



Antibacterial Activity of *Peucedanum ruthenicum*, *Johreniopsis seseloides* and *Cervaria cervariifolia* Extracts

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Abstract

Antimicrobial activity of the polar and nonpolar extracts of the roots and aerial parts of *Peucedanum ruthenicum* M. Bieb., *Johreniopsis seseloides* (C. A. Mey.) Pimenov and *Cervaria cervariifolia* (C. A. Mey.) Pimenov were examined against gram-positive and gram-negative bacteria. The polar extracts of the roots and aerial parts displayed no activity against bacteria, while the nonpolar extracts of the roots which contains coumarins, presented activity against *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Keywords: Antibacterial activity; Apiaceae; *Cervaria cervariifolia*; *Peucedanum*; *Johreniopsis*; *ruthenicum*; *seseloides*.

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1. Introduction

Plants play an important role for treatment of many infectious diseases. *Peucedanum ruthenicum* M. Bieb. *Johreniopsis seseloides* (C.A. Mey.) Pimenov and *Cervaria cervariifolia* (C.A. Mey.) Pimenov are placed in Apiaceae family [1]. The genus *Peucedanum* comprises about 100-120 species, mainly distributed in Europe and Asia. In Europe *peucedanum* comprises 29 species [2] while 4 species dedicated to Iran include: *P. glaucoprinosum*, *P. knappii*, *P. translucens* and *P. ruthenicum*, which are distributed in the north and central provinces

of Iran [3].

Some species of this genus have been used traditionally in the treatment of cold, cough due to pathogenic wind-heat, accumulation of phlegm, heat in the lung, anti-tussive, anti-asthma and as a remedy for angina [4, 5, 6]. Therefore, the extracts of *P. ruthenicum* was subjected to investigation for antibacterial properties.

Study on essential oil of *P. ruthenicum* fruits collected from Arak, was showed antibacterial activity against gram positive bacteria which may justify the use of this genus in traditional medicine and underline the importance of the bioactive ethnobotanical approach for discovery of the new antibacterial substances from plants [7].

Previous phytochemical studies on some of

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the species of *Peucedanum* have shown the presence of furanocoumarin and their glycoside derivatives, linear-type furanocoumarin glycosides and simple coumarin glycoside in plant extracts [8-10].

A phytochemical experiment on *P. ruthenicum* from Bulgaria showed the presence of peucedanin and a new coumarin (peuruthenicin) in the root and also rutin in the flower [11]. Several new coumarins such as Qianhuocoumarin I, have been reported from *P. praeruptorum* Dunn. [4]. Of seven coumarins: Imperatorin, bergapten, isopimpinellin, umbelliferon, byakangelicin, 7-methoxy coumarin and 5-hydroxy-8-methoxy psoralen, that isolated from methanol extract of *P. zenkeri* by chromatographic methods, only imperatorin, bergapten and isopimpinellin have shown antimicrobial activity [12]. Acetone extract of *P. nebrodense* has been shown antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *C. tropicalis* and *Streptococcus agalactia* [13]. *P. ostruthin* contained ostruthin (6-geranyl-7-hydroxy coumarin), imperatorin and umbelliferon, of which ostruthin showed strong activity against some species of mycobacteria (MICs 3.4 -107.4 μ M) and umbelliferon displayed only weak activity (MIC= 0.79 mM) [14]. Seed methanol extract of *P. graveolens* had moderate activity on *Salmonella thyphi* with zone inhibition diameter \leq 5-9 mm [15]. In addition some compounds such as: Coumarins, phenolic acids, terpenoids, flavonoids, fatty acids, normal alkanes and one carbohydrate were found in methanol extract of *P. ruthenicum* [16, 17, 18]. Genus *Johreniopsis* and *C. cervariifolia*, belong to tribe peucedanae, subfamily of Apioideae and family of Apiaceae. *Johreniopsis* genus has 4 species (*J. oligactis*, *J. scopari*, *J. seseloides* and *J. sticticaulis*) in Iran [3]. From methanol extract of *J. seseloides*, some compounds such as: Flavonoids, mannitol, coumarins and a

triterpen and have been detected. In methanol extract of *C. cervariifolia*, coumarins, terpenoid, flavonoids and a carbohydrate were identified [19]. These two coumarin containing plants also were subjected to study of antibacterial activity.

2. Materials and methods

2.1. Plant material

Aerial parts of *P. ruthenicum*, *J. seseloides* and *C. cervariifolia* were collected from Arak (Markazi province), Haris (Kurdistan province) and Golestan province in August 2004, respectively. The roots of the three plants were collected in October 2004. The plants were identified by Department of Biology, Faculty of Science, Tehran University, Tehran, Iran and were deposited with numbers (Hb.Akh.15487, Salimian 32 and Hb.Akh.16913) respectively in the herbarium of Dr. Akhiani at above address.

2.2. Extraction

For the preparation of polar and nonpolar extracts from aerial parts and roots of *P. ruthenicum*, *J. seseloides* and *C. cervariifolia*, they were shade dried at room temperature and powdered by electric blender. Powdered materials (100 g) were percolated with 3 \times 300 ml MeOH-H₂O mixture (80-20 v/v) for 3 \times 24 h separately. Extracts were concentrated under reduced pressure. The condensed products were re-extracted with 3 \times 100 ml chloroform. Chloroform solvent was evaporated to prepare nonpolar extracts (3.96, 3.66, 1.02 % for aerial parts and 2.50, 2.77, 1.51 % for roots, respectively). The amounts of residue or polar extracts were 11.20, 11.60 and 4.26 % for aerial parts and 12.10, 11.36 and 5.60 % for roots, respectively.

2.3. Antibacterial activity

Antibacterial activity of polar and nonpolar extracts were examined by cup plate method to determine the growth inhibition of the following bacteria: *Staphylococcus aureus* ATCC 29737, *S. epidermidis* ATCC 12229, *Pseudomonas aeruginosa* ATCC 9027,

Table 1. Antimicrobial activity of *P. ruthenicum*, *J. Seseloides* and *C. cervariifolia* extracts using cup plate method.

Samples	Strains extracts (100µg/cup)	Inhibition zone diameter (mm)			
		SA	SE	PA	EC
<i>P. ruthenicum</i> root	Polar	-	-	-	-
	Nonpolar	25	12	6	17
<i>P. ruthenicum</i> aerial part	Polar	-	-	-	-
	Nonpolar	-	-	-	-
<i>J. seseloides</i> root	Polar	-	-	-	-
	Nonpolar	16	19	5	17
	Polar	-	-	-	-
<i>J. seseloides</i> aerial part	Nonpolar	-	-	-	-
<i>C. cervariifolia</i> root	Polar	-	-	-	-
	Nonpolar	23	21	17	19
	Polar	-	-	-	-
<i>C. cervariifolia</i> aerial part	Nonpolar	-	-	-	-
DMSO (negative control)	-	-	-	-	-
Gentamycin (positive control)	4 µg/cup	19	12	19	18

SA: *Staphylococcus aureus*; SE: *Staphylococcus epidermidis*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*. - no effect

Escherichia coli ATCC 35218, which were obtained from Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences. Base plates were prepared by pouring 25 ml of melted Muller-Hinton-agar into sterile petridishes and allowed to set Muller-Hinton agar held of 48 °C, and was inoculated with a broth cultured 0.5 Mcfarland (1×10^8 cfu/ml) of the test organism and was poured over the base plates forming a homogenous top layer. Then some wells were prepared on the plates. DMSO (dimethyl sulfoxide) was used as a solvent to solve the extracts to obtain a 1% w/v concentration. This solution was loaded into the holes and incubated at 37 °C for 24 h. Antibacterial activity was expressed as the inhibition zone (mm) [19]. The activity of Gentamycin was included in this equation to adjust for plate-to-plate variation in the sensitivity of a particular bacterial strain.

Minimum inhibition of concentrations (MICs) of the extracts were determined against the tested microorganisms. The macrodilution technique in Muller-Hinton broth [20] was used against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, with dilutions of plant extracts from 0.0976 to 5 mg/ml of medium.

MICs values were taken as the lowest concentration of the extracts which completely inhibited bacterial growth after 24 h of

incubation at 37 °C. Gentamycin and DMSO with no extracts were used as the positive and negative controls, respectively. Minimum bactericidal concentration (MBC) was defined as the lowest concentration yielding negative subcultures or only one colony.

3. Results and discussion

The polar and nonpolar extracts of the roots and aerial parts of *P. ruthenicum*, *J. seseloides* and *C. cervariifolia* tested against 4 standard bacteria (gram positive and gram negative) strains. The zone inhibited diameters (ZIDs) of the extracts shown in Table 1 indicate that the polar and nonpolar phase of the aerial parts and the polar phase of the roots had no significant antibacterial activity against the tested bacteria. However the nonpolar phase of the roots showed inhibitory effect on tested bacteria. The MICs as well as minimum bactericidal concentrations (MBCs) of the extracts tested by the macrodilution technique in Muller-Hinton broth are presented in Table 2. *P. ruthenicum* nonpolar root extract displayed activity against *S. aureus* and *E. coli* with MICs 156.2 and 312.5 µg/ml, respectively while was inactive against *S. epidermidis* and *P. aeruginosa* with MICs of >1000 µg/ml.

J. seseloides nonpolar root extract presented activity against *S. epidermidis* and *E. coli* with MIC 312.5 µg/ml and *S. aureus* with MIC 625.0 µg/ml. *J. seseloides* extracts

Table 2. The minimum inhibition of concentrations (MICs) and minimum bactericidal concentration (MBC) of the nonpolar extracts tested by Macro-dilution technique.

Sample	Strain	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
		(µg/ml)			
<i>P. ruthenicum</i>	MICa	156.2	>1000	>1000	312.5
	MBCa	312.5	-	-	625.0
<i>J. seseloides</i>	MIC	625.0	312.5	>1000	312.5
	MBC	>1000	625.0	-	312.5
<i>C. cervariifolia</i>	MIC	156.2	312.5	312.5	156.2
	MBC	312.5	312.5	625.0	625.0

^aMean of MIC and MBC (n=3).

was inactive against *P. aeruginosa* with MICs of >1000 µg/ml.

C. cervariifolia extract showed activity against all the tested bacteria with MIC 156.2 µg/ml for *S. aureus* and *E. coli* and 312.5 µg/ml for *S. epidermidis* and *P. aeruginosa*. Previous studies on the antibacterial activity of the genus *Peucedanum* has shown that 3 of the coumarins isolated from *P. zenkeri* (imperatorin, bergapten and isopimpinellin) had antimicrobial activities [12]. *P. ostruthin* contained ostruthin (6-geranyl-7-hydroxy coumarin), imperatorin and umbelliferon, of those ostruthin had strong activity against some species of *Mycobacterium* (MICs range 3.4 to 107.4 µM) and umbelliferon had only weak activity (MIC= 0.79 mM) [14]. Methanol extract of *P. graveolens* seeds had moderate activity on *Salmonella typhi* with zone inhibition diameter of ≤5-9 mm [15].

In conclusion, this research displayed that diverse antibacterial effects of different extracts e.g. crude extracts such as methanol, polar or nonpolar ones, depends on the type or also amounts of coumarins that present in the extracts will show positive or negative antibacterial properties. However the polar phase of the aerial and root parts and the nonpolar phase of the aerial parts did not show antibacterial activity but the nonpolar extracts of the plants roots contain coumarins showed activity against some bacteria.

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