Heliyon 8 (2022) e12457

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Physico-chemodiversity variation between the most common calcareous red seaweed, Eastern Harbor, Alexandria, Egypt

Abeer A.M. El-Sayed, Mona M. Ismail *

National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt

ARTICLE INFO

Keywords:
Calcified algae
FTIR
GCMS
Protein fraction
Swelling capacity
Water retention capacity

ABSTRACT

The present study sought to detect the difference in physicochemical properties and the proximate compositions of common calcareous red seaweeds "*Corallina officinalis, Jania rubens*, and *Amphiroa rigida*". *J. rubens* was recorded highly water (WHC), and oil holding capacity (OHC) (1.619 ± 0.08 g g^{-1} DW, 3.1 ± 0.50 g g^{-1} DW, respectively) than those other species relating to the hydrophilic nature of polysaccharides, whereas swelling water capacity (SWC) was higher in *A. rigida* (5.25 ± 0.38 mL g^{-1} DW). A higher value of carbohydrates (49.88 ± 2.56% DW) was observed in *J. rubens*, Contrariwise, protein (36.99 ± 1.36% DW) and lipid contents (5.85 ± 0.49% DW) were higher in *C. officinalis*. Albumin and protamine protein fractions were higher in *J. rubens* specimens (45.11 ± 2.29 mg g^{-1} & 0.0014 ± 0.0007 mg g^{-1} DW, respectively) than other species. While globulin and glutilin (31.70 ± 1.90 mg g^{-1} DW & 41.93 ± 2.20 mg g^{-1} DW, respectively) were high in *A. rigida*. Contrariwise, insoluble protein fraction was high in *C. officinalis* (9.50 ± 0.50 mg g^{-1} DW). *J. rubens* specimens were recorded maximum values of the photosynthetic pigments. The different surface types and elemental analysis of three species were examined by Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX). From fourier transform infrared (FTIR) spectroscopy the S–S stretching peak of disulfides group at 462 cm^{-1} was the fingerprint of *J. rubens*. From GC-MS data *A.rigida* possesses 16 bioactive components with biological properties. As a result, *J. rubens* and *A. rigida* could be employed as an ingredient in functional foods and drug manufacture.

1. Introduction

Macroalgae (seaweed) are classified into three groups as red (Rhodophyta), green (Chlorophyta), and brown (Ochrophyta, Phaeophyceae) algae. Red seaweeds are characterized by red color due to the dominance of phycoerythrin and phycocyanin, which mask other pigments like Chlorophyll *a*, β-carotene, and other xanthophylls (Lewis and McCourt, 2004). They are the dominant species (660 spp.) in Mediterranean Sea comparing with green species (180 spp.) and brown species (280 spp.). Globaly, there are nearly 6000 species (Rashad and El-Chaghaby, 2020). They had higher carbohydrate and protein content than other groups (Ismail et al., 2017; El Zokm et al., 2021) and they are composed of calcium (25–33%) and magnesium carbonates (1.7–3.3%) (Blunden et al., 1977). Moreover, they have been used as a novel source in nutritional and medical industries due to their bioactive compounds, which are characterized by their antimicrobial, antiviral, antioxidant, anti-inflammatory, and anticancer properties (Ismail et al., 2020). In addition to they are renewable sources of phycocolloids, and thickening agents like agar and carrageenan which, have for various economic applications. Corallinaceae and Gigartinaceae species are one of the main biomass of red algae worldwide. Especially, the Corallinales is a distinctive and diverse order of algae, distributed in marine wave-exposed littoral and sublittoral habitats around the world (Johansen, 1976) and comprising both crustose and geniculate (articulated) forms. Ecologically, they are known to inhabit distinct zones in the littoral and sublittoral and provide niches for many other species (Kinzie and Buddemeier, 1996). Calcareous red seaweed (phylum Rhodophyta, class Florideophyceae, subclass Corallinophycidae) are receiving renewed attention across the ecological and geological sciences as important macroorganisms in the context of global environmental change, especially ocean acidification (OA). It is calcification in the cell wall of the coralline algae that enables provision of these ecosystem components (Nash et al., 2019). Primarily, they are composed of calcium (25–33%) and magnesium carbonates (1.7–3.3%) (Blunden et al., 1977).

The physicochemical properties of algae like water holding capacity (WHC), swelling water capacity (SWC) and oil holding capacity (OHC)

* Corresponding author.
E-mail addresses: mm.esmail@niof.sci.eg, mona_es5@yahoo.com (M.M. Ismail).

https://doi.org/10.1016/j.heliyon.2022.e12457
Received 28 July 2022; Received in revised form 27 September 2022; Accepted 10 December 2022

varied between species that are mainly responsible for their physiological effects (Elleuch et al., 2011).

The current study aims to detect the differences in the proximate compositions and physicochemical properties (WHC, SWC and OHC) of the three common red calcified seaweeds from Eastern Harbour, Alexandria coast, Egypt. In addition to describe their function groups and biological compounds by Fourier Transform Infrared (FTIR) and Gas Chromatography–Mass Spectrometry (GC-MS). Also, Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX) were used for morphological characterization, and elemental identification, respectively to assess their potential usage as a food ingredient and/or nature drugs.

2. Material and method

2.1. Collection and identification of calcareous species

Common calcareous seaweeds "*Corallina officinalis* Linnaeus, *Jania rubens* (Linnaeus) Lamouroux, and *Amphiroa rigida* J.V. Lamouroux" were handpicked from Eastern Harbour, Alexandria coast, Egypt (S1–S5: 29° 885′–29° 905′E - 31° 202–31° 211′ N) during spring 2020 and immediately washed with seawater to remove the extraneous foreign particles, sand particles and epiphytes. Then it was kept in an ice box containing ice until transported to the laboratory and washed with tap water to remove the salt on the surface of the sample. Some of the collected samples were preserved in 4% formalin for taxonomical identification according to (Aleem 1993; Jha et al., 2009). Other collected samples were dried by spreading on blotting paper at room temperature until constant weight, and ground into powder then preserved in dark at 4 °C for further investigation.

2.2. Morphological description

Morphological characterization (length, thickness, color, branching) was carried in the same day of collection according to Cassano et al. (2012).

2.3. Estimation the physicochemical properties

Swelling capacity (SWC) of the dried seaweeds was determined by following the method according to Gómez-Ordóñez et al. (2010) and expressed as mL g^{-1} DW using the following equation:

SWC = Initial volume of water (mL) - Volume of water after incubation (mL)

Water retention capacity (WHC) and oil holding capacity (OHC) were measured based on Yaich et al. (2011) a method as g g^{-1} DW using the following equations:

WHC = Wet weight of the sample (g) - Dry weight of the sample (g)

OHC = Initial volume of oil (g) - Volume of oil after incubation (g)

2.4. Chemical analysis of the selected species

2.4.1. The proximate compositions

Proximate analysis of the collected algae; carbohydrate, total protein, and lipid were done following Dubois et al. (1956); Lowry et al. (1951) and Daneshvar et al. (2018) methods, respectively.

2.4.2. The protein fractionation

Protein fractionation was carried out according to the method of El-Sheekh et al. (2014).

2.4.3. The pigment contents

Chlorophyll *a* (Chl-*a*), and total carotenoid content (TCC) in the acetone extracts were measured by a UV-VIS spectrophotometer at wavelengths of 630 and 664 nm; and 450 nm and expressed as (μg g^{-1} fresh wt) according to Jeffrey and Humphrey (1975); Chan and Matanjun (2017) method. Then they were calculated using the following equations:

$$\text{Chl } a = 11.47\,A664 - 0.40\,A630$$

$$\text{Carotenoid content} = \frac{A450}{2500}$$

Where A is the absorbance values of the sample at, 630, 664, and 470 nm, respectively; 2500 is the extinction coefficient in hexane).

The polar pigments phycoerythrin (PE), allophycocyanine (APC) and phycocyanin (PC) from the tested algae were extracted with phosphate buffer pH 6.8 (Beer and Eshel, 1985) and calculated as μg g^{-1} Fw using the following formula:

$$\text{PC} = \frac{(A615)\text{X}\ (0.475X\ A652)}{5.34}$$

$$\text{APC} = \frac{(\text{A652})\text{X}\ (0.208\text{X A615})}{5.09}$$

$$\text{PE} = \frac{(\text{A562}) - (2.41\text{X PC}) - (0.849\ \text{XAPC})}{9.62}$$

Where: A_{562}, A_{615} and A_{652} = absorbance values at 562, 615 & 652 nm, respectively.

2.5. Scanning electron microscopy (SEM) &energy dispersive X-ray spectroscopy (EDX)

The surface morphology of the selected algae was analyzed using a scanning electron microscopy (SEM, JEOL JSM 5500 LV) under a vacuum running at 25.0 kV and using a tungsten filament. The major elemental components of these seaweeds were determined using the attached to Hitachi SU 8020 UHR field emission scanning electron microscopy.

2.6. Fourier transform infrared (FTIR) spectroscopy

The main functional groups in the collected seaweeds were characterized using FTIR. The dried samples were ground with KBr powder and pressed into pellets for FTIR spectra measurement in the frequency range of 400–4000 cm^{-1}.

2.7. Gas Chromatography–Mass Spectrometry (GC–MS)

The phytochemical compositions of the methanolic algal extract were determined using GC–MS (Agilent 6890 GC coupled to an Agilent 5975 quadrupole mass detector). The spectrum of the unknown compounds was compared with the spectrum of the known compounds stored in the WileyRegistry8e Library. The name, molecular weight and peak area of the compounds of the test samples was confirmed.

2.8. Statistical analysis

All determinations were determined three time, the data were expressed as means of dry weight ± standard deviations. One-way analysis of variance (ANOVA) was carried out to assess for any significant differences between the means at ($P < 0.05$) level.

3. Results & discussion

3.1. Morphological description

The collected red species follow Order: Corallinales, Family: Corallinaceae. There are some significant differences between them like the length, thickness, branching and color of thallus as shown in Figure 1. *Corallina officinalis*; thallus up to 3 cm high, stiff, colour purple-red pink,

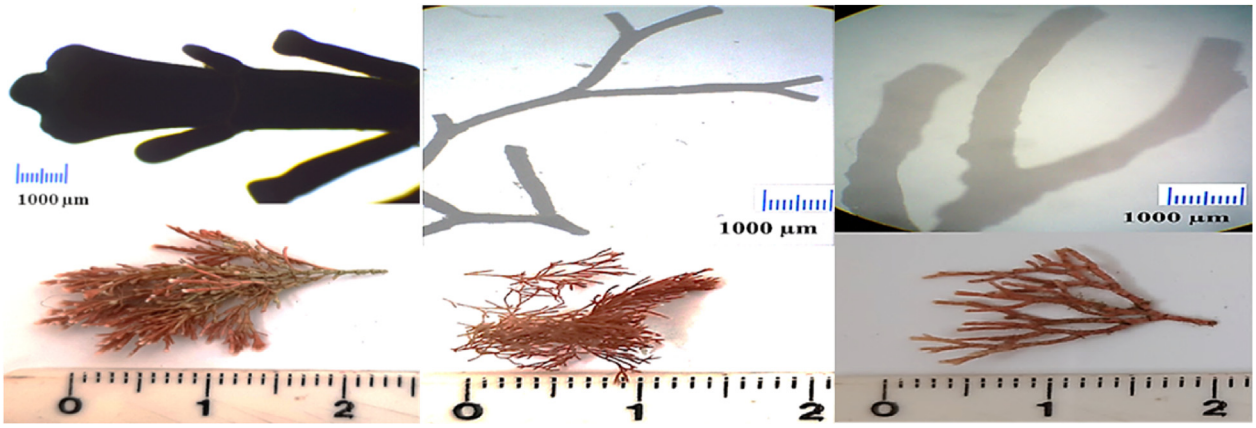

Figure 1. Morphological and microscopic description of the collected algal species.

erect, calcified and pinnately dichotomous branch. The stem appears jointed with bone like. The thallus of *Jania rubens* is characterized rose-pink in colour, up to 1–1.5 cm, tufted, calcified, segments and branching repeatedly dichotomously branched and not opposite. *Amphiroa rigida* thallus is thickener (1000–1200 µ) than the both collected species (200–500 µ), the length up to 2 cm, erect, dichotomously branched and pinkish red in colour (Cassano et al., 2012; Ismail and Osman, 2016).

3.2. The physicochemical properties

Table 1 shows the SWC, WHC, and OHC of *C. officinalis, J. rubens,* and *A. rigida*. The physicochemical content showed statistically significant ($p < 0.05$) differences between the three species. *J. rubens* was recorded highly WHC, and OHC (1.619 ± 0.08 g g^{-1} DW, 3.1 ± 0.50 g g^{-1} DW, respectively) than those other species. The WHC related to the hydrophilic nature of the charged algal polysaccharides such as agar and carrageenan as well as protein which links to the cell wall of polysaccharides of *J. rubens* (Benjama and Masniyom, 2011) and higher neutral sugar content found in the soluble dietary fibers of *J. rubens* (Aroyehun et al., 2019; Gómez-Ordóñez et al., 2010). However, higher OHC of *J. rubens* might be due to the physical trapping of oil by capillary attraction (Aroyehun et al., 2019). While, higher content of SWC (5.25 ± 0.38 mL g^{-1} DW) in *A. rigida*, this owing to the anatomy, the physiological characteristics of its fiber and chemical composition of this specie (Benjama and Masniyom, 2012). The values of SWC (5 ± 0.26 mL g^{-1} DW) of *C. officinalis* and the OHC of *J. rubens* (3.1 ± 0.50 g g^{-1} DW) were similar to SWC and OHC of *Gracilaria changii* (5.00 ± 0.28 mL g^{-1} DW & 3.11 ± 0.60 g g^{-1} DW, respectively) as reported by Chan and Matanjun (2017). The SWC of *A. rigida* (5.25 ± 0.38 mL g^{-1} DW) was nearly similar to *Gracilaria fisheri* (5.22 ± 1.14 mL g^{-1} DW was recorded by Benjama and Masniyom (2012). From our result, *J. rubens* might be utilized in food industry as a source for producing low-calorie food and improving of viscosity of formulated food.

3.3. Chemical analysis of the collected species

3.3.1. The proximate composition

The chemical composition of the tested red species (%DW) is presented in Table 2. Carbohydrates, protein, and lipid contents were significantly different between the tested red species at $P < 0.05$. The content of carbohydrates of the tested species was ranged from (42.02 ± 1.39–49.88 ± 2.56%), in that the maximum value of carbohydrates was observed in *J. rubens* (49.88 ± 2.56%), this might be relate to higher phycocolloid (agar and carrageenan) content in these cell wall (Parthiban et al., 2013). However, the minimum content of carbohydrates was found *in A. rigida* (42.02 ± 1.39%) this due to *A. rigida* thallus is thickener (1000–1200 µ) than the both collected species as illustrated in morphological study, the extensive growth of thallus of seaweeds and the high carbonate content in the calcareous species (Ismail et al., 2017).

The protein content of the selected algae in the range of protein value of red algae (10–47%) (Chan and Matanjun, 2017). Low protein concentration was observed in *A.rigida* (31.11 ± 1.56%), while the high protein value was recorded in *C. officinalis* (36.99 ± 1.36%). This variation in protein content of these red species may be related to spatial or temporal in nature, surrounding water quality (as salinity, temperature, and dissolved oxygen) (Parthiban et al., 2013), also, morphological, structural characteristics, different periods, and different locations (Ismail et al., 2017).

C. officinalis has recorded higher lipid content (5.85 ± 0.49), however *A.rigida* has lower value of lipid (4.43 ± 0.29) these difference related to different season of harvesting, habitat area of production, geographical origin and environmental conditions (Marinho-Soriano et al., 2006; Ismail et al., 2017).

The result explains *J. rubens* had the highest carbohydrates value, while *C. officinalis* had higher protein and lipid contents, so this indicate that *C. officinalis* may become a potential source of protein and lipid, on other hand, *A. rigida* had low concentration of carbohydrates, protein and lipid. Our results of total carbohydrates content (45.34 ± 2.12%) of tested red seaweeds was lower than total carbohydrates content was found by Ismail et al. (2017) (55.24 ± 17.12%),but our total protein and total lipid (34.21 ± 1.73% & 5.27 ± 0.39%, respectively) were higher than these reported by Ismail et al. (2017) for the red seaweeds of total protein & total lipid (19.86 ± 1.73% & 0.98 ± 0.24%, respectively), this variation of chemical constituents, may be due to environmental conditions, change of seasons, metabolic preferences, in addition to the phases growth of seaweed (Marinho-Soriano et al., 2006).

Table 1. The Physicochemical Properties of the selected seaweeds.

Spp.	SWC (mL g^{-1} DW)	WHC (g g^{-1} DW)	OHC (g g^{-1} DW)
C. officinalis	5 ± 0.26^{a}	1.321 ± 0.06^{a}	1.2 ± 0.05^{a}
J. rubens	3.25 ± 0.59^{b}	1.619 ± 0.08^{b}	3.1 ± 0.50^{b}
A. rigida	5.25 ± 0.38^{a}	1.403 ± 0.07^{a}	1.5 ± 0.09^{c}

Values are Mean ± standard deviation, n = 3 on DW. a, b &c values with different superscripts within the same column are significantly different between the tested seaweeds ($P < 0.05$).

Table 2. Proximate composition of the tested red species (%DW).

Spp.	Carbohydrate	Protein	Lipid
C. officinalis	44.21 ± 2.40^{a}	36.99 ± 1.36^{a}	5.85 ± 0.49^{a}
J. rubens	49.88 ± 2.56^{b}	34.52 ± 2.26^{b}	5.54 ± 0.40^{b}
A.rigida	42.02 ± 1.39^{c}	31.11 ± 1.56^{c}	4.43 ± 0.29^{c}

Values are Mean ± standard deviation, n = 3 on DW. a, b &c values with different superscripts within the same column are significantly different between the tested seaweeds ($P < 0.05$).

Table 3. Protein fractionation of the tested seaweed (mg g^{-1} dry weight).

Fractions	*C. officinalis*	*J. rubens*	*A. rigida*
Water-soluble "albumin"	35.86 ± 2.02[a]	45.11 ± 2.29[b]	24.54 ± 1.37[c]
Salt-soluble "globulin"	19.63 ± 0.70[a]	25.58 ± 1.56[b]	31.70 ± 1.90[c]
Alcohol- soluble "protamine"	0.0013 ± 0.0005[a]	0.0014 ± 0.0007[b]	0.0006 ± 0.0002[c]
Alkali- soluble "glutilin"	35.00 ± 1.28[a]	28.37 ± 1.01[b]	41.93 ± 2.20[c]
Insoluble protein	9.50 ± 0.50[a]	0.93 ± 0.09[b]	1.83 ± 0.19[c]
Total	100.00	100.00	100.00

Values are Mean ± standard deviation, n = 3 on DW. a, b &c values with different superscripts within the same column are significantly different between seaweeds ($P < 0.05$).

3.3.2. The protein fractionation

Protein fractionation of the three red species (mg g^{-1} dry weight) is summarized in Table 3. The algal protein fractionation contents were significantly different between species at ($P < 0.05$), albumin and protamine fractions which are soluble in water and ethyl alcohol, respectively were the highest in *J. rubens* specimens (45.11 ± 2.29 mg g^{-1} & 0.0014 ± 0.0007 mg g^{-1} DW, respectively) than other species. Globulin (31.70 ± 1.90 mg g^{-1} DW) and glutilin extractions by NaCl and NaOH, respectively (41.93 ± 2.20 mg g^{-1} DW, respectively) were high in *A. rigida*, while, insoluble protein fraction was high in *C. officinalis* (9.50 ± 0.50 mg g^{-1} DW), these variations may be related to many reasons as, method of fractionation, differences extraction conditions (such as incubation temperature, time, and ratio of solvent to biomass) also, the chemical composition, the morphology (especially thallus forms) of these species, as well as, different solubility and polarity of the solvents used (Caronni et al., 2021).

Jania rubens recorded the greatest "albumin" fraction than other species, this may be related to this species possess highly polar components which are soluble substance in a high polar solvents as H_2O, otherwise, H_2O has a high polarity index (9.0) (El Nemr et al., 2021). Moreover, albumin is the most significant in human nutrition due to its high digestibility; thus, the biological value of protein is determined by the amount of albumin fraction (El-Sheekh et al., 2014).

3.3.3. The pigment composition

The photosynthetic pigments content as chlorophyll a (Chl-*a*), and total carotenoid content (TCC) and polar pigments (phycoerythrin (PE), allophycocyanine (APC) and phycocyanin pc)) of three species of red seaweeds were illustrated in Figure 2. This figure shows that significant different between the selected species ($P < 0.05$). *J. rubens* specimens were recorded maximum values of the photosynthetic pigments (Chl-*a* 37.20 ± 1.50, TCC: 0.69 ± 0.05, PE: 41.98 ± 2.05, APC: 46.94 ± 2.58 and PC: 37.26 ± 1.29 μg g^{-1} Fw), conversely, minimum concentrations of Chl-*a*, TCC, PE, APC and PC (19.17 ± 0.30, 0.42 ± 0.02, 22.02 ± 0.88, 34.48 ± 1.05 and 18.16 ± 0.25 μg g^{-1} Fw, respectively) were observed in *A. rigida*. This variation may be related to chemical parameters (dissolved oxygen, pH, and nutrients), physical factors (as light density, depth, photoperiod, temperature and salinity), algal species (Ismail and Osman, 2016), also, the thallus morphology, thickness, and structure of photosynthetic system (Wu, 2016).

3.4. Scanning electron microscopy (SEM and EDX)

3.4.1. SEM

The three tested red algae were characterized by different surface types, which examine by the scanning electron microscopy (SEM) as Figure 3. *C. officinalis* surface consists of chambers with the calcified walls, have a symmetrical, and rounded outline. On other hand, the surface of *J. rubens* featured large, lobed and dichotomously branched cells. These data were in agreement with those reported by Pueschel et al. (2002). It can noticed that, *A. rigida* possesses a relative roughness surface with more ordered pores of homogeneous shapes and a cluster of heterogenic cells on some pore surfaces, consequently, the porous surface of *A. rigida* can trap the bioactive extract which may act as a second barrier to release the trapped extract this agreement with our present data of GC-MS (section 3.6) and with the results of El Nemr et al. (2021).

3.4.2. EDX

The results of energy-dispersive X-ray (EDX) of *C. officinalis, J. rubens* and *A. rigida* were identified as Figure 4 & Table 4, which give the elemental compositions (C, O, Mg, Al, Si, S, Ca, N, K and Fe) owing to mass percentages with their standard deviation. C, O, Mg, Al, Si, S, and

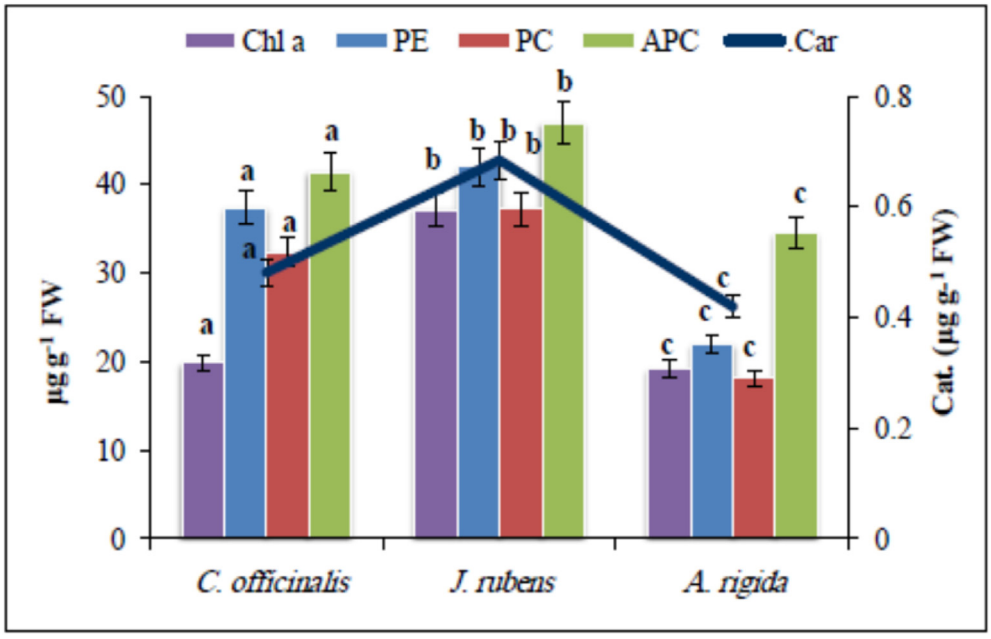

Figure 2. Pigment composition of the selected species, the error bars show the coefficient of variation (standard deviation as a percentage of means); the maximum value was 5% (three independent replicates), a, b and c means values with differ letters within the same column are significantly different between different tested red seaweeds ($P < 0.05$).

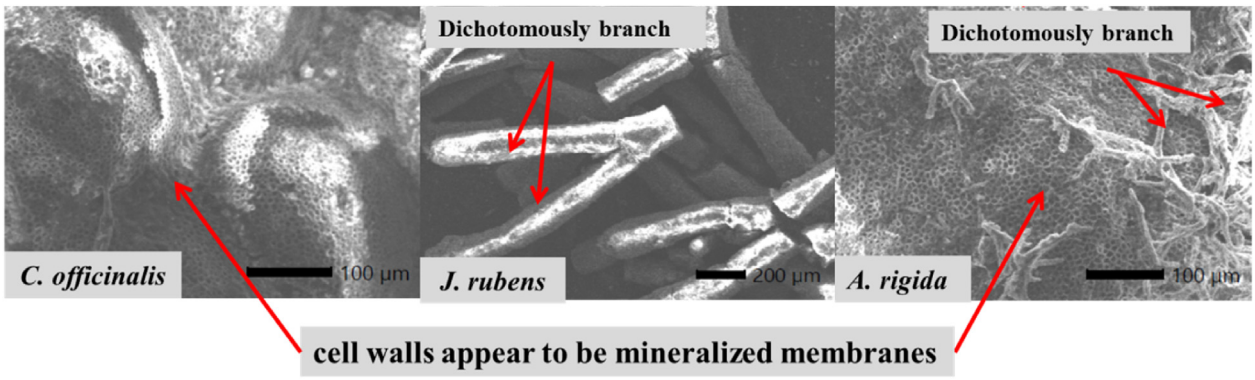

Figure 3. SEM of three tested red seaweeds: *C. officinalis*, *J.rubens* and *A.rigida*.

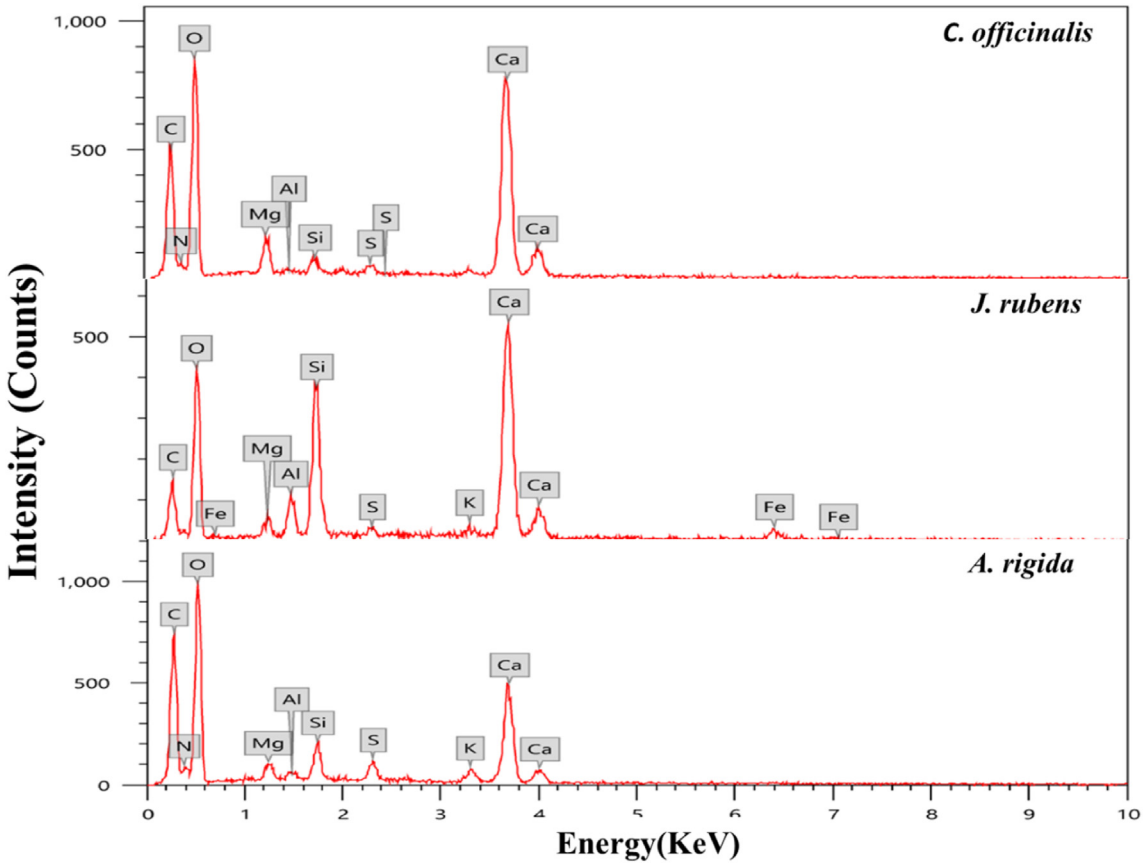

Figure 4. EDX of three tested red seaweeds species: *C. officinalis, J.rubens* and *A.rigida*.

Ca were the dominate elements in the three species with different percentage. On the other hand, N (3.06 ± 0.34% & 7.73 ± 0.37%) was detected in *C. officinalis* and *A. rigida*. Whereas K (0.66 ± 0.08% & 0.88 ± 0.05%) was observed in *J. rubens* and *A. rigida* and Fe (1.75 ± 0.16%) was noted only in *J. rubens*. This variation in the metals concentrations in the tested species related to seaweeds species, and their surface morphology and efficiency of forming coordination complexes with specialized transport ligand in their outer membranes.

It is noticed that from Figure 4 & Table 4 the presence of C, O, Mg and Ca, which affirm that these species was essentially a calcite containing Mg (Bouremmad et al., 2019). Fe is only found in *J. rubens*, besides; this species is distinguished by the presence of all the previous elements except the element N. This may be attributed to higher phycocolloid (agar and carrageenan) content in *J.rubens* cell wall (Parthiban et al., 2013) which can able to bind these elements on the surface of this species (Lima et al., 2019). Herein, having the above elements in available form is of great importance for the use of *J. rubens* as a nutrient/supplement and fresh seaweed for human consumption.

3.5. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectroscopy was used to identify the functional groups and predict the main chemical components present in three red species across various indicator bands (Figure 5). Appeared strong broad bands at 3282, 3291 and 3285 cm^{-1} in *C. officinalis, A. rigida* and *J. rubens*, respectively were assigned to N–H and O–H stretching vibrations corresponding to the amino acids and polysaccharides (Deepika, 2019). The absorption week bands at frequencies 2925, 2926 & 2923 cm^{-1} in *C. officinalis, A. rigida* and *J. rubens*, respectively can be attributed to the $–CH_3$ and $–CH_2$ stretching aliphatic vibrations of the chlorophyll compounds or C–H

Table 4. EDX data of three tested red seaweeds species.

Elements	*C. officinalis*	*J. rubens*	*A. rigida*
	Elemental Concentration % ±SD		
C	22.88 ± 0.23	16.58 ± 0.33	29.09 ± 0.23
N	3.06 ± 0.34	-	7.73 ± 0.37
O	53.59 ± 0.70	48.12 ± 0.88	49.99 ± 0.60
Mg	2.10 ± 0.09	1.03 ± 0.09	0.96 ± 0.06
Al	0.09 ± 0.04	2.35 ± 0.13	0.33 ± 0.04
Si	0.66 ± 0.05	8.24 ± 0.22	1.69 ± 0.07
S	0.49 ± 0.04	0.68 ± 0.06	1.18 ± 0.05
Ca	17.14 ± 0.25	20.58 ± 0.37	8.16 ± 0.15
K	-	0.66 ± 0.08	0.88 ± 0.05
Fe	-	1.75 ± 0.16	-
Total	100.00	100.00	100.00

stretching symmetric aliphatic vibration was supposedly pointed to the secondary amines (Kannan, 2014; Deepika, 2019). The appearance of week peaks 2282 cm^{-1} (in *C. officinalis*) & 2279 cm^{-1} in both *A. rigida* and *J. rubens* may assigned to the C–O stretching band which is a characteristic peak for carboxylic group (Rajasekar et al., 2013) or P–H stretching peak of phosphine compound (Kannan, 2014). Three medium bands were spotted at 1640, 1641 and 1644 cm^{-1} in *C. officinalis, A. rigida* and *J. rubens*, respectively may be due to C=O Stretching and N=O asymmetric stretching of esters and pectin complexes (Deepika, 2019). The intense and strong peaks 1401, 1395 and 1409 cm^{-1} in *C. officinalis, A. rigida* and *J. rubens*, respectively may be related to S=O stretching vibration (sulfonamide) in lignin or the C–H stretch vibration of alkanes (methyl) (Kannan, 2014; Raubbin et al.,2020). The appearance of moderate peaks at 1033 and 1036 cm^{-1} in *C. officinalis, A. rigida,* respectively as well as *J. rubens* possess strong and sharp band at 1039 cm^{-1} may be attributed to S=O stretching vibration of sulfonides in starch or polysaccharides compounds (Deepika, 2019; Ismail and Amer, 2020). The manifest of the intense and sharp signal at 871 cm^{-1} in all species may be due to out of plane deformation C–H bending mode of glucose & galactose (Kannan, 2014; Deepika, 2019). A weak and relative sharp bands at 712, 713 and 711 cm^{-1} for *C. officinalis, A. rigida* and *J. rubens* respectively were corresponding to out of plane N–H vibration of fatty acid (Kannan, 2014). *J. rubens* had weak absorption band at 535 cm^{-1} due to iodo and brominated compounds present in it only (Kannan, 2014). The presence of S–S stretching vibration (disulfides) at a medium and sharp band (462 cm^{-1}) is considered the fingerprint of *J. rubens* (Kannan, 2014).

From FTIR results *J. rubens* species was distinguish than other species with the presence of two peaks at 535 cm^{-1} and 462 cm^{-1} assigned to iodo and brominated compounds and disulfides group, respectively this agreement with the (Kannan, 2014).

3.6. Gas Chromatography–Mass Spectrometry (GC–MS)

A comparative study of compounds present in the tested algal methanol extracts through GC-MS was performed. The name, retention time, molecular weight, and biological activity of the components were reported (Table 5). A total of 21 biochemical compounds were detected. The maximum number of bioactive compounds was found in *A. rigida* (16 compounds) followed by *C. officinalis* (10 compounds) and *J. rubens* (7compounds). There are common peaks appeared at "14.06, 22.04, 26.24, 29.57 and 35.94" retention time in all tested algal methanolic extracts which were corresponding to the prevailing compounds "1-hexadecanol, methyl tetra decanoate, hexadecanoic acid, methyl ester, 9-octadecenoic acid (Z), methyl ester and 9-octadecenoic acid, 1,2,3-propanetriyl ester (E,E,E), respectively. There are many reports documented the biological properties of these compounds such as antimicrobial, antioxidant antiviral, anti-inflammatory, and medical properties (Adnan et al., 2019; Gollo et al., 2020). In addition to other fatty acid methyl ester, volatile organic and esters compounds like methyl stearate, 1-hexadecanol, 2-methyl-, Silicone oil, heptadecanoic acid, 9-methyl-, methyl ester, and 1,6-octadien-3-OL,3,7-dimethyl which present in different ratio in different species. These biological compounds are characterized by different biological activities such as antioxidant, antimicrobial, antitumor and anti-inflammatory as illustrated in Table 5

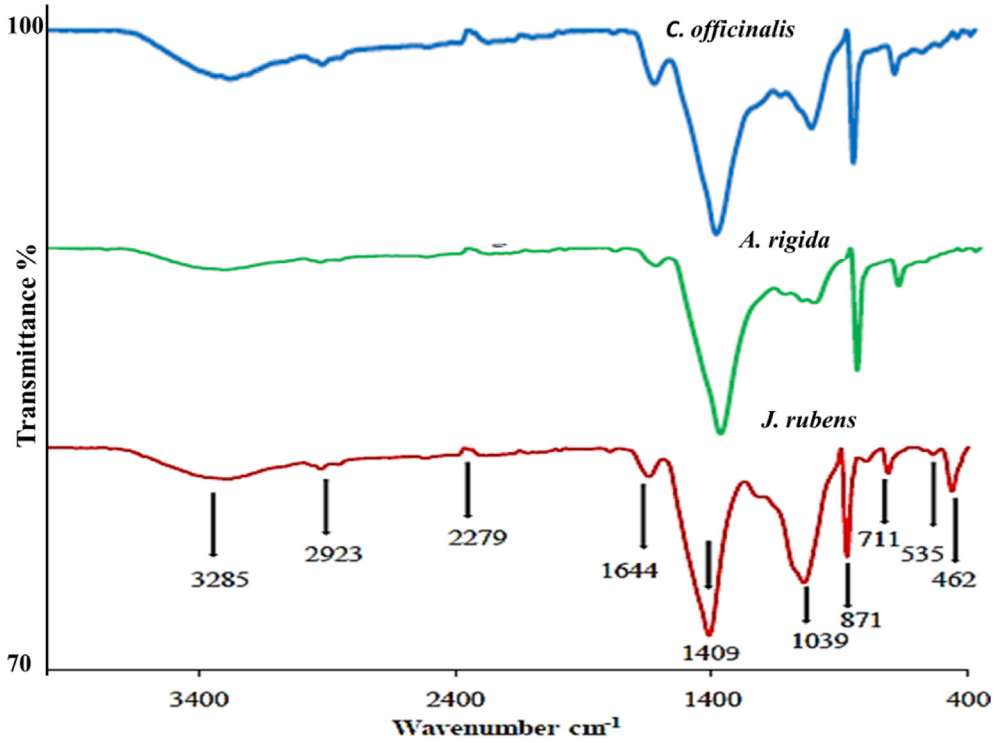

Figure 5. FTIR spectrum of the collected seaweeds.

Table 5. GC-MS analysis of the tested algae.

Bioactive compounds	R.T.	Area %			Molecular formula	M. Wt	Biological activity
		C. officinalis	*J. rubens*	*A. rigida*			
1,6-Octadien-3-OL,3,7-Dimethyl	7.25	10.48			$C_{10}H_{18}O$	154	Anti-inflammatory and anti-cancer properties (Ganesh and Mohankumar, 2017)
2-Decen-1-ol, (E)-	9.58	5.82			C10H20O	156	Antioxidant, and Antibacterial activities (Zine et al., 2021)
2-Decenal, (E)-	11.13	4.80			$C_{10}H_{18}O$	154	Antimicrobial & antioxidant activity (Zine et al., 2021)
1-Hexadecanol	14.06	4.27	2.10	1.2	$C_{16}H_{34}O$	242	anticancer, anti-inflammatory and antimicrobial, antioxidant activities (Ganesh and Mohankumar, 2017)
Cycloheptasiloxane, tetradecamethyl-	16.20			1.48	$C_{57}H_{104}O_6$	884	Antimicrobial activity (Mebude and Adeniyi 2017)
5-EICOSENE, (E)-	18.92			1.01	$C_{20}H_{40}$	280	antimicrobial activities (Ganesh and Mohankumar, 2017)
1-Hexadecanol, 2-methyl-	18.93	3.20	1.66		$C_{17}H_{36}O$	256	Anti-microbial (Ganesh and Mohankumar, 2017)
Methyl tetradecanoate	22.04	6.09	1.45	0.95	$C_{15}H_{30}O_2$	242	Antifungal and antioxidant activities (Gollo et al., 2020)
Cyclooctasiloxane, Hexadecamethyl-	20.09			2.21	$C_{16}H_{48}O_8Si_8$	592	Antimicrobial activity (Ganesh and Mohankumar, 2017)
Cyclononasiloxane, Octadecamethyl-	23.48			2.32	$C_{18}H_{54}O_9Si_9$	666	Antioxidant and Insecticidal Activities (Ganesh and Mohankumar, 2017)
Hexadecanoic Acid, methyl ester	26.24	25.60	26.25	17.60	$C_{17}H_{34}O_2$	270	Antioxidant, antiinflammatory and anthelmintic activities (Gollo et al., 2020; Adnan et al., 2019)
Cyclodecasiloxane, eicosamethyl-	26.51			1.09	$C_{20}H_{60}O_{10}Si_{10}$	740	Antimicrobial activity (Ganesh and Mohankumar, 2017)
1H-Purin-6-Amine, [(2-Fluorophenyl) Methyl]-	29.29			0.32	$C_{12}H_{10}FN_5$	243	Antimicrobial & antioxidant activity (Gollo et al., 2020)
9-Octadecenoic acid (Z)-, methyl ester	29.57	30.87	59.67	64.14	C19H36O2	296	Antimicrobial activity (Gollo et al., 2020; Adnan et al., 2019)
11-Octadecenoic acid, methyl ester	29.78			0.22	$C_{19}H_{36}O_2$	296	Antioxidant and antimicrobial activities (Gollo et al., 2020)
Methyl stearate	30.07			4.51	$C_{19}H_{38}O_2$	298	Anti-inflammatory & anthelmintic activity (Adnan et al., 2019)
Heptadecanoic acid, 9-methyl-, methyl ester	30.08	4.73	6.91		$C_{19}H_{38}O_2$	298	Many of biological activities (Zine et al., 2021)
Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11, 13,13,15,15-hexadecamethyl-	31.83			0.95	$C_{16}H_{50}O_7Si_8$	578	Antioxidant & Antimicrobial (Ganesh and Mohankumar, 2017)
Silicone oil	34.24			0.76	$C_{12}H_{10}FN_5$	243	Antimicrobial & antiviral (Örnek et al., 2014)
9,12-Octadecadienoic acid (Z,Z)-,	35.63			0.67	$C_{27}H_{54}O_4Si_2$	498	Many biological activity (Ganesh and Mohankumar, 2017)
9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	35.94	413	1.95	0.67	$C_{57}H_{104}O_6$	884	Different biological activities (Gollo et al., 2020)

(Ganesh and Mohankumar, 2017; Gollo et al., 2020; Nautiyal and Dubey, 2021; Zine et al., 2021).

4. Conclusion

The morphological description, physicochemical characteristics, and chemical composition of the tested calcified red algae *C. officinalis*, *J. rubens*, and *A. rigida* were valorized for comparing the difference between them. Thallus of *A. rigida* was thickener than other two species. Also GC-MS analyses displayed *A. rigida* had the highest number of bioactive components (16 compounds). Whereas *J. rubens* had higher WHC, OHC, carbohydrates, albumin fraction, minerals and photosynthetic pigments values than those two other species. Additionally, *J. rubens* specimen was distinguished from other species based on FTIR data due to the presence of iodo and brominated chemicals as well as the disulfides group. In view of these results, *A. rigida* and *J. rubens* were a good source of protein, minerals, and bioactive compounds with biological properties like antimicrobial, antioxidant and anti-inflammatory which can be recommended as nature supplementary for nutrional and pharmaceutical applications. Further studies are needed (amino acid and fatty acids) to improve our understanding of the nutritional value of these common algae.

Declarations

Author contribution statement

Abeer A.M. El-Sayed, Mona M. Ismail: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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