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Research Article

PROFILING THE CAROTENOIDS OF MICROALGA (*Scenedesmus obliquus*) EXTRACT BY HPLC AND ITS ANTIOXIDANT CAPACITY

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ABSTRACT. The extracts of the algae species *Scenedesmus obliquus* isolated from Kapulukaya Reservoir (Kırıkkale, Turkey) were analyzed to determine the composition, content and antioxidant properties of carotenoids. The HPLC analysis was applied to identify carotenoids species and to determine their contents. Methanol–acetonitrile–water (84:14:2, v/v/v) and methylene chloride (100%) used in the gradient solvent system resulted in an acceptable separation. Of the 22 resolution peaks assigned to carotenoids either tentatively or positively, the first eight were identified to be epoxy containing while the remaining ones as main carotenoids namely, all-*trans* forms of lutein, β-carotene, α carotene, zeaxanthin, 13-or 13'-*cis*-lutein, 9-or 9'-*cis*-lutein, *cis*-lutein, 13-or 13'-*cis*- β-Carotene, 9-or 9'-*cis*-β-Carotene, 9-or 9'-*cis*- α-carotene and *cis*-neoxanthin. Quantitatively, *S. obliquus* was predominated by all-*trans* isomers of lutein and β-carotene, being 83.74 % (2.52 mg g^{-1}) in total carotenoids (TC). Antioxidant capacity assays of DPPH and FRAP showed considerably low effects of *S. obliquus* extracts compared to standards. This was attributed to the lesser existence of *cis* isomers within derivatives. Multiple regression was utilised to partition the antioxidant effect of both assays and, revealed an estimation of FRAP dominating over DPPH. A need was arisen on the exploration of isomerisation mechanisms of the carotenoids compounds in order to better evaluate the species for a probable potential of further practical applications.

Keywords: *Microalgae, Scenedesmus obliquus, carotenoids, HPLC analysis, DPPH and FRAP assays.*

INTRODUCTION

Owing to their structural and functional roles, carotenoids are indispensable in photosyntetic life. Their task in plants is ample. During photosynthesis, they harvest the light, transfer the energy and protect the photosynthetic apparatus against photooxidative damage [1]. The antioxidant property is in general operated via scavenging singlet molecular oxygen and peroxyl radicals and increasing the stability of the photosynthetic apparatus [2]. Protective functions of carotenoids via their anti-oxidant activities [3, 4], have also been well accounted for the human [5]. The natural carotenoids are well known to have a unique complexity owing to a mixture of various isomers in their structure and chemical formations with other bioactive compounds. Therefore, they are preferred over

synthetic counterparts dominated by all-trans compounds [6] in direct human consumption.

Boosted by increasing results about the limitations of the use of synthetic carotenoids due to potential safety concerns [7, 8] microalgae have recently received particular attention as a natural source of carotenoids [9], amongst other bioactive compounds such as carbohydrates, lipids, proteins, alcaloids, fatty acids etc. that can be utilized for commercial use in a wide variety of industries (i.e. food and feeds, health, agriculture, cosmetic and energy (biofuel) [10, 11, 12] .

Numerous studies reported sufficient isolation of various types of antioxidant carotenoids compounds including β-carotene from *Dunaliella salina* [13, 14], zeaxanthin and lutein from *Scenedesmus almeriensis*, [15, 16] astaxanthin and lutein in *Chlorella zofingensis and Chlorella vulgaris* [17, 18, 19, 20], astaxanthin from *Haematococcus pluvialis* [21, 22] and *Chlorococoum* sp. [23], α-tocopherol from *Nanochloropsis oculata* [24]. Various technological and manipulative applications, such as environmental cues that induce carotenoid accumulation through the regulation of carotenogenesis, are still pursued on the aforementioned and other potential species to obtain compounds at desired content and efficacy [25]. In this study, *Scenedesmus obliquus* which has recently gained attention for its potential to be a model species [26, 27] are therefore explored by means of profiling its carotenoids and antioxidant activity performance.

MATERIALS AND METHODS

Collection and culturing of microalgae

Scenedesmus obliquus were isolated from fresh water samples of the River Kızılırmak in Kırıkkale Province (Turkey). The species identification was based upon molecular characterization, using 18S rRNA, ITS, rbcl and 16S molecular markers [19].

Basal Bold Medium (BBM) [28] was selected for the growth of *S. obliquus* using an illumination of 16:8 h light–dark cycle of 4000 lux light intensity under temperature conditions kept at 25 ±1 °C. Cell counting, chlorophyll *a* and dry biomass were measured to monitor the growth of cultures until the stationary phase, at the middle of which (Day 18) the harvesting was executed. Centrifugated biomass at 3000 rpm for 10 min. was lyopilizated for 48 hours at -83 °C and 1.33 Pa [29] and stored at -80 °C until used.

Extraction and profiling Carotenoids

The details of both the extraction procedure [30] and the analysis of carotenoids were given in a previous study by Aluç et al., 2018 [19]. Briefly, saponification was first applied to a 0.1 g microalgae sample using 3 mL hexane–ethanol–acetone–toluene (10:6:7:7, v/v) for 1h and then the separation of carotenoids was achieved by consecutive usage of hexane and sodium sulphate under dimmed light and nitrogen gas. Collected extracts dried by evoporation were subjected to HPLC analysis in mobile phase solvent. Two mobile phases (A and B) of methanol–acetonitrile–water (84:14:2, v/v/v) and methylene chloride (100%) with an arranged flow rate of 0.6 mL min^{-1} were used in a gradient manner from 100% A and 0% B. Reproducibility, the limits of detection (LOD) and quantification were calculated as described in Aluç et al. [19].

Antioxidant capacity assays

For the extraction of antioxidants, the microalgae biomass (0.1 g) was mixed with 1 ml methanol-toluene (3:1) in a volumetric flask. Following homogenization, insoluble biomass was separated from supernatant by centrifugation at 13500 rpm for 10 min. [30].

DPPH (2,2-Diphenyl-1-picrylhydrazyl) activity of the *S. obliquus* extract solutions was determined based on the method described by Blois, 1958 [31] Volumes of 62.5 µL from each extract solutions prepared with methanol at concentrations of 2, 10, 25, 50, 100 µg mL^{-1}, respectively, were added to a mixture of methanol (125 µL) and DPPH (62.5 µL) and then left for incubation in the dark at room temperature. The reaction values measured at 515 nm against methanol extract as blank were used to calculate the percentage radical scavenging activity as below.

$$\%\ \mathrm{RSC} = \frac{(\mathbf{A}\ \text{control} - \mathbf{A}\ \text{sample})}{\mathbf{A}\ \text{control}} \times 100$$

So that the control included DPPH and methanol while sample consisted of microlagae extract, DPPH and methanol. The EC_{50} values which were calculated from the plot of scavenging activity against the concentration of sample represented the half maximal potency of microalgal extract to scavenge DPPH radicals. For the Ferric Reducing Antioxidant Power Assay (FRAP), a 1 ml of *S. obliquus* extract solutions prepared at graded concentrations (2, 5, 10, 25, 50, 100 µg mL^{-1}) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (200 µL, 1%) and left for incubation at 50 ºC for 20 min. Trichloroacetic acid (TCA, 10%, 200 µL) was added to the mixture and centrifuged at 3750 rpm for 10 min. [32]. A 125 µL of the upper layer was separated and mixed with 25 µL distilled water and 20 µL $FeCl_3$ (0.1 %). The absorbance of the mixture was measured at 665 nm by a UV-spectrophotometer.

Statistics

Means of data were obtained from triplicate analysis during HPLC analysis. Results from antioxidant assays were obtained via triplicate experiments. Relationships between antioxidant activity and concentrations of algal extracts and standards were depicted by regression analysis. Stepwise multiple regression analysis was used to explore substantially the partial contribution of antioxidant activity at each assay (DPPH and FRAP), using STATISTICA (version 7) statistical software.

RESULTS AND DISCUSSION

Identification of carotenoid peaks

For the identification of carotenoids in *S. obliquus*, the photoisomerized lutein and β–carotene standards were subjected to HPLC analysis in order to obtain spectral characteristics and Q ratio values, according to which the peaks obtained from *S. obliquus* samples were designated. Considering the results obtained from these standards in the previous study by Aluç et al., 2018 [19], solvent selection was considered reliable, indicated by the range of retention time and Q ratio values of these standards [33, 34]. The used solvent system was efficient implied by the the retention factor (k values between 0.76 and 7.76) [34, 35] and the mobile phase used for the separation of carotenoid compositions was adequate decided by the separation factor (α) values always

being higher than 1. All peaks and standards also exhibited a high percentage of purities being higher than 90% [19].

The chromatogram of the extract solution from the *S.* o*bliquus* cells revealed 22 resolution peaks assigned to carotenoids (Figure 1 and Table 1). All-*trans* forms of lutein, zeaxanthin, α-carotene and β-carotene were assigned to peaks 12, 13, 19 and 20, respectively (Figure 1 and Table 1). They were all positively identified based on retention time and Q ratio values of standards except for all-*trans* α-carotene, designation of which was tentative and made by the comparison of retention time and absorption spectra values given in the literature (Table 1 and 2) [34, 35, 36]. The peaks 1 through 8 were again tentatively identified to be the epoxy-containing compounds relying upon spectral characteristics and retention behavior reported in the literature (Figure 1 and Table 2) [36, 37, 38].

As given in our previous study [19], during HPLC analysis of standards, an acceptable reproducibility was reached since RSD values were found to be lower than 2.37 and 4.76 for retention times and integrated areas, respectively. Linearities with their markedly high correlation coefficients (> 99%) also indicated that a good purity in the analysis was attained. The calculated LOD values were 0.013, 0.019, 0.024, 0.022 μg/ml for lutein, zeaxanthin, β-cryptoxanthin and β-carotene, respectively while the corresponding values of LOQ were found to be 0.041, 0.058, 0.071 and 0.068 μg mL^{-1}.

Table 1. *Retention time, retention factor (k), separation factor (α), peak purity and resolution of carotenoids in S. Obliquus.*

Peak no.	Compound	Retention tıme (min)	k	α	Peak purity (%)	Resolution
1	Auroxanthin	8.61	0.76	0.00	96.61	9.58
2	Auroxanthin	9.99	1.04	1.37	99.74	2.34
3	Neochrome	11.26	1.31	1.26	98.01	2.48
4	Neoxanthin	12.24	1.50	1.14	98.75	1.63
5	*Cis*-neoxanthin	13.82	1.82	1.22	99.93	2.97
6	Neoxanthin	14.39	1.94	1.06	99.81	0.95
7	Neoxanthin	15.47	2.16	1.12	99.49	1.68
8	Neochrome	16.52	2.37	1.10	96.54	1.91
9	*Cis*-neoxanthin	17.67	2.61	1.10	97.67	2.09
10	13-or 13'-*cis*-lutein	20.60	3.20	1.23	99.83	4.47
11	13-or 13'-*cis*-lutein	22.01	3.49	1.09	99.99	2.39
12	All-*trans*-lutein	23.47	3.79	1.09	96.08	3.40
13	All-*trans*-zeaxanthin	26.19	4.34	1.15	99.99	6.88
14	9-or 9'-*cis*-lutein	27.12	4.53	1.04	99.97	1.90
15	9-or 9'-*cis*-lutein	28.35	4.78	1.06	93.64	2.09
16	*Cis*-lutein	29.52	5.02	1.05	99.91	2.05
17	*Cis*-lutein	31.07	5.34	1.06	97.92	3.33
18	13-or 13'-cis- β-carotene	36.93	6.53	1.18	99.70	10.22
19	All-*trans*- α-carotene	37.77	6.70	1.03	98.40	1.80
20	All-*trans*- β-carotene	41.32	7.43	1.11	99.81	7.86
21	9-or 9'-*cis*- α-carotene	41.93	7.55	1.02	98.75	1.48
22	9-or 9'-*cis*- β-carotene	42.93	7.76	1.03	98.85	2.40

Table 2. Assignment data for all-*trans* and *cis* forms of carotenoids their amounts (mg g^{-1}) in *S. Obliquus.*

Peak no.	Compound	Retention time (min.)	λ (nm, inline)				λ (nm, reported)				Q-ratio[a]	Amount (mg g^{-1})
1	Auroxanthin	8.61	-	400	421	-	-	398	422[b]	-	0.38	0.000
2	Auroxanthin	9.99	-	400	422	-	-	398	422[b]	-	0.31	0.000
3	Neochrome	11.26	-	399	421	-	326	398	422	440[b]	0.32	0.000
4	*cis*-Neoxanthin	12.24	-	401	425	-	326	404	428	458[b]	0.24	0.000
5	*cis*-Neoxanthin	13.82	-	405	429	456	-	411	429	459[c]	0.35	0.000
6	Neoxanthin	14.39	-	418	439	-	-	418	446	470[b]	0.28	0.000
7	Neoxanthin	15.47	-	418	440	469	-	416	440	470[b]	0.23	0.000
8	Neochrome	16.52	-	400	422	448	332	398	422	446[b]	0.17	0.000
9	*cis*-Neoxanthin	17.67	-	415	436	465	-	411	434	464[b]	0.11	0.000
10	13-or 13'-*cis*-Lutein	20.60	331	-	442	468	-	415	440	464[b]	0.21	0.000
11	13-or 13'-*cis*-Lutein	22.01	331	-	440	467	-	416	440	464[b]	0.36	0.079
12	All-trans-lutein	23.47	333	-	446	473	-	423	446	470[d]	0.12	1.368
13	All-Trans-zeaxanthin	26.19	-	-	452	477	332	423	446	470[b]	0.16	0.008
14	9-or 9'-*cis*-Lutein	27.12	-	-	441	469	344	427	452	476[b]	0.15	0.000
15	9-or 9'-*cis*-Lutein	28.35	327	-	444	470	332	416	440	470[b]	0.14	0.016
16	*cis*-Lutein	29.52	328	-	442	470	332	416	440	470[b]	0.13	0.032
17	*cis*-Lutein	31.07	-	-	447	473	344	421	446	476[b]	0.70	0.004
18	13-or 13'-cis- β-carotene	36.93	343	-	451	-	344	422	452	476[b]	0.19	0.227
19	All-trans-α-Carotene	37.77	340	-	448	475	344	426	449	476[b]	0.18	0.000
20	All-trans-β-Carotene	41.32	344	-	454	479	350	430	458	482[b]	0.16	1.151
21	9-or 9'-*cis*-α-Carotene	41.93	-	-	444	470	344	421	446	470[b]	0.17	0.000
22	9-or 9'-*cis*-β-Carotene	42.93	346	-	449	474	344	428	452	476[b]	0.17	0.121
Total												3.008

a: A gradient mobile phase of methanol-acetonitrile-water (84:14:2, v/v/v) and methylene chloride (from 100:0, v/v to 45:55, v/v) was used.

b: A gradient mobile phase of methanol-acetonitrile-water (84:14:2, v/v/v) and methylene chloride (from 100:0, v/v to 45:55, v/v) was used by Inbaraj et al.(2006)

c: A gradient mobile phase of methanol-2-propanol (99:1, v/v) and methylene chloride (from 100:0, v/v to 70:30, v/v)was used by Chen et al., (2004)

d: A gradient mobile phase of methanol-2-propanol (99:1, v/v) and methylene chloride (from 100:0, v/v to 70:30, v/v) was used by Liu et al., (2004)

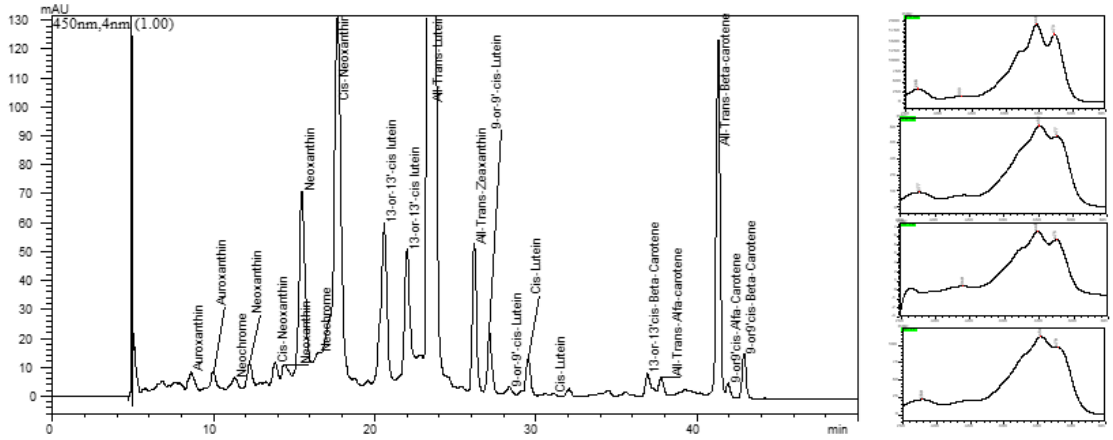

Fig. 1. *The HPLC chromatogram of the carotenoids obtained from Scenedesmus obliquus (left) and the profiles of all-trans carotenoids (right)*

Quantification of carotenoids

The *S. obliquus* eliquates contained 3.09 mg g^{-1} of total carotenoids (TC), dominated by the two main derivatives, trans and *cis* forms of lutein and β-carotene, with an equal contribution of each being 49.83% (1.49 mg g^{-1}). All-trans-zeaxanthin was represented by a small amount in TC, being 0.26 % (0.008 mg g^{-1}), (Table 2). In a previous study by Aluç et al. [19], another species of the same genus, *Scenedesmus regularis* was represented with a similar carotenoids composition whereas the total amount of carotenoids was markedly higher (7.14 mg g^{-1}) than the species *S. obliquus*, used in the present study.

Various factors are involved in the biosynthesis of carotenoids or the distribution of derivatives in algae. It is not only species-specific [5] but also may differ within the different strings of the same species [39]. Additionally, variations may emerge due to different culture conditions as stated either in studies of direct determination of carotenoids [40] or in those applying stress factors to increase carotenoids content within a potential [39, 41]. In spite of such facts, our findings can still be made comparable to those given in previous studies. For example, in a study carried out on 36 different microalgae biomass [40], a total carotenoid of 0.44±0.06 mg g^{-1} D.W. in ethanol/water extracts of *Scenedesmus obliquus* was reported, being comparatively lesser than that in our study. But, relatively high phenolic content was also found as to be 1.94±0.16 mg GAE. g^{-1} D.W. for the same species. Patnaik and Mallick, 2015 [26] conducted a research on the potential of *S obliquus* in β-carotene production under growth conditions optimized by the manipulations of citrate. Total carotenoids content was found to be around 2.80 mg g^{-1} under control and 2.5 mg g^{-1} under optimized conditions. The corresponding values of β-carotene were around 0.82 mg g^{-1}. and 0.57 mg g^{-1}. These findings are all similar or slightly less than that obtained in our study. In another study by Ho et al., 2014 [39], the cell growth and lutein increment in six *S. obliquus* isolates were enhanced under different light exposures. Lutein content varied between strings with the lowest value of 1.86 ± 0.11 mg g^{-1} (*S. obliquus* ESP-5) and the highest of 3.63±0.12 mg g^{-1} (*S. obliquus* FSP-3). Three strains of *Scenedesmus* sp. among four classes of chlorophyta were also presented to have concentrations ranging between 0.53–5.57 mg TC g^{-1} DCW [42]. Distinctively

high yield of TC (10.45 mg g^{-1} DCW) was also noted for *Scenedesmus* sp. [43] Guedes et al., 2011 [44] used a string (M2-1) of *S. obliquus* to determine the optimal antioxidant capacity in relations to the carotenoid content varying during growth phases under cross-combination of pH and temperature conditions. They found markedly higher lutein (203.57±1.41 mg mL^{-1}) and β-carotene (18.2±0.33 mg mL^{-1}) contents from the biomass at the early exponantial growth phase.

Antioxidant capacity

As a general rule, the background in antioxidant assays are to imitate the biochemical reactions within a cell. Yet, a wide variety of assays which has been evolved within this approach sustain some degree of complexity and complications during applications due to, amongst others, multifunctional nature of the natural antioxidants or phytochemicals [45]. The composition of the biological component, oxidizable substrate, the media used during oxidation experiment and methods to measure antioxidant activity can all vary, and multitude of tests are indeed needed to take account of the chemistry that would better respond to the targeted chemical [46]. Moreover, variations also occur in mechanisms viz., radical scavenging, metal ion transition, hydrogen peroxide decomposition etc. through which antioxidants exert their effect. Therefore, in this study, the assays were selected to measure antioxidant activity regarding two different mechanisms to reveal the whole picture as much as possible as suggested by Apak et al. [46] DPPH, represented the hidrogen atom transfer reactions or (HAT) and FRAP the single electron transfer reactions (ET). The antioxidant activities of the algal extract determined by DPPH and FRAP assays were evaluated against those of ASC and BHT. Concentrations of *S. obliquus* extract and both standard solutions produced positive and significant correlations in DPPH and FRAP assays, the latter being represented with stronger regression coefficients. (Figure 2a, b). The IC_{50} values calculated for DPPH activities followed an increasing order of ASC (IC_{50} 0.91 mg mL^{-1}) <BHT (IC_{50} 3.59 mg mL^{-1}) <*S. obliquus* extract (IC_{50} 115.62 mg mL^{-1}). ASC and BHT equivalent values of microalgae extract are also calculated as given in Table 3.

Table 3. *IC_{50} values of S. obliquus and standards (BHT and ASC) in DPPH assay, and equivalent BHT and ASC of S.obliquus in both assays.*

	IC_{50} (mg mL^{-1})	**Equivalent µmol g^{-1} DW (DPPH)**	**Equivalent µmol g^{-1} DW (FRAP)**
S. obliquus extract	115.617		
BHT	1.393	3.59	1.02
ASC	0.211	0.91	0.48

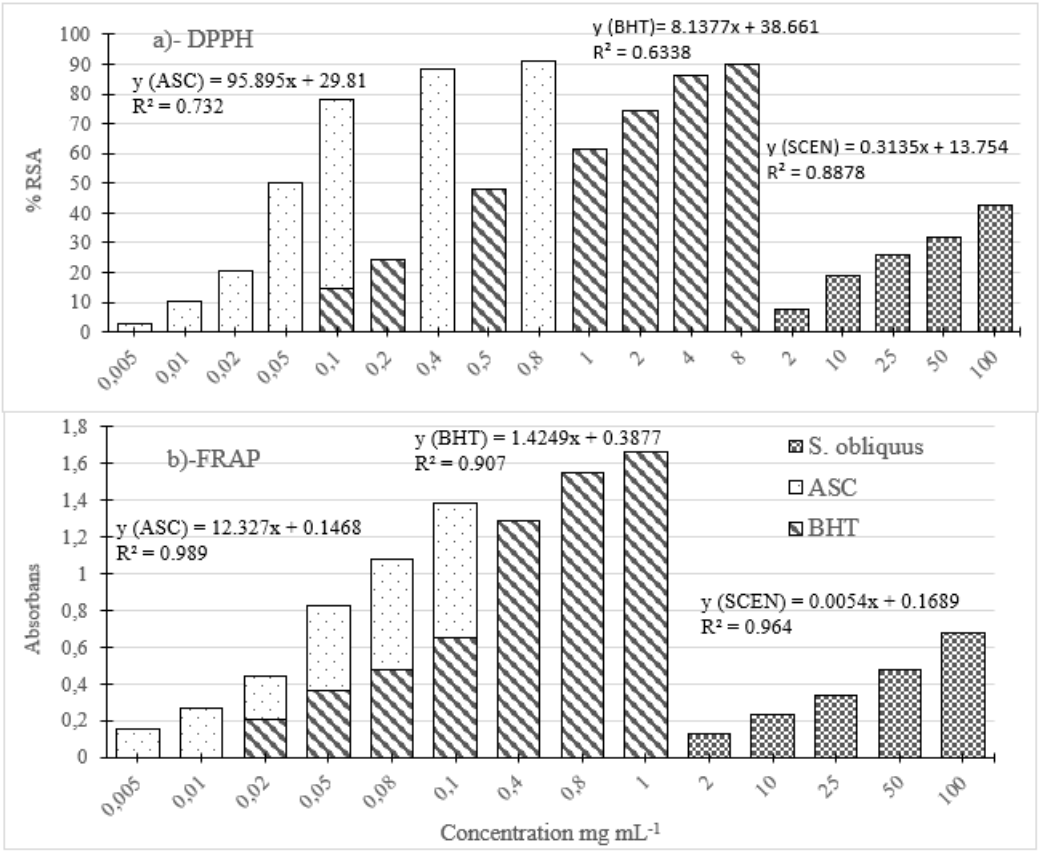

Fig. 2. *Collective display of DPPH radical sweeping (a) and FRAP inhibition (b) effect of S. obliquus extract. Column legends and x axis title are shown in graph b.*

In order to explore the respective contribution of each mechanism (DPPH and FRAP) on the antioxidant reactions, stepwise multiple regression analysis was applied. Despite the existance of collinearity, a well known problem encountered in multiple regressions, the analysis was proceeded to obtain substantive information. Each explanatory variable alone showed linearity on the carotenoid concentration but they differed from each other (Friedman ANOVA, p=0.0253). Thus, the aim was set to find out the best subset of explanatory variable(s) with the strongest contribution rather than attaining a forecasting statistic. This was achieved best with the (forward) stepwise elimination procedure since it allows the inclusion of all variables initially and then removes the variable that contributes the least (usually that with the smallest partial correlations) to explaining the response variable (Carotenoid concentration), until explanatory variable(s) remaining in the model having a significant partial correlation coefficient (Table 4) [47]. As for our data, it was found that DPPH mechanism was excluded but the FRAP mechanisms remained in the model indicating its significant contribution on the carotenoid concentration (R^2= 0.998, p = 0.035). Semipartial correlations of FRAP and DPPH were 0.965 and 0.865, respectively, indicating also the approval of selection. Cook's distance values were used to detect the influential data observed in linear regression, and analysis revealed that residuals of all observed cases were validated by their accepted values within the model except for case 1 (concentration of 2 mg mL^{-1}), which was excluded. Overal, it could therefore be concluded that the effect of antioxidant activity of

carotenoids in the *S. obliquus* occurred predominantly by the mechanisms exerted via reduction of metal ions (FRAP).

Table 4. *Multiple regression results between dependent (S. obliquus extract concentration) and independents (DPPH and FRAP values). Significant values were shown in bold*

	Beta	**Std.Err.**	**B**	**Std.Err.**	**t(2)**	**p-level**
Intercept			2.44	1.30	1.88	0.20
DPPH	0.32	0.13	0.75	0.31	2.45	0.13
FRAP	0.68	0.13	1.58	0.30	5.18	**0.04**

Regression Summary for Dependent Variable (*S. obliquus* extract concentration)
$R = 0.999$, $R^2 = 0.998$, Adjusted $R^2 = 0.997$
$F(2,2)=851.65$, $p<0.001$

It is also clearly understood that considering the DPPH assay, the methanolic extracts of *S. obliquus* exhibited markedly low radical scavenging activity compared to the standards, BHT and ASC. Considerably high amounts of carotenoid extracts (i.e. 100mg mL^{-1}) reached only to a maximum of 42.69% scavenging ability (IC_{50}:115.62 mg mL^{-1}) whereas standards of ASC (IC_{50}: 0.21 mg mL^{-1}) and BHT (IC_{50}:1.39) had strong scavenging effects around 80%) despite their small amounts i.e. 0.1 and 3.0 mg mL^{-1}, respectively.

The FRAP assay also generated similar results; the effect of carotenoid extract being the lowest (i.e. 0.68 at 100 mg mL^{-1}) followed by BHT and ASC (0.65 and 1.39 at 0.1 mg mL^{-1}), respectively. Higher antioxidant effects of standards compared to the sample may be an expected result as it is often found in previous studies. For example, Hu et al., 2008 [48] utilized DPPH assay and found that antioxidant capacities of the algal (*Dunaliella salina*) carotenoid extract was relatively lower than α-Tocopherol used as a standard. But, still the extent of the difference between algal carotenoids extracts and standards is so high and this may make our species, *Scenedesmus obliquus*, doubtful about its efficiency or its preference over chemical counterparts in practical applications. Despite considerable amount of total carotenoids, the composition consisting of only trans forms (All-*E* isomers) and/or lacking cis forms (Z isomers) might be the underlying reason for the relatively low antioxidant activity of *Scenedesmus obliquus.* As demonstrated by numerous studies [49, 50, 51], antioxidant activity showed variations between both isomers of the same compound as well as between different compounds of the carotenoid. In a well documented study by Honda et al., 2019 [52], the supporting results from researches are overvhelming to conclude that the Z isomers display antioxidant activity as well as bioavailability and functionality greater than or equal to those of all-*E* counterparts in carotenoids. Especially, Z isomers of lutein are explicitly evidenced in favour of higher antioxidant activities. Consequently, the results about antioxidant potential in our study are susceptable to the composition of carotenoids that has occurred in specific cultur conditions and timing of harvesting as much as extraction solution and the type of antioxidant assay deployed. The study of Guedes et al., 2011 [44] supports well the variations of compounds and changes in antioxidant activity throughout the

culture. They proved that antioxidant activity of *S. obliquus* showed variations during 17 days of incubation period, depending on the variations occurring in the rate of carotenoid compounds. Despite no isomer data was given, in another study conducted by Guedes et al., 2013 [53], particularly the high lutein content (*i.e.*, 2.69±0.09 mg g^{-1}) recorded in the *Scenedesmus obliquus* M2-1 extract was responsible for the observed high antioxidant capacity but together with the putative synergistic effect with β-carotene and neoxanthin recorded to a lesser amount. They also demonstrated that the intracellular extracts of *Scenedesmus obliquus* outperformed those of 18 species of cyanobacteria (prokaryotic microalgae) and 23 species of (eukaryotic) microalgae almost four fold the highest value in total antioxidant activity to scavenge ABTS. Hu et al., 2008 [48] found that the antioxidant activity of carotenoid extract from *D. Salina* was higher than the each all-trans forms carotenes such as all *trans*-zeaxanthin, all *trans*-lutein, all *trans*-β -carotene and all *trans*-α-carotene used as standards. They commented that this was caused by the cis forms of carotenoids existed additionally in the total carotenoids content of *D. salina*. Similar findings were presented also by other studies conducted on algae [54] and on vegetables and fruits [55]. Regarding the few but succesful findings present in the literature, the evaluation of our results on the antioxidant ability of this species could be made more sufficient by further attempts that would consider certain other factors such as culture conditions, extract solution, isomerization and different antioxidant assays.

CONCLUSION

The amount of total carotenoids that the string of a local species, *Scenedesmus obliquus*, contained could be considered as sufficient, relying upon comparisons to other species of *Scenedesmus* sp., or some other microalgal species. Very high domination of lutein and β-caroten in the composition of total carotenoids ascertained for the species might be due partly to conditions selected under study such as growth, extraction and harvesting time which were of significance in variations.

Relatively low antioxidant capacity of the extract compared to standards was attributed to the absence of *cis*-isomers of the compounds, inducing further studies that would encompass an understanding on the mechanism of isomerisation and its control for practical usage in order to maximize both the amount and effect of the targeted compound.

Conflict of Interest. The author declared that there is no conflict of interest.

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