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Composition of the essential oils, antioxidant and antibacterial activities of the methanolic extract of *Prangos uloptera*

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Abstract

Introduction: *Prangos uloptera* is a medicinal plant from the *Apiaceae* family which is native to Iran.
Objectives: In the current study, phytochemical constituents of the essential oils, antibacterial and antioxidant activities of methanolic extract of the aerial parts of *P. uloptera* were investigated.
Materials and Methods: To determine the main ingredients of the essential oils and to investigate the antioxidant and antibacterial activities of this plant, the gas chromatography-mass spectrometry (GC-MS) analysis, DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and microdilution method were conducted.
Results: The GC–MS analysis showed that the leading constituents of the essential oils were butyl octanoate (24.88%), (Z)-9-Octadecenoic acid (14.19%) and α-pinene (19.28%) in the flowers, fruit and leaves, respectively. The greatest amount of phenolic compound was observed in methanolic extract of the leaves (0.5 ± 0.11 mg GAEs/g). Likewise, the greatest level of flavonoids (0.074 ± 12.4 mg QEs/g) was identified in the leaves. Furthermore, DPPH assay showed the most radical scavenging activity (IC50 201.7 ± 20.39 µg/mL) in the methanolic extract of the leaves. The highest total antioxidant capacity (TAC) was observed in the fruit (0.004 ± 0.0005 g AAE/mL). The antibacterial activity of the plant extracts against the pathogenic fungal and bacterial species was investigated by broth microdilution method. Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) amounts were in the ranges of 312 to 10000 and 1000 to 10000 mg/ml, respectively.
Conclusion: The results of this research demonstrated that *P. uloptera* was a potent source of useful bioactive compounds, making it a promising candidate for further studies.

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Introduction

Pharmaceutical plants are regularly used in conventional medicine due to presence of essential oils and volatile constituents. Recently, the medicinal plants and their derivatives have attracted a lot of attention in scientific research in order to investigate their nutritional, pharmaceutical, and cosmetic applications (1-3). Moreover, various biological components such as polyphenols, alkaloids, flavonoids and terpenoids have been found to have promising antimicrobial, antioxidants and anticancer functions which are important in the prevention and treatment of diseases with microbial and oxidative stress origins (4, 5). With nearly 8000 species, Iran has rich flora. Most of species are native to the country and there is a well-established traditional use of medicinal plants (6).

Key point

The methanolic extract of *Prangos uloptera* had antioxidant and inhibition activities against the tested bacterial and fungal pathogens, therefore, it can be used as a potential antimicrobial candidate.

The genus *Prangos* from the *Umbelliferae* family comprises almost thirty species, fifteen of which are found in Iran (7). Some *Prangos* species are regarded as carminative, emollient, antifungal, antibacterial, antioxidant, cytokine release inhibitor, anti-flatulent, anti-HIV (human immunodeficiency virus) and anti-helminthic in traditional medicine (8). Previous investigations on phytochemical properties of different species of *Prangos* revealed the presence of coumarins and

volatile oils in different parts of this plant (6).

Prangos uloptera is a native plant which is widespread in mountainous parts of Iran. In traditional medicine, it is administered as a remedy for leukoplakia, and digestive disorders and also healing scars (9,10). Furthermore, it has aphrodisiac and coagulant properties (6). The phytochemical composition of its oils has been investigated for isolation and characterization of volatile constituents (6,11,12).

Objectives

In the present study, phytochemicals, antimicrobial and antioxidant potential of both essential oils and extracts of aerial parts of *P. uloptera* were investigated.

Materials and Methods

Study design

New plant materials (stems, leaves and fruit) were collected from the high altitudes of Aligudarz in Lorestan province, Iran, from April to May 2012. The plant was identified by the senior taxonomist Dr. Valiollah Mozaffarian at the Research Institute of Forests and Rangelands. The materials were dried in the shade.

Light Microscopy

Anatomical studies were conducted on the cross-sections of the stem. The stem was fixed using formaldehyde alcohol acetic acid solution. After fixation, the stem was washed with distilled water and hand-sectioned by a sharp blade. The segments were stained with methylene blue to be able to be photographed under Olympus microscope.

Isolation of the essential oils

The ground plant materials (400 g) were exposed to hydrodistillation by British-type Clevenger apparatus for 3-5 hours. The concentrated essential oil was weighed for yield determination. The obtained essential oil was covered with aluminum foil and stored at 4°C until subsequent analysis.

GC-MS analysis of the essential oil

The analysis of the essential oil by gas chromatography-mass spectrometry (GC-MS) was conducted by a 6890 Agilent gas chromatograph equipped with an Agilent 5973N mass spectrometer with a HP-5MS Agilent column (30 m × 0.25 mm I.D., 0.25μ film thickness). For GC-MS identification, an electron ionization system with ionization energy of 70 eV was utilized. First, the temperature of column was preserved at 60°C for 3 minutes and programmed to reach 246°C at a rate of 3°C/min. Helium was used as carrier gas at a flow rate of 1 mL/min. The temperature of injector was set at 250°C. Diluted samples (0.1 μL) were injected in the split (1:20) mode manually.

Preparation of the extracts

The plant materials were extracted using methanol solvent. Twenty grams were extracted with 200 mL methanol solvent by Soxhlet extractor for 72 hours. After completing the extraction, rotary evaporator was utilized to evaporate the methanol solvent. Then the extracts were kept in a dark place at 4°C until further examination.

Antioxidant activity

Total flavonoids

Flavonoid contents of the extracts of the aerial parts were measured using colorimetric method. Briefly, 2 g of aluminum chloride was dissolved using 200 mL of methanol. Then, 0.5 mL of 1% aluminum chloride solution was mixed with the identical volume of the extracts with different concentrations. The samples were vortexed and kept at room temperature for 1 hour. Absorbance values were determined at 420 nm and standard curves of flavonoids were plotted.

Total phenolic

Phenolic contents of the extracts were characterized by previously described method (13). Briefly, 90 mL distilled water was mixed with 10 mL of the Folin-Ciocalteu reagent. The 750 μL of the mixture was mixed with 100 μL of the extracts. The solution was vortexed and kept for 5 minutes at room temperature. Then 750 μL of Na_2CO_3 solution (6% w/v) was added and the combination was allowed to stand for 40 minutes. Absorbance values were determined at 725 nm and standard curves of phenolic content were plotted.

Total antioxidant activity

The total antioxidant capacity (TAC) of extracts was determined by previously described method (14) by ammonium phosphomolybdate reagent. 1 μL of reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate) was mixed with an aliquot of 0.1 μL of extract. The samples were vortexed and kept for 90 minutes in the boiling water. Absorbance values were determined at 695 nm and standard curves of the total antioxidant activity were plotted.

DPPH radical scavenging assay

The capability of the plant extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was investigated as described previously (15). Briefly, 1000 μL of DPPH (0.2 mM) in methanol was mixed with 200 μL of various concentrations of the extracts and 800 μL of Tris buffer (pH 7.4). In blank samples, the extract was replaced by methanol. The mixture was covered and shaken vigorously for 40 minutes. Absorbance values were determined at 517 nm. The inhibition percentage of DPPH free radicals [I (%)] was computed as follows:

$$I(\%) = 100 \times (A_{blank} - A_{sample}) / A_{blank}$$

A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the extract. In order to compare the extracts,

the half-inhibitory concentration (IC_{50}) that was described as the concentration of the sample needed to inhibit 50% of radicals, was used.

MIC and MBC methods

Antibacterial activities of the methanolic extracts of the stems and leaves against several gram-positive and gram-negative human bacterial and fungal pathogens (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 11775, *Bacillus cereus* PTCC 1154, *Candida albicans* PTCC 5188, *Candida glabrata* PTCC 5297 and *Kluyveromyces marxianus* PTCC 5027) were investigated as described previously by reference guideline of the clinical and laboratory standards institute (16,17). Minimum inhibitory concentration (MIC) of the methanolic extracts of leaves and stems were determined by broth microdilution method in 96-well microtiter plates. About 100 µL of sterile Mueller Hinton Broth was added to every well. Ten dilutions of the extract were prepared by two-fold serial dilutions starting from 1024 mg/mL. The inoculums of human pathogens and fungi were prepared (0.5 McFarland) and poured to each well. The plates were incubated at 37ºC for 18-24 hours based on each organism. Then 15 µL of 2,3,5-triphenyltetrazolium chloride (TTC) was added to every well and incubated at 37°C for 120 minutes. The growth of bacteria changed the color of TTC into pink, hence the wells that had no changes in color after the addition of TTC were MIC values. The minimum bactericidal concentration (MBC) is described as the lowest concentration at which no bacterial growth is observed. For the determination of MBC, a portion from each well that showed an antibacterial activity was aseptically subcultured on Mueller-Hinton agar and incubated for 24 hours at 37°C. The lowest concentration that demonstrated no bacterial growth in the subcultures was used as the MBC, and gentamicin (30 mg) was utilized as the positive control.

Results

Anatomical and morphological investigation

The sections were analyzed using a light microscope. The external layer was epidermis which was formed from a layer of rectangular shape cells. The outer surface of these cells was covered with a layer of cuticle. Collenchyma was observed at the angles of the stems, which strengthened the stem tissue. The next layer was the parenchymal layer, which consisted of several rows of almost compact cells. Secretory cavities were observed between the parenchymal cells. These cavities are the main characteristics of *Apiaceae* family. In the inner part, there were several rows of phloem. In the center, the brain tissue was clearly evident.

Yields of essential oils

The yields of the essential oils from different aerial parts were 0.065, 0.1 and 0.13% (w/w) for the leaves, fruit and flowers respectively.

Chemical compositions in essential oils

The chemical compositions of *P. uloptera* essential oils for the leaves (Table 1), fruit (Table 2) and flowers (Table 3) are shown. An entire of 134 compounds were recognized in these three parts. The mean concentrations of the leading components were (Z)-9-octadecenoic acid (14.19%), α-pinene (19.28%) and butyl octanoate (24.88%) for the leaves, fruit and flowers, respectively. The GC-MS chromatograms are shown in (Figure 1A-C).

Total phenols and flavonoids

Phenolic compounds are considered to possess a wide range of biological activities like antioxidant and antimicrobial activities. Total phenolic compounds in methanolic

Table 1. Chemical composition of essential oils from the leaves of *P. uloptera*

No.	Compounds	Retention time (min)	Content (%)
1	Acetone	2.23	0.39
2	2-Ethylhexyl acetate	2.65	2.05
3	α-Pinene	7.45	19.28
4	Camphene	7.87	7.06
5	Sabinene	8.53	0.31
6	β-Pinene	8.68	0.98
7	β-Myrcene	9.07	1.41
8	δ-3-Carene	9.78	0.86
9	Benzene, 1-methyl-2-[(1-methylethyl)thio]	10.33	0.54
10	Limonene	10.54	4.07
11	Verbenol	15.21	0.63
12	Acetic acid	17.95	0.49
13	Fenchyl acetate	18.41	4.72
14	Norborneol	21.33	9.38
15	Cis-2,6-dimethyl-2,6-octadiene	23.99	0.7
16	Copaene	25	0.85
17	Valencene	25.16	1.18
18	Geranyl acetate	25.28	0.69
19	β-Cubebene	25.58	0.65
20	Trans-caryophyllene	26.95	4.81
21	α-Pinene dimer	27.42	0.69
22	4,7,10-Cycloundecatriene, 1,1,4	28.25	0.96
23	5-Ethoxy-2-methyl-pyridine	28.88	1.52
24	α-Amorphene	29.21	1.57
25	β-Cubebene	29.41	2.13
26	Lyratyl propanoate	29.67	1.98
27	α-Selinene	30.11	1.39
28	β-Bisabolene	30.4	0.45
29	δ-Cadinene	31.02	0.46
30	Cyclohexanol, 3-ethenyl-3-methyl	31.23	0.52
31	1,5,8-p-Menthatriene	32.29	2.07
32	Spathulenol	33.47	7.3
33	α-Amorphene	34.06	4.27
34	Geranyl isovalerate	34.25	0.34
35	Unknown	34.48	0.77
36	Isospathulenol	35.63	0.94
37	Terephthalaldehyde	36.03	1.53
38	α-Cubebene	36.24	1.52

Table 2. Chemical composition of essential oils from the fruit of *P. uloptera*

No.	Compounds	Retention time (min)	Content (%)
1	2-Propanone	2.23	0.27
2	Acetic acid	2.61	0.83
3	α-Pinene	7.42	9.39
4	Camphene	7.86	3.27
5	β-Pinene	8.69	0.65
6	β-Myrcene	9.09	0.85
7	3-Carene	9.8	0.28
8	Benzene, 1-methyl-2-[(1-methylethyl)thio]	10.34	0.26
9	Limonene	10.53	1.24
10	1-Octanol	12.26	1.4
11	Benzene, 1-methoxy-4-(2-propeny	17.63	1.45
12	2-Ethylhexyl acetate	18.33	7.53
13	Fenchyl acetate	18.65	2.77
14	Hexyl isovalerate	19.46	1.27
15	Z-Citral	19.58	0.66
16	Anisaldehyde	20.17	0.32
17	Z-Citral	20.95	1.41
18	Norborneol	21.57	6.53
19	Cis-2,6-Dimethyl-2,6-octadiene	24.17	0.28
20	α-Copaene	25.18	0.39
21	Unknown	25.35	0.83
22	Geraniol	25.47	0.6
23	Valeric acid	26	0.32
24	Trans-caryophyllene	27.10	1.6
25	n-Octyl 2-methyl butyrate	27.59	2.32
26	α-Humulene	28.47	0.65
27	α-Santalol	29.03	0.29
28	Unknown	29.35	0.37
29	D-Germacrene	29.56	0.63
30	Unknown	29.79	0.62
31	β-Patchoulene	30.27	0.55
32	β-Bisabolene	30.57	0.17
33	Trans-α-bisabolene	31.9	0.34
34	1,5,5-Trimethyl-6-(3-methyl-buta-1,3-dienyl)-cyclohexene	32.57	2.28
35	Citronellyl valerate	32.96	0.49
36	(+) Spathulenol	33.68	4.6
37	(E)-3,7-dimethylocta-2,6-dien-1-yl butyrate	34.32	5.29
38	Geranyl isovalerate	34.44	0.18
39	2-Methylbicyclo[3.3.1]nonane	34.69	0.56
40	Cis-Carane	34.98	0.3
41	Cis-α-Copaene-8-ol	35.73	0.37
42	Trans-caryophyllene	35.97	0.36
43	β-Costol	36.35	0.91
44	7-Vinylbicyclo[4.2.0]oct-1-ene	36.63	0.28
45	Isoledane	36.78	0.61
46	α-Bisabolol	37.54	2.58
47	5,5-Dimethylcyclopentadiene	38.05	0.45
48	Vulgarol B	38.61	0.8
49	Heptyl octanoate	40.50	2.71
50	Hexadecanoic acid	47.54	5.61
51	Cis-α–Copaene-8-ol	48.18	0.35
52	6-amino-1H-indazole-5-carboxylic acid	49.38	0.34
53	(Z)-9-Octadecenoic acid	53.23	14.19
54	p-Tolualdehyde	53.58	2.35
55	(Z,Z)-9,12-Octadecadienoic acid	54.93	0.37
56	Thieno[2,3-b]pyridine 7-oxide	57.38	1.83
57	Pyrazole-4-carboxaldehyde	57.75	1.03
58	1-(1,5-dimethylhexyl)-4-(4-methylpentyl) cyclohexane	58.17	0.56
59	3-Amino-5-methylthio-1H-1,2,4-triazole	61.10	0.37

Table 3. Chemical composition of essential oils from the flowers of *P. uloptera*

No.	Compounds	Retention time (min)	Content (%)
1	Ethyl ether	2.25	1.10
2	Acetic acid	2.59	1.65
3	α-Pinene	7.39	7.74
4	Camphene	7.8	1.36
5	β-Pinene	8.67	0.56
6	P-Cymenene	10.33	0.53
7	Limonene	10.51	2.59
8	1-Octanol	12.31	5.35
9	p-Cresol	12.84	1.18
10	N-octyl formate	14.39	0.39
11	2H-pyranmethanol, tetrahydro-2	15.58	15.58
12	2-Ethylhexyl acetate	18.18	9.53
13	Fenchyl acetate	18.49	2.33
14	Hexyl isovalerate	19.27	0.89
15	2,4,4-trimethyl-4-vinyl-3-cyclo	21.13	1.75
16	Norborneol	21.35	6.13
17	Cis-2,6-dimethyl-2,6-octadiene	24.02	0.51
18	Copaene	25.02	0.43
19	Unknown	25.17	0.66
20	Geranyl acetate	25.3	0.26
21	Trans-caryophyllene	26.9	1.76
22	Butanoic acid	27.43	2.96
23	1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene	28.29	0.42
24	4,5,6,7-Tetrahydro-1H-indol-3(2H)-one	28.88	0.53
25	Unknown	29.20	0.72
26	1-Isopropyl-1-cyclohexen	29.66	1.62
27	α-Selinene	30.11	0.64
28	Unknown	32.38	1.22
29	1H-cycloprop[e]azulen-7-ol	33.47	7.04
30	(E)-3,7-dimethylocta-2,6-dien-1-yl butyrate	34.13	5.6
31	9-Methylbicyclo[3.3.1]nonane	34.86	0.47
32	α-Bisabolol	37.40	2.83
33	1-Methyl-5,6-divinyl-1-cyclohexene	37.92	0.73
34	4-Methyldodecanoic acid	39.51	0.9
35	Butyl octanoate	40.75	24.88
36	n-Hexadecanoic acid	46.82	1.19
37	Thieno[2,3-b]pyridine 7-oxide	57.16	0.74

extracts were identified by Folin-Ciocalteu method and represented as gallic acid equivalents (GAMEs). The outputs were presented as mg Quercetin equivalent per gram of dry weight of extract using the following equation:

$$R^2 = 0.9751,\ y = 0.0008x + 0.1649$$

The greatest amount of phenolic compounds was observed in the methanolic extract of the fruit (0.72 ± 0.01 mg GAEs/g) followed by that of the leaves (0.5 ± 0.11 mg GAEs/g) and the stem (0.06 ± 0.05 mg GAEs/g; Figure 2).

The total amount of flavonoids was presented as mg Quercetin equivalent per gram of dry weight of extract using the following equation:

$$R^2 = 0.978,\ y = 0.0454x + 0.0309$$

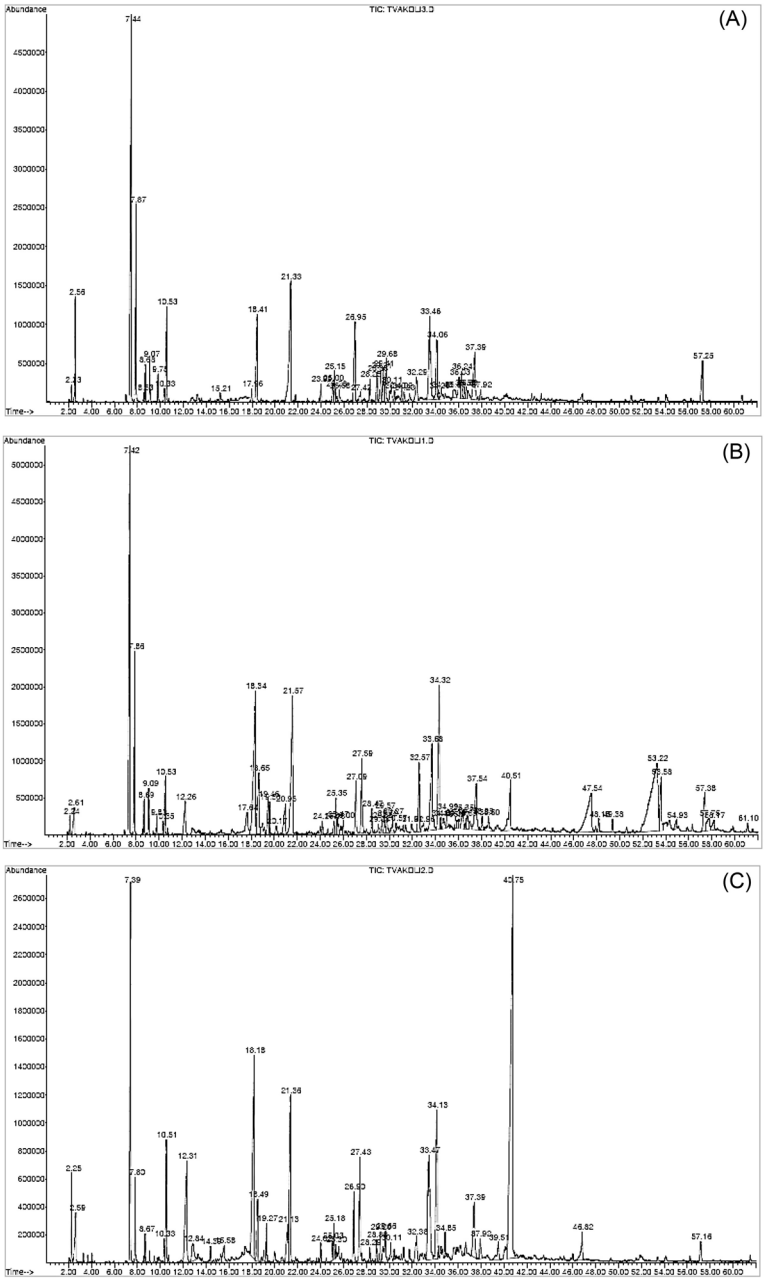

Figure 1. Chromatogram of essential oils from *P. uloptera* (A) leaves, (B) fruit and (C) flowers.

The methanolic extract of the leaves had the highest level of flavonoids (0.074 ± 0.023 mg QEs/g) followed by that of the fruit (0.062 ± 0.021 mg QEs/g). The least quantity was observed in the stem extract (0.012 ± 0.003 mg QEs/g; Figure 3).

Total antioxidant capacity

The results of the TAC were presented as mg ascorbic acid equivalent per gram of dry weight of extract using the following equation:

$$R^2 = 0.9988,\ y = 0.7633x + 0.0149$$

In the methanolic extract of aerial parts of *P. uloptera*, TAC was detected to be the highest in *the fruit* (0.004 ± 0.0005 TE/g). The results for the stem and leaves were (0.002 ± 0.0004 TE/g) and (0.002 ± 0.0001 TE/g), respectively (Figure 4).

DPPH

The stem showed the strongest DPPH radical-scavenging ability (IC_{50}: 201.7 ± 20.39 µg/mL), while the fruit showed the weakest DPPH radical-scavenging ability (IC_{50}: 63.8 ± 9.9 µg/mL). The result for the leaves was 101.77 ± 4 µg/mL (Figure 5).

Antibacterial activity

Table 4 shows MIC and MBC values of the methanolic

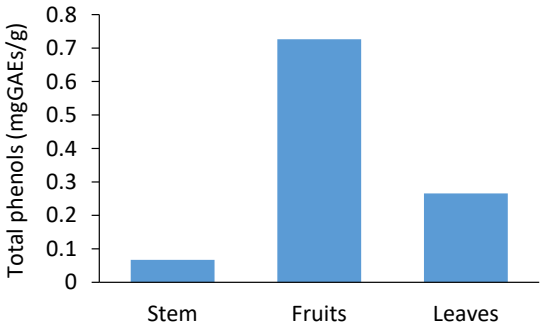

Figure 2. Total phenols of aerial parts of *P. uloptera* that was expressed as gallic acid equivalents (mg GAEs/g).

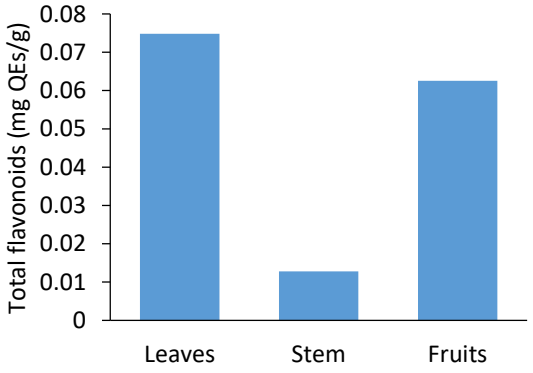

Figure 3. Total flavonoids of aerial parts of *P. uloptera* that was expressed as quercetin equivalent (mg QEs/g)

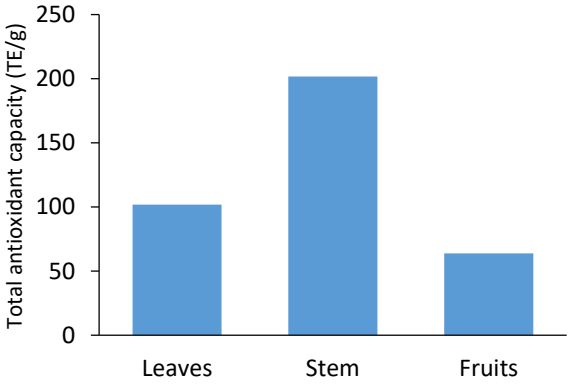

Figure 4. Total antioxidant capacity (TE/g) of aerial parts of *P. uloptera* methanolic extract

extract against whole tested microorganisms. The MIC values acquired in this research ranged from 312 to >10 000 mg/mL. The extract of the stem demonstrated the lowest MIC (1000 mg/mL) against *E. coli and Staphylococcus aureus*. The results indicated the greatest sensitivity of *these strains* (*S. aureus* and *E. coli)* amongst the investigated microorganisms. Moreover, *S. aureus* and *C. glabrata* were highly resistant against the extract of the

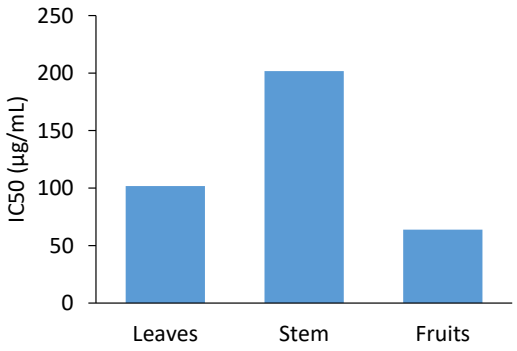

Figure 5. The IC_{50} values of DPPH scavenging effect of *P. uloptera* extracts (µg/mL).

leaves. Furthermore, *K. marxianus* was the most sensitive strain with MIC of 312 mg/mL. The lowest MBC value of 1000 mg/mL was observed from the extract of the stem against *E. coli* that indicated the greatest sensitivity of *this strain* amongst the investigated strains. Similarly, *E. coli* was detected to be the most resistant against the extract of the leaves with MBC of 1250 mg/mL.

Data analysis

The detection of individual compounds of the essential oils was based on comparison of their comparative retention times and mass spectra of peaks with those obtained from published data and library of GC-MS instrument (NIST and Wiley mass spectral Libraries).

Discussion

The efficiency of the essential oils obtained in this study was different between the aerial parts. In general, essential oil outputs were less than 1%. In this study, the flowers produced greater yields than the leaves and fruit. Various plant organs produce different quantities of essential oils, and this usually reflects the action of the oils in that plant organ (18). The chemical composition of the essential oils of *P. uloptera was* identified using GC–MS. Thirty-seven components were determined in the flowers. The major constituents of flowers were butyl octanoate (24.88%), 2-ethylhexyl acetate (9.53%), α-pinene (7.74%), 1H-cycloprop[e]azulen-7-ol (7.04% and 1-octanol (5.35%). Furthermore, 59 components were identified in the fruit. The major constituents of the fruit were (Z)-9-octadecenoic acid (14.19%), (9.39%) α-pinene, 2-ethylhexyl acetate (7.53%), hexadecanoic acid (5.61%) and (E)-3,7-Dimethylocta-2,6-dien-1-yl butyrate (5.29%). The components of flowers and fruit showed some similarities and differences. For example, α-pinene was a common component between the two parts. Forty-three components were identified in the leaves. The major constituents of leaves were α-pinene (19.28%), bicyclo[2.2.1]heptan-2-ol (9.38%), spathulenol (7.30%), camphene (7.06%) and trans-caryophyllene (4.81 %). In the current study, spathulenol was a major component (7.3%) in the leaves. Major differences between the fruit and

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (mg/mL) of the methanolic extract of *P. uloptera*

	Extract concentration (mg/mL)					
	Stem extract		Leaf extract		Positive control	
	MIC	MBC	MIC	MBC	MIC	MBC
P. aeruginosa	2500	>10000	2500	>10000	0.5	1
E. coli	1000	1000	1250	1250	0.5	1
S. aureus	1000	>10000	10000	>10000	8	128
B. cereus	5000	5000	2500	2500	128	128
C. albicans	2500	2500	2500	2500	64	128
C. glabrata	5000	>10000	>10000	>10000	128	128
K. marxianus	1250	>10000	312	>10000	1	8

flower components showed that most of the components underwent substantial quantitative and qualitative changes during fruiting. This was attributable to metabolic shifts during phenological stages. Abyshev and Denisenko reported considerable differences among the oils of aerial parts of *P. uloptera* at three phenological periods (11), which was in agreement with the discoveries of the current study. Rechinger et al identified major components in the essential oils of the aerial parts of *P. uloptera* (19). The differences in chemical ingredients of essential oils can be due to the variances in genetic and environmental factors, geographical situations, chemical polymorphic structure, plant maturation stage, nutritional condition of the plant, post collection processing, and extraction techniques (20). Comparing the main components of *P. uloptera* essential oils showed that β-caryophyllene and α-pinene were common compounds between the present study and the previous ones. We further investigated the antioxidant capacity of methanolic extract of aerial parts. The results of our study demonstrated that methanolic extract of *P. uloptera* exhibited great antioxidant activities. Among the aerial parts, the stem showed the highest antioxidant activity. Antimicrobial activities of the extracts of the leaves and the stem were investigated against common human pathogens. It is believed that plant extracts are further effective on gram-positive bacteria than gram-negative bacteria. The cell walls of gram-negative bacteria are less permeable to antimicrobial metabolites (21). However, we observed that both *E. coli* and *Bacillus cereus* were sensitive to the extract of the stem. In addition, the results of the MBC showed that the *E. coli* was the most sensitive strain. Generally, the extracts exhibited a good antibacterial activity against the investigated strains. Previous studies on *P. uloptera* showed that the dichloromethane root extracts exerted potent antibacterial activity against *S. aureus* and *Bacillus subtilis* with MIC values of 1 and 0.5 mg/mL, respectively. The methanol extracts of roots demonstrated modest antibacterial activities, with MIC ranging from 0.1 to 1 mg/mL. The antimicrobial activities are probably attributable to the existence of bioactive components like alkaloids, flavonoids, terpenoids, polyphenols, and quinine found in these methanolic extracts (22,23). In this regard, previous studies showed that α-pinene had significant antibacterial activities (24,25). It has been reported that antimicrobial activities of the herbal extracts correlate with their flavonoid contents (26). The highest flavonoid content was identified in the leaves, in which potent antibacterial activities against *K. marxianus* and *E. coli* were observed. Flavonoids exert antibacterial activities through creating complexes with diverse proteins inside the extracellular proteins or bacterial cell walls (26,27). Among the bacterial and fungal strains investigated in the current research, *E. coli* is known as the most prevalent etiological agent of diarrhea in developing countries (27). In addition, other strains like *B. cereus* and *S. aureus* are involved in food poisoning (28).

Conclusion

The essential oils of aerial parts were rich in bioactive components such as phenolic, flavonoid, anthocyanin, and tannin. The methanolic extract of *P. uloptera* showed potent anti-oxidant activities. It also showed remarkable inhibition activities against the tested bacterial and fungal pathogens. This effect was similar in gram-positive and gram-negative strains. It could be concluded that bioactive compounds were responsible for the biological activities of extracts. The results provided evidence for the traditional use of the medicinal plants against microbial diseases. Although these plants can act as a potential antimicrobial candidate, further studies are required to fully discover their beneficial roles.

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Authors' contribution

AV and NT were the principal investigators of the study. HA and HAV were included in preparing the concept and design. LH performed microbial tests. RH and RR analyzed the data and interpreted the results. RH and NTD revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

All authors declare that they have no conflict of interest.

Ethical issues

The Ethics Committee of Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Iran approved this study (Ref #150/1200/2180). Moreover, ethical issues (including plagiarism, data fabrication and double publication) have been completely observed by the authors.

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