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Research Article

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Biological activities of extracts from seaweed *Cystoseira tamariscifolia*: Antibacterial activity, antileishmanial activity and cytotoxicity

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ABSTRACT

The valuation of algal biomass is considered among the most interesting in the use of the marine international programs. On the other hand, Morocco, with its double Atlantic and Mediterranean coast, is a country deeply influenced by the sea, which implies the existence of thousands of marine species. This work involves the use of extracts of brown seaweed Cystoseira tamariscifolia in various biological activities such as: antibacterial activity, antileishmanial activity and cytotoxicity activity. Briefly, samples are obtained from the Soxhlet extraction successively using solvents of increasing polarity (hexan, ether and chloroform) , and they represent 0.22 % to about 5.67 % by weight of raw seaweed. The antibacterial activity was carried out by the method of diffusion in agar Legard Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae and Staphylococcus aureus. The antileshmanien activity was carried out in vitro by MTT colorimetric method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide) by Leishmania infantum, and cytotoxicity was achieved a larvae brine shrimp : Artemia salina (Brine shrimp). The overall results show that the products extracted from seaweed Cystoseira tamariscifolia exhibit remarkable biological activity.

INTRODUCTION

Of all time, men have used natural products for their biological properties, for therapeutic purposes, cosmetics ... For a long time, religious practices and medications were closely related , different treatments bases terrestrial, marine plants or organic extracts were thus associated with predominant deities . [1]

Extracts and biologically active molecules of natural origin now occupy a prominent place in our society because they are the basis of many active ingredients used in pharmacy. [2]

According to estimates, the total number of marine species is about half of the total biodiversity of the planet. Only 1% of identified marine species have been studied to date. The study of marine products began in the late 1960s and led to the isolation of approximately 10,000 substances. Many isolated extracts have pharmacological potential, much higher than that of natural products from plants and terrestrial organisms. [2]

Despite the large number of discoveries marine molecules, very few of them are on the market or expected to be commercialized soon. Nevertheless, they represent a source of inspiration for the discovery of new drugs. [2]

In the other hand, *Cystoseira tamarisifolia* is a macro algae presents an interesting biomass on the Moroccan coastal, Previous chemical studies on this alga showed the presence of méroditerpéniques majority molecules. Mention: the bifurcarénone, méditerranéols the A, B, C, D, the cystoseirols A, B and C, the strictakétal the baléarone, and the néobaléarone épinéobaléarone and méthoxybifurcarenone. [3-6]

The objective of this part is to try to exploit marine algae *Cystoseira tamariscifolia* as sources of pharmaceutical molecules by studying the pharmacological potential of their extracts, as a range of biological activities such as: antibacterial activities, antileishmanial activity and cytotoxicity.

EXPERIMENTAL SECTION

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) [7]. 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 Escherichia coli (ATCC 25922), Enterobacter cloacae (University Teaching Hospital Ibn Rushd), Klebsiella pneumoniae (University Teaching Hospital Ibn Rushd) and Staphylococcus aureus (ATCC 25923) 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.

Antileishmanial activity

The MTT method used is that described by A. Dutta (2005) [8] This is a colorimetric technique, which allows to determine the antileishmanial activity of promastigote forms of Leishmania . The principle of the test is based on the conversion of a tetrazolium salt MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan by enzymatic reduction .

The antileishmanial evaluations were performed on cultures of Leishmania infantum promastigotes. Promastigotes were maintained at 25 ° C by subculturing every five days using the RPMI 1640 phenol red-free , buffered with 25 mM $NaHCO_3$ (pH = 7.2) and supplemented with 20 % fetal bovine serum (FBS) . The parasites were incubated in culture flasks with a starting concentration of 5×10^5 parasites per ml .

The parasites were counted on Thoma cell and shared on a 96 well ELISA plate at a concentration of 2×10^5 parasites / well in 90 µl RPMI medium. The test products are dissolved in DMSO 1 % (to final concentrations of 20, 30, 40 and 50 µg/mL) and the solvent control (100 µL of parasitic cultivation + l0µL DMSO) are then added in triplicate in the wells for an additional 10 µl. After 72 hours incubation at 25 °C was added a 10 volume µl of MTT (5 mg/mL) and the plates are returned to the incubator. After 1 hour incubation at room temperature and 3 hours at 37 °C the developed Violete staining, the plate was centrifuged at 2000 rpm for 5 min and was added to a volume of 200 µl DMSO. The absorbance each well is measured at 492 nm on an ELISA spectrophotometer.

The determination of the concentration value of 50% inhibition IC_{50} is determined by linear interpolation of the curves showing percent viability based on the logarithm of concentration tested. It is noted that the percentage of viability is given by the relationship:

$$\% \text{ Viability} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

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. [9]

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 LD_{50} 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).

RESULTS AND DISCUSSION

Extraction
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 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 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)

Antibacterial activity
The test results of the antibacterial activity of the extracts H, E, C and A seaweed Cystoseira tamariscifolia are summarized in Table 2, and Tetracycline which was filed at the same concentration of the extracts of algae as an antibiotic control. The results of this work showed that all the extracts produced interesting zones of inhibition for both strains *Enterobacter cloacae* and *Klebsiella pneumoniae*. We also note that all extracts are active against Escherichia coli extract except H which did not show a remarkable activity. As wholes that the extracts have no activity against *Staphylococcus aureus*.

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

Bacteria	*E. coli*	*E. cloacae*	*K. pneumoni*	*S. aureus*
H	-	+	++	-
E	+	++	+	-
C	+	++	++	-
A	+	+	+	-
Tetracyclin	+++	+++	+++	+++

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

Antileishmanial activity
The product H, E, A and C were tested at concentrations of 20, 30, 40 and 50 µg/mL. The values of percentage viability of *Leishmania* in function of the concentration are given in Table 3.

Table 3: Results of the antileishmanial activity

Extract	Concentration	ABS_{sample}	$ABS_{control}$	Viability (%)
H	20 µg/mL	n.d	0.579	-
	30 µg/mL	n.d	0.579	-
	40 µg/mL	n.d	0.579	-
	50 µg/mL	n.d	0.579	-
E	20 µg/mL	0.552	0.579	4.690
	30 µg/mL	0.540	0.579	6.762
	40 µg/mL	0.528	0.579	8.8345
	50 µg/mL	0.512	0.579	11.597
C	20 µg/mL	0.575	0.579	0.719
	30 µg/mL	0.571	0.579	1.410
	40 µg/mL	0.568	0.579	1.928
	50 µg/mL	0.552	0.579	4.690
A	20 µg/mL	n.d	0.579	-
	30 µg/mL	n.d	0.579	-
	40 µg/mL	n.d	0.579	-
	50 µg/mL	n.d	0.579	-

n.d.: not detected

From Table 3, it is deduced that the percent viability increases with concentration. It is noted that the samples A and H have no antileishmanial activity, against the C and E samples exhibits activity. The concentration values of 50% inhibition IC_{50} obtained from the linear expressions of sustainability based on the logarithm of the two extracts tested for concentration greater than 100 µg/mL (Table 4).

Table 4: IC_{50} values of the antileishmanial activity for extracts *Cystoseira tamariscifolia*

Extract	IC_{50} (µg/mL)
H	n.d
E	>100
C	>100
M	n.d

Cytotoxicity test

The extracts were tested at 20, 40, 60 and 100 µg/mL by Brine Shrimp test described previously. The values of the percentages of mortality of larvae in the concentration function are given in Table 5.

Table 5: Results of cytotoxicity test Brine Shrimp

Extract	Concentration	NLT	NLP	% M
H	20 µg/mL	10	0	0
	40 µg/mL	10	0	0
	60 µg/mL	10	0	0
	100 µg/mL	10	0	0
E	20 µg/mL	10	2	20
	40 µg/mL	10	5	50
	60 µg/mL	10	7	70
	100 µg/mL	10	7	70
C	20 µg/mL	10	3	30
	40 µg/mL	10	4	40
	60 µg/mL	10	6	60
	100 µg/mL	10	8	80
M	20 µg/mL	10	1	10
	40 µg/mL	10	2	20
	60 µg/mL	10	3	30
	100 µg/mL	10	3	30

From this table, we deduce that the percentage of larval mortality increases as a function of concentration. This is well illustrated by the curves shown in Figure 1, which show the percentages of mortality according to the logarithm of the concentrations. LD50 values of the various extracts obtained by different linear expressions are displayed in Table 6.

The extract of H has no cytotoxic activity to extract and A has an LD_{50} value greater than 200 µg/mL. Extracts C and E where they are toxic have values of 43.38 µg/mL and 43.26 µg/mL successively. This compared to other products [10] toxicity, shows that all the samples are less than podophyllotoxin (2.4 µg/mL) and greater than digitalis (77.2 µg/mL) and strychnine sulfate (151 µg/mL).

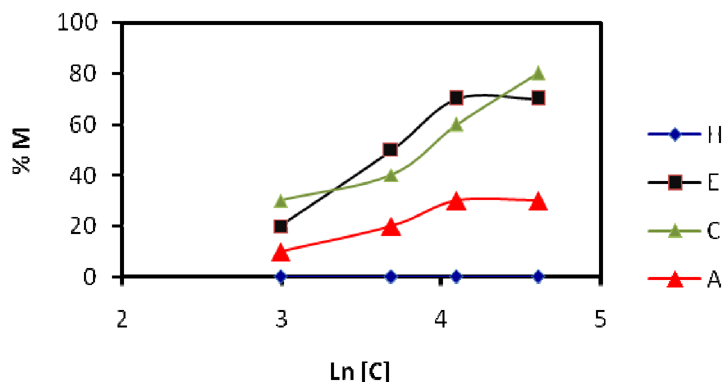

Figure 1: Curve representing the percentage of mortality as a function of logarithm of concentration

Table 6: 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
Podophyllotoxin	2.4
Digitalis	77.2
Strychnine sulphate	151

CONCLUSION

The biological study of the alga *Cystoseira tamariscifolia* through antibacterial activities, antileishmanial activity and cytotoxicity activity produces 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 study of different organic fractions of algae usually ethereal fraction and the chloroform fraction, which have been shown capable of providing biologically active molecules.

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