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Chemical and Antioxidant Analysis of *Spirulinaplatensis* Products Available in Some Local Market

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Abstract: A comparative study was conducted to evaluate quality of *Spirulinaplatensis* products like powder and tablets that were manufactured by different companies in Bangladesh. The powder form was collected from Bangladesh *Spirulina* Company Ltd. and was named as SP-1. On the other hand, the tablets were collected from Eureka International, Life Line International and Evergreen Enterprise's and were as named as SP-2, SP-3 and SP-4, respectively. The parameters investigated in this study were moisture content, ash content, acid insoluble and acid soluble ash content, crude fat content, nitrogen and protein content. Additionally, the amount of different solvent extracts, phytochemical screening, determination of total phenolic and flavonoids contents and antioxidant activity (DPPH radical scavenging activity), reducing power assay and total antioxidant activity and elemental analysis were also investigated. DPPH radical scavenging activity, reducing power assay and total antioxidant activity of all samples extract of four varieties showed significant/good/moderate activity, especially methanol and water extracts showed significant activity. All the four varieties of *Spirulinaplatensis* possessed good reducing power. Results of elemental analysis of four verities of *Spirulinaplatensis* showed significant difference. Analysis of mineral composition revealed the presence of sufficient amount of minerals such as sodium, potassium, iron and zinc. High levels of potassium were observed in all the samples. Heavy metals were also analyzed like lead, copper, chromium etc. Some of the samples were found to have contained lead in significant amounts. The reducing powers of the methanol and water extracts of four different samples (*Spirulinaplatensis*) were found to be significant in comparison with the standard ascorbic acid. The total antioxidant capacities of the methanol and water extracts of four different samples (*Spirulinaplatensis*) were found to be moderately significant in comparison with the standard ascorbic acid. From the result of this study, it is observed that different samples of *Spirulinaplatensis* contained different amount of physicochemical, chemical and antioxidant properties. However, among of the four products, SP-3 was found to have the best quality.

Keywords: *Spirulinaplatensis*, Proximate Composition, Phytochemicals

1. Introduction

Spirulinaplatensis is a blue-green alga (cyanobacterium) that has a long history and documented record of human consumption in many countries since ancient times. Spirulina was used traditionally by Mexicans during Aztec civilization for over 1000 years [1]. Its use as a food by the natives in the Lake Chad area has also been documented. Up to very recently, the interest in Spirulina was mainly in its nutritive value. However, it has gained immense attention for its nutraceutical value and bio-functional properties [2]. Many scientific studies have supported its role as antiviral, anti-mutagenic, and cholesterol lowering agent-primarily due to the presence of carotenoids, and other nutrients/antioxidants

in the tiny plants. It is also known to exhibit anti-inflammatory and anticancer properties [3]. Moreover, numerous data published in the primary scientific literature, including human and animal safety studies and work with malnourished children, attest to the safety of dietary Spirulina. Consequently, it has been listed by the US Food and Drug Administration under the category Generally Recognized as Safe (GRAS) [4-6].

Spirulina contains a high content of protein (up to 70%), along with high amounts of essential fatty acids, essential amino acids, minerals, vitamins (especially B_{12}), antioxidant pigments (phycobiliproteins and carotenoids) and polysaccharides [2, 7]. Consequently, the great efforts and extensive investigations have been turned to the development of nutraceuticals or functional food for preventing or managing various diseases since mid-1980's. It has become one of such nutraceutical food with diverse beneficial effects on an array of disease conditions. Numerous countries including the United States, Thailand, India, Taiwan, China, Pakistan, Myanmar, Greece, Chile, Japan, and Bangladesh are the largest commercial producers of *spirulina*. It is commonly available in two forms – tablets or capsules and powdered form. However, powder and tablets are the two most common forms of commercially available spirulina.

Bangladesh is very rich in natural and herbal products. Spirulina, the microscopic aquatic plants have been cultivated in Bangladesh since the past 40 years. Initially it started producing spirulina in laboratories of the Bangladesh Council of Scientific and Industrial Research (BCSIR) in 1978 together with the French government. Nowadays *spirulina* of local quality is getting popularity as a food supplement among consumers. There are many different spirulina products, only some of which are identified on labels of commercially available products. Spirulina is available in ditches, ponds, lake etc. in Bangladesh. It is commercially available both as herbal medicine as well as in the form of tablet, capsule and powder in the market since 1993.

Hundreds of published scientific studies have been carried out to disclose how *Spirulina* boosts the immune system and improves health. Detailed biochemical composition analyses have been conducted of spirulina grown either under laboratory conditions, collected in natural conditions or in mass culture system using different agro-industrial waste effluent. In Bangladesh, so far, the number of scientific research on spirulina is very scarce. It ismainly focused on the culture medium that is suitable for culturing a local strain of *spirulina* [8]. Millions of people in Bangladesh, India, Taiwan and Chile are consuming high concentration of arsenic through drinking water and are at risk of chronic arsenic poisoning for which there is no specific treatment. A placebo-controlled, double-blind study was conducted to evaluate the effectiveness of *spirulina* extract plus zinc in the treatment of chronic arsenic poisoning [9]. However, to the best of our knowledge, there has been no study on the spirulina products especially in the form of powder and tablet that are commercially available in Bangladesh.

The aim of the present study is to determine the chemical, antioxidant and phytochemical compositions of commercially available spirulina products (powder and tablets) in Bangladesh. Moreover, spirulina can also absorb heavy metals from the water where it is grown. Therefore, an additional experiment was also conducted to evaluate the presence of heavy metals in the *spirulina* products. The parameters investigated in this study were moisture, ash, acid insoluble and acid soluble ash, crude fat, nitrogen and protein. Additionally, phytochemicals (total phenolic and flavonoids contents), antioxidant activity (DPPH radical scavenging activity), reducing power were also investigated.

2. Materials and Methods

2.1. Collection and Preparation of Sample

Spirulinaplatensis samples were collected as powder and tablets from different local markets of Bangladesh. The powder form was collected from Bangladesh Spirulina Company Ltd. and was named as SP-1. On the other hand, the tablets were collected from Eureka International, Life Line International and Evergreen Enterprise's and was as named as SP-2, SP-3 and SP-4, respectively. After collection, the tablets were also ground separately in to fine powder with mortars. Subsequently, they were directly used in the research.

2.2. Proximate Analysis

The proximate analysis of the samples for moisture, ash and crude fat were done by the method of AOAC (2005). The Nitrogen was determined by micro-Kjeldahl method as described by Pearson (1976). The percentage was converted to crude protein by multiplying 6.25. All determination was performed in triplicates.

2.3. Mineral Analysis

Minerals such as Magnesium (Mg), Iron (Fe), Potassium (K), Sodium (Na), Zinc (Zn), Chromium (Cr), Lead (Pb), and Copper (Cu) were estimated by using titrimetric and spectrophotometric methods.

2.4. Phytochemical Analysis

Phytochemical analysis for total phenolic content of the extracts was determined by the modified Folin-Ciocaltu method [10]. And aluminum chloride colorimetric method was used for flavonoids determination [11].

3. Results and Discussion

3.1. Proximate Analysis of Different Samples of Spirulinaplatensis

The proximate analysis (in%) of different samples of *S.platensis* is shown in Table 1. The moisture contents of four samples (SP-1, SP-2, SP-3 and SP-4) were found to be 12.12%, 10.09%, 9.01%, and 10.60%, respectively. The

power form of *Spirulina*product (SP-1) was showed the highest amount of moisture whereas the tablet form (SP-2, SP-3 and SP-4) contains little bit less amount of moisture. However, SP-3 presented more less amount of moisture as compared to other products. The results obtained in this research are in agreement with other previous studies that have reported the moisture content in *spirulina* was between 5 and 10% [12-15]. The solid contents present in all the *Spirulina*products SP-1, SP-2, SP-3 and SP-4 were found to be 87.88%, 89.899%, 90.89% and 89.28% respectively. This finding was supported by the previous study [12]. Interestingly, these results are much higher than those reported in nature from different sites in Chadthat was 70% dry matter [16]. The ash contentsand organic matter of SP-1, SP-2, SP-3 and SP-4 were 6.20%, 8.34%, 9.96%, 8.88% and 93.8%, 91.66%, 90.03, and 91.12 respectively. A similar finding was observed by other study [12]. The acid soluble and insoluble ashcontents of SP-1, SP-2, SP-3 and SP-4were 98.55%, 74.66%, 77.459%, 78.82% and 1.45%, 25.34%, 22.54% and 21.17% respectively.

In addition, the fat contents of SP-1, SP-2, SP-3 and SP-4 were 1.92%, 2.34%, 1.41% and 0.86% respectively. These values are much lower than the finding of previous studies of *Spirulina*that reported the lipid values was ranged from 5.6 to 7% [17-18]. However, the other literature showed the lipid values ranging from 6 to 13% [19-20]. The protein contents of *Spirulina* products SP-1, SP-2, SP-3 and SP-4 were found to be 43.897%, 50.73%, 52.43% and 36.12%respectively. The highest amount of protein was found in SP-3 of this study. A study found that *Spirulina* protein content represented 58.2% [21], which is in agreement with our result. However, other authors reported a high amount of protein (65-70%) found in *Spirulina* powder [22-23]. The nitrogen content of *Spirulina* products SP-1, SP-2, SP-3 and SP-4 were found to be 7.024%, 8.12%, 8.37% and 5.77% respectively.

Table 1. *Proximate analysis of different samples of S.platensis.*

Sample No.	Moisture (%)	Solid (%)	Ash (%)	Organic matter (%)	Acid soluble ash (%)	Acid insoluble ash (%)	Fat (%)	Protein (%)	Nitrogen (%)
SP-1	12.12	87.88	6.20	93.8	98.55	1.45	1.92	43.897	7.024
SP-2	10.09	89.899	8.34	91.66	74.66	25.34	2.34	50.73	8.12
SP-3	9.01	90.89	9.96	90.03	77.46	22.54	1.41	52.43	8.37
SP-4	10.60	89.28	8.88	91.12	78.82	21.176	0.86	36.12	5.77

Note: SP-1= Powder form; SP-2; SP-3 and SP-4= Tablet forms

3.2. Mineral Contents of Different Samples of Spirulinaplatensis

The total mineral contents of *Spirulinaplatensis*is presented in Table 2. The sodium contents of SP-1, SP-2, SP-3 and SP-4 were found to be 2329.48, 2418.20, 2581.48 and 2362.20 ppm, respectively. On the other hand, the potassium and iron contents of SP-1, SP-2, SP-3 and SP-4 were found to be 4612.37, 5985.05, 4563.40 and 3377.95 ppm and 17.47, 15.11, 5.378 and 12.65 ppm, respectively. The zinc contents of SP-1, SP-2, SP-3 and SP-4 were found to be 43.85, 51.63, 96.20 and 63.89 ppm, respectively while the copper contents of were found to be 13.15, 16.69, 10.69 and 13.52 ppm, respectively. The amount of chromium of SP-1, SP-2, SP-3 and SP-4 were found to be 0.66, 2.85, 2.62 and 2.04 ppm, respectively. The lead content of SP-1, SP-2, SP-3 and SP-4 were found to be 3.58, 8.00, 6.66 and 6.08 ppm, respectively. These results are higher than the previous published study [13].

Table 2. *Mineral analysis of different samples of S.platensis.*

Name of sample	Na (ppm)	K (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Cr (ppm)	Pb (ppm)
SP-1	2329.48	4612.37	17.47	43.85	13.15	0.66	3.58
SP-2	2418.20	5985.05	15.11	51.63	16.69	2.85	8.00
SP-3	2581.48	4563.40	5.378	96.20	10.69	2.62	6.66
SP-4	2362.20	3377.95	12.65	63.89	13.52	2.04	6.08

Note: SP-1= Powder form; SP-2; SP-3 and SP-4= Tablet forms

3.3. Functional Nutrients Contents of Different Samples of Spirulinaplatensis

There are many functional nutrients present in *Spirulina*. This study measured total phenols and flavonoids. The total phenol and flavonoid contents were evaluated to investigate the effect of phenolic and flavonoid compounds on the antioxidant activities of the study extracts. Figure 1 shows the standard curve of gallic acid. The total phenolic contents of methanol extract in*Spirulina* products SP-1, SP-2, SP-3 and SP-4 were estimated to be 32, 42.94, 35.57 and 33.53 mg Gallic acid equivalent (GAE) per gm of dry extract, respectively. On the other hand, the total phenolic contents of water extract in *Spirulina* products SP-1, SP-2, SP-3 and SP-4 were found to be 42, 37.52, 43.13 and 35.64 mg Gallic acid equivalent (GAE) per gm of dry extract, respectively (Table 3). The results showed that the range of total phenol content in the methanol extract was 32 to 42.94 mg Gallic acid equivalent (GAE) per gm of dry extract and in the water extract it was in the range of 35.64 to 43.13 mg Gallic acid equivalent (GAE) per gm of dry extract. Another study reported [24] that the mean value of total phenol content in the carotenoid extract was in the range of 1.3-6.4mol gallic acid equivalents (GAE)/g dry weight gallic acid equivalents (GAE)/g dry weight (Table 3) which is supported our present study.

Table 3. *Determination of total phenolic content of the methanol and water extract of different samples of S.Platensis.*

Extract and concentration	Name of sample	TotalPhenolic content (mg)	Extract and concentration	Name of sample	TotalPhenolic content (mg)
Methanol extract (1mg/ml)	SP-1	32	Water extract (1mg/ml)	SP-1	42
	SP-2	42.94		SP-2	37.52
	SP-3	35.57		SP-3	43.13
	SP-4	33.53		SP-4	35.64

Table 4. *Determination of total flavonoids content of different samples (Spirulinaplatensis) of the methanol and water extract.*

Extract and concentration	Name of sample	TotalFlavonoid content (mg)	Extract andconcentration	Name of sample	TotalFlavonoid content (mg)
Methanol extract (1mg/ml)	SP-1	554.5	Water extract (1mg/ml)	SP-1	228.701
	SP-2	864.58		SP-2	126.3
	SP-3	540.88		SP-3	144.41
	SP-4	691.5		SP-4	136.709

Note:SP-1= Powder form; SP-2; SP-3 and SP-4= Tablet forms

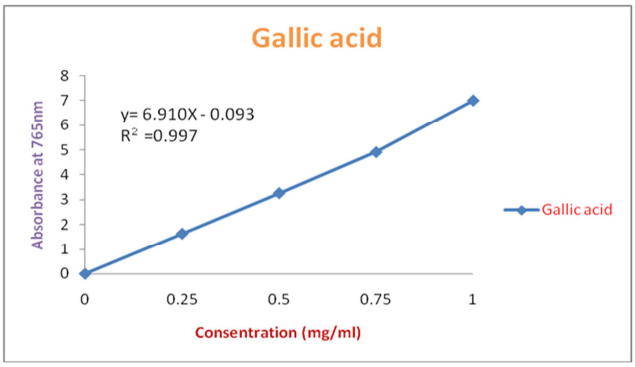

Figure 1. *Standard curve of gallic acid.*

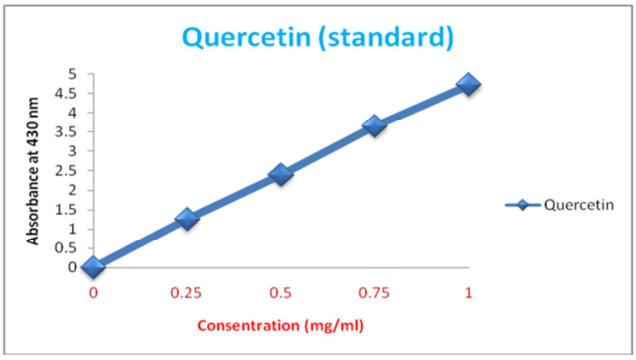

Figure 2. *Standard curve of quercetin.*

Figure 2 showed the standard curve of quercetin. The total flavonoid contents of methanol extract of *Spirulina* products SP-1, SP-2, SP-3 and SP-4 wereestimated to be 554.5, 864.58, 540.88 and 691.5mg respectivelyquercetin equivalent (QE) per gm of dry extract. On the other hand, the total flavonoid contents of water extract of *Spirulina* products SP-1, SP-2, SP-3 and SP-4 were found to be 228.701, 126.3, 144.41 and 136.709 mg respectivelyquercetin equivalent (QE) per gm of dry extract. The range of flavonoid content in the methanol extract of different sample of *Spirulinaplatensis*was in the range of 554.5 to 864.58 5mg quercetin equivalent (QE) per gm of dry extract whereas, in methanolextract its range was 126.3 to 228.701 5mg quercetin equivalent (QE) per gm of dry extract in water extract.

4. Conclusion

Spirulina can play an important role in human and animal nutrition due to the presence of plenty of minerals, proteins, fats and functional nutrients. The proximate analysis of *Spirulina* product both in powder and tablets form showed that it contains considerable amount of minerals, proteins fats and functional nutrients. It also present sufficient amount of phenolic content and flavonoid content. In contrast, it contain low amount of heavy metal like, zinc, copper, chromium and lead. Therefore, *Spirulina* product can be used as a food supplement for human with higher health benefits.

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