SHORT REPORT

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Flavonols and Antioxidant Activity of *Ammi visnaga* L. (Apiaceae)

Reguia Bencheraiet[1], Habiba Kherrab[1], Ahmed Kabouche[1], Zahia Kabouche[1*] and Maurice Jay[2]

[1]*Laboratoire d'Obtention de Substances Thérapeutiques, Département de Chimie, 25000 Constantine, Algérie*

[2]*Université Claude-Bernard, Laboratoire de phytochimie, Lyon, France*

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Abstract: Eleven flavonols have been isolated from the aerial parts of *Ammi visnaga* L. from which four aglycones, four monoglycosides, two diglycosides and one triglycoside. The flavonoid aglycones were distributed into one hydroxylated, quercetin (**1**) and three methoxylated, namely, rhamnetin (**2**), isorhamnetin (**3**) and rhamnazin (**4**). Among the monoglycosides, we found three 3-O-glucosides respectively linked to rhamnetin (**5**), isorhamnetin (**6**) and rhamnazin (**7**) and one 7-O-glucoside of isorhamnetin (**8**). The two diglycosides were 3-O-rutin of quercetin (**9**) and isorhamnetin (**10**) while the single trioside was quercetin 7,3,3'-O-triglucoside (**11**). These flavonols are reported for the first time from *A. visnaga* L. Free radical DPPH scavenging potential of the butanolic extract was investigated.

Keywords: *Ammi visnaga* L.; Apiaceae; flavonol glycosides; antioxidant capacity.

1. Plant Source

Ammi visnaga L. (Apiaceae) is a perennial herb widely distributed in the Mediterranean area. The genus *Ammi* comprises 3 species in the Algerian flora [1]. *Ammi visnaga* is used in Algerian folk medicine to treat vitiligo. Aerial parts of *Ammi visnaga* L. were collected during the flowering period in may (2005), at Annaba, Algeria. A voucher specimen was deposited at the Herbarium of the Laboratory under the code number LOST.Av.05.05.

1. Previous Study

Furanochromones (khellin and visnagin) have been reported from the fruits of *Ammi visnaga* (L.) LAM [2] but flavonoids have not been investigated in this species. Our species *Ammi visnaga* L. has not been the subject of any phytochemical study.

* Corresponding author: E-mail : zkabouche@yahoo.com. Tel/fax : 213-31-81885

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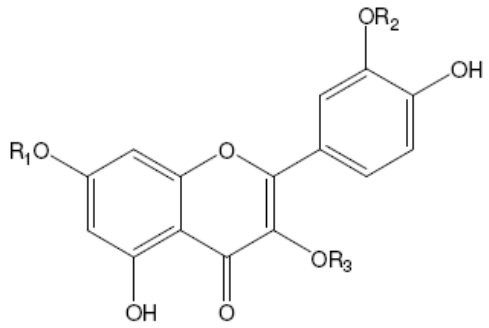
3. Present Study

Air dried aerial parts of *Ammi visnaga* (2500 g) were macerated three times with 70% MeOH solution. The hydro-alcoholic solution was concentrated under reduced pressure to dryness and the residue was dissolved in hot water (1000 mL) and kept in cold overnight. After filtration, the aqueous solution was successively extracted with EtOAc for one time and with n-BuOH for three times then the EtOAc and n-BuOH extracts were evaporated to dryness.

The butanolic extract (20 g) was subjected to a column chromatography on polyamid SC6 with a gradient of toluene-MeOH of increasing polarity. Preparative TLC on polyamid DC6 using the system toluene-MeOH-methylethylketone (4:3:3) and further flash column chromatography on Sephadex eluted with MeOH- H_2O (60:40) leaded to eleven compounds (**1-11**).

Acid hydrolysis of **5-11***:* Each compound (5 mg) was refluxed with 5% H_2SO4 (5 mL) in water for 1 h. The reaction mixture was diluted with water and fractionated by EtOAc. Each EtOAc-soluble fraction was concentrated and examined by TLC with authentic samples. Each remaining aqueous layer was adjusted to pH 7 with $NaHCO_3$ and filtered. The filtrate was concentrated and examined by TLC with authentic sugars.

Eleven flavonols have been identified by the use of spectroscopic techniques (NMR, UV, Mass) and acid hydrolysis for compounds (**5-11**), from which four aglycones, four monoglycosides, two diglycosides and one triglycosides. The flavonoid aglycones were distributed into one hydroxylated, quercetin (**1**) and three methoxylated, rhamnetin (**2**), isorhamnetin (**3**) and rhamnazin (**4**). Among the monoglycosides, we found three 3-O-glucosides of rhamnetin (**5**), isorhamnetin (**6**) and rhamnazin (**7**) and one 7-O-glucoside of isorhamnetin (**8**). The two diglycosides were, a 3-O-rutinosyl of quercetin (**9**) and isorhamnetin (**10**) while the single trioside was quercetin 7,3,3'-O-triglucoside (**11**).



	R_1	R_2	R_3
1	H	H	H
2	Me	H	H
3	H	Me	H
4	Me	Me	H
5	Me	H	Glc
6	H	Me	Glc
7	Me	Me	Glc
8	Glc	Me	H
9	H	H	Rut
10	H	Me	Rut
11	Glc	Glc	Glc

Figure 1. Isolated flavonoids

These flavonols are reported for the first time from *A. visnaga* L. and for *A. visnaga* (L.) LAM. however, only the aglycones (**1-4**) [3] and the monoglycoside isorhamnetin 3-O-glucoside (**6**) [4] and the diglycoside isorhamnetin 3-O-rutinoside (**10**) [4] have been reported from the genus.

Antioxidant activity : 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) by Hatano method: The antioxidant activity of the butanolic extract of *Ammi visnaga* (BEAV) was determined by the slightly modified method of Hatano [5]. One milliliter of a 0.2 mM DPPH methanol solution was added to 4 ml of various concentrations of the extract in methanol. The mixture was shaken vigorously and left to stand at room temperature. After 30 min, the absorbance of the solution was measured at 517 nm and the antioxidant activity calculated using the following equation:

Scavenging capacity % = 100 - [(Ab of sample - Ab of blank) x 100/Ab of control].

Methanol (1 mL) plus BEAV solution (4 ml) were used as a blank, while DPPH solution plus methanol was used as a negative control. The positive control was DPPH solution plus 1 mM rutin. Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition percentage against extract concentration.

Table 1. The free radical scavenging capacity in DPPH system (IC50, µg/mL) of the samples[a]

Sample	IC_{50} (µg/mL)
BEAV	8.77 ± 0.2
Rutin [b]	3.01 ± 0.2

[a] Values expressed are means ± S.D. of three parallel measurements.
[b] Reference compound.

As shown in table 1, BEAM possess a good antioxidant activity. This may be explained by the resence of quercetin.

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) by Blois method: The DPPH radical-scavenging activity of the BEAV was assayed by the slightly modified method of Blois [6]. After 30 min, the absorbance of the solution was measured at 660 nm and the antioxidant activity calculated using the following equation:

DPPH radical-scavenging activity % = [(Absorbance of the control – Absorbance of the sample) / Absorbance of the control] x 100

As shown in table 2, the BEAV markedly quenched the DDPPH radical by 78.7 % at a concentration of 200 µg/mLwhich confirms the result obtained with Hatano method.

Table 2. DPPH radical-scavenging activity of BEAV

Sample	**DPPH radical-scavenging activity %**				
	1µg/mL	**10µg/mL**	**50µg/mL**	**100µg/mL**	**200µg/mL**
BEAV	29.6	43.1	51.2	69.8	78.7

From these results, We can conclude that the butanolic extract of *Ammi visnaga* possess an equivalent high antioxidant activity.

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