



Coumarins from *Zosima absinthifolia* seeds, with allelopathic effects

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Abstract

Zosima absinthifolia belongs to the Apiaceae family and is found in Iran, Turkey, Iraq and different countries of the Caucasus, Middle East and Central Asia. The fruits are used as food flavoring and as a food spice in Iran. In the present work, an n-hexane extract of the plant seeds was purified by vacuum liquid chromatography and preparative TLC for affording a furanocoumarin named imperatorin and two known coumarins, 7-prenyloxy coumarin and auraptene. The compound structures were elucidated by UV, ¹H and ¹³C NMR data. Our results indicated that all three compounds, especially imperatorin exhibited fungi toxic activity against *Sclerotinia sclerotiorum*, a common plant pathogen. The compounds also displayed phytotoxic effects and stunted seed germination, shoot and root growth of lettuce. It could be concluded that the purified compounds play allelopathic roles for the plant and could protect the plant against pathogens and competing herbs.

Keywords: Allelopathy, auraptene, imperatorin, *Sclerotinia sclerotiorum*, 7-prenyloxy coumarin.

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INTRODUCTION

The genus *Zosima* (Apiaceae) consists of six biennial or perennial herbs in Iran. *Zosima absinthifolia* (Vent) Link. is the well-known species of the genus that is found in Iran, Turkey, Iraq and different countries of the Caucasus, Middle East and Central Asia (Rechinger and Hedge 1987). The plant grows in the steppes, fields and lime stone slopes from 400 to 2000 m and has grooved pubescent stems that reach up to 1 m in height. This widespread plant has tri-pinnate leaves, 10-25 rayed umbels, greenish to pale yellow flowers and elliptic to obovate fruits with tumid margin (Davis 1972). *Z. absinthifolia*, other than the *Heracleum* species, is commonly known as Golpar in Iran where its fruits are used for a food flavoring and as a food spice. Crushed seeds of the plant are used as a flavoring agent in

seasonings, pickles and cookies.

Our previous study showed that the essential oil of the *Z. absinthifolia* seed is dominated by octyl acetate (87.4%), octyl octanoate (5.0%) and 1-octanol (2.3%) which exhibits high antibacterial effects against gram-positive bacteria like *Bacillus subtilis* and *Bacillus pumilus* (Razavi and Nejad-Ebrahimi 2009). We also previously demonstrated that the extracts of *Z. absinthifolia* seeds exhibited antiproliferative, antioxidant and phytotoxic activity (Razavi et al. 2009).

Like other species of Apiaceae, it is assumed that *Z. absinthifolia* may have coumarins. In the present work, we investigated the coumarin compounds of the plant seeds and their allelopathic activity.

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MATERIAL AND METHODS

General experimental procedures

UV spectra was obtained using a Hewlett-Packard 8 435 UV/Vis spectrophotometer in methanol. The NMR spectra was recorded in CDCl₃ using a DRX-500 Avance instrument (500 MHz for ¹H and 125 MHz for ¹³C) using the residual solvent peak (δ 3.31 ppm) as the internal standard. Column chromatography was conducted with silica gel 230-400 mesh, Merck. Preparative TLC was performed on RP-18 GF254 plates (20 × 20 cm, Merck) and the observation of the plates was carried out under a UV CAMAG spectrometer 254 and 366 nm. Silica 60G was used for the vacuum liquid chromatography (VLC).

Plant materials

The seeds of *Zosima absinthifolia* (Vent) Link. were collected from Vanyar, East Azerbaijan province, Iran, in August 2005. The plant was identified by the Department of Biology, Faculty of Sciences, Mohaghegh Ardabili University. A voucher specimen (No: 1387-1) has been deposited at the Herbarium of the Faculty of Sciences, Mohaghegh Ardabili University.

Isolation and identification of compounds

The crushed seeds of *Z. absinthifolia* (150 g) were extracted successively, with n-hexane, dichloromethane and methanol using a soxhlet apparatus. The hexane extract (4 g) was subjected to vacuum liquid chromatography (VLC) fractionation on silica gel starting with 100% n-hexane followed with a step gradient of EtOAc-n-hexane mixtures (1:99; 5:95; 10:99; 20:80; 40:60; 60:40; 80:20; 100) and finally MeOH. Fractions 30 and 40% EtOAc were purified by preparative silica TLC using (CH₃)₂CO-CHCl₃, 3:97 and 4:96 as the mobile phase, respectively. Fraction 30% EtOAc was purified to yield compound 1 (25.2 mg, Rf 0.64, blue fluorescence). Fraction 40% EtOAc was purified to yield compound 2 (14.3 mg, Rf 0.68, olive fluorescent) and compound 3 (6 mg, Rf 0.75, blue

fluorescent).

The compounds were identified by comparing their UV, ¹H NMR and ¹³C NMR data with those of published data.

Phytotoxic assay

A phytotoxic assay was carried out with Lettuce (*Lactuca sativa* L. cv. *Varamin*) seeds evaluating the response of seed germination, shoot and root elongation of the seedlings with different concentrations of the compounds. The stock solutions of the purified compounds were prepared by dissolving them in the minimum volume of acetone. Three concentrations of each compound (1, 0.1 and 0.01 mg mL⁻¹) were obtained by dilution with deionized water. Parallel controls were performed with the same volume of acetone. All seeds were surface sterilized with sodium hypochloride (1%). Four replicates, with 25 seeds, were prepared for each treatment using sterile Petri dishes (90 mm) lined with one sterile filter paper (Whatman, number 2) with 5 mL of the different concentrations of the compounds added to each Petri dish. Prepared plates were then placed in a germination cabinet at 25 °C in the dark. After 1 w, in each treatment, the germination percentage was determined and the root and shoot length was measured (Jefferson and Pennacchio 2003).

Antifungal activity assay

The antifungal activity assay was performed on *Sclerotinia sclerotiorum* (Lib.) de Bary fungus that causes stem rot in many plants such as Rapeseed, Sunflower and Lettuce and it is one of the most prevalent plant pathogens. In this study, an isolate of *S. sclerotiorum* from rapeseed was used. The assay was assessed by means of a combination with the medium (PDA) at 3 concentrations (0.01, 0.1 and 1 mg/mL of each compound). The PDA was poured into Petri plates that were then inoculated with 4 mm plugs from 7 d old cultures. The control experiments had distilled water in place of essential oils. The cultures were incubated at

25°C for 7 d. The diameter of the radial growth of the fungi were measured at the end of incubation period and then used to determine the percentage inhibition of each compound using the formula:

$$\text{Mycelia growth inhibition (\%)} = [(Dc - dt)/Dc] \times 100(\%)$$

Where Dc = average diameter of fungal colony in the control and

Dt = average diameter of fungal colony in treatment group (Taiga et al. 2002).

Statistical analysis

In all assays, SPSS 11.5 software was used for the statistical analysis. The analysis of variance (ANOVA) followed by a Duncan test was used to see the difference amongst various the groups. The significance level was set at $P < 0.05$

RESULTS AND DISCUSSION

Our phytochemical analyses of the n-hexane extract of the seeds of the *Z. absinthifolia* afforded a linear furanocoumarin imperatorin (Compound 1, Harkar et al. 1984) and two simple coumarins, 7-prenyloxy coumarin (Compound 2, Bohlmann et al. 1968) and auraptene (Compound 3, Karyone and Matsuno 1953). The structure of the purified compounds (Fig.1) was elucidated by direct comparison of their respective UV, ^1H and ^{13}C NMR data with published data. The assignment of all ^1H and ^{13}C NMR signals for these compounds is presented in Table 1 and 2.

Imperatorin occur in many genera of the family Apiaceae such as *Angelica*, *Prangos*, *Heracleum*, *Peucedanum* and *Glehnia* (Buckingham 2005). Auraptene was isolated from some genera of Rutaceae like *Citrus* and occurrence of it within the the family Apiaceae is restricted to a few genera. There were 7-prenyloxy coumarins as well as occurring in some members of Apiaceae such as the *Heracleum dissectum* Ledeb., *Peucedanum stenocarpum* Boiss. & Reuter, *Seseli libanotis* (L.) Koch. and *Angelica ursine*

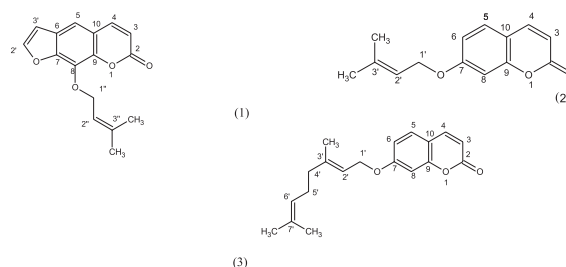


Fig. 1. Structures of imperatorin(1), 7-prenyloxy coumarin (2) and auraptene (3).

Table 1. ^1H NMR data of compounds 1-3. Data in the parentheses are coupling constant (J) in Hz.

Position	Chemical shift (δ) in ppm		
	1	2	3
3	6.28 d (9.4)	6.32 d (9.8)	6.39 d (9.7)
4	7.67 d (9.4)	8.17 d (9.4)	7.80 d (9.7)
5	6.85 S	7.30 d (8.4)	7.66 d (8.3)
6	-	6.86 d (8.4)	7.06 d (8.3)
8	-	7.03	7.65 S
1'	-	5.04 brd (6.7)	4.61 brd (6.7,13.3)
2'	6.87 d (2.3)	5.65 t (6.7,13.3)	5.51 t (6.7,13.3)
3'	6.89 d (2.3)	-	-
3' \times 2Me	-	1.76 s 1.78 s	1.88 s
4'	-	-	3.80 m
5'	-	-	4.88 m
6'	-	-	5.17 t
7' \times 2Me	-	-	1.81 s 1.85 s
1''	4.61 brd (6.3)	-	-
2''	5.51 t (6.3,13.6)	-	-
3''' \times 2Me	1.80 S 1.85 S	-	-

Table 2. ^{13}C NMR data of compounds 1-3.

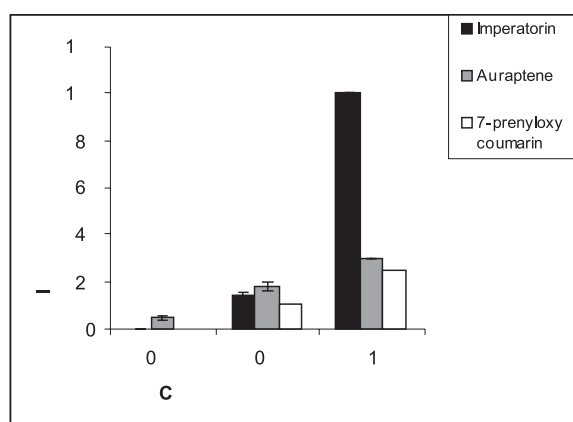
Position	Chemical shift (δ) in ppm		
	1	2	3
2	160.5	160.9	160.9
3	113.3	113.5	112.9
4	143.9	145.2	144.7
5	113.6	126.2	130.9
6	129.1	113.3	114.1
7	161.7	150.4	151.1
8	139.7	94.2	104.7
9	156.2	147.0	155.5
10	119.0	113.2	113.3
1'	-	70.5	65.8
2'?	148.5	120.2	120.1
3'	101.9	139.8	145.5
3' \times 2Me	-	25.9 S 26.9 S	18.5 S
4'	-	-	39.1
5'	-	-	28.2
6'	-	-	123.2
7'	-	-	131.5
7' \times 2Me	-	-	18.5 S 25.9 S
1''	65.8	-	-
2''	112.8	-	-
3''	131.5	-	-
3''' \times 2Me	18.7	-	-
	26.2		

Table 3. Phytotoxic activity of the imperatorin, auraptene and 7-prenyloxy on lettuce seeds coumarin purified from *Z. absinthifolia* seeds.

Concentration (mg/mL)	Germination (%)			Shoot length (mm)			Root length (mm)		
	I	A	P	I	A	P	I	A	P
0	84 ± 4.1 a	90 ± 3.5 a	97 ± 1.8 a	18.1 ± 1.5 a	19. ± 2.8 a	19.5 ± 2.1 a	24.4 ± 3.2 a	23.9 ± 2.3 a	49.4 ± 5.7 a
0.01	82 ± 3.1 a	48 ± 4.3 b	8 ± 3.2 b	17.5 ± 3.1 a	0 b	0 b	22.1 ± 1.8 a	2.5 ± 0.3 b	5.6 ± 0.6 b
0.1	72 ± 2.1 a	0c	0c	13.0 ± 1.1 b	0 b	0 b	18.5 ± 2.1 b	0 c	0c
1	80 ± 4.3 a	0c	0c	4.1 ± 0.3 c	0 b	0 b	3.4 ± 0.8 c	0 c	0 c

I= Imperatorin, A= Auraptene, P= 7- prenyloxy coumarin

Mean values (± SE) in the same column followed by the same letter are not significantly different at the 0.05 level according to the Duncan test.

**Fig. 2.** Antifungal activity of isolated compounds.

(Rupr.) Maxim (Buckingham 2005). To our knowledge, this is the first report on occurrence of imperatorin, auraptene and 7-prenyloxy coumarin in the genus *Zosima*.

Previous papers reported an occurrence of anglylon and O-angeloyl in some *Zosima* species such as *Z. korovinii* and *Z. absinthifolia* (Sklyar et al. 1981). The chemical analyses on the *Z. absinthifolia* seeds oil indicated octyl hexanoate and octyl acetate as characteristic compounds, as well as (Baser et al. 2000).

It was mentioned in literature that species of *Zosima* genus are very allied to *Heracleum* species in morphotaxonomical characters (Davis 1972).

Our finding together with those of Sklyar et al. (1982) demonstrated that there is a considerable similarity between *Zosima* and *Heracleum* species in phytochemical constituents as well as morphological characters (Khetwal and Pathak 1986, Niu et al. 2002).

The antifungal effects assay results indicated that all of three isolated compounds could reduce the growth of *S. sclerotiorum*. Imperatorin exhibited more fungi toxic activity than the other two compounds. An addition of 1 mg/mL of imperatorin to the PDA medium entirely inhibited the mycelia growth of *S. sclerotiorum*. The same concentration of auraptene and 7-prenyloxy coumarin reduce the mycelia growth of *S. sclerotiorum* to 30 and 25% than the control, respectively (Fig. 2).

The compounds also exhibited significant phytotoxic potential in the lettuce assay. The 7-prenyloxy coumarin and auraptene at a concentration of 0.1 mg/mL entirely stunt the seed germination, root and shoot growth of lettuce. Imperatorin did not exhibit significant effects on seed germination, but at concentration of 1 mg/mL reduced root and shoot growth of the lettuce to 12.5 and 22.2% than the control, respectively (Table 3).

In conclusion, the findings of the present work have revealed that isolated coumarins could play an allelopathic role in the plant. The compounds could serve as a phytotoxin and protect the plants against phytopathogen agents like *S. sclerotiorum* fungus, as well as. Thus, these compounds could be considered as allelochemicals that inhibit the growth of the competing plant surrounding the dominant plants produced by them.

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***Zosima absinthifolia* Tohumlarından Elde Edilen Allelopatik Etkili Kumarinler**
Özet

Apiaceae üyesi olan *Zosima absinthifolia*, İran, Türkiye, Irak ile Kafkaslar, Orta Dogu ve Orta Asya'nin degisik ülkelerinde bulunur. Meyveleri gida tatlandiricisi olarak ve İran'da baharat olarak kullanilir. Bu calismada; bitki tohumunun n-hekzan özütleri, imperatorin isimli bir furanokumarin ile 7-preniloksi kumarin ve orapten isimli iyi bilinen 2 kumarin elde etmek için, vakum sivi kromatografi ve preparatif TLC ile saflastirildi. Bilesik yapıları UV, ¹H ve ¹³C NMR verileri aracılığı ile elde edildi. Bulgularımız, tüm bitki bilesiklerinin, özellikle de imperatorinin, yaygın bir patojen olan *Sclerotinia sclerotiorum*'a karsi fungal toksik aktivitesi oldugunu gösterdi. Bilesikler ayrıca fitotoksik etki sergilediler ve tohum cimlenmesini, filiz ve kök gelişimini engellediler. Buradan, saflastirilan bilesiklerin bitkiler için allelopatik rol oynadıkları ve patojenlerle rakip otlara karsi bitkiyi koruyabilecegi sonucuna varilabilir.

Anahtar Kelimeler: Allelopati, imperatorin, orapten, *Sclerotinia sclerotiorum*, 7-preniloksi kumarin.