African Journal of Biotechnology Vol. 8 (7), pp. 1258-1262, 6 April, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco

Chiheb Ibtissam[1], Riadi Hassane[1*], Martinez-Lopez José[2], Dominguez Seglar Josè Francisco[3], Gomez Vidal Josè Antonio[3], Bouziane Hassan[1] and Kadiri Mohamed[1]

[1]Department of Biology, Faculty of Sciences, Tétouan, B. P: 2121, 93002, University Abdelmalek, Essâadi, Morocco.
[2]Department of Microbiology, Faculty of Pharmacy, Campus de Cartuja, CP: 18071, University of Granada, Spain.
[3]Department of Organic chemistry, Faculty of Pharmacy, Campus de Cartuja, CP: 18071, University of Granada, Spain.

Accepted 13 February, 2009

Antibacterial activity of methanolic extracts from 32 macroalgae (13 Chlorophyta and 19 Phaeophyta) from the Atlantic and Mediterranean coast of Morocco were evaluated for the production of antibacterial compounds against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Klebsiella pnomeuniae* ATCC 700603 and *E. faecalis* ATCC 29213. Our results indicate that these species of seaweed collected from the Atlantic and Mediterranean coast of Morocco present a significant capacity of antibacterial activities, which makes them interesting for screening for natural products.

Key words: Antibacterial activity, green and brown macroalgae, Moroccan coast, algal extract

INTRODUCTION

The production of antimicrobial activities was considered to be an indicator of the capacity of the seaweeds to synthesize bioactive secondary metabolites (González del Val et al., 2001). There are numerous reports of compounds derived from macroalgae with a broad range of biological activities, such as antibacterial (Berland et al., 1972; Biard et al., 1980a, b; Burkholder et al., 1960; Chenieux et al., 1980; De Lara-Isassi, 1991; Fenical and Paul, 1984; Freile-Pelegrìn and Morales, 2004; Magallanes et al., 2003; Oranday et al., 2004; Lima-Filho et al., 2002; Lustigman and Brown, 1991), antivirals (Richards et al .,1978), antitumorals (Espeche et al., 1984; Maruyama and Yamamoto, 1984; Mayer and Panick, 1984; Yamamoto et al., 1982), anticoagulant (Athukorala et al., 2006; Farias et al., 2000) and anti-fouling (Hellio et al., 2001; Hellio et al., 2004; Maréchal et al., 2004). Numerous substances were identified as anti-microbial agents from algae: chlorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocyclic compounds, phenolic inhibitors, etc (Espeche et al., 1984). Antibacterial activity was found to vary with season (Moreau et al., 1984).

The present study was undertaken in order to examine the antibacterial effects of crude methanol extracts obtained from 32 marine macroalgae species (13 Chlorophyceae and 19 Phaeophyceae) harvested from the Atlantic and Mediterranean coast of Morocco against pathogenic bacteria *Escherichia coli* ATCC 25922, *Staphylococcus. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *Klebsiella pnomeuniae* ATCC 700603 and *Enterococcus faecalis* ATCC 29213.

MATERIALS AND METHODS

Algal materials

Seaweeds were collected by hand picking using Scuba diving or snorkeling (1 – 4 m depth) and preserved on ice until further processing. The zones were investigated from 2003 to 2007 including 8 sampling stations of the Atlantic and Mediterranean coast of Morocco (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 at Abdelmalek Essâadi University, 93002 Tetouan, Morocco.

*Corresponding author. E-mail: hassaner@hotmail.com. Tel.: (212 039) 97 24 23. Fax: (212 039) 99 45 00.

Table 1. Harvesting sites of algal material.

Coast of Morocco	Site of collection	Coordinates
Atlantic Coast	Briech	35º31'42.20''N 6º00'26.25''O
	Rabat	34º01'56.78''N 6º50'41.30''O
	El-Jadida	33º15'50.61''N 8º30'30.81''O
Mediterranean Coast	Marina-smir	35º45'56.81''N 5º20'58.04''O
	Martil	35º37'10.22''N 5º16'15.79''O
	Sidi Abslam	35°33'46.60'' N 5º14'54.39''O
	Nador	33º37'00.23''N 3º43'59.82''O
Strait of Gibraltar	Ksar-sghir	35º50'52.58''N 5º33'39.04''O

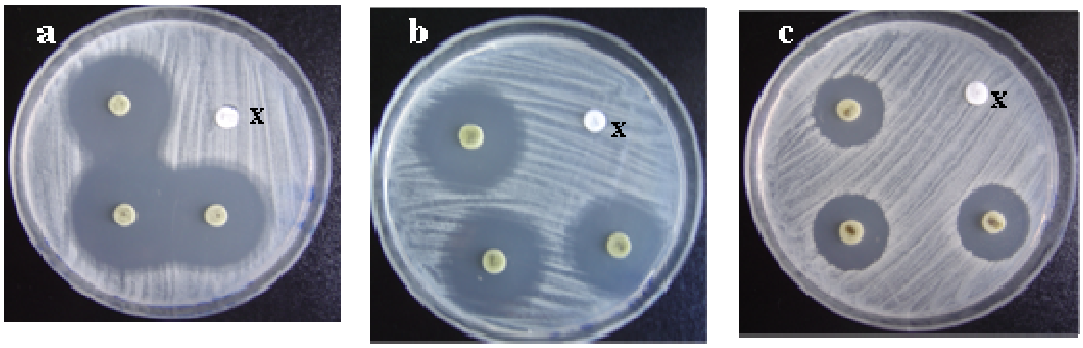

Figure 1. Inhibition zones obtained by the methanol extracts against *Staphylococcus aureus*: a: *Cystoseira mediterranea*, b: *U lactuca*, c: *Codium dichotomum*, X: negative control.

In the field, the algae were cleaned of epiphytes, washed thoroughly in sterile sea water. Then seaweeds were dried for 24 h under an artificial light in 24ºC and finally in a heater. The dry seaweeds were crushed in an electric mill until a fine powder was obtained. Every sample was preserved in a freezer until compound extraction.

Chemical extraction

The powder (5 g) of dried plant was extracted in soxhlet apparatus using methanol (200 ml) as solvent (8 h at 65ºC). The resulting extracts were concentrated to dryness in a rotary evaporator under reduced pressure. The extracts were diluted in 2 ml of pure (Sreenivasa-Rao and Parekh, 1981).

Antimicrobial activity test

Test was performed by the diffusion method (Burkholder et al., 1960), Sterile discs (BBLTM) de 6 mm were preparated by pipetting 25 µl of extract to each disc, placed on the agar Mueller-Hinton (pH 7.4± 0.2 a 25°C) and incubated at 37°C during 24 h overnight (Ballantine et al., 1987). Inhibition results are expressed as width of the clear halo surrounding each disc on cultivated agar plats. Methanol solvent (100 %) without algal extract was also used as a negative control.

All experiments were performed at least in triplicate. Representative halos were those measuring a diameter superior to 10 mm (Lima et al., 2002).

Microorganisms

The strains used were *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *K. pnomeuniae* ATCC 700603 and *E. faecalis* ATCC 29213. The organisms were obtained from the Department of Microbiology, Faculty of Pharmacy, University of Granada (ATCC: American Type Culture Collection).

RESULTS AND DISCUSSION

The antibacterial activity of crude methanol extracts of the 32 algal species is summarised in Figure 1 and Table 2. The antibacterial activity was classified from less active (+:10 mm ≥ Diameter of inhibition < 16 mm), to moderately active (++: 16 mm ≥ diameter of inhibition < 20 mm), to highly active (+++: diameter of inhibition ≥ 20 mm) and no active (- : diameter of inhibition < 10 mm) (Table 2). Twenty eight species showed antibacterial activity, no activity was observed from four methanolic extracts of *Fucus platycarpus*, *Phyllaria brevipes* (C. Agardh) E.C. Henry and G.R. South, *Codium bursa* (Olivi) C. Agardh and *Sargassum vulgare* C. Agardh.

The majority of algal extracts were active against one or two microorganisms. Most of them, 28 (87.5%) showed activity against *S. aureus*, 22 (68.75%) showed activity against *E. coli* and 16 (50%), 9 (28.12%) and 3

Table 2. Antibacterial activity of seaweed species

Species tested	Date	Locality	Target microorganisms				
			Eco	Sta	Ent	Kle	Entf
Chlorophyceae							
Ulva lactuca	15/08/2003	Ksar-sghir	++	+++	+	+	-
Ulva lactuca	23/02/2007	Ksar-sghir	-	-	-	-	-
Ulva fasciata	16/05/2005	Sidi abslam	+	+++	-	+	-
Ulva olivascens	20/08/2005	Nador	-	++	+	-	-
Enteromorpha linza	16/05/2005	Sidi abslam	+	+++	-	-	-
Enteromorpha compressa	4/8/2003	Ksar-sghir	+	+++	+	+	+
Enteromorpha compressa	23/02/2007	Ksar-sghir	-	-	-	-	-
Enteromorpha ramulosa	1/8/2003	Ksar-sghir	-	++	-	-	-
Enteromorpha intestinalis	1/8/2003	Ksar-sghir	+	+++	-	+	-
Cladophora prolifera	1/8/2003	Ksar-sghir	+	+++	+	+	-
Chaetomorpha linum	1/8/2003	Ksar-sghir	+	+++	-	-	-
Codium dichotomum	1/2/2004	Ksar-sghir	-	+++	-	-	-
Codium fragile	23/05/2005	Martil	-	+++	-	-	-
Codium bursa	15/03/2004	Ksar-sghir	-	-	-	-	-
Codium tomentosum	21/09/2003	Martil	-	+++	-	-	-
Phaeophyceae							
Cladostephus spongiosus	21/09/2003	Martil	+	+++	+	+	-
Stypocaulon scoparium	3/8/2003	Ksar-sghir	+	+++	-	+	-
Dictyota linearis	3/8/2003	Ksar-sghir	++	+++	-	+	-
Dictyopteris membranacea	21/09/2003	Martil	+	+++	-	+	-
Padina pavonica	1/8/2003	Ksar-sghir	+	+++	-	+	+
Laminaria ochroleuca	15/05/2005	Jadida	+	+++	-	+	-
Sacchoriza polyschides	10/8/2003	Ksar-sghir	+	+++	+	+	-
Sacchoriza polyschides	23/02/2007	Ksar-sghir	-	-	-	-	-
Phyllariopsis brevipes	28/09/2003	Marina smir	-	-	-	-	-
Fucus spiralis	20/04/2005	Ksar-sghir	-	+++	-	-	-
Fucus vesiculosus	11/7/2005	Briech	+	++	-	-	-
Fucus platycarpus	1/8/2003	Ksar-sghir	-	-	-	-	-
Sargassum vulgare	4/8/2005	Ksar-sghir	-	-	-	-	-
Bifurcaria bifurcata	16/08/2005	Rabat	+	++	-	-	-
Cystoseira tamariscifolia *Cystoseira mediterranea*	14/09/2003	Martil	+	+++	+	+	+
Cystoseira mediterranea	1/8/2003	Ksar-sghir	++	+++	+	+	-
Cystoseira compressa	23/02/2007	Ksar-sghir	-	-	-	-	-
Cystoseira crinita	1/8/2003	Ksar-sghir	-	+++	-	-	-
Cystoseira humilis	28/05/2005	Nador	+	+++	-	+	-
Cystoseira usneoides	12/4/2005	Nador	+	+++	-	+	-
Cystoseira usneoides	1/8/2003	Marina smir	+	++	+	-	-
	23/02/2007	Marina smir	-	-	-	-	-

-: No activity, +: 10mm≥ Diameter of inhibition<16 mm, ++: 16mm≥ Diameter of inhibition <20mm, +++: Diameter of inhibition ≥20mm). Micro-organisms: Eco: *Escherichia coli* ATCC 25922, Sta: *Staphylococcus aureus* ATCC 25923, Ent: *Enterococcus faecalis* ATCC 29213.

(9.37%) presented inhibition activity against *K. pnomeuniae*, *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 29213, respectively.

The methanol extracts of *Enteromorpha compressa* (Linnaeus) and *Cystoseira tamariscifolisa* (Hudson) Papenfuss showed activity against every bacterial strain

tested. In all 13 species tested of Chlorophyceae, the extract of *Ulva lactuca* (Linnaeus) has a larger inhibition diameter against *E. coli* (16 mm), *S. aureus* (30 mm) and *E. faecalis* ATCC 29212 (13 mm). However, the extract of *E. compressa* (Linnaeus) Nees is the only one that showed activity against *E. faecalis* ATCC 29213 (14 mm).

Among the 19 *Phaeophyceae* tested, the extract of *Cystoseira mediterranea* sauvageau exhibited the highest activity against *E. coli* (16 mm) and *S. aureus* (33 mm), *C. tamariscifolia* (Hudson) Papenfuss, *Dictyota linearis* (C. Agardh) Greville and *Padina pavonica* (Linnaeus) Thivy acted against *E. faecalis* ATCC 29212 (13 mm), *K. pnomeuniae* (15 mm) and *E. faecalis* ATCC 29213 (11 mm).

S. aureus was more sensitive than all the stocks, with the largest inhibition diameter and *E. faecalis* ATCC 29213 was more resistant against all the methanol extracts of the marine algae tested. It was reported that the Gram-positive bacterial strains were more susceptible to seaweeds extract than Gram-negative bacterial strains (Sreenivasa-Rao and Parekh, 1981; Pesando and Caram, 1984).

The algal extracts such as *Enteromorpha ramulosa* (Smith) Carmichael and *Dictyopteris membranacea* (Stackhouse) Batters were active against Gram-positive and Gram-negative bacteria (González et al., 2001).

Lima-Filho et al. (2002) found that the hexane extract of *Ulva fasciata* Delile does not inhibit to *S. aureus* and *E. coli*. In our study, the methanol extract of *U. fasciata* Delile produced an inhibiting effect against *S. aureus* and *E. coli.*

Another significant result of the present study was the non efficiency of the methanol extracts of *Sargassum vulgare* C. Agardh, which did not show antibacterial activity against *E. coli* and *S. aureus* growth. No growth inhibition of these two bacteria was also reported by Ballantine et al. (1987) when the hexane extracts of the same algae were tested.

Activity is classified according to the diameter of the inhibition zone around the point of application of the sample (- : No activity, +:10 mm ≥ diameter of inhibition < 16 mm, ++:16 mm ≥ diameter of inhibition < 20 mm, +++: diameter of inhibition ≥ 20 mm). Micro-organisms: Eco: *E. coli* ATCC 25922, Sta: *S. aureus* ATCC 25923, Ent: *E. faecalis* ATCC 29212, Kle: *K. pnomeuniae* ATCC 700603, Entf: *E. faecalis* ATCC 29213.

Moreau et al. (1984) reported that antibacterial activity is influenced by the reproductive state and seasonality. The results obtained in the present study confirm this suggestion. The most active particularly against *S. aureus*, came from fertile plants of *U. lactuca* Linnaeus collected in August. Almost no activity was detected from vegetative plants collected in winter.

Similarly, *E. compressa* (Linnaeus) Nees, *Saccorhiza polyschides* (Lightfoot) Batters, *Cystoseira mediterranea* Sauvageau and *Cystoseira usneoides* (Linnaeus) M. Roberts were quite inactive in winter but strongly active in summer versus *S. aureus* (Moreau et al., 1984) (Table 2).

Finally we conclude that macroalgae from the Moroccan coast are potential sources of bioactive compounds and should be investigated for natural antibiotics. This study has shown that the production of antibacterial substances by macroalgae is a regular occurrence among those found on the coast of Morocco. Biochemical analysis are currently undertaken to determine the structure and nature of these compounds.

ACKNOWLEDGEMENTS

We are especially very grateful to Dr. Conxi Rodriguez–Prieto (University of Girona, Faculty of Sciences, Department of Environmental Sciences) for useful discussion on nomenclature.

This work is partially supported by the Spanish Agency of International Cooperation and Development of the Foreign Ministry (AECID) (A/ 3952/05, A/ 5583/06)

REFERENCES

Athukorala Y, Ki-Wan Lee, Se-Kwon K, You-Jin J (2006). Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. Bioresour. Technol. 98(9): 1711-1716.

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.

Berland BR, Bonin DJ, Cornu AL, Maestrini SY, Marino JP (1972). The antibacterial substances of the marine alga *Stichochrysis immobilis* (Chrysophyta). J. Phycol. 8: 383-392.

Biard JF, Verbist JF, Le Boterff J, Ragas G, Lecocq M (1980a). Algues fixées de la côte atlantique française contenant des substances antibactériennes et antifungiques. Planta Med. Suppl: 136-151.

Biard JF, Verbist JF, Letourneux Y, Floch R (1980b). Cétols Diterpéniques à Activité Antimicrobienne de *Bifurcaria bifurcata*. Planta Med. 40: 288-294.

Burkholder R, Burkholder LM, Almodovar LR (1960). Antibiotic activity of some marine algae of Puerto Rico. Bot. Mar. 2: 149-156.

Chénieux JC, Verbist JF, Biard JF, Clement E (1980). Algues Fixées de la Côte Atlantique Française Contenant des Substances Antimitotiques. Planta Med. Suppl. pp. 152-162.

De Lara-Isassi G (1991). Propiedades antibióticas de algunas especies de algas marinas bentónicas. Hidrobiológica 1: 21-28.

Espeche ME, Fraile ER, Mayer AMS (1984). Screening of Argentine marine algae from antimicrobial activity. Hydrobiologia 116/117: 525-528.

Farias WRL, Valente AP, Pereira MS, Mourào PAS (2000). Structure and Anticoagulant Activity of Sulfated Galactans. J. Biol. Chem. 275(38): 29299-29307.

Fenical W, Paul VJ (1984). Antimicrobial and cytotoxic terpenoids from tropical green algae of the family Udoteaceae. Hydrobiologia 116/117: 135-170.

Freile-Pelegrìn Y, Morales JL (2004). Antibacterial activity in marine algae from the coast of Yucatan, Mexico. Bot. Mar. 47: 140-146.

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

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. Mar. Environ. Res. 52(3): 231-247.
Hellio C, Marechal JP, Véron B, Bremer G, Clare AS, Le Gal Y (2004). Seasonal variation of antifouling activities of marine algae from the Brittany coast (France). Mar. Biotechnol 6: 67-82.
Lima-Filho JVM, Carvalho AFFU, Freitas SM, Melo VMM (2002). Antibacterial activity of extracts of six macroalgae from the northeastern Brasilian coast. Braz. J. Microbiol 33: 311-314.
Lustigman B, Brown C (1991). Antibiotic production by marine algae isolated from the New York/New Jersey coast. Bull. Environ. Contam. Toxicol. 46: 329-335.
Magallanes C, Córdova C, Orozco R (2003). Actividad antibacteriana de extractos etanólicos de macroalgas marinas de la costa central del Perú. Rev. Peru. Biol. 10(2): 125-132.
Maréchal JP, Culioli G, Hellio C, Thomas-Guyon H, Callow ME, Clare AS, Ortalo-Magné A (2004). Seasonal variation in antifouling activity of crude extracts of the brown alga Bifurcaria bifurcata (Cystoseiraceae) against cyprids of Balanus Amphitrite and the marine bacteria *Cobetia marina* and *Pseudoalteromonas haloplanktis*. J. Exp. Mar. Biol. Ecol. 313: 47-62.
Maruyama H, Yamamoto I (1984). An antitumor fucoidan fraction from an edible brown seaweed, *Laminaria religiosa*. Hydrobiologia 116/117: 534-536.
Mayer AMS, Panick B (1984). Antitumor evaluation of marine algae in Argentina. Hydrobiologia 116/117: 529-533.
Moreau J, Pesando D, Caram B (1984). Antifungal and antibacterial screening of Dictyotales from the French Mediterranean coast. Hydrobiologia 116/117: 521-524.
Oranday MA, Verde MJ, Martinez- Lozano SJ, Waksman NH (2004). Active fractions from four species of marine algae. Int. J. Exp. Bot. pp. 165-170.
Pesando D, Caram B (1984). Screening of marine algae from the French Mediterranean coast for antibacterial and antifungal activity. Bot. Mar. 27: 381-386.
Richards JT, Kern ER, Glasgow LA, Overall JC, Deign EF, Hatch MT (1978). Antiviral activity of extracts from Marine algae. Antimicrob. Agents Chemother. 14(1): 24-30.
Sreenivasa-Rao P, Parekh KS (1981). Antibacterial activity of Indian seaweed extracts. Bot. Mar. 24: 577-582.
Yamamoto I, Takahashi M, Tamura E, Maruyama H (1982). Antitumor activity of crude extracts from edible marine algae against L-1210 leukemia. Bot Mar 25: 455-457.