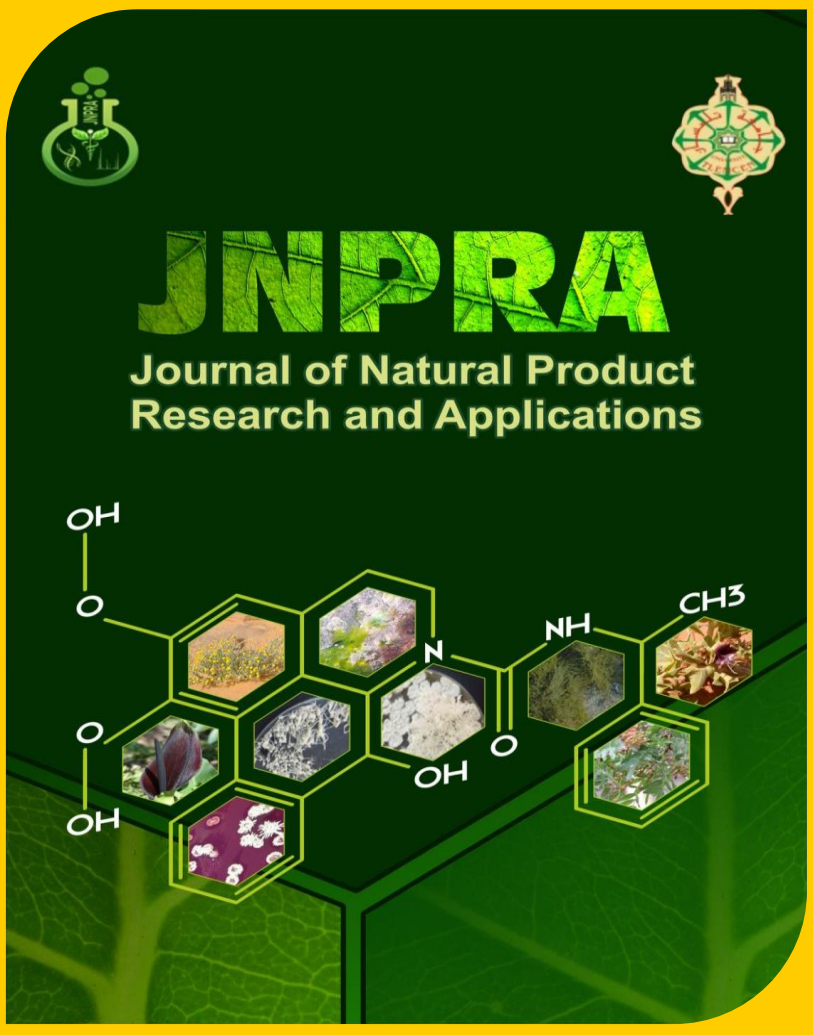
Phycochemistry study and antimicrobial activity of *Spirogyra* freshwater green microalgae from Algeria

Larbi BELYAGOUBI
Rachid CHAIBI
Hicham GOUZI
Fatima Zohra AISSAOUI
Zahrat El Oula BENAMAR
Nabila BELYAGOUBI-BENAHMMOU



Journal of Natural Product Research and Applications
Volume 3, Issue 2

Phycochemistry study and antimicrobial activity of *Spirogyra* freshwater green microalgae from Algeria

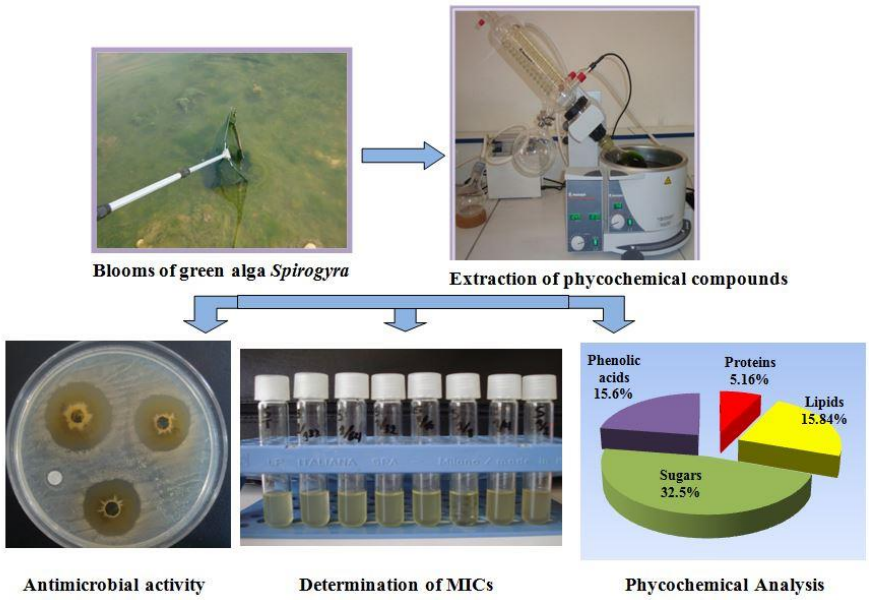
Larbi BELYAGOUBI[1,*], Rachid CHAIBI[2], Hicham GOUZI[2], Fatima Zohra AISSAOUI[3], Zahrat El Oula BENAMAR[3], Nabila BELYAGOUBI-BENAHMMOU[1]

[1]Natural Products Laboratory, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe, University Abou-Bekr Belkaïd, Tlemcen, 13000, Algeria; [2]Laboratory of Biological and Agricultural Sciences (LSBA), University of Amar Telidji (UATL), 03000 Laghouat, Algeria; [3]Département de Biologie, Université Amar Telidji, Laghouat, Algérie.
*Corresponding Author; Larbi BELYAGOUBI; E-mail: belyagoubi_larbi@yahoo.fr; Tel: +213-553-14-76-33; Fax : +213-43-21-55-34

Highlights

- Preparation of various organic crude extracts of *Spirogyra* sp.
- Quantitative phycochemical analysis of dry matter of *Spirogyra* algal biomass.
- Antimicrobial activity of organic extracts against microbial pathogen.
- The extracts of filamentous green alga *Spirogyra* sp. can be an interesting source of pharmaceutical substances used in the treatment of various human diseases.

Graphical Abstract



Abstract
In Algeria, algae have not been adequately explored for their potential as a source of bioactive substances. In this context the blooms of green alga *Spirogyra* sp. were collected from freshwater habitats in Oued El Mellah near M'sila province of Algeria. Hexane, acetone and ethanol crude extracts were screened for antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*. The contents of total soluble sugars, soluble proteins, total lipids and phenolic acids were also determined. The present findings revealed that the highest extraction yield was obtained from ethanol (4.93%) compared to acetone (3.67%) and hexane (2.33%). The contents of phycochemical compounds were 32.5% of soluble carbohydrates, 15.84% of lipids, 15.6% of phenolic acids and 5.16% of proteins. All extracts exhibited better and stronger antibacterial activities against the five pathogenic bacteria with inhibition zones diameter ranged from 13.5-29 mm for acetone extract, from 13-25 mm for ethanol extract and from 12.5-20.5 mm for hexane extract at 7.5 µL. The minimal inhibitory concentration (MIC) was in the range of 15-40 mg mL^{-1}. The acetone extract was more effective than others extracts with MIC value of 15 mg mL^{-1} for all bacteria. These findings indicate the presence of promising antibacterial compounds originating from microalgae, which can be exploited for the production of new antimicrobial agents.

Keywords: antimicrobial activity; Algae; Oued El Mellah; phycochemical componds; *Spirogyra*.

1. Introduction

The emergence of resistance pathogenic microorganisms to majority of antibiotics encourage the scientific community to explore and to develop the natural products-based drugs with a great therapeutic potential and lesser side effects as compared with synthetic drugs (Jaswir et al., 2014). Some microorganisms can contaminate foods and cause serious diseases in humans and some others can also induce foodborne illness such as fever, nausea, vomiting and diarrhea (Falaise et al., 2016).

Natural products such as plants and algae represent an inexhaustible treasure of bioactive molecules. Numerous studies focused to detect the antimicrobial compounds for goal to develop new drugs against microbial infections and to contribute in shelf-life extension and food safety preservation (Aloui and Khwaldia, 2016). Marine and freshwater microalgae provide a great chemical diversity of molecules, which have significant potential use as antimicrobial agents against Gram-positive and Gram-negative bacteria.

Spirogyra (Family of Zygnemataceae) is member of freshwater filamentous green algae. It is found in a small stagnant water bodies, ditches as well as the littorals of lakes and streams (Hainz et al., 2009). Its consumption is very widespread as food in many Asian countries. The species of this genus are producers of several bioactive compounds that show a broad spectrum of biological activities such as antimicrobial, antiviral, antioxidant, anti-inflammatory and cytotoxic (Kumar et al., 2015; Belyagoubi et al., 2022). Comparatively to marine algae, the chemical composition of metabolites in *Spirogyra* species has not entirely reported. Some studies described the presence of phenolic compounds like eriodictyol, isoquercetin, kaempferol, quercetin, hydroquinin, rutin, catechin, and tannic acid in aqueous *S. neglecta* extracts (Duangjai et al., 2016). Others phytochemicals like alkaloids, ketones, terpenes, phenolics, hydrocarbons, fatty acids, fatty alcohols, esters and sterols were also identified in different extracts of *S. longata* (Abdel-Aal et al., 2015). Some authors indicated that the genus of *Spirogyra* constitutes the biological material with great potentials and their biomass is a

promising source of chemicals, energy, fuels and food (protein, polysaccharides, lipid, unsaturated alcohols, alkynes etc.) (Kumar et al., 2015; Chang and Lee, 2017).

However, to our knowledge, there are no published studies focusing on the filamentous green alga *Spirogyra* sp. growing in different lakes in Algeria. The objective of this research was therefore to investigate both the broad-spectrum antimicrobial activity and the chemical composition of this microalga collected from Oued El Mellah.

2. Materials and methods

2.1. Algal material

The blooming of the freshwater green algae *Spirogyra* sp. was obtained from Oued El Mellah (depths of 0.3–1 m and a width of 67 m) (Algeria) from the top surface (Figure 1) and identified by Prof. Rachid CHAIBI from University of Laghouat, through some morphological characters. The collected blooms were brought to the laboratory and carefully rinsed with tap water and then with distilled water to remove all particles and parasites related. The samples were dried in the shadow for about 4 days (Figure 1C) then ground into fine powder in a clean mixer grinder; next passed through a 1.0 mm sieve and stored at room temperature until further analysis.

Figure 1. Filaments green and feel like wet soapy hair (A), Bloom greens (B) and dry algal biomass of *Spirogyra* sp. (C).

2.2. Preparation of algae extracts

The crude extracts were prepared by using the organic solvents (Hexane, Acetone and Ethanol) with increasing polarity at the ratio of 1:3 (w/v) for 48 h at room temperature and under dark condition with occasional shaking. The extracts were centrifuged at 3000 rpm for 10 min. The supernatants obtained were subjected to evaporation to dryness under reduced pressure using rotary evaporator at 47°C. The weighted crude extracts were suspended in the dimethyl sulfoxide (DMSO) and stored in a refrigerator at 4°C until further use.

2.3. Determination of yield

The percentage yield was calculated using the equation of Jaswir et al. (2014).

Yield (%) = (Mass of dried extract/ Mass of alga powdered) x 100

2.4. Biochemical estimations

Dry matter of *Spirogyra* algal biomass was used for the estimation of soluble sugars, soluble proteins, lipids and phenolic acids.

2.4.1. Estimation of soluble proteins

One hundred milligram of dry algal matter was mixed with 2 mL of distilled water. After grinding, suspensions were centrifuged at 15,000 for 5 min at 4°C. Supernatant was collected and stored frozen for soluble proteins assay. Total soluble proteins were determined quantitatively according to Bradford method (1976). 50 µL of the soluble protein extract were mixed with 3 mL Coomassie Blue reagent. After 5 min at room temperature, the absorbance was measured at 595 nm. The protein concentration was determined by using bovine serum albumin standard curve at maximum concentrations of 1 mg mL^{-1} (O.D = 0.754xC; R^2 = 0.997), and then expressed as mg protein in 100 mg of dry weight algae biomass.

2.4.2. Estimation of total soluble sugars

20 mg of crushed dried algal material were homogenized in 2 mL of 80% boiling ethanol at 80% and then centrifuged at 5000 trs/min for 20 min at 4°C. The pellet was re-extracted twice with 80% ethanol and the supernatants were pooled. Total soluble sugars were estimated by anthrone–sulphuric acid method of McCready et al. (1950) using 0.2 % anthrone in concentrated H_2SO_4 as reagent. The absorbance of green to dark green colour was read at 630 nm in UV-visible spectrophotometer. The amount of the total soluble sugars was obtained from a standard curve using known concentrations of glucose (O.D = 0.016xC; R^2= 0.999).

2.4.3. Estimation of total lipids

Lipid extraction is performed in the Bligh and Dyer (1959) method. The mixture of chloroform, methanol and deionized water (2:3:0.5, v/v/v) were added to 0.25g of dried algal. After homogenization, the mixture was centrifuged to obtain two phases. The clear aqueous phase was discarded and the chloroform phase was transferred to another flask and dried using a rotary evaporator under vacuum, at temperature of 40°C. The weight of the lipid was determined.

2.4.4. Extraction and quantification of phenolic acids

Dried algal powder (0.2 g) was finely powdered, mixed with 100 mL of distilled water and kept at room temperature with agitation. After 24 h, 1.5 g of NaOH was added at 20 mL of filtrate for 2h. After alkaline hydrolysis, the extract was acidified with 2N HCl and the free phenolic acids were extracted twice-using 5 mL of diethyl ether. Then, the combined solutions were dried and dissolved in methanol.

The total phenolic content (TPC) was determined by spectrophotometry, using gallic acid as a standard, according the method described by Singleton et al. (1999) with suitable modification. A solution of 0.5 mL methanolic extract, or gallic acid, was mixed with distilled water to complete 5 mL. Then, 0.25 mL of Folin Ciocalteu reagent was added and the mixture was vortexed. After 3 min, 0.5 mL of aqueous sodium carbonate (Na_2CO_3) was added. The tubes are incubated for 60 min. The absorbance was measured at 765 nm against a blank. Gallic acid was used as a standard for the calibration curve (O.D = 0.5511*C; R^2= 0.9997).

2.5. Antimicrobial activity of organic extracts

2.5.1. Microbial strains

The antimicrobial activity of organic solvent extracts (Hexane, Acetone and Ethanol) of freshwater green algal (*Spirogyra* sp.) was evaluated using six different microorganisms. Two Gram-positive strains (*Staphylococcus aureus* 43300 MRSA and *Listeria monocytogenes* ATCC 19115) and three Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853,

Escherichia coli ATCC 25922, and *Salmonella typhi* (Institut Pasteur, Algeria). One species of fungi, *Candida albicans* (IBMC Strasbourg, MNHN) was used as indicator microorganism to detect antifungal activity. All strains bacterial were maintained on slants with nutrient agar. *C. albicans* was grown in Sabouraud Dextrose Agar.

2.5.2. Agar disc diffusion method

Antimicrobial activity was carried out using the disc diffusion technique in Petri dishes, which is performed according to National Committee for Clinical Laboratory Standards guidelines (CLSI, Document M2-A9 2006, NCCLS-M27-A 1997) with slight modifications. Bacterial suspensions were prepared in Nutrient Broth (NB) and fungal suspension in Sabouraud Dextrose Broth (SDB), and then incubated at 37°C for 24 h and at 37°C for 24-48 h, respectively. The tested microorganism suspension was adjusted to a similar optical density to that of McFarland 0.5 (10^8 CFU mL^{-1} by MHB for bacteria and 10^6 CFU mL^{-1} for *Candida* by SDB). Then 0.15 mL of suspension of each culture was spread on the solid media plates using a sterile cotton swab and allowed to dry for 10 mn. Whatman No.1 sterile filter paper discs (6 mm diameter) were impregnated with three different volumes (2.5 µL disc, 5 µL disc and 7.5 µL disc) of algal extracts and the same volumes of Dimethylsulfoxide (DMSO) solvent was used as the negative control. The loaded discs were placed on the surface of the solidified agar medium prepared with Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for yeast and left for 30 min at room temperature for compound diffusion. Standard antibiotic disks were used as positive controls, ampicillin (10 µg disc) and chloramphenicol (30 µg) for bacteria, and Nystatin (100 µg) and Amphotericin B (50 µg) for yeast in order to control the sensitivity of the tested microorganism. The Petri dishes were kept at 37°C and incubated 24 h for bacteria and 24-48 h for fungus. After the incubation period, the inhibition zone diameters (IZDs) were measured, including the paper disk (in mm). The results were expressed as average of duplicates.

2.5.3. Determination of the minimum inhibitory concentrations (MICs)

The MICs was defined as the lowest concentration of filamentous green algal extracts which can inhibit the growth of microorganisms after 24 h at 37°C (Kim and Lee, 2008). Determination of MICs for testing the antimicrobial activity of the algal extract were made by the modified macrodilution broth method which is performed according to the procedure recommended by NCCLS (CLSI document M07-A9 2006). All tests were performed in Mueller Hinton Broth (MHB), and cultures of each strain were prepared overnight. Microorganism suspensions were adjusted in a spectrophotometer to give a final organism density about 5×10^5 CFU mL^{-1} in each tube. Serial dilutions were carried out in tubes (3 mL) to obtain the dilutions of the algal extracts with the concentrations ranging from 5 to 45 mg mL^{-1}. After incubation for 24 h at 37°C in normal atmosphere, the MIC was defined as the lowest concentration of the algal extracts at which the microorganisms did not exhibit visible growth in the tube. The growth of the microorganism was indicated by turbidity. All tests were done in duplicate and values of MIC are expressed in mg mL^{-1}. The solvent control test has been performed to study an effect of DMSO on the growth of a microorganism.

3. Results and discussion

3.1. Stream of El Mellah

The fauna and flora of stream is characterized by significant species diversity, closely related to biotope diversity. According to the Figure 2, we found that the filamentous green algae *Spirogyra* bloom (Figure 3), is considered as a shelter or an air of distribution and lay of several

aquatic species (zooplankton (microscopic animals), larval fish and other animals), beetles (e.g. *Bostrychidae*, *Carabidae*, *Dyticus*, …), dytic larva, red water mite, annelids (e.g. Leech), Ephemeroptera, Diptera, and fishes and their larvae (e.g. *Pseudophoxinus*) that graze on this algae.

Algae are considered the base of most aquatic food chains, so, they are essential indicators of ecosystem health and integrity. They affect the quality and the chemical properties of water (Huynh and Serediak 2006).

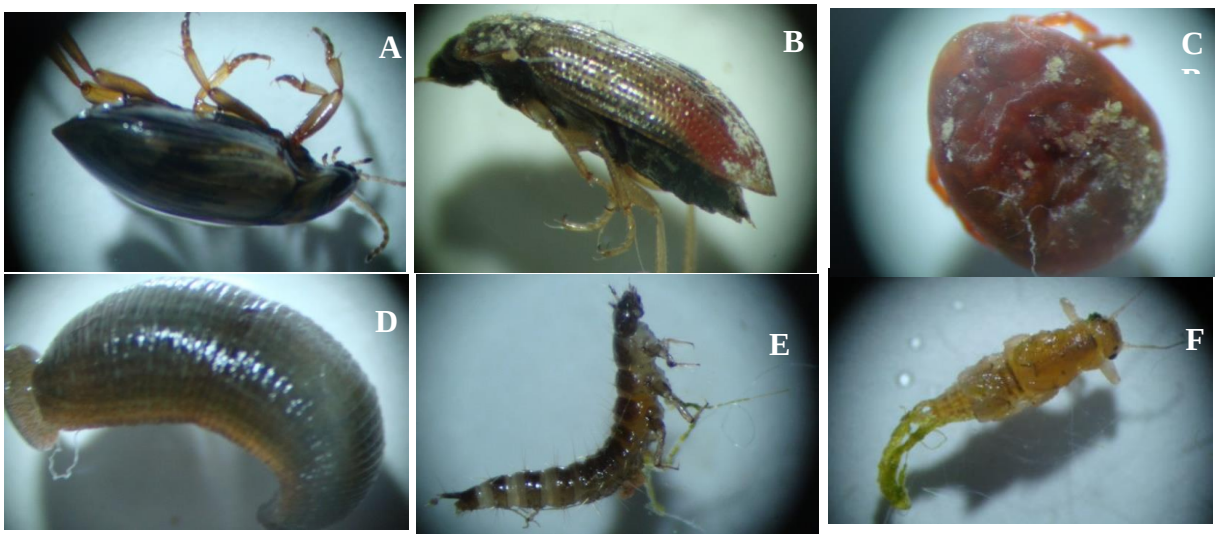


Figure 2. Species diversity of fauna found in filamentous green algae *Spirogyra* bloom (A- *Dyticus;* B- *Carabidae;* C- Red water mite; D- Leech; E- Dytic larva; F- Mayfly).

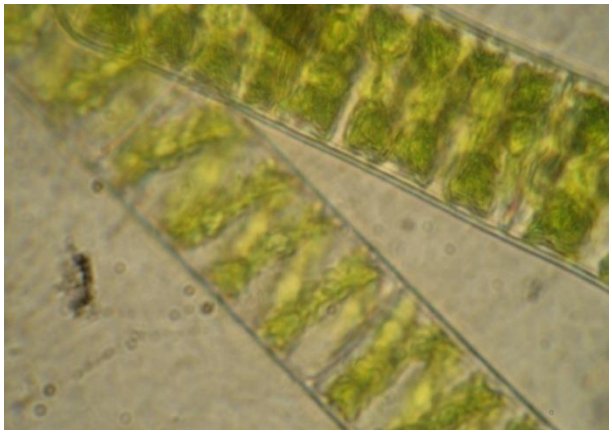


Figure 3. Filament of the alga *Spirogyra* observed under microscope (x400).

3.2. Extraction yields

The percentage yields of extracts obtained from dry green filamentous algae powder using different organic solvents are described in Figure 4. The highest extraction yield was obtained from ethanol (4.93%) compared to acetone (3.67%) and hexane (2.33%). In the study of Abdel-Aal et al. (2015), a low value of extraction yield of *S. longata* was reported with acetone (0.751%). It should be noted that the quantity and quality of chemical compounds present in

algae are vary according to the extraction solvent used. This suggestion can explain this difference between our results and the literature. In addition, some studies showed that the solvent polarity and extraction yield were directly proportional (Alassali et al., 2016). Herrero et al. (2005) also suggested that the polarity of solvents significantly affects the yield of *Spirulina* microalgae wherein the highest yield was recorded with ethanol as compared with other higher and lower polarity solvents. Similarly, Santoyo et al. (2009) considered ethanol to be the best solvent for the extraction of antimicrobial compounds from *Haematococcus pluvialis* algae.

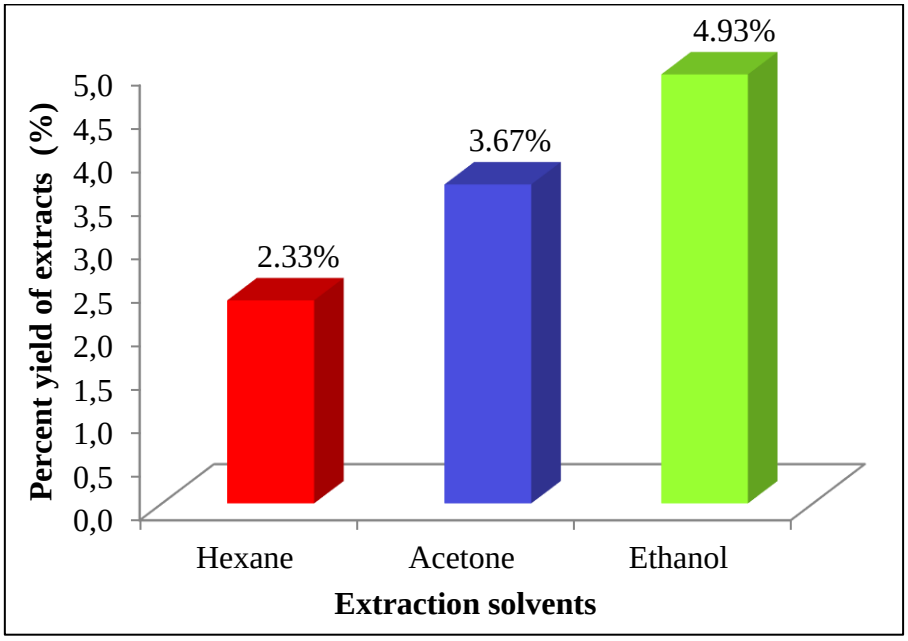


Figure 4. Variation in % crude extracts of the filamentous alga *Spirogyra* sp. with different organic solvents.

3.3. Biochemical composition

The biochemical composition of *Spirogyra* under present study is given in Figure 5. The content of carbohydrates was found to be in considerable high value in the filamentous green alga with average level of 32.5% of the dry weight (DW). Its occurrence is a function to the intensity of sunlight (El-Tawil and Khalil, 1983). This study showed also that *Spirogyra* contained significantly higher amounts of lipids and phenolic acids of 15.84% and 15.6%, respectively, but lower content of protein (5.16%). Previous phytochemical studied on *Spirogyra longata* reported that the crude extracts revealed the presence of phenolic compounds in petroleum ether, methylene chloride and chloroform extracts, with a total concentration of 0.654%, while they were absent in acetone and methanol extracts (Abdel-Aal et al., 2015). A study by Mitova et al. (1999) reported that the residue obtained after extraction of freshwater algae *Spirogyra* and *Mougeotia* biomass with chloroform and methanol contained mainly polysaccharides and proteins.

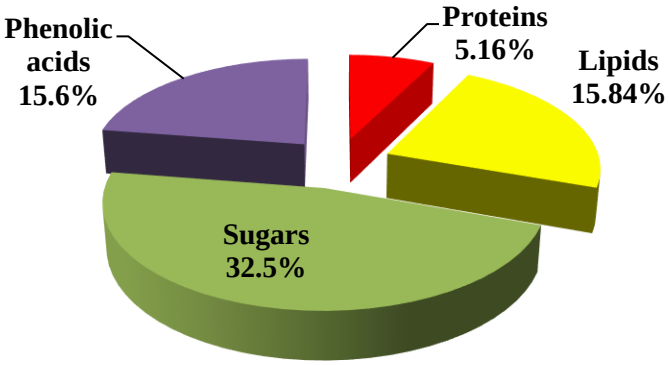


Figure 5. Mean percentages of carbohydrate, protein, lipid and phenolic acids of freshwater alga *Spirogyra*.

3.4. Evaluation of antimicrobial activity on some pathogenic microorganisms

The antimicrobial potential of extracts from *Spirogyra* sp. extracts obtained using different organic solvents was qualitatively and quantitatively assessed by the values of IZDs and minimum inhibition concentration (MIC). Table 1, shows the *in vitro* antimicrobial property of the extracts of five pathogenic bacterial strains and one yeast. Generally, when extract volumes increased, the inhibition zone diameters also increased significantly. The *Spirogyra* extracts obtained by organic solvents (hexane, acetone and ethanol) exhibited antimicrobial activity against all the tested pathogenic bacterial strains with the IZD values of 8–29 mm. This activity showed a selective effect against Gram-positive bacteria compared to Gram-negative bacteria. This remark is widespread in literature and can be probably due to the more complex structure of the cell wall of Gram-negative bacteria (Stirk et al., 2007).

Table 1. Antimicrobial activity of microalga *Spirogyra* organic extracts in agar diffusion assay (inhibition zone is expressed in mm) against microbial pathogens.

Solvent extracts	**Volume of extract (µL)**	**Diameter of zone of inhibition (mm)** [a]					
		Gram-positive bacteria		**Gram- negative bacteria**			**Fungus**
		S. aureus	*L. monocytogenes*	*E. coli*	*P. aeruginosa*	*S. typhi*	*C. albicans*
Hexane	2.5	9.5	10	14	9	10.5	–
	5	13.5	19	16	10.5	13.5	–
	7.5	16	20.5	18.5	12.5	14.5	–
Acetone	2.5	23.5	16.5	11	11	11.5	–
	5	25.5	21.5	12.5	12	18	–
	7.5	29	22.5	17.5	13.5	22.5	–
Ethanol	2.5	17	10.5	10	9	8	–
	5	19	12.5	11.5	11	10.5	–
	7.5	25	15.5	15.5	13	13.5	–
Negative control – DMSO (7.5 µL)		–	–	–	–	–	–
Positive control	– Ampicillin (10 µg)	34 *(S)*	30 *(S)*	24 *(S)*	– *(R)*	34.5 *(S)*	N.S
							25.66*(S)* [b]
	– Chloramphenicol (30 µg)	31 *(S)*	25 *(S)*	15 *(R)*	– *(R)*	30 *(S)*	N.S
							–*(R)* [c]
	– Tetracycline (30 µg)	14 *(R)*	13 *(R)*	15 *(R)*	11 *(R)*	15 *(R)*	N.S

[a] Expressed as the size of the inhibition zones (mm) as an average of duplicates ± SD, including diameter of paper disk (6 mm).

[b] Nystatin (100 µg disc) ; [c] Amphotericin B (50 µg disc); –: means that there is no inhibition zone; N.S: not suitable to test.

S: Microorganism classified as **S**usceptible to the antibiotic according to the recommendations of the Antibiogram Committee of the French Microbiology Society (Comité de l'antibiogramme de la Société Française de Microbiologie 2017); *R*: Resistant.

The inhibitory activity of the extracts was comparable to those of the reference drugs (ampicillin, chloramphenicol and tetracycline) used as positive controls. Antibiogram results of five bacteria strains have shown that almost all of these strains are resistant to the standards antibiotic tertracycline, while that we have to point out that there is variability in the antibiotic susceptibility for the two others antibiotics (ampicillin and chloramphenicol). The results showed also that the standard antibiotic ampicillin had a stronger activity than the tested samples (Table 1). In the negative control, DMSO had no inhibitory effect on the tested organisms. Remarkably, the highest antibacterial activities were found against Gram-positive bacteria *S. aureus* and *L. monocytogenes*, with the largest IZD for 7.5 µL of acetone extract (29 and 22.5 mm, respectively), followed by *S. typhi*, *E. coli* and *P. aeruginosa* with the IZDs of 22.5, 17.5 and 13.5 mm, respectively. Further, the hexane extract was found to be effective against *E. coli* with the largest IZD of 18.5 mm for 7.5 µL of extract. However, the fungus *C. albicans* exhibits the highest resistance to the *Spirogyra* extracts from the different solvents. This result agrees with the work of Jebasingh et al. (2011) which showed that the acetone was found best among several solvents used for extracting antibacterial substances from green and red seaweeds. Cox et al. (2010) reported that extraction of antimicrobials from different species of seaweeds were solvent dependent. This agrees with our result that acetone is suitable, in term of yield and antibacterial activity. In comparison to others studies reported the antimicrobial activity of *Spirogyra* species, the ethanolic extracts of *Spirogyra grantiana* showed the antibacterial activity against three organisms *i.e. Escherichia coli, Proteus vulgaris* and *Proteus mirabilis* with the zone of inhibition of 9, 10 and 9 mm, respectively (Prakash et al., 2011). A study by Ivanova et al. (2011) reported that the alga *Spirogyra crassa* (L.) Kutz present in two lakes near Sofia (Bulgaria) has an antibacterial effect on both Gram-positive and Gram-negative bacteria. Moreover, the crude methanol extracts of *Spirogyra hyaline* Cleve and *S. rhizoides* Randhawa showed strong antimicrobial activity against 14 bacterial and 20 fungal species, including 7 human-, 5 plant-pathogens and 8 saprophytes (Khalid et al., 2012). The remarkable differences between our results and others studies may be due to several factors such as the intraspecific variability in the production of bioactive molecules, the extraction protocols of these compounds and the choice of the antimicrobial power assay, which greatly affects the sensitivity of strains (Tuney et al., 2006). Another significant result of the present study is the absence of any antifungal efficiency of *Spirogyra* extracts. It did not show any antifungal activity against *C. albicans*. These results are in agreement with Kamenarska et al. (2000). These authors indicated that both volatiles components from *Spirogyra crassa* and *Spirogyra longata* show no activity against *E. coli* and *C. albicans.* Contrariwise, the total inhibitory growth against plant pathogenic fungi of cold water extract of *Spirogyra plena* was described in the study of Kamble et al. (2012).

3.5. MIC values of *Spirogyra* organic extracts

The MICs of obtained *Spirogyra* extracts determined by macrodilution broth method was shown in the Table 2. All three organic algae extracts showed broad-spectrum antimicrobial activities against five pathogenic bacteria. The algae extracts showed different inhibitory effects against microorganisms. As also observed with the disk diffusion method, the acetone extract was more efficient with MIC value of 15 mg mL^{-1} for all bacteria. In this assay, the lowest activities were obtained on ethanol extract against *L. monocytogenes* with MIC of 40 mg mL^{-1}. On the other hand, the highest MIC value of 30 mg mL^{-1} was found in hexane extract towards tested bacteria *L. monocytogenes* and *P. aeruginosa*. According to the results, algal extracts are slightly more active against Gram-positive than Gram-negative microorganisms. In the study of Pane et al. (2015), the algal extract of freshwater algae *Pseudokirchneriella subcapitata* showed interesting antimicrobial properties, which mostly inhibited the growth of isolated *S. aureus, P. aeruginosa, E. coli, and Klebsiella* spp. with MICs range of 1.6×10^9 to 1.2×10^{10}

cells mL^{-1}. Najdenski et al. (2013) reported that microalgae and cyanobacteria were tested for their antibacterial and antifungal agents' production on various pathogenic organisms, with MIC values ranging from 0.125 mg mL^{-1} to 12.5 mg mL^{-1}. However, the exact mechanism of their microbicidal effect is not entirely understood. The rupture of cellular membrane of microbial cells entrains leakage of intracellular molecules causing reduction in nutrient uptake or inhibition of cellular respiration (Smith et al., 2010). Genovese et al. (2012) reported that bioactivity of algae varies with geographical scale and seasonality, depending on the variation of the content of active metabolites and ultimately on physiological conditions.

Table 2. Minimum inhibitory concentration (MIC) of organic algae extracts.

Microbial strains	**MIC (mg mL^{-1})**		
	Hexane extract	***Acetone extract***	***Ethanol extract***
Gram-positive bacteria			
S. aureus	15	15	15
L. monocytogenes	30	15	40
Gram-negative bacteria			
E. coli	15	15	15
P. aeruginosa	30	15	15
S. typhi	15	15	15
Fungus			
C. albicans	NT	NT	NT
Negative control			
DMSO (45 mg mL^{-1})	–	–	–

N.T: not tested because algal extracts did not show the inhibitory effect by agar disc diffusion method; –: means that there is no inhibition.

4. Conclusion

Our preliminary results obtained from this study suggest that the extracts of filamentous green alga *Spirogyra* sp. can be an interesting source of natural antibiotics and could lead to develop of a new drug for the treatment of microbial infections and the control of foodborne pathogens in food products. However, further research needs to be done on the purification and identification of the bioactive phyco-constituents responsible for antimicrobial activity.

Acknowledgements

The authors would like to thank Dr. DJEBBAR Réda and Prof. BELKEBIR Aicha for their kind Scientific and Technical assistance and help during the biochemical analysis in their laboratories (Laboratory of Biology and Organism Physiology, Biological Sciences Institute, University of Sciences and Technology Houari Boumediene, Algiers, Algeria).

Author Contribution Statement

Larbi BELYAGOUBI: Experimentation and writing the original manuscript; **Rachid CHAIBI:** Collect of Alga; Experimentation; **Hicham GOUZI, Fatima Zohra AISSAOUI, and Zahrat El Oula BENAMAR:** Experimentation; **Nabila BELYAGOUBI-BENAHMMOU:** writing the original manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

ORCID:
Larbi BELYAGOUBI: 0009-0002-5047-7379.

References

Abdel-Aal, E. I., Haroon, A. M. & Mofeed, J. (2015). Successive solvent extraction and GC–MS analysis for the evaluation of the phytochemical constituents of the filamentous green alga *Spirogyra longata*. *Egyptian Journal of Aquatic Research*, 41, 233–46.

Alassali, A., Cybulska, I., Brudecki, G. P., Farzanah, R. & Thomsen, M. H. (2016). Methods for Upstream Extraction and Chemical Characterization of Secondary Metabolites from Algae Biomass. *Advanced Techniques in Biology & Medicine*, 4,163.

Aloui, H., Khwaldia, K. (2016). Natural antimicrobial edible coatings for microbial safety and food quality enhancement. *Comprehensive Reviews in Food Science and Food Safety*, 15, 1080–1103.

Belyagoubi, L., Belyagoubi-Benhammou, N., Atik-Bekkara, F., Abdelouahid, D.E. (2022). Influence of harvest season and different polarity solvents on biological activities, phenolic compounds and lipid-soluble pigment contents of *Spirogyra* sp. from Algeria. *Advances in Traditional Medicine*, 22, 359–369. https://doi.org/10.1007/s13596-021-00551-0

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911–917.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254.

Chang, S., & Lee, Y. (2017). Comparison of two chemical extraction methods for proteins and polysaccharides of *Spirogyra fluviatilis* in extracellular polymeric substances. International Symposium on Resource Exploration and Environmental Science. *IOP Conference Series: Earth and Environmental Science*, 64, 012122. doi :10.1088/1755-1315/64/1/012122.

CLSI (Clinical and Laboratory Standards Institute). (2006). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Ninth Edition. Clinical and Laboratory Standards Institute, Wayne, PA, CLSI document M2-A9.

CLSI. (2006). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard— Standard Edition. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute.

Comité de l'antibiogramme de la Société Française de Microbiologie. Recommandations (2017). V.1.0 Mars. 2017. [http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFMV1_0_MARS_2017.pdf].

Cox, C.S., Abu-Ghannam, N., & Gupta, S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds**.** *International Food Research Journal*, 17, 205–220.

Duangjaia, A., Limpeanchob, N., Trisat,K, & Amornlerdpison, D. (2016). *Spirogyra neglecta* inhibits the absorption and synthesis of cholesterol in vitro. *Integrative Medicine Research*, 5, 301–308

El-Tawil, B. A. H., & Khalil, A. N. (1983). Chemical constituents of some algal species from Abu-Qir Bay, Egypt. *Journal of the Faculty of Marine Science*, 3(1404H), 85–94.

Falaise, C., François, C., Travers, M. A., Morga, B., Haure, J., Tremblay, R., Turcotte, F., Pasetto, P., Gastineau, R., Hardivillier, Y., Leignel, V. & Mouget, J. L. (2016). Antimicrobial compounds from eukaryotic microalgae against human pathogens and diseases in aquaculture. *Marine Drugs*, 14, 159.

Genovese, G., Faggio, C., Gugliandolo, C., Torre, A., Spanò, A., Morabito, M. & Maugeri T. L. (2012). *In vitro* evaluation of antibacterial activity of *Asparagopsis taxiformis* from the Straits of Messina against pathogens relevant in aquaculture. *Marine Environmental Research*, 73, 1–6.

Hainz, R., Wöber, C. & Schagerl, M. (2009). The relationship between *Spirogyra* (Zygnematophyceae - Streptophyta) filament type groups and environmental conditions in Central Europe. *Aquatic Botany*, 91, 173–180.

Herrero, M., Martín-Álvarez, P. J., Señorans, F. J., Cifuentes, A. & Ibáñez, E. (2005). Optimization of accelerated solvent extraction of antioxidants from *Spirulina platensis* microalga. *Food Chemistry*, 93, 417–423.

Huynh, M. & Serediak, N. (2006). Algae Identification Field Guide. *Agriculture and Agri-Food Canada*, 40 pp.

Ivanova, A. J., Nechev, I., Tsvetkova, H., Najdenski, K. & Popov, S. S. (2011). Compounds with antibacterial activity from the freshwater alga *Spirogyra crassa* (L.) Kutz. Genet. *Plant Physiology*, 1, 31–37.

Jaswir, I., Tawakalit Tope, A. H., Raus, R. A., Ademola Monsur, H., & Ramli, N. (2014). Study on anti-bacterial potentials of some Malaysian brown seaweeds. *Food Hydrocolloids.* 42(P2), 275–279.

Jebasingh, S. E. J., Rosmary, S., Elaiyaaja, S., Sivaraman, K., Lakshmikandan, M., Murugan, A. & Raja, P. (2011). Potencial antibacterial activity of selected green and red seaweeds. *Journal of Pharmaceutical and Biomedical Sciences*, 5, 1–7.

Kamble, S., Rokde, A. & Chavan, A. (2012). Antifungal activity of algal extracts against plant pathogenic fungi. *An International Multidisciplinary Research Journal*, 2(3), 23–24.

Kamenarska, Z. G., Dimitrova-Konaklieva, S. D., Nikolova, C., Kujumgiev, A. I., Stefanov, K. L., Popov, S.S. (2000). Volatile Components of the Freshwater Algae *Spirogyra* and *Mougeotia*. Z. Naturforsch, C55, 495-499.

Khalid, M. N., Shameel, M. & Ghazala, B. (2012). Bioactivity and Phycochemistry of Two Species of *Spirogyra* Link (*Zygnemophyceae*) from Pakistan. *International Journal on Algae*, 14(3), 237–246**.**

Kim, I. H., Lee, J.H. (2008). Antimicrobial activities against methicillin-resistant *Staphylococcus aureus* from macroalgae. *Journal of Industrial and Engineering Chemistry*, 14, 568–572.

Kumar, J., Dhar, P., Tayade, A. B., Gupta, D., Chaurasia, O. P., Upreti, D. K., Toppo, K., Arora, R., Suseela, M. R. & Srivastava R. B. (2015). Chemical Composition and Biological Activities of Trans-Himalayan Alga *Spirogyra porticalis* (Muell.) Cleve. PLoS One, 10(2), 1–24.

McCready, R. M., Guggolz, J., Silviera, V. & Ownes, H. S. (1950). Determination of starch and amylase in vegetables, application to peas. *Analytical Chemistry*, 22, 1156–1158.

Mitova, M. Iv., Usov, A. I., Bilan, M. I., Stefanov, K. L., Dimitrova-Konaklieva, S. D., Tonov, D. P. & Popov, S. S., (1999). Sterols and polysaccharides in freshwater algae *Spirogyra* and *Mougeotia*. Z. *Naturforsch*, 54c, 1016–1020.

NCCLS (National Committee for Clinical Laboratory Standards). (1997). Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard. National Committee for Clinical Laboratory Standard, Wayne, PA, USA, M27-A.

Najdenski, H. M., Gigova, L. G., Iliev, I.I., Pilarski, P.S., Lukavsky, J., Tsvetkova, I.V., Ninova, M.S., & Kussovski, V.K. (2013). Antibacterial and antifungal activities of selected microalgae and Cyanobacteria. *International Journal of Food Science & Technology*, 48(7), 1533–1540.

Pane, G., Cacciola, G., Giacco, E., Mariottini, G. L. & Coppo E. (2015). Assessment of the Antimicrobial Activity of Algae Extracts on Bacteria Responsible of External Otitis. *Marine Drugs*, 13, 6440–6452.

Prakash, J. W., Antonisamy, J. M. & Jeeva, S. (2011). Antimicrobial activity of certain fresh water microalgae from Thamirabarani River, Tamil Nadu, South India. *Asian Pacific Journal of Tropical Biomedicine*, 1, S170-S173.

Santoyo, S., Rodriguez-Meizoso, I., Cifuentes, A., Jaime, L., García-Blairsy Reina, G., Señorans, F. J. & Ibáñez, E. (2009). Green processes based on the extraction with pressurized fluids to obtain potent antimicrobials from *Haematococcus pluvialis* microalgae. *LWT - Food Science and Technology*, 42, 1213–1218.

Singleton, V. L., Orthofer, R. & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and antioxidants and other substrates by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.

Smith, V.J., Desbois, A.P. & Dyrynda, E.A. (2010). Conventional and unconventional antimicrobials from fish, marine invertebrates and micro-algae. *Marine Drugs*, 8, 1213–1262.

Stirk, W.A, Reinecke, D.L. & Staden, J. (2007). Seasonal variation in antifungal, antibacterial and acetyl cholinesterase activity in seven South African seaweeds. *Journal of Applied Phycology*, 19, 271-276.

Tuney, I., Cadirci, B. H., Unal, D. & Sukatar, A. (2006). Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). *Turkish Journal of Biology*, 30, 171–175.