CHEMICAL AND RADICAL SCAVENGING ACTIVITY OF CONSTITUENTS FROM *Hammada scoparia* (Pomel) Iljin

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ABSTRACT: 1-Methylsalsolinol **1**, dopamine **2**, quercetin **3**, isorhamnetin **4** and quercetin 3-O-robinobioside **5** were isolated for the first time from ethanolic extract of *Hammada scoparia* (Pomel) Iljin. The complete NMR analysis of compound **1** was reported. The scavenging activity of the pure compounds **1**-**5** against DPPH (2, 2-diphenyl-1-picrylhydrazyl) was investigated. Results showed that quercetin, 1-methylsalsolinol and dopamine presented a potent antiradical activity in comparison with ascorbic and caffeic acids. The chemical composition of hexanic extract obtained from the leaves of this plant, was analysed by GC-MS. This analysis led to the identification of 35 constituents.

Key Words: *Hammada scoparia*; Chenopodiaceae; alkaloids; flavonoids; antiradical activity.

RESUME: Le 1-méthylsalsolinol **1**, la dopamine **2**, la quercétine **3**, l'isorhamnétine **4** et la quercétine 3-O-robinobioside **5**, ont été isolés pour la première fois de l'extrait éthanolique de *Hammada scoparia* (Pomel) Iljin. L'activité anti-radicalaire des composés isolés **1**-**5**, moyennant le test de DPPH (2, 2-diphényl-1-picrylhydrazyle), a été évaluée. Les résultats obtenus ont montré que la quercétine, le 1-méthylsalsolinol et la dopamine présentent une activité anti-radicalaire intense par comparaison avec les acides ascorbique et caféique. L'analyse par couplage CPG-SM de l'extrait hexanique a permis d'identifier 35 constituants.

Mots clés: *Hammada scoparia*; Chenopodiaceae; alcaloïdes; flavonoïdes; activité anti-radicalaire.

INTRODUCTION

Hammada scoparia (Pomel) Iljin (*Haloxylon scoparium* (Pomel) = *Arthrophytum scoparium* (Pomel) Iljin = *Haloxylon articulatum* subsp. *scoparium* (Pomel) batt.)(Chenopodiaceae) is distributed in North Africa, south-east Spain and Irano-Turanian region [1-3]. In Tunisia, it is used in traditional medicine to treat eye disorders [4, 5]. Aqueous extracts of this plant have been reported to show anti-cancer, anti-plasmodial and larvicidal activity [6, 7], but relatively little has been published on its phytochemistry [8-10].

In the present work, we report the identification of five minor compounds from the leaves ethanolic extract of *H. scoparia* and the analysis by GC-MS of the chemical composition of its hexanic extract. All compounds **1**-**5** were tested for their free radical scavenging activity.

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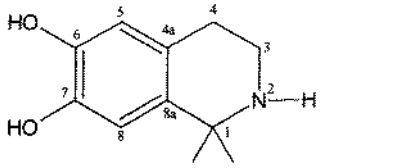

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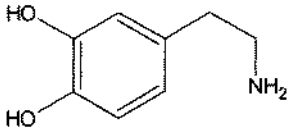

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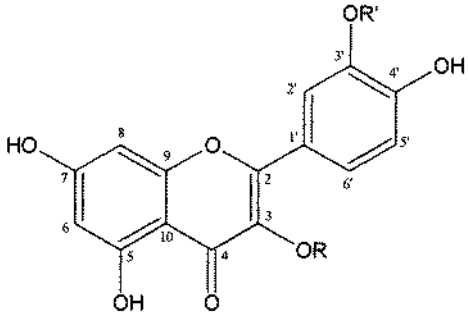

3 : R = H ; R' = H

4 : R = H ; R' = CH_3

5 : R = robinobioside ; R' = H

Robinobioside : Galactosyl-(6"→1'")-Rhamnosyl

EXPERIMENTAL

1- General experimental procedures

UV spectra were recorded on a Schimadzu 2100 UV-visible spectrophotometer. The IR spectra were recorded on Jasco FT-IR420 instrument. The mass spectra ESI were obtained with a Thermoquest AQA Navigator spectrometer. ^{1}H- and ^{13}C- NMR spectra were recorded in CD_3OD and DMSO-d_6 on a Bruker AC-270 (270MHz) and AC-300 (300MHz) instruments.

The GC-MS analyses were performed using a HP 5890 gas chromatograph coupled with HP59712 mass spectrometer. The GC was used with a fused-silica capillary column HP5MS (30m × 0.25mm i.d.). Helium was used as the carrier gas at a flow rate of 1.4ml.min^{-1}. The GC oven temperature was programmed from 180°C, 2min isothermal at a rate of 5°C min^{-1} and then was increased to 295°C, 15min isothermal. The injector temperature was 250°C. Samples were run in the electron impact mode at 70 eV with a 2.9s scan time over a 50-550 a.m.u. range resolution.

2- Plant material

Hammada scoparia (Pomel) Iljin was collected in November 2000 from Sfax (Tunisia). The leaves were carefully detached from the air-dried plants. A voucher specimen N° "LCSN101" has been deposited at the "Laboratoire de Chimie des Substances Naturelles", Faculté des Sciences de Sfax (Tunisie).

3- Extraction and isolation

Air-dried leaves (300g) of *Hammada scoparia* were extracted by soxhlet apparatus with hexan, CH_2Cl_2 and EtOH successively.

2.7g of hexanic extract (12.9g) were fractionated by Silica gel column (Merck, 230-400 mesh) eluting with increasingly gradient of hexan / CH_2Cl_2 (100:0 → 0:100). 14 fractions were collected A → N. The dichloromethanic extract (9.8g) afforded alkaloids previously described [8, 9] from *Hammada scoparia*.

2g of the ethanolic extract (9.3g) were subjected to Silica gel CC eluting with an increasingly polar gradient of CH_2Cl_2 / MeOH (100:0 → 0:100). Column fractions submitted to Silica gel preparative TLC (20×20) and to preparative paper chromatography (Whatman 3MM) afforded compounds **1** (20mg, 0.031 %), **2** (41mg, 0.063 %), **3** (15mg, 0.023 %), **4** (44mg, 0.068 %) and **5** (15mg, 0.023 %).

Compound **1** : White amorphous solid ; UV max (MeOH) : 211.8, 224.8, 286.6 nm; IR bands (KBr) : 3343 (ν_{OH}), 3185 (ν_{NH})cm^{-1} ; ESI (positive mode) : *m/z* 194 $[M + H]^+$; ^{1}H-NMR (300 MHz, CD_3OD) : δ : 1.67 (6H, *s*, 2 CH_3), 2.96 (2H, *t*, *J* 6.3 Hz, H-4), 3.45 (2H, *t*, *J* 6.3 Hz, H-3), 6.57 (1H, *s*, H-5), 6.70 (1H, *s*, H-8) ; ^{13}C-NMR (75 MHz, CD_3OD) : 147.03 (C-6), 146.70 (C-7), 130.17 (C-8a), 122.82 (C-4a), 116.60 (C-5), 113.21 (C-8), 58.61 (C-1), 39.31 (C-3), 29.05 (CH_3), 29.03 (CH_3), 26.51 (C-4).

4- Free-radical scavenging activity

The free radical-scavenging activity of compounds **1**-**5** on the DPPH radical was assessed using the method described by Brand-williams and al. [11]. Etbanolic sample solution (2ml) was mixed with 2ml of 0.6 10^{-4}M DPPH (2, 2-diphenyl-1-picrylbydrazyl hydrate, 95 %, Aldrich) ethanolic solution and then kept in the dark for 30min. The absorbance was measured at 517nm and the percent free radical inhibition (PI) was calculated. The measurements were performed at least in triplicate. The antioxidant activity of each sample was expressed in IC_{50} (µg / ml), concentration obtained from the inhibition curve. Known antioxidants, ascorbic (99 %, Aldrich) and caffeic (97 %, Aldrich) acids, were used for comparison.

RESULTS AND DISCUSSION

1- Isolated compounds

All the phenolic compounds **1**-**5**, known in literature, were isolated for the first time from *Hammada scoparia*. The structures of the isolated compounds were determined on the basis of spectral data such as UV, MS, ^{1}H- NMR, ^{13}C-NMR, HMQC, HMBC and NOESY and confirmed by comparison with published spectra and structures.

Compound **1**, identified as 1-methylsalsolinol, has been reported only from *Dioscorea batatas* [12]. The structure of **1** was assigned by the study of HMQC, HMBC (Table I) and NOESY (Figure 1) experiments. A complete set of ^{1}H- and ^{13}C-NMR resonance assignments for this compound in CD_3OD was given for reference in the experimental section (the original data of ^{1}H-NMR were obtained in D_2O).

Table I: ^{1}H and ^{13}C NMR assignments and ^{1}H-^{13}C long-range correlations of **1** by HMQC and HMBC in CD_3OD

N°	^{13}C NMR (ppm)	^{1}H NMR (ppm)	HMBC correlations
1	58.61		
3	39.31	3.45 (t, *J* = 6.3Hz)	26.51 (4), 58.61 (1), 122.82 (4a)
4	26.51	2.96 (t, *J* = 6.3Hz)	39.31 (1), 116.60 (5), 122.82(4a), 130.17 (8a)
4a	122.82		
5	116.60	6.57 (s)	26.51 (4), 130.17 (8a), 146.70 (7)
6	147.03		
7	146.70		
8	113.21	6.70 (s)	58.61 (1), 122.82 (4a), 147.03 (6)
8a	130.17		
CH_3	29.31	1.67 (s)	58.61 (1), 130.17 (8a)
CH_3	29.05	1.67 (s)	58.61 (1), 130.17 (8a)

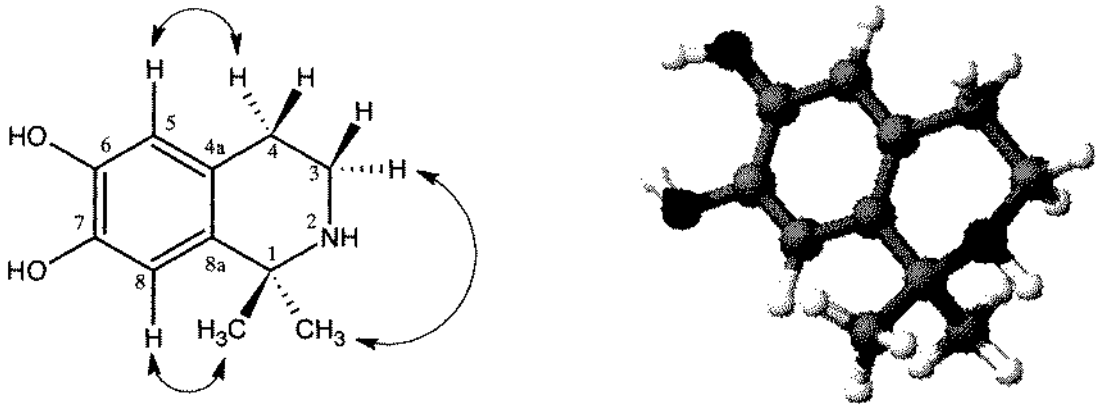

Figure 1: Significant NOESY correlations and 3D structure of compound **1**.

Compounds **2**, **3**, **4** and **5** were identified respectively, as dopamine [13], quercetin, isorhamnetin [14, 15] and quercetin 3-O-robinobioside [16].

2- Free-radical scavenging activity test

The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. The results of our experiments using DPPH test (Table II) demonstrated that ortho-diphenol compounds (1-methylsalsolinol **1**, dopamine **2** and quercetin **3**) possess a potent antiradical activity in comparison with ascorbic and caffeic acids.

In agreement with Chen et al. [17] and Bocco et al. [18], the antioxidant activity of natural flavonoids is governed by the number and the location of their aromatic hydroxyl groups. In the case of quercetin, the antioxidative effectiveness increased with the presence of five hydroxyl groups and an ortho-hydroxylation on B ring.

The methoxyl group at position 3' (which is shared by isorhamnetin **4**) or the glycosylation at position 3 (which is shared by quercetin 3-O-robinobioside **5**), reduced the antioxidant activity. This ascertainment was confirmed by 1-methylsalsolinol **1** and dopamine **2**. These alkaloids, having ortho-diphenol function, showed a potent radical scavenging activity (IC_{50} = 3.8 and 4.2 µg / ml, respectively).

Table II: Scavenging effects (IC_{50}) of *Hammada scoparia* constituents on DPPH free radicals.

Entry	Sample	IC_{50}[a] (µg / ml)
1	1-Methyl salsolinol	3.8 ± 0.4
2	Dopamine	4.2 ± 0.2
3	Quercetin	2.2 ± 0.1
4	Isorhamnetin	28.0 ± 0.4
5	Quercetin 3-O-robinobioside	6.3 ± 0.5
6	Caffeic acid	3.2 ± 0.1
7	Ascorbic acid	3.5 ± 0.2

Values are mean ± standard deviation of three replicate analyses.
[a] IC_{50} : the concentration of sample required for 50 % inhibition.

3- Analysis of chemical composition of the hexanic extract

The chemical constituents of hexanic extract obtained from leaves of *H. scoparia* were carried out by GC-MS. The identity of some compounds was established by comparison of their mass spectra with those in the spectrometer data bank (Chemstation HP NRS 75KL). In Table III, we reported the identification of 35 constituents.

Table III: Chemical constituents identified from the hexanic extract of *Hammada scoparia*.

Family	Compound	MW[a]	RT[b](min)	Area[c](%)
Hydrocarbons (Fraction A)	Nonadecan	268	14.17	12.50
	Tetracosan	338	16.41	1.33
	Dotriacontan	450	18.36	6.38
	Hexatriacontan	506	19.82	19.75
	Tritetracontan	604	20.95	1.67
	Tetratetracontan	618	21.75	1.72
Grass acids (Fraction B)	Hexadecanoic acid	256	7.35	3.70
	Octadec-9-enoic acid	282	9.91	3.58
	Octadecanoic acid	284	10.29	1.49
	Nonadecanoic acid	298	13.25	0.95
	Eicosanoic acid	312	16.14	1.27
Esters (Fraction D)	Tetradecanoic acid, ethyl ester	256	4.21	4.19
	Hexadecanoic acid, methyl ester	270	5.78	7.18
	Hexadecanoic acid, ethyl ester	284	6.80	16.68
	Octadec-9-enoic acid, methyl ester	296	8.16	5.16
	Octadecanoic acid, methyl ester	298	8.54	1.41
	Ethyl oleate	310	9.28	11.91
	Octadecanoic acid, ethyl ester	312	9.62	5.01
	Eicosanoic acid, methyl ester	326	11.54	0.84
	Docosanoic acid, methyl ester	354	14.55	1.62
	Tetracosanoic acid, methyl ester	382	17.42	1.67
	Hexacosanoic acid, methyl ester	410	20.09	0.38
	Octacosanoic acid, methyl ester	438	22.64	0.26
	Triacontanoic acid, methyl ester	466	25.05	0.19
Others (Fractions G and H)	β -Amyrin	426	25.30	1.40
	Octadecanal	268	21.52	2.01
	Octacosanol	410	22.95	8.13
	Hexacosanal	380	24.01	0.64
Sterols (Fraction K)	Cholesterol	386	22.89	2.44
	(3β, 5α) Cholestan-3-ol	388	23.01	2.49
	Ergosta-5,7,22-trien-3β-ol	396	23.96	1.42
	(3β, 24R)Ergost-5-en-3-ol	400	24.21	4.51
	Stigmasterol	412	24.67	11.85
	β-Sitosterol	414	25.42	19.46
	(3β, 5α) Stigmastan-3-ol	416	25.53	8.62

[a] : molecular weight ; [b] : retention time ; [c] : the area (%) was done relatively to every fraction.

β-Sitosterol and stigmasterol were found to be the major sterols. We have notice significant similarities between many fractions. It's the case of B and C fractions. The same is true for D, E and F fractions. While the differences between G, H, I and J fractions concern the octacosanol content ; it's maximum in G fraction but only traces of this compound were detected in H, I and J fractions. The latest fractions (L, M and N) contain compounds which are not identified by spectrometer data bank.

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