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Invasive Biomass Algae Valorization: *Rugulopteryx okamurae* as a Sustainable Source of Natural Antioxidants

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ABSTRACT

Pigments content, total phenolics (TPC) and total flavonoids (TFC), as well as the antioxidant activity (AA) of ethyl acetate (EtOAc), methanol, and chloroform extracts of *Rugulopteryx okamurae* were measured to find novel potential sources of natural pigments and antioxidants. The extraction of chlorophylls (a, b, c, and d), carotenoid, fucoxanthin, and phycobiliproteins was carried out using six solvents, namely acetone 90%, dimethyl sulfoxide-water (4: 1, v/ v), pure methanol, and pH 6.8 phosphate buffer, respectively. TPC and TFC were assessed using the Folin Ciocalteu and aluminum chloride assays, respectively, while, the antioxidant activity was assessed by using two different methods: DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and ABTS [2,20-azino-bis-(3-ethylbenzothiazoline-6- sulfonic) acid)]. The findings revealed a high content of chlorophyll a [263.87± 5.88µg/ g DW (dry weight)] and fucoxanthin (18.54± 0.73µg/ g DW) in *R. okamurae*. In addition, this species presented values as high as 17.67± 0.13mg GAE (gallic acid equivalent) / g and 42.99± 0.46mg QE (quercetin equivalent) / g, of phenolic and flavonoid contents, respectively. The antioxidant activity of *R. okamurae* extracts in both assays was highest in the most polar solvents: methanol≥ EtOAc> chloroform. There was a negative correlation between TPC and DPPH (r= -0,4888) test, and a moderate positive correlation with ABTS test (r= 0,4888), while there was a strong positive correlation between ABTS assay and carotenoid (r= 0,8735). In conclusion, the *R. okamurae* extract is a potential natural source of natural pigment and antioxidants.

INTRODUCTION

Non-native or introduced species are identified as one of the main threats to the resource values of the world's oceans and marine biodiversity **(Sagoff, 2005; Schaffelke *et al.*, 2006; Molnar *et al.*, 2008; Galil *et al.*, 2018; Vega *et al.*, 2023)**. They can cause significant ecological impacts, affecting natural habitats, trophic relations and community structures, thereby reducing the growth potential of indigenous species **(Viard & Comtet, 2015; Sempere-Valverde *et al.*, 2021)**. Additionally, huge blooms caused by certain invasions could result in major implications not only for economic sectors but also for human health **(Perrings, 2002; Davidson *et al.*, 2015; Kourantidou *et al.*, 2021)**.

The brown alga *Rugulopteryx okamurae* (E.Y.Dawson) I.K.Hwang, W.J.Lee and H.S.Kim was described for the first time from Japan as *Dilophus okamurae*; it is originating from the northwestern temperate Pacific Ocean, particularly Korea and Japan **(Guiry & Guiry, 2023)**. This alga was first documented in the Mediterranean Sea (Lagoon Thau, France) by **Verlaque *et al.* (2009)**. Nowadays, this species is considered one of the newest non-native example species **(Faria *et al.*, 2022a, b; Bernal-Ibáñez *et al.*, 2022)**. In recent years, there has been an increasing interest in the algal biodiversity of Morocco **(Hassoun *et al.*, 2015, 2016a, b, 2018, 2023)**. But the first population of the brown seaweed *R. okamurae* was detected on the Moroccan coast in the Strait of Gibraltar in August 2017 **(El Aamri *et al.*, 2018; El Madany *et al.*, 2024)**, and the first local bloom was observed in M'diq in 2018. The formation of algal blooms and invasive algae (such as *R. okamurae*) can be exploited as a biomass source for the isolation of bioactive substances for various uses **(Pinteus *et al.*, 2018; Pereira *et al.*, 2021; Agabo-García *et al.*, 2023; Vega *et al.*, 2023)**. This could serve as a management measure to find new bioactive compounds from widely disposable biomass and, then contribute to the attenuation of the harmful impacts generated by non-indigenous algae. In the case of *R. okamurae*, there are only a limited number of works on biochemical composition or bioactivities. Generally, terpenoids were almost the only substances extracted in this invasive species **(Suzuki *et al.*, 2002; Paula *et al.*, 2011; Casal-Porras *et al.*, 2021; Cuevas *et al.*, 2021; Vega *et al.*, 2023)**. The chemical composition of *R. okamurae* reveals that this species is rich in carbonated compounds (lipids, total carbon, CHOP, and CHO) and molecules, with a wide range of applications in pharmaceuticals (e.g., anti-inflammatory or antitumoral), biomaterial, antibacterial in addition to other utilities that were discovered **(Vega *et al.*, 2023)**.

The literature review exhibited no data on the pigments contents, phytochemicals contents, and antioxidant activities of the invasive alga *Rugulopteryx okamurae*. Hence, the originality of our work, which had as goals (i): To determine pigments contents (chlorophylls, fucoxanthin and phycobiliproteins), the total flavonoids, and phenolic contents in three various extracts of *R. okamurae*, and (ii): The evaluation of the antioxidant activities of these extracts through two different techniques, notably free radical scavenging assays DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and ABTS [2,20- azino- bis- (3- ethylbenzothiazoline- 6- sulfonic) acid)].

MATERIALS AND METHODS

1. Sampling

Rugulopteryx okamurae (Ochrophyta) was harvested in April 2021 from 0.5- 1m depth at the Tetouan coastal region, M'diq, Morocco, with an altitude of 35°40′59′39′N/5°19′0′18 W), as shown in Fig. (1). The identification of this brown alga was carried out in the laboratory of Applied Phycology-Mycology, Faculty of Science, Tetouan, based on the anatomical and morphological characteristics, using light microscopes (Olympus, Tokyo, Japan) and stereo-microscope OZL 451 KERN (China). After rinsing with clean water to remove salt and debris, *R. okamurae* was dried in an oven at 60°C for 24h and broken down into powder via a blender. Afterward, it was conserved in plastic bottles at room temperature (RT) until use.

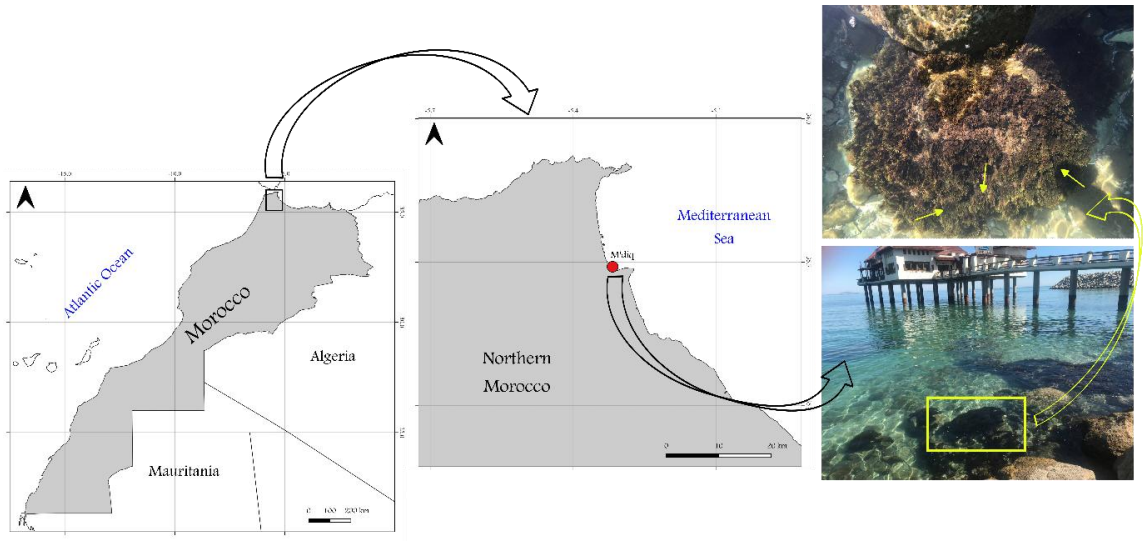

Fig. 1. Map sampling site (M'diq 35°40′59′39′N/5°19′0′18 W)

2. Pigment content

To determine the pigment content, chlorophylls (carotenoids, fucoxanthin, phycobiliproteins) & various solvents (10mL each) were used. Solvents were chosen on the basis of their affinity for each category of pigments, and the samples were shaken for 1.0min at room temperature. Mass of *R. okamurae* used in this study was 100mg of dry macroalgae (DW). The chlorophylls (a, b, c, and d), fucoxanthin and total carotenoids were extracted using acetone 90%, pure methanol and DMSO-water (4: 1, v/ v), respectively. The polar pigments phycobiliproteins (phycocyanin and phycoerythrin) were extracted using phosphate buffer of pH 6.8. The homogenates were centrifuged at 2500r/ min for 10min, and the obtained supernatant was processed by another centrifugation (5000r/ min for 5min), while the absorbance of the supernatants was measured spectrophotometrically measured as follows: chlorophylls at 630, 647, 664, 691, and 750nm, total carotenoids at 480 and 750nm, fucoxanthin at 480, 631,582, 665, and 750nm, as well as phycoerythrin and phycocyanin at 565, 618, and 750nm. The

concentrations were determined using equations provided by **Connan (2015)** and **Osório *et al.* (2020)**.

1. 100% Methanol (carotenoids)

1.1. Carotenoids (µg/ mL) = 4 × (A480 - A750)

2. 90% Acetone (chlorophylls)

2.1. Chl *a* (µg/ mL) = - 0.3319 × (A630 - A750) - 1.7485 × (A647 - A750) + 11.9442 × (A664 - A750)
- 1.4306 × (A691 - A750) (±0.0020)

2.2. Chl *b* (µg/ mL) = - 1.2825 × (A630 - A750) - 19.8839 × (A647 - A750) - 4.8860 × (A664 - A750)
- 2.3416 × (A691 - A750) (±0.0076)

2.3. Chl *c* (µg/ mL) = 23.5902 × (A630 - A750) - 7.8516 × (A647 - A750) - 1.5214 × (A664 - A750)
- 1.7443 × (A691 - A750) (±0.0075)

2.4. Chl *d* (µg/ mL) = - 0.5881 × (A630 - A750) + 0.0902 × (A647 - A750) - 0.1564 × (A664 - A750)
+ 11.0473 × (A691 - A750) (±0.0030)

2.5. Total Chl (µg/ mL) = Chl*a* + Chl*b* + Chl*c* + Chl*d*

3. DMSO-Water (4: 1, *v*/ *v*) (fucoxanthin)

3.1 Fucoxanthin (µg/ mL) = 7.69 × (A480 - A750) - 5.55 × [(A631 - A750) + (A582 - A750) - 0.297 × (A665 - A750)] - 0.377 × (A665 - A750)

4. Phosphate buffer (pH= 6.8) (phycoerythrin and phycocyanin)

4.1 Phycoerythrin (µg/ mL) = $\frac{A565-A750}{2.41\times10^6} \times 240{,}000 \times 10^3$

4.2 Phycocyanin (µg/ mL) = $\frac{A618-A750}{1.90\times10^6} \times 264{,}000 \times 10^3$

3. Total phenolic content (TPC), total flavonoids content (TFC), and antioxidant activities of R. okamurae extracts

3.1. Bioactive compounds extraction

The extraction of samples was made using ethyl acetate (EtOAc), methanol, and chloroform (different polarities) with comparison 1: 10w/ v, subsequently it was agitated over 72h at room temperature (RT). The extracts were concentrated by removing the solvent with a rotary vacuum evaporator (40°C). Three crude extracts were obtained at the end of the extraction operation. Afterward, they were subsequently weighed to compute the yield of the extraction for each solvent and conserved in a refrigerator (4°C) in airtight bottles until use for later tests.

3.2. Chemicals and reagents

2,2- Diphenyl- 1- picrylhydrazyl (DPPH), 2,20- azino- bis- (3- ethylbenzothiazoline- 6- sulfonic) acid (ABTS), dimethyl sulfoxide (DMSO), L- ascorbic acid and butylated hydroxytoluene (BHT) were purchased from Sigma (St. Louis, MO, USA). Folin- Ciocalteu phenol reagent and standards (quercetin, gallic acid) were obtained from Merck Life Science (Merck KGaA, Darm- stadt, Germany). Phosphate buffer (pH 6.8) was prepared with 10mM KH2PO4 and 10mM NaOH. All other chemicals were of analytical grade and obtained from Sigma.

3.3. Analysis and quantification of phenolic contents

TPC of *R. okamurae* extracts was measured by the Folin-Ciocalteu colorimetric method, as outlined by **Singleton and Rossi (1965)**. Briefly, 100µL of the diluted extracts were dosed 500µL of the Folin-Ciocalteu reagent (10%), after 5min at RT, 400µL of 7.5% saturated aqueous sodium carbonate (Na_2CO_3) was dosed and mixed. The obtained solution was then carefully homogenized and incubated for 2h at RT. The absorbance was calculated at 765nm after incubation. Gallic acid was employed to determine the standard curve (10– 100µg/ ml). The evaluation of the phenolic compounds was conducted in triplicate. The findings were represented as mg of gallic acid equivalents (GAE) per gram of *R. okamurae* extract.

TFC of *R. okamurae* extracts was assessed by the aluminum chloride ($AlCl_3$) method employing Quercetin as the standard compound. Shortly, 1.0ml of each sample was added to 1ml of 2% $AlCl_3$. These mixtures were incubated in the dark at RT and after 40min the absorbance was determined at 415nm. TFC was quantified as quercetin equivalent (QE) and given as mg QE/ g dry mass of the sample.

3.4. Antioxidant activity (DPPH and ABTS)

Antioxidant activity (AA) of the studied samples was assessed using two complementary techniques on the basis of the free radical scavenging activity.

The ABTS assay was conducted, following the method outlined by **Re *et al.* (1999)**. The ABTS radical cation (ABTS+•) was obtained by a reaction of 2.45mM $K_2S_2O_8$ (potassium persulphate) aqueous solution and 7mM ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) aqueous solution. This mixture was then stored for 12 to16 hours at room temperature to guarantee the complete development of the radical. ABTS+• solution was diluted with methanol until the absorbance at 734nm was around 0.7± 0.02. For the reaction, 0.1mL of the samples was added to 3.9mL of the diluted ABTS+•. The solution was incubated for 10 minutes at room temperature and darkness, then the absorbance was calculated at 734nm. The ABTS radical scavenging activity was calculated as the concentration of the sample required to achieve a 50% decrease in the sample absorbance (IC_{50}), using ascorbic acid as a standard, as follows:

$$\text{ABTS Scavenging effect \%} = \frac{A0 - A1}{A0} \times 100$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

The DPPH assay was carried out, following the method of **Brand-Williams *et al.* (1995)**, with a few minor modifications. For the reaction, 200µL of the samples was mixed with 1.8mL of the DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (0.11mM of DPPH in methanol). After 30 minutes of incubation at room temperature and darkness, the absorbance was determined at 517nm, using a blank consisting of methanol and DPPH. The butylated hydroxytoluene (BHT) was employed as a standard. DPPH scavenging activity was calculated as the concentration of sample required to achieve a 50% decrease in the sample absorbance (IC_{50}).

4. Statistical analysis

All data were analyzed using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA). Three replicates were conducted for each experiment, and the findings are presented as the mean of three measurements ± SD (standard deviation). Differences between groups were compared using a one-way analysis of variance (ANOVA), followed by Tukey test. The correlation matrix was performed on average values with Statgraphics package version XVII using R software (R Core Team, 2017). Differences were accepted as significant when $P< 0.05$.

RESULTS AND DISCUSSION

1. Pigment analysis

The contents of chlorophylls (a, b, c, and d), carotenoids, fucoxanthin, phycoerythrin, and phycocyanin of *R. okamurae* are shown in Table (1). The findings revealed that *Rugulopteryx okamurae* extracts were rich in chlorophyll a [263.87± 5.88µg/ g DW (dry weight)], while the content phycocyanin and phycoerythrin was low.

Table 1. Pigment content of *Rugulopteryx okamurae*

Pigment	Pigment content (µg. g -1 DW)
Chlorophyll a (Acetone 90%)	263.87± 5.88
Chlorophyll b (Acetone 90%)	ND
Chlorophyll C (Acetone 90%)	64.59± 4.09
Chlorophyll d (Acetone 90%)	27.85± 0.84
Total Chl (Acetone 90%)	356.3± 7.58
Carotenoid (100% Methanol)	21.48± 0.06
Fucoxanthin (DMSO-Water (4:1, v/v)	18.54± 0.73
Phycoerythrin PB (pH= 6.8)	0.21± 0.02
Phycocyanin PB (pH= 6.8)	0.15± 0.019

Data are mean ± standard deviation in micrograms of pigment per 1g of algae on a dry weight basis (DW). All determinations were the result of three replicates, each injected three times. SD: Standard deviation.

This is the first work focusing on the pigment constituents of this exotic macroalga *R. okamurae*. It was difficult to compare the obtained results with other reported studies. Table (1) shows that *R. okamurae* has the highest amount of chlorophyll C (64.59± 4.09µg/ g DW), which is a unique characteristic of brown seaweeds since they contain this pigment as a pigment in their photosynthetic membranes in place of chlorophyll b, which is commonly present in higher plants **(Juliadiningtyas *et al.*, 2017)**.

The concentrations of phycoerythrin and phycocyanin in *R. okamurae*, extracted using PB (pH= 6.8), were 0.21± 0.02 and 0.15± 0.019, respectively. These values are lower compared to those found in *Sargassum* sp. hexane and ethanol extracts **(Sanger *et al.*, 2022)**. It is generally known that phycoerythrin and phycocyanin pigments are more abundant in red seaweeds. In brown algae, fucoxanthin is one of the common carotenoids, as observed in other studies **(Miyashita & Hosokawa, 2017)**. Fucoxanthin accounted for 86% of the total carotenoids extracted from *R. okamurae* in this paper. It is higher than the values recorded by **Osório *et al.* (2020)** in *Undaria pinnatifida* and *Laminaria ochroleuca* (49 and 52% of the total carotenoids, respectively), however it is smaller than the finding for *Himanthalia elongate* (96% of the total carotenoids). The value of fucoxanthin was 18.54± 0.73µg/ g DW, higher than the result obtained by **Nunes *et al.* (2019)** for the species *Dictyota dichotoma*, *Halopteris filicina*, *Halo scoparia*, and *Padina pavonica* (12.2± 0.4, 17.3± 0.3, 10.1± 0.3, 10.1± 0.3µg/ g DW, respectively). Thus, the concentration of chlorophylls was higher than the carotenoids concentration and, particularly, than fucoxanthin concentration. The existence of this xanthophyll overlaps the occurrence of other pigments in a microscopic view. Indeed, brown macroalgae have been attracting a wide interest from the researchers due to their richness in this type of pigment, which has been characterized by its biological characteristics **(Maeda *et al.*, 2018)**. This carotenoid plays a significant role in their light harvesting and photoprotection **(Kim *et al.*, 2012)**. Furthermore, it is known by its interesting biological activities, particularly the antioxidant properties that are principally associated with single oxygen species quenching and the free radical scavenging **(Miyashita & Hosokawa, 2017)**. In this context, in the last year, several millions of tons per year of this algae biomass have been removed from the Moroccan shores. This means that a large quantity of biomass is available for the isolation of valuable bio-compounds that could contribute to manage the invasion, for example, the fucoxanthin represents high commercial and pharmaceutical values.

2. Total flavonoids contents and total phenolics

The TFC findings show that chloroform extract of the *R. okamurae* (42.99± 0.46mg QE/ g of extract) contains a greater quantity of flavonoid compared to EtOAc and methanol extracts. Statistically, the TFC of the chloroform extract of *R. okamurae* is significantly higher *($P < 0.05$)* than the TFC of the other extracts.

The TPC in *R. okamurae* extracts was measured according to the Folin Ciocalteu technique. Table (2) indicates that TPC of *R. okamurae* extracts is declining in the following order: EtOAc ≥ chloroform> methanol. In particular, the TPC of the EtOAc extract of *R. okamurae* (17.67± 0.13mg QE/ g of extract) is greater than the methanol and the chloroform extracts, however it is only significantly higher ($P < 0.05$) in comparison with the methanol extract.

Table 2. TPC and TFC of *Rugulopteryx okamurae* extracts

Extract	Polyphenol (mg GAE/ g of extract)	Flavonoid (mg QE/ g of extract)
EtOAc	17.67± 0.13 [a]	34.9± 0.38 [c]
Methanol	9.63± 0.49 [b]	37.28± 0.23 [b]
Chloroform	16.48± 0.63 [a]	42.99± 0.46 [a]

Data are expressed as mean ± SD (n= 3). Different superscript letters (a, b, and c) in same column indicate a significant difference (P< 0.05). GAE: Gallic acid equivalent, QE: Quercetin equivalent.

There is limited published data about the TPC and TFC of *R. okamurae,* and the methods used by authors vary, making it challenging to compare the results presented in this work. The concentration of phenolic compounds observed in *R. okamurae* was in the range of other brown seaweeds species, despite it did not attain the highest values detected in *Cystoseira* sp. or *Sargassum* sp. **(Connan *et al.,* 2004; Zubia *et al.,* 2009; Schneider *et al.,* 2020)**. In comparison to other macroalgae in Moroccan shore, the value of TPC and TFC in *Ericaria amentacea*, *Cystoseira humilis*, *Fucus spiralis* and *Bifurcaria bifurcate* in 70 % ethanol are less than our results in *R. okamurae* (TPC up 17.67± 0.13mg GAE/ g of extract and TFC up 42.99± 0.46mg QE/ g of extract**) (Grina *et al.,* 2020)**. By comparing these findings with the existing studies on *R. okamurae,* the reported values are substantially higher than those recorded by **Lee *et al.* (2021)**, who demonstrated that the values of TPC of the ethanolic extract of *R. okamurae* were 6.75± 0.12 PGE mg/ g; phloroglucinol equivalent, (PGE) in Korea (Jeju-do). A recent paper reported by **López-Hortas *et al.* (2023)** for Bolonia beach (Tarifa, Spain) concerning *R. okamurae* extracts from different drying methods, namely microwave hydrodiffusion and gravity (MHG), showed a total phlorotannins content of 3.0± 0.3mg phloroglucinol/ g DW, which are smaller than our result. In the literature, **Vega *et al.* (2023)** recorded a value of TPC of *R. okamurae* aqueous extract close to our finding. In fact, the variation of TPC and TFC can be due to several reasons, such as extraction method, environmental conditions and portion of the seaweed employed.

3. Antioxidant activity

The antioxidant characteristics of *R. okamurae* were assessed by ABTS and DPPH assays. The results presented as IC_{50} values are listed in Table (3). The antioxidant activity is linked on the redox characteristics of the extracts which facilitate their activity as decreasing agents; such capacity is normally related to the presence of reductants which exert antioxidant activity through destroying the free radical chain by inhibiting peroxide formation or providing a hydrogen atom **(Kumaran & Joel karunakaran, 2006)**. Invasive macroalgae are rich in phenolic molecules. These molecules demonstrated many biological effects, including antioxidant effects. All three extracts of *R. okamurae* demonstrated considerable antioxidant activity, with considerable variability among the organic extracts and the different methods employed (Table 3). Eminently, the methanol extract was the most active in regard to antioxidant activities with IC_{50} values of

17.79± 0.75mg/ mL and 15.99± 0.49mg/ mL obtained by DPPH and ABTS assays, respectively. Comparing the two methods used (Table 3), the ABTS scavenging activity assay was the more sensitive with regards to IC_{50}. The chloroform extract showed the highest concentration of flavonoids and phenolic compounds, however had low antioxidant activity. These findings are in line with previous works **by Sanger *et al.* (2019)** and **Grina *et al.* (2020)**. However, the antioxidant activities were not associated only to phenolic compounds. For instance, the methanolic extract had high antioxidant activity, but with low phenolic content. It is possible that the activities started by other hydrophilic constituents, such as peptides, fucoidan and Maillard reaction products, and it may be correlated to pigments and polysaccharides **(Chattopadhyay *et al.*, 2010)**. The TFC values of *R. okamurae* showed a different trend when compared to the TPC values, which suggests that the other phenolic compounds are involved in the antioxidant activity, while flavonoids are not involved. For example, there are works demonstrating that a phenolic compound known as phlorotannin, extracted from brown algae, has high antioxidant activity, which suggests that phenolic compounds contribute to the antioxidant activity of algae **(Li *et al.*, 2011; Wang *et al.*, 2012)**. In addition, a positive correlation was noticed between the antioxidant activity in the ABTS test and the carotenoid, suggesting the intervention of carotenoid (natural antioxidant) in the antioxidant capacity (Fig. 2).

Table 3. Antioxidant activity of *Rugulopteryx okamurae* extracts

Antioxidant properties (Mean IC50 value mg/ mL ± Standard Deviation)		
Seaweed extract	DPPH	ABTS
EtOAC	22.82± 1.26 [a]	15.75±0.65 [a]
Methanol	17.79± 0.75 [b]	15.99± 0.49 [a]
Chloroform	35.32± 2.01 [c]	27.03± 1.5 [b]
BHT (µg/ mL)	50.78± 8.45 [d]	-
Ascorbic acid (µg/ mL)	-	67.42± 0.3 [c]

Data are expressed as mean ± SD (n= 3). DPPH: 2,2-diphenyl-1-picrylhydrazyl radical, ABTS: 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic) acid, IC50: Concentration of sample providing 50% inhibition. Different superscript letters (a, b, and c) in same column indicate a significant difference *(P*< 0.05).

There are few reports concerning the antioxidant activity of *R. okamurae*. Normally, brown algae display higher antioxidant capacity than green or red ones **(Zubia *et al.*, 2007; Vega *et al.*, 2020)**, which is in line with our findings. **Schneider *et al.* (2020)** in their study reported even lower values than those found in this study. Moreover, **López-Hortas *et al.* (2023)** in their study reported lower inhibition percentage of DPPH (6.1± 0.1%) for the extracts using methods, namely microwave hydrodiffusion and gravity (MHG), which are close to our findings. In addition, the DPPH radical

scavenging activity recorded from the methanol extract of *R. okamurae* in our work (IC_{50}= 17.79± 0.75mg/ mL) was lower than that of *Sargassum* sp. from North Sulawesi (IC_{50}= 2.684± 0.256mg/ mL**) (Sanger *et al.,* 2022)**. Additionally, when compared to the brown seaweed *Padina durvillei*, its methanol extracts demonstrated weaker antioxidant activity in DPPH test (EC_{50} at 54.1mg/ mL) compared to the methanol extract of *R. okamurae* in this work. The antioxidant activity of *R. okamurae* is high than many other seaweed species, e.g., *Ulva expansa*, *Rhizoclonium riparium*, *Caulerpa sertularioides*, *Spyridia filamentosa*, *Gracilaria vermiculophylla*, and *Codiumisabelae* **(Osuna-Ruiz *et al.,* 2016)**. **Vega *et al.* (2023)** have studied the antioxidant capacity of *R. okamurae* aqueous extract; however, the difference in the result expression units made it difficult to compare their results with those of the present work. In addition, numerous works demonstrated that *R. okamurae* is rich in phenolic compounds, which are involved in various activities, including antioxidant, anti-inflammatory and antibacterial properties **(Cuevas *et al.,* 2021; Vega *et al.,* 2023)**.

4. Correlation study

The correlations matrix between the chosen investigated variables is presented in Table (4). Significant negative and positive correlations were found between the investigated dependent variables. The strongest positive correlations were the following: (TPC, TFC), (ABTS, carotenoid), (DPPH, phycoerythrin), (DPPH, chlorophyll C), (chlorophyll a, chlorophyll d), (chlorophyll a, fucoxanthin), (chlorophyll C, phycoerythrin), (total Chl, chlorophyll d), (chlorophyll d, fucoxanthin), (phycocyanin, phycoerythrin), (total Chl, fucoxanthin) and (total Chl, phycocyanin). On the other hand, the most important negative correlations were (TPC, Total Chl), (TPC, phycocyanin), (TFC, chlorophyll d), (TFC, total Chl), (TFC, phycocyanin), (DPPH, carotenoid), (ABTS, chlorophyll C) & (ABTS, phycoerythrin). The remaining correlations were low or insignificant. Similar correlations among phenolics and both antioxidants and pigments contents were found in other studies **(Osuna-Ruiz *et al.,* 2016; Vega *et al.,* 2020)**. Interestingly, the good correlation observed between ABTS and carotenoid contents suggests that carotenoid may be implicated in this activity of this alga. In addition, the low positive correlation between TPC, TPC/ABTS, DPPH, suggests that phenolic and other non-phenolic compounds present in extract exhibit antioxidants activity.

Table 4. Correlation coefficients among studied parameters

	TPC	TFC	DPPH IC50	ABTS IC50	Chlorophyll a	Chlorophyll C	Chlorophyll d	Total Chl	Carotenoid	Fucoxantin	Phycoerythrin	Phycocyanin
TPC		0,9925	-0,4888	0,4888	-0,7407	-0,598	-0,815	-0,9881	0,0022	-0,8002	-0,7348	-0,9833
***P*-value**		0,0783	0,6748	0,6748	0,469	0,5919	0,3934	0,0981	0,9986	0,4095	0,4745	0,1163
TFC			-0,3782	0,3782	-0,8175	-0,4952	-0,8799	-0,9995	-0,1204	-0,8677	-0,6461	-0,9536
***P*-value**			0,7531	0,7531	0,3908	0,6702	0,3152	0,0199	0,9231	0,3312	0,5528	0,1946
DPPH IC50				-1	-0,2241	0,9915	-0,107	0,3491	-0,8735	-0,132	0,9509	0,6392
***P*-value**				0	0,8561	0,0829	0,9317	0,773	0,3237	0,9157	0,2003	0,5585
ABTS IC50					0,2241	-0,9915	0,107	-0,3491	0,8735	0,132	-0,9509	-0,6392
***P*-value**					0,8561	0,0829	0,9317	0,773	0,3237	0,9157	0,2003	0,5585
Chlorophyll a						-0,0955	0,993	0,835	0,6702	0,9956	0,0885	0,6062
***P*-value**						0,9391	0,0756	0,3709	0,5324	0,0596	0,9436	0,5854
Chlorophyll C							0,023	0,4679	-0,8028	-0,0021	0,9831	0,7337
***P*-value**							0,9853	0,69	0,4067	0,9986	0,1173	0,4755
Chlorophyll d								0,8943	0,5776	0,9997	0,2059	0,6962
***P*-value**								0,2953	0,608	0,016	0,868	0,5098
Total Chl									0,1513	0,8828	0,622	0,9438
***P*-value**									0,9033	0,3113	0,5727	0,2145
Carotenoid										0,598	-0,6799	-0,1839
***P*-value**										0,592	0,524	0,8822
Fucoxantin											0,1812	0,6779
***P*-value**											0,884	0,5258
Phycoerythrin												0,8458
***P*-value**												0,3582
Phycocyanin												
***P*-value**												

CONCLUSION

The present study has showed that the brown macroalga *R. okamurae* constitutes a promising raw material for pigment production. It has been observed that *R. okamurae* compounds are suitable for characterizing antioxidant activity. The use of *R. okamurae* compound as a new resource could be a suitable alternative for valorizing the algal biomass, helping us to decrease the negative impact of this invasive alga in the Moroccan Mediterranean. This study gives value to this invasive algae, transforming a challenge into an opportunity for obtaining new products with remarkable potential for uses in several fields of research and in various industries. However, further research and improvement will be required in the future to extract other molecules from *R. okamurae* in Morocco. In addition, it would be useful to test other types of activity on *R. okamurae*, such as anti-inflammatory, antibacterial and anticancer activity.

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