

Morphological, Indumentum and Chemical Characteristics and Analysis of the Volatile Components of the Flowers of *Rhododendron ponticum* L. subsp. *ponticum* (Ericaceae) of Turkish Origin[§]

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Abstract: Morphological, chemical and indumentum characteristics of *Rhododendron ponticum* L. subsp. *ponticum* collected from Bartın province have been investigated. A detailed description of the species has been prepared and compared with that published in the Flora of Turkey of Davis in a tabular form. The taxonomic and morphological characteristics of the plant material have been described and illustrated by drawings. Indumentum characteristics were also investigated. Volatiles of the flowers were trapped by a dynamic headspace SPME setup and their hexane extract were analyzed by GC/MS. Main components of the headspace trapped volatiles of flowers were characterized as α -pinene (44.5 %), β -pinene (10.8 %), linalool (4 %) and limonene (3.3 %) whereas main components in volatiles of the hexane extract were linalool (19.6 %), phenylethyl alcohol (19.1 %), myrtenol (10.1 %), citronellol (9.4 %) and phenylacetaldehyde (7.8%).

Key words: *Rhododendron ponticum* subsp. *ponticum*, ericaceae; morphology; indumentum; chemistry, GC-MS HS-SPME. © 2018 ACG Publications. All rights reserved.

1. Introduction

More than 850 species of *Rhododendron* L. (Orman Gülü) are distributed in the Northern Hemisphere. The genus *Rhododendron* distributed in North Eastern and West Anatolia is represented by 9 species two being endemic and altogether 12 taxa, 4 hybrids and 1 form [1-5].

These are *Rhododendron. luteum* Sweet (Sarı çiçekli ormangülü), *R. ungerii* Trautv. (Ak Ormangülü), *R. smirnowii* Trautv. (Kızıl Kumar) and., *R. caucasicum* Pallas (Kafkas Ormangülü), *R. ponticum* L. subsp. *ponticum* (Mor Çiçekli Ormangülü), *R. ponticum* subsp. *ponticum* forma *album*, *R. x sohadzeae* Charadze & Davlianidze, *R. x rosifaciens* R.Milne, *R. x davisianum* R.Milne, *R. x filidactylis* R.Milne [1-5].

Leaves and flowers of *Rhododendron* (especially *R. luteum* and *R. ponticum*) contain toxic compounds (andromedotoxin). Honey from the flowers of *Rhododendron* is locally known as ‘deli bal,

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acı bal, tutar bal” (Mad honey). It has antihypertension activity and when consumed it inflicts neurotoxicity by causing light-headedness and hallucinations. *Rhododendron* species are also used as decorative plants [6-10]. When consumed, consciousness disorders similar to drunkenness are observed. Overdose is lethal. Leaves of *R. luteum* (Kumar leaf) contain tannin, essential oil, ericolin, arbutin and andromedol derivatives. Although used as diuretic and analgesic in rheumatic pains its infusion can be dangerous due to andromedol derivatives. A decoction of *R. luteum* and *R. ponticum* leaves is externally used to treat fungal foot infectious in Giresun province (Dereli, Çalca Eğriambar). *R. luteum* is also used as ornamental due to its showy flowers [6-10].

R. ponticum is an evergreen plant, growing up to 10 m in Northern and Eastern Anatolian mountains. This plant known as “Mor çiçekli ormangülü, Alp gülü, Komar, Karaağu, Kara Kumar, Kumar” [6-10].

The volatile profile of some *Rhododendron* species have been investigated. 1-Methyl-2-pyrrolidone was reported as main compound in the CH₂Cl₂ extract flowers of *Rhododendron ponticum* L. [11].

This report concerns morphological, chemical and indumentum characteristics of *Rhododendron ponticum* subsp. *ponticum* collected from Bartın province. A detailed description of the species has been prepared and compared with that published in the Flora of Turkey of Davis in a tabular form. The taxonomic and morphological characteristics of the plant material have been described and illustrated by drawings. Indumentum characteristics were also investigated. Volatiles of the flowers and their hexane extract were trapped by a dynamic Headspace Solid-Phase Microextraction setup and were analyzed by GC-MS (HS-SPME-GC-MS).

This is the first report on the morphological, indumentum characteristics and headspace volatiles and hexane extract of the flowers of this plant.

2. Materials and Methods

2.1. Plant Material

Aerial parts were collected in May 2011, A4 Bartın: Hasan kadı beldesi, Akbay köyü, 300 m. Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy, Anadolu University in Eskişehir, Turkey (ESSE 14428).

2.2. Morphological and Anatomical Studies

The plant material was identified as *R. ponticum* subsp. *ponticum* using the Flora of Turkey and the East Aegean Islands (Stevens 1978). Herbarium specimens were used for description and detailed morphological drawings. A Leitz SM-LUX binocular microscope with drawing tube was used for anatomical drawings. A wild M5 A stereo microscope with drawing tube was utilized for morphological drawing.

2.3. Headspace-Solid Phase Micro Extraction (HS-SPME) Analysis

60 min sampling was carried out on live plant materials using a blue fibre Polydimethylsiloxane-Divinylbenzene (PDMS/DVB - 65µm) – Blue (supplied by Supelco Bellefonte, USA). The fiber was directly desorbed in GC/MS for 10 min.

Headspace-SPME procedure for the extract: The volatiles were trapped by SPME. SPME fiber coated with PDMS/DVB - 65µm was used with a sampling time of 15 min-50 °C. Thermal desorption at 250°C for 10 min.

2.4. GC/MS Conditions

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60m x 0.25mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min.). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1°C/min., at splitless mode. The injector temperature was at 250 °C. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

The components of essential oils were characterized by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRI). The results of analysis are shown in Table 2.

3. Results and Discussion

3.1. Morphological Observations

Rhododendron ponticum subsp. *ponticum* (Figure 1) is a large shrub, to 10 m Stem with variable indumentum when young, but not tomentose, glabrous-glandular; terminal bud *ca.* 1.5-2 cm. Petiole 1-1.7 cm, glabrous-glandular; lamina elliptic-obovate, 6-12.5×2-4.5 cm, base acute, margin repant-integer, obtuse-acute at apex, coriaceous, glabrous-glandular hairs, fine venation usually flat above.



Figure 1. *Rhododendron ponticum* subsp. *ponticum*. a—plant; b—leaf; c-- bracts; d—bracteols; e-- flower; f-- pistil; g-- stamen; h-- capsule; i-- nutlets.

Inflorescence terminal, racemous, 7-25 flowered, axis 5-7.5 cm, peduncle 1.5-8 cm; pedicels 2-3 cm, glabrous glandular hairs, bracts 6-10×2-3.5 cm, ovate-linear, deciduous and bracteols 1-2.5 cm, pubescent-eglandular. Calyx 5 lobes, ovate-linear, lobes 0.6-4 mm, glabrous-glandular. Corolla 2.8-3.6 cm, purplish-pink, upper with yellow spots, campanulate, 2-8×3-6 cm, 1-2 cm across, 5 lobes, acute-rounded, lobes 1.5-2.5 cm, usually glabrous outside, pubescent towards base inside, tube 1.2-1.6 cm., Stamens 6-10, 2.5-4 cm. Anther dorsifixed, pink, 2.3-3 mm. Filament 2.2-3.7 cm, pink, base pubescent. Ovary 4-5×1.5-2 cm, cylindric, 5-locular, glabrous-eglandular; style 3-4 cm, glabrous-glandular; stigma capitate, pink -pale brown. Capsule 1.5-3 cm, septicidal capsule; seeds *ca.* 2.5 mm (Figure 1). Flowering period: (3-)5-6(-8). Habitat: Usually in *Fagus orientalis* forest, rarely in other types of forest or above tree line. Altitude: (s.l.-)150-1800 (-2100) m. Distribution in Turkey: N. Turkey. E. Balkans, Caucasus, Georgia, Lebanon. Euxine element [3].

Table 1. Morpho-metrical characters of *Rhododendron ponticum* subsp. *ponticum* (Figure 1)

Morpho-metrical characters		Flora of Turkey	The findings of this study
Indumentum of stem		variable when young, but not tomentose	Glabrous, glandular
Petiole	indumentum	--	Glabrous, glandular
	size	(6-)10-17×(2.8-)3.5-4.5 cm	6-12.5×2-4.5 cm
	base	--	Acute
Lamina	margin	--	Repand-integer
	apex	--	Obtuse-acute
	indumentum	Coriaceous, initially tomentose below but soon glabrescent, glandular hairs persistent	Glabrous, glandular
Inflorescences	Number of flowers	5-20	7-25
	shape	Terminal	Terminal racemous
	axis	1.3-7 cm	5-7.5 cm
Peduncle	size	1.3-7 cm	1.5-8 cm
	size	2-2.5 cm	2-3 cm
Pedicel	shape	Usually glabrous, sometimes with glandular hairs or pubescent	Glabrous, glandular
Bracts	shape	Ovate-linear	Ovate-linear, deciduous
	size	2.5-3.5 cm	6-10×2-3.5 cm
	indumentum	--	Pubescent, eglandular
	Shape	--	Ovate -linear, deciduous
Bracteols	Size	2.5-3.5 cm	1-2.5 cm
	Indumentum	--	Pubescent, eglandular
	Shape of lobes	--	Ovate-linear
Calyx	Size of lobes	0.6-1(-3) mm	0.6-4 mm
	Number of lobes	--	5
	Indumentum	--	Glabrous, glandular

<i>Table 1 Continued..</i>				
	colour	Purplish-pink, upper with yellow spots	Same	
	shape	Campanulate	Campanulate	
	size	--	2.8-3.6 cm	
	Shape of lobes	Acute-rounded	Acute-rounded	
Corolla	Number of lobes	--	5	
	Size of lobes	1.5-2.5 cm	1.5-2.5 cm	
	Across of lobes	1.5-2.5 cm	1-2 cm	
	Size of tube	1.5-2.5 cm	1.2-1.6 cm	
	Indumentum	Usually glabrous outside, pubescent towards base, inside pubescent	Same	
		number	10	6-10
	Stamen	size	--	2.5-4 cm
shape		--	Dorsifixed	
Anther		colour	--	Pink
	size	--	2.3-3 mm	
	size	--	2.2-3.7 cm	
Filament	colour	--	Pink	
	<i>Table 1 Continued..</i>			
	indumentum	--	Base pubescent	
	size	--	4-5×1.5-2 cm	
Ovary	shape	5-locular	Cylindrical, 5-locular	
	indumentum	Glabrous	Glabrous, eglandular	
	size	3-4 cm	3-4.5 cm	
Style	indumentum	Glabrous	Glabrous, glandular	
	shape	Capitate	Same	
Stigma	colour	--	Pink -Pale brown	
	shape	Septicidal capsule	Same	
Capsule	size	1.6-2.8 cm	1.5-3 cm	
	Seeds	size	c.2 mm	2.5 mm

Leaves elliptic-obovate, glabrous-glandular hairs on surfaces. Flowers are pink- purple. Flowers are usually with 5 fragments. Flowering period, usually in summer months [3].

To the best of our knowledge, there is no morphological study on *R. ponticum* subsp. *ponticum*. Morphological and morphometric descriptions are compared with those in the Flora of Turkey as shown in Table 1 and Figure 1. Most of our findings were in agreement with those features published in the Flora of Turkey. However, lower limits of leaf, pedicel, style and fruit dimensions were found to be higher in our findings. This is possibly due to the number of specimens investigated and to the ecological reasons. Here, we also report for the first time the base, margin, shape of lamina, type of inflorescence, calyx tooth shape and number, corolla length, shape and number of lobes, stamen length, shape colour and length of anther, colour and length of filament, ovary size and shape, style colour, stigma shape and colour, seed colour characteristics [3].

3.2. Indumentum

Indumentum characteristics of stem, leaf, pedicel calyx, corolla, ovary and style were investigated by anatomical studies in comparison with those given in the Flora of Turkey in Table 1. Covering trichomes are simple with 1-2 cells; glandular hairs are emergence. Indumentum characters deviated from those reported by Davis [3]. In addition to those characters published in Flora of Turkey, we also observed glabrous-glandular on the stem and glabrous-glandular on the pedicel. Covering trichomes on the leaves are glabrous-glandular. Bracts, bracteols and ovary are glabrous-eglandular. Calyx and style are glabrous-glandular (Figure 2).

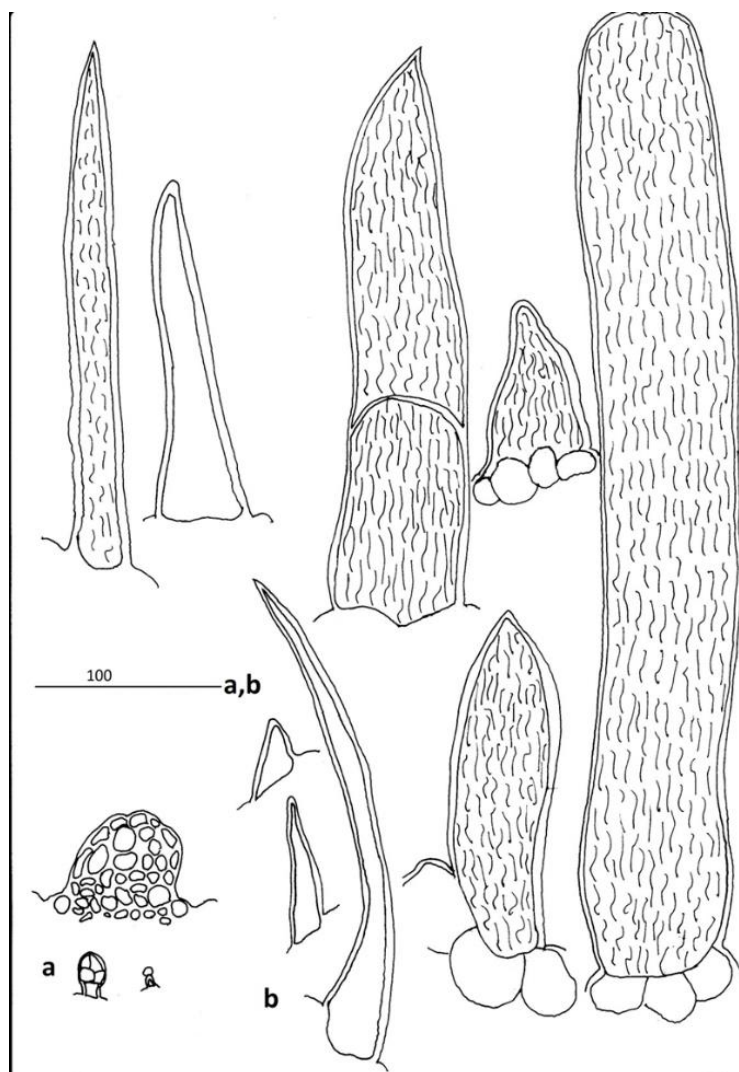


Figure 2. In light microscope of hairs of *Rhododendron ponticum* subsp *ponticum*. a- glandular hairs (emergence); b- covering hairs

3.3. Composition

Essential oils of some *Rhododendron* species have been investigated. Mostly terpenes such as α -humulene, caryophyllene, limonene, α - or -pinene comprise the main constituents of the essential oils. A study reported mostly non-terpenic hydrocarbons, alcohols, esters and ketones as the major components [11]. The main poisonous constituents of *Rhododendron* plants, grayanotoxins (andromedotoxins), were not detected in any of the extracts. These diterpenes are unstable on heating and have low vapor pressure, hence they require derivatization (TMS) before the GC analysis. Andromedotoxin has been isolated from the waste material in the production of *Rhododendron* essential oils [11].

Table 2. Composition of the flower volatiles of *R. ponticum* subsp. *ponticum*

RRI ^a	RRI ^b	Compounds	A %*	B %	ID**
1032	1025 ^{c,d} 1032 ^g	α-Pinene	44.5	1.3	RRI, MS
1118	1117 ^{c,d}	β-Pinene	10.8	-	RRI, MS
1203	1212 ^{c,d}	Limonene	3.3	-	RRI, MS
1205		3-Hexanol	-	2.7	RRI, MS
1213	1013-1039 ^f 1213 ⁿ	1,8-Cineole	0.8	-	RRI, MS
1223		2-Hexanol	-	3.5	MS
1266	1249 ^c	(<i>E</i>)- β -Ocimene	1.3	-	MS
1267		3-Octanone	1.4	-	RRI, MS
1280	1268 ^e 1282 ^d	<i>p</i> -Cymene	1.8	-	RRI, MS
1360	1360 ^{n,t}	1-Hexanol	0.8	0.5	RRI, MS
1400	1400 ⁿ	Tetradecane	1.1	0.3	RRI, MS
1418		(<i>Z</i>)-2-Hexen-1-ol	-	0.1	RRI, MS
1450	1450 ^c	<i>trans</i> -Linalool oxide (<i>Furanoid</i>)	-	0.6	MS
1452	1452 ^h	<i>p</i> -Cymenene	0.6	-	MS
1453	1444-1452 ^d	1-Octen-3-ol	2.9	6.5	RRI, MS
1466	1466- 1480 ^d	α -Cubebene	0.2	-	MS
1478	1445-1448 ^c	<i>cis</i> -Linalool oxide (<i>Furanoid</i>)	0.3	1.7	MS
1496	1496 ^s	2-Ethyl-1-hexanol	1.1	-	MS
1497	1488 ^d	α -Copaene	1.4	0.5	MS
1532	1515-1532 ^d	Camphor	1.4	-	RRI, MS
1535	1523 ^d 1535 ^k	β -Bourbonene	-	0.5	RRI, MS
1541	1541 ^{n,s}	Benzaldehyde	-	1.0	MS
1541		Isolongifolene	0.8	-	RRI, MS
1548	1548 ^t	(<i>E</i>)-2-Nonenal	-	0.2	MS
1553	1538 ^c -1553 ^{c,g}	Linalool	4.0	19.6	RRI, MS
1562	1562 ^t	Octanol	-	0.2	RRI, MS
1583	1583 ^r	Longifolene	1.7	0.1	MS
1589	1576 ^d	β -Ylangene	-	0.4	MS
1612	1608- 1612 ^c	β -Caryophyllene	1.3	0.5	RRI, MS
1621		2-Octen-1-ol	-	0.6	MS
1641	1641 ^t	Methyl benzoate	-	0.3	MS

Table 2 Continued..					
1648	1648 ^k	Myrtenal	1.0	1.6	MS
1658	1663 ^s	Phenyl acetaldehyde	-	7.8	MS
1704	1689 ^d	γ -Muuroolene	-	0.3	MS
1706	1695 ^{c,e} -1698 ^e	α -Terpineol	1.6	2.2	RRI, MS
	1706 ^{c,k,g}				
1719	1700-1701 ^e	Borneol	0.5	0.3	RRI, MS
1725	1720-1725 ^d	Verbenone	0.7	-	RRI, MS
1726	1711 ^h	Germacrene D	-	0.6	MS
	1722-1726 ^c				
1733	1740 ^m	Valencene	0.5	-	RRI, MS
1740	1723 ^d	α -Muuroolene	0.2	-	MS
1758	1758n	(<i>E,E</i>)- α -Farnesene	0.7	-	MS
1772	1763 ^{f,g}	Citronellol	0.7	9.4	RRI, MS
	1772n				
1773	1755 ^d	δ -Cadinene	0.5	-	MS
	1750-1752 ^e				
1776	1763 ^d	γ -Cadinene	0.4	-	MS
	1748-1749 ^e				
1802	1790-1804 ^{d,h}	Myrtenol	2.1	10.1	MS
1849	1849-1927 ^d	Calamenene	0.3	-	MS
1857	1839-1857 ^{d,g}	Geraniol	-	1.5	RRI, MS
1896	1896 ^s	Benzyl alcohol	-	1.8	MS
1937	1937 ^g	Phenylethyl alcohol	0.5	19.1	MS
2045	1988 ^p	Isopropyl myristate	0.3	-	MS

A: Headspace volatiles of the fresh flowers; B: Headspace volatiles of the hexane extract; RRI^a: RRI Relative retention indices experimentally calculated against *n*-alkanes; RRI^b: RRI from literature (c [13]; d [14]; e [15]; f [16]; g [17]; h [18]; k [19]; m [20]; n [21]; p [22]; r [23]; s [24]; t [25]) for polar column values; *% calculated from FID data; **ID: Identification Method; Identification method based on the relative retention indices (RRI) of authentic compounds 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 [26], MassFinder [27] and Wiley [28] libraries.

In previous studies, headspace volatiles were reported from flowers of *R. luteum*. Main odour components were found as β -caryophyllene (34.0%), methyl benzoate (11.7%), (*E*)- β -ocimene (10.4%) and α -pinene (10.0%) [12].

Headspace volatiles of fresh flowers of *Rhododendron ponticum* and the hexane extract were analyzed by gas chromatography/mass spectrometry. The volatiles were trapped by SPME in a dynamic headspace set up. A blue -Polydimethylsiloxane/Divinylbenzene (PDMS / DVB fibre was used.

Thirty -four and thirty-one volatile compounds were identified in both of the fresh flowers and hexane extract representing 91.5 % and 95.8 % of the total volatiles respectively.

Volatiles of *R. ponticum* subsp. *ponticum* were trapped by blue SPME fibre over a period of 1.5 hours. Main components were identified as α -pinene (44.5 %), β -pinene (10.8 %), linalool (4 %) and limonene (3.3 %).

Volatiles of the hexane extract of *R. ponticum* were trapped on an HS-SPME Blue fibre. Main components were identified as linalool (19.6 %), phenylethyl alcohol (19.1 %), myrtenol (10.1 %), citronellol (9.4 %) and phenylacetaldehyde (7.8%).

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