ANTIMICROBIAL ACTIVITY OF DIFFERENT FRACTIONS OBTAINED FROM *GELIDIUM SESQUIPEDALE* AND *LAMINARIA OCHROLEUCA*

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ABSTRACT

Antimicrobial activities of two species of marine algae, Phaeophyceae (*Laminaria ochroleuca*) and Rhodophyceae (*Gelidium sesquipedale*) were investigated. General components, lipids, proteins, and polysaccharides of the algae were analyzed. Using three strains of gram positive, *Bacillus* sp., *Staphylococcus aureus*, *Streptococcus feacalis*, and two strains of Gram negative, *Escherichia coli*, *Pseudomonas* sp., and two yeasts *Candida albicans* and *Candida tropicalis*, antimicrobial activity was determined by paper-disc method on the four fractions. The Lipid fraction from *Laminaria ochroleuca* and *Gelidium sesquipedale* showed a strong activity on the test strains with somewhat higher activity against Gram positive than Gram negative strains. The protein fraction of *Gelidium sesquipedale* showed a high activity against *S. feacalis*, while the protein fraction of *Laminaria ochroleuca* showed no activity. For the antifungal activity, lipid fraction of *Laminaria ochroleuca* and protein fraction of *Gelidium sesquipedale* showed a moderate activity against *Candida albicans*.

Keywords: *Laminaria ochroleuca*, *Gelidium sesquipedale*, lipid, protein and polysaccharide fractions, antimicrobial activity.

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INTRODUCTION

Algae are group of marine plants attaching global attention in terms of research work and commercial exploitation. The seaweed has touched new horizons like marine pharmacology, bioremediation, seaweed tissue culture etc. Marine algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antifeedant, antihelmintic and cytotoxic agents and the bioactive substances included alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols (Cabrita*et al.,* 2010) and marine macro-algae are considered as the actual producers of some bioactive compounds with high activity (Shimizu, 1996). Hence they have drawn great attention recently (Abdel-Raouf*et al.,* 2008; Ibraheem*et al.,* 2008; Al-Haj*et al.,* 2009; Bazes*et al.,* 2009; Vallinayagam*et al.,* 2009;Cabrita*et al.,* 2010). Recently, lipid constituents have been recognized as potentially important factors in the processes of signal transduction and endomembrane transport (Simons and Toomre 2000). However, algae represent valuable sources of a wide spectrum of complex lipids, and their components have promising potential applications especially in the food, cosmetic, and pharmaceutical industries (Hossain *et al.,* 2005). Lipids of marine macroalgae possess antibacterial, antiviral, antitumor, anti-inflammatory, antiprolierative and antioxidant activity (Gerasimenko*et al.,* 2010; Goeke*et al.,* 2010; El Baz *et al.,* 2013). The main components of brown and red seaweed are usually polysaccharides, which may have storage and structural functions. Cell walls of algae are composed of a variety of polysaccharides including alginic acid and alginates, carrageenans and agar, laminarans, fucoidans, ulvans and derivatives (Balboa *et al.,* 2013; Usov*et al.,* 2013).Laminaran is the principal storage polysaccharide of brown seaweed (e.g., *Laminaria* or*Saccharina* spp.) and their content can represent up to 32%–35% dry weight (Kraan, 2012). Fucoidans and laminarans are considered as the main water-soluble polysaccharides of brown algae (Kardoso*et al.,* 2014).

Their antimicrobial activity depends on some factors, such as their distribution, molecular weight, charge density, sulphate content (in sulphated polysaccharides), and structural and conformation aspects. In addition, oligosaccharides obtained by depolymerization of seaweed polysaccharides also induce protection against viral, fungal and bacterial infections in plants Vera *et al.,* 2011).

The aim of the present work is to evaluate the biological activityof the different fractions of two marine algae *Gelidium sesquipedale* and *Laminaria ochroleuca* collected fromthe Atlantic coast in El Jadida-Morocco.

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

toApril from SidiBouzid-El Jadida 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, dichloromethane, dichloromethane /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.

Lancelot fractionation protocol

In parallel, in order to determine the nature of the charge fractions of biological activity, a biochemical fractionation is performed on the powders of the alga *Laminaria ochroleuca*and*Gelidium sesquipedale* according to the protocol of Lancelot based on differential extraction (Bourdier, 1985).

- Lipid extraction is carried out with a chloroform / methanol 2/1.
- Extraction of the compound with low molecular weight (amino acids, organic acids, monosaccharides) is by hot ethanol.
- Proteins were precipitated by trichloroacetic acid, while the polysaccharide remains in supernatant.

Microbial strains

The strains used to evaluate the antimicrobial activity were obtained fromthe 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 tubecontaining 5 ml broth. An overnight culture yielded a suspension of 10^6 bacteria/ml. Thissolution 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 culturemedia (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 incubatedovernight 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 $P < 0,05$

RESULTS AND DISCUSSION

Antimicrobial activity in the different fractions obtained by the fractionation of Lancelot

Gelidium sesquipedale

Biochemical fractionation Lancelot (Lancelot and Mathot, 1985) of red algae *Gelidium sesquipedale* yielded four fractions (lipid, protein, polysaccharide and low molecular weight compounds).The tests of antimicrobial activity performed on these four fractions are summarized in Table 1.

The antimicrobial activity evaluated in the different fractions obtained by separation of Lancelot showed that the protein and lipid fractions have a significant antibacterial activity 15mm and 16mmagainst*Streptococcus faecalis* respectively. The polysaccharide fraction has demonstrated also an activity of 14mm against*Streptococcus faecalis*, by cons, low molecular weight compounds have shown no activity against all strains used. The protein fraction obtained from fractionation Lancelot showed antibacterial activity of 15mm against*Streptococcus faecalis* but in the aqueous extract no activity was detected.

Laminaria ochroleuca

Four fractions (lipid, protein, polysaccharide and low molecular weight compounds) were obtained by the biochemical fractionation of Lancelot. The tests of antimicrobial activity of the four fractions are summarized in Table 2.

The lipid fraction showed antibacterial activity of 15 mm, 14 mm and 13 mm against*Streptococcus faecalis*, *Staphylococcus aureus* and *Candida albicans* respectively. by cons, the polysaccharide fraction and the protein fraction showed a moderate activity against*Bacillus* sp. However, the low molecular weight compounds indicated no activity against all strains used.

Table 1: Antimicrobial activity in the different fractions of *Gelidium sesquipedale* obtained by method of biochemical fractionation Lancelot.

Different fractions	Inhibition diameter (mm)							
	Gram positive bacteria			Gram negative bacteria		Yeast		
	S. aureus	*S. faecalis*	*B.* sp.	*P.* sp.	*E. coli*	*C. albicans*	*C. neoformans*	*C. tropicalis*
Lipid	10±0.577	16±1.00	11±0.577	-	-	10±0.577	-	-
LMW	-	-	-	-	-	-	-	-
Polysaccharide	11±0.577	14±0.577	10±0.577	-	-	9±0.577	-	-
Protein	13±0.577	15±0.000	10±0.577	9±0.577	9±0.577	12±0.577	10±0.577	-

S. aureus: Staphylococcus aureus, B. sp.*: Bacillus* sp.*; E. coli: Escherichia coli; S. faecalis: Streptococcus faecalis; P.* sp.*: Pseudomonas* sp.*; C. albicans: Candida albicans; C. tropicalis: Candida tropicalis*. LMW : Low molecular weight compounds

Table 2: Antimicrobial activity in the different fractions of *Laminaria ochroleuca* obtained by method of biochemical fractionation Lancelot.

Different fractions	Inhibition diameter (mm)							
	Gram positive bacteria			Gram negative bacteria		Yeast		
	S. aureus	*S. faecalis*	*B.* sp.	*P.* sp.	*E. coli*	*C. albicans*	*C. neoformans*	*C. tropicalis*
Lipid	14±0.577	15±0.000	10±0.577	-	-	13±0.577	-	-
LMW	-	-	-	-	-	-	-	-
Polysaccharide	-	-	10±0.577	-	-	-	-	-
Protein	-	-	11±1.000	-	-	-	-	-

S. aureus: Staphylococcus aureus, B. sp.*: Bacillus* sp.*; E. coli: Escherichia coli; S. faecalis: Streptococcus faecalis; P.* sp.*: Pseudomonas* sp.*; C. albicans: Candida albicans; C. tropicalis: Candida tropicalis*. LMW : Low molecular weight compounds

CONCLUSION

In conclusion, seaweeds or marine algae are a valuable source of natural antimicrobial compounds as their crude extracts and fractions exhibit antimicrobial activity. The results indicate that the lipid fraction of both algae have an antimicrobial activity, but, only the protein fraction of red algae *Gelidium sesquipedale* showed an antimicrobial activity.

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