ANTIMICROBIAL EFFECT OF TWO MARINE ALGAE *GELIDIUM SESQUIPEDALE* AND *LAMINARIA OCHROLEUCA* COLLECTED FROM THE COAST OF EL JADIDA-MOROCCO

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ABSTRACT

Marine algae are known to produce a wide variety of bioactive secondary metabolites and several compounds have been derived from them for prospective development of novel drugs by the pharmaceutical industries. However, algae of the Atlantic coast have not been adequately explored for their potential as a source of bioactive substances. In this context *Gelidium sesquipedale* and *Laminaria ochroleuca* isolated from Atlantic coast of El Jadida-Morocco, were evaluated for their potential bio activity.Extracts of the algae selected for the study were prepared using hexane, dichloromethane, dichloromethane/methanol (50:50), methanol and water, and assayed for antibacterial activity against *Escherichia coli* ATCC 10536, *Pseudomonas* sp. ATCC 10430, *Staphylococcus aureus* ATCC 6538, *Bacillus* sp. CIP 104717 and *Streptococcus faecalis* ATCC 19433 and for antifungal activity against *Candida albicans* ATCC 60193, *Candida tropicalis* ATCC 127581 and *Cryptococcus neoformans* ATCC 11576. It was found that dichloromethane/methanol was most effective followed by methanol for the preparation of algal extract with significant antibacterial activities (P=0, 05), respectively. Results also indicated that the hexane, dichloromethane and water extracts of both algae were no active against all tested bacterial strains.

Keywords: Antimicrobial activity, *Gelidium sesquipedale*, *Laminaria ochroleuca*, seaweeds extracts,El Jadida-Morocco

No: of Tables : 2 **No: of References:25**

INTRODUCTION

About 30,000 species of algae are found the world over which occur at all places where there is light and moisture and are found in abundance in sea. They supply oxygen to the biosphere, are a source of food for fishes, cattle and man. Algae are also used as medicine and fertilizers. A majority of red algae and almost all the genera of brown algae except *Bodanella, Pleurocladia* and *Heribaudiella* occur in salt water. Marine waters comprise a high diversity of microbial life, predominantly including bacteria, fungi, viruses, spores, and actinomycetes (Harder, 2009). These organisms also settle on marine animals and plants, besides occurring in sea surface, and form unique associations with their hosts (Singh and Reddy, 2014). Seaweeds are part of highly productive ecosystems and are habitats of numerous bioactive compounds producing microorganisms. Bioactive compounds obtained from associated microorganisms are known for broad range of biological effects such as antimicrobial, antisettlement, antiprotozoan, antiparasitic, and antitumors (Egan *et al.,* 2001, 2008 ; Penesyan *et al.,* 2011; Lee *et al.,* 2013). Mostly, bioactive compounds producing microorganisms are evolved through high competitive environment due to nutrient and space limitation on their host surface that led them to produce allele chemicals capable of preventing secondary colonization (Egan *et al.,* 2001, 2008).These bioactives compounds are secondary metabolites and have exceptional molecular structure as compared to those produced by terrestrial microorganisms (Fenical, 1993; Clardy *et al.,* 2006; Penesyan *et al.,* 2009, 2010). The bioactivities of such secondary metabolites indicate that they possess pharmaceuticals, industrial, agricultural, and biotechnological applications (Armstrong *et al.,* 2001; Penesyan *et al.,* 2011). Recently, seaweed-associated microorganisms have significantly been investigated for analysis of microbial communities and identification of their bioactivities (Molinski *et al.,* 2009; Penesyan *et al.,* 2009, 2011, 2013a, b; *Burke et al.,* 2011; *Bondoso et al.,* 2013; Goecke *et al.,* 2013; Hollants *et al.,* 2013; Tebben *et al.,* 2014). In the present study, we evaluated the antimicrobial activities of dichloromethane, methanol, hexane, dichloromethane-methanol and water extracts of two marine algae collected from the coast of El Jadida–Morocco for targeting isolation of bioactive molecules for searching broad range bioactives.

MATERIEL AND METHODS

Algal materials

Seaweeds *Gelidium sesquipedale* and *Laminaria ochroleuca* were collected at low tide and during the spring tide by hand-picking in the period of March to April 2009 from Sidi Bouzid coast (33°-33°16'09''N, 8°30'-8°45'W). The algae were cleaned, washed in distilled water, then dried at room temperature and crushed until a fine powder was obtained.

Preparation of extracts

The powder of dried algae was extracted in different solvents methanol, hexane, dichloro methane /methanol and water

as described by Caccamese and Azolina (1979). The resulting extracts were concentrated to dryness in a rotary evaporator under reduced pressure (at 45°C) until a crude extract was obtained and was conserved at 4°C.

Microbial strains

The strains used to evaluate the antimicrobial activity were obtained from the Collection of Institute Pasteur of Paris (CIP) and from American Type Culture Collection (ATCC) and they were encountered in human pathology. The Gram-positive bacteria included: *Staphylococcus aureus* (ATCC 9144), *Bacillus* sp. (CIP 104717), and *Streptococcus faecalis* (ATCC 19433). Gram-negative bacteria used were *Pseudomonas* sp. (ATCC 19433) and *Escherichia coli* (ATCC 10536), while the fungi used were *Candida albicans* (ATCC 60193), *Candida tropicalis* (ATCC 127581) and *Cryptococcus neoformans* (ATCC 11576).

Antimicrobial bioassays

Antibacterial assays were carried out using the agar disk-diffusion assay (Bauer *et al.,* 1966). Three colonies of each bacterium were removed with a wire loop from the original culture plate and were introduced into a test tube containing 5 ml broth. An overnight culture yielded a suspension of 10^6 bacteria/ml (evaluated by the absorbance value of 0.5 at 620 nm). This solution was diluted 100-fold and the bacterial density was then adjusted to 0.2 × 10^4 cells/ml with sterile water to inoculate Petri dishes containing culture media (12 ml Mueller-Hinton agar, 3 mm thick). Plates were dried for about 30 min before inoculation and were used within four days of preparation. Organic extracts were tested using paper disks (6 mm diameter) impregnated with the solution (500µg/disk), while aqueous extract was tested according to the well assay (Chabbert, 1963) using a solution of extracts (concentration of 500µg/50µl) in each well (well volume is 100µl). After the temperature was equalized at 4°C, the microorganisms were incubated overnight at 37°C. Inhibition zones were then measured.

For fungicidal activity, zones of inhibition were determined after 24 h of incubation at 27°C. Discs impregnated with standard antibiotics were used. Streptomycin was used (at 100µg/ml) as reference in the test of antibacterial activity and Amphotericin B (at 200µg/ml) was used in the antifungal activity. In addition, control disks were prepared with each solvent and all tests were performed in triplicate. Representative halos were those measuring a diameter superior to 10 mm (Lima *et al.,* 2002).

Statistical analysis

The antimicrobial activities of the data are expressed as means ±SD. The statistical analysis was performed using Tukey test at P=0.05 using the Software Package for Social Sciences (SPSS, version 20.0, IBM Inc., USA). All tests were considered to be statistically significant at $P<0.05$.

Table 1: Antimicrobial activity of red algae *Gelidium sesquipedale*

Microorganisms tested		Extracts prepared in					Positive control	
		Hexane	DC	DC/ MeOH	MeOH	Water	Streptomycin 100µg/ml	Amphotericin B 200µg/ml
Gram positive bacteria	*Staphylococcus aureus*	-	-	18±0.00	-	-	20±0.50	
	Streptococcus faecalis	-	-	18±1.15	-	-	22±0.00	
	Bacillus sp.	-	-	11±0.57	-	-	14±0.57	
Gram negative bacteria	*Escherichia coli*	-	-	-	-	-	13±0.00	
	Pseudomonas sp.	-	-	14±0.57	-	-	19±0/57	
Fungi	*Candida albicans*	-	-	-	-	-		18±0.57
	Cryptococcus neoformans	-	-	11±0.00	-	-		16.67±0.21
	Candida tropicalis	-	-	-	-	-		20±0.00

Data are expressed as the mean ± standard deviation (SD) of three replicates. - : No activity, DC: dichloromethane, MeOH: Methanol

Table 2: Antimicrobial activity of brown algae *Laminaria ochroleuca*

Microorganisms tested		Extracts prepared in					Positive control	
		Hexane	DC	DC/ MeOH	MeOH	Water	Streptomycin 100µg/ml	Amphotericin B 200µg/ml
Gram positive bacteria	*Staphylococcus aureus*	-	-	14±0.00	14±0.00	-	20±0.50	
	Streptococcus faecalis	-	-	16±1.15	-	-	22±0.00	
	Bacillus sp.	-	-	11±0.57	-	-	14±0.57	
Gram negative bacteria	*Escherichia coli*	-	-	13±0.57	-	-	13±0.00	
	Pseudomonas sp.	-	-	16±0.57	-	-	19±0.57	
Fungi	*Candida albicans*	-	-	-	-	-		18±0.57
	Cryptococcus neoformans	-	-	8±0.57	-	-		16,67±0.21
	Candida tropicalis	-	-	7±1.00	-	-		20±0.00

Data are expressed as the mean ± standard deviation (SD) of three replicates. -: No activity, DC: dichloromethane, MeOH: Methanol

RESULTS AND DISCUSSION

Gelidium sesquipedale

The results of screening tests are shown in table 1. The positive activity was assessed by the diameter of the inhibition zones.
In the present study, the antibacterial activity is not uniformly distributed in the various extracts; dichloromethane / methanol extract exhibited greater inhibition against gram-positive bacteria.
Dichloromethane/methanol (50/50) extract of *Gelidium sesquipedale* exhibited strong inhibition against *Staphylococcus aureus* and *Streptococcus faecalis* with inhibition zone of 18 mm, which was significantly higher than all other extracts ((*P*=0, 00)<0,05), whereas, inhibition zone obtained against *Pseudomonas* sp. was 14 mm. This alga showed a moderate activity against *Cryptococcus neoformans* with an inhibition diameter less than 13 mm. However, no antimicrobial activity was detected in extracts prepared in hexane, dichloromethane, methanol and water.

Laminaria ochroleuca

These results presented in table 2 show that dichloromethane/methanol extract presents a high activity against all strains used compared with all other extracts ((*P*< 0,000)<0,05). An inhibition diameter of 16 mm of dichloromethane/methanol extract was observed against *Streptococcus faecalis* and *Pseudomonas* sp. While, inhibition diameter of 14 mm and 13 mm were obtained against *Staphylococcus aureus* and *Escherichia coli,* respectively. Methanolic extract of this alga inhibited *Staphylococcus aureus* (inhibition diameter of 13 mm). These results are in agreement with those obtained by Chiheb *et al.* (2009) who found that methanolic extract of *Laminaria ochroleuca* inhibit *Staphylococcus aureus* with a diameter of inhibition rather than 20 mm. However, these results are in contrast with those of Salvador *et al.* (2007) who mentioned that methanolic extract of this alga, collected from Atlantic coast of Spain, doesn't show activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

CONCLUSION

In conclusion, the results of the present study revealed that *Laminaria ochroleuca* and *Gelidium sesquipedale* are the potential producers of antibacterial activity. Therefore, it should be thoroughly investigated for natural sources bioactive compounds properties. Thus, these bioactive compounds may provide high-quality drug candidates for pharmaceutical applications. In our ongoing program, we are in progress to isolate and characterize active compounds.

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