Original article

Antimicrobial activity of essential oils of *Ferulago angulata* subsp. *carduchorum*

Mojtaba Taran[1], Hamid Reza Ghasempour[2], Ebrahim Shirinpour[2]

[1]*Microbiology Laboratory, Biology Department of Razi University, Kermanshah, Iran*
[2]*Plant Tissue Culture, Biotechnology and Physiology Laboratory, Biology Department of Razi University, Kermanshah, Iran*

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Abstract

Introduction and objective: *Ferulago angulata* is an important medicinal plant of Iran. The essential oil of seeds aerial parts of this plant contains variety of components with different therapeutical effects. The purpose of this study was to provide the first examination of the antibacterial and antifungal effects of essential oils obtained from aerial parts and seeds of *F. angulata* subsp. *carduchorum* which is endemic to Shahoo Mountains (west of Iran).

Materials and methods: Chemical composition of the essential oils of seeds and aerial parts of *F. angulata* subsp. *carduchorum* was analysed by the Gas Chromatography-mass spectrometry (GC-MS). The susceptibility of the microorganisms to essential oils was determined by minimum inhibitory concentration (MIC) using microdilution method.

Results: The GC-MS analysis of the essential oils has led to the identification and quantification of 57 components, the most abundant of them were cis-ocimene (27.9%) for aerial parts and α-pinene (76.1%) for seeds. Other compounds present (α-pinene, β-pinene, 4-terpineol, α-terpineol and caryophyllene oxide) have been reported to have antimicrobial effects on bacteria and fungi. Among different bacteria, *Staphylococcus aureus* (MIC= 15µg/ml) and *Listeria monocytogenesis* (MIC=137µg/ml) disclosed a high sensitivity to essential oils of aerial parts and seeds respectively. These essential oils showed weak activity against fungus (*Candida albicans*).

Conclusion: This study showed that the essential oil of seeds and aerial parts of *F. angulata* subsp. *carduchorum* have antimicrobial activity against different infectious microbes.

Keywords: *Ferulago angulata*, Antifungal, Antibacterial, Essential oil

Introduction

The genus *Ferulago* belongs to Apiaceae family [1]. *Ferulago* species are used in folk medicine for their sedative, tonic, digestive and anti-parasitic effects [2,3]. Antibacterial and antifungal activities have previously been investigated for some *Ferulago* species as *F. thyrsiflora, F. sylvatica, F. nodosa* and *F. longistylis,* and inhibitory effects for microorganisms have

been observed [4-6]. *F. angulata* (referred to locally as Chavir) is a perennial shrub with the height 60-150cm [7,8] that grows 1900-3200m (above sea level) [1]. The *F. angulata* have two subspecies; subsp. *angulata* (Schlecht) that is wide spread in Turkey, Iraq and Iran, and subsp. *carduchorum* which is endemic to the Shahoo Mountains of west Iran [1].

Data in the existing literature states that oils of *F. angulata* are predominantly ferulagone, β-hydroxy-13-epi-manoyl oxide, α-pinene, 2,5-dimethoxy-p-cymene, p-cymene, methyl carvacrol, trans-chrysanthenyl acetate, γ-terpinene (Z)-β-ocimene, α-pinene, myrcene, (Z)-β-ocimene, terpinolene, 2,4,5-trimethyl-benzaldehyde and α-phellandrene [9-16]. Some of these compounds have both antibacterial and antifungal activities [16-19]. The part of the plant, its stage of development, and its geographical origin can significantly influence the composition of the oils obtained from these species [20,21]. The aim of the present work was to investigate and compare the antimicrobial effects of the aerial parts and seed oils of *F. angulata* subsp. *carduchorum* that grow wild in the Shahoo Mountains.

Materials and methods

Plant material and isolation procedures

The aerial parts and seeds of *F. angulata* subsp. *carduchorum* were collected at May and October, 2004 respectively from of Shahoo Mountains, Kermanshah province west of Iran. The voucher specimen (No. 2414) is deposited in the herbarium of agricultural faculty of Razi University, Kermanshah, Iran. The aerial parts were cut into pieces and air-dried for even days at room temperature (17-22°C). 100g of aerial parts were powdered, mixed with1200ml of distilled water and the essential oils hydrodistilled in a Clevenger-type apparatus (Clevenger 1928) according to the British method for three hours. This procedure was carried out on 50g of seeds in 1000ml distilled water. The essential oil were dried over anhydrous Na_2SO_4 and stored at 4°C in the dark [16]. Gas chromatography (GC) and Gas Chromatography-mass spectrometry (GC-MS) have been reported in the literature [16].

Microorganisms

The antimicrobial activity was individually tested against a panel of microorganisms, including *Staphylococcus aureus* (ATCC 6884) *Listeria monocytogenesis* (ATCC 29843), *Escherichia coli* (from clinical sample), *Pseudomonas aeruginosa* (ATCC 29399), *Sallmonela typhi* (from clinical sample), and *Candida albicans* (ATCC 11236). Bacterial strains were cultured overnight at 37ºC in Mueller Hinton broth (Merck, Germany). Yeast was cultured at 28ºC in Sabouraud dextrose broth (Merck, Germany). To determine antimicrobial activities, Minimum Inhibitory Concentration (MIC) method was employed. The MIC of essential oils against the test microorganisms were determined by the micro dilution method [22].

Results

The essential oil yields were 0.63% (v/w) for aerial parts and 3.2% for seeds based on the dry weights of the samples. Chemical composition of the essential oils of the aerial parts and seeds from Shahoo ecotype of *F. angulata* are reported in table1. In the essentials oils of the aerial parts and seeds of *F. angulata* 57 components were identified (31 and 26). The major aerial parts and seeds volatiles were α-pinene (25.7%, 7.29%), cis-ocimene (27.9%, 76.1%), bornyl acetate (3.9%, 1.69%), γ-terpinene (0.1%, 2.88%), germacrene D (22.3, 0.5%), and trans-ocimene (3.3%, 1.4%).

The most abundant volatile was cis-ocimene (27.9%) for aerial parts and α-pinene (76.1%) for seeds. Some

components such as terpinolene, 4-terpineol, α-terpineol, β-bourbonene, β-caryophllene, allo-aromadendrene, α-humulene and caryophyllene oxide were only found in aerial parts essential oil and some components such as α-terpinene, p-cymene, α-terpinolene, trans-verbenol, γ-curcumene were only found in essential oil of seeds. The results of antimicrobial activity are reported in table 2.

Table 1: Chemical composition (%) of identified compounds in the oil of *F. angulata* aerial parts and seeds

Component	RI[a]	Aerial parts	Seeds	Component	RI[a]	Aerial parts	Seeds
α-thujene	926	0.2	0.06	Geraniol	1237	-	-
α-pinene	937	25.7	7.29	Tymol	1269	-	-
Camphene	950	1.6	0.51	Bornyl acetate	1275	3.9	1.69
Dimethyl- bicycle (3,1) Hepta-2(8), 3- diene	952	-	-	Myretenyl acetate	1285	-	-
sabinene	970	2.1	.017	Ipsdienol	1298	-	-
β-pinene	977	1.7	0.23	δ-elemene	1342	-	0.02
Myrcene	983	2.0	1.05	Benzyl isovaletrate	1365	-	-
α-phellandrene	1003	0.2	0.72	Methyl eugenol	1374	-	-
3-carene	1011	1.9	0.03	α-copaene	1383	-	-
α-terpinene	1013	-	0.02	β-cubenone	1384	1.0	0.05
Ortho-cymene	1016	-	-	β-bourbonene	1393	0.8	-
p-cymene	1018	-	1.4	β-cederene	1424	-	0.07
Cis- ocimene	1031	27.9	76.11	β-caryophyllene	1427	0.9	-
Trans- ocimene	1040	1.3	2.26	Allo-aromadendrene	1449	0.1	-
γ-terpinene	1053	0.1	2.88	α-humulene	1459	0.2	-
α-terpinolene	1063	-	0.59	Γ-curcumene	1478	-	0.34
Terpinolene	1083	0.1	-	γ-muurolene	1480	.01	-
Linalool	1085	-	-	Germacrene D	1487	22.3	0.5
1,3,8-p-menthatriene	1112	0.1	0.03	γ-elemene	1500	1.1	0.25
Cis- epoxy ocimene	1114	-	-	Cyclogermacrene	1501	-	-
Allo-ocimene	1120	1.6	2.38	δ-cadinene	1523	0.4	0.16
Cis-verbenol	1131	-	-	Epigloubol	1531	-	-
Trans-verbenol	1135	-	0.26	Cadrol	1562	-	-
p-mentha-1,5-dien-8-ol	1151	-	-	Spathulenol	1576	0.3	0.13
4-terpineol	1168	0.1	-	Caryophyllene oxide	1582	0.1	-
α-terpineol	1178	0.2	-	Aromadendrene oxide	1650	0.1	-
				Neoclvenoxid-alkohol	1684	0.1	-

[a]RI: Retention index

The antibacterial activity ranged from no inhibition to MIC 15µg/ml. Of the selected strains of five bacteria, Gram-positive strains (*S. aureus* and *L. monocytogenesis*) were the most susceptible to the essentials oils of aerial parts and seeds respectively, followed by *E. coli* showed the lowest susceptibility to both essential oils. The essential oil of aerial parts showed higher antibacterial and antifungal activity than that of the seeds. The quantitative and qualitative analysis of the constituents of the oils showed significant differences. The chemical composition of the essential oil of *F. angulata* from ecotype Shahoo is peculiar and rather different from those of other origins [9,17].

Table 2: The minimum inhibitory concentration (MIC, µg/ml) of *F. angulata* essential oils of aerial parts and seeds against bacteria and fungi

Microorganism	Essential oil (MIC, µg/ml)	
	Aerial parts	Seeds
S. aureus	15	$>4\times10^3$
S. typhi	$>2\times10^4$	No inhibition
E. coli	$>1.9\times10^3$	$>4\times10^3$
P. aeruginosa	$>9.5\times10^2$	$>4\times10^3$
L. monocytogenesis	170	137
C. albicans	$>1.9\times10^3$	$>4\times10^3$

The results are the conclusion of three replicates

Discussion

The antibacterial and antifungal activities of these oils were tested on Gram-positive bacteria (*S. aureus, L. monocytogenesis*), Gram-negative bacteria (*E. coli, P. aeroginosa, S. typhi*) and fungi (*C. albicans*). In the literature, the antibacterial and antifungal effects of α-pinene, β-pinene, 4-terpineol, α-terpineol and caryophyllene oxide have been reported [18-20]. In fact, aerial parts essential oil showed significantly more α-pinene and β-pinene, than those of seeds and some antimicrobial components found only in essential oil of aerial parts, from comparison of antimicrobial values between oils, we observed that oil of aerial parts gave a higher inhibition activity against all tested microorganisms.

The stronger antimicrobial activity of this essential oil is likely due to the presence of these mentioned components. The essential oil of *F. angulata* shows stronger antimicrobial activity than that of other species [4,5]. As far as the mechanism of the antimicrobial activity, the complex and variable chemical composition of essential oils, which can include many different molecules, prevents the understanding of the mechanism of action. This study showed a potent antimicrobial activity of *F. angulata* essential oil against different infectious microbes, is noteworthy, but needs further survey to evaluate the suitability of this remarkable antimicrobial property.

Conclusion

Our study showed that the seeds essential oil and aerial parts of *F. angulata* subsp. *carduchorum* have antibacterial and anti-Candida activities.

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Address for correspondence:
Hamid Reza Ghasempour, Plant Tissue Culture, Biotechnology and Physiology Lab., Biology Department of Razi University, 67149-67346, Kermanshah, Iran
Tel: +98831 4274545; Fax: +98831 4274555
Email: ghasempour2009@gmail.com; Hamidrezaghasempour@yahoo.com