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Original Article

SCREENING OF ANTIBACTERIAL AND ANTIFONGICAL ACTIVITY IN MARINE MACROALGAE AND MAGNOLIOPHYTEA FROM THE COAST OF TUNISIA

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ABSTRACT

Objective: The present study was conducted to evaluate the antimicrobial and antifongical activity of Hexane, ethyl acetate and methanol extracts of thirteen marine spices from Tunisian coastline (Chebba and Sfax): five *pheophytea*, five *cholorophytea* and three *Magnoliophytea*.

Methods: These spices were tested against eight human pathogenic bacteria: Gram- (*Escherichia coli, DH5 (alpha), listeria monocytogéne, Salmonella enterica, Agrobacterium tumefaciens, Pseudomonas aerigunosa)*, Gram+ (*Staphylococus aureus, Micrococcus luteus)*, and two human pathogenic yeast (*Candida tropicalis, saccharomyces cerevisiae)* and a fungi (*Aspergillus niger)*, using the agar disk diffusion assay method.

Results: The results of thirteen marine spices extracts collected from the coast of Tunisia have shown significant antimicrobial activity and the maximum inhibitory activities were observed in the brown algae, it was more active on bacteria than the fungi.

Conclusion: The results of the present study revealed that the seaweeds and *magnoliophytea* from Tunisia appear to have immense potential as a source of antibacterial and antifongical compounds, they could be used in treating diseases caused by these organisms test.

Keywords: Antibacterial activity, Antifungal activity, Disc diffusion method.

INTRODUCTION

The marine environment is an exceptional reservoir of bioactive natural compounds, which exhibit structural/ chemical features not found in terrestrial natural products [1].

These compounds are synthesized by different metabolic pathways those observed in terrestrial environments. Among marine organisms, algae, marine *magnoliophytea* that synthesize a wide variety of secondary metabolites chemically active, which are used for defense against other organizations predators or colonizers. These active metabolites produced by several species of and macro marine micro algae, show antibacterial and anti-inflammatory properties, which are effective in the therapeutic field [2-9]. Several studies indicated that there has bioactivity in marine organisms origins [10-16]. The synthesis of substances Side by marine algae is influenced by seasonal variation and geographical distribution [17, -19]. With the emergence of resistant bacteria to several forms of certain antibiotics, of research new active molecules has become a necessity.

The purpose of this work was to evaluate the antibacterial and antifungal activity of ten Mediterranean marine macroalgae and three *Magnoliophytea* collected from the coast of Chebba and Sfax (Tunisia), with solvents of increasing polarity extraction (Hexane, Ethyl Acetate, Methanol) against eight human pathogenic bacteria and three human pathogenic yeast in order to discover new antibacterial or / and antifungal metabolites.

MATERIALS AND METHODS

Sample collection

Fresh ten seaweeds: five *pheophytea* (*Dictyota dichotoma, Cystoseira crinita, Cystoseira barbata, Dictyopteris membranaceae and Sargussum vulgare*), five *cholorophytea* (*Flabellia petiollata, Anadypméne stellata, codium fragile, Halimeda tuna and Ulva rigida*) and three *Magnoliophytea*: *Cymodocea nodosa, Posidonea oceanica and Halophila stipulacea*, were collected at Tunisian coastline (Chebba and Sfax), during Mars 2013 by hand picking using Scuba diving or snorkeling (1 – 4 m depth) and preserved on ice until further processing. These species were identified at the National Institute of Oceanology of S fax (INSTM). After collection, the samples were rinsed with fresh seawater and distilled water to remove associated debris and epiphytes. Then seaweeds were dried for 24 h under an artificial light in 24 °C and finally in a heater. The dry seaweeds were crushed in an electric mill until a fine powder was obtained.

Preparation of extracts

For extraction of bioactive in shade dried seaweeds, 600 g of finely powdered algal material were packed in small bags (5x 10 cm) of Whatman filter paper and all bags were sealed and soaked three times in an organic solvent bath for steeping during 24h. The extraction was carried out, separately, with different organic solvents in the order to increase polarity: Hexane, ethyl acetate and methanol. The organic extracts were concentrated to solvent free by evaporation in a rotary vacuum evaporator rotavable at 45 °C. The extracts obtained for each species were stored at 4 °C for testing different biological activities.

Microorganisms

The strains used were Eight bacteria: Gram-positive *Escherichia coli* (ATCC 8739), *Escherichia coli* DH5 (alpha), *listeria monocytogéne* (BUG 496), *Salmonella enteria* (ATCC 43972*), Agrobacterium tumefaciens, Pseudomonas aerigunosa* (ATCC 49189), Gram-negative*: Staphylococus aureus* (ATCC6538), *Micrococcus luteus* (LB 14110); Antifungal activity was determined against the filamentous fungi *Aspergilus niger*, unicellular yeast *saccharomyces cerevisiae* and *Candida tropicalis* R2 CIP203 (CTR2), which is a strain resistant to both reference polyene antifungal major: amphotericin B and nystatin. were obtained from the Microbiology Departement, Faculty of science, S fax.

Disk diffusion method

The antibacterial activity of extracts was performed using the agar-disk diffusion method [20]. The suspensions of organisms were initially adjusted with sterile distilled water to a density equivalent to the 0.5 McFarland standards. 0.20 ml of a 24 h-broth culture (106 cfu/ml) of the bacteria species were spread on the surface of gelled

sterile Mueller-Hinton Agar plates (pH 7, 4 ± 0, 2 à 25 °C) at 37 °C for 24h prior to use. Several colonies of similar morphology of the respective bacteria were transferred into API suspension medium. The different extracts were prepared and then absorbed onto the sterile disks (20 and 30 µl) and the same volume of solvent was used as the negative control. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones surrounding the disks. The experiment was performed in triplicate.

Anti fongical activity

For screening the antifungal activity of algae extracts, the agar-disk diffusion method was used as previously described [21]. Three strains (*saccharomycete, Candida tropicali*s and *Aspergillus niger*). All strains were first grown on Sabouraud chloramphenicol agar plate at 30°C for 18-24h. Several colonies were transferred into API suspension medium and adjusted to 2 McFarland turbidity standards with a densimat. The inocula of the respective yeast was streaked into Sabouraud chloramphenicol agar plates at 30°C using a sterile swab and then dried. A sterile filter disk, diameter 5 mm (Whatman paper # 3) was placed in the plate. An amount of 10 µl of the extract were dropped on each paper disc (10 mg/ disk). The treated Petri dishes were incubated at 30 °C for 18- 24h. The antifungal activity was evaluated by measuring the diameter of the inhibition zones around the disks. The same volume of solvent was used as the negative control. Each experiment was carried out in triplicate and the mean diameter of the inhibition zones was recorded.

RESULTS

Anti bacterien test

Currently, nosocomial infections are a major problem in public health. The bacteria is considered among the bacteria most responsible for these infections. the deadliest bacteria we can cite *Staphylococcus aureus* (gram+) who shares with *Escherichia coli* (gram-) the forefront of germs responsible for nosocomial infections. To this worrying situation it is essential to find new antibiotics active from natural resources. The results of antibacterial tests of the species studied are given in table 1**,** present inhibition diameters obtained on bacteria pathogens references. The antibacterial activity was classified from less active (+: Diameter of inhibition 10 mm) to moderately active (++:10 mm Diameter of inhibition 15 mm), to highly active (+++Diameter of inhibition 15 mm) and no active (-: resistant).

Table 1: Antimicrobial activity of macroalgae extracts

Microorganisms									
Algea	**Extractant**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Gram		-	-	-	-	+	+	-	-
Phéophytea									
I	Hexane	-	+	-	+	-	+	-	-
	Ethyl Acetate	-	+	+	+	+	++	-	-
	Methanol	-	+	+	+	++	++	-	-
II	Hexane	-	-	-	-	+	-	-	-
	Ethyl Acetate	-	-	+	-	++	-	-	-
	Methanol	-	-	-	-	+++	+	-	-
III	Hexane	-	-	-	-	-	-	-	-
	Ethyl Acetate	-	-	-	+	-	-	+	-
	Methanol	-	-	-	-	+	+++	-	-
IV	Hexane	-	-	-	-	-	+	-	--
	Ethyl Acetate	-	+	-	-	+	-	-	-
	Methanol	+	-	-	-	+++	-	-	-
V	Hexane	-	-	-	-	-	-	+	+
	Ethyl Acetate	-	+	-	-	-	+	-	-
	Methanol	++	-	-	-	++	+++	-	+
Chlorophytea									
VI	Hexane	-	-	-	-	-	+	-	-
	Ethyl Acetate	+	+	+	-	+	+	-	-
	Methanol	-	-	-	-	++	-	-	-
VII	Hexane	-	-	+	-	-	-	-	-
	Ethyl Acetate	+	-	-	+	++	-	-	-
	Methanol	+	-	-	-	++	+	-	-
VIII	Hexane	-	-	-	-	-	-	-	-
	Ethyl Acetate	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-
IX	Hexane	-	-	-	-	-	-	++	-
	Ethyl Acetate	+	-	-	-	+	-	-	-
	Methanol	++	-	+	-	++	+	-	-
X	Hexane	-	-	+	-	-	-	-	-
	Ethyl Acetate	+	+	-	-	-	-	-	-
	Methanol	-	-	-	-	+++	-	-	+
Magnoliophytea									
XI	Hexane	-	-	-	-	-	-	-	-
	Ethyl Acetate	-	+	-	-	-	+	-	-
	Methanol	+	+	+	-	+	+	-	-
XII	Hexane	-	-	-	-	-	-	-	-
	Ethyl Acetate	-	-		-	-	+	-	-
	Methanol	-	+	+	-	+	++	-	-
XIII	Hexane	-	-	-	-	-	-	-	-
	Ethyl Acetate	-	-	+	+	-	-	-	-
	Methanol	-	-	-	-	++	++	-	-

I -*Dictyota dichotoma (Dd*) from Chebba II- *Cystoseira crinita (Cc)* from Chebba III- *Cystoseira barbata* (*Cb*) from Sfax IV- *Dyctéopteris menbranacea (Dm)* from Sfax V- *Sargussum vulgare (S* v) from Sfax VI- *Flabelia petiolata (Fp)* from Chebba VII -*Anadyoméne stelleta (As)* from Chebba *VIII-Halimeda tuna (Ht)* from Sfax *IX- Ulva rigida (Ur)* from Sfax *X-Codium fragile (Cf)* from Sfax *XI-Posidonea oceanica (Po)* from Chebba *XII-Cymodocea*

nodosa (Cn) from Sfax *XII-Halophila stipulacea (Hs)* from Sfax*;* 1. *Escherichia coli, 2. Escherichia coli DH5(alpha), 3. listeria monocytogéne, 4. Salmonella enterica, 5. Staphylococus aureus, 6. Micrococcus luteus, 7. Agrobacterium tumefaciens, 8. Pseudomonas aerigunosa.*

Table 2: Antifongical activity of macroalgae extracts

Algea	Extractant	Micro organisms		
		1	2	3
Phéophytea				
I	Hexane	-	-	-
	Ethyl Acetate	-	-	-
	Methanol	-	-	-
II	Hexane	-	-	-
	Ethyl Acetate	++	-	-
	Methanol	+	-	+
III	Hexane	-	-	-
	Ethyl Acetate	-	-	-
	Methanol	-	-	++
IV	Hexane	-	-	-
	Ethyl Acetate	-	+	-
	Methanol	+	-	-
V	Hexane	-	-	+
	Ethyl Acetate	-	-	-
	Methanol	-	-	+
Chlorophytea				
VI	Hexane	-	-	-
	Ethyl Acetate	-	-	-
	Methanol	-	-	-
VII	Hexane	-	-	-
	Ethyl Acetate	-	-	-
	Methanol	-	-	-
VIII	Hexane	-	-	-
	Ethyl Acetate	-	-	-
	Methanol	-	-	-
IX	Hexane	-	-	-
	Ethyl Acetate	-	-	-
	Methanol	-	-	-
X	Hexane	-	-	-
	Ethyl Acetate	-	-	-
	Methanol	-	-	-
Magnoliophytea				
XI	Hexane	-		-
	Ethyl Acetate	-	+	-
	Methanol	-	+	-
XII	Hexane	-	-	-
	Ethyl Acetate	-	-	-
	Methanol	+	-	++
XIII	Hexane	-	-	-
	Ethyl Acetate	-	-	-
	Methanol	-	-	-

I -*Dictyota dichotoma(Dd*) from Chebba II- *Cystoseira crinita(Cc)* from Chebba III- *Cystoseira barbata*(*Cb*) from Sfax IV- *Dyctéopteris menbranacea (Dm)* from Sfax V- *Sargussum vulgare (S* v) from Sfax VI- *Flabelia petiolata (Fp)* from Chebba VII -*Anadyoméne stelleta (As)* from Chebba *VIII-Halimeda tuna (Ht)* from Sfax *IX- Ulva rigida (Ur)* from Sfax *X-Codium fragile (Cf)* from Sfax *XI-Posidonea oceanica (Po)* from Chebba *XII-Cymodocea nodosa (Cn)* from Sfax *XII-Halophila stipulacea (Hs)* from Sfax*;* 1. *Candida tropicalis, 2. saccharomyces cerevisiae, 3. Aspergillus niger.*

Antifongical activity

Algal extracts are subjected to screening antifongical activity. The fungi studied are harmful to crops and even humain health. These results are shown in the following table 2

DISCUSSION

Antimicrobial activity

In this study, all algal extracts tested showed an important antibacterial activity. Our results showed that the methanol extracts caused better halo-zones then followed by the ethyl acetate than Hexane for all strains. The type of extraction solvent had a big influence on the antimicrobial properties of obtained extracts, suggesting that antimicrobial activity depends on both algal species and the efficiency of the extraction method. Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than hexane and ethyl acetate, whereas others report that chloroform extraction is better than methanol and benzene [22]. A good number of authors [23] have documented the antimicrobial potency of organic extracts and of some compounds isolated from marine algae of the genus of *Cystoseira*: such as terpenes [24, 25]. From our results it seems that the antibacterial actions of the organic extracts were more pronounced with brown algae are the best actives. The best activities were detected against *S. aureus* and *micrococus* for most algal species or *magnoliophytea* (*Dd, Cc, Dm, Sv, Cb, Cf, Fp, As, Ur, Cn, Po, Hs*) species and against S. aureus for Cc species. these results are correlated with those of [26]. who reported that The seaweed extracts are responsible for its activity against Gram (+) bacteria, especially *Bacillus subtilis* and *Staphylococcus aureus* and There are some reports regarding the antimicrobial activity of seaweeds from the Aegean Sea, Turkey [27–29] The previous reports showed that the algal extracts were generally more effective against Gram (+)

than Gram (–) bacteria, probably due to the more complex structure of the cell wall of Gram (–) bacteria. Antibacterial activity found by other authors in Mediterranean seaweeds was considerably larger; it ranged between 13% [30] and nearly 50% [31, 32] of the species tested.

The Gram-negative bacteria (*E. coli, Salmonella, and Listeria*) reveal themselves more resistant to the majority of algal extracts obtained with a few exceptions. There are some reports regarding the antimicrobial activity of seaweeds, [33] reported showed that Of the 14 brown algae tested, only nine species showed a positive activity against at on a Gram+ bacterium and Of the 13 green algae tested, the presence of a positive activity on a Gram+ bacterium was observed in seven species. Diameter of inhibition higher than 15 mm was observed in the chloroformic extract of *C. clathrata* against S. aureus ssp. aureus, and in the acetonic extract of *R. riparium* against *S. aureus ssp. aureus* and that of E. compressa toward S. aureus, and for the green algae tested, no activity was detected against the Gram-negative bacteria.

The most sensitive to the action of algal extracts bacterial strains are strains gram (+) essentially *Staphylococcus aureus* and *Micrococcus luteus* which correlates with previous studies [34, 22].

The difference in results for antimicrobial activity in these marine algae shows the involvement envirnnementals factors in the metabolism of algae and significant way affects the presence of such bioactive compound responsible for these activities. Finally we conclude that macroalgae from Tunisian coast are potential sources of bioactive compounds and should be investigated from natural antibiotics. This study has shown that the production antibactérial subtance by macroalgae is a regular occurrence among those found on the coast of Tunisia. Biochemical analyses are currently undertaker to determine the structure and nature of these compounds.

Antifongical activity

After the results of the biological screening, we can note that low activity towards the fungi and human pathogenic yeast. Only 5 algal extracts that showed antifungal activity against 13 extracts for antibacterial activity (considering only the upper zones of inhibition to 10 mm). These results show that the extracts are more active on bacteria on champignon. Similar results were obtained by [35], they found that 61% of algae screening conducted inhibit bacteria gram positive against 15% for gram-negative and 19% are active in fungies. Similar findings have been reported by other groups [36]. For after this study we also found that the *magnoliophytea* have an important antifungal than the antimicrobial activity, these results are in accordance with [37], reported a high level of antifungal activity, coupled with a low level of antibacterial activity, exhibited by the *magnoliophytea*. For the antifungal activity, our results showed that the brown algae, a diameter of inhibition ranging between 10 and 15 mm was observed in the ethyl acetate extract of *Cystoseira crinita* against *Candida tropicalis*, and in the methanolic extract of *Cystoseira barbata* toward *Aspergillus niger*. For the green algae studied, no activity was detected against fungi. From the species tested, those belonging to *Phaeophyceae* were the most active in comparison with *Chlorophyceae*; the same result was reported by [38] and [39]. The seaweeds belonging to the genus of *Cystoseira* possess a wide variety of compounds with different biological activities. These results are of interest as we are dealing with an extract and not a pure product; therefore the antimicrobial activity may be due to different compounds and related to the presence of bioactive metabolites.

CONCLUSION

Marine organisms have several active chemicals such as antioxidant and antimicrobial Compounds. Marine organisms are currently undergoing detailed investigations with the objective of isolating biologically active molecules along with the search for new compounds.

This study reports the presence of antibacterial and antifongical compounds in the algae and magnoliophytea collected from the coast of Chebba and Sfax.

We hope that the present results will provide a starting point of investigations aimed at exploiting new natural pharmaceutical substances present in the extracts of the species collected from the coast of Tunisia.

CONFLICT OF INTERESTS

Declared None

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