Umbelliprenin from *Ferula persica* Roots Inhibits the Red Pigment Production in *Serratia marcescens*

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The chloroform extract of *Ferula persica* var. *persica* roots was found to inhibit red pigment production of *Serratia marcescens*. A bioguided fractionation study by preparative thin layer chromatography (PTLC) detected a fraction (R_f = 0.71, petroleum ether/EtOAc, 2:1 v/v), which was effective on depigmentation of *Serratia marcescens*. Using conventional spectroscopy methods, the active fraction was identified as umbelliprenin. Neither the chloroform extract nor the isolated umbelliprenin fraction showed any antibacterial activity against the test strain at a certain concentration. In contrast, they exhibited depigmentation zones on culture plates.

Key words: Ferula persica, *Serratia marcescens*, Umbelliprenin

Introduction

Serratia marcescens is a Gram-negative bacterium that causes diseases in plants and in a wide range of both invertebrates and vertebrate hosts (Grimont and Grimont, 1978). It is an opportunistic human pathogen and in the last three decades there has been a steady increase in nosocomial *S. marcescens* infections that can be life threatening (Haddy *et al.*, 1996; Hejazi and Falkiner, 1997 and references cited therein). Environmental *S. marcescens* strains are often red, due to the production of prodigiosin (Thomson *et al.*, 2000). It is well-known that there are some antibiotics affecting pigmentation in bacteria. Cefoxitin, erythromycin, tobramycin, co-trimoxazole, imipenem and nitrofurantoin showed an inhibitory effect on pigmentation in a *S. marcescens* strain isolated from urine (Ang-Kucuker *et al.*, 2000). During our investigation on the screening of Iranian plants for their antibacterial activity we surprised with the depigmentation activity of a chloroform extract of *Ferula persica* roots (Umbelliferae) in *S. marcescens*. Members of the genus *Ferula* are widespread throughout central Asia. The roots of *F. persica* are used for the treatment of diabetes in folk medicine (Afifi and Abu-Irmaileh, 2000). In this study, we have tested the depigmentation activity of the chloroform extract of *Ferula persica* roots. We have detected a component of the coumarin group that inhibits red pigment production in *S. marcescens*.

Materials and Methods

Plant collection

Ferula persica Willd. var. *persica* was collected from the north of Tehran, Iran, at an altitude of 2000 m in May 2002 and identified. A voucher specimen of the plant (No. 6523) was deposited in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Siences (Iran).

Extraction, chromatography and spectroscopy

The roots of the plant were air-dried at room temperature, pulverized and extracted with chloroform (Merck) by maceration for 72 h. The chloroform extract of *F. persica* was fractionated by Preparative Thin Layer Chromatography (PTLC) 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 using chloroform. The pure compound was identified using conventional spectroscopy. ^{1}H NMR and ^{13} C NMR spectra were measured in

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$CDCl_3$ with TMS as an internal standard using a Varian 400 Unity *plus* spectrometer. Melting points were taken on a Reichert-Jung apparatus.

Antimicrobial and depigmentation activities of the chloroform extract and its TLC fractions

A disk diffusion method was used to assay the extract of *Ferula persica* and its TLC fractions bactericidal and the depigmentation activities against *S. marcescens* on Muller-Hinton agar plates. This strain was a clinical isolate from Shariati Hospital, University of Tehran (Iran). The identification and antibiotic susceptibility of this strain were carried out using conventional methods. A single colony of *S. marcescens* was grown overnight in Muller-Hinton liquid medium on a rotary shaker (200 rpm) at 35 °C. The inocula were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5 MacFarland standard and were applied to the plates along with the disks containing the chloroform extract and its TLC fractions. After incubation at 35 °C for 18 h, the zones of growth inhibition and depigmentation were measured. The assays were performed in triplicate. The fraction of TLC, which was effective in depigmentation of *S. marcescens*, was also further purified and tested at 0.3, 0.6, 0.9, 1.2 and 1.5 μmol. Erythromycin (15 μg) and nitrofurantoin (300 μg) standard disks were purchased from Padtan Teb Co., Iran, and used as control.

Results and Discussion

The TLC analysis of the chloroform extract of *F. persica* showed at least 9 compounds, which were visualized under UV at 254 nm. The antimicrobial activities of the chloroform extract of *F. persica* roots and each of the fractions were tested for the nitrofurantoin/erythromycin resistant strain of *S. marcescens* by a disk diffusion method. Neither the crude extract nor the umbelliprenin fraction isolated by the PTLC method showed any antibacterial activity against the test strain at concentrations tested. In contrast, on the plates, zones of depigmentation were observed with the chloroform extract of *F. persica* roots and the umbelliprenin fraction. The active component of the root extract involved in inhibition of red pigments production had R_f = 0.71 on PTLC. The structure of

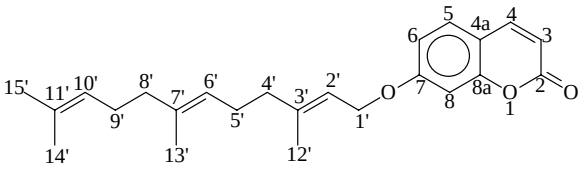

Fig. 1. The structure of umbelliprenin.

umbelliprenin (Fig. 1) was confirmed by ^{1}H NMR, ^{13}C NMR spectra and its melting point.

The chemical and spectroscopic data of umbelliprenin (M_r 366) are as follows: White crystals. – 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 Hz, 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′).

The inhibitory effect of the umbelliprenin fraction on the pigmentation of *S. marcescens* was also shown at various concentrations (Table I). As

Table I. Effect of the *Ferula persica* roots extract and active component, umbelliprenin, on pigmentation in *Serratia marcescens.*

Compounds	Inhibition zone [mm]	Depigmentation zone [mm]
Root extract [mg]		
0.5	–	–
1	–	–
2	–	–
4	–	–
8	–	9
Umbelliprenin [μmol]		
0.3	–	–
0.6	–	8
0.9	–	10
1.2	–	15
1.5	–	21
Control disks [μg]		
Erythromycin 15	–	18
Nitrofurantoin 300	–	17

shown this coumarin as well as positive controls led to depigmentation of *S. marcescens*. The bleaching effect of the umbelliprenin was concentration depended for *S. marcescens*. The highest concentration tested was 1.5 μmol, but a bleaching effect was observed at 0.6 μmol of umbelliprenin.

Umbelliprenin has been reported in some plants (Abu-Mustafa *et al.*, 1971; Gonzalez *et al.*, 1993; Nassar *et al.*, 1995; Filippini *et al.*, 1998; Ngwendson *et al.*, 2003; Iranshahi *et al.*, 2003). This is the first report of the inhibitory effect of a coumarin derived from *F. persica* on pigmentation in *S. marcescens*. Pigment formation by some strains of *S. marcescens* was noted by Bizio as early as 1823 (Grimont and Grimont, 1978). Several pigments in this species were later described. The best known, prodigiosin, is a nondiffusible red pigment attached to the inner membrane (Vinas *et al.*, 1983; Paruchuri and Harshey, 1987). While environmental *S. marcescens* strains are often red, the strains associated with hospital outbreaks are mostly non-pigmented (Carbonell *et al.*, 2000; Kurz *et al.*, 2003). Also it has been reported that in contrast to the pigmented *S. marcescens*, the non-pigmented variant caused infection in an animal model (Ang-Kucuker *et al.*, 2000). This phenomenon of a natural substance on the pigmentation of *S. marcescens* may or may not have significance in the clinical treatment of infections caused by *S. marcescens*. However, the interaction of some natural products such as umbelliprenin as well as erythromycin and nitrofurantoin may increase the risk of pathogenesis of *S. marcescens*.

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