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Brown Seaweed *Bifurcaria bifurcata*: Bioguided Fractionation of Extracts by Antibacterial Activity and Cytotoxicity Test

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In this present work, we screened for the biological activities and fractionation of extracts of the seaweed *Bifurcaria bifurcata* the Moroccan coast (Casablanca). Selecting *Bifurcaria bifurcata* was based on the results of biological activity on the extracts obtained after Soxhlet extraction with solvents of increasing polarity such as hexane, ether, chloroform and water. We followed two biological activities: (1st) the antibacterial activity in the agar diffusion test against *Klebsiella pneumoniae* and *Enterobacter cloacae*, and (2nd) cytotoxicity assay by brine shrimp test (*Artemia salina*). After these preliminary tests, we selected the ether extract of this seaweed for fractionation, where we collected 11 fractions per gradient of Petroleum ether (PE) and Ethyl acetate (EA). The collected fractions from the ether extract also tested for antibacterial activity and cytotoxicity test. The overall results show that the fractions from ether extract of *Bifurcaria bifurcata* exhibit remarkable biological activity.

Key words: *Bifurcaria bifurcata*, antibacterial activity, cytotoxicity assay, extraction, fractionation.

Within the past 40 years various research groups have shown an increasing interest in marine natural products chemistry. This area is particularly attractive not only because a small percentage of the over 200 000 species supposed to be present in the seas and oceans (in contrast with terrestrial organisms) have been chemically investigated, but also because of the fascinating structural peculiarity and unusual molecular arrangements of many marine compounds[1-2].

Over 10000 new structures have been identified between 1974 and 2014, confirming considerable development in marine chemistry. The reason for this uninterrupted interest can undoubtedly be ascribed to an awareness of the great contribution that chemical data can give to exploring important pharmacological properties[3]. It is appropriate to point out that many articles have been published concerning extracts of marine organisms and seaweeds that dealt with spectral-structure correlations as well as their bioactivity[4].

On the other hand, brown seaweeds are rich in fractions and/or compounds that could potentially be exploited as various biological applications. Over the years, these compounds and the fractions with potential pharmacological, nutraceutical, functional food and cosmeceutical properties have been isolated from brown seaweeds[5].

Bifurcaria bifurcata is a brown alga belonging to the *Cystoseiraceae* family. This species, it is a widespread species on the Moroccan Atlantic coast between Tangier and Dakhla[6]. Several biological studies have been carried on

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extracts of this species there is work done on the antioxidant activities[7], antimicrobial activities[7], antiprotozoal activities[8] and other biological valuations value[9-11].

In this study was based on the preliminary results on the biological activities of the extracts *Bifurcaria bifurcata* such as: antibacterial activity against *Klebsiella pneumoniae* and *Enterobacter cloacae*, and the cytotoxicity assay by testing brine shrimp test against *Artemia salina*. The ethereal extract was presented a remarkable activity, then fractionation this extract is performed by a gradient from petroleum ether to ethyl acetate. Then, we tested fractions obtained by the previous two biological activities.

MATERIALS AND METHODS

Extract preparation

After harvesting the seaweed *Bifurcaria bifurcata* in the south of Casablanca (Morocco) in the period of low tide, it is washed with water and dried for one day at room temperature and arbitrary of light, then it is dried in an oven at 60 ° C for 3 days.

The extracts H, E, C and A of seaweed *Bifurcaria bifurcata* obtained from the Soxhlet extraction successively with solvents of increasing polarity: hexane, ether, chloroform and water.

Antibacterial activity

The method used is the well diffusion agar described by N. Debbache *et al.* (2014)[12]**.** This method can quickly observe effects of a substance by bacterial growth. Screening for antibacterial activity of the extracts was determined by agar well diffusion method. The extracts was dissolved in dimethyl sulfoxide (DMSO) 5%. Ten microliter of crude extract (2 mg/mL) was loaded onto well (diameter 6 mm). Fresh colonies of *Streptococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* on supplemented MH agar were inoculated in supplemented MH broth and incubated overnight under aerobic condition. The bacterial suspensions were adjusted to McFarland standard No. 0.5 and spreaded onto supplemented MH agar plates. The seeded plates and incubated at 37 °C for 24 h under aerobic condition. The diameters of the inhibition zones were measured and the mean was recorded. Experiments were done in triplicate. Bacterial culture with 1% DMSO was used as negative control. In addition, Tetracyclin and Streptomycin used a positive control.

Cytotoxicity test

The cytotoxicity test Brine Shrimp used in this work is described by A. Shaukat *et al*. (2014)[13]. Briefly, this test determines the toxic activity of the extracts tested their effects on the larvae of brine shrimp: *Artemia salina*. It also allows for determining the median lethal dose LD_{50} which can determine the power of toxicity compared to other products of references. Test samples are dissolved in 2% DMSO, with concentrations of 20, 40, 60 and 100 µg / ml and, immediately determined volumes of the prepared solution are added to tubes containing the larvae of Artemia salina. The tubes are placed in a chamber at room temperature and the results were read after 24 hours is by counting under a dissecting microscope. If the lamp contains dead larvae, the percentage mortality is corrected using the following formula:

$$\%M = \frac{NLP}{NLT} \times 100$$

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

Fractionation the extracts of the brown seaweed *Bifurcaria bifurcata* was done on an open silica gel column with gradient solvent of petroleum ether / ethyl acetate. Fractions obtained were tested for the previous two biological activities.

RESULTS AND DISCUSSION

After harvesting and drying seaweed *Bifurcaria bifurcata* four extracts H, E, C and A are obtained from successive Soxhlet extractions with solvents of increasing polarity: hexane, ether, chloroform and water. Once the extracts were obtained, it was determined their colors and returns relative to the initial amount of dry seaweed. Data for samples obtained are given in the table 1.

First, after obtaining extracts of the seaweed *Bifurcaria bifurcata*, we started by

preliminary tests on extracts H, E, C and A, where we have made the antibacterial activity and the test cytotoxicity. The test results of the antibacterial activity of the extracts H, E, C and A seaweed *Bifurcaria bifurcata* are summarized in Table 2, and tetracycline and streptomycin which was filed at the same concentration of the extracts of seaweed as an antibiotic control. On the other hand, the results of cytotoxicity test (Brine Shrimp) are shown in Table 3, the above results are the values of samples of each lethal dose 50 LD_{50}, thus the value of the positive control Strychnine sulphate.

The results obtained during these activities showed that the ether extract of the seaweed *Bifurcaria bifurcata* was an important activity. Hence, for the antibacterial activity, this extract was presented inhibition diameter of over 15 cm against two bacteria, thus, he gave in cytotoxicity test, a value of the lethal dose of LD_{50} = 40.46 µg/mL

This extract, which has a yield of 0.92% was fractionated on open silica gel column with gradient solvent of Petroleum ether / Ethyl acetate (PE/EA), then 11 fractions were recovered. Figure 1 shows the fractions obtained during fractionation of the ether extract, and Table 4 gives the yields and colors of these fractions.

Table 1. The different extracts of *Bifurcaria bifurcata* with yield and color

Extract	Color	Yield (%)
H	Dark green	4.04
E	Green – yellow	0.92
C	Green	1.43
A	Brown	8.66
Marc (*)	Brown	84.4

*After evaporation

Table 2. Antibacterial activity of various extracts of seaweede *Bifurcaria bifurcata*

Bacteria	*E. cloacae*	*K. pneumoni*
H	++	++
E	+++	+++
C	++	++
A	+	+
Tetracycline	+++	+++
Streptomycin	+++	+++

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 *Bifurcaria bifurcata*

Extract or Product	LD_{50} (µg/mL)
H	21.8
E	40.46
C	37.05
M	>>200
Strychnine sulphate	151

n.d.: not detected

Table 4. Yields and colors of the various fractions from the ether extract of the brown seaweed *Bifurcaria bifurcata*

Fraction	Color	Yield (%)
F_1	Yellow - orange	1.89
F_2	Green	1.41
F_3	Black - Grey	1.42
F_4	Black - Green	34.02
F_5	Yellow	15.87
F_6	Green	11.63
F_7	Brown	5.02
F_8	Brown	4.20
F_9	Green - Grey	2.41
F_{10}	Green	1.46
F_{11}	Dark green	10.70

Table 5. Antibacterial activity against *Klebsiella pneumoniae* and *Enterobacter cloacae* for different fractions from the ether extract of *Bifurcaria bifurcata*

Fraction	*K. pneumoni*	*E. cloacae*
F_1	++	+
F_2	-	-
F_3	+	-
F_4	++	++
F_5	+	++
F_6	+	+
F_7	-	-
F_8	++	+
F_9	+	-
F_{10}	++	-
F_{11}	+	+

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 *Bifurcaria bifurcata*

Fraction	LD_{50} (µg/mL)
F_1	12.53
F_2	n.d
F_3	23.14
F_4	44.46
F_5	61.49
F_6	38.40
F_7	n.d
F_8	19.28
F_9	15.20
F_{10}	10.42
F_{11}	31.06

n.d.: not detected

The various fractions from the ether extract of the brown seaweed *Bifurcaria bifurcata* tested by the antibacterial activity against of *Klebsiella pneumoniae* and *Enterobacter cloacae*, and the cytotoxicity test Brine Shrimp. The results of these activities are mentioned respectively in Table 5 and table 6. While the results show that most of the fractions exhibit remarkable activities (For two activities: antibacterial activity and cytotoxicity test), where the fraction F4, F5 and F6 had important activities and fractions F2 and F7 show no activity.

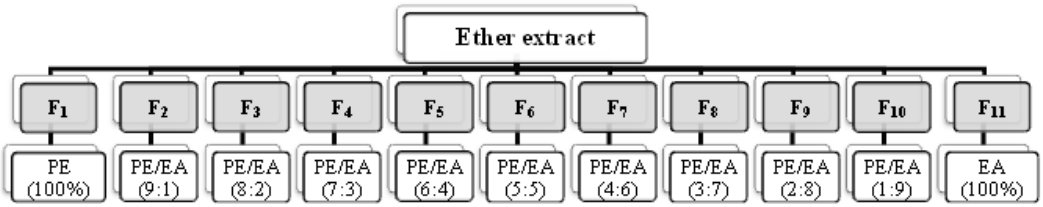

Fig. 1. Fractionation ether extract of the brown seaweed *Bifurcaria bifurcata*

CONCLUSION

Bioguided fractionation of extracts of the brown seaweed *Bifurcaria bifurcata* through antibacterial activities and cytotoxicity activity has positive results. These results obtained in this work showed that the seaweed *Bifurcaria bifurcata* has great potential and could be the subject of several pharmaceutical and biological applications and this by the chemical study of important fractions of seaweed usually ethereal fraction, which have been shown capable of providing biologically active compounds.

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