

Chemical composition and antibacterial activity of essential oil from leaves, stems and flowers of *Prangos ferulacea* (L.) Lindl. grown in Iran

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Essential oils from the leaves, stems and flowers of *Prangos ferulacea* (family *Umbelliferae*) growing in Esfahan, Iran, were obtained by hydrodistillation using a Clevenger-type apparatus and their chemical composition and antibacterial activity analysed by GC-MS. All the oils consisted mainly of oxygenated monoterpenes and a small percentage of sesquiterpene compounds. In the oil from the leaf, 10 components were identified, dominated by oxygenated monoterpenes. The three major constituents identified (representing 65.1% of the oil) were linalool (36.7%), caryophyllene oxide (16.3%) and α -pinene (12.1%). In the stem oil, 11 compounds were identified, with oxygenated monoterpenes again predominating. The two major constituents identified (representing 29.3% of the oil) were 1,8-cineole (19.0%) and α -pinene (10.3%). Of the 17 compounds found in the flower oil, the five main components identified (representing 74.1% of the oil) were oxygenated monoterpenes: linalool (19.0%), lavandulyl acetate (16.0%), 1,8-cineole (14.5%), α -pinene (12.4%) and geranyl isobutyrate (12.2%). The oils were tested against four Gram-positive or Gram-negative bacteria. Antibacterial activity was measured using a dilution method. It was found that oil from leaves, stems and flowers of *P. ferulacea*, and especially that of leaves, exhibited interesting antibacterial activity.

Key words: *Prangos ferulacea*, umbelliferae, essential oil, linalool, antibacterial activity.

INTRODUCTION

Of the fifteen species of the genus *Prangos* (family *Umbelliferae*) found in Iran, five are endemic: *P. gaubae*, *P. crossoptera*, *P. tuberculata*, *P. cheilanthifolia* and *P. cattigonoides* [1, 2]. A survey of the literature revealed that the oil composition of *P. latiloba* [3], *P. pabularia* [4], *P. hissarica*, *P. seraiuschanica*, *P. fedtschenkoi* [5], *P. ferulacea* [6, 7], *P. uechtritzi* [8, 9], *P. bornmuelleri* [10], *P. heyniae* [11], *P. uloptera* [12], *P. asperula* [13] and *P. platychlaena* [14] have been reported. The main constituents of the aerial parts of *P. uloptera* were found to be β -caryophyllene (18.2%), germacrene D (17.2%) and limonene (8.7%), whereas the seed oil comprised mainly α -pinene (41.5%) and β -cedrene (4.0%) [12]. Analysis of the aerial parts of *P. asperula* showed δ -3-carene (16.1%), β -phellandrene (14.7%), α -pinene (10.5%), α -humulene (7.8%), germacrene-D (5.4%), δ -cadinene (4.2%) and terpinolene (4.0%) to be the major components of the oil [13]. Aerial parts of *P. uechtritzi* contained δ -carene (3.39%) and *p*-cymene (3.38%) [8, 9], while α -pinene (40.82%), nonene (17.03%), phellandrene (11.14%), δ -carene (7.39%),

and *p*-cymene (4.90%) were identified as major components of *P. platychlaena* [14]. Study of the chemical composition and antibacterial activity of essential oil from aerial parts of *P. ferulacea* (L.) Lindl grown in Iran showed its primary constituents to be α -pinene (36.6%), β -pinene (31.9%) and β -phellandrene (11.7%) [15]. Some *Prangos* species have been used in folk medicine as emollient, carminative [16], tonic, antifatulent, anthelmintic, antifungal and antibacterial agents [17, 18]. Chemical investigations on the components of the genus *Prangos* have resulted in the isolation of various coumarins, alkaloids, flavonoids and terpenoids [19]. According to the literature, leaves, stems and flowers of *P. ferulacea* have not been the subject of any investigation, and this paper is the first such phytochemical study on this plant.

EXPERIMENTAL

Plant material

The sample of *Prangos ferulacea* was collected during the flowering stage in June 2005 from the Province of Esfahan, in the centre of Iran. Voucher specimens were deposited at the Herbarium (Voucher No. 6014) of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

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Oil isolation

Fresh leaves (80 g), stems (90 g) and flowers (70 g) of *P. ferulacea* were subjected to separate hydro-distillation for 3 h using a Clevenger-type apparatus. After decanting and drying over anhydrous sodium sulphate, the corresponding yellowish coloured oils were recovered from the leaves, stems and flowers in yields of 0.9, 0.8 and 1.1% (w/w), respectively.

Analysis

GC analysis of the oils was performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless injector (250°C). N₂ was used as carrier gas (1 mL/min), and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was maintained at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min.

GC/MS analysis was performed using a Hewlett-Packard 6890/5973 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was maintained at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min, and kept constant at 220°C for 5 min. The flow rate of helium as the carrier gas was 1 mL/min. MS was taken at 70 eV.

Identification of the constituents of each oil was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and the authentic samples [20–22]. Relative percentage amounts were calculated from the peak area using a Shimadzu C-R4A Chromatopac without correction factors.

Antibacterial activity

A collection of four microorganisms was used, including the Gram-positive bacteria *Staphylococcus aureus* (ATCC 1112), *Staphylococcus epidermidis* (ATCC 1114) and *Bacillus cereus* (ATCC 1015) and the Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 1310), identified by the Research Centre of Science and Industry, Tehran, Iran.

Microorganisms (obtained from enrichment culture of the microorganisms in 1 mL of Mueller-Hinton broth, incubated at 37°C for 12 h) were cultured on Mueller-Hinton agar medium.

The following method was used to measure antibacterial activity: 40 µL of diluted essential oil (40 µL oil in 2 mL DMSO 10%) was added to a 200 µL microbial suspension (1 loop from medium in physiological serum that compared with a 0.5 McFarland standard) in well 1 in a microplate, and 100 µL from this well was added to a 100 µL

microbial suspension in well 2, and this continued until 8 wells in the microplate were filled. Microplates were incubated at 37°C for 24 h [23].

RESULTS AND DISCUSSION

Chemical components identified in the three oils of *P. ferulacea* and their percentage compositions are listed in Table 1.

Table 1. Percentage composition of the leaf, stem and flower oils of *Prangos ferulacea*.

Compound	RI*	Leaf Oil	Stem Oil	Flower Oil
α-pinene	953	12.1	10.3	12.4
sabinene	970	0.5	0.4	1.4
p-cymene	1014	-	-	1.4
1,8-cineole	1033	8.9	19.0	14.5
linalool	1087	36.7	3.7	19.0
α-campholenal	1126	2.3	0.7	7.0
camphor	1136	1.6	-	1.3
α-terpineol	1189	-	2.8	-
myrtenal	1190	-	0.5	0.5
lavandulyl acetate	1289	1.2	-	16.0
β-caryophyllene	1410	-	-	0.6
γ-elemene	1433	-	-	0.6
germacrene D	1480	-	0.5	1.6
δ-cadinene	1524	-	-	1.4
geranyl isobutyrate	1530	1.2	-	12.2
germacrene B	1556	-	-	0.3
caryophyllene oxide	1581	16.3	4.2	-
β-eudesmol	1648	-	0.7	-
α-cadinol	1650	-	-	6.4
kusinol	1674	0.9	0.5	1.6

* Retention indices as determined on a DB-5 column using the homologous series of *n*-alkane.

The leaf oil consisted of 10 identified compounds representing 81.7% of the oil composition. The main compounds were linalool (36.7%), caryophyllene oxide (16.3%) and α-pinene (12.1%). Another notable constituent was 1,8-cineole (8.9%).

In the stem oil, 11 compounds were identified, representing 43.3% of the oil composition. The main compounds were 1,8-cineole (19.0%) and α-pinene (10.3%).

Linalool (19.0%), lavandulyl acetate (16.0%), 1,8-cineole (14.5%), α-pinene (12.4%) and geranyl isobutyrate (12.2%) were the main compounds among the 17 constituents representing 98.2% of the total components detected in the flower oil.

Oxygenated monoterpenes represented the most abundant constituent of the oil of leaves, stems and flowers (63.3%, 37.4% and 74.7%, respectively). Linalool was the main constituent of the leaf and flower oils (36.7% and 19.0%, respectively), and 1,8-cineole (19.0%) of the stem oil.

The literature survey of the chemical composition of *P. asperula* showed δ -3-carene, β -phellandrene, α -pinene and α -humulene to be the major components of the oil [13].

Dried aerial parts of *Prangos uechtritzi* contained δ -carene (3.39%) and *p*-cymene (3.38%) [8, 9], while α -pinene (40.82%), nonene (17.03%), phellandrene (11.14%), δ -carene (7.39%), and *p*-cymene (4.90%) were identified as major components of *P. platychnaena* [14]. The main compounds of the *P. ferulacea* aerial parts were α -pinene (36.6%), β -pinene (31.9%) and β -phellandrene (11.7%) [15]. In our previous investigation [24] the oil of *P. ferulacea* collected from north of Tehran, Iran, contained α -pinene, δ -3-carene, β -pinene and epi- α -bisabolol as main compounds and was found to be rich in sesquiterpenes hydrocarbons, while in the present study, the stem, leaf and flower oils of the plant, collected from Lorestan Province, Iran, contained mostly oxygenated monoterpenes.

The oil of *P. ferulacea* aerial parts collected from Lorestan province, Iran, and of *P. acaulis* from Iran were rich in regard to oxygenated monoterpenes (77.8% and 86.2%, respectively) [15,25]. Our results, compared with our previous investigation on oils of the *Prangos* genus, also showed the oils of these parts to be dominated by oxygenated monoterpenes.

The antibacterial assays showed that the oils of leaves, stems and flowers of *P. ferulacea* inhibited the growth of all the bacteria. Leaves, stems and flowers of *P. ferulacea* were further tested for Gram-positive and Gram-negative bacteria. The results of the bioassays (Table 2) showed that the three oils exhibited moderate to strong differences in antimicrobial activity.

Table 2. Antibacterial activity of leaves, stems and flowers of *Prangos ferulacea* oils based on dilution method and using DMSO*.

Bacterial Species	Gram +/-	Leaf Oil	Stem Oil	Flower Oil	DMSO
Staphylococcus aureus ATCC 1112	+	0.5	2	0.5	>4
Staphylococcus epidermidis ATCC 1114	+	0.25	0.5	>4	>4
Bacillus cereus ATCC 1015	+	1	2	0.5	>4
Pseudomonas aeruginosa ATCC 1310	-	0.0625	0.5	1	>4

* Values are the mean MIC (ppm).

In the antimicrobial screening, the oil of *P. ferulacea* leaves exhibited particularly strong activity, especially for the Gram-positive organisms,

although that of stem and flower oils was also interesting. (Leaf-oil MIC values for *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus cereus* were 0.0625 ppm, 0.25 ppm, 0.50 ppm and 1.00 ppm, respectively.) In previous studies, antibacterial activity of the essential oils of aerial parts of *P. ferulacea* in Iran appeared strong for Gram-positive bacteria, especially *Staphylococcus aureus* [15], while the *P. ferulacea* in Turkey was active against *Staphylococcus aureus* [23]. Our previous article addressed the antibacterial activity of leaf oils against a Gram-negative strain.

CONCLUSIONS

1. The chemical composition and antibacterial activity of essential oil from leaves, stems and flowers of *Prangos ferulacea* (L.) Lindl grown in Iran were investigated by hydrodistillation using a Clevenger-type apparatus and analysed by GC-MS.

2. The leaf oil consisted of 10 identified compounds representing 81.7% of the oil composition. The main compounds were linalool (36.7%), caryophyllene oxide (16.3%) and α -pinene (12.1%). Another notable constituent was 1,8-cineole (8.9%).

In the stem oil, 11 compounds were identified, representing 43.3% of the oil composition. The main compounds were 1,8-cineole (19.0%) and α -pinene (10.3%).

Linalool (19.0%), lavandulyl acetate (16.0%), 1,8-cineole (14.5%), α -pinene (12.4%) and geranyl isobutyrate (12.2%) were the main components among the 17 constituents characterized in the flower oil, representing 98.2 % of the total components detected.

3. Oxygenated monoterpenes represented the most abundant constituents of the oil of leaves, stems and flowers (63.3%, 37.4% and 74.7%, respectively).

4. The oils were tested against four Gram-positive or negative bacteria using a dilution method. It was found that oils from leaves, stems and flowers of *P. ferulacea*, and especially that of leaves, exhibited interesting antibacterial activity.

5. Comparing these results with investigations on oils of other species of the *Prangos* genus showed they are also dominated by oxygenated monoterpenes.

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ХИМИЧЕН СЪСТАВ И АНТИБАКТЕРИАЛНА АКТИВНОСТ НА ЕТЕРИЧНИ МАСЛА ОТ ЛИСТА, СТЬБЛА И ЦВЕТОВЕ ОТ *Prangos ferulacea* (L.) Lindl. ОТ ИРАН

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(Резюме)

Получени са етерични масла от листа, стъбла и цветове от *Prangos ferulacea* (L.) Lindl. растящи в Исфахан, Иран чрез хидродестилация с използване на оборудване тип Clevenger и е анализиран техния химичен състав чрез ГХ-МС и е изследвана антибактериалната им активност. Всички масла се състоят главно от кислородсъдържащи монотерпени и малък процент от сескитерпенови съединения. В маслото от листа са идентифицирани 10 компонента с преобладаване на кислородсъдържащи монотерпени. Идентифицираните три главни съставки (представляващи 65.1% от маслото) са линалол (36.7%), кариофилен оксид (16.3%) и α -пинен (12.1%). В маслото от стъбла са идентифицирани 11 съединения като отново преобладават кислородсъдържащи монотерпени. Двете главни идентифицирани съставки (представляващи 29.3% от маслото) са 1,8-цинелол (19.0%) α -пинен (10.3%). От 17-те съединения намерени в маслото от цветове, петте главни идентифицирани компоненти (представляващи 74.1% от маслото) са кислородсъдържащи монотерпени: линалол (19.0%), лавандулилacetат (16.0%), 1,8-цинелол (14.5%), α -пинен (12.4%) и геранилизобутират (12.2%). Маслата бяха тествани срещу грам-положителни и грам-отрицателни бактерии. Антибактериалната активност е измерена използвайки метода на разреждане. Намерено е, че маслото от листа, стъбла и цветове от *Prangos ferulacea* и особено това от листа показва интересна антибактериална активност.