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Antimicrobial activity of aqueous and ethanol extracts of two marine algae collected from Algerian west coast

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Abstract

From medicinal point of view, marine environment is a diversified source of several biologically active compounds that are relatively untapped. Exploitation of marine resources may provide valuable leads which carry economic and scientific potential. Therefore, the present study was conducted to evaluate the antimicrobial activity of for marine algae harvested from Algerian west coast extracts in ethanol and water. Antimicrobial activities of the extracts were assessed against Gram (+) bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram (–) bacteria (*E. coli*, *pseudomonas aeruginosa*) and two fungal strain *Fusarium oxysporum f.* sp. *Albedinis* and *Pénicillium* sp by the disk diffusion method. According to the results, the highest values of performance in crude extract are those for ethanol in both species *Stypocaulon scoparium* and *Halopitys incurvus* with percentages of 5.6% et.4% respectively. The ethanolic extract of *Stypocaulon scoparium* (brown seaweed) elicited remarkable antibacterial activity against all pathogenic bacteria screened, followed by *Halopitys incurvus* (red seaweed). All the aqueous extracts used in this study were exhibited significant antifungal activity.

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Introduction

Algae's are very large and important aquatic diverse groups found in marine and fresh water. Algae are divided into three classes, chlorophyceae (green algae), phaeophyceae (brown algae) and Rhodophyceae (red algae) (Gamal, 2010).

In recent years there has been an increase of the resistance of microorganisms to antibiotics that are usually used in the treatment of some diseases. To overcome this problem, new therapeutic drugs from natural products have been explored (Sasidharan *et al.*, 2010).

Thus, marine organisms appear as an efficient alternative source of new drugs and algae have been extensively documented for their capacity to provide a rich source of primary and secondary metabolites (Turney *et al.,* 2006).

Actually, there are several substances obtained from algae that are already in use in traditional medicine for a long time (Taskin *et al.* 2007).

Although there are numerous studies published on antimicrobial activity of algae extracts, but are very limited that report results of algae collected from the west coast of Algeria.

Based on the above rationale this research study's objectives were: (i) to establish an improved procedure to obtain extracts from seaweeds and (ii) to test the antimicrobial activity of the prepared extracts on Gram positive and Gram negative bacteria, as well as on a two fungal strain. It is important to notice that extracts were prepared from 2 species of seaweed collected from Algerian west coast.

Materials and methods

Collection of seaweeds Samples

The seaweeds are collected from Ain Franin coastal area in South Oran, Algéria during January 2016.The identification of the investigated marine algae was kindly verified by F.A.O the species identification guide for fishery purposes.

Once harvested, marine algae were stored in plastic bags and placed on ice for transport to the laboratory. They were cleaned from epiphytes, extraneous matters and necrotic were removed. These seaweeds samples were collected in sterilized polyethylene bags. After that the samples were washed thoroughly with seawater then sterile double distilled water, air dried, cut into small pieces and then ground in a tissue grinder until reach fine powder form and stored in airtight bottles **(**Guiry *et al.*, 2011**).**

Preparation of algae extracts

Seaweed samples were pulverized and 5 g of each were sequentially extracted as reported by Bouhlal and *al.*, (2010) in 100 mL ethanol in a Soxhlet apparatus for about 8 hours using filter paper for the cartridge. Final extracts were evaporated under reduced pressure with a roto-evaporator of type HAHNVAPOR HS-2005V-N.

Antibacterial activity determination

To evaluate the antimicrobial activity of the seaweed extracts, the following microorganisms were tested: Gram negative- *Escherichia coli* (ATCC 8739) *Pseudomonas aeruginosa* (ATTC 10145); Gram positive-*Basillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), and the fungal strains- *Fusarium oxysporum f.* sp. *Albedinis* and *Pénicillium*. Bacterial and fungal strains were obtained from Environmental Monitoring Network, Department of Biology, Faculty of Science, University of Oran, Algeria. All bacteria were cultivated and stored in Nutrient Agar (NA) and for the fungal strains that used either Sabouraud Broth or Agar at 4°C.

The Muller-Hinton agar medium was used for antibacterial assay. The agar diffusion method was used to assess the antimicrobial activity of the extracts:

The inoculation of all agar plates with bacterial strains as follows: Sterile cotton swab was dipped in cultures of the microorganisms; (containing bacterial cultures incubated for 24 hours at 37 ° C) were adjusted to 0.5 of McFarland standard (1.5 x 10^8 CFU/mL); then,

seaweed extracts were applied directly on seeded agar plates using the drop method (20 µL) (Boulekbache-Makhlouf *et al.,* 2012).

Then the prepared extracts are poured in to the well in the standard concentration. All the plates were incubated for 24 hours at 37°C.

Then the presence of zone of inhibition could be measured on the plates. All tests were performed in triplicate, and clear zones greater than 10 mm were considered as positive results (Lima-Filho *et al.*, 2002).

Antifungal activity

Poisoned food technique (Schmitz, 1930) was employed to screen the antifungal efficacy of seaweed extracts. Sabouraud agar amended with seaweed extracts (5%) was autoclaved and poured into sterile Petri plates.

Fungal disc of 6 mm diameter were cut with the help of sterile cork borer from the periphery of 5 days old culture of *Fusarium oxysporum f.* sp. *Albedinis* and *Pénicillium* and the disk were transferred aseptically on Sabouraud agar poisoned with seaweed extracts.

A plate only with Sabouraud and fungal disc was considered as control and the diameter of growth of fungus in this plate was used as a control for the calculation of percent inhibition of test fungus.

Inhibition percentage

The inhibition percentage was calculated measuring the radial growth of the fungus grown on control and amended plates, using the following formula (Harlapur *et al.*, 2007 Harlapur):

I% = 100 x (C – T) / C

Where, I% = inhibition percentage of pathogen growth, C = average radial growth in control plates and T = average radial growth in plates amended with seaweed extracts.

Results

Marine algae are considered as source of bioactive compounds to produce great variety of secondary metabolites characterized by a broad spectrum of biological activities. In the present study antibacterial activity of different type of seaweeds collected along the Ain Franin coastal area in South Oran, Algéria were carried out. All the extracts were taken using ethanol and water as solvent.

Table 1. Antibacterial activity of seaweed species extracts against different pathogen strains (Inhibition zone in mm).

Extracts / Bacteria	*Halopitys incurvus*		*Stypocaulon scoparium*	
	Ethanol	Aqueous	Ethanol	Aqueous
E. coli	10	-	12	-
Pseudomonas Aeruginosa	22	-	12	-
Bacillus subtilis	10	-	14	-
Staphylococcus aureus	18	-	12	-

Antibacterial and Antifungal activity determination

The results of the screening of antibacterial and antifungal activities against bacteria and yeast are summarized in Tables 1 and 2.

Stypocaulon scoparium (brown algae) and *Halopitys incurvus* (red algae) showed a positive activity against at least one bacteria test.

An important activity (diameter of inhibition higher than 15 mm) was observed in the ethanolic extract of *Halopitys incurvus* which inhibited *Pseudomonas aeruginosa* and *Staphylococcus aureus* followed by the ethanolic extract of *Stypocaulon scoparium* (dimeter of inhibition higher than 10 mm) was observed with al bacteria strains tested.

Table 2. Antifungal activity of *halopitys incurvus* and *stypocaulon scoparium* against different fungal pathogens (Inhibition percentage**.**

Champignon / Extrait	*Halopitys incurvus*		*Stypocaulon scoparium*	
	Ethanol	Aqueous	Ethanol	Aqueous
Fusarium oxysporum f. sp.albedinis	48.71%	61.53%	42.3%	60.25%
Pénicillium .sp	10.58%	3.59%	8.23%	1.17%

This can be explained by the effect of the metabolites secreted by the two algae, which belong to the Rhodophyceae and pheophyceae. Among the extracts tested, aqueous extract of *Halopitys incurvus* and *Stypocaulon scoparium* showed better performance for reducing the *Fusarium oxysporum f. sp.albedinis* growth (61% and 60%) (Figure 1).

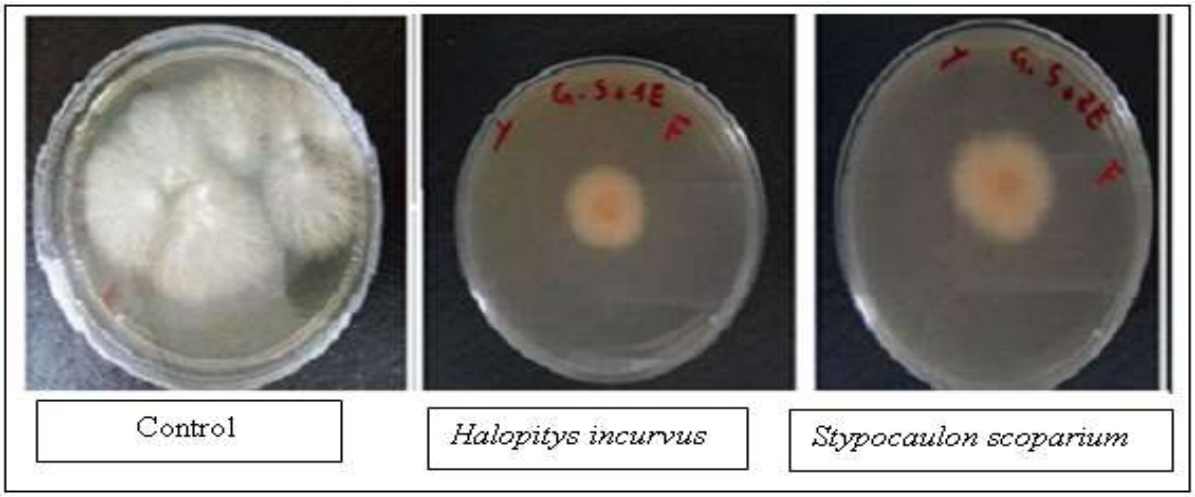

Fig. 1. Effect of aqueous seaweed extracts on mycelial growth of *Fusarium oxysporum f.* sp. *albedinis.*

Discussion

Seaweed has been proven to be a potential source of antibacterial compounds towards both Gram-negative or Gram-positive pathogenic bacteria (Kolanjinathan *et al.*, 2009).

Taskin *et al.*, (2007) reported that ethanolic extract of eight seaweed species belonging to Chlorophyta, Phaeophyta and Rhodophyta exhibited broad spectrum activity of both antibacterial and antifungal activities. In this study, the brown seaweed was found to be more active than the red seaweed.

The results were similar with the study by Lavanya and Veerappan (2011) which reported that the brown seaweed extracts showed higher activity than the red seaweed extracts. Nagayama *et al.*, (2002) suggested that the strong antibacterial activities from brown seaweed may be due to the compounds such as phlorotannins, eckol and eckol related-compounds that have strong bactericidal activity.

The percent reduction over control in ethanol extract of *Halopitys incurvus* was 48.71% compared with that of Stypocaulon scoparium which did not exceed 42.3%.Lowest mycelia growth (*Pénicillium* sp.) was reported in *Stypocaulon scoparium* at 1.17% Figure), followed by *Halopitys incurvus* at 3.53% in the aqueous compared to ethanol extracts 10%.

Can be explained by the probability that water with a polarity lower than that of ethanol could extract molecules of inhibitory character mainly antifungal. The difference may due to the efficiency of the extraction methods to recover the active metabolites, solvents used (Turney *et al.,* 2006), susceptibility of strains (Perez *et al.*, 1990) and seasonal variation (Vidyavathi and Sridhar, 1991).

Some studies concerning the effectiveness of solvent used for extraction reported that methanol extraction yielded higher antifungal activity than n-hexane and ethyl acetate (Sastry, Rao, 1994), whereas others reported that chloroform is better than methanol and benzene (Febles *et al*., 1995). In this study, antifungal activity of was higher aqueous extract compared to organics extract (ethanol).

Conclusions

From the study, it can be concluded that the antibacterial activity of seaweeds ethanolic extracts was found to be high for gram positive and gram negative strains. On the other hand for the fungus *Fusarium oxysporum f.* sp. *albedinis* the results were better with aqueous extract so we can conclude that the activity varies according to the seaweeds species and the type of solvents used for extraction.

Different solvents with different polarity may result in extraction of different types of biologically active compound from seaweeds. These bioactive compounds may go and bind to the cell wall of the microbes leading to inhibition of its growth.

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