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Antimicrobial Activity of the Marine Algal Extracts against Selected Pathogens

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ABSTRACT

The inhibitory effect of *Ulva lactuca* (Chlorophyta), *Dilophus spiralis* (Phaeophyta) and *Jania rubens* (Rhodophyta) marine algae species has been evaluated against 2 Gram-positive bacterial (*Streptococcus pyogenes* and *Micrococcus luteus*); 2 Gram-negative bacterial (*Shigella flexneri* and *Vibrio cholerae*) and 2 fungal (*Candida albicans* and *Aspergillus niger*) isolates using aqueous and six organic extracts (methanol, ethanol, chloroform, acetone, ethyl acetate and hexane). Data revealed that the *M. luteus* bacteria was the most sensitive pathogen by showing the highest zone of inhibitions (ZIs) of 17 mm with the lowest Minimum Inhibitory Concentration (MIC) of 26.7 µgmL^{-1} and the lowest Minimum Bactericidal Concentration (MBC) of 53.3 µg mL^{-1} with chloroform *D. spiralis* extract. Whereas, aqueous extracts were not active against all selected pathogens regardless of the examined algae species. Based upon data presented herein, chloroform *D. spiralis* extract was the most active against examined pathogens. Thereby, future performance research in *D. spiralis*is requested due to their high effectiveness as a cheap antimicrobial agent.

Keywords: Algae, Antimicrobial activity, Minimum Bactericidal Concentration (MBC), Minimum Inhibitory Concentration (MIC).

INTRODUCTION

The occurrence of approximately 9.000 macroalgae species around the oceans worldwide has been demonstrated (Wajahatullah *et al*., 2009). However, their identification covered only very little of it. Previousely, Garson (1989) reported that algae could be divided into three main groups (phyla): Green algae (Chlorophyta); red algae (Rhodophyta) and brown algae (Phaeophyta) (Sambamurty, 2005; Wajahatullah *et al*., 2009). Algae exhibited great potential due to their importance as a useful bioindicator for heavy metals pollution in ecosystems and its multiusage for many other purposes (medicinal, antimicrobial…*e.g.*) (Sode *et al*., 2013; Oumaskour *et al.,* 2013; Abo-State *et al*., 2015; Kausalya and Rao, 2015).

Many investigations revealed that macroalgae have a broad range and potential use in pharmacology researches as antibacterial (Zbakh *et al*., 2012; Malingin *et al*., 2012; Jeyaseelan *et al*., 2012; Alghazeer *et al*., 2013; Oumaskour *et al.*, 2013; Abo-State *et al*., 2015) or/and antifungal (Karabay-Yavasoglu *et al*., 2007; Oumaskour *et al.*, 2013; Abo-State *et al*., 2015; Kausalya and Rao, 2015). Their inhibitory effects is related to the presence of bioactive compounds as secondary metabolites *e.g.* phenol and carotenoids compounds (Malingin *et al*., 2012) or due to the presence of saponins, flavonoids, tannins and cardiac glycosides (Jeyaseelan *et al*., 2012).

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Thereby, the current investigation focused on algae utility as antibacterial and antifungal agents alsoreported in many investigations (Zbakh *et al*., 2012; Oumaskour *et al.,* 2013; Abo-State *et al*., 2015; Kausalya and Rao, 2015; Hamza *et al*., 2015). So, the most potent algae will be handled in the future research as a cheaper source for antimicrobial treatment.

MATERIALS AND METHODS

Collection and Preparation of Algae Samples

Algal samples for *Ulva lactuca* (Chlorophyta), *Dilophus spiralis* (Phaeophyta) and *Jania rubens* (Rhodophyta) species were collected from Latitude at 4 km North Lattakia - Syria (35° 33′ 790″ N longitude, 35° 43′ 996″ E) along the Syrian coast of the Mediterranean Sea. Algae were identified by taxonomical study in the Division of Plant Biotechnology at the Atomic Energy Commission of Syria (AECS) in Damascus-Syria. Algae samples were harvested by hand with disposable gloves; biomass were washed with seawater where the algae were collected. Then two successive washing with double-distilled water (ddH_2O) has been done. Biomass were drained, then transported to Whatman filter paper for eliminating attached water and facilitating their drying. Algal samples were shade dried for two weeks, powdered by special electric mill and stored separately in polyethylene bags until analysis.

Preparation of Algal Extracts

The marine algal extracts of *U. lactuca*, *D. spiralis* and *J. rubens* were prepared using aqueous and six solvents (methanol, ethanol, chloroform, acetone, ethyl acetate and hexane). All solvents were purchased from Sigma-Aldrich-Germany. The extraction had been performed as follows: 1 g of shade-dried, pulverized algae was subjected to extraction in 100 mL solventuntil complete solubility. Then, extracts were filtered with Whatman filter papers. Extracts were kept at laboratory temperature for 2 hours to evaporate the solvent. All extracts were then kept in tightly fitting stopper bottles and stored in 4°C. The concentration of each extract was considered 10 mg mL^{-1}.

Phytochemical Assay

Phytochemical algal screening (Alkaloids, flavonoids, saponins, terpenoids, tannins, steroids, carbohydrates, proteins and phenols) was performed according to standard procedures described by Lala (1993), El Baky *et al*. (2008) and Arthanan and Kumar (2013).

Microorganisms and Growth Conditions

Six pure clinical isolates of 2 Gram-positive (*S. pyogenes* and *M. luteus*)*;* 2 Gram-negative (*S. flexneri* and *V. cholerae*) bacterial and 2 fungal (*C. albicans* and *A. niger*) pathogens were collected from the Microbiology and Immunology division, Department of Molecular Biology and Biotechnology of Atomic Energy Commission of Syria (AECS) in Damascus - Syria.

Bacterial culture was done by inoculation trypticase soy broth (TSB, Difco, BD, Spars, MD) at 37°C for 24 hours for all tested bacteria.

After growth, samples were centrifuged (1,000 xg 15 min 4°C) and resuspended in sterile Phosphate-Buffered Saline (PBS). The turbidity of each bacterial suspension was adjusted equivalent to a no. 0.5 McFarland standard and then inoculated on Mueller-Hinton agar (Oxoid, UK) at 37°C for about 18-24 hours. The bacterial cultures standardize to approximately 10^6 CFU mL^{-1} (Fagbohun *et al*., 2013). The exact counts were assessed retrospectively by viable counts on trypticase soy agar plates (TSA,

Difco, BD, Spars, MD) at 37°C for 18 hours. Whereas, all tested fungal inoculations were done by incubation on Potato Dextrose Agar (PDA) and incubated at 28±3°C for 2 days.

Antimicrobial Activity Assay

The Disc-Diffusion Assay

The disc-diffusion method was adopted to examine the antimicrobial activity as previously reported (Bauer *et al*., 1966). Ciprofloxacin (100 mg mL^{-1}) (Bayer, Istambul, Turkey) and Amphotericin B (40 mg mL^{-1}) (Sigma-Aldrich, St. Louis, USA) were used as standard for antibacterial and antifungal activity, respectively. The sterilized disc filter papers (Whatman no. 1 of 6 mm diameter) were inculated with 100 µL of extract dilutions (10 mg mL^{-1}) and subjected to the culture plates previousely cultivated with 10^6 CFU mL^{-1} of bacterial culture, then inculation was done at 37°C for 18 hours. Whereas, paper discs that were inculated with 20 µL of 10 mg mL^{-1} Ciprofloxacin and 20 µL of 40 mg mL^{-1} Amphotericin B, were used as standard for antibacterial and antifungal activity, respectively for comparison. Negative control was done using paper disc that was inculated with 10 µL methanol only. Antimicrobial activity was determined by measuring the zone of inhibition (mm) around each paper disc. For each extract, duplicate trials were conducted against each organism.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Microdilution broth susceptibility test was assessed according to Ríos-Dueñas *et al*. (2011). Three replicates of serial dilutions of extract (10 mg mL^{-1}), Ciprofloxacin as an antibacterial (100 mg mL^{-1}) and Amphotericin B (40 mg mL^{-1}) as an antifungal were prepared in LB broth medium in 96-well microtiter plates, using a series concentrations of the 6 tested solvents of the examined algae from 0.166 to 40 µL in a final volume of 100 µL per well. One hundred microlitres of freshly grown bacteria standardized 106 CFU mL^{-1} in LB broth were added to each well. Positive control was achieved with the same conditions, but without extract. Negative control was also made with the same conditions but without adding the pathogen. The plate was shaken and incubated for 24 hours at 37°C. The lowest concentration that completely inhibited the growth was recorded and interpreted as the *MIC* and is expressed in µg mL^{-1} or mg mL^{-1}.

Whereas, *MBC* was determined by plating 0.010 mL of each well that showed no visible growth on Mueller-Hinton agar plates (Oxoid) and incubating for 18–24 hours. The *MBC* was defined as the lowest concentration that kills 99.9% of the initial inculations, so the lowest concentration showing no growth after inculations is considered the *MBC*.

Statistical Analysis

All statistical analyses were performed using Statview 4.5 statistical package (Abacus, 1996) at the 5% significance level (P= 0.05). Where, data were subjected to Analysis of Variance (ANOVA) for the determination of differences in means between different tested solvents against selected isolates for each algae species. Differences between means were tested for significance by Fisher's Least Significant Difference (PLSD) test. Data are expressed as mean±Standard Deviation (SD).

RESULTS

Phytochemical Test

Phytochemical screeningof *U. lactuca*, *D. spiralis* and *J. rubens* aqueous, methanol, ethanol, chloroform, acetone, ethyl acetate and hexane extracts has been performed. Phytochemical algal screening showed the absence of proteins from all examined algae species, regardless of tested solvents (Table

1). For *U. lactuca,* flavonoids and phenols were presented with all solvents (Table 1). Whereas, in *D. spiralis,* tannins and phenols were presented with all solvents. While, for *J. rubens,* bioactive compounds were presented in a similar manner with aqueous and ethanol extracts. In this regards, alkaloids, flavonoids, saponins, tannins, steroids, carbohydrates and phenols were presented with aqueous and ethanolic *J. rubens* extracts (Table 1).

Estimated Zone of Inhibitions (ZIs)

Inhibitory activity of algae had been evaluated against 4 bacterial and 2 fungal isolates. Algal crude extracts were active against examined pathogens in different degrees (Table 2). Our data showed that the aqueous algal extracts showed no activity against all examined pathogens regardless studied algae species (data not presented herein).

From the data presented in Table 2, variance analysis showed that solvent, isolate and interaction solvent with isolate effect's on *ZIs* values were significantly different ($P \leq 0.001$).

In this regards, *ZIs* values ranged between 6-17 mm for Gram-positive, 0-12 mm for Gram-negative bacterial and between 2–14 mm for fungal isolates (Table 2). In this respect, the highest *ZIs* against Gram-

Table 1. Algal phytochemical analysis using different examined solvents.

Chemical components	Aqueous	Methanol	Ethanol	Chloroform	Acetone	Ethyl acetate	Hexane
U. lactuca							
Alkaloids	- [a]	+ [b]	-	-	+	-	-
Flavonoids	++ [c]	+	+	+	+	+	+
Saponins	+	+	+	+	-	++	+
Terpenoids	-	+	+	+	-	+	+
Tannins	++	+	+	-	-	-	+
Steroids	-	-	+	-	++	+	-
Carohydrates	-	+	-	+	-	-	-
Proteins	-	-	-	-	-	-	-
Phenols	+	+	+	+	+	++	++
D. spiralis							
Alkaloids	-	+	+	-	-	-	-
Flavonoids	-	+	+	-	-	-	-
Saponins	-	-	-	+	-	+	+
Terpenoids	-	+	+	-	-	+	-
Tannins	+	+	++	+	+	+	+
Steroids	-	-	-	-	+	-	-
Carohydrates	-	+	+	-	-	+	-
Proteins	-	-	-	-	-	-	-
Phenols	+	++	++	+	+	+	++
J. rubens							
Alkaloids	+	+	+	+	+	-	+
Flavonoids	+	+	+	-	+	+	-
Saponins	+	+	+	-	-	+	+
Terpenoids	-	-	-	-	-	-	-
Tannins	+	+	+	+	-	-	+
Steroids	+	-	+	-	-	+	-
Carohydrates	+	+	+	-	+	-	-
Proteins	-	-	-	-	-	-	-
Phenols	+	+	+	+	-	-	+

[a] Absent; [b] Present, [c] Higher presence.

Table 2. Algal antimicrobial activity using disc-diffusion method (Zone of inhibition in mm). [a]

Zone of Inhibitions (ZIs) (mm)							
Microorganisms	Methanol	Ethanol	Chloroform	Acetone	Ethyl acetate	Hexane	Control
U. lactuca							
S. pyogenes	7.0±0.29^{Bd}	6.0±0.15^{Cd}	9.0±0.22^{Ac}	6.0±0.18^{Cd}	9.0±0.23^{Ab}	7.0±0.20^{Bd}	22.0±0.44
M. luteus	10.0± 0.25^{Ab}	7.0±0.25^{Cc}	9.0±0.16^{Bc}	10.0±0.15^{Ab}	7.0±0.18^{Cd}	9.0±0.25^{Bc}	23.0±0.32
S. flexneri	8.0±0.11^{Dc}	8.0±0.15^{Db}	9.0±0.20^{Cc}	6.0±0.17^{Ed}	10.0±0.12^{Ba}	11.0±0.14^{Aa}	31.0±0.2
V. cholerae	6.0±0.15^{Ee}	9.0±0.25^{Ba}	11.0±0.17^{Ab}	8.0±0.25^{Cc}	9.0±0.21^{Bb}	7.0±0.25^{Dd}	19.0±0.15
C. albicans	12.0±0.25^{Aa}	9.0±0.45^{Da}	11.0 ±0.34^{Bb}	10.0±0.44^{Cb}	9.0±0.17^{Db}	10.0±0.62^{Cb}	17.0±0.16
A. niger	8.0±0.48^{Dc}	8.0±0.27^{Db}	12.0±0.66^{Aa}	11.0±0.35^{Ba}	8.0±0.18^{Dc}	10.0±0.42^{Cb}	15.0±0.34
D. spiralis							
S. pyogenes	15.0±0.2^{Bb}	16.0±0.15^{Aa}	13.0±0.09^{Db}	11.0±0.27^{Eb}	11.0±0.17^{Ea}	14.0±0.17^{Ca}	22.0±0.44
M. luteus	17.0±0.19^{Aa}	15.0± 0.16^{Cb}	16.0±0.2^{Ba}	14.0±0.25^{Da}	11.0±0.07^{Ea}	14.0±0.17^{Da}	23.0±0.32
S. flexneri	7.0±0.09^{Bd}	6.0±0.07^{Cd}	8.0±0.15^{Ac}	5.0±0.08^{De}	4.0±0.06^{Ec}	6.0±0.07^{Cc}	31.0±0.2
V. cholerae	9.0±0.11^{Ac}	7.0±0.12^{Cc}	6.0±0.09^{Dd}	8.0±0.07^{Bc}	5.0±0.08^{Eb}	7.0±0.07^{Cb}	19.0±0.15
C. albicans	4.0±0.09^{Cf}	5.0±0.05^{Be}	6.0±0.11^{Ad}	6.0±0.07^{Ad}	2.0±0.07^{De}	4.0±0.12^{Cd}	17.0±0.16
A. niger	5.0±0.07^{Be}	4.0±0.1^{Cf}	4.0±0.03^{Ce}	6.0±0.06^{Ad}	3.0±0.09^{Dd}	3.0±0.08^{De}	15.0±0.34
J. rubens							
S. pyogenes	11.0± 0.0^{Ad}	8.0±0.12^{Cd}	7.0±0.1^{Dd}	9.0±0.14^{Bc}	10.0±0.12^{Ac}	6.0±0.05^{Ed}	22.0±0.44
M. luteus	15.0± 0.09^{Aa}	13.0±0.24^{Ba}	13.0±0.15^{Ba}	12.0±0.13^{Ca}	11.0±0.07^{Db}	9.0±0.19^{Eb}	23.0±0.32
S. flexneri	11.0±0.09^{Bd}	12.0±0.14^{Ab}	9.0±0.06^{Dc}	6.0±0.12^{Ee}	10.0±0.13^{Cc}	8.0±0.09^{Ec}	31.0±0.2
V. cholerae	9.0± 0.1^{Ae}	8.0±0.16^{Bd}	6.0±0.09^{De}	7.0±0.07^{Cd}	6.0±0.07^{Dd}	0.0±0.0^{Ee}	19.0±0.15
C. albicans	13.0±0.11^{Ac}	11.0±0.07^{Cc}	12.0 ±0.15^{Bb}	10.0±0.16^{Db}	13.0±0.2^{Aa}	10.0±0.16^{Da}	17.0±0.16
A. niger	14.0±0.12^{Ab}	13.0±0.08^{Ba}	9.0±0.07^{Ec}	10.0±0.22^{Cb}	13.0±0.15^{Ba}	9.0±0.05^{Db}	15.0±0.34

[a] Figures sharing same lowercase letter (column) and capital letter (row) are not significantly different at *P*= 0.05 probability by Fisher's PLSD test. $LSD_{0.05}$ Solvent and isolate: 0.664, 0.664 and 0.655 for *U. lactuca, D. spiralis* and *J. rubens* respectively.

positive bacteria (17 mm) was recorded for methanol *D. spiralis* extract; followed by chloroform *D. spiralis* (16 mm). Whereas, for fungi the highest *ZIs* value was found to be 14 mm with methanol *J. rubens* extract.

Estimated Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

In the current investigation, *MIC* values as useful parameters have been estimated in order to screen algal inhibitory effects (Table 3). As mentioned in Table 3, analysis of variance revealed that the effect of different solvents on *MIC* was significantly different (P≤ 0.001) for the three algae species. In this respect, the lowest *MIC* values were recorded in the case of *D. spiralis* (Table 3). In this regards, *MIC* varied between 26.7 µg mL^{-1} (methanol against *S. pyogenes* and with chloroform against *M. luteus*) and > 10 mg mL^{-1} (hexane against *M. luteus, S. flexneri* and *V. cholerae*). Whereas, for fungi this value varied between 106 µg mL^{-1} (methanol against the both fungal strains and with acetone and hexane against *C. albicans*) with *U. lactuca* extract and 10 mg mL^{-1} against the both fungal strains with hexane *J. rubens* extract.

Moreover, algal extracts efficiency in killing the pathogens has been screened by *MBC* estimation (Table 4). As presented in Table 4, the effect of solvents was significantly different (P≤ 0.001) for *D. spiralis* algae species on *MBC* values. In this regards, the highest antimicrobial activity was recorded against *M. luteus* isolate (53.3 µg mL^{-1}) with chloroform *D. spiralis* extract. Whereas, *U. lactuca* extracts showed no activity against all tested pathogens regardless of the examined solvents. While, *J. rubens* extracts were less potent in killing the examined pathogens compared to *D. spiralis* extract (Table 4). As for fungi, algal extracts exhibited different

Table 3. Algal Minimum Inhibitory Concentration (MIC) values using different examined solvents. [a]

Minimum Inhibitory Concentration (MIC)							
Microorganisms	ethanol	Ethanol	Chloroform	Acetone	Ethyl acetate	Hexane	Control
U. lactuca			(µg mL^{-1})				
S. pyogenes	213.3^{Ca}	320.0^{Aa}	266.7^{Ba}	320.0^{Aa}	266.7^{Ba}	160.0^{Da}	5.0
M. luteus	106.7^{Bb}	213.3^{Ac}	133.3^{Bc}	186.7^{Ab}	213.3^{Ab}	133.3^{Ba}	4.0
S. flexneri	80.0^{Bb}	186.7^{Ac}	106.7^{Bc}	133.3^{Bc}	213.3^{Ab}	106.7^{Bc}	0.4
V. cholerae	106.7^{Cb}	213.3^{Bc}	268.0^{Aa}	213.3^{Bb}	213.3^{Bb}	133.3^{Ca}	0.8
C. albicans	106.7^{Bb}	266.7^{Ab}	133.3^{Bc}	106.7^{Bc}	266.7^{Aa}	106.7^{Bc}	2.0
A. niger	106.7^{Cb}	266.7^{Ab}	213.3^{Bb}	213.3^{Bb}	266.7^{Aa}	133.3^{Ca}	2.0
D. spiralis			(µg mL^{-1})				
S. pyogenes	26.7^{Be}	33.3^{Bd}	40.0^{Bc}	60.0^{Bc}	160.0^{Ad}	40.0^{Bc}	5.0
M. luteus	33.3^{Ae}	46.7^{Ad}	26.7^{Bc}	33.3^{Ac}	66.7^{Ae}	33.3^{Ac}	4.0
S. flexneri	133.3^{Bc}	133.3^{Bc}	133.3^{Bb}	133.3^{Bb}	213.3^{Ac}	133.3^{Bb}	0.4
V. cholerae	80.0^{Cd}	160.0^{Bc}	133.3^{Bb}	133.3^{Bb}	266.7^{Ab}	160.0^{Bb}	0.8
C. albicans	266.7^{Ba}	266.7^{Ba}	213.3^{Ca}	266.7^{Ba}	320.0^{Aa}	266.7^{Ba}	2.0
A. niger	213.3^{Cb}	213.3^{Cb}	213.3^{Ca}	266.7^{Ba}	320.0^{Aa}	266.7^{Ba}	2.0
J. rubens			(mg mL^{-1})				
S. pyogenes	2.1^{Bc}	2.5^{Bc}	3.3^{Bc}	2.9^{Bd}	2.1^{Bc}	7.5^{Ab}	5.0
M. luteus	4.2^{Bb}	5.0^{Bb}	5.8^{Ba}	10.0^{Aa}	3.3^{Cb}	>10.0^{Aa}	4.0
S. flexneri	4.2^{Cb}	5.8^{Cb}	5.8^{Ca}	8.3^{Bb}	3.3^{Db}	>10.0^{Aa}	0.4
V. cholerae	6.7^{Ba}	8.3^{Ba}	6.7^{Ba}	10.0^{Aa}	4.2^{Ca}	>10.0^{Aa}	0.8
C. albicans	3.3^{Bb}	5.0^{Bb}	5.0^{Bb}	4.2^{Bc}	3.3^{Bb}	10.0^{Aa}	2.0
A. niger	3.3^{Cb}	4.2^{Bb}	4.2^{Bb}	5.8^{Bc}	5.4^{Ba}	10.0^{Aa}	2.0

[a] Figures sharing same lowercase letter (column) and capital letter (row) are not significantly different at *P*= 0.05 probability by Fisher's PLSD test. $LSD_{0.05}$ Solvent and isolate: 48.257, 38.683 and 1.553 for *U. lactuca, D. spiralis* and *J. rubens* respectively.

mortality degrees (Table 4). In this respect, *MBC* values recorded for *D. spiralis* ranged between 266 µg mL^{-1} (Chloroform against both fungal strains) – 320 µg mL^{-1} (for the five other solvents against the both fungal strains). Whereas, for *J. rubens* this value ranged between 4.2 mg mL^{-1} (methanol against *A. niger*) - 10 mg mL^{-1} (hexane against the both fungal strains) (Table 4).

DISCCUSION

In the current investigation, algal qualitative phytochemical assay indicated a variance in presenting chemical components, according to algae species and tested solvents. In this regards, alkaloids, flavonoids, tannins, carbohydrates and phenols were presented with absence of steroids in methanol *U. lactuca*, *D. spiralis* and *J. rubens* extracts. Manchu *et al*. (2014) reported the presence of coumarins in aqueous *U. lactuca* extract; carbohydrates, steroids, proteins, terpenoids and phytosterols with saponins and flavonoids absence in chloroform *U. lactuca* extract. Whereas, flavonoids, glycosides, steroids, terpenoids and phytosterols were presented in ethanolic *U. lactuca* extract. While, in acetonic *U. lactuca* extract, flavonoids, glycosides, quinones, coumarins and steroids were presented. However, Chandrasekaran *et al*. (2014a) reported that the ethyl acetate extract among different algal (green, red and brown) extracts, had the strongest bioactive compounds, including terpendois, tannins and phenolic components compared to the other examined solvents.

Karabay-Yavasoglu *et al*. (2007) reported the antimicrobial activity of *J. rubens* (1, 2 and 4 mg disc^{-1}) against 5 Gram-positive, 4 Gram-negative bacterial and *C. albicans* fungal strains. The previous study revealed that methanol and chloroform extracts (4 mg

Table 4. Algal Minimum Bactericidal Concentration (MBC) values using different examined solvents.[a]

Minimum Bactericidal Concentration (MBC)							
Microorganisms	Methanol	Ethanol	Chloroform	Acetone	Ethyl acetate	Hexane	Control
U. lactuca			(µg mL^{-1})				
S. pyogenes	NA[b]	NA	NA	NA	NA	NA	10.0
M. luteus	NA	NA	NA	NA	NA	NA	8.0
S. flexneri	NA	NA	NA	NA	NA	NA	0.8
V. cholerae	NA	NA	NA	NA	NA	NA	1.5
C. albicans	NA	NA	NA	NA	NA	NA	4.0
A. niger	NA	NA	NA	NA	NA	NA	4.0
D. spiralis			(µg mL^{-1})				
S. pyogenes	66.7^{Bd}	80.0^{Bc}	106.7^{Bd}	93.3^{Bc}	213.3^{Ac}	66.7^{Bc}	10.0
M. luteus	66.7^{Bd}	66.7^{Bc}	53.3^{Be}	66.7^{Bc}	106.7^{Ad}	66.7^{Bc}	8.0
S. flexneri	186.7^{Bb}	186.7^{Bb}	213.3^{Bc}	213.3^{Bb}	266.7^{Ab}	213.3^{Bb}	0.8
V. cholerae	133.3^{Bc}	213.3^{Bb}	160.0^{Bb}	186.7^{Bb}	320.0^{Aa}	213.3^{Bb}	1.5
C. albicans	320.0^{Aa}	320.0^{Aa}	266.7^{Ba}	320.0^{Aa}	320.0^{Aa}	320.0^{Aa}	4.0
A. niger	320.0^{Aa}	320.0^{Aa}	266.7^{Ba}	320.0^{Aa}	320.0^{Aa}	320.0^{Aa}	4.0
J. rubens			(mg mL^{-1})				
S. pyogenes	3.8^{Bb}	4.2^{Bc}	4.2^{Bb}	4.2^{Bc}	4.2^{Bb}	10.0^{Aa}	10.0
M. luteus	5.0^{Bb}	6.7^{Bb}	6.7^{Ba}	10.0^{Aa}	6.7^{Ba}	>10.0^{Aa}	8.0
S. flexneri	5.0^{Bb}	6.7^{Bb}	8.3^{Ba}	10.0^{Aa}	6.7^{Ba}	>10.0^{Aa}	0.8
V. cholerae	8.3^{Ba}	10.0^{Aa}	8.3^{Ba}	10.0^{Aa}	6.7^{Ba}	>10.0^{Aa}	1.5
C. albicans	5.0^{Bb}	6.7^{Bb}	6.7^{Ba}	6.7^{Bb}	6.7^{Ba}	10.0^{Aa}	4.0
A. niger	4.2^{Cb}	6.7^{Bb}	6.7^{Ba}	8.3^{Bb}	6.7^{Ba}	10.0^{Aa}	4.0

[a] Figures sharing same lowercase letter (column) and capital letter (row) are not significantly different at *P*= 0.05 probability by Fisher's PLSD test. $LSD_{0.05}$ solvent and isolate 40.762 for *D. spiralis*; 1.667 and 1.760 for solvent and isolatein the case of *J. rubens,* respectively. [b] No Activity

disc^{-1}) were the most active extracts compared to the other tested extracts. In this respect, *ZIs* values ranged between 11-21 mm for Gram-positive and between 8-13 mm for Gram-negative bacteria with methanolic extract. Whereas, no inhibitory effect was observed against the *C. albicans* fungal isolate regardless of concentration or tested extracts.

Whereas, Zbakh *et al*. (2012) investigated the antibacterial activity of methanolic extracts of 20 species of marine benthic algae collected from the Mediterranean Moroccan coasts, against 3 bacterial isolates. The previous study showed that Rhodophyceae out to examine algae had remarkable inhibitory effects with *ZIs* value ranging between 20-24 mm. Moreover, *U. rigida* green algae showed activity against *Enterococcus faecalis* with *ZIs* of 15 mm. Alghazeer *et al*. (2013) reported the methanolic and aqueous extracts of 19 marine algal species collected along the western coast of Libya against 4 Gram-positive and 4 Gram-negative bacteria. *Cystoseira crinite* (Phaeophyceae) of the 19 examined algae, was the most potent against tested isolates. The previous study revealed that the observed *ZIs* values with methanolic extract of *U. lactuca* ranged between 11 mm (*S. aureus* and *Bacillus subtilis*) and 14 mm (*P. aeruginosa* and *Klebsiella* spp). Whereas, in *J. rubens* extracts these values ranged between 11 mm (*S. aureus, B. subtilis* and *P. aeruginosa*) and 13 mm (*S. typhi*). The previous study showed that the *Cauler paracemosa* (Chlorophyceae) methanol extract had the highest *ZIs* (16 mm) against both the *Klebsiella* spp., and *S. typhi*. Whereas, Oumaskour *et al.* (2013) investigated the antimicrobial activity of 23 red marine algae collected from the Atlantic

coast of Morocco, against 10 Gram-positive and 2 Gram-negative bacterial and 3 fungal isolates using 6 solvents and water extracts. The previous study showed that the highest *ZIs* value was recorded with methanol and methanol–dichloromethane (50:50) extracts. Indeed, the same study revealed that *S. aureus* ssp. *aureus* was the most sensitive isolate.

Recently, Abo-State *et al*. (2015) studied the antimicrobial effects of hexane, chloroform, ethyl acetate, ethanol (70%) and water extracts of seven cyanobacteria species collected from Egypt against 8 bacterial, 2 fungal and 3 yeast pathogens. The previous study showed that *ZIs* ranged between 11-30 mm. In this respect, the highest observed antibacterial activity was recorded with chloroformic extract of *Anabaena flosaquae* against *K. pneumonia* isolate. Indeed, hexane and water extracts showed no inhibitory effects. Whereas, for fungi, these values recorded to be 11 mm against *Aspergillus terreus.*There was no effect observed against *Tirchoderma viride*. As for yeast, these values ranged between 11-13.5 mm. Whereas, Kausalya and Rao (2015) investigated the antimicrobial activity of *Sargassum polycystum* and *S. tenerrimum* species against12 bacterial and 6 fungal isolates. The previous study revealed that the highest *ZIs* was recorded to be 19 mm for methanolic extract of *S. polycystum* against both *A. niger* and *Rhizopus stolonifer* and also with ethanol extract against *R. stolonifer.* As for *S. tenerrimum* this value was recorded to be 10 mm with ethanol extract against *A. niger, Mucor racemosus* and *R. stolonifer* pathogens.

In our case study, all over, the estimated *ZIs* for tested algae against bacterial isolates varied between 6-11 mm for *U. lactuca* (green); between 4-17 mm for *D. spiralis* (brown) and between 6-15 mm for *J. rubens* (red); with no inhibitory effect against *V. cholera* isolate. This observation stated that the lowest inhibition was recorded for *U. lactuca* (Chlorophyta) compared with the two other tested algae members (Phaeophyta and Rhodophyta) with all tested solvents. This observation could be related to the abaundance of phenols components in Phaeophyta extracts making them more potent against tested isolates compared to the Chlorophyta. On the other hand, this phenomenon could be related to the occurrence of other bioactive components which were not analyzed in the current investigation. However, Elnabris *et al*. (2013) reported the antibacterial activity of methanolic extracts of 4 algae (*U. lactuca* and *Enteromorpha compressa*) (Chlorophyta), *Padina pavonica* (Phaeophyta) and *J. rubens* (Rhodophyta) against 4 gram-negative and 2 Gram positive isolates. The previous study showed that *U. lactuca* extract exhibited the most inhibitory effect with *ZIs* of 9.8, 9.3, 5.8, 4.8 and 3.3 mm for *K. pneumonia*, *S. aureus*, *P. vulgaris*, *B. subtilis* and *P. aeruginosa*, respectively; followed by *E. compressa.* Nonetheless, methanol *U. lactuca* was inactive against *E. coli*. Whereas, *P. pavonica* and *J. rubens* extracts showed the lowest antibacterial activity. Moreover, the same investigation revealed that the highest activity of *P. pavonica* extract was found against *K. pneumoniae*, while that of *J. rubens* was against *S. aureus*, with *ZIs* of 6.6 and 2.3 mm, respectively. These observed differences in algal biological activity in the current work compared to other investigations could be related to a potential activity of the given algae related to their bioactive compounds that act as a secondary metabolites.

Overall, chloroform extract of the three examined algae showed the highest antibacterial activity compared tothe other examined solvents. Other investigations, however, reported that the methanol extract was the most potent against all tested pathogens (Lavanya and Veerappan, 2011; Elnabris *et al*., 2013). Recently, Hamza *et al*. (2015) reported the antibacterial activity of methanol/methylene chloride *U. lactuca, Codiumto mentosum* and *Hypnea musciformis* collected from the Suez Canal, Egypt. The latest study showed that *U. lactuca* showed inhibitory activity only

against *S. typhimurium*, *K. pneumonia*, *E. coli*, *Shigella boydii* and *S. aureus* with *ZIs* ranging between 6-9 mm. Evaluation of algal antimicrobial effect has also been performed based on *MIC* and *MBC* valuesestimation. In this regards, estimated *MIC* values ranged between 26.7 µg mL^{-1}- >10 mg mL^{-1} for bacteria. Whereas, it varied between 106 µg mL^{-1}–10 mg mL^{-1} for fungal isolates. As for *MBC* values, they varied between 53.3 µg mL^{-1}-> 10 mg mL^{-1} for bacterial and between 266.7 µg mL^{-1}–10 mg mL^{-1} for fungal isolates.

Alghazeer *et al*. (2013) reported that *MIC* of the methanol and aqueous extracts for *Bacillus* spp., were 50 and 200 mg mL^{-1}(*C. racemosa*), 50 and 25 mg mL^{-1} (*C. crinita*), and 100 and 25 mg mL^{-1} (*G. latifolium*), respectively.

Whereas, Chandrasekaran *et al*. (2014a) reported the inhibitory effect of *U. lactuca* (125, 250 and 500 µg $disc^{-1}$) against *E. faecalis* bacteria using methanol, ethanol, hexane, chloroform and ethyl acetate solvents. The previous investigation showed that *MIC* values ranged between 250 µg mL^{-1} with ethyl acetate and 500 µg mL^{-1} for the other tested extracts. While, *MBC* were varied between 500 µg mL^{-1} with ethyl acetate and 1,000 µg mL^{-1} for the other tested extracts. Moreover, Chandrasekaran *et al*. (2014b) studied *S. wightii* antibacterial activity against 10 bacterial isolates. The latest study revealed that *MIC* ranged between 250 µgmL^{-1} (chloroform and ethyl acetate) and 500 µg mL^{-1} (hexane, methanol and acetone) against *S. pyogenes*isolate. While, *MBC* were varied between 500 µg mL^{-1} (ethyl acetate) and 1,000 µg mL^{-1} (chloroform, hexane, methanol and acetone).

Based upon data presented in the current study, *D. spiralis* (Phaeophyta) brown algae was the most active against examined pathogens. Other investigations revealed that the *Cystoseira crinita* a brown algae displayed the strongest inhibitory effects against all tested pathogens (Alghazeer *et al*., 2013).

We could suppose that the chloroform *D. spiralis* extract slightly stimulates free radicals induction (hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals) compared to the other tested solvents. These free radicals play an important role in cytoplasmic membrane, proteins and DNA pathogen destruction. However, their solubility in water makes it more potent not only against pathogens, but also against cancer cells (Jeeva *et al*., 2012). These phenomena could explain by the fact that in water extract, antioxidants appeared with no free radicals (Cushnie and Lamb, 2005; De Sousa *et al*., 2007).

CONCLUSIONS

Overall, the antimicrobial algal effectiveness could be classified in the following order: *D. spiralis*> *J. rubens*> *U. lactuca.* ofalgal extracts, aqueous extracts showed no inhibitory effect against all examined pathogens regardless of the studied algae. Otherwise, *D. spiralis* was the most potent by showing the highest *ZIs* and lowest *MIC* and *MBC* values compared to the other two tested algae. Future researches in *D. spiralis* are needed to determine their fractions and investigate their biological activity separately.

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فعالیت ضد میکروبی عصاره جلبک دریایی در برابر پاتوژن های انتخاب شده

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چکیده

اثر بازدارندگی گونه های *Ulvalactuca* (Chlorophyta), *Dilophusspiralis* (Phaeophyta) و *Janiarubens*(Rhodophyta) marine algae بر ۲ باکتری گرم مثبت *Streptococcus pyogenes* و *Micrococcus luteus* ، ۲ باکتری گرم منفی *Shigella flexneri*و *Vibrio cholera* و ۲ جدایه قارچ *Candida albicans* و *Aspergillus niger* با استفاده از عصاره های آبی و شش عصاره آلی (متانول، اتانول، کلروفرم، استون، اتیل استات و هگزان) بررسی شد. داده ها نشان داد که *M. luteusbacteria* با نشان دادن بالاترین نقطه بازدارندگی (ZIs) ۱۷mm با کمترین حداقل غلظت مهاری (MIC) of 26.7 µgmL-1 و کمترین حداقل غلظت باکتریایی (MBC) of 53.3 µg mL-1 با عصاره کلروفرم *D. spiralis* بیشترین حساسیت را دارا می باشد. اگرچه عصاره های آبی، صرف نظراز گونه های جلبک مورد بررسی، در برابر تمام پاتوژن های انتخاب شده فعال نبودند. بر اساس داده های ارائه شده، عصاره کلروفرم *D. spiralis* بیشترین فعالیت را در برابر پاتوژن های مورد بررسی داشت. از همین رو، به دلیل اثربخشی بالای *D. spiralis* به عنوان عامل ضد میکروبی ارزان، تحقیقات بیشتری مورد نیاز می باشد.