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ПОРІВНЯННЯ ХІМІЧНОГО СКЛАДУ ТА БІОЛОГІЧНОЇ АКТИВНОСТІ ПОЛІСАХАРИДІВ БУРИХ ВОДОРОСТЕЙ, ЧЕРВОНЕ МОРЕ, ЄГИПЕТ, ХУРГАДА¹

Показано, що сульфатовані полісахариди з водоростей можуть мати широке застосування у медичних цілях. Хімічний склад та біологічні функції різних полісахаридів були визначені для трьох видів бурих водоростей з єгипетського Червоного моря: *Dictyopteris polypodioides*, *Polycladia turrica* та *Turbinaria decurrens*. Виявлено, що вихід неочищених полісахаридів був вищим, ніж вихід альгінату та фукоїдану в діапазоні від 43,64 до 61,90 %. У *D. polypodioides* виявлено фукоїдан, який має максимальний вміст вуглеводів — 56,89 %. Найвищий вміст сульфату (22,44 %) зареєстровано у сирих (неочищених) полісахаридах, екстрагованих з *P. turrica*. Усі функціональні групи досліджуваних зразків були підтверджені інфрачервоним спектром Фур'є (FTIR). Для кількісного визначення антиоксидантної активності екстрагованих полісахаридів залежно від застосованого методу, типу полісахаридів і виду водоростей експериментально було проведено три типи аналізів. Протидіабетична активність *T. decurrens*-неочищеного полісахариду була високою і становила 85,85 % в аналізі із застосуванням α -глюкозидази. Крім того *T. decurrens*-неочищений полісахарид мав найвищу проти-артритну активність (89,89 %). Найвищим значенням активності проти зайвої ваги (95,25 %) характеризувався *T. decurrens*-фукоїдан. Виділені екстракти полісахаридів проявляли і протибактеріальну активність. Найбільш активний екстракт *T. decurrens*-неочищений полісахарид був перевірений

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на цитотоксичність щодо клітин печінки людини та визнаний безпечним. Встановлена залежність між активністю екстрагованих полісахаридів та їхнім хімічним складом. Біологічно активні протестовані полісахариди можуть бути використані в медичних цілях для розробки нового терапевтичного втручання.

Ключові слова: альгінат, фукоїдан, протибактеріальний, проти-діабетичний, проти зайвої ваги, *Phaeorhysae*.

Macroalgae are among the main producers of biomass in marine ecosystems, which also have a variety of bioactive metabolites with useful applications in the food and pharmaceutical industries [30, 61]. Additionally, they are regenerative, simple to cultivate, non-toxic, and have no negative side effects [33]. Especially, brown algae are widely distributed throughout the tropics, subtropics, and occasionally in temperate marine ecosystems [25].

On focus, sulfated polysaccharides (SPs) are the primary biochemical structure among the several bioactive chemicals that are pertinent to the algal taxonomic position. High levels of polysaccharides, which are absent in terrestrial plants, are mostly replaced by sulfate in the macroalgal cell wall, which is what gives it its distinctive appearance. According to phylum, species, various locations, and harvest time, the chemical composition and diversity of the algal polysaccharides vary [34, 36].

SPs are complex and diverse anionic macromolecules that can make approximately 4 to 76 % of the dry weight of macroalgae. In particular, alginate, laminarin, and fucoidan are only a few examples of the polysaccharides from brown algae that account for more than 50 % of the total dry weight of the algae and may even reach 70 % in some species [42].

While alginate is among the most widely used brown seaweed polysaccharides by a variety of industries, it is crucial to identify and study new sources for obtaining alginate to meet the rising demand for alginate, particularly sodium alginate, around the world [18]. The extraction process of alginate is based on the transformation of an insoluble combination of salts of alginic acid into a soluble salt (alginate), which is suitable for water extraction. On the other side, fucoidans are SPs found in the cell walls of brown seaweed species composed of fucose as the main monosaccharide, but accompanied by very variable amounts of other monosaccharides like galactose, xylose, mannose, rhamnose, and/or glucuronic acid [34].

There is a wealth of information available about the bioactivity of fucoidans, including their antiviral, anticoagulant, anticancer, antioxidant, anti-inflammatory, and anti-coagulant properties [34, 64]. Numerous investigations have sought to explore the structural details of fucoidans, but it has been very difficult to identify a common characteristic shared by all of the fucoidans that have been studied to date [57, 64]. Typically, fucoidans are extracted from brown algae in a multi-step, hot acid process. However, the extraction parameters have a substantial impact on the structural and compositional characteristics of the fucoidan polysaccharides, as well as on their bioactivity [64]. Furthermore, alginate and fucoidan also have beneficial qualities such as biocompatibility, non-toxicity, biodegradability, and functional adaptability with dif-

ferent matrices and substrates [18]. The alginate and fucoidan extracted from *Turbinaria* species may be valuable in medical uses due to their antioxidant and anticancer properties [69].

Generally, a challenge for the use of macroalgae in food and medicine is that seaweed harvesting is often seasonal so it should be kept and maintained to provide year-round industrial processes. Information on post-harvest storage techniques and conditions for algal raw materials, as well as their impact on the stability and concentration of biologically active chemicals, are crucial step. According to [50], there are very few studies on the impact of freezing and long-term storage at low temperatures on the amount of bioactive substances in algae. However, the effects of cryopreservation allow one to rely on a high quality and level of these substances while maintaining the original properties of the material. Regarding the amounts of polysaccharides, amino acids, and total phenols, air drying and freezing are viable processes that allow for a shelf life of at least 365 days [48].

Therefore, the aim of the present study was to extract and characterize crude sulfated polysaccharides, crude alginate and fucoidan from three brown seaweed species collected from the Egyptian Red Sea, Hurghada and to evaluate their antioxidant, anti-diabetic, anti-obesity, anti-arthritis, and antimicrobial activities, and also to determine the cytotoxicity effect of the most active sample.

Material and Methods

Chemicals. All chemicals were purchased from the Sigma-Aldrich Co (Darmstadt, Germany).

Reference bacterial strains. The bacterial indicator strains (*Bacillus subtilis* ATCC 6051, *Aeromonas hydrophila* ATCC 13037, *Pseudomonas aeruginosa* ATCC 9027, *P. fluorescens* ATCC 13525, *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae* ATCC 13813, *Vibrio damsela* ATCC 33539, *V. fluvialis* ATCC 33812, *Escherichia coli* ATCC 8739, and *Klebsiella pneumoniae* ATCC 13883) were kindly provided by the Marine Microbiology Department, the NIOF, Alexandria, Egypt.

Seaweed collection and identification. In front of the National Institute of Oceanography and Fisheries (NIOF) in the city of Hurghada, the Red Sea, Egypt, between latitudes 27°17'13"N and longitudes 33°46'21"E, three brown seaweeds (*Dictyopteris polypodioides* (De Candolle) J.V. Lamouroux, *Polycladia myrica* (S.G. Gmelin) Draisma, Ballesteros, F. Rousseau & T. Thibaut, and *Turbinaria decurrens* Bory) were collected. The collected species were cleaned with seawater before being transported in an ice tank to the Taxonomy and Biodiversity of Aquatic Biota Laboratory at the NIOF in Alexandria, Egypt for morphological description and identification in accordance with [3, 51] and confirmed using the Algae Base website [25]. They were washed with tap and distilled water, dried in the shade, ground into a powder, and stored at 4°C for additional analyses.

Seaweed polysaccharides extraction and yield. According to [17], water-soluble polysaccharides were isolated. For the extraction of crude alginate, the

procedure described in [10] was used. Crude fucoidan was extracted by the method [60], with a few adjustments. The extraction process was carried out for 12 h at 25°C. The yields of the extracted samples were reported as a percentage of the alga's initial dry weight (% DW).

Polysaccharides characterization. The total content of carbohydrate, protein, sulfate, and total organic carbon content were determined in all extracted polysaccharide samples. According to [16], the total carbohydrates were calculated using d-glucose as the reference. The total proteins were identified using bovine serum albumin (BSA) as the reference protein [43]. As well, barium chloride-gelatin was used to estimate the sulfate content [40]. The amount of total organic carbon was measured using the acid/dichromate titration assay [23]. All the estimated chemical parameters were expressed as a percentage of the extracted polysaccharides (%). The Fourier Transform Infrared (FTIR) spectroscopy was carried out using a Perkin Elmer spectrophotometer.

Biological activities of the extracted polysaccharides in vitro. Antioxidant activities. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The DPPH radical scavenging activity of the SPs methanol extract (100 µg/ml) was assessed using the technique [67]. The radical scavenging efficiency was determined using the following formula and measured at 550 nm:

$$\text{Antioxidant activity} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}}$$

where, A_{sample} is the absorbance of the tested solutions, while A_{control} is the absorbance of the control sample, and A_{blank} is the absorbance of the blank solutions. The ascorbic acid standard was used to calculate the results and the GraphPad Prism software was used to determine the LC_{50} (µg/ml) values, or the concentration of SPs extracts that could scavenge 50 % of DPPH free radical.

Hydrogen peroxide radical scavenging activity. The hydrogen peroxide radical scavenging test was carried out at 230 nm. Standardization was done using ascorbic acid [26]. The fraction of SPs that can scavenge free radicals was determined using the following equation:

$$\text{Free radical scavenging (Hydrogen peroxide)\%} = \left[\frac{(A_c - A_s)}{A_c} \right] \times 100$$

where, A_c is the absorbance of control and A_s — the absorbance of sample.

Total antioxidant activity. Using the method described in [58], the total antioxidant capacity (TAC) of various extracted SPs (100 µg/ml methanol) was calculated. The obtained results are expressed in equivalent mg of extract weight (mg AsAE per g SPs) and were calculated using an ascorbic acid standard curve.

Antidiabetic activity. Inhibition of α -amylase activity. Following a procedure outlined in [27], the inhibition of α -amylase of several SPs extracts was determined. The non-linear regression curve was used to get the LC_{50} value

(µg/ml), or the concentration of extracts or standards that inhibit 50 % of the enzyme activity. In this case, acarbose was used as standard.

Inhibition of α-glucosidase activity. The method [15] was used to assess the α-glucosidase inhibition using acarbose as a standard to detect the absorbance at 540 nm. Additionally, the non-linear regression curve was used to calculate the LC₅₀ value (µg/ml).

Anti-obesity pancreatic lipase inhibitory assay. According to the approach [39], the lipase inhibitory activity of various SPs and Orlistat (a reference drug) extract was assessed using a Multiplate Reader at 750 nm. The following equation was used to estimate the results:

$$\text{Inhibitory activity} = \left[1 - \frac{As}{Ac} \right] \times 100$$

where *As* is the absorbance in the presence of SPs substance and *Ac* is the absorbance of control.

Anti-arthritic activity. The anti-arthritic activity was assessed using the albumin denaturation test and a microplate reader set at 660 nm. As a reference drug, diclofenac sodium was used to calculate the percentage of protein denaturation inhibition:

$$\% \text{ Inhibition of protein denaturation} = \left[1 - \frac{A1}{A2} \right] \times 100$$

where *A1* is the absorbance of control, *A2* — the absorbance of test/standard sample with albumin solution, and the LC₅₀ value was established as the concentration to inhibit 50 % of protein denaturation under the assay conditions.

Antibacterial activity. Using the well diffusion method, the antibacterial efficacy of all examined SPs dissolved in sterile distilled water was evaluated against ten bacterial pathogens [4]. The diameter of the inhibitory zone around each well measured in millimeters was used to represent positive results [28].

*Cytotoxic evaluation of *T. decurrens* crude SPs.* Preliminarily, the crude SPs from *T. decurrens* was initially chosen because it had the highest bioactivity in the majority of experiments and was tested as a cytotoxic agent. The WISH cells (Human amnion; normal liver cells) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Additionally, the Sigma (St. Louis, Missouri, USA) supplied the trypan blue dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO). Moreover, fetal bovine serum, DMEM, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution, l-glutamine, gentamicin, and 0.25 % trypsin-EDTA were purchased from the Lonza.

Cell line propagation. The Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated fetal bovine serum, 1 % l-glutamine, HEPES buffer, and 50 µg/ml gentamicin was used to propagate the cells. All cells were sub-cultured twice a week and kept at 37°C in a humidified atmosphere with 5 % CO₂.

Cytotoxicity evaluation using viability assay. For the cytotoxicity experiment, the WISH cells were seeded in 96-well plates at a density of 1×10^4 cells per well in 100 μ L of cultural media. After 24 h of seeding, fresh medium with various concentrations of the test sample was introduced. Serial two-fold dilutions of the investigated chemical component were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C for 24 h in a humidified incubator with 5 % CO₂. For each concentration of the test material, three wells were used. Control cells were cultured with or without DMSO and without the test sample. The experiment was shown to be unaffected by the minimal amount of DMSO (a maximum of 0.1 %) contained in the wells. A colorimetric approach was used to calculate the viable cell yield after incubation.

Briefly, the Roswell Park Memorial Institute (RPMI) 1640 medium without phenol red was added to the 96-well plates in 100 L increments, and 10 L of the 12 mM MTT stock solution (5 mg of MTT in 1 ml of phosphate-buffered saline (PBS)) was added to each well, including the untreated controls. Afterwards, the 96-well plates were incubated for 4 h at 37°C with 5 % CO₂. After removing an 85 μ L aliquot of the media from each well, 50 μ L of DMSO was added, mixed well with the pipette, and incubated at 37°C for 10 min. The number of viable cells was then determined by measuring the optical density at 590 nm using a microplate reader (SunRise, TECAN, Inc., USA) according to [24], and the percentage of viability was calculated as follows:

$$\text{Viability} = \left[\frac{OD_t}{OD_c} \right] \times 100$$

where OD_t is the mean optical density of wells treated with the SPs samples and OD_c is the mean optical density of untreated cells.

To determine the relation between sample concentrations and surviving cells is plotted to get the survival curve of the cell line after treatment with *T. decurrens* crude SPs. The 50 % inhibitory concentration (LC₅₀), or the dosage needed to cause toxic effects in 50 % of intact cells, was detected by using Graph-Pad Prism software (San Diego, CA, USA) [46].

Statistical analysis. Data were reported as the mean and standard deviation (SD) for all experimental works that were carried out in triplicate ($n = 3$). To ascertain their relationship, the Pearson correlation was performed between the chemical composition of the separated polysaccharides and the bioactivities.

Results and Discussion

Identification of the collected seaweeds. Initially, seaweeds richen with alginate and fucoidan were selected from the Egyptian part of the Red Sea. They were identified as *D. polypodioides*, *P. myrica* and *T. decurrens* (Figure 1). Many other researches monitored them in their studies. Several biological activities were detected in the same species previously [35]. Fucoidan was isolated

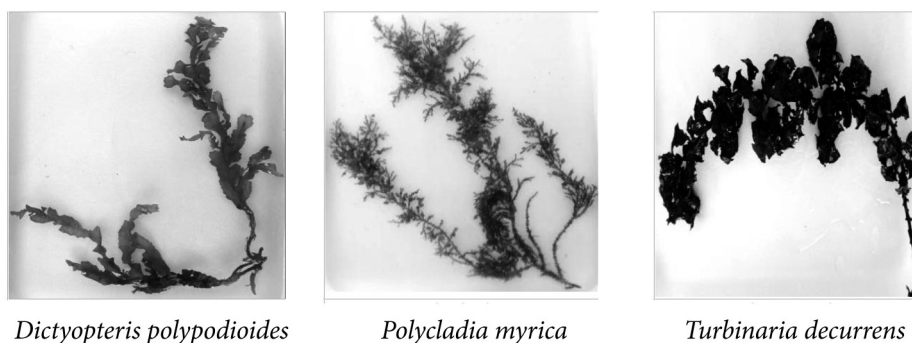


Fig. 1. General features of the collected algal species

from both *Cystoseira barbata* and *Fucus virsoides* [14]. Further, the bioactivity of alginate from *Dictyopteris polypodioides* was reported [1].

Yield of extracted algal SPs. The data presented in Table 1 reveal various ratios of different SPs yields from three algal species under investigation in comparison to their crudes. The performed investigations showed that the yield of crude SPs recorded the highest values of all. Its values ranged from 61.90 % to 43.64 % for *T. decurrens* and *P. myrica* dry weight, respectively. In this case, *D. polypodioides* had 46.97 % as a yield of its dry weight. Additionally, the highest alginate yield was recorded in *T. decurrens* (41.90 % DW) followed by *D. polypodioides* (36.97 % DW), whereas the lowest percentage was in *P. myrica* (33.64 % DW). Moreover, *D. polypodioides* showed the highest fucoidan value (22.51 % DW) followed by *P. myrica* (22.12 %), whereas the lowest value was observed in *T. decurrens* (15.37 % DW). This finding confirms that *T. decurrens* was a good source for alginate and *D. polypodioides* was a good source for fucoidan. The variation in the yield of algal SPs may be related to the algal species and their morphological form [18]. The estimated yield of crude SPs was higher than that obtained from *Sargassum euryphyllum* (39.49 %) and *S. aquifolium* (23.13 %), both collected from the same area [29].

Basically, alginate makes up to 40 % of brown algal dry weight and is the major polysaccharide in the cell wall with species-specific composition [41].

Table 1
Yield of the extracted crude SPs (%) from different algal species

Species	Yield of the different extracted SPs (% DW)		
	Crude SPs	Alginate	Fucoidan
<i>D. polypodioides</i>	46.97±2.8	36.97±2.12	22.51±1.24
<i>P. myrica</i>	43.64±9.4	33.64±1.45	22.12±2.23
<i>T. decurrens</i>	61.90±4.10	41.90±1.10	15.37±1.33

The obtained results are less or more similar to [37], which suggests that the alginate yield ranged from 20.19 % to 49.8 % dry weight for *Laminaria ochroleuca* and *Saccorhiza polyschides*, respectively. On the other hand, it has been shown [52] that the total yield of fucoidan isolated from *S. polycystum* is 4.51 %. In the Egyptian part of the Red Sea, fucoidan production varied from 9.085 % in *Sargassum linifolium* to 13.04 % in *Stypopodium schimperi* [18].

Characterization of the extracted algal SPs

Chemical composition. The chemical composition of the extracted SPs is given in Table 2. In some details, the highest total organic carbon (TOC) value was registered in fucoidan of *P. myrica* (28.33 %), followed by *D. polypodioides*-fucoidan (27.64 %), and *T. decurrens*-alginate (25.01 %). In addition, carbohydrate content in all tested SPs ranged from 37.27 % (*D. polypodioides*-crude SPs) to 56.89 % (*D. polypodioides*-fucoidan). Generally its values were higher in the case of fucoidan compared to others. Sulfate content showed moderate values with obvious fluctuations. The lowest one was 13.71 %, which was detected in *D. polypodioides*-alginate and the highest value — 22.44 % in *P. myrica*-crude SPs. Protein content was characterized by very low values, which ranged between 0.0001 % (*T. decurrens*-alginate) and 0.009 % (*D. polypodioides*-crude SPs).

According to findings from the literature, crude polysaccharides from *Sargassum* spp. have a comparable ratio of the total carbohydrate content (44.09—

Table 2

Chemical composition of the extracted SPs from different algal species under investigation in comparison to their crudes

Species	Parameter, %			
	Total organic carbon	Carbohydrate	Sulfate	Protein
Crude SPs				
<i>T. decurrens</i>	17.15 ^b	43.64 ^a	21.63 ^a	0.003 ^a
<i>P. myrica</i>	21.28 ^a	46.90 ^a	22.44 ^a	0.008 ^a
<i>D. polypodioides</i>	17.29 ^b	37.27 ^b	19.89 ^a	0.009 ^a
Alginate				
<i>T. decurrens</i>	25.01 ^a	41.89 ^b	18.97 ^a	0.0001 ^b
<i>P. myrica</i>	23.91 ^a	46.96 ^a	15.98 ^b	0.001 ^a
<i>D. polypodioides</i>	19.32 ^b	43.64 ^a	13.71 ^a	0.003 ^a
Fucoidan				
<i>T. decurrens</i>	14.20 ^b	53.69 ^a	20.03 ^a	0.001 ^b
<i>P. myrica</i>	28.33 ^a	55.07 ^a	17.32 ^b	0.001 ^b
<i>D. polypodioides</i>	27.64 ^a	56.89 ^a	15.03 ^b	0.004 ^a

Note. Different letters at the same row mean significant difference at $P \leq 0.05$.

57.43 %) and sulfate content (13.58—18.26 %), but no protein was found [29]. Later, it has been found [38] that the polysaccharides extracted from different brown algal species significantly differed in the total content of carbohydrates (from 32.15 % in *Turbinaria conoides* to 59.95 % in *Padina minor*). The content of sulfate varied from 9.74 % to 14.22 % depending on algal species. The fucoidan isolated from *T. decurrens* included 54.86 % of the total sugar, 23.51 % of sulfate, and 3.4 % of protein [45].

FTIR profile of SPs. For all illustrated FTIR spectra, shown in Figure 2, a wide peak in the range 3395 to 3470 cm^{-1} is due to the stretching vibrations of O-H and a small peak at 2925—2946 cm^{-1} for the extracted crude SPs and alginates represents the stretching of C-H [17]. For the extracted fucoidan from *D. polypodioides*, and *T. decurrens* this peak does not appear. Sharp peaks were observed in the range from 1618—1640 cm^{-1} , which were attributed to carboxylate O-C-O asymmetric stretching or can be assigned to the amide groups of protein or to the carbonyl stretching groups. In general, the FTIR spectrum shows an intense band at 1594.70 cm^{-1} reflecting the asymmetrical stretching of carboxylate (COO-) confirming the high content of uronic acid in the extract [9]. The peaks appear at 1413—1466 cm^{-1} for the crude of both *P. myrica* (SPs and alginates) and *D. polypodioides* (SPs, alginate and fucoidan) and indicate C-H bending vibration of polysaccharide. These bands appeared as broad bands at 1408—1409 cm^{-1} for crude SPs and alginate from *T. decurrens* may be assigned to C-OH deformation vibration with the contribution of O-C-O symmetric stretching vibration of the carboxylate group. Weak and short bands at 1249 cm^{-1} in crude SPs of *P. myrica* and *D. polypodioides* are due to the presence of sulfated ester groups (S = O). Weak and broad bands at 1070—1090 cm^{-1} in all samples except *T. decurrens*-SPs represented the S = O stretch of the sulfated polysaccharides or the C-N stretching of aromatic amine group. The bands at 1120—1124 cm^{-1} of *T. decurrens* crude SPs and *P. myrica* alginates are allocated to C-O groups of polyols of polysaccharides. The spectrum also showed the most intense band at 1047.07 cm^{-1} corresponding to the C = O group [21].

Additionally, the spectrum displayed three peaks between 950 and 750 cm^{-1} providing further evidence of the existence of uronic acid. With the exception of *P. myrica* crude SPs, all samples showed weak and broad bands between 597 and 619 cm^{-1} , which may have been caused by C = C-H stretching vibration. The FTIR spectra of the alginates produced from *L. ochroleuca* and *S. polyschides* resembled those of commercial alginate [37].

The bands at 1618—1640 cm^{-1} related to carbonyl (C = O) group in alginate. The bands appearing at 1413—1466 cm^{-1} in the extracted alginates are assigned to carboxyl (COOH) group present in the alginate [59]. The short band at 1249 cm^{-1} can be assigned to the C-O stretching vibration. Bands at approximately 1.070—1.090 cm^{-1} in algal species correspond to mannuronic (M) and guluronic (G) units, respectively [55]. The bands at 1120—1124 cm^{-1} in the tested algae correspond to C-O and C-C-H groups present in mannuronic and guluronic acids forming the alginate. The bands at 1.070 and 1.090 cm^{-1} in the tested algae correspond to S = O and C-C-H groups. Multiple very small peaks observed in the range of 1070—1124 cm^{-1} represent the S = O stretch of the sul-

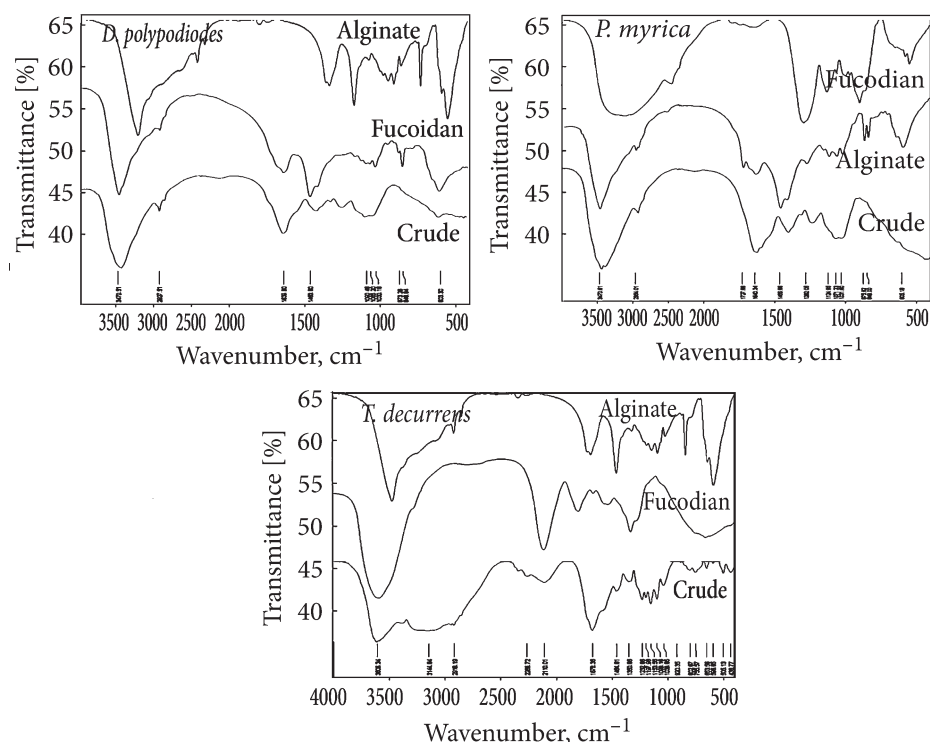


Fig. 2. Different FTIR profiles of the extracted polysaccharides from brown algal species under investigation.

fated polysaccharides or the C-N stretching of the aromatic amine group [47]. The band around 1.000 cm^{-1} is attributed to alcohol groups in the alginate. The bands at $597\text{--}619\text{ cm}^{-1}$ are related to the vibration of the C = C-H stretching [11].

Biological activity of the tested SPs

Antioxidant activity of the extracted algal SPs. The antioxidant activity of three algal SPs was estimated with sharp accuracy via three assays. Table 3 reveals that the SPs activities detected by the DPPH radical scavenging assay were very promising compared to ascorbic acid as a known antioxidant substance. The lowest value (54.2 %) was detected in *D. polypodioides*-fucoidan, whereas the highest value (88.71 %) was recorded in *T. decurrens*-crude SPs. Different LC_{50} values were calculated for all treatments. Obviously, the LC_{50} of most treatments was lower than ascorbic acid's LC_{50} ($69.25\text{ }\mu\text{g/mL}$), which confirmed their strong antioxidant activity and potential to be a radical scavenger agent.

Further, the antioxidant activity was also estimated via the H_2O_2 radical scavenging assay. The data presented in Table 3 exhibit the lowest activity (32.50 %) in the case of alginate from *D. polypodioides*, whereas the highest activity (68.89 %) was detected in the case of *P. myrica*-alginate. The antioxidant activity obtained by ascorbic acid (40.25 %) was lower than that of the majority

of SPs, which demonstrated the highest H₂O₂ radical scavenging activity of these SPs.

The LC₅₀ ranged between 60.48 µg/mL for alginate of *P. myrica* and 83.68 µg/mL for fucoidan of *T. decurrens*.

Moreover, the antioxidant activity was also estimated by TAC assay (Table 3), with the lowest activity (8.20 mg/g AsA equivalent) in the case of alginate of *P. myrica* and the highest activity (18.62 mg/g AsA equivalent) — in the case of *P. myrica*-crude SPs. The antioxidant activity conducted using ascorbic acid (13.25 mg/g AsA equivalent) showed a value lower than most of all algal SPs, but it was much higher than others. These observed variations in antioxidant activity of the experimented SPs were related to the assay and type of SPs used. In general, the highest antioxidant activity was detected in the tested crude SPs

Table 3

Antioxidant activity of algal SPs (crude SPs, alginate and fucoidan) estimated via DPPH radical scavenging, TAC and H₂O₂ radical scavenging assays in comparison to ascorbic acid as a powerful antioxidant

Polysaccharide	Antioxidant activity			
	<i>P. myrica</i>	<i>D. polypodioides</i>	<i>T. decurrens</i>	Ascorbic acid
DPPH radical scavenging %				
Crude Sps, LC ₅₀ (µg/mL)	72.75 ^b 62.48	69.36 ^c 65.53	88.71 ^a 51.24	69.25
Alginate, LC ₅₀ (µg/mL)	68.19 ^b 66.66	66.2 ^b 68.66	72.66 ^a 62.56	
Fucoidan, LC ₅₀ (µg/mL)	60.92 ^a 74.61	54.2 ^b 83.86	64.05 ^a 70.97	
H ₂ O ₂ radical scavenging %				
Crude Sps, LC ₅₀ (µg/mL)	67.82 ^a 61.44	42.50 ^c ND	53.52 ^b 83.68	40.25
Alginate, LC ₅₀ (µg/mL)	68.89 ^a 60.48	32.50 ^c ND	53.50 ^b 81.27	
Fucoidan, LC ₅₀ (µg/mL)	66.30 ^a 62.85	41.31 ^c ND	51.96 ^b 83.68	
TAC (mg/g ASA)				
Crude SPs	18.62 ^a	8.94 ^b	16.61 ^a	13.25
Alginate	8.20 ^b	6.25 ^b	15.77 ^a	
Fucoidan	15.72 ^a	10.29 ^b	16.87 ^a	

Note. Bold values refer LC₅₀. Small superscript letters refer to significant difference between the three extracted seaweed polysaccharides. Different letters at the same row mean significant difference at $P \leq 0.05$.

compared with crude alginate and fucoidan, which may be due to their composition and nature.

The antioxidant activity of sulfated polysaccharides from brown algal species was estimated by several researchers, and as a result, different values were obtained depending on the season, type of algae, and location of the collection. The sodium alginate derived from *D. polypodioides* showed the highest antioxidant activity with LC_{50} (20 $\mu\text{g/mL}$) in the DPPH test [1]. It has been found that the DPPH assay produced powerful antioxidant effects for the SPs from *S. euryphyllum* (59.15 %) and *S. aquifolium* (65.25 %) compared to ascorbic acid (67.33 %), whereas the H_2O_2 radical scavenging activity of the crude SPs from the two *Sargassum* species was less effective than that assessed for ascorbic acid [29]. On the other hand, the highest activity via DPPH (61.2 %), reducing ability (67.56 %), and the total antioxidant activity (65.3 %) were obtained at 1000 g/mL of fucoidan in evaluating the antioxidant activity of *S. polycystum* fucoidan using various assays [52].

Antidiabetic activity of the tested algal SPs. The anti-diabetic ability of the isolated SPs was determined by two effective assays and their data are presented in Figure 3. Firstly, the α -glycosidase assay scored high activity with the range of 62.50 % and 85.85 % for *D. polypodioides*-alginate and *T. decurrens*-crude SPs, respectively, whereas acarbose showed activity (63.63 %). The calculated LC_{50} of α -glycosidase assay for most treatments (14.0—19.5 $\mu\text{g/mL}$) except *D. membranacea*-alginate (20.5 $\mu\text{g/mL}$) was lower than standard concentration (19.64 $\mu\text{g/mL}$).

Secondly, α -amylase assay exhibited rather lower activity values than those gained by the first assay. In addition, acarbose showed activity (55.45 %) in the later assay. The estimated LC_{50} values of α -amylase of most isolated SPs (16.1—19.6 $\mu\text{g/mL}$) exhibited less or more similar behavior of α -glucosidase compared with the value of standard drug 29.84 $\mu\text{g/mL}$. These findings proved the potential anti-diabetic activity of different extracted SPs.

In the same context, alginates from *Laminaria digitata* and *Undaria pinnatifida* appeared to be strong inhibitors of α -amylase activity with LC_{50} of 0.075 ± 0.010 — 0.103 ± 0.017 mg/mL [68]. Overall, this finding shows that alginates are strong α -amylase inhibitors, which may delay the release of glucose from starches and reduce postprandial hyperglycemia. In addition, it has been found [54] that fucoidan treatment reduced insulin-induced 2-deoxy-D-[3H] glucose absorption by up to 51 % compared to control cells.

Anti-obesity pancreatic lipase inhibitory assay of the isolated SPs. For different extracted SPs, anti-obesity activity is illustrated in Figure 4. In particular, results of such an assay showed the lowest value as 62.81 % in the case of alginate of *D. polypodioides*, whereas the highest value (95.25 %) was detected for *T. decurrens*-fucoidan. In addition, Orlistat exhibited moderate activity (89.35 %) when compared to the algal SPs.

Complementarily, the LC_{50} ranged from 22.77 $\mu\text{g/mL}$ (*T. decurrens*-fucoidan) to 79.61 $\mu\text{g/mL}$ (*D. polypodioides*-alginate), whereas Orlistat had its effect at 55.96 $\mu\text{g/mL}$ as LC_{50} . However, all the extracted SPs from *T. decurrens* sho-

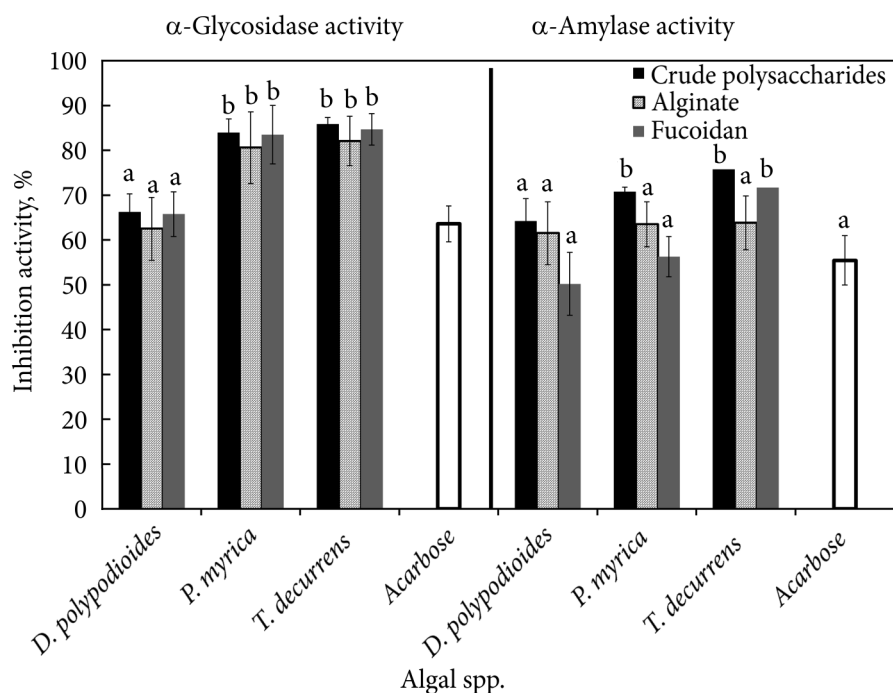


Fig. 3. Antidiabetic activity for different extracted polysaccharides via inhibition of α -amylase and α -glucosidase activity. Different letters mean significant difference at $P \leq 0.05$

wed the lowest values revealing the potential of these polysaccharides as molecules for anti-obesity applications. Seaweeds are a promising source of anti-obesity agents, especially alginates, fucoidans, fucoxanthin, and phlorotannins [65]. Alginates, in particular, have been demonstrated to have inhibitory activity against pancreatic lipase and the source and chemical form of the compound may affect the inhibitory activity of the substance. Practically, the inhibition of pancreatic lipase by alginates is substrate specific [12]. The fucoidans extracted from *U. pinnatifida* reduced lipid accumulation by stimulating lipolysis and demonstrating their anti-obesity properties [54].

Anti-arthritis activity. The data in Figure 5 illustrate the anti-arthritis activity of different extracted SPs via a protein denaturation assay. The crude SPs of *T. decurrens* showed the highest activity (89.89 %), followed by fucoidan of *T. decurrens* (88.25 %) and alginate of *T. decurrens* (86.32 %). The lowest values were recorded for all SPs of *D. polypodioides*, whereas the SPs of *P. myrica* exhibited moderate values between the two when compared with diclofenac sodium as a standard anti-arthritis agent. The LC_{50} values of crude SPs of *P. myrica* (14.75 μ g/mL) were only slightly lower than the LC_{50} of diclofenac sodium (15.12 μ g/mL) and other SPs treatments, so these tested polysaccharides have promising anti-arthritis properties.

It has been proven [2] that fucoidan extracts from *U. pinnatifida*, *L. japonica*, *Fucus vesiculosus*, *Ascophyllum nodosum*, and *Macrocystis pyrifera* have the

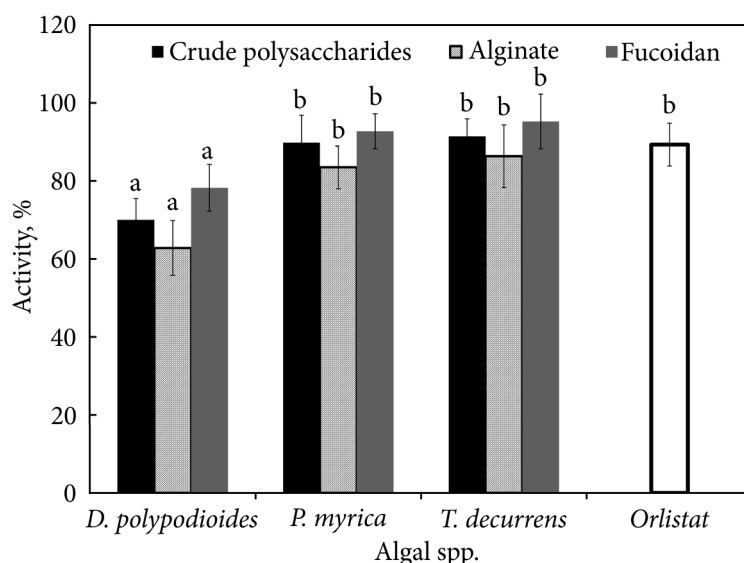


Fig. 4. Anti-obesity activity for different extracted SPs via pancreatic lipase inhibitory assay in vitro. Different letters mean significant difference at $P \leq 0.05$

anti-inflammatory effects. Fucoidan nanoparticles were confirmed to have an anti-oxidative and anti-inflammatory impact against the nephropathy of streptozotocin-induced diabetes in rats [66].

Antibacterial activity. Brown seaweed extracts are utilized in aquaculture feed as preventative and/or therapeutic treatments due to they have antibacterial effect against fish diseases [56]. In particular, the use of brown seaweed extracts encourages immunological system of fish and shrimps [62].

Complementarily to the biological activities determined, the antibacterial activity of all SPs samples was detected by using the well cut-diffusion technique. The data in Figure 6 and Table 4 suggest that few positive activities were recorded. The crude SPs of *D. polypodioides* showed considerable values against *E. coli* ATCC 8739 (22 mm) followed by *S. aureus* ATCC 25923 (20 mm). In addition, its fucoidan showed low inhibition activity towards *S. agalactiae* ATCC 13813, *S. aureus* ATCC 25923, *V. fluvialis* ATCC 33812, and *K. pneumoniae* ATCC 13883. Furthermore, the crude SPs of *P. myrica* showed moderate activity towards *B. subtilis* ATCC 6051 (18 mm), *E. coli* ATCC 8739 (16 mm), *S. aureus* ATCC 25923 (18 mm), and *K. pneumoniae* ATCC 13883 (18 mm) and very low activity against *A. hydrophila* ATCC 13037 (12 mm). The crude SPs of *T. decurrens* showed low activity (12 mm) only against *B. subtilis* ATCC 6051 and *K. pneumoniae* ATCC 13883. Strangely, the alginate of three seaweeds did not show any activity at all against the references tested bacteria. Thus, the sulfate content of SPs may show how effective they are at fighting bacteria. It has been found [8] that that highly sulfated polysaccharides have the strongest antimicrobial properties. On the other hand, the antimicrobial activity of

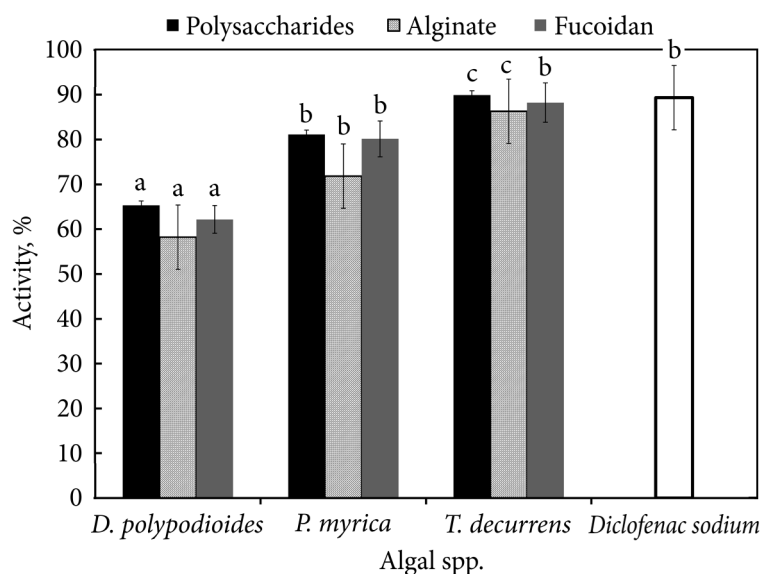


Fig. 5. Anti-arthritis activity for the different extracted SPs *in vitro*. Different letters mean significant difference at $P \leq 0.05$

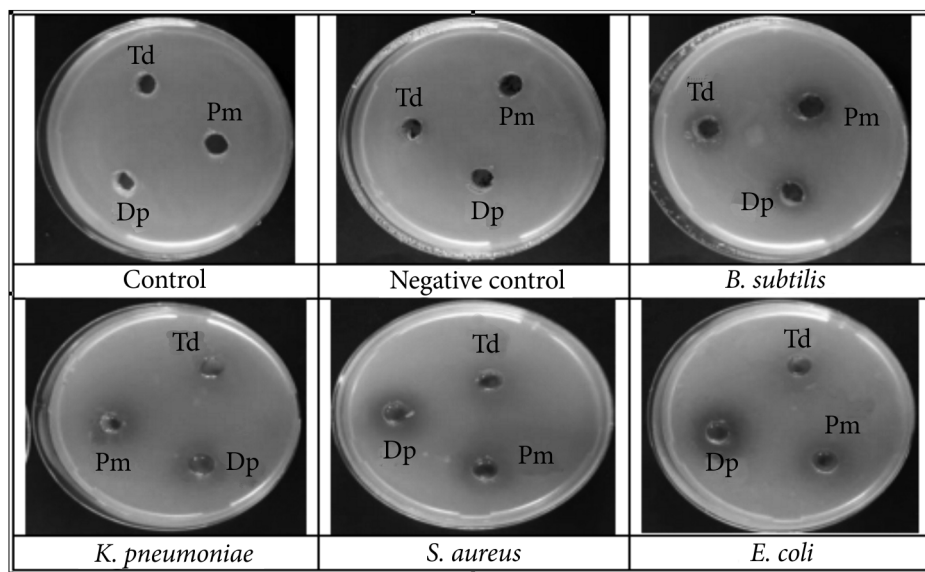


Fig. 6. A side of antibacterial activities detected by the most potent algal crude SPs against bacterial pathogens. Td – *T. decurrens*, Dp – *D. polypodioides*, and Pm – *P. myrica*

the brown algal SPs may be related to the collection season and sites [38]. In relation to this, the antibacterial agent fucoidan, which was isolated from *S. polycystum*, was investigated for its potential to treat human and animal harmful microorganisms [53]. The fucoidans from *Fucus vesiculosus* appeared to have a

significant bacteriostatic effect on the growth of tested bacterial strains and *E. coli* was the most susceptible bacterium [6].

Cytotoxic evaluation of *T. decurrens* crude SPs. As shown in Figure 7, the cytotoxicity of *T. decurrens* crude SPs was further evaluated towards normal liver cells due to this extract exhibited more bioactivity than other SPs extracts in most the estimated activities. Both viability and inhibitory percentages (%) were determined for detecting the safe concentration, upon which it will be restricted and then forbidden. Specifically, even at 31.25 µg/mL the cells keep their viability completely. Above such crude SPs concentration, cellular viability decreased gradually with the increase of SPs concentrations. At 500 µg/mL, the inhibitory effect against cells (58.24 %) started to be high and then the cells started to be extensively affected. This finding was consistent with that made by [5], who found that the crude polysaccharides of *Portieria hornemannii* and *Spyridia hypnoides* were cytotoxic at higher concentrations but promoted cell growth at lower ones. Generally, according to toxicity grade (Table 5), the tes-

Table 4

Screening of antibacterial activities (mm) for different algal SPs against human and fish bacterial pathogens via well cut-diffusion technique

Pathogens	Extract code / Activity (mm)								
	Alginate			Fucoidan			Crude SPs		
	<i>Pm</i>	<i>Dp</i>	<i>Td</i>	<i>Pm</i>	<i>Dp</i>	<i>Td</i>	<i>Pm</i>	<i>Dp</i>	<i>Td</i>
<i>B. subtilis</i> ATCC 6051	ND	ND	ND	ND	ND	ND	18	16	12
<i>E. coli</i> ATCC 8739	ND	ND	ND	ND	ND	ND	16	22	ND
<i>S. agalactiae</i> ATCC 13813	ND	ND	ND	ND	14	ND	ND	ND	ND
<i>S. aureus</i> ATCC 25923	ND	ND	ND	ND	12	ND	18	20	ND
<i>V. damsela</i> ATCC 33539	ND	ND	ND	ND	ND	ND	ND	14	ND
<i>V. fluvialis</i> ATCC 33812	ND	ND	ND	ND	12	ND	ND	14	ND
<i>K. pneumoniae</i> ATCC 13883	ND	ND	ND	ND	12	ND	18	16	12
<i>P. aeruginosa</i> ATCC 9027	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>P. fluorescens</i> ATCC 13525	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>A. hydrophila</i> ATCC 13037	ND	ND	ND	ND	ND	ND	12	14	ND

Note. *Td* — *T. decurrens*, *Dp* — *D. polypodioides*, *Pm* — *P. myrica*. ND — not detected.

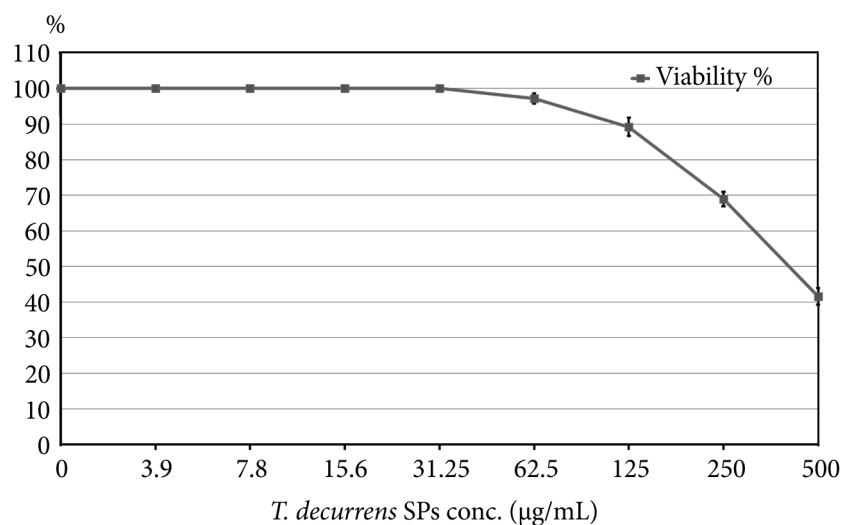


Fig. 7. Cytotoxic evaluation of *T. decurrens* crude SPs by viability and inhibitory % assay

ted crude SPs is a Grade 1 so it is consistently nontoxic towards healthy human liver cell and safe for medical uses [20].

Surprisingly, the majority of studies on alginates tended to focus on their nanocomposites, films, hydrogels, etc. This indicates that there is less information than there should be about the biological activities of alginate as a raw polysaccharide. As a result, we view this research as a collective and comprehensive study of biological evaluation.

Correlation analysis. The correlation analysis proved that there is a strong correlation between the sulfate content of different isolated polysaccharides and the estimated biological activities (Table 6). Generally, the biological efficiency of algal polysaccharides is affected by a variety of parameters such as type, purity, structure, molecular weight, and attached groups. The most crucial factors affecting their biological activities, such as those that affect their capacity to scavenge free radicals and their antiviral activity, are specifically their sulfate

Table 5

Cytotoxicity grade depending on the cell viability percentage of (Feng et al., 2017)

Grade	Viability, %
0	0
1	75—99
2	50—74
3	25—49
4	1—24
5	0

Table 6

Correlations between the chemical composition of SPs and their biological activities

	Sulfate	Protein	TOC	Carb	DPPH	TAC	H ₂ O ₂	α-glucosidase	α-amylase	Anti-arthritis
DPPH _(c)		-0.95	-0.66							
DPPH _(a)	0.99	-0.92	0.85	-0.54						
DPPH _(f)	0.97	-0.97	-0.71	-0.99						
TAC _(c)	0.99	-0.46	0.64	0.99						
TAC _(a)	0.97	-0.87	0.78	-0.63	0.99					
TAC _(f)	0.92	-0.99	-0.60	-0.96	0.99					
H ₂ O _{2(c)}	0.96		0.89	0.97		0.92				
H ₂ O _{2(a)}		-0.72	0.81	0.57	0.99					
H ₂ O _{2(f)}		-0.78			0.60	0.72				
α-glucosidase _(c)	0.92	-0.69		0.91		0.96	0.77			
α-glucosidase _(a)	0.86	-0.96	0.99		0.78	0.71	0.88			
α-glucosidase _(f)	0.87	-0.99	-0.51	-0.93	0.97	0.99	0.79			
α-amylase _(c)	0.72	-0.90		0.70	0.72	0.80	0.50	0.94		
α-amylase _(a)	0.89	-0.98	0.99		0.82	0.75	0.84	0.99		
α-amylase _(f)	0.98	-0.76	-0.95	-0.95	0.90	0.82		0.75		
Anti-arthritis _(c)	0.78	-0.86		0.76	0.66	0.85	0.58	0.96	0.99	
Anti-arthritis _(a)	0.99	-0.98	0.94		0.98	0.95			0.92	

Table 6 (continued)

	Sulfate	Protein	TOC	Carb	DPPH	TAC	H ₂ O ₂	α -glucosidase	α -amylase	Anti-arthritis
Anti-arthritis _(f)							-0.49	-0.70		
Anti-obesity _(c)	0.93	-0.68		0.92		0.96	0.79	0.99	0.93	0.96
Anti-obesity _(a)	0.88	-0.98	0.99		0.81	0.74	0.86	0.99	0.99	0.91
Anti-obesity _(f)	0.91	-0.99	-0.58	-0.95	0.98	0.99	0.73	0.99	0.81	

Note. Bold values are significant correlations at $P < 0.05$ level; c — crude SPs, a — alginate, f — fucoidan.

content, location, and monosaccharide composition [31, 32, 34]. Since it was hypothesized that the sulfate group would activate the hydrogen atom of the anomeric carbon, the polysaccharide's capacity to donate hydrogen would increase [7].

High-sulfated polysaccharides are more effective at scavenging sulfur than low sulfated polysaccharides [13]. In addition, depending on their sulfate concentration, the sulfated polysaccharides could inhibit the lipopolysaccharide-induced inflammatory response in the macrophage (RAW cells) [63]. It has been found [49] that the anti-obesity properties of polysaccharides were related to their sulfate concentration, explaining that the more sulfated sample derived from fucoidan had an anti-adipogenic activity that might regulate obesity *in vivo*. On the other hand, proteins represent contaminants during the extraction process and the purifying procedure is a crucial step. The obtained results demonstrate a negative relationship between the polysaccharide protein content and all the observed activities.

The correlation of the total organic carbon and the total carbohydrate content of different extracted polysaccharides with the estimated biological activities showed a fluctuation pattern according to the types of polysaccharides and biological assays. This observation is in line with [19].

As shown from Table 6, the antioxidant activity of the studied extracted polysaccharides was positively correlated with the anti-diabetic activity meaning these isolated SPs play a protective role against diabetes-related oxidative stress and might be the reason for the major contribution of the increased level of α -glucosidase and amylase inhibition. A similar observation, which confirmed that polysaccharides' anti-diabetic efficiency was due to their antioxidant capabilities, which may play a role in maintaining glucose homeostasis was made [44].

According to the present study, there is a strong correlation between anti-obesity activity and the antioxidant and anti-diabetic activities of the tested algae (Table 6). Polysaccharides are

potent natural antioxidants that may lessen oxidative stress related to diabetes, obesity, and other disorders, as well as oxidative damage in food [22]. Additionally, antioxidant polysaccharides can be used to treat obesity [65]. Algal SPs are the primary contributors to antioxidant and anti-arthritic activities and each assay, although only anti-arthritic of crude and alginate, exhibited a good correlation with the DPPH and TAC [7].

Conclusion

Due to their vast range of important uses today, seaweeds have drawn a lot of interest. The brown algae in particular are a practical and significant source of sulfated polysaccharides. In the present study, the SPs isolated from the Egyptian seaweeds (*Dictyopteris polypodioides*, *Polycladia myrica*, and *Turbinaria decurrens*) inhabiting the Red Sea, contained potent antioxidant, anti-diabetic, anti-obesity, anti-arthritic, and antibacterial properties without having any cytotoxic effects on liver cells. The obtained original data confirm the potential application of these seaweeds as a nutraceutical raw source that can be safely applied by the food industry and/or other sectors, especially since they have a wide array of medicinal, biomedical, and commercial applications.

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COMPARISON OF CHEMICAL COMPOSITION AND BIOACTIVITIES OF VARIOUS POLYSACCHARIDES OF BROWN SEAWEEDS, THE RED SEA, EGYPT, HURGHADA

Sulfated polysaccharides (SPs) from algae have been shown to be effective in a number of biological applications. Therefore, the chemical composition and different biological functions of various SPs were determined for three brown seaweed species from the Egyptian Red Sea: *Dictyopteris polypodioides*, *Polycladia myrica*, and *Turbinaria decurrens*. It has been found that the yield of crude SPs was higher than that of alginate and fucoidan with a range of 43.64 % to 61.90 %. Fucoidan, which has the maximum carbohydrate content of 56.89 %, was found in *D. polypodioides*. The crude SPs of *P. myrica* had the greatest sulfate content of 22.44 %. All functional groups of the examined samples were confirmed by the Fourier Transform Infrared spectrum (FTIR). Experimentally, three applicable assays were used to quantify the antioxidant activity of the extracted SPs depending on the method used, the type of polysaccharides, and algal species. The anti-diabetic activity of *T. decurrens*-crude SPs was highly active scoring 85.85 % in the α -glucosidase assay. The anti-obesity activity showed the highest value (95.25 %) for *T. decurrens*-fucoidan. Besides, *T. decurrens*-crude SPs showed the highest anti-arthritis activity (89.89 %). In addition, a few positive records of antibacterial activity were detected. Furthermore, the most potent *T. decurrens*-crude SPs extract was tested for cytotoxicity against human liver cells and found to be safe. The activity of the isolated SPs and their chemical composition were shown to be correlated. Conclusively, the bioactivities recorded herein by the tested SPs pose promising medicinal applications towards developing a new therapeutic intervention.

Keywords: alginate, fucoidan, antibacterial, anti-diabetic, anti-obesity, *Phaeophyceae*.