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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF THE METHANOL EXTRACT OF CHARA HISPIDA FRESHWATER GREEN ALGAL OF CHARACEAE

Salamat Asmaa[1]*, Siroua Karima[1], Kenz Abdelkbir[1], Ainane Ayoub[1], Talbi Mohammed[1], Elkouali M'hamed[1], Ainane Tarik [1,2]

[1]Faculty of Sciences Ben Msik, Laboratory of Analytical Chemistry and Physical Chemistry of Materials, University of Hassan II, BP 7955 Casablanca 20,660, Morocco.

[2]Superior School of Technology – Khenifra (EST-Khenifra), University of Sultan Moulay Slimane, PB 170, Khenifra 54,000 Morocco.

*salamatasmaa@gmail.com

Abstract

Chara Hispida is a freshwater green alga in the division Charophyta, have been chosen on the basis of their medicinal properties and the frequency of its biomass around the Moroccan Middle Atlas (Beni Mellal region). Our work aims to evaluate the antioxidant and antimicrobial activities of the methanol extract of Chara Hispida, Characean algae. The results obtained showed a clear anti-free radical activity of the Methanol extract by the DPPH test and the ABTS test. The antimicrobial activity is tested against two Gram-negative bacteria (Escherichia Coli and Pseudomonas Aeruginosa), and two Gram-positive bacteria (Staphylococcus Aureus and Bacillus Cereus) using the diffusion method. The results showed that methanol extracts produced interesting inhibition zones against three bacterial strains Escherichia Coli, Pseudomonas Aeruginosa and Bacillus Cereus. For Staphylococcus Aureus they have no antibacterial activity.

Keywords: *Chara Hispida, Characeae, Charophyta, Charales, green algae, freshwater, antibacterial activity, antioxidant activity.*

Introduction

Global interest is increasing in studying traditional systems of medicine and exploring their effectiveness day by day. The therapeutic use of green algae is one of these systems due to its efficaciousness in treating some diseases and as an effective and inexpensive source of medicine by using various techniques such as pharmacology properties to evaluate green algae or characeae medically. The various molecules that make up the algae are the main source of potential functional components. The Flora of Charales (Charophyta) has many uses in the food, cosmetics and pharmaceutical industries, especially in today's consumer demand for natural products. Besides, Algae are environmentally friendly, and because there is no shortage of raw materials or limited seasonal changes, there are many algae biomass. So the aquatic environment produces a wide variety of species, including fresh algae, the only living family of the order Charales, a group that grows excessively and occurs in high concentrations, the massive accumulation of green algae has a fairly large biomass **[1]**. Therefore, it's particularly relevant to this research to evaluate the pharmacological properties of the charophytic algae, *Chara hispida*, from the Middle Atlas of Morocco and its value as a huge source of active biomolecules of biological interest.

Chara Hispida belongs to the largest species of charophytic flora, most of which have important pharmacological and ecological effects. It's a hardy and often highly encrusted species, visible all year, easy to spot and commonly found in North Africa (Atlas) as well as Europe and Western Asia. It colonises stagnant to slightly flowing, permanent, rather deep, oligomesotrophic to eutrophic, neutral to carbonate waters, often of phreatic origin, not very turbid and rarely polluted, on a varied substrate, in sunny conditions. Exists in many types of environments from forest ponds, ponds, marsh ditches, gravel pits, canals, quiet parts and annexes of watercourses to artificial basins in parks and castles **[2-3]**.

Recently, there has been a growing interest of characeae about their biological properties and their ability to reduce the incidence of certain diseases are involved **[4-5]**. This article on the valorisation of *Chara Hispida* of the Moroccan Middle Atlas (Beni Mellal region) is the subject of the first work that will be carried out on this species, since no study on the Characeae of our country has been carried out. To appreciate this algal biomass, antioxidant and antibacterial properties will be two tests to consider, due to their potential source of natural bioactive compounds for new demand in medicine and food industry**.**

The main objective of the present research was the evaluation of antioxidant capacities of the methanol extract of *Chara Hispida* biomass and to evaluate its antimicrobial properties. Then, the estimation of antioxidant properties was analysed using DPPH· and ABTS· assays, compared to ascorbic acid. The Antibacterial activity was tested in vitro against two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) at comparable concentrations of penicillin and novobiocin antibiotics.

Material and methods

Extract Preparation

Chara Hispida is a freshwater green alga was collected in the Middle Atlas of Morocco (Beni Mellal region) in the period of low tide, washed, cut into small pieces and dried for three days at room temperature. Parched algae were then grounded with an electric mill to obtain a particle size less than 63 µ m. then we stored the powder in glass bottles, away from light and humidity.

The identification of the species was made by Mr T. AINANE, Professor Chemist at the Superior School of Technology-Khenifra (EST-Khenifra), University of Sultan Moulay Slimane.

Fourteen grams of characeae powder was extracted with methanol using Soxhlet extraction. The procedure was last 6h and repeated three times to attain max of fragment. Finally, the solvent was ejecting using a rotary evaporator and the diluted sample was used to evaluate the antibacterial antioxidant activity.

Evaluation of antioxidant activity

DPPH radical-scavenging activity

According to the method described by Talla et al. (2017) **[6]**, the antioxidant activity of the methanol extract of *Chara Hispida* was determined by DPPH (1.1-diphenyl-2-pyridyl hydrazine) test. The liquid of DPPH- 60 µM (2.3 mg in 100 ml ethanol) is prepared and stored in the dark at +4 °C. The ethanolic solution of each sample (50 µl) at different concentrations is placed in a haemolysis tube and 1.95 ml of the DPPH- solution is added. After 30 minutes incubation at room temperature, the absorbance is measured at 517 nm. A sample containing 50 µl of ethanol and DPPH–mixtures were used as negative control. Ascorbic acid was used as a positive control. The percentage inhibition is calculated according to the following formula:

$$IC\% = \left(\frac{A_0 - A_i}{A_0}\right) \times 100$$

With

A_0: the absorbance of the negative control;
A_i: the absorbance of the sample.

ABTS Radical-Scavenging Activity

The determination of antioxidant activity by the ABTS test was performed according to the method reported by Salar (2019) **[7]**. The cationic radical $ABTS^{\cdot+}$ is generated by mixing a 2.45 mM solution of potassium persulfate ($K_2S_2O_8$) and a previously prepared 7 mM solution of ABTS with equal volume. The mixture is stored in the dark and at room temperature for 16 hours before use. The solution obtained is diluted with ethanol to obtain an absorbance of 0.7 to 0.8 at 734 nm. Twenty µl samples at different concentrations (10 µl) are added to 1.49 ml of the $ABTS^{\cdot+}$ solution.

The reading is taken at 734 nm at 0 min (A0) for the negative control and after 30 min (Ai) for each run. Ascorbic acid and trolox are used as positive controls. The percentage inhibition is calculated according to the following formula:

$$IC\% = \left(\frac{A_0 - A_i}{A_0}\right) \times 100$$

With

A_0: the absorbance of the negative control;
A_i: the absorbance of the sample.

Similarly, we calculate the median inhibitory concentration IC^{50} for the radical scavenging activity of ABTS.

Evaluation of antibacterial activity

We employed the solid diffusion method (Muller-Hinton medium) for antibacterial activity described by Bennamara & Abourriche (2020) **[8]**.

The samples were prepared by dissolving different amounts of the extracts in 80% aqueous methanol to the desired concentration. Aqueous methanol (80%) was used as a negative control. Penicillin G (10 units) and Novobiocin (5 mg) were used as positive controls. Four bacterial strains to be tested (Table 1) were cultured in a test tube containing 5 ml nutrient broth and incubated at 37°C for 24 hours, and then 200 µ L of bacterial suspension was inoculated into a 90 mm diameter Petri dishes containing 20 ml of previously cast and solidified Muller Hinton agar. For each bacterium, the inoculation was carried out in three Petri dishes. A fourth Petri dish cultures were reserved for the negative control.

Sterile Watt man paper discs N°1 and 6 mm diameter were placed in Petri dishes and soaked with 50 µ l of extract. Three concentrations were tested for each extract (10 mg/ml, 50 mg/ml and 100 mg/ml). The Petri dishes were incubated at 37 °C for 24 hours. Antibacterial activity was evaluated by measuring the inhibition zone.

Results and discussion

Extraction

After the extract was obtained, it was determined their colours and returns on the initial amount of dry algae. Data for samples obtained are given in the table 2.

Evaluation of antioxidant activity

The anti-free radical activity of the methanol extract of *Chara Hispida* was determined according to the process of reduction of the DPPH radical. The latter is a free radical that accepts hydrogen radical to become a stable molecule, whose colour changes from purple to yellow **[9]**.

The median IC50 inhibitory concentration for the radical scavenging activity of DPPH was 18.73 mg/ml (Table 3), which was obtained by plotting the percent inhibition against the concentrations used as shown in Figure 1. The IC50 value for ascorbic acid was 0.08 mg/ml. The results below for these algae may have led to its good trapping activity of DPPH.

The ABTS assay is based on a single electron transfer and the methodology used is the same as that described previously.

The percentage of inhibition by the ABTS assay of the methanol extract of *Chara Hispida* was around 2,014 mg/ml (Table 4). The results obtained show that the evaluation of antioxidant activity by the ABTS test is more efficient than DPPH reduction **[10]**.

These results partly explain the ability of our extracts to capture or trap free radicals or reactive oxygen species (DPPH, ABTS, etc.). In general, both assays are widely used to investigate the antioxidant activity of synthetic and natural compounds due to their simplicity and efficiency **[11-14]**.

Evaluation of Antibacterial Activity

Antibacterial activity is tested against two Gram-negative bacteria (*Escherichia Coli and Pseudomonas Aeruginosa*), and two Gram-positive bacteria (*Staphylococcus Aureus* and *Bacillus Cereus*) using the diffusion method. The results showed that methanolic extracts produced interesting inhibition zones against three bacterial strains *Escherichia Coli*, *Bacillus Cereus* and *Pseudomonas Aeruginosa*. For *Staphylococcus aureus* they have no antimicrobial activity (Table 5).

Freshwater algae contain a large amount of biological active compounds functioning as antioxidants, antimicrobial, antifungal and antiviral activity **[15-17]**. In the majority of papers, researchers focused on terrestrial plants (fruits, flowers, seeds, leaves, stems, bark and roots) to determine antioxidant substances; however, the source of natural antioxidants is not only terrestrial plants, but also aquatic plants that are rich in natural antioxidant compounds **[18-19]**. Green algae are potential sources of natural bioactive compounds, containing organic and inorganic substances such as polysaccharides, lipids, proteins, carotenoids, phenolic compounds, vitamins, and minerals, the levels of which play an important role in the high antioxidant activity of algae **[20]**. According to our research, there is no reported work on the biological activities of the alga *Chara Hispida*. Below, we summarise the main works related to the study of green algae families of Characeae: The literature reveals that the activity of green algae has been verified on various antioxidant tests as well as on various bacterial strains. According to a scientific research on different biological tests, we find the antioxidant test of freshwater algae *Microspora sp.* which showed the highest value of antioxidant activity in an extract of methanol by B. Akar and all. **[15]** Research on algae *Chara* [Green algae] showed maximum inhibition zone in *Pseudomonas Syringae* (12 mm) whereas in *Xanthomonas Campestris* and *Agrobacterium Tumefaciens* the inhibition zone was 10 mm **[21]**. and many studies show that green algae have a higher antibacterial activity that has been carried out to know the medicinal properties and importance.

Conclusion

As a conclusion, aquatic plants have demonstrated their biological power, the methanol extract of the algae species *Chara Hispida* of the Characeae family against the two tests, antioxidant and antibacterial, revealed interesting inhibition zones as well as a clear anti-free radical activity.

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Table 1. Bacterial strains

Strain name	Gram	Code
Escherichia Coli	-	25222
Pseudomonas Aeruginosa	-	27853
Staphylococcus Aureus	+	25923
Bacillus Subtilis	+	6633

Table 2. The Extract of *Chara Hispida* with yield and colour

Extract	Colour	Yield (%)
Me OH	Brown	1.83

Table 3. Values of antiradical experience by DPPH

Methanol Extract			
Concentration (mg/ml)	**Absorbance**	**% Inhibition**	**DPPH IC_{50} (mg/ml)**
1	0.515	5,850	18,728
3	0.469	14,260	
5	0.426	22,121	
7	0.385	29,616	
9	0.369	32,541	
10	0.327	40,219	
20	0.219	59,963	
Blank		0.547	

Table 4. Values of the anti-radical experiment by ABTS

Methanol Extract			
Concentration (mg/ml)	**Absorbance**	**% Inhibition**	**ABTS IC_{50} (mg/ml)**
0.5	0.695	7,086	2,014
1	0.692	7,531	
3	0.607	18.85	
5	0.523	30,036	
7	0.423	43,405	
9	0.382	48.93	
10	0.33	55,838	
20	0.065	91,266	
Blank		0.748	

Table 5. Diameters of the inhibition zones in (mm) of the methanol extract of *Chara Hispida*

Concentrations (g/l)	10	50	100
Bacillus Subtilis (+)	6.78	11.89	10.78
Pseudomonas Aeruginosa (-)	5.89	5	8.11
Staphylococcus Aureus (+)	-	-	-
Escherichia Coli (-)	10	10.22	11

Key: (–): No inhibition, low inhibition: less than 10 mm diameter inhibition, medium inhibition.

Figure 1. Anti-radical activity by DPPH of the methanol extract of *Chara Hispida*

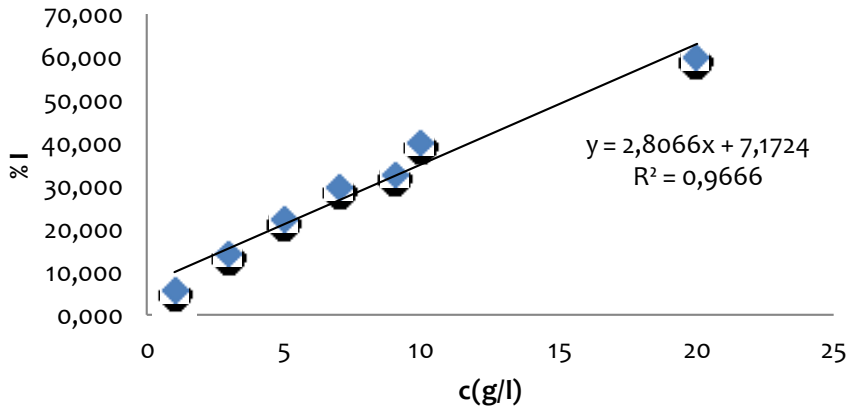

Figure 2. Anti-radical activity by ABTS of the methanolic extract of *Chara Hispida*