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GC/MS analysis and antibacterial potential of macroalgae extracts harvested on Moroccan Atlantic coast.

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Abstract

Marine Marine algae synthesize a wide variety of chemically active secondary metabolites, which are an important source of products used by humans for therapeutic purposes. The objective of this study is to evaluate the antibacterial efficacy of the seaweed extracts at three different harvesting sites located on the coast of Moroccan Atlantic, to select active species and best site of collect. For that, agar well diffusion assay was used to determine the antibacterial potential of the extractable matter against multi-resistant bacteria, two gram negative bacterium (*Escherichia coli* ATCC 10536 and *Proteus mirabilis* NIH) and two gram positive bacteria (*Staphylococcus aureus* CECT976 and *Staphylococcus aureus* ATCC 25923), subsequently the MIC was determined. In addition, the chemical constituents of selected macroalgae have been investigated.

Obtained results demonstrated that antibacterial activity of algae was influenced by solvent extraction and harvested site, the extracts of *Pterosiphonia complanata* collected from Sidi Bouzid were more efficiency against the strains studied in comparison with the same algae harvesting in two other sites. Compared to standards antibiotics, this algae extracted in dichloromethane/methanol, exhibited a stronger activity with (MIC of 0.02µg/ml against *Proteus mirabilis* (NIH), 0.3µg/ml against *Staphylococcus aureus* CECT 976 and 1.8 µg/ml against the *Staphylococcus aureus* ATCC 25923.

GC/MS analysis the Dichloromethan/Methanolic extract of *Pterosiphonia complanata* collected of Sidi Bouzid site indicated presence of two major components including 3- n-Hexadecanoic acid (palmitic acid) (15.68%) and Neophytadiene (12. 35%).

Keywords: *Pterosiphonia complanata*, Algae extracts, Antibacterial activity, MIC, GC/MS

1. Introduction

The resistance of pathogenic bacteria to existing antibiotics has become a global epidemic [1]. To date, biofilm formation is a common feature of bacterial infections. In most cases, the presence of biofilms leads to the spread of infections and more than 60% of infections seem to be occurred with the presence of biofilms [2]. In pharmacology, the utilization of natural bioactive compounds is known to be an efficacious procedure. Bioactive compounds extracted from plants, animals, and microorganisms are known to have therapeutic and biological properties. They can be utilized in the treatment of various diseases and infections [3]. More than 75% of drugs utilized to treat infectious diseases are derived from natural sources [4]. The marine environment is home to a taxonomically diverse ecosystem. Organisms such as algae contain pharmacologically active compounds such as polysaccharides, fatty acids, phlorotannins, terpenes and peptides that resist bacterial invasion. Algae have been shown to produce secondary metabolites different from those produced by terrestrial organisms [5]. Therefore, they have been shown to be a source of biomedically interesting compounds [6].

Seaweeds extract are prolific producers of biologically active compounds. Such ability was developed as defense against numerous organisms, which coexist and interact in the same complex environment [7]. According to Smit [8], the discovery of metabolites with biological activity from algae increased substantially in the last three decades. These substances exhibit an appreciable number of distinct biological activities such antimicrobial activity [9,10,11]. antitumoral, antiviral, antifungal, insecticidal, cytotoxic, phytotoxic and antiproliferative actions [12,13].The majority of these compounds are terpenes and polyphenols [14,15].Marine algae have shown promise as candidates in novel antibacterial drug discovery.

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The Moroccan coast is particularly rich in algal biodiversity and constitutes are serve of species of considerable economic, social and ecological potential. Inventories of benthic algae off the coast of El Jadida show the presence of 143 species, including 23 Chlorophyceae, 25 Phaeophyceae and 95 Rhodophyceae [16,17].

Several studies have been reported on the antibacterial activity of marine algae; however, available information on their antibacterial activity in different solvents depending on the harvest sites is limited. The present investigation aimed to evaluate the antibacterial activity of Atlantic macroalgae extracts, collected from the tree different site, against four multi-resistant bacteria in order to discover potential antibacterial metabolites. The identification of the most potential antibacterial extracts was performed using retention times and mass spectra in the GC/MS analysis.

2. Materials

2.1. Algal materials

Algae were collected by handpicking at low tide (2019), in different locations from the coast of Moroccan Atlantic (Figure 1). The collected algae were cleaned, washed in distilled water and then dried and crushed to a fine powder. The algae investigated for this study were present in the three harvest sites and were identified as:

Green algae: *Ulva rigida;*

Brown algae: *Bifurcaria bifurcata, Fucus spiralis, Saccorhiza polyschides, Sargassum muticum;*

Red algae: *Gelidium corneum, Pterosiphonia complanata.*

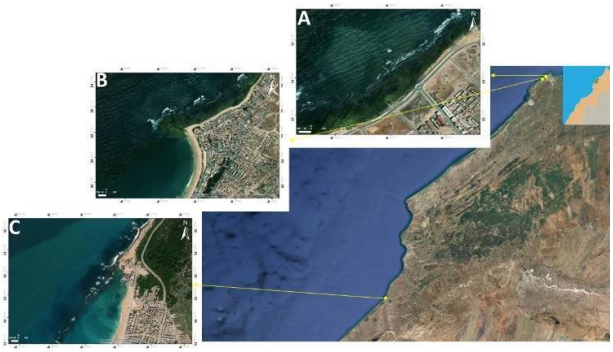

Figure 1: Localization of the collection site

A: Saada site; **B:** Sidi Bouzid's site **C:** Souiria Kdima's site (Google Earth Pro, 2020).

Saada (N 33°.14'47.53" N°; 8°32'31.9" W) and Sidi Bouzid (33°13'19.97" N; 8°33'27.57" W), particularly rich in algal biodiversity due to the presence of Upwelling phenomena.

Souiria Kdima (32°04'99.4" N; 009°34'28.8" W), is less rich in algae with a weak Upwelling in comparison with others sits.

2.2. Preparation of algal extracts

The powder from each species was dissolved in different solvents, namely methanol, dichloromethane/methanol (50:50) and dichloromethane, as described by Caccamese and Azolina [18]. The Final product were concentrated by drying in a rotary evaporator under reduced pressure at 45°C until a crude extract were obtained, this extract was stored at 4°C until utilization.

2.3. Microorganisms tested

To evaluate the antibacterial activity, strains with human pathogencity were used: two positive Gram+ bacterial belonging to the Staphylococcaceae family and two negative Gram bacterial strains from the Enterobacteriaceae family. *Staphylococcus aureus* (CECT 976) were obtained from the Spanish Type Culture Collection, the *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 10536). They are procured from American Type Culture Collection and *Proteus mirabilis* NIH was obtained from the National Institute of Hygiene, Rabat, Morocco.

2.3.1. Antibacterial bioassay

Antibacterial assay was carried out using the agar plate diffusion assay [19]. One colonies of each bacterium was taken with a wire loop from the original culture plate and were inoculated into a test tube filled with 5 ml of sterile physiological water, the bacterial density was adjusted to 10^6 bacteria/ml with sterile water to inoculate petri dishes who contain Mueller-Hinton agar culture media. Using paper disks, 40 µl of organic extracts (10 µg/ml) were tested (6 mm diameter). After the temperature was regularized at 4 °C, the petri dishes were incubated for the duration of a night at 37 °C. After incubation, diameters of inhibitory zones were measured. Discs impregnated by 40 µl of standard antibiotics (10 µg/ml, streptomycin and tetracycline). In addition, control disks were prepared with every used solvent as negative, control; all tests were performed in triplicate.

2.3.2. Determination of minimum inhibitory concentrations (MIC)

The determination of the minimum inhibitory concentration (MIC) was carried out as stated to the microtitre technique as maintained by Sarker [20], with some modifications. Briefly, 100 µl of seaweed extract were added to the serial dilutions and performed to achieve final concentrations ranged from 8.10-7 to 75 mg/ml. Then we add a 100 µl of Mueller Hinton broth (BMH) that inoculated with 100 µl of a

bacterial suspension to reach a concentration of 10^6 CFU/ml. After incubation at 37 °C for 18–24 h, the antibacterial activity was examined using resazurin (7-hydroxy-3H-phenoxazin-3-one) as colored indicator. Streptomycin was used as positive control and BMH without bacterial suspension as negative one. All experiments were performed in triplicate.

2.4. GC-MS analysis

The chemical composition of *Pterosiphonia complanata* extract was characterized by gas chromatography (GC) (Agilent 7890 A Series) coupled to mass spectrometry (MS) equipped with a 123-BD11 column of dimension 15 m × 320 µm × 0.1 µm and a multimode injector. 4 µl of the soluble extract in chloroform was injected into the column by 1:5 split mode using helium as carrier gas at 3 ml min^{-1}. With a gain factor of 5, the detection was done using full scan mode between 30 and 1000 m/z. The temperatures of the ion source was 230 °C and the quadrupoles was 150 °C. The oven temperature was maintained at 30 °C for 3 min and then increased at 10 °C min^{-1} until 250 °C. NIST 2017 MS Library was used for identification. The quantity of every compound was estimated by comparison of peak area with a known quantity of internal standard.

2.5. Statistical analysis

All experimental data were analyzed with SPSS Version 22 statistical software and were presented as mean ± SD. The significant level was set at P-Value at 0.05, and these were performed using one-way ANOVA test.

3. Results and Discussion

3.1. Antibacterial activity

Antibacterial activity of seven algae were collected in tree sites and extracted in different solvent (dichloromethane, dichloromethane/methanol and methanol) and evaluated using agar disc-diffusion assay. The results obtained are showed that only dichloromethane/methanol extracts of five species (*Gelidium corneum*, *Fucus spiralis*, *Bifurcaria bifurcata*, *Ulva rigida* and *Pterosiphonia complanata*), were shown to be efficacious against all bacterial strains tested, with an inhibitory diameter that varied according on harvesting area. Extracts from *Sargassum muticum* and *Saccorhiza polyschides* are inactive at the three sites.

Against *Proteus mirabilis* (NIH) Gram negative bacteria, antibacterial activity of organic algal extracts at 10 µg/ml represented in Figure 2.

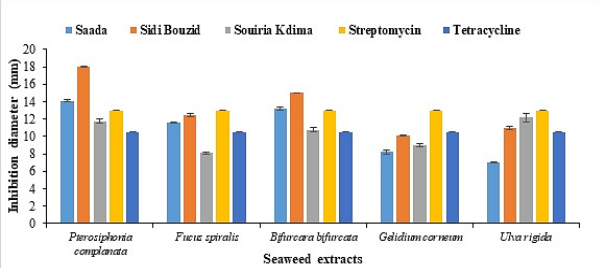

Figure 2: Anti-*Proteus mirabilis* (NIH) activity of dichloromethane/methanol extracts of algae harvested in three sites.

According to the results presented in Figure 2, the largest inhibition zone was obtained by Dichloromethane/methanol extracts of algae harvested at the site of Sidi Bouzid *Pterosiphonia complanata* (18 mm), followed by *Bifurcaria bifurcata* (15 mm). These activities were higher than that obtained by tetracycline (10.5 mm) and streptomycin (13 mm).

On the Saada site, antibacterial activity was less important (14 mm) for *Pterosiphonia complanata* and (13 mm) for *Bifurcaria bifurcata*, low activity was obtained with algae collected on Souiria kdima sites.

Among the five active seaweed extracts tested against *Escherichia coli* ATCC 10536 strain (Figure 3), the extract of *Pterosiphonia complanata* harvested at the site of Sidi Bouzid showed the highest activity with an inhibition diameter of 13 mm compared to the controls Streptomycin (8 mm) and Tetracycline (10 mm). Followed by inhibition zone of 12 mm revealed by the extract of *Bifurcaria bifurcata*, *Gelidium corneum* and *Fucus spiralis* on the same harvest site. On the site of Saada, only, the extract of *Bifurcaria bifurcata* shows the best activity with an inhibition diameter of 13 mm.

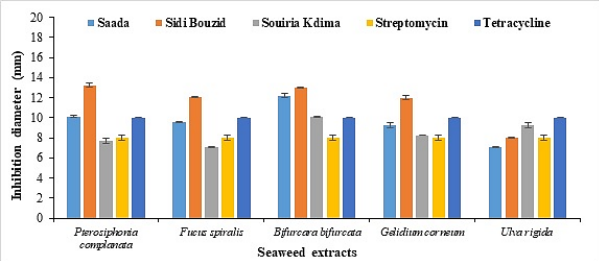

Figure 3: Anti- *Escherichia coli* ATCC 10536 activity of dichloromethane/methanol extracts algae harvested in the three sites.

Against Gram-positive bacteria, activity obtained with *Staphylococcus aureus* CECT 976 was represented in **figure 4**.

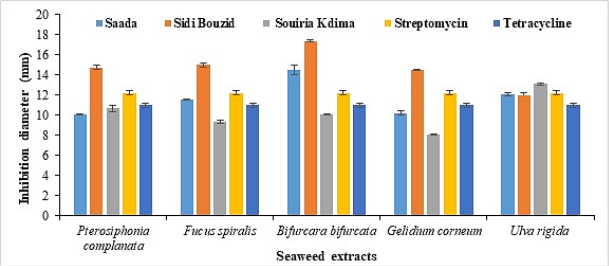

Figure 4: Anti-*Staphylococcus aureus* CECT 976 activity of dichloromethane/methanol extracts of algae harvested in three sites.

The obtained results show that the highest activity were recorded by the extract of algae collected on the Sidi Bouzid site, *Bifurcaria bifurcata* with an inhibition diameter of 17 mm, followed by *Fucus spiralis* (15 mm). Wile, antibacterial activity against *Staphylococcus aureus* of *Bifurcaria bifurcata* harvested on the Saada site was less important with an inhibition diameter of 14 mm. in comparison with the tetracycline and streptomycin controls. On the site of Souria kdima, the best activity was obtained by the extract of *Ulva rigida* with an inhibition diameter of 13 mm. All these activities always remain superior to that obtained with antibiotics controls tetracycline (11 mm) and 12.25 mm for streptomycin.

The results of antibacterial activity of seaweeds organic extracts against *Staphylococcus aureus* (ATCC 25923) are summarized in Figure 5.

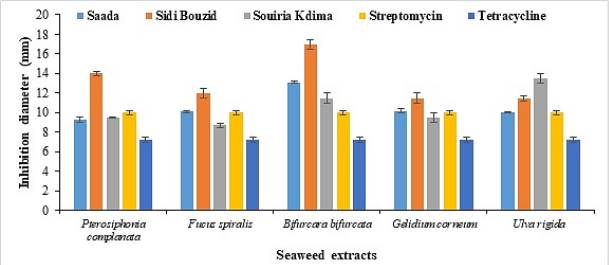

Figure 5: Anti-*Staphylococcus aureus* ATCC 25923 activity of dichloromethane/methanol extracts algae harvested in the three sites.

Extracts of five active algae harvested on the different sites showed a significant antibacterial activity against the bacterial strain *Staphylococcus aureus* ATCC 25923. The extract of *Bifurcaria bifurcata* harvested at the site of Sidi Bouzid showed the highest activity (17 mm), followed by the extract of *Pterosiphonia complanata* (14 mm) collected on the same site. On the site of Saada, the highest activity was obtained by the extract of *Bifurcaria bifurcata* (13 mm). For the extracts harvested on Souiria kdima site, the extract of *Ulva rigida* shows the highest activity with an inhibition diameter of 14 mm. The activity obtained by all extract tested was interesting in comparison to the controls Streptomycin (10 mm) and Tetracycline (7.25 mm).

These results exhibit that efficiency of algal extracts harvested from the site of Sidi Bouzid indicate highest antibacterial activity. Indeed, our results confirm this observation: *Pterosiphonia complanata* (Red algae) and *Bifurcaria bifurcata* (Brown algae) exhibited a higher activity against *Proteus mirabilis* and *Staphylococcus aureus* with a concentration of 10 µg/ml.

From these results, it can be seen that the different seaweed extracts collected on the different sites showed slightly less important activity against Gram (-) bacteria *Proteus mirabilis* NIH and Gram (+) bacteria *Staphylococcus aureus*. However, these results revealed that Gram (+) bacteria were more sensitive to these extracts than Gram (-) with inhibition values of 18 mm, a significant difference between the extracts of the different algae was observed. *Escherichia coli* was the most resistant strain. However, compared to the controls, the algae extracts studied show an important or similar activity as streptomycin and tetracycline, with the highest values was observed in the extract of *Pterosiphonia complanata*, *Fucus spiralis* and *Bifurcaria bifurcata* prepared in the dichloromethane/methanol mixture

3.2. Minimum inhibitory concentrations (MIC)

The lowest concentration of the antibacterial agent, which did not show any visible growth was considered as the MIC, her determination was made only for extracts that showed highest antibacterial activity against different strain how were collected at the site of Sidi Bouzid. The concentration of the test product was ranged from 8.10^{-7} to 75 mg/ml.

These results showed that the extracts of *Pterosiphonia complanata* and *Bifurcaria bifurcata* were more efficiency against *Proteus mirabilis* NIH and *Staphylococcus aureus* (CECT 976), respectively. The best MIC was 0.02 mg/ml of *Pterosiphonia complanata* against *Proteus mirabilis* NIH.

3.3. Chemical components of the *Pterosiphonia complanata* extract

For his high activity, the chemical composition of the extract of *Pterosiphonia complanata* was expressed in percentage of the total area of peaks separated using GC and identified by mass spectroscopy **(table 2).**

thus the total detected compounds of were 80.49%. The major constituents of *Pterosiphonia complanata* extract were palmitic acid (15.68%) and Neophytadiene (12.35%). The most of the identified components have been found to possess antimicrobial activity, which may be responsible for the antibacterial potential reported in this study.

Table 1. The minimum inhibitory concentrations (MIC) dichloromethane/methanol extracts of algae harvested at Sidi Bouzid site

Bacteria Pathogens	MIC µg/ml of Extracts algae with Standard Antibiotic						
	F. spiralis	***B. bifurcata***	***U. rigida***	***G. corneum***	***P. complanata***	**Tetracycline**	**Streptomycine**
S. aureus (CECT 976)	2,7±1,27	0,3±0,0	39,5±14,84	5.4±0,0	0,2±0,14	15,62± 0,0	7,8±0,0
S. aureus(ATCC 25923)	10,65± 4,7	1,8±0,0	21.8± 0,0	21.8± 0,0	7,3± 0,0	62,5±0,0	15,62± 0,0
E. coli (ATCC 10536)	1875±0,0	1875±0,04	7500±0,0	3750±0,0	29±0,0	250±0,0	125±0,0
***P. mirabilis* (NIH)**	3,6±0,0	1,8±0,0	21,8±10,1	39,5±14,84	0,02±0,0	39,5±14,84	1,95±0,0

Table 2. Chemical composition of *Pterosiphonia complanata* separated by GC and identified using mass spectroscopy.

Sample	RT (min)	Name of the Compound	Molecular Formulae	Peak Area %	MW(g/mol)
1	18.35	Z-5-Nonadecene	$C_{19}H_{38}$	0.1	266
2	18.51	Pentadecane	$C_{15}H_{32}$	0.20	212
3	18.76	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O_7Si_7$	0.15	519
4	19.98	7-Hexadecene, (Z)-	$C_{16}H_{32}$	0.19	224
5	20.39	Benzene, 1,1'-(1,3-propanediyl)bis	$C_{15}H_{16}$	1.49	196
6	20.74	1,2-Diphenylcyclopropane	$C_{15}H_{14}$	0.43	194
7	21.03	8-Heptadecene	$C17H_{34}$	0.35	238
8	21.18	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	0.39	593
9	21.35	Heptadecane	$C_{17}H_{36}$	6.15	240
10	21.54	1,2-Diphenylcyclopropane	$C_{15}H_{14}$	3.8	194
11	22.82	Benzene, 1,1'-(3-methyl-1-propene-1,3-diyl)bis	$C_{16}H_{16}Se_2$	0.61	366
12	22.52	1,4-Diphenyl-1,3-butadiene	$C_{16}H_{14}$	1.23	206
13	22.76	Tetradecanoic acid	$C_{14}H_{28}O_2$	9.83	228
14	22.88	Neophytadiene	$C_{20}H_{38}$	12.35	278
15	23.15	Benzene, 1,1'-(1-methyl-2-butynylidene)bis	$C_{17}H_{16}$	3.81	220
16	23.82	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.65	270
17	24.6	n-Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	15.68	256
18	25.53	2,4,6-Tris(perfluorononyl)-1,3,5-triazine	$C_{30}F_{57}N_3$	0.38	1485
19	27.74	3-Benzyloxy-1,2-diacetyl-1,2-propanediol	$C_{14}H_{18}O_5$	2.91	266
20	28.71	3-Methyl-2-phenylindole	$C_{15}H_{13}N$	0.68	207
21	30.18	1,4-Benzenedicarboxylic acid, bis2-ethylhexyl) ester	$C_{24}H_{38}O_4$	1.77	390
22	30.7	Cholesta-3,5-diene	$C_{27}H_{44}$	0.95	268
23	31.18	1,1':3',1''-Terphenyl, 5'-phenyl	$C2_4H1_8$	1.96	306
24	32.16	Cholesterol	$C_{27}H_{46}O$	5.87	386
25	32.27	5-Phenylvaleric acid, but-3-yn-2-y	$C_{11}H_{14}O_2$	1.11	178
26	32.38	7-Phenyl-n-heptanol	$C_{13}H_{20}O$	3.46	192
27	34.31	Pyrimidine, 2-chloro-4-methyl-6-phenyl	$C_{11}H_9ClN_2$	0.05	204
28	35.23	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	$C_{35}H_{62}O_3$	0.17	530
29	36.05	1,4,5,6-Tetrahydro-6-[phenylmethyl]	$C_9H_{12}N_4S$	0.87	208
30	36.14	Norfluoxetine	$C_{16}H_{16}F_3NO$	2.75	295
31	40.29	Benzene, 1,1'-[1-(2,2-dimethyl-3-butenyl)-1,3-propanediyl]bis	$C_{21}H_{26}$	0.55	278
Total				80.89	

RT: retention time of the identified compounds; MW: molecular weight of the identified compounds

Generally, the chemical classes highlighted in this study were previously observed in the literature regarding macroalgae from other environments [26,27], and several studies have been reported on the antimicrobial activity of marine algae, however, available information on their antibacterial activity in different solvents depending on the harvest sites is limited.

An abundance of molecules with pharmaceutical applications has been produced from marine algae in recent decades. worldwide, over one thousand pharmacologically active compounds extracted from

marine algae were characterized with potential efficacy against bacteria, fungi, viruses, cancer, hypertension, high cholesterol, and other diseases [28,29].

To develop new drugs and healthy foods, algae have received significant attention in the search for new bioactive compounds. Many marine algae compounds show anti-bacterial activities such as polysaccharide [29], lyengaroside [30], polyhydroxy lated fucophlorethol [31], bromophenols [32], guaianesesquiterpene [33], lactone malyngolide [34], cycloeudesmol [35], polyphenolic compound [36], halogenated compound [37] and quinone metabolite [38]. Several other researchers have also extensively studied the antibacterial activities of compounds derived from algae [39].

The solvents always have stronger efficiency in extracting anti-bacterial compounds. However, the Methanolic extract was more effective than chloroform. These results absolutely showed the presence of antibacterial substances in algae which triggers and stimulate the activity against pathogens [40]. These results are in agreement with those reported previously concerning extracting antibacterial substances such as brominated phenols, hydroquinones, phenols, sesterpenoids and polyphenols from species of Rhodophyceae, Phaeophyceae, and Chlorophyceae [41].

Table 3. Major phytochemical compounds in the *Pterosiphonia complanata* obtained by GC and identified by mass spectroscopy.

Sample	RT (min)	Name of the Compound	Molecular Formulae	Peak Area %	MW (g/mol)	Compound Nature	Activity	Reference
9	21.35	Heptadecane	$C_{17}H_{36}$	6.15	240	Alcane	Antibacterial, antifungal	[21]
13	22.76	Tetradecanoic acid	$C_{14}H_{28}O_2$	9.83	228	Fatty acids	Antibacterial, antifungal	[22]
14	22.88	Neophytadiene	$C_{20}H_{38}$	12.35	278	Diterpenes	Antibacterial, antifungal	[23]
17	24.6	n-Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	15.68	256	Fatty acids	Antimicrobial, antiinflammatory	[24]
24	32.16	Cholesterol	$C_{27}H_{46}O$	5.87	386	Steroid	antimicrobial,anti-inflammatory,	[25]

RT: retention time of the identified compounds; MW: molecular weight of the identified compounds

Antibacterial activity of red, brown and green algae against both Gram positive and Gram negative bacteria has been established by several scientists. The antibacterial activity was recorded with *Gracilaria edulis* extract. *Calorpha peltada* was showed inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus faecalis* in comparison with the ampicillin antibiotics [42].

However, it is reported that the strong antibacterial activity of seaweeds resides in the algal species selected, the efficiency of the extraction method and the solvents being used [43, 44].

In this study, it was reported that the methanol and mixture of dichloromethane-methanol- extract of brown and red algae showed stronger antibacterial activity against Gram- and Gram+ bacteria, when compared to other studied solvents. Likewise, the most of the active algae are those harvested at the Sidi Bouzid site in comparison with the tetracycline and streptomycin antibiotics.

Red algae are the most important source of biologically active metabolites. They are rich in secondary metabolites than green and brown algae [45].

The halogenated compounds extract from red algae exhibited various biological activities, which include antifungal, antibacterial, cytotoxic, and anti-inflammation activity. In addition, the red algae also produce polyether, terpenoid, and acetogenin with some nucleic acid derivatives, amino acid, acetate, and shikimate[46].

Bouhlal [47], has shown that out of 26 species of Red algae tested, 25 species indicate an antibacterial activity at least on one of the microorganisms examined. From the 25 active species, 12 species had shown an antibacterial activity against *E. coli* ATCC 25922, 8 species was active against *K. pneumoniae* ATCC700603, *E. faecalis* ATCC29213, and *E. faecalis* ATCC29212, and 25 species inhibited the growth of *S. aureus* ATCC25923. The extracts showed the greatest inhibition on *S. aureus* and *E. coli*, while the extract of *H. musciformis* showed the strong inhibition against all the microorganisms tested, with inhibition diameters ranging from 15 to 35 mm.

Several studies have detected the antibacterial activities from red algae, the study of Etahiri [10] indicated that the methanolic extracts of *H. musciformis*, *H. incurvus*, *G. latifolium*, *S. coronopifolius*, and *P. cartilagineum* showed good inhibition against the bacterium *S. aureus* (ATCC

6538) and the result was negative for the *A. armata* extract on this bacteria.

This work shows that antibacterial activity in *P. complanata* was steady over time, and that there are others compounds in addition to 3, 4, 6-tribromo-5-methoxymethyl-benzene-l, 2-diol Etahiri et al. (2007) responsible of antibacterial activity.

In most of the surveys on antibacterial activities from seaweeds reported in the literature the higher frequency of activity against bacteria has also been observed [48,49,10]. Etahiri [10] reported that algae extracts collected in the spring were importantly more active than those collected in the winter.

Differences between the results of the present investigation and those of other studies may be due to the organic solvents used for the extraction of bioactive compounds and the differences in the assay methods, the geographical zone and the seasonal production of bioactive compounds [50]**.**

Environmental factors have a very important effect on the bioactivity of algae. Faced with the global change in the environment, which is intensifying more and more, marine macroalgae living fixed on rocky coastal areas adapt to these new environmental conditions under pain of disappearing or to see their area of distribution regress [51]. These macroalgae adapt (or not) by producing original primary and / or secondary metabolites that may vary with the growth cycle and with the bioactivity of the algae [52], adaptations playing a very important role in the structuring of coastal communities. Depending on the physicochemical and biological conditions of their biotope (temperature, salinity, microbiological and chemical pollution, predation intensity) [53].

Several studies have demonstrated the richness of algae harvested in the Doukkala coast as biologically active secondary metabolites that can be a major source of original molecules [10,54].

4. Conclusions

Marine algae collected of coast of Doukkala possess several active chemicals compounds responsible of antibacterial activity, which are currently under detailed investigation with the aim of isolating biologically active molecules as well as searching for new compounds of health and environmental interest.

This study reports the presence of antibacterial compounds in marine algae collected at different sites. Results obtained shows the implication of the intrinsic factors of the species and environmental factors in the algae metabolism and which significantly affect the presence of this or that bioactive compound responsible for this activity. This study demonstrated that bioactivity of the seaweed collected from different site varied within a species and may be influenced by the geographic distribution. Indeed, the site of Sidi Bouzid remains the best site and the richest in marine species, and *Pterosiphonia complanata* was always a promising source of antibacterial agents.

5. Conflicts of interest

The authors declare no conflicts of interests.

6. References

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