



# Autecology Essential Oil Composition, Antibacterial, Anti Candidacies and Ethnopharmacological Survey of *Ferula Gummosa* L. As Anti Infection to Treat Of Vaginal Infections in Traditional Medicine of Razavi Khorasan Province (North East of Iran)

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## Abstract

**Objective:** *Ferula gummosa* Boiss. (Apiaceae family), which has been used in traditional medicine of Iran as anti vaginal infection, anti-sinusitis, sedative, and anti-inflammation.

**Materials and Methods:** In this research, the gums of plant root were collected from the Heidary nature reserve in Razavi Khorasan Province (Iran) in August 2012. The ethnopharmacological data about traditional uses of plant were obtained from the rural healers (women 67-75 year) of this region. Essential oil of the plant root gum was obtained by hydrodistillation (Clevenger apparatus) and was analyzed by gas chromatography-mass spectrometry. Antibacterial activity of plant ethanolic extract was studied in vitro against *Candida albicans* and 9 Gram-positive and negative bacteria using well method and the minimum inhibitory concentration (MIC) assay.

**Results:** Results showed that a total of 39 components have been identified in the plant sampl oil, representing 81% of the total oil and  $\beta$ -pinene (19.88%), guaiol (8%), shyobunone (6.96%), delta-cadinene (4.65%),  $\alpha$ -pinene (3.16 %),  $\beta$ -phellandrene (3.28%) and myrtenol (2.8%), were the main essential oil composition, respectively. The results from antibacterial screening (Table 2), were showed that *C. albicans* ( $25.2 \pm 1.6$ ), *Staphylococcus epidermidis* ( $23.6 \pm 0.7$  mm), *Staphylococcus aureus* ( $21.3 \pm 0.2$  mm), *Escherichia coli* ( $16.5 \pm 0.8$  mm), *Bacillus cereus* ( $19.5 \pm 0.1$  mm), *Enterococcus faecalis* ( $17.2 \pm 0.8$  mm) and *Pseudomonas aeruginosa* ( $17.8 \pm 0.2$  mm) inhibition zone and MIC (35.4-112  $\mu$ g/ml) were the most sensitive pathogens to the plant extract respectively, which followed *Shigella* ( $12.3 \pm 0.3$  mm) and *Klebsiella pneumonia* ( $12.5 \pm 0.2$  mm) were found to be moderate sensitive bacteria and then the *Salmonella typhymorium* which completely was resistant to plant root extract.

**Conclusion:** According to these results, it can be concluded that the extract of *F. gummosa* L. have suitable antimicrobial and anti-Candidacies activity, which can be used as natural anti-infection to treat of many infection diseases, especially in vaginal infection.

**Keywords:** *Candida*, Ethnopharmacology, Essential, *Ferula*, Oils, Vaginitis

## Introduction

Endemic aromatic plants in mountain regions have been used for centuries as a sources of anti-inflammation, anti-oxidant, anti-fungal and antimicrobial (1-3). Hence due to increasing

prevalence of multi drug-resistant strains of pathogens, the antimicrobial effects of some aromatic plants and their constituents have been recognized for many years (4).

*Ferula* genus (Apiaceae) consists of 133 species,

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are aromatic herbs which distributed throughout the Mediterranean area and central Asia (5). *Ferula gummosa* Boiss. (Barije in Persian), is a wild plant indigenous to Iran, growing in the northern and western parts of the country (6,7). In Iranian traditional medicine, the gums and root extract of *Ferula* species, which has been used for the treatment of stomach pain, sinusitis, vaginal infection and wound-healing remedy (4,6). Although phyto chemical data about essential oil composition of *F. gummosa* L. was limited, but according to some recent studies, the presence of terpenoides ( $\alpha$ -pinene and  $\beta$ -pinene, thujone, terpinolene, camphor,  $\alpha$ -humulene, camphene,  $\beta$ -caryophyllene, cadinene, verbenone, 1,8-cineole, geraniol, menthol, linalool and camphor), flavonoids and alkaloids in *Ferula* extract from Iran and Turkey (4,7) as the anti-inflammation, anti-spasmodic, anti-fungal (*Clostridium perfringens*, *Candida albicans* and *Aspergillus niger*) and antimicrobial activity against many Gram-positive and negative bacteria were reported (8-12).

Hence according to traditional uses of *F. gummosa* L. as anti-infection and anti-inflammation in many regions of Iran, the present study was carried out in ecological requirement investigation, ethnopharmacology, essential oil composition, antibacterial and anti-*Candida* survey of *F. gummosa* L. as anti-infection and anti-inflammation to treat of vaginal infections in traditional medicine of Razavi Khorasan Province (North east of Iran).

## Materials and Methods

Heidary nature reserve from Neyshabour in Razavi khorasan province (North east of Iran), is located in Southeast of this province, in of latitude of 36°35' 78" N and longitude of 58°36' 83" E with Clay loam soils. Its average height is 600-2350m from sea levels, semi-dry climate, rainfall of about 305 mm/year and a mean temperature  $-2/8$  °C (January-February) and  $17/3$  °C (July-August).

The roots of *F. gummosa* Boiss. were collected in August 2012 from Heidary nature reserve at height of 2020 meters above sea level. The plant was identified at the Islamic Azad University of Gorgan branch. A voucher specimen was deposited and identified at the Herbarium of the Research Center of Medicinal Plants of Gorgan, Iran and kept under voucher specimen number RCH 1349.

The current ethnopharmacological survey was carried on 3 native-born rural Kord practitioners (especially bonesetters) randomly chosen from among the most famous elderly traditional medicine practitioners living in Heydar-Abad villages. Data about the medicinal plant uses have been collected through interviews with the practitioners. Each practitioner was interviewed about local name, the current diseases, its medicinal effects and plant part used and other methods of its preparation.

The root of the plant was washed and dried at room temperature for 6 days. Essential oil was

obtained by hydrodistillation in Clevenger-type apparatus for 5 h according to Mazandarani et al., (13). The oil was kept in refrigerated (4 °C) and protected from direct light until the analysis time.

The essential oil was analyzed by GC/MS. The GC/MS analysis was carried out on a Shimadzu GC/MS (QP5050). The capillary conditions were as follows; carrier gas, helium with a flow rate of 1.7 ml/min; injected 0.1  $\mu$ l of the essential oil and ionization potential 70eV. The initial temperature of column was 60 °C (held 1 min) then heated to 280°C with a rate 3 °C/min then heated to 250 °C and kept constant for 4 min. The same condition of temperature programming was used for n-alkenes mixture to calculate the retention indexes. The identification of each component was studied by mass spectral data, literature, and NIST computer library. The relative percentage of the oil constituent was calculated.

The bacterial strains were obtained from the Microbiology Laboratory, Golestan University of Medical Sciences. The ethanol extract of plant root were individually tested against two strains of Gram-positive and Gram-negative bacteria: *Shigella dysenteriae* (PTCC1188), *Pseudomonas aeruginosa* (PTCC1430), *Escherichia coli* (PTCC1399), *Staphylococcus aureus* (PTCC1431), *Bacillus cereus* (PTCC1015), *Salmonella typhimurium* (ATCC1596), *Staphylococcus epidermidis* (PTCC1114), *Enterococcus faecalis* (PTCC1393), *Klebsiella pneumoniae* (PTCC1291) and *C. albicans* (PTCC5027).

At a first screening, the plant extract were tested against the above mentioned bacteria. Minimal inhibitory concentrations (MICs) were determined by the agar serial dilution method at concentration ranging from 0.93 to 60  $\mu$ g/ml. Two fold serial dilutions were made from essential oil in molten Mueller Hinton agar (Pronadisa-Madrid) cooled to 45-50°C in a water bath. The essential oil was dispersed in mixture using dimethyl sulfoxide (DMSO). The amount of 0.01 mL of every bacterial suspension, equivalent to McFarland tube No. 0.5 (108 CFU/ml), inoculated on the agar of every well. The culture plates were then incubated at 37°C for 24 h. The MIC was defined as the lowest concentration at which no visible growth was observed (14). The Mueller Hinton agar were contained DMSO without essential oil was used a negative control while gentamicin was used as positive control.

## Statistical analysis

The statistical method of ANOVA was used for comparison of the effectiveness of the anti-*Candida* activities of essential oils and drugs.  $P < 0.05$  were considered significant.

## Results

### Essential oil composition

Essential oil analysis showed in table 1, which approximately 40 compounds with 0.92% yield (w/w) and  $\beta$ -pinene (19.88%), guaiol (8%), shyobunone (6.96%), delta-cadinene (4.65%),

$\alpha$ -pinene (3.16 %),  $\beta$ -phellandrene (3.28%), and myrtenol (2.8%) were the most components, which identified in plant essential oil in heidary nature reserve region.

The results from antibacterial screening (Table 2), were showed that *C. albicans* ( $25.2 \pm 1.6$ ) *S. epidermidis* ( $23.6 \pm 0.7$  mm), *S. aureus* ( $21.3 \pm 0.2$  mm), *E. coli* ( $16.5 \pm 0.8$  mm), *B. cereus* ( $19.5 \pm 0.1$  mm), *E.*

*faecalis* ( $17.2 \pm 0.8$  mm) and *P. aeruginosa* ( $17.8 \pm 0.2$  mm) inhibition zone and MIC ( $35.4$ - $112$   $\mu$ g/ml), respectively were the most sensitive bacteria to the plant extract, followed *Shigella* ( $12.3 \pm 0.3$  mm) and *K. pneumonia* ( $12.5 \pm 0.2$  mm) were found to be moderate sensitive bacteria and then the *Salmonella typhimurium* which completely was resistant to plant root extract, respectively.

**Table 1.** Essential oil composition of *Ferula gummosa* Boiss. In Sarvelayt region (2020 m)

No	Compounds	Percent (%)	RT
1	hexanal	0.35	3.45
2	$\alpha$ -pinene	3.16	5.69
3	$\beta$ -Pinene	19.88	6.75
4	p-cymene	0.57	7.80
5	$\beta$ -Phellandrene	3.28	7.98
6	Pinocarveol	1.46	11.55
7	galbanolene	0.59	11.81
8	terpineol	0.32	12.04
9	2-cyclohexane-1-one,4-(1-methylethyl)	1.20	12.22
10	myrtenol	2.80	12.47
11	fenchyl acetate	0.44	13.03
12	benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-	2.05	13.59
13	propanedinitrile	0.84	13.76
14	6-Methyl-bicyclo(4,2,0)octan-7ol	0.10	14.57
15	bornyl acetate	0.25	14.875
16	myrtenyl acetate	0.44	15.17
17	thiophene 2,5-bis (1,1-dimethylethyl)	0.19	15.33
18	cypionic acid	0.10	15.67
19	$\alpha$ -terpinenyl acetate	2.55	16.54
20	1,2,4-metheno, 1H-indene	0.20	17.21
21	$\alpha$ -copaene	0.29	17.36
22	$\beta$ -elemene	0.36	17.69
23	benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy	0.83	18.23
24	gumma-murolene	0.27	18.67
25	cedrene-V6	0.88	19.41
26	linalyl isovalerate	0.40	19.52
27	$\alpha$ -cedrol	2.84	20.27
28	$\alpha$ -muurolene	1.48	20.50
29	shyobunone	9.59	20.85
30	delta-cadinene	4.65	21.02
31	9-cedranone	1.58	21.18
32	citronellyl propionate	0.77	22.24
33	germacrene-D-4-OL	1.11	22.44
34	2-cyclopentane-1-carboxylic acid	2.13	22.62
35	guaial	8.03	22.94
36	$\alpha$ -cadinol	1.17	24.27
37	$\alpha$ -bisabolol	0.84	24.97
38	Illudol	0.82	25.19
39	calarene	0.61	25.55
Total		80.03	

RT: Retention time

**Table 2.** The in vitro antibacterial and anti-Candida activity and the MIC values in root extract of *Ferula gummosa* L. from Sarvelate region in Khorasan Razavi Province (North east of Iran)

Microorganisms	Sarvelayt region (2020 m)		Gentamycin
	Inhibition zone (mm) (mean $\pm$ SD)	MIC ( $\mu$ g/ml)	
<i>Staphylococcus aureus</i>	$21.3 \pm 0.2$	35.4	16.7
<i>Staphylococcus epidermidis</i>	$23.6 \pm 0.7$	42.6	14.7
<i>Bacillus cereus</i>	$19.5 \pm 0.1$	49.2	16.5
<i>Enterococcus faecalis</i>	$17.2 \pm 0.8$	73.8	9.6
<i>Escherichia coli</i>	$16.5 \pm 0.8$	72.8	11.0
<i>Pseudomonas aeruginosa</i>	$17.8 \pm 0.2$	80.5	9.0
<i>Klebsiella pneumonia</i>	$12.5 \pm 0.2$	119.2	-
<i>Salmonella typhimurium</i>	$12.3 \pm 0.3$	112.0	11.0
<i>Shigella dysenteriae</i>	$12.6 \pm 0.7$	92.5	11.0
<i>Candida albicans</i>	$25.2 \pm 1.6$	62.0	12.8

MIC: Minimum inhibitory concentration; SD: Standard deviation

### **Ethnopharmacological survey of plant in Sarvelayat region (Around Heidary nature reserve)**

Sarvelayat region is Kordish (Kurmanji) in Khorasan Razavi, which have long history in plant using and had good potential for growth of many wild aromatic medicinal plants, such as *F. gummosa* Boiss., which in the Kurmanji language is called, "Qasny." Ethnopharmacological results showed that the root and gums of *F. gummosa* has been used in traditional medicine of this region as antiseptic, anti-spasmodic, anti-inflammatory, Anticonvulsant, epilepsy and analgesic to treat of headache, migraine, wound and ulcers. According to their traditional medicine, the powders of *Ferula* root in combination of *Teucrium polium* and *Achillea millefolium*, which has been used as anti-spasmodic to treat of rheumatic, abdominal pain, diarrhea and dysmenorrhea. The infusion of *Myrtus communis* (leaves), *Mentha longifolia* (aerial parts), *Stachys byzantina*, *Tribulus terrestris*, *Satureja montana*, *Thymus* spp., *Zataria multiflora*, *Origanum vulgare*, *Lawsonia inermis*, *Artemisia sieberi*, *Peganum harmala* with the root powders or smoking of *F. gummosa* and *P. harmala* L. which has been used as disinfectant solution to treat of vaginal infection (15).

### **Discussion**

The resistance of pathogenic fungi, including *C. albicans* and non-*Candida* species isolated from patients, against antifungal agents has increased (16).

Around 75% of adult women have at least one episode of vaginal infection during their life, with prevalence of *C. albicans* in 70-90% and in order to in many traditional medicine derived from plant sources are still being used (15,17,18). Wild aromatic medicinal plants, especially in Mountain endemic regions are renewable in nature major source of new anti-oxidant and anti-pathogenic compounds (19).

Such as many traditional medicine of Iran, Razavi Khorasan Province in North east of Iran is blessed with semi-dry climate, a rich source region of medicinal plants, such as *F. gummosa* L. with long history of traditional uses, which has been used as anti-inflammation, sedative and anti-infection to treatment of many current infection diseases, especially to treat of vaginal infection.

In this study, ethnopharmacological survey of plant showed that the combination of merged powders of *M. communis*, *M. longifolia*, *S. byzantina*, *Tribulus terrestris*, *S. montana*, *O. vulgare*, *L. inermis*, *A. sieberi* and *P. harmala* with *F. gummosa* roots, which has been used as disinfectant solution to treat of inflammation and vaginal infection. According to these results in Tables 1 and 2 showed that  $\beta$ -pinene (19.88%), guaialol (8%), shyobunone (6.96%), delta-cadinene (4.65%),  $\alpha$ -pinene (3.16 %),  $\beta$ -Phellandrene (3.28%) and myrtenol (2.8%) were the most essential oil composition of plant, which had the highest inhibition zone values against *C. albicans* ( $25.2 \pm 1.6$  mm), *S. epidermidis* ( $23.6 \pm 0.7$  mm), *S. aureus* ( $21.3 \pm 0.2$  mm), *B. cereus* ( $19.5 \pm 0.1$  mm), *E.*

*faecalis* ( $17.2 \pm 0.8$  mm), *P. aeruginosa* ( $17.8 \pm 0.2$  mm) and *E. coli* ( $16.5 \pm 0.8$  mm), with lowest MIC value ( $16-72.8$   $\mu\text{g/ml}$ , respectively against *C. albicans* and other tested bacteria.

In many researches, which have been shown that the  $\alpha$ -pinene,  $\beta$ -pinene, acetone, menthol, myrthenole, and benzene are most anti pathogenic fractions of *F. gummosa*, *Foeniculum vulgare*, *Trachyspermum ammi*, *S. montana*, *Cuminum cyminum* and *A. sieberi*, which were evaluated (18,20).

Although phytochemical data about essential oil composition of *F. gummosa* L. was limited, but some identified compounds, such as: thujone, terpinolene, camphor,  $\alpha$ -humulene, camphene,  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -caryophyllene, cadinene, verbenone, 1,8-cineole and camphor were the main oil constituents, which had good anti-oxidant and antibacterial activity against Gram-positive and Gram-negative bacteria, which have reported in thyme, pennyroyal, lemon, *Ficus lyrata* and *Z. multiflora* from Iran and Brazilian medicinal plants against some *Candida* species (12,20-23). So In many literatures are described that the antibacterial activity of *F. gummosa* Boiss. can be depended to their terpenoids and flavonoids in essential oil or extract (24-26).

Briefly, similar to another researches, our results in Table 2 indicated that *C. albicans*, *S. epidermidis*, *S. aureus* and followed *B. cereus* and *P. aeruginosa* were sensitive pathogens against plant extract in Neyshabour region, which can caused of boils, sores and wounds are considered as a main pathogen of causing hospitalized patients infections, diarrhea and dysentery in humans (27,28).

Also in another works to similar our ethnopharmacological survey, the components such as: terpinolene, terpinene 4-ol,  $\gamma$ -terpinene,  $\alpha$ -pinene, 1,8-cineole, borneol,  $\beta$ -pinene, Chamazulene and thujone were the most anti-bacterial compounds of *A. millefolium*, *Thalictrum minus*, *Perovskia abrotanoides*, *Ditrichia* sp. and *Eucalyptus* spp. (28-31), because in previous research these compounds have strong antibacterial activity and which have been used in traditional medicine of many countries to healing wound, treat of rheumatic pain, arthritis, urinary tract infection, cold, flu, leishmanious and vaginal infection (10,32-36).

Due to confirming of our ethnopharmacological findings, another researches were showed that the plant extracts of *Z. multiflora*, *Thymus kotschyianus*, *C. cyminum*, *S. byzantina*, *T. terrestris*, *S. montana* and *P. harmala* were found to be active against *C. albicans* tested with MIC values ranging from 150 to 2300  $\mu\text{g/ml}$  and the growth inhibition zone ranging from 16 to 55 mm, which have showed significant activity against *C. albicans* ( $P < 0.05$ ) (37).

In conclusion, these results suggested the presence of high antibacterial and anti-inflammation components in *F. gummosa* essential and confirmed the traditional application of this plant as anti-infection and anti-inflammation to treatment of many infection disease such as vaginal infection.

## Conclusion

Our results demonstrate that the roots extract of *F. gummosa* L. could have good potentials to prevent or controlling on bacteria and *C. albicans*, which can produces of many infectious diseases, such as vaginal infection, so these data which can confirming of the traditional uses of this plant with its terpenoides such as:  $\alpha$ -pinene,  $\beta$ -pinene, myrtenol, 1,8-cineole, camphor, borneol, terpinolene,  $\gamma$ -terpinene and thujone and it is which can be possess more antibacterial activity especially against infectious pathogen.

Hence, we believe that the plant root gums absolutely had anti-infection and anti-inflammation effects to treat of many infectious diseases against vaginal infection and additional in vivo studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antimicrobial agent in topical or clinical applications and further studies are necessary to evaluate the in vivo effects of active compounds of this plant. In addition, investigations confirm that higher plants used as anti-infective phyto medicines may serve as a valuable source for novel antibiotics.

## Ethical issues

Ethical of this research work was approved by Gorgan Branch, Islamic Azad University.

## Conflict of interests

We declare that we have no conflict of interests.

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