

Volatiles profiling, phytotoxic activity, and antioxidant potentiality of *Hammada scoparia* (Pomel) Iljin extracts from southern Tunisia

OLFA KAROUS^{1,2*}, HAIFA YOUSFI AICHI^{1,2}, IMTINEN BEN HAJ JILANI^{1,2}, ZEINEB GHRABI-GAMMAR^{1,2}

¹ Université de Carthage, Institut National Agronomique de Tunisie (INAT), Département d'Agronomie et Biotechnologie Végétale, 43 Avenue Charles Nicolle, 1082 Cité Mahrajène, Tunis, Tunisie

² Université de Manouba, Faculté des Lettres, des Arts et des Humanités de la Manouba, LR 18ES13 Biogéographie, Climatologie Appliquée et Dynamiques Environnementales (BiCADE), 2010 Manouba, Tunisie

*Corresponding author: karous-olfa@hotmail.fr

Abstract - The present study was conducted to determine phenolic content evaluate biological properties in terms of antioxidant potentiality of *Hammada scoparia* (Pomel) Iljin collected from the sector of Ouled Dabbeb of the governorate of Tataouine (Southern Tunisia), and to investigate, in vitro and in situ the phytotoxic activity of its extracted water. Also, we aimed to assess the volatile profile of water extract of areal part of this specie using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS).

Total phenolic, flavonoid and condensed tannins content of methanolic and water extracts was determined by using Folin-Ciocalteu reagent, Aluminum chloride and vanillin methods, respectively. We recorded high content in total phenols for both methanolic and water extracts of areal parts of *H. scoparia* (260mg GAE/g DW). Flavonoids and condensed tannins content levels varied significantly according to the polarity of the used solvent with methanol extract an interestingly high in Total Flavenoids (662, 46±5.08).

Thirty-two volatile compounds were identified representing (98.6%) of the total water extract of *H.scoparia* including Fatty acid methyl ester (54.64%) and oxygenated monoterpenes (17.6%) as major compounds.

The highest free radical scavenging activity of methanolic extract of *H.scoparia* obtained by DPPH method was with the highest tested concentration (50 µg.mL⁻¹), resulting in 91.02 ±0.652% of inhibition with IC₅₀=1.56 (µg.mL⁻¹) more effective than synthetic antioxidant BHT (IC₅₀=13.45 (µg.mL⁻¹)).

Phytotoxic activity of water extract of the areal parts of *H.scoparia* was assessed *in vitro* (10,20, and 40 g L⁻¹) and *in situ* (40 g L⁻¹) based on the initial shoot and root growth of two crops (radish and wheat) and two weeds (lamb's quarters and Ray grass). The results showed that the root growth of different target species was more sensitive to the phytotoxic effect with an average percentage of inhibition varying from 75.06 to 96% than shoot length with inhibition ranging between 19.11% and 100% in all cases. Except for the dicotyledonous weed lamb's quarters, the extent of the phytotoxic activity length was significantly decreased *in situ*. Our findings are the first to report the phytotoxic activity of water extract of *H. scoparia* and its volatile profile.

Keywords: *Hammada scoparia*, volatile profile, HS-SPME/GC-MS, phytotoxic activity, Phenolic compounds

1- Introduction

Hammada scoparia (Pomel) Iljin = (*Haloxylon scoparium* (Pomel) Bge. = *Haloxylon articulatum* ssp. *scoparium* (Pomel) Batt. = *Arthrophytum scoparium* (Pomel) Iljin) belongs to the *Chenopodiaceae* family and is locally known as "rimth" in Tataouine Tunisia. As quoted by the International Plant Names Index and World Checklist of Selected Plant Families 2019, the distribution of this species extends to North Africa (Morocco, Algeria, Libya, Egypt), Sinai, Lebanon -Syria, Palestine and Western Sahara. It is a highly branched, halophytic shrub with succulent, spindly, segmented branches growing in a wild state in Southern Tunisia. This Saharo-Mediterranean species



develops in bioclimates that range from the upper arid among the gypseous steppe associations especially with *Artemisia herba alba*, to the lower Saharan where it is found in association with *Arthrophytum schmittianum* (Boucherit et al., 2018).

Traditionally, it is used to prepare snuff powder (Neffa) to which it owes its degradation. A previous ethnobotanical study (Le Floc'h, 1983) conducted in southern Tunisia revealed that it is widely used in traditional medicine as an antiseptic to hasten wound healing, while bark powder reduces scars. The local population also noted the use of this species for ethnoveterinary purposes. In fact, the water infusion is applied locally on the skin of livestock against scabies and as disinfectants after mowing or taken orally as appetiser and digestive.

This plant has been reported to possess has anti-cancer properties as well as antiplasmodial, larvicidal and anti-leishmaniasis activities (Sathiyamoorthy et al., 1999; El Rhaffari et al., 2002), anti-molluscicidal activity (Mezghani- Jarraya et al., 2009), and anti-leukemic agent (Bourogaa et al., 2011). However relatively there is no much research that has been published on the bioactivities of this species and its application in agriculture pest management. Therefore, in this study, in addition to the evaluation of its biological properties in terms of antioxidant potentiality and its chemical characteristics we aim to investigate for the first time the phytotoxic activity of water extract of *H.scoparia* *in vitro* and *in situ*. Besides, it would be a first to assess the composition of volatile compounds extracted from water extract of this studied species by headspace extraction.

2. Materials and methods

2.1. Plant material

The aerial parts of *H. scoparia* were collected in March 2016 from the sector Ouled Dabbeb Tataouine, Southern Tunisia. A voucher specimen (STOD52) was deposited at the herbarium of the National Agronomic Institute of Tunisia. The aerial parts were air dried at room temperature ($20\pm 2^{\circ}\text{C}$) for one week, ground in Retsch blender mill (Normandie-Labo, Normandy, France), sieved through 0.5 mm mesh screen to obtain a uniform particle size. Dry plant material was stored at -20°C until use.

2.2. Preparation of Extracts

The methanolic extract was obtained from 25 g of powder using soxhlet (70°C for 8h). After filtration, the organic phase was concentrated under reduced pressure at $45-50^{\circ}\text{C}$, using rotavapor R-114 (Buchi, France). The dry residue was stored at 4°C .

The aqueous extract was obtained by soaking 40g of powder in 1 L distilled water at 24°C for 24 h on an orbital shaker to give a concentration of $40\text{ g}\cdot\text{L}^{-1}$. In order to avoid the fermentation or microbial growth, the extract was subsequently filtered through for layers of cheesecloth and centrifuged for 20 min at 10,000 rpm to remove particulate material. A subsequent filtration was performed through 0.22 μm syringe filters. Each extraction was done in triplicate.

2.2.1. Analysis of total phenolic content (TPC)

TPC quantification of plant extract was carried out using the Folin–Ciocalteu reagent (Singleton et al., 1999), with some modification (Dewanto et al., 2002). An aliquot (125 μL) of a suitable diluted sample was mixed with 500 μL of deionized water and 125 μL of the Folin–Ciocalteu reagent. After a 5 min rest period at room temperature, 1.25 mL of 7% Na_2CO_3 was added to the mixture. The solution was then adjusted with deionized water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark for 90 min at 23°C , the absorbance versus prepared blank was read at 760 nm. TPC were expressed as mg gallic acid equivalents/g of dry weight (mg GAE/g DW). All measurements were performed in triplicate.

2.2.2. Analysis of flavonoids contents (TFC)

TFC was quantified according to Dewanto et al. (2002). Briefly, 250 μL of sample appropriately diluted was mixed with 75 μL NaNO_2 (sodium nitrite, 5%) for 6 min. After, 150 μL of 10% aluminium chloride (AlCl_3) and 500 μL of 1 mol/l NaOH were added to the mixture. The final volume was adjusted to 2.5 ml with distilled water. The absorbance of the mixture was determined at 510 nm. Total flavonoid content was expressed as mg catechin equivalents/g of dry weight (mg CE/g DW).

All samples were analysed in triplicates.

2.2.3. Analysis of Total condensed tannins (TTC)

TTC was quantified using the modified vanillin assay described by Sun et al.(1998). 3 mL of 4% methanol vanillin solutions and 1.5 mL of concentrated H₂SO₄ were added to 50 µL of suitably diluted sample. The mixture was allowed to stand for 15 min, and the absorbance was determined at 500 nm against methanol as a blank. TTC was expressed as mg (+)-catechin equivalent/g of dry weight (mg CE/g DW). All samples were analysed in triplicates.

2.2.3. Volatile profile of water extract of *H.scoparia* assessed by (HS-SPME/GC–MS)

The headspace solid phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS) method was used to determine volatile and semivolatile compounds of different origin, such as aldehydes and alcohols in aqueous extract of *H. scoparia* using an Agilent 7890A GC (Agilent Technologies, Colorado Springs, USA) and a BenchTOF-dx mass spectrometer (ALMSCO International, Llantrisant, UK).

HS-SPME of the preheated sample (40 °C, 5 min) was done under agitation for 1 min at 500 rpm using a 50/30 µm DVB/Carboxen/PDMS stable flex™ fiber (Supelco, Bellefonte, PA, USA), followed by desorption (5 min) at 250 °C onto a 60 m DB-WAX capillary column (0.25 mm i.d. –0.25 µm film thickness). The oven temperature was set at 40 °C for 1 min and then increased at 5 °C·min⁻¹ until it reached 250 °C and held for 1 min. The helium flow rate was maintained constant at a flow rate of 1 mL min⁻¹. All analyses were carried out in triplicate injection. Selected ions were used for quantification of the individual components. Compound identification was performed by injection of commercial standards, by spectra comparison using the Wiley Registry 7th Edition Mass Spectral Library (Wiley and Sons Inc., Weinheim, Germany) and the National Institute Standards and Technology (NIST) 2005 Mass Spectral Library and by calculation of linear retention indexes (LRI) relative to a series of alkanes (C6–C20).

2.2.5. Assessment of antioxidant activity (DPPH radical scavenging activity)

The antioxidant activity of the *H. scoparia* was measured by bleaching of the purple-coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato et al. (1998). 1 mL of different concentrations of the extracts was added to 0.5 mL of a 0.2 mmol DPPH methanolic solution. The mixture was shaken vigorously and kept at room temperature for 30 min. The absorbance of the resulting solution was then measured at λ = 517 nm. The antiradical activity was expressed as IC₅₀ (µg. mL⁻¹). The percentage inhibition of the DPPH radical was calculated by the following equation.

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

A₀ is the absorbance of the control at 30 min, and A₁ is the absorbance of the sample at 30 min. BHT was used as a positive control. All determinations were carried out three times.

2.2.6. Assessment of phytotoxic activity in vitro

Aqueous extracts of *H. scoparia* was diluted with distilled water to prepare 10, 20, and 40 g.L⁻¹ concentrations. They were tested on two crops (radish (*Raphanus sativus*) and wheat (*Triticum durum*) and two weeds lamb's quarters (*Chenopodium album*) and ray grass (*Lolium italicum*). Seeds were surface sterilized with 0.525 g.L⁻¹sodium hypo-chlorite for 15 min, then rinsed four times with deionized water. Ten of pregerminated target seeds (germinated in the darkness at 25°C for 1–3 days after overnight soaking) were placed on the filter paper 9cm diameter Petri dish. Seeds watered with distilled water were used as control. The Petri plates were then placed in a growth chamber with 400µmol photons m⁻²s⁻¹ photosynthetically active radiation (PAR) at 24/22 °C for 14/10 h light and dark periods respectively. Treatments were arranged in a completely randomized design with three replications. Roots and Hypocotyl/Coleoptile length of target species were measured at 7th days after sowing. Data were transformed to percent of control for analysis.

2.2.7. Assessment of phytotoxic activity in situ

To determine whether the phytotoxic effects of *H.scoparia* waterextract would be maintained in 'soil', additional trial was carried out in an incubator set at 25°C with 14/10 h, day/night. Thus, nursery trays (7 x 11 grids, each square 3 cm x 3 cm) were filled with sand soil, five pregerminated of each target seeds tested *in vitro* were planted/square just under the soil surface then sprayed with distilled water to humidify the soil. After three days, 5 mL of aqueous extract of *H. scoparia* was added at 40 g L⁻¹/square. Distilled water was used for the control. Treatments were arranged in a completely randomized design with three replications and data were transformed to percent of control for analysis.

2.8. Statistical Analysis

Data were expressed as means± SD. For statistical analysis, one way analysis of variance (ANOVA) was applied followed by Turkey's test. Difference was considered significant at $p < 0.05$ (SPSS, version 20).

3. Results and discussion

3.1. Volatile profile

Our results demonstrate that the water extract of *Hammada scoparia* was a rich source of different secondary metabolites (Tables 1).

Thirty two compounds were identified by headspace solid phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS) representing (98.6%) of the total water extract of *H. scoparia*.

Components were clustered (Table 1) in a homologous series of Fatty acid methyl ester, oxygenated monoterpenes, monoterpene hydrocarbons aromatic compounds, and others. Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester, Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl) propyl ester and Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester were the three main fatty acids methyl esters FAMES that constitute the most abundant compounds in *H. scoparia* water extract (54,64%). Secondly, monoterpenes constituted 17.6% of the volatiles emitted from the aerial parts *H. scoparia*. Among them, 14.3% are oxygenated monoterpenes including mainly Linalool, Nerol Oxide, Nerol, cis Rose oxide (-)-Borneol, 1,8-Cineole. However, monoterpene hydrocarbons were detected at 2.9% of the total volatiles and represented by two compounds o-Cymene, p-Cymene.

Table 1: Relative percentage of components identified in the water extract of areal parts of *H. scoparia*

| Peak # | Common Name | CAS Number | RT | Area (%) |
|----------------------------------|---|------------|-----------------|-------------|
| Fatty acids methyl esters | | | 37,95 | 56,3 |
| 36 | Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester | | 39,32 | 21,8 |
| 38 | Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester | | 39,8 | 16,8 |
| 37 | Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester | | 39,52 | 16,1 |
| 44 | Benzoic acid, 4-(1,1-dimethylethyl)-, ethenyl ester | | 44,68 | 0,7 |
| 17 | Sulfurous acid, cyclohexylmethyl nonyl ester | | 26,43 | 0,9 |
| Oxygenated monoterpenes | | | 30,37 | 14,3 |
| 25 | Linalool | 78-70-6 | 29,94 | 4,5 |
| 19 | Nerol Oxide | | 27,72 | 3,2 |
| 33 | Nerol | 106-25-2 | 38,24 | 1,9 |
| 12 | cis Rose oxide | 16409-43-1 | 23,69 | 1,6 |
| 28 | (-)-terpinen-4-ol | 20126-76-5 | 32,9 | 1,7 |
| 32 | (-)-Borneol | 464-45-9 | 37,41 | 0,6 |
| 6 | 1,8-Cineole | 470-82-6 | 18,29 | 0,4 |
| 31 | (-)- α -terpineol | 10482-56-1 | 34,8 | 0,4 |
| Monoterpene hydrocarbons | | | 23,72 | 2,9 |
| 9 | o-Cymene | 527-84-4 | 20,76 | 1 |
| 18 | p-Cymene | 99-87-6 | 26,68 | 1,9 |
| Aromatic compounds | | | 31,70 | 11,4 |
| 34 | β -Damascenone | 23726-93-4 | 38,35 | 0,7 |
| 35 | 1,1,6-Trimethyl-1,2-dihydronaphthalene | 30364-38-6 | 38,62 | 1,2 |
| 5 | Isoamyl acetate | 123-92-2 | 18,14 | 9,5 |
| Other | | | 32,19708 | 15,2 |
| 20 | 2-Ethyl-1-hexanol | 104-76-7 | 28,11 | 4,2 |
| 46 | Diisobutyl phthalate | 84-69-5 | 48,96 | 4 |
| 13 | 3-Hexen-1-ol | | 24,62 | 0,9 |
| 40 | 2-Phenylethanol | 60-12-8 | 41,55 | 0,6 |
| 43 | 4,6-di-tert-Butyl-m-cresol | 497-39-2 | 44,34 | 0,5 |
| 11 | 1-Hexanol | 111-27-3 | 23,47 | 0,5 |
| 4 | 2-Heptanone | | 17,36 | 0,5 |
| 8 | Bicyclo[4.2.0]octa-1,3,5-triene | | 20,29 | 0,5 |
| 26 | Cyclopropane, pentyl- | | 30,25 | 0,4 |
| 42 | Benzene, 1,3-bis(1,1-dimethylethyl)-5-methyl- | | 43,66 | 0,3 |
| 24 | 1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl | | 29,692 | 0,9 |
| 10 | 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, acetate | | 21,23 | 0,8 |
| 45 | 3-Allyl-6-methoxyphenol | | 45,03 | 0,7 |
| 29 | 1,4-Methanoazulen-7-ol, decahydro-1,5,5,8a-tetramethyl-, [1s-(1 α ,3 α ,4 α ,7 β ,8 α)]- | | 33,72 | 0,4 |

3.3. Total phenolic, flavonoids and tannins contents

As described in Table 2, we recorded roughly an equal high total phenolic content for both methanolic and water extracts of areal parts of *H. scoparia* more or less around of 260mg GAE/g. The obtained results were four time higher than those quantified in the water extract of the same plant collected from Sfax, Tunisia (Bourogaa et al., 2012). However, our data were quite close to those reported in previous studies conducted in Algeria on the aerial part of the same studied plant species (336.756±0.855 mg GAE/g DW in the WE of the 228.582±0.689 mg GAE/g DW in ME) (Salah and Bordjiba, 2018). This could confirm the richness of this plant on polyphenols.

Contrary to the total phenolic contents, flavonoids and condensed tannins content levels significantly varied according the polarity of the used solvent (Table 2). Indeed, methanol extract was more significantly effective than water in extracting these two classes of secondary metabolites.

H.scoparia was interestingly high in total flavonoid with 662.46±5.08 and 260.615 ±3.66 (mg GAE/g DW) for methanolic and water extracts respectively. These amounts were significantly greater than those found by (Salah and Bordjiba, 2018) who reported that the TFC of methanol and water extracts of *H.scoparia* collected from south Algeria were of the order of 17.056± 0.108 mg and 12.99±0.117 mg RE/g DM respectively). Such variations could be due to various biotic (organ and physiological stage) and abiotic (environmental, handling, solvent extraction) factors that affect significantly the flavonoids content of plants (Kumar et al., 2018). In addition, such richness in flavonoids was previously confirmed by the study of Jarraya et al.(2009) who isolated from the WE of this plant species some flavonoids namely quercetin, isorhamnetin and quercetin 3-O-robinobioside. Considering the known capacity of flavonoids to absorb the most energetic solar wavelengths (i.e., UV-B and UV-A); this high TFC could contribute to explain the resistance of this hardy to harsh conditions of Sahara (Brunetti et al., 2013). Nevertheless, tested extracts of *H.scoparia* extracts have a low levels of tannins (4, 12±2.08 and 2.18 ± 0, 23mg CE/g DW) respectively.

Table 2: Total phenolic (TPC) flavonoids (TFC) and tanins (TTC) contents, antioxidant activity

| | TPC (mg GAE/g DW) | TFC(mg CE/g DW) | TTC(mg CE /g DW) | IC ₅₀ (µg/mL) |
|-----|----------------------------|--|------------------------|--------------------------|
| ME | 259.44 ^a ±16.65 | 652.61 ^a ±6.65 | 4,12±2.08 ^a | 8,8 |
| WE | 260.615 ^a ±3.66 | 105.91 ^b ±2.58 ^b | 2.18±0.23 ^b | – |
| BHT | – | – | – | 13,45 |

Values are means ±SD of three determinations. Different letters in the same column indicate significant different difference (*p<0.05). ME: methanolic extract, WE: water extract, BHT: Butylatedhydrotoluene.

3.4. DPPH free radical scavenging assay

The highest free radical scavenging activity of methanolic extract of *H.scoparia* obtained by DPPH method was with the highest tested concentration (50 µg.mL⁻¹), resulting in 91.02 ±0.652% of Inhibition With IC₅₀=1.56 (µg.mL⁻¹) the methanolic extract of *H.scoparia* has proved to be more effective than synthetic antioxidant BHT (IC₅₀=13.45 (µg/mL)).

This finding confirms the potent antioxidant activity of methanolic extract of the same species collected from Sfax, Tunisia with IC₅₀=2.6 (µg.mL⁻¹)(Bourogaa et al., 2012). Such important antioxidant activity is likely mediated by the presence of phenolic components. This assumption is further supported by the high content of total phenolic compounds and flavonoids present in methanolic extract (Table3). In a separate study (Bouaziz et al., 2016) reported that the fractionation of the crude extract of *H. Scoparia* afforded a major pure alkaloid: N-methylisosalsoleN which exhibited a potent scavenging ability thanks to its hydroxyl group (Jang et al., 2009).

3.4. Assessment of phytotoxic activity (aqueous extract) *in vitro* and *in situ*

Phytotoxic activity of water extract of the aerial parts of *H.scoparia* was assessed *in vitro* and *in situ* based on initial shoot and root growth of two crops (radish and wheat) and two weeds (lamb's quarters and Ray grass) to investigate its extensibility and possibility for use as natural weed inhibitors .

Except for wheat hypocotyl growth that was the most resistant to the different treatments with the water extract of *H.scoparia*, a considerable allelopathic effect on the radicle and shoot development of the target species were exhibited by the various concentrations of tested extract under laboratory conditions. This effect was statistically significant (P<0.05) already at the lowest concentration compared to the control (table 3). Furthermore, it could be noted that the extent of the inhibitory action was concentration dependent only for the root length of the two target dicotyledonous species (radish and lamb's quarters).

Conversely, seedling growth inhibition of wheat and ray grass inhibition was not directly related to the concentration applied.

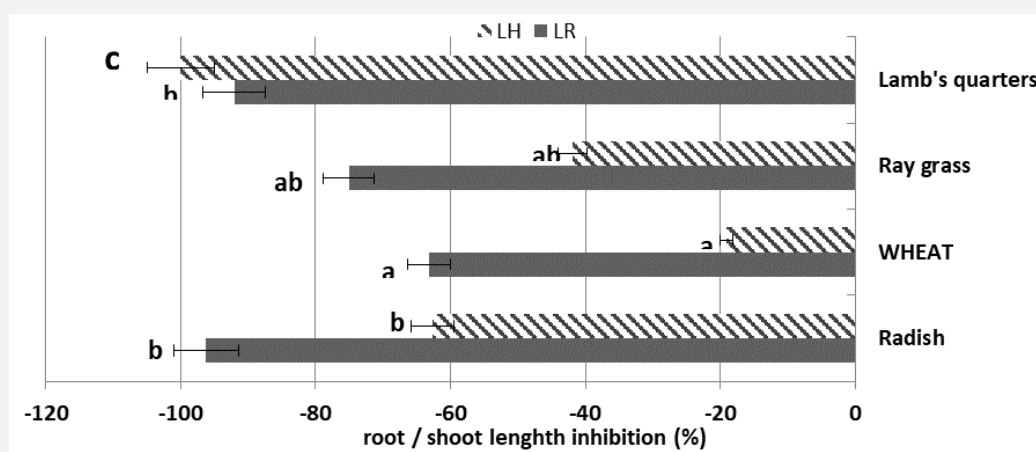
As shown in Figure 1, it can be stated that the root growth of different target species was more sensitive to the phytotoxic effect of *H.scoparia* water extract with an average percentage of inhibition varying from 75.06 to 96% than shoot length with inhibition ranging between 19.11 and 100% in all cases. These results agree with the finding that water extracts of allelopathic plants generally have more pronounced effects on radicle, rather than hypocotyl/coleoptile, growth (Turk and Tawaha, 2002). This may be attributable to the fact that radicles are the first to come in contact with allelochemicals. Moreover, treatment with the higher tested concentration bring out a low sensitivity of Monocots receiving species to the phytotoxic effect of *H.scoparia* compared to the two other dicotyledonous target plants expressed through a significant inhibition of the seedling growth of radish and lamb's quarters that exceed 80% versus 41% and 58% for wheat and ray grass respectively. Previous studies have attributed this different sensitivity of various plant species to inhibitory effect to physiological and biochemical characteristics of each species, the seed structure, seed coat penetrability and seed size (Hoagland and Williams, 2004). Furthermore, as Robert et al. (1999) argue, receiving plants differ in their tolerance and capacity to detoxify phenolic and other allelochemicals. Added to that, certain phenolic acids implicated in allelopathy also have functional importance to internal physiology.

Except for the dicotyledonous weed lamb's quarters, the extent of the phytotoxic activity length was significantly decreased *in situ*. In fact we recorded reduction by half of inhibitory effect on root length compared to its decline by 75% on hypocotyl growth of wheat. Such response might be explained by the fact that phytotoxic activity is influenced by soil processes like adsorption on the soil components and degradation by microorganisms (Inderjit, 2005).

Table 3: Effect of *H.scoparia* WE treatments the radicle and shoot growth of target species *in vitro* and *in situ*

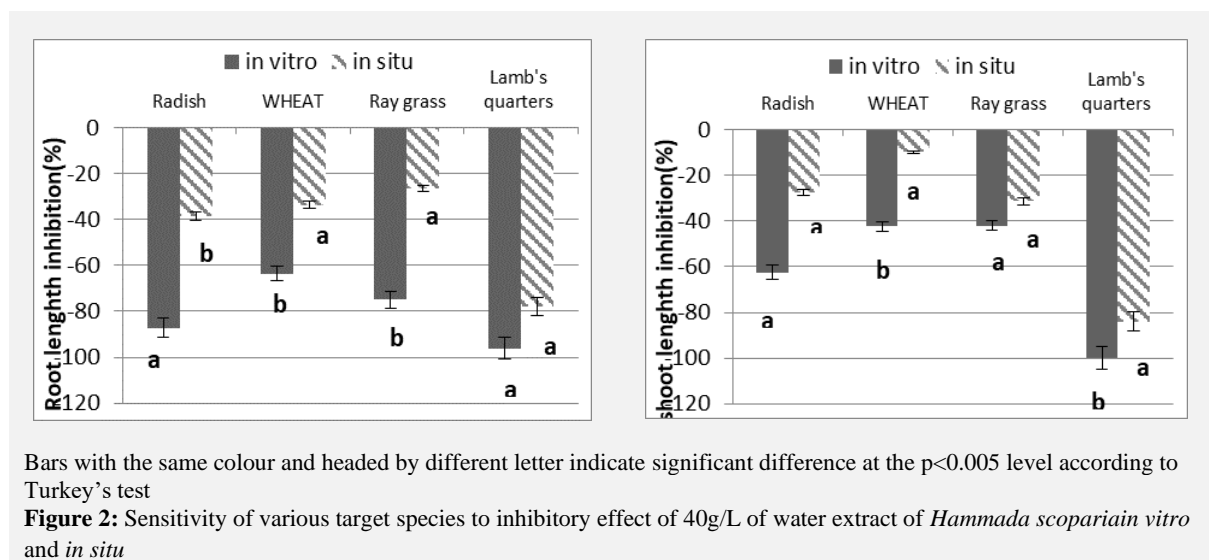
| | Crops | | | | Adventices | | | |
|---------|--------------------|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------------|-------------------|
| | Wheat (Monocot) | | Radish (Dicot) | | Ray Grass (Dicot) | | Lamb's quarters (Dicot) | |
| | LR (cm) | LS (cm) | LR (cm) | LS (cm) | LR (cm) | LS (cm) | LR (cm) | LS (cm) |
| Control | 14,37 ^a | 8,41 ^a | 8,77 ^a | 2,28 ^a | 3,86 ^a | 1,11 ^a | 2,64 ^a | 0,96 ^a |
| 10g/L | 4,33 ^b | 4,52 ^a | 1,51 ^b | 0,82 ^b | 2,47 ^{ab} | 0,92 ^a | 0,53 ^b | 0,36 ^b |
| 20g/L | 3,72 ^b | 4,08 ^a | 1,17 ^b | 0,72 ^b | 2,17 ^{ab} | 0,64 ^b | 0,47 ^b | 0,12 ^c |
| 40g/l | 5,29 ^b | 7,58 ^a | 0,7 ^c | 0,85 ^b | 0,96 ^b | 0,64 ^b | 0,1 ^b | 0 ^c |

Means with the same letter in a column are not significantly different at $p < 0.05$. Data are mean \pm S.D of three replicates



Bars with the same colour and headed by different letter indicate significant difference at the $p < 0.005$ level according to Turkey's test

Figure 1: Sensitivity of radicle and shoot growth of various target species to inhibitory effect of 40g/L of water extract of *Hammada scoparia* *in vitro*



An extensive literature survey showed no previous information on the allelopathic abilities of either *H.scoparia* or species of the same genus, whereas previous study (Pal, 2013) elucidated the survival of desert plants as *H. scoparia* under harsh conditions is in conjunction with their strong allelopathic behaviour.

Such results indicate that there is a chemical basis for the differential allelopathy exhibited by *H.scoparia*. This is likely regulated by several allelochemicals present in its water fraction. This assumption is further supported by the detection of volatile organic compounds including esters, terpenoids and aroma alcohols, that the phytotoxic effect was well studied (Yu et al., 2015). Among major metabolites identified in this study Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester, Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester and Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester, three fatty acids methyl esters FAMES that have a high affinity for fatty compounds (Wang et al. 2015) thanks to their construction, and therefore, they easily penetrate the plant cuticle and also improve the adhesion of the spray mixture to the leaf surface. An earlier study (Synowiec et al., 2017) revealed that common lambsquarters was the most susceptible to the sunflower FAME treatment. In the cited study, authors confirmed that the herbicidal effect of commercial herbicides can be significantly improved by the addition of FAMES to a tank mixture.

Furthermore, the possible additive, synergistic and antagonistic effects of multiple allelochemicals need to be considered, especially for identified oxygenated monoterpenes including Nerol, (-)-Borneol and (-)-terpinen-4-ol whose phytotoxic activity was shown in previous studies (Jassbi et al., 2010; Morimoto et al., 2009; Verdeguer et al., 2009). In this context, Jassbi and co-workers (2012) attributed the phytotoxicity of the monoterpenes to their chemical structures rather than to their water solubility.

The allelopathic effect of such volatile components implied the possibility that it might establish its dominance by releasing plant growth inhibitory compounds to suppress neighbouring plants' development to favour itself.

Although *in situ* experiments suggest that the observed phytotoxic responses of *H.scoparia* may operate in natural conditions, more research is needed to isolate and identify the allelochemicals involved, as well as how biotic and abiotic factors influence its effect on representative receptor plants in natural conditions.

Conclusion

This is the first report on the chemical composition and phytotoxic activity of water extract of *Hammada scoparia*. The results confirm the strong antioxidant activity of this plant with a positive correlation with its phenolic content. Moreover, we found that the tested aqueous extract possessed effective activity to inhibit seedling growth of two weed species *in vitro* and *in situ*. Such findings indicate the potential value of this desert plant to be exploited for the generation of species-targeted herbicides.

Acknowledgement

This work was supported by grants from the “Tunisian Ministry of Higher Education and Scientific Research.

References

- Bouaziz A, Mhalla D, Zouari I, Jlaïel L, Tounsi S, Jarraya R, Trigui M (2016)** Antibacterial and antioxidant activities of Hammada scoparia extracts and its major purified alkaloids. South African Journal of Botany 105, 89-96.
- Boucherit H, Benabdeli K, Benaradj A, Boughalem M (2018)** Phytoécologie de "Hammada scoparia" dans la région de Naâma (Algérie occidentale). Botanica Complutensis 42
- Bourogaa E, Nciri R, Jarraya R, Racaud-Sultan C, Mohamed D, El Feki A (2012)** Antioxidant activity and hepatoprotective potential of Hammada scoparia against ethanol-induced liver injury in rats. Journal of physiology and biochemistry 69
- Brunetti C, Di Ferdinando M, Fini A, Pollastri S, Tattini M (2013)** Flavonoids as Antioxidants and Developmental Regulators: Relative Significance in Plants and Humans. International journal of molecular sciences (14) 3540-3555
- Dewanto V, Wu X, Adom K, Liu RH (2002)** Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem (50), 3010–3014
- EL RHAFFARI L, HAMMANI K, BENLYAS M, ZAID A (2002)** Traitement de la leishmaniose cutanée par la phytothérapie au Tafilalet Biol Sante (1) 45–54
- Hanato T, Kagawa H, Yasuhara T, Okuda T (1998)** Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharm Bull (36) 2090–2097
- Inderjit (2005)** Soil Microorganisms: An Important Determinant of Allelopathic Activity. Plant and Soil - PLANT SOIL 274: 227-236
- Jang, JW, Kay, CS, You, CR, Kim, CW, Bae, SH, Choi, JY, Yoon, SK, Han, CW, Jung, HS, Choi, IB (2009)** Simultaneous Multitarget Irradiation Using Helical Tomotherapy for Advanced Hepatocellular Carcinoma With Multiple Extrahepatic Metastases. International Journal of Radiation Oncology* Biology* Physics 74(2), 412-418
- Jarraya R, Damak M (2001)** Alkaloids extracted from the leaves of Hammada scoparia (Pomel) Iljin. Journal de la Société Chimique de Tunisie 4, 941–948
- Jarraya R, Bouaziz A, Hamdi B, Ben Salah A, Damak M (2008)** N-methylisosalsoline from Hammada scoparia. Acta Crystallographica Section E 64, 1714
- Jassbi AR, Zamanizadehnajari S, Baldwin I (2010)** Phytotoxic Volatiles in the Roots and Shoots of Artemisia tridentata as Detected by Headspace Solid-phase Microextraction and Gas Chromatographic-mass Spectrometry Analysis. Journal of chemical ecology 36, 1398-1407
- Jassbi AR, Asadollahi M, Masroor, M, Schuman, M, Mehdizadeh, Z, Soleimani, M (2012)** Chemical Classification of the Essential Oils of the Iranian Salvia Species in Comparison with Their Botanical Taxonomy. Chemistry & Biodiversity (9) 1254-1271
- Kumar V; Roy BK (2018)** Population authentication of the traditional medicinal plant Cassia tora L based on ISSR markers and FTIR analysis. Sci Rep (8) 10714
- Le Floc'h E (1983)** Contribution à une Etude Ethnobotanique de la Flore Tunisienne, Programme Flore et Végétation Tunisiennes. Tunisia, Imprimerie Officielle de la République Tunisienne, 39–255.
- Mezghani-Jarraya R, Hammami H, Ayadi A, Damak M (2009)** Molluscicidal activity of Hammada scoparia (Pomel) Iljin leaf extracts and the principal alkaloids isolated from them against Galba truncatula. Memórias do Instituto Oswaldo Cruz 104, 1035–1038
- Morimoto M, Cantrell CL, Libous-Bailey L, Duke SO (2009)** Phytotoxicity of constituents of glandular trichomes and the leaf surface of camphorweed, Heterotheca subaxillaris. Phytochemistry 70(1), 69-74
- Pal S (2013)** Physiological Conjunction of Allelochemicals and Desert Plants. PLOS ONE, doi:10.1371/journal.pone
- Roberts, R, Roberts, C W, Johnson J J, Kyle D E, Krell T, Coggins JR, Coombs GH, Milhous WK, Tzipori S, Ferguson DJ, Chakrabarti D, McLeod, R (1999)** Evidence for the shikimate pathway in apicomplexan parasites. Nature (393) 801-805

- SalahB, Bordjiba O (2018)** Phytochemical study and in vitro antioxidant activities of hammada scoparia extracts from southeastern Algeria Asian. Journal of Pharmaceutical and Clinical Research 11, 187
- Sathiyamoorthy, P, Lugasi-Evgi, H, Van-Damme, P, Abu-Rabia, A, Gopas, J, Golan- Goldhirsh, A, (1997)** Larvicidal activity in desert plants of the Negev and Bedouin market plant products. International Journal of Pharmacology (35) 265–273
- SingletonVL, OrthoferR, Lamuela-Raventos, RM (1999)** Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol(299) 152-178
- SynowiecA, Halecki, W, Wielgusz, K, Byczyńska, M, Czaplicki, S,(2017)** Effect of Fatty Acid Methyl Esters on the Herbicidal Effect of Essential Oils on Corn and Weeds. Weed Technology 31
- SunB, Richardo-da-Silvia JM, Spranger I (1998)**Critical factors of vanillin assay for catechins and proanthocyanidins .J Agric Food Chem (46) 4267–4274
- Turk MA and Tawaha AM (2002)** Inhibitory effects of aqueous extracts of barley on germination and growth of lentil Pakistan. Journal of Agronomy (1)28–30
- VerdeguerM, Blázquez M A, Boira H(2009)**Phytotoxic effects of Lantana camara, Eucalyptus camaldulensis and Eriocephalus africanus essential oils in weeds of Mediterranean summer crops. Biochemical Systematics and Ecology 37(4), 362-369
- Wang W, Wei H, Du Z, Tai X, Wang G (2015)** Formation and characterization of fully dilutable microemulsion with fatty acid methyl esters as oil phase. ACS Sust Chem Engin (3)443–450
- WCSP (2019).** *World Checklist of Selected Plant Families* Facilitated by the Royal Botanic Gardens, Kew Published on the Internet; <http://wcpssciencekew.org/> Retrieved 01 **November 2019**
- Yu P, Su Y, Dong C, Yao C, Ding Y, Zhou X (2015)** Preliminary proteomic analysis of tobacco leaves affected by volatile organic compounds from floral scent of rose .Plant Growth Regulation (75) 689-694