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Phytochemical Screening and Biological Activity of Leaves and stems extract of *Hammada Scoparia*

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Abstract

The antioxidant and antibacterial activities of alcoholic extract from wild Moroccan *Hammada Scoparia* leaves and stems were assessed. Phytochemical analysis revealed the presence of active ingredients such as Phenols, Flavonoids, Tannins, Alkaloids, Steroids and Saponins. Total content of phenol and flavonoid was quantitatively estimated in leaf and stem extracts of *Hammada Scoparia*. Total phenoilc content in the leaf and stem was found to be 50,08±1,09and 14,3±0,5 (mg EAG/g DM), while the total flavonoid content in the leaf and stem were found to be 28,8±0,07 and 5,1±0,2 (mg EQ/g DM) respectively. The antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl radical-scavenging DPPH assay, iron-reducing-power FRAP assay and inhibition test of the cation radical ABTS assay, the antioxidant activity showed that leaves extracts exhibited strong antioxidant activity. A linear relationship has been found between the total phenolic content and the antioxidant activity of the studied extracts. The extracts exhibited strong antibacterial activity against *Citrobacter freundii* and *Acinetobacter baumanii*. The stem extracts was more active than the leaf extracts against test bacteria with Inhibition zone diameters ranging from 7 to 20 mm and the leaf extracts from 7 to 13 mm. *Hammada Scoparia* leaves and stems had antiradical and antimicrobial activity that may be useful in pharmacy and phytotherapy.

Keywords: Antibacterial, Antioxidant, DPPH, *Hammada Scoparia*, MBC, Phytochemical, TPC.

1.Introduction

Because of its particular geographical situation, Morocco benefits from a bioclimate allowing a rich and diverse vegetation. The valorization of these plant resources in the form of dry plants, essential oils or extracts is a good alternative to diversify agricultural production. It also remains an employment and income generating activity for the rural population and a source of foreign exchange for the country's economy. *Hammada Scoparia* is a medicinal plant, belonging to the family Chenopodiaceae, which comprises more than one hundred genera grouping about a thousand species. This plant is distributed in the salt soils, lives in an arid or semi-arid climate and originates from the dry regions of the Middle East and Maghreb. Currently, it is distributed in southeastern Spain, North Africa and parts of Iran, Turkey, Iraq and Syria [1]. In Morocco, the plant grows spontaneously in the south. *Hammada Scoparia* has several synonyms: *Hammada scoparia* (Pomel) Iljin., *Arthrophytam scoparium* (Pomel) Iljin., *Salsola articulata* av., *Haloxylon articulatum* (Cav.) [2]. research on the species *Hammada Scoparia* has shown that the latter is rich in alkaloids [3], saponosides [4], and flavonoids [5]. Recently, two alkaloids, carnegine and N-methylisosalsoline, were isolated from leaf extracts of *Hammada Scoparia [6]*. Traditionally, infusion of powder from the aerial part of *Hammada Scoparia* is used for its antidiabetic, antiseptic and anti-inflammatory effect [7]. The plant is used to treat eye disorders. It is also used against scorpion stings. The stems are used as a mordant for dyeing wool in traditional weaving [8]. The researchers have demonstrated that *Hammada Scoparia* has antiproliferative activity and a larviciding effect [9]. It also showed an antidiabetic effect and anticoagulant activity in laboratory animals [10]. Therefore, this research was conducted to evaluate the presence of antioxidant compounds (Polyphenol and flavonoid) and antioxidant activity in hydroalcoholic extract of leaf and stem of *Hammada Scoparia*, which may have the potential to be developed as natural compound-based products by the nutraceutical and pharmaceutical industries.

2. Materials and methods

2.1 Plant material

Hammada Scoparia was collected from its natural habitat in the region of Tazarine (a small town in southern Morocco), province of Zagora, Souss-Massa, during the month of September 2016. The region is known for its Saharan desert climate type, characterized by aridity. The temperature can reach 45 ° C in summers, while in winter it varies between 10 °C and 18 °C.

2.2 Preparation of the extract

The crude extracts of stems and leaves of *Hammada Scoparia*were obtained by extraction in an ultrasonic bath for one hour at room temperature, using solvent of water-ethanol (20: 80, v: v).

2.3 Phytochemical screening

The qualitative assay of the major metabolite of families such as Triterpenoids, Steroids, reducing sugars, Saponins, Alkaloids, Flavonoids, Tannins and Proteins, was focused on two crude hydro-alcoholic extracts of leaves and stems, all based on the staining reactions and precipitation [11, 12].

2.4. Total phenolic and flavonoid content

2.4.1 Determination of total phenolic content

The amount of total phenolic contents was determined according to Folin-Ciocalteu method as described by Lister and Wilson [13]. Briefly, 0.5 ml of sample solution was mixed with 2.5 ml of Folin-Ciocalteu reagent diluted with distilled water 1:10, followed by the addition of 4 ml of Na_2CO_3 (7.5 %, w/v). The mixture is then incubated in a water bath at

45°C for 30 min and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer against a blank sample. The standard curve of Gallic acid is obtained under the same conditions as above using a range of concentrations. The total phenolic content was measured as Gallic acid equivalents (mg GAE/g DM).

2.4.2 Determination of flavonoids content

Flavonoid contents were measured using a modified colorimetric method [14]. 0.25 mL of extract solution was added to a test tube containing 1.25 mL of distilled water. Sodium nitrite solution (5%, 0.075 mL) was added to the mixture and maintained for 5 min. Then, 0.15 mL of 10% aluminum chloride was added. After 6 min, 0.5 mL of 1 M sodium hydroxide was finally added. The mixture was diluted with 0.275 mL of distilled water. The absorbance of the mixture at 510 nm was measured immediately in comparison to a standard curve prepared by quercetin. The flavonoid contents were expressed as mg quercetin equivalent (QE)/g of dried material.

2.4.3 Determination of tannins content

Contents of tannins was determined by the procedure of Sun et al. [15]. Five hundred microliters of extract solution was mixed with 3mL of 4% vanillin–methanol solution and 1.5mL hydrochloric acid. The mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm, while the final result was expressed as mg catechin equivalent (CE)/g dry matter.

2.5. Antioxidant activity

2.5.1. Free radical scavenging activity (DPPH)

The free radical scavenging activity of the plant extracts was measured by 1.1-diphenyl-2-picryl-hydrazil (DPPH) [16], with some modifications. Briefly, 0.2 mM solution of DPPH in ethanol was prepared and 0.5 ml of this solution was added to 2.5 ml of plant extract and was allowed to stand at room temperature for 30 min, and then absorbance was read at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The IC_{50} value is used to express the results obtained by DPPH it is defined as the antioxidant quantity needed to lower the radical to 50%. It is inversely related to the antioxidant capacity. The low values of IC_{50} indicate higher extract antioxidant power efficiency.

2.5.2. ABTS radical scavenging assay

The scavenging activity of extracts against ABTS radical was determined [17]. Briefly the stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate ($K_2S_2O_8$) in equal volumes were allowed to stand in the dark for 12-16 h at room temperature. Prior to assay, ABTS solution was diluted in ethanol to give an absorbance of 0.7 ± 0.02 at 734 nm. 2 ml of the resulting solutions was allowed to react with 200µl of the plant extracts with different concentrations, reaction mixture was vortexed and absorbance was measured at 734 nm after 30 min. The same was done for the trolox of various concentrations. The percentage inhibition of $ABTS^{+}$ by the different extracts was calculated and compared with trolox. Inhibition Concentration (IC_{50}) parameter was used for the interpretation of the results from $ABTS^{\cdot+}$ method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC_{50} value. It is defined as the amount of sample necessary to decrease the absorbance of $ABTS^{\cdot+}$ by 50 %.

2.5.3 Determination of reducing antioxidant power (FRAP)

The ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) method [18] was used to measure the reducing capacity of the plant extracts with a slight modification, which involves the presence of extracts to reduce the ferricyanide

complex to the ferrous form. Various concentrations of extracts from the stock solutions and the standard (Trolox) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 min. Then 2.5 ml of trichloroacetic acid (10% w/v) was added to the reaction mixture. Afterwards, it was centrifuged at 3000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with deionized water (2.5 ml) and 0.5 ml of ferric chloride (0.1% w/v). The absorbance was measured at 700 nm at the reaction time of 30 min. The reducing power of the extracts was represented as trolox equivalent (mg TE/g DM).

2.6 Evaluation of antibacterial activity

The ten microbial strains to be tested (*Enterococcus faecalis, Salmonella sp, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Staphylococcus epidermidis, Citrobacter freundii, Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa*) were cultured in petri dishes containing nutrient agar. After 24 hours of incubation at 37 °C, bacterial suspensions with an optical density of 1 McFarland were prepared for each strain in 5 ml of sterile water. Evaluation of the antibacterial activity of the extracts of stems and leaves of *Hammada Scoparia* was carried out using two different methods:

2.6.1 Solid-state diffusion technique (disk method)

The method of diffusion from a solid disk was used to demonstrate the antibacterial activity of these microorganisms regarding to the extracts.

The organic extracts are solubilized in DMSO. The sui**Table** Muller Hinton agar is poured into petri dishes and inoculated with freshly prepared pure microbial suspension. A sterile Whatman paper disk is impregnated with 30 µl of extract (reconstituted according to the desired concentration) and then deposited on the seeded agar; the dishes are incubated at 37 °C for 24 hours. A uniform diffusion of the extract is observed as soon as the impregnated discs are applied, and after 24 hours of incubation, the presence around the discs of a circular inhibition zone in which there is no growth of microorganisms denotes the sensitivity of these to this extract. The larger the zone of inhibition, the more sensitive the germ [19].

2.6.2 Microdilution technique in a solid medium

Dilutions of the extracts are prepared at $1/10^{e}$, $1/25^{e}$, $1/50^{e}$, $1/100^{e}$, $1/200^{e}$, $1/300^{e}$ and $1/500^{e}$ in a 0.2% agar solution. In test tubes each containing 13.5 ml of autoclave sterilized Mueller Hinton medium and cooled to
45 °C, aseptically added 1.5 ml of each of the dilutions so as to obtain final concentrations of 1/100 , 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 (v / v). The tubes were stirred in order to disperse the extract well into the culture medium before pouring them into the petri dishes. Controls containing the culture medium plus the 0.2% agar solution alone are also prepared [20]. The lowest concentration of extract inhibiting any growth visible to the naked eye after 16 to 20 hours of incubation at 37 °C is called the minimum inhibitory concentration denoted MIC. [21]We determined the minimum bactericidal or MBC concentration by inoculating a sample of the non-growth boxes on Mueller-Hinton agar. The lowest extract concentration at which 99.99% of the bacteria are killed, after 24 hours of incubation at 37 °C corresponds to MBC [21]. Each test was repeated three times to minimize the experimental error.

3. Results and Discussions

The calculated yields of extracts from dry weight of leaves and stems part of *Hammada Scoparia* plants in hydroalcoholic solvents are shown in **Table** 1. The yields of leaves extracts (18%) were higher than stems extracts (10.55%). These indicated that the polar substances in the leaves of *Hammada Scoparia* are more than the stems.

Table 1: Yields of water/ethanol extracts of *Hammada Scoparia* (**Values are given as means of three replicates ± SD)**

Extract	Yield (%)
Leaves	18,00±0,24
Stems	10,55±0.14

The results of the phytochemical screening carried out on the leaf and stems extracts of *Hammada Scoparia* are mentioned in **Table** 2. Analysis of these results shows that the extracts studied are very rich in phenolic compounds such as flavonoids, catechic tannins and anthocyanins. It turns out that the extract of leaves and stems of *Hammada Scoparia* are also rich in alkaloids, saponins and sterols. On the other hand, the preliminary analysis shows the absence of quinons, proteins, coumrins and reducing sugar. The presence of different phyto-constituents in leaf and stem extracts may be responsible for the medicinal properties of *Hammada Scoparia*. For example, alkaloids have been reported as, anti-inflammatory [18] antimicrobial [22]. Similarly, sterols derived from plants are known to have a cardiotonic effect and also possess antibacterial and insecticidal properties [23]. The presence of phenolic compounds (total phenols, tannins and flavonoids) provides pharmacological activities such as anti-cancer [24], antioxidant [25], antimicrobial [26] and anti-inflammatory [27], which may suggest association with the species here studied.

Table 2: Phytochemical screening of water/ethanol extracts obtained from of *Hammada Scoparia*

Compound	**Leaves**	**Stems**
Sterols, polyterpenes	+ +	+ +
catechic tannins	+ +	+ +
hydrolysable tannins	+ +	+
Flavonoids	+ + +	+ + +
Saponins	+ +	+
Reducing sugar	–	–
Coumarins	–	–
Alkaloids	+ +	+ +
Quinons	–	–
Proteins	–	–

(+) = Present; (++) = Abundant; (+++) = Very abundant; (-) = Absent

The determination of the TPC, TFC and TTC, of the hydro-alcoholic extract of *Hammada Scoparia* stems and leaves, showed that the leaves (TPC: 50.08 EAG/g DM, TFC: 28.812 EQ/g DM, and TTC: 9.247 mg ECa/g DM) are richer in those compounds than the stems (TPC: 14.310 EAG/g DM, TFC: 5.134 EQ /g DM, and TTC: 4.377 ECa/g DM). The hydroalcoholic extract (70%) of the aerial part of *Hammada Scoparia* was studied by Miguel et *al.* The following values was found (TPC: 12.338 mg EAG / g DM, TFC: 4.500 mg QE / g DM) [28]. Also, the results reported by Rached et *al.*, On the TPC assay and the TFCs of the aerial part of *Hammada Scoparia*, showed that this part contained TPC: 37.31 mg EAG / g DM, TFC: 12.3 mg RE / g DM [29].

Table 3: Total phenolic content (TPC), flavonoid content (TFC) and catechic tannins (TTC) of water/ethanol extracts of *Hammada Scoparia*

Parts	TPC (mg EAG/g DM)	TFC (mg EQ/g DM)	TTC (mg ECa/g DM)
Leaves	50,08±1,092	28,812±0,075	9,247±0,369
Stems	14,310±0,533	5,134 ±0,200	4,377±0,214

Values are given as means of three replicates ± SD

Evaluation of the antioxidant activity of the species *Hammada Scoparia* was carried out, using three different techniques, DPPH, ABTS, and FRAP. **Table** 4 shows the results obtained. Compared with the positive test of the trolox, the results obtained showed an interesting activity of the species *Hammada Scoparia*. The inhibition of the DPPH radical showed that the extract obtained from the leaves inhibited 50% of the DPPH radical at a concentration of 7.06 µg/ml while that of the stem at a concentration of 23.416 µg/ml. The study of Miguel et al showed that the IC50 of the inhibition of the DPPH radical by the hydroalcoholic extract (70%) of the aerial part of *Hammada Scoparia* is of the order of 1.867 µg / ml [28]. According to Bakchiche and Gherib the IC50 to inhibit DPPH radical by the Hydroalcoholic extract (70%) of *Hammada Scoparia* is 0.044 µg / ml [30]. The results obtained by the inhibition test of the cation radical ABTS, showed that the hydro-alcoholic extract of the leaves (IC50 = 63.6 µg/ml) presents a more advantageous antiradical potential than that obtained by the stem (IC50 = 138.9 µg/ml). Studies by Miguel et *al* reported that the antioxidant activity by ABTS test of the hydro-alcoholic extract of the aerial part of *Hammada Scoparia* was in of the order of, IC50 = 1.018 µg/ml [28]. Evaluation of antioxidant potency by the FRAP test revealed a similar profile to the DPPH and ABTS test. The FRAP test also showed that the leaves (67.2 mg Trolox equivalent/g DM) are richer in antioxidant compounds than the stem (15.2 mg Trolox equivalent/g DM). The results obtained by the DPPH, ABTS, FRAP tests and the TPC, TFC, TTC assays were in agreement. Indeed, according to Mi-YaeShon et *al.*, The antioxidant activity is mainly due to the presence of the phenolic compounds [31].

Table 4: Antioxidant activity of *Hammada Scoparia* leaves and stems extracts. (Values are given as means of three replicates ± SD)

Extracts / Assay	Leaves	Stems	Trolox
DPPH (IC_{50} µg/ml)	7,060±0,091	23,416±0,194	1.279±0.019
ABTS (IC_{50} µg/ml)	63,683±0,645	138,949±0,408	25.298±0.274
FRAP (mg equivalent Trolox/g DM)	67,286±2,380	15,205±0,677	-

The experimental results presented in the figure 1 show that the extracts of two parts were studied (stems and leaves) of the plant *Hammada Scoparia* are active against the major of pathogen strains tested manifested with inhibition zone diameter superior 7 mm. The stems extract tested of *Hammada Scoparia* demonstrated a high antibacterial activity against the *Citrobacter freundii* represented with inhibition zone diameter (20mm) compared with the strains *Acinetobacter baumanii, Enterococcus faecalis, Staphylococcus epidermidis* and *Salmonella* sp strains with medium inhibition zone diameter (9 mm to10 mm), the strains *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis* and *Staphylococcus aureus* with slightly medium diameter (7 mm to 8 mm). The leaves extract of Hammada Scoparia also show antibacterial effect against the bacteria tested. A high effect against the bacteria *Citrobacter freundii* with high inhibition zone diameter (13mm) and medium effect against *Staphylococcus*

epidermidis, *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumanii*, *Salmonella* sp, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Proteus mirabilis* with inhibition zone diameter between 7mm to 9mm. Comparing with *Hammada Scoparia* stems extract and leaves extract the results show the same antibacterial effect against three strains the *Proteus mirabilis*, *Escherichia coli* and *Staphylococcus epidermidis*, while there is no antibacterial effect was detected against *Pseudomonas aeruginosa* strain.

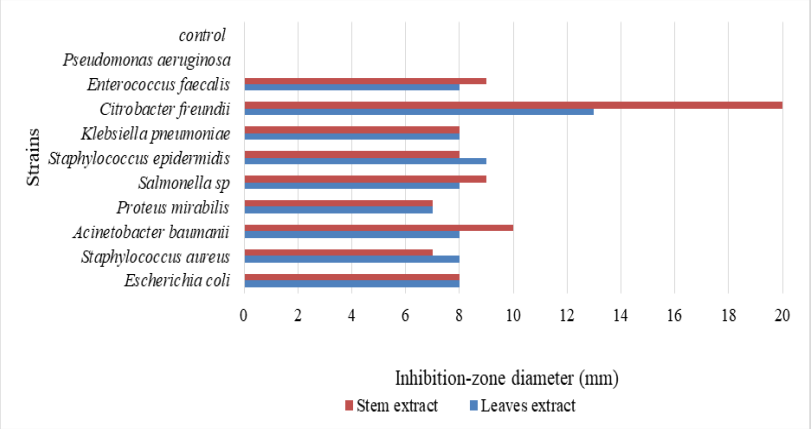

Figure 1: Inhibition zone diameters determined with the Disc-Diffusion Method of *Hammada Scoparia*.

Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by the agar dilution method of the stems extract and leaves extract of *Hammada Scoparia*, the results are summarized in the **Table** 5 and 6. According to the results obtained, the stem extract is bactericidal (CMB/CMI=1) for *Acinetobacter baumanii*, *Escherichia coli*, *Salmonella sp*, *Klebsiella pneumoniae*, *Enterococcus faecalis*. But it's only bacteriostatic (CMB/CMI≥2) for *Citrobacter freundii*, *Staphylococcus aureus, and Staphylococcus epidermidis*. Concerning leaf extract, the results show that it is bactericidal for *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae.* But it is bacteriostatic for *Salmonella sp*, *Citrobacter freundii.*

Table 5: Minimum inhibitory (MIC) and minimum bactericidal (MBC) and rapport CMB/CMI of the stems extract of Hammada Scoparia

Strains	**CMB**	**CMI**	**CMB/CMI**
Acinetobacter baumanii	*1/250*	*1/250*	*1*
Escherichia coli	*1/100*	*1/100*	*1*
Salmonella sp	*1/250*	*1/250*	*1*
Klebsiella pneumoniae	*1/100*	*1/100*	*1*
Citrobacter freundii	*1/1000*	*1/3000*	*3*
Staphylococcus aureus	*1 /100*	*1/250*	*2.5*
Staphylococcus epidermidis	*1/100*	*1/250*	*2.5*
Enterococcus faecalis	*1/100*	*1/100*	*1*

We also observed that neither leaf nor stem extract had an effect on *Proteus mirabilis* and *Pseudomonas aeruginosa.* In the present study we have shown the antibacterial activity of two parts of *Hammada Scoparia* plant against different stains recognized by these noxious effects for humans. Highest antibacterial activity has being demonstrated against the different an opportunistic pathogenic strains that causes disease in healthy people especially *Citrobacter freundii* which recognized as causative agents of severe gastrointestinal infections [32]. Previous study was proved the

antibacterial activity of the extract of leaves against an array of different micro-organisms which support our study [33]. In another hand in this study, the stems extract of *Hammada Scoparia* show an important antibacterial activity comparing with leaves extract.

Table 6: Minimum inhibitory (MIC) and minimum bactericidal (MBC) and r*apport CMB/CMI of* the leaves extract of *Hammada Scoparia*

Strains	**CMB**	**CMI**	**CMB/CMI**
Acinetobacter baumanii	*1/100*	*1/100*	*1*
Escherichia coli	*1/100*	*1/100*	*1*
Salmonella sp	*1/100*	*1/250*	*2.5*
Klebsiella pneumoniae	*1/250*	*1/100*	*0.4*
Citrobacter freundii	*1/1000*	*1/2000*	*2*

4. Conclusion

The hydroalcoholic extract of *Hammada Scoparia* leaf displayed higher antiradical activity than the stem extract, the two parts of the plants may constitute a good source of polyphenols, the results demonstrate that stem may be a good antimicrobial agent against bacteria responsible for human gastrointestinal infections, The results of this study confirm that *Hammada Scoparia* has antioxidant and antimicrobial potential and may be useful in pharmaceutical and phytotherapeutic applications.

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