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Original Article

Composition and antimicrobial activity of oleogumresin of *Ferula gumosa* Bioss. essential oil using Alamar Blue™

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Abstract

Essential oils and their components are becoming increasingly popular as naturally occurring antimicrobial agents. In this work the chemical composition and antimicrobial activity of *Ferula gumosa* essential oils were determined. The essential oil components were identified by GC/MS analysis. The minimum inhibitory concentration (MIC) of essential oil against five selected pathogenic microorganisms was determined using micro-plate Alamar Blue™ assay (MABA). A total of 22 components have been identified in the oil, representing 97.1% of the total oil. The main group was monoterpene hydrocarbons (88.4%) including sabinene (40.1%), α-pinene (14.3%) and β-pinene (14.1%). This oil inhibited the growth of *Escherichia coli* (MIC=0.25µl/ml), *Pseudomonas aeruginosa* (MIC=50 µl/ml) *Staphylococcus aureus* (MIC=3.125 µl/ml), *Salmonella enteritidis* (MIC=6.25 µl/ml) and *Listeria monocytogenes* (MIC=1.56 µl/ml). The tested gram-positive bacteria were more sensitive than the gram-negative bacteria. According to these results, it can be concluded that the essential oils of *Ferula gumosa* have suitable antimicrobial activity and can be used as natural preservatives in food industry.

Keywords: *Ferula gumosa*; Antimicrobial activity; Essential oil

INTRODUCTION

The antimicrobial effects of some plants and their derivatives such as essential oils and oleoresins have been recognized for many years (1,2). Essential oils (EOs) are also used as flavoring agents in food industry (3,4). Numerous studies have reported the antimicrobial activity and chemical composition of essential oils (3-6).

Ferula genus (Apiaceae) consists of 133 species distributed throughout the Mediterranean area and central Asia (3,7). *Ferula gumosa* Boiss., Barije in Persian, is a wild plant indigenous to Iran, growing in the northern and western parts of the country (8). *F. gumosa* have shown several pharmacological activities, including antispasmodic, expectorant, anticonvulsant, anticatarrhal, anti-nociceptive and antimicrobial activity (9-13). However, the interesting point to note is that the main constituents of all *F. gumosa* essential oils are α-pinene and β-pinene, therefore the minor constituent may play an important role in its antimicrobial activity. The antimicrobial activity and chemical constituents of *F. gumosa* fruits has been reported before (8). Similarly, antimicrobial activity of *F. gumosa* fruits essential oil was studied against bacterial laboratory ATCC standards using the disk diffusion method (8). It was reported that the fruits oil had activity against gram-positive bacteria and *Escherichia coli*. There are a few substances in *F. gumosa* fruits oil that have antimicrobial activity against *Pseudomonas aeruginosa*. The main constitu-

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ents were β-pinene (50.1%), α-pinene (18.3%) (8). Little work has been performed to study the antimicrobial activity of *F. gumosa* oleogumresine essential oil. The current study reports the results of detailed chemical composition analysis and antimicrobial activities of the essential oil of *F. gumosa* obtained from its oleogumresine.

MATERIALS AND METHODS

Plant material

The essential oil used in this study was obtained from *F. gumosa* Boiss. oleogumresin which purchased from market.

Extraction and isolation of the essential oil

Oleogumresin of the plant was exhaustively extracted by hydrodistillation for 3 h using a Clevenger-type apparatus. Oil was obtained in a yield of 5% (v/w) based on weight of sample. For collecting the oil, it was diluted with pentane and after the evaporation of solvent the oil was stored in a sealed glass vial in the dark place at 4 °C for analysis and bioassay tests (14).

GC Analysis

Gas chromatography analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with FID detector and a BP-1 capillary column (39 m × 0.25 mm; film thickness 0.25 μm). The carrier gas was nitrogen with a flow rate of 2 ml/min, the oven temperature for first 4 min was kept at 60 °C and then increased at a rate of 4 °C/min until reached to the temperature of 280 °C, injector and detector temperatures were set at 280 °C.

GC/MS Analysis

The mass spectra were recorded on a Hewlett Packard 6890 MS detector coupled with Hewlett Packard 6890 gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The gas chromatography condition was as mentioned previously. Mass spectrometer condition was as follow: ionized potential 70 eV, source temperature 200 °C.

The identification of the constituents was based on computer matching against the library spectra Wiley 275L built up using pure substances and components of known constituents, MS literature data and evaluation of fragmentation patterns of compounds and confirmed by their gas chromatography retention times. The percentage of composition of the essential oil computed from gas chromatography peak areas without using correction factors. A series of hydrocarbon standards (C_9-C_{18}) were also used to calculate kovats indices from the gas chromatography analysis. Kovats Indices were calculated by the Kovates equations (15-17).

Tested microorganisms

For the bioassays, Test strains were selected among common foodborne pathogens and spoilage bacteria: two gram-positive *Staphylococcus aureus* PTCC 1337, *Listeria monocytogenes* (foodborn pathogen) IRCC 1294 and three gram-negative rods *E. coli* PTCC 1338, *P. aeruginosa* PTCC 1074 and *Salmonella enteritidis* RIICC 1624. The tested microorganisms were identified by using microbiological identification tests. All of microorganisms were maintained at -60 °C in Brain-Heart Infusion (BHI) containing 17% (v/v) of glycerol. Before testing, the suspensions were transferred to nutrient broth (NB) and cultured overnight at 37 °C. The 0.5 Mcfarland standard was used to adjust the turbidity of the inoculum to obtain concentration of 1.5×10^8 CFU/ml. Serial doubling dilutions of this suspension in 0.1% peptone (w/v) solution in sterile water were inoculated on nutrient agar (NA), to check the viability of the preparations (18).

Antimicrobial assay

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the oils were determined using the micro-plate Alamar Blue™ assay (MABA) (1-3,19). Serial doubling dilutions of essential oil was performed in 96-well micro-plate. Dimethyl Sulfoxide (DMSO) solution (160 μl) that consist of 20 μl essential oil and 140 μl Muller Hilton Broth (MHB) was added to the wells of row A, while the remaining wells in rows B to H received 80 μl of MHB. Serial two-fold dilutions were prepared vertically in

the plate. Excess dilutions (80 µl) were discarded from row H. The inoculum (20 µl) containing 1.5×10^8 CFU/ml of each microorganism, and 20 µl of Alamar Blue^{Tm} (Aqeous solution, Serotec, UK) was added to the wells as a growth indicator (1). Overnight broth cultures were prepared in NB and adjusted so that the final concentration in each well following inoculation was approximately 5.0×10^5 cfu/ml. The concentration of each inoculum was confirmed using viable counts on Tryptic Soy Agar (TSA) plates for bacteria. Positive and negative growth controls were included in each test. A microbial susceptibility control test was performed with ciprofloxacin as a standard antibacterial agent and DMSO was used as negative control.

The plates were incubated at 37 °C in ambient air and examined at 24 and 48 h. The color change from blue to red was taken as indication of bacterial growth as described by Baker et al (4). The MIC considered as a concentration of Essential oil (µl per ml of medium) in the first well where the mixture remained blue.

In order to confirm MIC_S and to establish MBC_S, 10 µl of broth was taken from each well and inoculated on TSA plates. After aerobic incubation at 37 °C overnight, the number of surviving organisms was determined. The MIC considered as the lowest concentration which resulted in a significant decrease in inoculum viability (> 90%), the MBC considered as the concentration which 99.9% or more of the initial inoculum was killed (5).

Each experiment was repeated three times for essential oil at each test concentration and for each microorganism. The modal MIC and MBC values were reported (20).

RESULTS

Table 1 shows the results of GC-MS analysis of the *F. gumosa* essential oils. The compounds are listed in order of their retention time on the HP-5MS column. A total of 22 components have been identified in the oil, representing 97.1% of the total oil. The main group was monoterpene hydrocarbons (88.4%) including sabinene (40.1%), α-pinene (14.3%) and β-pinene (14.1%). Moreover the other main components of this oil were *p*-cymene (8.5%) and α-thujene (8.1%). The oil contained a lower percentage of limonene (2.2%), Terpinene-4-ol (1.5%), myrtenal (1.3%) and Gamma-Terpinene (1.1%) (7,21,22).

Table 1. Constituents of *F. gummosa* oleogum-resine essential oil.

Compound	Percent (%)	RT	KI
Unknown	0.65	4.13	922
α-thujene	8.12	4.30	930
α -pinene	14.28	4.50	939
camphene	0.35	4.77	954
sabinene	40.08	5.59	975
β –pinene	14.11	5.64	979
myrcene	0.29	5.83	991
δ – 3 -careen	0.30	6.33	1002
α –terpinene	0.62	6.52	1017
p-cymene	8.46	6.83	1025
limonene	2.23	6.92	1029
γ – terpinene	1.12	7.82	1060
terpinolene	0.38	8.79	1089
nopinone	0.32	10.57	1140
trans – verbenol	1.10	10.70	1145
Unknown	1.28	11.56	1171
Unknown	0.30	11.72	1173
terpinene – 4- ol	1.48	12.21	1177
myrtenal	1.33	12.88	1196
α–longipinene	0.48	19.75	1371
α–copaene	0.47	20.13	1377
β–bourboene	0.42	20.48	1388
β–elemene	0.01	20.85	1391
Unknown	0.76	23.02	1453
garmacrene-d	0.41	23.65	1485
α–amorphene	0.64	25.71	1514

RT: Retention Time
KI: Kovats Index

Table 2 shows the MIC of *F. gummosa* against each microorganism tested. This oil inhibited the growth of *E. coli* (MIC=12.5 µl/ml), *P. aeruginosa* (MIC=50 µl/ml), *S. aureus* (MIC=1.56 µl/ml), *S. entritidis* (MIC=6.25 µl/ml) and *L. monocytogenes* (MIC=1.56 µl/ml). The gram-positive microorganisms were more sensitive compare to tested gram-negative microorganisms. *E. coli* and *S. enteritidis* among gram-negatives and *L. monocytogenes* among gram-positives were the most susceptible strains.

Table 2. Antimicrobial activity expressed as MIC and MBC of *F. gummosa* essential oil against each microorganisms tested by the broth micro-dilution method.

Microorganisms		Essential oil		Ciprofloxacin	
		MIC (µl/ml)	MBC (µl/ml)	MIC (µg/ml)	MBC (µg/ml)
Gram-positive bacteria	*S. aureus*	1.56	25.0	0.50	8.00
	L. monocytogenes	1.56	12.5	1.00	4.00
Gram-negative bacteria	*E. coli*	12.50	25.0	0.03	0.25
	P. aeruginosa	50.00	50.0	0.25	8.00
	S. enteritidis	6.25	12.5	0.50	2.00

DISCUSSION

The major components of *F. gummosa* fruit essential oil collected from Isfahan, were β-pinene (43.7%), α-pinene (27.2%) and myrcene (3.4%) (8). Different quantities of the same component [β-pinene (82.0%), α-pinene (5.4%) and myrcene (3.4%)] were reported in fruit essential oil of *F. gummosa* collected from Tehran (13).

The antimicrobial activity of the oil may be associated with the relatively high α-pinene (14.9%) and β-pinene (14.1%) content. It has been reported that these components have significant antimicrobial activities (23). Antibacterial activities of α-thujene, 3-carene and terpinene-4-ol against *E. coli*, *P. aeruginosa* and *S. aureus* have also been reported (24).

In the present study the oil killed 99.9% or more of *E. coli* (MBC=2.5 µl/ml), *P. aeruginosa* (MBC=100 µl/ml), *S. aureus* (MBC=2.5 µl/ml), *S. enteritidis* (MBC=12.5 µl/ml) and *L. monocytogenes* (MBC=12.5 µl/ml). Although the MIC and MBC results varied between test organisms, in most cases the MIC was equivalent to the MBC, indicating a bactericidal action of the oils.

The GC-MS analysis showed that the major constituents of the oil examined in our study were monoterpene hydrocarbons. The bacteriostatic properties of the oil are suspected to be associated with the high α-pinene and β-pinene content, which has been tested previously and was found to have a significant antibacterial activity (23,25).

CONCLUSIONS

From the bioassays, we can conclude that *F. gumosa* oil possessed antibacterial activity against gram-positive as well as gram-negative bacteria. These observations lead us to suggest that this oil is a broad spectrum antimicrobial agent and has potential for use as an antimicrobial agent in food industries.

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