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Volatile Oil Constituent and Biological Activity of *Gundelia Tournefortii* L. From Iran

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A B S T R A C T

Gundelia tournefortii L. is an important food source and a well-known medicinal plant in Iran. In the present study, the Hydro-distilled volatile oil from the aerial parts of G. tournefortii was investigated by GC-MS and GC. A total of 41 compounds representing 96.2% of the volatile oil were identified. The main constituents were thymol (11.2%), γ-terpinene (9.8%), germacrene D (6.6%) and *p*-cymene (6.3%).

The antimicrobial activities of different extracts of *G. tournefortii* were examined against five Gram-positive and four Gram-negative bacteria. The antioxidant activities of different extracts were evaluated with DPPH radical scavenging activity and their relationship with the phenolic composition were also determined spectrophotometerically.

Methanol extract of *G. tournefortii* showed the strongest antioxidant activity ($IC_{50=}$ 40.3 µg/ml) and the highest total phenolic content (103.4 mgGA/g extract). Ethyl acetate extract demonstrated antibacterial effect against *Bacillus cereus, Bacillus pumilus* and *Bacillus subtilis* strains in concentration of 7.5 mg/ml.

The data of this study suggests that the essential oil and extracts from *G. tournefortii* has great potential for application as a prospective source of antioxidant and antimicrobial agent in pharmaceutical and food industries.

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Introduction

Gundelia tournefortii is a medicinal plant of Asteraceae family, native to Asian-temperate zones of Western Asia, namely Iran, Turkey, Cyprus, Egypt, Jordan, Turkmenistan and Azerbaijan. The *G. tournefortii* is used widely in traditional medicine for treatment liver disease, diabetes, gastric pain, chest pain; hear stroke, diarrhea, vitiligo, and bronchitis [1]. The different parts of *G. tournefortii* are used as food sources.

Antibacterial compounds such as antibiotics had been available for decades. Nowadays multi-drug resistant (MDR) human pathogens are considered among the most important health threatening problems worldwide [2]. Application of new natural antibacterial such as plant extracts has been recently gained increasing attention [3, 4].

Free radicals or reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl, hydroxyl radicals, and peroxynitrite can damage the body by cellular or oxidative stress. This leads to the development of diseases like cardiovascular, diabetes, cancer, and cirrhosis. Free radicals generated in the body can be removed by its own natural antioxidant defense systems that include catalase, glutathione peroxidase, superoxide dismutase, etc. Endogenous antioxidant defense are not completely efficient, therefore dietary and natural antioxidants are required to reduce the effect of oxidative stress due to excessive free radicals occurring in our system [5-7].

The chemical components of the extracts and essential oils allowed their use in traditional medicine and as food preservatives. Recently, there has been a growing interest in substances exhibiting antimicrobial and antioxidant properties that are provided to human as pharmaceutics and nutraceutics. It been well-known that plant essential oils and extracts have antioxidant and antimicrobial effects [8-10]. There are study on volatile oil of *G. tournefortii* from Turkey but there is not any study on the volatile oil of *G. tournefortii* from Iran.

The aim of the present study was to identification of volatile oil composition of *G. tournefortii* from Iran as well as investigate the antimicrobial and antioxidant capacities and total phenolic content of chloroform, ethyl acetate, methanol and aqueous extracts of *G. tournefortii*.

Materials and methods

Chemicals

2,2-diphenyl,1-picrylhydrazyl (DPPH), Butylated hydroxyl toluene (BHT), Folin–Ciocalteu reagent (FCR), and gallic acid were obtained from Sigma Chemical Co. All other chemicals were of analytical grade.

Plant material

G. tournefortii plants were collected from Mariwan, Kurdistan province, Iran, in April 2014. They were identified and authenticated and a voucher specimen (MPH- 1124) was deposited at the Herbarium of the Institute of Forests and Rangelands Researches, Sanadaj-Iran.

Preparation of the extracts

The dried and powdered aerial parts of the plant (250 g) were powdered and extracted successively with chloroform, ethyl acetate, methanol and aqueous (3 × 1 L, rt for 48 h). The extracts concentrated in vacuum at 45°C using a rotary evaporator and the residues obtained were stored in a freezer until further tests.

Extraction of the volatile oil

The essential oil of air dried plant (150 g) was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus as previously reported [9]. The obtained essential oil was dried over anhydrous sodium sulfate and stored at 4 °C until tested and analyzed.

Analysis of the essential oil

GC analysis was carried out on a Thermoquest-Finnigan Trace gas chromatograph with a flame ionization detector (FID). The analysis was performed on fused silica capillary DB-5 column (60 m × 0.25 mm; film thickness 0.25 µm). The oven temperature was programed from 40°C to

260°C at the rate of 4°C/min, and finally held isothermally for 10 min. Nitrogen was used as carrier gas at a flow rate of 1.1 mL/min. The injector and detector temperatures were kept at 250°C and 280°C, respectively. GC-MS analysis was carried out on a Thermoquest-Finnigan gas chromatograph equipped with above mentioned column, used under the same conditions coupled to a TRACE mass spectrometer. Helium was used as the carrier gas at a flow rate of 1.1 mL/min with a split ratio of 1:100. The quadrupole mass spectrometer was scanned over 45–465 amu with an ionizing voltage of 70 eV. Ion source and interface temperatures were kept 200°C and 250°C, respectively.

The constituents of the volatile oil were identified by calculation of their retention indices under temperature programmed conditions for homologous series of *n*-alkanes (C_6-C_{24}) and the essential oil on a DB-5 column under the same chromatographic conditions. In the identification process of individual compounds, their mass spectra was compared by internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [11, 12].

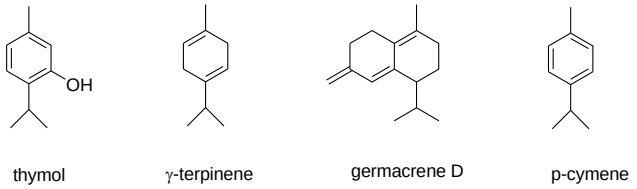

Fig. 1. The structure of major compounds from *G. tournefortii* volatile oil.

Measurement of free radical-scavenging activity (DPPH assay)

The capacity of *G. tournefortii* extracts to scavenge DPPH· were determined according to the technique reported by Mohammadi *et al.* [5]. BHT was used for comparison and the absorbance of the reaction mixture was measured at 517nm. Sample concentration providing 50% inhibition (IC_{50}) was obtained from plotting the inhibition percentage against sample (extract solution) concentrations.

Determination of total phenolics

The total phenolics content (TPC) of the plant extract was determined according to the Folin-Ciocalteu procedure [13]. Total phenols content was expressed as mg gallic acid equivalents per g plant extract (mg gallic acid/g Sample).

Antimicrobial activity

The extracts of *G. tournefortii* were tested individually against a range of 9 bacteria, including *Escherichia coli* ATCC 25922 (American Type Culture Collection number), *Klebsiella pneumoniae* ATCC 10031, *Bacillus cereus* PTCC 1015 (Persian Type Culture Collection number), *Bacillus pumilus* PTCC 1274, *Bacillus subtilis* ATCC 465, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29737 and *Pseudomonas aeruginosa* ATCC 85327. The antimicrobial activity of extracts was determined by the disk diffusion method using Mueller–Hinton Agar plates with determination of inhibition zones. Also the MIC values were determined by the broth microdilution assay [14].

Results and discussion

Hydrodistillation of dried aerial parts of *G. tournefortii* afforded a light yellow color volatile oil in 0.3% yield (v/w %) relative to dry weight of plant. Forty one compounds representing 96.2% of the volatile oil were identified. The compounds were identified by GC–MS and quantified by GC–FID. The compounds are listed according to their elution from the DB-5 column (Table 1). The major compounds were thymol (11.2%), γ-terpinene (9.8%), germacrene D (6.6%), *p*-cymene (6.3%), α -terpineol (6.2%), carvacrol (6.1%) and *β* -caryophyllene (5.8%).

Table 1. Percentage composition of the volatile oils of *Gundelia tournefortii.*

Compounds	[a]RI	Percentage
α-Thujene	924	0.6
α-Pinene[c]	932	0.6
Camphene[c]	946	2.2
Sabinene	969	0.7
3-Octanon	979	0.8
2-Pentyl furan	984	0.5
Myrcene	988	1.1
α-Phellandrene	1002	[b]t
α-Terpinene	1014	1.6
***p*-Cymene**	1020	6.3
Limonene[c]	1024	t
1,8-Cineole	1026	2.8
3-Octen-2-one	1030	0.2
***cis*-Ocimene**	1032	4.6
Benzene acetaldehyde	1036	2.3
γ-Terpinene	1054	9.8
2-Methyl benzaldehyde	1066	1.2
Terpinolene	1086	t
Linalool	1095	0.4
3-Cyclohexan-1-ol	-	1.7
α-Terpineol	1186	6.2
Thymol methyl ether	1232	2.4
Carvacrol methyl ether	1241	5.2
Thymol	1289	11.2
Carvacrol	1298	6.1
2,4-Decadienal	1315	0.7
β-Bourbonene	1387	1.1
β-Caryophyllene	1428	5.8
α-Humulene	1452	0.9
Germacrene D	1484	6.6
Bicyclogermacrene	1500	4.1
***cis*-α-Bisabolene**	1506	0.3
***cis*-Nerolidol**	1531	0.4
Spathulenol	1577	2.9
Caryophyllene oxide	1582	1.4
***α*-Cadinol**	1652	0.4
2-Pentadecanone	1697	0.2
Benzyl benzoate	1759	1.8
Nonadecan	1900	0.4

Continue of Table 1.

Compounds	[a]RI	Percentage
Nonacosane	2900	0.3
Monoterpene hydrocarbons	-	27.5
Oxygenated monoterpenes	-	35.0
Sesquiterpene hydrocarbons	-	18.8
Oxygenated sesquiterpene	-	5.3
***n*-Alkan**	-	8.5
Total	-	96.2

[a]RI: retention indices relative to C_6-C_{24} *n*-alkanes on the DB-5 column, [b]t: trace, [c]the identification was also confirmed by co-injection with an authentic samples.

Inspection of the literature revealed that the volatile oil composition of the *G. tournefortii* from Iran is qualitatively similar to *G. tournefortii* from Turkey with some variation. The amount of major compounds, thymol and germacrene D in *G. tournefortii* from Turkey and Iran are (24.5%, 11.2%, 0.5% and 6.6%) respectively. The different in environmental conditions leads to the variation in amount of volatile compounds [15]. These results can be used for further study on the taxonomy of *Gundelia* genus.

The antioxidant and antimicrobial activities of different *Gundelia* species, including *O. narbonense L., O. Brachystachys* and *O. sintenisii L*. have been studied by different researchers [16-20]. Similar to the other species, the extracts of *G. tournefortii* showed significant antioxidant and antimicrobial effect.

Free radical scavenging capacities of different extracts of *G. tournefortii* measured by DPPH assay are shown in Table 2. According to the results the highest scavenging activity was found for methanol extract ($IC_{50=}$ 42.3 µg/ml), followed by aqueous ($IC_{50=}$ 72.3 µg/ml), ethyl acetate (IC_{50}= 76.1 µg/ml), and chloroform (IC_{50}= 101.4 µg/ml) extracts. The total phenolics of various extracts of the plant were measured using Folin–Ciocalteu's assay. The highest phenolic content was found for the methanol extract (103.4 mg GAE/g sample) and the lowest was found for the chloroform extract (46.3 mg GAE/g sample). The results showed that the extent of antioxidant activities of extracts is in accordance with the amounts of phenolics existing. It is widely accepted that the antioxidant activity of a plant extract is correlated to its phenolic content [21]. Coruh et al. investigated the antioxidant activities of *G. tournefortii* from turkey and found IC_{50} values of 73 µg/mL for DPPH scavenging of the methanolic extract while in *G. tournefortii* from Iran it is 42.3 µg/mL.

The different extracts of *G. tournefortii* were tested against four Gram-negative and five Gram-positive bacteria. The results indicated that the extracts had moderate to high inhibitory activity against the *Bacillus cereus*, *Bacillus pumilus,* and *Bacillus subtilis* (Table 3). The most sensitive microorganism was *Bacillus pumilus* with inhibition zones of 20 mm and MIC values of 7.5 mg ml^{-1} for ethyl acetate extract. According to study by Aburjai the methanol extract of *G. tournefortii* from Jordan acted as antibacterial against multi drug resistant *Pseudomonas aeruginas* and *Escherichia coli* [18]. Methanolic extract of *G. tournefortii* in combination with penicillin G and erythromycin inhibited the growth of *Pseudomonas aeruginosa* including a resistant strain [1].

Table 2. Antioxidant activities and total phenolics content of the different extracts from *Gundelia tournefortii*.

Extracts	DPPH assay IC_{50}(µg/ml)	TPC mg gallic acid/g Sample
Chloroform extract	101.4±0.2	46.3±0.4
Ethyl acetate extract	76.1±0.2	68.1±1.1
Methanol extract	42.3±0.1	103.4±1.3
Aqueous extracts	72.3±0.2	85.3±0.8
BHT	24±0.2	

Values were the means of three replicates **±** standard deviation.

Table 3. *In vitro* antibacterial activities of the different extracts from *Gundelia tournefortii*.

Microorganism	Sample						
	Chloroform extract	**Ethyl acetate extract**	**Methnol extract**	**Aqueous extract**	**Tetracycline[b]**	**Gentamicin[c]**	**Ampicillin[d]**
Bacillus pumilus	12[a] (15)[b]	20(7.5)	14(15)	14(15)	nt	nt	15(15)
Bacillus subtilis	13(15)	18(7.5)	14(15)	12(>15)	21(3.2)	-(nt)	14(15)
Staphylococcus aureus	12(15)	10(>15)	13(15)	13(15)	20(3.2)	-(nt)	13(15)
Bacillus cereus	14(15)	18(7.5)	18(7.5)	14(15)	nt	nt	nt
Klebsiella pneumoniae	11(15)	13(15)	10(15)	17(15)	nt	nt	nt
Enterococcus faecalis	9(-)	10(>15)	11(15)	10(>15)	nt	nt	nt
Escherichia coli	10(15)	14(15)	11(15)	11(15)	- (nt)	23(3.2)	12(15)
Staphylococcus epidermidis	14(15)	11(>15)	11(>15)	12(15)	34(1.6)	- (nt)	19(15)
Pseudomonas aeruginosa	-	-	-	-	nt	nt	nt

a: Zone of inhibition (in mm) includes diameter of the disc (6 mm), b: Minimum inhibitory concentration values as mg ml^{-1}, (–): Inactive, (7 – 13): moderately active, (> 14): highly active, nt: not tested

Conclusion

The main constituents of *G. tournefortii* volatile oils were thymol (11.2%), γ-terpinene (9.8%), germacrene D (6.6%) and *p*-cymene (6.3%). The high antioxidant activity of methanol extract of *G. tournefortii* is correlated to its phenolic content. Also the high antioxidant activity and good antimicrobial inhibitory effect of the extracts of *G. tournefortii* supports its potential as a prospective source of antimicrobial and antioxidant agent in pharmaceutical and food industries.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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