

Chemical Analysis and Biological Potentials of Extracts from *Colpomenia sinuosa*

Reem Monal Al Monla, Zeina T. Dassouki, Hala Gali-Muhtasib¹, Hiba R. Mawlawi

AZM Center for Research in Biotechnology and Its Applications, Doctoral School for Sciences and Technology, Laboratory of Applied Biotechnology (LBA3B), Lebanese University, Tripoli, ¹Department of Biology, Center for Drug Discovery, American University of Beirut, Beirut, Lebanon

ABSTRACT

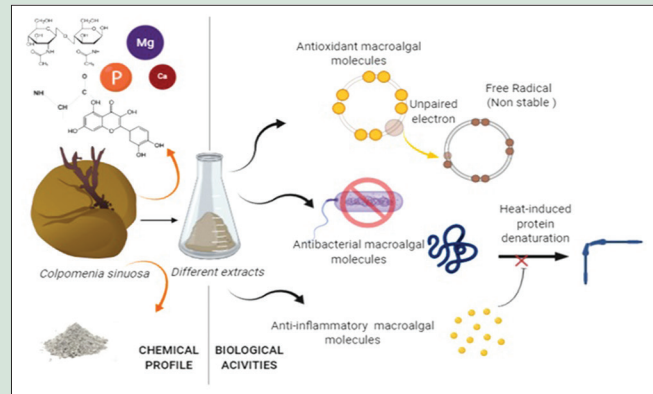
Background: *Colpomenia sinuosa* is a brown alga rich in molecules of pharmacological, nutraceutical, and functional properties. Despite its therapeutic potentials, this alga is poorly studied in this area of the Mediterranean. **Objectives:** The study objective was to investigate the physicochemical properties and biological potentials of different extracts of the brown seaweed *C. sinuosa*. **Materials and Methods:** Crude extracts and fractions were obtained by different types of solvent extractions. Proximal analysis was adopted to reveal the ash content. Protein and carbohydrate contents were determined by phenol-sulfuric acid method and Lowry and Bradford methods, respectively. The mineral content was assessed by atomic absorption spectroscopy, where the total phenolic content (TPC) was determined by Folin-Ciocalteu reagent, antioxidant properties by radical scavenging assay, and antimicrobial activity by disc diffusion method. Finally, the anti-inflammatory activity was determined by albumin denaturation test. **Results:** Proximal analysis revealed high levels of total ash content ($20.03 \pm 0.79\%$), protein content ($10.77 \pm 0.18\%$), total lipids ($4.35 \pm 0.212\%$), and total carbohydrates ($40.367 \pm 0.421\%$). Magnesium concentration (16.79 ± 0.721 mg/g) was highest among macro minerals. The TPC of fractions and extracts ranged from 17.78 ± 0.092 to 39.02 ± 0.199 mg gallic acid equivalents/g dry weight. The highest scavenging activity reached $84.1\% \pm 0.83$ at $750 \mu\text{g/mL}$. Maximal anti-inflammatory activity was noted in aqueous extract ($59.38 \pm 4.35 \mu\text{g/mL}$). **Conclusion:** This significant scavenging activity increased in polar extracts obtained by Soxhlet extraction. The aqueous extracts showed the highest 2,2-diphenyl-1-picrylhydrazyl scavenging activity and highest albumin denaturation inhibition, whereas the highest bactericidal activity was found in the methanolic extracts. Our results represent the first report on Lebanese *C. sinuosa* extract as a promising source of bioactive compounds with high antioxidant potential.

Key words: Anti-inflammatory, antimicrobial, antioxidant, biochemical analysis, Phaeophyta

SUMMARY

Colpomenia sinuosa was collected and analyzed to identify its pigment concentration and organic and inorganic contents. Different extraction systems were used to obtain the extracts of different polarities and characteristics. These were later tested to explore their biological activities on different aspects: Antioxidant, anti-inflammatory, antimicrobial, and

antifungal. Based on the data provided by this study, *C. sinuosa* extracts, specifically aqueous extract, can be considered a potential source of significant therapeutic antioxidant and anti-inflammatory activities.



Abbreviations Used: *C. sinuosa*: *Colpomenia sinuosa*; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TCA: Trichloroacetic acid; DM: Dichloromethane:methanol; M: Methanol; Aq: Aqueous; Chl: Chlorophyll; Fucx: Fucoxanthin; Cart: Carotenoids; GAE: Gallic acid equivalents; DW: Dry weight; TPC: Total phenolic content; IC₅₀: Half-maximal inhibitory concentration.

Correspondence:

Prof. Hiba R. Mawlawi,
AZM Center for Research in Biotechnology and
Its Applications, Doctoral School for Sciences
and Technology, Laboratory of Applied
Biotechnology (LBA3B), Lebanese University,
Tripoli, Lebanon.

E-mail: hiba.mawlawo@ul.edu.lb

Prof. Hala Gali-Muhtasib,

Department of Biology, Center for Drug
Discovery, American University of Beirut,
Riad El Solh 1107 2020, Beirut, Lebanon.

E-mail: amro@aub.edu.lb

DOI: 10.4103/pr.pr_91_19

Access this article online

Website: www.phcogres.com

Quick Response Code:



INTRODUCTION

Oceans offer an enormous reservoir for novel compounds considering that marine organisms comprise about half of the global biodiversity.^[1] Marine algae are considered as one of the most prolific sources of biologically active metabolites.^[2,3] Thus, the investigation of different macroalgae species for novel active molecules is of great interest.

Seaweeds are exposed to major environmental stress.^[4] Macroalgae counteract this oxidative stress by generating multiple antioxidant defense mechanisms to neutralize the harmful reactive free radicals.^[5] The secondary metabolites present in brown, red, and green algae have

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Al Monla RM, Dassouki ZT, Gali-Muhtasib H, Mawlawi HR. Chemical analysis and biological potentials of extracts from *Colpomenia sinuosa*. Phcog Res 2020;12:272-7.

Submitted: 13-Oct-2019

Revised: 26-Dec-2019

Accepted: 11-Mar-2020

Published: 14-Aug-2020

diverse functions and can act as antimicrobials, or anti-inflammatory, antifungal, and antitumor agents.^[6] They are used by pharmaceutical industries in drug development to treat many diseases.

Phaeophyta are exclusively photoautotrophic marine macroalgae; they form a huge group of over 1500 species.^[7] Focus on brown algae (Phaeophyta) has significantly increased because it contains a wide range of various bioactive compounds including phenols.^[8,9]

Brown algae have higher concentrations of polyphenols in comparison to red and green algae. Many researchers showed a correlation between the phenolic compounds of a macroalga and its antioxidant potential, as antioxidants play a crucial role in cellular defense against stresses.^[10]

The Lebanese coast is a great area for investigating diverse algal biomass due to many factors including its proximity to the Suez canal.^[11,12] Multiple species of brown algae from this area have been previously investigated and were found to have great biological potential.^[13-15]

Colpomenia sinuosa is a brown algae that belongs to the order of Scytosiphonales.^[16] It occurs abundantly in the Mediterranean region.^[17] Several studies carried out on this alga document its biological significance and its cosmopolitan distribution in the world's oceans.^[10,18-20]

The aim of the present study is to analyze the physicochemical properties and the potential use of *C. sinuosa* as a source of bioactive compounds with antioxidant, anti-inflammatory, and antibacterial effects. To our knowledge, this species has never been studied in this area of the Mediterranean previously. This seaweed was assessed by preparing extracts based on the following three variables: polarity, temperature of extraction process, and fractionation.

MATERIALS AND METHODS

Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, Lowry, Bradford, bovine serum albumin (BSA), sulfuric acid, D-glucose, and gallic acid.

Marine algal material

C. sinuosa samples were collected from the Lebanese North coast of the Mediterranean region at a depth of 3–5 m. Fresh seaweeds were rinsed under tap water and polished to remove associated epiphytes, salt, sand, micro-organisms, and other materials. Then, it was air dried in a shady place at room temperature (25°C–30°C) on an absorbent paper, and then ground to a fine powder.^[21]

Physico-chemical characterization

Proximal analysis

Analysis of total lipid, moisture content, total ash, acid-insoluble ash, water-soluble ash, and sulfated ash was done according to the methods described by the Association of Official Analytical Chemists.^[22] The percentage of ash was calculated with reference to the air-dried algae.

Total carbohydrate extraction and quantification

This protocol starts with hydrolysis by concentrated acid (11 M H₂SO₄) at 37°C for 1 h, followed by diluting the acid strength to 1 M and boiling the reaction for 2 h. Quantification was calculated using Dubois colorimetric phenol-sulfuric acid method. The absorbances were read at 490 nm in a spectrophotometer, and concentrations were determined by glucose standards.^[23]

Protein extraction and quantification

Extraction procedures were started with 1 g of freeze-dried algae, using 30 mL of ultra-pure water, for 12 h, and the samples were grinded with a homogenizer for 5 min. The samples were then centrifuged, and the supernatant was stored at 4°C. Then, 40 mL of 0.1 M NaOH was added

to the residue for 1 h with shaking. The samples were centrifuged again, and all supernatants were pooled for further precipitation and solubilization. Precipitation: 25% trichloroacetic acid (TCA) (2.5:1) was added to the supernatant and kept in the ice bath for 35 min. This was followed by centrifugation; the supernatant was discarded and the pellet was rinsed with 10% cold TCA, and then the samples were centrifuged and the pellet was kept. This was followed by solubilization of the pellet in 5% TCA (5:1) and centrifugation (20 min, 15,000 ×g, and 20°C). Protein quantification was done using both the Bradford and Lowry methods, with BSA as standard.^[24]

Elemental analysis

One gram of powdered seaweed was ashed at 500°C for 2 h. The cooled ash was moistened with distilled water and then dissolved with 3 mL 1:1 nitric acid and 1.95 mL deionized water. The solution was heated at 100°C till dry. The residue was then ashed at 500°C for 1 h. The ash was then cooled, dissolved with 10 mL HCl, and then filtered with ashless filter paper into a volumetric flask. Atomic absorption spectrometer was used for macro and microelement analyses.^[22] For each element measured, a standard calibration curve was prepared.

Estimation of pigment

Five hundred milligrams of algal material was taken and ground by a pestle and a mortar with 10 mL of 80% acetone in order to extract and quantify algal pigments. The homogenate was centrifuged, and the supernatant was stored. The residue was re-extracted with 80% acetone till it became colorless. The extract was utilized for chlorophyll (Chl) estimation. The absorbance was read at 645 and 663 nm in an ultraviolet-spectrophotometer.^[25]

Estimation of Chl: The amount of Chl present in the algae was estimated by the method of Arnon.^[25] The absorbance was measured at 645 nm and 663 nm in a spectrophotometer.

Arnon's (1949) equations:

$$\text{Chl a } (\mu\text{g/mL}) = 12.7 (A_{663}) - 2.69 (A_{645})$$

$$\text{Total Chl } (\mu\text{g/mL}) = 20.2 (A_{645}) + 8.02 (A_{663}).$$

Chl c1 + c2 content was determined using the following equation according to Jeffrey and Humphrey.^[26]

$$\text{Chl c1 + c2 } (\mu\text{g/g}) = 24.36 (A_{630}) + 3.73 (A_{664})$$

Where A = Absorbance at respective wavelength = Volume of extract (mL), W = weight of the sample (g).

Estimation of carotenoid: Carotenoid concentration was estimated by the method of Kirk and Allen.^[27] The same Chl extract was measured at 480 nm in the spectrophotometer to estimate the carotenoid content.

$$\text{Carotenoids } (\mu\text{g/g}) = A_{480} + (0.114 \times A_{663}) - (0.638 \times A_{645})$$

Where A = Absorbance at respective wavelength.

Estimation of fucoxanthin: The amount of fucoxanthin was estimated by the method of Seely *et al.*^[28] The same Chl extract was measured in the spectrophotometer to estimate the fucoxanthin content.

$$\text{Fucoxanthin } (\text{mg/g}) = (A_{470} - 1.239 [A_{631} + A_{581} - 0.3 \times A_{664}] - 0.0275 [A_{664}]) / 141$$

Where A = Absorbance at particular wavelength, V = Total volume of the pigment extract.

Marine algal solvent extraction

Four types of solvent extractions were followed using three different solvents. These include cyclohexane, dichloromethane: methanol (DM, 1:1), methanol (M), and finally water or aqueous (Aq) extract. For crude maceration, 20 g of the sample was macerated for 3 days in 200 mL in the solvent at room temperature in an orbital shaker. Soxhlet maceration was done using the same extraction solvents but

with Soxhlet extractor at elevated temperatures for 6 h. For sequential maceration, the samples were sequentially extracted by soaking in various solvent systems starting with cyclohexane, DM (1:1, v/v), M, and Aq [Supplementary Figure 1]. Each solvent extraction process was conducted for 3 days. The extracts were then concentrated using a rotary evaporator. For the water extracts, the samples were lyophilized.^[29]

Total phenol content

The total phenolic content (TPC) of the extract was determined by the Folin–Ciocalteu method.^[30] Folin–Ciocalteu reagent determines total phenols, producing blue color by reducing heteropolyphosphomolybdate-tungstate anions.^[31] An aliquot of the extract (100 μ L) was mixed with 0.75 mL of Folin–Ciocalteu's phenol reagent (diluted ten folds with deionized water) and allowed to stand for 5 min at room temperature; 0.75 mL of 7.5% sodium bicarbonate solution was added to the mixture and the absorbance was measured at 725 nm after 90 min at room temperature. The extract absorbance values were compared with a gallic acid standard curve for estimating the concentration of TPC in the sample. The TPC was expressed as mg of gallic acid equivalents (GAE) per gram of powder on dry weight basis.

2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay

The scavenging effects of samples for DPPH radical were monitored according to the method of the previous report.^[32] Seaweed extracts and gallic acid (standard) were aliquoted into series of concentrations (100–750 μ g/mL). 1 mL of freshly prepared 0.16 mM methanolic DPPH solution was added and incubated in dark for 30 min. The absorbance was measured at 517 nm. All the tests were performed in triplicates. The percentage of free radical scavenging was calculated using the following formula:

$$\text{Free radical scavenging (\%)} = \frac{([\text{control OD} - \text{sample OD}]/\text{control OD})}{100}$$

Inhibition of protein denaturation

Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi and Sakat *et al.* with slight modification.^[33] Five hundred microliters of 1% bovine serum albumin was added to 100 μ L of the plant extract. This mixture was kept at room temperature for 10 min, followed by heating at 51°C for 20 min. The resulting solution was cooled down to room temperature and the absorbance was recorded at 660 nm. Diclofenac sodium was taken as a positive control. The experiment was carried out in triplicates.

Antimicrobial and antifungal testing

Antibacterial tests of algal extracts were performed *in vitro* using the disc diffusion method in Petri dishes. Sterile discs of 6.4 mm in diameter were impregnated with 25 μ L of seaweed extract of concentration 1 mg/mL deposited on the surface of agar medium (Mueller–Hinton Agar, pH 7.4 \pm 0.2 at 25°C) previously inoculated with bacteria strains and incubated at 37°C for 24 h. The results were expressed by measuring in millimeter the diameters of the inhibition halos of bacterial growth around the disc.^[34] Six bacterial and two pure cultures of fungal strain were obtained from the Health and Environment Microbiology Lab of EDST, AZM Center, Lebanon: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Candida albicans*, and *Aspergillus*.

Statistics

All assays were carried out in triplicates, and data of each result were expressed as mean \pm standard deviation. Statistical analysis was performed by ANOVA and two-tailed Student's *t*-test. $P \leq 0.05$ was taken to indicate statistical significance.

RESULTS

Physicochemical profile of *Colpomenia sinuosa*

The results obtained from the physicochemical evaluation will be used for standardization of raw materials and helps in the quantification of the seaweed, which adds to its nutritive value. Proximal analysis shows that carbohydrates were found to be the most abundant organic compound of the seaweed [Table 1].

Analysis of macro and microelements of *Colpomenia sinuosa*

Table 2 presents the concentrations of the macrominerals and microminerals studied. Magnesium (Mg) was found to be the most abundant macromineral in *C. sinuosa* with a concentration reaching 16.79 mg/g. The studied algae had an adequate Fe concentration, with minimal trace minerals of Copper (Cu) and Zinc (Zn) [Table 2].

Estimation of pigment concentration

Chls at high concentrations possess excellent antioxidant activities.^[35] Carotenoids are recognized as potent antioxidants preventing many human chronic diseases.^[36] *C. sinuosa* is rich in Chl, a pigment showing the highest concentration (10.98 μ g/mL). Fucoxanthin was the pigment with the lowest concentration (3.56 μ g/g) [Table 3].

Total phenolic content of *Colpomenia sinuosa* different extracts

Figure 1 shows that the Aq extraction with Soxhlet apparatus at elevated temperatures yielded the maximal phenolic content (39.02 \pm 0.199 mgGAE/g). Both crude Aq extract and Aq fraction showed a lower concentration. Cyclohexane solvent was omitted from further analysis due to very low yield. Water extracted the highest amounts of phenols among all other solvents, specifically by Soxhlet extraction in comparison to room temperature. Hence, polar extracts using high temperature extraction showed the highest phenolic content.

2,2-diphenyl-1-picrylhydrazyl scavenging activity of *Colpomenia sinuosa* different extracts

The results of Figure 2 reveal that all the extracts show a dose-dependent inhibition (100–750 μ g/mL). A very significant antioxidant activity was noted in the Aq extracts of *C. sinuosa* [Figure 2c], with the Aq soxhlet extract revealing the highest DPPH scavenging

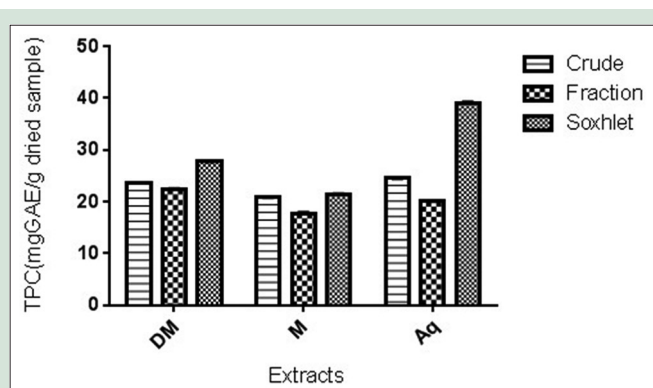


Figure 1: Folin–Ciocalteu total phenolic content assay of *Colpomenia sinuosa* extracts. Values represent the mean \pm standard deviation of three replicates ($n = 3$). DM: Dichloromethane:methanol (1:1), M: Methanol, Aq: Aqueous, TPC: Total phenolic content

activity among all ($84.1\% \pm 0.83$) and with the lowest IC_{50} value of 163.4 ± 1.08 [Supplementary Figure 2]. Thus, it is relevant that the

Table 1: Physico-chemical analysis of *Colpomenia sinuosa*

Physicochemical parameters	Results (%w/w)
Humidity	6.70±0.25
Total ash	20.03±0.79
Acid-insoluble ash	5.2±1.85
Water-soluble ash	4.11±0.10
Sulfated ash	0.66±0.10
Total lipids	4.35±0.212
Total carbohydrate	40.367±0.421
Soluble protein by Lowry	10.77±0.18
Soluble protein by Bradford	9.87±0.13

Values represent the mean±SD of three replicates ($n=3$). SD: Standard deviation

Table 2: Elemental analysis of *Colpomenia sinuosa*

Trace elements	Concentration in <i>Colpomenia sinuosa</i> (mg/g)
Mg mineral	16.79±0.721
Ca mineral	6.814±0.11
K mineral	1.21±0.04
Fe trace mineral	0.521±0.08
Zn trace mineral	0.112±0.007
Cu trace mineral	0.042±0.006

Values represent the mean±SD of three replicates ($n=3$). Mg: Magnesium; Ca: Calcium; K: Potassium; iron; Zn: Zinc; Cu: Copper; SD: Standard deviation

Table 3: Pigment content of *Colpomenia sinuosa* using acetone extraction

Type of pigment	Concentration
Total chlorophylls (mg/g)	1.17±0.52
Chl a (mg/g)	0.98±0.34
Chl c1+c2 (µg/g)	0.245±0.03
Cart (mg/gDS)	0.533±0.02
Fucx (µg/gDS)	3.7±0.001

Data represent the mean of three replicates±SE ($n=3$). Chl: Chlorophyll; Cart: Carotenoids; Fucx: Fucoxanthin; DS: Dry sample; SE: Standard error

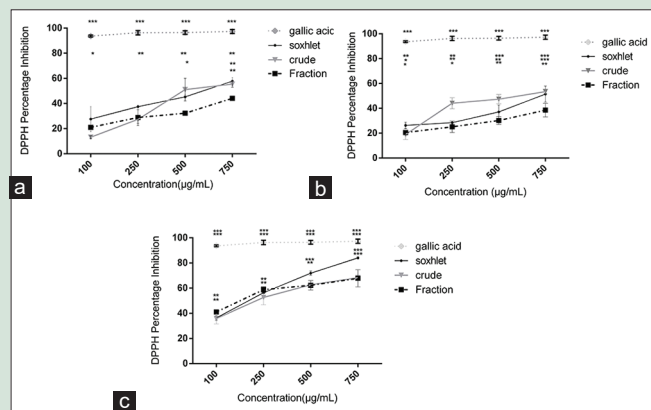


Figure 2: 2,2-diphenyl-1-picrylhydrazyl scavenging activity of different concentrations of *Colpomenia sinuosa* extracts (100, 250, 500, and 750 µg/mL). (a) Effect of DM extracts on 2,2-diphenyl-1-picrylhydrazyl scavenging activity: Crude, Soxhlet, and fraction. (b) Effect of M extracts on 2,2-diphenyl-1-picrylhydrazyl scavenging activity: Crude, Soxhlet, and fraction. (c) Effect of Aq extracts on 2,2-diphenyl-1-picrylhydrazyl scavenging activity: Crude, Soxhlet, and fraction. Values represent the mean ± standard deviation of three replicates ($n=3$). $P < 0.05^*$, $P < 0.01^{**}$, $P < 0.0001^{***}$. DM: Dichloromethane: Methanol (1:1), M: Methanol, Aq: Aqueous

polar extracts of *C. sinuosa* exhibited the most potent DPPH scavenging activity.

Albumin denaturation inhibition by *Colpomenia sinuosa*

The extracts of *C. sinuosa* were compared to the standard diclofenac sodium. Dose-dependent inhibition was observed in all extracts. Based on Figure 3, Aq soxhlet extract exerting the highest inhibition effect, followed by DM sox. Aq sox showed the lowest IC_{50} value of 407.6 ± 1.09 µg/ml [Supplementary Figure 2]. All extracts used revealed a lower activity than that of commercial standard diclofenac sodium.

Antibacterial effect of *Colpomenia sinuosa* extracts

Table 4 shows that the DM crude extracts showed potent activity against *P. vulgaris* and *P. aeruginosa*. Moreover, the Aq extract did not show any antibacterial activity with the exception of the Soxhlet extract against *P. aeruginosa* (12 mm).

Antifungal activity of *Colpomenia sinuosa* extracts

Regarding the antifungal activity, no significant effect was recorded [Table 5]. Mainly, the M crude and fraction extracts showed minimal activity against both strains tested (5 mm in *C. albicans* and 6 mm in *Aspergillus*).

DISCUSSION

The ash content of the *C. sinuosa* was found to be 20.03% [Table 1], suggesting that it is rich in minerals and is of high nutritional importance. In addition, *C. sinuosa* collected from the Lebanese coast had higher percentage of total carbohydrate content in comparison to some brown algae collected from other Mediterranean areas.^[37-39]

Among the analyzed extracts, those with the highest phenolic content also showed relevant antioxidant and albumin denaturation inhibition activities. The maximal antioxidant activity ($84.1 \pm 0.83\%$), anti-inflammatory activity ($59.37 \pm 4.35\%$), and phenolic concentration (39.02 ± 0.199 mgGAE/g) were obtained by the Soxhlet Aq extract at a concentration of 750 µg/mL.

To compare our data with other Lebanese brown algae, the TPC and antioxidant activity of the brown algae *Padina pavonica* and *Sargassum vulgare* collected from the North Lebanese coast were found to be

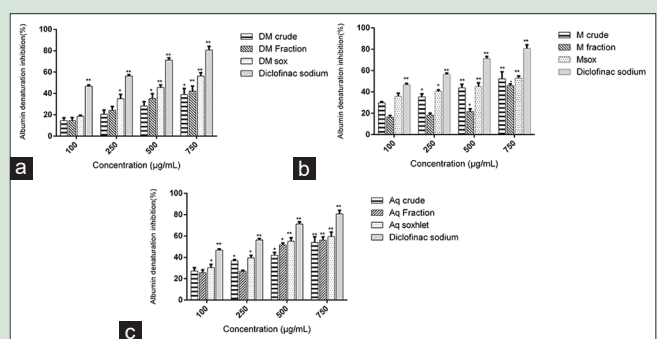


Figure 3: Albumin denaturation inhibition by *Colpomenia sinuosa* extracts (100,250,500,750 µg/ml). (a) Effect of DM extracts on percentage of protein denaturation: Crude, Soxhlet, and fraction. (b) Effect of M extract. (c) Effect of Aq extract. Values represent the mean ± standard deviation of three replicates ($n=3$). $P < 0.05^*$, $P < 0.01^{**}$. DM: Dichloromethane: Methanol (1:1), M: Methanol, Aq: Aqueous

Table 4: Antibacterial activity of *Colpomenia sinuosa* extracts by disc diffusion method

<i>Colpomenia sinuosa</i>	Bacterial strains: Mean zone of inhibition (mm)					
	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Enterococcus faecalis</i>
Extracts						
DM						
Fraction	-	-	-	-	-	-
Crude	-	10±0.57	13±1.52	8±1.72	-	-
Soxhlet	-	-	-	-	-	-
M						
Fraction	-	10±2.57	-	-	8±0.57	8±0.57
Crude	-	10±2.5	12±1.73	10±2.31	10±1.32	12±1.21
Soxhlet	-	-	-	-	-	-
Aq						
Fraction	-	-	-	-	-	-
Crude	-	-	-	-	-	-
Soxhlet	-	-	12±2.45	-	-	-

Data represent the mean of three replicates±SE (n=3). SE: Standard error; DM: Dichloromethane:methanol; M: Methanol; Aq: Aqueous

Table 5: Antifungal activity of *Colpomenia sinuosa* extracts by disc diffusion method

<i>Colpomenia sinuosa</i>	Fungal strains: Mean zone of inhibition (mm)	
	<i>Candida albicans</i>	<i>Aspergillus</i>
Extracts		
DM		
Fraction	-	-
Crude	-	-
Soxhlet	-	-
M		
Fraction	5±0.577	6±2.082
Crude	5±1.528	6±2.61
Soxhlet	-	-
Aq		
Fraction	-	-
Crude	-	-
Soxhlet	-	-

Data represent the mean of three replicates±SE (n=3). DM: Dichloromethane:methanol (1:1); M: Methanol; Aq: Aqueous; SE: Standard error

distinctly lower than that of *C. sinuosa*. Thus, it is relevant that the phenolic content of all *sinuosa* extracts is significantly higher.^[40] When comparing our results with *C. sinuosa* collected from other areas, it was found that *sinuosa* from the Aegean sea had lower phenolic content in the methanolic extract in comparison to our studied algae. Moreover, *C. sinuosa* collected from the Brazilian coast showed a higher phenolic content (85.30 mgGAE/g) than that we collected from the Lebanese coast, however it revealed lower antioxidant potentials.^[41]

Aqueous extract obtained by Soxhlet extraction had the highest DPPH scavenging activity. These results are in agreement with literature.^[42] In addition, our findings are in agreement with reports, showing that the aqueous solvent is an optimal choice to extract biologically active polyphenols.^[43,44]

Denaturation of albumin is a well-documented cause of inflammation. Anti-inflammatory drugs have shown the ability to thermally induced protein denaturation.^[45] Maximal inhibition of albumin denaturation occurred in the aqueous extracts, especially at high temperatures. This activity may be due to many polyphenols.^[46] In comparison to other brown algae, *C. sinuosa* exhibited a significantly higher anti-inflammatory activity than that of *Sargassum wightii* of India.^[47] To our knowledge, the albumin denaturation potentials of *C. sinuosa* have not been studied till date.

Thus, we can demonstrate a correlation between the antioxidant activities, anti-inflammatory potentials of the extracts, and their TPC. Hence,

extracts from *C. sinuosa* with high level of polyphenolic compounds showed good biological potentials in *in vitro* systems.

We also showed that the crude extracts possess higher antioxidant and higher phenolic content than that of fractionated extracts. This suggests the possible presence of synergism among the bioactive compounds in the extracts.^[48,49]

Antibacterial assay showed that methanol solvent had the greatest potential among all the solvents used. Previous reports selected methanol as the optimal solvent for the extraction of antimicrobial compounds from different seaweeds,^[50] which also correspond with this study.

Despite the correlation between the antioxidant activity and the TPC, we believe that there is no relation between phenolic content and antimicrobial or antifungal activity.

Data about *C. sinuosa* from other areas were collected, and it was noticed that *C. sinuosa* from the Aegean Sea have similar profile to that extracted from the Lebanese coast. However, Brazilian *colpomenia* had a lower activity against *S. aureus* and *P. vulgaris*. *C. sinuosa* of both areas exerted no activity against *E. coli* and *C. albicans*.^[51] However, we could not compare antibacterial activity to other brown algae in Lebanon due to the lack of published data. Regarding the antifungal activity, it was found that *P. pavonica* and *S. vulgare* also lack antifungal activity against *C. albicans*.^[40]

CONCLUSION

This study is the first report describing the biochemical and biological properties of extracts from *C. sinuosa* collected from the Lebanese coast. Total carbohydrates are the most abundant organic compounds, with moderate protein concentration and high mineral content. *C. sinuosa* aqueous crude extract obtained by Soxhlet extraction had the highest TPC and had the strongest antioxidant and anti-inflammatory activities. Fair antimicrobial activity was noted. The results obtained suggest that the studied seaweed is a promising source of bioactive compounds with high antioxidant potential.

Acknowledgements

The authors would like to thank the research assistant Ahmad Mostafa and the masters student Rayan Dergham for their support.

Financial support and sponsorship

The financial grant was received from the Lebanese university. Also,

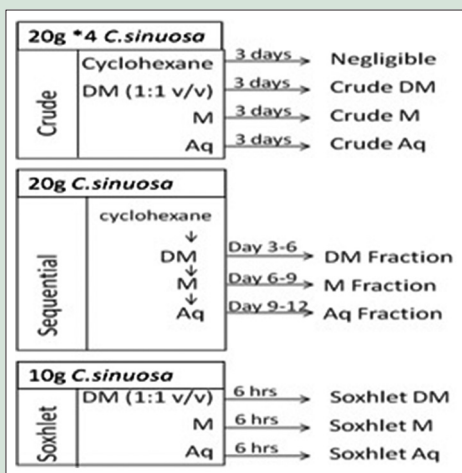
the Doctoral School for Sciences and Technology laboratories are acknowledged.

Conflicts of interest

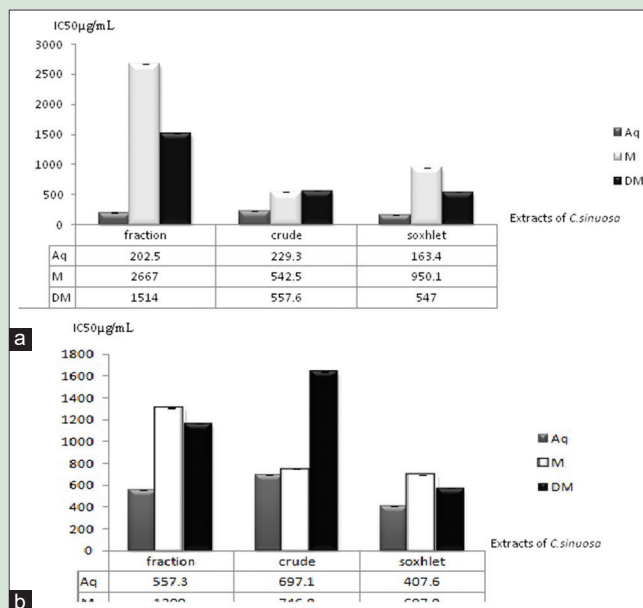
There are no conflicts of interest.

REFERENCES

- Aneiros A, Garateix A. Bioactive peptides from marine sources: Pharmacological properties and isolation procedures. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004;803:41-53.
- Kim SK, Pangestuti R. Biological activities and potential health benefits of fucoxanthin derived from marine brown algae. *Adv Food Nutr Res* 2011;64:111-28.
- Wijesekara I, Yoon NY, Kim SK. Phlorotannins from *Ecklonia cava* (Phaeophyceae): Biological activities and potential health benefits. *Biofactors* 2010;36:408-14.
- Langebartels C, Wohlgenuth H, Kschieschan S, Grün S, Sandermann H. Oxidative burst and cell death in ozone-exposed plants. *Plant Physiol Biochem* 2002;40:567-75.
- Lezcano V, Fernandez C, R. Parodi E, Morelli S. Antitumor and antioxidant activity of the freshwater macroalgae *Cladophora surera*. *J Appl Phycol* 2018;30:2913-21.
- Ianora A, Boersma M, Casotti R, Fontana A, Harder J, Hoffmann F, *et al.* New trends in marine chemical ecology. *Estuar Coast* 2006;29:531-51.
- Fedorov SN, Ermakova SP, Zvyagintseva TN, Stonik VA. Anticancer and cancer preventive properties of marine polysaccharides: Some results and prospects. *Mar Drugs* 2013;11:4876-901.
- Heffernan N, Smyth T, Soler-Villa A, Fitz Gerald R, Brunton N. Phenolic content and antioxidant activity of fractions obtained from selected Irish macroalgae species (*Laminaria digitata*, *Fucus serratus*, *Gracilaria gracilis* and *Codium fragile*). *J Appl Phycol* 2014;27:519-30.
- Lordan S, Smyth TJ, Soler-Villa A, Stanton C, Ross RP. The α -amylase and α -glucosidase inhibitory effects of Irish seaweed extracts. *Food Chem* 2013;141:2170-6.
- Kelman D, Posner EK, McDermid KJ, Tabandera NK, Wright PR, Wright AD. Antioxidant activity of Hawaiian marine algae. *Mar Drugs* 2012;10:403-16.
- Harmelin JG. High xenodiversity versus low native diversity in the South-Eastern Mediterranean: Bryozoans from the coastal zone of Lebanon. *Mediterr Mar Sci* 2016;17:417-39.
- Kanaan H, Belous O, Chokr A. Diversity investigation of the seaweeds growing on the Lebanese coast. *J Mar Sci Res Dev* 2014;5:1-12.
- Karaki N, Faour T, Sebaaly C, Chahine N, Zichenko A, Rachid S, *et al.* The antioxidant and anticoagulant activities of polysaccharides isolated from the brown algae *Dictyopteris polypodioides* growing on the Lebanese coast. *JAPS* 2013;3:43-51.
- Haddad M, Zein S, Hazimeh G, Karaki R, Krivoruchko E, Makhour Y, *et al.* Structural characteristics, antitumor and antioxidant properties of polysaccharides isolated from the brown algae *Styopodium schimperi* growing on the Lebanese coast. *ARJMD* 2017;17:36-43.
- Sari-Chmayssem N, Taha S, Mawlawi H, Guégan JP, Jeftić J, Benvegna T. Extracted and depolymerized alginates from brown algae *Sargassum vulgare* of Lebanese origin: Chemical, rheological and antioxidant properties. *J Appl Phycol* 2016;28:1915-29.
- El Asri O, Ramdani M, Latrach L, Haloui B, Ramdani M, Afifal ME. Comparison of energy recovery after anaerobic digestion of three Marchica lagoon algae (*Caulerpa prolifera*, *Colpomenia sinuosa*, *Gracilaria bursa-pastoris*). *Sustainable Materials and Technologies* 2017;11:47-52.
- Cotton AD. The appearance of *Colpomenia sinuosa* in Britain. *Bull Miscellaneous Infr Kew* 1908;1:73-7.
- Lee KM. Cryptic diversity and biogeography of the widespread brown alga *Colpomenia sinuosa* (Ectocarpales, Phaeophyceae). *Bot Mar* 2012;56:15-25.
- Lekameera R, Pandian V, Thirugnanasambandan S. Evaluating antioxidant property of brown alga *Colpomenia sinuosa* (DERB. ET SOL). *Afr J Food Sci* 2008;2:126-30.
- Manam VK, Subbiah M. Biological synthesis of silver nanoparticles from marine alga *Copomenia sinuosa* and its *in vitro* ant-diabetic efficacy. *Am J Biopharm Biochem Life Sci* 2014;3:1-7.
- Palanisamy S, Vinosha M, Marudhupandi T, Rajasekar P, Prabhu NM. Isolation of fucoxanthin from *Sargassum polycystum* brown algae: Structural characterization, *in vitro* antioxidant and anticancer activity. *Int J Biol Macromol* 2017;102:405-12.
- AOAC International. Official methods of analysis of AOAC International. 17th ed. Gaithersburg, MD, USA: AOAC International; 2002.
- Kostas ET, Wilkinson SJ, White DA, Cook DJ. Optimization of a total acid hydrolysis based protocol for the quantification of carbohydrate in macroalgae. *J Algal Biomass Util* 2015;7:21-36.
- Barbarino E, Lourenço SO. An evaluation of methods for extraction and quantification of protein from marine macro-and microalgae. *J Appl Phycol* 2005;17:447-60.
- Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. *Plant Physiol* 1949;24:1-5.
- Jeffrey SW, Humphrey GF. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem Physiol Pflanz* 1975;167:191-4.
- Kirk JT, Allen RL. Dependence of chloroplast pigment synthesis on protein synthesis: Effect of actidione. *Biochem Biophys Res Commun* 1965;21:523-30.
- Seely GR, Duncan MJ, Vidaver WE. Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. *Mar Biol* 1972;12:184-8.
- Tye KY, Gan SY, Lim SH, Tan SE, Chen CA, Phang SM. Comparison of visual observation and emission intensity of resazurin for antimicrobial properties of hexane, dichloromethane, methanol and water extracts from a brown alga, *Turbinaria ornata*. Bahler J, editor. *Cogent Biol* 2016;2:1.
- Dahmoune F, Nayak B, Moussi K, Remini H, Madani K. Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. *Food Chem* 2015;166:585-95.
- Athukorala Y, Kim KN, Jeon YJ. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food Chem Toxicol* 2006;44:1065-74.
- Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem* 1995;43:27-32.
- Govindappa M, Farheen H, Chandrappa CP, Channabasava, Rai RV, Raghavendra VB. Mycosynthesis of silver nanoparticles using extract of endophytic fungi, *Penicillium* species of *Glycosmis mauritiana* and its antioxidant, antimicrobial, anti-inflammatory and tyrosinase inhibitory activity. *Adv Nat Sci Nanosci Nanotechnol* 2016;7:035014.
- Balouiri M, Sadiki M, Ibsouda SK. Methods for *in vitro* & evaluating antimicrobial activity: A review. *J Pharm Anal* 2016;6:71-9.
- Lanfer-Marquez UM, Barros RM, Sinnecker P. Antioxidant activity of chlorophylls and their derivatives. *Food Res Int* 2005;38:885-91.
- Rao AV, Rao LG. Carotenoids and human health. *Pharmacol Res* 2007;55:207-16.
- El-Said GF, El-Sikaily A. Chemical composition of some seaweed from Mediterranean Sea coast, Egypt. *Environ Monit Assess* 2013;185:6089-99.
- Polat S, Ozogul Y. Biochemical composition of some red and brown macro algae from the Northeastern Mediterranean Sea. *Int J Food Sci Nutr* 2008;59:566-72.
- Ngo DH, Kim SK. Sulfated polysaccharides as bioactive agents from marine algae. *Int J Biol Macromol* 2013;62:70-5.
- Khaled N, Chbani A, Mawlawi H. Antioxidant and antifungal activities of *Padina pavonica* and *Sargassum vulgare* from the Lebanese Mediterranean Coast. *AEB* 2012;6:42-8.
- Martins LD, Ramlov F, Nocchi N, Gestinari LM, dos Santos BF, Bento L, *et al.* Antioxidant properties and total phenolic contents of some tropical seaweeds from the Brazilian coast. *J Appl Psychol* 2012;25:1179-87.
- Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn. – An *in vitro* approach. *J King Saud Univ Sci* 2014;26:267-75.
- Pietta PG. Flavonoids as antioxidants. *J Nat Prod* 2000;63:1035-42.
- Shukla S, Mehta A, Mehta P, Bajpai VK. Antioxidant ability and total phenolic content of aqueous leaf extract of *Stevia rebaudiana* Bert. *Exp Toxicol Pathol* 2012;64:807-11.
- Umapathy E, Ndebia E, Meeme A, Adam B, Menziwa P, Nkeh-Chungag B, *et al.* An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute. *J Med Plant Res* 2010;4:789-95.
- Naz R, Ayub H, Nawaz S, Islam ZU, Yasmin T, Bano A, *et al.* Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. *BMC Complement Altern Med* 2017;17:302.
- Hemalatha S, Fazeela Begum SM. Characterization, *in silico* and *in vitro* determination of antidiabetic and anti-inflammatory potential of ethanolic extract of sargassum wightii. *AJPCR* 2017;10:297-301.
- Costa LS, Fidelis GP, Cordeiro SL, Oliveira RM, Sabry DA, Câmara RB, *et al.* Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomed Pharmacother* 2010;64:21-8.
- Sousa M, Maria dos Santos Pires K, Barroso de Alencar D, Sampaio A, Saker-Sampaio S. α - and β -carotene and α -tocopherol in fresh seaweeds. *Ciênc Tecnol Aliment* 2008;28:953-8.
- González del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I, *et al.* Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int Microbiol* 2001;4:35-40.
- Demirel Z, Yilmaz F, Karabay-Yavasoglu UN, Ozdemir G, Sukatar A. Antimicrobial and antioxidant activity of brown algae from the Aegean Sea. *J Serbe Chem Soc* 2009;74:619-28.



Supplementary Figure 1: Extraction process of *Colpomenia sinuosa*



Supplementary Figure 2: The half maximal inhibitory concentration of (a) 2,2-diphenyl-1-picrylhydrazyl scavenging activity of *Colpomenia sinuosa* extracts. (b) Albumin denaturation inhibition by *Colpomenia sinuosa* extracts