


# In Vitro Evaluation of Antioxidant and Antimicrobial Effects of *Hammada scoparia* Extracts

Moha Afrokh <sup>1,2</sup>, Saida Tahrouch <sup>2</sup>, Fadma Fahmi <sup>2</sup>, Abdelkader Chibani <sup>3</sup>, Nabila Chahboun <sup>1,4,5</sup>, Abdelkader Zarrouk <sup>1,\*</sup> , Mohamed Tabyaoui <sup>1</sup>

<sup>1</sup> Laboratory of Materials, Nanotechnology and Environment, Faculty of Sciences, Mohammed V University in Rabat, Av. Ibn Battouta, P.O. Box 1014 Agdal-Rabat, Morocco

<sup>2</sup> Laboratory of Plant Biotechnology, Faculty of Sciences, Ibn Zohr University, Box. 8106, Agadir, Morocco

<sup>3</sup> Nutrition, Health and Environment, Department of Biology, Faculty of Sciences, University Ibn Tofail, Kenitra, Morocco

<sup>4</sup> Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Science, Ibn Tofail University, BP 133, Kenitra, 14000, Morocco

<sup>5</sup> Institute of Nursing Professions and Health Techniques, Annex Kenitra, Morocco

\* Correspondence: [azarrouk@gmail.com](mailto:azarrouk@gmail.com);

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**Abstract:** This study describes, firstly, the quantification of phenolic compounds in the methanolic extract of *Hammada scoparia* (HS) and carries out, on the one hand, a comparative study on the method of evaluation of the antioxidant activity using four methods namely: 2,2 diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging test, 2,2'-azino-bis-(3-ethylbenzothiazoline -6-sulfonic acid) (ABTS) radical cation scavenging test, ferric reducing antioxidant power assay (FRAP) and  $\beta$ -carotene bleaching assay, and on the other hand d to evaluate the antimicrobial activity with different organic solvents. The results obtained show that the extract of this plant has a stronger antioxidant activity for the FRAP and  $\beta$ -carotene bleaching method, with the same value of  $IC_{50}$  ( $0.042 \pm 0.002$  mg/mL) against  $IC_{50}$  ( $0.021 \pm 0.001$  mg/mL) for the standard antioxidant by the free radical scavenging test DPPH. In addition, our extract contains high levels of total phenols ( $79.50 \pm 3.33$   $\mu$ g GAE/mg), total flavonoids ( $21.16 \pm 4.50$   $\mu$ g QE/mg), and total condensed tannins ( $55.00 \pm 7.66$   $\mu$ g CE/mg). The results of this study showed good agreement between the antioxidant activity and the phenolic content of the plant material studied. Moreover, the organic extracts show good effectiveness against Gram-positive bacteria, particularly the butanol extract, which shows an inhibition zone of 13 mm at 150 mg/mL against *Staphylococcus coagulase-negative* bacteria. While it was moderate against other bacteria: *Enterococcus faecalis* (10 mm at 150 mg/ mL) and *Streptococcus spp*, *Escherichia coli*, and *Proteus mirabilis* with the same zone of inhibition (09 mm at 150 mg/ mL) as well as against the yeast, *Candida albicans* (08 mm at 150 mg/ mL).

**Keywords:** *Hammada scoparia*; phenolic compounds; antioxidant activity; antimicrobial activity.

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## 1. Introduction

Aromatic and medicinal plants constitute a very important natural resource whose valorization requires inventories and ethnobotanical surveys of this heritage to understand it better. Their activities depend on the presence of various bioactive natural compounds belonging to different chemical classes. Generally, these natural products are intended for the development of new drugs [1], which help individuals regain and improve their physiological

equilibrium [2]. In Morocco, these plants are natural resources that make up a large part of the national economy [3].

The species *Haloxylon scoparium* Pomel (Syn. *Hammada scoparia* (Pomel) Iljin) belongs to the most important genus (*Haloxylon*) of the family Amaranthaceae [4] and grows wild in the desert and semi-desert areas in the Mediterranean and the Near East regions [5]. In Morocco, it is locally known as "Remth" [6].

*Hammada scoparia* is a small, highly branched shrub distributed in North Africa, southeastern Spain, and parts of Iran, Turkey, Iraq, and Syria [7]. This species is known for its medicinal properties and is used in traditional medicine [8]. In North Africa, it is used to prevent various diseases, including hepatitis, antidiabetic, antioxidant, anti-inflammatory, photoprotective, obesity [9-11] and protect against cardiovascular disease, atherosclerosis, hypertension [12], antimutagenic [13] and green synthesis of silver-doped nickel and copper nanoparticles from *Hammada scoparia* showed moderate antimicrobial activity against four bacterial strains (*E. coli*, *S. Typhimurium*, *S. aureus*, and *E. faecalis*) and low activity or was ineffective against a strain of yeast (*C. albicans*) [14]. Also, the extract of the *Hammada scoparia* plant showed excellent inhibitory activity against steel corrosion in an acidic medium [15]. It is known in traditional medicine for the treatment of diabetes. It has shown a revival of interest with its use by cancer patients. In Morocco, this species is widely used in traditional medicine for treating wounds and pimples, as well as for hair care [1].

For this, we planned to carry out the present study, whose aim is to evaluate the antioxidant activity using four methods: DPPH free radical scavenging test, ABTS radical cation scavenging test, ferric reducing antioxidant power test (FRAP) and  $\beta$ -carotene bleaching test and finally to evaluate the antimicrobial activity of the aerial part of HS harvested in Guelmim in the south of Morocco.

## 2. Materials and Methods

### 2.1. Chemicals and plant material.

All solvents were of analytical or HPLC grade and purchased from Professional Labo (Casablanca, Morocco). The chemical reagents used in this work are classified as follows: 2,2'-azino-bis (3-ethyl benzthiazoline-6-sulphonic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH 90%),  $\beta$ -Carotene, aluminum chloride ( $\text{AlCl}_3$ ), ferric chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), potassium persulphate ( $\text{K}_2\text{S}_2\text{O}_8$ ), potassium ferricyanide  $\text{K}_3\text{Fe}(\text{CN})_6$ , Folin-Ciocalteu's phenol reagent, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), sodium hydroxide (NaOH), Gallic acid, Ascorbic acid, Quercetin, Catechin, and Vanillin were sourced from Professional Labo (Casablanca, Morocco).

*Hammada Scoparia* was harvested fresh from the Guelmim area during March randomly; the geographical location of the harvest site is as follows: (Latitude : N: 28°59'52.186'', Longitude : W: 10°2'13.631'' and Altitude: 365 m), allowed to dry in the shade, protected from moisture and stored carefully in a dry and ventilated area within the Laboratory of Plant Biotechnology of the Faculty of Sciences in Agadir. The aerial part of this species was then milled with an electric mill until a powder was obtained.

### 2.2. Extraction protocol.

A mass of 300 g of the aerial parts of the dry plant was macerated in 1.5 L of a MeOH/water mixture (70/30, v/v) for 24 hours. The procedure was repeated three times, and

the filtrates were combined before being concentrated to dryness under reduced pressure in a rotavapor. The residue is added with 300 mL of distilled water and left to stand overnight. After filtration, an aqueous solution is obtained, which is then subjected to liquid-liquid extraction with solvents of increasing polarity: dichloromethane, ethyl acetate, and n-butanol.

The three recovered organic phases are dried over anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), filtered, concentrated to dryness, and weighed. The residue obtained is stored at 4 °C in tinted bottles, away from light, until used.

### 2.3. Quantification of the phenolic compound content.

#### 2.3.1. Total phenolic content.

The phenolic content of the methanol extract was quantified using the Folin-Ciocalteu method [16]. Briefly, 100  $\mu\text{L}$  of extract or standard antioxidant (gallic acid (GA)) was mixed with 500  $\mu\text{L}$  of the Folin-Ciocalteu reagent (10 times diluted in distilled water). After 2 minutes of incubation, 2.00 mL of the 20%  $\text{Na}_2\text{CO}_3$  solution was added. The mixture was left to settle for 30 minutes in the dark, and the absorbance was read at 765 nm using a LLG-uniSPEC 2 Ultraviolet-Visible spectrophotometer against a blank without extract. The optical densities of the prepared solutions were used to plot the calibration curve for GA. All measurements are performed in triplicate.

#### 2.3.2. Total flavonoid content.

The total flavonoid content of the extract was determined by the colorimetric method using aluminum trichloride, as described by Quettier-Deleu *et al.* (2000) [17]. A sample of 1.00 mL of extract or standard quercetin (Q) (prepared in methanol) is added to 1.00 mL of a freshly prepared solution of  $\text{AlCl}_3$  (2% in methanol (mass/volume)). After 10 minutes of reaction, the absorbance is read with a spectrophotometer (LLG-uni spec2spectrophotometer) at 430 nm. The concentration of total flavonoids was calculated using a calibration range established with standard quercetin prepared in methanol (mass/volume). Results are expressed as milligrams of quercetin equivalent (QE) per gram of extract (mg QE/g of extract). All the tests are reproduced three times.

#### 2.3.3. Total condensed tannin content.

The quantification of condensed tannins (proanthocyanidins) in our extracts was determined according to Sun *et al.*'s 1998 method. In the presence of hydrochloric acid, the condensed tannins depolymerize and, upon reaction with vanillin, turn into red anthocyanins, which can be measured spectrophotometrically at 500 nm [18]. Fifty  $\mu\text{L}$  of the sample or diluted standard, 3.00 mL of 4% vanillin solution in methanol (mass/volume), and 1.50 mL of concentrated HCl were added. The mixture was allowed to stand for 15 minutes, and the absorption was measured at 500 nm against a water/methanol mixture (v/v) as a blank. A calibration curve was produced in parallel under the same operating conditions using catechin (C). The contents of condensed tannins are expressed in milligrams of catechin equivalent per gram of extract (mg CE/g extract). The sample was analyzed in triplicate.

#### 2.4. Evaluation of antioxidant activity.

##### 2.4.1. DPPH free radical scavenging assay.

The evaluation of the antioxidant activity is carried out by the Scavenger method of the DPPH<sup>•</sup> radical (2,2-diphenyl-1-picrylhydrazyl). It is a relatively stable free radical, purple in color, with a maximum absorption band at 517 nm in methanol. In the presence of a hydrogen-donating antioxidant (HA), it is reduced to 2,2-diphenyl-1-picrylhydrazine (DPPH-H) and changes color to yellow. 1.80 mL of a methanolic solution of DPPH of concentration 0.004% (w/v) and 0.20 mL of various concentrations of methanolic extract or of standard were added. After 30 minutes in the dark, the absorbance of the mixture is measured at 517 nm spectrophotometrically. The decrease in absorbance indicates an anti-radical effect of the test substance [19]. The IC<sub>50</sub> (50% inhibitory concentration), also called EC<sub>50</sub> (Efficient concentration 50), is the concentration of the sample (or standard) tested necessary to reduce 50% of the DPPH<sup>•</sup> radical. The IC<sub>50</sub> is calculated graphically from the percentage inhibition at different concentrations of the extracts tested [20]. IC<sub>50</sub> values were determined graphically by linear regression. A low IC<sub>50</sub> value indicates high antioxidant activity. The experiment was done in triplicate.

##### 2.4.2. Ferric reducing antioxidant power assay (FRAP).

The reducing power of iron (Fe<sup>3+</sup>) in the extracts is determined according to the method described by Oyaizu [21]. One milliliter of the extract at different concentrations is mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a solution of potassium ferricyanide K<sub>3</sub>Fe(CN)<sub>6</sub> at 1%. The whole is incubated in a water bath at 50°C for 20 min, then 2.5 mL of 10% trichloroacetic acid is added to stop the reaction, and the tubes are centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of supernatant is combined with 2.5 mL of distilled water and 0.5 mL of an aqueous solution of (FeCl<sub>3</sub> · 6H<sub>2</sub>O) at 0.1%. The reading of the absorbance of the reaction medium is done at 700 nm against a similarly prepared blank, replacing the extract with distilled water, making it possible to calibrate the device (UV-VIS spectrophotometer). The positive control is a solution of a standard antioxidant, whose absorbance was measured under the same conditions as the samples. The experiment was done in triplicate.

##### 2.4.3. ABTS radical cation scavenging assay.

The solution of ABTS radical cation (ABTS<sup>•+</sup>) is prepared by mixing 2 mM of ABTS with 70 mM of a solution of potassium persulfate (v/v). The mixture is left to stir for 24 hours in the dark and at room temperature before use. This solution is then diluted with methanol to obtain an absorbance of 0.700 (± 0.02) at 734 nm. 2 mL of this solution and 200 µL of extract or positive control are added after 30 min; the absorbance at 734 nm is noted [22]. The experiment was done in triplicate.

##### 2.4.4. β-Carotene bleaching assay.

The antioxidant activity of the aqueous solution was determined by a β-carotene/linoleic acid system [23]. Briefly, 1 mL of β-carotene solution (2 mg/mL in chloroform), 40 µL of linoleic acid, and 400 µL of Tween 20 were transferred to a round-bottom flask. The chloroform from the mixture was evaporated using a stream of nitrogen.

Then, distilled water (100 mL) was slowly added to the residue and vigorously stirred to give a stable emulsion. A 2.5 mL aliquot of this emulsion was added to 500  $\mu$ L of the methanolic solution of *Hammada scoparia* prepared at different concentrations. To the control reaction mixtures, 500  $\mu$ L of distilled water was added. Absorbance was measured immediately at 470 nm. The tubes were placed in a 50°C water bath, and the absorbance was measured after 120 min. The experiment was done in triplicate.

## 2.5. Evaluation of antimicrobial activity.

### 2.5.1. Microorganisms.

Microorganisms used in this study are pathogenic species obtained from Oued Nouné Laboratory for Medical Biology Analysis, Guelmim, Morocco, and consisted of five bacterial species, namely *Enterococcus faecalis*, *Proteus mirabilis*, *Streptococcus spp*, *Escherichia coli*, *Staphylococcus coagulase-negative*, and a yeast: *Candida albicans*. Purity, viability, and identification of the organisms were checked by plating, Gram staining, and the Vitek 2 System (Biomérieux Vitek).

### 2.5.2. Solid medium diffusion method.

Antimicrobial activity was assessed using the diffusion method on Mueller-Hinton (MH) and Sabouraud (SB) agar media according to CLSI recommendations [24]. Thus, the media (MH) and (SB) are uniformly inoculated with a sterile swab using a saline suspension (NaCl 0.9%) of the strain to be studied, previously adjusted to the 0.5 McFarland standard (equivalent to  $1 \times 10^8$  cfu/mL). The inoculum can be adjusted by adding either sterile physiological water if it is too strong or culture if it is too weak. This solution represents the bacterial inoculum that will be used for the entire study. Whatman absorbent paper discs 5 mm in diameter are sterilized in an autoclave (121°C for 15 minutes). They are soaked in the vegetable waters to be tested (introduced into the bottles containing the evaporated vegetable waters of different concentrations). The discs thus prepared are deposited on the media (MH) and (SB) after 24 hours of incubation at 37°C for bacteria and 48 hours at 25°C for yeasts. The diameter of the inhibition zones is measured using a ruler. The manipulations are repeated 3 times for each test.

### 2.5.3. Determination of minimum inhibitory concentration (MIC).

The minimum inhibitory concentration (MIC) is determined using the 96-well microdilution technique with brain-heart broth: Brain Heart Infusion (BHI) [25]. A range of concentrations is prepared by diluting the extract in a base-2 series. 25  $\mu$ L of the dilutions is distributed in wells 1 to 10 of the microplate. Wells 11 and 12, respectively, represent the bacterial culture control (culture medium and inoculum) and the sterility control of the BHI culture medium (nutrient broth only). The microbial inoculum is prepared in 5 to 10 mL of sterile physiological water from pure 24-hour cultures. The suspension density, measured on a densitometer, is adjusted to 0.5 MacFarland ( $10^8$  CFU/mL). The microplate wells are inoculated with a microbial suspension, except the 12th column, which serves as a negative control.

5  $\mu$ L of the microbial suspension, except for the 12<sup>th</sup> column, serves as a negative control. 70  $\mu$ L of BHI medium are distributed in the 96 wells of the microplate. The plates are

then incubated at 37°C for 24 hours. After incubation, any growth is indicated by turbidity at the bottom of the well. The MIC is defined as the minimum concentration of extract for which no growth visible to the naked eye is observed.

#### 2.5.4. Determination of minimum bactericidal concentration (MBC).

The minimum bactericidal concentration (MBC) was determined by plating directly 100 µL of the contents of wells with concentrations above the MIC. The MBC was determined after incubation for 24 hours at 37°C, and the lowest concentration that completely inhibited growth was considered the MBC. The antibacterial effect was judged to be bactericidal or bacteriostatic, depending on the ratio of MBC/MIC. Indeed, if  $MBC / MIC \leq 4$ , the effect is bactericidal, and if  $MBC/MIC > 4$ , the effect is bacteriostatic [26].

### 3. Results and Discussion

#### 3.1. Total phenolic, flavonoid, and tannin contents.

Plant-derived natural products, whether as pure compounds or standardized extracts, have attracted attention for their many health benefits and offer numerous opportunities for new drug discovery [27-29]. Natural products possess structural complexity, diversity, and chirality with attractive functions and biological activities that have significantly impacted drug development initiatives [30].

Preliminary tests to estimate the phenolic content in the aerial parts of *Hammada scoparia* (Pomel) Iljin enable assessment of the biochemical quality of this plant species. In other words, these tests revealed the presence of a significant amount of these secondary metabolites and suggested that the plant may have medicinal importance. The phytochemical constituents of HS were extensively investigated, and the structures of the main bioactive molecules were determined. It is a plant particularly rich in alkaloids (carnegine and N-methylisosalsole) and flavonoids (isorhamnetin triglycerides), as reported by Bourogaa *et al.* and Ben Salah *et al.* [31,32]. Qiu *et al.* [33] determined the potential renoprotective effects of isorhamnetin in a type 2 diabetic rat model. Indeed, plants rich in phenolic metabolites exhibit biological activities such as antiviral, antithrombotic, anticancer, antiallergic, antimicrobial, hepatoprotective, and antihypertensive. As shown in previous studies, plants rich in phenolic metabolites exhibit biological activities such as antiviral, antithrombotic, anticarcinogenic, antiallergic, antimicrobial, hepatoprotective, and antihypertensive [34,35]. Additionally, many natural compounds with anticancer properties are phenols and flavonoids that can influence cell cycle progression [36-38].

The results of the assay of the phenolic compounds of the aerial part of *Hammada scoparia* are grouped in Table 1.

**Table 1.** Contents of phenolic compounds in the aerial parts of *Hammada scoparia*

The phenolic compounds	Total phenolic (µg GAE /mg)	Total flavonoids (µg QE/mg)	Total condensed tannin (µg CE/mg)
Content	79.50 ± 3.33	21.16 ± 4.50	55.00 ± 7.66

According to our results, it appears that the content of total phenols in our methanolic extract of HS (79.50 ± 3.33 µg GAE/mg of extract) has a lower content than that of Benkherara *et al.* (2018) (228.582 ± 0.689) µg GAE /mg Dry Matter(DM) [39] and better than that of

Bouaziz *et al.* (2016) ( $59.75 \pm 1.80$ )  $\mu\text{g GAE/mg}$  [40]. While the total flavonoid content of the methanolic extract of the same species is ( $21.16 \pm 4.50$   $\mu\text{g QE/mg}$  of extract), this value is higher than those of Benkherara *et al.* 2018 ( $17.056 \pm 0.108$ )  $\mu\text{g Rutin equivalent (RE)/mg DM}$  [39] and lower than those of Alghazeer *et al.* ( $81.07 \pm 6.14$ ) ( $\mu\text{g RE/mg DM}$  [41]. In addition, it is important to emphasize that our methanolic extract of HS has a content of condensed tannins higher than those Belhadj Tahar *et al.* of the fraction butanol ( $2.862 \pm 0.012$ )  $\mu\text{g CE/mg DM}$  [42], i.e. around  $55.00 \pm 7.66$   $\mu\text{g CE/mg}$  of extract and also higher than those of Benkherara *et al.* ( $0.958 \pm 0.052$ )  $\mu\text{g CE/mg DM}$  [39]. In general, phenolic compounds are among the most effective antioxidant sources, and they are dominant in plants, as their content can be extracted more readily, and a direct relationship between total phenolic content has been highlighted, highlighting the importance of structure-activity relationships in the field of natural antioxidants [43].

### 3.2. Evaluation of antioxidant activity.

This study evaluates the antioxidant activity of the extract from the aerial parts of *Hammada scoparia* (Pomel) Iljin using four methods: DPPH, ABTS, FRAP, and the  $\beta$ -carotene bleaching test. These methods are distinguished by their mechanisms of action and would be complementary to the study of the antioxidant potential of plants (Table 2).

#### 3.2.1. DPPH Free radical scavenging activity.

The lower IC<sub>50</sub> value indicated the plant extract's higher antioxidant activity [44].

According to the values reported in Table 2, it is important to emphasize that the extract of *Hammada scoparia* (has a very powerful antioxidant power( $0.047 \pm 0.007$  mg/mL) but remains less important than the standard antioxidants, ascorbic acid ( $0.021 \pm 0.001$ mg/mL). By comparing our results with those of the literature, let us quote, for example, the IC<sub>50</sub> of our methanolic extract of *Hammada scoparia* ( $0.047 \pm 0.007$  mg/mL) is higher than those obtained by Bouaziz *et al.* ( $28 \pm 0.70$   $\mu\text{g/mL}$ ) [40] but this lower than those of Bakchiche *et al.* ( $114 \pm 1.50$   $\mu\text{g/mL}$ ) [45], in other words, our extract has a lower antioxidant power than those of Bouaziz *et al.* [40] and powerful to those of Bakchiche *et al.* [45].

#### 3.2.2. Ferric reducing antioxidant power assay (FRAP).

According to the values grouped in Table 2, it is clearly apparent that the *Hammada scoparia* extract has excellent antioxidant power ( $0.042 \pm 0.002$  mg/mL) but remains less important than the positive control, ascorbic acid ( $0.022 \pm 0.004$  mg/mL). It is suggested that the reducing power of our extract is due to the presence of hydroxyl groups in the phenolic compounds, which can serve as electron donors. Therefore, antioxidants are considered reducers and inactivators [29].

#### 3.2.3. ABTS radical cation scavenging test.

In light of the research results reported in Table 2, we see that the extract of *Hammada scoparia* has good antioxidant activity ( $0.046 \pm 0.005$  mg/mL) but remains lower than that of the reference antioxidant, ascorbic acid ( $0.031 \pm 0.002$  mg/mL). Moreover, the antioxidant effect of the methanolic extract of *Hammada scoparia* is closer to that of Benkherara *et al.* ( $40.506 \pm 0.110$ ) mgTrolox E/g DM [39].

### 3.2.4. $\beta$ -Carotene bleaching test.

According to the values in Table 2, it is important to note that the extract of *Hammada scoparia* has strong antioxidant activity ( $0.042 \pm 0.002$  mg/mL) but remains lower than that of the reference antioxidant, ascorbic acid ( $0.027 \pm 0.005$  mg/mL). In addition, the IC<sub>50</sub> values in the table below demonstrate that our extract is a strong natural antioxidant. Generally, the results of such a study vary depending on the nature of the extraction solvent, its polarity, and the analytical methods used.

**Table 2.** Antioxidant activity of *Hammada scoparia* extract using DPPH, ABTS, FRAP, and  $\beta$ -Carotene bleaching methods.

Sample/Standard	Antioxidant activity tests			
	DPPH IC <sub>50</sub> (mg/mL)	FRAP IC <sub>50</sub> (mg/mL)	ABTS IC <sub>50</sub> (mg/mL)	$\beta$ -Carotene bleaching IC <sub>50</sub> (mg/mL)
Methanolic extract	$0.047 \pm 0.007$	$0.042 \pm 0.002$	$0.046 \pm 0.005$	$0.042 \pm 0.002$
Ascorbic acid	$0.021 \pm 0.001$	$0.022 \pm 0.004$	$0.031 \pm 0.002$	$0.027 \pm 0.005$

The antioxidant activity of the sample varies depending on the solvent used and, in particular, the analytical method. Recent studies have shown that there is no universal method to quantitatively and accurately assess antioxidant activity [31]. In addition, the results of these in vitro tests have given us an idea of the relative antioxidant activity of the extract from the aerial parts of HS. This study confirmed the medicinal potential of the leaves and stems of this halophyte plant species and agrees with the medicinal potential of the Amaranthaceae family demonstrated by several authors [32-34].

The most common tests used are the DPPH and ABTS tests, both based on and, therefore, named after an electron-deficient radical [46] as a stable free radical. DPPH does not need to be freshly prepared like the ABTS radical cation. However, the ABTS test is less prone to interference from colored samples because it uses a higher wavelength [47]. Both are artificial radicals and not physiologically relevant; therefore, only direct reactions of the radical with the antioxidant compound studied are measured [48]. The underlying reaction mechanisms cannot be attributed solely to hydrogen atom transfer (HAT) or single-electron transfer (SET), as both occur in varying proportions depending on the concentration and structure of the test compound, the solvent, and pH. In general, the transfer of electrons is very fast, while the transfer of hydrogen is relatively slow. This initial electron transfer is much faster in the ABTS assay due to the sterically hindered DPPH radical site, which is difficult for phenols to access [49]. Again, this confirms the higher reactivity of the ABTS radical cation [47]. The other test studied is the FRAP test, which is based on a SET reaction. In the presence of an antioxidant, during FRAP testing, Fe<sup>3+</sup> is reduced to Fe<sup>2+</sup>, respectively, resulting in a color change that can be monitored photometrically and is directly correlated to phenolic content and antioxidant capacity [48]. Moreover, the FRAP assay has high sensitivity and accuracy but suffers from non-physiological measurement conditions at low pH [50]. However,  $\beta$ -carotene is nonpolar and highly lipophilic due to its conjugated hydrocarbon structure without a polar functional group [51]. The free radical of linoleic acid is attacked by  $\beta$ -carotene so that it undergoes rapid bleaching as it loses the double bonds and, therefore, its orange color [52].

### 3.3. Evaluation of antimicrobial activity.

#### 3.3.1. Sensitivity test.

The values in Table 3 represent the zone of inhibition measured in millimeters, including the diameter of the paper disc, which was used as the criterion for measuring HS's antimicrobial activity. These results revealed that the extracts studied are slightly more effective against Gram-positive than Gram-negative bacteria, with a diameter of 7-13 mm and 13 mm for the butanol extract.

**Table 3.** Sensitivity of the strains studied to different extracts of HS solvents.

Strains		Zones of inhibition (mm)								
		Dichloromethane extract (mg/mL)			Ethyl acetate extract (mg/mL)			Butanolic extract (mg/mL)		
		50	100	150	50	100	150	50	100	150
Bacteria	<i>Staphylococcus coagulase-negative</i>	0	0	0	0	0	07	07	10	13
	<i>Streptococcus spp</i>	0	0	0	0	0	0	0	07	09
	<i>Enterococcus faecalis</i>	0	0	0	0	0	0	0	08	10
	<i>Escherichia coli</i>	0	0	07	0	0	08	0	07	09
	<i>Proteus mirabilis</i>	0	0	0	0	0	0	0	07	09
Yeasts	<i>Candida albicans</i>	0	0	0	0	0	0	0	0	08

On the other hand, activity has been reported against *Candida albicans* yeast, with a zone of inhibition of 08 mm for the butanol extract. Moreover, Lamchouri *et al.* [53] reported no inhibitory effect of the organic extracts studied (petroleum ether, chloroform, ethyl acetate, methanolic, and aqueous) against the microorganisms tested, except for *Staphylococcus aureus* with the ethyl acetate extract (7-12 mm). In contrast to our results, those obtained by Awaad *et al.* [54], showed good antimicrobial activity of the ethanolic extract of *Hammada scoparia* against several pathogenic bacteria, including *Enterococcus faecalis* (13 mm), *Escherichia coli* (15 mm), *Moraxella lacunata* (12 mm), *Proteus mirabilis* (12 mm), *Serratia marcesens* (10 mm), *Pseudomons aeruginosa* (09 mm), *Salmonella typhi* (12 mm), *Bacillus subtilis* (11 mm), *Micrococcus luteus* (12 mm), *Sarcina ventricull* (09 mm), *Staphylococcus aureus* (10 mm) and pathogenic fungi including *Candida albicans* (10 mm), *Candida tropicalis* (0 mm), *Aspergillus flavus* (09 mm), *Aspergillus fumigates* (08 mm), *Penicillium chrysogenum* (10 mm). However, the plant extracts had higher phenolic content, which did not correlate with the antibacterial effects. The extracts could be combined to achieve broader antibacterial effects [55].

#### 3.3.2. Determination of antimicrobial parameters (MIC and MBC).

The antimicrobial parameters (MIC and MBC) are collated in Tables 4-6.

**Table 4.** Antibacterial parameters of butanolic extract.

Strains		Butanolic extract (mg/mL)		
		MIC	MBC	MBC/MIC
Bacteria	<i>Escherichia coli</i>	50	50	1
	<i>Staphylococcus coagulase-negative</i>	50	100	2
	<i>Enterococcus faecalis</i>	100	100	1
	<i>Proteus mirabilis</i>	100	100	1
	<i>Streptococcus spp</i>	100	100	1
Yeasts	<i>Candida albicans</i>	100	-	-

**Table 5.** Antibacterial parameters of ethyl acetate extract.

	Strains	Ethyl acetate extract (mg/mL)		
		MIC	MBC	MBC/MIC
Bacteria	<i>Escherichia coli</i>	100	100	1
	<i>Staphylococcus coagulase-negative</i>	100	100	1
	<i>Enterococcus faecalis</i>	-	-	-
	<i>Proteus mirabilis</i>	-	-	-
	<i>Streptococcus spp</i>	-	-	-
Yeasts	<i>Candida albicans</i>	-	-	-

**Table 6.** Antibacterial parameters of dichloromethane extract.

	Strains	Dichloromethane extract (mg/mL)		
		MIC	MBC	MBC/MIC
Bacteria	<i>Escherichia coli</i>	50	50	1
	<i>Staphylococcus coagulase-negative</i>	-	-	-
	<i>Enterococcus faecalis</i>	-	-	-
	<i>Proteus mirabilis</i>	-	-	-
	<i>Streptococcus spp</i>	-	-	-
Yeasts	<i>Candida albicans</i>	-	-	-

According to the results collated in the tables above, the minimum inhibitory concentrations are high for the organic extracts of Hammada scoparia, ranging from 50 to 100 mg/mL. Similarly, MBC oscillates between the same values of 50 and 100 mg/mL. Through these results, it is important to emphasize that the butanol extract is bactericidal ( $MBC/MIC \leq 4$ ) against all microorganisms except *Staphylococcus coagulase-negative*, while the Ethyl acetate extract is bactericidal against *Escherichia coli* and *Staphylococcus coagulase-negative*. At the same time, the dichloromethane extract is bactericidal, but only against *Escherichia coli*. A study on the same species from Tunisia, conducted by Bouaziz et al. [40], demonstrated that different alcoholic and organic extracts of Hammada scoparia exhibit antibacterial activity against a range of bacteria, except the hexane extract. For example, the sensitivity of *Escherichia coli* ATCC 25922 to hydroethanolic extracts (1: 9; v/v), dichloromethane, methanol, and the alkaloid "Carnegine" isolated from crude plant extract,  $18 \pm 0.5$  mm,  $16 \pm 0.5$  mm,  $18 \pm 0.6$  mm,  $22 \pm 1.1$  mm, respectively. At the same time, the sensitivity of *Enterococcus faecalis* ATCC 29212 to the same extracts is  $8 \pm 0.1$  mm,  $12.3 \pm 0.4$  mm,  $12 \pm 0$  mm, and  $18 \pm 0.6$  mm, respectively. However, the MIC values of carnegine ranged from 0.125 to 0.5 mg/mL, and the MBC was from 0.25 to 2 mg/mL. The most sensitive microbe for their study was Gram-negative *Escherichia coli*. In addition, they were obtained for N-methylisosalsole, with large MIC (0.5 to 4 mg/mL) and MBC (2 to  $\geq 10$  mg/mL).

Distinctive phenolic compounds have been reported to have different effects on Gram-negative and Gram-positive bacteria [56] because they have distinct mechanisms of action. The antibacterial mechanism of phenolic compounds is mainly attributed to their ability to generate hydrogen peroxide, which, when coupled with their capacity to complex metal ions, inhibits the activity of essential enzymes and destabilizes bacterial membranes, increasing their permeability [57]. The antimicrobial effect of plant extracts is generally due to phenolic compounds with free hydroxyl groups, such as flavonoid compounds, which can act in several ways: forming bonds with extracellular and soluble proteins, glutamate, and phosphate of bacteria; altering cellular peptidoglycan; disrupting bacterial membrane permeability; inhibiting vital enzyme pathways; binding to the active site of enzymes; forming hydrogen bonds with enzymes; and altering enzyme metabolism [58]. However, Gram-negative bacteria are generally more resistant to antimicrobials than Gram-positive bacteria because they have an additional outer membrane that can protect them from antimicrobial compounds [59]. Additionally, outer membrane proteins of Gram-negative bacteria, such as porins, allow the

passage of small molecules, which can serve as potential entry points for hydrophilic antibiotics, so mutations or downregulation of porins can confer antimicrobial resistance [60]. Although there is no such selective barrier in Gram-positive bacteria, they use other strategies, such as thickening their peptidoglycan cell wall to prevent antibiotics from reaching their targets or producing enzymes that degrade antibiotics [61].

#### **4. Conclusion**

Recent interest in phenolic compounds has grown significantly due to their antioxidant capacity and potential benefits for human health. In addition, in scientific research, particular interest has been shown in herbal products known in traditional medicine to have beneficial effects on microbial infections. At the end of this work, it turns out that the extract of *Hammada scoparia* has a stronger antioxidant activity for the FRAP and  $\beta$ -Carotene bleaching method, with the same value of  $IC_{50}$  ( $0.042 \pm 0.002$  mg/mL) against  $IC_{50}$  ( $0.021 \pm 0.001$  mg/mL) for the standard antioxidant by DPPH free radical scavenging Test. In contrast, the contents of phenolic compounds are high, with contents of total phenols ( $79.50 \pm 3.33$   $\mu$ g GAE/mg), total flavonoids ( $21.16 \pm 4.50$   $\mu$ g QE/mg), and total condensed tannins ( $55.00 \pm 7.66$   $\mu$ g CE/mg). In addition, the organic extracts show moderate efficacy against gram-positive bacteria, especially the butanol extract, which shows a zone of inhibition of 13 mm against *Staphylococcus coagulase-negative*. Thus, the results of this study provide informative data on the traditional use of *Hammada scoparia* as an antiseptic and could serve as natural antioxidants.

#### **Author Contributions**

Methodology, S.T. and M.T.; software, M.A. and A.Z.; validation, S.T. and M.T.; formal analysis, M.A.; investigation, M.A., F.F., A.C., and N.C.; resources, M.A., F.F., A.C., and N.C.; writing—original draft preparation, M.A., F.F., A.C., N.C., and A.Z.; writing—review and editing, M.A., S.T., M.T., and A.Z.; visualization, M.A.; supervision, S.T. and M.T. All authors have read and agreed to the published version of the manuscript.

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#### **Data Availability Statement**

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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## Conflicts of Interest

The authors declare no conflict of interest.

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