

Full Length Research Paper

The antibacterial potential of the seaweeds (*Rhodophyceae*) of the Strait of Gibraltar and the Mediterranean Coast of Morocco

Bouhlal Rhimou^{1,2*}, Riadi Hassane², Martínez José³ and Bourgougnon Nathalie¹

¹Université européenne de Bretagne (UEB), Laboratoire de Biotechnologie et Chimie Marines (LBCM), Centre de recherche Yves Coppens, Université de Bretagne-Sud. Vannes, France.

²Laboratoire de Diversité et Conservation de Systèmes Biologiques (LDICOSYB), Faculté de Science, Université Abdelmalek Essaâdi, Tétouan, Maroc.

³Laboratory of Microbiology, Faculty of Pharmacy, University of Grenade, Spain.

Accepted 8 July, 2010

The antibacterial activity of extracts from 26 marine *Rhodophyceae* (8 Ceramiales, 7 Gelidiales, 9 Gigartinales, 1 Bonnemaisoniales and 1 Rhodymeniales) was studied to assess their potential in the pharmaceutical industry. Their bioactivity was analysed from crude methanolic extracts of dried samples against three gram-positive bacteria and two gram-negative bacteria using the disc diffusion technique. The samples were collected from Gibraltar and the Moroccan Mediterranean coast. Of the macroalgae analysed, 96% of extracts were active against at least one of the five test microorganisms. *Staphylococcus aureus* was the most susceptible microorganism. Methanolic extracts of all seaweed extracts tested in the present study exhibited a broad spectrum of antibacterial activity with inhibition diameters ranging from 10 to 35 mm. An extract of *Hypnea musciformis* exhibited high antibacterial activity against all the bacteria tested. The results of the present study confirmed the potential use of seaweed extracts as a source of antibacterial compounds.

Key words: Antibacterial activity, methanolic extracts, pathogenic bacteria, *Rhodophyceae*.

INTRODUCTION

Several macroalgae produce bioactive metabolites in response to ecological pressures such as competition for space, deterrence of predation and the ability to reproduce (König et al., 1994). In a marine environment, where all surfaces are constantly exposed to the threat of surface colonisation, sessile organisms remain relatively free from biofouling. These sedentary organisms control epibionts in particular marine bacteria by effective antifouling mechanisms (Hellio et al. 2001 and Bazes et al. 2006).

Macroalgae are a rich source of natural bioactive products although little has been done to define an ecological role for these compounds (Ballantine et al., 1987; De Nys et al., 1995; Shanmugam and Mody, 2000; Suzuki et al., 2001; Vairappan et al., 2001b). They may, therefore possess chemical defences to prevent the colonisation of their surface. The use of marine natural products capable

of inhibiting bacteria development offers rich pharmacological potential (Kornprobst, 2005). Numerous reports show macroalgae to present a broad range of biological activities such as antibacterial (Fenical and Paul, 1984; Mtolera and Semesi, 1996; Gonzalez et al., 2001; Selvin and Lipton, 2004; Karabay-yavasoglu et al., 2007; Salvador et al., 2007), antifungal (Moreau et al., 1984; Tariq, 1991; Mayer, 2002; Mayer et al., 2007, 2009) and antiviral (Bourgougnon et al., 1993, 1994; Hudson et al., 1999; Serkedjieva 2000, 2003; Mayer, 2002; Ghosh et al., 2004; Zandi et al., 2007) activities.

The ability of seaweeds to produce secondary metabolites of antimicrobial value, such as volatile components (phenols, terpenes) (Ozdemir et al., 2004; König et al., 1999a; Awad, 2004; Glombitza et al., 1985; Xu et al., 2003, Karabay-yavasoglu et al., 2007; Vairappan et al., 2001a,b; Vairappan, 2003), steroids (Awad, 2000), phlorotannins (Nagayama et al., 2002) and lipids (Ballantine et al., 1987; Rossel and Srivastava, 1987; Freile-Pelegri and Morales, 2004) has already been studied.

*Corresponding author. E-mail: rhimou.bouhlal@univ-ubs.fr.

In contrast to the brown and green algae, the red algae are more known to produce halogenated metabolites, particularly bromine and iodine (McConnell and Fenical, 1977; Fenical, 1981; König et al., 1999b). The orders of Nemaliales, Gigartinales, Ceramiales, Rhodymeniales and Cryptonemiales have been shown to be engaged in biological halogenations yielding a diverse array of organic compounds (McConnell and Fenical, 1977).

The Moroccan Coast is particularly rich in algal biodiversity and constitutes a reserve of species of considerable economic, social and ecologic potential. Today however, only the *Gelidium sesquipedale* species is exploited in Morocco. Between 1995 and 2000, the extraction of agar-agar increased by 7968 at 12.068 tons per year. If other horizons could be prospected and other algae developed, the pressure on the traditional species could decrease. Nevertheless, little is known about the antimicrobial activity of algae from the coast of Morocco, with the exception of some studies carried out on the Atlantic coast and reported in the work of Abourriche et al. (1999), Etahiri et al. (2003), Moujahidi et al. (2004) and Souhaili et al. (2004).

The purpose of our work is to research the new antibiotic compounds to be extracted from benthic extracts of *Rhodophyceae* of the Mediterranean Coast of Morocco for a possible valorisation and exploitation in a spirit of sustainable development. In the present investigation, a successful attempt was made to determine the antibacterial activity of methanolic extracts from marine algae belonging to five orders: Ceramiales, Gelidiales, Gigartinales, Rhodymeniales and Bonnemaisoniales against five pathogenic terrestrial bacteria.

MATERIALS AND METHODS

The collection of seaweeds

Seaweeds were collected by hand using Scuba diving or snorkelling (1-4m depth) and preserved on ice until further processing. Twenty-six were sampled between 2003 and 2006 at various sites, along the Mediterranean coast (Marina smir, Nador) and on the Strait of Gibraltar (Ksar-sghir, Dalya, Belyounech) (Table 1). The taxonomic identification of species was done by experts in these fields, using standard literature and taxonomic keys. Voucher specimens of all species tested are deposited in the herbarium of our Laboratory of Applied Algology-Mycology, Department of Biology, Faculty of Sciences, Abdelmalek Essâadi University, 93002 Tetouan, Morocco (Table 1). Seven species belong to the Gelidiales order, nine to Gigartinales order, eight to Ceramiales order, one to Bonnemaisoniales order and one to Rhodymeniales.

Preparation of extracts

After collection, the samples were rinsed with sterile seawater to remove associated debris and necrotic parts. Epiphytes were removed from the algae and the surface microflora was removed by washing the algal samples for 10 min with 30% ethanol. The samples were shade dried, cut into small pieces and powdered in a mixer grinder. The powder obtained was preserved cold (-12°C). Samples (5 g) were extracted with methanol solvents for 8 h using a

soxhlet apparatus. The resulting organic extracts were concentrated to dryness under reduced pressure at 30 - 35°C with a rotary evaporator. Each residue was weighed and stored in sealed vials in a freezer until being tested. All extracts were stored at (-4°C) (Ozdemir et al., 2004).

Bacterial strains

The strains used were, three gram-positive bacteria *Enterococcus faecalis* (ATCC 29212), *Enterococcus faecalis* (ATCC 29213) and *Staphylococcus aureus* (ATCC 25923) and two gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 700603). The bacteria strains were obtained from the Microbiology Department, Faculty of Pharmacy, University of Grenade, Spain. All cultures were kept on Brain Heart Infusion (BHI, Sigma) agar plates and stored at 4°C, except the initial stock cultures which were stored at -80°C in BHI broth containing 20% glycerol.

Antibacterial activity by disc diffusion assay

The screening of the antibacterial activity of the methanolic extracts was performed by the disc diffusion technique in agar-plated petri dishes (Hellio et al., 2000). Sterile discs (BBLTM) of 6 mm were used. Methanol extracts (25 µl) were loaded on each of these discs, allowed to dry overnight at room temperature for 24 h to evaporate methanol, and then tested in antibacterial activity. A bacterial suspension (number 0.5 in McFarland scale about 1.5×10^8 bacteria ml⁻¹) was spread on Mueller-Hinton (pH 7.4) agar using a cotton swab. After incubation for 24 h at 37°C, the antibacterial activity was quantified as the diameter (mm) of the growth inhibition zones. Control paper discs with the solvent (100%) were tested for every assay and showed no antibacterial activity. All extracts were tested in three discs.

Statistical analysis

The data were statistically analysed by applying a one-way ANOVA for comparison of mean values. All tests were considered to be statistically significant at $P < 0.05$.

RESULTS

Out of 26 species of *Rhodophyceae* tested, 25 species (96%) showed an antimicrobial activity on at least on one of the micro-organisms. Concerning the distribution of this activity among the collected groups of seaweeds, our results show that activity one is present with all the orders of seaweeds (Table 2). Of the 25 active species, 12 of the species had an antibacterial activity against *E. coli* ATCC 25922, 8 of species against *K. pneumoniae* ATCC 700603, *E. faecalis* ATCC 29213, *E. faecalis* ATCC 29212 and 25 species inhibited the development of *S. aureus* ATCC 25923.

Crude extracts of *Hypnea musciformis*, *Halopitys incurvus* and *Gelidium pusillum* showed an inhibitory action against the five bacteria tested. The extracts of *H. incurvus* and *G. pusillum* showed the greatest inhibition on *S. aureus* and *E. coli*, moderate inhibition on *E. faecalis* and *K. pneumoniae*, whereas the extract of *H. musciformis* showed the highest inhibition on all the

Table 1. List of marine algae.

Order	Marine algae	Date of collection	Site of collection	Position
Ceramiales	<i>Alsidium corallinum</i>	15/05/2005	Nador	35°11'11.98"N 2°55'30.75"O
	<i>Centroceras clavulatum</i>	15/05/2005	Marina smir	35°45'56.81"N 5°20'58.04"O
	<i>Callithamnion granullatum</i>	03/10/2003	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Ceramium rubrum</i>	10/08/2003	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Halopitys incurvus</i>	15/06/2006	Dalya	35°54'24.20"N 5°28'18.84"O
	<i>Osmundea pinnatifida</i>	08/05/2006	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Pterosiphonia complanata</i>	14/05/2006	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Boergeseniella thuyoides</i>	12/08/2006	Ksar sghir	35°50'52.58"N 5°33'39.04"O
Gelidiales	<i>Gelidium attenatum</i>	14/05/2006	Dalya	35°54'24.20"N 5°28'18.84"O
	<i>Gelidium latifolium</i>	15/06/2006	Dalya	35°54'24.20"N 5°28'18.84"O
	<i>Gelidium pusillum</i>	11/01/2004	Marina smir	35°45'56.81"N 5°20'58.04"O
	<i>Gelidium pulchellum</i>	16/08/2005	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Gelidium sesquipedale</i>	12/08/2006	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Gelidium spinulosum</i>	12/05/2005	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Pterocladea capillacea</i>	15/06/2006	Dalya	35°54'24.20"N 5°28'18.84"O
Gigartinales	<i>Chondrocanthus acicularis</i>	15/05/2005	Marina smir	35°45'56.81"N 5°20'58.04"O
	<i>Caulacanthus ustulatus</i>	15/05/2005	Marina smir	35°45'56.81"N 5°20'58.04"O
	<i>Gracilaria confervoides</i>	12/08/2006	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Gracilaria multipartita</i>	12/08/2006	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Gymnogongrus patens</i>	12/05/2005	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Hypnea musciformis</i>	08/05/2006	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Plocamium cartilagineum</i>	14/05/2006	Bel younech	35°54'34.87"N 5°23'41.91"O
	<i>Plocamium coccineum</i>	10/08/2003	Ksar sghir	35°50'52.58"N 5°33'39.04"O
	<i>Sphaerococcus coronopifolius</i>	10/04/06	Bel younech	35°54'34.87"N 5°23'41.91"O
Bonnemaisoniales	<i>Asparagopsis armata</i>	15/06/2006	Dalya	35°54'24.20"N 5°28'18.84"O
Rhodymeniales	<i>Rhodymenea pseudopalmata</i>	16/08/2005	Ksar sghir	35°50'52.58"N 5°33'39.04"O

Table 2. Antimicrobial activities of the seaweeds species by disc-diffusion technique.

Order	Methanolic extracts	<i>E. coli</i> ATCC 25922	<i>K.pneumoniae</i> ATCC 700603	<i>E. faecalis</i> ATCC 29212	<i>E. faecalis</i> ATCC 29213	<i>S. aureus</i> ATCC 25923
Ceramiales	<i>A. corallinum</i>	11.67±0.57	10.00±0.00	-	-	16.67±0.57
	<i>C. clavulatum</i>	-	-	-	-	15.00±0.00
	<i>C. granullatum</i>	-	-	-	-	19.00±0.00
	<i>C. rubrum</i>	16.00±0.00	-	12.33±0.57	-	25.33±0.57
	<i>H. incurvus</i>	15.33±0.57	11.67±0.57	11.67±0.57	10.00±0.00	24.67±1.15
	<i>O. pinnatifida</i>	-	-	-	-	-
	<i>P. complanata</i>	9.00±0.00	-	14.33±0.57	16.00±0.00	20.67±0.57
	<i>P. thyoides</i>	-	-	-	-	13.00±0.00
Gelidiales	<i>G. attenatum</i>	11.67±1.52	10.67±0.57	-	10.00±0.00	19.00±0.00
	<i>G. latifolium</i>	-	-	-	-	14.67±0.57
	<i>G. pusillum</i>	16.33±0.57	9.00±1.00	12.67±0.57	9.00±0.00	26.67±0.57
	<i>G. pulchellum</i>	8.67±0.57	-	15.00±1.00	-	20.00±0.00
	<i>G. sesquipedale</i>	7.67±0.57	-	-	-	18.0±0.00
	<i>G. spinulosum</i>	19.67±0.57	-	15.33±0.57	-	32.67±0.57
	<i>P. capillacea</i>	-	-	-	-	13.00±0.00
Gigartinales	<i>C. acicularis</i>	10.33±2.08	15.67±1.15	-	8.33±0.57	16.33±0.57
	<i>C. ustulatus</i>	-	9.00±0.00	-	-	14.33±0.57
	<i>G. confervoides</i>	-	-	-	-	13.00±0.00
	<i>G. multipartita</i>	-	10.00±0.00	-	-	17.67±0.57
	<i>G. patens</i>	-	-	-	-	10.00±0.00
	<i>H. musciformis</i>	20.67±0.57	19.00±0.00	24.33±1.15	15.33±0.57	35.00±1.73
	<i>P. cartilagineum</i>	8.00±0.00	-	11.67±0.57	13.00±0.00	23.33±0.57
	<i>P. coccineum</i>	-	-	-	-	15.67±0.57
	<i>S. coronopifolius</i>	-	-	-	9.00±0.00	13.33±0.57
Bonnemaisoniales	<i>A. armata</i>	-	-	-	-	20.33±0.57
Rhodymeniales	<i>R. pseudopalmata</i>	-	-	-	-	12.67±0.57

The activity is classified according to the diameter of the zone of inhibition (in mm ±SD) around the sample's point of application. Weak inhibition: ≤10 mm, 10 mm < moderate inhibition ≤ 15 mm, highest inhibition: > 15 mm, - : no activity.

microorganisms tested, with inhibition diameters ranging from 15 to 35 mm.

All extracts of algae tested except *Osmundea pinnatifida* were effective against the gram-positive strain *S. aureus*, which was highly inhibited as by the extracts *H. musciformis*, *Gelidium spinulosum*, *G. pusillum* and *Pterosiphonia complanata*; the diameter of the inhibition zone was 35, 32, 26 and 20 mm, respectively (Figure 1). Rizvi and Shameel (2005) reported that the methanolic extract of the alga *O. pinnatifida* did not inhibit the *S. aureus* and *K. pneumoniae* bacteria. This result is in agreement with the present study. The diameter of the zone of inhibition varies from one algal extract to the other and for the same extract, from one microorganism to the other (Figure 2).

In our findings, all the extracts were active on *S. aureus* ATCC 25923 with the extract of *H. musciformis* showing the greatest inhibition on all the bacteria tested except on the bacteria *E. faecalis* ATCC 29213, on which it showed a moderate inhibition (Table 2). The inhibition effects

from extracts are classified as greatest at weak inhibition as follows, for *E. coli*: *H. musciformis*, *G. spinulosum*, *G. pusillum*, *Ceramium rubrum*, and *H. incurvus* > *Alsidium corallinum*, *Gelidium attenatum*, and *Chondracanthus acicularis* > *Pterosiphonia complanata*, *Gelidium pulchellum*, and *Plocamium cartilagineum*. For *K. pneumoniae*: *H. musciformis*, and *C. acicularis* > *H. incurvus*, and *G. attenatum* > *A. corallinum*, *Gracilaria multipartita*, *Caulacanthus ustulatus*, and *G. pusillum*. For *E. faecalis* ATCC 29212: *H. musciformis*, and *G. spinulosum* > *G. pulchellum*, *P. complanata*, *G. pusillum*, *C. rubrum*, *P. cartilagineum*, and *H. incurvus*. For *E. faecalis* ATCC 29213: *P. complanata*, and *H. musciformis* > *P. cartilagineum*, *H. incurvus*, and *G. attenatum* > *G. pusillum*, *Sphaerococcus coronopifolius*, and *C. acicularis*. For *S. aureus*: *H. musciformis*, *G. spinulosum*, *G. pusillum*, *C. rubrum*, *H. incurvus*, *P. cartilagineum*, *P. complanata*, *Asparagopsis armata*, *G. pulchellum*, *G. attenatum*, *C. granullatum*, *G. sesquipedale*, *G. multipartita*, *A. corallinum*, *C. acicularis*,

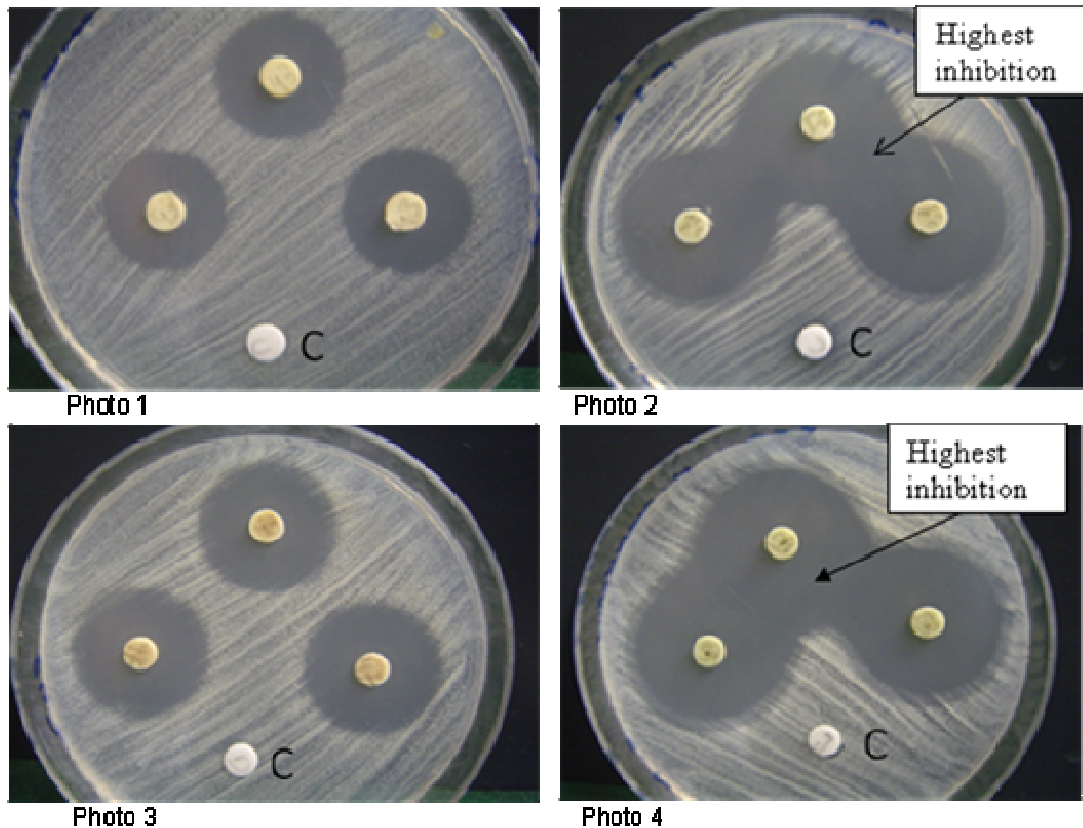


Figure 1. The effect of the methanolic extracts of *P. complanata* (Photo 1), *G. spinulosum* (Photo 2), *G. pusillum* (Photo 3) and *H. musciformis* (Photo 4) on *S. aureus*. C: negative control.

and *Plocamium coccineum* > *Centroceras clavulatum*, *Gelidium latifolium*, *Caulacanthus ustulatus*, *S. coronopifolius*, *Boergesenella thuyoides*, *Pterocladia capillacea*, *Rhodymenia pseudopalmata*, and *Gymnogongrus patens*.

According to these results, it appears that the bacterium *S. aureus* is more sensitive to the methanolic extracts compared to the other bacteria tested, whose great zones of inhibition were shown by the extract of *H. musciformis* and *G. spinulosum*.

DISCUSSION

The antibacterial activity was screened against widely-distributed *E. coli*, *E. faecalis*, *S. aureus* and *K. pneumoniae* pathogens, which cause serious problems for human health. The bacterial strain *S. aureus* is responsible for food poisoning, suppurating located infections and blood-poisoning for debilitated subjects. This study has demonstrated that algae extracted by a methanol solvent showed the greatest inhibition diameters. These results are in agreement with the observations of Vlachos et al. (1996), Gonzalez et al. (2001), Ozdemir et al. (2004), Karabay-Yavasoglu et al. (2007), Taskin et al. (2007)

and, Kandhasamy and Arunachalam (2008), who reported that extracts prepared with methanol showed the best activity. Taskin et al. (2007) reported that the highest activity was shown on *E. coli* ATCC (29998) (32.00 ± 1.73 mm) and *E. faecalis* ATCC (8043) (21.66 ± 0.57 mm) by a methanolic extract of *Corallina officinalis* red algae. Kandhasamy and Arunachalam (2008) observed that methanolic extract of *H. musciformis* is active on the *K. pneumoniae* (13 ± 0.59), *S. aureus* (12 ± 0.69) and *E. faecalis* (12 ± 0.75) bacteria. These results, however, are in contrast with those of Bansemir et al. (2006), who mentioned dichloromethane as the most suitable solvent and those of Lima-Filho et al. (2002), who reported that hexane was the best solvent for extracting antibacterial substances from algae.

Magallanes et al. (2003) reported that the bacterium *E. faecalis* ATCC (29212) was inhibited by the ethanolic extract of *Grateloupia doryphora* (15.7 mm), whereas the bacterium *E. coli* ATCC 25922 was inhibited by ethanolic extracts of *Rhodophyceae* from *Grateloupia doryphora*, *Rhodymenia flabellifolia*, *Gracilariopsis lemaneiformis*, *Ahnfeltiopsis durveillaei*, *Porphyra columbina* and *Cryptopleura* sp. While Sastry and Rao (1994) showed that the methanolic extract of *Gracilaria corticata* red algae was negative on the bacteria *E. coli* ATCC 25922, a chloroform

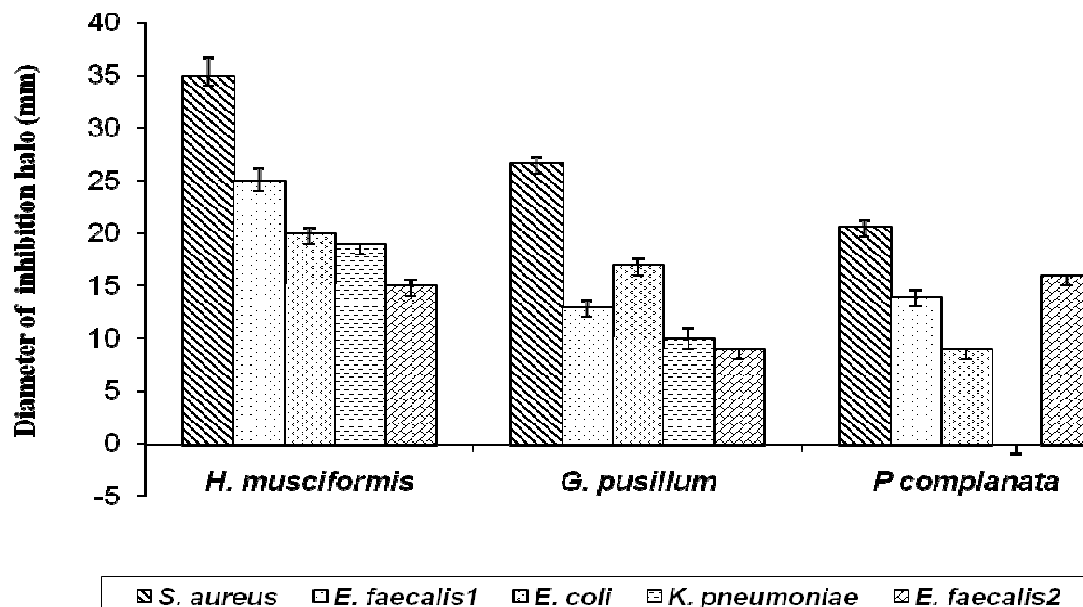


Figure 2. Diameter averages (mm) of the zones of inhibition of methanolic extracts of *H. musciformis*, *G. pusillum* and *P. complanata* against five tested bacterial strains. *E. faecalis 1*: *E. faecalis* ATCC 29212 and *E. faecalis 2*: *E. faecalis* ATCC 29213.

extract had moderate bactericidal activity and a benzene extract had a weak inhibition potential. The three extracts: methanolic, chloroform and benzene of *Acanthophora delilei* did not exhibit any inhibition on this bacterium.

Several studies have detected the antibacterial activities from red algae, the study of Gonzalez et al. (2001) reported that the methanolic extracts of *H. incurvus* and *P. capillacea* did not cause any inhibition of the bacterium *S. aureus* MB5393. Etahiri et al. (2003) mentioned that the methanolic extracts of *H. incurvus*, *H. musciformis*, *P. cartilagineum*, *S. coronopifolius* and *G. latifolium* showed an inhibition on the bacterium *S. aureus* (ATCC 6538) and the result was negative for the extract of *A. armata* on this bacteria. The methanolic extracts from *H. incurvus*, *S. coronopifolius*, and *A. armata* did not show any activity on the bacterium *E. coli* (ATCC 10536). The methanolic extract of the seaweed *H. musciformis* exhibited strong antibacterial activity against the gram positive and seven gram negative bacteria (Siddiqui et al., 1993). Bansemir et al. (2006) investigated the antibacterial activities of extracts from 26 algae species prepared with dichloromethane against five fish-pathogenic bacteria. He reported that the most active algal species was *A. armata* against all tested bacteria. The dichloromethanolic extracts of *H. incurvus* and *C. rubrum* showed their highest activity against the marine bacterium *Pseudomonas anguilliseptica*, while the extract of *P. cartilagineum* showed a weak activity on these five fish-pathogenic bacteria. The extract methanol-dichloromethane (1:1) of *H. musciformis* inhibited the growth of gram positive strains (*Bacillus cereus*, *Bacillus subtilis* and *Micrococcus luteus*), to the extent of 66.0% at

30°C, whereas all the gram positive bacteria were susceptible at 20°C (Selvin and Lipton, 2004). The antibacterial activity of methanol/toluene (3:1) extract from *G. pusillum* and *S. coronopifolius* did not show any inhibition on strains of *E. coli* and *B. subtilis* (Ballesteros et al., 1992). On the other hand, the observations of Caccamese et al. (1980, 1981) reported that, methanol-toluene (3:1) extracts from *S. coronopifolius* and *P. cartilagineum* inhibit the bacteria strain *B. subtilis*.

The higher frequency of activity against gram-positive bacteria has also been observed in most of the surveys on antimicrobial activities from seaweeds reported in literature (Gonzalez et al., 2001; Etahiri et al., 2003). The present investigation revealed that *S. aureus* was more sensitive than all the extracts, with the largest inhibition diameter. It was reported that the gram-positive bacterial strains were more susceptible to seaweed extract than gram-negative bacterial strains (Ballantine et al., 1987). Similar results were obtained by Ballantine et al. (1987), Rossell and Srivastava (1987) and Magallanes et al. (2003).

In the literature, the majority of the compounds responsible for the antibacterial activity at the marine algae are of terpenic (König et al., 1999a; Etahiri et al., 2001; Wang et al., 2007; Smyrniotopoulos et al., 2008; Vairappan et al., 2008; Chakraborty et al., 2010), phenolic (Xu et al., 2003; Oh et al., 2008; Etahiri et al., 2007) and lipidic nature (Findlay and Patil, 1986; Sidqqiui et al., 1993; Al-Fadhli et al., 2006; Paul et al., 2006). Two bromoditerpenes were isolated from *S. coronopifolius*, 12 S-hydroxybromosphaerodiol and bromosphaerone; these compounds showed an antibacterial activity against the

bacteria gram positive like *S. aureus* with an inhibiting minimal concentration of 0.104 and 0.146 μM , respectively (Etahiri et al., 2001). The phenolic compound 3,4,6-tribromo-5-methoxymethyl-benzene-1, 2-diol which was found at the species *P. complanata* proved to have an antibacterial activity against several positive and negative pathogenic bacteria gram. Its inhibiting minimal concentration against the bacterium *S. aureus* was 2.8 $\mu\text{g/ml}$ (Etahiri et al., 2007), and thus, the fatty-acids which were found in the extract methanolic of the alga *H. musciformis* exhibited strong antibacterial activity against the gram positive and seven gram negative bacteria (Sidqqiui et al., 1993).

Differences between the results of the present investigation and those of other studies may be due to the organic solvents used for the extraction of bioactive compounds and the differences in the assay methods, the geographical zone and the seasonal production of bioactive compounds. Salvador et al. (2007) studied the antimicrobial activities of 82 marine algae in fresh and lyophilized forms and according to a seasonal variation, they reported that red algae had both the highest values and the broadest spectrum of bioactivity. The highest percentages of active extracts of *Phaeophyceae* and *Rhodophyceae* were found in the autumn, whereas, they were found in the summer for *Chlorophyceae*. Etahiri et al. (2003) showed that algae extracts collected in the spring were significantly more active than those collected in the winter.

According to these reports, and taking into account the results detailed in the present contribution, it appears that the seaweeds from our coasts possess significant bioactive capacities, and thus deserve a place in marine biotechnology programmes to examine the properties of natural products. The extracts of *H. musciformis*, *G. spinulosum*, *C. rubrum*, *H. incurvus*, *P. complanta* and *G. pusillum* showed a real potential with good yields. These results suggest the possibility of using marine algae extracts in therapy as natural alternatives to antibiotics currently in the market, and clearly show that seaweeds from the Mediterranean coast of Morocco are valuable source of biologically active compounds. Further research is underway to determine the structure and nature of these antibacterial substances.

ACKNOWLEDGEMENTS

This work was supported by the SAIC (Spanish Agency of International Culture) Morocco-Spain, Project n° A/4931/06 and a grant from the Unesco-Oreal Organization.

REFERENCES

Abourriche A, Charrouf M, Berrada M, Bennamara A, Chaib N, Francisco C (1999). Antimicrobial activities and cytotoxicity of the brown alga *Cystoseira tamariscifolia*. *Fitoterapia*, 70: 611-614.

Al-Fadhli A, Wahidulla S, D'Souza L (2006). Glycolipids from the red

alga *Chondria armata* (Kütz) Okamura. *Glycobiology*, 16: 902-915.

Awad NE (2000). Biologically active Steroid from the green alga *Ulva lactuca*. *Phytother. Res.* 14: 641-643

Awad NE (2004). Bioactive brominated diterpenes from the marine red alga *Jania rubens* (L.) Lamx. *Phytother. Res.* 18: 275-279.

Ballantine DL, Gerwick WH, Velez SM, Alexander E, Guevara P (1987). Antibiotic activity of lipid-soluble extracts from Caribbean marine algae. *Hydrobiologia*, 151/152: 463-469.

Bansemir A, Blume M, Schröder S, Lindequist U (2006). Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture*, 252: 79-84.

Bazes A, Silkina A, Defer D, Bernède-Baudin C, Quéméner E, Braud JP, Bourgougnon N (2006). Active substances from *Ceramium botryocarpum* used as antifouling products in aquaculture. *Aquaculture*, 258: 664-674.

Bourgougnon N, Roussakis C, Kornprobst JM, Lahaye M (1994). Effects *in vitro* of sulphated polysaccharide from *Schizymenia dubyi* (Rhodophyta, Gigartinales) on a non-small-cell bronchopulmonary carcinoma line (NSCLC-N6). *Cancer Lett.* 85: 87-92.

Bourgougnon N, Lahaye M, Chermann JC, Kornprobst JM (1993). Composition and antiviral activities of a sulphated polysaccharide from *Schizymenia dubyi* (Rhodophyta, Gigartinales). *Bioorgan. Med. Chem. Lett.* 3: 1141-1146.

Chakraborty K, Lipton AP, Raj RP, Vijayan KK (2010). Antibacterial labdane diterpenoids of *Ulva fasciata* Delile from southwestern coast of the Indian Peninsula. *Food Chem.* 119: 1399-1408.

De Nys R, Steinberg PD, Willemsen P, Dworjanyn SA, Gabelish CI, King RJ (1995). Broad spectrum effects of secondary metabolites from the red alga *Delisea pulchra* in antifouling assays. *Biofouling*, 8: 259-271.

Etahiri S, Bultel-Poncé V, Elkouri AE, Assobhei O, Zaoui D, Guyot M (2003). Antibacterial activities of marine algae from the Atlantic coast of Morocco. *Mar Life* 13: 3-9.

Etahiri S, Bultel-Poncé V, Caux C, Guyot M (2001). New bromoditerpenes from the red alga *Sphaerococcus coronopifolius*. *J. Nat. Prod.* 64:1024-1027

Etahiri S, El kouri A, Bultél-Poncé V, Guyot M, Assobhei O (2007). Antibacterial bromophenol from the marine red alga *Pterosiphonia complanata*. *J. Nat. Prod. Com* #2 (7): 749-752.

Fenical W, Paul VJ (1984). Algae in medicine and pharmacology. *Hydrobiologia*, 116/117: 135-170.

Fenical W (1981). Natural halogenated organics, chap 12, Elsevier oceanography series, pp. 375-393.

Freile-Pelegrin Y, Morales JL (2004). Antibacterial activity in marine algae from the coast of Yucatan, Mexico. *Botanica Marina*, 47: 140-146.

Findlay JA, Patil DA (1986). Antibacterial constituents of the red alga *cystoclonium purpureum*. *Phytochemistry*, 25: 548-550.

Hellio C, Bremer G, Pons AM, Le Gal Y, Bourgougnon N (2000). Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany, France. *Appl. Microbiol. Biotechnol.* 54: 543-549.

Hellio C, De La Broise D, Dufossé L, Le Gal Y, Bourgougnon N (2001). Inhibition of marine Bacteria by extracts of macroalgae: potential use for environmentally friendly antifouling paints. *Marine Environ. Res.* 52: 231-247.

Ghosh P, Adhikari U, Ghosal PK, Pujol CA, Carlucci MJ, Damonte EB, Ray B (2004). *In vitro* anti-herpetic activity of sulphated polysaccharide fractions from *Caulerpa racemosa*. *Phytochemistry*, 65: 3151-3157.

Glombitza KW, Sukopp I, Wiedenfeld H (1985). Antibiotics from algae XXXVII. Rhodomelol and Methylrhodomelol from *Polysiphonia lanosa*. *Planta Med.* pp. 116-120.

Gonzalez Del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I, Vicente F, Portillo E, Jiménez Del Rio M, Reina GG, Peláez F (2001). Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int. Microbiol.* 4: 35-40.

Hudson JB, Kim JH, Lee MK, De Wreede RE, Hong YK (1999). Antiviral compounds in extracts of Korean seaweeds: Evidence for multiple activities. *J. Appl. Phycol.* 10: 427-434.

Kandhasamy M, Arunachalam KD (2008). Evaluation of *in vitro* anti-

- bacterial property of seaweeds of southeast coast of India. Afr. J. Biotechnol. 7: 1958-1961.
- Karabay-Yavasoglu NU, Sukatar A, Ozdemir G, Horzum Z (2007). Antimicrobial activity of volatile components and various extracts of the red alga *Jania rubens*. Phytother. Res. 21: 153-156.
- König GM, Wright AD, Linden A (1999a). *Plocamium hamatum* and its monoterpenes: chemical and biological investigations of the tropical marine red alga. Phytochemistry, 52: 1047-1053.
- König GM, Wright AD, De Nys R (1999b). Halogenated monoterpenes from *Plocamium costatum* and their biological activity. J. Nat. Prod. 62: 383-385.
- König GM, Wright AD, Stiche O, Angerhofer CK, Pezzuto JM (1994). Biological activities of selected marine natural products. Planta Med. 60: 532-537.
- Kornprobst JM (2005). Substances naturelles d'origine marine, Tome 1: Généralités, micro-organismes, algues. Editions Tec & Doc, Paris, pp. 598.
- Lima-Filho JVM, Carvalho AFFU, Freitas SM, Melo VMM (2002). Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian coast. Braz. J. Microbiol. 33: 311-313.
- Magallanes C, Cordova C, Orozco R (2003). Antibacterial activity of ethanolic extracts of marine algae from central coast of Peru. Rev. Peru Boil. 10: 125-132.
- Moujahidi A, Bencharki B, Hilali L, Bagri A, Najim L (2004). Activités antibactérienne et antifongique des extraits d'algues marines d'origine marocaine. Rev. Biologie et Santé Vol. 2, No. 2.
- Mayer AMS (2002). Current marine pharmacology contributions to new drug development in the biopharmaceutical industry. Pharmaceut. News, 9: 479-482.
- Mayer AMS, Rodríguez AD, Berlinck RGS, Hamann MT (2007). Marine pharmacology in 2003-2004: Marine compounds with anthelmintic antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action, Comparative Biochemistry and Physiology Part C: Toxicol. Pharmacol. 145: 553-581.
- Mayer AMS, Rodríguez AD, Roberto BGS, Hamann MT (2009). Marine pharmacology in 2005-2006: Marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. Biochimica and Biophysica Acta (BBA)-General Subjects, 1790: 283-308.
- McConnell O, Fenical W (1977). Halogen chemistry of the red alga *Asparagopsis*. Phytochemistry, 16: 367-374.
- Moreau J, Pesando D, Caram B (1984). Antifungal and antibacterial screening of Dictyotales from the French Mediterranean coast. Hydrobiologia, 116/117: 521-524.
- Mtolera MSP, Semesi AK (1996). Antimicrobial activity of extracts from six green algae from Tanzania. Current Trends in Marine Botanical Research in East African Region: pp. 211-217.
- Nagayama K, Iwamura Y, Shibata T, Hirayama I, Nakamura T (2002). Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. J. Antimicrob. Chemother. 50: 889-893.
- Oh KB, Lee JH, Chung SC, Shin J, Shin HJ, Kim HK, Lee HS (2008). Antimicrobial activities of the bromophenols from the red alga *Odonthalia corymbifera* and some synthetic derivatives. Bioorgan. Med. Chem. Lett. 18: 104-108.
- Ozdemir G, Karabay NU, Dalay MC, Pazarbasi B (2004). Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytother. Res. 18: 754-757.
- Paul NA, De Nys R, Steinberg PD (2006). Chemical defence against bacteria in the red alga *Asparagopsis armata*. Marine Ecol. 306: 87-101.
- Rizvi MA, Shameel M (2005). Pharmaceutical biology of seaweeds from the Karachi coast of Pakistan. Pharmaceut. Biol. 43: 97-107.
- Rossel KG, Srivastava LM (1987). Fatty acids as antimicrobial substances in brown algae. Hydrobiologia, 151/152: 471-475.
- Salvador N, Garreta AG, Lavelli L, Ribera MA (2007). Antimicrobial activity of Iberian macroalgae. Scientia Marina, 71: 101-113.
- Sastry VMVS, Rao GRK (1994). Antibacterial substances from marine algae: successive extraction using Benzene, Chloroform and Methanol. Botanica Marina, 37: 357-360.
- Selvin J, Lipton AP (2004). Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the peninsular coast of India. J. Marine Sci. Technol. 12: 1-6.
- Serkedjieva J (2000). Antiherpes virus effect of the red marine alga *Polysiphonia denudata*. Z Naturforsch. 55c: 830-835.
- Serkedjieva J (2003). Antiviral activity of the red marine alga *Ceramium rubrum*. Phytother. Res. 18: 480-483.
- Shanmugam M, Mody KH (2000). Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. Curr. Sci. 79(12).
- Siddiqui S, Shyum SBN, Usmanhany K, Shameel M (1993). Antibacterial activity and fatty acid composition of the extract from *Hypnea musciformis* (Gigartinales, Rhodophyta). Pak. J. Pharm. Sci. 6: 45-51.
- Smyrniotopoulos V, Vagias C, Rahman MM, Gibbons S, Roussis V (2008). Brominated diterpenes with antibacterial activity from the red alga *Sphaerococcus coronopifolius*. J. Nat. Prod. 71(8): 1386-1392.
- Souhaili N, Lagzouli M, Faid M, Fellat-Zerrouk K (2004). Inhibition of growth and mycotoxins formation in moulds by marine algae *Cystosiera tamariscifolia*. Afr. J. Biotechnol. 3: 71-75.
- Suzuki M, Daitoh M, Vairappan CS, Abe T, Masuda M (2001). Novel halogenated metabolites from the Malaysian *Laurencia pannosa*. J. Nat. Prod. 64: 597-602.
- Tariq VN (1991). Antifungal activity in crude extracts of marine red algae. Mycol. Res. 95: 1433-1440.
- Taskin E, Ozturk M, Taskin E, Kurt O (2007). Antibacterial activities of some marine algae from the Aegean Sea (Turkey). Afr. J. Biotechnol. 6: 2746-2751.
- Vairappan CS, Daitoh M, Suzuki M, Abe T, Masuda M (2001a). Antibacterial halogenated metabolites from the Malaysian *Laurencia* species. Phytochemistry, 58: 291-297.
- Vairappan CS, Suzuki M, Abe T, Masuda M (2001b). Halogenated metabolites with antibacterial activity from the Okinawan *Laurencia* species. Phytochemistry, 58: 517-523.
- Vairappan CS (2003). Potent antibacterial activity of halogenated metabolites from Malaysian red algae, *Laurenciamajuscula* (Rhodomelaceae, Ceramiales). Biomol. Eng. 20: 255-259.
- Vairappan CS, Suzuki M, Ishii T, Okino T, Abe T, Masuda M (2008). Antibacterial activity of halogenated sesquiterpenes from Malaysian *Laurencia* spp. Phytochemistry, 69: 2490-2494.
- Vlachos V, Critchley AT, Von HA (1996). Establishment of a protocol for testing antimicrobial activity in southern African macroalgae. Microbios, 88: 115-123.
- Wang G, Tang W, Bidigare RR (2007). Terpenoids as therapeutic drugs and pharmaceutical agents. Natural products: Drug discovery and therapeutic medicine. pp: 197-277.
- Xu N, Fan X, Yan X, Li X, Niu R, Tseng CK (2003). Antibacterial bromophenols from the marine red alga *Rhodomela confervoides*. Phytochemistry, 62: 1221-1224.
- Zandi K, Fouladvand M, Pakdel P, Sartavi K (2007). Evaluation *in vitro* antiviral activity of a brown alga (*Cystoseira myrica*) from the Persian Gulf against *Herpes simplex* virus type 1. Afr. J. Biotechnol. 6: 2511-2514.