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Natural Pigment of Selected Blue-Green Algae and Their Antioxidant and Antimicrobials Activities

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ABSTRACT

The Islamic dietary and consumption system is distinct and unique compared to other ethnic dietary systems. The global community has only recently begun to recognise the significance of the Muslim requirement for halal food and other consumption. This study is focusing on exploring photosynthetic pigments in microalgae that is highly potential to be utilised as a natural and halal food colorant. Carotenoid is the pigment of interest in this research as an alternative to the synthetic food colorant in the market. The argument that the synthetic food dyes contain harmful carcinogenic substances had led to a search for natural colorants as an alternative. In contrast with health threatening substances that is found in synthetic colorants, Cyanobacteria possesses health-promoting phytocompounds thus making it a good alternative. With regards to the said concerns, we have proposed the idea to study the behaviour of algae and environmental factor that affects the production of carotenoids. In this study, blue-green algae (cyanobacteria) were chosen as the candidates due to its resemblance of higher plants. Carotenoid pigments were extracted from them and subjected to further analysis via High-Performance Liquid Chromatography. Thus, studies on carotenoids have revealed that their biosynthesis and distribution in microalgae are influenced by various factors, including species-specific traits and environmental conditions. The finding found the potential applications in different fields, paving the way for further research and practical use.

Keywords: Natural colourant; Halal pigment; Halal science; Carotenoids; Blue-green algae

1 Introduction

Microalgal biomass, insects and mycoprotein, is being considered by scientists, engineers, and investors as a future food to combat malnutrition. Habib et al., (2008) suggested that future foods will have a high protein content, a low reliance on chemicals such as fertilisers and hormones, and a low carbon footprint (Habib et al., 2008). Experts says that the future food will be in the form of powders, tablets, and capsules made from microalgae biomass. In the last few years, the number of snacks and drinks containing microalgae has doubled, especially in western countries. Bakery, meals, and chocolate confectionery are the most popular new products introduced (Kratzer and Murkovic, 2021). The most common colourants used in

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dairy products are carotenoids and anthocyanins, but the blue pigment provided by phycocyanobilins and the pH-stable shades provided by betacyanins have allowed these chemical groups to be more widely incorporated (Luzardo-Ocampo et al., 2021). The researcher predicts that the cost of producing microalgae will drop, microalgae will be cultivated for protein production, and a slew of newly developed microalgae products will hit the market in the near future (Kratzer and Murkovic, 2021).

2 Materials and Methods

2.1 Growth Medium Preparation

Microalgae has been cultured in standard growth medium for blue-green microalgae named Bold Basal Medium (BBM) and BBM Modified which contains two-times and three-times nitrate concentration. The growth medium has been prepared following protocols outlined by Bischoff and Bold (1963).

2.2 Carotenoid Extraction

The extraction procedure was carried out following method described by Othman (2009). 0.1 gram of each powdered sample was rehydrated with distilled water and extracted with a mixture of acetone and methanol (7:3) at room temperature and left overnight. The mixture was then centrifuged for 5 minutes at 8000 rpm and the supernatant was collected in a 50 ml centrifuge tube (Fisherbrand, UK). The supernatant was stored at 4ºC in the dark before analysis. To extract carotenoids, hexane was added to the supernatants and the mixture was allowed to separate. Then, the upper layer containing the carotenoids was collected. The combined upper phase will then be dried to completion under a gentle stream of oxygen-free nitrogen.

2.3 High–Performance Liquid Chromatography (HPLC) Analysis

The HPLC analysis of carotenoids was performed following the procedure described by previous authors (Othman, 2009; Radzali et al., 2016), on an Agilent model 1200 series comprised of a quaternary pump with autosampler injector, micro-degassers, column compartment equipped with a thermostat and a diode array detector. The column used was a ZORBAX Eclipse XDB-C18 end-capped 5µm, 4.6x150mm reverse phase column (Agilent Technologies, USA). The eluents used were (A) acetonitrile: water (9:1 v/v) and (B) ethyl acetate.

2.4 Antioxidant Activity by Using DPPH Assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich) assay has been employed in this study, where the degree of decolourisation is associated with the antioxidant concentration of the sample (Gorinstein, Böhm, Schaich, Özyürek, & Güçlü, 2013). The antioxidant assay has been done following the DPPH microplate protocol described by Prieto (2012).

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2.5 Antibacterial Assay

Sterilized filter paper discs (6 mm diameter) have been prepared accordingly. 10 µl of sample at different concentrations have been pipetted on the discs using a micropipette making the final concentrations of 10, 20, and 30 µg/µl each disc. Then, 10 µl of tetracycline is used as positive control, while 10 µl of sample solvent (DMSO) is used as negative control, which did not indicate any inhibition. The extract discs and control discs have been arranged on the media plates streaked with the bacteria, with all being prepared in triplicates and sealed with parafilm. The plates are then incubated for 24 hours at 37 °C. After a 24-hour incubation period, the bactericidal effects of carotenoid are assessed by measuring the inhibition zones (mm) marked by a transparent ring around the disc.

3 Results

3.1 Carotenoid Profile of Microalgae

Four species of microalgae were evaluated by means of quantitative and qualitative analysis for carotenoid profiling as detailed in Table 1. Carotenoid analysis performed by HPLC system detected five major carotenoid peaks: β-carotene, zeaxanthin, neoxanthin, lutein and violaxanthin. As a result, the carotenoid content and composition range were divided into two groups as detailed in Table 1. Each of the microalgae species produced either two or three types of carotenoids. However, β-carotene was found in all species meanwhile the other carotenoid was identified only in certain species. *Phormidium* sp., was found to have the highest total carotenoid content (697.49 ± 12.69 µg/g DW) which is significantly higher by at least 14 times in comparison to other species tested. In contrast, the lowest total carotenoid concentration was found in *Synechococcus* sp. (23.32 ± 30.4 µg/g DW).

Table 1: *Species of Microalgae*

Species	Total Carotenoid (µg/g DW)	β-carotene (µg/g DW)	Zeaxanthin (µg/g DW)	Neoxanthin (µg/g DW)	Lutein (µg/g DW)	Violaxanthin (µg/g DW)
Species with 3 carotenoid pigments						
Pseudanabaena sp.	51.96 ± 68.47	4.05 ± 2.02	46.99 ± 66.45	nd	0.92 ± 0	nd
Species with 2 carotenoid pigments						
Alkalinema sp.	23.57 ± 8.19	0.68 ± 0.03	nd	22.89 ± 8.16	nd	nd
Phormidium sp.	697.49 ± 12.69	13.58 ± 0.11	nd	nd	nd	683.91 ± 12.69
Synechococcus sp.	23.32 ± 30.4	22.3 ± 30.4	nd	nd	1.02 ± 0	nd

3.2 Carotenoid Antibacterial Activity

The antibacterial activities of carotenoid have been observed after the incubation period by measuring the inhibition zone shown via a transparent ring area around the disc. The result of the test has been tabulated in Table 2. From all antibacterial assays performed, carotenoid

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has shown antibacterial activities against two gram positive bacteria species which are *Bacillus subtilis* at concentrations of 20 and 30 µg/ml and *Bacillus cereus* at concentrations of 30 µg/ml.

Table 2: *Antibacterial effects of carotenoid*

Bacteria species	**Carotenoid concentration**			**Tetracycline**
	10 g/L	**20 g/L**	**30 g/L**	
Bacillus subtilis	-	+	+	+++
Bacillus cereus	-	-	+	+++
Corynebacterium diphtheriae	-	-	-	+++
Escherichia coli	-	-	-	+++
Pseudomonas aeruginosa	-	-	-	+++
Proteus mirabilis	-	-	-	+++
Staphylococcus aureus	-	-	-	+++
Salmonella enteritidis	-	-	-	+++
Shigella sonnei	-	-	-	+++

-: No antibacterial activity, inhibition zone (i.z) of sample < i.z of DMSO + 1 mm
+: Low antibacterial activity, i.z of sample 2-3 mm > i.z of DMSO
++: Clear antibacterial activity, i.z of sample 4-10 mm > i.z of DMSO
+++: Strong antibacterial activity, i.z of sample > i.z of DMSO + 10 mm

4 Conclusions

Biosynthesis of carotenoids and the distribution of their derivatives in microalgae is governed by various factors. Although it varies species-specific, it can also differ in different environments and conditions of the same species Furthermore, the antibacterial activities are influenced by the concentration, purity and source of the sample. These medical properties are specifically responsible for their ability to act as a preservative that can inhibit the growth of food-borne pathogens and prevent food spoilage, as well as enhancing the flavors and appearance of the food or beverages.

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