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Anti-HCV Potential of the Medicinal Roots of Khella and Celery Plants

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Abstract

Khella (*Ammi visnaga* (L.) Lam.) and celery (*Apium graveolens* L.) are two valuable medicinal plant species with a wide-ranging health and therapeutic benefits, including hepatoprotective and antiviral properties. Thus, in this work, the total ethanolic extracts of the roots of the aforementioned species were studied for their antiviral potential against Hepatitis C virus (HCV) using the *in vitro* luciferase assay. The obtained data demonstrated the noteworthy inhibitory activity of khella roots against HCV (IC_{50}= 9.5 µg/ml), while no noticeable inhibition was shown by celery roots. Besides, three phytosterols (**I–III**) and one furanochromone (**IV**), with previously reported antiviral properties, were isolated and identified from khella roots for the first time. These data could pave the way towards further phytochemical and biological investigation of such medicinal roots as potential sources of natural anti-HCV agents.

Keywords
Ammi visnaga, *Apium graveolens*, Hepatitis virus C, Medicinal roots, Secondary metabolites.

1. Introduction

Hepatitis C virus (HCV), first identified in 1989, is the major etiological agent of non-A non-B hepatitis [1]. It is an enveloped, positive stranded RNA virus belonging to the family Flaviviridae [2]. HCV infection occurs principally through blood or blood-derived products and is regarded as the major cause of chronic liver diseases, infecting more than 170 million people worldwide and often leading to liver cirrhosis, hepatic failure, and hepatocellular carcinoma [3–5].

The use of medicinal plants dates back to the origin of human civilization on earth, with many of them was used to treat viral infections in the past. However, the first recognized interest in the development of medicinal plants as antiviral agents was the efforts of the Boots drug company (Nottingham, England) to screen 288 plant species for their anti-influenza activities [6]. Afterwards, researchers have turned to the plant kingdom to search for new antiviral drug candidates due to the unwelcome adverse effects of the existing antiviral agents as well as the growing phenomena of drug resistance. In this respect, a vast range of plant secondary metabolites, such as silymarin, epigallocatechin gallate, and naringenin that belong to the flavonoid family, have been reported to possess outstanding antiviral activities, namely against HCV infections [7]. Allicin and ajoene; two organosulfur compounds isolated from garlic (*Allium sativum* L., Family Amaryllidaceae) bulbs have been also found to exhibit wide-ranging antiviral activities [8], while the ethanolic extract of garlic bulbs is known to possess beneficial hepatoprotective effects [9, 10]. Likewise, the seeds' extract of celery (*Apium graveolens* L., Family Apiaceae) was described as a potent liver-protecting agent [11, 12], whereas the natural flavonoidal molecule, apigenin, isolated from celery leaves has been shown to inhibit HCV replication by decreasing mature microRNA122 levels [13, 14]. In the same vein, the two common furanochromones, khellin and visnagin from khella (*Ammi visnaga* (L.) Lam., Family Apiaceae) fruits have been also reported to exert antiviral activities against the mammalian viruses, herpes simplex virus-1 (HSV-1) and vesicular stomatitis virus (VSV) [12, 15, 16]. Despite the fact that the two plants under study, namely *A. visnaga* and *A. graveolens*, have been described to show hepatoprotective and/or antiviral activities, the roots of these medicinal plant species remain unexplored yet. As a result, the root extracts of these plants were searched herein for their *in vitro* antiviral potential against HCV in cultured cells using the luciferase assay [17], followed by phytochemical analysis.

2. Experimental

2.1. Plant material

The fresh roots of *A. visnaga* and *A. graveolens* were collected in 2018 and identified by Prof. Mahmoud Abdel Hady (Faculty of Agriculture, Minia University, Egypt). Voucher samples were added to the herbarium section of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Egypt under the numbers Mn-Ph-Cog-039 (*A. visnaga*) and Mn-Ph-Cog-040 (*A. graveolens*). The collected roots were then air-dried in the shade, converted to fine powders (400 g for *A. graveolens* and 7 kg for *A. visnaga*), and macerated in 95% ethanol, followed by concentration under vacuum to viscous brown residues (*A. visnaga*: 80 g and *A. graveolens*: 22 g).

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2.2. Evaluation of anti-HCV activity

Testing of the anti-HCV potential of the total root extracts of the plants under study was carried out according to the method mentioned by Abdelaleem *et al.* [18]. Briefly, HCV replicon cells (Vero E6 cells) were inoculated in 48-well plates at 26 × 10^4 cells/well one day before the test (Reblikon, Mainz, Germany) and the total plant extracts were then added at a concentration range of 1–200 µg/ml. After three days, a cell culture lysis reagent was employed for harvesting and lysis of the cells. A luciferase assay was adopted to determine the luciferase activity and the obtained luminescence was measured by using a plate reader (the obtained data reflect the level of expression of the HCV replicon) [19]. Then, the MTS assay was employed to test for any cytotoxicity caused by the phytocompounds of the tested samples [20]. Cyclosporin was added as a positive control for the inhibition of HCV replication. IC_{50} values of the tested extracts were finally determined.

2.3. Phytochemical study of *A. visnaga* roots

2.3.1. Fractionation of the total extract

The total ethanol extract of *A. visnaga* roots was suspended in dist. H_2O and successively extracted with petroleum ether, dichloromethane, and ethyl acetate to afford three fractions: [fraction I (25 g), fraction II (11 g), and fraction III (8 g)], respectively, along with the mother liquor [fraction IV (36 g)].

2.3.2. Isolation of compounds (I–IV)

The petroleum ether fraction I (25 g) was initially gross fractionated using vacuum liquid chromatography (VLC) in a glass column (6 × 30 cm) packed with silica gel for TLC (250 g) and a vacuum pump was used to help elution. Elution was performed using petroleum ether and then with petroleum ether–ethyl acetate gradient mixtures (10%, 20%, 40%, 60%, 80%, and 100% ethyl acetate) and the effluents were obtained in fractions (200 ml each). Each fraction was concentrated under reduced pressure, examined by TLC on precoated silica gel GF_{254} plates, and similar fractions were lastly added together to give seven subfractions (I_1–I_7). Compound **I** (70 mg) was obtained by direct precipitation from subfraction I_2 that was eluted with petroleum ether–ethyl acetate (90:10). Likewise, the treatment of subfractions I_4 and I_6, obtained with petroleum ether–ethyl acetate (60:40) and (20:80), with methanol afforded compounds **II** (74.7 mg) and **III** (53.4 mg) as white precipitates, respectively. In the same way, the dichloromethane fraction II (11 g) was gross fractionated using the VLC technique in a glass column (6 × 30 cm) containing silica gel for TLC (250 g) and elution was carried out with dichloromethane and then dichloromethane–methanol gradient mixtures (10%, 15%, 20%, 40%, 60%, 80% and 100% of methanol). The effluents were divided into 100 ml-fractions which were concentrated, checked by TLC, and added together on the basis of their TLC behavior to provide four subfractions (II_1–II_4). Among them, the subfraction II_2 (1.5 g) was further purified by silica gel column chromatography using dichloromethane–methanol gradient mixtures (0%, 5%, 10%, and 15%) to give subfractions II_2-1: II_2-4. Further purification of the subfractions II_2-1 (140 mg) on silica gel for column chromatography using gradient mixtures of dichloromethane–methanol (99:1, 98:2, and 97:3) yielded compound **IV** (40.4 mg) from the mobile phase system dichloromethane–methanol (99:1).

3. Results and discussion

3.1. Anti-HCV activity

Of the tested plants, the total ethanolic extract of *A. visnaga* roots showed the most potent activity against HCV with an IC_{50} value of 9.5 µg/ml, while *A. graveolens* root extract was inactive.

3.2. Phytochemical study of *A. visnaga* roots

Previous chemical examination of *A. visnaga* plants has reported the existence of several important chemical principles that chiefly involved γ-pyrones like visnagin and khellin as well as varied phenolics like flavonoids and coumarins [12, 21]. According to the current literature, the aerial parts and the fruits were the most studied organs from *A. visnaga*, while little phytochemical attention has been given to the roots [21]. Consequently, the chemical composition of *A. visnaga* roots was investigated herein, leading to the isolation of compounds **(I–IV)** from the petroleum ether and dichloromethane fractions of their total ethanolic extract. Identification of the purified metabolites was achieved via ^{1}H- and ^{13}C-NMR, DEPT-Q [on a Bruker Avance III HD 400 MHz spectrometer (Switzerland)], EI-MS, and ESI-MS [on a Thermo Scientific mass spectrometer (USA)] analyses as well as the comparison with the previously reported data in the literature. According to the data mentioned in **Tables 1** and **2** (supplementary material Figures S1–S22), structures of compounds **(I–IV)** were elucidated as: a mixture of *β*-sitosterol (**Ia**) and stigmasterol (**Ib**) [22–24], stigmasterol 3-*O*-*β*-glucopyranoside-6'-*O*-stearate (**II**) [25–27], stigmasterol 3-*O*-*β*-glucopyranoside (**III**) [25, 28], and visnagin (**IV**) [29–31] (**Figure 1**). Of these, *β*-sitosterol (**Ia**) and visnagin (**IV**) were previously isolated from the aerial parts and the fruits of *A. visnaga*, respectively [16, 21], whereas this is the first isolation of both metabolites from the roots. In contrast, compounds (**Ib**), (**II**), and (**III**) are reported herein for the first time in khella plants. Previous literature works have shown the antiviral potential of phytosterols such as *β*-sitosterol, stigmasterol, and their derivatives against a variety of human viruses [32–37]. Likewise, the natural furanocoumarin derivatives, khellin and visnagin have been also reported to possess antiviral properties against some mammalian viruses, e.g. HSV-1 and VSV [15, 16]. These data might propose the contribution of these natural metabolites to the observed anti-HCV potential of *A. visnaga* roots, which should inspire further research on the probable antiviral properties of each of these individual molecules against HCV.

Conclusion

This work evaluated the anti-HCV potential of two important Apiaceae plants that are commonly used as food and/or medicinal species, of which the total extract of khella roots revealed a noteworthy inhibitory activity (IC_{50}= 9.5 µg/ml), while celery roots were inactive. Additionally, four natural metabolites, with previously reported antiviral properties, were isolated and identified from khella roots for the first time. These findings could embolden further phytochemical and biological exploration of these roots as a source of potential natural anti-HCV agents.

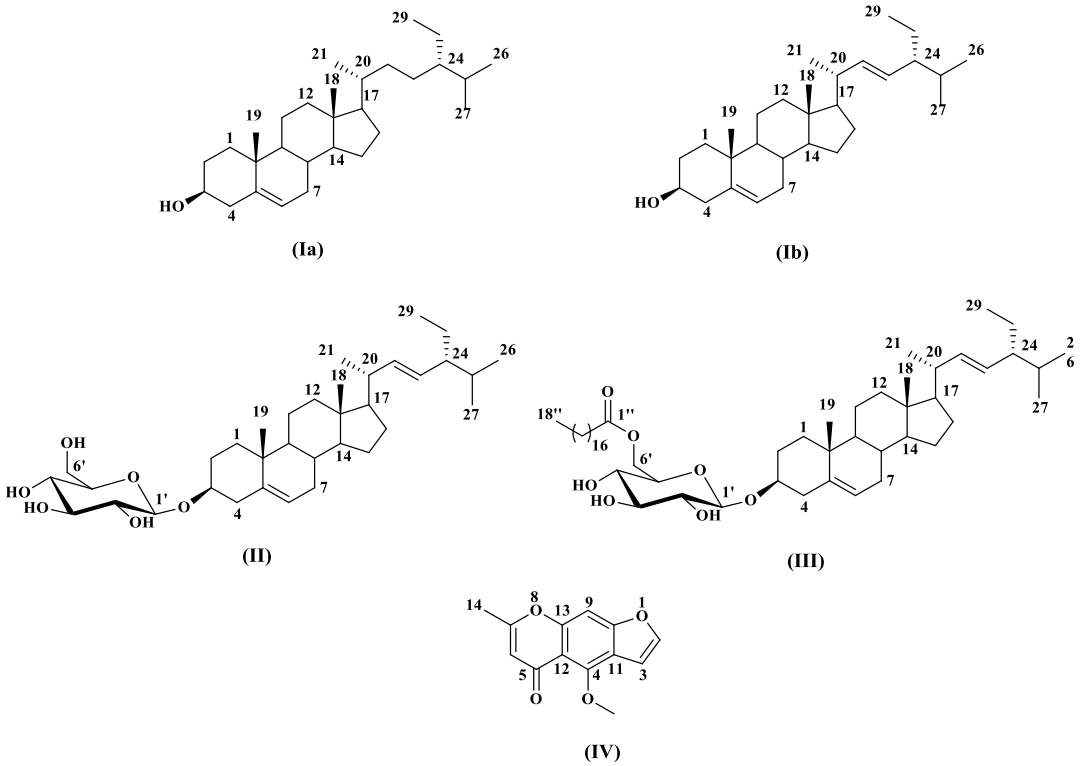

Figure 1: Chemical structures of the isolated compounds (**I-IV**) from *A. visnaga* roots.

Table 1: ^{1}H-NMR spectral data of the isolated compounds (**I–IV**)*

No.	Compound I	Compound II	Compound III	Compound IV
	δ_H (Intg., Mult., *J* in Hz)			
H-2				7.72 (1H, d, 2.1)
H-3	3.46 (1H, m)	3.64 (1H, m)	3.26 (1H, m)	7.26 (1H, d, 4.7)
4-OCH$_3$				4.07 (3H, s)
H-6	5.27 (1H, d, 5.2)	5.32 (1H, br d, 4.9)	5.27 (1H, br s)	5.99 (1H, s)
H-9				7.12 (1H, s)
H-14				2.28 (3H, s)
H-18	0.63 (3H, s)[a], 0.61 (3H, s)[b]	0.66 (3H, s)	0.61 (3H, s)	
H-19	0.94 (3H, s)	0.96 (3H, s)	0.92 (3H, s)	
H-21	0.84 (3H, d, 6.5)	0.91 (3H, d, 6.5)	0.84 (3H, d, 5.8)	
H-22	5.05 (1H, m)	4.87 (1H, m)	5.04 (1H, m)	
H-23	4.94 (1H, m)	4.42 (1H, t, 5.7)	4.91 (1H, m)	
H-26	0.71 (3H, d, 6.8)	0.82 (3H, d, 5.8)	0.73 (3H, d, 8)	
H-27	0.79 (3H,d, 7.4)	0.81 (3H, d, 6.6)	0.81 (3H,d, 7.1)	
H-29	0.75 (3H, t, 8.1)	0.84 (3H, t, 6.4)	0.77 (3H, t, 7.8)	
Other CH$_2$ groups	1.01–2.24	2.40–0.99 (m)	0.99–2.40 (m)	
H-1'		4.21 (1H, d, 7.8)	4.29 (1H, d, 7.6)	
H-2'		2.91 (m)		
H-3'		3.07 (m)		
H-4'		3.05 (m)	3.40–3.45 (m)	
H-5'		2.99 (m)		
H-6'		3.66 (m)		
H-2''			2.25 (2H, t, 8)	
H-18''			0.80 (3H, t, 7.1)	
Other (CH$_2$)$_n$ of fatty acid			1.18-1.50 (m)	

[a]Signals for *β*-sitosterol. [b]Signals for stigmasterol.
*Compounds **I** and **III** ($CDCl_3$, 400 MHz). Compound **II** (DMSO-d_6, 400 MHz). Compound **IV** (MeOD, 400 MHz). **I** [24–26], **II** [27–29], **III** [27, 30], and **IV** [31–33].

Table 2: ^{13}C-NMR spectral data of the isolated compounds (**I-IV**)

No.	Compound I	Compound II	Compound III	Compound IV
			δ_C	
C-1	37.3	37.3	37.3	–
C-2	31.7	33.8	29.7	145.9
C-3	71.8	77.4	79.8	105.1
C-4	42.3	36.7	39.8	156.0
C-5	140.8	140.9	140.4	179.5
C-6	121.7	121.7	122.1	109.6
C-7	31.9	31.8	31.9	165.8
C-8	31.9	31.9	31.9	–
C-9	50.2	49.6	50.2	94.5
C-10	36.5	36.7	36.7	158.5
C-11	21.1	23.1	21.1	116.3
C-12	39.8[a], 39.7[b]	38.8	38.9	112.3
C-13	42.3	42.3	42.3	153.4
C-14	56.8[a], 56.9[b]	55.9	56.8	
C-15	24.3[a], 24.5[b]	24.3	25.0	
C-16	28.2[a], 28.9[b]	29.7	28.3	
C-17	56.0[a], 56.1[b]	56.7	56.2	
C-18	12.1	12.3	12.0	
C-19	19.4	19.1	19.4	
C-20	36.1[a], 40.5[b]	35.5	36.2	
C-21	18.7[a], 21.2[b]	20.2	18.8	
C-22	34.0[a], 138.3[b]	138.2	138.3	
C-23	26.2[a], 129.3[b]	129.3	129.3	
C-24	45.9[a], 51.3[b]	50.1	45.8	
C-25	29.2[a], 31.9[b]	29.2	29.4	
C-26	19.8[a], 19.0[b]	19.6	19.0	
C-27	19.1[a], 21.2[b]	19.3	19.8	
C-28	23.1[a], 25.4[b]	25.9	23.1	
C-29	12.2	12.1	11.9	
C-1'		101.3	101.3	
C-2'		73.9	73.2	
C-3'		77.2	76.2	
C-4'		70.6	70.5	
C-5'		77.2	73.7	
C-6'		61.6	63.7	
OCO			174.3	
Other $(CH_2)_{16}$ of fatty acid			22.7–34.3	
CH_3-18'' (fatty acid)			14.2	
CH_3-14				18.5
OCH_3-4				60.4

[a]Signals for *β*-sitosterol. [b]Signals for stigmasterol.
*Compounds **I** and **III** ($CDCl_3$, 400 MHz). Compound **II** (DMSO-d_6, 400 MHz). Compound **IV** (MeOD, 400 MHz). **I** [24–26], **II** [27–29], **III** [27, 30], and **IV** [31–33].

Conflict of Interest

The authors declare that there is no conflict of interest regarding this study.

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