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Chemical Composition and Anticandidal Activity of Essential Oils Obtained from Different Parts of *Prangos heyniae* H. Duman & M. F. Watson

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Abstract: The chemical compositions and anticandidal activities of the essential oils from the aerial parts, inflorescence, and roots of the endemic *P. heyniae* H. Duman & M. F.Watson were evaluated. Hydrodistillation was used to isolate the essential oils and the chemical analyses were performed both by GC-FID and GC/MS, respectively. Forty-four compounds constituting 94.4% of aerial parts, thirty-nine compounds constituting 99.5% of inflorescence, and twenty-five compounds representing 100.0% of root essential oil were characterized. The main compounds of the aerial parts oil were elemol (36.2%), β-bisabolenal (14.1%), and germacrene D (12.7%). Main compounds of inflorescence oil were β-bisabolenal (21.6%) germacrene D (15.6%), and germacrene B (12.4%) while the roots oil were α-pinene (44.8%), limonene (15.3%), *β*-pinene (12.5%), and δ-3-carene (10.2%), respectively. The *in vitro* anticandidal activity of the essential oils were evaluated against several *Candida* strains by using partly modified CLSI broth microdilution method M27-A2. The tested essential oil showed relatively weak effects against pathogenic *Candida* strains compared to standard antifungal agents.

Keywords: Apiaceae; essential oil; GC/MS; *Prangos heyniae*; anticandidal activity

1. Introduction

Apiaceae species are rich in essential oils. Essential oils in this family could be found in different organs such as fruits, roots, flowers, leaves and stems [1]. The genus *Prangos* Lindl. belonging to Apiaceae consists about 35 species worldwide [2,3]. The genus *Prangos* is represented by 20 taxa where 12 are endemic, which are within the important medicinal and aromatic plants in Turkey [4].

Some *Prangos* species are known as "çakşir otu" [5] are used in Traditional Anatolian Medicine for their emollient, carminative, tonic, antiflatulent, anthelmintic, and antifungal properties [6]. *Prangos* liquid extracts are used to stop bleeding and heal scars when applied topically [7]. Roots

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of the *Prangos* species are also used as an aphrodisiac, similarly the roots of the *Ferula* and *Ferulago* species [8,9]. In Serbia and the surrounding regions, the root extracts of *P. ferulacea* (L.) Lindl, are used in the treatment of intestinal wounds, and especially haemorrhoids [6,10]. In Uzbekistan, the herbal preparations of *P. tschimganica* O.Fedtsch are used in the treatment of leucoplakia disease [11]. In Turkey, *Prangos* species are used as culinary food and feed [6,12,13].

Prangos species showed in vitro biological activities such as antiviral, anti-cancer, anti-inflammatory, anti-diabetic and neuroprotective [14]. This genus contains coumarin glycosides, alkaloids, flavonoids, and terpenoids among others. Hydrodistilled essential oils (EOs) obtained from different parts of the plant contain monoterpene and sesquiterpene hydrocarbons [15]. According to previous work, EOs from *Prangos* species demonstrated varying degrees of anti-candidal activity [14].

In the present study it was aimed to determine the chemical compositions of the EOs obtained from different parts of endemic *P. heyniae* H. Duman & M. F.Watson. *In-vitro* anticandidal activity of the EOs was also studied. To the best of our knowledge this is the first study in this scope.

2. Materials and Methods

2.1. Plant Material

P. heyniae was collected between Hadim-Bozkır (Konya) at an altitude of 1600 m in Turkey on May 2020. Collected plant samples were identified and the voucher specimens are kept at the Herbarium of Faculty of Pharmacy of Anadolu University, Turkey (ESSE No:15797). The plants were identified by one of us (O.Tugay).

2.2. Isolation of Essential Oil

Aerial parts, inflorescence and roots of the plant were distilled for 3 h using a Clevenger-type apparatus. The essential oils were stored at 4°C in the dark until analysed. Oils yields (w/v) were 0.29%, 0.26% and 0.73%, respectively.

2.3. Analysis of the Essential Oil

The oils were analyzed by capillary Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC/MS) using a Agilent GC-MSD system (Mass Selective Detector-MSD) (Agilent Technologies Inc., Santa Clara, CA) [16]

2.4. Anticandidal Activity

Standard strains of *Candida albicans* ATCC 90028, *C. utilis* NRRL Y-900, *C. tropicalis* ATCC 750, *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as microorganism test panel.

Anticandidal effects of the compounds were screened by using partly modified CLSI (formerly NCCLS) liquid micro dilution method M27-A2. Standard antifungals and EOs dissolved in sterile 50% DMSO (CLSI document M27-A2). Amphotericin B and ketoconazole were used as standard agents [17].

3. Results and Discussion

3.1. Chemical Investigation of Essential Oil

The EOs of aerial parts, inflorescence and roots of *P. heyniae* were analysed by GC-GC/MS. As a result, forty-four, thirty-nine and twenty-five components were identified representing 94.4%, 99.5% and 100.0% of the oils respectively. The resulting ratio of main components and oil yields were shown in Table 1.

Table 1. Composition of the essential oils of *Prangos heyniae*

RRI[a]	RRI[b]	Compounds	Aerial Parts %	Inflorescence %	Roots %
1032	1008-1039 [c]	α-Pinene	0.5	3.2	**44.8**
1076	1043-1086 [c]	Camphene	0.1	0.4	4.4
1093	1056-1106 [c]	Hexanal	-	-	0.1
1118	1085-1130 [c]	*β*-Pinene	0.1	0.3	**12.5**
1132	1098-1140 [c]	Sabinene	0.1	0.1	1.1
1159	1122-1169 [c]	δ-3-Carene	-	-	**10.2**
1174	1140-1175 [c]	Myrcene	-	0.3	3.6
1176	1148-1186 [c]	α -Phellandrene	-	-	0.6
1188	1154-1195 [c]	α-Terpinene	-	-	0.3
1195	1167-1197 [c]	Dehydro-1,8-cineole	1.2	0.4	-
1203	1178-1219 [c]	Limonene	0.4	1.6	**15.3**
1213	1188-1233 [c]	β-Phellandrene	0.1	-	0.3
1244	1213-1249 [c]	Amylfuran	t	-	0.1
1246	1211-1251 [c]	(*Z*)-β-Ocimene	-	-	t
1255	1222-1266 [c]	γ-Terpinene	t	-	0.9
1266	1232-1267 [c]	(*E*)-β-Ocimene	t	-	tr
1280	1246-1291 [c]	*p*-Cymene	0.1	-	0.4
1286	1286[e,f]	Isoterpinolene	-	-	0.3
1290	1261-1300 [c]	Terpinolene	-	-	0.8
1497	1462-1522 [c]	α-Copaene	0.3	0.5	-
1535	1496-1546 [c]	β-Bourbonene	t	1.1	-
1589	1547-1589 [c]	β-Ylangene	t	t	-
1590	1549-1597 [c]	Bornyl acetate	t	t	0.4
1600	1565-1608 [c]	β-Elemene	0.6	1.5	-
1602	1550-1603 [c]	β-Copaene	-	0.5	-
1611	1564-1630 [c]	Terpinen-4-ol	-	-	t
1612	1569-1632 [c]	β-Caryophyllene	2.2	1.2	0.1
1651	1612-1654 [c]	γ-Elemene	-	3.6	-
1661	1624-1668 [c]	Alloaromadendrene	-	0.5	-
1687	1637-1689 [c]	α-Humulene	1.4	4.7	-
1690	1665-1691 [c]	*trans*-Verbenol	0.1	-	-
1704	1655-1704 [c]	γ-Muurolene	t	0.3	-
1726	1676-1726 [c]	Germacrene D	**12.7**	**15.6**	-
1741	1698-1748 [c]	β-Bisabolene	0.8	5.5	-
1748	1670-1740 [c]	*p*-Mentha-1,5-dien-8-ol	0.3	-	-
1755	1692-1757 [c]	Bicyclogermacrene	0.5	4.6	-
1773	1722-1774 [c]	δ-Cadinene	0.8	1.4	-
1776	1735-1782 [c]	γ-Cadinene	0.1	0.3	-
1797	1750-1800 [c]	Selina-3,7(11)-diene	0.5	-	-
1827	1770-1834 [c]	(*E*,*E*)-2,4-Decadienal	-	-	0.1
1853	1778-1854 [c]	Germacrene B	-	**12.4**	-
1945	1949[d]	1,5-Epoxy-salvial(4)14-ene	0.8	0.3	-
1957	1884-1964 [c]	Cubebol	0.2	-	-
2000	2000[d]	Isocaryophyllene oxide	0.4	-	-
2008	1936-2023 [c]	Caryophyllene oxide	4.5	2.1	0.2
2057	2014-2062 [c]	Ledol	-	0.6	-
2071	2003-2071 [c]	Humulene epoxide-II	1.8	1.6	-
2096	2043-2103 [c]	Elemol	**36.2**	3.8	-
2144	2074-2150 [c]	Spathulenol	1.4	1.5	-
2185	2147-2199 [c]	γ-Eudesmol	3.0	-	-
2187	2136-2200 [c] 2192[d]	T-Cadinol	-	0.8	-
2209	2153-2209 [c] 2209 [d]	T-muurolol	0.6	0.6	-

Table 1 continued..

RRI[a]	RRI[b]	Compounds	Aerial Parts %	Inflorescence %	Roots %
2250	2186-2250 [c]	α-Eudesmol	2.6	0.6	-
2255	2180-2255 [c]	α-Cadinol	3.6	1.9	-
2257	2196-2272 [c]	β-Eudesmol	0.7	-	-
2278	2231[f]	Torilenol	-	0.4	-
2361	2380 [d]	β-Bisabolenal	**14.1**	**21.6**	0.4
2369	2351-2402[c]	Eudesma-4(15), 7-dien-1β-ol	0.2	-	-
2563	2574 [d]	β-Bisabolenol	0.4	1.4	-
2607	2613 [d]	14-Hydroxy-δ-cadinene	0.2	-	-
2931	2862-2945[c]	Hexadecanoic acid	0.8	0.7	-
2942	2942[f]	Osthol	-	1.6	3.1
		Monoterpene hydrocarbons	1.4	5.9	95.5
		Oxygenated monoterpenes	1.6	0.4	-
		Sesquiterpenes hydrocarbons	19.9	53.7	0.1
		Oxygenated sesquiterpenes	56.6	15.6	0.2
		Others	14.9	23.9	4.2
		Total %	94.4	99.5	100.0
		Oil Yields (%)	0.29	0.26	0.73

RRI[a]: RRI Relative retention indices experimentally calculated against n-alkanes; RRI[b]: RRI from literature (c [18]; d [19]; e [28]; f [20]) for polar column values; % calculated from FID data; t; Trace (<0.1 %); Identification Method: tR, Identification based on comparison with co-injected with standards on a HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries.

The main components in the aerial parts were detected as elemol (36.2%), β-bisabolenal (14.1%) and germacrene D (12.7%) while in the inflorescence were found β-bisabolenal (21.6%) germacrene D (15.6%) and germacrene B (12.4%). Major compounds of the root oil were detected as α-pinene (44.8%), limonene (15.3%), *β*-pinene (12.5%) and δ-3-carene (10.2%).

The EO of aerial parts of *P. heyniae* was constituted mainly by hydrocarbon monoterpenes 1.4%, oxygenated monoterpenes 1.6%, hydrocarbon sesquiterpenes 19.9% and oxygenated sesquiterpenes represents 56.6% while EO from inflorescence has hydrocarbon monoterpenes 5.9%, oxygenated monoterpenes 0.4%, hydrocarbon sesquiterpenes 53.7% and oxygenated sesquiterpenes 15.6%. The roots oil of the plant was dominated by hydrocarbon monoterpenes 95.5%, hydrocarbon sesquiterpene 0.1%, oxygenated sesquiterpene 0.2%. In earlier studies, the main compounds of the fruit EOs of *P. heyniae*, collected from two localities, were reported as β-bisabolenal (53.3% - 18.0%), β-bisabolenol (14.6% - 2.3%), β-bisabolene (12.1% - 10.1%), germacrene D (13.5%) and germacrene B (9.4%) [19]. In other study, fruit EOs of the *P. heyniae* were β-bisabolenal (70.7%), β-bisabolene (14.4%) and β-bisabolenol (8.4%). In a study by Ozek et al., the aerial parts EOs of *P. heynia* were reported as the main compound of elemol (29.5%), germacrene D (12.1%) and unidentified compound [21]. Also in addition in our research we found that β-bisabolenal (14.1%) was main compound. There are differences in the aerial parts of EOs of *P. heyniae* compared to other species, especially in terms of the major components. *P. heyniae* contains elemol (36.2%), β-bisabolenal (14.1%) and germacrene D (12.7%) while another some *Prangos* species such as *P. acaulis* (DC) Bornm., *P. coryombosa* Boiss., *P. ferulaceae* (L.) Lindl, *P. serpentinica* (Rech.f., K.Rasbach, Reichst. & Bennert) Herrnst. & Heyn, Boiss, *P. uechtritzii* Boiss. & Hausskn. and *P. uloptera* DC. contain α-pinene, limonene, myrcene, β-pinene, δ-3-carene, α-terpinolene, caryophyllene, γ-curcumene, β-elemene, spathulenol, kessane, β-phellandrene, α−terpinolene, α-pinene, δ-3-carene, α-phellandrene, *trans*-β-ocimene as main compounds [22-28]. The different composition of essential oils can be thought to be due to the edaphic and climatic differences of the habitats.

There are few studies on chemical composition of root essential oil of *Prangos* species. In several previous studies have been reported that EO composition of *P. denticulata* Fisch. & Mey., *P.*

ferulacea and, *P. latiloba* Korov roots. The main compounds of the oils were determined as δ-3-carene (49.3%) and (Z)-3,5-nonadiyne-7-ene (20.4%) for *P. denticulata*, δ-3-carene for *P. ferulacea* and spathulenol (29.5%), 1,8-cineol (19.4%), *p*-cymene (17.0%), and α-bisabolol (15.3%) for *P. latiloba* [29-31]. δ-3-carene the main compound of *P. heyniae* roots (10.2%), has been reported as the main compound of *P. denticulate* and *P. ferulacea* root oil.

3.2. Anticandidal Activity of Essential Oil

The anticandidal activity data of the EOs are given Table 2. The EOs showed weak inhibitory effects against the tested microorganisms in comparison with the ketoconazole and amphotericin-B. Best inhibition was observed against *Candida utilis* with a concentration of 0.125 mg/mL in essential oil of the aerial parts. The anticandidal effects of *P. heyniae* EOs are reported here for the first time.

Table 2. Anticandidal activity (MIC, µg/mL) of *P. heyniae* essential oils

***Candida* sp.**	**Strain**	**Aerial Parts**	**Root**	**S1**	**S2**
C. albicans	ATCC 10231	500	500	0.5	0.06
C. utilis	NRRL Y-900	125	250	0.5	0.06
C. albicans	ATCC 90028	500	500	0.5	0.03
C. tropicalis	ATCC 750	500	500	1	0.03
C. parapsilosis	ATCC 22019*	500	500	0.5	0.06
C. krusei	ATCC 6258*	500	500	0.5	0.25

S1: Amphotericin B, S2: Ketoconazole, *CLSI quality control strains

In consequence of this finding, as far as we know, roots and inflorescence essential oil components of *P. heyniae* were studied first time. According to test results, different parts of *P. heyniae* essential oils contain elemol, β-bisabolenal, germacrene, β-bisabolenal, germacrene D, germacrene B, α-pinene, limonene, *β*-pinene and δ-3-carene. Essential oils showed weak to moderate effects on the tested *Candida* strains between the MIC values of 125 to 500 µg/mL. This is the first report on the anticandidal effect of *P. heyniae* essential oil.

3.3. Chemtaxonomic Evalaution

The secondary metabolites are widely exploited groups of compounds utilized for chemotaxonomic classification. Although essential oil composition is affected by edaphic and environmental conditions, it could be considered as a useful tool to distinguish morphologically difficult and taxonomically problematic taxa. *P. heyniae* was separated from closely related *P. corymbosa* by using morphological features such as leaf morphology, sexual differentiation of the inflorescence and hair type of petals. Besides morphological differences, the presence of Elemol in *P. heyniae* and the complete absence of these compounds in *P. corymbosa* strengthen the taxonomic position as independent species [33]. Likewise, β-bisabolenal was found to be the main component in *P. heyniae* in both present and previous studies [19, 21], and the absence of any *Prangos* species as a major component in the literature could be considered one of the evidence of chemical separation. (Table 3).

Table 3. The main compounds reported in EOs of *Prangos* species

Taxa	Plant part	Compounds (content)	Ref.
P. acaulis	Ap	δ-3-carene(25.5%) α-terpinolene(14.7%) α-pinene (13.6%)	[26]
	Ap	cis-sesquisabinene hydrate (25.6%) α-pinene (12.5%)	[23]
P. asperula	Fr	sabinene (20.6%) β-phellandrene (19.0%) γ-terpinene (9.0%)	[25]
	L	2,3,6-trimethyl benzaldehyde (18.4%) δ-3-carene (18.0%) α-pinene (17.4%)	[32]
P. asperula subsp. *haussknechtii*	Fr	δ-3-carene (16.1%) b-phellandrene (14.7%) α -pinene (10.5%) α -humulene (7.8%)	[15]
P. coryombosa	Ap	β-elemene (22%) spathulenol (12.5%) kessane (10.7%)	[33]
P. denticulate	Fr	sabinene (26.1%) *p*-cymene (19.7%)	[30]
	R	δ-3-carene (49.3%) (*Z*)-3,5-nonadiyne-7-ene (20.4%)	
P. ferulaceae	Ap	β-phellandrene (20.3%) α−terpinolene (15.2%) α-pinene (11.5%)	[28]
	Ap	β-pinene (43.1%) α-pinene (22.1%) δ-3-carene (16.9%)	[34]
	Ap	β -pinene (22.9%) 6-3-carene (16.0%)	[35]
	Se	β -pinene (33.0%) α -pinene (10.1%)	
	Ap	(*E*)- β-ocimene (43.1%) (*Z*)- β-ocimene (15.8%)	[36]
	Fr	chrysanthenyl acetate (26.53%), limonene (19.59%), α-pinene (19.50%),	[37]
P. gaubae	Ap	germacrene D (26.7%) caryophyllene oxide (14.3%) (*E*)-caryophyllene (13.8%)	[38]
P. heyniae	Ap	β-bisabolenal (1.4-70.7%) elemol (3.4-46.9%) kessane (26.9%) β-bisabolene (14.4%)	[21]
	Fr	β-bisabolenal (53.3 and 18.0%) β-bisabolenol (14.6 and 2.3%) germacrene D (13.5)	[19]

Table 3 continued..

Taxa	Plant part	Compounds (content)	Ref.
P. latiloba	Ap	α-pinene (25.1%) limonene (16.1%) myrcene (9.5%)	[39]
	Fl	limonene (18.3%) myrcene (10.4%) (*E*)-β-ocimene (7.8%)	[40]
	L	limonene (17.4%) myrcene (9.4%) α-pinene (6.1%)	
	S	limonene (13.5%) myrcene (8.6%) α-phellandrene (4.9%)	
P. pabularia	Fr	α-pinene (4.2 and 23.9%) α-humulene (16.6 and 15.5%) bicyclogermacrene (16.1 and 7.9%) spathulenol (10.6 and 5.7%)	[41]
	L	spathulenol (16.0%) α-bisabolol (14.3%)	[42]
	Fr	α-pinene (33.87%) spathulenol (9.32%) α-santalene (7.05%)	
	Um	α –pinene (21.46%) α -santalene (6.36%) p-methoxyacetophenone (5.39%)	
P. peucedanifolia	Fl	β-pinene (35.5%) α−pinene (22.1%) β-phellandrene (12.5%)	[43]
	L	*m*-cresol (50.3%)	
P. platychlaena	Fr	δ-3-Carene (3.3%) *p*-cymene (3.3%)	[44]
	L	(*E*)-β-Ocimene (25.93%) Bornyl acetate (24.58%) α-Pinene (5.84%)	[45]
	S	Bornyl acetate (25.49%) (E)-β-Ocimene (22.94%) α-Pinene (9.5%)	
	Fl	(*E*)-β-Ocimene (28.5%) Bornyl acetate (24.18%) γ-Terpinene (14.15%)	
P. scabra	Fr	β-elemene (23.3%) (*Z*)-β-farnesene (16.2%) γ-cadinene (10.0%)	[46]
	Fl	epi-globulol (21.9%) β-elemene (19.7%) caryophyllene oxide (9.0%)	
P. serpentinica	Ap	β−carryophyllene (26.4%) δ-3-carene (6.1%) linalool (5.7%) α-phellandrene (5.3%)	[27]
P. uechtritzii	Ap	α-pinene (40.8%) nonene (17.0%) β-phellandrene (11.1%)	[44]

Table 3 continued..

Taxa	Plant part	Compounds (content)	Ref.
P. uloptera	Ap	β-caryophyllene (27.1%)	[47]
		caryophyllene oxide (15.9%)	
		α-pinene (12.4%)	
	Fl	saferole (21.6%)	
		α-pinene (20%)	
	Fr	α-terpinene (35.5%)	
		trans-anethole (23.5%)	
P.turcica	Fr	α-humulene (11.0%)	[48]
		germacrene D (10.6%)	
		naphthalene (8.5%)	

Ap: Aerial part, S: Stem, L: Leaves, Fl: Flower, Um: Umbel, Fr: Fruit, Se: Seed

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