Journal of Microbiology, Biotechnology and Food Sciences

Zbakh et al. 2012 : 2 (1) 219-228

SHORT COMUNICATION

ANTIBACTERIAL ACTIVITY OF BENTHIC MARINE ALGAE EXTRACTS FROM THE MEDITERRANEAN COAST OF MOROCCO

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ABSTRACT

Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. The Moroccan marine biodiversity including macroalgae remains partially unexplored in term of their potential bioactivities. Antibacterial activity of methanolic extracts from 20 species of macroalgae (9 Chlorophyta, 3 Phaeophyta and 8 Rhodophyta) collected from Moroccan Mediterranean coasts was evaluated against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. The extracts of the studied Rhodophyceae inhibited considerably the growth of the three tested bacterial strains and gave inhibition zones between 20 and 24 mm. The results indicate that these species of seaweed present a significant capacity of antibacterial activities, which makes them interesting for screening for natural products.

Keywords: Antibacterial activity, Macroalgae, Moroccan Mediterranean coast, Algal extract

INTRODUCTION

Traditional and modern medicines have relatively exhausted most of their resources in land plants. However, the marine environment by dint of its biological and chemical diversity can be a source of new types of agents against cancer and infectious diseases (Bazes *et al.,* 2006; Chew *et al.,* 2007; Mayer *et al.,* 2007). During the last decades, numerous novel compounds have been isolated from marine organisms and many of these substances have been demonstrated to possess interesting biological activities (Duarte *et al.,* 2001; Faulkner, 2002; Ely *et al.,* 2004; Dubber and Harder, 2008).

Marine macroalgae are the most interesting algae group because of their broad spectrum of biological activities such as antimicrobial (Chiheb *et al.,* 2009, Bouhlal *et al*.,2010), antiviral (Bouhlal *et al*., 2010, Bouhlal *et al.,* 2011, Kim and Karadeniz, 2011), antifungal (de Felício *et al.,* 2010), anti-allergic (Na *et al.,* 2005), anticoagulant (Dayong *et al.,* 2008), anticancer (Kim *et al.,* 2011), antifouling (Bhadury and Wright, 2004) and antioxidant activities (Devi *et al.,* 2011). They produce a wide variety of chemically active metabolites in their surroundings as an aid to protect themselves against other settling organisms (Bhadury and Wright, 2004). There are numerous reports of macroalgae derived chemical compounds that have a broad range of biological activities, some of which have been used in pharmaceutical industries.

The algal richness of Moroccan coasts is undeniable in terms of diversity and quantity (Kazzaz and Riadi, 2000). The antimicrobial potential of macroalgae from Mediterranean Moroccan coasts remains partially unexplored. Many chemically unique compounds of marine algae with antimicrobial activity have been isolated and a number of them are under investigation and/or are being developed as new pharmaceuticals such as brominated phenols, sterols, terpenoids, polysaccharides, peptides, proteins, acrylic acid, terpenes, chlorophyllides, phenols and heterocyclic carbons etc. (Bhacuni *et al*. 2005; Li *et al*., 2007; Bouhlal *et al.,* 2011; Priyadharshini *et al*., 2011). The present study was undertaken to examine the antibacterial effect of methanolic crude extracts of 20 species of marine benthic algae (9 Chlorophyceae, 3 Phaeophyceae and 8 Rhodophyceae), collected from the Mediterranean Moroccan coasts, against pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*.

MATERIAL AND METHODS

Algal material

The algae were collected between 1 and 4 m of depth, conserved on ice until use. Sampling was conducted between May 2005 and June 2008 on the coast of Ksar Seghir (35°50´52.58"N 5°33´39.04"0), Martil (35°37´10.22"N 5°16´15.79"0) and the lagoon of Nador (33º37'00.23''N 3º43'59.82"0). Taxonomic identification of species was performed with the aid of standard literature and determination keys. Voucher specimens of all species identified are deposited in the herbarium of our laboratory (Applied Algology-Mycology Laboratory, Department of Biology, Faculty of Science, Abdelmalek Essaâdi University, 93002 Tetouan, Morocco).

Chemical extraction

The algae samples were rinsed with sterile seawater to remove sand and epiphytes and washed with water. Then, they were dried on air ambient and finally in an oven (35-40 °C). The dried seaweeds were crushed by an electric grinder and the obtained powder was then stored at -12 °C until the extraction step. The powder of dried seaweeds (5 g) was extracted with methanol (200 ml) for 8 h at 65°C using the Soxhlet apparatus. The obtained extracts were concentrated under vacuum using a rotary evaporator. The residues were then diluted in 2 ml of pure methanol (Sreenivasa-Rao and Parekh, 1981).

Antibacterial activity test

Antibacterial tests of algal extracts were performed *in vitro* using the disc diffusion method (Sreenivasa-Rao and Parekh, 1981) in Petri dishes. Sterile disks (BBLTM) of 6 mm in diameter were impregnated with 25 µl of seaweeds extract, deposited on the surface of agar medium (Mueller-Hinton Agar, pH 7.4 ± 0.2 at 25 °C) previously inoculated with bacteria strains and incubated at 37 °C for 24 h (Ballantine *et al.,* 1987). The results are expressed by measuring in millimeter the diameters of the inhibition halos of bacterial growth around the disk. Methanol (100%) without seaweed extract was used as negative control. All tests were performed in triplicate, and clear halos greater than 10 mm were considered as positive results (Lima-Filho *et al.,* 2002).

Bacteria strains

Bacteria strains used in this study are: *E. coli* ATCC 25922 ($Gram^-$), *S. aureus* ATCC 25923 ($Gram^+$) and *E. faecalis* ATCC 29212 ($Gram^+$). They were obtained from the Department of Microbiology, Faculty of Pharmacy - University of Granada, Spain (ATCC: American Type Culture Collection).

RESULTS AND DISCUSSION

The antibacterial activity of crude methanol extracts of 20 species of Moroccan marine algae is shown in the Table 1. The antibacterial activity is ranked from no activity (-: inhibition diameter <10 mm), low (+: inhibition diameter between 10 and 15 mm), moderate (++: inhibition diameter between 15 and 20 mm) and high activity (+++: diameter inhibition ≥ 20 mm).

Among the algae tested, seventeen species have shown antibacterial activity, the highest activity was shown by crude extracts of *Ulva lactuca*, *Gracilaria bursa-pastoris* and *Chaetomorpha linum*. The majority of the algal extracts were active against at least one or two microorganisms. Fifteen extracts (75%) were moderately to highly active against *S. aureus*, seven (35%) against *E. coli* and finally two extracts (10%) inhibited *E. faecalis*. Methanol extracts of *Ulva rigida*, *Enteromorpha compressa*, *musciformis Hypnea*, *Caulerpa prolifera*, *Asparagopsis armata* and *Cladostephus spongiosus* showed inhibitory activity against all strains tested.

Among the nine species tested of Chlorophyceae, the extract of *Caulerpa prolifera* has the larger diameters of inhibition against *S. aureus* (23 mm), *E. coli* (16 mm) and *E. faecalis* (13 mm). However, extracts of *Ulva rigida* (Fig 1), *Enteromorpha compressa* and *Caulerpa prolifera* were the only ones to present inhibitory activity against *E. faecalis*. Their respective inhibition diameters are 15 mm, 13 mm and 12 mm.

The results obtained with extracts of *Ulva rigida* and *Caulerpa prolifera* from the Mediterranean Moroccan coasts are similar to those obtained with the same species from the Canary Islands against $Gram^+$ bacteria (González *et al.,* 2001). Similar results were also obtained with extracts of ethanol, ethanol/methanol and hexane of *Caulerpa prolifera* from Mexico and the Caribbean (Ballantine *et al.,* 1987; Freile-Pelegrin and Morales, 2004).

Table 1 Antibacterial activity of marine algae extracts from different localities of the Mediterranean Moroccan coasts

Algae	locality	strains		
		Eco.	*Ent*	*Sta*
Chlorophyceae				
Ulva rigida	Ksar Seghir	+	++	+
Ulva lactuca	Nador	+	-	+
U. olivascens	Nador	++	-	++
Enteromorpha compressa	Ksar Seghir	+++	+	+
E. linza	Nador	+	-	++
E. intestinalis	Nador	+	-	++
Chaetomorpha linum	Nador	+	-	+
Caulerpa prolifera	Nador	++	+	+++
Codium dichotomum	Martil	-	-	+++
Phaeophyceae				
Cystoseira humilis	Nador	+	nt	++
C. compressa	Nador	-	-	+++
Cladostephus spongiosus	Martil	+	+	+++
Rhodophyceae				
Gymnogongrus patens	Ksar Seghir	+++	-	++
Plocamium coccineum	Ksar Seghir	+	-	++
Asparagopsis armata	Ksar Seghir	+	+	++
Centroceras clavulatum	Nador	++	-	++
Gracilaria confervoïdes	Nador	+	-	++
G. bursa- pastoris	Nador	+	-	+
Hypnea musciformis	Nador	+++	+++	+++
Alsidium corallinum	Nador	++	-	++

Legend: -: no activity; +: 10mm< inhibition diameter <15 mm; ++: 15mm< inhibition diameter <20mm; +++: inhibition diameter ≥ 20mm). Strains: Eco: *Escherichia coli* ATCC 25922, Sta: *Staphylococcus aureus* ATCC25923, Ent: *Enterococcus faecalis* ATCC 29213, nt: not tested.

Extract of *Cladostephus spongiosus* showed a broad inhibitory activity against *E. coli* (13 mm), *E. faecalis* (12 mm) and *S. aureus* (24 mm). Negative responses of *Cystoseira compressa*, *C. humilis* and *Cladostephus spongiosus* extracts are consistent with those reported by González *et al.* (2001) concerning samples from the Canary Islands.

The extract of *Hypnea musciformis* (Rhodophyceae) inhibited considerably the growth of the three strains tested and gave inhibition zones between 20 and 24 mm. In the case of the extract of *Asparagopsis Armata*, the inhibition diameters were smaller than those of *Hypnea*

musciformis and varied between 12 and 15 mm. *S. aureus* appeared more sensitive than the other two strains with larger inhibition zones.

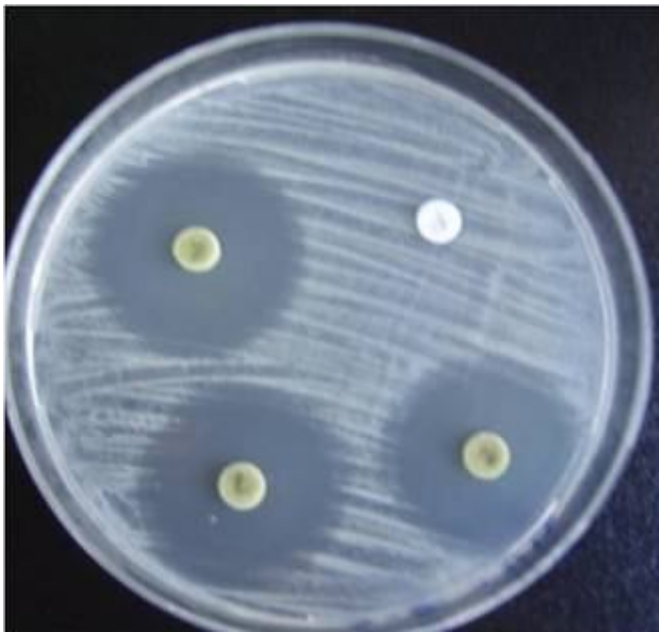

Figure 1 Inhibition zones of *Ulva rigida* methanolic extract against *Enterococcus faecalis* in triplicate and negative control

Nonetheless, *E. Faecalis* was highly resistant against the majority of the algal extracts used. It was reported that $Gram^+$ bacteria are more efficiently inhibited by algal extracts than $Gram^-$ bacteria (Sreenivasa-Rao and Parekh, 1981; Pesando and Caram, 1984). Studies on *Hypnea musciformis* from the Indian coast showed that methanol-dichloromethane extracts are more effective against $Gram^+$ bacteria strains than $Gram^-$ bacterial strains (Selvin and Lipton, 2004).

CONCLUSION

The Mediterranean coasts are a source of great biological diversity with an almost unexplored potential to provide significant therapeutic, as well as nutritional, benefits for humans. The investigation and exploitation of the potential of marine algae will have significant health implications for current and future generations, not only for local people inhabiting in the Mediterranean border countries but also for people from all around the world.

From all these results, we can conclude that macroalgae from Mediterranean Moroccan coasts represent a potential source of bioactive compounds and must be studied for the production of natural antibiotics. Biochemical analysis are being undertaken to determine the structure and nature of compounds responsible of the bioactivity of the extracts with high antibacterial activity. Not only the presence of a particular compound which makes these organisms, interesting but also their huge diversity and the possibility of not only harvesting them but also of growing them at different conditions, leading to an enrichment of some bioactive compounds.

Acknowledgments: Authors greatly thank the Prof. Jose Martinez Lopez from the Faculty of Pharmacy (University of Grenada, Spain) for providing gratefully the bacteria strains and the Dr. Conxi Rodriguez–Prieto from the Department of Environmental Sciences of the Faculty of Sciences (University of Girona, Spain) for his help in discussions of results and for providing documentation on the systematic of phycoflora.

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