African Journal of Biotechnology Vol. 11(104), pp. 16831-16837, 27 December, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3761
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Screening of antibacterial and antifungal activities in green and brown algae from the coast of Sidi Bouzid (El Jadida, Morocco)

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Accepted 23 April, 2012

Six organic extracts prepared with different solvents (methanol, acetone, hexane, chloroform and dichloromethane-methanol) and aqueous extract of 27 species of marine algae belonging to the Chlorophyta and Phaeophyta were studied for antibacterial and antifungal activities against pathogenic microorganism: eight Gram-positive bacteria: *Bacillus cereus*, *Bacillus thuringiensis, Bacillus subtillus, Clostridium sporogenes, Staphylococcus aureus, Mycobacterium smegmatis, Streptococcus faecalis* and *Bacillus* sp, two Gram-negative bacteria: *Escherichia coli* and *Pseudomonas* sp and against fungi: *Candida tropicalis* and *Cryptococcus neoformans*. The best activity was observed in methanolic extract followed by acetonic extract and that prepared with methanol–dichloromethane. Of the 27 species tested, those belonging to Phaeophyceae were the most active in comparison with Chlorophyceae. The Gram-positive bacteria presented a sensibility superior to the Gram-negative and *S. aureus* ssp. *aureus* was the more sensitive

Key words: Macroalgae, algal extracts, antibacterial activity, antifungal activity, pathogenic microorganism.

INTRODUCTION

More than 150 000 macroalgae species are found in oceans of the globe, which include green, brown and red algae, but only a few of them are identified (Bansemir et al., 2006). These organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents (Blunt et al., 2006). During the last four decades, numerous novel compounds have been isolated from marine organisms and many of these substances have been demonstrated to possess interesting biological activities (Faulkner 1984, 2002) such as antibacterial (Barreto and Meyer, 2006; Berland et al., 1972; Biard et al., 1980; Burkholder et al., 1960; Chenieux et al., 1980; Etahiri et al., 2003; Fenical and Paul, 1984; Freile-Pelegrìn and Morales, 2004; Oranday et al., 2004; Lima-Filho et al., 2002; Lustigman and Brown, 1991), antiviral (Richards et al., 1978; Barbosa et al., 2004), antitumoral (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 antifouling (Hellio et al., 2001, 2004, Maréchal et al., 2004) activities.

The antimicrobial activity from algae was largely studied and numerous substances were identified as antimicrobial agents, which include chlorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocyclic compounds, phenolic inhibitors, alkaloids, etc (Espeche et al., 1984; Etahiri et al., 2001, 2007; Rosell and Srivastava, 1987). This study is a part of a program aimed at the isolation and characterization of bioactive compounds from marine algae collected from the Atlantic coast of Morocco. The algae were collected from the coast of Sidi Bouzid (Figure 1), located at 3 km from the city of El Jadida. It is characterized by a strong rebound of a frozen ocean current,

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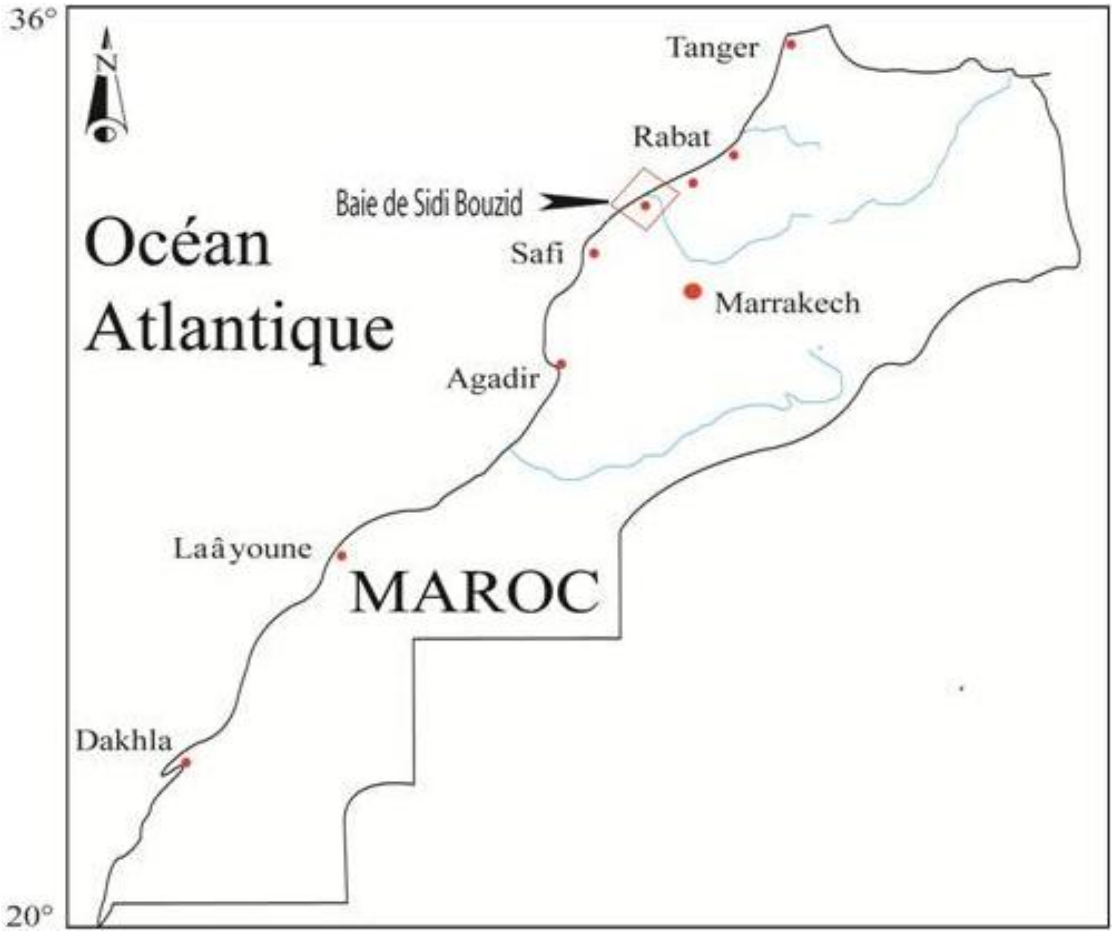

Figure 1. Localisation of the collection site of Sidi Bouzid.

and rich in minerals called upwelling and that makes the wealth of this region algal (quantity and quality); hence the importance of studying algae harvested from this area.

In this study, we report the antimicrobial activity of methanol, acetone, hexane, chloroform and dichloro-methane-methanol extracts of 27 marine algae to select the most active species which could be utilized for the purification of antimicrobial compounds.

MATERIALS AND METHODS

Algal materials

Seaweeds were collected by hand-picking in the period of March to April 2009 from Sidi Bouzid coast (33°-33°16'09''N, 8°30'-8°45'W). 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; Green algae: *Ulva lactuca* C. Agardh, *Enteromorpha compressa* (Linnaeus) Kutzing, *Codium tomentosum* Stackhouse, *Enteromorpha linza* (Linnaeus) J. Agardh, *Ulva crispa* Lightfoot, *Codium elongatum* (Turner) Greville, *Codium adherens* A. Agardh, *Bryopsis balbisiana* (Hudson) C. Agardh, *Enteromorpha clathrata* (Roth) J. Agardh, *Enteromorpha intestinalis* Link, *Enteromorpha muscoides* (Clement y Rubio) Cremades in cremades and Perez-Cicera, *Rhizoclonium riparium* (Roth) Harvey and *Ulva rigida* (Kützing) and Brown algae: *Bifurcaria bifurcata* Ross, *Cystoseira humilis* Kützing, *Cystoseira tamariscifolia* (Hudson) Papenfuss, *Fucus spiralis* Linnaeus, *Sacchorhiza bulbosa* (Lightfoot) Batters, *Laminaria ochroleuca* de la Pylaie, *Colpomenia sinuosa* (Mert) Derbes and Solier, *Sargassum vulgare* C. Agarth, *Spatoglossum schroederi* (C. Agarth) Kutzing, *Dictyopteris polydioides* (De Candolle) lamouroux, *Halopteris scoparia* (Linnaeus) Sauvageau, *Padina pavonica* (Linnaeus) Thivy, *Dictyota dichotoma* (Hudson) Lamouroux, *Cystoseira ericoides* C. Agarth.

Preparation of extracts

The powder of dried algae was extracted in different solvents methanol, acetone, ethanol, chloroform, hexane, dichloromethane, methanol and water as described by Caccamese and Azolina (1979). The resulting extracts were concentrated to dryness in a rotary evaporator under reduced pressure (at 45°C) until a crude extract was obtained, and was conserved at 4°C.

Microbial strains

The strains used to evaluate the antimicrobial activity were obtained from the Collection of Institute Pasteur (CIP) and from American Type Culture Collection (ATCC). The Gram-positive bacteria included: *Staphylococcus aureus* (ATCC 9144), *Staphylococcus*

Table 1. Antibacterial activity of Brown algae against gram positive bacteria .

Brown algae	Extract	Gram-positive bacteria									
		B. c	***B. t***	***B.s1***	***B. s2***	***C. s***	***S. a***	***S. a* ssp. *a***	***M.s***	***S. f***	***B.* sp.**
Bifurcaria bifurcata	MeoH	+	++	+	-	+++	-	+++	-	-	-
	Ac	++	+++	+++	-	++	-	-	-	-	-
	Ch	-	-	-	-	-	-	T	-	-	-
	Hex	+	+	+	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	+++	+	++	-	-	+++	-	-	+	+
Fucus spiralis	MeoH	+	-	-	-	+++	-	+++	-	-	-
	Ac	+	++	-	-	++	-	-	-	++	-
	Ch	-	-	-	-	-	-	-	-	-	-
	Hex	-	-	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	+	-	-	-	-	+	-	-	+	+
Laminaria ochroleuca	MeoH	-	-	-	-	-	-	++	-	-	-
	Ac	-	-	-	-	-	-	+++	-	-	-
	Ch	-	-	-	-	-	-	+	-	-	-
	Hex	-	-	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	+	-	-	-	ND	++	-	-	+	+
Cystoseira tamariscifolia	MeoH	-	-	-	-	++	-	++	-	-	-
	Ac	-	-	-	-	+	-	+++	-	-	-
	Ch	-	-	-	-	-	-	++	-	-	-
	Hex	-	-	-	-	+	-	-	-	-	-
	Water	-	-	-		-	-	-	-	-	-
	DC/MeoH	+	-	-	-	-	+	-	-	-	-
Cystoseira humilis	MeoH	+++	+++	+++	+++	++	+++	+++	+++	-	-
	Ac	++	+++	++	++	++	+++	++	++	-	-
	Ch	-	-	-	-	-	+	-	-	-	-
	Hex	+	+	+	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	+++	++	++		-	+++	-	-	-	-
Sargassum vulgare	MeoH	+	-	-	-	+	-	+++	-	-	-
	Ac	+	-	-	-	++	-	+++	-	-	-
	Ch	-	-	-	-	-	-	-	-	-	-
	Hex	-	-	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	-	-	-		-	-	-	-	-	-
Colpomenia sinuosa	MeoH	-	-	-	-	-	++	-	-	+++	+++
	Ac	-	-	-	-	-	++	-	-	-	-
	Ch	-	-	-	-	-	++	-	-	-	-
	Hex	-	-	-	-	-	+	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	-	-	-	-	-	-	-	-	-	-

Table 1. Contd.

Brown algae	Extract	Gram-positive bacteria									
		B. c	***B. t***	***B.s1***	***B. s2***	***C. s***	***S. a***	***S. a* ssp. *a***	***M.s***	***S. f***	***B.* sp.**
Spatoglossm schroedrii	MeoH	++	-	++	-	-	+	-	-	+	+
	Ac	-	-	+	-	-	++	-	-	++	-
	Hex	-	-	-		-	+	-	-	-	-
	Ch	-	-	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	+	-	-	-	-
	DC/MeoH	-	-	-	-	-	-	-	-	-	-
Sacchorhiza bulbosa	MeoH	-	-	-	-	-	-	-	-	-	-
	Ac	-	-	-		-	-	-	-	-	-
	Hex	-	-	-		-	-	-	-	-	-
	Ch	-	-	-		-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	++	-	++	-	-	++	-	-	++	+++

MeoH, Methanol; Ac, acetone; Ch, chloroform; Hex, hexane; DC, dichloromethane. *B. c*, *Bacillus cereus; B.t, Bacillus thuringiensis; B.s1*, *Bacillus subtillus1*; *B.s2, bacillus subtillus2; C.s, Clostridium sporogenes; S.a, Staphylococcus aureus; S. a* ssp. *a, Staphylococcus aureus* ssp. *aureus; S .f, Streptococcus faecalis; M.s*, *Mycobacterium smegmatis; B. sp*, *Bacillus* sp. - : No activity, + : < 10 mm, ++: < 15 mm, +++: > 15 mm, T: trace, ND: not determined.

aureus ssp. *aureus* (ATCC 6538), *Bacillus* sp. (CIP 104717), *Streptococcus faecalis* (ATCC 19433), *Bacillus cereus* (CIP 783), *Bacillus thuringiensis* (ATCC 10792), *Bacillus subtillus 1* (ATCC 9372), *Bacillus subtillus 2* (ATCC 6633), *Clostridium sporogenes* (CIP 7939) and *Mycobacterium smegmatis* (CIP 7326). Gram-negative bacteria used were *Pseudomonas sp (*ATCC 19433*)* and *Escherichia coli* (ATCC 10536*)*, while the fungi used were *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 (Bauer et al., 1966). 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 0.2×10^4 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. The organic extracts were tested using paper disks (6 mm diameter) impregnated with the solution. After the temperature was equalized at 4°C, the microorganisms were incubated overnight at 37°C. Diameters of inhibitory zones were then measured.

For fungicidal activity, zones of inhibition were determined after 24 h of incubation at 27°C. Discs impregnated with standard antibiotics such as chloramphenicol, streptomycin and the tetracycline were used at 50 or 100 µg/ml as reference in the test of antibacterial activity. Amphotericin B at 200 µg/ml was 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 (Lima et al., 2002).

RESULTS AND DISCUSSION

The results of the screening of antibacterial and antifungal activities against bacteria and yeast are summarized in Tables 1 to 3. We reported only the species showing a positive activity against stains tested.

Antibacterial activity against Gram positive bacteria

Brown algae

Of the 14 brown algae tested, only nine species showed a positive activity against at least one bacteria test. An important activity (diameter of inhibition higher than 15 mm) was observed in the methanolic extract of *C. humilis* which inhibited eight Gram positive bacteria. The acetone extract of this species showed an inhibition of *B. thuringiensis* and *S aureus*. Also, the extract prepared in the mixture of dichloromethane and methanol showed an inhibition of *B. cereus* and *S aureus*. A similar activity was obtained in the presence of the methanolic extract of *B. bifurcata* against *C. sporogenes* and *S. aureus* ssp. *aureus*. Moreover, the acetonic extract inhibited *B. thuringiensis* and *B subtillus1*. For extract prepared in the dichloromethane/methanol (DC/MeoH) mixture, activity was observed toward *B. cereus* and *S aureus*. In addition, we noted that the methanolic extracts of *F. spiralis*, *S. vulgare* and *C. sinuosa* showed inhibition of some bacteria used in this test.

Table 3: Antimicrobial activity of green algae against gram positive bacteria.

Green algae	Extract	Gram-positive bacteria									
		B. c	***B. t***	***B.s1***	***B. s2***	***C.s***	***S. a***	***S. a* ssp. *a***	***M.s***	***S. f***	***B.* sp**
Ulva lactuca	MeoH	++	-	++	-	-	++	-	-	-	-
	Ac	-	-	-	-	-	+	-	-	-	-
	Chl	-	-	-	-	-	+	-	-	-	-
	Hex	-	-	-	-	-	-	-	-	-	-
	water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	-	-	-	-	-	-	-	-	-	-
Enteromorpha intestinalis	MeoH	+	-	-	-	-	++	-	-	+	+
	Ac	-	-	-	-	-	++	-	-	-	-
	Ch	-	-	-	-	-	++	-	-	-	-
	Hex	-	-	-	-	-	T	-	-	-	-
	water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	-	-	-	-	-	-	-	-	-	-
Enteromorpha clathrata	MeoH	+	-	-	-	-	++	-	-	-	-
	Ac	-	-	-	-	++	-	++	++	-	-
	Ch	-	-	-	-	+	-	+++	-	-	-
	Hex	-	-	-	-	-	-	-	-	-	-
	water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	-	-	-	-	-	-	-	-	-	-
Enteromorpha muscoides	MeoH	-	-	-	-	-	+	-	-	-	-
	Ac	+	-	-	-	-	-	++	-	-	-
	Ch	+	-	-	-	-	-	++	-	-	-
	Hex	-	-	-	-	-	-	T	-	-	-
	water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	-	-	-	-	-	-	-	-	-	-
Rhizoclonium riparium	MeoH	-	-	-	-	-	+	-	-	-	-
	Ac	-	-	-	-	-	-	+++	-	-	-
	Ch	-	-	-	-	-	-	+	-	-	-
	Hex	-	-	-	-	-	-	++	-	-	-
	water	-	-	-	-	-	-	-	-	-	-
	DC/MoH	-	-	-	-	-	-	-	-	-	-
Ulva rigida	MeoH	-	-	-	-	-	+	-	-	-	-
	Ac	++	-	-	-	-	-	++	++	-	-
	Ch	T	-	-	-	-	-	-	T	-	-
	Hex	-	-	-	-	-	-	T	-	T	-
	water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	-	-	-	-	-	-	-	-	-	-
Enteromorpha compressa	MeoH	-	-	-	-	-	+	-	-	-	-
	Ac	+	-	++	-	-	+++	-	-	+	-
	Ch	-	-	+	-	-	+	-	-	-	-
	Hex	-	-	-	-	-	+	-	-	-	-
	water	-	-	-	-	-	-	-	-	-	-
	DC/MeoH	-	-	-	-	-	-	-	-	-	-

MeoH, methanol; Ac, acetone; Ch, chloroform; Hex, hexane; DC, dichloromethane. *B. c, Bacillus cereus; B. t, Bacillus thuringiensis; B.s1, Bacillus subtillus1; B.s2, Bacillus subtillus2; C.s, Clostridium sporogenes; S. a, Staphylococcus aureus; S. a* ssp. *a, Staphylococcus aureus* ssp. *aureus; S .f, Streptococcus faecalis; M.s, Mycobacterium smegmatis; B. sp, Bacillus sp.* - : No activity, + : < 10 mm, ++: < 15 mm, +++: > 15 mm, T: trace, ND: not determined.

Green algae

Of the 13 algae tested, the presence of a positive activity on a Gram$^+$ bacterium was observed in seven species. Diameter of inhibition higher than 15 mm was observed in the chloroformic extract of *C. clathrata* against *S. aureus* ssp. *aureus*, and in the acetonic extract of *R. riparium* against *S. aureus* ssp. *aureus* and that of *E. compressa* toward *S. aureus.*

Antibacterial activity against Gram negative bacteria

For brown algae, concerning the bacteria Gram-negative, only the methanolic extract of *C. sinuosa* showed an inhibition with a diameter superior to 15 mm against *Pseudomonas* sp. A weak activity was observed in the dichloromethane/methanol extract of *B. bifurcata*, *S. spiralis*, *L. ochroleuca* and *S. Bulbosa*. For the green algae tested, no activity was detected against the Gram-negative bacteria.

Antifungal activity

For brown algae, a diameter of inhibition ranging between 10 and 15 mm was observed in the dichloromethane/ methanol extract of *L. ochroleuca* against *C. neoformans*, and in the methanolic extract of *C. sinuosa* and *S bulbosa* toward *C. tropicalis*. For the green algae studied, no activity was detected against fungi. Of the 27 species tested, those belonging to Phaeophyceae were the most active in comparison with Chlorophyceae; the same result was reported by Caccamese et al. (1980) and Pesando and Garam (1984). This difference in antibacterial activity of algae exists because macroalgae produce a great variety of secondary metabolites. Hornsey and Hide (1974) reported that 151 species of marine algal crude extracts exhibited activity against pathogenic bacteria but variation may exist in antibacterial activity of algae. The difference may due to the efficiency of the extraction methods to recover the active metabolites, solvents used (Turney et al., 2006), susceptibility of strains (Perez et al., 1990) and seasonal variation (Vidyavathi and Sridhar, 1991). In view to this, various solvents and water were applied in this study, with the aim to select the best solvent yielding maximum amount of bioactive compounds responsible for the antimicrobial activity.

Some studies concerning the effectiveness of solvent used for extraction reported that methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate (Sastry and Rao, 1994), whereas others reported that chloroform is better than methanol and benzene (Febles et al., 1995). In this study, antimicrobial activity of organics extract was higher compared to aqueous extract. In addition, this result shows that Gram-positive bacteria present a sensibility superior to that of the Gram-negative. The majority of seaweed tested towards *E. coli*, showed a low or no activity. Febles et al. (1995) noticed a very low activity towards *E. coli* by seaweeds harvested on the Atlantic Coast. Allen and Danwson (1960), Rao and Parekh (1981), Vidyavathi and Sridhar (1991) also reported the same result that the Gram-positive bacterial strains were more susceptible to seaweeds extract than Gram-negative strains. In general, antibiotic substances appear to have more inhibitory effect towards Gram-positive than to Gram-negative bacteria. Result obtained in this study reveals that *S. aureus* ssp. *aureus* was more sensitive than all the strains; with the largest inhibition diameter.

Conclusion

This study reports the presence of antibacterial compounds in the algae collected from the site of Sidi bouzid. The antibacterial activity of algae depends on the class of algae; a number of active species was more important in the class of Phaeophyceae than Chlorophyceae. As for the extraction used, in the majority of the cases, methanol followed by acetone were the solvents that enhanced the activity of the extracts towards the bacterial strain used for the antibiotic test. As a result, it can be concluded that macroalgae from the coast of Sidi Bouzid are potential sources of bioactive compounds and should be investigated for isolation of natural antibiotics.

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