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

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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. pneumoniae, 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 widespread 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 pneumoniae, Salmonella typhi, Serratia marccens, Shigella dysenteriae, Staphylococcus aureus, Staphylococcus epidermis, 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 F254, 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.

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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 Muller-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 Muller-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. 1H NMR and 13C NMR spectra were measured in CDCl3 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 antibacterial activity. 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. epidermis by the disk diffusion method. Bioactivity-guided fractionation of this extract led to the isolation of compound I as white crystals of Rf = 0.71 which on the basis of 1H NMR, 13C NMR spectra and melting point (7-10) its structure was found to be umbelliprenin.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Chloroform extract (mg/disk)</th>
<th>Umbelliprenin</th>
<th>Gentamycin</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>4</td>
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<tr>
<td>Bacillus cereus</td>
<td>-</td>
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<tr>
<td>Bacillus subtilis</td>
<td>-</td>
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<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
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<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>-</td>
<td>14</td>
<td>16</td>
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<tr>
<td>Enterobacter cloacae</td>
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<tr>
<td>Klebsiella pneumoniae</td>
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<tr>
<td>Salmonella typhi</td>
<td>-</td>
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<td>16</td>
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<tr>
<td>Serratia marcescens</td>
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<tr>
<td>Shigella dysenteriae</td>
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<td>7</td>
<td>14</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
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<td>13</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
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</table>

All microorganisms were clinically isolated from Shariati Hospital, University of Tehran, Iran. * Compound per paper disk
Antibacterial compound from Ferula persica roots

This compound at concentration of 500 µg/ml (Table 1) showed highest activity against B. subtillis, B. cereus, E. coli, K. pneumoniae, S. typhi, S. aureus, S. epidermis 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. epidermis 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. pneumoniae, S. typhi, S. aureus and S. epidermis. 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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REFERENCES