Medicinal & Aromatic Plants

Mneimne et al., Med Aromat Plants 2016, 5:3
http://dx.doi.org/10.4172/2167-0412.1000242

Research Article Open Access

Chemical Composition and Antimicrobial Activity of Essential Oils Isolated from Aerial Parts of *Prangos asperula* Boiss. (Apiaceae) Growing Wild in Lebanon

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Abstract

Prangos species have been commonly used in traditional medicine in East Mediterranean and Middle Eastern countries. The hydrodistilled essential oils by HDC from different fresh aerial parts (stems and leaves, flowers and fruits) of *Prangos asperula Boiss*., growing wild in Lebanon were analyzed by gas chromatography-mass spectrometry. Forty two, 46 and 4 compounds representing 75.5%, 86.9% and 99.8% of the total fresh stems and leaves, flowers and fruits oils were, respectively, identified. The main compounds characterizing these oils were nerolidol (15.2%), *p*-menth-3-ene (13.3%), β-myrcene (9.2%) in stem and leaves; *p*-menth-3-ene (14.9%), nerolidol (14.7%), β-phellandrene (7.9%) in flowers; sabinene (43.5%), β-phellandrene (36.1%), α-phellandrene (11.9%) and α-terpinene (8.3%) in fruits. The antimicrobial activity of the total essential oil evaluated by growth inhibition and MIC values revealed variable levels of susceptibility in the tested bacteria and fungi. *S. aureus* displayed highest sensitivity (15.06 mm, MIC 5.0 µl), followed by *E. coli* (11.80 mm, MIC 10.0 µl), *A. fumigatus* (9.16 mm, MIC 10.0 µl), *T. mentagrophytes* (7.3 mm, MIC 25 µl), *S. enteritidis* (3.8 mm, MIC 25 µl) and *C. albicans* (1.96 mm, MIC 50 µl). The oil displayed a remarkable activity against both *S. aureus* being more effective than the antibiotic Norfloxacine (10 µg) and *T. mentagrophytes* which was completely resistant to the antifungal Nystatine (100 µg). The findings confirm the traditional use and promising potential of the antibacterial properties of this plant oil. This opens the possibility for further research on other biocidal activities and investigations of individual antimicrobial and antifungal component.

Keywords: *Prangos asperula*; Essential oils; Nerolidol; Sabinene; Antimicrobial activity

Introduction

Prangos is a perennial genus of the *Apiaceae* family distinguished by its winged fruits [1]. It consists of about 30 worldwide species with a diversity center being situated in the Irano-Turanian phytogeographic region [2]. *Prangos* species have been commonly used in traditional medicine in East Mediterranean and Middle Eastern countries. Numerous studies have cited *Prangos* species as carminative, wound healing, haemostasis, antiflatulent, antihaemorrhoidal, anthelmintic, antispasmodic and antimicrobial in a wide range of diseases [3-9]. *Prangos* species are also indicated in the treatment of leucoplakia and digestive disorders and to have anti-HIV and antioxidant activities [4,10,11]. Similarly to *Ferula* and *Ferulago*, the roots have aphrodisiac properties [12,13]. More recently, insecticidal properties against Mediterranean flour moth (*Ephestia kuehniella*) found in stored food products, such as cereals are also displayed [14].

Recent phytochemical investigations on different species have led to the isolation of coumarins, flavonoids, alkaloids, terpenoids and other compounds from the different plant parts which have attracted considerable attention due to their pharmacological properties supporting the traditional use [4,5,13,15-23].

Prangos asperula Boiss., commonly known as *Farsh Al Dabe'e*, is the only native *Prangos* species that grows wild in the upper mountainous regions of Lebanon (Tannourine, Dahr el Baydar, Ehden, Shouf, Kefraya, Rachaiya). *P. asperula* has been traditionally used as remedy of skin diseases, wounds infections and as carminative against aerocoly and in the treatment of diabetes [24]. Studies on the plants growing wild in Lebanon have reported a remarkable antimicrobial activity of aerial parts against some Gram-positive and Gram-negative bacteria and interesting antiproliferative effects of leaves essential oil on renal adenocarcinoma cell line [25,26].

Forty-two terpenes were identified in *P. asperula* essential oil, representing 92.1% of total oil. Sabinene (20.6%), β-phellandrene (19.0%), γ-terpinene (9.0%), α-pinene (8.4%), and α-phellandrene (6.1%) were the most representative constituents. Other interesting oil components were δ-3-carene, p-cymene and α-bisabolol. Sabinene, α-pinene, α-phellandrene and δ-3-carene were tested for their cytotoxic activity on tumor cell *in vitro* models. None of the compounds was found active. It was concluded that major and minor constituents may act in synergy with the other cytotoxic components of the essential oils [24,26]. Other investigations on the fruit oil of the plant from Iran demonstrated that δ-3-carene, β-phellandrene, α-pinene, α-humulene, germacrene D and δ-cadinenewere the major components of the essential oil of fruits [27], while δ-3-carene, α-terpinolene, α-pinene, limonene, 2,3,6-trimethyl benzaldehyde, bornyl acetate, osthol and *cis*-chrysanthenyl acetate were the most representative constituents of the aerial parts essential oil [28,29]. In the latter study, investigators were also able to isolate in the hexane extract of the fruits the

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Received January 30, 2016; **Accepted** March 09, 2016; **Published** March 14, 2016

Citation: Mneimne M, Baydoun S, Nemer N, Apostolides NA (2016) Chemical Composition and Antimicrobial Activity of Essential Oils Isolated from Aerial Parts of *Prangos asperula* Boiss. (Apiaceae) Growing Wild in Lebanon. Med Aromat Plants 5: 242. doi:10.4172/2167-0412.1000242

prenylated coumarin osthol recognized to be effective the prevention of atherosclerosis, suppression of hepatic lipids, antitumor and anti-inflammatory among its major bioactivities.

This study concerns a comparative analysis of essential oils composition from different aerial plant parts of *P. asperula* growing wild in Lebanon and assessment of their antimicrobial activity.

Material and Methods

Plant material

Aerial parts of the wild growing *P. asperula* were collected at random from Tannourine in the North of Lebanon in July 2014, at 1750 meters. The species identification was performed using the determination keys of the New Flora of Lebanon and Syria [30]. A voucher specimen (RCED 2015-295) was deposited at the herbarium of the Research Center for Environment and Development, Beirut, Arab University, Lebanon.

Essential oil isolation

The essential oils from fresh leaves and stems, flowers and fruits *of P. asperula* were hydrodistillled by Clevenger-type apparatus for three hours [31]. The volatile oils were dried using anhydrous sodium sulphate and then stored in sterile sealed vials in the dark at 4°C until analysis. The yield percentages of oils were calculated based on fresh weight of plant parts.

Bacterial and fungal strains

Certified bacterial and fungal strains (Medi Mark, Europe) were used. They were two pathogenic Gram+ bacteria: *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923); two Gram negative bacteria: *Escherichia coli* (ATCC 8739), *Salmonella enteritidis* (ATCC 13076); two fungal strains: filamentous *Aspergillus fumigatus* (ATCC 1022), dermatophyte *Trichophyton mentagrophytes* (ATCC 9533); and the yeast *Candida albicans* (ATCC 10231).

GC and GC-MS analyses

Gas chromatography with mass spectrometry (GC-MS) was used to identify essential oils compounds. The analysis was performed by Agilent Technologies 7890 gas chromatography equipped with a Flame Ionization Detector (FID) and a HP- 5 MS 5% capillary column (30 m x 0.25 mm x 0.25 µm film thickness). Mass spectra were recorded at 70 eV of electron energy and a mass range of 50-550 m/z. The carrier gas was Helium at a flow of 0.8 ml/min. The initial column temperature was 60°C programmed to increase to 280°C at a rate of 4°C/min. The split ration was 1:40. The injector temperature was set at 300°C. The purity of helium gas was 99.99%. A sample of 1 ml was injected manually in the split mode. Components identification was based on retention indices and comparison with mass spectral data of authentic standards and computer matching with Wiley 229, NIST 107, NIST 21 libraries as well as by comparing the fragmentation patterns of the mass spectra with those reported in the literature.

Antimicrobial activity by disc diffusion method

The antibacterial and antifungal activity of total essential oil was carried out by disc diffusion method using 100µl of suspension containing 10^6 CFU/ml of bacteria spread on Mueller-Hinton agar medium (Merck). Sterile 6 mm diameter filter paper discs (Whatman No. 3) were impregnated with 10 µl of essential oil and were placed on the agar. Standard reference discs of the antibiotics Norfloxacin (10 µg) and Nystatin (10 µg) were used as standard antibacterial or antifungal agents and positive controls. Each test was run in triplicate and the mean values were considered. A blank disc was used as a negative control. The bacterial cultures were incubated at 37°C for 24 h. Whereas, *Candida albicans* and *Trichophyton mentagrophytes* were incubated at 27°C for 48 h and 5 days, respectively. The diameters (mm) of growth inhibition zones around discs were measured using a caliper.

Minimum Inhibitory Concentration (MIC) by agar dilution method

MICs were determined by agar dilution method approved by NCCLS (1997). A series of four concentrations of oil (5, 10, 25 and 50 µl) with 0·5% (v/v) Tween-20 were added to a one ml of microbial suspension containing approximately 10^6 CFU/ml of each organism in Mueller Hinton broth. Tween-20 (Sigma) was used to enhance the solubility of essential oil in broth. 100 µl of each mixture was spread on Mueller Hinton agar plates. The plates inoculated with bacteria were incubated at 37°C for 24 h. MICs determined as the lowest concentration of oil inhibiting the visible growth of each microorganism on agar plates were determined. All tests were performed in triplicates.

Results

Composition of essential oil

The yields of oils of stems and leaves, flowers and fruits were 0.09%, 0.22 % and 0.21%, respectively. 42 compounds were identified in the oil from leaves and stems representing 75.5% of the total, 46 compounds in the flowers oil representing 86.9% of total and 4 compounds in the fruits oil representing 99.8% of total (Table 1).

The main components of stems and leaves oil were nerolidol (15.2%), *p*-menth-3-ene (13.3%), β-myrcene (9.2%), β-farnesene (4.8%), cis-β-ocimene (3.5%), α-farnesene (3.4%), α-phellandrene (3.2%), α-bisabolol (3.1%), α-caryophyllene (3%), neo-allo-ocimene (1.9%), α-bergamotene (1.8%), cis-α-bisabolene (1.3%), o-ocymol (1.2%), 2-thujene (1.1%) and safranal (1.1%). Hydrogenated monoterpenes dominated the chemical composition (34.1%), followed by oxygenated sesquiterpenes (19.4%) and hydrogenated sesquiterpenes (19.1%), while oxygenated monoterpenes (2.9%) formed a minor share of this oil.

The main components of flowers oil were *p*-menth-3-ene (14.9%), nerolidol (14.7%), β-phellandrene (7.9%), α-caryophyllene (5.1%), β–farnesene (5.1%), neo-allo-ocimene (5.1%), α-phellandrene (4.3%), α-farnesene (4.2%), β-trans-ocimene (3.9%), α-bisabolol (2.7%), α-terpinene (2.7%), α-bergamotene (1.6%), α-terpinolene (1.4%), IR-α-pinene (1.2%), *cis*-β-ocimene (1.2%), α-cedrene (1.1%) and (+)-4-carene (1.1%). Similarly to the stems and leaves, hydrogenated monoterpenes formed the major share of constituents (44.7%) followed by hydrogenated sesquiterpenes (22.3%), oxygenated sesquiterpenes (18.7%) and oxygenated monoterpenes (1.2%).

Finally the major components of fruits were sabinene (43.5%), β-phellandrene (36.1%), α-phellandrene (11.9%) and α-terpinene (8.3%). Absolute dominance of hydrogenated monoterpenes is detected forming the sole group of identified compounds (99.8%), while oxygenated monoterpenes, hydrogenated sesquiterpenes and oxygenated sesquiterpenes were completely absent in the fruits oil.

Antimicrobial activity

The antimicrobial activity of the total essential oil evaluated by the disc diffusion method revealed variable levels of susceptibility in the tested bacteria and fungi (Table 2). The results showed that *S. aureus* had the highest sensitivity (15.06 mm) which was also more effective

RT	Compound	Content %		
		Stems & Leaves	Flowers	Fruits
7.37	2-Thujene	-	0.4	-
7.38	3-Thujene	0.1	-	-
7.51	1R-α-Pinene	-	1.2	-
7.51	1S-α-Pinene	0.5	-	-
7.90	4-Carene	-	0.1	-
7.91	Camphene	0.1	-	-
8.62	Sabinene	0.8	-	-
8.62	β-Phellandrene	-	7.9	-
8.85	Sabinene	-	-	43.5
9.16	β-Myrcene	9.2	-	-
9.51	α-Phellandrene	3.2	4.3	-
9.66	2-Thujene	1.1	-	-
9.74	α-Phellandrène	-	-	11.9
9.88	α-Terpinolene	-	1.4	-
9.88	2-Carene	0.4	-	-
10.10	α-Terpinene	-	-	8.3
10.11	O-Ocymol	1.2	-	-
10.25	p-Menth-3-ene	13.3	14.9	0
10.48	β-Phellandrene	-	-	36.1
10.54	β-Trans-ocimene	-	3.9	-
10.87	Cis-β-ocimene	3.5	-	-
10.88	β-Cis-ocimene	-	1.2	-
11.21	α-Terpinene		2.7	-
12.31	(+) -4-Carene	-	1.1	-
14.32	Neo-allo-ocimene	1.9	5.1	-
14.91	Cis-allo-ocimene	-	0.5	-
17.02	Terpinen-4-ol	0.1	0.6	-
18.97	Trans-Pinocarveol	-	0.1	-
34.38	Copaene	-	0.1	-
34.55	Calarene	0.1	-	-
35.01	β-Elemene	0.1	0.1	-
35.47	α-Cedrene	0.1	-	-
35.71	Caryophyllene	0.4	-	-
35.72	β -Caryophyllene	-	0.4	-
36.18	Gamma-Elemene	0.1	-	-
36.19	Aromadendrene	-	0.1	-
36.45	δ-Cadinene	0.1	-	-
36.46	D-Cadinene	-	0.1	-
36.59	α-Caryophyllene	3	5.1	-
36.83	β-Farnesene	4.8	5.1	-
36.97	Trans-β-Farnesene	0.1	-	-
37.05	2-Dehydro-1,8 cineole	0.5	-	-
37.06	Santanol (CAS)	-	0.4	-
37.15	Gamma-Muurolene	0.5	0.4	-
37.24	α-Cedrene	-	1.1	-
37.24	β-Himachalene	0.6	-	-
37.32	Curcumene	0.3	0.4	-
37.44	Germacrene D	0.1	0.1	-
37.59	α-Bergamotene	1.8	1.6	-
37.71	Cis-α-Bisabolene	0.3	0.3	-
37.83	α -Farnesene	3.4	4.2	-
38.08	βSesquiphellandrene	0.6	-	-
38.23	Trans-.Gamma.-Bisabolene	0.6	0.5	-
38.42	Cis-α-Bisabolene	1.3	0.9	-
38.63	α-Bulnesene	-	0.1	-
38.64	α-Patchoulene	0.1	-	-
38.83	Nerolidol	15.2	14.7	-
39.06	β-Farnesene	0.1	-	-
39.07	α-Himachalene	-	0.1	-
39.13	Isocaryophyllene	0.1	-	-
39.14	Longifolene	-	0.1	-
39.30	Safranal	1.1	-	-
39.63	Safranal	-	0.5	-
39.83	α-Bulnesene	-	0.3	-
40.02	Gamma-Himachalene	-	0.1	-
40.12	Gamma-Cadinene	-	0.2	-
40.19	Epi-cis-.β.-Santalol	-	0.2	-
40.30	β-Selinene	0.3	-	-
40.31	(E,Z)-α-Farnesene	-	0.2	-
40.53	α-Bisabolol	3.1	2.7	-
40.70	Cis-Gamma-Bisabolene	0.1	-	-
40.80	Longifolene	0.1	0.1	-
40.96	Farnesol	-	0.2	-
	Hydrogenated Monoterpenes	**34.1**	**44.7**	**99.8**
	Oxygenated Monoterpenes	**2.9**	**1.2**	**-**
	Hydrogenated Sesquiterpenes	**19.1**	**22.3**	**-**
	Oxygenated Sesquiterpenes	**19.4**	**18.7**	**-**
	Total	**75.5**	**86.9**	**99.8**

Table 1: Yield percentages and chemical composition of essential oils from different aerial parts of *Prangos asperula Boiss*. from Lebanon.

Microorganisms		*P. asperula* essential oil	*Norfloxacine 10 µg*	*Nystatine 100 µg*	*P. asperula* essential oil
		Growth Inhibition Zone(mm)			Minimum Inhibitory Concentration MIC/MFC (µL)
Gram Positive Bacteria	*E. faecalis*	R	24.8 ± 0.6	-	-
	S. aureus	15.0 ± 0.3	9.3 ± 0.2	-	5.0
Gram Negative Bacteria	*E. coli*	11.8 ± 0.2	29.2 ± 0.2	-	10.0
	S. enteritidis	3.8 ± 0.4	31.2 ± 0.5	-	25.0
Fungi	*A. fumigatus*	9.1 ± 0.8	-	11.8 ± 0.5	10.0
	T. mentagrophytes	7.3 ± 0.2	-	R	25.0
Yeast	*C. albicans*	1.9 ± 0.1	-	8.9 ± 0.2	50.0

Table 2: The mean ± SD growth inhibition zone and MIC/MFC values of the essential oil of *Prangos asperula Boiss*. growing wild in Lebanon.

than the antibiotic Norfloxacine (9.30 mm). The susceptibility of *E. coli* came in second place (11.80 mm) followed by *A. fumigatus* (9.16 mm), *T. mentagrophytes* (7.3 mm), *S. enteritidis* (3.8 mm) and *C. albicans* (1.96) being the least sensitive among the responsive microorganisms. The oil failed to show any response on *E. faecalis* which was completely resistant to the volatile oil. It may be of a high importance to note that the relatively substantial susceptibility of *T. mentagrophytes* (7.3 mm) to the oil which was completely resistant to the antifungal Nystatine (10 µg). The MIC/MIF values determined by mean of agar dilution method were 5.0 µl in *S. aureus* followed by *E. coli* and *A. fumigatus* (10 µl), *S. enteritidis* and *T. mentagrophytes* (25 µl) and *C. albicans* (50 µl).

Discussion

The composition of essential oils in this study strongly illustrate

that major differences exist between the compositions of essential oils from different aerial parts of *P. asperula*. The oil of fresh fruits appeared as a major source for both sabinene (43.5%), and β-phellandrene (36.1%), while aerial parts (stems, leaves and flowers) may together be considered as moderate sources for nerolidol (15.2% and 14.7%, respectively) and *p*-menth-3-ene (13.3 % and 14.9%, respectively). It is interesting to note that β-myrcene (9.2%) seemed to characterize the oil of stems and leaves only.

Although, a direct comparison between the composition of combined stems and leaves oils in this study with that reported of leaves is not legitimate [26], the most representative constituents of leaves oil in the later study were sabinene (20.6%), and β-phellandrene (19.0%) with no presence of nerolidol or p-menth-3-ene which were the major constituents of our oil (15.2%, 13.3%, respectively). Moreover, only β-phellandrene as the second dominant constituent of the fresh fruit oil herein (36.1%) was also reported among the most representative compounds of the dry fruit of the plant growing in Iran (14.7%) [29]. No presence of sabinene which was the major component of our fresh fruits oil (43.5%) was noted.

In general terms, neither of our oils displayed the presence of 2,3,6-trimethyl benzaldehyde or δ-3-carene which were reported as the major constituents in the aerial parts oil of the plant from Iran (18.4% and 18.0%, respectively) [29].

Comparisons with previous studies on the essential oils of other *Prangos* species showed considerable quantitative and qualitative variations in yield and composition. In the oils of the flowers/umbels of several other species, the main compounds reported were: β-pinene (35.58%); α-pinene (22.13%) and β-phellandrene (12.54%) in *P. peucedanifolia* [32], α-pinene (33.87%) in *P. pabularia* [33], epi-globulol (21.1%) and β-elemene (19.7%) in *P. scabra* [18], α-pinene (31.78%) and β-bourbonene (15.9%) in *P. uloptera* [19].

Substantial differences were even also found in three different studies on flowers/umbels oil of *P. ferulacea* from Iran: β-pinene (20.6 %) [34], linalool (19.0%); lavandulyl acetate (16.0%); 1,8-cineole (14.5%); α-pinene (12.4%) and geranyl isobutyrate (12.2%) [35], *α*-pinene (42.2%) and c*is*-ocimene (36.3%) [8].

Thus, both the major constituents of the flowers/umbels oil of *P. asperula* in this present study, *p*-menth-3-ene (14.9%) and nerolidol (14.7%),which were not previously reported in any of the above mentioned species seem to be distinctive to our plant.

In the fruits oil, similarly to our results sabinene (26.1%) was reported as a major constituent in P. denticulata [13]. On the contrary, α-pinene representing the main constituent of *P. ferulacea*; *P. pabularia* and *P. uloptera* (63.1%, 21.46%, 14.98%, respectively) was absent in our plant [8,19,33]. In addition, neither of the following reported major constituents was noted in our results: chrysanthenyl acetate (26.53%), limonene (19.59%), α-pinene (19.50%) in *P. ferulacea* [7]; β-elemene (19.9%), β-farnesene (16.2%) in *P. scabra* [19], *α*-humulene (16.6%); bicyclogermacrene (16.1%); spathulenol (10.6%) in *P. pabularia* [17], germacrene-D-4-ol (42.8%); α-cadinol (18.5%) in *P. bornmuelleri* [36], γ-terpinene (30.22% and 33.27%), α-pinene (16.71% and 12.83%) in crushed and whole fruits yielded oils of *P. ferulacea,* respectively [37], β-bisabolenal (53.3%) and β-bisabolenol (14.6%) in *P. heynlae* [15], and *p*-cymene (10.9%) in *P. uechtritzii* [16].

In conclusion, the above mentioned variability in oil composition from different species cited and *P. asperula*, sufficient to allow distinction of different chemotypes, are the result of an adaptive process of the plant to a particular ecological conditions (geographical regions, climatic conditions, period of collection, plant part, state of plant- dry or fresh- and extraction methods).

Further, the antimicrobial activity of the tested oil is in line with the findings of a previous study testing the oil of the plant from Lebanon against several microorganisms all of which exhibited remarkable activity supporting the traditional use of plant in the treatment of wide range of diseases and confirming its promising potential of the antibacterial properties of this plant oil [25]. Some of the main compounds are reported to possess antimicrobial activities. In particular, nerolidol, *β*-phellandrene, sabinene are reported to possess antibacterial and antifungal activity [38-40].

The findings can further underline the importance of the ethno botanical approach to select plants that contain new antimicrobial substances, particularly when considering the increased development of resistance of bacteria, fungi and yeast to antibiotics.

The strong inhibitory activity of the oils, particularly on *S. aureus* and *T. mentagrophytes*, may be related to hydrogenated monoterpene components which constituted 34.1%, 44.7%, 99.8% of the oil of stems and leaves, flowers and fruits, respectively. However, it is difficult to attribute the activity of a complex mixture to a single or group of constituents especially when some evidence that minor components have a critical part to play in antimicrobial activity, possibly by producing a synergistic effect with various components [24,26,41-42].

Conclusion

P. asperula is one of Lebanese indigenous plants which possess many medicinal properties. The study presents for the first time knowledge on the composition of the oils of stems and leaves, flowers and fruits which appear as unique and substantially different from previously reported oils. The antimicrobial activity suggests a potentiality for a new source of antimicrobial compounds to be applied in pharmaceutical industry. Further studies are needed for a better understanding of the biological properties of the oils and their constituents.

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Citation: Mneimne M, Baydoun S, Nemer N, Apostolides NA (2016) Chemical Composition and Antimicrobial Activity of Essential Oils Isolated from Aerial Parts of *Prangos asperula* Boiss. (Apiaceae) Growing Wild in Lebanon. Med Aromat Plants 5: 242. doi:10.4172/2167-0412.1000242