Potential control of toxic cyanobacteria blooms with Moroccan seaweed extracts

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Abstract

Marine macroalgae have acquired a considerable attention as a new promising source of diverse bioactive compounds that can be used in the biocontrol of harmful cyanobacteria blooms (cyanoHABs). In this work, we evaluated the potential algicidal activities of fourteen species of seaweed collected from the coast of Souiria Laqdima, Morocco, extracted with methanol, and screened in solid and liquid medium against the growth of the toxic cyanobacteria *Microcystis aeruginosa* and the feed microalgae *Chlorella* sp. After isolation of unicellular *M. aeruginosa* and *Chlorella* sp. strains the algicidal activity was tested for the first time by the agar diffusion technique in solid medium and the counting technique in liquid medium. The results in solid medium revealed that the algicidal activity was limited to *M. aeruginosa*. The extract of *Bornetia secundiflora* showed the highest growth inhibition activity against *Microcystis* (27.33±0.33 mm). Whereas, the extracts of *Laminaria digitata, Halopytis incurvus, Ulva lactuca* and *Sargasum muticum* were not showed any zone of inhibition. While, in liquid medium the results indicated that all methanolic extracts of different macroalgae tested have a significant inhibitory effect on *M. aeruginosa* compared to that of the negative control. The maximum inhibition rates of *M. aeruginosa* were revealed by the extracts of *Bifurcaria tuberculata, Codium elongatum* and *B. secundiflora.* Moreover, the extracts of *B. secundiflora* recorded the maximum inhibition rate of *Chlorella* sp. Overall the results highlight the properties of the extracts from macroalgae and their potential use in the control of toxic cyanobacteria species.

Keywords: CyanoHABs, *Microcystis*, Algicidal activity, Seaweeds extracts, Biocide effect, Biocontrol.

Introduction

In recent years, Harmful Algal Blooms (HABs) have been a serious problem for the aquatic ecosystems, the aquaculture industry as well as for the human health (Jeong et al. 2000). Due to the undesirable effects caused by these HABs, extensive research into the topic of the control or mitigation of cyanobacterial blooms has been conducted. Regarding the control of HABs several strategies have been developed which include the use of chemical agents such as copper sulphate (Seder-Colomina et al. 2013). Mechanical control of HABs involves the use of filters, pumps, and barriers (Visser et al. 2005). Biological agents constitute another alternative strategy to the control of HABs. This control can be carried out by microorganisms and herbivorous fishes (silver and bighead carp) (Demeke 2016). Although these methods are useful, they are associated with non negligible deficiency, including nonselective toxicity to many aquatic organisms (Jeong et al. 2000), and high cost (Gao and Xie 2011). Furthermore, some biological methods, such as the introduction of new species in the aquatic environment involves potential biological invasion risks with an imbalance in the trophic chain (Park et al. 2009; Zhu et al. 2010). Thereby the control of cyanobacterial blooms by alternative approachs is of utmost importance. Wherefore, several natural and natural-based compounds from many aquatic and terrestrial plants and seaweeds have been isolated and tested for controlling harmful algae and cyanobacteria (Schrader 2003; Tazart et al. 2018; Tebaa et al. 2018). These compounds involve a variety of bioactive molecules such us rutacridone epoxide from the roots of *Ruta graveolens* (Meepagala et al. 2005); ethyl 2-methylacetoacetate isolated from an emergent macrophyte *Phragmites communis* (Li and Hu 2005); N-phényl-2- naphtalène-amine myristate d'isopropyle from the roots of *Potamogeton maackianus* (Wu et al. 2007); α-linolenic acid, oleic acid, and palmitic acid purified from *Botryococcus braunii* (Chiang et al. 2004).

Seaweeds are amongst the most dominant organisms in marine ecosystems; they have the ability to produce a variety of bioactive compounds with diverse biological activity, namely antibacterial, antifungal and antioxidant (Abdel-Latif et al. 2018; Lezcano et al. 2018; Soliman et al. 2018; Kazir et al. 2019). Moreover, several marine macroalgae have been found to inhibit bloom-forming microalgae (An et al. 2008; Wang et al. 2015, 2018; Zerrifi et al. 2018). Algicidal agents such as, 9-hexadecenoic acid, 2,3-dihydroxypropyl ester purified for the first time from the methanol extract of *Ulva prolifera* (Sun et al. 2016b); α-linolenic acid, oleic acid, and palmitic acid obtained from *B. braunii* (Chiang et al. 2004); and gossonorol, 7,10-epoxy-

ar-bisabol-11-ol, glycerol monopalmitate, stigmasterol, 15-hydroxymethyl-2, 6,10, 18, 22, 26,30-heptamethyl-14-methylene-17-hentriacontene, 4 hydroxyphenethyl alcohol, and margaric acid purified from the ethanolic extract of the red alga *Gracilaria lemaneiformis* (Sun et al. 2017) have been reported.

The most important worldwide freshwater bloom-forming cyanobacteria species are *Microcystis* species, known as producer of various microcystins congeners (Catherine et al. 2013). To our knowledge very little information is known on the control of *Microcystis* harmful blooms by using algicidal compounds extracted from seaweeds (Zerrifi etl al. 2018). Our study reports the effects of methanolic extracts of different macroalgae, isolated from Souiria Laqdima, Morocco, on the growth of prokaryotic cells *"M. aeruginosa",* a cyanobacteria species that commonly form fresh waters Cyano-HABs in Morocco, qualitatively in solid medium by the application of paper disk diffusion as initial screening and quantitatively in liquid medium by cells counting. In addition to the prospecting of possible biocidal action of macroalgae extracts on the growth of prokaryotic cyanobacteria, we tried to see if this effect is selective or not; for this reason, some tests will be carried out on the eukaryotic microalga *"Chlorella* sp." (Chlorophyceae).

Materials and methods

Sample Collection.

According to their ecological interest, availability, accessibility, facilitates harvesting from the coastal area and their potent antifungal or antibacterial activities, 14 seaweeds species belonging to Chlorophyta, Phaeophyta and Rhodophyta were collected from Jorf Lihoudi Sea in Souiria Laqdima, located 30 km south of Safi, Morocco, during January 2017. All samples were brought to the laboratory in plastic bags containing seawater to prevent evaporation, and then washed with seawater and distilled water to remove epiphytes, sand and other extraneous matter. After identification of each species, the macroalgae were cut into small pieces <5 mm x 5 mm marked and stored at a temperature of -20°C for ulterior lyophilization.

Preparation of algal extracts.

Macroalgae tissues were subjected to methanol extraction following the method previously described by Sahnouni et al. (2016) with minor modifications. Each 20 g of lyophilized sample was soaked in 100 ml of methanol at room temperature for 1 day with agitation and filtered through no. 2 filter paper under reduced pressure. This extraction procedure was repeated three times, and the extracts were combined. The combined filtrates

were concentrated by rotary evaporation at 45-50°C. The resulting dried extracts were then dissolved in dimethlysulfoxide (DMSO) and kept at 4°C until further use.

Microalgae media and growth conditions.

BG 13 and Z8 media were used as the base for solid and liquid media. The pH of both media was adjusted to 9.0. Solid media were prepared on a base of BG 13 medium consolidate with 0.4% of agarose, the medium composition and the agarose was autoclaved separately at 121°C for 15 min and then mixed together after cooling (Shirai et al. 1989). Microalgae growth was performed in a culture chamber with the following conditions: 26±2°C under light intensity of 4000 lx.m $^{-2}$ s $^{-1}$, with a light/dark cycle of 15 h/9 h.

Isolation of unicellular *M. aeruginosa* strain.

A natural cyanobacterial bloom that consisted of over 95% of *M. aeruginosa* was collected from the eutrophic reservoir Lalla Takerkoust (31°21′36″ N; 8°7′48″ W), Morocco in October 2015. The morphotypes of *M. aeruginosa* were identified individually using a microscope according to their colonial morphologies. In order to break up the colonies in culture and separate them into single cells, the obtained buoyant cells in the top layer of *M. aeruginosa* complex culture were suspended in sterilized water. This procedure was repeated five times to wash the cells. Afterward, 2 mL of the washed cells were vigorously shaken by a vortex mixer for 1 to 2 min and serially diluted 10-fold in sterilized distilled water before plating, poured into BG 13 medium with 0.4% agarose, allowed to solidify, and incubated for 7 to 10 days (first culture). When the colony formation was observed, the *Microcystis* cells in the colonies were transferred to fresh agarose medium and incubated again (second culture). The cells in the second culture were inoculated into Z8 medium and cultured for 7 to 14 days (Shirai et al. 1989).

Isolation of *Chlorella* sp. strains.

Water sampling was carried out in a basin within the Faculty of Sciences Semlalia (Marrakech) and the sample was concentrated with 30 µm plankton net. In order to obtain pure isolates, a series of successive subculturings was carried out on solid Z8 medium. Colonies with a macroscopic appearance similar to *Chlorella* sp. confirmed under a microscope, were selected and maintained in batch cultures on Z8 liquid medium.

Screening for algicidal activity.

Algicidal activity assays on solid media.

The potencies of seaweeds methenolic extracts against the two tested species was assessed qualitatively by the application of paper disk diffusion. Sterile filter paper discs, 9 mm in diameters (Whatman No. 1), were loaded with 20 µl of the different extracts and air dried. The crude extracts were dissolved in 0.2% DMSO because the growth of algae was not influenced at this solvent concentration, as confirmed in a previous study (Kamaya et al. 2003). Discs impregnated with cooper sulphate and DMSO were used as positive and negative controls respectively. The discs were placed on BG13 with 4 % of agarose medium and Z8 medium inoculated respectively with *M. aeruginosa* and *Chlorella* sp. (inoculum was prepared from a culture of 7 days). The plates thus prepared were stored in a refrigerator at 4 ° C for at least four hours to allow diffusion of the bioactive substances contained in the extracts into the solid medium while arresting the growth of the test microalgae. All the experiments were repeated three times to validate the findings statistically.

Algicidal activity assays on liquid media.

The algicidal activity in liquid medium of methanolic extracts against the two tested species was performed in polystyrene 6-well macroplates. 10 µL of extract were added to 5 mL of microalgal culture to final concentrations of 0, 0.3 and 0.6 mg/mL, respectively (Those two extract concentrations were shown to be the most affective dose used for microalgae growth control by seaweeds extracts (Sun et al. 2016a)). Equal volumes of DMSO and copper sulphate were used respectively as negative and positive control. In addition, an untreated microalgal culture was used as negative control for achievement of the different calculations necessary for the results treatment. The initial density of *M. aeruginosa* and *Chlorella* sp. cultures was adjusted to 3×10^6 cells/mL (the exponential growth phase).

Determination of microalgae growth and inhibition rates.

The effects of different methanolic extracts on the tested microalgae were determined by estimation of growth and inhibition rates. This was done by repeated cells counting using a hemocytometer under a microscope (Sbiyyaa et al. 1998). Inhibition rate (IR inhibition rate %) was calculated by the following equation (1) :

$$\mathrm{IR}(\%) = ((\mathrm{N0} - \mathrm{NS})/\mathrm{N0}) \times 100 \qquad (1)$$

In which N0 and NS (cells/mL) are the cell densities in the control and treatment samples, respectively.

Moreover, growth rate was calculated using the following equation (2) (Xu et al. 2010):

$$\mu = (\ln N2 - \ln N1)/\Delta t \quad (2)$$

where, μ is the average growth rate; N2 and N1 represent the cell concentrations at the end and at the beginning of the experiment, respectively and Δt indicates the time period of the experiment.

Statistical analysis.

The experiments were repeated three times (n=3) with each independent assay. Statistical differences between experimental groups and the control were analysed by applying a one-way and two-way ANOVA. Post hoc differences between group means were tested with the Tukey test. Values of P lower than 0.05 were considered significant. Statistical analyses were performed using the computer software Sigma Plot 12.5 for Windows.

Results.

Isolation of unicellular *M. aeruginosa* strain.

After two weeks of incubation time, blue-green colonies had grown in BG 13 media with 0.4 % of agarose. From the 3rd week, these colonies begin to change their colours to white and the cells forming these white colonies lost their ability to develop new colonies in a subsequent culture. In addition, *M. aeruginosa* strains becomes able to form a mat in the solid medium (figure 1).

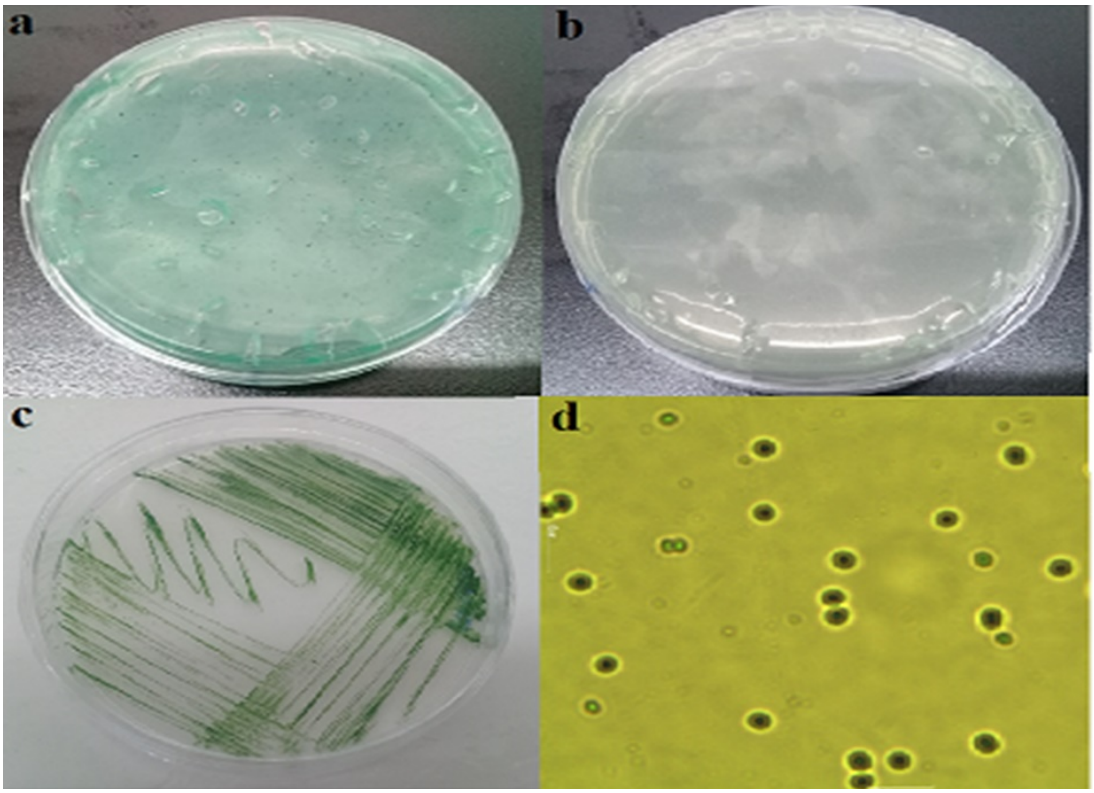

Figure 1. Colonies of *M. aeruginosa* on an agarose 0.4% plate a) After two weeks of incubation; b) After four weeks of incubation. c) Unicellular *M. aeruginosa* in BG13 medium with 0.4 % of agarose plate. d) Microscopic observation of unicellular *M. aeruginosa* isolated (Gr. x 100).

Isolation of *Chlorella* sp. strain.

After a series of successive subcultures, the colonies with a macroscopic appearance approaching to *Chlorella* were observed under a microscope for identification and maintenance in batch culture on Z8 liquid medium. Thereafter, monospecific strain of *Chlorella* was purified (figure 2).

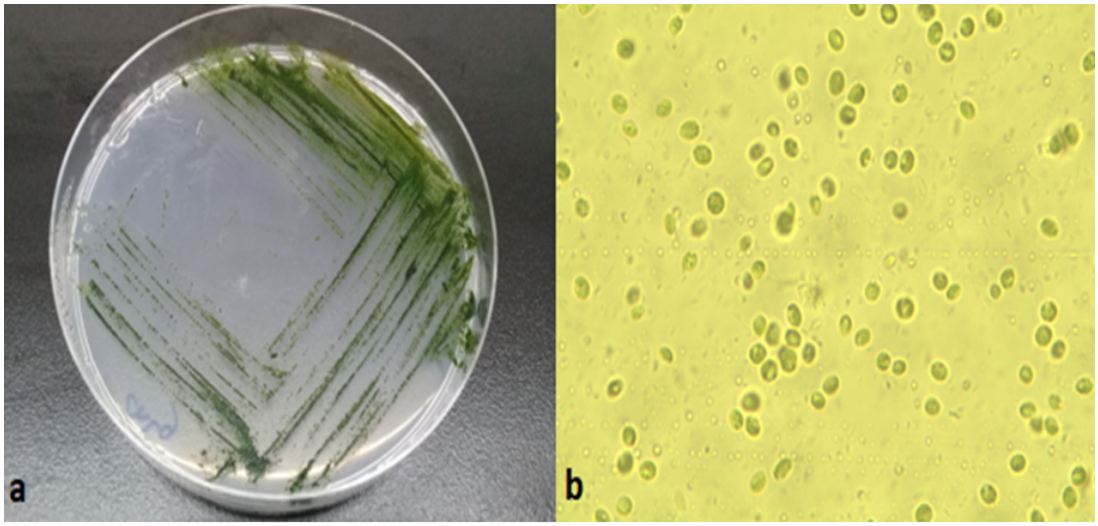

Figure 2. a). Colonies of isolated *Chlorella* sp. in Z8 medium. b). Microscopic observation of *Chlorella* sp. isolated (Gr. x 100).

Evaluation of algicidal activity on solid media

Table 1. Inhibition of microalgae in solid media assays, in the presence of methanolic extracts of Moroccan seaweeds. Diameter (mm) of the area of growth inhibited by the seaweed extracts.

	Zone of inhibition (mm)	
Treatments	***M. aeruginosa***	***Chlorella* sp.**
Codium elongatum	14.33 ± 0.33***	0±0 ns
Laminaria digitata	0±0 ns	0±0 ns
Cystoseira ericoides	16 .33 ± 0.33***	0±0 ns
Bifurcaria tuberculata	11 ± 0.00***	0±0 ns
Geledium pulchellum	16.67 ± 0.33***	0±0 ns
Laurencia pinnatifida	17.33 ± 0.33***	0±0 ns
Cystoseira tamarisafolia	13.33±0.33***	0±0 ns
Halopytis incurvus	0±0 ns	0±0 ns
Plocamium coccineum	11±0***	0±0 ns
Ulva lactuca	0±0 ns	0±0 ns
Rhodymenia pseudopalmata	11.33±0.33***	0±0 ns

Bornetia secundiflora	27.33±0.33***	0±0 ns
Enteromorpha intestinalis	11.33±0.33***	0±0 ns
Sargassum muticum	0±0 ns	0±0 ns
Copper sulphate	45.33 ± 0***	62,67 ± 0***
DMSO	0±0	0±0

***P< 0.001 indicate significant differences compared with the negative control. ns mean no significant differences compared with the negative control. Each value representing mean ± SD of 3 replicates.

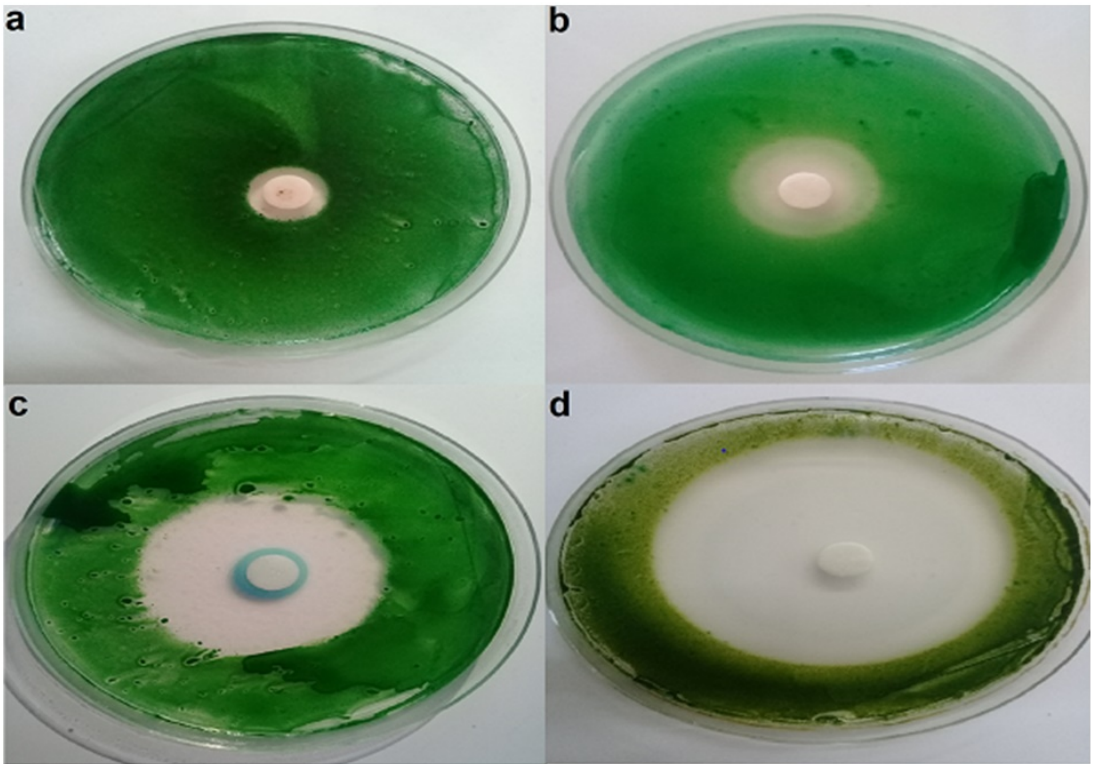

Figure 3. Algicidal activity of methanolic extracts against *M. aeruginosa* a). *G. pulchellum* b). *B. secundiflora*. c). copper sulphate d). Copper sulphate against *Chlorella* sp.

After 7 days of incubation, the measurement of the inhibition zones has been done and the results of algicidal activity in solid medium are summarized in table 1 and figure 3. DMSO and copper sulphate were used as negative and positive control respectively; the negative control (DMSO) never showed algicidal activity while copper sulphate showed strong activity against both tested microalgae (45.33 ± 0 and 62.67 ± 0 mm against *M. aeruginosa* and *Chlorella* sp. respectively). However, ten extracts out of fourteen from different species of Moroccan seaweeds showed algicidal activity against *M. aeruginosa.* In contrast no extracts showed algicidal activity against *Chlorella* sp. The most important activity against *M. aeruginosa* (e.g. diameter of growth inhibition higher than 27 mm) was observed in the extract

of the red macroalgae *B. secundiflora*, whereas moderate activity against *M. aeruginosa* was observed with the extracts of *L. pinnatifida, G. pulchellum, C. ericoides* and *Codium elongatum* (the diameter of inhibition zone was 17.33 ± 0.33 mm, 16.67 ± 0.33 mm, 16.33 ± 0.33 mm and 14.33 ± 0.33 mm, respectively). The lower inhibitory effect for *M. aeruginosa* was recorded in the methanolic extracts of *B. tuberculata* and *P. coccineum* (11 ± 0.00 mm). The extracts of *L. digitata, H. incurvus, U. lactuca* and *S. muticum* did not reveal any inhibitory activity against the cyanobacterium.

Evaluation of algicidal activity on liquid media

Inhibitory rates of seaweeds methanolic extracts on microalgae tests

The inhibition rates of the fourteen macroalgal crude extracts tested at the 2 concentrations (0.3, 0.6 mg/mL) against *M. aeruginosa* and *Chlorella* sp. growth is shown as the IR (%) in tables 2 and 3 respectively. Copper sulphate as positive control showed strong inhibition of *M. aeruginosa* and *Chlorella* sp., during the entire follow-up period the IR was more than 86% for both tested concentrations, while DMSO, as negative control, did not show any inhibitory effect on both tested microalgae (IR<0.5%). The results indicate that all methanolic extracts of different macroalgae had a significant inhibitory effect on *M. aeruginosa* compared to the negative control. At the first day of treatment, the macroalgal extracts of *B. secundiflora*, *C. elongatum, E. intestinalis, B. tuberculata* and *S. muticum* showed strong growth inhibition (IR>50% for both tested concentrations) on *M. aeruginosa*. The lowest inhibition rate was recorded by the methanolic extract of *C. tamarisafolia* (IR< 32 % for both concentrations used). The IR of *B. secundiflora* extract was a maximum of 80.65 ± 0.23 % and 73.63± 0.19 % at 0.6 mg/mL and 0.3 mg/mL respectively, on the first day; thereafter, it began to decrease gradually but was maintained at more than 21 % all the time. At the end of the experiment, the maximum inhibition rates were revealed by the extracts of *B. tuberculata, C. elongatum* and *B. secundiflora.* The IR reached at a concentration of 0.3 mg/mL was 24.2±0.24 %, 22.24±0.24 % and 21.28±0.22 % respectively. The higher concentration (0.6 mg/mL), of *P. coccineum* showed the most important inhibitory effect on *M. aeruginosa* (26.57±0.41 %). Whereas at a concentration of 0.3 mg/mL the lowest inhibition rates were observed in the extract of the brown seaweeds *C. ericoides* (8.25±0.48 %) and *S. muticum* (9.95±0.16 %). This last species showed the lowest effect at 0.6 mg/mL too (11.96±0.02 %). The effects of methanolic macroalgae extracts tested against *Chlorella* sp. varied according to the macroalgae species from which the extracts were obtained. Firstly, the extract of *R. pseudopalmata, E.*

intestinalis and *S. muticum* promotes the growth of *Chlorella* at the first day of treatment. In the days after an inhibition that does not exceed 3% begins to appear. In the other hand, *C. ericoides* and *C. elongatum* have the maximum inhibition rate at the first day (16.64±2.81 % and 11.87±1.16 % at 0.6 mg/mL, 13.99±0.49 % and 11.39±0.66 % at 0.3 mg/mL respectively). These inhibitions rates began to decrease gradually with the treatment by *C. ericoides* and increase with the use of *C. elongatum* extract but was maintained at less than 25 %. The maximum inhibition rate was revealed at the last day of treatment by the extracts of *B. secundiflora* (42.9±1.75 % and 35.95±0.11% at 0.6 mg/mL and 0.3 mg/mL respectively).

Treatments	Concentration (mg/mL)	Time (days)					
		0	1	2	3	4	5
Codium elongatum	0.3	0±0	46.23±0.4***	50.18±0.24***	40.86±0.2***	25.09±0.04***	22.24±0.24***
	0.6	0±0	66.82±0.12***	52.69±0.24***	44.86±0.03***	31.92±0.04***	25.1±0.22***
Laminaria digitata	0.3	0±0	46.65±0.62***	33.37±3.84***	23.09±0.33***	20.24±0.38***	14.67±0.2***
	0.6	0±0	49.66±0.13***	39.53±2.61***	27.22±0.14***	21.9±0.13***	15.32±0.12***
Cystosiera ericoides	0.3	0±0	39.71±2.4***	26.23±0.54***	17.43±0.18***	13.32±0.13***	8.25±0.48***
	0.6	0±0	60.47±0.45***	38.49±4.27***	25.71±0.28***	20.83±0.66***	16.27±0.16***
Bifurcaria tuberculata	0.3	0±0	57.27±0.81***	51.51±1.13***	41.07±0.05***	31.24±0.34***	24.2±0.24***
	0.6	0±0	64.88±0.17***	52.13±1.83***	41.57±0.11***	34.08±1.18***	26.54±0.05***
Gelidim pulchellum	0.3	0±0	39.5±0.53***	38.03±0.61***	26.2±0.65***	20.83±0.22***	18.06±0.33***
	0.6	0±0	57.31±0.68***	47.57±0.28***	33.37±0.75***	27.77±0.08***	20.61±0.35***
Laurentia pinnatifida	0.3	0±0	45.53±1.05***	29.85±5.02***	22.79±0.59***	14.53±0.3***	10.01±0.19***
	0.6	0±0	49.46±0.52***	38.49±0.24***	34.4±1.42***	20.76±0.13***	15.03±0.23***
Cystosiera tamarisafolia	0.3	0±0	30.03±1.81***	34.16±1.4***	31.08±0.22***	22.03±0.5***	14.06±0.16***
	0.6	0±0	31.43±0.96***	36.84±0.39***	32.99±0.09***	24.24±0.28***	23.51±0.14***
Halopytis incurvus	0.3	0±0	39.32±0.35***	39.42±3.28***	26.23±0.15***	21.17±0.16***	11.78±0.23***
	0.6	0±0	44.5±1.26***	38.07±1.87***	27.99±0.29***	22.24±0.16***	15.12±0.05***
Plocamium coccineum	0.3	0±0	48.09±0.09***	40.32±2.17***	29.53±0.66***	21.72±0.17***	16.48±0.12***
	0.6	0±0	47.81±0.64***	54.02±2.24***	46.22±0.47***	33.56±0.14***	26.57±0.41***
Ulva lactuca	0.3	0±0	47.41±1.06***	42.31±2.3***	31.19±0.32***	22.32±0.13***	13.27±0.24***
	0.6	0±0	55.64±1.55***	53±0.49***	37.16±0.48***	28.62±0.22***	19.79±0.36***
Rhodymenia pseudopalmata	0.3	0±0	44.32±0.86***	31.08±1.43***	26.11±0.38***	15.43±0.1***	10.9±1.94***
	0.6	0±0	41.94±1.62***	37.47±0.08***	28.32±0.28***	21.19±0.4***	14.96±0.27***
Bornetia secundiflora	0.3	0±0	73.63±0.19***	60.72±2.44***	39.49±0.58***	26.85±0.61***	21.28±0.22***
	0.6	0±0	80.65±0.23***	78.57±0.33***	51.98±0.88***	32.64±0.5***	23.25±0.26***
Enteromorpha intestinalis	0.3	0±0	60.55±0.51***	35.58±2.08***	23.09±0.2***	17.88±1.26***	13.12±0.24***
	0.6	0±0	67.83±1.25***	44.11±0.32***	34.36±0.41***	23.8±0.04***	17.36±0.06***
Sargassum muticum	0.3	0±0	55.62±0.59***	37.88±0.84***	26.26±0.48***	14.81±0.43***	9.95±0.16***
	0.6	0±0	63.52±0.08***	40.29±1.11***	28.49±0.73***	19.83±0.59***	11.96±0.02***
Copper sulphate (Positive control)	0.3	0±0	86.06±1.77***	90.54±0.27***	94.82±0.07***	95.95±0.27***	96.47±0.36***
	0.6	0±0	90.17±0.3***	96.21±0.46***	96.4±0.38***	97.8±0.67***	97.35±0.69***

DMSO (Negative control)		0±0	0.48±1.1	0.29±1.3	0.11±0.53	0.38±0.25	-2.62±0.35

Table 2. Inhibitory rates of Moroccan seaweeds methanolic extracts on *M. aeruginosa.*

Each value representing mean ± SD of 3 replicates, ***P< 0.001 indicate significant differences compared with the negative control.

Table 3. Inhibitory rate of Moroccan seaweeds methanolic extracts on *Chlorella* sp.

Treatments	Concentration (mg/mL)	Time (days)					
		0	**1**	**2**	**3**	**4**	**5**
Codium elongatum	0.3	0±0	11.87±1.16***	33.41±0.25***	32.78±0.22***	26.03±0.21***	24.72±0.12***
	0.6	0±0	11.39±0.66***	31.69±0.12***	31.92±0.89***	21.64±0.18***	20.17±0.26***
Laminaria digitata	0.3	0±0	11.94±0.92***	33.45±0.48***	36.28±0.38***	25.22±0.09***	24.27±0.3***
	0.6	0±0	6.87±1.34***	23.64±0.38***	21.56±0.73***	11.78±0.42***	11.43±0.25***
Cystosiera ericoides	0.3	0±0	16.64±2.81***	28.89±0.52***	24.9±1.95***	19.15±0.56***	12.01±0.11***
	0.6	0±0	13.99±0.49***	21.73±0.07***	19.18±0.12***	17±0.4***	14.3±0.13***
Bifurcaria tuberculata	0.3	0±0	12.68±0.33***	29.75±0.38***	33.22±0.26***	25.31±0.12***	19.86±0.17***
	0.6	0±0	-0.87±0.42***	17.37±0.22***	20.34±0.23***	15.59±0.27***	14.29±0.18***
Gelidim pulchellum	0.3	0±0	2.95±0.21	16.83±0.16***	18.13±0.82***	16.7±0.35***	8.22±0.28***
	0.6	0±0	0.64±0.43***	13.74±0.43***	17.66±0.2***	12.68±0.18***	15.09±0.2***
Laurentia pinnatifida	0.3	0±0	-0.75±0.55	18.33±0.24***	24.32±2.88***	21.44±0.49***	8.85±0.28***
	0.6	0±0	-3.12±0.5	5.32±0.39***	11.54±0.3***	13.02±0.25***	10.35±0.35***
Cystosiera tamarisafolia	0.3	0±0	6.47±0.06	37.54±6.01***	26.18±2.55***	20.45±9.92***	8.66±0.26***
	0.6	0±0	-1.88±0.27***	29.67±0.04***	19.42±0.3***	23.87±0.9***	21.33±0.11***
Halopytis incurvus	0.3	0±0	6.37±0.66	36.68±0.27***	33.01±0.3***	20.47±0.56***	21.25±0.3***
	0.6	0±0	3.07±0.5***	26.15±0.36***	22.67±0.82***	18.43±0.11***	11.77±0.54***
Plocamium coccineum	0.3	0±0	6.75±1.04***	30.31±0.66***	17.16±0.52***	13.42±0.44***	2.89±0.17
	0.6	0±0	0.23±1.28***	18.77±0.45***	22.83±1.58***	10.59±0.26***	12.45±0.1***
Ulva lactuca	0.3	0±0	4.73±0.6	16.51±0.26***	16.07±0.27***	11.66±0.07***	10.69±0.18***
	0.6	0±0	-3.07±1.05	11.95±0.19***	11.75±1.26***	8.53±0.17***	6.5±0.21***
Rhodymenia pseudopalmata	0.3	0±0	-15.94±0.19	-4.47±0.63***	0.17±0.11	1.14±0.31	2.04±0.26
	0.6	0±0	-18.87±0.38***	-6.08±0.26***	-3.53±0.27***	2.23±0.08***	2.28±0.25
Bornetia secundiflora	0.3	0±0	6.7±0.42***	23.21±0.42***	24.28±2.07***	36.95±1.21***	42.9±1.75***
	0.6	0±0	2.89±0.72***	8.33±0.14***	18.66±0.42***	20.96±0.08***	35.95±0.11***

Enteromorpha intestinalis	0.3	0±0	2.61±0.23	2.61±3.76	-0.11±0.26	-2.8±0.27	0.39±0.26
	0.6	0±0	-18.11±1.01***	3.24±1.6***	0.03±0.68	-1.76±0.29	-0.78±0.2
Sargassum muticum	0.3	0±0	-6.11±0.65	2.71±2.61	1.72±0.06	-2.2±0.32	1.82±0.43
	0.6	0±0	-2.04±0.32***	-2.11±2.79	-2.21±0.21***	-5.51±0.23***	-3.12±0.31***
Copper sulphate (Positive control)	0.3	0±0	35.09±0.29***	65.7±0.2***	84.56±0.07***	94.68±0.05***	95.06±0.19***
	0.6	0±0	35.09±0.29***	65.7±0.2***	84.56±0.07***	94.68±0.05***	95.06±0.19***
DMSO (Negative control)		0±0	3.01±0	4.28±0.9	2.29±1.6	-0.46±0.13	-0.95±1.917

2 Each value representing mean ± SD of 3 replicates, ***P< 0.001 indicate significant differences compared with the negative control.

Effect of seaweeds methanolic extracts on growth rates of microalgae

The experimental results expressed in growth rates (μ) of the strain *M. aeruginosa* and *Chlorella* sp. at the various macroalgal extracts treatment are shown in figure 3 and 4. Under standard culturing conditions, the growth rates were about 0.74 /day for *M. aeruginosa* and 0.41/day for *Chlorella* sp., which corresponds to a generation time of ~ 1.0 and ~2.0 days respectively. The DMSO has no effect on the growth rates of both tested microalgae whereas copper sulphate decreased significantly the growth of *M. aeruginosa* and the culture of *Chlorella* died with this treatment. The results show that μ of both microalgae tests decreased significantly after treatment with the majority of the seaweed extracts tested. The extracts of *B. tuberculata, C. elongatum* and *B. secundiflora* have the strongest inhibitory effect on μ of tested cyanobacteria. On the other hand, the extract of *S. muticum* have no significant effect on the growth of both tested microalgae. *R. pseudopalmata* and *E. intestinalis* has no significant effect against *Chlorella* sp.

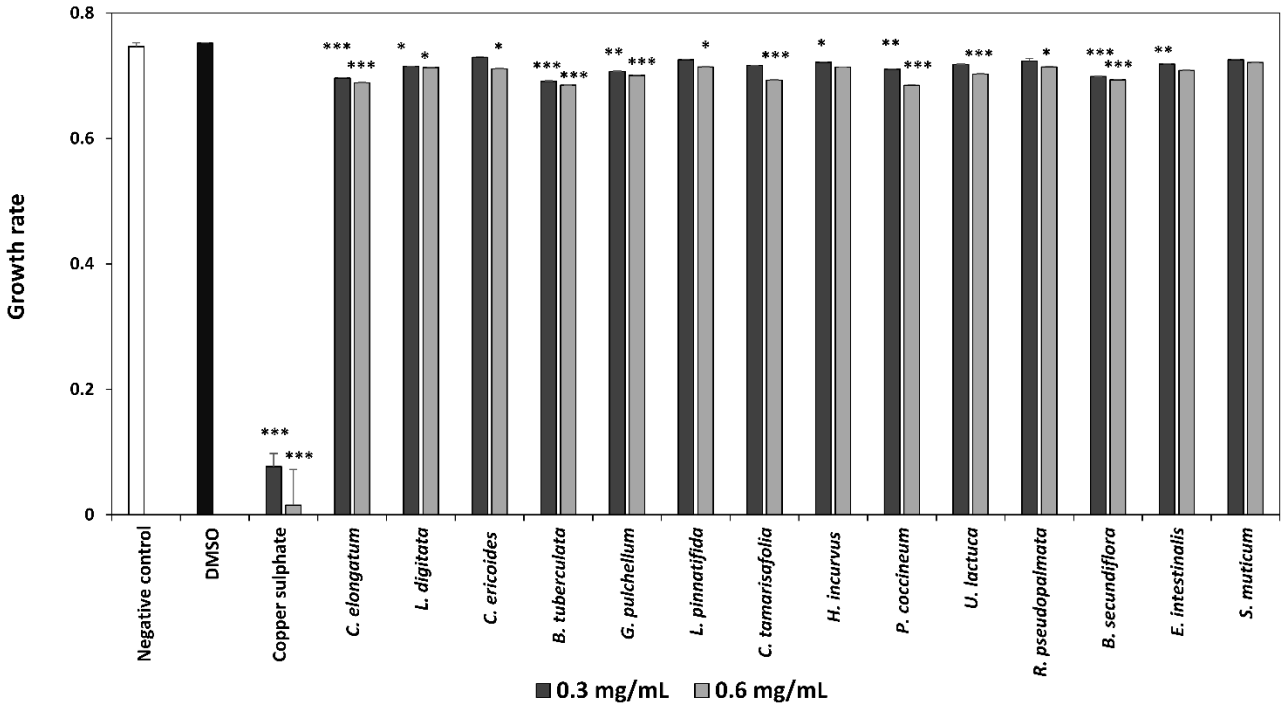

Figure 4. Effect of Moroccan seaweeds methanolic extracts on the growth rate of *M. aeruginosa.* Each value representing mean ± SD of 3 replicates, ***P< 0.001; **P< 0.005 and *P< 0.05 indicate significant differences compared with the negative control.

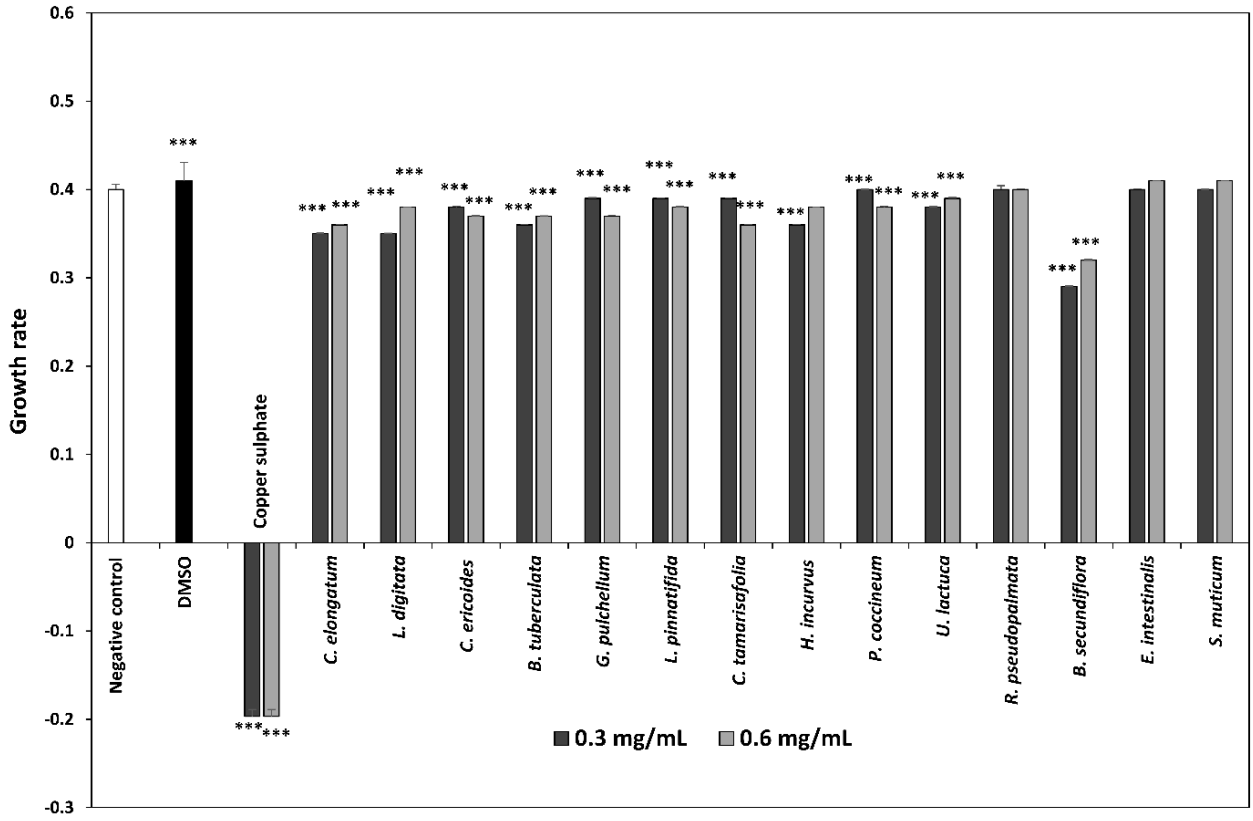

Figure 5. Effect of Moroccan seaweeds methanolic extracts on the growth rate of *Chlorella* sp. Each value representing mean ± SD of 3 replicates, ***P< 0.001; indicate significant differences compared with the negative control.

Discussion

This present study includes the first description of the algicidal activity in solid and liquid medium of 14 seaweeds from the coast of Morroco. In solid media the methanolic extracts of *L. digitata, H. incurvus, U. lactuca* and *S. muticum* did not show any inhibition against the Gram-negative bacteria *M. aeruginosa*. Whereas, Kumaresan et al. (2018) observed that aqueous extract of the genus *Sargassum (S. wightii*) showed a moderate antibacterial activity against gram negative bacteria (13 mm against *Escherichia coli* and 10 mm against *Salmonella typhi*). Furthermore, Mishra (2018) reported that the methanolic extracts of *U. lactuca* and seaweed of the genus *Sargassum* have a moderate effect against *M. aeruginosa.*

In addition, we found that the methanolic extracts of *B. secundiflora, L. pinnatifida* and *G. pulchellum* showed a strong activity against *M. aeruginosa* (27.33±0.33 mm, 17.33 ± 0.33 mm and 16.67 ± 0.33 mm, respectively). Our results are similar to those found by Al-Enazi

et al. (2017) who investigated the antibacterial activities of three seaweeds collected from Alharra, Umluj, Kingdom of Saudi Arabia. The highest activities were obtained against the negative bacteria *Klebsiella pneumonia* by seaweeds of genus *Laurencia (L. catarinensis)* (23.40 ± 0.58 mm). In another study, the methanol extract of *Laurencia iliformis* was the most active against *P. aeruginosa, S. aureus, S. pyogenes* and *E.coli*. The methanolic extract of *U. reticulata* also showed a strong effect against *P. aeruginosa* (Begum et al. 2018). Li et al. (2018) reported that the ethanolic extracts of *S. fusiforme, U. pertusa* and *G. furcate* reveal the maximum inhibitory activity against Gram-negative bacteria *Escherichia coli* at 10 mg/ mL (10 mm, 7 mm and 7 mm, respectively). Salvador et al. (2007) observed that 18 taxa of seaweeds, including *E. intestinalis, Gelidium spinosum, Codium fragile, B. secundiflora, C. tamariscifolia, Ulva rigida* did not show antimicrobial activity against any Gram negative bacteria assayed. Although, in the present study *B. secundiflora*, *G. pulchellum* and *C. elongatum* showed a strong activity against Gram-negative bacteria *M. aeruginosa*, *C. tamariscifolia* and *E. intestinalis* exhibited moderate anti-cyanobacterial activity. However, the strong algicidal activity of tested seaweeds could be explained by the presence of metabolites and natural bioactive compounds groups, such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids that have been reported as bacterial inhibitors (Rodrigues et al. 2015). Pérez et al. (2016) suggested that the higher antibacterial activity of seaweeds was related to their ability to bind with bacterial proteins such as enzymes and cell membranes.

In liquid medium, all methanolic extracts of different macroalgae tested have a significant algicidal activity against the toxic cyanobacteria *M. aeruginosa* and the feed microalgae *Chlorella* sp. Several studies have investigated the antialgal property of seaweeds in liquid medium (Schwartz et al. 2017; Wang et al. 2018). Our results in liquid medium revealed that the extracts of *B. tuberculata, C. elongatum* and *B. secundiflora* have the strongest inhibitory effect on μ and the maximum inhibition rates of tested cyanobacteria. These results are in agreement with the observations of Zerrif et al. (2018) who reported that the methanol extract of the *C. elongatum* collected from the coast of Morocco showed a significant progressive reduction in growth of the filamentous cyanobacterium *Phormidium* sp. While, we found that the extract of *S. muticum* had no significant effect on the growth rate and the inhibitory rate of *M. aeruginosa*. Contrariwise, Renjun et al. (2012) revealed that the methanol extract of the genus *Sargassum* (*S. thunbergii*) caused a stronger inhibitory effects on the growths of red tide microalgae tests such as *Heterosigma akashiwo*, *Skeletonema costatum* and *Prorocentrum micans*. In another report, Sun et al. (2016b) demonstrated the algicidal

activity of methanolic extract of *Ulva prolifera* in liquid medium against red tide microalgae; also, they showed that this extract has no effect on feed microalgae**.**

Furthermore, the decrease in growth rates of *M. aeruginosa* strain treated by the extracts of *B. secundiflora, C. elongatum* and *B. tuberculata.* compared to the control, might be explained by the presence of algicidal compounds. The potential of seaweeds as a source of active compounds against microalgae forming harmful blooms (HABs) has been confirmed in different studies. Recently, Sun et al. (2018) indicated that three sesquiterpenoids (gossonorol, 7,10-epoxy-ar-bisabol-11-ol and cyclonerodiol) had selective antialgal activity against the growth of different red tide microalgae such us *Amphidinium carterae, H. akashiwo, Karenia mikimitoi,* and *Phaeocystis globosa*. (6E,9E,12E)-(2-acetoxy-β-D-glucose)-octadecatrienoic acid ester isolated from *Ulva intestinalis* displayed strong algicidal activity with IC50 values of 4.9 and 14.1 µg/mL for *H. akashiwo* and *P. micans*, respectively (Sun et al. 2016a).

Conclusion

After screening of 14 Moroccan seaweeds species for their algicidal activity, our results revealed that seaweeds are potential producers of anti-algicidal compounds. For that reason, they should be thoroughly investigated as natural sources of bioactive substances. In order to understand the potential inhibitory effect of these seaweeds extracts, characterization of the extracts and study of the effective compounds would be the next step of our work. Moreover, further researches will need to be conducted against other seaweeds and/or phytoplankton in macrocosms and natural field conditions, with a focus on the study of the nature and stability of the specific compounds and their potentially synergistic interactions in the aquatic ecosystem.

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