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Original Article

ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENTS OF SEVEN BROWN SEAWEED FROM DJIBOUTI COAST

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ABSTRACT

Objective: This study focuses on the antioxidant potential of Djibouti brown seaweed and their phenolic contents.

Methods: We evaluated the antioxidant potential by DPPH method (1,1-diphenyl-2-picrylhydrazyl) and their phenolic contents of seven Djibouti seaweed: *Cytoseira myrica, Padina pavonica, Sargassum fluitans, Sargassum ilifolium, Sargassum sp, Turbinaria triquetra* and *Turbinaria turbinata*. Also, we searched the secondary metabolites of these seaweeds.

Results: We obtain a higher antioxidant activity at 60,7±0,9 % and a higher phenolic content at 199,01±0,5 µg equivalent phloroglucinol (PGE)/g dry matter for *Padina pavonica*. A good linear correlation (R^2 = 0,898) is observed between the antioxidant activity and the phenolic content of the seaweed studied. Also, two *Padina pavonica* collected in two different locations have different biochemical concentrations and antioxidant activity, suggesting the influence of the marine environment on the biosynthesis of secondary metabolites and the biological activities of seaweed.

The present study shows the presence of tannins, saponosides, flavonoids and steroid-terpenes.

Conclusion: The species studied show interesting antioxidant activities and can be consumed to prevent oxidative stress.

Keywords: Brown seaweed, Antioxidant activity, Polyphenols, Djibouti

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INTRODUCTION

Free radicals are molecules with an odd or unpaired electron in the outer orbital of their atomic structures, which makes them very reactive. These radicals are produced during cellular respiration and the human body owns a way to neutralize them. However, it happens that the production of these molecules becomes uncontrolled and settles what is called oxidative stress [1]. In this case, free radicals cause degradation of important biological substrates such as DNA, proteins and lipids, leading to the appearance of pathogens of degeneressances such as cancers and cardiovascular diseases [2].

Macroscopic seaweed, classified according to their colors (green, red, brown and blue), have a richness in diversified antioxidant molecules. They also have recognized the commercial value in various fields, such as food, cosmetics, textiles, paper mills, pharmaceuticals and medicine. They are an important source of polysaccharides (agars, carrageenans, alginates) used as emulsifiers, thickeners and stabilizers in the food industry. Almost all the production of macro seaweed is shared between three Asian countries namely China (62.8%), Indonesia (13.7%) and the Philippines (10,6%) [3]. The three African countries (Tanzania, Madagascar and South Africa) with an algal farming system represent only 0.093% of world production.

Located at the meeting point of the Red Sea and the Gulf of Aden, and undergoing the oceanographic influences of the Indian Ocean, the Red Sea and the Arabian Sea, Djibouti is proud of its fauna and diverse and unique flora. There are multitudes of seaweed species on these coastlines. Despite this abundance of marine organisms, their valuation is very low or non-existent. One of the impediments to this non-exploitation lies in the absence of scientific studies that would lead to the invention of effective recovery techniques. Therefore, we selected seven Djiboutian algal species which are *Cytoseira myrica, Padina pavonica, Sargassum fluitans, Sargassum ilifolium, Sargassum sp, Turbinaria triquetra* and *Turbinaria turbinata*. These seaweeds are known for their biological activities such as antibacterial [4-9], anti inflammatory [10] and antiviral [11]. Also some biomolecules have already been isolated as diterpenes [12], saponins [13], steroids [14, 15], polyphenols [16], alginates [17], fucoidans [18] and carotenoids [19]. We will evaluate here the potential of the antioxidant effects and their contents in phenolic compounds of these seven brown seaweeds of Djibouti.

MATERIALS AND METHODS

Seaweeds materials

The seven brown seaweed was collected off the coast of Khor Ambado and Moucha Island, East of Djibouti (fig. 1). A part is used to prepare a herbarium paper for identification and the remains have been dried in the open air for three days. The identities of the studied species are brought together in fig. 2.

Preparation of extracts

After grinding of the algal materials, 75 mg of the powder obtained is macerated in 1.5 ml of 50% methanol in an eppendorff. The whole is stirred for 3 h at a 40 °C. The tubes are centrifuged at 5000 rpm and the supernatants are recovered and then evaporated to dryness.

The crude extracts were stored in a freezer at-20 °C prior to analysis.

Phytochemical screening

The dried extracts were subjected to qualitative tests for the detection of biomolecules according to the procedures described by [20].

Determination of phenolic content

The content of the phenolic compounds of the seaweed is determined according to the folin-Ciocalteu method adapted by [21] of the Folin-Denis method adapted by [22].

It consists to pour 20 µl of the sample (or standard) into wells of a 96-well microplate. Then 10 µl of Folin-Ciocalteu reagent and 40 µl of Na_2CO_3 at 200 g/l are added. The mixture is homogenized and

then kept in a water bath at 70 °C. for 10 min. The absorbance reading is performed at 620 nm.

Phloroglucinol is used as a standard for the calibration curve and phenolic compound levels are expressed as mg phloroglucinol equivalent per gram of dried algal matter (mg PGE/g).

Comparison of the ^{1}H NMR of two *Padina pavonica*

Two *Padina pavonica* collected in two different places (Khor ambado and Moucha Island) were extracted as described previously. The two crude extracts thus obtained are dissolved in D2O and was subjected to a 1H NMR acquisition on a Bruker Avance 400. Their 1H spectra were compared.

Measurement of antioxidant activity

The antioxidant activity test is carried out according to the method of DPPH (1,1-diphenyl-2-picrylhydrazyl) on microplates previously described by [23]. Briefly, 22 µl of extract (or controls) is added 200 µl of DPPH at 25 mg/l in ethanol. The plates are closed, covered with aluminum foil and kept at room temperature. After incubation (2 h), the optical density reading is performed at 540 nm. The percentage of inhibition of free radicals is calculated according to the following equation:

Inhibition percentage rate = [(As-Ai)/As] X100;

Whereas is the absorbance of DPPH alone, and Ai is the absorbance of DPPH in the presence of various extracts. Ascorbic acid (Vitamin C) is used as a positive control.

Statistical analysis

All tests are performed in triplicate and their values are expressed as a mean±standard deviation. All analyzes are performed at the 95% level of significance.

RESULTS AND DISCUSSION

After evaporation of methanol and removal of water by freeze-dryer, the yield of each extract is calculated. The values obtained are 4,1%; 3,8%; 6,2%; 5,7%; 6,2%; 7% and 0,1% respectively for *Cytoseira myrica, Padina pavonica, Sargassum fluitans, Sargassum ilifolium, Sargassum sp, Turbinaria triquetra* and *Turbinaria turbinate* (table 1). The best yield and the lowest yield are obtained for the genus Turbinaria respectively for *Turbinaria triquetra,* collected at Khor Ambado, and for *Turbinaria turbinate,* collected at Moucha Island.

In addition, the main types of secondary metabolites namely terpenes, alkaloids, flavonoids, tannins and saponins have been sought in the extracts. The result of their presence or not is gathered in table 2. Alkaloids and terpenes are very little or not present in the analyzed samples. In contrast, saponosides are found in all species except *Sargassum sp.* Also, a strong presence of tannins is observed in *Turbinaria triquetra* extract (table 2).

The crude extracts were also tested for their antioxidant activity by the DPPH method. The percentages of eliminations of radicals go from 47±2% for *Sargassum sp.* to 60.7±0.9% for *Padina pavonica* (fig. 3). The latter has a good antioxidant activity compared to the positive control which is vitamin C with antioxidant activity of 83±0.7%. Hydroalcoholic extracts of seaweed from different countries have already shown these good abilities to fight against free radicals, as per example in China [24], in Malaysie [25] and in Algérie [26].

Brown algae are rich in alginate and these substances can be at the origin of their antioxidant powers. Indeed it is shown the link between the alginate content and the antioxidant activity [27]. Their mechanism of action lies in their ability to give protons [28].

In this same mode of action, phenolic compounds, in particular, seaweed phlorotannins, possess antiradical activities. For this, we determined the contents of phenolic compounds of seven seaweed extracts (table 1). The highest content of phenolic compounds is obtained for *Padina pavonica* 199.01±0.5 µg PGE/g of dry algal matter. This species is also the one with the highest antioxidant activity (fig. 3). As a result, a representation of the values of antioxidant activities according to the phenolic contents is carried out. We obtain a linear relationship between the two factors measured with a correlation coefficient R^2= 0,898 (fig. 4). This linear relationship shows that antioxidant activity is dependent on the content of phenolic compounds. This dependence is obtained often for terrestrial and marine plants [29].

In addition, two species of *Padina pavonica* collected in two places (PP1 at Moucha Island and PP2 at Khor Ambado) have been compared both on their antioxidant powers and on their phenolic contents. We obtain a better antioxidant activity and a higher phenolic content for the species collected at Khor ambado. The latter has a percentage of antioxidant activity of 60.7±0.9% and a content of 199.01±0.5 µg PGE/g of dry algal matter against 57.6±1.7% and 124.88±1.2 µg PGE/g of dry algal matter for the same species collected at Moucha Island. The difference is significant and beyond the confidence interval.

On the other hand, we injected the two extracts of the same species in NMR and recorded the chemical shift of 1H (fig. 5). We compared the area of 5.5 to 6.5 pm corresponding to phenolic protons of phlorotannins [30]. We observe an absence of notable peaks for PP1 and a peak around 5.75 pm for PP2. The phenols in the crude extract of PP2 would be larger and detectable in NMR. The amount of phenol produced by an alga would depend on the ecosystem where it grows. In fact, unlike primary metabolites, plants produce secondary metabolites including phenolics according to their state of health or in response to external attacks among others [31].

CONCLUSION

Cell damage caused by oxidative stress brings out serious chronic diseases such as cancer, diabetes and cardiovascular disease. The consumption of foods or food supplements rich in antioxidant substances can help prevent these pathologies.

Djibouti enjoys a coastline and has a variety of macroscopic algae, especially brown algae. In this study, we evaluated the antioxidant power of seven species of brown algae collected at Moucha Island and Khor ambado: *Cytoseira myrica, Padina pavonica, Sargassum fluitans, Sargassum ilifolium, Sargassum sp, Turbinaria triquetra* and *Turbinaria turbinata*.

The species *Padina pavonica* has both the best antioxidant activity and the highest phenolic content.

The present study shows the presence of tannins, saponosides, flavonoids and steroid-terpenes.

These species can be valued for their antioxidant actions. However, a quantitative analysis of minerals, proteins and lipids is necessary to select candidate species for effective dietary supplements.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declare none

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