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EVALUATION OF THE ANTIBACTERIAL AND CYTOTOXICITY ACTIVITIES OF *CYSTOSEIRA GIBRALTARICA* BY BIOGUIDED FRACTIONATION

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Abstract

The purpose of this work is to study the antibacterial and cytotoxic activity by bioguided fractionation of extract of the seewead *Cystoseia Gibraltaria* screened from Moroccan coastlines. The observed extract was obtained using a soxhelt extractor with solvent characterized by increased polarities such as: hexane, ether, chloroform and methanol. The extracts are prepared from the seaweed *Cystoseira Gibraltarica* and each extract evaluated against the bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the yeast *Candida albicans* and the fungus *Aspergillus niger* for the follow-up of the atibaterial activity in an agar diffusion test, and for Cytotoxicity test with Artemia test (*Artemia salina*). Then, on a chromatographic column, using also solvents with increasing polarity such as petroleum ether, toluene, ethyl ether, dichloromethane, ethyl acetate, methanol, we received 6 fractions ester extract of *Cystoseira Gibraltarica*. The obtained extracts were tested in second time by antibacterial and cytotoxic activity. In short, it can be said that the tested seaweed *Cystoseira Gibraltarica* has great potential and can be regarded as a source of biologically active compounds.

Keywords: *Cystoseia Gibraltaria, antibacterial activity, cytotoxicity assay, extraction, fractionation.*

Introduction

At the word echelle, the marine environment has become a research focus for discovering different proprieties of natural products due to its biodiversity. Compared with the terrestrial environment, the marine environment is relatively undeveloped, the maintenance of the biodevirsity of the marine products is essential for the human being, for food, cosmetic or therapeutic purposes [1-2]. The marine products have a great economic importance for the country. The knowledge of the marine environment is relatively recent insofar as the development of in situ exploration of the underwater environment dates from the 1940's, while man has been interacting with the terrestrial environment and plants for about 3000 years [3]. Contrary to terrestrial organisms, there is no ethnopharmacological guide for the search of marine organisms of potential interest: they have, with a few known exceptions, a traditional use (medicine, poisons...) [4]. However, it should be recalled that the most powerful toxins known to date are of marine origin (palytoxin, tetrodotoxin, saxitoxin, all molecules of the Biotox plan - biological and chemical terrorism), most probably because they must remain effective despite their great dilution in the environment [5]. The brown algae have a great potential and could be the subject of several pharmaceutical and biological applications and have been shown capable of providing biologically active molecules. [6-8]

In this work, we are interested in the biological activity study of an algal species collected from the area of Casablanca Morocco *Cystoseia Gibraltaria*, antibacterial activity and the cytotoxicity assay. In order to expose pharmacological potential of these algal species.

Materials and Methods

Extract preparation

The observed extracts were prepared by extracting *Cystoseira Gibraltarica* by soxhelt extraction method using solvents of increasing polarity. Before the preparation of this extracts we took the *Cystoseira Gibraltarica* seaweed fresh in the period of low tide and it is rinsed with fresh water, then we dehydrated in two phases which are: the first phase was a drying in the open air, protected from the light at room temperature during a day. And the second phase was a drying in the oven at a temperature of 60°C Dry for 3 days.

After sohxelt extraction with the 4 solvents hexane, ether, chloroform and methanol, we obtained the extracts object of the tests antibacterial and cytotoxicity activities [9].

Antibacterial activity

The prepared extracts of *Cystoseira Gibraltarica* were tested against five microorganisms, bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, yeast: *Candida albicans* and fungi: *Aspergillus niger*, using The good diffusion agar method described by Ainane et al. (2020) [10] it is the most commonly used technique to observe bacterial growth ,and then the agar well diffusion method to assess antibacterial activities, The extracts were dissolved in 5% dimethylsulfoxide (DMSO). Ten microliters of crude extract (2 mg/mL) was loaded into a 6 mm diameter well. (6 mm diameter). During night hours and under aerobic conditions we incubated the selected microorganisms on supplemented MH agar. The bacterial suspensions were adjusted to McFarland standard No. 0.5 and spread on plates of supplemented MH agar at temperature 37 °C for one day in aerobic condition, the seeded plates were incubated. The negative control was bacterial culture with 1% DMSO in addition to the positive control was tetracycline and streptomycin. The result was based on the averages of inhibition area which was measured and recorded for analysis.

Cytotoxicity test

The cytotoxicity test is described by Yadav al. (2020) [11] ,this test allows us to evaluate the viability of the cells within the prepared solutions, by extension it allows to determine the cell mortality of the brine shrimp larvae: *Artemia salina* induced by the prepared extracts, it also allows to specify the median lethal dose LD50 which corresponds to the amount that produces the death of these larvae compared to other control products. The fragments that are dissolved in 2% DMSO at concentrations of 20, 40, 60 and 100 µg/ml were placed a certain volume of the prepared solution was immediately placed in the tube with the

Artemia larvae in a room temperature chamber, the tubes were placed and left for 24 hours before reading the results, and this was done by counting under a dissecting microscope, and using formula below to specify the percentage of mortality, If there are dead larvae in the test tube.

% M= (NLP/ NLT)×100

With:

- % M: percentage mortality.
- NLP: number of dead larvae in the presence of the product tester.
- NLT: Number of dead larvae in the Presence of Witness (solvent).

Fractionation and biological screening

The bioguided fractionation was carried out on a chromatographic column of the ethyl ether extract of *Cystoseira Gibraltarica* by solvents of increasing polarity, Petroleum ether, Toluene, Ethyl ether, Dichloromethane, Ethyl acetate, and Methanol. The objective of this step is to carry out the antibacterial and cytotoxicity activities tests of the prepared fractions [12].

Results and discussion

In the first stage of the tests of this study, using extraction by soxhlet with solvents of increasing polarity, extracts of *Cystoseira Gibraltarica* obtained. the table n°1 shows the result of this test by determining in function of the quantity of the initial dry seaweed their color and their output.

Secondly, on the extracts obtained, we carried out preliminary object tests to determine the bacterial activity and cytotoxicity. Table 2 shows the antibacterial activity of the extracts against the bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the yeast *Candida albicans* and the fungi *Aspergillus niger* by using benzoic acid as a control and by depositing the same concentration as the extract of alga control. Then the results of the cytotoxicity test are presented in Table 3 which represents the extracts with the value of the lethal dose of 50 LD50.

The results obtained from the bacterial activity and cytotoxicity test reveals that the extract hexane does not show any inhibition against *P. aeruginosa* and *A. niger*, as well as the extract methanol does not show any inhibition against *P. aeruginosa*, *C. albicans* and *A. niger,* that the ethyl ether extract presents an antibacterial activity with an average diameter of 10 to 15 mm and a diameter of more than 15 mm against *E.coli*.

Also, in the cytotoxicity test, ethyl ether extract give a lethal dose value of LD50 = 57.5 µg/mL and a maximum value of LD50 = 70.8 µg/mL given by Chloroform extract.

As shown in Figure 1, we performed the fractionation of the ether extract on silica gel with a solvent gradient to obtain six fractions of petroleum ether, toluene, ethyl ether, dichloromethane, ethyl acetate, and methanol, mentioning the yields and colors of these fractions in Table 4.

Then, we proceeded to the test of antibacterial activity against the bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the yeast *Candida albicans* and the fungus *Aspergillus niger*, also the cytotoxicity test on the shrimp test. The tables 5 and 6 illustrate the results obtained, so the tested fraction F4 on average present an inhibition between 10 and 15 mm and a value of LD50= 44.63 µg/mL, which approves that thIS fraction present significant antibacterial and cytotoxic activities.

Conclusion

Cystoseira Gibraltarica shows antibacterial and cytotoxic activity according to the results obtained. this biomass can be the object of an added value in various pharmaceutical and biological applications. Further studies are needed to characterize and identify the active substances present in these species of algae.

References

1. Ashraf, S. A., Adnan, M., Patel, M., Siddiqui, A. J., Sachidanandan, M., Snoussi, M., & Hadi, S. (2020). Fish-based bioactives as potent nutraceuticals: Exploring the therapeutic perspective of sustainable food from the sea. Marine drugs, 18(5), 265, 1-20.
2. Alves, A., Sousa, E., Kijjoa, A., & Pinto, M. (2020). Marine-derived compounds with potential use as cosmeceuticals and nutricosmetics. Molecules, 25(11), 2536, 1-38.

3. Abdoul-Latif, F. M., Oumaskour, K., Boujaber, N., Ainane, A., Mohamed, J., & Ainane, T. (2021). Formulations of a cosmetic product for hair care based on extract of the microalga Isochrysis galbana: in vivo and in vitro activities. Journal of Analytical Sciences and Applied Biotechnology, 3(1), 15-19.
4. Ainane, T., Abourriche, A., Kabbaj, M., Elkouali, M., Bennamara, A., Charrouf, M., Lemrani, M. (2014). Biological activities of extracts from seaweed Cystoseira tamariscifolia: Antibacterial activity, antileishmanial activity and cytotoxicity. J. Chem. Pharm. Res, 6, 607-611.
5. Chia, M. A., Kramer, B. J., Jankowiak, J. G., Bittencourt-Oliveira, M. D. C., & Gobler, C. J. (2019). The individual and combined effects of the cyanotoxins, anatoxin-a and microcystin-LR, on the growth, toxin production, and nitrogen fixation of prokaryotic and eukaryotic algae. Toxins, 11(1), 43, 1-17.
6. Kumar, B. R., Mathimani, T., Sudhakar, M. P., Rajendran, K., Nizami, A. S., Brindhadevi, K., & Pugazhendhi, A. (2021). A state of the art review on the cultivation of algae for energy and other valuable products: application, challenges, and opportunities. Renewable and Sustainable Energy Reviews, 138, 110649.
7. Ainane, T., Abourriche, A., Bennamara, A., Talbi, M., & Lemrani, M. (2018). Activité anti-leishmanienne des extraits d'une algue brune Bifurcaria bifurcata de la côte atlantique du Maroc. Phytotherapie, 16(2), 68-73.
8. Bouhadi, M., Ainane, A., M'hammed, E. L., Talbi, M., Cherifi, O., El Yaacoubi, A., & Ainane, T. (2019). Role of the macroalgae Corallina officinalis in alleviating the toxicity of hexavalent chromium on Vicia faba L. Journal of Analytical Sciences and Applied Biotechnology, 1(2), 60-64.
9. Venturi, F., Sanmartin, C., Taglieri, I., Andrich, G., & Zinnai, A. (2017). A simplified method to estimate Sc-CO2 extraction of bioactive compounds from different matrices: Chili pepper vs. tomato by-products. Applied Sciences, 7(4), 361, 1-12.
10. Ainane, A., Abdoul-Latif, F. M., Abdoul-Latif, T. M., & Ainane, T. (2020). Evaluation of biological activities of two essential oils as a safe environmental bioinsecticides: case of Eucalyptus globulus and Rosmarinus officinalis. Przegląd Naukowy Inżynieria i Kształtowanie Środowiska, 29, 544-556.
11. Yadav, A. R., & Mohite, S. K. (2020). Toxicological Evaluation of Psidium guajava Leaf Extracts using Brine Shrimp (Artemia salina L.) Model. Research Journal of Pharmaceutical Dosage Forms and Technology, 12(4), 258-260.
12. Ainane, T., & Abourriche, A. (2014) Brown Seaweed Bifurcaria bifurcata: Bioguided Fractionation of Extracts by Antibacterial Activity and Cytotoxicity Test. Biosciences Biotechnology Research Asia, 11(3), 1081-1085.

Table 1: The different extracts of *Cystoseira Gibraltarica* with yield and color.

Extract	Color	Yield (%)
Hexane	Black - green	7.27
Ethyl ether	Green – orange	1.42
Chloroform	Dark green	1.33
Methanol	Brown - Black	9.06
Marc (*)	Brown - black	80.25

(*) (After evaporation)

Table 2: Antibacterial activity of various extracts of *Cystoseira Gibraltarica.*

Bacteria	*E.coli*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. niger*
Hexane	+	-	+	+	-
Ethyl ether	+++	++	++	++	+
Chloroform	+++	+	++	++	++
Methanol	+	-	+	-	-
benzoic acid	+++	+++	+++	+++	+++

Key: -: no inhibition, +: less than 10mm diameter inhibition, + + inhibition diameter between 10 and 15mm, + + + greater than 15mm diameter inhibition.

Table 3: Values of LD_{50} test Brine shrimp the extracts of *Cystoseira Gibraltarica.*

Extract or Product	LD_{50} (µg/mL)
Hexane	n.d
Ethyl ether	57.5
Chloroform	70.8
Methanol	>>100
Pyridine	3.4

n.d.: not detected

Figure 1. Fractionation of the ethyl ether extract of *Cystoseira Gibraltarica* by different solvents with increasing polarity on a chromatographic column.

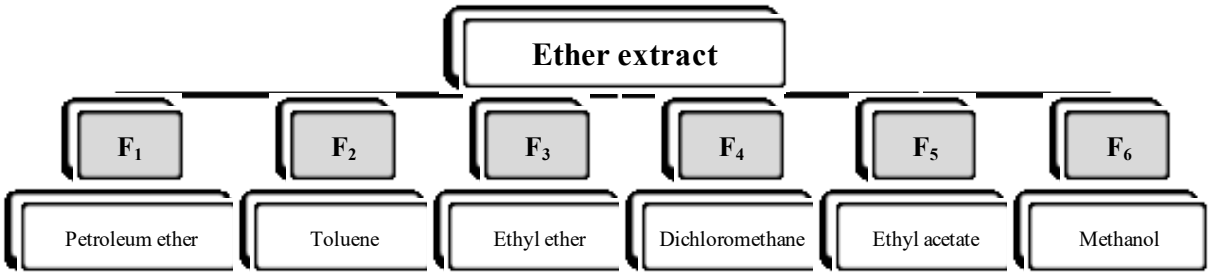

Table 4. Yields and colors of the various fractions from the Ethyl ether extract of the *Cystoseira Gibraltarica.*

Fraction	Color	Yield (%)
F1	Yellow - orange	1.89
F2	Green	1.41
F3	Yellow	1.42
F4	Black - Green	34.02
F5	Brown	15.87
F6	Green - Grey	11.63

Table 5: Antibacterial activity for different fractions from the ether extract of *Cystoseira Gibraltarica.*

Bacteria	*E.coli*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. niger*
F1	++	+	-	-	-
F2	+	+	-	+	-
F3	++	+	+	+	++
F4	++	+	++	++	++
F5	+++	++	++	+	+
F6	+	-	-	+	-

Key: -: no inhibition, +: less than 10mm diameter inhibition, + + inhibition diameter between 10 and 15mm, + + + greater than 15mm diameter inhibition.

Table 6. Values of LD_{50} test Brine shrimp for different fractions from the ether extract of *Cystoseira Gibraltarica.*

Fraction	LD_{50} (µg/mL)
F1	84.34
F2	n.d
F3	63.49
F4	44.63
F5	65.93
F6	n.d

n.d.: not detected