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ANTIOXIDANT ACTIVITIES, TOTAL PHENOL AND FLAVONOID CONTENTS OF TWO SEAWEEDS FROM ATLANTIC COAST OF MOROCCO: *CYSTOSEIRA SPINOSA* AND *CYSTOSEIRA MAURITANICA*

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Abstract

This study aims to examine the total phenol and flavonoid contents of the two seaweeds: *Cystoseira spinosa* and *Cystoseira mauritanica* from the Atlantic coast of Morocco, as well as to assess the antioxidant activities of their extracts. To perform this experiment, the antioxidant activities was evaluated in vitro spectrophotometrically using four different methods, such as ABTS, 1,1-diphenyl-2-picrylhydrzyl (DPPH), ferric reducing antioxidant power and total antioxidant capacity assays. The total content of phenols and flavonoids is measured using the Folin-Ciocalteu test and the aluminum chloride colorimetric method. The total contents, whether in phenols or flavonoids, as well as the antioxidant activities of the extracts of *C. Spinosa* and those of *C. mauritanica* are almost the same; there is a very small difference between them. The phenol contents varied from 121.04 to 30.21 mg GA E / g of dry extract. Total flavonoids varied between 198.52 and 11.28 mg RE / g dry weight. The extract showed significant ABTS + and DPPH scavenging activity, with IC50 values ranging from 2.5 to 55.2 mg / ml and 0.39 to 2.54 mg / ml respectively. In addition, all the extracts showed good antioxidant iron reducing power with EC50 values varying between 0.25 and 3.41 mg / ml. Analysis of the total antioxidant capacity revealed that *C. Spinosa* extract had high activity with a value of 251 mg VitCE / g dry weight. Methanol extract has low antioxidant activity in all four tests. These results show that the *cystoseira* species is a rich source of phenols and antioxidant compounds which can be used for pharmacological interests.

Keywords: *Antioxydant activities, Total phenol, Flavonoid, Cystoseira, Atlantic coast.*

Introduction

Currently the need for new drugs is great, due to populations and pathologies evolution. One of the ways of innovation is based on the discovery of original active ingredients, which can come from synthetic chemistry, extraction from biological sources, and finally, more recently, biotechnologies based on genetic engineering [1-2]. As far as extraction is concerned, plants have been and still are widely exploited, but the current trend is to assess the relevance of other living groups, such as insects, micro-organisms or marine organisms, as a source of active molecules, with the idea that new groups will deliver different chemical contents, and therefore a greater exploitable variety [3-5]. Marine macro- and micro-organisms occupy a prominent place in this field: very numerous in terms of species, subject to a specific environment very different from that of terrestrial organisms, they are as easily accessible as at the beginning of this research, in the 1950s [6].

Cystoseira is a genus of worldwide distribution with about 80% of the species occurring along the Mediterranean and adjoining Atlantic coasts [7]. It was created in 1820 by the Swedish phycologist Carl Adolph Agardh and it has described as seaweed, very branched, which can reach, for the largest, more than 1 meter in height. The Cystoseires have a strong heritage value in the Mediterranean. *Cystoseira* forests are home to a large number of species. This species is considered to be an efficient biological indicator of pure water. Indeed, this species seems to be very sensitive to pollution because a strong regression is observed around large cities [8].

The place of molecules of marine origin particularly seaweeds in the therapeutic arsenal is still modest, a fact which must be put into perspective due to the short period of corresponding research, and the difficulties of currently developing a new drug [9]. For the manufacturer, these products do not have a different status from those obtained from other processes, only efficiency and innovation count. It is precisely on this point that molecules of marine origin, resulting from original and still largely unexploited metabolisms, stand out and show all their interest.

Phenolic compounds are secondary metabolites of plants that play a role in the growth, reproduction and protection of the plant against predators and pathogens. They act as phytoalexins, anti-appetite, pollinator attractants, pigmentation contributors, antioxidants and protectors against UV rays. They also contribute to the nutritional and sensory characteristics of fruits and vegetables. The majority of phenolic compounds derive from the shikimate pathway. One of the two flavonoid cycles come from another pathway, that of acetate / malonate. Phenolic compounds can be single or composed of several aromatic rings. Nowadays the term “polyphenol” is used to designate complex and even the simplest phenolic compounds [10-11]. In the other hand, flavonoids (from flavus, "yellow" in Latin) are almost universal pigments in plants which are partly responsible for the coloring of flowers, fruits and sometimes leaves; nearly 6,500 flavonoids divided into 12 classes are known. The existence of different structural classes of flavonoids would be a function of modifications of the heterocycle; different types of nuclei are distinguished: flavones, flavonols, flavanones, flavanonols, flavans, flavan-3-ols, flavylium, chalcones, aurones, isoflavones, isoflavonols, isoflavans, pterocarpans, coumaronochromones, 3-arylcoumarins, ... [12]. To date, nearly 6,500 flavonoids have been identified. They have a common biosynthetic origin therefore, have a basic fifteen-carbon backbone, which consists of two phenyl rings, rings A and B, connected by a three-carbon bridge (C6-C3-C6 structure). The C3 bridge between rings A and B is commonly cyclized to form ring C [13-14].

The objective of this part is to try to exploit two seaweeds *Cystoseira spinosa* and *Cystoseira mauritanica* as sources of pharmaceutical molecules by studying the antioxidant potential of their extracts.

Materials and methods

Extract Preparation

After harvesting the two seaweeds *Cystoseira spinosa* and *Cystoseira mauritanica* in the south of Casablanca (Atlantic coast of 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.

Determination of total phenolic content

The total phenol content of the extracts was determined by the Folin-Ciocalteu method [15]. The reaction is prepared by adding the 0.5 ml of a known dilution of the extract and 2 ml of 7% sodium carbonate solution in 2.5 ml of 10% (v / v) Folin-Ciocalteau reagent. Then, the absorbance readings at 760 nm take place after 2 hours of reaction at room temperature in the dark conditions. The calibration curve was constructed using gallic acid (as standard) and the total phenol contents were expressed in milligrams of gallic acid equivalents per gram of dry weight of extract (mg GAE / g DW).

Determination of total flavonoids contents

Flavonoids are considered the most important natural phenols. They have a broad spectrum of biological and chemical effects, including radical scavenging activities. *Cystoseira*'s total flavonoid content was measured by aluminum chloride colorimetric assay [16].

In a volumetric flask containing 4 ml of distilled water, 1 ml of sample or rutin (standard solution) was added. Then 0.30 ml of 5% NaNO2 was added. After five minutes, 0.3 ml of 10% AlCl3 was added to react for 6 minutes. After that, 2 ml of NaOH (1 M) was added and the total was made up to 10 ml with distilled water. Then, the solution was mixed and the absorbance was measured at 510 nm. The calibration curve was constructed with rutin as a standard, and the total flavonoid contents were expressed in mg of rutin equivalents per gram of dry weight of each extract (mg RE / g DW).

In vitro antioxidant activities

ABTS Activity

ABTS is a screening method for antioxidant activity, generally used to assess the antioxidant capacity of natural products. The ABTS radical was generated from the oxidation of ABTS by potassium persulfate using the method described by Adedapo et al. [17]. After incubation in the dark for 12 h and at room temperature of the radical which was prepared by mixing equal amounts of 7 mM ABTS and 2.4 mM potassium persulfate. Then 1.5 ml of the solution was mixed with methanol to obtain an absorbance of 0.7 at 734 nm. In addition, 1 ml of extract or standard prepared in methanol at different concentrations was added to 1 ml of the methanolic solution of ABTS. Finally, and after 7 min, the absorbance was measured at 734 nm. The percentage of ABTS trapping activity of the extract or standard was calculated using the formula:

$$I\ (\%) = (1- (A_s / A_c)) * 100$$

where I (%) is the percent inhibition, A_c and A_s are the absorbances of the negative control and the sample (Trolox served as the positive control), respectively.

The IC50 values show the concentration causing 50% inhibition of the ABTS radical.

DPPH radical scavenging activity

DPPH is a stable radical, widely used to determine the antioxidant effect of natural and synthetic compounds. The capacity of the extracts to scavenge the DPPH radical was measured using the method described by Ichikawa et al. (2019) [18]. 0.1 ml of different concentrations of each extract or standard was added to 1.5 ml of ethanolic solution containing 0.1 mmol of DPPH (2, 2-diphenyl-1 picrylhydrazyl). After 30 min of incubation at room temperature in the dark, the absorbance of the mixture (0.1 ml of different concentrations of each extract (BHT as positive control) or standard added to 1.5 ml of ethanolic solution containing 0, 1 mmol of DPPH (2, 2-diphenyl-1 picrylhydrazyl)) was measured at 517 nm. The percent inhibition was calculated by following the previous formula (1). The IC50 values show the concentration causing 50% inhibition of the DPPH radical.

Ferric Reducing antioxidant power (FRAP)

The reducing power of the extracts tested was determined according to procedure of Jones et al. (2020) [19]. 200 µl of extract were mixed with 500 µl of phosphate buffer (0.2 M, pH 6.6) and 500 µl of potassium ferricyanide [K3Fe (CN) 6] 1%. The solution was incubated at 50 ° C for 20 min. After having acidified the mixture with 500 µl of 10% trichloroacetic acid (TCA), it was centrifuged at 3000 rpm for 10 min. The supernatant of the solution (2.5 ml) was mixed with 500 µl of distilled water and 100

µl of FeCl3 (0.1%) and the absorbance was measured at 700 nm.

Total antioxidant capacity assays

The test was based on the reduction of Mo (VI) to Mo (V) and the subsequent formation of a green phosphate Mo (V) complex at acidic pH [20]. A total volume of 25 µL of extract was added to 1 mL of reagent solution (0.6 mol / L of sulfuric acid, 28 mmol / L of sodium phosphate and 4 mmol / L of ammonium molybdate). The mixtures were incubated at 95 ° C for 90 min, then cooled to room temperature. The absorbance was measured at 695 nm. The total antioxidant activity was expressed in ascorbic acid equivalence number (mg Vit C E / g DW).

Results and discussion

The distribution of phenolic compounds in the species *Cystoseira* (figure 1) demonstrated that Ethyl ether from *C. spinosa* and chloroform from *C. mauritanica* contained the highest amounts 121.04 mg GAE / g dry weight and 98.33 mg GAE / g dry weight of extract, respectively. While the lowest phenol content was observed in methanol extracted from *C. spinosa* (30.21 mg GAE / g DW) (Figure 1). In all extracts, the levels of phenolic compounds were almost equal in *C. spinosa* and *C. mauritanica*. The total flavonoid content was determined in comparison with the rutin standard and the results were expressed in terms of mg RE / g dry weight extracted.

As presented in figure 2, the total flavonoid content of two seaweeds extracts ranged from 11.28 to 198.52 mg RE/g. The ethyl ether extracts of *C. spinosa* contained significantly a higher concentration of flavonoids (198.52 mg of RE/g) than the other tested extract. Comparing the flavonoid concentration of *C. spinosa* and *C. mauritanica*, all extracts of *C. spinosa* (except hexane) had greater concentration of flavonoids than *C. mauritanica* extract obtained using the same solvent.

The antioxidant activities of *Cystoseira* extracts were measured by four different test systems among them ABTS, DPPH, ferric reducing power, and total antioxidant capacity assays. All the extracts showed a notable effect which varied significantly between species**.**

As shown in Figure 3, the two essential extracts of the species *Cystoseira* showed a correlation between the antiradical effect and the concentration. Chloroform from *C. spinosa* and *C. mauritanica* extracts were more effective in reducing ABTS than all other extracts. While the lowest values were recorded with methanol extracts in both species. Compared to Trolox pure reference antioxidant.

Free radical scavenging capacity was defined as the concentration of antioxidant needed to reduce the initial DPPH 50% radical concentration (IC50). The highest antioxidant activity was indicated by the lowest IC50 value.

Table 1 shows the DPPH radical scavenging activity of *C. spinosa* and *C. mauritanica* extracts. In general, all the extracts inhibited the DPPH radical as follows: Ethyl ether> Hexane> chloroform> methanol. These results indicate that the extracts of *Cystoseira spinosa* had a higher activity than that of *Cystoseira mauritanica* compared to same solvent extracts. We found that the Ethyl ether extract of *C. spinosa* had the largest radical trapping capacity in all samples tested with an IC50 value of 0.387 mg / ml, followed by Hexane (0.854 mg/ml) then chloroform (1,025 mg/ml) and methanol (2.541 mg/ml). With *C. mauritanica*, we found also that the extract of Ethyl ether had the greatest capacity with an IC50 value of 0.387 mg/ml followed by hexane (0.748 mg/ml), then chloroform (1.245 mg/ml) and finally methanol (2.112 mg/ml).

The FRAP test is used to assess the reducing power of the extracts studied. Reducing capacity is generally associated with the presence of antioxidant agents which exert its effect by breaking free radical chains via the donation of hydrogen atoms [21]. Therefore, the reducing power assay is often used to assess the ability of extracts to convert Fe^{3+} to Fe^{2+}.

The results of Table 2 showed that the Ethyl ether extract of *C. mauritanica* had the strongest ferric reducing power than all the other extracts with an EC_{50} value of 0.255 mg/ml. While the Methanol exract from *C. mauritanica* had the lowest ferric reducing power with an EC_{50} value of 3.410 mg/ml.

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The total antioxidant capacity of the extracts of the two *Cystoseira* species studied was determined by the phosphomolybdenum method which is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the subsequent formation of a phosphate complex, green Mo (V) at acidic pH [22].

The results, expressed in ascorbic acid equivalents (vit C) are shown in Figure 4. They confirm that the most solvent extraction of antioxidant capacity was Ethyl ether extract of *C. spinosa* with 251.17 mg ascorbic acid equivalent to / g dry weight. In the Hexane and Methanol extracts, the antioxidant capacity was significantly higher in *C. mauritanica* than *C. spinosa*.

It is important to use many tests to highlight the chemical composition of the extract which acts by different mechanisms. Antioxidant activities were assessed using four different methods based on various mechanisms of action. The present study serves to demonstrate that extracts of *C. spinosa* and *C. mauritanica* had good antioxidant effects. Our study found that the ethyl ether extract of *C. spinosa* contained the highest content of total phenols (121.04 mg GAE / g DW), therefore ethyl ether is the best solvent which can be used to extract phenolic compounds from seaweed. Also in our study the values of the flavonoid contents are high varying from 65.24 to 198.52 mg RE / g in the extracts of ethyl ether of *C. spinosa*. The ABTS radical assay was generated by potassium persulfate in order to determine its property of donating hydrogen. In this study, there is a correlation between the anti-radical effect of *Cystoseira* extracts and the concentrations. The high percentage of ABTS trapping activity based in this study can be attributed to a high content of phenolics. An antioxidant is considered to be active against free radicals if the IC50 is less than 5 mg / ml [23]. All the extracts studied have an IC50 of less than 5 mg / ml, therefore all the extracts tested constitute a possible good source of antioxidant compounds. In addition, all extracts with high scavenging activity should have a low IC50 value [24]. In this study, we found that the ethyl ether extract of *C. mauritanica* showed the highest reducing power property. The determination of phosphomolybdenum is often used as an indicator of the antioxidant capacity of plant extracts [24]. *Cystoseira* extract had significant antioxidant activity in vitro. *Cystoseira* ethyl ether extract showed significant antioxidant activity in the DPPH test. According to the literature, phenolic compounds may contribute to the powerful antioxidant [25-26] and they are considered to be anticancer, anti-inflammatory, antiviral and antibacterial agents due to their antioxidant and anti-free radical properties [27].

Conclusion

In the present study, extracts of the two seaweeds from the Atlantic coast of Morocco, *C. spinosa* and *C. mauritanica*, were studied and compared in terms of their total phenol and flavonoid content as well as their antioxidant activities. The results showed that both seaweeds are rich in phenol and flavonoids, also they contain significant antioxidant capacities. All the results obtained during these tests have enabled us to discover new ways of valuing the algal biomass of Morocco, giving new products and materials in various fields of application and proved to be possible its use in traditional medicine.

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Tableau 1. DPPH radical scavenging activity (mg/ml) of different extracts from two seaweeds compared to that of BHT (IC50 = 0.127).

Seaweed / Extract	Hexane	Ethyl ether	Chloroform	Methanol
Cystoseira spinosa	0.854	0.425	1.025	2.541
Cystoseira mauritanica	0.748	0.387	1.245	2.112

Tableau 2. Ferric reducing power capacity (mg/ml) of two seaweeds compared to that of quercetin (EC50=0.034).

Seaweed / Extract	Hexane	Ethyl ether	Chloroform	Methanol
Cystoseira spinosa	0.621	0.287	1.154	3.241
Cystoseira mauritanica	0.741	0.255	1.286	3.410

Figure 1. Total phenolic compounds of different extracts from two seaweeds. Results were expressed as mg GAE/g dry weight.

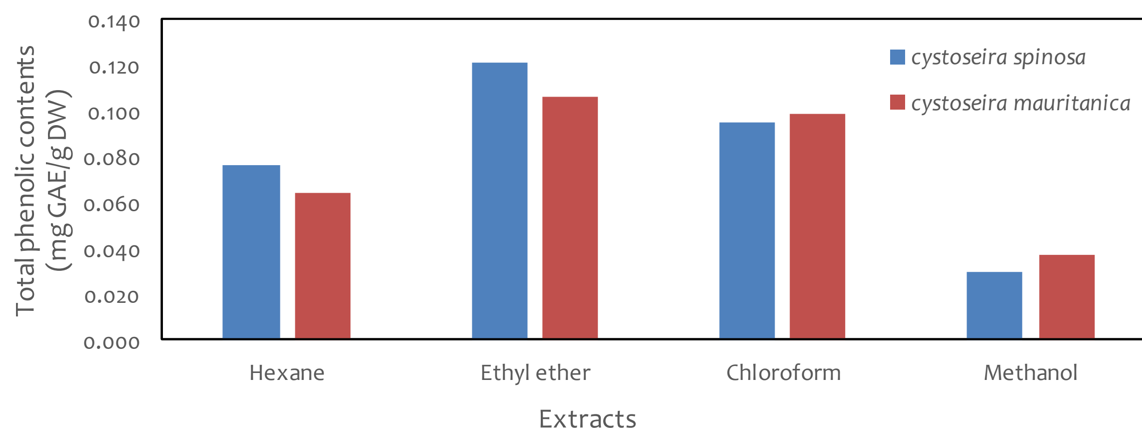

Figure 2. Total flavonoid content of different extracts from two seaweeds. Results were expressed as mg RE/g dry weight.

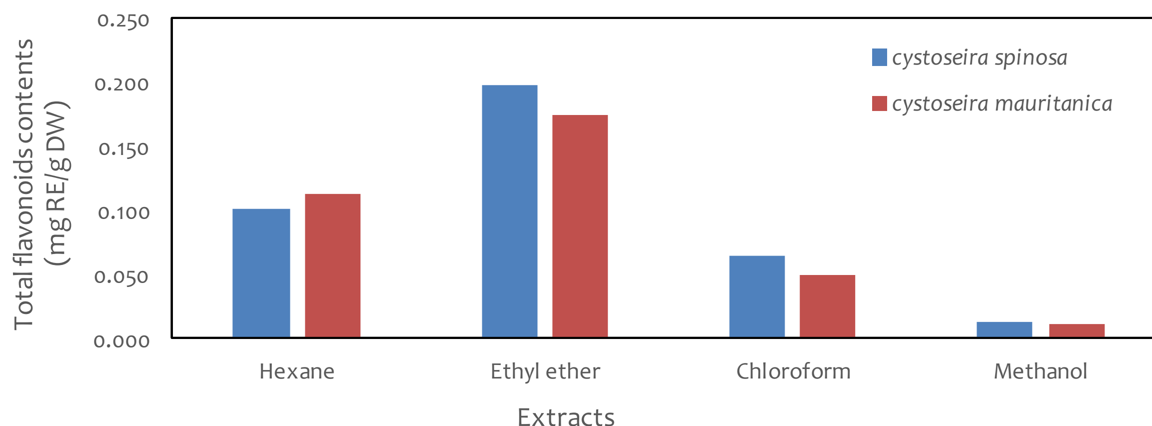

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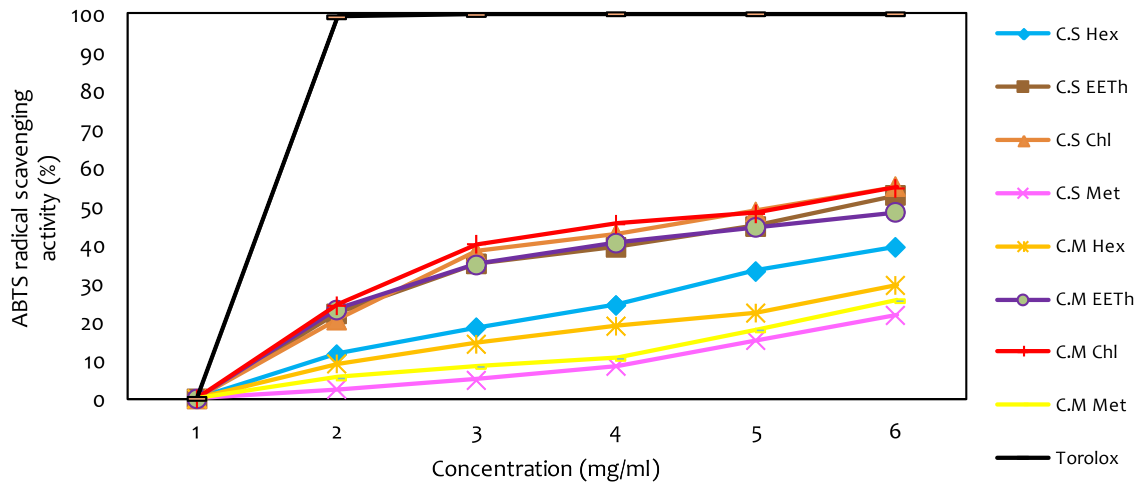
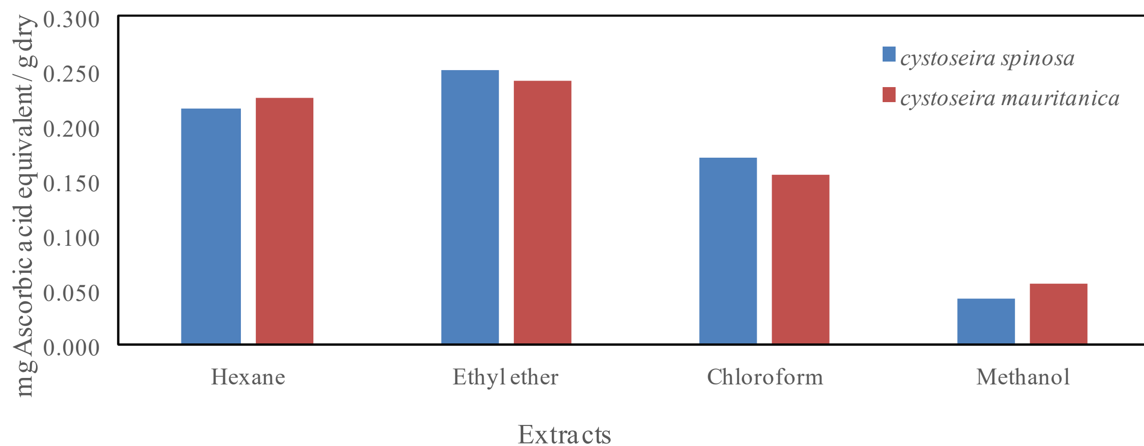
Figure 3. Dose-dependent ABTS radical scavenging activity of the studied extracts.



C.S: C. Spinosa; C.M: C. Mauritanica; Hex: Hexane; EETh: Ethyl Ether,; Chl: Chloroform; Met: Methanol.

Figure 4. Total antioxidant capacity of different extracts from two seaweeds. Results were expressed as mg vit C E/g dry weight.



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