CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *FERULAGO BERNARDII* TOMK. AND M. PIMEN.

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ABSTRACT

The chemical composition of the essential oil of the aerial parts of *Ferulago Bernardii* from Iran was analysed by GC and GC/MS. Sixty constituents were found representing 87.9% of the oil. The main constituents of the essential oil were 2,4,5-trimethyl-benzaldehyde (21.2%), *α*-pinene (17.0%), spathulenol (5.0%), *cis*-chrysanthenyl acetate (4.4%) and caryophyllene oxide (3.2%). Antimicrobial activity of the essential oil of *Ferulago Bernardii* by the broth dilution method in comparison with Gentamycin and Fluconazole as standard showed weak activity against *Staphylococcus aureus*, *Bacilus subtilis*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. The essential oil did not show any activity against *Pseudomonas aeruginosa*.

Keywords: *Ferulago Bernardii*, Apiaceae, Essential oil composition, 2,4,5-trimethyl-benzaldehyde, *α*-pinene, Antimicrobial activity

INTRODUCTION

Ferulago W. D. Koch, is a perennial genus of the Apiaceae distinguished by the presence of persistent bracts and bracteoles (1). The genus *Ferulago* is represented by 40 species in the world. Eight species exist in Iran of which three are endemic. *Ferulago Bernardii* Tomk. & M. Pimen. is a glabrous plant distributed in southeast Turkey and west of Iran (1,2).

Ferulago, *Ferula* and *Prangos* species are used since ancient times in folk medicine for their sedative, tonic, digestive, aphrodisiac properties and have been used in the treatment of intestinal worms and hemorrhoids in different regions of Turkey (3,4). Moreover the plants of the genus *Ferulago* have been employed against ulcers, snake bite, as well as headache and diseases of the spleen (5).

The chemical compositions of some *Ferulago* species have previously been reported. Coumarins from *F. meoides*, *F. turcomanica*, *F. granatensis*, *F. aucheri*, *F. asparagifolia*, *F. nodosa*, *F. capillaris* and *F. brachyloba* (6-13), flavonoids from *F. aucheri* and *F. asparagifolia* (10,11), *β*-sitosterol from *F. granatensis* (9), aromatic compounds from *F. aucheri* (10), monoterpenes from *F. nodosa* (12), phenylpropanoid and sesquiterpene aryl esters from *F. antiochia* also have been reported (14).

A number of *Ferulago* species have previously been investigated for their essential oil compositions and antimicrobial activities (3,5,15-23). The chemical composition of the essential oil of *F. Bernardii* has not yet been described. In this paper the chemical composition of this species is reported. In addition, since the essential oils of some other *Ferulago* species have shown remarkable antimicrobial activities (3,5,22), in vitro activity of the essential oil of *F. Bernardii* against some microorganisms in comparison with Gentamycin and Fluconazole by the broth dilution method was determined.

MATERIALS AND METHODS

Plant Material and Isolation Procedure:

The aerial parts of *F. Bernardii* in fruiting stage were collected in September 2001 from west of Iran, the height of Ariz (in the road of Sanandaj to Marivan), ca. 2140 m. The plant was identified by the department of Botany of the Research Institute of Forests and Rangelands (TARI), Tehran. A voucher specimen No. 71608 has been deposited at the Herbarium of TARI.

The aerial parts were cut into pieces, air-dried for 7 days at room temperature, crushed and hydrodistilled for 4 h using a Clevenger-type

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apparatus to yield an essential oil (0.2%). The oil was dried over anhydrous Na_2SO_4 and kept refrigerated until used.

Analysis of the Essential oil:
The essential oil was analysed by GC and GC/MS.
GC: Capillary GC carried out using a thermoquest 2000 GC chromatograph with DB-1 (30m×0.25 mm×0.25µm) column. Oven temperature was performed as follows: 50ºC for 1 min and finally heated to 265ºC with a 2.5ºC/min rate and then kept constant at 265ºC for 30 min; injector temperature 250ºC; carrier gas, He (1.5 ml/min); split ratio of 1:25 and a flame ionization detector. Quantitative data were obtained from FID area percentage data.
GC/MS: The essential oil was also analysed by a Thermoquest 2000 with a quadrupole detector, on capillary column DB-1 (see GC), carrier gas: He, flow rate: 1.5 ml/min. The column was held at 50 ºC for 1 min and programmed up to 265 ºC at a rate of 2.5 ºC/min and then kept constant at 265 ºC for 30 min. MS were taken at 70 eV.

Identification of essential oil components:
Retention indices were calculated by using retention times of n-alkanes (C_8-C_{20}) that were injected after the oil at the same temperature and conditions. Compounds were identified by comparison of their retention indices (RI) with those reported in the literature (24,25) and their mass spectrum with the Wiley library (26).

Biological Activity:
The antibacterial and antifungal activities of the essential oil were determined against *Staphylococcus aureus* (ATCC 29737), *Bacilus subtilis* (ATCC 12711), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 14053) and *Aspergillus niger* (ATCC 16404) by the broth dilution method (27,28). The bacteria and *C. albicans* were maintained on Nutrient broth (Difco) and adjusted to 1×10^6 and 1×10^4 organism/ml, respectively. Inoculum for *A. niger* was prepared as a spore suspension from a 5 days old agar-surface culture and adjusted to a concentration of approximately 1×10^4 in a final volume of cultures.
The susceptibility of the strains to *F. Bernardii* essential oil was performed by determination of the minimum inhibitory concentrations (MICs) of the isolates using the broth dilution method. First stock solution of the sample of concentration of 10^5 µg/ml was prepared in DMSO. The test sample was further diluted in sterile water to obtain serial dilution concentrations from 250µg/ml to 2000 µg/ml. Then 0.5 ml of each concentration was added to 0.5 ml of double-strength medium broth for test strain. Inoculum which was prepared as described above was introduced to each test tube. The cultured tubes were incubated at 37 ^oC for bacteria for 24h, at 30 ^oC for 24h for *C.* albicans and at 25 ^oC for 3 days for *A. niger*. The lowest concentration at which no growth was observed, recorded as MICs. Culture media with different concentration of Gentamycin and Fluconazole were used as control and DMSO (40µl) was used as negative control. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Water distilled of essential oil from aerial parts of *F. Bernardii* was analysed by means of GC and GC/MS.
The chemical composition of the essential oil is represented in Table 1. Sixty constituents were found representing 87.9% of the oil; 2,4,5-trimethyl-benzaldehyde (21.2%) and *α*-pinene (17.0%) were the main components, with the appreciable amounts of spathulenol (5.0%), *cis*-chrysanthenyl acetate (4.4%), caryophyllene oxide (3.2%), *trans*-verbenol (2.5%) and 2,4,6-trimethyl-benzaldehyde (2.1%). The oil was characterized by large amounts of oxygenated monoterpene (46.4%) and monoterpene hydrocarbons (23.7%), small amounts of oxygenated sesquiterpenes and sesquiterpene hydrocarbons (13.1% and 3.8%, respectively).
Major components of the hydrodistilled of essential oils of eight *Ferulago* species are listed in Table 2. The main component identified in the essential oils of *F. angulata* (Schlecht.) Boiss. (21), and of *F. Bernardii* Tomk. & M. Pimen. from Iran, is *β*-phellandrene (32.0%) and 2,4,5-trimethyl-benzaldehyde (21.2%), respectively. While oils of *F. asparagifolia* Boiss., *F. thirkeana* (Boiss.) Boiss. and *F. trachycarpa* (Fenzl) Boiss. from Turkey are rich in 2,3,6-trimethyl-benzaldehyde (38.9%), ferulagone (63.5%) and (*Z*)-*β*-ocimene (30.7%), respectively (3,15,16). The essential oils of *F. nodosa* (L.) Boiss, *F. sylvatica* (Besser) Reichenb and *F. thyrsiflora* (Sibth. & Sm.) Koch from Greece contain *α*-pinene (31.12%), spathulenol (13.02%) and spathulenol (31.07%), respectively (5).
The major component of each eight *Ferulago* species is different and only in two species namely, *F. sylvatica* and *F. thyrsiflora*, the major component (spathulenol) is similar. Also the main component of *F. Bernardii*, 2,4,5-trimethyl-benzaldehyde, was found in only *F. thyrsiflora* in high amount (21.2% and 17.25% respectively). At the same time four species, *F. angulata*, *F. Bernardii*, *F. nodosa* and *F. thirkeana* had *α*-pinene in high concentration. In two species of *F. asparagifolia* and *F. trachycarpa*, in addition to major component (2,3,6- trimethyl - benzaldehyde

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and (*Z*)-*β*-ocimene respectively), myrcene (18.2% and 27.7% respectively) were found in high amounts.

Antimicrobial activity was assayed with the oil of *F. Bernardii* (Table 3). The oil showed weak antimicrobial activity against *Bacilus subtilis* (MICs <125 µg/ml), *Escherichia coli* (MICs <125 µg/ml), *Staphylococcus aureus* (MICs = 250 µg/ml), *Candida albicans* (MICs = 500 µg/ml) and *Aspergillus niger* (MICs = 250 µg/ml). The essential oil did not show activity against *Pseudomonas aeruginosa* (MICs >1000 µg/ml).

Finally, this is the first report on the chemical composition and antimicrobial activity of the essential oil of *F. Bernardii*. As it could be seen, the essential oil of *F. Bernardii* showed antimicrobial activity against some Gram (+) and Gram (-) bacteria and fungi.

Table 1: Chemical composition (%) of identified compounds in the oil of *Ferulago Bernardii*

No.	Compounds	RI[a]	%	No.	Compounds	RI[a]	%
1	hexanal	781	0.5	35	**2,4,6-trimethyl-benzaldehyde**	**1279**	**2.1**
2	***α*-pinene**	**922**	**17.0**	36	*cis*-pinocarvyl acetate	1298	0.7
3	camphene	941	0.6	37	**2,4,5-trimethyl-benzaldehyde**	**1334**	**21.2**
4	thuja-2,4(10)-diene	943	0.6	38	linalyl isobutanoate	1356	0.3
5	sabinene	960	0.4	39	*α*-copaene	1364	1.0
6	*β*-pinene	964	1.3	40	*β*-bourbonene	1370	0.3
7	myrcene	981	0.6	41	*α*-cedrene	1390	0.1
8	*p*-cymene	1007	0.4	42	*β*-caryophyllene	1402	1.3
9	limonene	1017	1.4	43	neryl acetone	1424	0.1
10	*β*-phellandrene	1026	0.9	44	*α*-humulene	1433	0.1
11	(*Z*)-*β*-ocimene	1034	0.1	45	valencene	1472	0.3
12	*m*-cymenene	1066	0.3	46	*α*-muurolene	1480	0.2
13	*α*-terpinolene	1072	0.1	47	7-epi-α-selinene	1494	0.3
14	n-nonanal	1078	0.2	48	kessane	1503	1.7
15	2*Z*-heptenyl acetate	1080	0.1	49	(*Z*)-nerolidol	1514	0.1
16	*α*-campholenal	1098	1.6	50	*cis*-sesquisabinene hydrate	1521	0.3
17	*trans*-pinocarveol	1115	1.9	51	*cis*-muurol-5-E-4-α-ol	1530	1.3
18	*cis*-verbenol	1118	0.5	52	germacrene B	1532	0.2
19	***trans*-verbenol**	**1125**	**2.5**	53	bornyl angelate	1535	0.4
20	pinocarvone	1130	1.3	54	**spathulenol**	**1553**	**5.0**
21	menthofuran	1135	0.3	55	**caryophyllene oxide**	**1556**	**3.2**
22	*p*-mentha-1,5-dien-8-ol	1143	2.0	56	humulene epoxide II	1575	0.4
23	terpinen-4-ol	1153	0.3	57	caryophylla-4(14), 8(15)-dien-5α-ol	1602	0.4
24	*m*-cymen-8-ol	1155	0.2	58	valeranone	1633	0.3
25	myrtenal	1159	0.8	59	khusinol	1648	0.2
26	*α*-terpineol	1165	0.4	60	(*Z*)-*γ*-atlantone	1663	0.2
27	verbenone	1170	0.6		monoterpene hydrocarbons		23.7
28	myrtenol	1172	0.5		oxygenated monoterpenes		46.4
29	*trans*-carveol	1194	0.8		sesquiterpene hydrocarbons		3.8
30	carvone	1206	0.1		oxygenated sesquiterpenes		13.1
31	thymoquinone	1223	0.4		others		0.9
32	***cis*-chrysanthenyl acetate**	**1242**	**4.4**		not identified		12.1
33	bornyl acetate	1262	1.7		**Total identified**		**87.9**
34	*trans*-pinocarvyl acetate	1273	1.4				

[a] RI: Retention index on DB-1 column

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Table 2. Major components of the essential oils of eight *Ferulago* species

	F. angulata	*F. asparagifolia*	*F. Bernardii*	*F. nodosa*	*F. sylvatica*	*F. thirkeana*	*F. thyrsiflora*	*F. trachycarpa*
Hydrodistillation (yields of essential oil)	2.5%	7%	0.2%	3%	0.1%	4.1%	0.8%	7.3%
α-pinene	**9.1**	4.7	**17.0**	**31.12**	-	**9.0**	2.69	4.6
sabinene	2.1	0.5	0.4	3.14	-	0.4	0.15	6.7
β-pinene	7.1	0.3	1.3	1.65	-	0.5	0.16	0.6
myrcene	-	**18.2**	0.6	-	-	0.8	6.8	**27.7**
α-phellandrene	**13.8**	2.0	-	-	-	-	-	1.0
p-cymene	-	1.6	0.4	4.06	-	-	-	0.2
limonene	-	2.0	1.4	5.23	3.86	0.6	0.5	3.0
β-phellandrene	**32.0**	1.7	0.9	-	-	-	-	2.8
(*Z*)-*β*-ocimene	-	1.0	0.1	-	-	-	0.7	**30.7**
terpinolene	5.5	t[a]	0.1	-	1.25	-	-	6.0
α-campholenal	-	-	1.6	6.35	-	0.1	-	-
β-elemene	-	0.2	-	-	-	0.9	-	0.3
cis-chrysanthenyl acetate	-	-	4.4	3.37	-	-	-	-
carvacrol	-	0.5	-	-	5.11	-	**13.75**	-
2,3,4-trimethyl-benzaldehyde	-	4.2	-	-	-	-	-	-
2,3,6-trimethyl-benzaldehyde	-	**38.9**	-	-	-	-	-	-
2,4,5-trimethyl-benzaldehyde	-	-	**21.2**	-	-	-	**17.25**	-
α-copaene	-	-	1.0	6.1	2.6	-	-	0.1
β-patchoulene	-	-	-	-	6.6	-	-	-
Z-caryophyllene	-	-	-	-	7.18	-	-	-
germacrene D	1.4	3.6	t	-	-	**14.0**	-	3.3
bicyclogermacrene	-	3.0	-	-	-	-	-	2.4
spathulenol	0.7	0.7	5.0	-	**13.02**	-	**31.07**	-
caryophyllene oxide	-	-	3.2	-	7.52	-	-	-
ferulagone	-	-	-	-	-	**63.5**	-	-
ent-3*β*-hydroxy-13 epimanoyl oxide	-	-	-	-	-	-	6.59	-
hexadecanoic acid	-	-	-	-	7.3	-	-	-

[a] t: trace

Table 3. Antimicrobial activity of *Ferulago Bernardii* (MIC[a] values: µg/ml)

Microorganisms	MICs		
	Essential oil (µg/ml)	Gentamycin (µg/ml)	Fluconazole (µg/ml)
Staphylococcus aureus (ATCC[b] 29737)	250	17	NT[c]
Bacilus subtilis (ATCC 12711)	<125	30	NT
Escherichia coli (ATCC 8739)	<125	10	NT
Pseudomonas aeruginosa (ATCC 9027)	>1000	22	NT
Candida albicans (ATCC 14053)	500	NT	12
Aspergillus niger (ATCC 16404)	250	NT	10

[a] MIC: Minimum Inhibitory Concentrations
[b] ATCC: American Type Culture Collection
[c] NT: Not tested

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