

Component Analysis and Antimicrobial Activity of the *Plocamium cartilagineum* Extract

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ABSTRACT

The extracts of nine seaweed species in three different solvents (methanol, dichloromethane/methanol and dichloromethane) were examined *in vitro* for their antimicrobial properties against two pathogenic microorganisms: *Pectobacterium carotovorum* and *Fusarium* sp. responsible of root rot in sugar beet and resulting in yield shortages and economic losses. This study showed that dichloromethane extract from *Plocamium cartilagineum* has the maximum inhibition against growth of both pathogens under study, with a zone value ranging from 17.5 to 34mm, respectively. These results suggest that red seaweed *Plocamium cartilagineum* contains compounds which could be considered for controlling root rot in sugar beet. Thus, the composition of dichloromethane extract from *Plocamium cartilagineum* was determined by gas chromatography/mass spectroscopy (GC/MS) analysis. The main compounds detected were selenocyanic acid (20.77%), oleic acid (10.52%), and 9, 12, 15 octadecatrienoic acid (Z, Z, Z) (9.30%).

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the world's two major sugar crops. It is a herbaceous dicotyledonous plant belonging to the family Chenopodiaceae (Ghazy *et al.*, 2021). It was attacked by *P. carotovorum* formerly known as *Erwinia carotovora*, the cause of bacterial soft rot (Wright, 1998). It's one of the most important diseases causing significant losses (Glosek-Sobieraj *et al.*, 2019). *P. carotovorum* has the widest host range of all the soft rot bacteria (Yahiaoui-Zaidi *et al.*, 2010; Lakhdar *et al.*, 2018), favored in moisture and at a moderate temperature (Terta *et al.*, 2010). These bacteria produce extra cellular enzymes, such as pectate-lyases, pectinases, cellulases and proteases, resulting in tissue maceration and rot symptoms (Saubeau *et al.*, 2014).

Moreover, several soil-borne fungi including *Fusarium* sp. produce wilt and root rot disease in almost all the infected plants (**Khan *et al.*, 2017; Melo *et al.*, 2020**). On its path to the cell wall, the fungus *Fusarium* sp. initially reaches the cell interior where it should be able to degrade the suberin, which covers the outer organs of the plant, followed by the middle lamella, with the effect of the pectinolytic enzymes (**Lotfi *et al.*, 2021**).

The *P. carotovorum* species is generally the cause of soft rot symptoms in some crops, generating physical, physiological, and chemical changes that lead to severe damage to the quality of sugar (**Schaad, 2001**), and subsequently resulting in serious yield losses in the field (**Metzger, 2018; Ghazy *et al.*, 2021**). *P. carotovorum* is the largest competitor of agricultural crops that severely reduces the production in the range of 25–50% (**Lakhdar *et al.*, 2018**). In addition, it causes additional economic losses in storage and during processing (**Terta *et al.*, 2010**) due to the accumulation of invert sugar that reduces sugarbeet quality (**Metzger, 2018**).

Notably, seaweeds are one of the important living resources of the marine environment (**Pushparaj *et al.*, 2014; Aly *et al.*, 2019**). They are one of the main biological agents that have been studied for the control of phytopathogenic diseases (**Paulert *et al.*, 2009**). The seaweed extracts have complex composition and deliver various benefits (**Wierzbowska *et al.*, 2015**); in the last three decades, the detection of metabolites in the biological activities of macroalgae has significantly increased (**O’Keeffe, 2019**). This was associated with valuable bioactive compounds (**Pushparaj *et al.*, 2014**); this vast phytochemical array includes those exhibiting antifungal and antibacterial properties (**Agarwal *et al.*, 2021**). The antimicrobial potential of seaweeds has been proved and recorded (**Jiménez *et al.*, 2011; Mukherjee & Patel, 2019; Lotfi *et al.*, 2021**). Thus, seaweeds have been exploited in the search for novel antimicrobial compounds (**Vallinayagam *et al.*, 2009**). In this context, **Bouhraoua *et al.* (2018)** postulated that, the red seaweed extracts showed an important inhibition against *Fusarium culmorum*, and added that the red seaweeds have the highest effect against *Bipolaris sorokiniana*, the causative agent of helminthosporalblight. Furthermore, **Lakhdar *et al.* (2018)** reported that, the red seaweed extracts collected off the coast of Sidi Bouzid can be used in the treatment of plant diseases.

Seaweeds form a rich source of different growth hormones, such as auxins, cytokinins and gibberellins (**Craigie, 2011**) and are reported as effective bio-stimulants, which can increase the yield of different crops (**Craigie, 2011; Lotfi *et al.*, 2021**).

This growing interest in seaweeds “as alternatives” is a consequence of the negative impact of the use of pesticides on the environment and health (**Chanthini *et al.*, 2012**). There is an urgent need to develop more effective, sustainable and environmentally friendly tools for pathogen control to replace the use of chemical products with more ecologically sound alternatives (**Al-Ani *et al.*, 2012; O’Keeffe, 2019**). Thus, the focus of this research was to evaluate the use of seaweed extracts as biopesticides against two

plant pathogens; namely, *P. carotovorum* and *Fusarium* sp. to reduce the root rot in sugar beet.

MATERIALS AND METHODS

1. Seaweeds

The seaweeds were collected from Sidi Bouzid on the Moroccan Atlantic coast, south of the city of El Jadida (Lat 32° 15' to 33° 15'; Long 7° 55' to 9° 15'). They were thoroughly washed and packaged in polyethylene bag and then dried and crushed into a fine powder. These species belong to three groups, including **Chlorophyceae** *Ulva lactuca*; **Phaeophyceae** *Bifurcaria bifurcata* and *Fucus spiralis*, and **Rhodophyceae** *Hypnea musciformis*, *Plocamium cartilagineum*, *Gracilaria cervicornis*, *Halopitys incurvus*, *Ellisolandia elongate* and *Coralline officinalis*.

2. Extraction procedures

For the extracts from the powdered seaweeds, three solvents were used: methanol, dichloromethane/methanol (v/v) and dichloromethane, following the method of **Caccamese and Azolina (1979)**. The extracts were extensively dried in a rotary evaporator under reduced pressure until attaining a crude extract that was kept in a dry place before use.

3. Strains studied

The pathogen *P. carotovorum* (CCMM B1158T) was obtained from CNRST, Rabat- Morocco. While, pathogen *Fusarium* sp. was isolated from the infected sugar beet tubers collected from the Doukkala region and identified in mycology laboratory at the Department of Phytopathology, Agronomic and Veterinary Institute Hassan II, Rabat, Morocco.

4. Assay of antimicrobial activity in culture media

The technique used for the evaluation of the antibacterial and antifungal activities of seaweed extracts is the diffusion in agar medium, using cellulose discs (6 mm), according to the technique described by **Bauer et al. (1966)**.

4.1. Preparation of the inoculum

The multiplication of *P. carotovorum* was carried out in petri dishes containing nutrient agar as culture medium and incubated at 26 °C. The bacterial suspension was prepared from a young culture (24-48 hours) of *P. carotovorum*; five colonies were taken and mixed with sterile physiological water at 9‰. To prepare the bacterial concentration of the inoculum, we used the method of comparison of the bacterial density with that of a reference tube McFarland (Absorbance at 625nm). The bacterial density was equal to 10⁸ CFU / mL and was adjusted to 10⁶ CFU / mL for further use in the test. For the

experiment using *Fusarium* sp., it was performed using 7- day culture, and the concentration of inoculum was adjusted to 10^6 CFU / mL.

4.2. *In vitro* assay

Antibacterial and antifungal activities of the extracts were determined using the disc diffusion method (Bauer *et al.*, 1996), each disc received 20 μ l of the extract (50 mg/ml) after evaporation of the solvent, it was placed on the surface of a petri dish containing Muller Hinton agar previously inoculated by the suspension of the strain studied. After incubation at 26°C for 24 hours for *P. carotovorum* and 48 hours for *Fusarium* sp., the activity was evaluated by measuring the diameter of inhibition in mm. For the control, streptomycin (10 μ g/disc for bacteria) and amphotericin B (10 μ g/disc for fungus) were used; the results were expressed as diameters (mm) of the zones of inhibition produced around the discs. All tests were performed in triplicates.

The susceptibility of these strains to extracts was estimated in comparison with the inhibitory effect of streptomycin as an antibacterial standard (11mm) and amphotericin B as an antifungal standard (23 mm), using an arbitrary scale of 4 levels.

Note: the values attributed to the scale were estimated from the values determined during the test on the inhibitory effect of the standards: streptomycin and amphotericin B against the phytopathogens *P. carotovorum* and *Fusarium* sp.

Antibacterial activity

Diameter \leq 5mm : No significant antibacterial activity.

5 <Diameter \leq 11mm : Moderate antibacterial activity.

11 <Diameter \leq 15mm : Significant antibacterial activity.

Diameter >15mm : Very significant antibacterial activity.

Antifungal activity

Diameter \leq 12mm : No significant antifungal activity.

12 <Diameter \leq 23mm : Moderate antifungal activity.

23 <Diameter \leq 30mm : Significant antifungal activity.

Diameter >30mm : Very significant antifungal activity.

5. GC-MS analysis

The GC analysis was carried out with a Thermo gas chromatograph (model 8000). A non-polar Hewlett-Packard OV-17 capillary column (25 m long \times 0.25 mm i.d., Film thickness 0.25 μ m) was employed for the analysis. The column temperature program was 60°C for 6min, with 5°C increases per minute to reach 150°C, which was maintained for 10min. The carrier gas was helium at a flow rate of 2 ml/min (splitless mode). The detector and injector temperatures were maintained at 250 and 225°C, respectively.

RESULTS

1. Antibacterial and antifungal activities of the seaweed extracts in culture media

1.1. Antibacterial activity of organic seaweed extracts on the growth of *P. carotovorum*

The results of the antibacterial test of each extract (Methanol, Methanol/Dichloromethane, and Dichloromethane) against *P. carotovorum* are represented in Table (1).

Table 1. Antibacterial activity of seaweed extracts against *P. carotovorum*

Seaweed	Diameter of inhibition zone (mm)		
	Meth	Meth/Dich	Dich
<i>Ellisolandia elongata</i>	14±0.4	12±0.8	11±1.5
<i>Corallina officinalis</i>	10±0.6	10.5±1.3	12.5±0.4
<i>Plocamium cartilagineum</i>	11±0.4	13±1.4	17.5±0.5
<i>Hypnea musciformis</i>	11±0.8	12±0.7	9±1.6
<i>Halopitys incurvus</i>	10±0.3	11.5±0.9	13±0.8
<i>Fucus spiralis</i>	8±0.9	10±1.4	9±0.5
<i>Gracilaria cervicornis</i>	8±0.6	7±1.8	9±0.6
<i>Bifurcaria bifurcata</i>	10±1.2	10±0.8	9±0.3
<i>Ulva lactuca</i>	7±0.7	7.5±0.5	7±1.3
Streptomycin (control)	11±0.9		

*Meth : Methanol, *Dich : Dichloromethane, *Meth/Dich : Methanol/Dichloromethane

In comparison with the inhibitory effect of streptomycin (11 mm), these results showed that the dichloromethane extract from *Plocamium cartilagineum* has the highest activity against *P. carotovorum* (17.5 mm). While, the dichloromethane extracts from *Corallina officinalis* and *Halopitys incurvus* have an inhibition zone values ranging from 12.5 to 13 mm, respectively.

However, the extracts of methanol/dichloromethane (v/v) from the three red seaweeds, *Ellisolandia elongata*, *Hypnea musciformis* and *Plocamium cartilagineum*, showed a moderate antibacterial activity with the following inhibition zone diameters: 12, 12 and 13.5mm. The other methanol/dichloromethane (v/v) seaweed extracts have a weak inhibitory effect against the growth of *P. carotovorum*, with inhibition zone ranging from 7 to 11.5 mm.

It was noted that the methanolic extract from *Ellisolandia elongate* inhibited the growth of *P. carotovorum* with an inhibition zone diameter of 14 mm, while the methanolic extracts of other seaweeds showed a moderate activity ≤ 11 mm.

Eminently, the inhibitory effect of the red seaweeds on the bacterium *Pectobacterium* was widely reported, which coincides with the results of the current experiment. In their study, **Lakhdar *et al.* (2018)** recorded the significant antibacterial activity of Rhodophyceae against *Pectobacterium brasiliensis*. In addition, **Vera *et al.* (2012)** noted that the red seaweeds enhanced plant protection against *P. carotovorum*.

1.2. Antifungal activity of seaweed extracts against the growth of *Fusarium* sp.

The results of the antifungal test of each extract (Methanol, Dichloromethane and Methanol/Dichloromethane) against *Fusarium* sp. are presented in Table (2).

Table 2. Antifungal activity of seaweed extracts against *Fusarium* sp.

Seaweed	Diameter of inhibition zone (mm) against <i>Fusarium</i> sp.		
	Meth	Meth/Dich	Dich
<i>Ellisolandia elongata</i>	24±0.5	17±1.3	16±0.4
<i>Corallina officinalis</i>	9±1.2	11±0.2	12±0.6
<i>Plocamium cartilagineum</i>	29±0.4	30±0.4	34±1.2
<i>Hypnea musciformis</i>	16±0.7	22±0.6	25±0.7
<i>Halopitys incurvus</i>	9±0.5	9±1.4	10±0.4
<i>Fucus spiralis</i>	9±0.2	9±0.5	7±0.5
<i>Gracilaria cervicornis</i>	30±0.8	29±0.2	22±0.4
<i>Bifurcaria bifurcata</i>	11±0.3	24±0.8	27±1.2
<i>Ulva lactuca</i>	22±1.4	30±0.3	31±0.5
Amphotericin B 10µg/disque	23±0.7		

*Meth : Methanol, *Dich : Dichloromethane, *Meth/Dich : Methanol/ Dichloromethane

The antifungal standard (amphotericin B) has a potential antifungal activity against the growth of *Fusarium* sp., with an inhibition zone diameter of 23mm. All the seaweed extracts showed an inhibitory effect against the strain *Fusarium* sp. in comparison with the inhibitory effect of amphotericin B.

The highest activity against *Fusarium* sp. was obtained by dichloromethane extract from *Plocamium cartilagineum*, with an inhibition zone diameter of 34mm. In addition, the dichloromethane extract from *Ulva lactuca* showed very significant antifungal activity (31mm).

The methanolic extracts from the three red seaweeds, *Ellisolandia elongata*, *Plocamium cartilagineum* and *Gracilaria cervicornis*; the dichloromethane extracts from *Hypnea musciformis* and *Bifurcaria bifurcata*, and the extracts of methanol/dichloromethane (v/v) from *Plocamium cartilagineum*, *Gracilaria cervicornis*, *Bifurcaria bifurcata* and *Ulva lactuca* have significant inhibition zone values ranging from 24 to 30mm. On the other hand, the remaining seaweed extracts recorded an inhibitory effect < 23 mm against the growth of *Fusarium* sp.

The dichloromethane extract from *Plocamium cartilagineum* showed the highest antimicrobial activities against *P. carotovorum* and *Fusarium* sp., with inhibition zone values of 17.5mm and 34 mm, respectively. These results concur with those of **Mabrouki et al. (2018)** who reported that, the dichloromethane extract from *Plocamium cartilagineum* has the most effective inhibitory (89%) against *Sclerotium rolfsii*. Therefore, the dichloromethane extract from *Plocamium cartilagineum* was evaluated with GC/MS analysis to determine the components responsible for their inhibitory effect against these phytopathogenic microorganisms.

2. GC-MS analysis

The structural determination by gas chromatography, coupled with mass spectrometry (GC-MS) was carried out on the dichloromethane extract from *Plocamium cartilagineum* to determine their components. This extract showed the highest activity *in vitro* against the growth of *P. carotovorum* pathogen agent of soft rot on sugar beet.

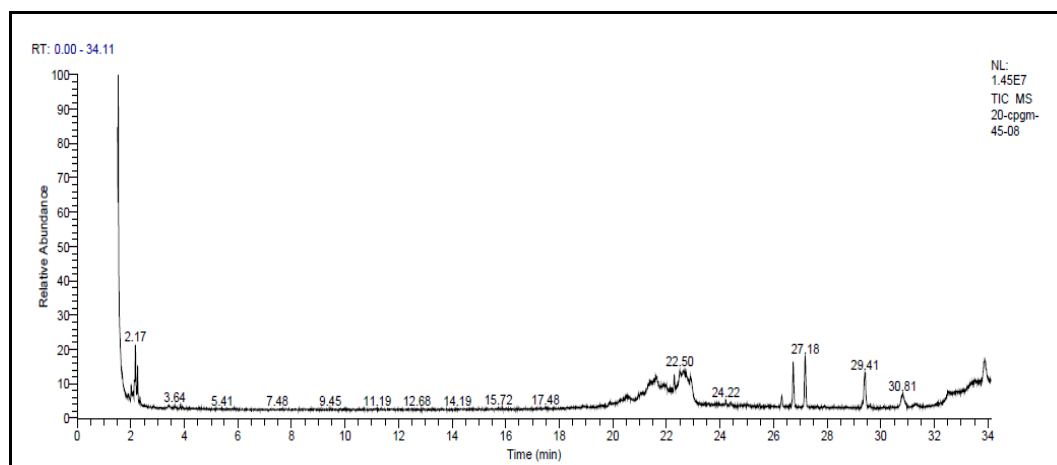


Fig.1. Chromatogram of the dichloromethane extract from *Plocamium cartilagineum*

The chromatogram in Fig. (1) shows the presence of peaks, with retention times between 0 and 34.11 minutes, corresponding to the elution times of the chemical compounds present in the extract.

Table 3. Characteristics of the compounds present in the dichloromethane extract from *Plocamium cartilagineum*

No.	Compound	Molecular formula	Retention time (min)	Area %
1	Oxetane, 2-methyl-4-propyl-	C ₇ H ₁₄ O	3.41	0.60
2	Methyl 8,11,14-heptadecatrienoate	C ₁₈ H ₃₀ O ₂	19.87	0.52
3	Methyl 8,11,14-heptadecatrienoate	C ₁₈ H ₃₀ O ₂	19.94	0.46
4	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	20.51	0.88
5	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	20.57	0.75
6	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	20.95	0.70
7	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	21.04	1.28
8	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	21.36	2.38
9	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	21.63	3.31
10	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	21.90	1.11
11	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	21.98	1.05
12	Z,Z,Z-1,4,6,9-Nonadecatetraene	C ₁₉ H ₃₂	22.29	2.14
13	OleicAcid	C ₁₈ H ₃₄ O ₂	22.50	3.80
14	OleicAcid	C ₁₈ H ₃₄ O ₂	22.64	6.72
15	9-Octadecenoic acid, (E)-	C ₁₈ H ₃₄ O ₂	22.91	3.71
16	trans-(2-Chlorovinyl)dimethylethoxysilane	C ₆ H ₁₃ ClOSi	24.22	0.72
17	trans-(2-Chlorovinyl)dimethylethoxysilane	C ₆ H ₁₃ ClOSi	24.39	0.54
18	16-Heptadecen-2,5,8-trione	C ₁₇ H ₂₈ O ₃	25.02	0.51
19	Selenocyanicacid	C ₈ H ₇ NSe	26.30	2.04
20	Selenocyanicacid	C ₈ H ₇ NSe	26.73	8.47
21	Selenocyanicacid,	C ₈ H ₇ NSe	27.18	10.26
22	Heptadecane	C ₁₇ H ₃₆	29.41	8.42
23	8,9-Dehydrothymol isobutyrate	C ₁₄ H ₁₈ O ₂	30.82	3.88
24	à-L-Fucopyranose 1,2:3,4-bis(benzeneboronate)	C ₁₈ H ₁₈ B ₂ O ₅	31.30	1.25
25	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	C ₂₁ H ₃₈ O ₂	32.53	2.28
26	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	33.25	0.64
27	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	33.31	0.43
28	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	33.39	0.85
29	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	33.50	1.57
30	ë-Pentachlorocyclohexene	C ₆ H ₅ Cl ₅	33.88	7.04

The elemental chemical composition and the chemical formula of each compound was determined by mass spectrometry, in comparison with the Mainlib, Replib and Nistdemo databases. 16 chemical compounds were identified in this analysis (Table 3). The main compounds detected were selenocyanic acid (20.77%), oleic Acid (10.52%), and 9, 12, 15 octadecatrienoic acid (Z, Z, Z) (9.30%).

In the literature, several studies have shown that fatty acids have antimicrobial activity. **Mc. Gaw *et al.* (2002)** demonstrated that fatty acids exhibited antibacterial activity. Moreover, **Agoramoorthy *et al.* (2007)** and **Jayalakshmi *et al.* (2021)** mentioned that lauric, palmitic, linoleic, oleic, stearic and myristic acids possessed antibacterial potential.

DISCUSSION

In Morocco, *P. carotovorum* (*Erwinia carotovora*) is well known as a soft rot pathogen (**Snaiki *et al.*, 2005**) that causes a reduction in sugar levels (**Snaiki *et al.*, 2005**; **Ghazy *et al.*, 2021**). This was reflected on the final product, specifically sucrose and purity percentages (**Ghazy *et al.*, 2021**). Sugar quality is an important parameter for sugar beet industry (**Ghazy *et al.*, 2021**). Meanwhile, several soil-borne fungi including *Fusarium* sp. cause wilt and root rot disease in almost all the infected plants, and they responsible for considerable plant yield losses compared to other microorganisms (**Khan *et al.*, 2017**). The fungus *Fusarium* sp. reaches initially the cell interior, where it should be able to degrade the suberin, which covers the outer organs of the plant, followed by the middle lamella, with the effect of the pectinolytic enzymes and finally the cell wall (**Melo *et al.*, 2020**).

In order to use the natural products for controlling this disease, seaweed extracts were used and their efficacy against sugar beet root rot caused by *P. carotovorum* and *Fusarium* sp. was evaluated. Therefore, nine seaweeds were used, and their antibacterial and antifungal activities against the growth of *P. carotovorum* and *Fusarium* sp. were determined. The highest activity against both strains of *P. carotovorum* and *Fusarium* sp. was obtained by dichloromethane extract from *Plocamium cartilagineum*, with an inhibition zone diameter of 17.5 and 34mm, respectively, in comparison with antibacterial standard (11 mm) and antifungal standard (23 mm). The other seaweed extracts showed weak antimicrobial activities on the growth of *P. carotovorum* (7 to 14mm and 9 to 31 mm) against *Fusarium* sp.

Studies on the antimicrobial activities of the red seaweed extracts have proved an important inhibitory effect. **Lakhdar *et al.* (2018)** demonstrated that, the Rhodophyceae showed the highest activity against *Pectobacterium brasiliensis*. Another study showed that ι -, κ -, and λ -oligocarrageenans from the red seaweeds provided tobacco plants enhanced protection against *P. carotovorum* and *Botrytis cinerea* likely due to a sustained induction of phenylalanine ammonia lyase activity, which determined the accumulation of phenylpropanoid compounds with potential antimicrobial activities (**Vera *et al.*, 2012**).

Bhuyar *et al.* (2020) showed that the red seaweed *Kappaphycus alvarezii* indicated scope for deriving bioactive compounds which are potential inhibitors of pathogenic bacteria. A study was carried out showing that the mixture of the two seaweeds: *Cystoseira compressa* and *Padina pavonica* have a significant cooperative antibacterial effect with an inhibition diameter value of 16 mm (**Abdeldjebbar *et al.*, 2021**). Additionally, **Agarwal *et al.* (2021)** concluded that *Gelidium pusillum* is a rich source of antibacterial. **Furthermore, Rhimou *et al.* (2010)** showed that the red seaweed *Hypnea musciformis* exhibited high antibacterial activity. In this context, **Etahiri *et al.* (2001)** mentioned that the red seaweed *Sphaerococcus coronopifolius* has significant antibacterial activity.

The results of the study effected by **Caprena *et al.* (2021)** confirmed that three red seaweeds (*Chondrus crispus*, *Mastocarpus stellatus* and *Gigartina pistillata*) have good antimicrobial properties. While, **Oumaskour *et al.* (2019)** found that the red seaweed *Bornetia secundiflora* collected from the Atlantic coast of Sidi Bouzid-El Jadida presented an important antibacterial activity.

In their study, **Rhimou *et al.* (2013)** have tested eighteen red marine seaweeds of Atlantic-Mediterranean for the production of antibacterial compounds. This study showed that the most significantly active and the highest rates of biological activity were found in five species, *Pterosiphonia complanata*, *Sphaerococcus coronopifolius*, *Plocamium cartilagineum*, *Asparagopsis armata* and *Boergeseniella thuyoides*. These same results are found in the study of **Fonseca (2021)** who observed that, the red seaweed *Plocamium cartilagineum* has an antimicrobial potential against different pathogenic microorganisms. In addition, **Martorell *et al.* (2020)** postulated that *Plocamium cartilagineum* and *Gymnogongrus turquetii* showed very significant results.

To determine the components of dichloromethane extract from *Plocamium cartilagineum*, which recorded the most antibacterial and antifungal activities *in vitro* against *P. carotovorum* and *Fusarium* sp., respectively, pathogens agent of soft rot on sugar beet, a structural determination using gas chromatography coupled with mass spectrometry (GC-MS) was carried out. The results showed the presence of 16 chemical compounds (Table 3). The main compounds detected were selenocyanic acid (20.77%), oleic acid (10.52%), and 9, 12, 15 octadecatrienoic acid (Z, Z, Z) (9.30%). These results are in agreement with a study which demonstrated that fatty acids have antibacterial activity (**Mc. Gaw *et al.*, 2002**) and lauric, palmitic, linoleic, oleic, stearic and myristic acids are have potential antibacterial agents (**Jayalakshmi *et al.*, 2021**).

The presence of fatty acids (**Bhuyar *et al.*, 2020**) can be used as key intermediates of biologically active compounds as the: 1, 2, 5 Thiadiazole-3-carboxamide, 4- [(2-chloroethyl) amino]-N-(2-hydroxyethyl)] which can be used for antimicrobial.

Somalraju *et al.* (2021) evaluated the effect of selenium (Se) on plant emergence from seed pre-treated with selenium and the seed decay pathogens *P. carotovorum*. In addition, **Agoramoorthy *et al.* (2007)** demonstrated that bioactive fractions, linoleic acid

and oleic acid from *Pelagonium* sp. possessed antibacterial activity against *Mycobacterium aurum*.

This experiment showed that the red seaweed *Plocamium cartilagineum* has the highest effect inhibitory among the 9 studied seaweeds against the growth of both pathogens, *Pectobacterium carotovorum* and *Fusarium* sp.

The components of dichloromethane extract from *Plocamium cartilagineum* was identified by gas chromatography/mass spectroscopy (GC/MS) analysis. Selenocyanic acid (20.77%), oleic acid (10.52%), and 9, 12, 15 octadecatrienoic acid (Z, Z, Z) (9.30%), could possibly be responsible of the highest antibacterial and antifungal activities of the extract. Thus, the red seaweed was proved potentially promising for disease control and for sustainable agriculture technology.

Based on the current findings, *Plocamium cartilagineum* from the coast of Sidi Bouzid could be used in the treatment of the disease under investigation.

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