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Screening Of The Antifungal Activity Of 22 Seaweed From The Coast Of El Jadida Morocco Against *Bipolaris sorokiniana.*

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ABSTRACT

Algae are a great source of complex natural products that could be a promising source of new bioactive compounds that can help plant survival by providing protection against stress imposed by pathogens. The coast of El Jadida (SidiBouzid) is particularly rich in algal biodiversity, it contains 143 algal species, with a considerable economic, social and ecologic potential. The aim of this study is to screen the algal species in order to use it for antifungal preparation. The inhibitory effect of 22 marine algae species has been evaluated against *Bipolarissorokiniana* causative agent of helminthosporal blight using five organic extracts (Methanol, Butanol, Methanol/Dichloromethane (50/50), Dichloromethane, and Hexane). Of the 22 species studied, those belonging to the Rhodophyceae and Phaeophyceae were the most active, while Chlorophyceae have a low inhibition. Maximum inhibition of *Bipolarissorokiniana* was obtained by extracts prepared in methanol and dichloromethane/methanol and the red alga *Gracilaria* sp., represents the important inhibition of the pathogen.

Keywords: Algae, *Bipolarissorokiniana*, helminthosporal blight, coast of El Jadida (SidiBouzid), antifungal activity.

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INTRODUCTION

On the globe, more than 150 000 macroalgae species are found in oceans. In Morocco there are 451 species present on the two marine facades (Atlantic and Mediterranean) including green, brown and red algae [1, 2, 3]. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient, pigments and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit for human health and plant disease [4, 5]. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae [5, 6, 7, 8, 9, 10, 11, 12, 13, 14]. The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective mechanisms and compounds [15]. Many of the seaweeds possess bio-active components which inhibit the growth of some of the Gram positive and negative bacterial pathogens [16], how causes high rate of mortality in human population and aquaculture organisms [17]. Nowadays there is an increasing demand for biodiversity in the screening programs for selecting therapeutic and phyto drugs from natural products.

Spot blotch, caused by the fungus *Bipolarissorokiniana* (Sacc.) Shoemaker, is one of the most important diseases of wheat (*Triticumaestivum L*.) worldwide [18]. This fungus can infect several parts of the plant, including the coleoptiles, crowns, culms, leaves and roots [19]. On the leaves, symptoms are small dark-brown lesions that may expand into oval-shaped black blotches [19, 20].

The present study aims to screen the antifungal properties of five extracts from 22 algae collected from the coast of El Jadida (Sidibousid) against *Bipolarissorokiniana*.

MATERIALS AND METHODS

Algal materials

Seaweeds was collected by hand-picking during March to April 2016 from the coast of El Jadida (33 ° 33 °16’09’’N, 8 °30’ 8 °45’W) (figure 1). The algae were cleaned, washed in distilled water, then dried at room temperature and crushed to a fine powder.

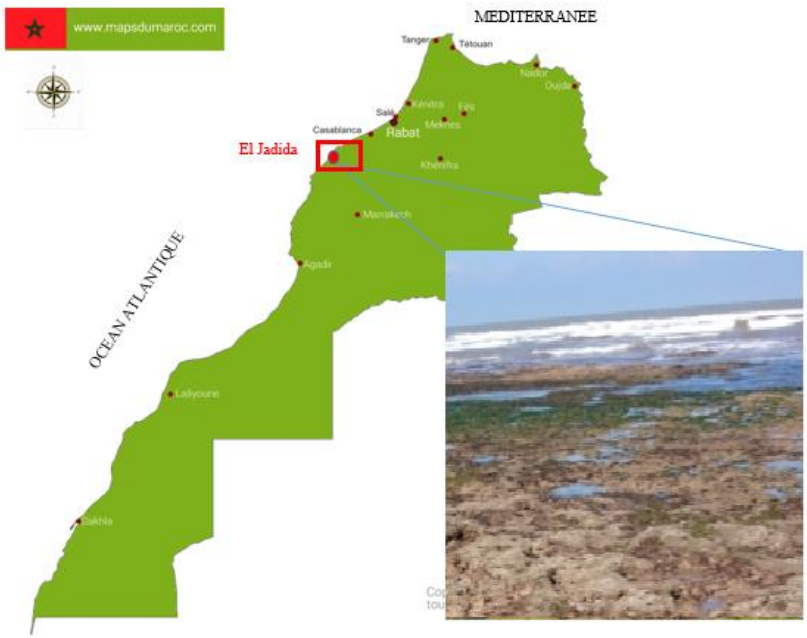

Figure 1: Localization of the collection site of SidiBouzid

The algae investigated were identified from fresh species as; **Chlorophyceae**: *Codiumelongatum, Enteromorpharamulosa, Ulva sp1, Ulva sp2*. **Phaeophyceae**:*Bifurcariabifurcata, Fucusspiralis, Laminariadigitata, Cystoseirahumilis, Sargassummuticum, Sargassum vulgaris*and **Rhodophyceae**: *Osmundeapinnatifida, Gelidium Sp1, Hypneamusciformis, Plocamiumcartilagineum, Gelidiumpulchellum, GracilariaSp, Ellisolandiaelongata, Ellisolandiaofficinalis, Bornetiasecundiflora, Gelidium Sp2, Gracilariacervicornis and Halopitysincurvus.*

Preparation of extracts:

Each powder from dried algae was extracted in different solvents, namely Methanol, Butanol, Methanol/Dichloromethane (50/50),Dichloromethane, and Hexane, as described by CaccameseetAzzolina [21].The resulting extracts were concentrated by drying in a rotary evaporator under reduced pressure (at 45°C). A crude extract obtained was stored at 4°C until utilization

Antifungal strain

The strain (*Bipolarissorokiniana*) used to evaluate the antifungal activity was obtained from laboratory of phytopathology of National Institute of Agronomic Research (INRA), Settat-Morocco.

Antifungal bioassays

Antifungal assays were carried out using the agar disc-diffusion assay [22]. Three colonies of each fungus were removed with a wire loop from the original culture plate and were introduced into a test tube containing 5 ml of Nutrient broth. An overnight culture yielded a suspension of 10^6 spore per ml (evaluated by the absorbance value of 0.5 at 620 nm). This solution was diluted 100-fold and the fungal density was then adjusted to 0.2×10^4 spores per mL with sterile water to inoculate petri dishes containing Mueller-Hinton agar culture media. Plates were dried for about 30 min before inoculation and were used within 4 days of preparation. The organic extracts were tested using cellulose disks (6 mm diameter) impregnated with the solution. After the temperature was equalized at 4 °C, the microorganisms were incubated overnight at 24 °C during 36 hours. Diameters of inhibitory zones were then measured. Discs impregnated with standard antifungal such, amphotericine B (50µg/ml) were used as reference and discs impregnated with each solvent are used like control. All tests were performed in triplicate [23].

Antifungal efficiency of extracts was evaluated according to the following scale:

- Ø ≤ 10 mm: No-significant antifungal activity
- 10<Ø ≤ 15 mm: Moderate antifungal activity
- 15<Ø ≤ 20 mm: Significant antifungal activity
- Ø>20 mm: Very significant antifungal activity

Statistical analysis:

The data were statistically analyzed by applying a one-way ANOVA for comparison of mean values. All tests were considered to be statistically significant at *P<0.05.

RESULTS AND DISCUSSION

Antifungal activity

The results of the antifungal test of each extracts (Methanol, Butanol, Methanol/Dichloromethane (50/50),Dichloromethane, and Hexane) against *Bipolarissorokiniana* are summarized in table 1 to 3.

Of the sex brown algae tested, the majority of species showed a positive activity against *Bipolarissorokiniana*. An important activity was observed in the methanol extract of *Fucusspiralis*(29 mm), followed by dichloromethane/methanol extract of *Laminariadigitata*(23 mm) and Butanol extract of *Fucusspiralis*(22,5mm). Significant inhibition with diameter of inhibition higher than 20 mm was observed in

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the dichloromethane and dichloromethane/methanol extract of *Fucusspiralis* wish is successively 20 and 20,5 mm compared to the antifungal control (table 1).

Table 1: Antifungal activity of brown seaweeds extracts against *Bipolaris sorokiniana*

	Diameter of inhibition (mm) against *Bipolaris sorokiniana*				
	MeOH	**But**	**DC/MeOH**	**DC**	**Hex**
Bifurcaria bifurcata	16±1	17,5±2	19,5±2	16±2	16±2
Fucus spiralis	29±3	22,5±2	20,5±2	20±1,5	16,5±2
Laminaria digitata	16±0	12±1	23±3	16,5±1	13,5±1
Cystoseira humilis	18±1	10±1	13,5±1	17±3	14±1
Sargassum muticum	16,5±2	15±2	11,5±1	14±1	15,5±2
Sargassum vulgaris	14,5±1	12±1	12±1	14±1	12,5±1
Amphotericine B (50 ug/mL)	21±1				

MeOH: Methanol, But : Butanol, DC/MeOH: Dichloromethane/Methanol, DC: Dichloromethane,Hex : Hexane

The same results were observed for the red algae, the majority of the species showed a positive activity against *Bipolarissorokiniana*(table 2), an important activity was observed in the methanolic extract of *Gracilaria*sp with a diameter of inhibition of 33 mm, followed by the dichloromethane extract of *Ellisolandiaelongata* (26 mm), the hexane extract of *Gracelaria*sp. (23.5 mm), the methanol extract of *Gelidium*sp2 and the dichloromethane / methanolic extract of *Gracelaria*sp (23 mm).Significant activity against *Bipolarissorokoniana*obtained in the hexane extract of *Ellisolandiaelongata*, the methanolic extract of *Gracelariacervicornis* and *Halopitysincurvus,* the dichloromethane / methanolic extract of *Gelidium*sp1 and the butanolic extract of *Bornitiasecundiflora* (table 2).

Table 2: Antifungal activity of red seaweeds extracts against*Bipolaris sorokiniana*

	Diameter of inhibition (mm)against *Bipolaris sorokiniana*				
	MeOH	**But**	**DC/MeOH**	**DC**	**Hex**
Osmundea pinnatifida	15±1	18±1	16±2	15±2	19,5±2
***Gelidium* sp1.,**	19,5±2	17,5±1	21,5±2	11,5±1	10,5±1
Hypnea musciformis	11±1	17±1	19,5±2	12±1	11±1
Plocamium cartilagineum	12,5±1	18,5±2	16±1	18±2	19±2
Gelidium pulchellum	18±2	12,5±1	13±1	16,5±2	11,5±1
***Gracilaria* sp.,**	33,5±3	18±2	23±2	15±1	23,5±3
Ellisolandia elongata	19±2	11±1	14±1	26±3	22,5±3
Ellisolandia officinalis	10,5±1	11±1	14±1	14±1	11,5±1
Bornetia secundiflora	16±2	20,5±1	15,5±2	11±1	10,5±1
***Gelidium* sp2.,**	23±3	14,5±1	12±2	9,5±1	14,5±1
Gracilaria cervicornis	21,5±1	15,5±2	19±3	12±2	15±1
Halopitys incurvus	21±2	14±2	11±1	9,5±1	11,5±1
Amphotericine B (50 ug/mL)	21±1				

MeOH: Methanol, But : Butanol, DC/MeOH : Dichloromethane/Methanol, DC: Dichloromethane,Hex : Hexane

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Table 3: Antifungal activity of green seaweeds extracts against *Bipolaris sorokiniana*

	Diameter of inhibition (mm)against *Bipolaris sorokiniana*				
	MeOH	**But**	**DC/MeOH**	**DC**	**Hex**
Codium elongatum	10±1	11±1	11±2	11±1	16±1
Enteromorpha ramulosa	20,5±2	19±2	20±1	10±1	10±1
***Ulva* sp1.,**	9±1	10±1	20,5±3	12±2	14±2
***Ulva* sp2.,**	7±1	12±1	8,5±1	14±1	8±1
Amphotericine B (50 ug/mL)	21±1				

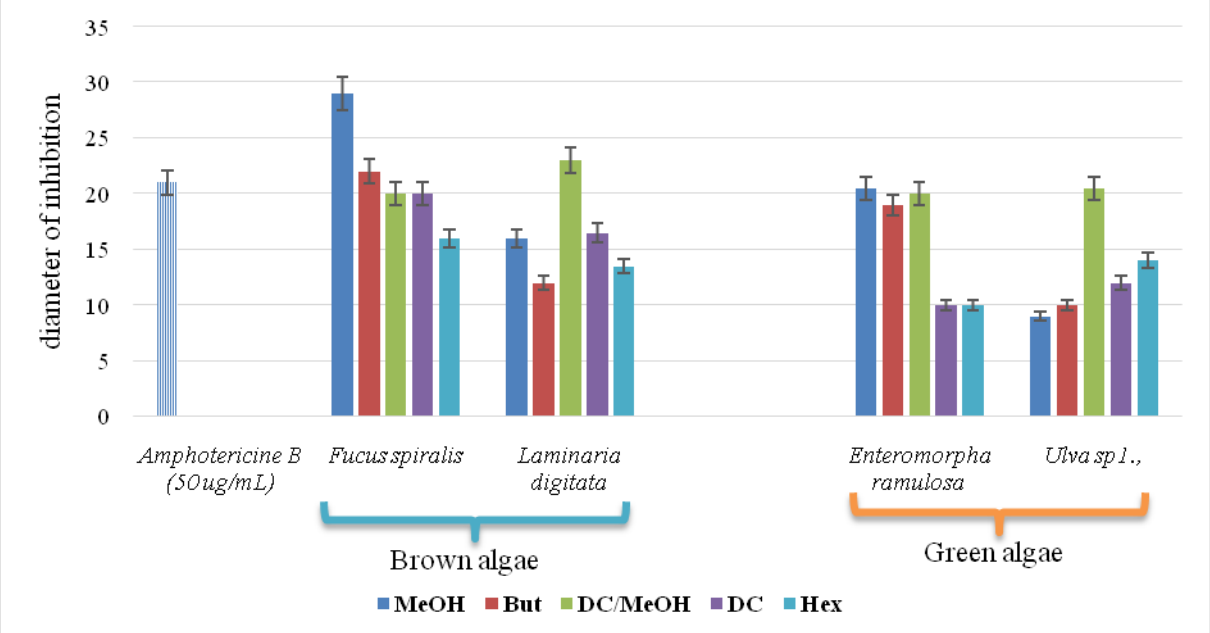
MeOH: Methanol, But : Butanol, DC/MeOH : Dichloromethane/Methanol, DC: Dichloromethane,Hex : Hexane

Of the four green algae tested, the best activity against *Bipolarissorokiniana* was observed in methanolic extract of *Enteromorpharamulosa* and dichloromethane/methanol extract of *Ulva*sp1 (20,5 mm), followed by the dichloromethane/methanol extract of *Enteromorpharamulosa* with diameter of inhibition equal to 20 mm (table 3)

Results make it possible to conclude that the majority of the brown algae represent a moderate activity (approximately 15 mm), while only 2 species (*Fucusspiralis and Laminariadigitata*) represent an activity greater than or equal to 20 mm in diameter, on the other hand the majority of red algae (*Gelidium sp., Gelidium sp2., Gracilaria sp., Gracilariacervicornis, Ellisolandiaelongata and Halopitysincurvus*) have an antifungal activity greater than or equal to 20 mm in at least one solvent used.

These results are in affirmation with the results of Pesando and Garam[22], which shows that in the 12 species tested, those belonging to phaeophyceae were the most active against *Bipolarissorokoniana* in comparison with Rhodophyceae and chlorophyceae; the same result was reported by Kumar C.S *et al.,*[5] and Lakhdar*et al.,*[23].

Algal species with significant antifungal activity against *Bipolarissorokoniana*(activity greater than or equal to the activity of the reference antifungal agent Amphotericin B at 50 µg / ml)were represented in figures 2 and 3.



MeOH: Methanol, But :Butanol, DC/MeOH : Dichloromethane/Methanol, DC: Dichloromethane,Hex : Hexane

Figure 2: Brown and green algae with inhibition diameter against *Bipolarissorokoniana*is greater than or equal to 20 mm.

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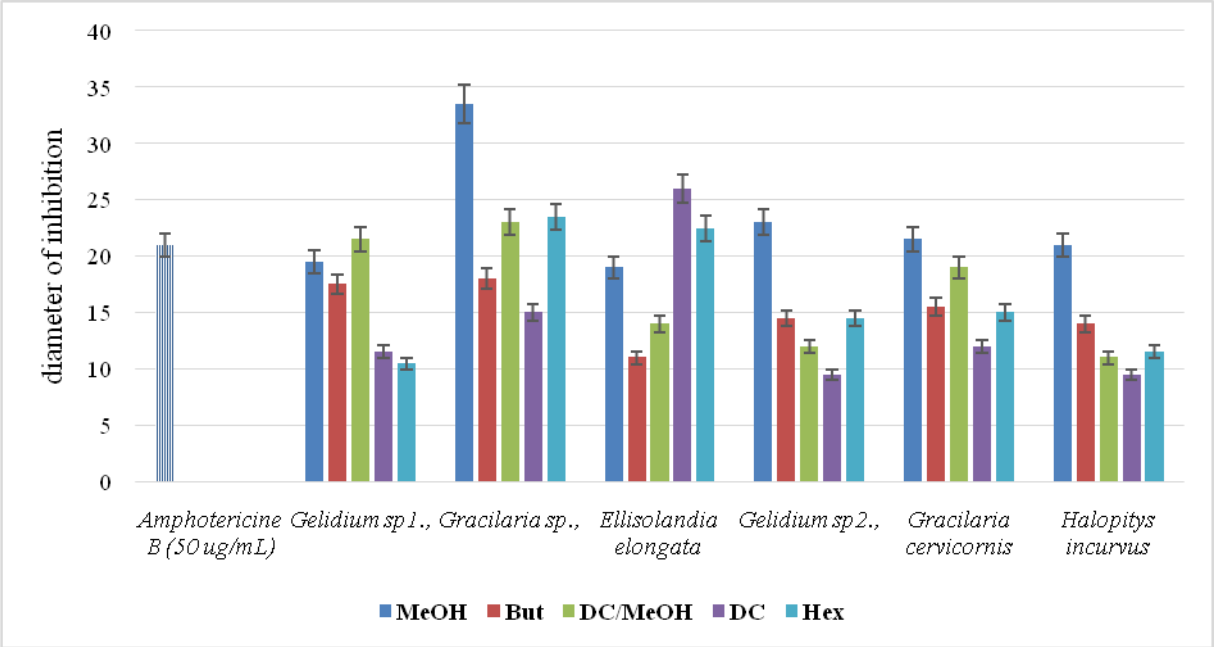

MeOH: Methanol, But :Butanol, DC/MeOH : Dichloromethane/Methanol, DC: Dichloromethane,Hex : Hexane

Figure 3: Red algae with inhibition diameter greater than or equal to 20 mm against *Bipolarissorokoniana.*

According to the two figures above (figure 1 and 2), it is also concluded that the best solvents of extraction are the methanol and the mixture dichloromethane/methanol. This is why these two solvents are selected to make the extractions for the continuation of work to make.

Marine organisms such as marine algae are source material for structurally unique natural products with pharmacological and biological activities. That seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids has exhibits different biological activities[24-26]. Depending upon their solubility and polarity, different solvents shows the different antimicrobial activity.

Sastry and Rao [27], reported that methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate, whereas others reported that chloroform is better than methanol and benzene [28].

According to Shanmughapriya [29], the extract from all species of Chlorophyta, Rhodophyta and Phaeophyta taxa were active against the yeast *Candida albicans*. Previous researchers have found that the extract of *Ulvalactuca* had the stronger antimicrobial activity, inhibiting the tested bacteria and fungi at low concentrations[30].

Pandian*et al.,* [31], showed that the methanolic extract of *Acanthaphoraspicifera* had a higher antibacterial and antifungal activity compare to other two extracts. The antibacterial and antifungal activity of methanolic extracts showed similar activity to that of ciprofloxacin and amphotericin respectively. Ballesteros *et al.,*[32],observed large antimicrobial activity of methanolic algae extracts from 71 species on the Mediterranean area, finding antifungal activities in tests with *Dictyota*, *Padina*and *Sargassum* species.

Regarding our results on fungi species, the methanol extract of *Gracilaria*sp, *Fucusspiralis* and *Enteromorpharamulosa*, dichloromethane/methanol extract of *Enteromorpharamulosa* and *Ulva* sp1 were those that showed the best inhibition against *Bipolarissorokiniana*, probably due to the polarity of these solvent and their substances in different concentrations responsible for antifungal activity, as it is observed in table 1 to 3.Tuney*et al.* [33], pointed out results that may be related to the presence of bioactive metabolites soluble in ethanol, which may also have occurred with dichloromethane and methanol extracts of the algae analyzed in this study.

These difference of activity may be due to the efficiency of the extraction methods to recover the active metabolites, solvents used [33],susceptibility of strains [34] and seasonal variation [35]. In view to this, various solvents were applied in this study, with the aim to select the best solvent yielding maximum amount of bioactive compounds responsible for the antimicrobial activity.

This study made it possible to select algae (*Fucusspiralis, Laminariadigitata, Gracilaria sp., Ellisolandiaelongata, Gelidiumsp2.,*), which show important activity against *Bipolarissorokoniana*. These selected algae will be used for the fractionation and determination of the compounds responsible for the antifungal activity.

CONCLUSION

This study reports the presence of antifungal compounds against *Bipolarissorokoniana* in the algae collected from the site of Sidibouzid, activities depends on the class of algae. A number of active species was more important in the class of Phaeophyceae than Rhodophyceae. For the extraction used, in the majority of the cases, methanol followed by dichloromethane/methanol were the solvents which gives the best results. However, the bioactive compounds present in these extracts, which vary widely in their chemical polarity, must be purified for better results.

Finally, it can be concluded that extracts of algal species used in the present investigation are potential sources of bioactive compounds and should be investigated for natural antifungal compounds. Research is in progress to identify and purify these antifungal substances.

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