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ANTIMICROBIAL AND ANTIRADICAL PROPERTIES OF *HAMMADA SCOPARIA* (POMEL) ILJIN

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Abstract

Background: *Hammada scoparia* (Pomel) Iljin (HS), is commonly used by traditional healers in Morocco against microbial and fungal infections. We studied antimicrobial, antifungal and antiradical effects of organic extracts *in vitro* in order to confirm traditional utilization after phytochemical screening.
Materials and methods: Aerial parts of HS have been extracted by hydro-distillation using Clevenger-type apparatus, and the chemical composition was realized by Gas Chromatography coupled with Mass Spectroscopy (GC/MS). The antioxidant activity has been evaluated using DPPH test, while the antimicrobial tests of HS extract were conducted on twenty-eight bacterial strains and antifungal on twelve fungal strains.
Results: Chemical characterization of HS essential oils (EO) confirmed the presence of carvacrol (82,28%), p-cymene (2,52%), γ-terpinene (2,18%) and Z-caryophyllene (2,04%). Antimicrobial tests of HS extract showed a moderate antibacterial activity without antifungal effect. In addition, HS exhibited a very powerful antiradical activity with IC_{50} = 1,2 mg/ml compared to that of ascorbic acid (IC_{50} = 0,5 mg/mL) and butylated hydroxyanisole (0,8 mg/mL).
Conclusion: To our knowledge, this is the first demonstration that HS directly inhibits the growth of microorganisms *in vitro*, and further validates its traditional use as an antiseptic by traditional Moroccan healers.

Keywords: polyphenolics, *Hammada scoparia*, antimicrobial, antifungal, antioxidant.

List of abbreviations: HS: *Hammada scoparia* (Pomel) Iljin, GC/MS: Gas Chromatography coupled with Mass Spectroscopy, DPPH: 2, 2- diphenyl-picrylhydrazine, EO: Essential oils, IC_{50}: Median Inhibition Concentration (concentration that reduces the effect by 50%), IK: Kovats's indices, BHA: Butylated hydroxyanisole, PI %: Inhibition percentage, RI: Retention index S.E.M: Standard Error of the Mean, ANOVA: Analysis of Variance, HSSM: Hydromethanol extract obtained by soxhlet of *H. scoparia*, HSSA: Hydroacetone extract obtained by soxhlet of *H. scoparia*, HSMM: Hydromethanol extract obtained by maceration of *H. scoparia*, HSMA: Hydroacetone extract obtained by maceration of *H. scoparia*, GAE: Gallic acid equivalent DW: Dry weight, QE: Quercetin equivalent, DI: Inhibition diameters, A. ACID: Ascorbic acid

Introduction

Hammada scoparia (Pomel) Iljin is presented under several names (Lamchouri et al., 2012): *Haloxylons coparium* (Pomel) Bge, *Haloxylon articulatum* subsp. scoparium (Pomel) Batt Arthrophytum scoparium (Pomel) Iljin). In Morocco, the species is recognized under the name: "Remth". *Hammada scoparia* belongs to the *Amaranthaceae* family which encloses 1300 species distributed over 120 genera, including the genus *Hammada*. This genus encompasses more than 150 species (Boulanouar et al., 2013). *Hammada scoparia* is a shrub with thin stems, which blacken after drying. It is also characterized by short-sized inflorescences and fruits with brightly coloured wings (often pink or red) (Boulos, 1999). The species is found in Moroccan arid and semi-arid regions, and other Mediterranean regions, especially south-eastern Tunisia, Spain and parts of Turkey (El-Shazly and Wink, 2003). It is also found in the Middle East; namely in Iran, Syria and Iraq (Iranian-Turanian region).

H. scoparium is used to treat eye disorders (Ben Salah et al., 2002). In Morocco, powdered aerial parts of this plant prepared as an infusion are used for their antidiabetic, antiseptic and anti-inflammatory effects (Ziyyat et al., 2014). In Oman, the species teams are used as a mordant for wool dyeing in traditional weaving.

Studies conducted by earlier researchers (Ajabnoor et al.,1984; Awaad et al., 2001) have proved that ethanol extract of *H. scoparium* showed antidiabetic and anticoagulant activities *in vivo* (in animal models). Other studies have shown that *H. scoparia* has several biological properties, such as larvicidal activity (Sathiyamoorthy *et* al., 1997), anticancer activity (Bourogaa et al., 2014), antiplasmodial activity (Sathiyamoorthy et al.,1999) and molluscicidal activity (Mezghani et al., 2009). This species is also used as an anti-leukemic agent (Bourogaa et al., 2011).

From a chemical point of view, *Hammada scoparia* is rich in polyphenols, especially in flavonol triglycosides: isorhamnetin 3-O-β-D-xylopyranosyl-α-L-rhamnopyranosyl-β-D-galactopyran-oside, isorhametin 3-O-D-galactopyranoside, isorhametin 3-O-α-L-rhamnopyranosyl -α-L-rhamnopyranosyl - β-D-alactopyranoside (Ben Salah et al., 2002). Moreover, ethanol extracts of this herb are rich in alkaloids. Thorough studies enabled us to identify carnegine and N-methylisosalsoline. The latter is endowed with molluscicidal activity (Mezghani et al., 2009). Qualitative phytochemical analyses of *H. scoparia's* aerial parts revealed the presence of alkaloids, cardiac glycosides, anthraquinones, flavonoids, saponins, coumarins, sterols, tannins and essential oils (EO) (Ajabnoor et al., 1984).

The present study focuses on valorization of *H. scoparia*. a highly popular species in Tata region known for its curative properties against diabetes mellitus, cancers, snake bites, and so on. The present study includes a phytochemical study and an assessment of biological activities of the herb in order to confirm the therapeutic uses of this species.

Material and methods

Plant material and EO extraction

The samples (stems, leaves, and flowers) of *Hammada scoparia* (Pomel) were harvested in June 2016 from the wild (spontaneous stands) in the region of Tata (Douar Oasis Tamsouloujou - Moroccan Anti-Atlas). Identification of this species was carried out in the Botany and Plant Ecology Laboratory of the Scientific Institute in Rabat by Professor IBN TATOU MOHAMED. A voucher specimen (Number RAB 110963) was deposited in the herbarium of the Institute.

Aerial parts of the plant were harvested and air-dried for thirteen (13) days. Then, essential oils were obtained by hydro-distillation process for three hours with 100 g of the dry matter in a Clevenger-type apparatus which is recommended by the French Pharmacopoeia.

Analysis and chemical characterization of *Hammada scoparia's* essential oils

The chromatographic analysis of EO samples was carried out using a Thermo-Electron-type gas chromatograph (Trace GC Ultra) coupled to a Thermo-Electron Trace MS system mass spectrometer (Thermo Electron: Trace GC Ultra; Polaris Q MS). Fragmentation is carried out by an electronic impact (70 eV intensity). The chromatograph is equipped with a DB-5 column (5% phenyl methyl-siloxane) (30m × 0.25mm × 0.25µm), a flame ionization detector (FID) supplied by a mixture of H_2 / Air gas. The temperature of the column increases with a gradient of 4 ° C/min from 50 to 200 ° C. for 5 min. The injection mode is split (leak 1/70, flow rate ml/min), nitrogen is used as a carrier gas with a flow rate of 1 ml/min.

Identification of EO chemical composition was performed through the comparison of compounds' Kovats (IK) indices with those of standard products known from the literature (Kovats 1965, Adams, 2007). This step was supplemented by a comparison of Kovats's indices of the compounds as well as their mass spectra with those gathered in reference documents (Adams, 2007, National Institute of Standards and Technology, 2014). Kovats's indices compare the retention time of any product with the retention time of a linear alkane containing the same carbon number. They are determined by injecting a mixture of the alkanes (C7-C40 standard) under the same operating conditions.

Phytochemical screening:

It is a qualitative study in which detection of chemical families is performed through compounds solubility tests, precipitation and turbidity reactions. It can also be performed through the observation of a specific colour change or an examination under ultraviolet light.

This phytochemical study was conducted on *H. scoparia's* aerial parts. Dry samples of the plant were ground into a fine powder, followed by characterization tests of different chemical groups, carried out according to the protocols of N'Guessan, 2009.

Assessment of polyphenol contents of *Hammada scoparia* (Pomel): Solid-liquid Extraction

This step consisted extracting the maximum amount of polyphenol molecules from the aerial parts of the plant by maceration and soxhlet extraction. Water-methanol or water-acetone mixtures can be used as solvents. The latter accelerates and increases extraction yields. Figure 1 summarizes the solid-liquid extraction protocol.

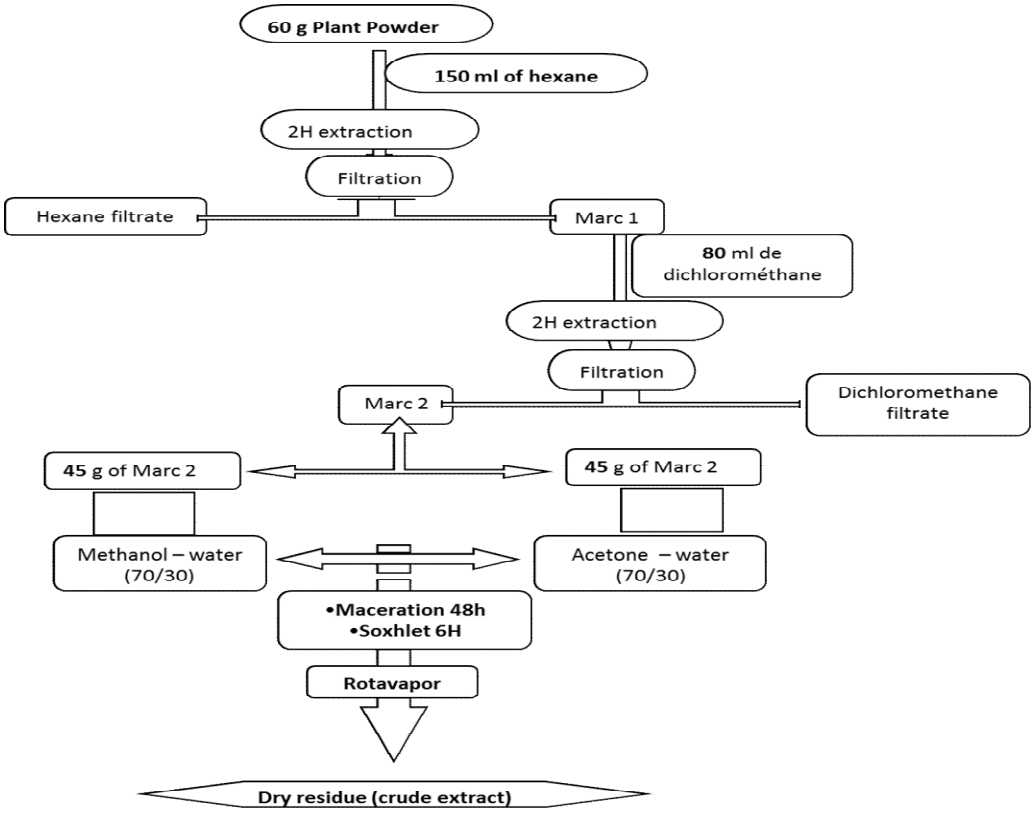


Figure 1: Steps used for the extraction of polyphenols from *H. Scoparia*

Quantitative analysis of phenolics
Total phenolic content

Total phenolics from *H. scoparia* stems, leaves and flowers extracts were assayed by Folin-Ciocalteu's method with UV-Visible spectrophotometry (Singleton et al., 1999). The reagent is composed of phosphomolybdic acid ($H_3PMo_{12}O_4$) and phosphotungstic acid ($H_3PW_{12}O_{40}$) which are reduced by the phenols oxidation into tungsten blue oxide (W_8O_{23}) and molybdenum oxide (Mo_8O_3). The phenolic contents are estimated by UV spectroscopy at a wavelength $\lambda = 760$ nm. Gallic acid was used as a standard.

The flavonoid content

Aluminum chloride ($AlCl_3$) forms a very stable complex with hydroxyl (OH) groups of phenols. This yellow complex absorbs the visible light at 433 nm. The phenols are estimated by UV spectroscopy at a wavelength $\lambda = 433$ nm (Chia-chi et al., 2002) and quercetin is used as a standard.

Antimicrobial Essay

To evaluate the antimicrobial activity of *Hammada scoparia'*s extracts, twenty-eight (28) bacterial strains and twelve (12) fungal strains (see Tables 1 and 2) were chosen.

These selected microorganisms are pathogenic strains known for their high resistance, invasiveness and toxicity in humans. They are frequently encountered in many infections in Morocco that pose clinical and therapeutic problems.

Antibacterial activities were performed on bacterial strains isolated from the hospital: Mohamed V-Meknes Provincial Hospital, whereas antifungal activities were investigated on isolates from fungal culture collections of the parasitology and mycology laboratories of Ibn Sina Hospital - Rabat.

Table 1: List of Bacterial Strains Tested

Gram-positive Cocci	Gram-negative Bacilli	Gram-Positive Bacilli
Streptococcus agalactiae (St. B)	*Escherichia coli (sauvage) (E. coli)*	*Listeria sp*
Streptococcus groupe D (non-enterococcus) (St. D)	*Escherichia coli BLSE (E. coli BLSE)*	*Corynebacterium sp*
Streptococcus porcinus (St. porcinus)	*Klebsiella pneumonie ssp pneumonie (K. pneumonie)*	

Streptococcus acidominimus (St. Acidominimus)	*Klebsiella oxytoca (K. oxytoca)*
Staphyloccocus epidermidis (S.epidermidis)	*Citrobacterkoseri (C. koseri)*
Staph aureus BLACT (S.aureus BLAC)	*Enterobacter aerogenes (E. aerogenes)*
Staph aureus STAIML/ MRS/ mecA/ HLMUP/ BLACT. (S.aureus MRS/BLAC)	*Enterobacter cloacae (E. cloacae)*
Staphyloccocushaemolyticus (S. haemolyticus)	*Salmonella sp*
Enterococcus faecalis (E. faecalis)	*Shigella sp*
Enterococcuss faecium (E. faecium)	*Serratia marcescens (S. marcescens)*
	Yersinia enterolitica (Y. enterolitica)
	Proteus mirabilis (P. mirabilis)
	Pseudomonas putida (P. putida)
	Pseudomonas aerogenosa (P. aerogenosa)
	Pseudomonas fluorescence (P. fluorescence)

Table 2: List of fungal Strains Tested

G E N U S	**S P E C I E S**
yeasts	*Candida albicans*
	Candida tropicalis
	Candida glabrata
	Candida dubliniensis
	Candida sp
	Rhodotorularubra
	Trichosporonsp
	Cryptoccocusneoformans
molds	*Aspergillus niger*
	Fusarium sp
	Pénicillium sp
Dermatophytes	*Trichophyton mentagrophytes*

Disk diffusion method

The antimicrobial activity was determined by the disk diffusion method (NCCLS, 1997). Briefly, a-90 mm diameter Petri-dish containing 30 ml of the Mueller-Hinton agar culture medium is firstly inoculated by swabbing with a 0,5-McFarland bacterial inoculum prepared in sterile physiological water.

Then, for the antifungal activity, the fungal inocula were prepared in sterile physiological saline and adjusted using Malassez's cell (hemocytometer). Final inocula were adjusted to10^5 conidia/ml for molds and dermatophytes. For yeasts, it was adjusted to 10^6 cells/ml. They were then flood-inoculated on Sabouraud-chloramphenicol medium.

Whatman paper disks (6 mm) previously loaded with different volumes of *H. scoparia's* (Pomel) extracts (10, 20, 30, 40 µl) are immediately incubated at + 4 ° C for 15 minutes and then deposited in the center of the culture medium.

A suitable antibiotic disk was applied onto each Petri-dish to serve as positive control against the bacterial and fungal strains. Blank disks were used as negative controls.

Incubation periods and temperatures vary as follows: 24 h at 37 ° C for bacteria, 48 h at 28 ° C for yeasts, seven (7) days for molds and two (2) weeks for dermatophytes at 28 ° C.

Inhibition diameters of the microbial growth were measured, the means were calculated (the operation is performed in triplicate) and the standard deviations were determined.

Antiradical activity by DPPH test

Antiradical activity of *H. scoparia*'s (Pomel) various extracts were evaluated *in vitro* by the DPPH test. DPPH (2,2-diphenyl-1-picrylhydrazyl) which is initially purple-coloured, turns yellow in the presence of free radical scavengers. It is subsequently reduced into 2, 2- diphenyl-picrylhydrazine. This colour change allows the kinetics of discoloration at 515 nm. For this purpose, volumes containing quantities equivalent to those of the various extracted functional compounds (2 mg/ml) were incubated in a 0,0024 g/l concentration of DPPH solution prepared in ethanol. The absorbance at 515 nm was regularly recorded. For each test, the coefficient of discoloration kinetics was determined, and the concentration values that inhibit or reduce 50% of the initial DPPH concentration (IC_{50}) were graphically determined by linear regression (Hassan et al., 2018).

Results for each tested compound are expressed as DPPH reduction percentage (PI %) in comparison to those obtained by the standards (vitamin C and BHA).

PI% = [(control abs -sample abs) / control abs] x 100

Statistical analysis

All data are presented as the mean(± S.E.M) of the indicated number of experiments. Results were analyzed by one-way or two-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparison post-test performed using Graph Pad Prism version 5.0 for Windows (GraphPad software Inc. San Diego. CA). Statistical significance was set at $p<0.05$.

Results

Phytochemical study

Results of the phytochemical screening of *Hammada scoparia*'s extracts obtained by qualitative characterization reactions are summarized in Table 3. This phytochemical screening enabled us to demonstrate the presence of alkaloids, saponosides, triterpenes and sterols, flavonoids, gallic tannins and mucilages. As for catechic tannins, anthocyanins and leucoanthocyanins, the tests were negative.

Table 3: Results of the characterization reactions of the different chemical groups sought in the powder of *H. scoparia*.

Chemical group			Results	Characteristics
Polyphenols	**Tannins**	Total tannins	+++	Black-green
		Gallic tannins	+++	Black-Blue
		Catechic tannins	_	No precipitate
	Flavonoïds	Flavonones	+++	Purlish-pink
		Anthocyanes	-	
		Leucoanthocyanes	-	
Alkaloïds			+++	Precipitate
			+++	Precipitate
Triterpenes and sterol			+++	Brownish ring and greenish supernatant
Mucilage			++	Flaky interface
Saponosids			+++	

++ = abundant - = absent + = weak reaction

Yield and chemical composition of *H. scoparia's* EO:

Essential oil extraction by hydrodistillation of *Hammada scoparia*'s aerial parts yielded 0,52 %. GC and GC/MS analyses of this EO enabled us to determine the chromatographic profiles. Identification of each compound was performed using the Kovats' index, by comparison of retention times of compound's peaks, with those of the known authentic standards available in the authors' laboratory, and also by comparing their mass spectra with known compounds or compounds published by Adams. Chemical composition results of *H. scoparia* collected in Tata region in June 2016 are shown in Tables 4A and 4B.

Table 4A: Composition of *H. scoparia* Essential Oil from Southern Morocco

Compounds	**Retention index (RI)**	**Percentage (%)**
Monoterpenes		91.98
Monoterpenes hydrocarbons		8.01
α-thujene	930	0.39
α-Pinene	939	0.58
Sabinene	975	0.12
β –Pinene	979	0.62
α –Phellandrene	1002	0.16
α-Terpinene	1017	0.46
p-Cymene	1024	2.52
β -Phellandrene	1029	0.14
Z- β-Ocimene	1037	0.02
E- β-Ocimene	1050	0.03
γ-Terpinene	1059	2.18
p-Mentha-2,4(8)-diene	1088	0.64
Terpinolene	1088	0.15
OxygenatedMonoterpenes		83.97
1-Octen-3-ol	979	0.07
3-octanol	991	0.09
α -pineneoxide	1099	0.03
Cis-Thujone	1102	0.02
6-Champenol	1113	0.09
Camphor	1146	0.13
Borneol	1169	0.26
Terpinen-4-ol	1177	0.32
para-cymen-8-OL	1182	0.03
α –Terpineol	1188	0.05
Cis-dihydrocarvone	1192	0.08
Trans-dihydrocarvone	1200	0.06
Trans-carveol	1216	0.02
Thymol	1290	0.44
Carvacrol	1298	82.28
Sesquiterpenes		5.9
Sesquiterpenes hydrocarbons		3.91
α-Cubebene	1351	0.02
Z-Caryophyllene	1408	2.04
E-Caryophyllene	1419	0.04
β-Duprezianene	1422	0.04
cis-thujopsène	1431	0.54
ɤ-élémène	1436	0.04
Aromadendrene	1441	0.03
α -Humulene	1454	0.06
Allo-aromadendrene	1460	0.13
β-acoradiene	1470	0.41
α-Neocallitropsene	1476	0.34
D-germacrene	1481	0.06
δ-Cadinene	1523	0.16

Table 4B: Composition of *H. scoparia* Essential Oil from Southern Morocco

Compounds	**Retention index(RI)**	**Percentage(%)**
Oxygenated Sesquiterpenes		1,99%
Thymol-methyl ether	1235	0.74
Cis-chrysanthenyl acetate	1265	0.03
Cis-verbenyl acetate	1282	0.03
Iso Bornyl acetate	1285	0.06
Thymol acetate	1352	0.02
Spathulenol	1578	0.59
Caryophylleneoxide	1583	0.27
Presilphiperfolan-8-ol	1586	0.04
1,10-di-epi-Cubenol	1619	0.02
Selina-3,11-dien-6α-ol	1644	0.05
Valerianol	1658	0.08
7-epi-α-Eudesmol	1663	0.06

Fifty-three (53) constituents, representing 97,88% of the EO, were identified. These compounds are: monoterpene hydrocarbons (8,01 %) represented by para-cymene (2,52%)and gamma-terpinene (2,18%), oxygenated monoterpenes (83,97%)mainly represented by carvacrol (82,28%). The monoterpene alcohols are in a small amount (<1%) and include 1-Octen-3-ol, 3-octanol, 6-Champenol, borneol, terpinen-4-ol, para-cymen-8-ol, α-terpineol, trans-carveol and thymol. Sesquiterpene derivatives are mainly represented by Z-caryophyllene (2,04%). The other constituents were detected with less than 0,6%.

At the end of this analysis. we can conclude that oxygenated monoterpenes are the most abundant. With about (83,97%) of the total EO content. Thus, concerning the main compounds and due to the lack of studies on the species, *Hammada scoparia*'s EOs mainly consist of carvacrol (82,28%), p-cymene (2,52%), γ-terpinene (2,18%) and Z-caryophyllene (2,04%).

Extraction of polyphenolic contents of *H. scoparia***:**
Extraction of polyphenolics

Several extracts were obtained by soxhlet and maceration from the aerial parts of *H. scoparia.* Extraction solvents were two mixtures of solvents: 70% methanol/water and 70% acetone/water.
According to the results presented in Figure 2, the yields of *H. scoparia's* hydro-methanol extracts obtained by soxhlet are higher (HSSM = 21,9%) than those of hydro-acetone extracts (HSSA = 13,55%), while the yields of the extracts obtained by maceration are almost the same, whatever the solvent was (HSMM = 6,85% and HSMA = 5,55%).

It should also be noted that the highest yields are obtained by soxhlet with hydro-methanol solvent (methanol 70%). These results may be justified on one hand, by the difference in solvents polarity, since methanol is more polar than acetone.

Therefore, it allows the extraction of a high rate of polyphenolic-type polar compounds. On the other hand, the temperature difference (soxhlet: hot extraction/maceration: cold extraction) can be another reason.

In general, extraction yield depends on the organ used for the extraction, the drying conditions, metabolite content, extraction method, nature of the solvent used for extraction or fractionation, and the polarity of the solvent.

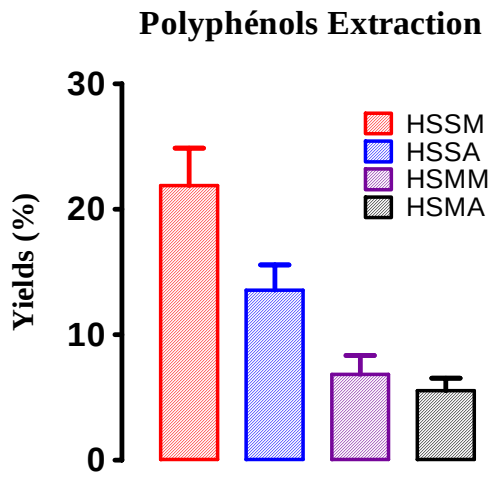


Figure 2: Percentage of extraction of total polyphenols from *Hammada scoparia.* HSSM: Hydromethanol extract, HSSA: Hydroacetone extract, HSMM: Hydromethanol , HSMA: Hydroacetone extract.

Quantitative analysis of *H. scoparia's* **phenolics**
Total phenol content

The objective of crude extracts quantitative study by means of spectrophotometric assays was to determine the total content of polyphenols in the plant's extracts. Polyphenolic contents were expressed as mg gallic acid equivalent (GAE) per g of dry weight (DW). They are determined by the following equation: y = 0,095x + 0,003.

According to Figure 3, the following polyphenolic contents were recorded for *H. scoparia*: 7,46 mg GAE / g DW; 4,80 mg GAE / g DW; 2,38 mg GAE / g DW and 1,9 mg AGE / g DW respectively with the extracts HSSM, HSSA, HSMM and HSMA. Extracts obtained by soxhlet represent the highest content of polyphenolics. In comparison to maceration extraction, soxhlet extraction with methanol remains the best method to obtain a maximum content of polyphenols.

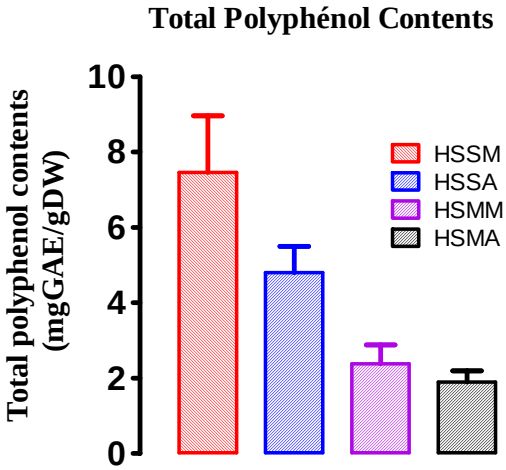


Figure 3: Total polyphenolic contents from *Hammada scoparia* in mg GAE/g DW.

Flavonoid contents

Flavonoid contents are expressed as mg quercetin equivalent (QE) per g of dry weight (DW). They are determined by this equation: y = 0,073x -0,081.

We recorded for *H. scoparia* (Figure 4): 0,74 mg and 0,37 mg EQ / g DW respectively, with hydro-methanol and hydro-acetone extracts (HSSM and HSSA). The hydro-methanol extract (HSSM) represents the highest content of flavonoids. The same observation was made in the case of extraction by maceration, while knowing that the highest content is recorded with soxhlet extraction (HSSM).

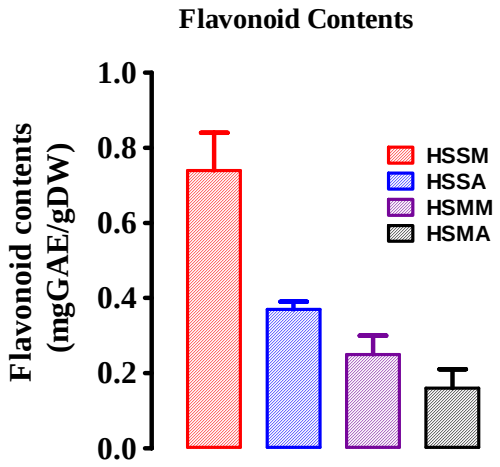


Figure 4: Total Flavonoid contents from *Hammada scoparia* in mg EQ/g DW of *H. scoparia.*

Antimicrobial activity
Antibacterial activity

In this study, we tested the effect of hydro-methanol soxhlet extract (HSSM) and the hydro-methanol macerate (HSMM) of *H. scoparia* against microbial strains. The results of this antimicrobial evaluation are shown in Table 5.

Table 5 indicates that the methanol extract of *Hammada scoparia* showed moderate activity against *Staphylococcus epidermidis, Pseudomonas putida, Escherichia coli BLSE, Klebsiella oxytoca, Enterobacter cloacae* and *Enterococcus faecium* with the following inhibition diameters: 11, 11, 12, 13, 11, 11 mm for 40 µl/disk, respectively. Activity was recorded against *Streptococcus agalactiae, Escherichia coli* (wild), *Acinetobacter baumannii* and *Staphylococcus haemolyticus* with the following inhibition diameters: 9, 8, 10, 8 mm with 40µl/disk. On the other hand, the plant's hydro-methanol extract was active only from 40 µl of the extract towards *Listeria sp* (DI = 9 mm), *Shigella spp* (DI = 11 mm) and *Serratia marcescens* (DI = 9 mm).

Table 5: Antibacterial activity of *H. scoparia*

Bacteria	Extracts used	Diameters of inhibition Zone (DI) in (mm)										Antibiotics
		HSSM				HSMM				Control		
		10(µl/disk)	20(µl/disk)	30(µl/disk)	40(µl/disk)	10(µl/disk)	20(µl/disk)	30(µl/disk)	40(µl/disk)	NC	PC	
	Concentration in (mg/ml)/disk	5	10	15	20	5	10	15	20	-	-	-
Gram-positive Cocci	*St. B*			7.0 ± 0.0	9.0 ± 0.5			7.0 ± 0.0	9.0 ± 1.0	0	21.0	*Cefotaxime*
	St. D					8.0 ± 0.3	9.0 ± 0.3	10.0 ± 0.5	12.0 ± 0.6	0	30.3	*Co-trimoxazole*
	St. porcinus				9.0 ± 0.3					0	29.0	*Chloramphenicol*
	St. Acidominimus									0	18.6	*Amoxicilline*
	S. epidermidis		7.0 ± 0.3	10 ± 0.6	11.0 ± 0.6					0	12.0	*Gentamicine*
	S. aureus BLAC									0	16.0	*Gentamicine*
	S. aureus MRS/BLAC									0	17.7	*Vancomycine*
	S. haemolyticus	7 ± 0.3	7.5 ± 0.0	8.0 ± 0.3	8.0 ± 0.5			7.0 ± 0.0	10.0 ± 0.6	0	20.3	*Co-trimoxazole*
	E. faecalis									0	17.0	*Gentamicine*
	E. faecium		7.0 ± 0.5	10.0 ± 0.5	11.0 ± 0.6		8.0 ± 0.3	10.0 ± 0.3	12.0 ± 1.0	0	24.0	*Imipeneme*
Gram-negative Bacilli	*E. coli*	7 ± 0.0	7.5 ± 0.5	7.5 ± 0.3	8.0 ± 0.0	7.0 ± 0.0	10.0 ± 0.3	11.0 ± 0.6	12.0 ± 0.3	0	17.3	*Imipeneme*
	E. coli BLSE	7 ± 0.3	10 ± 0.6	11.0 ± 1.0	12.0 ± 1.0	8.0 ± 0.3	9.0 ± 0.6	10.0 ± 0.3	11.0 ± 0.0	0	19.3	*Imipeneme*
	K. pneumonie					7.0 ± 0.0	7.5 ± 0.3	8.0 ± 0.0	11.0 ± 0.0	0	31.3	*Imipeneme*
	K. oxytoca		7.0 ± 0.0	10.0 ± 0.6	13.0 ± 0.6					0	16.5	*Norfloxacine*
	C. koseri									0	30.0	*Ciprofloxacine*
	E. aerogenes									0	20.3	*Imipeneme*
	E. cloacae	7 ± 0.0	9.0 ± 0.5	10.0 ± 0.5	11.0 ± 0.3	7.0 ± 0.0	9.0 ± 0.5	11.0 ± 0.0	12.0 ± 0.6	0	11.7	*Gentamicine*
	Salmonella sp									0	10.3	*Amoxicilline*
	Shigella sp				11.0 ± 0.3			10.0 ± 0.0	12.0 ± 0.3	0	31.0	*Co-trimoxazole*
	S. marcescens				9.0 ± 0.0		7.0 ± 0.0	11.0 ± 1.0	12.0 ± 1.0	0	22.3	*Chloramphenicol*
	Y. enterolitica								8.0 ± 0.3	0	30.0	*Co-trimoxazole*
	P. mirabilis					7.0 ± 0.3	8.0 ± 0.3	9.0 ± 0.5	10.0 ± 0.0	0	18.6	*Gentamicine*
	P. putida		8.0 ± 0.6	10.0 ± 0.6	11.0 ± 0.5	7.0 ± 0.3	7.5 ± 0.0	8.0 ± 0.3	9.0 ± 0.5	0	15.7	*Gentamicine*
	P. aerogenosa	7 ± 0.0	8.0 ± 0.6	9.0 ± 0.6	10.0 ± 0.3					0	26.6	*Ceftazidime*
	P. fluorescence					7.0 ± 0.5	8.0 ± 0.5	9.0 ± 0.6	10.0 ± 0.0	0	10.7	*Gentamicine*
	A. baumannii			7.0 ± 0.0	10.0 ± 0.5					0	17.7	*Ceftazidime*
Gram-Positive Bacilli	*Listeria sp*				9.0 ± 0.6					0	28.3	*Gentamicine*
	Corynebacterium sp					11.0 ± 0.5	12.0 ± 0.6	15.0 ± 1.0	17.0 ± 1.5	0	26.3	*Penicilline G*

HSSM: Hydromethanol soxhlet extract of *H. scoparia*, HSMM: Hydromethanol macerate of *H. scoparia.* NC: Negative Control. PC: Positive Control.

In addition, the hydro-methanol macerate of *Hammada scoparia* showed moderate activity against *Escherichia coli* (wild), *Klebsiella pneumonia* ssp. *pneumonia*, *Proteus mirabilis*, *Escherichia coli* ESBL, *Pseudomonas fluorescence*, *Serratia marcescens*, *Streptococcus* group D (non-enterococcus), *Enterococcus Faecium*, *Enterobacter cloacae* with inhibition diameters as follows: 12, 11, 10, 11, 10, 12, 12, 12, 12 mm for 40µl/disk, respectively. Low activity was remarked against Pseudomonas putida (DI = 9 mm to 40µl/disk). Moreover, the methanol extract of this plant showed activity only from 30 µl of the extract against *Streptococcus agalactiae*, *Shigella spp.* and *Staphylococcus haemolyticus*. Contrariwise, hydromethanol macerate of *Hammada scoparia* showed good activity against *Corynebacterium* sp (DI = 17 mm with 40 µl/disk), whereas no sensitivity was observed for the other strains.

To sum up, results from inhibition tests of *H. scoparia's* extracts against bacteria showed moderate antibacterial activity. In the absence or rarity of work on this species, we conclude according to our results, that hydro-methanol macerates gave a moderate activity compared to the hydro-methanol extract obtained by Soxhlet. In addition, *H. scoparia's* hydro-methanol macerate showed good activity against *Corynebacterium spp.* with 17 mm as inhibition diameter with 40 µl/disk.

Antifungal activity

The results of fungal inhibition tests were negative. Neither the soxhlet extracts nor the macerates (hydro-methanol and hydro-acetone) showed antifungal activity against the selected fungi. This lack of inhibitory effect may be due to the low concentrations tested, the extraction method and/or the solvents used.

The chemical composition of plant extracts may be influenced by many factors, including the species to which the plant belongs (taxonomy), the plant material (leaves, flowers, twigs, fruits, inflorescence, aerial part, and so on) used for extraction as well as the extraction process. Combination of these various parameters seems to be an explanation for the observed variations in antimicrobial activity.

Antioxidant activity
Estimation of the antioxidant power by DPPH method

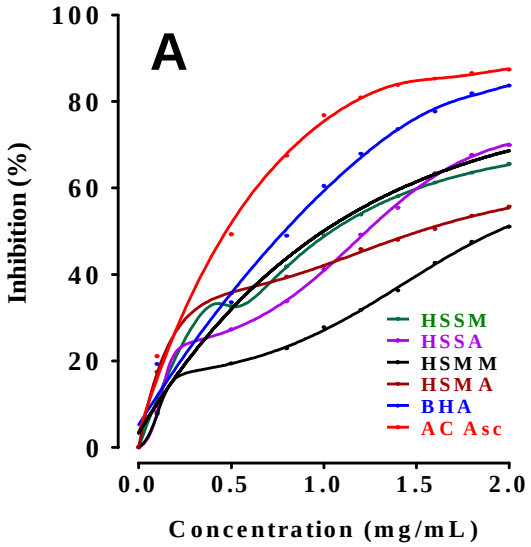
In addition, the selected extracts were also evaluated for their antioxidant activities. The DPPH radical method is one of the most widely used substrates for the rapid and direct evaluation of antioxidant activity due to its stability and the simplicity of the analysis (Bozin *et al.*, 2008).

To better characterize the antioxidant power, we introduced the IC_{50} parameter. IC_{50} values of the extracts were determined using the linear regression curve: $y = ax + b$.

Figures 5 (A) and (B) report the results of crude extracts antioxidant power assessed by the DPPH free radical scavenging method.

In general, all tested extracts exerted a more or less significant decrease of the absorbance at 517 nm according to their concentrations.

The standard antioxidants used in this study were ascorbic acid and BHA (2mg/ml). They showed a very powerful anti-radical activity with IC_{50}values equal to 0,5 and 0,8 mg/ml respectively, for ascorbic acid and BHA. For *H. scoparia.* HSSM and HSMA extracts are the most active, with IC_{50} values equal to 1,2 and 1,4 mg / ml respectively.



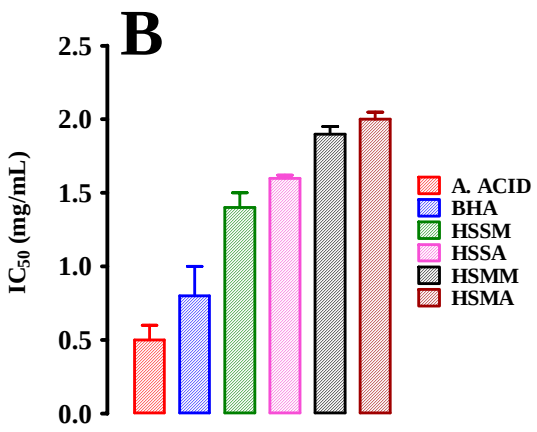


Figure 5: (A) Percentage inhibition of DPPH free radical according to the concentrations of the extracts of *H. scoparia*, ascorbic acid and from BHA (2 mg/ml). (**B)** IC_{50} of ascorbic acid, BHA and extracts of *H. scoparia*.

Discussion

Generally, the chemical groups detected in our studies confirm the work of Ezzeddine et al., 2012, Jarraya et al., 2005, on the same species (*H. scoparia* (Pomel) Iljin.

The coupling of gas chromatography with mass spectrometry (GC/MS) has identified in the essential oil of *Hammada scoparia*, 53 compounds. Due to lack of previous studies on the EO of *Hammada scoparia*, this essential oil could be characterized by its main compounds discovered in this study: carvacrol (82.28%). p-cymene (2.52%). γ-terpinene (2.18%) and Z-caryophyllene (2.04%).

The gap between polyphenolic and flavonoid contents could be attributed to the solubility difference of phenolic compounds due to the solvents polarity. It could also be explained by the extraction temperature and the chemical nature of phenolic compounds. The choice of the extraction method can also play an important role in polyphenolic and flavonoid extractions and quantification processes.

Polyphenol extraction yields, as well as total phenols and flavonoids contents, remain moderate in comparison to the work of Mohammedi (Mohammedi, 2012) in Algeria. This fact can be explained by the high polarity and the high hydro-solubility of phenolic compounds of this species.

For the antimicrobial activity carried out on the plant species, *Hammada scoparia*, and by the lack of work on this species, it can be said that according to our results, the methanolic macerate showed a moderate antibacterial activity compared to the methanolic extract obtained by Soxhlet. It should also be noted that the methanolic macerate of *Hammada scoparia* exhibited good activity with respect to *Corynebacterium spp.* with an inhibition diameter of 17 mm for taking 40 µl of extract. Furthermore, for the antifungal potential evaluated on the extracts and macerates prepared and used in this study, the lack of inhibitory effect may be due to the low concentration tested, the extraction method or the solvents used. The raised percentage of carvacrol in the aerial part of *H. scoparia* could justify the antimicrobial activity observed for the extracts tested. This major compound has also been identified as an innovative therapeutic agent for the treatment of infectious diseases (Seyed, 2015). In general, depending on the concentrations of the extracts evaluated in this study, gram-positive bacteria are more sensitive to the extracts than Gram-negative bacteria. Indeed, in a Langroodi study (Langroodi et al., 2018), the effect of *Berberis vulgaris* extracts on sprout growth in broth for heart and brain infusion was examined. Results obtained suggest the probability of Seed growth rates decrease when the concentration of extracts increases, due to high amounts of carvacrol. Their results were consistent with this study.

Mechanisms of the antimicrobial effects of active ingredients is undoubtedly very complex. Among the hypotheses advanced, inhibition of nucleic acid synthesis, alteration of the functions of the cytoplasmic membrane, sequestration of substrates necessary for microbial growth, and inhibition of microbial energy metabolism have been proposed (Fei et al., 2011; Coppo et al., 2014; Magi et al., 2015; Abbaszadeh et al., 2014).

With regard to the antiradical power, the hydro-méthanol Soxhlet extracts are more active than the hydro-acetone Soxhlet extracts, hydro-methanol macerate and hydro-acetone macerate. This is probably due to the complexity of the polyphenolic substances in these crude extracts, and/or to the synergy between the compounds for a better antioxidant activity (Vermerris and Nicholson, 2006; Scalbert et al., 2005; Patricia et al., 2014).

In this study focused on *H. scoparia*'s soxhlet extracts and macerates, the ratio of polyphenolic content/IC_{50} is much higher than 1, while the ratio of flavonoid content/IC_{50} is less than 1. According to Mohammedi (2012) and Djeridane (2006), the polyphenols represent a large group of phenolic compounds in the genus *Hammada*.

Conclusion

Phytochemical screening enabled us to demonstrate the presence of alkaloids, saponosides, triterpenes, sterols, flavonoids, gallic tannins and mucilages in the extracts used.

The oxygenated monoterpenes were the most abundant constituents, approximately 83.97% of *Hammada scoparia*'s EO. Due to lack of previous studies on *Hammada scoparia*'s EO, this EO could be characterized by its main compounds discovered in this study: carvacrol (82.28%), p-cymene (2.52%), γ- terpinene (2.18%) and Z-caryophyllene (2.04%).
It should also be noted that the highest extraction yields were obtained by the soxhlet method with 70% methanol as solvents.

These results are justified by the increase of the solvent polarity (methanol is more polar than acetone) and equally, by the increase in temperature (temperature (soxhlet) > temperature (maceration)). Thus, polyphenols extraction yields, contents of total phenols and flavonoids remain moderate compared to other studies. This can be explained by the high polarity and hydrosolubility of the phenolic compounds present in this plant species.

Results of bacterial growth inhibition tests by H. *scoparia's* extracts showed moderate antibacterial activity. Hydromethanol macerates exhibited a moderate activity compared to the hydromethanol extract obtained by soxhlet. In addition, *Hammada scoparia's* hydromethanol macerate showed good activity against *Corynebacterium* sp. with 17 mm inhibition diameter for 40 µl. All scientific results obtained in this work confirm the therapeutic use of *H. scoparia* in traditional medicine by the Moroccan population.

Conflict of interest:

All the authors declare that they have no actual or potential conflict of interest, including any financial, personal or other relationships with people or organizations.

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