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Antibacterial activity of four species of Algerian algae

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ABSTRACT

Antibiotic resistance in Bacteria is one of the emerging health related problem in the world nowadays. Plants and among them algae are valuable natural sources effective against infectious agents. Methanolic extracts of four marine algae of Algeria coast were investigated for antibacterial activity against six pathogenic bacteria (*Bacillus subtilis, Listeria innocua, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*). Susceptibility assays using disc diffusion and broth microdilution test for the determination of minimum inhibitory concentration (MIC) were employed to asses the antibacterial activity of methanolic extracts of algae. All algae extracts showed antibacterial activity against four of the six pathogenic bacteria tested with MIC values ranged between 0.25-3mg/ml. Extract of *Rhodomela confervoïdes* exhibited the highest activity against *Bacillus subtilis* (24 mm). *Cystoseira tamariscifolia* exhibited the highest activity against *Listeria innocua* (19.67mm)

KEYWORDS

Algae;
Polyphenols;
Active metabolites;
Antibacterial activity.

INTRODUCTION

Marine organisms, as algae, are a rich source of biologically active metabolites[2,4]. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry[13]. The cell extracts, and active constituents of various algae, have been shown to have antibacterial activity *in vitro* against Gram positive and Gram negative bacteria[12]

In this investigation antibacterial activity of four marine algae of Algeria coast belonging to families such as Rhodophyceae (*Rhodomela confervoïdes*), Chlorophyceae (*Ulva lactuca*), Phaeophyceae (*Cystoseira tamariscifolia* and *Padina pavonica*) was studied against pathogenic bacteria (*Bacillus subtilis ATTCC 6633, Listeria innocua CLIP 74915, Staphylococcus aureus ATCC 6538, Escherichia coli NAR, Klebsiella pneumonia E47, Pseudomonas aeruginosa ATCC27853*.

Tuney and al.[12] and Bansamir and al.[1] have shown that cell extracts and active constituents of various algae have antibacterial activity against Gram positive and Gram negative bacteria

MATERIALS AND METHODS

Algae materials

Marine algae were hand collected from the submerged marine rocks of Bejaia coats in north Algeria

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during March 2009. Seaweeds were identified by from botanic laboratory (University of Béjaia). Epiphytes and sediment were removed by washing first in sea water and then in fresh water. The algae were transported to the laboratory in polyethylen bags at ice temperature.

Preparation of methanolic extract of algae

The samples of algae were dried at room temperature, powdered, and sieved. The powder was dissolved with methanol (1/10 w:v) and kept at room temperature, under agitation for overnight. The solution was centrifuged at 2220g for 10 minutes (sigma). The supernatant, which contains polyphenol extract was recovered. The residue was dissolved twice in methanol (1/10 w:v). The supernatants were recovered and filtered through 4 layered cheese cloths and concentrated in kika labortechnik rotavapor. The dried sample was dissolved in methanol and stored at 4°C until further use[3]

Tested microorganisms

Tested microorganisms were obtained from applied microbiology laboratory (university of Bejaia). Six human pathogenic microorganisms, such as : *Bacillus subtilis* (ATCC 6633), *Listeria innocua* (CLIP 74915), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (N.A.R), *Klebsiella pneumonia* (E 47), *Pseudomonas aeruginosa* (ATCC 27853) were used in the present study for evaluation of the antimicrobial activity of algae extracts.

The pathogenic bacteria were cultured individually on selective broth at 37°C for 18h, before inoculation for assay[5]. 100µl of broth culture, which contain 10^8UFC/ml were used for inoculate Muller-Hinton agar medium[7,8]

Antibacterial assays

Antibacterial activity was evaluated by agar diffusion method[10]. Spots which contain 25µl of crude extract of algae were applied on Muller-Hinton agar medium, sowed with bacteria (10^8UFC)[6].

Inhibition zones, around spots, were evaluated after incubation during 24h at 37°C[6]. The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the algae extract required for complete inhibition of growth of the bacteria being tested after incubation at 37°C for 18h or 24h. All the essays were carried out in triplicate. Methanol (50%) without algal extract was used as a negative control and in this case, no antibacterial activity was observed.

RESULTS

Antibacterial activity

The values of Diameters of microbial inhibition zones (mm) recorded with the different extracts are reported

TABLE 1 : Antibacterial activity of methanolic extracts of Algerian algae against pathogenic bacteria strains

Marine algae	Dilution (mg/25µl)	Microbial inhibition zone diameter (mm)					
		B. subtilis	*L. innocua*	*S. aureus*	*E. coli*	*K.pneumoniae*	*p.aeruginosa*
Cystoseira tamariscifolia	1,25	00,00±00,00^{a}	19,67±00,57^{jkl}	18,33±00,57^{jk}	18,00±00,00^{mn}	15,67±00,57^{fg}	18,00±00,00^{lmn}
	0,625	00,00±00,00^{a}	17,66±00,57^{gh}	15,67±00,57^{ef}	17,00±00,00^{jk}	17,00±00,00^{hij}	17,66±00,57^{kl}
	0,312	00,00±00,00^{a}	16,67±00,57^{f}	15,33±00,57^{de}	16,00±00,00^{h}	17,00±00,00^{hij}	16,33±00,57^{jk}
	0,156	00,00±00,00^{a}	17,00±00,00^{fg}	14,67±00,57^{d}	18,00±00,00^{mn}	19,33±00,57^{k}	15,67±00,57^{ghi}
Padina pavonica	1,25	19,33±00,57^{f}	13,00±00,00^{c}	13,33±00,57^{c}	12,33±00,57^{e}	00,00±00,00^{a}	12,33±00,57^{b}
	0,625	09,33±00,57^{b}	00,00±00,00^{a}	16,33±00,57^{fg}	16,33±00,57^{hi}	00,00±00,00^{a}	15,33±00,57f^{gh}
	0,312	00,00±00,00^{a}	00,00±00,00^{a}	16,66±00,57^{gh}	14,00±00,00^{f}	00,00±00,00^{a}	16,33±00,57^{ij}
	0,156	00,00±00,00^{a}	00,00±00,00^{a}	12,66±00,57^{c}	16,00±00,00^{h}	00,00±00,00^{a}	13,33±00,57^{b}
Rhodomela confervoides	1,25	22,67±00,57^{ig}	20,33±00,57^{l}	16,33±00,57^{fg}	18,33±00,57^{no}	17,33±00,57^{ij}	16,33±00,57^{ij}
	0,625	22,33±00,57^{i}	18,00±00,00^{h}	15,66±00,57^{ef}	17,33±00,57^{kl}	16,33±00,57^{gh}	14,67±00,57^{ef}
	0,312	24,00±00,00^{kl}	18,00±00,00^{h}	11,33±00,57^{b}	16,00±00,00^{h}	16,33±00,57^{gh}	16,33±00,57^{ij}
	0,156	23,33±00,57^{gk}	19,33±00,57^{ijk}	15,67±00,57^{ef}	18,67±00,57^{o}	12,33±00,57^{d}	15,67±00,57^{ghi}
Ulva lactuca	1,25	00,00±00,00^{a}	16,33±00,57^{f}	16,00±00,00^{efg}	14,33±00,57^{f}	00,00±00,00^{a}	15,00±00,00^{efg}
	0,625	00,00±00,00^{a}	16,33±00,57^{f}	13,00±00,00^{c}	00,00±00,00^{a}	00,00±00,00^{a}	00,00±00,00^{a}
	0,312	00,00±00,00^{a}	13,00±00,00^{c}	13,00±00,00^{c}	00,00±00,00^{a}	00,00±00,00^{a}	00,00±00,00^{a}
	0,156	00,00±00,00^{a}	13,00±00,00^{c}	00,00±00,00^{a}	00,00±00,00^{a}	00,00±00,00^{a}	00,00±00,00^{a}

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on TABLE 1

Crude extracts of the four species of algae showed antibacterial activity (Figure 1). No activity was observed from four methanolic extracts : *Cystoseira tamariscifolia* against *Bacillus subtilis*, *Padina pavonica* against *Klebsiella pneumoniae*, *Ulva lactuca* against *Bacillus subtilis* and *Klebsiella pneumoniae*. The majority of algal extracts were active against four or five microorganisms.

All the extracts showed activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Listeria innocua*.

The extract of *Rhodomela confervoïdes* exhibited the highest activity against *Bacillus subtilis* (24 mm). *Cystoseira tamariscifolia* exhibited the highest activity against *Listeria innocua* (19.67mm). all the extracts exhibited a lowest activity than the polyphenol standards TABLE 2

TABLE 2 : Antibacterial activity of some polyphenol standards

Standard polyphénol	Dilution (mg/25 µl)	Microbial inhibition zone diameter (mm)					
		B. subtilis	*L. innocua*	*S. aureus*	*E. coli*	*K.pneumoniae*	*p.aeruginosa*
Cafeic acid	1,25	25,00±00,00^{mn}	24,33±00,57^{n}	37,33±00,57^{r}	24,00±00,00^{q}	23,33±00,57^{l}	23,33±00,57^{r}
	0,625	22,33±00,57^{hi}	24,67±00,57^{n}	25,67±00,57^{p}	26,33±00,57^{r}	22,67±00,57^{l}	24,33±00,57^{s}
	0,312	23,33±00,57^{gk}	18,33±00,57^{hi}	19,33±00,57^{l}	17,33±00,57^{l}	19,00±00,00^{k}	18,33±00,57^{mn}
	0,156	17,67±00,57^{de}	19,00±00,00^{ij}	19,33±00,57^{l}	16,66±00,57^{ijk}	17,33±00,57^{ij}	18,00±00,00^{lmn}
Gallic acid	1,25	20,67±00,57^{g}	28,33±00,57^{o}	27,33±00,57^{q}	20,00±00,00^{p}	19,33±00,57^{k}	18,67±00,57^{no}
	0,625	19,33±00,57^{f}	22,67±00,57^{m}	24,33±00,57^{o}	18,33±00,57^{no}	19,33±00,57^{k}	21,33±00,57^{q}
	0,312	18,33±00,57^{e}	20,33±00,57^{l}	15,33±00,57^{de}	18,33±00,57^{no}	16,67±00,57^{hi}	19,33±00,57^{op}
	0,156	15,33±00,57^{c}	19,00±00,00^{i}	17,33±00,57^{hi}	16,33±00,57^{hi}	17,33±00,57^{ij}	16,00±00,00^{hij}
T tannic acid	1,25	25,33±00,57^{n}	20,33±00,57^{b}	27,33±00,57^{q}	24,00±00,00^{q}	14,33±00,57^{e}	26,33±00,57^{t}
	0,625	24,66±00,57^{lmn}	15,33±00,57^{e}	24,33±00,57^{o}	17,67±00,57^{lm}	15,33±00,57^{f}	27,33±00,57^{u}
	0,312	21,67±00,57^{h}	18,33±00,57^{hi}	15,33±00,57^{de}	18,00±00,00^{mn}	17,67±00,57^{j}	21,33±00,57
	0,156	19,67±00,57^{fg}	15,00±00,00^{de}	17,33±00,57^{hi}	17,00±00,00^{jk}	14,33±00,57^{e}	17,67±00,57^{lmq}
Catechin	1,25	20,33±00,57^{g}	18,33±00,57^{hi}	20,33±00,57^{m}	17,00±00,00^{jk}	17,33±00,57^{ij}	17,33±00,57^{kl}
	0,625	19,33±00,57^{f}	19,33±00,57^{jk}	18,33±00,57^{jk}	09,33±00,57^{d}	16,67±00,57^{hi}	15,33±00,57^{fgh}
	0,312	18,33±0,57^{e}	20,00±00,00^{kl}	18,00±00,00^{ij}	06,33±00,57^{c}	19,33±00,57^{k}	16,67±00,57^{jk}
	0,156	18,33±00,57^{e}	19,00±00,00^{ij}	19,00±00,00^{kl}	04,00±00,00^{b}	18,67±00,57^{k}	14,33±00,57^{e}
Quercetin	1,25	17,33±00,57^{d}	14,33±00,57^{d}	17,33±00,57^{hi}	17,33±00,57^{kl}	12,00±00,00^{d}	18,33±00,57^{mn}
	0,625	20,33±00,57^{g}	20,00±00,00^{k}	22,33±00,57^{n}	15,33±00,57^{g}	17,33±00,57^{ij}	20,00±00,00^{p}
	0,312	25,00±00,00^{mn}	16,33±00,57^{fl}	19,33±00,57^{l}	18,00±00,00^{mn}	11,00±00,00^{c}	23,33±00,57^{r}
	0,156	24,33±00,57^{lm}	18,33±00,57^{hi}	19,00±00,00^{kl}	16,00±00,00^{h}	14,00±00,00^{b}	23,33±00,57^{r}

NT: Not tested

*** Methanol used as a negative control has shown no bacterial activity.**

Determination of minimum inhibitory concentration (MIC)

TABLE 3 shows the MIC results of four algal extracts against six bacteria strains

The highest MIC (l < 3mg/ml) is observed for *E.coli*, *L.innocua* and *K.pneumoniae* with *Cystoseira tamariscifolia* and *Rhodomela confervoïdes* extracts. The lowest MIC (>0.25mg/ml) is obtained for *S.aureus* with *Rhodomela confervoïdes* extract.

Among the standards tested, gallic acid and tannic acid showed the lowest MIC against *L.innocua*, *S.aureus* and *P.aeruginosa* for the first one (0.1mg/ml), and against *B.subtilis* and *P.aeruginosa* for tannic acid (TABLE 3)

DISCUSSION

The main objective of this study was to evaluate the ability of algal extracts of Bejaia coast, to inhibit the growth of pathogenic bacteria, with the aim to use them in the future as alternative to common antibiotics

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TABLE 3 : Determination of minimum inhibitory concentration of extract

Marine algae	MIC (mg/ml)					
	B.subtilis	*L.innocua*	*S.Aureus*	*E.Coli*	*K.pneumoniae*	*p.aeruginosa*
Cystoseira tamariscifolia	NT	>3	1,2	>3	>3	1,2
Padina pavonica	>3	>3	1,2	>3	NT	1,2
Rhodomela confervoides	0,5	>3	0,25	>3	>3	1,2
Ulva lactuca	NT/NT	>3	1,2	>3	NT	1,2

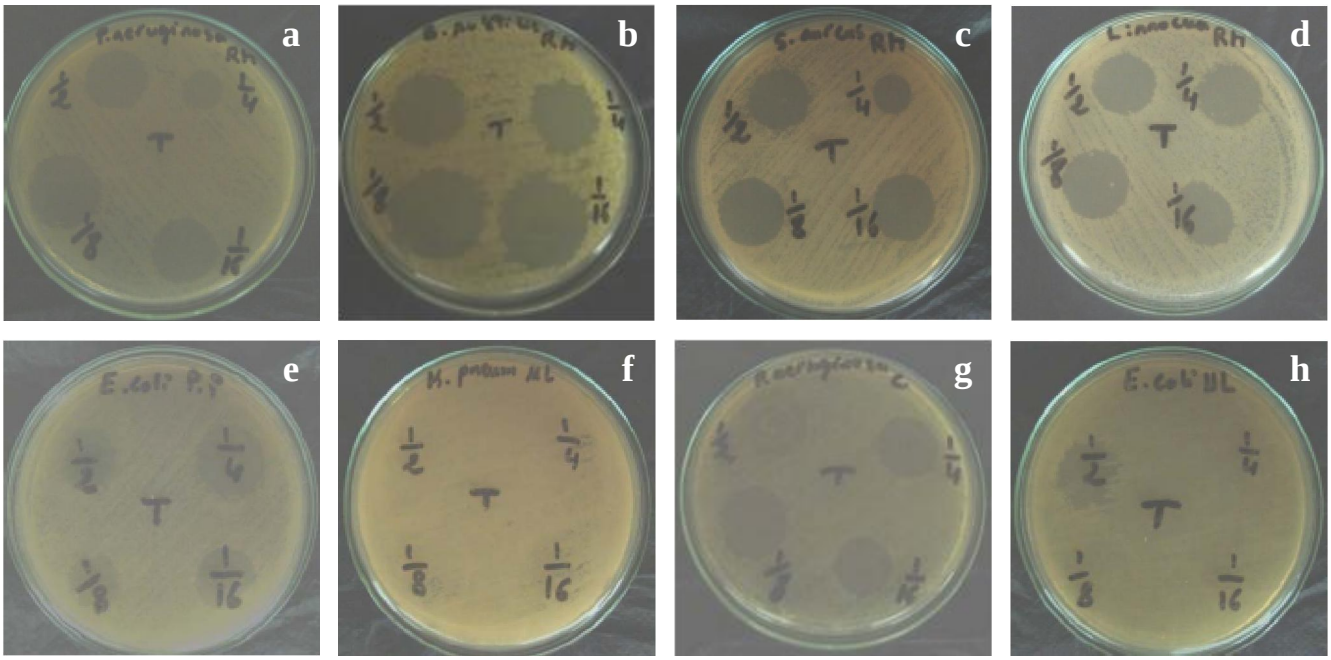

Figure 1 : Photos of some inhibition zones obtained with methanolic extracts: *Rhodomedela confervoides* vs *P. aeruginosa* (a,d), *B. subtilis* (b), *S.aureus* (c) *Ulva lactuca* vs *E. coli* (h), *K. pneumoniae* (f) *Cystoseira tamariscifolia* vs *P. aeruginosa* (g); *Padina pavonica* vs *E. coli* (e).

in human therapeutic.

Most active species were *Rhodomela confervoïdes* and *Cystoseira tamariscifolia* respectively against *B.subtilis* (inhibition zone˜22 at 23mm) and L.innocua (20mm) for *Rhodomela*, and against *L.innocua* (19.67mm) and *S.aureus* (18.33mm) for *Cystoseira*.

No activity was observed against *B.subtilis* with *Cystoseira tamariscifolia* and *Ulva lactuca* extracts. The same result was obtained with the extracts of *Padina pavonica* and *Ulva lactuca* against *K.pneumoniae*.

The high activity of seaweeds belonging to the Rhodophyceae agrees with the results of previous studies using other test microorganisms[7,8]. The result of the present study revealed that Gram positive organisms were generally more susceptible to the crude extracts of algae, which agrees with the results of others studies[11]

The more susceptibility of the Gram positive bacteria to algal extract is due to the differences in their cell wall structure and their composition[9]. In Gram negative bacteria, the presence of a thick murine layer in the cell wall prevents entry of the inhibitors.

The results obtained in this study suggest that algal extracts of Bejaia coast have a good antibacterial activity against pathogenic bacteria which makes them interesting for programs screening natural products. This ability is not restricted to one order or division within the macroalgae: all of them offer opportunities for producing new types of bioactive compounds.

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