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Biological screening and bioguided fractionation of extracts of the brown alga *Cystoseira tamariscifolia* the Moroccan coast: Antibacterial activity and cytotoxicity test

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ABSTRACT

In this study, we screened for the fractionation of extracts of the brown algae *Cystoseira tamariscifolia* the Moroccan coast. Selecting this alga 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: (a) the antibacterial activity in the agar diffusion test against *Klebsiella pneumoniae* and *Enterobacter cloacae*, and (b) cytotoxicity assay to brine shrimp (*Artemia salina*). After these preliminary tests, we selected the ether extract of this alga for fractionation, where we collected 11 fractions per gradient of petroleum ether / ethyl acetate. 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 *Cystoseira tamariscifolia* exhibit remarkable biological activity.

KEYWORDS

Cystoseira tamariscifolia;
Biological activity;
Extraction;
Fractionation.

INTRODUCTION

Marine brown algae algae are widespread throughout the world, this means adaptability through their reproduction and response to various environmental conditions. This assumes that the brown algae contains chemicals defense against the many dangers they face (mobile predators and invading microorganisms)[1-4]. Then *Cystoseiracee algae*, are a genus of brown algae marine belong to the class Phaeophyceae, order of Fucale, they comprise 50 to 55 species[5], *Cystoseira tamariscifolia* which is also called *Cystoseira erricoides* is a robust plant 10 to 50 cm long, olive green color, producing a rough feel to the touch, it is a widespread species on the Moroccan Atlantic coast between Tangier and Eljadida and Mediterranean coasts. Several biological studies have been carried on extracts of this species there is work done on the antibacterial activities[6], antifungal activities[7], antileishmanial activities[8] and other biological valuations value[9-12].

Our study was conducted in order to continue the previous work done on the biological activities of the extracts of the species *Cystoseira tamariscifolia* for a valuation of fractions obtained from the screening bioguided on the active extracts which has biological activity important, where we tested two biological ac-

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tivities such as: antibacterial activity against *Klebsiella pneumoniae* and *Enterobacter cloacae*, and the cytotoxicity assay by testing brine shrimp test against (*Artemia salina*).

EXPERIMENTAL

Harvesting algae and extract preparation

After harvesting the seaweed *Cystoseira tamariscifolia* 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 three days.

The extracts of algae *Cystoseira tamariscifolia* obtained from the Soxhlet extraction successively with solvents of increasing polarity: hexane, ether, chloroform and water.

Antibacterial activity

The method used, method or well diffusion agar described by C. Perez et al. (1990) [13]. This method can quickly observe effects of a substance by bacterial growth. Screening for antibacterial activity of the extracts of *Cytoseira tamariscifolia* was determined by agar well diffusion method. The extracts was dissolved in dimethyl sulfoxide (DMSO) 5%. Ten microliter of crude extract (250 mg/mL) was loaded onto well (diameter 6 mm). Fresh colonies of *Enterobacter cloacae* and *Klebsiella pneumoniae* (University Teaching Hospital Ibn Rushd) 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 used as a positive control.

Cytotoxicity test

To achieve this toxicological study to look for toxic compounds from the extracts, we relied on the BS test "Brine Shrimp" developed by P.i Vanhaecke in 1981 [14]. This test is used to determine the toxic activity of the products tested their effects on larval saltwater shrimp: *Artemia salina*. It also allows the determination of the concentration which kills 50% of *Artemia nauplii* in 24 hours under standardized conditions. This concentration is known as the lethal dose LD50 which can determine the power of toxicity compared to other products of references. The samples to be tested are dissolved in 2% DMSO. Determined volumes of the prepared solution were added to Petri dishes containing larvae of *Artemia salina*. The boxes 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 alga *Cystoseira tamariscifolia* 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 *Cystoseira tamariscifolia* four extracts H, E, C and A are obtained from successive Soxhlet extractions with solvents of increasing polarity: hexane, ether, chloroform and

TABLE 1: The different extracts of *Cystoseira tamariscifolia* with yield and color.

Extract	Color	Yield (%)
H	Dark green	0.36
E	Green – yellow	0.22
C	Green – yellow	0.25
A	Brown	5.67
Marc (*)	Brown	93

(*) (After evaporation)

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.

TABLE 2 : Antibacterial activity of various extracts of algae *Cystoseira tamariscifolia*.

Bacteria	*E. cloacae*	*K. pneumoni*
H	+	++
E	++	++
C	++	+
A	+	+
Tetracyclin	+++	+++

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 tamariscifolia*.

Extract or Product	DL50 (µg/mL)
H	n.d
E	43.38
C	43.26
M	>>200
Strychnine sulphate	151

n.d. : not detected

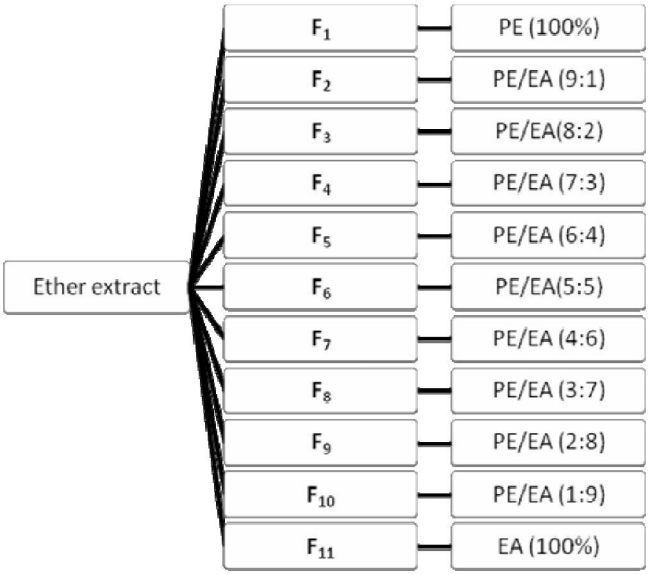

Figure 1 : Fractionation ether extract of the brown alga *Cystoseira tamariscifolia*.

First, after obtaining extracts of the alga *Cystoseira tamariscifolia*, we started by preliminary tests on extracts H, E, C and A, where we have made the anti-

TABLE 4 : Yields and colors of the various fractions from the ether extract of the brown alga *Cystoseira tamariscifolia*.

Fraction	Couleur	Yield (%)
F_1	Yellow – orange	1.74
F_2	Green – Grey	1.30
F_3	Black – Grey	1.32
F_4	Yellow	11.3
F_5	Black – green	14.6
F_6	Green	10.7
F_7	Brown	4.62
F_8	Brown	3.86
F_9	Green – Grey	2.22
F_{10}	Green	3.34
F_{11}	dark green	9.84

TABLE 5 : Antibacterial activity against *Klebsiella pneumoniae* and *Enterobacter cloacae* for different fractions from the ether extract of *Cystoseira tamariscifolia*.

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.

bacterial activity and the test cytotoxicity. The test results of the antibacterial activity of the extracts H, E, C and A seaweed *Cystoseira tamariscifolia* are summarized in TABLE 2, and tetracyclin which was filed at the same concentration of the extracts of algae 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 LD50, thus the value of the positive control Strychnine sulphate.

The results obtained during these activities showed that the ether extract of the alga *Cystoseira tamariscifolia* was an important activity. This extract,

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which has a yield of 0.22% 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.

The various fractions from the ether extract of the brown alga *Cystoseira tamariscifolia* 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, where the fraction F4, F5 and F8 had important activities and fractions F2, F3 and F7 show no activity.

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

Fraction	DL50 (µg/mL)
F_1	13.23
F_2	n.d
F_3	n.d
F_4	24.56
F_5	53.71
F_6	8.40
F_7	n.d
F_8	39.17
F_9	10.25
F_{10}	n.d
F_{11}	23.39

n.d.: not detected

CONCLUSION

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

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