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Nutritional profile, bioactive compounds, and antioxidant activity of microalgal strain, *Amphora* sp., isolated from the Cape coastal waters, South Africa

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Abstract

Microalgae represent a potential source of renewable nutrition, and there is growing global interest in algae-based dietary supplements. However, the selection of suitable and indigenous microalgae species is a fundamental requirement in developing value-added bioactive compounds in the food industry. The proximate composition, fatty acids, amino acids, and mineral profile, as well as bioactive compounds and antioxidant activity of an unexplored diatom strain, *Amphora* sp. WCA23.2, isolated from the Cape coastal waters, South Africa, were evaluated as a potential nutraceutical. The *Amphora* sp. WCA23.2 biomass had 44.2% ash, 28% carbohydrates, 15% protein, and 4% lipids. The fatty acid profile revealed that the diatom accumulates a significant amount of omega-7-monounsaturated fatty acid palmitoleic acid (24.50 mg/g), while the amino acid profile demonstrated that it contained all the nine essential amino acids. The antioxidant activities of the diatom extracts showed that the methanolic extract displayed the highest DPPH radical scavenging activity (1.90±0.11 mg GAE/g dry weight (DW)) and the lowest IC_{50} in all the antioxidant indices evaluated. These results suggest that *Amphora* sp. WCA23.2 biomass and its extracts can be utilized as a potential source of ingredients and nutraceuticals in food systems for humans.

Keywords: diatom; marine microalgae; bioactive compounds; antioxidant activity; nutritional composition.

Practical Application: This study has demonstrated that *Amphora* sp. WCA23.2 possesses abundant nutritional values such as protein, carbohydrates, amino acids, and minerals as well as important bioactive compounds and antioxidants that may be very useful in the food and nutraceutical industries.

1. Introduction

Food security is an urgent global problem as approximately one billion people are currently undernourished, a number that is expected to increase by an additional two to three billion people by 2050, thus requiring a projected 70% increase in food production (Ranganathan et al., 2018). However, increasing global food production has been identified as a burdensome challenge in both developed and developing countries. Problems with current food production systems include the production of greenhouse gases from land clearing, animal livestock and fertilizer production, and nutrient run-off from fertilizer damaging marine and terrestrial ecosystems (Tilman et al., 2011). Therefore, there is an urgent need to provide a well-balanced diet with sufficient calories and nutrients that are essential for good health while circumventing the current challenges encountered with the current food production systems. In this regard, a significant increase in food production as well as innovative approaches to provide alternate sustainable food sources to fulfill the global food crisis are imperative. One of these innovative approaches as an alternative solution has been noted to be the commercial production and utilization of marine microalgae as a basic food commodity; thus, in recent years, microalgae have attracted major interest as a source of natural nutrients (Mekkawy et al., 2020).

Microalgae occupy both littoral and benthic habitats throughout the sea waters as phytoplankton, with the most abundant phytoplankton species being the diatoms. The diatoms are among the most productive microalgae and are the predominant primary producers in marine ecosystems (Obata et al., 2013). Bioactive compounds found in diatoms have been investigated in the food industry for different applications, especially in human health and as food supplements (Gügi et al., 2015). Diatoms and other microalgae are well known for their unique chemical composition that includes their components with proven health benefits, making them valuable as nutrient-enhancing ingredients for foods (Borowitzka, 2013). Over the years, they represent promising sources of sustainable bioactivities with past literature reflecting a growing interest in algae-based dietary supplements in the form of whole biomass (Zhou et al., 2022). Notably, the bioactive molecules that can be identified and extracted in diatoms have scientifically been proven to possess therapeutic properties that can be beneficial to human health (Wong et al., 2022). Their high nutritional value led to an increased interest in the development of foods enriched in microalgae as the number of microalgae-containing foods launched into the market is increasing every year (Lafarga, 2019). Additionally, they are natural sources of antioxidants, which have considerable abilities to accumulate in various intracellular compartments, protecting the cell (Sansone & Brunet, 2019).

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In view of the above, the purpose of this study was to investigate the nutritional profile, bioactive compounds, and antioxidant activity of a microalgal strain, *Amphora* sp. The diatom *Amphora* sp. WCA23.2, a novel strain isolated from the coastal waters in Cape Town, South Africa, was evaluated for its potential in the development of food and feed products with high nutritional and functional status. According to the available literature, this is the first comprehensive study to fully explore the nutritional profile of an *Amphora* specie and its extract with emphasis on its proximate composition, its amino acid and fatty acid profile, and its antioxidant content/potential. The well-balanced nutritional composition and significant antioxidant activities of the microalgae and its extracts suggest its potential as a readily available alternative in the development of functional food/feed ingredients for both humans and animals.

2. Materials and methods

2.1. Sample preparation

The marine diatom culture, *Amphora* sp. WCA23.2 (Gene Accession Number: MW721231), was originally isolated from Slangrivier, Western Cape, South Africa, and grown by the Council for Scientific and Industrial Research (CSIR), South Africa. The biomass was provided in a lyophilized form by the CSIR for this study and was stored at -18°C before analysis.

2.2. Proximate composition

Moisture, protein, fat, ash content, and total carbohydrate of the biomass were determined in accordance with the standard methods of the Association of Official Analytical Chemists (AOAC, 2005).

2.3. Fatty acid methyl esters profile by gas chromatography-mass spectrophotometry

Lipids were transesterified from the biomass to fatty acid methyl esters (FAMEs) (Burja et al., 2007). The resultant fatty acid methyl esters were identified by gas chromatography-mass spectrophotometry (GC-MS) (Agilent Technologies, Santa Clara, CA, USA). Methanolic-HCl transesterification of lipids was performed, and the pooled organic layer was prepared for GC-MS analysis. The GC-MS system was coupled with a CTC Analytics PAL autosampler. Separation of the fatty acids was performed on a ZB-5MS GUARDIAN with dimensions of 30 m, 0.25 mm ID, and 0.25 µm film thickness. Helium was used as a carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 280°C, and the split ratio was set at 10:1. The oven temperature was programmed as follows: 100°C for 1 min, 180°C at a rate of 25°C/min for 3 min, 200°C at 4°C/min for 5 min, 280°C at 8°C/min for 7 min, and 310°C at a rate of 10°C/min and held for 5 min. The mass selective detector was operated in full scan mode, and the source and quad temperatures were maintained at 230 and 150°C, respectively. The transfer line temperature was maintained at 280°C. The mass spectrometer was operated under electron impact mode at an ionization energy of 70 eV, scanning from 35 to 500 *m/z*.

2.4. Amino acid composition

The amino acid content of the biomass was determined using the Pico-tag method (Bidlingmeyer et al., 1984). Liquid chromatographic separation was performed using reverse-phase ultra-performance liquid chromatography (UPLC) (LC-30A liquid chromatograph, Shimadzu Corporation, Tokyo, Japan) with a mobile phase consisting of AccQ – Tag Ultra eluent A & B with gradient separation at a flow rate of 0.7 mL/min. The column used was AccQ-Tag Ultra C18 1.7 µm 2.1×100 mm at a temperature of 49°C, coupled with a photodiode-array detection (PDA) detector, at an absorbance of 260 nm.

2.5. Mineral content

Approximately 0.5 g of biomass was placed in a beaker with 1 mL nitric acid (HNO_3). The mixture was heated at 50°C on a hot plate to allow the sample to be digested by HNO_3 in the fume hood. After acid digestion, the beaker was carefully removed from the hot plate and the contents were left to cool for 30 min, also allowing the acid to evaporate. After evaporation of the acid, the digested samples were transferred to a 50-mL volumetric flask with deionized water (1–5% acid concentration). Mineral elements (calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, and zinc) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500s, Agilent Technologies, Waldbronn, Germany).

2.6. Extraction of bioactive compounds

Amphora sp. WCA23.2 biomass was sonicated in a sonicator (Scientz-1500F, 18 mm tip) with a power of 20 W for 15 min. For each extraction, 1 g of dried biomass was extracted with 100 mL absolute methanol, hexane, and water in an orbital shaker (MaxQ 6000, Thermo Fisher Scientific Inc., USA) for 24 h. The extracts were centrifuged at 100×*g* for 10 min at 4°C, and the supernatants were separated. Methanolic and hexane extracts were filtered with Whatman's filter paper (grade 1) and concentrated under reduced pressure in a rotary evaporator at 50°C. Water extracts were filtered and lyophilized. All extracts were stored at -20°C until analysis.

2.7. Bioactive compounds and antioxidant activity

The total carotenoid content was estimated spectrophotometrically according to the method of Lichtenthaler (1987). Briefly, the extracts were diluted with 90 % (v/v) methanol in water and absorbances were measured at 470, 652, and 665 nm. The total phenolic content (TPC) was determined by the Folin-Ciocalteu procedure, and the absorbance was measured at 750 nm (Goiris et al., 2012). The antioxidant capacity of the extracts was investigated by different assays, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric reducing ability of plasma (FRAP), and total antioxidant capacity (TAC). The DPPH assay was performed based on the method described by Brand-Williams et al. (1995) and measured at an absorbance of 515 nm. The free radical scavenging activity of extracts was determined by the ABTS radical cation decolorization assay at an absorbance

of 734 nm (Re et al., 1999). Furthermore, the antioxidant compounds were monitored by using the FRAP assay and absorbance was measured at 593 nm (Benzie & Strain, 1996), while the TAC of the extracts was determined according to the method of Prieto et al. (1999) at an absorbance of 695 nm. The antioxidant activities and standards are defined as inhibition concentration 50 (IC_{50}) values (the concentration of extract causing 50% inhibition of absorbance).

2.8. Statistical analysis

All experiments were conducted in triplicate, and the results are presented as average±standard deviation (SD) on a dry weight (DW) basis. The Graph Pad Prism version 5.0 software for Windows (USA) was used to analyze the experimental data. Differences between groups were considered significant when $p<0.05$.

3. Results and discussion

The proximate analysis of *Amphora* sp. biomass revealed that the strain had a moisture content of 8.8% v/w which is in line with the ideal moisture content recommendation for microalgae, i.e., less than 10% (Becker, 1994). The *Amphora* sp. WCA23.2 biomass was found to be made up of 4% w/w lipids, in contrast to the reported values for *Spirulina* and *Chlorella* that range from 10 to 50% (Chandra et al., 2019; Darwish et al., 2020; Muys et al., 2019; Rasheed et al., 2020). The lipid content of diatoms reported by Bhattacharjya et al. (2020) was highest in *Thalassiosira* sp. (52%), followed by *Skeletonema* sp. (44%) and *Chaetoceros* sp. (22%).

The lipid content of *Amphora* sp. WCA23.2 was relatively lower than the value of 11.14% DW which was published for another strain of *Amphora* (Boukhris et al., 2017). It is believed that this disparity in lipid content within the same genus may be due to differences in geographical location and/or processing method.

The protein content of the *Amphora* biomass in this study was found to be 15% (w/w) of DW, which is slightly lower than diatoms *Chaetoceros calcitrans* (18.8%) and *Skeletonema costatum* (approximately 15%) (Bastos et al., 2022). A study by Cui et al. (2021) reported that the protein content of *Phaeodactylum tricornutum* was 36.6%. It is worth mentioning that the composition of microalgae is largely influenced by growth conditions and phase (Mata et al., 2016).

Carbohydrate was found to be the second most prominent component of *Amphora* sp. WCA23.2 biomass amounting to 28% w/w of DW. This is significantly higher than in commercially produced nutrient-rich microalgae such as *Nannochloropsis* sp. (9.6% w/w) and *Dunaliella* sp. (14.6% w/w) (Kent et al., 2015) and the diatom *Phaedodactylum tricornutum* (11% w/w) (Niccolai et al., 2017). Studies have shown that under ideal growth conditions, most microalgae tend to accumulate carbohydrates rather than lipids, which are synthesized more during stressed conditions as the biosynthetic pathways for both classes of compounds compete for precursor metabolites (Cheng et al., 2017; Debnath et al., 2021).

Ash was found to be the most abundant chemical component in this study, with a value of 44.2% w/w. It is generally known that ash content varies for microalgal species; however, diatoms are usually known to have an ash content of above 40%, as a result of their high silica levels that are accumulated in their membranes, resulting in a distinct external feature called frustule (Fox & Zimba, 2018). This recorded ash cum mineral content has also been ascribed to the environmental factors as well as the processing methods of the microalgal biomass (Qazi et al., 2022). Bastos et al. (2022) reported high ash contents of 28.7% and 50.5% in diatom *S. costatum* and *C. calcitrans*, respectively, while Lee et al. (2009) reported an ash content of 55.6% in *Amphora coffeaeformis*.

The biomass contains considerable levels of all the essential minerals for the body including iron, magnesium, manganese, potassium, calcium, phosphorus, and zinc. The high ash content of *Amphora* sp. WCA23.2 also signifies that the diatom adequately contains some of the essential minerals required for human nutrition, and thus it meets the recommended daily allowances (RDA) for an adult male for these minerals. For instance, the RDA for iron, zinc, and calcium are 8, 11, and 1,000–1,200 mg/day, respectively (West Suitor & Murphy, 2013), which are below the recorded values for *Amphora* sp. WCA23.2 in this study – iron (298 mg/100 g), zinc (12 mg/100 g), and calcium (2205 mg/100 g). In addition, the ash content recorded in this study was significantly higher than those of most land plants which are between 5% and 10% (Gebhardt, 2002). Furthermore, the mineral elemental composition recorded in this study was compared favorably with previously published work on microalgae, as highlighted in Table 1. In this regard, these results suggest that *Amphora* sp. WCA23.2 might be highly beneficial therapeutically due to its mineral contents, especially in addressing defects such as osteoporosis and anemia.

The fatty acid profile of the diatom showed that it included two monounsaturated fatty acids (MUFAs), seven saturated fatty acids (SFAs), and four polyunsaturated fatty acids (PUFAs) (Table 2). MUFAs were the dominant class of fatty acid in *Amphora* sp. WCA23.2, with a total of 25 mg/g, followed by SFAs with a total of 20.8 mg/g, while PUFAs were the lowest with an amount of 0.9 mg/g, as can be observed in Table 2. According to Maltsev and Maltseva (2021), the C16:1 and C18:1 MUFAs are usually found in microalgae and cyanobacteria in significant

Table 1. Mineral element content of *Amphora* sp. WCA23.2 (mg/100 g DW).

Mineral	mg/100 g	Other microalgae (Tibbetts et al., 2012)
Calcium	2025.00±3.61	300–2,100
Magnesium	1095.00±9.54	100–1,100
Phosphorus	929.00±6.56	1,700–3,000
Potassium	1147.00±4.58	600–1,200
Sodium	1293.00±12.77	700–1,100
Copper	5.60±0.17	1.2–65
Iron	298.00±7.21	100–700
Magnesium	18.00±1.00	3.7–59.2
Zinc	12.00±0.71	23.9–370

Table 2. Identification of fatty acids (mg/g DW) in *Amphora* sp. WCA23.2*.

Fatty acids	mg/g
Caproic acid	0.43±0.01[e]
Capric acid	0.24±0.03[c]
Myristic acid	4.07±0.07[j]
Pentadecanoic acid	1.12±0.01[i]
Palmitic acid	13.73±0.34[k]
Stearic acid	0.71±0.10[h]
Lignoceric acid	0.50±0.01[f]
Oleic acid	0.56±0.02[g]
Palmitoleic acid	24.50±0.66[l]
Linoleic acid	0.37±0.02[d]
Arachidonic acid	0.17±0.01[a]
Eicosapentaenoic acid	0.18±0.01[a]
Eicosatrienoic acid	0.21±0.01[b]

*Mean±standard deviation (n=3). Mean values in columns with different superscript letters are significantly different at p<0.05.

quantities. Scientific evidence has since suggested that dietary MUFAs are beneficial to human health, especially as they enhance a healthy blood lipid profile, mediate blood pressure, and positively modulate insulin sensitivity as well as glycemic control (Gillingham et al., 2011). The fatty acid profile of *Amphora* sp. in this study is also remarkable as its major constituent fatty acid, palmitoleic acid (POA, C16:1Δ9), has been identified as an omega-7 MUFA with various beneficial effects in humans including the improvement of metabolic syndrome indications, the reduction of inflammation, protection against cardiovascular diseases, and the inhibition of oncogenesis (Wu et al., 2012). It is noteworthy that the palmitoleic acid content in this study (24.50 mg/g) which amounts to ~2.5% w/w was compared favorably with the 3.6% w/w recorded by Zhou et al. (2021) in their study which was focused primarily on the production of the fatty acid from the oleaginous *Scheffersomyces segobiensis*.

Table 3 represents the amino acid composition of *Amphora* sp. WCA23.2 biomass in g/100 g protein DW. The biomass consisted of nine essential and nine non-essential amino acids with the most abundant amino acid being glutamine (7.87 mg/100 g protein), in contrast to the lowest, cysteine, with an amino acid content of 0.08 mg/100 g protein. Similar patterns of AA profile have been recorded in many microalgae (Brown, 1991). In terms of the contributions of the essential amino acids to the amino acid requirements (WHO, 2007), valine contributed 118%, followed by isoleucine (107%), threonine (100%), leucine (82%), phenylalanine (68%), histidine (55%), methionine (52%), and lysine (41%) (WHO, 2007). Tryptophan (1.49 g/100 g) exceeds the WHO/FAO/UNU amino acid requirements. *Amphora* sp. exhibited levels of essential amino acids that meet the nutritional requirement recommended by the WHO for adults and children (2–5 years) (WHO, 2007).

The methanol extract of *Amphora* sp. displayed the highest total carotenoid content (1.62±1.12 mg/g DW), followed by the hexane extract (0.85±0.95 mg/g DW), whereas the aqueous extract contained the lowest quantity (0.52±0.54 mg/g DW) (Table 4). These results are considerably lower than the findings

Table 3. Amino acid (AA) composition of *Amphora* sp. WCA23.2 biomass g/100 g protein DW#.

Amino acid	*Amphora* sp.	FAO (1985) Adults/Children
Histidine	1.04	1.9
Threonine	3.39	3.4
Lysine	2.39	5.8
Methionine	1.31	2.5**
Valine	4.13	3.5
Isoleucine	3.01	2.8
Leucine	5.40	6.6
Phenylalanine	4.28	6.3*
Tryptophan	1.49	1.1
Total essential AA	**26.44**	
Serine	3.12	
Arginine	3.82	
Glycine	4.47	
Aspartic acid	6.32	
Glutamic acid	7.87	
Alanine	4.20	
Proline	3.39	
Cysteine	0.08	
Tyrosine	3.32	
Total non-essential AA	**36.59**	

#Amino acid requirement by FAO g/100 g (WHO, 2007) for adults and children aged 2–5 years; *Phenylalanine+Tyrosine; **Methionine+Cysteine.

Table 4. Carotenoid content and TPC of *Amphora* sp. WCA23.2 extracts*.

Extraction solvent	Carotenoid mg/g DW	Total phenolic content mg GAE/g DW
Methanol	1.62±0.10[a]	1.90±0.11[a]
Aqueous	0.52±0.03[b]	0.33±0.06[b]
Hexane	0.85±0.03[c]	0.23±0.05[b]

*Results are presented as mean±SD (n=3). Mean values in the same column with the same letter superscripts are not significantly different at $p<0.05$.

of Ahmed et al. (2014) that reported the carotenoid content of some microalgal species extracts such as *Tetraselmis* sp. (5.8 mg/g DW), *Dunaliella tertiolecta* (1.1 mg/g DW), and *Isochrysis* sp. (5.0 mg/g DW). Goiris et al. (2012) also previously reported the carotenoid content in diatom *P. tricornutum* and *Nannochloropsis* sp. (6.1 and 2.2 mg/g DW, respectively). Similarly, the highest phenolic content was found in the methanol extract at 1.9 mg GAE/g DW, followed by the aqueous extract at 0.3 mg GAE/g DW and hexane extract at 0.2 mg GAE/g DW. These results are similar to those reported by Lee et al. (2009), for *A. coffeaeformis* as well as in line with those reported by Goiris et al. (2012) for *C. calcitrans* (1.8 mg GAE/g DW) and *Nannochloropsis* sp. (1.4 mg GAE/g DW). The TPC results of this study are marginally higher than the TPC value reported by Hossain et al. (2016) in *Spirulina* (1.78 mg GAE/g). Phenolic compounds and terpenoids play the most significant role as strong antioxidants in diatoms, translating into biological activities such as anti-atherosclerotic, anti-inflammatory, and anticarcinogenic activities (Saxena et al., 2021).

The inhibition percentages of the DPPH radicals by the *Amphora* sp. extracts and the vitamin C standard were found to be concentration-dependent (200–1000 µg/mL) as shown in Figure 1. All the tested microalgal extracts possessed the ability to scavenge DPPH at various degrees. However, the methanol extract at 1,000 µg/mL displayed the highest scavenging effect at 44%, followed by the aqueous extract at 32.7% and hexane extract (25.6%). These results are slightly higher than the activity recorded for the methanolic extracts of *A. coffeaeformis* (22.7%) and *Navicula* sp. (31.6%) as reported by Lee et al. (2009). Furthermore, these results are considerably higher than those reported by Hemalatha et al. (2013), which showed the methanol extract of *Chlorella marina* had an inhibition of 23.08% and *Dunaliella salina* (17.66%). Of noteworthy importance is that these results are significantly higher than those reported by Chu et al. (2010) for *Spirulina*, commercial microalgae, which showed <40% DPPH scavenging activity. The DPPH results of this study showed notable activities, especially in the methanolic extracts, indicating a higher efficacy for scavenging of free radicals.

Figure 2 displays the ABTS radical scavenging activity of *Amphora* sp. extracts and ascorbic acid standard. The methanol extracts (200–1,000 µg/mL) showed the highest antioxidant activity ranging from 48.42 to 63.62%, followed by the aqueous extracts (32.03–47.93%), while the hexane extracts displayed the lowest activity (19.92–31.59%). The IC_{50} value of 22.30 was obtained in this study, which is almost 11 times higher than the standard (1.83). Present results indicate that the free radicals ABTS scavenging activity of the extracts might be due to the presence of high-molecular-weight phenolic compounds and derivatives.

The FRAP radical scavenging activity of *Amphora* sp. extracts and the standard Trolox is illustrated in Figure 3. It can be seen that the FRAP activity is considerably lower than the standard Trolox (71–83 µmol TE/g). The methanol extracts contained the highest FRAP activity (3.47–7.91 µmol TE/g), while the lowest FRAP activity was found in the hexane extracts (1.35–4.76 µmol TE/g). The results in this study are similar to the reducing power of *Chlorella* (6.37–9.32 µmol TE/g), reported by Goiris et al. (2012). Goiris et al. (2012) reported a significantly higher reducing power activity for *Chlorella vulgaris* (42–64.65 µmol TE/g), *Haematococcus pluvialis* – green phase (41.34 µmol TE/g), *Isochrysis* ISO-T (46.69 µmol TE/g) and *Isochrysis* sp., (53.73 µmol TE/g), *Nannochloropsis oculata* (40.68 µmol TE/g), *Nannochloropsis* sp., (40.80 µmol TE/g), *P. tricornutum* (48.90 µmol TE/g), and *Tetraselmis* sp. (46.58 µmol TE/g). The results in this study are similar to the reducing power of *Chlorella* (6.37–9.32 µmol TE/g), reported by Goiris et al. (2012). Hajimahmoodi et al. (2010) reported FRAP activity of 0.56–31.06 µmol TE/g for the hexane extract of *Microchaete tenera* and the aqueous extract of *C. vulgaris*, respectively. FRAP activity was higher in the aqueous extract than in the hexane extract, and this trend is in agreement with Goiris et al. (2012) and Hajimahmoodi et al. (2010).

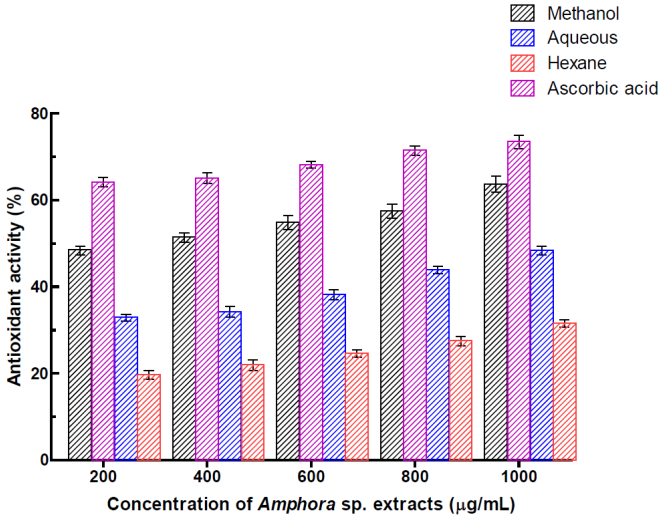

Figure 2. ABTS radical scavenging activity of *Amphora* sp. extracts and ascorbic acid standard. Each value represents mean±SD (n=3).

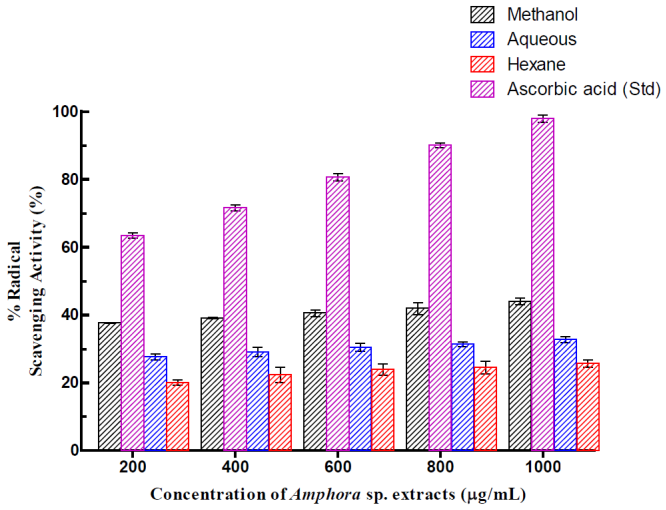

Figure 1. DPPH radical scavenging activity of *Amphora* sp. extracts and ascorbic acid standard. Each value represents mean±SD (n=3).

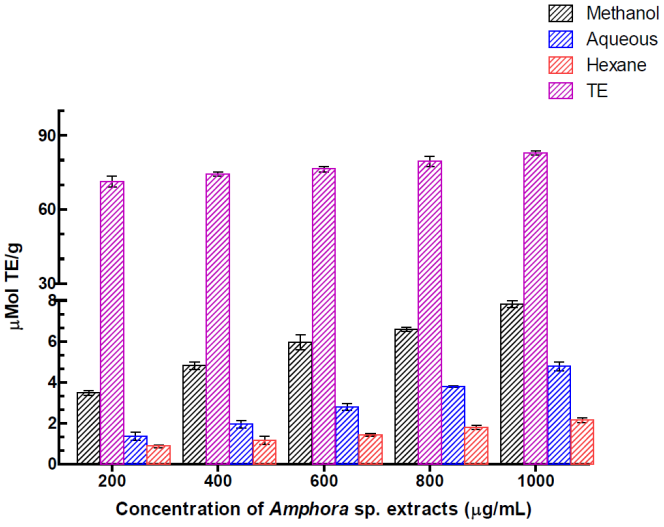

Figure 3. FRAP radical scavenging activity of *Amphora* sp. extracts and standard Trolox. Each value represents mean±SD (n=3).

TAC results demonstrate that the methanol fraction at 1000 µg/mL demonstrated the highest ability for reducing Fe^{3+}, 1.9 mg/g amino acid equivalent (AAE) at almost two times that of the aqueous extract (1.12 mg/g AAE) and approximately five times higher than the hexane extract (0.39 mg/g AAE) (Figure 4). The results of this study exhibited similar results compared to Hemalatha et al. (2013), who reported between 0.6 and 1.03 mg/g AAE for methanolic extracts of *C. marina, D. salina, and Navicula clavata.* The IC_{50} value of the standard was 8.47 µg/mL, while the extract showed an IC_{50} value of 49.72 µg/mL. It is well known that the yield of chemical extraction depends on the temperature and extraction time, the type of solvents, and the chemical composition of the sample. The antioxidant compounds of microalgae could have different polarities; thus, the antioxidant capacity of microalgae is strongly influenced by the extracting solvent (Ahmed et al., 2014). Thus, the findings from the antioxidant study demonstrate promising results indicating that *Amphora* sp. WCA23.2 could be important active ingredients for applications in the functional food, fortified nutraceuticals, and supplement sectors.

The IC_{50} values are presented in Table 5. A lower value of IC_{50} indicates a high antioxidant activity. The methanol extract exhibited an IC_{50} value of 26.78 µg/mL for the DPPH scavenging activity, while the ascorbic acid standard displayed a high antiradical activity with an IC_{50} value of 5.51 µg/mL. Boukhris et al. (2017) reported an IC_{50} value of 0.23 µg/mL for the DPPH scavenging activity for the ethanol extract of *Amphora* sp. WCA23.2. Similarly, with regards to ABTS scavenging ability, the IC_{50} value of 22.30 µg/mL recorded for the most active extract from the diatom was lower than 1.83 µg/mL observed for the standard antioxidant. Furthermore, the IC_{50} values for FRAP and TAC were also expectedly found to be lower than the respective standard compounds. It is suggested that the lower IC_{50} values relative to the different standard compounds are due to the fact that the standard compounds are highly purified compounds with established antioxidant activities, while the extracts are partially purified fractions that contain antioxidants and many other extraneous substances.

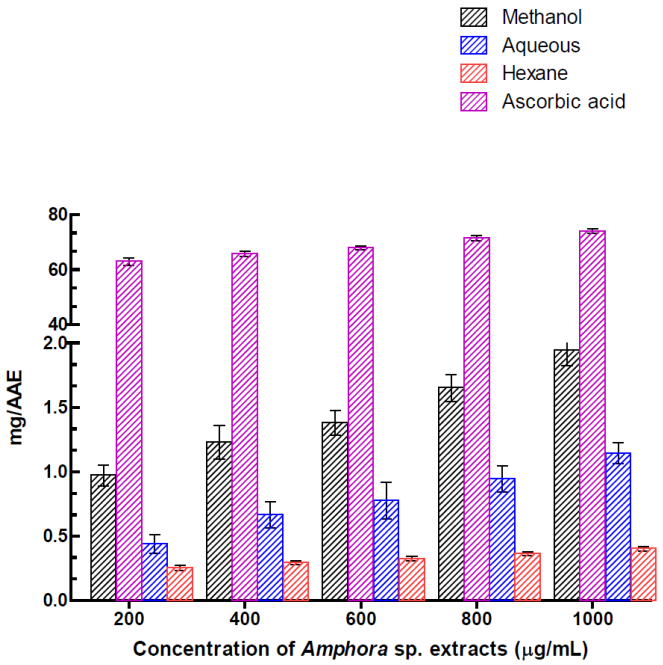

Figure 4. TAC of *Amphora* sp. extracts and ascorbic acid standard. Each value represents mean±SD (n=3).

Table 5. IC_{50} values for antioxidant capacities of *Amphora* sp. extract compared to standards.

Antioxidant activity	IC_{50} (µg/mL) extract	IC_{50} (µg/mL) standard
DPPH	26.78±0.17	5.51±0.58
ABTS	22.30±0.74	1.83±0.33
FRAP	49.64±0.63	6.59±1.64
TAC	49.72±1.24	8.47±1.29

4. Conclusions

The *Amphora* sp. WCA23.2 biomass was recorded to contain a significant amount of protein and carbohydrate, with a remarkable amino acid, fatty acid, and mineral profile. This is especially important as there is an urgent need to obtain protein and other important nutrients from alternative sources. It is quite notable that the concentration of five out of the nine essential amino acids in *Amphora* sp. WCA23.2 meets the nutritional guidelines (WHO, 2007), suggesting that microalgal proteins can be used in the production of foods, especially as a supplement. In addition, the antioxidant assays demonstrated that the methanol extracts consisted of the highest DPPH scavenging activity and a similar trend was observed for the ABTS assay. The appreciable antioxidative activities are a potential candidate as a natural antioxidant source in the food industry. This study has shown that *Amphora* sp. WCA23.2 has the potential to be used as a food and nutraceutical source. However, there is still a lot of scientific investigation to be carried out in order to unravel the full potential of this microalgae with the aim of its applications in the food and pharmaceutical industry. For instance, it is necessary to fully identify and enumerate the antioxidant compounds in the diatoms with the aim of meeting the huge demand for alternative sources of natural antioxidants and healthy foods.

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