antioxidants. J.Am. Oil Chem. Assoc. 1984 Oct 11*; 61*:928-31.
10. Zhishen J, Mengeheng T, Jianming W. The determination of flavonoid contents in mulbery and their scavenging effects on superoxide radicals. Food Chem. 1999 March 1: 64 (4): 555-59.
11. Das NP, Pereira TA. Effects of flavonoids on thermal auto oxidation of palm oil: structure activity relationship. J. Oil Chem. 1990 Aug 11; 67: 255–8.
12. Lowry OH. Rosenbrough NJ, Farr AL, Randal R.J. Protein measurement with the Folin phenol reagent. J.Biol. Chem. 1951 Nov 1; 193: 265–75.
13. Hedge JE, Hofreiter BT. Carbohydrate Chemistry. New York: Academic Press; 1962
14. Mohamed AA, Khalil AA, El-Beltagi. Antioxidant and antimicrobial properties of kaffmaryam (*Anastaticahierochuntica*) and doum palm (*Hyphaenethebaica*). Grasas Y Aceite2010 March 1;61: 67–75.
15. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of barks of *Azadirachtaindica*, *Terminaliaarjuna*, *Acacia nilotica*,

and *Eugenia jambolana*. FoodChem. 2007 Feb 1; 104(3): 1106–14.
16. Choi Y, Jeong HS, Lee J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chem. 2007 March 7; 103(1): 130–8.
17. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohani DP. Comparative antioxidant activity of individual herbal components used in ayurvedic medicine. J. Phytochem. 2007 May 1; 63 (1): 97–04.
18. Salla ACV, Margarites AC, Seibel FI, Holz LC, Brião VB, Bertolin TE, Colla LM,Costa JAV. Increase in the carbohydrate component of the microalgae Spirulina in culture by nutrient starvation and the addition of residue of whey protein concentrate. Bioresource Technol. 2016 June 1; 209: 133-41.
19. Cea-Barcia G, Buitrón G, Moreno G, Kumar G. A cost-effective strategy for the bio-prospecting of mixed microalgae with high carbohydrate content: Diversity fluctuations in different growth media. Bioresource. Technol. 2014 May 5; 163: 370-3.

Reviewers of this article

Dr. Gyan Prakash Mishra

Senior Scientist, ICAR-Indian Institute of Vegetable research, PB 01, Varanasi,Uttar Pradesh, India,Pin: 221305

Mr. Anubrata Paul M.Sc. Biotech (Research)

Department of Biotechnology, Natural Products Research Laboratory, Centre for Drug Design Discovery & Development (C-4D) , SRM University, Delhi-NCR,Sonepat.

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript