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ANTIBACTERIAL ACTIVITY OF THE EXTRACTS OF RHODOPHYCEAE FROM THE ATLANTIC AND THE MEDITERRANEAN COASTS OF MOROCCO

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ABSTRACT

Hundred eight of organic extracts from eighteen red marine algae of Atlantic-Mediterranean have been tested for the production of antibacterial compounds. These extracts were obtained for two methods, maceration and using soxhlet. This study shows that most of the algal extracts were significantly active. The highest rates of biologically activity were found in five species, *Pterosiphonia complanata*, *Sphaerococcus coronopifolius*, *Plocamium cartilagineum*, *Asparagopsis armata* and *Boergeseniella thuyoides*. Among the methanolic and chloroforme-methanolic extracts showed the greatest biologically active.

Keywords: Red algae; antibacterial activity; marine bacteria; terrestrial bacteria, organic extracts

INTRODUCTION

Marine plants have been recognised as producers of biologically active substances. The Moroccan coast is particularly rich in algal biodiversity and constitutes a reserve of species of considerable economic, social and ecologic potential. Subsequent surveys of antibacterial activity in algae have been reported from the Mediterranean (**Chiheb *et al.*, 2009; Bouhlal *et al.*, 2010a**) and the Atlantic (**Abourriche *et al.*, 1999; Etahiri *et al.*, 2003, 2007; Souhaili *et al.*, 2004**) from Morocco, India (**Selvin and Lipton, 2004; Choudhury *et al.*, 2005; Kandhasamy and Arunachalam, 2008**), China (**Jing-Wen and Wei-ci, 1984; Xu *et al.*, 2003**), Japan (**Nagayama *et al.*, 2002; Mori *et al.*, 2003**), Malaysia (**Vairappan, 2003; Vairappan *et al.*, 2008**), Spain (**Ballesteros *et al.*, 1992; Febles *et al.*, 1995; Salvador *et al.*, 2007; Plaza *et al.*, 2009**) and from USA (**Fenical and Paul, 1984; Ballantine *et al.*, 1987; Rosell and Srivastava, 1987; Lustigman and Brown, 1991**).

The marine algae produce many active substances in order to be defended with respect to the bacteria and other micro-organisms (**Fenical and Sims, 1974; Glombitza and Lentz, 1981; Tariq, 1991; Chang *et al.*, 1993; Vairappan, 2003; Xu *et al.*, 2003; Kladi *et al.*, 2004; Cardozo *et al.*, 2007; Pinteus *et al.*, 2011; Rebecca *et al.*, 2012; Genovese *et al.*, 2012**).

Some of these substances were studied (terpenes, phenols, fatty-acids .....) for their biological properties (**Rosell and Srivastava, 1987; Noguchi *et al.*, 1979; Suzuki *et al.*, 1989; Suzuki *et al.*, 2001; Takeda *et al.*, 1990a,b; Che, 1991; Etahiri *et al.*, 2001; Vairappan *et al.*, 2001a, 2001b; Nagayama *et al.*, 2002; Xu *et al.*, 2003; Kuda *et al.*, 2005a, 2005b; Duan *et al.*, 2006; Wang et al., 2005; Chew *et al.*, 2008; Ganesan *et al.*, 2008; Kumar *et al.*, 2008; Smyrniotopoulos *et al.*, 2008; Wang *et al.*, 2009; Wang *et al.*, 2010**). Within Phycophytes, the red algae Rhodophytes are known to present many biological activities and in particular antibacterial, antioxidant and antiviral (**Bouhlal *et al.*, 2010b, 2011**).

The purpose of our work is to research of new antibiotic compounds to be extracted from benthic extracts of Rhodophyceae of the Atlantic-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, from marine algae belonging to five orders: Ceramiales, Corallinales**,** Gelidiales, Gigartinales and Bonnemaisoniales.

MATERIAL AND METHODS

Collection of seaweeds

Seaweeds were collected by hand using Scuba diving or snorkeling (1-4m depth) and preserved on ice until further processing. Eighteen were sampled between 2006 and 2007 at various sites, along the Mediterranean coast (Amsa, Nador), on the Strait of Gibraltar (Ksar-sghir, Dalya, Belyounech) and on the Atlantic coast (Sidi Bouzid) (**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**). Six species belong to the Gelidiales order, three to Gigartinales order, five to Ceramiales order, three to Corallinales order and one to Bonnemaisoniales.

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). In the first case (Soxhlet), powder 5g of alga is extracted by 200 ml from solvent (methanol 100%, chloroform/methanol (3: 2), dichloromethane (100%) during 8h of extraction. For the maceration, 5g of the powder is soaked in 50 ml of solvent (methanol 100%, chloroform/methanol (3: 2), dichloromethane 100%) during 72h.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**).

Table 1 The species of algae collected along the coast of Morocco Atlantic-Mediterranean

Seaweeds		Collection site	Collection date
Orders	**Species**		
Bonnemaisoniales	*Asparagopsis armata* Harvey C	Ksar sghir	19/07/2007
Ceramiales	*Alsidium corallinum* C. Agardh	Nador	10/07/2006
	Ceramium rubrum C. Agardh	Ksar sghir	31/07/2007
	Halopitys incurvus (Gomel) Kützing	Sidi bouzid	02/08/2007
	Boergeseniella thuyoides (Harvey) Kylin C	Ksar sghir	31/07/2007
	Pterosiphonia complanata (Clemente) Falkenberg C	Ksar sghir	24/03/2007
Corallinales	*Corallina meditteranea* J.E. Areschoug S	Ksar sghir	15/08/2007
	Corallina officinalis Linnaeus	Sidi bouzid	02/08/2007
	Jania rubens (Linnaeus) J.V. Lamouroux	Amsa	14/07/2007
Gelidiales	*Gelidium attenatum* (Turner) Thuret P	Dalya	15/06/2006
	Gelidium pulchellum (Furner) Kützing C	Sidi bouzid	18/08/2007
	Gelidium pusillum (Stackhouse) Le Jolis C	Ksar sghir	31/07/2007
	Gelidium sesquipedale (Clemente) Thuret S	Ksar sghir	12/08/2007
	Gelidium spinulosum (C. Agardh) J. Agardh P	Ksar sghir	26/06/2007
	Pterocladia capillacea (S.G. Gmelin) Bornet S	Ksar sghir	15/08/2007
Gigartinales	*Hypnea musciformis* (Wulfen) J.V. Lamouroux	Ksar sghir	19/07/2007
	Plocamium cartilagineum (Linnaeus) P.S. Dixon C	Belyounech	14/05/2006
	Sphaerococcus coronopifolius Stackhouse	Belyounech	13/04/2006

Bacterial strains

Six marine bacteria were supplied by the Drs of the Broise and Dufosse of the University Laboratory of Biodiversity and Microbial Ecology (LUBEM). Eleven terrestrial bacteria were supplied by the Laboratory of Biotechnology and Chemistry Marines (5 bacteria) and The Pasteur institute (six bacteria) (Table 2). Bacterial strains are regularly preserved on solid medium (Marine Agar, DIFCO or Muller Hinton, MH) prepared in the Petri dish (every other week) and on slant agar (once both months) (Marine Agar, DIFCO or Muller Hinton). The strains were also kept in glycerine (20 %) in - 80°C.

Table 2 Strains of bacteria

Marine bacteria	Gram	References
Cytophaga	-	1M6
Micrococcus	+	5J6
Bacillus	+	4J6
Pseudoalteromonas	-	3J3
Pseudoalteromonas	-	3J6
Paracoccus	-	4M6
Terrestrial bacteria		
Escherichia coli	-	AL52
Escherichia coli	-	HB 101
Escherichia coli	-	K-12
Escherichia coli	-	CIP 54.117
Escherichia coli	-	ATCC 25922
Hafnia alvei	-	CIP 57.31
Pseudomonas aeruginosa	-	ATCC27853
Staphylococcus epidermidis	+	CIP 53.124
Staphylococcus haemolyticus	+	CIP 81.56
Staphylococcus simulans	+	CIP 81.64
Staphylococcus hominis	+	CIP 81.57

Antibacterial activity by disc diffusion assay

The screening of the antibacterial activity of the organic 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 (30 µl) were loaded on each of these discs, allowed to dry overnight at room temperature for 24 h to evaporate solvent, and then tested in antibacterial activity. A bacterial suspension (10^6 CFU/mL) was spread on Mueller-Hinton (pH 7.4) agar or on Marine Difco-Agar using a cotton swab. After incubation for 24 h at 37°C for the terrestrial bacteria and at 48h or 96h at 22°C for the marine bacteria.

The antibacterial activity was quantified as the diameter (mm) of the growth inhibition zones. Control paper discs with the solvents (100%) were tested for every assay and showed no antibacterial activity. Four positive controls: polymyxine B, lysozyme, ampicilline, tetracycline, were also tested on bacterial strains. All the tests of inhibition were carried out in triplicate.

Determination of the minimum inhibitory concentrations

Determination of the minimum inhibitory concentrations (MICs) was carried out for the bacteria by the macrodilution method for testing the antimicrobial activity of the algal extract. The extracts concentration-tested were 2,5 ; 1,25 ; 0,62 ; 0,31 ; 0,15 ; 0,075 ; 0,037 and 0,018 $mg.mL^{-1}$. Microorganisms (2.10^6 CFU/mL) were placed in a liquid medium consisting either of MH (Mueller Hinton) medium for *Escherichia coli* AL52 (Gram -), *Staphylococcus epidermidis* (Gram +) and *S. haemolyticus* (Gram +), or marine broth medium for *Pseudoalteromonas* 3J3 (Gram -), *Bacillus* 4J6 (Gram +), containing the algal extracts for testing. After incubation for 24 h at 37 °C for terrestrial bacteria and for 72h at 22 °C for marine bacteria, MIC represents the lowest concentration that inhibits the organism's growth.

RESULTS AND DISCUSSION

Antibacterial activity by disc diffusion assay

The organic solvents (100%) used as negative control showed no inhibitory action of the growth of bacteria studied (Table 3-6). The organic extracts showed variable degrees of inhibition of bacteria. Some strains were more resistant to algae extracts, these are strains of Gram-negative terrestrial bacteria (*Escherichia coli* HB101, *Escherichia coli* ATCC 25922, *Escherichia coli* K12, *Escherichia coli* CIP 54117, *Hafnia alvei*, *Pseudomonas aeruginosa*) and of Gram-negative marine bacteria, *Paracoccus* 4M6, 3J6 *Pseudoalteromonas* and a Gram-positive marine bacteria Micrococcus 5J6.

The extracts of seaweeds *Alsidium corallinum, Corallina meditteranea, Corallina officinalis, Ceramium rubrum, Gelidium pulchellum, Gelidium sesquipedale, Jania rubens, Pterocladea capillacea* present a spectre of activity more restricted with low diameters of inhibition in the range from 7 to 11.5 mm. Extracts CH/M (macer) of *Ceramium rubrum* and *Gelidium pulchellum* is effective on the bacteria *Escherichia coli* AL52 with inhibition zones of 12.5 and 15.5 mm respectively (Table 3).

Twenty-nine seaweed extracts present no activity on the strains tested, these extracts are *Alsidium corallinum* (DMsox, DMmacer, CH/Msox), *Corallina Meditteranea* (Msox, DMsox), *Corallina officinalis* (DMsox), *Ceramium rubrum* (Mmacer, DMsox, DMmacer, CH/ Msox), *Gelidium attenatum* (DMsox, DMmacer), *Gelidium pulchellum* (Mmacer, DMmacer), *Gelidium sesquipedale* (DMsox, DMmacer), *Halopitys incurvus* (Mmacer , DMsox), *Hypnea musciformis* (DMsox), *Jania rubens* (Mmacer, DMsox, DMmacer, CH/Msox), *Pterocladea capillacea* (Mmacer, DMsox, DMmacer, CH/Msox) and *Boergeseniella thuyoides* (DMsox, DMmacer) .

The evaluation of the antibacterial activity showed inhibitory effects of 77 extracts towards at least one of the strains studied (Table 3). Obtained results, it appears that the dichloromethanolic extracts are little active compared to the methanol extracts and chloroform-methanol. In this case, the extracts which presented an average activity are those of DM (maceration) of *Plocamium cartilagineum* on BM4, BM5, BM6, BT16 with 13.00, 14.5, 12.0, 15.5 mm respectively, and a highest inhibition is present in the extract DM (maceration) of *Plocamium cartilagineum* on BM2 with 17.00 mm.

In the case of methanol extracts, an moderate activity is revealed by the extracts of M (macer) of *Gelidium attenatum* on BT14 and BT16 with 15.00 mm and on BT17 with 14.5 mm, M (sox) of *Halopitys incurvus* on BT14, BT16 with 14.0, 12.5 mm respectively, M (macer) of *Gelidium pusillum* on BT17 with 13.5 mm, M (macer) of *Hypnea musciformis* on BT15, BT17 with 15.0, 12.0 mm respectively, M (macer) of *Plocamium cartilagineum* on BM6 with 12.0 mm, M (sox) of *Plocamium cartilagineum* on BM4 with 12.0 mm, M (sox) of *Pterosiphonia complanata* on BT10, BT13 by 15.0, 12.5 mm respectively, M (macer) of *Pterosiphonia complanata* on BM5, BM6, BT7, BT10 by 14.0, 14.5, 12.0, 13.5 mm, M (sox) of *Sphaerococcus coronopifolius*, on BM2, BM4 and BM6 with 14.0, 14.5 and 12.5 mm respectively, M (sox) of *Boergeseniella thuyoides* on BM4 with 14.0 mm, M (macer) of *B. thuyoides* on BM2 with 14.0 mm. High activity is found in methanol extracts of *Pterosiphonia complanata* M (sox) on BM1, BM2, BM4, BM5, BM6, BT14, BT15, BT16, BT17 with 20.0, 30.5, 27.0, 20.5, 26.5, 24.5, 19.0, 19.0, 21.5 mm respectively, M (macer) on BM1, BM2, BM4, BT14, BT15, BT16, BT17 with 20.5, 27.0, 18.5, 26.5, 16.5,

20.0, 18.5 mm, M (sox) of *Boergeseniella thuyoides* on BM2 and BM5 with 16,5 and 16,0 mm, M (sox) of *Halopitys incurvus* on BT10 with 16.0 mm, M (macer) of *Gelidium attenatum* inhibits the growth of BM1 by a zone of inhibition of 19.5 mm, M (macer) of *Hypnea musciformis* on BT14 with 19.0 mm, M (sox) of *Halopitys incurvus* on BT10, 16.0 mm, M (macer) of *Hypnea musciformis* on BT14, with 19.0 mm.

In the case of chloroform-methanol extracts, the moderate activity was shown by extracts CH/M (macer) of *Ceramium rubrum* on BT10 with 12.5 mm, CH/M (macer) of *Gelidium pulchellum* on BT10 with 15.5 mm, CH/M (macer) of *Gelidium pusillum* on MB2 and BT10 with 13.5 and 12.00 mm respectively, DM (sox) of *Gelidium pusillum* on BT10 with 12.5 mm, CH/M (macer) of *Gelidium spinulosum* on BM2 and BM4 with 15.5 and 12.5 mm respectively, CH/M (sox) of *Halopitys incurvus* on BT10 with 12.0 mm, CH/M (macer) of *Plocamium cartilagineum* on BM2, BM4, BM6, BT16 with 14.0, 15.0, 12.0, 15.0 mm respectively, CH/M (sox) of *Pterosiphonia complanata* on BM5, BT10, BT15 by 14.0, 13.5, 14.5 mm respectively, CH/M (macer) of *Pterosiphonia complanata* on BM5, BT15, BT16, BT17 by 14.5, 14.5, 15.5, 15.5 mm respectively, CH/M (sox) of *Sphaerococcus coronopifolius* on BM4 with 12.5 mm, CH/M (macer) of *Sphaerococcus coronopifolius* on BM2, BM4 and BM6 with 15.0, 12.5, 13.0 mm respectively, CH/M (macer) of *Boergeseniella thuyoides* on BM5 with 13.5 mm. The high activity is shown by the extracts of *Pterosiphonia complanata*, CH/M (sox) on BM2, BM4, BT7, BT16, BT17 with 30.0, 25.0, 24.5, 16.5, 21.0, 20.0 mm respectively and CH/M (macer) on BM1, BM2, BM4, BM6, BT10, BT14 by 19.5; 32.0, 26.5, 30.0, 16.0, 20.0 mm respectively. Then, *Boergeseniella thuyoides* CH/M (macer) on BM2 with 17.0 mm, CH/M (sox) on BM2 and BM4 with 17.0, 16.5 mm respectively.

In some species, the antibacterial activity is comparable from one bacterium to another while extractions are different. Indeed, there is a similar activity in extracts as, M (sox, macer) *P. cartilagineum* on BM2, BT14, BT16, CH / M (sox, macer) of *G.attenatum* on BM2 and BT10, M (sox, macer) of *G. attenatum* on BM4 and BM5, M (sox, macer) of *G. pusillum* on BM2, BM4, BT10, BT15, M (sox, Macer) and DM (sox, macer) of *G. spinulosum* on BM2, CH / M (sox, macer) of *B.thuyoides* on BM2, BM4, M (sox, macer) of *P. complanata* on BM1, BT9, BT14, BT16, CH / M (sox, macer) of *P. complanata* on BM2, BM4, BM5, BT12, BT13, M (sox, macer) of *S. coronopifolius* on M6, BT14, BT15, BT16, DM (sox, macer) of *S. coronopifolius* on BM2, BM4, BM6 and CH / M (sox, macer) of *S. coronopifolius* on BM4, BT14, BT16, respectively, obtained after the hot extraction (using soxhlet) and the cold extraction (by maceration).

Conversely, to the other species such as *Asparagopsis armata* and *Pterosiphonia complanata*, extracts obtained by Soxhlet gave better inhibition than those obtained by maceration especially for extraction by solvents methanol and chloroform-methanol. For the species *Plocamium cartilagineum*, the extract DM obtained after maceration has good inhibitory activity on bacteria, BM2, BM4, BM5 and BT16. Some algae extracts showed better inhibition by extraction with soxhlet ((eg. extracts M (sox) and CHM (sox) of *A. Armata*, M (sox) *B thuyoides*., M (sox) of *H. incurvus*)). Extraction by maceration is more effective for others ((eg extract DM (maceration) of *P. cartilagineum*, M (maceration) of *H. Musciformis*, M (maceration) of *G. attenatum*)).

This difference in activity may reflect the sensitivity of the active substances or not to heat. Indeed, some compounds can be degraded directly by heat: on the other hand, the other substances require an increase in temperature so that they are extracted. We notice that there is no specificity extracts towards Gram-positive or Gram-negative bacterial strains. However, we can notice that there is a general influence, which depends on the sensitivity of the strains studied to extract. The marine bacteria *Pseudoalteromonas* 3J3, *Bacillus* 4J6 and the terrestrial bacteria *Escherichia coli* AL52, *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* are the most sensitive to extracts with regard to other bacteria.

Table 3 Inhibition of the growth of the terrestrial and marine bacteria by extracts obtained by the method of hot extraction (soxhlet = Sox) and the method of cold extraction (maceration = Macer), Weak inhibition: ≤10 mm, 10 mm < moderate inhibition ≤ 15 mm, highest inhibition: > 15 mm. - : no activity.

Solvent		Extraction	BM1 G-	BM2 G-	BM3 G-	BM4 G+	BM5 G-	BM6 G+	BT7 G-	BT8 G-	BT9 G-	BT10 G-	BT11 G-	BT12 G-	BT13 G-	BT14 G+	BT15 G+	BT16 G+	BT17 G+
A. armata	M	Sox	8.5±0.7	**14.5±0.7**	8.0±0.0	**13.0±0.0**	**12.5±0.7**	11.5±0.7	-	-	-	8.0±0.0	-	-	-	8.0±0.0	10.0±1.4	-	7.5±0.7
		Macer	-	9.0±0.0	-	-	8.0±0.0	-	-	-	-	9.5±0.7	-	-	-	10.5±0.7	**12.0±1.4**	-	-
	DM	Sox	-	8.0±0.0	-	8.5±0.7	-	-	-	-	-	11.0±0.0	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	**12.0±0.0**	**20.0±0.0**	9.0±0.0	**18.0±0.0**	**14.5±0.7**	**14.0±0.0**	-	-	-	**12.0±0.0**	-	-	-	**20.0±0.7**	**26.02±0.7**	8.0±0.0	9.0±0.0
		Macer	-	10.0±0.0	-	8.0±0.0	-	-	-	-	-	8.5±0.7	-	8.0±0.0	9.0±0.0	**17.5±0.7**	**30.0±0.0**	11.5±0.7	**19.5±0.0**
A. corallinum	M	Sox	-	10.5±0.7	-	7.5±0.7	-	-	-	-	10.0±0.4	-	-	-	-	-	-	-	NT
		Macer	-	11.5±0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	-	-	NT	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	11.0±0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. mediterranea	M	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	7.0±0.0	-	-	-	-	-	-	-	-	-	-	-	-	-
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	10.0±0.0	-	10.5±0.7	-	-	-	-	-	-	-	-	-	9.5±0.7	9.0±0.0	7.0±0.0	9.0±0.0
	CH/M	Sox	-	-	NT	-	-	-	-	-	-	8.0±0.0	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	10.0±0.0	-	-	-	-	-	-	-
C. officinalis	M	Sox	-	-	-	10.0±0.0	-	-	-	-	-	7.0±0.0	-	-	-	-	-	-	-
		Macer	-	-	-	9.0±0.0	-	-	-	-	-	-	-	-	-	-	-	8.0±0.0	-
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	NT	7.0±0.0	-	-	-	-	-	9.0±0.0	-	-	-	-	-	-	-
	CH/M	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	9.0±0.0	-	-	-
		Macer	-	8.5±0.7	-	-	-	-	-	-	-	10.0±0.0	-	-	-	-	-	-	-
C. rubrum	M	Sox	-	-	-	-	8.5±0.7	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	10.0±0.0	-	-	-	-	-	-	-	**12.5±0.7**	-	-	-	-	-	-	-

Table 4 Continued

Solvent		Extraction	BM1^{G-}	BM2^{G-}	BM3^{G-}	BM4^{G+}	BM5^{G-}	BM6^{G+}	BT7^{G-}	BT8^{G-}	BT9^{G-}	BT10^{G-}	BT11^{G-}	BT12^{G-}	BT13^{G-}	BT14^{G+}	BT15^{G+}	BT16^{G+}	BT17^{G+}
G. attenatum	M	Sox	-	-	-	9.5±0.7	9.5±0.7	-	-	-	-	8.5±0.7	-	-	-	-	-	-	-
		Macer	**19.5±0.7**	10.0±0.0	-	9.5±0.7	9.0±0.0	-	-	-	-	7.5±0.7	-	-	-	**15.0±0.0**	9.0±0.0	**15.0±0.0**	**14.5±0.7**
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	-	8.0±0.0	NT	-	-	-	-	-	-	8.0±0.0	-	-	-	-	-	-	-
		Macer	-	8.5±0.7	NT	10.5±0.7	-	-	-	-	-	8.0±0.0	-	-	-	-	-	-	-
G. pulchellum	M	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	9.0±0.0	-	7.0±0.0	7.5±0.7
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DM	Sox	-	-	-	-	-	-	-	-	-	7.5±0.7	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	9.5±0.7	-	7.0±0.0	-
		Macer	-	7.5±0.7	-	-	8.0±0.0	-	-	-	-	**15.5±0.7**	-	-	-	11.0±0.7	8.0±0.0	9.5±0.7	9.5±0.7
G. pusillum	M	Sox	-	11.0±0.0	-	10.0±0.0	10.0±0.0	-	-	-	-	9.0±0.0	-	-	-	**15.5±0.7**	8.5±0.7	8.5±0.7	8.0±0.0
		Macer	-	10.0±0.0	-	9.0±0.0	-	-	-	-	-	8.0±0.0	-	-	-	11.0±0.0	8.5±0.7	9.5±0.7	**13.5±0.7**
	DM	Sox	-	-	-	-	-	9.0±1.4	-	-	-	**12.5±0.7**	-	-	-	-	-	-	-
		Macer	-	10.0±0.0	-	9.5±0.7	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	NT	8.5±0.7	NT	8.0±0.0	-	-	-	-	-	9.5±0.7	-	-	-	-	-	-	-
		Macer	-	**13.5±0.7**	-	10.5±0.7	-	-	-	-	-	**12.0±0.0**	-	-	-	10.5±0.7	7.0±0.0	8.0±0.0	10.5±0.7
G. sesquipedale	M	Sox	-	10.0±0.0	-	10.0±0.0	9.0±0.0	-	-	-	-	8.5±0.7	-	-	-	-	-	-	-
		Macer	-	-	-	9.0±0.0	-	-	-	-	-	-	-	-	-	8.5±0.7	-	-	-
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	7.5±0.7	-	7.0±0.0	-
		Macer	-	7.5±0.7	-	8.0±0.0	-	-	-	-	-	9.0±0.0	-	-	-	-	-	-	-
G. spinulosum	M	Sox	-	7.5±0.7	-	8.0±0.0	-	-	-	-	-	-	-	-	-	8.0±0.7	-	-	-
		Macer	-	8.5±0.7	-	10.0±0.0	7.5±0.7	**13.0±0.0**	-	-	-	8.5±0.7	-	-	-	10.0±0.7	8.5±0.7	8.5±0.7	10.0±0.0
	DM	Sox	-	9.5±0.7	-	11.5±0.7	-	8.0±0.0	-	-	-	10.0±0.0	-	-	-	-	-	-	-
		Macer	-	8.5±0.7	-	9.0±0.0	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	-	8.5±0.7	-	10.0±0.0	-	-	-	-	-	-	-	-	-	9.0±0.7	-	-	8.0±0.0
		Macer	-	**15.5±0.7**	-	**12.5±0.7**	8.0±0.0	-	-	-	-	11.0±1.4	-	-	-	10.5±0.7	9.5±0.7	10±0.0	10.0±0.0

Table 5 Continued

	Solvent	Extraction	BM1^{G-}	BM2^{G-}	BM3^{G-}	BM4^{G+}	BM5^{G-}	BM6^{G+}	BT7^{G-}	BT8^{G-}	BT9^{G-}	BT10^{G-}	BT11^{G-}	BT12^{G-}	BT13^{G-}	BT14^{G+}	BT15^{G+}	BT16^{G+}	BT17^{G+}
H incurvus	M	Sox	-	-	-	9.0±0.0	-	-	-	-	-	16.0±1.4	-	-	-	**14.0±1.4**	-	**12.5±0.7**	9.5±0.7
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.5±0.7
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	7.0±0.0	-	-	-
	CH/M	Sox	-	8.0±0.0	-	-	-	-	-	-	-	**12.0±1.4**	-	-	-	10.0±0.0	-	8.5±0.7	9.5±0.7
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	10.5±0.7	-	9.0±0.0	9.0±0.0
H. musciformis	M	Sox	-	8.0±0.0	-	9.0±0.0	8.0±0.0	-	-	-	-	8.0±0.0	-	-	-	-	9.0±1.4	-	-
		Macer	11.0±0.0	8.5±0.7	-	8.0±0.0	-	-	-	-	-	-	-	-	NT	**19.0±1.4**	**15.0±0.0**	11.0±1.4	**26.0±1.4**
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11.5±0.7	-
	CH/M	Sox	NT	9.5±0.7	NT	9.0±1.4	-	-	-	-	-	7.5±0.7	-	-	-	-	-	-	9.5±0.7
		Macer	-	-	-	-	-	-	-	-	-	9.0±0.0	-	-	-	9.5±0.7	-	-	**12.0±1.4**
J. rubens	M	Sox	-	11.5±0.7	-	**12.5±0.7**	8.0±0.0	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	8.5±0.7	-	-	-	-	-	-	-	**12.5±0.7**	-	-	-	-	-	-	-
P. capillacea	M	Sox	-	-	-	8.5±0.7	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH/M	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Macer	-	-	-	-	-	-	-	-	-	8.5±0.7	-	-	-	-	-	-	-
P. cartilagineum	M	Sox	-	11.5±0.7	-	**12.0±0.0**	11.0±0.0	8.0±0.0	-	-	-	9.5±0.7	-	-	-	8.5±0.7	8.5±0.7	8.5±0.7	-
		Macer	-	10.0±0.0	-	9.5±0.7	-	**12.0±0.0**	-	-	-	8.0±0.0	-	-	-	8.0±0.0	7.0±0.0	8.0±0.0	-
	DM	Sox	NT	9.0±0.0	NT	10.0±0.0	-	10.5±0.7	-	-	-	9.5±0.7	-	-	-	8.0±0.0	-	9.0±0.0	NT
		Macer	-	**17.0±0.0**	-	**13.0±0.0**	**14.5±0.7**	**12.0±0.0**	-	-	-	10.0±0.0	-	-	-	10.5±0.7	9.5±0.7	**15.5±0.7**	10.0±0.0
	CH/M	Sox	-	9.5±0.7	-	11.5±0.7	-	9.0±0.0	-	-	-	8.5±0.7	-	-	-	8.0±0.0	-	8.0±0.0	-
		Macer	-	**14.0±0.0**	-	**15.0±0.0**	9.0±0.0	**12.0±0.0**	-	-	-	11.0±1.4	-	-	-	10.5±0.7	9.5±0.7	**15.0±0.0**	9.5±0.7

Table 6 Continued

Solvent	Extraction	BM1 G-	BM2 G-	BM3 G-	BM4 G+	BM5 G-	BM6 G+	BT7 G-	BT8 G-	BT9 G-	BT10 G-	BT11 G-	BT12 G-	BT13 G-	BT14 G+	BT15 G+	BT16 G+	BT17 G+
P. complanata	Sox	**20.0±1.4**	**30.5±0.7**	11.5±0.7	**27.0±1.4**	**20.5±0.7**	**26.5±0.7**	-	-	8.5±0.7	**15.0±0.0**	-	7.5±0.7	**12.5±0.7**	**24.5±0.7**	**19.0±1.4**	**19.0±1.4**	**21.5±0.7**
M	Macer	**20.5±0.7**	**27.0±0.0**	NT	**18.5±0.7**	**14.0±0.0**	**14.5±0.7**	**12.0±0.0**	-	8.0±0.0	**13.5±0.7**	-	9.0±1.4	10.5±0.7	**26.5±0.7**	**16.5±0.7**	**20.0±0.0**	**18.5±0.7**
DM	Sox	-	**16.0±1.4**	-	**13.0±00**	-	-	-	-	-	10.5±0.7	-	-	-	-	8.0±0.0	-	-
	Macer	NT	**12.0±0.0**	NT	9.0±0.0	-	-	-	-	-	7.0±0.0	-	-	-	-	-	10.5±0.7	-
CH/M	Sox	NT	**30.0±0.0**	NT	**25.0±0.0**	**14.0±0.0**	**24.5±0.7**	**13.5±0.7**	-	8.5±0.7	**14.5±0.7**	-	10.0±0.0	11.5±0.7	-	**16.5±0.7**	**21.0±1.4**	**20.0±0.0**
	Macer	**19.5±0.7**	**32.0±1.4**	11.5±0.7	**26.5±0.7**	**14.5±0.7**	**30.0±0.0**	10.5±0.7	8.5±0.7	10.0±0.0	**16.0±0.0**	9.0±0.0	9.5±0.7	10.5±0.7	**20.0±0.0**	**14.5±0.6**	**15.5±0.7**	**15.5±0.7**
B. thuyoides	Sox	-	**16.5±0.7**	-	**14.0±0.0**	**16.0±1.4**	-	-	-	-	-	-	-	-	-	-	7.0±0.0	-
M	Macer	-	**14.0±0.0**	-	9.0±0.0	-	-	-	-	-	-	-	-	-	9.5±0.7	-	7.5±0.7	-
DM	Sox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Macer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CH/M	Sox	-	**17.0±0.0**	-	**16.5±0.7**	**13.5±0.7**	-	-	-	-	-	-	-	-	11.5±0.7	-	8.0±0.0	-
	Macer	-	**17.0±1.4**	-	**15.0±1.4**	-	-	-	-	9.0±1.4	11.0±0.0	-	-	-	9.0±0.0	-	-	9.0±1.4
S. coronopifolius	Sox	-	**14.0±0.0**	-	**14.5±0.7**	11.5±0.7	**12.5±0.7**	-	-	-	10.0±0.0	-	-	-	8.0±0.0	9.0±0.0	8.0±0.0	-
M	Macer	-	**12.5±0.7**	-	10.5±0.7	-	**13.0±0.0**	-	-	-	8.0±0.0	-	-	-	9.5±0.7	10.0±0.0	9.5±0.7	10.0±0.0
DM	Sox	NT	8.5±0.7	NT	10.0±0.0	-	9.0±1.4	-	-	-	8.5±0.7	-	-	-	-	8.0±0.0	-	-
	Macer	-	9.0±0.0	-	9.0±0.0	-	9.0±0.0	-	-	-	-	-	-	-	8.0±0.0	10.0±0.0	7.0±0.0	-
CH/M	Sox	-	10.0±0.0	-	**12.5±0.7**	10,5±0,7	10.5±0.7	-	-	-	8.5±0.7	-	-	-	8.0±0.0	10.0±0.0	7.0±0.0	-
	Macer	-	**15.0±0.0**	-	**12.5±0.7**	-	**13.0±0.0**	-	-	-	**12.0±0.0**	-	-	-	9.0±0.0	9.0±0.0	8.0±0.0	10.5±0.7
PolymyxineB (5µg/ml)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysozyme (5µg/ml)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ampicilline (5µg/ml)		NT	**22.5±0.5**	NT	8.0±0.3	NT	NT	-	-	-	-	-	-	NT	**18.0±0.1**	**14.0±0.4**	11.5±0,8	**14.0±0.7**
Tetracycline (5µg/ml)		NT	**30.0±0.7**	NT	**22.0±0.8**	NT	NT	-	-	-	-	-	-	NT	10.0±0.6	**16.0±0.9**	-	-
CH/M		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DM		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

BM1 : *Paracoccus 4M6* ; BM2 : *Pseudoalteromonas 3J3* ; BM3 : *Pseudoalteromonas 3J6* ; BM4 : *Bacillus 4J6* ; BM5 : *Cytophaga 1M6* ; BM6 : *Micrococcus 5J6* ; BT7 : *Escherichia coli HB101*; BT8 : *Pseudomonas aeruginosa ATCC 27853* ; BT9 : *Escherichia coli ATCC 25922* ; BT10 : *Escherichia coli AL52* ; BT11 **:** *Escherichia coli K12* ; BT12 : *Escherichia coli* (CIP 54.117) ; BT13 : *Hafnia alvei* (CIP 57.31), BT14 *Staphylococcus epidermidis* (CIP 53.124) ; BT15 : *Staphylococcus haemolyticus* (CIP 81.56); BT16 : *Staphylococcus simulans* (CIP 81.64) ; BT17 : *Staphylococcus hominis* (CIP 81.57). NT: No Tested. Bacteria, G+: Gram-positive; G- : Gram-negative, Macer: maceration, Sox: Soxhlet, CH/M : Chloroform-methanol, DM : Dichloromethano

Some inhibitory activities of algal extracts are as important as those obtained by commercial antibiotics (tetracycline, ampicillin, polymyxin B, lysozyme). It may be noted that strains of *Escherichia coli* HB101, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Escherichia coli* AL52, *Escherichia coli* K12, *Escherichia coli* (CIP 54117), *Hafnia alvei* are resistant to lysozyme, polymyxin B, tetracycline, and ampicillin.

Determination of minimum inhibitory concentration (MIC)

The results of the MIC of methanol extracts and chloroform-methanol (obtained using soxhlet) of algae are presented in Table 4. The controls were realized with ampicillin and tetracycline on the five strains sensitive to algae extracts (Table 7), all strains of terrestrial bacteria showed resistance to ampicillin and tetracycline. However, strains of marine bacteria showed sensitivity levels to tetracycline at 2 µg.mL^{-1} for *Pseudoalteromonas* 3J3 and 1 µg.mL^{-1} for *Bacillus* 4J6.

The results of the CMI on the marine bacterium *Pseudoalteromonas* 3J3 Gram-negative (Table 4) show that 1.25 mg.mL^{-1}, the active extracts are methanolic extracts of *G. pusillum* and *S. coronopifolius*. In 0.62 mg.mL^{-1}, the active fractions are those of methanolic *H. musciformis*, *J. rubens* and that of chloroform-methanol fraction of *S. coronopifolius*. The extract M of *P. complanata* showed an MIC at 0.31 mg.mL^{-1}. The MIC of the methanol extracts of *P. complanata* and *S. coronopifolius* on the Gram-positive marine bacterium *Bacillus* 4J6 are 0.62 mg.mL^{-1} and 2.5 mg.mL^{-1} respectively.

Table 7 Determination of MIC in mg.mL^{-1} for two marine bacteria and three terrestrial bacteria, P 3J3: *Pseudoalteromonas* 3J3, B 4J6: *Bacillus* 4J6, EC_{AL52}: *Escherichia coli* AL52, S e: *Staphylococcus epidermidis*, S h: *Staphylococcus haemolyticus*.

Extracts	*Bacteria strains*				
	P 3J3	*B 4J6*	*EC_{AL52}*	*S e*	*S h*
A. armata (CH/M)	> 5	> 5	> 5	> 5	> 5
A. armata (M)	> 5	> 5	> 5	> 5	> 5
A. corallinum (M)	> 5	> 5	> 5	NT	NT
C. officinalis (M)	> 5	> 5	> 5	NT	NT
C. rubrum (M)	> 5	> 5	> 5	NT	NT
G. attenatum (M)	>5	> 5	**1.25**	NT	NT
G. latifolium (M)	> 5	> 5	NT	NT	NT
G. pulchellum (M)	> 5	> 5	> 5	NT	NT
G. pusillum (M)	**1.25**	> 5	> 5	NT	NT
G. spinulosum (M)	**2.5**	5	> 5	NT	NT
H. incurvus (M)	NT	5	**0.018**	NT	NT
H. musciformis (M)	**0.62**	NT	> 5	NT	NT
J. rubens (M)	**0.62**	> 5	> 5	NT	NT
P. cartilagineum (CH/M)	**2,5**	> 5	> 5	> 5	> 5
P. cartilagineum (M)	**2.5**	5	2,5	> 5	> 5
P. complanata (CH/M)	**0.31**	**0.62**	5	> 5	> 5
P. complanata (M)	> 5	> 5	> 5	> 5	> 5
B. thuyoides (CH/M)	> 5	> 5	> 5	> 5	> 5
B. thuyoides (M)	**2.5**	> 5	> 5	> 5	> 5
S. coronopifolius (CH/M)	**0.62**	> 5	**1.25**	**1.25**	**0.31**
S. coronopifolius (M)	**1.25**	**2.5**	**0.62**	**1.25**	**0.62**
Ampicillin (en µg.mL^{-1})	-	-	-	-	-
Tetracycline (en µg.mL^{-1})	2	1	-	-	-

The results of the CMI on the terrestrial bacteria show that in 1.25 mg.mL^{-1}, the methanol extracts of *G.attenatum* and *S. coronopifolius* are respectively active on bacteria *E.coli* AL52 and *S. epidermidis* and the chloroform-methanol extract of second specie is active on these same bacteria. In 0.62 mg.mL^{-1}, the active extracts are from the extract M of *S.coronopifolius* on *E. coli* AL52 and *S. haemolyticus*. In 0.31 mg.mL^{-1}, the active fraction is the extract CH / M of *S.coronopifolius*. The extract M of *H.incurvus* show a CMI of 0.018 mg.mL^{-1} on *E.coli* AL52.

In conclusion, we found that the extracts of the species *A. armata, G. pusillum, G. spinulosum, P. cartilagineum, H. musciformis, H. ncurvus, P. complanata, B. thuyoides, C. rubrum, G. pulchellum and S. coronopifolius* are promising and should be studied in the search for active principles.

The compounds responsible for the antibacterial activity are secondary metabolites belonging to the family of terpenes and phenols (**Blunt *et al.*, 1984 ; Findlay and Patil, 1986 ; Etahiri *et al.*, 2001 ; Vairappan *et al.*, 2001a,b; Mayer and Hamann, 2002, 2005; Vairappan, 2003; Xu *et al.*, 2003; Etahiri *et al.*, 2007; Mayer *et al.*, 2007, 2009, 2011; Smyrniotopoulos *et al.*, 2008 ; Oh *et al.*, 2008 ; Jung *et al.*, 2009; Wendy *et al.*, 2009**).

The antibacterial activity was examined towards a wide distribution of marine bacteria as *Paracoccus, Pseudoalteromonas, Bacillus, Micrococcus* and *Cytophaga*. These bacteria are responsible for the formation of a typical, structured biofilm, are also linked to many problems caused on the submerged structures.The colonization of hulls of ship to a decrease of the speed of the ship and an increase of the consumption. **Callow (1986)** showed that a biofilm of a millimeter thick can reduce the speed of a ship of 15%. In addition, the increase in fuel consumption also results in an increase in pollution. Maintenance costs related to bio-fouling are estimated for the U.S. Navy, to 360 million dollars per year (**Lenwood *et al.*, 1999; Hellio, 2000**). The search for new molecules antifouling non-toxic from marine algae is an original field of investigation.

The search for antibacterial molecules was also carried out towards of pathogenic bacteria such as *E. Coli, E. faecalis, S. aureus, P. aeruginosa, H. alvei* and *K. pneumoniae*. The latter pose significant problems for human health. The bacterial strain *S. aureus* is responsible for food poisoning, nosocomial infections (**Michel and Gutmann, 1997; Broseta *et al.*, 2006**), and in certain cases extremes of sepsis in patients grafted with cardiac prosthesis (**Broseta *et al.*, 2006**).

The study of the CMI reveals the sensitivity of bacteria to the lowest concentration of algal extract. In our study, the important values of CMI, concern to extracts: CH / M of *S. coronopifolius* on *Pseudoalteromonas* 3J3and *S. haemolyticus* with an MIC of 0.62 and 0.31 mg.mL^{-1} respectively, the extract M of *S. coronopifolius* on *E.coli* AL52 and *S. haemolyticus* with an MIC of 0.62 mg.mL^{-1}, the extract M of *P. complanata* on *Bacillus* 4J6 and *Pseudoalteromonas* 3J3with an MIC of 0.62 and 0.31 mg.mL^{-1} respectively, the extract M of *H. incurvus* on *E coli* AL52 with a MIC of 0.018 mg.mL^{-1} and the extract M of *H. musciformis* and *J. rubens* on *Pseudoalteromonas* 3J3 with an MIC 0.62 mg.mL^{-1}.

The study of **Broseta (2006)** showed that the extract chloroform-methanol (1: 1) of *Pterosiphonia complanata* give a high inhibition on *Staphylococcus aureus* with an MIC of 2.8 µg.mL^{-1}. In our study, the antibacterial activity of dichloromethanolic extracts does not prove interesting, while the methanol extracts and chloroform-methanol showed good antibacterial activity. It appears that the nature of the solvent plays a role in the extraction of active substances. The observations of **(Ballantine *et al.*, 1987; Vlachos *et al.*, 1996; Gonzalez *et al.*, 2001; Ozdemir *et al.*, 2004; Etahiri *et al.*, 2007; Karabay-yavasoglu *et al.*, 2007; Taskin *et al.*, 2007; Kandhasamy and Arunachalam, 2008; Dubber and Harder., 2008)**, support this idea and our results. Indeed, **Ballantine *et al.* (1987)** state that chloroform-methanol extract (2:1) of *Asparagopsis taxiformis* presents an antibacterial effect interesting on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. **Taskin *et al.* (2007)** reported that the highest activity was shown in *E. coli* (ATCC 29998) (32.00 ± 1.73 mm) and *E. faecalis* (ATCC 8043) (21.66 ± 0.57 mm) by a methanol extract obtained from *Corallina officinalis*.

Kandhasamy and Arunachalam (2008) showed the inhibitory effect of the methanol extract of *Hypnea musciformis* on bacteria *K. pneumoniae* (13 ± 0.59 mm), *S. aureus* (12 ± 0.69 mm) and *E. faecalis* (12 ± 0.75 mm). **Dubber and Harder (2008)** reported that the methanol extract of *Ceramium rubrum* at a concentration of 10 mg.mL^{-1} showed high antibacterial activity against pathogenic bacteria of fish and marine bacteria such as *Pseudoalteromonas marina, Pseudoalteromonas* sp, *Bacillus licheniformis, Bacillus* sp. However, these results are different from those brought to light by **Bansemir *et al.* (2006)** where they mention the solvent dichloromethane as the most suitable for extracting active molecules. These authors studied the activity of 26 seaweed extracts prepared from dichloromethane against five pathogenic bacteria of fish. They reported that the species with the active extracts towards the bacteria tested is *Asparagopsis armata*. The dichloromethanolic extracts of *Halopitys incurvus, Ceramium rubrum* and *Gracilaria cornea* showed a higher activity towards the marine bacterium *Pseudomonas anguilliseptica* while the extract of *Plocamium cartilagineum* showed a weak activity on the pathogenic bacteria of the fish (**Bansemir *et al.*, 2006**).

Several works are accesses on the study of the antibacterial activity of seaweeds belonging to the same genres or the species of our study. **Sastry and Rao (1994)** showed that the chloroform-methanol extract of *Gracilaria corticata* was more active towards *Staphylococcus aureus, Echerichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella paraphyti, S. typhi,* and *S typhimurium*. **Gonzalez *et al.* (2001)** reported that methanolic extracts of *Halopitys incurvus, Sphaerococcus coronopifolius,* and *Asparagopsis armata* presented no activity on the bacterium *E. coli* (ATCC 10536). While a methanolic extract of *Hypnea musciformis* had shown a high antibacterial activity against Gram-positive and Gram-negative bacteria (**Siddqiui *et al.*, 1993**). **Jing-Wen and Wei-ci (1984)** showed that methanol-toluene (3:1) extracts of *Gelidium divaricatum, Corallina officinalis, Gracilaria* sp, *Ceramium kondoi, Ceramium boydenii* were not effective on *Bacillus subtilis, Escherichia coli*. However, the extract of *Polysiphonia urceolata* is active on *Escherichia coli* and *Plocamium telfairiae* on *Bacillus subtilis*. The studies of **Selvin and Lipton (2004)** showed that the methanol-dichloromethanolic (1:1) extract of *Hypnea musciformis* inhibits the growth of 66,0 % of the strains Gram-positif (*Bacillus cereus, Bacillus subtilis Micrococcus luteus*) at 30°C.Whereas all the Gram-positive bacteria was sensitive in 20°C. **Ballesteros *et al.* (1992)** concluded that methanol / toluene (3:1) extract of *Gelidium pusillum* and *Sphaerococccus coronopifolius* showed no inhibition on the strain of *Escherichia coli* and *Bacillus subtilis*. The observations of **Caccamese et al. (1980, 1981)** report that methanol-toluene (3:1) extract of *Sphaerococccus coronopifolius* and *Plocamium cartilagineum* prevent the growth of the bacterium *Bacillus subtilis*. In another study on the antimicrobial activity of extracts of *Asparagopsis armata, Ceramium deslongchampsii, Gelidium spinulosum, Gracilaria dura, Jania rubens, Osmundea truncata, Plocamium cartilagineum* and *Sphaerococcus coronopifolius* (**Salvador *et al.*, 2007**), it has been evidence that the methanol-toluene (3:1) extract inhibited the growth of three Gram-positive bacteria: *Bacillus subtilis, B. cereus, Staphylococcus aureus* and two Gram-negative bacteria, *Escherichia coli, Pseudomonas aeruginosa*. The methanol-toluene extract of *Corallina elongata* harvested in winter gave no activity (**Ballesteros *et al.*, 1992; Bansemir *et al.*, 2006; Salvador *et al.*, 2007**). In our study, extracts of *Pterocladea capillacea, Gelidium sesquipedale, Osmundea pinnnatifida* showed no antibacterial activity, this result is in agreement with the studies of **Gonzalez *et al.* (2001)**, **Rizvi and Shameel (2005)** and **Salvador *et al.* (2007)**. On the other hand, the methanol extracts of *C. rubrum, G. pusillum, G. pulchellum, G. spinulosum, H. musciformis* gave better results towards certain bacteria. This variation of the activity in these species may be due to one of the factors such as the seasonal variation, the geographical variation of site of collection, the genetic variability, or the bacterial resistance.

It has been observed in most studies on the antibacterial activities of algae that the frequency of the activity was higher against Gram-positive bacteria than Gram-negative (**Etahiri et al., 2007; Rosell and Srivastava, 1987; Ballantine et al., 1987; Gonzalez et al., 2001; Magallanes *et al.*, 2003**). **Etahiri *et al.* (2003)** mention that the methanol extracts of *Halopitys incurvus, Hypnea musciformis, Plocamium cartilagineum, Sphaerococcus coronopifolius* and *Gelidium latifolium* are active on the bacterium *S. aureus* (ATCC 6538). However, the result was negative for the extract of *Asparagopsis armata* on this bacterium. Contrary to our study, **Gonzalez *et al.* (2001)** reported that methanol extracts of *Halopitys incurvus* and *Pterocladea capillacea* present no inhibition on the bacterium *S. aureus* MB5393. Current research has indicated that the four Gram-positive bacteria *S. aureus, S. epidermidis, S. haemolyticus, Bacillus* 4J6 and two Gram-negative bacteria *E. coli* AL52 and *Pseudoalteromonas* 3J3, were more sensitive to extracts from other bacteria tested in this study. While bacteria, *Paracoccus* 4M6, *Pseudolateromonas* 3J6, *Micrococcus* 5J6, *E. coli* HB101, *P. aeruginosa, E. coli* K12, *E. coli* CIP 54117 and *H. alvei, K. pneumoniae* appear to be more resistant.

The majority of the compounds responsible for the antibacterial activity detected in marine algae are most likely terpene and phenolic nature and little lipid nature (**Konig *et al.*, 1999a,b; Ciavatta *et al.*, 2001; Xu *et al.*, 2003; Wang *et al.*, 2005; Oh *et al.*, 2008; Chakraborty *et al.*, 2010**). Two bromoditerpenes were isolated from *Sphaerococcus coronopifolius*, this is the 12 S-hydroxybromospha-erodiol and bromosphaerone. These compounds have shown antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus* with minimum inhibitory concentration of 0.104 and 0.146 µM (**Etahiri *et al.*, 2001**). The phenolic compound 3, 4, 6-tribromo-5-methoxymethyl-benzene-1,2-diol purified from chloroform-methanol extract of the species *Pterosiphonia complanata* proved active against several pathogenic Gram-positive bacteria and Gram-negative. Its minimum inhibitory concentration against the bacterium *Staphylococcus aureus* was 2.8 µg.mL^{-1} (**Etahiri *et al.*, 2007**). Fatty acids found in the methanol extract of *Hypnea musciformis* exhibited high antibacterial activity against Gram-positive bacteria (eg *Staphylococcus aureus*) and Gram-negative (eg *Escherichia coli*) (**Siddqiui *et al.*, 1993**). Furanones isolated from the fraction dichloromethanolic from *Delisea pulchra* (Bonnemaisoniales) (**De Nys et al., 1995; Dworjanyn *et al.*, 2006**) allow inhibition of the growth of marine bacteria at a concentration of 10.0 µg.mL^{-1}. **Devi *et al.* (1997)** have demonstrated the existence of inhibitors of marine

bacteria in the fractions dichloromethanolic of *Acanthophora muscoides* (Ceramiales) at a concentration of 50.0 $\mu g.mL^{-1}$.The antimicrobial activity of red algae is generally correlated with the presence of halogenated compounds (**De Nys *et al.*, 1995**). Monoterpenes isolated from the dichloromethane fractions of *Plocamium costatum* (Plocamiales) (**Konig *et al.*, 1999a**) and *Laurencia rigida* (Ceramiales) (**Konig and Wright, 1997**) show antimicrobial activity at concentrations of 1.0 $\mu g.mL^{-1}$.

CONCLUSION

In conclusion, the antibacterial activity of an algal extract varies from an extract to the other and from one bacterial strain to another. The most promising extracts are methanol extracts and chloroform-methanol of *A armata, P complanata, P cartilagineum, S coronopifolius* and *B thuyoides.*

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