Mutagenicity and Antimutagenicity of Polysaccharide and Phycocyaninextracts of *Arthrospira platensis* (IFRPD 1182) and *Arthrospira maxima* (IFRPD 1183)

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ABSTRACT

Arthrospira spp. are a filamentous blue-green algae that contain a high protein content (60%) and an impressive list of vitamins, amino acids and minerals. *Arthrospira* spp. are also a great source of polysaccharide and phycocyanin pigments that maintain body balance and boost the human immune system. The mutagenicity and antimutagenicity of these algae and their natural products are promising as the starting point for the discovery of novel, potentially bioactive compounds. Consequently, the aim of the study was to investigate the mutagenicity of water and 80% methanol extracted from *Arthrospira platensis* (IFRPD 1182) and *Arthrospira maxima* (IFRPD 1183) as well as the antimutagenic properties of the polysaccharide and phycocyanin obtained from both types of algae against four standard indirect mutagens (Trp-P-1, Trp-P-2, MeIQx and PhIP). The results revealed that the water extracts and 80% methanol extracts from both algae had no mutagenic effects on *Salmonella* Typhimurium strains TA98 and TA100 at all concentrations tested (300, 600, 1,200 and 2,500 µg/plate). This indicated that the extracts were safe and had no effects on the genetic material in cells. The phycocyanin extracted from both *Arthrospira* spp. was more effective in inhibition of mutagen than the polysaccharide extract from both algal types by 64–69%. Furthermore, the phycocyanin extracted from *Arthrospira* demonstrated protection against mutagenicity induced by toxic substances in grilled and smoked food products.

Keywords: Antimutagenicity, phycocyanin, polysaccharide, *Arthrospira*

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INTRODUCTION

Currently, serious diseases such as heart disease and cancer are a major public health problem all over the world. Treatments are not yet effective in reducing the incidence of these diseases and they also rely on advanced technology and are expensive (Coleman, 2009). Epidemiological studies and laboratory studies show that almost all types of vegetables are effective in preventing cancer and human mutations (Surh and Ferguson, 2003). Plants and algae are the best sources to obtain a

large number of diverse antioxidants, antimicrobial agents and bioactive compounds in various amounts (Aruoma, 1994). During the past decade, scientists have shown widespread interest in the application of some algal species for therapeutic purposes. Some algae possess promising biological activity such as anticancer, antimicrobial and antioxidant activities (Belay, 2002). For instance, a study by Abeer *et al.* (2015) showed that *Arthrospira platensis* water extracts contained antiproliferative properties in human colon carcinoma cells (HCT116) and hepatocellular carcinoma cells (HEPG2). It has been reported that Japanese women who consume algae regularly have a lower incidence of breast cancer than American women and the incidence of breast cancer can be up to six times lower in Japanese women (Reddy *et al*., 1980). Several types of algae have been tested for therapeutic purposes (Salazar, 1998). In this respect, *Arthrospira* spp. are one of the more promising algae due to their high essential nutrient contents including proteins, vitamins, fatty acids, minerals and pigments (Habib *et al*., 2008). *Arthrospira* spp. are cyanobacteria contain cylindrical filaments which are known as trichromes and vary greatly in length, ranging from 1 to 12 µm in diameter. These filaments are typically spiral-shaped or straight. The cell wall of *Arthrospira* spp. is composed of polysaccharides, proteins and peptidoglycans that are more easily digested, unlike the cellulose cell wall found in many other nutritional algae (Eykelenburg, 1977).

Arthrospira maxima and *Arthrospira platensis* were classified in the genus *Arthrospira* which contain essential polysaccharides (monosaccharides linked by glycosidic bonds). Depending on their structure, algal polysaccharides can have functional properties which are believed to have a role in stimulating the immune system and DNA repair mechanisms (Pang *et al*., 1988; Baojiang, 1994; Zhang *et al*., 1994) which can use in food and cosmetics and in pharmacological and nutraceutical applications (Chaiklahan *et al*., 2013). *Arthrospira* spp. also contain a high amount of phycocyanin (20% of total algal protein), which has been widely used as a pigment in food and cosmetic applications; recently, it has also been reported to have anti-aging, antioxidant, anti-inflammatory and anti-cancer properties (Silveira *et al*., 2007; Seo *et al*., 2013).

The investigation and discovery of mutagenic, antimutagenic, anticancer and antioxidant properties of algae which are beneficial to human health are therapeutically important. Several types of algae have been tested for therapeutic purposes, but some species of algae have been found to contain toxins known as microcystins. However, *Arthrospira* does not produce toxins at harmful levels (Carmichael *et al*., 2000). However, there is a limited understanding of the adverse effects and consequently, there is a need to quantify the bioactive compounds in these algae. Therefore, our aim was to assess the mutagenicity of the algal extracts and the antimutagenicity of polysaccharide and phycocyanin extracts from *Arthrospira platensis* (IFRPD 1182) and *Arthrospira maxima* (IFRPD 1183) and also to quantify the valuable bioactive compounds from both types of algae. The results obtained from this research will establish the safe use of the algae and also evaluate the mutagenicity and the antimutagenicity potential of *Arthrospira platensis* (IFRPD 1182) and *Arthrospira maxima* (IFRPD 1183) which are being developed by the Institute of Food Research and Product Development Kasetsart University.

MATERIALS AND METHODS

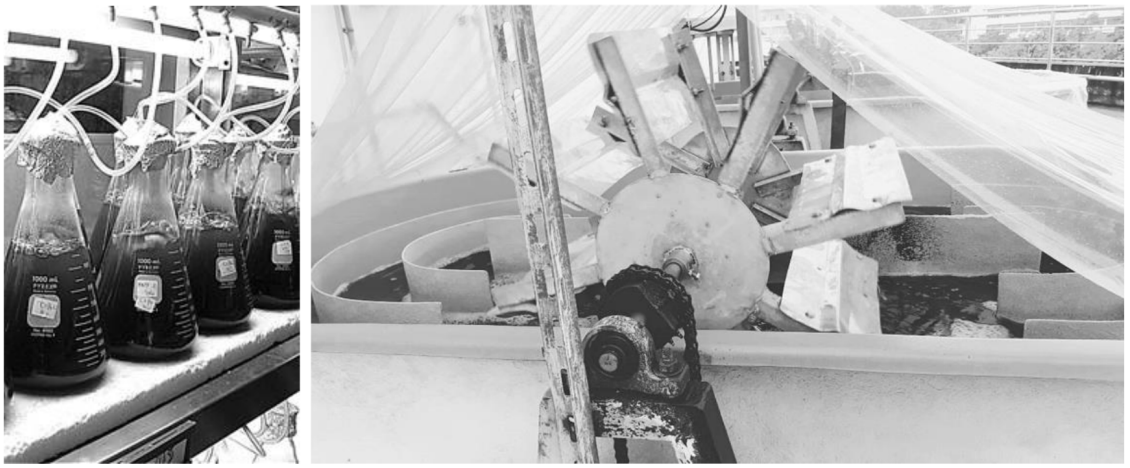
Algal Stock Culture

To prepare a stock culture*, Arthrospira platensis* (IFRPD 1182) and *Arthrospira maxima* (IFRPD 1183), which were collected from the Institute of Food Research and Product Development, Kasetsart University, Bangkok were transferred in 200 ml flasks containing 100 ml Zarrouk medium and cultured at 30°C under fluorescent light (12 Klux) for 16 h/day with 1–2% CO_2 until the optical density reached 1.0 (OD 560 nm) (Figure1a) (Zarrouk, 1996).

Algal Preparation

A portion (10%) of the algal stock culture was inoculated in a Raceway (capacity 6 L) containing Zarrouk medium and cultured for 10–15 days or until

the optical density reached 1.0 (OD 560 nm; Figure 1b). Then, the algal sample was filtered using a plankton net (30–50 µm) and washed with distilled water to remove the culture medium. After this, the sample was incubated in a hot-air over at 60–70°C for 6–8 h (Boyd, 1992). The algal products were then ground into fine powder which was suitable for further extraction.



(a) (b)

Figure 1 *A. platensis* stock culture: (a) the algae stock culture were inoculated in Raceway flasks (capacity 6 L) containing Zarrouk medium at 30°C under fluorescent lighting (12 KLux) for 16 h/day with 1–2% CO_2 (b)

Algal crude extract preparation

The algal powder was dissolved in 80% methanol or water with a ratio of 1:5 (W/V). The solution was mixed in a shaker for 8 h and sonicated for 15 mins. Then, the solution was filtered through filter paper (Whatman no.1). The solvents of the solution were evaporated using a rotary evaporator under low pressure at 45ºC. The weight of the crude extract samples was recorded. Then, the crude extract samples were redissolved to a concentration of 0.1 g/ml using dimethylsulfoxide (DMSO). The redissolved solution was kept in a deep freezer at -20°C (Vonshak, 1997).

C-phycocyanin extraction

C-phycocyanin was extracted from *Arthrospira* powder using 0.01 M sodium phosphate buffer (pH 7.0) at 25°C for 6–8 h. The samples were collected using centrifugation at 6,000 rpm for 10 min, then freeze-dried and the samples were kept at 4°C for further study. The C-phycocyanin content was measured using spectrophotometry at 615 and 652 nm and the C-phycocyanin concentration (C-PC) was calculated using the equation (Silveira *et al.,* 2007):

$$C - PC = \frac{(OD_{615} - 0.474\ (OD_{652}))}{5.43}$$

where: is the C-phycocyanin concentration in mg mL^{-1}, is the optical density of the sample at 615 nm and is the optical density at 652 nm.

Polysaccharide extraction

Polysaccharide was extracted from *Arthrospira* powder using sterilized water and an extraction temperature of 100°C for 1 h, followed

by centrifugation at 4,000 rpm for 20 min, before the solvents of the solution were evaporated using a rotary evaporator under low pressure (23 mbar) at 40°C. After that, five times the volume of 95% ethanol was added to the concentrated solution, followed by centrifugation at 4,000 rpm for 10 min, and then the sample was freeze-dried and kept at 4°C for further study. The polysaccharide content of the samples was measured using phenol-sulfuric acid and the percentage yield of the polysaccharide was determined (Dubois *et al.,* 1956).

Proximate composition and carotenoid content of *Arthrospira*

Determination of protein content in *Arthrospira*

The protein content of *Arthrospira* was estimated using the Kjeldahl method (AOAC, 2012). A sample (0.5–1 g) was digested in a Kjeldhal digestion flask, then 12 g of K_2SO_4, 1 ml of $CuSO_4$ and 20 ml H_2SO_4 were added. The solution was digested for 1–2 h until the sample turned slightly blue. After cooling, H_2O was added to the hydrolysates before transferring them to the distillation unit where they were titrated with 0.1 N HCl until the solution was slightly violet. With the volume and concentration of HCl needed, the percentage of nitrogen and then the percentage of protein in the sample were calculated using the formulae:

Nitrogen (%) = 14 × (v1 – v2) × Normality of HCL × 100 / Weight of Sample (g) × 1000

where: v1 is the volume of 0.2 N HCl titrant used in the sample and v2 is the volume of 0.2 N HCl titrant used as the blank

Protein (%) = Nitrogen (%) × conversion factor (6.25)

Determination of lipid content in *Arthrospira*

The lipid content of *Arthrospira* was estimated using acid hydrolysis (AOAC, 2005). A sample (8 g) was homogenized with 50 ml of distilled water, 8 ml of HCl and then boiled for 90 min and left to cool. The sample was placed in a Mojonnier flask and washed with 7 ml ethyl alcohol and 25 ml ether, followed by shaking for 1 min. Then, 25 ml petroleum ether was added and the homogenate was centrifuged at 650 rpm for 20 min, after which, the supernatant was transferred and the lipid was allowed to separate and dry.

The lipid yield was calculated using the formula:

Lipid content = $w2 \times 100$ $w1$

where: $w1$ is the weight of the sample before drying and $w2$ is the weight of the sample after drying.

Determination of moisture content

A crucible was dried in the hot-air oven at 105°C for 20 min and then transferred to a desiccator to cool, after which the crucible was weighed. An amount of sample (0.5 g) was added to the crucible and dried at 105°C for 5–6 h. After that, the crucible was transferred to the desiccator to cool. Finally, the crucible was reweighed after the sample had dried using the equation:

Moisture (%) = (W1-W2) / W1 × 100

where: $w1$ is the weight of the sample before drying and $w2$ is the weight of the sample after drying.

Determination of fiber content

A sample (1–2 g) of the dried and defatted sample was placed in a beaker containing 200 ml (1.25%) H_2SO_4 and then boiled for 30 min. Then, the solution was filtered through a Buchner funnel and washed with hot distilled water and the fiber was collected. After that, the fiber was transferred to a beaker containing 200 ml (1.25%) NaOH, boiled for 30 min and then filtrated using a Buchner funnel and washed with hot distilled water. The residue was collected and transferred to sintered glass. The residue was washed in 15 ml (95%) alcohol. The sintered glass was dried in the hot-air oven at 110°C for 2 h and then weighed. Then it was incinerated in a muffle furnace at 300°C for 5–6 h. Finally, the sintered glass was reweighed after incineration. The fiber content

was calculated according to the formula:

Fiber (%) = (Weight of sintered glass + fiber before incineration) – (Weight of sintered glass after incineration) ×100 / Weight of sample

Determination of ash content

A crucible was placed in the oven at 100°C for 1h and then transferred to a desiccator to cool, after which, the crucible was weighed. A sample (about 2 g) was added to the crucible and then incinerated in a fume hood. After that, the crucible was transferred to a furnace at 500–550°C for 6–8 h. The crucible was then cooled in the desiccator. Finally, the crucible and sample were reweighed after incineration and the amount of ash determined using the equation:

Ash (%) = Weight of the crucible with sample after incinerating – Weight of crucible × 100 / Weight of the sample

Determination of carotenoid content

A sample (0.02–0.05 g) was placed in a 50 ml beaker and added with 10 ml of (90%) ethanol and 1ml of (60%) KOH, followed by sonication for 5 min. Then, the beaker was placed in a water bath to break the cells for 5 min before being cooled down at room temperature, prior to centrifugation at 3,000 rpm for 10 min to pelletize the solid. The yellow extract was transferred to a Kjeldahl flask and 15 ml diethyl ether and 15 ml (9%) NaCl were added. The solution was vortexed and held at room temperature until it separated into two layers. The yellow-colored upper layer solution was removed to a beaker, where the final volume was adjusted to 25 ml by adding diethyl ether, followed by adding sodium sulphate anhydrous to remove water from the solution. Then, the solution was poured into a test tube and the test tube was covered with aluminum foil and kept in the dark. Absorbance of the yellow-colored extracted was recorded at 450 nm using a spectrophotometer. The carotenoid content was calculated using the equation:

Carotenoid content (mg/g cell dry weight) = A450 × 25 × 1,000 / 260 × mg cell dry weight.

where A450 is the absorbance of the solution at 450 nm.

Bacterial Strains for Antimutagenicity Test

Salmonella Typhimurium strains TA98 and TA100 were kindly supplied by Dr. Wannee Kusumran, National Cancer Institute, Thailand. The bacterial strains were maintained and routinely checked for presence of genetic markers according to the standard protocol as described by Maron and Ames (1983). The bacteria were inoculated in nutrient broth. Each culture (10 µl) was pipetted into flask containing Oxoid nutrient broth No.2. The flasks were kept in an incubator at 37°C for 16 h.

Antimutagenicity test

Phycocyanin or polysaccharide extracted from *Arthrospira* (1.5 g) was mixed with 30 ml (120 mM) NaCl and the mixture was digested under conditions similar to those occurring during stomach digestion (Ferruzzi *et al.*, 2006; Dawilai *et al*., 2013). The pH of the solution was adjusted to 2.0 using 1.0 M HCl and added with 2ml pepsin and then incubated in a shaking water bath at 37°C for 2 h. After that, the pH of the mixture was adjusted to 5.5 using 0.9 M $NaHCO_3$. After incubation, the solution was evaluated for antimutagenicity in the metabolic activation using the Ames test (Maron and Ames, 1983).

A sample (0.05 ml, using 50 ng/50 µl) of the indirect mutagen was incubated with 0.05 ml of the sample extract, added with 0.7 ml phosphate buffer (pH 7.0), 0.1 ml S-9 mix enzyme and 0.1 ml *Salmonella* Typhimurium strain TA 98. The mixture was incubated in a shaking water bath at 37°C for 20 min. Then, 2 ml of top agar (agar containing 10% 0.5 M L-histidine–HCl-0.5 mM biotin mixture) was added into each test tube, mixed using a vortex mixer for 10 sec and poured onto a glucose agar plate. The culture plate was incubated at 37°C for 48 h after which, the number of revertant colonies was counted. Each sample was repeated with three

replicates. The tube containing DMSO was set to determine spontaneous revertants. Antimutagenicity was evaluated from the percentage inhibition of mutagenicity, which was calculated according to the formula:

$$\text{Inhibition (\%)} = 100 \times (C_0 - S) / (C_0 - C_{100})$$

where C_0 is the number of mutant colonies of the positive control (indirect mutagen) without the testers, C_{100} is the number of mutant colonies of the negative control (DMSO, spontaneous mutation) and S is the number of mutant colonies of the positive control with the testers.

In the present study, the indirect mutagens used in this experiment were Trp-P-1 (3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole), Trp-P-2 (3-amino-1-methyl-5H-pyrido[4,3-b]indole), MeIQ (2-amino-3,4-dimethylimidazo[4,5-f]quinoline) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine).

The percentage inhibition of the samples was evaluated according to Karori *et al*. (2014) as shown in Table 1.

Table 1 Criteria to evaluate the percentage inhibition of the mutagen

% Inhibition	Activity
> 60 %	Very strongly active
41−60 %	Strongly active
21−40 %	Moderately active
10−20%	Weakly active
< 10%	Not active

Mutagenic activity test

In the mutagenicity test, the main procedures were similar to those of the antimutagenicity tests. According to Kangsdalampai *et al.*, (1997), the testers (at least three different concentrations as shown in Table 3) were added to test tubes. Then, 750 µl of $NaPO_4$-KCl buffer was added to each test tube and 100 µl of *Salmonella* Typhimurium (TA98) was also added. The test tubes were placed in a shaking water bath at 37ºC for 20 min. A sample of 2 ml of top agar (agar containing 10% 0.5M L-histidine–HCl-0.5 mM biotin mixture) was pipetted into each test tube and placed in a vortex mixer for approximately 10 sec and afterwards was poured onto a culture plate. Each culture plate was incubated at 37°C for 48 h, after which, the number of mutation colonies was counted. Each sample was repeated with four replicates and the assays were set in duplicates per dose. The mutagenicity was evaluated from the following criteria:

1) The extracted sample concentrations and the growth of the colonies had to show a dose response relationship; that is, as the extracted sample concentration increased, the mutant colony increased.

2) The number of revertants had to be higher than the number of spontaneous revertants in at least two extracted sample concentrations.

3) In addition, the number of revertants had to be twice as high as the spontaneous mutations in at least one extracted sample concentration.

RESULT AND DISCUSSION

Table 2 shows the estimated content of the valuable nutrients and other components of *Arthrospira platensis* IFRPD 1182 and *Arthrospira maxima* IFRPD 1183. These included the phycocyanin,

polysaccharide, protein, lipid, carotenoid, moisture, fiber and ash contents. The results showed that *Arthrospira* contained a high protein content (61–64%) but lower lipids (4–5%) and moisture content, with the fiber and ash contents in the range 7–8%. For other valuable substances, *Arthrospira* was also rich in phycocyanin and polysaccharide which varied in the range 10–13% and the carotenoid content was in the range 0.3–0.4%. Based on the results, *Arthrospira* is the best source of nutrients and is particularly rich in protein. This finding was consistent with Ciferri (1993) who reported that *Arthrospira* had an average protein content of 56–77% of its dry weight and also accounted for 40% of the total protein in the cell (Zhou *et al.*, 2005). This protein content is higher than in other protein sources such as meat, fish, milk, eggs, soybeans and other grains (Richmond, 1980; 1986). Since *Arthrospira* lacks a cellulose cell wall, it is readily absorbed by the human digestive system and provide its abundance of nutrients (Shimamatsu, 2004). However, the phycocyanin content of the two *Arthrospira* strains analyzed in this study was about 20% of the total protein in the algae. The differences could be explained by a variation of either age, species of algae or the concentration of nitrogen in the algal culture (Ruengjitchatchawalya *et al.*, 2002). Therefore, the composition of each algae may vary according to the culture conditions and the method of analysis. In this study, *Arthrospira maxima* IFRPD 1183 contained substantially higher protein and phycocyanin contents than *Arthrospira platensis* IFRPD 1182, indicating that the amount of phycocyanin varied according to the amount of protein present in the algae. Considering the overall composition, Spirulina is a rich source of proteins and essential minerals such as calcium, sodium, magnesium, iron, potassium, phosphate and copper, as well as being a source of pro vitamin A such as carotene. In addition, *Arthrospira* spp. contain a low level of saturated fatty acids (Bujard *et al.*, 1970), but most of the fatty acids found in *Arthrospira* are essential fatty acids such as oleic acid and linoleic acid. Belay (2013) reported that *Arthrospira platensis* contains a higher amount of 49% gamma linolenic acid (GLA) compared to the 10–20% of GLA in *Arthrospira maxima*. Therefore, it is suitable to use as a food supplement in cases of essential unsaturated fatty acid deficiency (Hudson and Karis, 1974).

Table 2 Nutritional components of phycocyanin, polysaccharide, protein, lipid and content of moisture, fiber, ash and carotenoids in *Arthrospira platensis* IFRPD 1182 and *Arthrospira maxima* IFRPD 1183 (g/100 g dry weight)

Algal type	Phycocyanin	Polysaccharide	Protein	Lipid	Moisture	Fiber	Ash	Carotenoids
A. platensis IFRPD 1182	10.63 ± 0.59	12.37 ± 0.51	61.15 ± 1.23	4.23 ± 0.51	6.94 ± 0.32	7.36 ± 0.24	7.86 ± 0.55	0.34 ± 0.01
A. maxima IFRPD 1183	13.12 ± 1.05	10.16 ± 0.23	64.70 ± 0.75	5.49 ± 0.46	7.35 ± 0.47	6.83 ± 0.28	7.40 ± 0.21	0.39 ± 0.02

Mutagens are substances that are capable of inducing DNA damage, altering the genetic code and thus increasing the frequency of mutation, which is an initiation event causing cancer development. Therefore, health scientists use mutagenic tests in some substances as a screening test for carcinogenic potential. Typically, about 80–90% of mutagens are also carcinogenic (McCann *et al.,* 1975; Sugimura *et al.,* 1976). Since it is believed that the efficacy of carcinogenicity is directly related to the efficiency of mutagenesis, the mutagenicity of the two algal extracts was evaluated using the Ames assay to

screen for carcinogenic potential. Table 3 shows the mutation evaluation of extracts of the two algal strains at different concentrations of 300, 600, 1,200 and 2,500 µg/plate using water and 80% methanol solvent toward *Salmonella* Typhimurium (TA 98 and TA 100). Based on the number of revertants, the algal extracts from both strains did not produce any mutation effect toward those bacteria and this indicated that the algal extracts were safe. Similarly, Ciferri and Tiboni (1985) reported that *Arthrospira* showed no mutagenic and carcinogenic effects on mice when they were fed with *Arthrospira* for 4 months. Gabriela *et al.* (2015), reported that *Arthrospira* sp. exhibited negative results using the *Salmonella* Typhymurium test and showed neither chronic nor acute toxicities, making it safe for human consumption.

Table 3 Mutagenicity test results of algal extracts using *Salmonella* Typhimurium TA98 and TA100

Algal type	Solvent	Concentration (µ/plate)	Mean revertants/plate	
			TA98	TA100
A. platensis IFRPD 1182	Water	0	24	72
		300	22	83
		600	26	75
		1,200	41	97
		2,500	34	77
A. maxima IFRPD1183	80% Methanol	0	32	87
		300	30	74
		600	25	93
		1,200	40	111
		2,500	47	104
Positive control 1 (Trp-P-1)		-	584	-
Positive control 2 (Aflatoxin B1)		-	-	751

The incidence of some diseases such as tumors or cancers, which are caused by some mutagens is reduced by the use of natural antimutagens (Satish *et al.,* 2013). Thus, the phycocyanin and polysaccharide extracts obtained from *Arthrospira maxima* IFRPD 1183 and *Arthrospira platensis* IFRPD 1182 were evaluated for their antimutagenic activities against indirect mutagens (Trp-P-1,Trp-P-2, MeIQx and PhIP) and the results are shown in Table 4.

Table 4 shows that the phycocyanin extracts from both algae had a moderate-to-very-strong inhibitory effect on mutagenesis induced by 4 mutagens, and the inhibitory activity increased with increasing concentrations of phycocyanin. Based on the criteria for evaluation of mutagenesis inhibition by Karori *et al.* (2014), the results indicated that at concentrations of 325, 625 and 2,500 µg/plate, *Arthrospira maxima* IFRPD 1183 was more effective in inhibiting mutagenesis than *Arthrospira platensis* IFRPD 1182.

Table 4 Antimutagenicity of phycocyanin obtained from *Arthrospira* species using *Salmonella* Typhimurium TA 98 against 4 mutagens

Tester	Concentration (µg/plate)	Number of revertants/plate (% Inhibition)			
		Trp-P-1	Trp-P-2	MeIQx	PhIP
Phycocyanin obtained from	325	470 ± 11 (22)	820 ± 43 (15)	1,024 ± 54 (6)	373 ± 18 (10)
A. Platensis IFRPD 1182	625	385 ± 11 (37)	674 ± 30 (31)	777 ± 40 (29)	285 ± 21 (32)
	1,250	336 ± 32 (46)	501 ± 19 (49)	640 ± 17 (42)	242 ± 17 (44)
	2,500	267 ± 29 (58)	334 ± 47 (67)	331 ± 33 (71)	222 ± 23 (49)
Phycocyanin obtained from	325	418 ± 18 (31)	895 ± 15 (7)	870 ± 28 (20)	336 ± 45 (19)
A. maxima IFRPD 1183	625	296 ± 6 (52)	828 ± 42 (14)	724 ± 26 (34)	324 ± 6 (22)
	1,250	257 ± 15 (59)	726 ± 27 (25)	389 ± 18 (66)	269 ± 26 (37)
	2,500	234 ± 20 (63)	341 ± 6 (66)	224 ± 32 (81)	195 ± 13 (56)
Solvent control (distilled water)		25 ± 3	25 ± 3	25 ± 3	25 ± 3
Positive control		595 ± 20	963 ± 54	1,083 ± 116	410 ± 19

The mutagens used in this experiment belong to the heterocyclic aromatic amines (HAAs) group, which are formed during high-temperature food preparation and are considered by the International Agency for Research on Cancer (IARC, 1993) as possible human carcinogens (class 2B). Meat or pork cooked using high temperature such as by grilling or frying over an open flame could produce HAAs. The high consumption of these grilled foods over a long time may be relevant to renal cell carcinoma risk through mechanisms related to the cooking compounds PHhiP and MeiQx (Melkonian *et al.,* 2016). Our current findings showed that phycocyanin extracted from both types of algae contained antimutagenic activities against indirect mutagens. Furthermore, other pigments found in the algae such as carotene, beta carotenoids, xanthophyll, zeaxanthin and chlorophyll have been proven to be natural antimutagens (Dashwood *et al.,* 1991). Phycocyanin has been recently reported to display a variety of therapeutic properties including antioxidant activity. This may have been due to the ability of phycocyanin to remove hydroxyl radicals and peroxyl radicals that are caused by mutagens, in which the covalently-linked tetrapyrrole chromophore phycocyanobilin has a role in the radical scavenging activities of the phycocyanin (Bhat and Madyastha, 2000; Bhat, 2001; Patel *et al.,* 2006). The antimutagen and antioxidant activities found in *Arthrospira* sp. are usually associated with anticancer properties because the mutagen and oxidative stress can lead to cancer incidence. Therefore, this implies that phycocyanin obtained from the *Arthrospira* sp. has a therapeutic effect to reduce the risk of cancer incidence induced by toxic substances in grilled and smoked food products. This finding was consistent with Liangqian *et al.* (2017), who reported that phycocyanin has anti-cancer activity by inducing cancer cell apoptosis and autophagy and also blocks the cancer cell cycle, which can restrict the proliferation of cancer cells and kill the cancer cells, while it has no side effects on normal tissue and can be used as a promising anti-cancer treatment.

Polysaccharides extracted from both algae were also evaluated for their inhibitory effect on mutagenesis induced by Trp-P-1, Trp-P-2, MeIQx and PhIP, respectively, and the results are shown in Table 5. In this study, polysaccharides extracted from both *Arthrospira* species could exhibit antimutagenic activity, which was considered mild-to-moderate inhibition against the mutagens. Similarly, Kurd and Samavati (2015) reported that polysaccharides extracted from *Arthrospira* had scavenging activities on DPPH and free radicals. In the present study, polysaccharide at lower concentrations of 325 and 625 µg/plate did not display any antimutagenic activities toward any mutagen tested, but the inhibition efficiency increased with increasing concentration to 1,250–2,500 µg/plate, for which the rated varied from mild to moderate inhibition. This result was in line with Forouzan and Vahid (2015) who reported that polysaccharide extracted from *Artrospira* sp. was capable of scavenging hydroxyl radicals, and the antioxidant activities increased with increased concentration. Zhang *et al.* (2001) also reported that polysaccharide obtained from *Arthrospira* sp. had potential to prevent tumor development in animals tested using enhanced hematopoietic cell proliferation which resulted in enhanced levels of hemoglobin, red and white blood cells and also increased nucleated cells in the bone marrow of animals tested. One of the mechanisms is upregulated Bcl-2 expression of hematopoietic cells by promoting endogenous cytokines secretion (Liu and Zhang, 2002). Our results suggested that the polysaccharides obtained from *Arthrospira* sp. which had antimutagenic activities against the indirect mutagens should be explored as potential candidates for the development of natural antimutagenic drugs and nutraceutical products.

Table 5 Antimutagenicity of polysaccharide obtained from *Arthrospira* species using *Salmonella Typhimurium* TA98 against 4 mutagens

Tester	Concentration (µg/plate)	Number of revertants/plate (% Inhibition)			
		Trp-P-1	Trp-P-2	MeIQx	PhIP
Polysaccharide obtained	325	543 ± 26 (9)	855 ± 17 (12)	996 ± 26 (8)	392 ± 9 (5)
from *A. Platensis* IFRPD	625	561 ± 8 (6)	839 ± 19 (13)	912 ± 18 (16)	383 ± 21 (7)
1182	1,250	479 ± 2 (20)	806 ± 12 (17)	814 ± 18 (23)	357 ± 4 (14)
	2,500	461 ± 21 (24)	734 ± 47 (24)	835 ± 27 (23)	325 ± 24 (22)
Polysaccharide obtained	325	562 ± 9 (6)	895 ± 13 (7)	969 ± 23 (11)	381 ± 19 (8)
from *A. maxima* IFRPD 1183	625	560 ± 6 (6)	881 ± 13 (9)	995 ± 8 (8)	357 ± 22 (14)
	1,250	547 ± 8 (8)	772 ± 9 (20)	902 ± 32 (17)	334 ± 42 (20)
	2,500	499 ± 19 (17)	762 ± 31 (21)	866 ± 30 (21)	313 ± 18 (25)
Solvent control (distilled water)		25 ± 3	25 ± 3	25 ± 3	25 ± 3
Positive control		595 ± 20	963 ± 54	1083 ± 116	410 ± 19

The relationship between antimutagenic activity and the phycocyanin and polysaccharide contents was evaluated using Pearson's correlation. It was found that the antimutagenicity potential was significantly correlated with the amount of phycocyanin and polysaccharide (r = 0.910 and 0.864, respectively) in Table 6 . Similarly, Estrada *et al.* (2001) reported that an increase in the amount of phycocyanin was related to enhanced antioxidant efficiency.

Table 6 Relationship between antimutagenic activity and phycocyanin, polysaccharide content in *Arthrospira* species using Pearson's correlation coefficient

Antimutagenic activity and phycocyanin content				**Antimutagenic activity and polysaccharide content**			
		Antimutagenic activity	Phycocyanin			Antimutagenic activity	Polysaccharide
Antimutagenic activity	Pearson's correlation	1	0.910*	Antimutagenic activity	Pearson's correlation	1	0.864*
	Sig (2-tailed)		0.044		Sig (2-tailed)		0.038
	N	16	16		N	16	16
Phycocyanin	Pearson's correlation	0.901	1	Polysaccharide	Pearson's correlation	0.795	1
	Sig (2-tailed)	0.44			Sig (2-tailed)	0.038	
	N	16	16		N	16	16

*Correlation is significant at the 0.05 level (2-tailed)

CONCLUSION

Arthrospira platensis IFRPD 1182 and *Arthrospira maxima* IFRPD 1183 are nutrient-rich, are an excellent source of protein (60%) and essential minerals. In addition, they also contain polysaccharides and phycocyanin, which help to balance and boost the immune system in the human body. Water extracts and 80% methanol extracts from both *Arthrospira* species showed no mutant activity against *Salmonella* Typhimurium strains TA98 and TA100 at all concentrations tested. Therefore, *Arthrospira* extract is safe and not harmful to genetic material. The anti-mutagenic properties of phycocyanin obtained from both *Arthrospira* species at concentrations of 625 mg/plate upward demonstrated strong antimutagenic activity against mutagens (Trp-P-1, Trp-P-2 MeIQx and PhIP) which are contaminants of fried and grilled food. The polysaccharides extracted from both algae were effective at 1,250 mg/plate and can be considered to have only mild-to-moderate inhibition activities against the mutagens. Phycocyanin in *Arthrospira* species can reduce the risk of mutation as a result of the toxins in the smoked roast. Based on our findings, *Arthrospira* species contained high nutritional value and essential bioactive compounds, which should establish these species as a future multifunctional food source. Therefore, the intake of nutritional substances extracted from *Arthrospira* that include natural antimutagens may play an important role in improving human health and disease prevention and may be an attractive source of potential nutraceuticals and pharmaceuticals.

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