BIOASSAY-GUIDED ISOLATION AND IDENTIFICATION OF AN ANTIBACTERIAL COMPOUND FROM *FERULA PERSICA* VAR. *PERSICA* ROOTS

[1]AHMAD-REZA SHAHVERDI, [2]MEHRDAD IRANSHAHI, [1]ROOHOLLAH MIRJANI, [1]HOSSEIN JAMALIFAR, [3]GHOLAMREZA AMIN, [4]ABBAS SHAFIEE

[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, [2]Department of Pharmacognosy, Faculty of Pharmacy, Mashad University of Medical Sciences, Mashad, [3] Central Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, [4] Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

The antibacterial activities of the chloroform and water extracts of *Ferula persica* var. *persica* (Apiaceae) roots were studied by the disk diffusion method. While the chloroform extract of *F. persica* roots showed antibacterial activity, the water extract of the roots at the concentrations that tested did not show any activity. By bioassay-guided fractionation of the chloroform extract of the roots by preparative thin layer chromatography (PTLC) a compound was found which was active against some bacteria. By conventional spectroscopy methods the active fraction was identified as umbelliprenin. This coumarin was mostly active against *B. subtillis*, *B. cereus*, *E. coli*, *K. ponumoniae*, *S. typhi*, *S. aureus*, and *S. epidermilis*.

Keywords: Umbelliprenin, *Ferula persica*, Apiaceae, *O*- Prenylated coumarin, Antibacterial activity

INTRODUCTION

In a program searching for antibacterial agents, an extract which was prepared from the roots of *Ferula persica* var. *Persica* (Apiaceae) was selected for investigation. Members of the genus of *Ferula* are widespeared throughout Central Asia, especially in Iran. The roots of *F. persica* have been used for treatment of diabetes in traditional medicine (1). The chemistry of this genus has been studied by different groups, and various germacrances, sulphur derivatives, coumarins and flavonoids have been isolated from this plant (2-5), However no report on the antibacterial effects of *F. persica* has been published in the literature. This paper reports on the bio-assay guided isolation and antibacterial evaluation of umbelliprenin, a known *O*–prenylated coumarin, from the roots of *Ferula persica* var. *persica* (Fig.1). The antibacterial effects of the compound and gentamycin and erythromycin as reference drugs were determined against 13 species of microorganisms: *Bacillus cereus, Bacillus subtilis, Citrobacter ferundi, Echerichia coli, Enterobacter cloacae, Klebsiella ponumoniae, Salmonella typhi, Serratia marcecens, Shigella dysenteriae, Staphylococcus aureus, Staphylococcus epidermilis, Streptococcus fecalis* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Plant Materials and Chemicals

Ferula persica var. *persica* was collected from the north of Tehran, Iran, at an altitude of 2000m, in May 2002 and was identified by Dr. Gholamreza Amin. A voucher specimen of the plant (No. 6523) was deposited in the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences and the roots of the plant were used for antibacterial investigations. Silica gel and organic solvents which were used during this study were from Merck (Germany). Standard disks (erythromycin and gentamycin) were purchased from Padtan Teb Co. (Tehran, Iran) and used as positive controls.

Extraction and chromatography

The chloroform and water extracts of *Ferula persica* roots were prepared separately. The air-dried roots of the plant were pulverized and extracted three times by maceration in water or chloroform for 72h at room temperature. The combined solvent extracts were evaporated to yield a brownish viscous residue. Preparative Thin Layer Chromatography (PTLC) was carried out on silica gel (60 F_{254}, Merck) using petroleum ether / ethyl acetate (2:1) as the solvent system. The fractions were visualized under UV at 254 nm and eluted by chloroform.

Correspondence: Ahmad-Reza Shahverdi, Department of Pharmaceutical Biotechnology, Faculty of Pharmacy , Tehran University of Medical Sciences, Tehran, P.O. Box: 14155-6451, Iran, E mail: shahverd@sina.tums.ac.ir

Antibacterial assay of TLC fractions

Approximately 5 mg of each TLC fractionated compounds was dissolved in *n*-hexane (200 µl), and its antibacterial activity against *S. epidermis* was determined by disk diffusion bioassays using disk which had 500 µg of compound. Disks containing *n*-hexane were used as a negative control in all experiments. The antibacterial activity of the chloroform extract of *F. persica* roots and its active fractions were further studied against different test strains which were isolated from patients of Shariati Hospital, University of Tehran, Iran (Table 1). The identity of the isolates were determined by conventional morphological, as well as biochemical, methods (6). A single colony of bacteria was grown overnight in Mùller-Hinton liquid medium on a rotary shaker (200 rpm) at 35°C. The inocula were prepared by dilution of the overnight cultures with 0.9% NaCl to a 0.5 MacFarland standard and applied to the Mùller-Hinton agar (MHA) plates along with the disks containing the extract and the umbelliprenin at concentrations which are indicated in Table 1. After incubation at 35 °C for 18 hrs, the zones of growth inhibition were measured. The assays were performed in triplicate.

Spectroscopy

The active compound was identified using conventional spectroscopy. ^{1}H NMR and ^{13}C NMR spectra were measured in $CDCl_3$ with TMS as an internal standard using a Varian 400 Unity *plus* spectrometer. Melting point was taken on a Reichert-Jung apparatus and is uncorrected.

RESULTS AND DISCUSSION

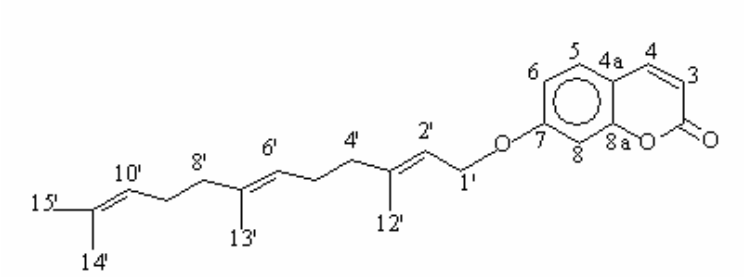
Table 1 shows the activity of the chloroform extract of *F. persica* against tested organisms which was remarkable at higher concentrations of the extract. In this investigation, the tested concentrations of the water extract did not show any antibacteri alactivity. TLC analysis of the chloroform extract of *F. persica* (petroleum ether - ethyl acetate, 2:1) showed the presence of at least nine compounds, which were visible under UV light at 254 nm and the activity of each of the fractions was tested against *S. epidermilis* by the disk diffusion method. Bioactivity-guided fractionation of this extract led to the isolation of compound I as white crystals of $R_{f=}$ 0.71 which on the basis of ^{1}H NMR, ^{13}C NMR spectra and melting point (7-10) its structure was found to be umbelliprenin.

M.P. 58 °C. - ^{1}H NMR: δ 7.63 (d, *J* = 9.6 Hz, H-4), 7.36 (d, *J* = 7.2 Hz, H-5), 6.84 (dd, *J* = 7.2, *J* = 2Hz, H-6), 6.81 (d, *J*= 2 Hz, H-8), 6.22 (d, *J* = 9.6 Hz, H-3), 5.45 (t, *J* = 7 Hz, H-2'), 5.06 (q, *J*= 7 Hz, H-6' and H- 10'), 4.6 (d, *J*= 7 Hz, H-1'), 1.95 - 2.15 (m, H- 4', H-5', H-8' , H-9'), 1.77 (s, H-12'), 1.67 (s, H-13'), 1.59, 1.6 (2s, H-14', H-15')- ^{13}C NMR: δ 161.1 (C-2), 112.8 (C-3), 143.3 (C-4), 112.3 (C-4a), 128.6 (C-5), 118.4 (C-6), 162 (C-7), 101.5 (C-8), 155.7 (C-8a), 65.4 (C-1'), 113 (C-2'), 142.1 (C-3'), 39.4 (C-4'), 26 (C-5'), 124.2 (C-6'), 135.1 (C-7'), 39.5 (C-8'), 26.6 (C-9'), 123.4 (C-10'), 131.1 (C-11'), 16.6 (C-12'), 15.9 (C-13'), 25.7 (C-14'), 17.5 (C-15').

Table 1. The antibacterial activity of the chloroform extract of *Ferula persica* roots and its active constituent

Organisms	Chloroform extract (mg/disk)				Umbelliprenin	Gentamycin	Erythromycin
	0.5	1	2	4	500 µg*	10µg	30µg
Bacillus cereus	-	-	-	-	10	21	23
Bacillus subtilis	-	-	8	12	22	30	35
Citrobacter ferundi	-	-	-	8	-	15	-
Echerichia coli	-	14	16	20	10	10	-
Enterobacter cloacae	-	-	-	-	-	18	-
Klebsiella ponumoniae	-	-	-	-	12	16	-
Salmonella typhi	-	-	-	16	12	16	10
Serratia marcecens	-	-	-	-	-	-	13
Shigella dysenteriae	-	-	7	14	-	17	14
Staphylococcus aureus	-	-	-	-	12	17	24
Staphylococcus epidermilis	-	13	14	17	11	25	20
Streptococcus fecalis	-	-	-	-	-	-	13
Pseudomonas aeruginosa	-	-	-	-	-	22	-

All microorganisms were clinically isolated from Shariati Hospital, University of Tehran, Iran.
* Compound per paper disk



Compound I

This compound at concentration of 500 µg /ml (Table 1) showed highest activity against *B. subtillis*, *B. cereus*, *E. coli, K. ponumoniae, S. typhi,S. aureus, S. epidermilis* and was not active against several other bacteria which were used in this investigation. The crude extract of *F. persica* did not show antibacterial activity against any of the strains at the lowest concentration (500 µg/ml), whilst at the higher concentrations showed good antibacterial activity, especially against *S. epidermilis* and *E. coli* and was not active against other bacteria at all tested concentrations.

CONCLUSION

The chloroform extract of *Ferula persica* var. *persica* roots showed good antibacterial activity. Umbelliprenin was identified as antibacterial component of the *Ferula persica* var. *persica* roots and was mostly active against *B. subtillis*, *B. cereus*, *E. coli*, *K. ponumoniae*, *S. typhi*, *S. aureus* and *S. epidermilis*. To the best of our knowledge, this is the first report on the antibacterial activity of the umbelliprenin against numerous Gram positive and Gram negative bacteria.

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