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Microalgae as Potential Antioxidants: Assessment of Antioxidant Capacities in Microalgae from Selected Regions of Peninsular Malaysia

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ABSTRACT

Antioxidants play critical roles in cellular defence mechanisms in both enzymatic and non-enzymatic forms within the intracellular and extracellular environments. While microalgae are recognised as a rich source of antioxidants, limited information is available on species native to Peninsular Malaysia that contain high antioxidant capacity for future applications. This study aimed to assess the antioxidant capacity of both enzymatic and non-enzymatic antioxidants in microalgae, particularly those cultivated locally in Malaysia, which are still scarce. Nineteen microalgae species collected from Kedah, Pahang, Terengganu and Johor were used in this study. Algal samples were cultured and harvested during the early stationary phase and then subjected to antioxidant assays. Enzymatic antioxidants were assessed using catalase, ascorbate peroxidase and superoxide dismutase (SOD) assays. Non-enzymatic antioxidants were evaluated through the quantification of ascorbate, α-tocopherol, and carotenoids. The findings reveal significant interspecies variation of microalgae in the types and quantities of antioxidants. Notably, *Neochloris conjuncta* and *Mychonastes ovahimbae* exhibited the highest levels of enzymatic antioxidants (p<0.05) and SOD. *Hematoccous* sp. had the highest concentration of ascorbic acid, while *Chlorella vulgaris* from Terengganu contained

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the most α-tocopherol, both with statistical significance (p<0.05). The data suggest that *Chlorella vulgaris* from Terengganu possesses considerable potential as a renewable source of antioxidants for diverse industrial applications, including food ingredients.

Keywords: Ascorbate, α-tocopherol, carotenoids, catalase, enzymatic, microalgae, non-enzymatic

INTRODUCTION

Microalgae, a varied collection of photosynthetic microorganisms, has been recognised as a potential source of compounds with numerous applications. Due to their ability to combat oxidative stress, a physiological state associated with a variety of chronic diseases, aging, and cellular degeneration, antioxidants have become a focal point of interest in the scientific community. Biosynthesised by organisms and having bioactive properties, these compounds are of particular interest.

Numerous recent studies have highlighted the remarkable antioxidant capabilities of microalgae, indicating that these microscopic organisms may possess the same or even greater antioxidant qualities than traditionally recognised sources such as fruits and vegetables (Alwi, Ismail, Hatta, Buyong, & Mohamad, 2015; Alwi, Ismail, Hatta, Buyong, Jamil, et al., 2015; Hawksworth, 2020; Hossain et al., 2020; Le et al., 2017; Noor et al., 2007). The synergistic presence of carotenoids, tocopherols, phenolic compounds, and other bioactive chemicals positions microalgae as a potential goldmine for antioxidant research. Frequently, the capacity of these organisms to produce these chemicals exceeds that of terrestrial plants, possibly because of their evolutionary adaptations that thrive in harsh environments. Microalgae are gaining increasing attention due to their extensive ecological range, diverse characteristics, and minimal land requirement. These organisms have remarkable survival and growth rates, permitting them to thrive in a variety of demanding environments, including those typically regarded as unsuitable for numerous plant species (Darvehei et al., 2018). This characteristic enhances their efficacy in biotechnological applications, particularly when land availability is limited.

Carotenoids, a prominent class of bioactive substances abundant in microalgae, have recently received significant attention owing to their wide variety of health benefits. The 40-carbon chained lipophilic compounds are commonly found in microalgae inhabiting marine and freshwater environments. Based on their chemical composition, carotenoids can be classified into two categories: carotenes, which consist solely of hydrocarbons, and xanthophylls, which are distinguished by the presence of oxygenated functional groups (Lietz et al., 2012). Higher plants and microalgae can produce a variety of xanthophylls, including violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, and lutein. However, certain xanthophylls, such as loroxanthin, astaxanthin, canthaxanthin, diatoxanthin, diadinoxanthin, and fucoxanthin, are generated exclusively by certain organisms, including

green microalgae, diatoms, and brown algae (Ahmed, 2015). Multiple scientific studies (Ali et al., 2014) have shown that microalgae possess significant antioxidant potential primarily due to the potent activity of these carotenoids.

Peninsular Malaysia is recognised as a centre for marine research due to its advantageous geographical location and abundant marine biodiversity. While Peninsular Malaysia is known for its abundant microalgal species, there is a significant research gap concerning the antioxidant properties of these species within the region. This gap presents a critical opportunity to explore and understand the factors influencing antioxidant capacities in microalgae, ultimately guiding the selection of species and regions for future product development. Addressing this gap is crucial for unlocking the untapped potential of microalgae as valuable sources of antioxidants and natural sources, which could have far-reaching implications for various industries and contribute to the sustainable utilisation of Peninsular Malaysia's rich aquatic biodiversity. Thus, this study aims to examine the relatively unexplored area of the antioxidant properties of microalgae. By shedding light on this overlooked aspect, the research seeks to elucidate the potential of microalgae as a natural and safe source of antioxidants and highlight the vast diversity of unexploited microalgal species in the region. The findings of this study are expected to contribute significantly to our understanding of the antioxidant capacities of microalgae and inform future research and product development.

In this study, 19 species of microalgae was used to determine the antioxidant properties includes *Chlorella* sp., different strains of *Chlorella vulgaris* (a, b, c, and d), *Neochloris conjuncta*, *Nephrochlamys ovahimbae*, *Mychonastes ovahimbae*, *Desmodesmus brasiliensis*, *Desmodesmus abundans*, *Nannochloropsis oceanica* (strains a and b), *Dicloster acuatus*, *Navicula pelliculosa*, *Tetraselmis chui*, *Isochrysis galbana*, *Botryosphaerella sudetica*, and *Hematociccus* sp. Their distribution and uniqueness additionally guide the selection of microalgae for this study. An overview of the geographical spread of microalgae species discussed across the Association of Southeast Asian Nations (ASEAN) countries is shown in Figure 1.

In addition to the visual representation in Figure 1, Table 1 provides a detailed summary of the species of microalgae, their distribution, and unique characteristics. This table offers a comprehensive overview of the data presented in this study. Based on the data presented in Table 1, the distribution patterns of microalgae across different regions may offer insights into why only certain species were identified in our study. Due to their proximity to the coast, the coastal or littoral zones of Peninsular Malaysia serve as essential microalgal reservoirs in addition to the terrestrial environments. These locations serve as abundant reservoirs and offer unique environmental conditions that can influence microalgal adaptation and interactions. The pH of bark, particularly in terrestrial environments, resembles the complex metabolic processes utilised by microalgae (Alwi, Ismail, Hatta,

Buyong, & Mohamad, et al., 2015; Alwi, Ismail, Hatta, Buyong, Jamil, et al., 2015). The involvement of these pathways in the production of antioxidants suggests that coastal areas have a great deal of potential as microalgal sources. In this investigation, enzymatic antioxidants was determined using assays for catalase, ascorbate peroxidase (APX), and SOD. Simultaneously, non-enzymatic antioxidants were evaluated by quantifying α-tocopherol, ascorbic acid, and carotenoids. Our objective is to elucidate the potential of these enzymatic and non-enzymatic antioxidants across a spectrum of industries.

Given the rising global demand for natural antioxidants and the acknowledged biodiversity of Peninsular Malaysia, it is crucial to investigate the antioxidant capabilities of its native microalgae species. This study seeks to address the existing knowledge gap by concentrating on the untapped potential of Peninsular Malaysian microalgae as valuable antioxidant sources. In addition, it aims to elucidate the significance of these microalgae in the broader fields of health, nutrition, and ecological balance.

Figure 1. Global distribution of microalgae species across ASEAN countries

Table 1
Distribution and uniqueness of microalgae species to ASEAN countries

Microalgae	Potential ASEAN distribution	Uniqueness
Chlorella sp. and *C. vulgaris*	Thailand, Malaysia, Indonesia, and the Philippines have all reported the presence of *Chlorella* species due to their vast freshwater resources.	As one of the most researched microalgae, its potential in biofuel, nutrition, and wastewater treatment is globally recognised. Its presence in ASEAN countries can contribute to local biotechnological developments.

Table 1 *(continue)*

Microalgae	Potential ASEAN distribution	Uniqueness
Neochloris conjuncta	Limited data exist, but freshwater bodies in countries like Malaysia or Indonesia might host this species.	It is not widely discussed in ASEAN literature, which can signify a potential new area of research.
Desmodesmus brasiliensis and *Desmodesmus abundans*	The "*brasiliensis*" suggests a Brazilian origin, but these species can be found in various freshwater habitats, potentially including countries like Vietnam, Thailand, or the Philippines.	Their resilience to environmental stressors might have implications for bioremediation efforts in the region.
*Nannochloropsis oceanica**	Coastal countries like the Philippines, Indonesia, and Malaysia might host marine species like *Nannochloropsis* sp.	They are known for their high lipid content, making them prime candidates for biofuel research.
*Navicula pelliculosa**	Both marine and freshwater habitats across ASEAN countries, potentially Thailand, Vietnam, and the Philippines.	As a diatom, its silica cell wall offers unique biotechnological applications.
*Tetraselmis chui**	Marine environments, potentially in countries with vast coastlines, like the Philippines and Indonesia.	Popular in aquaculture, ASEAN, being a hub for aquaculture, can benefit from its cultivation.
Nephrochlamys ovahimbae	The specific ASEAN distribution is not clear from the last update. However, considering the diversity of freshwater habitats in countries like Laos, Cambodia, or Myanmar, it is conceivable they may host this or related species.	As it is not a commonly discussed microalgae in global literature, its presence and potential benefits in the ASEAN region could be an uncharted research area.
Mychonastes ovahimbae	The specific ASEAN distribution for this species is not clear. Freshwater habitats across countries, especially Indonesia and Malaysia, with their numerous lakes and ponds, might be places to explore.	Its rarity in common literature could make it an interesting candidate for detailed research in the ASEAN context.
Dicloster acuatus	It is likely in freshwater systems of countries like Vietnam, Thailand, or the Philippines.	Any unique metabolic or ecological properties discovered could affect regional biotechnological applications.
*Isochrysis galbana**	It is a marine microalga, so coastal countries such as Indonesia, the Philippines, and Malaysia might be hosting this species with their vast marine ecosystems.	It is known for its nutritional value, especially in aquaculture, a major industry in many ASEAN countries.
Botryosphaerella sudetica	Freshwater habitats in countries with tropical climates, such as Malaysia, Thailand, and Indonesia, might be probable areas.	Detailed research on this species in the ASEAN context can shed light on any region-specific properties or benefits.
Hematococcus sp.*	Given the variety of this genus, it is conceivable that they can be found in both freshwater and marine environments in countries like Malaysia, Indonesia, and the Philippines.	*Hematococcus* is known for producing astaxanthin, a powerful antioxidant. Its cultivation and exploration in the ASEAN region can offer insights into the natural sources of this compound.

Note. *Samples were collected from coastal areas within the respective ASEAN countries. The focus on coastal regions reflects the specific scope of this study and its relevance to understanding microalgae distribution in coastal environments

MATERIALS AND METHODS

Microalgae Cultivation

Freshly collected microalgae samples, including *C. vulgaris*, *N. conjuncta*, *N. ovahimbae*, *M. ovahimbae*, *D. brasiliensis*, *D. abundans*, *N.oceanica*, *D. acuatus*, *N. pelliculosa*, *T. chui*, *I. galbana*, *B. sudetica*, and *Hematociccus* sp, were cultured and maintained at the SATREPS-COSMOS Laboratory, Universiti Malaysia Terengganu. The microalgae cells were cultured in three replicates of conical flasks, with a concentration of 1×10^5 cells/ml, using Bold's Basal Media (Sigma, Germany) (Kanz & Bold, 1969). Throughout the study, the cultures were exposed to continuous light-emitting diode lamps (2,000 lux) at 24 ± 2°C.

Microalgae Harvesting

Cultures were harvested during the early stationary growth phase, specifically on day 13, as described by Zakaria et al. (2020). A Beckman Coulter Allegra X-30R Centrifuge (Germany) was used to harvest the microalgal cultures. The cultures were centrifugated at 11180 × *g* for 10 min at 4°C. The pellets were subsequently used for phytochemical and antioxidant activity analyses.

Enzymatic Antioxidant Assays

Using a method adapted from Price et al. (1994), the specific activity of SOD was analysed. 0.1 g of freshly collected samples underwent homogenisation in 2 ml of 0.1 M phosphate buffer (pH 7.0, Bioenno Tech, USA) with the use of a pre-chilled mortar and pestle and then centrifuged at 10,000 × *g* (Hettich Universal 32R, Germany) at 4ºC for 10 min (Beauchamp & Fridovich, 1971). To 1.0 ml of buffer solution containing 50 mM phosphate buffer (Bioenno Tech, USA) with 0.1 mM ethylenediaminetetraacetic acid (EDTA, pH 7.8, GBiosciences, USA), 0.1 mM nitro blue tetrazolium (NBT, Sigma-Aldrich, USA), 0.048 mM xanthine oxidase (Sigma-Aldrich, U.S.A), and 0.05 mM xanthine (Sigma-Aldrich, U.S.A), 100 L of unprocessed extract was added. At 560 nm, the SOD activity was analysed spectrophotometrically (Shimadzu UV-1601, Japan). The amount of SOD needed to reduce the rate of NBT reduction by 50% is defined as one unit, and the specific activity of SOD was calculated as units/mg protein.

To calculate units of SOD activity in assayed fraction:

The rate of ΔAbs 560 increased from 2.5-5.5 min = Rate B

Initial rate of ΔAbs 560 from 0-2.0 min = Rate A

$$\therefore \% \text{ Decline in } A_{560} \text{ increase} = \frac{A - B}{A} \times 100\%$$

$$\therefore \text{Units of SOD activity /mg protein} = \frac{\text{\% Decline in A560 increae}}{50\% \times \text{mg protein}}$$

$$= \text{Unit SOD/mg protein}$$

Catalase Activity Test (CAT) Assay

CAT-specific activity was assessed using the procedure by Claiborne (1985). About 0.15 g of the sample was homogenised with 1.0 ml of 50 mM phosphate buffer (pH 7.4), cleaned in a pre-chilled mortar, and pestled. Following this, the homogenate underwent centrifugation (Eppendorf 5840R, Switzerland) at 11180 × *g* at 4°C for 10 min. The mixture contained 3.0 ml of 19 mM hydrogen peroxide (H_2O_2) (Fisher Scientific, USA) in 50 mM phosphate buffer (pH 7.0, Bioenno Tech, USA) and 100 µl of the extract. The change in absorbance rate was observed at 240 nm using a spectrophotometer (Shimadzu UV-1800, Japan). CAT-specific activity was determined in µmol of H_2O_2 consumed per min per mg of protein.

APX Assay

APX-specific activity was evaluated using the method outlined by Nakano and Asada (1981). Approximately 0.15 g of sample was homogenised using a pre-chilled mortar and pestle with 1.0 ml of 1 mM of ascorbic acid (Scharlau, Spain) in a 100 mM phosphate buffer (pH 7.0; Bioenno Tech, U.S.A) at 0–4°C. This homogenate was centrifuged (Eppendorf 5840R, Switzerland) at 11180 × g at 4°C for 10 min. The reaction mixture contained 1.5 ml of 100 mM phosphate buffer (pH 7.0, Bioenno Tech, USA), 0.5 ml of 3 mM ascorbic acid (Scharlau, Spain, 0.1 ml of 3 mM EDTA (GBioscience, U.S.A), 0.3 ml of distilled water, and 0.2 ml of 1.5 mM H_2O_2 was added into 200 µl of the enzyme extract. The absorbance rate of the reaction mixture was measured at 290 nm using the spectrophotometer (Shimadzu UV-1800, Japan).

Non-enzymatic Antioxidant Assays

α-tocopherol Assay

The extraction of α-tocopherol was done following the protocol developed by Hodges et al. (1996). This procedure was conducted under subdued lighting and on an ice bed. Fresh microalgae samples weighing 0.15 g were homogenised in a mixture containing 1.5 ml acetone (Emsure, Germany) and fine sand. Subsequently, the homogenate was mixed with 0.5 ml hexane (Emsure, Germany), vortexed for 30 s, and centrifuged at 11180 × *g* for 10 min. The supernatant was then carefully decanted, and the extraction process was repeated twice. The assay formulation was prepared in alignment with the guidelines provided by Kanno and Yamauchi (1997). Subsequently, 0.5 ml of the hexane-extracts were combined

with 0.4 ml of 0.1% (w/v) 3-(2-pyridyl)-5,6-diphenyl-1,2,4 triazine, solubilised in ethanol (PDT, Emsure, Germany) and 0.4 ml 0.1% (w/v) ferric chloride (Sigma-Aldrich, U.S.A). The total volume was adjusted to 3.0 ml using absolute ethanol (Emsure, Germany). After gentle agitation, the mixture was allowed to stand for 4 min to facilitate colour development. Then, 0.2 ml of 0.2 M orthophosphoric acid (Sigma-Aldrich, USA) was added and left to stabilise for 30 min at ambient temperature prior to spectrophotometric measurement at 554 nm.

Ascorbic Acid Assay

All procedures were conducted under subdued light conditions to prevent photodegradation. Fresh samples weighing 0.15 g were ground using a pre-chilled mortar and pestle in 1.0 ml of 10% trichloroacetic acid (TCA, Emsure, Germany) under ice-cold conditions (Jagota & Dani, 1982). The mixture was centrifuged at 3044 × *g* for 10 min at 4°C using an Eppendorf 5840R (Switzerland) centrifuge. After that, 300 µl of the supernatant was diluted with 1,700 µl of distilled water and treated with 200 µl of 10% Folin reagent (Sigma-Aldrich, USA). The mixture was softly agitated and allowed to stand on the bench in subdued light for 10 min prior to the absorbance reading at 760 nm. Following the same procedure described above, 300 µl ascorbic acid was introduced into the solution, and the quantity of ascorbic acid in the sample was determined using the standard curve.

Carotenoids Assay

The carotenoid amounts were quantified using the Lichtenthaler method (1987). Samples were processed under dim light conditions and on a cold bed of ice to ensure sample integrity. Fresh leaf tissue samples weighing 0.02 g were homogenised with 3.0 ml of 80% (v/v) acetone (Emsure, Germany) The mixture was centrifuged at 11180 × *g* for 10 min. Subsequently, the absorbances of the collected supernatant were assessed at three distinct wavelengths: 663.2, 646.8, and 470 nm.

RESULTS AND DISCUSSION

Enzymatic Antioxidant Assay

Results of the concentration of enzymatic antioxidant capacity (CAT, APX, and SOD) towards 19 species of microalgae collected along the Peninsular Malaysia coastal area were shown as follows (Figures 2-5).

Figure 2 illustrates that microalgal species from Terengganu exhibit the highest catalase activity compared to those from Kedah, Pahang, and Johor. Notably, *C. vulgaris* (strain c) demonstrated the highest activity, with 1.405 units/mg protein, followed by *N. oceanica* (strain b) and *D. acuatus*. On the contrary, species from Johor, Kedah, and Pahang displayed low CAT activities and were not significantly different (p>0.05).

Figure 3 depicts APX activity varied from 0.025 to 1.078 units/mg protein. The maximum APX activity was assayed from *N. oceanica* (strain a), followed by *C. vulgaris*, and *Hemotococcus* sp. However, the species with the lowest APX activity was *C. vulgaris*, but different strains with 0.025 units/mg protein. *N. oceanica* demonstrated superior enzymatic antioxidant activities, particularly in CAT and APX. However, it lagged in SOD activities (Figure 4). The SOD activity of these microalgae ranged considerably, from 23.985 to 0.375 (units/mg protein), with *N. conjuncta* and *M. ovahimbae* manifesting significantly higher SOD activities compared to the other species examined. In contrast, the SOD activities in the remaining species were considerably lower and significantly different from those two species.

In essence, microalgae, being photosynthetic organisms, generate reactive oxygen species (ROS) through both enzymatic and non-enzymatic pathways. Scientific research has firmly established the detrimental effects on cellular structure and functions due to increased oxidative stress. Antioxidant enzymes such as ascorbate oxidase, peroxidase, catalase, and ascorbate peroxidase serve as cellular detoxifying agents in cells (Hakiman & Maziah, 2009). In this study, highly significant variances in mean squares for CAT, APX, and SOD were observed across species, indicating species-specific enzymatic antioxidant defence mechanisms.

Several factors, such as the microalgae's aquatic habitat and environmental stressors like metal exposure or pH fluctuations, may contribute to the variations in antioxidant

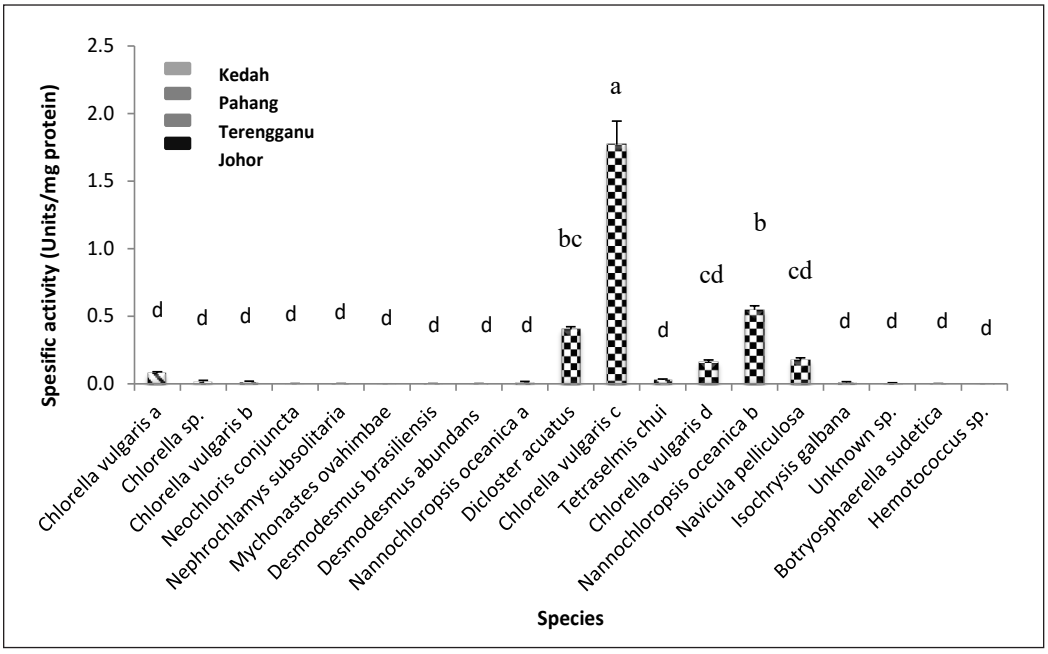

Figure 2. Catalase activity in different species of microalgae collected from selected regions in Peninsular Malaysia

Note. Data are means ± standard errors. Different letters in the graph represent significantly different at $p<0.05$

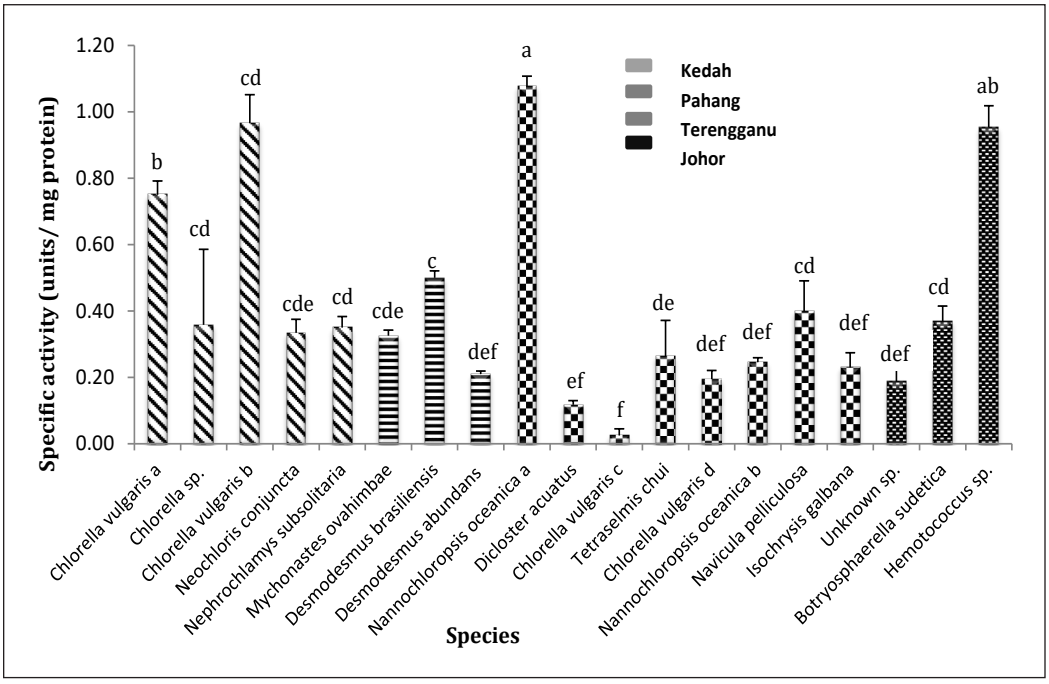

Figure 3. Ascorbate peroxidase (APX) activity in different species of microalgae collected from selected regions in Peninsular Malaysia

Note. Data are means ± standard errors. Different letters in the graph represent significantly different at $p<0.05$

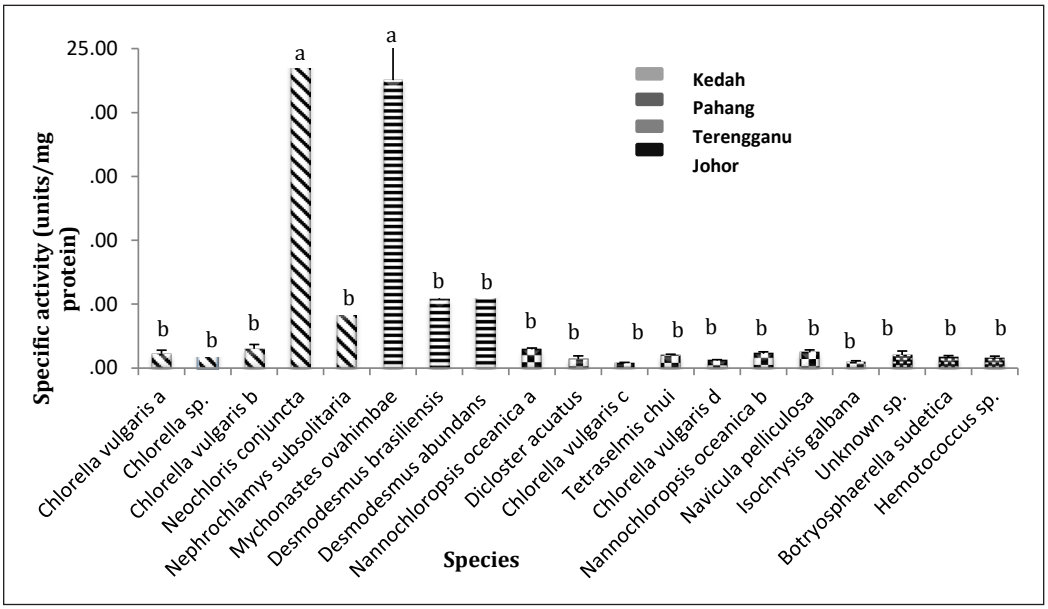

Figure 4. Superoxide dismutase (SOD) activity in different species of microalgae collected from selected regions in Peninsular Malaysia

Note. Data are means ± standard errors. Different letters in the graph represent significantly different at $p<0.05$

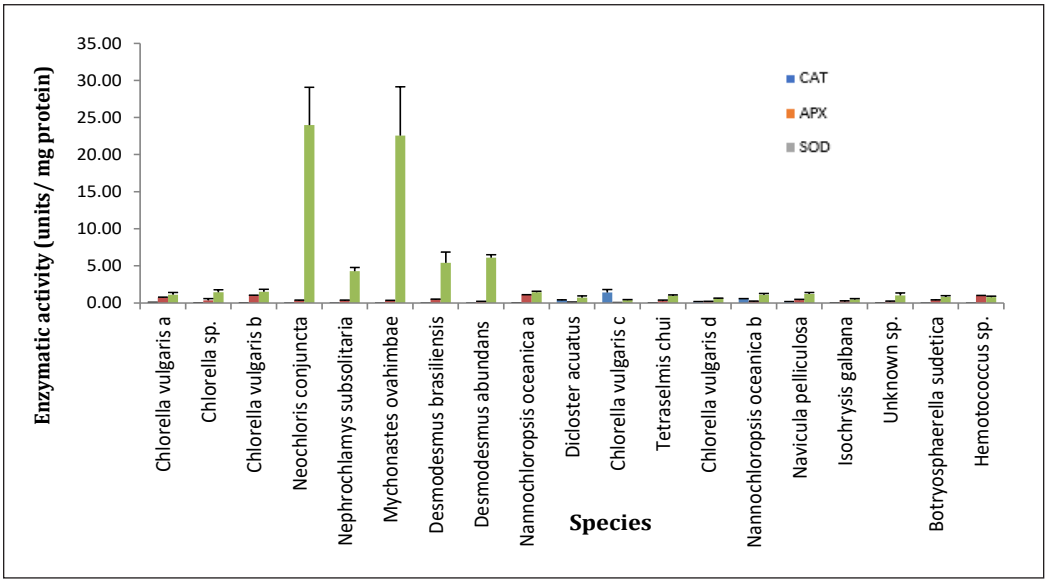

Figure 5. Comparison of enzymatic antioxidant activities (catalase [CAT], ascorbate peroxidase [APX], and superoxide dismutase [SOD]) in different species of microalgae from selected regions in Peninsular Malaysia

enzyme activities (Gauthier et al., 2020). For example, the elevated CAT activity in *C. vulgaris* from Terengganu, a marine environment, may attributed to these environmental variables.

APX is predominantly localised in the chloroplasts and cytoplasm of cells, and it has a greater affinity for H_2O_2 than CAT, making it crucial for ROS detoxification (Gauthier et al., 2020). APX activities of microalgae were the highest in green microalgae such as *N. oceanica* and *C. vulgaris*, which is similar to Hakiman and Maziah (2009), who said APX activities were found to be higher in the leaves extracts than roots and stems extract due to the location of APX in the chloroplast. Green microalgae tend to contain higher chloroplast than brown microalgae or blue-green microalgae. It suggests higher APX activity corresponds to greater antioxidant potential in these green microalgae species. Variation in enzymatic antioxidant activities among different microalgae species indicates that each species uniquely develops its antioxidant mechanisms in response to its surroundings.

SOD is an important antioxidant enzyme found in all subcellular compartments of aerobic organisms that are susceptible to ROS-mediated oxidative damage (Danouche et al*.,* 2020). Elevated SOD activities in *N. conjuncta* and *M. ovahimbae* may reflect their adaptive responses to environmental stress, as suggested by Kumar et al. (2014). Antioxidant enzymes like SOD are important in eliminating ROS generated within microalgae as part of their response to diverse physical and chemical stressors. These two species likely arise from environmental stress conditions. However, compared to other plants like citrus, microalgae produce lower SOD activities (23.99 units/mg

protein) compared to citrus (284.00 units/mg protein) (Arbona et al., 2003). Despite that, microalgae are way more convenient to be cultivated in a very short time. Microalgae, as photosynthesising plant cells, can experience photooxidative damage under extremely high light and oxygen conditions, which require microalgae to produce cells that possess protective, antioxidative mechanisms, and compounds that lead to the production of various antioxidants (Kumar et al., 2014). It necessitates the production of cells that possess protective antioxidative mechanisms and compounds, leading to the generation of various antioxidants. Therefore, higher SOD and APX activities in our microalgae samples imply higher antioxidant activity, indicating that these species can produce significant antioxidant defences under stress conditions.

Non-enzymatic Antioxidant Assay

Figures 6–9 represent the non-enzymatic antioxidant assay in microalgae species collected from selected places in Peninsular Malaysia.

Our analysis of non-enzymatic antioxidants revealed distinct patterns varying with species. According to Figure 6, the highest ascorbate content was found in a *Hematococcus* sp. collected from Johor, followed by *Nephrochlamys subsolitaria* from Kedah. Meanwhile, *C. vulgaris* from Terengganu was found to be the highest in α–tocopherol, as per Figure 7, and *N. pelliculosa* exhibited the highest in carotenoid levels, as illustrated in Figure 8. Figure 9 further accentuates that α–tocopherol predominates as the most abundant antioxidant in most of the analysed microalgae, with *C. vulgaris* containing the highest levels.

Although the samples were derived from various locations, the underlying factors causing the observed differences in antioxidant properties across species remain unclear. The possibility of this explanation is due to abiotic stresses that can induce antioxidant properties in Malaysian indigenous microalgae and cyanobacterium (Azim et al., 2018). These variations could stem from morphological differences between species or be influenced by the environmental conditions in their respective habitats. Previous research suggests that elevated oxidative stress can stimulate antioxidant activity in microalgae (Chokshi et al., 2017). Moreover, microalgae exposed to specific environmental stressors, such as metal contamination, fluctuating pH levels, and nutrient scarcity, tended to exhibit higher ascorbate, carotenoids and α-tocopherol content (Gauthier et al., 2020).

It is noteworthy that α-tocopherol, also known as vitamin E, was the predominant antioxidant in our study. It could be attributed to its crucial role in protecting cellular structures from oxidative damage by scavenging free radicals (Szewczyk et al., 2021).

The higher α-tocopherol content in *C. vulgaris* from Terengganu could respond to specific environmental stressors prevalent in the Terengganu coastal waters, such as high ultraviolet radiation, fluctuating salinity, and temperature variations. Additionally, Terengganu's well-known petroleum extraction activities (offshore drilling) may introduce

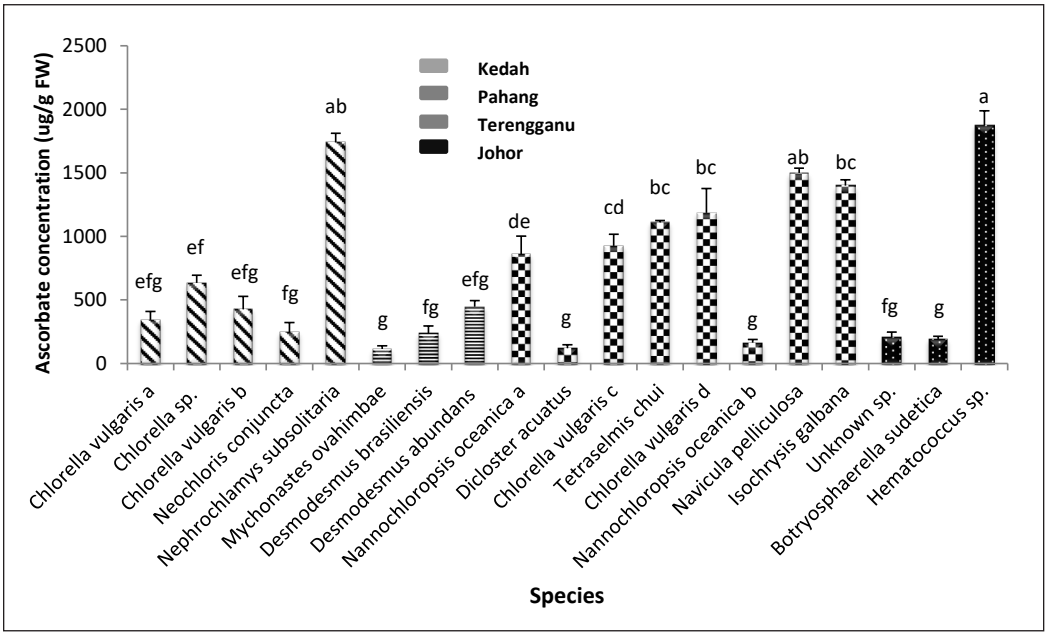

Figure 6. Ascorbate concentration (µg/g FW) in different species of microalgae collected from selected regions in Peninsular Malaysia

Note. Data are means ± standard errors. Different letters in the graph represent significantly different at $p<0.05$

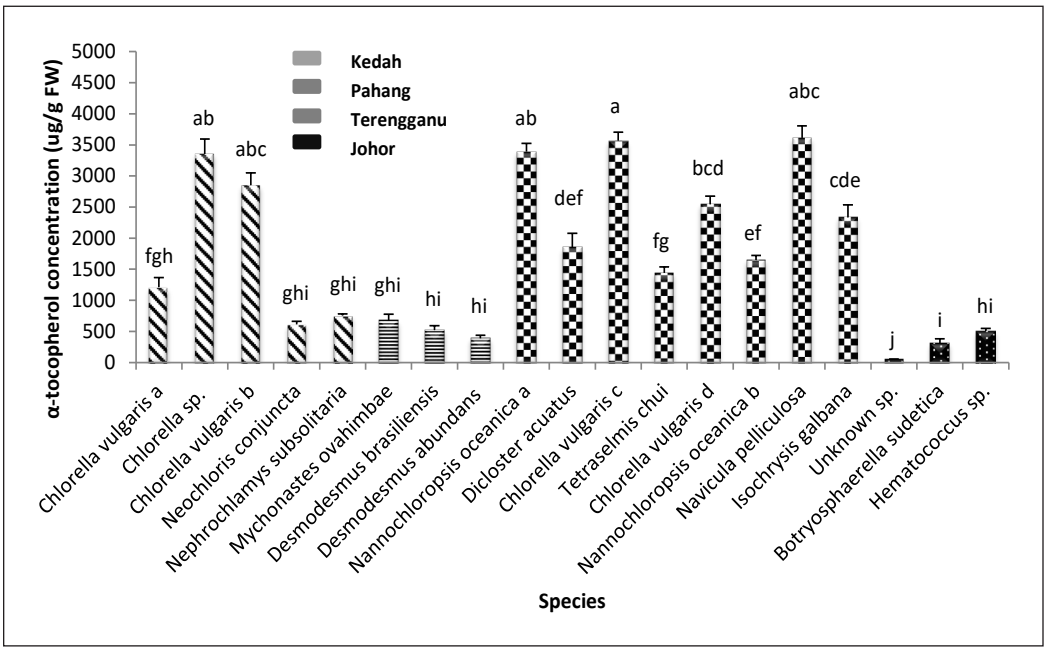

Figure 7. α-tocopherol concentration (µg/g FW) in different species of microalgae collected from selected regions in Peninsular Malaysia

Note. Data are means ± standard errors. Different letters in the graph represent significantly different at $p<0.05$

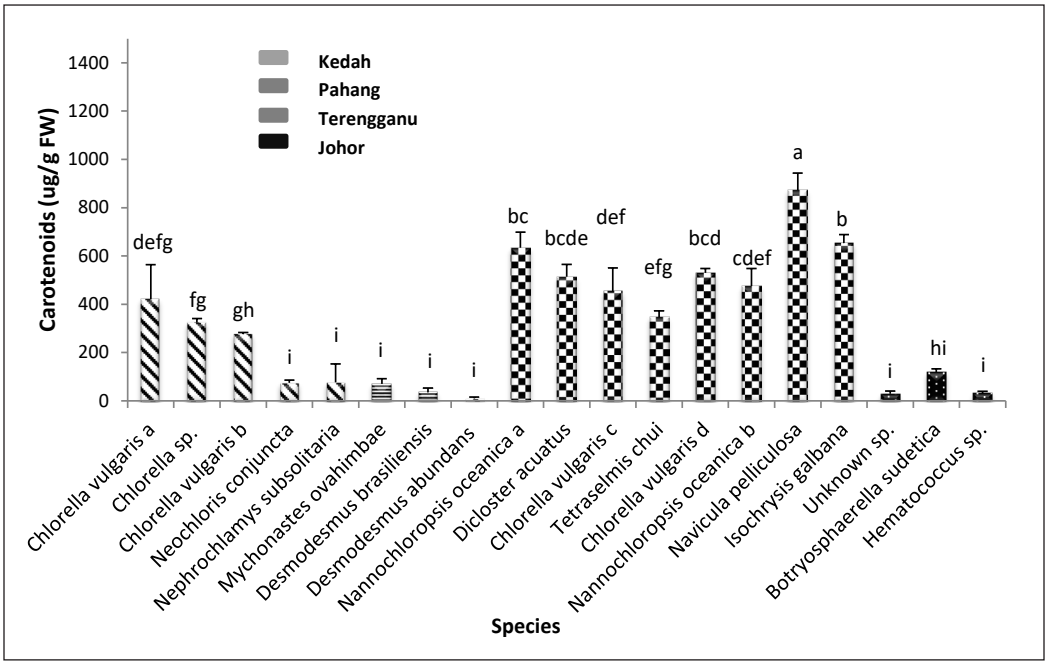

Figure 8. Carotenoids content (ug/g FW) in different species of microalgae collected from selected regions in Peninsular Malaysia

Note. Data are means ± standard errors. Different letters in the graph represent significantly different at $p<0.05$

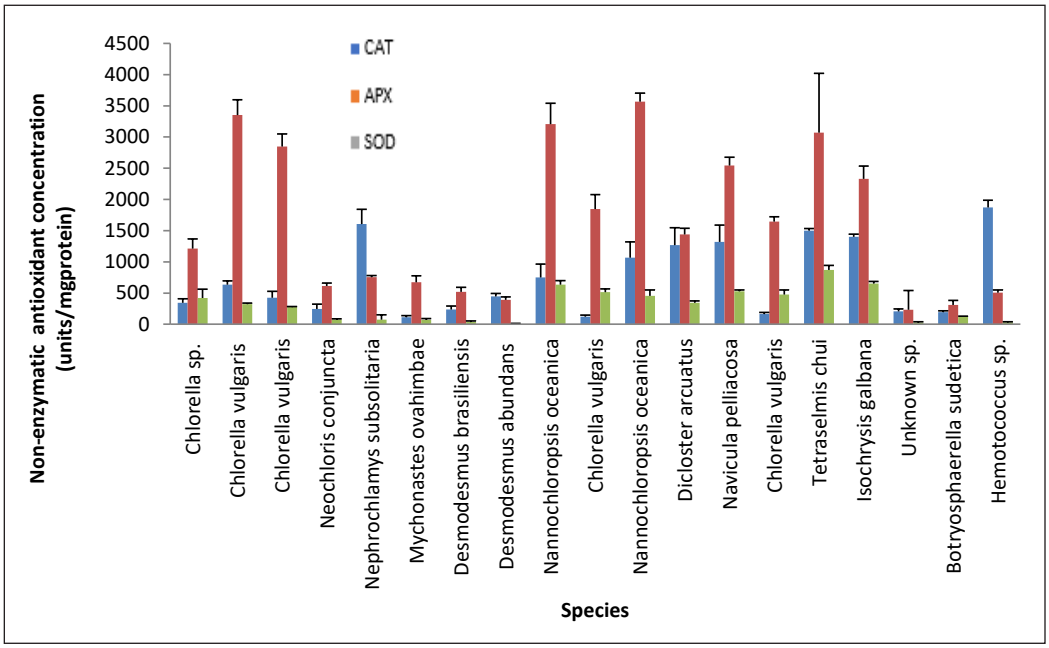

Figure 9. Comparison of non-enzymatic antioxidant (ascorbate, α-tocopherol, and carotenoids) concentration (µg/g FW) in different species of microalgae collected from selected regions in Peninsular Malaysia

metal contaminants and other pollutants into the marine environment, inducing oxidative stress in microalgae and thereby enhancing the synthesis of α-tocopherol as a protective mechanism (Azim et al., 2018).

Additionally, research has shown that tocopherols can be extracted, purified, or concentrated from higher plant substances, including *Spirulina*, *Dunaliella tertiolecta*, and *Chlorella* sp. (Ogbonna, 2009). Furthermore, the oceanographic conditions in Terengganu, including nutrient availability and water quality, may create a conducive environment for the synthesis of α-tocopherol in microalgae. The presence of specific nutrients and the overall quality of the water can significantly influence the metabolic pathways involved in antioxidant production (Poot-Delgado & Pkplodkov, 2016; Xiao et al., 2023). Therefore, the abundant α-tocopherol content in microalgae from Terengganu might reflect an adaptive response to these environmental factors.

Despite these insights, the exact factors contributing to the variations in antioxidant properties among different species of microalgae in Peninsular Malaysia remain speculative. This study unequivocally underscores the potential applications of these microalgae based on their valuable properties.

However, to gain a more comprehensive understanding, it is suggested that the perimeter of the sampling area be expanded with additional parameters such as the physical properties of microalgae, pH changes, metal exposure, weather conditions, water salinity, and nutrient content.

CONCLUSION

This study successfully screened and identified the antioxidant composition and capacity of various microalgae species collected from the selected coastal areas of Peninsular Malaysia. The findings demonstrate that the antioxidant profiles are species-specific and possibly influenced by their habitat. Specifically, *N. conjuncta* and *M. ovahimbae* exhibited the highest enzymatic antioxidant activities, particularly in SOD concentrations. Additionally, *Hematococcus* sp. showed the highest ascorbic acid content, while *C. vulgaris* from Terengganu was found to have the richest concentration of α-tocopherol. The results indicate that microalgae from Terengganu, particularly, are abundant in antioxidants, highlighting the potential of this region as a valuable source of natural antioxidants. These findings align with the study's objective to explore and compare the antioxidant capacities of different microalgae species from various regions, providing insights into their potential applications in health and wellness.

Overall, this study underscores the significant diversity of antioxidant compounds in microalgae and their promising potential as natural sources of antioxidants. Future research should focus on the isolation and detailed characterisation of these compounds, as well as the exploration of their bioactivity and possible commercial applications.

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