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Full Length Research Paper

Antifungal activity of methanolic extracts of four Algerian marine algae species

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Since ancient times antimicrobial properties of seaweeds have been recognized. In this study, antifungal activity of four species of marine algae of Bejaia coast (Algeria) was explored. This activity was evaluated by agar diffusion method. The minimum inhibitory concentrations were also determined for all the strains. All the extracts used in this study exhibited antifungal activity. The highest inhibiting effect was noted for *Rhodomela confervoides* (red algae) and *Padina pavonica* (brown algae), respectively against *Candida albicans* (diameter of inhibition zone: 24 mm) and *Mucor ramaniannus* (diameter of inhibition zone: 26 mm) for the first one and *Candida albicans* (diameter of inhibition zone: 26 mm) for the second one. *Aspergillus niger* showed resistance against majority of methanolic extracts. The evaluation of minimum inhibitory concentrations showed that extracts of *Padina pavonica*, *Rhodomela confervoides* and *Ulva lactuca* were very efficient against *Mucor ramaniannus* and *Candida albicans*. These results suggest that seaweeds collected from Algerian coast present a significant capacity which makes them interesting for screening for natural products.

Key words: Marine algae, antifungal activity, methanolic extracts, natural substances.

INTRODUCTION

Since the finding of antibacterial and antifungal activities in many species of marine algae from different part of the world and the isolation of some active compounds from them (Hornsey and Hide, 1974; Reichelt and Borowitzka, 1984), marine algae have become recognized as potential sources of antibiotic substances (Rao, 1991). Algeria has a high species diversity of marine algae but there are only few reports on the screening of Algerian algae for antibacterial and antifungal activities.

This study aimed to determine the antifungal activity of methanolic extracts of four Algerian marine algae, on three strains of fungi *Aspergillus niger* (939N), *Candida albicans* (ATCC 1024) and *Mucor ramaniannus* (NRRL1829).

MATERIALS AND METHODS

Algae materials

Four species of marine algae belonging to families such as *Rodophyceae* (*Rhodomella confervoides*), *Chlorophyceae* (*Ulva lactuca*) and *Phaeophyceae* (*Cystoseira tamaricifolia* and *Padina pavonica*) were collected from Bejaia coast in March 2009. The algal samples were thoroughly washed to remove all attached materials and dried under shade.

Preparation of methanolic extract of algae

The samples of marine algae are dried at room temperature under shade and powdered. The powder was dissolved with methanol (1/10w.v) and soaked overnight. The solvent extracts were centrifuged at 2220 g for 10 min (sigma). The supernatant which contains polyphenols was recovered.

The pellet was dissolved twice in methanol (1/10 w.v). The supernatants were filtered through layered cheese cloths and concentrated in Kika labortechnik Rotavapor. The dried extract were dissolved in methanol and stored at 4°C before testing (Cho et al., 2007).

Tested microorganisms

Fungal microorganisms used in this study were obtained from applied microbiology laboratory (University of Bejaia). Three fungal species such as *A. niger* (939N), *C. albicans* (ATCC 1024) and *Mucor ramaniannus* (NRRL 1829) were cultured individually on selective broth at 28°C for 24 h, before inoculation for assay (Karabay-Yavasoglu et al., 2007).

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Table 1. Inhibition zones (expressed in mm) in presence of methanolic extracts of algae.

Marine algae	Dilution (mg/25 µl)	Diameter of inhibition zone (mm)		
		C. albicans	*A. niger*	*M. ramaniannus*
Cystoseira tamariscifolia	1.25	16.67±00.57^{cd}	08.00±00.00^{b}	21.00±00.00^{e}
	0.625	17.33±00.57^{de}	08.00±00.00^{b}	19.00±00.00^{c}
	0.312	16.33±00.57^{c}	08.00±00.00^{b}	18.00±00.00^{b}
	0.156	17.67±00.57^{ef}	08.00±00.00^{b}	19.00±00.00^{c}
Padina pavonica	1.25	26.00±00.00^{k}	18.00±00.00i	15.00±00.00^{a}
	0.625	25.00±00.00^{j}	20.00±00.00^{k}	19.00±00.00^{c}
	0.312	24.00±00.00^{i}	19.00±00.00^{j}	15.00±00.00^{a}
	0.156	25.00±00.00^{j}	20.00±00.00^{k}	22.00±00.00^{f}
Rhodomela confervoides	1.25	23.00±00.00^{h}	11.00±00.00^{d}	26.00±00.00^{j}
	0.625	24.00±00.00^{i}	11.00±00.00^{d}	25.00±00.00^{i}
	0.312	24.00±00.00^{i}	11.00±00.00^{d}	24.00±00.00^{h}
	0.156	22.00±00.00^{g}	11.00±00.00^{d}	25.00±00.00^{i}
Ulva lactuca	1.25	14.33±00.57^{b}	11.00±00.00^{d}	15.00±00.00^{a}
	0.625	12.33±00.57^{a}	15.00±00.00^{g}	19.00±00.00^{c}
	0.312	16.33±00.57^{c}	13.00±00.00^{f}	15.00±00.00^{a}
	0.156	18.33±00.57^{f}	13.00±00.00^{f}	22.00±00.00^{f}

All values are mean (n=3) (standard deviation). Means with the same letter in the same column are not significantly different ($p<0.05$).

One hundred milliliter of broth culture which contain 10^6 ufc/ml were used for the inoculation of Muller- Hinton medium (Salvador et al., 2007).

Antifungal assay

Antifungal activity was evaluated by agar diffusion method (Suay et al., 2000). The agar plates inoculated with the test microorganisms were incubated for 1 h before placing extract, following this spots of 25 ul of crude extract of algae were applied on agar medium. After incubation at 28°C±0.1 for 48 h, all plates were observed for zones of growth inhibition, and the diameters of these zones were measured in millimeters. All tests were performed on sterile conditions in duplicate and repeated three times. Nystatine was used as positive control, and methanol as negative control (Karabay-Yavasoglu et al., 2007). The minimum inhibitory concentration (MIC) was also determined for each tested microorganism. The MIC value was taken as the lowest concentration of extract which inhibited the growth of the test microorganisms after 48 h at 28°C (Patra et al., 2009).

Statistical analysis

Data were subjected to analysis of variance using the Statistica 5.5 package (StatSoft „97 edition). Where statistical differences were noted, differences among packages were determined, using the low significant difference (LSD) test. Significance was defined at $P < 0.05$.

RESULTS

Antifungal activity

The results of antifungal activity seaweeds methanolic extracts are summarized in Table 1 and Figure 1. Methanol without algae extract was used as negative control, no antifungal activity was observed in this case. Extracts of the four species of Algerian algae showed antifungal activity against every fungal strain tested in this study. Methanolic extract of *Cystoseira tamariscifolia* (Phaeophyceae) showed the lowest activity against *A. niger* (8 mm±00.00), the same strain was moderately sensitive to extract of *Rhodomela confervoides* (Rhodophyceae) (11 mm±00.00). *M. ramaniannus* was the most sensitive strain against all the extracts used in this study.

Polyphenol standards and nystatin exhibited highest activity than the majority of dilution extracts. However, no activity was observed with the catechin against *A. niger* (Table 2).

Determination of minimum inhibitory concentration

The antifungal activity of methanolic extracts of algae, assessed by the determination of the minimum inhibitory concentration (MIC) is shown in Table 3. The highest MIC was obtained with methanolic extract of cystoseirae tamarisciflora against the three strains used in this study. Extracts of *Padina pavonica*, *Rhodomela confervoides* and *Ulva lactuca* were more efficiency against the three fungi (*C. albicans, A. niger* and *M. ramanaiannus*), because MIC values, in this case, was very low compared with that of *Cystoseira tamarisciflora*, excepted in the case of *A. niger.* Nystatin used as a positive control presented the lowest MIC value against

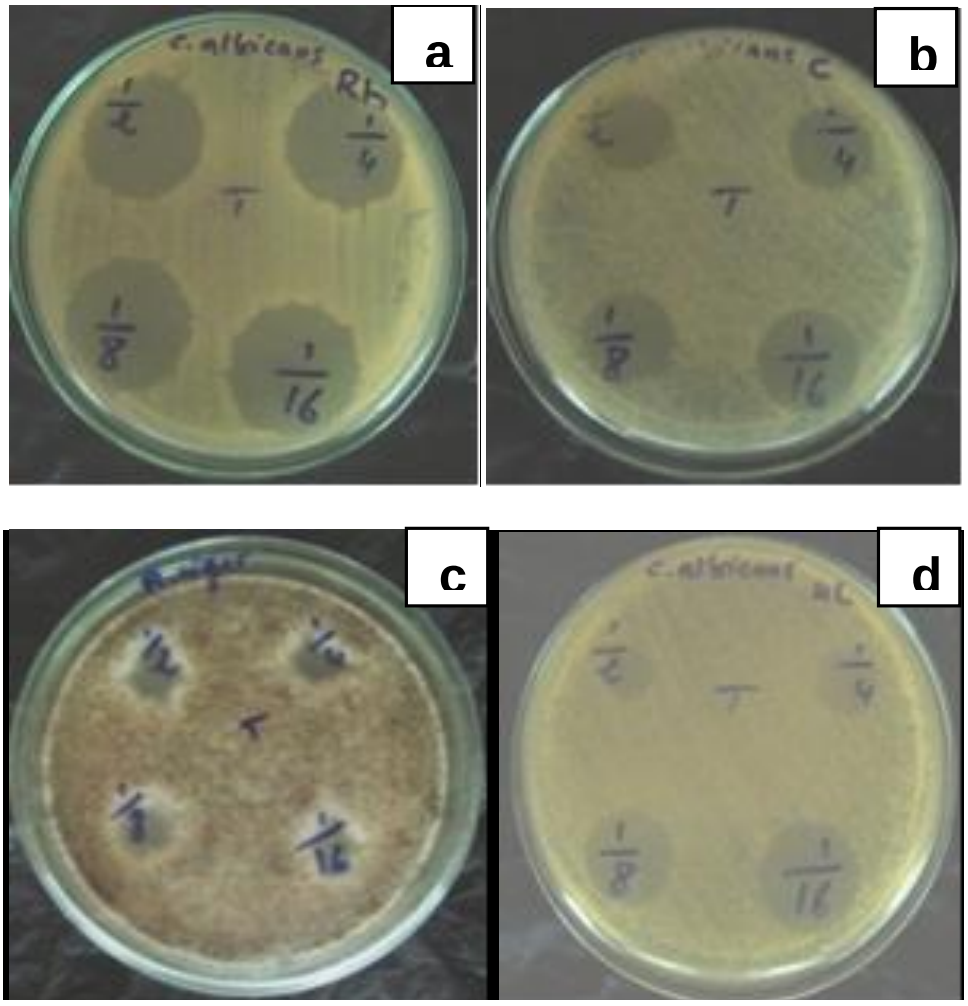

Figure 1. Antifungal activities of methanolic extracts of the four species of algae. a) Extract of *Rhodomela conforvoides* against *Candida albicans.* b) Extract of Cystoseira tamariscifolia against *Candida albicans.* c) Extract of *Padina pavonica* against *Aspergilus niger*. d) Extract of *Ulva lactuca* against *Candida albicans.*

all the strain tested in this study (Table 4). Polyphenol standards except quercetin showed low MIC values against *C. Albicans* and *M. ramaniannus* however MIC value was high concerning *A. niger* which presented resistance against all algae methanolic extracts and ploy phenol standards.

DISCUSSION

Extract of the four species of Algerian algae showed antifungal activity against every fungal strain. In the study by Gonzalez et al. (2001) no antifungal activity was found against *C. albicans* (MY 1055) *Saccharomyces cerevisae* (w303) and *Aspergillus fumigatus* (MF5667) with extracts of 40 species of marine algae; except the extract of *Cympolya barbata* (green algae) and *Asparagopsis taxiformis* (red algae) which exhibited antifungal activity against three strains cited previously. In our study, methanolic extracts of the four species of algae: *Cystoseira tamarisciflora* (brown algae), *Padina pavonica* (brown algae), *Rhodomela confervoides* (red algae) and *Ulva lactuca* (green algae) exhibited antifungal activities against the three strains used in this study. Zovko et al. (2012) obtained the same results against fungal strains with a high activity of algal extracts against *C. albicans*. Gao et al. (2011) showed that a few extracts of marine algae have not only an antifungal activity but a toxicity towards cancer cells.

In another study, the authors (Tuney et al., 2006) found that ethanolic extract of *Padina pavonica* were active against *C. albicans,* however methanolic and acetonic extracts of the same algae were inactive against *C. albicans.* In our study, methanolic extract of these algae exhibited a good activity against *C. albicans.* These data seemed to indicate that efficiency of algal extracts

Table 2. Antifungal activity of polyphenol standards and nystatin.

Phenolic standard	Dilution (mg/25 µl)	Diameter of inhibition zone (mm)		
		C. albicans	*A. niger*	*M. ramaniannus*
Caffeic acid	1.25	16.67±00.57^{cd}	12.00±00.00^{e}	29.00±00.00^{m}
	0.625	17.33±00.57^{e}	12.00±00.00^{e}	27.00±00.00^{k}
	0.312	16.33±00.57^{c}	12.00±00.00^{e}	23.00±00.00^{g}
	0.156	17.67±00.57^{ef}	12.00±00.00^{e}	23.00±00.00^{g}
Gallic acid	1.25	32.67±00.57^{op}	10.00±00.00^{c}	41.00±00.00^{s}
	0.625	30.33±00.57^{n}	10.00±00.00^{c}	40.00±00.00^{r}
	0.312	21.67±00.57^{g}	10.00±00.00^{c}	39.00±00.00^{q}
	0.156	17.33±00.57^{de}	10.00±00.00^{c}	39.00±00.00^{q}
Tannic acid	1.25	38.67±00.57^{s}	25.00±00.00^{n}	28.00±00.00^{l}
	0.625	34.33±00.57^{q}	26.00±00.00^{o}	25.00±00.00^{i}
	0.312	32.33±00.57^{o}	21.00±00.00^{l}	21.00±00.00^{e}
	0.156	30.33±00.57^{n}	20.00±00.00^{k}	21.00±00.00^{e}
Catechin	1.25	40.33±00.57^{t}	00.00±00.00^{a}	43.00±00.00^{t}
	0.625	38.33±00.57^{s}	00.00±00.00^{a}	41.00±00.00^{s}
	0.312	36.33±00.57^{r}	00.00±00.00^{a}	40.00±00.00^{r}
	0.156	33.33±00.57^{p}	00.00±00.00^{a}	39.00±00.00^{q}
Quercetin	1.25	28.67±00.57^{lm}	16.00±00.00^{h}	15.00±00.00^{a}
	0.625	29.33±00.57^{m}	16.00±00.00^{h}	18.00±00.00^{b}
	0.312	28.33±00.57^{l}	16.00±00.00^{h}	20.00±00.00^{d}
	0.156	28.33±00.57^{l}	16.00±00.00^{h}	21.00±00.00^{e}
Nystatin (ATF)	1.25	NT	NT	NT
	0.625	18.33±00.00^{f}	19.00±00.00^{j}	38.00±00.00^{p}
	0.312	21.67±00.57^{g}	20.00±00.00^{k}	36.00±00.00^{o}
	0.156	21.67±00.57^{g}	23.00±00.00^{m}	33.00±00.00^{n}

All values are mean (n=3) (standard deviation). Means with the same letter in the same column are not significantly different ($p<0.05$).

Table 3. MIC values (mg /ml) of the four marine algae against fungal strains.

Marine algae	MIC (mg/ml)		
	C. albicans	*A. niger*	*M. ramaniannus*
Cystoseira tamariscifolia	2.2	2.8	1.8
Padina pavonica	0.6	1.2	0.1
Rhodomela confervoides	0.1	1.2	0.1
Ulva lactuca	0.6	1.2	0.1

against microorganisms is influenced by factors such as location and seasonality (Febles et al., 1995). The differences between our results and the others may be due to several factors, for example the inter specific variability in the production of secondary metabolites (Febles et al., 1995) which may be related to seasonal variations (Moreau et al., 1988; Itoh and Shinya, 1994; laturnus et al., 1996). These differences may be also due to extraction protocols to recover the active metabolites and the assay methods. (Karthikaidevi et al., 2009)

Antifungal activity of seaweeds also varied with the species from different division. Several authors showed

Table 4. Estimation of MIC values of polyphenol standards and nystatin (mg/ml).

Phenolic standard	MIC (mg/ml)		
	C. albicans	*A. niger*	*M. ramaniannus*
Caffeic acid	0.6	1.2	0.1
Gallic acid	0.6	>3	0.4
Tanic acid	0.1	1.8	0.1
Catechin	0.8	NT	0.6
Quercetin	>3	>3	1.2
Nystatin	<0.1	<0.1	<0.1

that *Rhodophylea* (red algae) showed highest antimicrobial activity than *Phaephila* (brown algae). Effectively, our results confirm this observation: *Rhodomela confervoides* (red algae) exhibited a strongest activity against *C. albicans* and *M. ramaniannus* than *Cystoseira tamariscifolia* (brown algae) (Padmakumar and Ayyakkannu, 1997).

Conclusion

Methanolic extracts of the four species of algae used in this study exhibited activities against *C. albicans, A. niger* and *M. ramaniannus*. *Calbicans* and *M. ramaniannus* were more sensitive against all the extracts than *A. niger.* The active compounds in the species that showed strong antifungal activity in our study remain to be identified.

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