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Research Article

ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF TWENTY-THREE MARINE RED ALGAE FROM THE COAST OF SIDI BOUZID (EL JADIDA-MOROCCO)

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ABSTRACT

Six organic and aqueous extracts of twenty-three red marine algae collected along the Atlantic coast of Morocco were studied for anti-inflammatory and antimicrobial activities. Anti-inflammatory activity was evaluated as inhibition rate of two enzymes (phospholipase A2 and elastase). Antimicrobial activity was tested against ten gram positive bacteria : *Bacillus cereus*, *Bacillus thuringensis, Bacillus subtillus1 and Bacillus subtillus 2, Clostridium sporogenes, Staphylococcus aureus, Staphylococcus aureus ssp aureus, Mycobacterium smegmatis, Streptococcus feacalis* and *Bacillus sp,* against two gram negative bacteria : *Escherichia Coli* and *Pseudomonas sp* and against fungi *: Candida tropicalis, Candida albicans* and *Cryptococcus neoformans*. The result shows that 100% of inhibition of phospholipase A2 activity was obtained by *Asparagopsis armata, Chondrus crispus* and *Gelidium sesquipedale* extracts, while the inhibition of elastase was obtained with *Corallina elongata, Chondrus crispus, Gelidium sesquipedale* and *Laurencia pinnatifida* extracts which give an inhibition greater than 95%. The positive antibacterial activity with diameter inhibition more than 10 mm was found in methanolic and methanol–dichloromethane (50:50) extract. Also, a gram-positive bacteria presents a sensibility superior to the gram-negative and *Staphylococcus aureus ssp aureus* was the more sensitive. The purification and the determination of chemical structure of active compounds from red algae are under investigation.

Keywords: Marine red algae, Anti-inflammatory activity, Antibacterial activity, Antifungal activity, Moroccan coast.

INTRODUCTION

Macroalgae are a rich source of natural bioactive products, although a little has been done to define an ecological role for these compounds [1]. They may, therefore, possess chemical defenses to prevent the colonization of their surface. The use of marine natural products able to inhibit bacteria development offers a rich pharmacological potential [2]. Numerous reports show that macroalgae present a broad range of biological activities such as antibacterial [3,4,5], antifungal [6], antiviral [7] and anti-inflammatory [8].

The ability of seaweeds to produce secondary metabolites of antimicrobial value, such volatile components as phenols, terpenes [3], steroids [9], phlorotannins [10], lipids [11] and anti-inflammatory value such as retinol which inhibited the phospholipase A2 [12] has already been studied.

In contrast to the brown and green algae, the red algae are more known to produce halogenated metabolites, particularly bromine and iodine [13,14]. 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 [13].

The Moroccan coast is particularly rich in algal biodiversity and constitutes a reserve of species of considerable economic, social and ecologic potential. However, only the *Gelidium sesquipedale* specie is exploited in Morocco to extract agar-agar. 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 [15,16,17,18].

To date, many chemically unique compounds of marine origin with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals compounds [19].

In the present study, we evaluated the anti-inflammatory and the antimicrobial activities of methanol, acetone, chloroform, hexane, dichloromethane-methanol and water extracts of twenty-three marine algae collected from the coast of El Jadida–Morocco. The aim of this work is to select algae with the best activity to use it for purification of active compounds.

MATERIALS AND METHODS

Algal materials

Seaweeds were collected by hands picking in period of March-April 2009 from Sidi Bouzid coast (33°-33°16'09''N, 8°30'-8°45'W) figure 1, the algae were cleaned, washed in distilled water, then dried at room temperature and crushed until a fine powder was obtained.

Algae investigated were identified as : *Asparagopsis armata* Harvey, *Bornetia secundiflora* (J. Agarth) Thuret, *Calliblepharis ciliata* (Hudson), *Caulacanthus ustulatus* (Mert) Kûtzing, *Chondrus crispus* Stackhouse, *Corallina elongata* Ellis and Solander, *Corallina officinalis* Linnaeus, *Gelidium latifolium* (Greville) Bornet and Thuret, *Gelidium sesquipedale* (Clemente) Thuret, *Gigartina acicularis* (Roth) Lamouroux, *Gigartina pistillata* (S.G.Gmelin) Stackhouse, *Gigartina teedi* (Roth) Lamouroux, *Gracilaria multipartida* (Clement y Rubio) Harvey, *Gracilaria verrucosa* (Hudson) Papenfuss, *Halopitys incurvus* (Hudson) Batters, *Hypnea musciformis* (Wulfen) Lamouroux, *Laurencia pinnatifida* (Hudson) J. V. Lamouroux, *Palmaria palmata* (Linnaeus) Weber & Mohr, *Plocamium cartilagineum* (Linnaeus) Dixon, *Sphaerococcus coronopifolius* Stakhouse, *Pterosiphonia complanata* (Clemente) Sauvageau, *Chondria dasyphylla* (Woodward) C. Agardh and *Acrosorium venulosum* (Zanardini) Kylin.

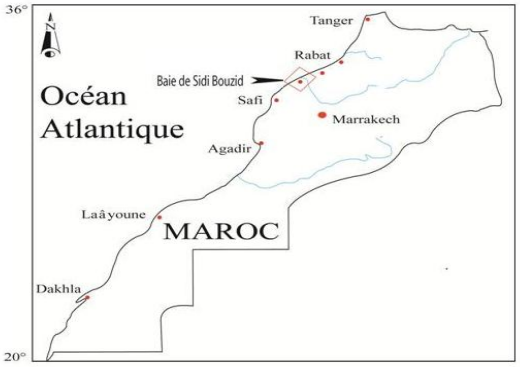

Fig. 1: Localisation of the collection site of Sidi Bouzid

Chemical extracts

The powder of dried algae was extracted in different solvents: methanol, acetone, chloroform, hexane dichloromethane/methanol

(50:50) and water [20]. The resulting extracts were concentrated to dryness in a rotary evaporator under reduced pressure (at 45°C) until to obtain a crude extract and were conserved at 4°C.

Anti-inflammatory activity

PLA2 inhibition assay

Bioassay was based on a colorimetric bioassay [21]. Each extract (10 µg dissolved in DMSO (10 µL) was incubated in 96 well plates for 1 h at 25°C, with *Apis mellifera* venom PLA2 (Sigma, 2 µL of a 1 mg/mL DMSO stock solution). Substrate solution (198 µL) containing L- α-phosphatidylcholine (L- α-lecithin, 3.5 mM), red phenol (0.055 mM), NaCl (100 mM), $CaCl_2$ (10 mM) and Triton (7 mM) at pH 7.6 were added. Manoalide was used as a positive control. Colorimetric measurements were made as duplicates at time 0 and after 5 min there after read at 550 nm.

Elastase Inhibition assay

This activity is measured by the calorimetric method [22]. Bioassay was monitored by measuring the inhibition of the amidolysis of N-succinyl-alanyl-alanyl-prolyl-leucyl p-nitroanilide (Sigma) by the elastase (EC 3.4.21.36 Type II-A) from porcine pancreas (Sigma) at 410 nm. The reaction was carried out in 0,2M Tris-HCl buffer (pH 8.0) containing 200µl elastase (0.2mg/ml), 100µg of each extract prepared in 10µl of DMSO was added to the reaction mixture, and the elastase inhibition was assessed at 25 °C. The reaction mixture was pre-incubated for 10 min before addition of 2 µl the substrate (7.2 µg/100µl of DMSO). The change in absorbance was measured at 410 nm in a 96-well reader.

Antimicrobial activity

Microbial strains

The strains used to evaluate the antimicrobial activity were obtained from Collection of Institute Pasteur (CIP) and from American Type Culture Collection (ATCC).

Gram-positive bacteria: *Staphylococcus aureus* (ATCC 9144), *Staphylococcus aureus ssp aureus* (ATCC 6538), *Bacillus sp* (CIP 104717), *Streptococcus faecalis* (ATCC 19433), *Bacillus cereus* (CIP 783), *Bacillus thuringensis* (ATCC 10792), *Bacillus subtillus1* (ATCC 9372), *Bacillus subtillus2* (ATCC 6633), *Clostridium sporogenes* (CIP 7939) and *Mycobacterium smegmatis* (CIP 7326).

Gram-negative bacteria: *Pseudomonas sp (*ATCC 19433*)* and *Escherichia coli* (ATCC 10536).

Fungi: *Candida albicans* (ATCC 60193), *Candida tropicalis* (ATCC 127581) and *Cryptococcus neoformans* (ATCC 11576).

Antimicrobial bioassays

Antibacterial assays were carried out using the agar disk-diffusion assay [23]. Three colonies of each bacterium were removed with a wire loop from the original culture plate and were introduced into a test tube containing 5 mL broth. An overnight culture yielded a suspension of 10^6 bacteria/mL (evaluated by the absorbance value of 0.5 at 620 nm). This solution was diluted 100-fold and the bacterial density was then adjusted to 0.2 x 10^4 cells/mL with sterile water to inoculate Petri dishes containing culture media (12 mL Mueller-Hinton agar, 3 mm thick). Plates were dried for about 30 min before inoculation and were used within four days of preparation.

Organic extracts were tested using paper disks (6 mm diameter) impregnated with the solution (500µg/disk), while aqueous extract was tested according to the well assay [24] using a solution of extracts (concentration of 500µg/50µl) in each well (well volume is 100µl). After the temperature was equalized at 4°C, the microorganisms were incubated overnight at 37°C. Inhibition zones were then measured.

For fungicidal activity, zones of inhibition were determinated after 48h of incubation at 27C°.

Discs impregnated with standard antibiotics such chloramphenicol, streptomycin and the tetracycline are used at 50 or 100µg/ml as reference in the test of antibacterial activity. The amphotericine B at 200µg/ml is used in the antifungal activity. In addition, Control disks were prepared with each solvent and all tests were performed in triplicate. Representative halos were those measuring a diameter superior to 10 mm [19].

RESULTS AND DISCUSSION

Anti-inflammatory activities

Phospholipase A2 and Elastase inhibition by dichloromethane/methanol (50:50) extracts from algae were presented in figure 2 and 3, but only species which gave positive inhibition were reported. Total inhibition of PLA2 was observed in the extracts of *Asparagopsis armata, Chondrus crispus and Gelidium sesquipedale*. Also, extract of *Corallina elongata, Chondria dasyphylla*, *Laurencia pinnatifida, Gigartina acicularis, Pterosiphonia complanata* and *Palmaria palmata* showed antiphospholipase activity with an inhibition percentage higher to 70%. A similar result was reported by Mayer et al. [8] who demonstrated an anti-phospholipase A2 inhibition in 10 species of algae on the 29 species tested. However, Ktari [25] showed that the extract of *Corralina elongata* does not inhibit phospholipase A2.

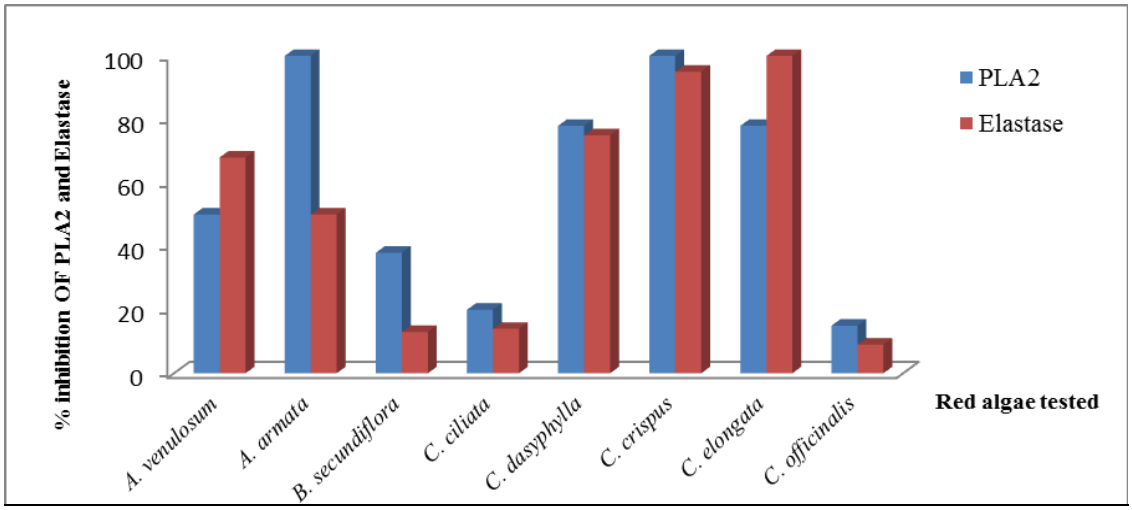

Fig. 2: Percentage inhibition of PLA2 and Eiastase of red algae tested

Concerning the elastase inhibition, among the 23 species tested, only *Corallina elongata, Chondrus crispus, Gelidium sesquipedale* and *Laurencia pinnatifida* which give inhibition greater than 95%. Furthermore, *Chondria dasyphylla, Acrosorium venulosum, Hypnea musciformis, Gigartina pistillata, Palmaria palmata* and *Pterosiphonia complanata* showed an activity included between 50 and 90%. A

weak activity with an inhibition percentage less than 40% was observed in extracts of *Bornetia secundiflora, Corallina officinalis, Calliblepharis ciliata, Halopitys incurvus* and *Asparagopsis armata*. A product with a major antielastase activity was isolated by Bultel et al. [26] from the algae *Hypnea musciformis* harvested from the coast of El Jadida.

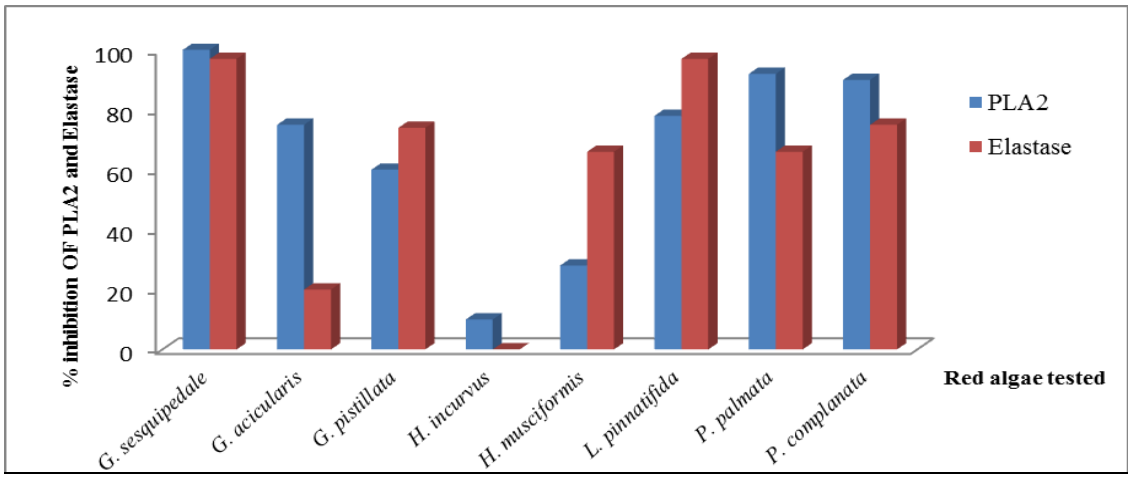

Fig. 3: Percentage inhibition of PLA2 and Elastase of red algae

This first study concerning the research of phospholipase A2 and elastase inhibition from marine red algae collected from the coast of Morocco demonstrated that some species are promising for a research of antiphospholipase A2 and antielastase compounds and could be a potential source of anti-inflammatory components.

Table 1: Antibacterial activity of Red seaweed species against Gram positive Bacteria

Algae	Solvents of extraction	Antibacterial activity	
		Gram positive bacteria	Diameter of inhibition (mm)
Asparagopsis armata	MeOH	*B. c ; C. s*	++
	Ac	*S. a ssp*	+++
	Ch	*S. a ssp*	++
	DC/MeOH	*B. c*	++
		B. t ; B. S1	+++
Corallina elongata	MeOH ; Ch, Hex ;	*S. a ssp*	++
	Ac	*S. a ssp*	+++
		S. f	++
Gigartina acicularis	Ch	*S. a ssp*	++
Gracilaria multipartita	MeOH ; Ac	*S. a ssp*	++
Halopitys incurvus	MeOH	*C. s*	++
	Ac	*S. a ssp*	+++
	DC/MeOH	*C.s*	++
		B.c	+++
		S.a	++
Pterosiphonia complanata	MeOH	*B. c ; B. S1; S. a sp*	+++
	Ac	*B. S1; S. a ssp*	++
	DC/MeOH	*B. t*	++
		B. c; S. a ssp	+++
Chondria dasyphylla	MeOH	*S. f; B. sp*	+++
	MeOH; Ac; Ch	*S. a*	++
Bornetia secundiflora	MeOH; Ac; Ch	*S. a ssp*	++
Caulacanthus ustulatus	MeOH	*S. a ssp*	++
Gélidium latifolium	MeOH, Ac	*S. a ssp*	++
	Hex	*B. S1*	++
Gigartina teedi	MeOH; Ch	*S. a ssp*	++
Gracilaria verrucosa	MeOH	*S. a ssp*	+++
	Ac	*S. a ssp*	++
Hypnea musciformis	MeOH	*S. a ssp*	++
Placamium cartilagineum	MeOH	*S. a ssp*	++
Sphaerococcus coronopifolius	MeOH; Ch; Hex	*S. a ssp*	++
	Ac	*S. a ssp*	+++
Gigartina pistillata	Dc/MeOH	*B. sp; S. f*	++
Corallina officinalis	Dc/MeOH	*B.sp; S. f*	++
Chondrus crispus	Dc/MeOH	*B. c ; S. a*	++
Gelidium sesquipedale	Dc/MeOH	*B.c ; S. a; S. f*	+++
Laurencia pinnatifida	Dc/MeOH	*S. a ssp ; B. sp; S. f*	++

Staphylococcus aureus : S. a, Staphylococcus aureus ssp aureus : S. a ssp, Bacillus sp : B. sp, Streptococcus faecalis : S. f, Bacillus cereus : B. c, Bacillus thuringensis : B. t, Bacillus subtillus1: B. S1, Bacillus subtillus2 : B. S2, Clostridium sporogenes C. S and Mycobacterium smegmatis : M. S.

MeoH: Methanol, Ac: Acetone, Ch: Chloroform, Hex: Hexane, DC: Dichloromethane

Antimicrobial activity

The result of screening tests is shown in table 1. The positive activity was assessed by the diameter of the inhibition zones. This activity was classified from less active (+<10), moderately active (++<15mm) and to highly active (+++>15). Only algae with inhibition zones rather than 10 mm are reported.

In the present study, the antibacterial activity is not uniformly distributed in the various extracts; methalonic extract exhibited greater inhibition against gram-positive bacteria. These results are in agreement with the observations of Taskin et al. [27], Kandhasamy and Arunachalam [28] and Nanthini et al. [29] who reported that the extracts prepared with methanol showed the best activity; however, these results are in contrast with the observations of Mhadhebi et al. [30] who found that the methanolic extracts of algae didn't show any antibacterial activity.

Methanolic extracts of *Corallina elongata, Halopitys incurvus, Gracilaria verrucosa* and *Sphaerococcus coronopifolius* inhibited *Staphylococcus aureus ssp aureus* with a diameter of the inhibition rather than 15 mm, while methanolic extract of *Pterosiphonia complanata* was active against *Bacillus cereus*, *Bacillus subtillus* 1 and *Staphylococcus aureus ssp aureus.* For methanolic extract of *Chondria dasyphylla,* the activity was obtained against *Streptococcus faecalis* and *Bacillus sp.*

Inhibition zone greater than 15 mm was also observed in dichloromethane: methanol (50:50) extract of *Halopitys incurvus* against *Bacillus cereus*. For *Asparagopsis armata,* inhibition was obtained against *Bacillus thuringensis* and *Bacillus subtillus* 1, while the similar extract of *Pterosiphonia complanata* showed activity toward *Bacillus cereus* and *Staphylococcus aureus ssp aureus*. *Gelidium sesquipedale* give a comparable activity, but against *Bacillus cereus, Staphylococcus aureus* and *Streptococcus faecalis.*

These results are in agreement with those obtained by Febles et al. [31]; however they are in contrast with those of Sastry et al. [32] who mentioned that chloroform as the most suitable solvent for extracting antibacterial substances from algae.

For aqueous extracts, no antibacterial activity was detected due to the chemical nature of extracted molecules like proteins.

In addition, this study demonstrated that *Staphylococcus aureus ssp aureus* was more sensitive than all strains with the largest inhibition diameter. Rao and Parekh [33], Vidyavathi and Sridhar [34] also reported that gram positive bacterial strains were more susceptible to seaweeds extracts than gram negative bacterial strains [10,35]. Concerning antifungal activity, only few extracts showed very low activity against fungi strains.

This study reports the presence of anti-inflammatory and antibacterial compounds in the algae from the coast of El Jadida. The anti-inflammatory activity was obtained in extract prepared in dichloromethane/methanol (50:50) while antibacterial activity was found predominantly in extracts prepared in methanol and dichloromethane/methanol.

We showed that many extracts of marine algae considerably inhibited the growth of the bacterium *Staphylococcus aureus ssp aureus*.

Disparities reported by different workers for activity of some algae may be due to different preservation of algae before extraction, different solvents of extraction, and to the different susceptibilities among bacterial strains. Such discrepancies in activity could be also be explained by geographical variations and a wide variety of habitats exist for algae in the marine environment; so it is difficult to compare results obtained with algae collected in different areas. For anti-inflammatory activity, very few screening was done with Morocco algae extracts.

CONCLUSION

In conclusion, the results of the present study revealed that marine red alga are the potential producers of anti-inflammatory and antibacterial activity with some few exceptions. Therefore, it should be thoroughly investigated for natural sources bioactive compounds properties. In our ongoing program, we are in progress to isolate and characterize active compounds.

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