Antimicrobial Activity of *Ferula halophila* Peşmen

**Summary**

Water, methanol extract and fractions obtained from methanol extract (chloroform, ethyl acetate, butanol and remainder) of the aerial and underground parts of *Ferula halophila* were evaluated for antimicrobial activities. Cefazolin, ciprofloxacin and fluconazole were used as standards. Antimicrobial activities of the extracts against Staphylococcus aureus ATCC 25923, Methicillin-resistant Staphylococcus aureus (MRSA) (clinical isolate), Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Bacillus cereus RSKK 1122, Bacillus subtilis ATCC 6633, Enterococcus faecalis ATCC 29212 and Candida albicans ATCC 10231 were investigated by using disc diffusion method. It was observed that the chloroform extract of aerial and underground parts showed activity against Gram-positive MRSA, S. aureus, B. cereus, and B. subtilis. While the extracts of the aerial parts showed less effect. None of the extracts was found active against the Gram-negative bacteria E. coli and P. aeruginosa or against the Gram-positive bacterium E. faecalis. Similarly, extracts of aerial and underground parts had no antifungal activity against C. albicans. The activity of the extracts and fractions of aerial and underground parts of *Ferula halophila* was less than that of the standards.

**Key Words:** *Ferula halophila*, Umbelliferae, antimicrobial, activity.

INTRODUCTION

The genus *Ferula* was separated into six genera by Korovin\(^1\). It is one of the biggest genera of the family Umbelliferae (Apiaceae) with its more than 170 species. *Ferula* species are distributed throughout Asia, North Africa and southern Europe. Species are mainly found in Central Asia from the Canary Islands to Mongolia.\(^2\) Eighteen species are recorded in Turkey and nine of them are endemics.\(^3\)

*Ferula halophila* Peşmen is an endemic species, growing in Central Anatolia, Konya Cihanbeyli-Yavlan Tuzlas.\(^3\) The members of the genus are well known...
in Turkey; the aerial and underground parts are traditionally used as food and aphrodisiac.

There have been many investigations of the coumarin and volatile oil content, bioactivity and microbiological activity of some Ferula species, but no chemical or bioactivity studies have been conducted on F. halophila. We thus aimed to conduct antimicrobial investigations of Ferula halophila, an endemic species in Turkey. This species is also found in the vulnerable category, indicating plants with the potential of extinction, according to the Red Data Book of Turkish Plants.

EXPERIMENTAL

Plant Material

Research materials were collected from the below mentioned localities; voucher specimens are deposited in Ankara University Faculty of Pharmacy Herbarium (AEF 21139, AEF 23140).

B4: Konya: Yavflan tuzlası, Salty steppe, 800-900 m., 18/05/2004., AEF, 23139!

Preparation of Plant Extracts

100 g aerial and 150 g underground parts of F. halophila were ground and extracted with methanol for 4 h using magnetic stirrer (Velp Scientifica C15) at room temperature, and the extraction was repeated three times with fresh methanol. Extracts were filtered and evaporated till dryness and dispersed in methanol : water (1 : 9). They were then partitioned with chloroform, ethyl acetate and butanol in a separating funnel (each with 30 ml x 3). Each fraction was evaporated to dryness.

2.26 g of chloroform, 0.43 g of ethyl acetate and 0.58 g of butanol fractions were obtained from aerial parts and 19.84 g of chloroform, 1.41 g of ethyl acetate and 2.38 g of butanol fractions from the underground parts.

The remaining aqueous parts were also used for the antimicrobial activity studies. Lyophilized extracts were also prepared. 10 g aerial and underground parts were extracted with 100 ml of water for 4 h using magnetic stirrer (Velp Scientifica C15), filtered, frozen (Labconco Shell Freezer Model 117) and lyophilized (Labconco Freeze Dry System/Freezeone ® 4.5).

Test Microorganisms

The following microorganisms were used for testing antibacterial activity:


The microorganisms were obtained from Refik Saydam National Type Culture Collection of Turkey.

Media

Mueller-Hinton agar (Difco, Detroit, MI, USA) was used for bacteria, and MHA supplemented with 2% glucose and 0.5 µg/ml methylene blue (GMB) was used for C. albicans.

Disc Diffusion Method

Antimicrobial activities of the samples were determined using the disc diffusion technique. Solutions of concentrations (1/9 w/v) of extracts were prepared with sterile distilled water, and the solvents used for the extraction and 0.02 ml of these portions were impregnated on sterile paper discs. Then all discs were left to dry overnight. Cefazolin (30 µg), ciprofloxacin (5 µg) and fluconazole (25 µg) were used as standards. Colonies, chosen from fresh cultures of the microorganisms used for microbiological studies, were taken into Tryptic Soy Broth (Oxoid) and incubated for 2-4 h at 35°C (0.5 McFarland standard). After 10 min all discs were dispensed on the
surface of the inoculated agar plates. Petri dishes were incubated for 16-18 h at 35˚C for bacteria and 24 h at 35˚C for C. albicans and inhibition zones were measured.

**RESULTS and DISCUSSION**

Ethyl acetate fraction of aerial parts also showed activity against B. subtilis along with these fractions. The chloroform fraction of underground parts showed the most significant activity against these bacteria (Table 1).

Antimicrobial activities of the extracts prepared from the aerial and underground parts of F. halophila Peflmen were investigated against various bacteria and fungi.

First methanol extracts were prepared, then extracted with chloroform, ethyl acetate and butanol. Antimicrobial activity of the aqueous extract and aqueous waste remainder of the fractioning were also investigated. The zones they produced were compared with the zones produced by standards in mm. Aerial and underground extracts showed no activity against E. coli, P. aeruginosa, F. faecalis and C. albicans (Table 1).

While only the chloroform fraction of aerial parts was active against S. aureus, methanol, aqueous extracts and ethyl acetate and chloroform fractions of the underground parts showed activity. In addition, ethyl acetate fraction of the aerial parts showed activity against S. aureus (MRSA) (Table 1).

Methanol extract of aerial parts and chloroform fraction of underground parts showed activity against B. cereus. Methanol and aqueous extracts and ethyl acetate and chloroform fractions also showed activity.

**Table 1. Antimicrobial activity of Ferula halophila Peşmen**

<table>
<thead>
<tr>
<th>Extracts/Drugs</th>
<th>ATCC 25923</th>
<th>MRSA</th>
<th>ATCC 25922</th>
<th>ATCC 27853</th>
<th>ATCC 6633</th>
<th>RSKK 1122</th>
<th>ATCC 29212</th>
<th>ATCC 10231</th>
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The Umbelliferae family is found to be rich in coumarins. In a study conducted on the stems and roots of Prangos pabularia, a species belonging to another genus of the Umbelliferae family, coumarins and δ-pyrene derivatives were isolated from stems and roots, and antibacterial activity was investigated. It was determined that oxypeucedanin and imperatorin showed slight activity against E. coli and C. albicans. Furthermore, four compounds (oxypeucedanin, oxypeucedanin methanolate, imperatorin and osthol) showed activity against S. aureus (MSSA, MRSA), E. coli and P. aeruginosa. However, the tested compounds showed weak activities, whereas osthol showed significant activity, against S. aureus (MRSA) and P. aeruginosa compared to other compounds.

In our study, extracts prepared from aerial and underground parts of Ferula halophila and fractions obtained from these extracts showed antibacterial activity against S. aureus, S. aureus (MRSA), B. subtilis, and B. cereus; however, no activity against E. coli, P. aeruginosa, E. faecalis, and C. albicans was seen.

The activities of the extracts and fractions of aerial and underground parts of Ferula halophila were observed to be less compared to those of cefazolin, ciprofloxacin and fluconazole.

CONCLUSION

The aim of this study was to establish whether or not the Ferula halophila species possessed antibacterial activity. Considering the above-mentioned studies, we can conclude that the antimicrobial activity of Ferula halophila that was demonstrated to be present may result from coumarins. These studies are certainly not sufficient; further studies should be conducted to isolate the active substances and their activities should then be investigated.

REFERENCES

11. Iranshahi M, Arfa P, Ramezani M, Jaafari MR, Sadeghian H, Bassarello C, Piacente S, Pizza C. Sesquiterpene coumarins from Ferula szovitsiana and in vitro antileishmanial activity of 7-prenyloxycoumarins against promastigotes,
