Original Article

TIPS Trends in Pharmaceutical Sciences 2021: 7(4): 233-242.

The Antimicrobial Activity of Two Marine Red Algae Collected from Algerian West Coast

Souhila Saim[1*], Fatima Sahnouni[1], Djilali Bouhadi[1,2], Soumia Kharbouche[1]

[1]Faculty of Natural and Life Sciences, Laboratory of Research in Geo-Environment and Spatial Development GEDE, Department of biology. University of Mustapha Stambouli,29000 Mascara, Algeria. [2]Bioconversion Laboratory, Microbiology Engineering and Health Safety, Faculty of Natural Sciences and Life, University of Mascara, Algeria.

Abstract

In this present study, extracts of two marine red algae (*Asparagopsis taxiformis*, *Hypnea musciformis*), harvested from Algerian West Coast (Oran), were investigated for their antimicrobial activity against Human pathogenic bacteria including antibiotic-resistant organisms and three fungi. Their antimicrobial activities from crude methanolic extracts were evaluated by using the paper disc agar diffusion method at different concentrations (0.5 to 2 mg/ml). Methanolic extract of *A. taxiformis* showed highest antibacterial activity compared to that of *H. musciformis*. This activity was dependent of the used concentration of methanolic extracts. Methanolic extract of *A. taxiformis* elicited remarkable antimicrobial activity against all Human pathogenic bacteria except *Salmonella* sp., *Serratia* sp. and *P. aeruginosa*. However, this extract showed moderate antifungal activity against *Candida albicans* and *Penicellium* sp. On the other hand, results obtained show that methanolic extract of *H. musciformis* exhibit a low activity-which was effective only at 0.2 and 1.5 mg/ml. However, for its antifungal activity, a potent activity against *C. albicans*, *Aspergillus* sp. and *Penicellium* sp. was recorded. The present finding confirms that marine algae can be further studied and used as a possible source of antimicrobial compounds in the medical field.

Keywords: Algerian West Coast, antimicrobial activity, *Asparagopsis taxiformis*, *Hypnea musciformis*, marine red algae.

Please cite this article as: Saim S, Sahnouni, F, Bouhadi D, Kharbouche S. Antimicrobial activity of two marine red algae collected from Algerian West Coast. Trends in Pharmaceutical Sciences. 2021;7(4):233-242.doi:10.30476/tips.2021.89827.1078

1. Introduction

Every year, infectious diseases cause a high rate of the worldwide death toll (1). Rapid appearances of drug-resistant pathogens and escalating emergence of new infections diseases have been widely reported (2). For these threats and challenges to be countered, many researchers have focused on investigating natural and safe antimicrobial agents for both the food and medical industries (3).

Besides plant extracts, marine algae are considered as source of bioactive compounds to produce great variety of secondary metabolites characterized by a broad spectrum of biological activities. They comprise one of the commercially important marine renewable resources (4). Indeed, they compose a natural source of a variety of drugs for pharmaceutical, food and cosmetic applications including carotenoids, terpenoids, steroids,

Corresponding Author: Souhila Saim, Laboratory of Geomatic, Ecology and Environment, Faculty of Natural Sciences and Life, University of Mascara, Algeria.
Email: saimsouhila@gmail.com

Received: 08/07/2021; Accepted: 01/12/2021

amino acids, phlorotannins, phenolic compounds, halogenated ketones, alkanes and cyclic polysulphides (5, 6). Also, they contain different vitamins, minerals, trace elements, proteins, iodine, bromine and bioactive substances (7). Recently, researchers have reported that seaweed extracts compounds show a variety of biological activities including: antibacterial (8, 9), antifungal (10),antiviral (11, 12), antitumorals (13), antioxidant(14), anti-inflammatory (15) and anticancer (16). Concerning antibacterial activity, several workers have reported that the seaweed extracts exhibit inhibitory activity against pathogenic bacteria. However, data on biological activities of seaweed from the Algerian coast are very scarce.

The aim of this study was to assess the antibacterial and antifungal activity of methanolic crude extracts of two marine red algae (*Asparagopsis taxiformis*, *Hypnea muscuformis*) from the western coast of Algeria. This activity targeted Human pathogenic bacteria including antibiotic-resistant forms and three fungi.

2. Materail and Methods

2.1. Algal collection and preparation

The marine red algae, object of this study, were collected from Algerian West Coast (Oran) during March 2016. The algal species were identified by experts in these fields, using standard literature and taxonomic keys. The collected algal samples were cleaned well with seawater to remove impurities matter such as epiphytes, sand particles, The seaweeds were transported to the laboratory in sterile polythene bags and were washed with distilled water several times, spread on plates at room temperature and in the dark for three weeks (17). Dried samples were cut into small pieces and ground with a blender into powder to be analyzed to be analyzed before extraction.

2.2. Preparation of the extract

Seaweed samples were pulverized and 100 grams of dried material were added to 100 ml of methanol and left for 24 h at room temperature with stirring at 200 rpm. The solvent extracts were then filtered and the filtrate was concentrated by rotary evaporation at 45-50 °C. We dried the filtered solvent under reduced pressure at 40 °C, and used the resultant deposits as crude extracts. The resulting extracts were then dissolved in dimethlysulfoxide (DMSO) and kept at +4 °C until further use.

2.3. Preliminary Phytochmical Tests

This analysis determined the presence or absence of different compounds: alkaloids, saponins, flavonoids, tannins, steroids and terpenoids for both estracts using standard qualitative methods.

2.3.1. Flavonoids test

Appearance of orange colour confirms the presence of flavonoids after addition of sulfuric acid on the extracts (18).

2.3.2. Terpenoids test

Addition 2 ml chloroform and 3 ml sulfuric acid to extracts, apperance of a reddish brown colour confirms the presence of terpenoids (19).

2.3.3. Saponins test

The presence of saponins were confirmed by appearance of bubbles after shaking of the extracts with distilled water (18).

2.3.4. Alkaloids test

Formation of yellow cream precipitate after adding Mayers reagent to the extracts and indicates the presence of alkaloids (19).

2.4. Culture and Maintenance of microorganisms

The seaweed extracts were tested against a panel of clinical isolates via:

Staphylococcus aureus (*Sa63*), *Bacillus subtilus* (*Bs64*), *Clostridium* sp. (*Cl17*), *Streptococcus* sp. (*St16*), *Vibrio proteolyticus* (*Vp15*), *Pseudomonas fluorescence* (*Pf14*), *Pseudomonas aerugenosa* (*Pa62*), *Klebseilla pneumonia* (*Kp13*), *Escherichia coli* (*Ec61*), *Salmonella* sp (*Sa10*), *Klebseilla* sp (*Kl12*), *Serratia* sp. (*Se11*), *Aspergillus* sp, *Penicillium* sp. *Candida albicans.*

Clinical isolates were procured from Hospital of Mascara-Algeria. They were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by sub-

culturing regularly on the same medium and stored at 4 °C.

2.5. Antibiotic susceptibility testing

The antimicrobial susceptibility of the collected bacteria was assessed using the modified Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (20).The following concentrations of antibiotics were tested: Tétracycline TE (30 µg), Céfazoline CZ (30 µg), Pénicilline P (10 µg), Oxacilline OX (5 µg), *Spiramycine* SP (10 µg) Amoxicillin AM (5 µg), Aztréonam ATM (30 µg), Colistine C (10 µg), Gentamicine GEN(15 µg). The antibiotic disks were then applied to the prepared plates and incubated at 37 °C for 18 h then, the diameter of the growth inhibition zones was measured (CASFM, 2015). The multiple-antibiotic resistances (MAR) index was calculated for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (21).

2.6. Antibacterial assay

To evaluate the antimicrobial activity of the seaweed extracts, the following microorganisms were tested: Gram positive stains: *Staphylococcus aureus* (*Sa63*), *Bacillus subtilus* (*Bs64*), *Clostridium* sp. (*Cl17*), *Streptococcus* sp. (*St16*) and Gram negative stains: *Vibrio proteolyticus* (*Vp15*), *Pseudomonas fluorescence* (*Pf14*), *Pseudomonas aerugenosa* (*Pa62*), *Klebseilla pneumonia* (*Kp13*), *Escherichia coli* (*Ec61*), *Salmonella* sp. (*Sa10*), *Klebseilla* sp. (*Kl12*), *Serratia* sp. (*Se11*), also, fungal stains: *Aspergillus* sp, *Penicillium* sp. and *Candida albicans*.

The sensibility tests were performed according to National Committee for Clinical Laboratory Standards ,1993. Antimicrobial activity was evaluated using the agar diffusion technique in Petri dishes (22, 23). Briefly, all bacterial isolates were suspended in saline to a turbidity equivalent to 0.5 McFarland (1.5 x 10^8 CFU/ml) and 0.1 ml standardized inoculum suspension was swabbed uniformly on MHA plates. Sterile filter paper discs, 6 mm in diameters (Whatman No. 1), were loaded with 20 µl of the different extracts and air dried. The crude extracts were dissolved in 10% diméthylsulfoxyde (DMSO). Discs impregnated with methanol and with DMSO were used as negative controls. The discs were placed on Muller Hinton agar plates (Merck, Darmstadt, Germany) inoculated with each of the previously mentioned microorganisms (approximately 10^7-10^8 bacteria and fungus/ml). Plates were incubated for 24 h at 35 °C. After incubation, the diameter of complete inhibition zones was measured and means and standard deviations of triplicate were calculated. The extracts were tested at different concentrations (2 mg/ml, 1.5, 1 mg/ml and 0.5 mg/ml).

Zones of inhibition were determined after 24-48 h at 37 ºC for bacteria and 25 ºC for fungi. The tests were carried out in triplicates. The data were represented as the mean±standard deviation (SD).

2.7. Statistical Analysis

All the experiments were carried out in triplicates and values were expressed as mean±SD. Graphics were made using Microsoft Office Excel 2007.

Statistical analysis The data were statistically analysed by applying an one-way ANOVA carried out with the Statistical Program for Social Sciences 16.0 (SPSS, USA, ver. 16.0). Pearson's correlation analysis was done to correlate the phytochemicals content and antioxidant potential in the samples

3. Results and Discussion

Macroalgae have been extensively investigated for the past 30 years. A Recent report suggests that their extracts possess bioactive compounds of great medicinal value.

The present study is aimed to provide data on *in vitro* antioxidant and antibacterial activity of three red algae (*A. taxiformis*, *H. musciformis*) harvested from Algerian west coast (Oran).

3.1. Extraction and fractionation

The yield of the extraction procedure, expressed as the weight percentage of collected dry matter relative to the initial algae powder is as follows: extraction yield is different between two spices of algae. In our result, we find that of the alga *Hypnae musciformis* (9.8%) give the Higher

Table 1. Preliminary phytochemical screening of crude extracts of red algae.

	Flavonoïds	Tannin	Saponin	anthraquinons	terpénoïds	Coumarins
A.taxiformis	+	+	+	+	+	-
H. musciformis	+	+	ND	-	+	-

(+): Positive test (-): Negative test ND: not determined.

extraction followed by the crude extract of the alga *A. taxiformis* (4.5%). The yield of the methanolic extract of the *H. musciformis* alga in the present study was higher compared to subsequent studies by Kajal *et al.* (24) who obtained 4.8%. in comparaison with the result of Mellouk *et al.* (25) for *A. taxiformis*, yield extraction was lower than his funding who worked on the same specie collected on the coast of Oran in northwest Algeria at Bousfer beach (Coralès)

The yield is only relative and depends on the method and the conditions under which the extraction was carried out. The extraction method also affects the total content of phenolic and flavonoid compounds and antioxidant activity (26).

3.2. Phytochemical screening

The result of qualitative phytochemical screening of the crude extract of two algae (*H. musciformis* and *A. taxiformis*) was carried out in order to assess the presence of bioactive substances which might have antibacterial potency. The qualitative analysis of our extracts (Table 1) shows that algae extracts contain six major groups of chemical compounds these are polyphenols, flavonoids, tannins as well as terpenoids and anthraquinone. We note the absence of anthraquinone in the second alga and coumarin in both species of algae may be because of using only methanol as an eluent, some bioactive compounds were not extracted (27). For *A. taxiformis*, the data are scarce.

Preliminary phytochemical analysis revealed a slight variation between the tested species. Our results are partially in agreement with those obtained by Alghazeer *et al.* (28) who worked on metabolic extract of *H. musciformis* collected from the western coast of Libya, the found in their result that the extract of these spcies have not flavonoids, terpenes, anthraquinones and coumarins. Also, as the finding of Mellouk *et al.* (25) who revealed that the presence of bioactive molecules in extracts from *A. taxiformis* may be are the source of antioxidant agents.

3.3. Sensitivity study for different antibiotics

As shown in the table 2, the majority of antibiotics are ineffective. The proportions of resistance varied significantly between different

Table 2. Effect of antibiotics on pathogenic bacteria.

Pathogenic bacteria	Antibiotics								
	P	OX	AX	ATM	C	GN	TE	SP	CZ
E. coli (LRSE 61)	R	R	S	R	S	R	S	NT	R
Serratia sp. (CHU 11)	R	NT	S	S	NT	S	R	NT	NT
Salmonella sp. (CHU 10)	R	S	R	S	NT	I	R	NT	R
Klebsiella sp. (CHU 12)	NT	NT	R	R	NT	S	R	R	R
K. pneumoniae (CHU 13)	NT	NT	R	R	NT	R	S	I	R
P. aeruginosa (LRSE 62)	R	R	R	S	R	S	R	R	R
P. fluorescens (CHU14)	R	R	R	R	R	I	NT	R	NT
V. proteolyticus (CHU 15),	NT	NT	R	R	NT	S	I	R	R
Clostridium sp. (CHU 17)	R	NT	NT	R	NT	I	NT	I	R
S. aureus (LRSE 63)	R	R	NT	R	NT	S	R	I	R
B. subtilis (LRSE 64)	R	NT	NT	R	S	S	I	NT	R
Streptococcus sp. (CHU 16)	NT	NT	NT	R	NT	R	NT	NT	R

P : penicillin ; OX : oxacillin ; AX : amoxicillin ; C : colistin ; GEN : Gentamycin ; TE tetracyclin ;ATM Aztreonam ;Sp :spiramicyn ;Cz :cefazidin, NT : not tested, R: resistant; S: sensitive

bacteria. The resistance towards penicillin, oxacillin and cefazolin was observed for all clinical isolates. *S. aureus* (*Sa63*) was the major resistant bacteria towards almost of antibiotics followed respectively by *Pseudomonas aerugenosa* (*Ps62*), *Pseudomonas fluorescens* (*Pf14*), *E.coli* (*Ec61*), *Klebsiella pneumonia* (*Kp13*), *Salmonella* sp (*Sa10*) and *B. subtilis* (*Ba64*).

3.4. Antibacterial Activity of Algal Extracts

In the current work, methanol was chosen for extraction method. This is justified by the fact that, in most cases, seaweeds extracts obtained with methanol have higher antimicrobial activity than that of extracts obtained with other organic solvents viz: n-hexane, acetone and ethyl acetate (29, 30) .Our results are partially in agreement with those obtained by Kladi (31) who demonstrated that red algae have a potential source of antibacterial compounds towards both Gram negative or Gram-positive pathogenic bacteria

As shown in figure 1, results showed that methanolic extract of *A. taxiformis* exhibited remarkable antibacterial activity against all Human pathogenic bacteria screened in this study except *Salmonella* sp (*Sa10*), *Serratia* sp (*Se11*) and *P.aeruginosa* (*Ps62*). Likewise, we noticed, this extract successfully prevented the growth of gram positive bacteria more significantly than the gram negative strains. Among the test microorganisms *S. aureus* was found most sensitive against methanolic extract of *A. taxiformis* with inhibition diameter of 22 mm±0.2. Equally, *S. aureus* (*Sa63*), *B. substilis* (*Ba64*), *Streptococcus* sp (St16), *E.coli* (*Ec61*), *Klebsiella* sp (*Kl12*), *K. pneumoniae* (*Kp13*), *P. fluorescens* (*Pf14*) and *V. proteolyticus* (*Vp15*) were inhibited by methanolic extract of *A. taxiformis* at different extract concentrations with inhibition diameters ranging from 10±0.2 to 21±0.5 mm (figure 1). However, *Clostridium* sp. was inhibited until 1 mg/ml.

These results were comparable to those obtained by Manilal *et al.* (26) who reported that methanol extract of *A. taxiformis* exhibited highest activity against multidrug resistant clinical human pathogens viz, *Bacillus subtilis*, *Staphylococcus* aureus, *Staphylococcus epidermidis*, *Micrococcus luteus*, non-haemolytic *Streptococcus*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. According to published data, ethanol extracts of *A. taxiformis* has inhibitory influences on *Vibrio alginolyticus*, *Vibrio vulnificus* and *Aeromonas salmonicida* sub sp. and *Salmonicida* (32).

As observed from figure 2, the methanolic extract of *A. taxiformis* showed moderate antifungal activity against *Candida albicans* and *Penicellium* sp. This activity was observed only from 2 and 1.5 mg/ml. Nonetheless, no activity was recorded against *Aspergillus* sp. These results are partially in agreement with those obtained by Genovese *et al.* (33) who reported that ethanol extract of *A. taxiformis*, collected from, Sicilian coast of the Straits of Messina (Italy) showed bacteri-

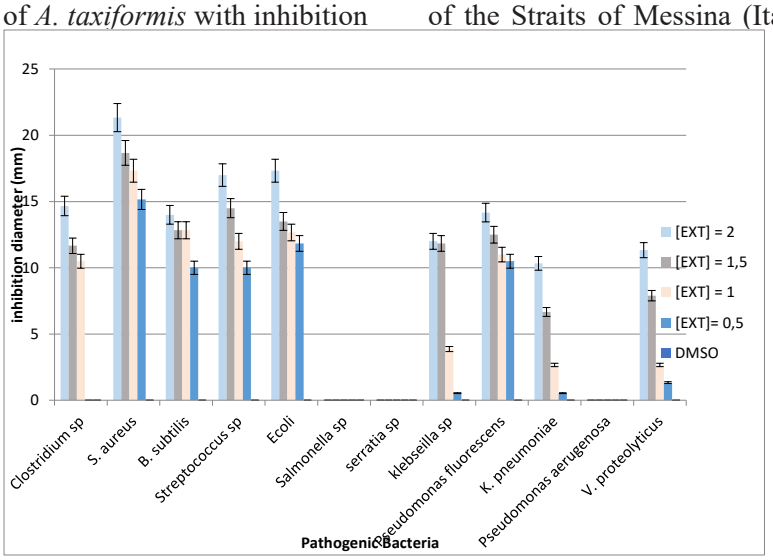

Figure 1. Antibacterial activity of extracts of *A. taxiformis.*

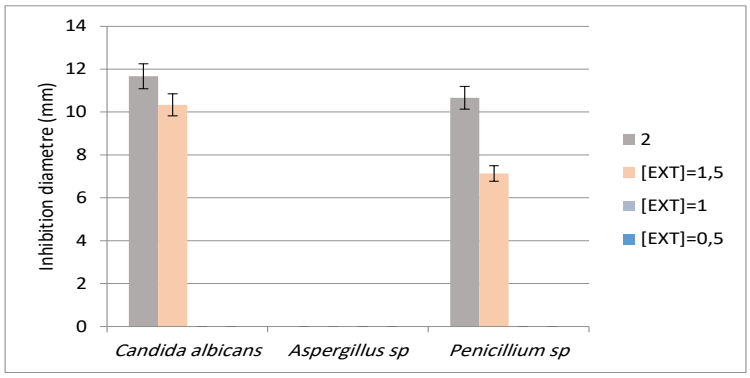

Figure 2. Antifungal activity of extracts of *Asparagopsis taxiformis*.

cidal activity against yeast (*Candida albicans*, *C. parapsilisis*) and moulds (*Aspergillus fumigates* and *Aspergillus terreus*).

Earlier studies have demonstrated the genus *Asparagopsis* as the richest bio-resource of potential secondary metabolites, especially halogenated metabolites and aromatic volatile organic compounds with strong antimicrobial activity (34). Also, Kazlowska (35) acknowledged microbicidal property of *A. taxiformis* was due to volatile metabolites such as halomethanes, haloacetone and acrylates.

Compared with methanolic extract of *A. taxiformis*, results obtained showd that methanolic extract of *H. musciformis* exhibit a low activity which was effective only at 0.2 and 1.5 mg/ml. *Bacillus subtilus* (BA64), *Klebseilla* sp. (KL12) and *Clostridium* sp. (CL17) were the most susceptible microorganisms whose inhibition zones were respectively around 0.8±0.2 mm and 17±0.5 mm at 0.2 mg/ml. Similarly in the present study, *Serratia* sp. (Se11)., *Salmonella* sp. (Sa10), *Klebsiella* sp. (Kl12), *Klepsiella pneumonia* (Kp13) and *Vibrio proteolyticus* (Vp15) were found to be moderately sensitive to *H. musciformis* given that they were inhibited only at 2 mg/ml. No activity was detected against *E. coli*, *P. aeruginosa* and *P. fluorescens* (figure 3).

These results are partially in agreement with Alghazeer *et al.* (25) who concluded in their study that methanolic extract of *H. musciformis* inhibited simultaneously the growth of gram positive and gram negative such as *E. coli* and *Pseudomonas aeruginosa*. Bouhlal *et al.* (36) and Kandhasamy *et al.* (37) reported that methanolic of *H. musciformis* exhibited strong antibacterial activity against the gram positive and gram negative bacteria (38). Also, it found that *H. musciformis* exhibited potent activity not only against Gram positive bacteria (*Staphylococcus aureus*, *Micrococcus luteu* and *Bacillus subtilis*) but also against Gram negative (*Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Serratia marcescens*, *Vibrio fischeri* and *Vibrio alginolyticus*).

Contrastingly, this study reveals that methanolic extract of *H. musciformis* showed a potent

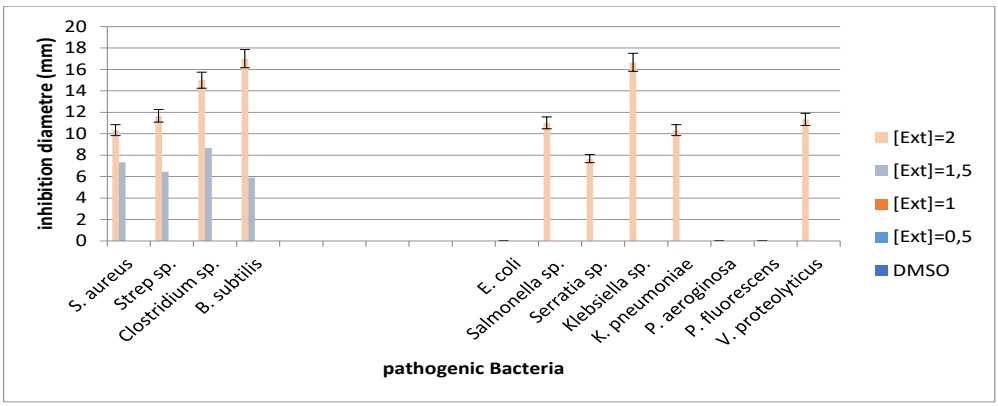

Figure 3. Antibacterial activity of extract of *Hypnae musciformis*.

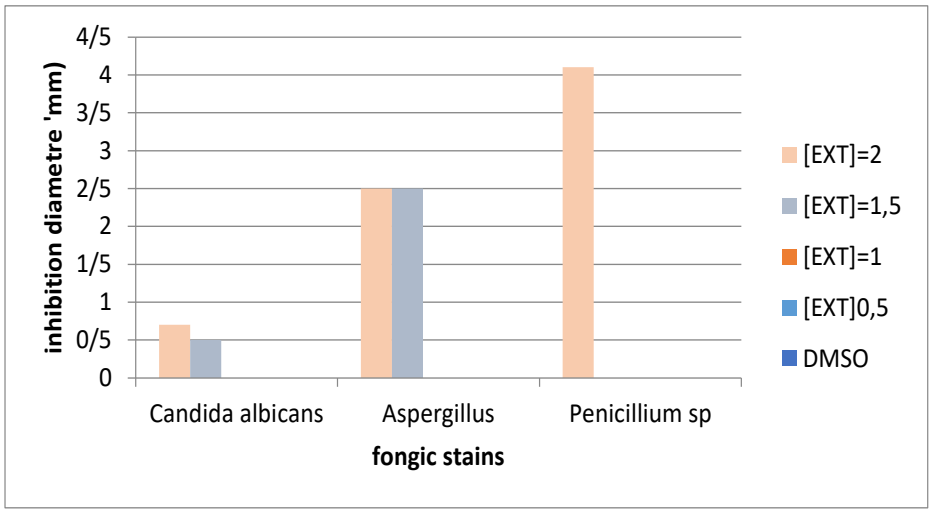

Figure 4. Antibacterial activity of extract of *Hypnae musciformis.*

activity against *C. albicans*, *Aspergillus* sp. and *Penicellium* sp. These results are not in agreement with those obtained Salvador *et al.* (39) and Taskin *et al.* (40) who described antimicrobial activity of *H. musciformis* against a panel of microorganisms and they found that methanolic extract of *H. musciformis* showed no activity on *Candida albicans*.

Padmakumar *et al.* (41) highlighted antimicrobial potential represented by species of *Rhodophyceae algae* (*Hypnea musciformis*, *Falkenbergia rufolanosa* and *Laurencia obtusa*), harvested from French Mediterranean Coast. They noted that of several microorganisms were clearly inhibited by *F. rufolanosa* and *L. obtusa*, whereas *H. musciformis* showed a very weak activity towards bacteria and fungi including *Candida albicans*.

In another study carried by Namvar *et al.* (42), ethanol extract of *H. musciformis* exhibited activity against *Aspergillus* sp. and other dermatophytes, but not against *A. niger* and *C. albicans*. These disparities between our findings with those of previous studies for the same algae may be due to a number of factors such as: organic solvents used, assay methods, seasonal variation as well as otherintrinsic factors such as geographic, climatic and genetic factors.

In all case, the antimicrobial effect of both methanol extracts were found to be dose-dependent as it was observed that the inhibition zone increased as the concentration of methanol extracts increased. Furthermore, no inhibition of test microorganism's growth was observed in the presence of 10% DMSO in disc diffusion method.

Similarly in the present study, we found that Gram positive bacteria were the most sensitive to methanol extract of *A. taxiformis* and *H. musiformis.* This higher frequency had already been observed in most of the surveys on antimicrobial activities from seaweeds (43, 44).

At the end, it is imperative to point out that the differences recorded between our results and the results obtained in previous studies may be due to several factors: algal specie, seasonal variation, and solvent type, efficiency of the extraction method and the susceptibilities of the target strains.

4. Conclusion

It can be concluded in the present contribution that red algae from Algerian West Coast possess biologically active compounds which are effective in inhibiting the growth of both pathogenic bacteria and fungi. Therefore, screening of their natural bioactive substances should be thoroughly determined to evaluate actual substances responsible for this antimicrobial property.

Acknowledgements

This study was supported by the Algerian Ministry of Higher Education and Scientific Research.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Zheng, W., Sun, W., & Simeonov, A. Drug repurposing screens and synergistic drug-combinations for infectious diseases. *British J Pharm. 2018;175(2), 181-191.*
2. Luepke KH, Suda KJ, Boucher H, Russo RL, Bonney MW, Hunt TD, et al. Past, Present, and Future of Antibacterial Economics: Increasing Bacterial Resistance, Limited Antibiotic Pipeline, and Societal Implications. *Pharmacotherapy*. 2017 Jan;37(1):71-84. doi: 10.1002/phar.1868. Epub 2016 Dec 27. PMID: 27859453.
3. Silver LL, Bostian KA. Discovery and development of new antibiotics: the problem of antibiotic resistance. *Antimicrob Agents Chemother.* 1993;37(3):377-383. doi:10.1128/AAC.37.3.377
4. Domettila C, Brintha TSS, Sukumaran S, Jeeva S. Diversity and distribution of seaweeds in the Muttom coastal waters, south-west coast of India. *Biodivers J.* 2013;4(1):105-110
5. Taskin E, Ozturk M, Taskin E, Kurt O. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *Afr J Biotechnol.* 2007;6 (24):2746-2751.
6. Guedes AC, Amaro HM, Malcata FX. Microalgae as sources of carotenoids. *Mar Drugs.* 2011;9(4):625-644. doi:10.3390/md9040625
7. Jeeva S, Antonisamy JM, Domettila C, Anantham B, Mahesh M. Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar India. *Asian Pac J Trop Biomed.* (2012);2 (1), S30–S33.
8. Ramkissoon A, Ramsubhag A, Maxwell A, Jayaraman J. In vitro antimicrobial activity of common species of seaweeds native to Trinidadian coasts. *Algol Stud*. 2015;147:4-66.
9. Damonte EB, Matulewicz MC, Cerezo AS. Sulfated seaweed polysaccharides as antiviral agents. *Curr Med Chem.* 2004 Sep;11(18):2399-419. doi: 10.2174/0929867043364504. PMID: 15379705.
10. Cox S, Abu-Ghannam N, Gupta S. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *Food Res Int*. 2010;J:17, 205-20.
11. Menaa F, Wijesinghe U, Thiripuranathar G, Althobaiti NA, Albalawi AE, Khan BA, et al. Marine Algae-Derived Bioactive Compounds: A New Wave of Nanodrugs? *Mar Drugs.* 2021 Aug 26;19(9):484. doi: 10.3390/md19090484. PMID: 34564146; PMCID: PMC8469996.
12. Namvar F, Tahir PM, Mohamad R, Mahdavi M, Abedi P, Najafi TF, Rahmanand HS, et al. Biomedical properties of edible seaweed in cancer therapy and chemoprevention trials: a review. *Nat Prod Commun.* 2013 Dec;8(12):1811-20. PMID: 24555303.
13. Lavanya R, Veerappan N. Antibacterial potential of six seaweeds collected from Gulf of Mannar of Southeast Coast of India. *Adv Biol Res.* 2011;5(1):38-44.
14. Thanigaivel S, Vijayakumar S, Gopinath S, Mukherjee A, Chandrasekaran N, Thomas J. In vivo and in vitro antimicrobial activity of Azadirachta indica (Lin) against Citrobacter freundii isolated from naturally infected Tilapia (Oreochromis mossambicus). *Aquac*. 2015;437:1:252-255.
15. Balboa EM, Conde E, Moure A, Falqué E, Domínguez H. In vitro antioxidant properties of crude extracts and compounds from brown algae. *Food Chem*. 2013 Jun 1;138(2-3):1764-85. doi: 10.1016/j.foodchem.2012.11.026. Epub 2012 Nov 16. PMID: 23411309.
16. Thinh PD, Menshova RV, Ermakova SP, Anastyuk SD, Ly BM, Zvyagintseva TN. Structural characteristics and anticancer activity of fucoidan from the brown alga Sargassum mcclurei. *Mar Drugs.* 2013 May 6;11(5):1456-76. doi: 10.3390/md11051456. PMID: 23648551; PMCID: PMC3707154.
17. Yang Y, Liu D, Wu J, Chen Y, Wang S. In vitro antioxidant activities of sulfated polysaccharide fractions extracted from Corallina officinalis. *Int J Biol Macromol.* 2011 Dec 1;49(5):1031-7. doi: 10.1016/j.ijbiomac.2011.08.026. Epub 2011 Sep 1. PMID: 21896282.
18. Kavitha J, Palani S. Phytochemical screening, GC-MS analysis and antioxidant activity of marine alga Chlorococcum humicola. World *J Pharm Pharm Sci.* 2016;5(6):1154-1167
19. Karthick N, Kumar RM. Evaluation of phytochemical analysis and antimicrobial activity of different solvent extracts of Sargassum weightii. *Int J Plant Sci.* 2014;4(4):96-99.
20. CLSI. CLSI; 2012. Performance standards for antimicrobial disc susceptibility tests;approved standard – 11th ed. document M02-A11.
21. Jayachandran M, Chung SSM, Xu B. A critical review on diet-induced microbiota changes and cardiovascular diseases. *Crit Rev*

Food Sci Nutr. 2020;60(17):2914-2925. doi: 10.1080/10408398.2019.1666792. Epub 2019 Sep 25. PMID: 31552753.
22. Lee KW, Kim YJ, Lee HJ, Lee CY. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J Agric Food Chem.* 2003 Dec 3;51(25):7292-5. doi: 10.1021/jf0344385. PMID: 14640573.
23. Boulekbache-Makhlouf L, Slimani S, Madani K. Total phenolic content, antioxidant and antibacterial activities of fruits of Eucalyptus globulus cultivated in Algeria. *Ind Crops Prod*. 2013;41:85-89. 10.1016/j.indcrop.2012.04.019.
24. Chakraborty K, Praveen NK, Vijayan KK, Rao GS. Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to Turbinaria spp. (Phaeophyta, Sargassaceae) collected from Gulf of Mannar. *Asian Pac J Trop Biomed.* 2013 Jan;3(1):8-16. doi: 10.1016/S2221-1691(13)60016-7. PMID: 23570010; PMCID: PMC3609390.
25. Mellouk Z, Benammar I, Krouf D, Goudjil M, Okbi M, Malaisse W. Antioxidant properties of the red alga Asparagopsis taxiformis collected on the North West Algerian coast. *Exp Ther Med.* 2017 Jun;13(6):3281-3290. doi: 10.3892/etm.2017.4413. Epub 2017 May 2. PMID: 28587401; PMCID: PMC5450726.
26. Manilal A, Sugathan S, Kiran S, Selvin J, Shakir C, Gandhimathi R, Panikkar MV. Biopotentials of Seaweeds Collected from Southwest Coast of India. *J Mar Sci Tech-Taiw.* 2009;17:67-73. doi:10.51400/2709-6998.1979.
27. Balakrishnan CP, Jenifer P, Esakkilingam M. Algal documentation and Phytochemical studies of red algae Gracilaria corticata of Manapad Coast, Tamil Nadu. *J Pharmacogn Phytochem*. 2013;2(4):193-7.
28. Alghazeer R, Fauzi W, Entesar A, Fatiem W, Gammoudi SA. Screening of antibacterial activity in marine green, red and brown macroalgae from the western coast of Libya. Nat Sci. 2013;5(1):7-14.
29. Lesueur D, Serra D, Bighelli A, Hoi TM, Ban NK, Thai TH, et al. Chemical composition and antibacterial activity of the essential oil of Michelia foveolata Merryll ex Dandy from Vietnam. *Flavour Fragr* J. 2007;22:317-321.
30. Sastry V, Rao G. Antibacterial Substances from Marine Algae: Successive Extraction Using Benzene, Chloroform and Methanol. *Botanica Marina*. 1994;37(4): 357-360. https://doi.org/10.1515/botm.1994.37.4.357
31. Kladi M, Vagias C, Roussis V. Volatile halogenated metabolites from marine red algae. *Phytochem Rev.* 2004;3:337-66
32. Furnari G, Giaccone, G. SERIO D. Alien macrophytes in the Mediterranean Sea: a review. 2004;1:153-202.
33. Genovese G, Leitner S, Minicante SA, Lass-Flörl C. The Mediterranean red alga Asparagopsis taxiformis has antifungal activity against Aspergillus species. *Mycoses*. 2013 Sep;56(5):516-9. doi: 10.1111/myc.12065. Epub 2013 Feb 26. PMID: 23437896.
34. El baroty G, Moussa M, Shallan MA, Ali MA, Sabh AZ, Shalaby E. Contribution to the aroma, biological activities, minerals, protein, pigments and lipid contents of the red alga: Asparagopsis taxiformis (Delile) Trevisan. *J Appl Sci Res*. 2007;3:1825-34.
35. Kazłowska K, Hsu T, Hou CC, Yang WC, Tsai GJ. Anti-inflammatory properties of phenolic compounds and crude extract from Porphyra dentata. *J Ethnopharmacol.* 2010 Mar 2;128(1):123-30. doi: 10.1016/j.jep.2009.12.037. Epub 2010 Jan 4. PMID: 20051261.
36. Rhimou B, Riadi H, Martínez J, Nathalie B. The antibacterial potential of the Seaweeds (Rhodophyceae) of the Strait of Gibraltar and the Mediterranean Coast of Morocco. *Afr J Biotechnol*. 2010;9:6365-6372.
37. Kandhasamy M, Kantha A. Evaluation of in vitro antibacterial property of seaweeds of southeast coast of India. *Afr J Biotechnol*. 2010;7:1958-61.
38. Moreau J, Pesando D, Caram B. Antifungal and antibacterial screening of Dictyotales from the French Mediterranean coast. In: Bird C.J., Ragan M.A. (eds) Eleventh International Seaweed Symposium. *Developments in Hydrobiology.* 1984;22. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-6560-7_106
39. Taskin E, Caki Z, Ozturk M. Assessment of in vitro antitumoral and antimicrobial activities of marine algae harvested from the eastern Mediterranean Sea. *Afr J Biotechnol*. 2010;9:4272-4277.
40. Salvador N, Gómez Garreta A, Lavelli L, Ribera MA. Antimicrobial activity of Iberian mac-

roalgae. *Sci Marina*. 2007;71(1):101-14.
41. Padmakumar K, Ayyakkannu K. Seasonal Variation of Antibacterial and Antifungal Activities of the Extracts of Marine Algae from Southern Coasts of India. *Botanica Marina.* 1997;40:507-516. doi: 10.1515/botm.1997.40.1-6.507.
42. Guedes AC, Amaro HM, Malcata FX. Microalgae as sources of carotenoids. *Mar Drugs.* 2011;9(4):625-44. doi: 10.3390/md9040625. Epub 2011 Apr 20. PMID: 21731554; PMCID: PMC3124977.
43. González del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I, et al. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int Microbiol.* 2001 Mar;4(1):35-40. doi: 10.1007/s101230100006. PMID: 11770818.
44. Etahiri S, Bultel-Poncé V, Elkouri AE, Assobhei O, Zaoui D, Guyot M. Antibacterial activities of marine algae from the Atlantic coast of Morocco. *Mar Life.* 2003;13:3-9.