INTRODUCTION
Macroalgaes 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 antioxidant [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, Ceramiaceae, Rhodymniaceae, 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 species 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°-3°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 secundifora (J. Agarth) Thuret, Calliblepharis ciliata (Hudson), Caulacanthus ustulatus (Mert) Küting, Chondrus crispus Stackhouse, Corallina elongata Ellis and Solander, Corallina officinalis Linnaeus, Gelidium latifolium (Greville) Bornet and Thuret, Gelidium sesquipedale (Clement) Thuret, Gigartina acicularis (Roth) Lamouroux, Gigartina pistillata (S.Gmelin) Stackhouse, Gigartina teedi (Bot) Lamouroux, Gracilaria multipartita (Clement y Rubio) Harvey, Gracilaria verrucosa (Hudson) Papenfuss, Haloptilus incurvus (Hudson) Batters, Hypnea musciformis (Wulfen) Lamouroux, Laurencia pinnatifida (Hudson) J.V. Lamouroux, Palmaria palmata (Linnaeus) Weber & Mohr, Plocamium cartilagineum (Linnaeus) Dixon, Sphaerococcus coronopifolius Stackhouse, Pterosiphonia complanata (Clemente) Sauvageau, Chondria dasypylla (Woodward) C. Agardh and Acrosorium venulosum (Zanardini) Kylin.

Chemical extracts
The powder of dried algae was extracted in different solvents: methanol, acetone, chloroform, hexane, dichloromethane-methanol.
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[0x0]-
[0x0]night at 37°C.
[0x0]t culture yielded a
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[0x0],
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[0x0]ning culture media (12 mL
[0x0]ypnea
[0x0]s
[0x0]d
[0x0])
[0x0].
[0x0]µg/ml as
[0x0]ctivity. The amphotericine B at
[0x0]-
[54x75]Laurencia pinnatifida
[54x325]127581) and
[54x335]Fungi:
[54x350]Escherichia coli
[54x360]Gram
[54x384]9372),
[54x403]Streptococcus faecalis
[54x412]Bacillus cereus
[54x421]Gram
[54x436]Culture Collection (ATCC).
[54x470]Microbial strains
[54x500]410
[54x509]was pre
[0x0]the elastase inhibition was assessed at 25 °C. The reaction mixture
[0x0]prepared in 10µl of DMSO
[0x0]8.0) containing
[0x0]410 nm
[0x0]elastase (EC 3.4.21.36 Type II
[0x0]was monitored by measuring the inhibition of the amidolysis of N
[0x0]This activity is measured by the calorimetric method
[0x0]Elastase
[0x0]there
[0x0]measurements were made as duplicates at time 0 and after 5 min
[0x0]there after read at 550 nm.
[0x0]Elastase Inhibition assay
This activity is measured by the calorimetric method [22]. Bioassay
[0x0]7.2
[0x0]µg/100µl of DMSO).
[0x0]°
[0x0]C, with
[0x0]negative bacteria:
[0x0]positive bacteria:
[0x0]Bacillus sp
[0x0]Bacillus subtilis
[0x0]and
[0x0]Bacillus subtillus2
[0x0]Pseudomonas sp
[0x0]Candida tropicalis
[0x0]Cryptococcus neoformans
[0x0]Ktari [8] also tested a
the extract in 96 well plates for 1 h
[0x0]bioassay
[0x0]The change in absorbance was
[0x0]Bioassay was based on a colorimetric bioassay [21]. Each extract (10
[0x0]µg dissolved in DMSO (10 µL) was incubated in 96 well plates for 1 h
[0x0]at 25°C with Apis mellifera venom PLA2 (Sigma, 2 µL of a 1 mg/mL
[0x0]DMSO stock solution). Substrate solution (198 µL) containing L- α-
[0x0]phosphatidylcholine (L- α- lecithin, 3.5 mM), red phenol (0.055 mM),
[0x0]NaCl (100 mM), CaO2 (10 mM) and Triton (7 mM) at pH 7.6 were
[0x0]added. Manoalide was used as a positive control. Colorimetric
[0x0]preparation was incubated for 10 min before addition of
[0x0]was
to obtain a crude extract and were conserved at 4°C.

Antimicrobial activity
Microbial strains
The strains used to evaluate the antimicrobial activity were obtained
from Collection of Institute Pasteur (CIP) and 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 thuringiensis (ATCC 10792), Bacillus subtilius1 (ATCC 9372),
Bacillus subtilius2 (ATCC 6633), Clostridium sporogenes (CIP 7939) and
Mycobacterium smegmato (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 10^6 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 determined after
48h of incubation at 27°C.

Discs impregnated with standard antibiotics such chloramphenicol,
streptomycin and the tetracycline were 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 as 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 than 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 Corallina elongata does not
inhibit phospholipase A2.

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
muscomformis, 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.

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

<table>
<thead>
<tr>
<th>Algae</th>
<th>Solvents of extraction</th>
<th>Antibacterial activity</th>
<th>Diameter of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gram positive bacteria</td>
<td></td>
</tr>
<tr>
<td>Asparagopsis armata</td>
<td>MeOH</td>
<td>B. c; C. s</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Ac</td>
<td>S. a ssp</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Ch</td>
<td>S. a ssp</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>DC/MeOH</td>
<td>B. c</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. t; B. S1</td>
<td>+++</td>
</tr>
<tr>
<td>Corallina elongata</td>
<td>MeOH; Ch; Hex</td>
<td>S. a ssp</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Ac</td>
<td>S. a ssp</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. f</td>
<td>++</td>
</tr>
<tr>
<td>Gigartina acicularis</td>
<td>Ch</td>
<td>S. a ssp</td>
<td>++</td>
</tr>
<tr>
<td>Gracilaria multipartita</td>
<td>MeOH; Ac</td>
<td>S. a ssp</td>
<td>++</td>
</tr>
<tr>
<td>Halopitys incurvus</td>
<td>MeOH</td>
<td>C. s</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Ac</td>
<td>S. a ssp</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>DC/MeOH</td>
<td>C. s</td>
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<tr>
<td></td>
<td></td>
<td>B. c</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. a</td>
<td>++</td>
</tr>
<tr>
<td>Pterosiphonia complanata</td>
<td>MeOH</td>
<td>B. c; B. S1; S. a sp</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Ac</td>
<td>B. S1; S. a ssp</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>DC/MeOH</td>
<td>B. t</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. c; S. a ssp</td>
<td>+++</td>
</tr>
<tr>
<td>Chondria dasiphylla</td>
<td>MeOH</td>
<td>S. a</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>MeOH; Ac; Ch</td>
<td>S. a</td>
<td>++</td>
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<td></td>
<td></td>
<td>S. a</td>
<td>++</td>
</tr>
<tr>
<td>Bornetia secundiflora</td>
<td>MeOH; Ac</td>
<td>S. a ssp</td>
<td>++</td>
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<tr>
<td>Caulacanthus ustulatus</td>
<td>MeOH</td>
<td>S. a ssp</td>
<td>++</td>
</tr>
<tr>
<td>Gellidium latifolium</td>
<td>MeOH; Ac</td>
<td>S. a ssp</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Hex</td>
<td>B. S1</td>
<td>++</td>
</tr>
<tr>
<td>Gigartina teedi</td>
<td>MeOH; Ch</td>
<td>S. a ssp</td>
<td>++</td>
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<tr>
<td>Gracillaria verrucosa</td>
<td>MeOH</td>
<td>S. a ssp</td>
<td>++</td>
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<td></td>
<td>Ac</td>
<td>S. a ssp</td>
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<td></td>
<td></td>
<td>S. a</td>
<td>++</td>
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<tr>
<td>Hypnea musciformis</td>
<td>MeOH</td>
<td>S. a ssp</td>
<td>++</td>
</tr>
<tr>
<td>Placodium cartilagineum</td>
<td>MeOH</td>
<td>S. a ssp</td>
<td>++</td>
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<td></td>
<td></td>
<td>S. a</td>
<td>++</td>
</tr>
<tr>
<td>Sphaerococcus coronopilus</td>
<td>MeOH; Ch; Hex</td>
<td>S. a ssp</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Ac</td>
<td>S. a ssp</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. f</td>
<td>++</td>
</tr>
<tr>
<td>Gigartina pistillata</td>
<td>Dc/MeOH</td>
<td>B. sp; S. f</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. sp; S. f</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. a</td>
<td>++</td>
</tr>
<tr>
<td>Corallina officinalis</td>
<td>Dc/MeOH</td>
<td>B. c; S. a</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. f</td>
<td>+++</td>
</tr>
<tr>
<td>Chondrus crispus</td>
<td>Dc/MeOH</td>
<td>B. c; S. a</td>
<td>++</td>
</tr>
<tr>
<td>Gelidium sesquipedale</td>
<td>Dc/MeOH</td>
<td>B. c; S. a; S. f</td>
<td>+++</td>
</tr>
<tr>
<td>Laurencia pinnatifida</td>
<td>Dc/MeOH</td>
<td>S. a ssp; B. sp; S. f</td>
<td>++</td>
</tr>
</tbody>
</table>

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 subtilis 1: B. S1; Bacillus subtilis 2: 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 (+<15mm), 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; methanol extract obtained greater inhibition against gram-positive bacteria. These results are in agreement with the observations of Taskin et al. [27], Randhasamy 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 Mhdhebi et al. [30] who found that the methanolic extract 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 subtilis 1 and Staphylococcus aureus ssp aureus. For methanolic extract of Chondria dasypylla, 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 thuringiensis and Bacillus subtilis 1, while the similar extract of Pterosiphonia complanata showed activity toward Bacillus cereus and Staphylococcus aureus ssp aureus. Gelidium sesquipedale gave a comparable activity, but against Bacillus cereus, Staphylococcus aureus and Streptococcus faecalis.

These results are in agreement with those obtained by Fehles et al. [31]; however they are in contrast with those of Sastry et al. [32] who mentioned that chloroform is 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], Vidyavati 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 from 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 algae 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.

REFERENCES