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Original article

Bioscreening of Oxypeucedanin, a Known Furanocoumarin

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Abstract

Objective(s)
As an important class of natural products, coumarins exhibit many biological activities and the diversity of their bioactivity is so huge that the pharmacological promiscuity has been applied on their case. Oxypeucedanine also named as prangolarin is a linear furanocoumarin with an oxygenated prenylated substitution at C-5 of the nucleus. To our knowledge, there are few reports on pharmacological and biological activities of this compound. In the present work, we focused on some bioactive aspects of it.
Materials and Methods
In the present work, the compound was purified from *Prangos uloptera* using TLC and its phytotoxic, antibacterial, antifungal, antioxidant and cytotoxic effects were evaluated by Lettuce assay, disc diffusion method, mycelia radial growth, DPPH and MTT assays, respectively.
Results
Our results revealed that oxypeucedanin exhibit considerable phytotoxic activity and might play an allelopathic role for plants. The compound indicated high cytotoxic activity with IC_{50} value of 314 µg/ml. No antipathogenic and antioxidant activity were found for oxypeucedanin in this study.
Conclusion
We conclude that oxypeucedanin (found in some vegetables) can be considered as an antiproliferative agent.

Keywords: Allelopathy, Antiproliferative agent, Cytotoxic activity, Oxypeucedanin, Phytotoxic activity, *Prangos uloptera*

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Introduction

In natural habitats, plants are surrounded by an enormous number of potential enemies. Nearly all ecosystems contain a wide variety of bacteria, fungi, viruses, competing herbs, insects and other herbivorous animals. By their nature, plants can not avoid the pathogens and herbivores simply by moving away; they protect themselves in other ways. One of the most important mechanisms of plants against enemy organism is the production of a wide group of compounds known as secondary metabolites (1).

These compounds may have other important functions as well, such as structure support or serving as pigments.

On the other hand, plant secondary metabolites named as natural products as well, are associated with health benefits for humans, and possess various pharmacological properties (2).

Coumarins comprise a large group of compounds widely distributed in the plant kingdom especially in families Apiaceae, Rutaceae, Fabaceae and Asteraceae. The compounds are divided into two subgroups: simple coumarins and furanocoumarins. These compounds exhibit many biological effects and the diversity of their bioactivity is so huge that the pharmacological promiscuity has been applied on their case (3).

Oxypeucedanine is a linear furanocoumarin with an oxygenated prenylated substitution at C-5 of the nucleus. The compound named as prangolarin as well, was isolated from *Prangos*, *Hippomarathrum*, *Angelica* and *Ferulago* genera of Apiaceae and *Ruta* genus of Rutaceae family (2). However, there are few reports on pharmacological and biological activities of this compound. In the present work, we described some bioactivity aspects of this compound (Figure 1).

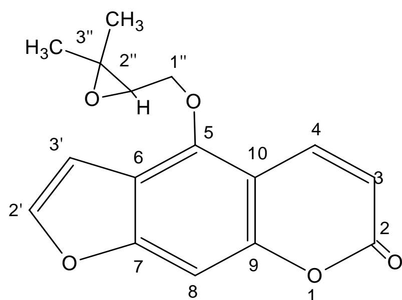

Figure 1. Structure of oxypeucedanin

Materials and Methods

General experimental procedures

UV spectra were obtained using a Hewlett-packard 8 435 UV/Vis spectrophotometer in ethanol. NMR spectra were recorded in $CDCl_3$ on a Bruker 200 NMR spectrometer (200MHz for ^1H) using the residual solvent peak (δ 3.31 ppm) as internal standard. Preparative TLC was performed on RP-18 GF_{254} plates (20×20 cm, Merck) and observation on plates was carried out under UV CAMAG spectrometer 254 nm.

Plant materials

The leaves of *P. uloptera* were collected from Mishov Dagh Mountains, east Azarbaijan province, Iran, in September 2006. The plant was identified by the department of biology, Faculty of Sciences, University of Mohaghegh Ardabili. A voucher specimen (No: 1386-1) has been deposited at the herbarium of the Faculty of Sciences, University of Mohaghegh Ardabili.

Extraction and isolation

Dried and powdered leaves of *P. uloptera* (270 g) were soxhlet extracted successively, with n-hexane (Hex.), dichloromethane (DCM) and methanol (MeOH). Hexane extract (2 g) were subjected to vacuum liquid chromatography fractionation on silica gel starting with 100% Hex, followed by step gradient of ethyl acetate (EtOAc)–Hex. mixtures (1:99; 5: 95; 10:99; 20:80; 40:60; 60:40; 80:20; 100) and finally MeOH. Fractions 14-16 (80%, 100% EtOAc and 100% MeOH, respectively) were purified by preparative silica TLC using $(CH_3)_2$ CO-$CHCl_3$, 5:95 as the mobile phase to yield oxypeucedanine (9.3 mg, Rf: 0.55; olive green fluorescence).

The compound was identified by comparing its 1D (1H NMR, ^{13}C NMR, Dept) and 2D (COSY, HMBC and HMQC) NMR data with those of published data (4).

Phytotoxic assay

Lettuce (*Lactuca sativa* L. CV. Varamin) seeds were used to test germination response to different concentration of oxypeucedanin. The stock solution of the purified compound was prepared by dissolving it in the minimum volume of acetone. Four concentrations of

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each compound (1, 0.1, 0.01 and 0.001 mg ml^{-1}) were obtained by dilution with deionized water. All seeds were surface sterilized with sodium hypochloride (1%). Four replicates, each of 25 seeds, were prepared for each treatment using sterile petri dishes (90 mm) lined with one sterile filter paper (Whatman, number 2). Five ml of different concentration of the compound was added to each plate. Prepared plates were then placed in a germination cabinet at 25 °C in the dark. Germination was deemed to occur only after the radicle had protruded beyond the seed coat by at least 1 mm. After 1 week, in each treatment, germination percentage was determined and shoot and root length was measured. The IC_{50} value, which is the concentration of oxypeucedanin that reduced 50% of the growth parameters, was calculated as mg/ml (5).

Antibacterial assay

The antibacterial activities of oxypeucedanin were determined against two common plant pathogens, *Xanthomonas campestris* (PTCC 1473) and *Erwinia cartovorum* (PTCC 1675) by the disc diffusion method. Muller-Hinton agar (MHA) (oxoid) was used for bacterial strains. The filter paper discs (6 mm in diameter) were individually impregnated with 10 µl of 100 mg/ml of the compound stock solution and then placed onto the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37 °C for 24 hr. The diameters of inhibition zones were measured in millimeters. All the tests were performed in duplicate. Gentamicin (30 µg) served as positive control (6).

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, lettuce and is one of the most prevalent plant pathogens. In this study, an isolate of *Sclerotinia sclerotiorum* from rapeseed was used. The stock solution of the purified compound was prepared by dissolving it in the minimum volume of acetone. Four concentrations of each compound (1, 0.1, 0.01 and 0.001 mg ml^{-1}) were obtained by dilution with deionized water. The assay was assessed by means of combination with the medium (potato dextrose agar-PDA) at the compound concentrations. The PDA was poured into petri plates and then inoculated with 4 mm plugs from 7 days old cultures. The control experiments had distilled water in place of essential oils. The cultures were incubated at 25 ºC for 7 days. 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:

Mycelia growth inhibition (%)= [(dc-dt)/dc]×100(%)

Where dc= average diameter of fungal colony in the control and dt= average diameter of fungal colony in treatment group (7).

Cytotoxicity assay

Hela cell line (Pasteur, C123) was grown in RPMI 1640 (Gibco, No 51800-019) medium. Cell line was maintained in a humidified atmosphere of 5% CO_2 at 37 °C. The stock solutions of oxypeucedanin were prepared by dissolving the compound in DMSO (100 µl). The final concentrations of the compound were 50, 100, 200 and 400 µg/ml. Cells were plated in the appropriate media on 20-well plates in a 500 µl total volume at a density of 6×10^5 cell/ml. Due to usage of spectrophotometer for measurement of UV absorbance instead of micro plate reader, 20-well plates were used for cell culturing. The 20-well triplicate wells were treated with media containing different concentration of the compound. The plates were incubated at 37 °C in 5% CO_2 for time course of 16 hr. The MTT test was used for evaluating cell viability. The amount of MTT converted to formazan is a sign of the number of viable cells. Media- only treated cells served as the indicator of 100% cell viability. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the control in the MTT assay. Viability percentage was

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evaluated as $OD_{treatment}/OD_{contro}$. Morphological study of cell shape changes was performed by direct microscopic analysis (6).

Antioxidant assay
Serial dilutions were carried out with the stock solutions (1 mg/ml) of oxypeucedanine to obtain concentrations of 0.5, 0.25, 0.175, 0.087, 0.043, 0.021, 0.010, .005, 0.002 and 0.001 mg/ml. Diluted solutions (5 ml each) were mixed with 5 ml of 2,2- diphenyl- 1-picryl hydrazyl (DPPH, Sigma) and allowed to stand for 3 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in duplicate and the average absorption was noted for each concentration. After measurement of reduction percentage of DPPH as: [(Control UV absorbance-Sample UV absorbance/Control UV absorbance)×100], the RC_{50} value, which is the concentration of the test material that reduced 50% of free radical concentration, was calculated as mg/ml (8).

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

Results
On the lettuces assays, oxypeucedanin showed considerable phytotoxic activity and inhibited seed germination, as well as shoot and root growth of lettuce at concentrations higher than 0.1 mg/mL. The IC_{50} values of the compound were calculated as 0.21, 0.59 and 0.62 mg/ml, respectively (Table 1).

The results of antibacterial and antifungal assay indicated that the compound had no effect on plant pathogen bacteria, *Xanthomonas compestris* and *Erwinia cartovorum*, and *Sclerotinia sclerotorium*, common plant pathogen fungi.

Regarding the results of DPPH assay, oxypeucedanin has a weak antioxidant potential with RC_{50} value of 51.25 mg/ml.

Our results also showed that oxypeucedanin exhibited strong cytotoxic activity against hela cell line. The effect appeared at the concentrations higher than 100 µg/ml. The IC_{50} value of the compound was calculated as 314 µg/ml (Figure 2). It was also found that whereas untreated cells exhibited normal shapes with clear outline and strike intercellular connections, the oxypeucdedanin-treated cells were round in shape and exhibited loose intercellular connections. The treated cells had significantly more granules in cytoplasm (Figure 3).

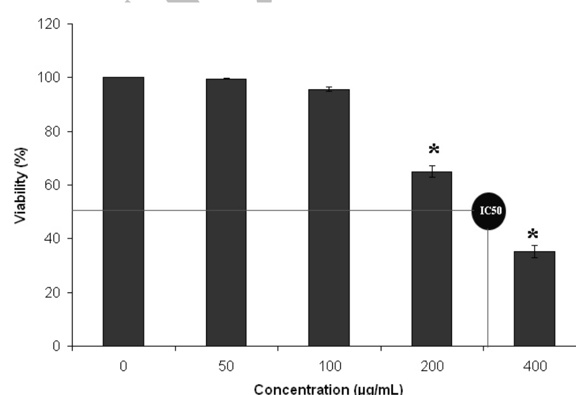

Figure 2. Cytotoxic activity of different concentrations of oxypeucedanin on hela cell line incubated for 16 hr. The evaluation was performed using MTT cell viability assay. Bars indicate standard error of mean (SEM) and the asterisks indicate statistically significant difference at $P< 0.05$.

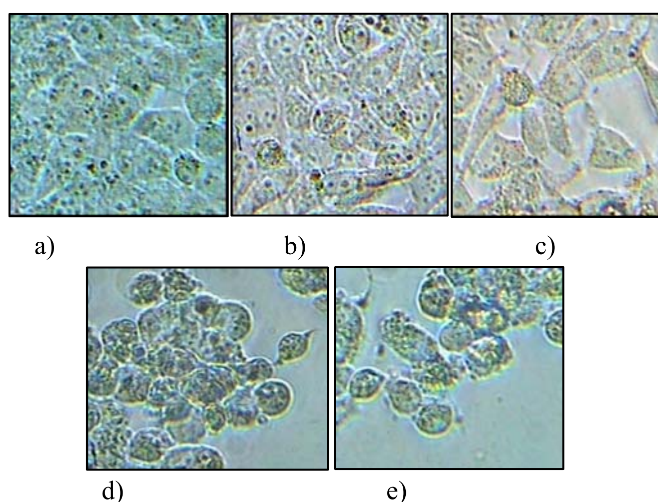

Figure 3. Effects of oxypeucedanin on morphology of Hela cells cultured in PMR II for 16 hr. (a) effects of 0 µg/ml, (b) effects of 50 µg/ml, (c) effects of 100 µg/ml, (d) effects of 200 µg/ml, (e) effects of 400 µg/ml.

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Table1. Phytotoxic activity of the Oxypeucedanin.

Concentration (mg/ml)	Germination (%)	Shoot length (mm)	Root length (mm)
0	82.6±2.3[a]	23.84±1.26[a]	38.85±3.43[a]
0.001	76.0±1.3[b]	21.87±1.15[a]	31.74±3.19[a]
0.01	78.6±2.8[b]	23.97±2.29[a]	33.97±3.06[a]
0.1	66.6±2.6[b]	16.39±2.39[b]	16.25±3.14[b]
1	68.0±0.8[b]	5.00±0.90[c]	9.52±0.87[b]
IC_{50} value (mg/ml)	0.21	0.59	0.62

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.

Discussion

The results presented here indicated that oxypeucdedanin may serve as an allelopatic role for the plant. The compound may be released from leaves to decompose and stunt growth of surrounding plants to avoid competition. There are some other reports on allelopathic potential of furanocoumarins such as imperatorin, ammirin, chalepin and psoralen (9). This finding leads us to the conclusion that furanocoumarins can be considered as allelochemicals. The current trend is to find a biological solution to minimize the perceived hazardous impacts from synthetic herbicides in agriculture production and investigation on allelochemicals that could lead to the development of natural herbicides.

Due to non antibacterial and antifungal activity of oxypeucedanin, it could be also concluded that the compound could not be considered as anti-pathogenic agent in the plant. It was also previously shown that the compound may be metabolized by some plant pathogen fungi like *Fusarium sambucinum*, so it is assumed that plant pathogen fungi may have resistance against oxypeucedanin (10).

On the other hand, our findings revealed that oxypeucedanin exhibit considerable cytotoxicity against Hela cell line. There are some reports on antiproliferative effect of the compound against some human tumor cell lines such as melanoma, prostate carcinoma DU145, etc (11). It has been previously pointed out that the compound is abundant in some vegetables such as parsley and celery (12). Thus, high consumption of this vegetable could be associated with a reduced risk of cancer. We also previously isolated 8-geranyloxy psoralen from *P. uloptera* and showed that this compound possesses cytotoxic activity against Hela cell line with IC_{50} value of 0.79 mM (13). Another study of our group also demonstrated that the dichloromethane extract of *P. uloptera* roots indicated high cytotoxic activity and caused apoptosis with DNA fragmentation (14). It was assumed that this bioactivity caused by *P. uloptera* extract might be attributed to the presence of furanocoumarins in this plant.

A survey of literature revealed that oxypeucedanin displays different biological activities. It was reported to have antiarrhitmic, channel blocker and antiestrogenic activity (15-17).

Conclusion

In conclusion oxypeucedanin can be regarded as a bioactive agent in plants. It can be found in some vegetables and may be considered as an antiproliferative agent for chemo-preventative purpose. Due to allelopathic activity, it may be a candidate for production of a new generation of bio-herbicides that are more ecologically friendly.

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References

1. Taiz L, Zeiger E. Plant physiology. 3 th ed. Sunderland: Sinauer Associates; 2002.
2. Buckingham J. Dictionary of natural products on CD-ROM. Bocakaton:Chapman & Hall/CRC Press; 2005.
3. Rahman AU. Studies in natural products chemistry, Part D. Amesterdam: Elsevier sciences; 2000.
4. Gupta BK, Wali BK, Vishwapaul K, Handa KL. Coumarins from *Prangos pabularia*. Indian J Chem 1964; 2:464-466.
5. Razavi SM, Zahri S, Zarrini G, Nazemiyeh H, Mohammadi S. Biological activity of quercetin-3-O-glucoside, a known plant flavonoides. Russ J Bioorganic Chem 2009; 35:376-378.
6. Razavi SM. Zahri S, Zarrini G, Nazemiyeh H, Mohammadi S, Ghasemi KA. A furanocoumarin from *Prangos uloptera*, biological effects. Nat Prod Res 2009; 23:1522-1527.
7. Razavi SM, Imanzadeh GH, Davari M. Coumarins from *Zosima absinthifolia* seeds, with allelopatic effects. EurAsia J Biosci 2010; 4:17-22.
8. Razavi SM, Nazemiyeh H, Delazar A, Hajiboland R, Kumarssamy YV, Nahar L, *et al*. Coumarins from the aerial parts of *Prangos uloptera*. Braz J Pharmacogn 2008; 18:1-5.
9. Anya AL, Rubalcora MM, Orega RC, Santana CC, Monteruhio PNS, Bautist BE, *et al*. Allelochemicals from *Sataurantus perforatus*, a Rutaceae tree of the *Yucatan Penisula*, Mexico. Phytochemistry 2005; 66:484-487.
10. Desjardins AE, Spencer GF, Plattner RD. Tolerance and metabolism of furanocoumarins by the phytopatogenic fungus *Giberella pulicaris* (*Fusarium sambucinum*). Phytochemistry 1989; 28:2963-2969.
11. Kang TJ, Lee Y, Singh R, Agrawal R, Yim DS. Anti-tumor activity of oxypeucedanin from *Ostericum koreanum* against human prostate carcinoma Du145 cells. Acta Oncol 2009; 48:895-9000.
12. Chaudhary SK, Ceska O, Tetu C, Warrington PJ, Ashwood MJ, Poulton GA. Oxypeucedanin, a major furanocoumarin in parsley, *Petroselinum crispum*. Planta Med 1986; 52:462-464.
13. Razavi SM, Zahri S, Zarrini G, Nazemiyeh H, Mohammadi S, Abolghasemi MA. A furanocoumarin from *Prangos uloptera*, biological effects. Nat Prod Res 2009; 23:1522-1527.
14. Zahri S, Razavi SM, Hasanzadeh F, Mohammadi S. Induction of programmed cell death by *Prangos uloptera*, a medicinal plant. Biol Res 2009; 42:517-522.
15. Soon EJ, Ah PJ, Hee CB, Kyung CS, Keun KD, Geun KY. Effects of oxypeucedanin on hkv1.5 and action potential duration. Biol Pharm Bull 2005; 28:657-660.
16. Piao XL, Yoo HH, Kim HY, Kang TL, Hwang GS, Perk JH.Estrogenic activity of furanocoumarins isolated from *Angelica dahurica*. Arch Pharm Res 2006; 29:741-745.
17. Harmala P, Vourela H, Tornquist K, Hiltonen R. Choice of solvent in the extraction of *Angelica archangelica* roots with reference to calcium blocking activity. Planta Med 1992; 58:176-183.

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