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Antioxidants activity of the cyanobacterium, *Arthrospira* (Spirulina) *fusiformis* cultivated in a low-cost medium

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Spirulina **species are known to have a good nutritional profile and antioxidant properties against reactive oxygen species. However, little is known about the antioxidant contents and the scavenging ability of *Arthrospira fusiformis,* cultivated under various conditions. This study aimed at evaluating the content of antioxidants (total phenols, total flavonoids, β-carotene, and lycopene) and the activity of *A. fusiformis* produced using low-cost culture (LCMA) and standard culture (Zarrouk) media. The results revealed that *A. fusiformis* is rich in antioxidants and it possesses high scavenging and chelating activities. Interestingly, the LCMA was superior over the Zarrouk medium as it resulted in spirulina with a higher amount of antioxidants and lower EC_{50} values. In this context, production of natural antioxidants can be maximized through the use of cost-saving, inorganic culture medium.**

Key words: *Arthrospira fusiformis*, spirulina, total phenols, total flavonoids, carotenoids, scavenging activity, low-cost culture (LCMA) medium, Zarrouk medium.

INTRODUCTION

Arthrospira, commonly known as spirulina is a filamentous helical shaped cyanobacterium belonging to the family Oscillatoriaceae (Kumar et al., 2005; Rasool et al., 2006). It occurs naturally in warm alkaline lakes of the tropical and sub-tropical countries (Germán Chamorro-Cevallos and Vázquez-Sánchez, 2008; Habib et al., 2008; Shalaby and Shanab, 2013; Kumari et al., 2015). Spirulina is proven to be toxicologically free (Germán Chamorro-Cevallos and Vázquez-Sánchez, 2008; Gutiérrez-Salmeán et al., 2015) and it has been cultivated massively in several countries especially those in the Asian and American continent and used as protein and vitamins supplement in the diets (Rasool et al., 2006; Belay, 2008; Salamatullah, 2014). Spirulina is a rich source of protein (about 50 to 70%), essential amino acids, vitamins, minerals and unsaturated fatty acid (Pandey et al., 2010;

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Gutiérrez-Salmeán et al., 2015). More interestingly, it possesses the antioxidant and antiradical properties being attributed by phytonutrients such as phenolics, phycocyanin, tocopherol and β-carotene (Colla et al., 2007; Shalaby and Shanab, 2013; Al-Dhabi and Valan Arasu, 2016; Ismaiel et al., 2016). Thus, consumption of spirulina improved the resistance of consumers against oxidative stress. Several studies have pointed out the efficiency of spirulina as an anti-viral and anticancer (Kumar et al., 2005), anti-inflammation (Rasool et al., 2006), and anti-allergic and antibacterial (Belay, 2008). It is further reported that the antioxidant activity in spirulina extract against lipid peroxidation is even more powerful than that of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Chopra and Bishnoi, 2008; Tarko et al., 2012).

Among the *Arthrospira* species, *Arthrospira platensis* and *Arthrospira maxima* are the most studied and cultivated for human food, dietary supplement, and animal feed additive (Belay, 2008). There is scarce information regarding the potential of *Arthrospira fusiformis* especially the nutritional and bioactive composition. However, some studies have confirmed the anti-inflammatory and anti-cancer properties of *A. fusiformis* (Mathew et al., 1995; Rasool et al., 2006; Deng and Te-Jin, 2010). More recently, Mulokozi (2016) suggested that the cultivated *A. fusiformis* can replace a significant amount of the fishmeal in tilapia feeds. Nevertheless, antioxidant and nutritional contents may vary due to factors such as culture conditions, culture media, analysis methods, type and source of the organism (Habib et al., 2008; Gutiérrez-Salmeán et al., 2015; Al-Dhabi and Valan Arasu, 2016).

Spirulina can be cultivated under laboratory conditions as well as outdoor for large-scale systems. The outdoor culture systems rely mainly on Zarrouk medium (Belay, 2008; Madkour et al., 2012; Tarko et al., 2012) though it is highly expensive. Thus, efforts have been made to develop a more convenient and a less expensive culture media (Raoof et al., 2006; Chen, 2011; Gami et al., 2011; Madkour et al., 2012), which can produce high-quality spirulina biomass comparable to the standard culture medium. Cultivation trials of spirulina conducted in Tanzania used the culture medium termed as OFER, which composes fewer analytical grade chemicals as compared to those of Zarrouk medium (Mulokozi, 2016). In joining the effort to reduce the cost of production and maximizing spirulina biomass, in this study a new culture medium, namely, LCMA was introduced. The LCMA was formed by mixing a low-cost inorganic fertilizer (NPK10-20-20), two analytical grade chemicals from Zarrouk medium (sodium chloride and sodium bicarbonate) and drops of trace element solution. Moreover, the aim of this study was to assess the antioxidant components (total phenols, total flavonoids, β-carotene, and lycopene) and antioxidant properties in the fresh and dried extracts of *A. fusiformis* cultivated in the cost-effective medium, and compared the results to the standard culture medium.

MATERIALS AND METHODS

Preparation of culture media and spirulina cultivation

The strain of *A. fusiformis* used in this study was obtained from the stock kept at the Institute of Marine Sciences, University of Dar es Salaam, Tanzania. The algal sample was previously collected from Lake Big Momela, Tanzania, and purified according to Mulokozi (2016). The stock was maintained in 2000 ml conical flasks in standard culture medium (Zarrouk). On the beginning of this study, spirulina was cultivated in two synthetic media (Table 1) whereby Zarrouk was used as a standard medium (Kumari et al., 2015) and LCMA as an alternative low-cost medium for mass culture. NPK10-20-20 fertilizer was obtained from authorized dealers of agricultural inputs farmers in Tanzania at Kariakoo Market. The analytical grade chemicals for Zarrouk medium were purchased from laboratory equipment and chemical supplier in Zanzibar (Zan-Lab Equipment).

The experiment was carried out in 10 L aquaria containing 1900 ml of culture media and 100 ml (0.038 g/L dry weight) of spirulina. Three aquaria were set for each Zarrouk and LCMA. The culture was incubated for 30 days in a growth chamber at the Department of Botany, University of Dar es Salaam at a temperature range of 28 to 30°C. Light emitting diodes (LEDs) supplying 4.5 Klux light intensity at the surface of the vessels with a photoperiod of 12/12 h light/dark cycle were used as a source of light. Aerators fixed on the air pump were used to supply air in the cultures. On harvest, some of the spirulina concentrates was kept fresh in the refrigerator for further analysis and part of spirulina concentrates were oven dried at 60°C overnight. The dry sample was ground to make powder and then stored in the refrigerator.

Preparation of spirulina extracts

Fresh and dry biomass of spirulina (0.5 g) from each LCMA and Zarrouk media were placed in the conical flasks and then soaked in 100 ml of 95% ethanol. The sample was continuously stirred to ensure complete extraction. The extracts were centrifuged for 10 min then filtered using Whatman No. 1 filter paper. Ethanol was evaporated from the supernatant in a rotary evaporator (Gmbh & Co.KG, Germany) under reduced pressure at 40°C. Extraction was repeated three times until the desired concentration of extract was obtained. The obtained extracts were stored in a refrigerator at 4°C until further analyses.

Yield of extracts

The yield of ethanolic extracts of spirulina samples was calculated based on the following equation:

$$\text{Yield (\%)} = (W_2 \times 100) / W_1$$

Where W_1 is a weight of spirulina before evaporation/extraction and W_2 is the weight of extract after evaporation.

Determination of antioxidants levels and activity in spirulina extracts

Total phenolic compounds were estimated by Folin-Ciocalteu calorimetric method adapted from Pal et al. (2010). In brief, 1 ml of ethanolic extract was mixed with 1 ml of Folin-Ciocalteus's reagent

Table 1. Chemical composition of Zarrouk and LCMA culture media.

Component	Concentration (g/L	
	Zarrouk	LCMA
$NaHCO_3$	18	10
NaCl	1	1
$MgSO_4 \cdot 7H_2O$	0.2	-
NaEDTA	0.08	-
$CaCl_2 \cdot 2H2O$	0.04	-
$NaNO_3$	2.5	-
K_2SO4	1	-
K_2HPO_4	0.5	-
$FeSO_4 \cdot 7H_2O$	0.01	-
NPK10-20-20 complex	-	0.5
Micronutrient	1 ml	1 ml
Distilled water	1 L	-
Boiled, cool tap water	-	1L (0.024 g/L N, 0.001 g/L P, 0.005 g/L K

Micronutrients composition (g/L): H_3BO_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $ZnSO_4 \cdot 4H_2O$, 0.222; Na_2MoO_4, 0.0177; $CuSO_4 \cdot 5H_2O$, 0.08.

(Sigma-Aldrich, St. Louis USA) and incubated at room temperature. After 3 min, 1 ml of 7.5% (w/v) sodium carbonate was added to the mixture. The reaction was kept in the dark for 2 h and the absorbance was read at 725 nm using UV-spectrophotometer (Jenway 6305, UK). Standard solution of gallic acid was used to obtain a standard curve and total phenols were expressed as milligram of Gallic Acid Equivalent per gram of extract (mg GAE/g). Analysis for each extract was done in quadruplicate and the results were expressed as mean ± standard deviations (SD).

Total flavonoids concentration was determined by calorimetric assay according to Bonvehí et al. (2001). One milliliter of spirulina extract was diluted with 4.3 ml of 80% ethanol containing 0.1 ml 10% aluminium nitrate and 0.1 ml of aqueous potassium acetate (1 M). The mixture was left for 40 min at room temperature and then absorbance was read at 415 nm. Total flavonoids content expressed as Rutin Equivalents milligrams per gram of extract (RE mg/g) was calculated using rutin as standard.

The carotenoids contents, namely, β-carotene and lycopene were determined according to the method described by Barros et al. (2007). Only the dried samples of spirulina were used, as the method requires dry samples. Briefly, 100 mg of extract was shaken vigorously with 10 ml acetone-hexane for 1 min then filtered through Whatman No. 1 filter paper. The absorbance of filtrate was measured at 453, 505 and 663 nm and the contents of β-carotene and lycopene were calculated according to the following equations:

$$\text{Lycopene (mg/100 mg)} = -0.0458A_{663}+0.372A_{505}-0.0806A_{453}$$
$$\beta\text{-carotene (mg/100 mg)} = 0.216_{A663}-0.304A_{505}+0.452A_{453}$$

The mean value of three assays was used to estimate contents of carotenoids and results were presented as mg/g of extract.

DPPH radical scavenging assay

The ability of spirulina extracts to scavenge the stable radical 2,2-diphenyl-picrylhydrazyl (DPPH) was assessed based on the modified method of Batool et al. (2010). Briefly, a series of ethanolic extracts (0.01, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) were prepared. A measure of 500 µl of extract was mixed with 1 ml solution of DPPH (0.125 µM in 95% ethanol). The mixture was shaken vigorously and incubated in dark room for 30 min and the absorbance was measured at 515 nm using UV-spectrophotometer. The DPPH radical scavenging activity (RSA) of spirulina extracts was calculated as percent of DPPH inhibition using the following equation:

$$\%RSA = ((A_{DPPH}-AE) / A_{DPPH}) \times 100$$

where A_{DPPH} is the absorbance of DPPH solution and AE is the absorbance of extract containing DPPH.

The percentage of DPPH RSA was plotted against spirulina extract concentrations (mg/ml) to determine the amount of extract necessary to inhibit (scavenge) initial concentration of DPPH radical by 50% (EC_{50}). The lower EC_{50} value indicated higher scavenging activity of an extract.

Ferrous ions chelating assay

The ability of spirulina extracts to chelate Fe (II) ions was determined according to the method described by Pal et al. (2010). In short, different concentrations (0.01, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) of spirulina extracts and ethylene diamine tetra acetic acid (EDTA) were prepared. On start of experiment, 0.1ml of 2 mM $FeCl_2$ was added to the reaction mixture containing 10 mg of 0.1 M Tris-HCl and 0.2 ml 5 mM ferrozine and the aqueous ethanolic extracts. The reaction was left for 10 min at room temperature then the absorbance was measured at 562 nm against a blank in UV-spectrophotometer. The effective concentration (EC_{50}) of each extract at which 50% of ferrous ions were chelated, was obtained by interpolation from linear regression graph. EDTA was used as standard chelator.

Statistical analyses

All measurements were carried out in at least three replicates and the results presented as mean ± standard deviation. Data of the extract yields and antioxidants were analyzed by using

Table 2. The yields, contents of antioxidants, EC_{50} values of DPPH scavenging and Fe^{2+} chelation in the Spirulina extracts.

Sample	Yield (%)	Phenolics (mg GAE/g)[b]	Flavonoids (RE mg/g)[b]	β-carotene (mg/100 mg)[a]	Lycopene (mg/100 mg)[a]	EC_{50} DPPH scavenging (mg/ml)	EC_{50} Fe^{2+} chelation (mg/ml)
ZM fresh	6.98±0.00	137.65±4.39	5.10±0.12	NA	NA	0.3	0.078
LCMA fresh	6.83±0.01	151.45±0.70	8.30±0.141	NA	NA	0.202	0.068
ZM Dry	16.21±0.32	292.17±5.50	11.25±0.5	0.17±0.00	1.06±0.01	0.26	0.014
LCMA Dry	21.63±0.04	409.28±28.78	13.25±0.5	0.89±0.00	1.28±0.02	0.11	0.001

[a]Values are the mean ± SD (n = 3); [b]values are mean ± SD (n = 4). ZM: Extracts from Zarrouk medium; LCMA: extracts from LCMA medium. NA: not applicable (the determination was done for dry samples only).

Paleontological Statistical programme (PAST ver. 2.17, Natural History Museum, University of Oslo, Norway). A two-sample *t*-test was used to see if there is significant difference in the yields, total phenolics, flavonoids and carotenoids between the two culture media. The differences between means at 5% (*P*-values less than 0.05) were considered significant.

RESULTS AND DISCUSSION

Yield of spirulina extracts

The extract yields of spirulina samples are shown in Table 2. The highest (21.63 ± 0.04%) and lowest (6.83 ± 0.01) yields for LCMA medium were recorded in dry and fresh extracts, respectively. In the fresh extracts, standard culture medium (Zarrouk) recorded significantly higher yield than LCMA ($p < 0.001$, $t = 22.958$). Similarly, there was significant difference ($p < 0.0001$, $t = -28.709$) among dried sample extracts with LCMA's extracts recording higher yield (21.63 ± 0.04%) as compared to Zarrouk's extracts (16.21 ± 0.32%). The highest yield recorded in LCMA suggests that LCMA is the best medium for the yield of spirulina biomass. The yields of the current study are higher than previously reported by Shalaby and Shanab (2013).

Antioxidant contents

Table 2 also shows the antioxidant contents in spirulina extracts. The total phenolics among other antioxidants were the most abundant. All the extracts analyzed were found to have significant amount of total phenols, flavonoids and carotenoids, which are evidence for protection of human body and other spirulina consumers against oxidative damage. With regard to total phenolics, it was shown that the dried sample extracts for spirulina grown in LCMA medium contained notably higher levels (409.28 ± 28.78 mg GAE/g) as compared to other extracts. There was significant variation in phenolics between dry ($p = 0.0002$, $t = -7.8769$) and fresh extracts ($p = 0.0013$, $t = -5.6934$). Differences in phenolic contents among extracts may be caused by several factors as previous reported (El-Baky et al., 2009; Tarko et al., 2012; Salamatullah, 2014; Ismaiel et al., 2016). For instance, Tarko et al. (2012) stated that the composition of growth media used for cultivating the selected species of *Arthrospira* influenced the synthesis of bioactive components and antioxidant properties. However, Wu et al. (2013) reported that organisms may produce phenols as a defensive mechanism against disease and other stress especially when nutrient is depleted. The authors also recorded higher phenolic contents in the jujube tree planted in natural unfertilized area and they linked the observations with limitation of nutrient resources. Studies on *Spirulina* species (Salamatullah, 2014; Ismaiel et al., 2016), associated pH rise in the culture media to the increased production of phenols so as to alleviate the oxidative stress induced by the rising pH. In the current study, pH level (not reported here) was higher in LCMA medium in few days before harvest, and it was lower for Zarrouk medium; this might have influenced the variation in total phenols.

The current results on phenols are incomparable to previous reports due to the differences in methods and extraction solvent used. For instance, the study by Machu et al. (2015), water extract of *A. platensis* recorded the highest level of 43.2 mg/g GAE, which is lower than that obtained in this study. Another study by Shalaby and Shanab (2013), on *Spirulina platensis,* recorded phenolics of 282.76 g/100 mg, which are more or less similar to the current study (for Zarrouk's phenolics). However, Bhattacharya and Shivaprakash (2005) reported that higher phenolic contents as compared to the current study whereby *Spirulina laxissima* was found to contain the highest intracellular phenolics (4.46 g/100 mg), while *S. platensis* contained the highest extracellular phenolics (0.3 g/100 mg).

For the total flavonoids, there was significant difference in total flavonoids between dried ($p = 0.0013$, $t = -5.6569$) and fresh extracts ($p < 0.0001$, $t = -35.054$). The dried spirulina extracts from LCMA medium contained higher flavonoids than the dried extract of Zarrouk medium and the fresh extracts. In general, all extracts were found to have lower total flavonoids than the total phenols implying that large part of polyphenol compounds in

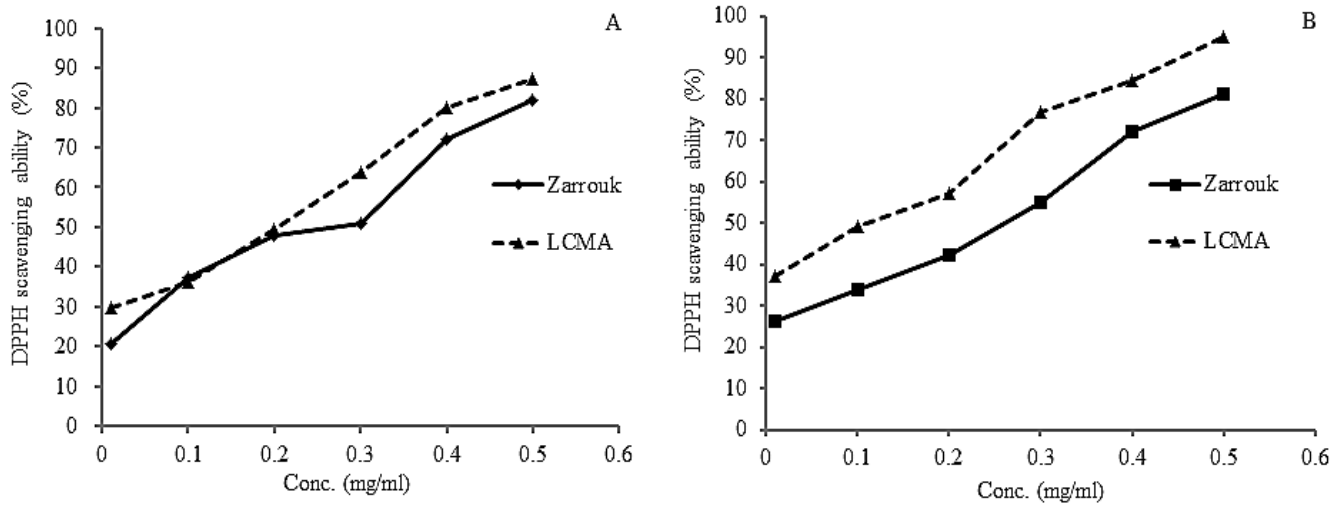

Figure 1. Scavenging activity (%) of spirulina ethanolic extracts on DPPH by fresh spirulina extracts (A) and dry extracts (B).

spirulina is phenolic substance instead of flavonoids. Other studies working on different sources of flavonoids (El-Baky et al., 2009; Salamatullah, 2014) also recorded lower amount of the flavonoids as compared to total phenols. As it is for phenolic compounds, the amount of flavonoid is also affected by the growth media (El-Baky et al., 2009).

Carotenoids are important groups of pigments found in some plants and algae. They possess strong antioxidant properties (Rao and Agarwal, 2000; Pal et al., 2010) and thought to be an anti-cancer agents (Gutiérrez-Salmeán et al., 2015). Beta carotene is a fat soluble pigment and is known as precursor of vitamin A in mammals (Pal et al., 2010), it bio-transforms into vitamin A once absorbed (Gutiérrez-Salmeán et al., 2015). In the current study, the content of β-carotene was higher in spirulina extract cultured in LCMA medium as compared to Zarrouk medium (Table 2). The reason for such variation may be due to the differences in the composition and amount of nutrients used for preparing the culture media. The investigations by Tarko et al. (2012) reveled that β-carotene contents of different strains of spirulina was strongly influenced by the growth medium whereby the standard Zarrouk medium was superior over the low-cost medium. In the current study, although the values of β-carotene are lower than reported by Tarko et al. (2012) but the LCMA recorded higher content than Zarrouk medium. Moreover, the β-carotene contents reported in the current study are much lower than previously reported (Bhattacharya and Shivaprakash, 2005; Belay, 2008; Gutiérrez-Salmeán et al., 2015) for other species of spirulina. Contrary, the content of β-carotene for *S. laxissima* reported by Bhattacharya and Shivaprakash (2005) was much lower than those in the current study. Earlier study demonstrated that the β-carotene is the most fluctuating pigment among other carotenoids, and the variation can even be more than 40 times, that is, from 10 to 400 mg/100 g (Tarko et al., 2012).

For lycopene content, this study is the first found lycopene in spirulina (*A. fusiformis*). Spirulina extracts cultured in LCMA medium was found to have significantly ($p < 0.0001$, $t = -20.863$) higher lycopene contents than that of Zarrouk. The amount of lycopene reported here are slightly higher than that present in edible mushrooms (Barros et al., 2007; Robaszkiewicz et al., 2010). Variations in lycopene contents are associated with variety of factors such as climatic conditions (Wawrzyniak et al., 2005) geographic location, fertilizer used and plant variety (Bhumsaidon and Chamchong, 2016).

Radical scavenging activity (RSA) using DPPH

The DPPH is a stable free radical, which possesses a characteristic absorption at 515 nm, the absorption decreases regularly upon exposure to radical-scavenging species. A lower absorbance indicates high radical scavenging activity of an extract (Barros et al., 2007). DPPH was selected to evaluate the antioxidant activities of spirulina extracts because it is the most effective and standard method for assessing the radical scavenging activity of a particular extract (Amarowicz et al., 2004; Maisuthisakul et al., 2007). Figure 1 shows the DPPH RSA of both fresh and dry spirulina extracts cultured in Zarrouk and LCMA media. The dry extract from LCMA exhibited strongest scavenging activity than all other extracts thereby quenching 95% of the DPPH radicals at the concentration of 0.5 mg/ml. The scavenging activities of other extracts at 0.5 mg/ml were 87, 81 and 72% for fresh spirulina in LCMA, dry and fresh spirulina in Zarrouk media, respectively. Though the extracts showed good scavenging activities, there was no significant variation

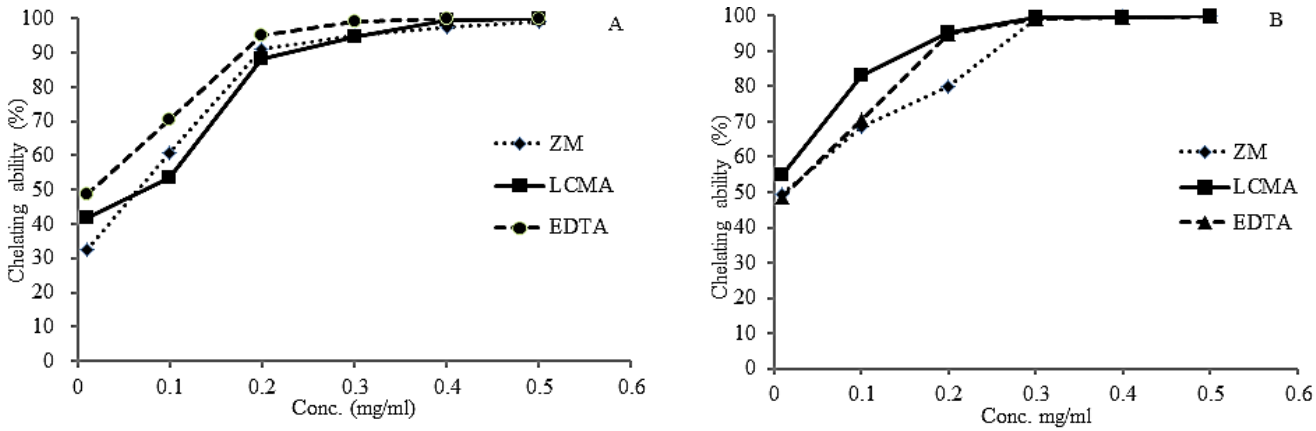

Figure 2. Ferrous (II) ion chelating ability of fresh spirulina extracts (A) and dry extracts (B). ZM: Zarrouk medium.

for both fresh and dried sample extracts ($p > 0.05$). Comparing with previous studies, spirulina extracts of the current study show stronger scavenging activity than reported by Chopra and Bishnoi (2008) for *A. platensis.* The result for dry extract cultured in LCMA is comparable to Miranda et al. (1998) who also reported a 95% antioxidant activity at 0.5 mg for *A. maxima* methanolic extract. The effective concentration (EC_{50}) usually corresponds to antioxidant activity of the particular extract (Maisuthisakul et al., 2007), the lower the EC_{50} value the powerful the antioxidant activity. The lowest EC_{50} (0.11 mg/ml) was recorded in dry spirulina extracts cultured in LCMA, which also contained high total phenols, flavonoids and carotenoids (Table 2). The EC_{50} values for extracts of fresh spirulina from LCMA, dry and fresh spirulina from Zarrouk media were 0.202, 0.26 and 0.3 mg/ml, respectively. However, the extracts with high contents of antioxidants did not always have lower EC_{50} value as it was evident for dry spirulina extract from Zarrouk medium which had higher contents of phenols and flavonoids but weak antioxidant activity, that is, higher EC_{50} value compared to fresh extract from LCMA. The reason for lower scavenging activity of the extracts with high antioxidants could be related to the types of phenols and flavonoids present in the extracts (Salamatullah, 2014). Differences in the strength of scavenging abilities between spirulina extracts of the current study might be due to differences in the compositions of culture media. For instance, Hussein et al. (2015) worked on antioxidants in the wild and domesticated mushroom extracts, reported that nutrients in the substrate had influence on the scavenging ability of the mushrooms. They suggested that higher scavenging ability of the domesticated mushroom was caused by high nutrient in the substrate (Hussein et al., 2015).

Ferrous ions chelating ability of spirulina extracts

The chelating power of extracts increased with increasing concentrations (Figure 2). At 0.5 mg/ml concentration of fresh extracts of spirulina and standard chelator (Ethylene diamine tetra acetic acid, EDTA), chelating ability was in the order: EDTA (99.91%) > LCMA (99.87%) > Zarrouk medium (99.25%). The standard metal chelator showed an outstanding chelating capacity (EC_{50} = 0.016 mg/ml) compared to extracts from LCMA (EC_{50} = 0.068 mg/ml) and Zarrouk medium (EC_{50} = 0.072 mg/ml). For the dry samples, spirulina extracts showed powerful chelating power than the synthetic metal chelator (Figure 2B). Of the spirulina extracts, dried extract from LCMA medium revealed strong chelating power whereby it chelated 55% of the ferrous ions at lowest concentration (0.01 mg/ml). The EC_{50} value for dry extract of spirulina grown in LCMA was less than 0.01 mg/ml as the 50% of ions were chelated before attaining the initial concentration. The EC_{50} values for extract cultured in Zarrouk medium was 0.014 mg/ml while that of standard metal chelator (EDTA) was 0.016 mg/ml. In general, it was observed that both fresh and dried spirulina extracts possess strong chelating activity against the ferrous ions as they chelated more than 50% of the ions at lower concentration of 0.1 mg/ml. However, there was no significant difference in chelating power between the dry samples as well as between the fresh sample extracts ($p > 0.05$).

Conclusion

The current study revealed that both fresh and dried extracts of *A. fusiformis* are rich source of antioxidants with a substantial amount of total phenols, flavonoids, β-carotene, and lycopene. Thus, consumption of spirulina as diet or feed/food additive is important for health. Spirulina extracts grown in the newly formulated low-cost medium resulted in higher antioxidants and stronger scavenging activity as compared to the standard culture medium. This implies that the production of natural antioxidants from spirulina can be maximized through the

use of cost-saving culture medium, the LCMA. However, it was noted that sometimes the extracts with higher antioxidants do not always express powerful antioxidant activity. This calls for further studies to assess the types of phenols and flavonoids in spirulina extracts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Al-Dhabi NA, Valan-Arasu M (2016). Quantification of phytochemicals from commercial spirulina products and their antioxidant activities. Evidence-Based Complementary and Alternative Medicine 2016:1-8.

Amarowicz R, Pegg R, Rahimi-Moghaddam P, Barl B, Weil J (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry 84:551-562.

Barros L, Ferreira MJ, Queiros B, Ferreira IC, Baptista P (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chemistry 103:413-419.

Batool F, Sabir SM, Rocha J, Shah AH, Saify ZS, Ahmed SD (2010). Evaluation of antioxidant and free radical scavenging activities of fruit extract from *Zanthoxylum alatum*: a commonly used spice from Pakistan. Pakistan Journal of Botany 42:4299-4311.

Belay A (2008). Spirulina *(Arthrospira*): Production and quality assurance. In: *Spirulina* in Human Nutrition and Health*, (*Gershwin M, Belay A (eds.), Boca Raton: CRC Press, London. pp. 1-26

Bhattacharya S, Shivaprakash M (2005). Evaluation of three Spirulina species grown under similar conditions for their growth and biochemicals. Journal of the Science of Food and Agriculture 85:333-336.

Bhumsaidon A, Chamchong M (2016). Variation of lycopene and beta-carotene contents after harvesting of gac fruit and its prediction. Agriculture and Natural Resources 50:257-263.

Bonvehí JS, Torrent MS, Lorente EC (2001). Evaluation of polyphenolic and flavonoid compounds in honeybee-collected pollen produced in Spain. Journal of Agricultural and Food Chemistry 49:1848-1853.

Chen YC (2011). The effect of shifts in medium types on the growth and morphology of *Spirulina platensis* (*Arthrospira platensis*). Journal of Marine Science and Technology 19:565-570.

Chopra K, Bishnoi M (2008). Antioxidant profile of Spirulina: a blue-green microalga. In: *Spirulina* in Human Nutrition and Health, Gershwin M, Belay A (eds.), Boca Raton: CRC Press, London. pp. 101-118.

Colla LM, Furlong EB, Costa JAV (2007). Antioxidant properties of spirulina *(Arthospira*) *platensis* cultivated under different temperatures and nitrogen regimes. Brazilian Archives of Biology and Technology 50:161-167.

Deng R, Te-Jin C (2010). Hypolipidemic, antioxidant, and anti-inflammatory activities of microalgae spirulina. Cardiovascular Therapeutics 28:33-45.

El-Baky HA, El Baz FK, El-Baroty GS (2009). Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects. African Journal of Biotechnology 8:7059-7067.

Gami B, Naik A, Patel B (2011). Cultivation of spirulina species in different liquid media. Journal of Algal Biomass Utilization 2:15-26.

Germán Chamorro-Cevallos BLB, Vázquez-Sánchez AJ (2008). Toxicologic Studies and Antitoxic Properties of Spirulina. In: Spirulina in Human Nutrition and Health, (Gershwin M, Belay A (eds,),. Boca Raton: CRC Press, London. pp. 28-44.

Gutiérrez-Salmeán G, Fabila-Castillo L, Chamorro-Cevallos G (2015). Nutritional and toxicological aspects of spirulina (*Arthrospira*). Nutricion Hospitalaria 32:34-40.

Habib MAB, Parvin M, Huntington TC, Hasan MR (2008). A review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish. Food and Agriculture Organization of the United Nations (FAO), Rome.

Hussein J, Tibuhwa D, Mshandete A, Kivaisi A (2015). Antioxidant properties of seven wild edible mushrooms from Tanzania. African Journal of Food Science 9:471-479.

Ismaiel MMS, El-Ayouty YM, Piercey-Normore M (2016). Role of pH on antioxidants production by *Spirulina (Arthrospira) platensis*. Brazilian Journal of Microbiology 47:298-304.

Kumar M, Sharma MK, Kumar A (2005). *Spirulina fusiformis*: a food supplement against mercury induced hepatic toxicity. Journal of Health Science 51:424-430.

Kumari A, Pathak AK, Guria C (2015). Cost-effective cultivation of *Spirulina platensis* using NPK fertilizer. Agricultural Research 4:261-271.

Machu L, Misurcova L, Vavra Ambrozova J, Orsavova J, Mlcek J, Sochor J, Jurikova T (2015). Phenolic content and antioxidant capacity in algal food products. Molecules 20:1118-1133.

Madkour FF, Kamil AEW, Nasr HS (2012). Production and nutritive value of *Spirulina platensis* in reduced cost media. The Egyptian Journal of Aquatic Research 38:51-57.

Maisuthisakul P, Suttajit M, Pongsawatmanit R (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chemistry 100:1409-1418.

Mathew B, Sankaranarayanan R, Nair PP, Varghese C, Somanathan T, Amma BP, Amma NS, Nair MK (1995). Evaluation of chemoprevention of oral cancer with *Spirulina fusiformis*. Nutrition and Cancer 24:197-202.

Miranda M, Cintra R, Barros S, Mancini-Filho J (1998). Antioxidant activity of the microalga Spirulina maxima. Brazilian Journal of Medical and Biological Research 31:1075-1079.

Mulokozi D (2016). Spirulina (*Arthrospira spp*) culture and its use as a protein source for Rufiji tilapia (*Oreochromis urolepis urolepis* Norman, 1922) Mariculture. M. Sc. Thesis. University of Dar es Salaam, Tanzania. P 116.

Pal J, Ganguly S, Tahsin KS, Acharya K (2010). In vitro free radical scavenging activity of wild edible mushroom, *Pleurotus squarrosulus* (Mont.) Singer. Indian Journal of Experimental Biology 48(12):1210-1218.

Pandey J, Pathak N, Tiwari A (2010). Standardization of pH and light intensity for the biomass production of *Spirulina platensis*. Journal of Algal Biomass Utilization 1:93-102.

Rao AV, Agarwal S (2000). Role of antioxidant lycopene in cancer and heart disease. Journal of the American College of Nutrition 19:563-569.

Raoof B, Kaushik B, Prasanna R (2006). Formulation of a low-cost medium for mass production of Spirulina. Biomass and Bioenergy 30:537-542.

Rasool M, Sabina EP, Lavanya B (2006). Anti-inflammatory effect of *Spirulina fusiformis* on adjuvant-induced arthritis in mice. Biological and Pharmaceutical Bulletin 29:2483-2487.

Robaszkiewicz A, Bartosz G, Ławrynowicz M, Soszyński M (2010). The role of polyphenols, β-carotene, and lycopene in the antioxidative action of the extracts of dried, edible mushrooms. Journal of Nutrition and Metabolism pp. 1-9.

Salamatullah A (2014). Characterization of extraction methods to

recover phenolic-rich antioxidants from blue green algae (spirulina) using response surface approaches. M. Sc. Thesis. Univerity of Nebraska, Lincoln. 94 p.

Shalaby EA, Shanab SM (2013). Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis.* Indian Journal of Geo-Marine Sciences 42:556-564.

Tarko T, Duda-Chodak A, Kobus M (2012). Influence of growth medium composition on synthesis of bioactive compounds and antioxidant properties of selected strains of *Arthrospira* Cyanobacteria. Czech Journal of Food Science 30:258-267.

Wawrzyniak A, Marciniak A, Rajewska J (2005). Lycopene content of selected foods available on the polish market and estimation of its intake. Polish Journal of Food and Nutrition Sciences 14:195-200.

Wu CS, Gao QH, Kjelgren RK, Guo XD, Wang M (2013). Yields, phenolic profiles and antioxidant activities of *Ziziphus jujube* Mill. in response to different fertilization treatments. Molecules 18:12029-12040.