Original Article

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Analysis of the essential oil chemical profiles of two Orobanche species: *O. laxissima* Uhlich & Rätzel and *O. ramosa* L.

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Abstract

The Orobanche genus (broomrapes) is well-known as an herbaceous parasitic plant with a long history in traditional Asian medicine and folklore foods. The volatile components of two Orobanche species, *O. laxissima* and *O. ramosa* (branched broomrape), from two different areas, East Azerbaijan and Fars provinces, were analyzed in this study using hydrodistillation extraction and gas chromatography-mass spectrometry (GC-MS). Essential oils from *O. laxissima* and *O. ramosa* aerial parts consisted of fourteen and eight compounds, respectively, which represented 97.15% and 99.8% of total oil compositions. *O. laxissima* essential oil contains carvacrol (53.35%), thymol (9.65%), and trans-caryophyllene (5.17%), while, as a substantial difference, *O. ramosa* essential oil mostly contains carvacrol (81.53%), α-terpinolene (4.57%), and trans-caryophyllene (3.6%). As a significant difference, *O. laxissima* essential oil includes a significant content of aliphatic hydrocarbons, including tricosane (5.46%), tetracosane (5.62%), and docosane (1.13%), whereas *O. ramosa* essential oil excludes these components. The monoterpene, and sesquiterpene profiles of both plants were similar, especially for α-terpinolene, spathulenol, and hotrienol. Although these essential oils contained similar phytochemical profiles, the differences may serve as marker references for future research on Orobanche species.

Keywords: Orobanchaceae, Broomrapes, Essential oil, *Orobanche laxissima*, *Orobanche ramosa*

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1. Introduction

Orobanchaceae is one of the largest flowering plant families that exhibit parasitic behavior. Among the 90 Orobanchaceae plants, Orobanche (broomrapes), a genus with over 200 species, is an important member of this genera. Broomrapes are parasitic chlorophyll-less species that are predominantly distributed in the northern hemisphere (1, 2). Broomrapes are valued due to their environmental impact on agriculture as well as their therapeutic applications in medicine. Additionally, several species, such as *O. cooperi*, *O. fasciculata*, and *O. crenata*, are used as edible vegetables in various countries (3, 4). Among the most important broomrapes, *O. coerulescens*, *O. cernua*, *O. pycnostachya*, *O. yunnanensis*, *O. megalantha*, *O. ombrochares*, and *O. alba* are well-known for their traditional use, particularly in the treatment of inflammation, neurological diseases, and pain (5).

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Despite extensive use of these plants, challenging morphological distinctive characteristics made numerous concerns in species determination, which led to a greater recognition of the better taxonomic relationships established based on secondary metabolites in the last decade(6). Chemotaxonomical analyses were employed as a rapid method to identify species; this approach is based on the quantitative and qualitative detection of numerous secondary metabolites, such as essential oils (7-9). As a result, studying the composition of essential oils is a common practice in plant physiology and biochemistry (10). Despite the distinctive aromas of numerous Orobanche species, research on the compositions of their essential oils remains scarce. In this study, we investigated the chemical composition of essential oils from two fragrant broomrape species, *O. laxissima* and *O. ramosa* by gas chromatography-mass spectrometry (GC-MS).

2. Material and methods

2.1. Plant Materials

The aerial parts of both spices were collected at the flowering period between April to May 2022. *O. laxissima* was gathered from the Aynalo protected forest, near the Kaleybar city in the East Azerbaijan province of Iran (38°53'14.3"N 46°47'53.8"E, altitude 1422m). *O. ramosa* was collected from the garden of Shiraz School of Pharmacy (29°41'02.9"N 52°33'27.6"E, altitude 1745m). The plants were registered in the herbarium of the Medicinal Plants Processing Research Center with the voucher specimen numbers (MPPRC-03-03 and MPPRC-03-04), respectively.

2.2. Extraction of the Essential Oils

The air-dried aerial parts of *O. laxissima* and *O. ramosa* (500 g of each plant) were powdered and used for hydrodistillation. After 3-hour distillation, the essential oils were separated and dried using sodium sulphate. All samples were stored in the dark at -20 °C for GC-MS analysis.

2.3. Analysis of the Essential Oils

An Agilent 7890A5975CMSD instrument with a DB-5 column (60 m × 0.25 mm i.d., 0.25 µm film thickness; resolution 2000 amu/s) was used for essential oils analysis. A flow of helium with 1 mL/min was used as the mobile phase with a 2 min delay. The initial temperature of the column was 60 °C for 3 min., which it increased with a 5 °C/min rate up to 220 °C and stopped at 280 °C for 10 min. The injector temperature was adjusted at 250 °C with a 1:50 split ratio. The ionization and EM voltages were 70 eV and 3000 V, respectively. 1 µL of essential oils were used for injection with a 30-800 amu scan range, and C7-C30 n-alkanes standards were used with the chromatography conditions above on the same DB-5 column. The essential oil compounds were identified based on comparison of their relative Kovats indices and NIST NBS54K Library mass spectra matching (8, 9, 11, 12).

3. Results and discussion

Although variations in the chemical composition of essential oils demonstrate a broad spectrum of plant fragrances, some aroma similarities between species within the same genus are common. In many cases, similarities or differences between inter-genus fragrances may represent genetic, environmental, and adaptations (13). Thus, the study of molecular composition of essential oils in species within a genus is considered one of the most important methods for botanical identification of the members of the genus. The utilization of these methods has great significance in various fields, including therapeutics, cosmetics, and agriculture.

This study identified fourteen and eight compounds in the essential oils of *O. laxissima* and *O. ramosa*, respectively, accounting for approximately 97.15% and 99.8% of the total volatile compositions (Table 1). Carvacrol, α-terpinolene, and trans-caryophyllene are the major components of both plants' essential oils (Figures 1-2). Nevertheless, *O. ramosa* essential oil does not contain thymol, whereas it is a significant component of *O. laxissima* essential oil. The content of two sesquiterpene hydrocarbons, spathulenol and caryophyllene oxide, showed more similarities in both species (Table 1). Conversely, *O. laxissima* essential oil has significant quantities of aliphatic hydrocarbons such as docosane, tricosane, and tetracosane, whereas *O. ramosa* does not have any aliphatic hydrocarbons. There are few studies that have been carried out on the essential oils of Orobanche spp., and very limited information regarding their composition is accessible. An investigation on *O. cernua* revealed that more than thirty percent

Table 1. The essential oil content of *Orobanche laxissima* and *Orobanche ramosa*.

			Orobanche laxissima	*Orobanche ramosa*		
	Component	Chemical formula	Percentage in sample (%Q)	Percentage in sample (%Q)	[a]Measured Ki	[b]Literature Ki
1	α-Terpinolene	$C_{10}H_{16}$	3.27	4.57	1100	1100
2	Hotrienol	$C_{10}H1_{6}O$	1.06	2.3	1105	1109
3	Thymol	$C_{10}H1_{4}O$	9.65	-	1293	1297
4	Carvacrol	$C_{10}H1_{4}O$	53.35	81.53	1305	1307
5	trans-Caryophyllene	$C_{15}H_{24}$	5.17	3.6	1425	1423
6	Aromadendrene	$C_{15}H_{24}$	0.75	-	1444	1444
7	Viridiflorene	$C_{15}H_{24}$	1.35	-	1500	1494
8	Spathulenol	$C_{15}H_{24}O$	3.22	2.19	1584	1576
9	Caryophyllene oxide	$C_{15}H_{24}O$	4.55	2.96	1590	1596
10	Tetradecanoic acid	$C_{14}H_{28}O_2$	-	1.1	1769	1766
11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1.81	1.7	1962	1961
12	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	0.76	-	2132	2134
13	Docosane	$C_{22}H_{46}$	1.13	-	2200	2200
14	Tricosane	$C_{23}H_{48}$	5.46	-	2300	2300
15	Tetracosane	$C_{24}H_{50}$	5.62	-	2401	2400
	Monoterpene derivatives (%)		67.33	88.4		
	Sesquiterpene hydrocarbons (%)		15.04	8.75		
	Fatty acid and derivatives (%)		2.57	2.8		
	Aliphatic hydrocarbons (%)		12.21	0		
	Total (%)		97.15	99.8		

[a]Ki= Kovats retention index as determined on DB-5 column using homologous series of n-alkanes.
[b]Kovats retention indices reported in the National Institute of Standards and Technology Database (NIST) and literatures.

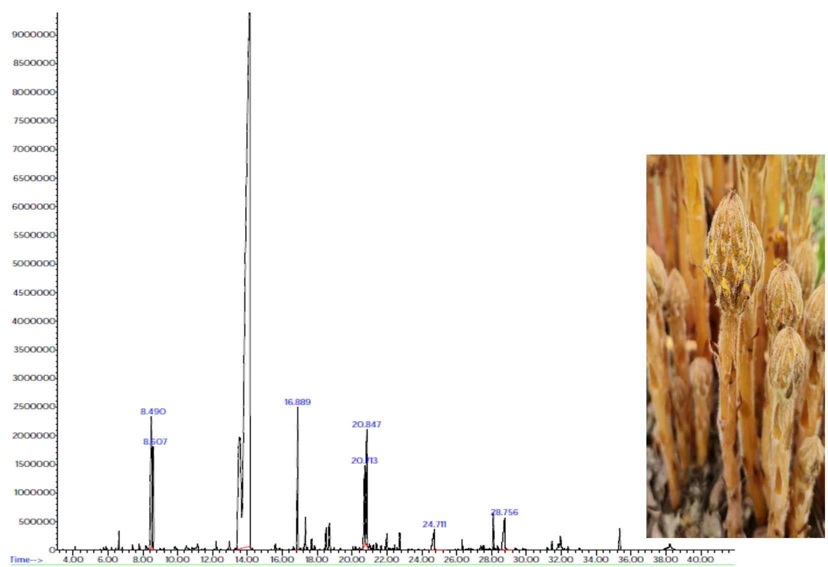

Figure 1. The arial parts of *Orobanche laxissima* and the GC-MS chromatogram of its essential oil.

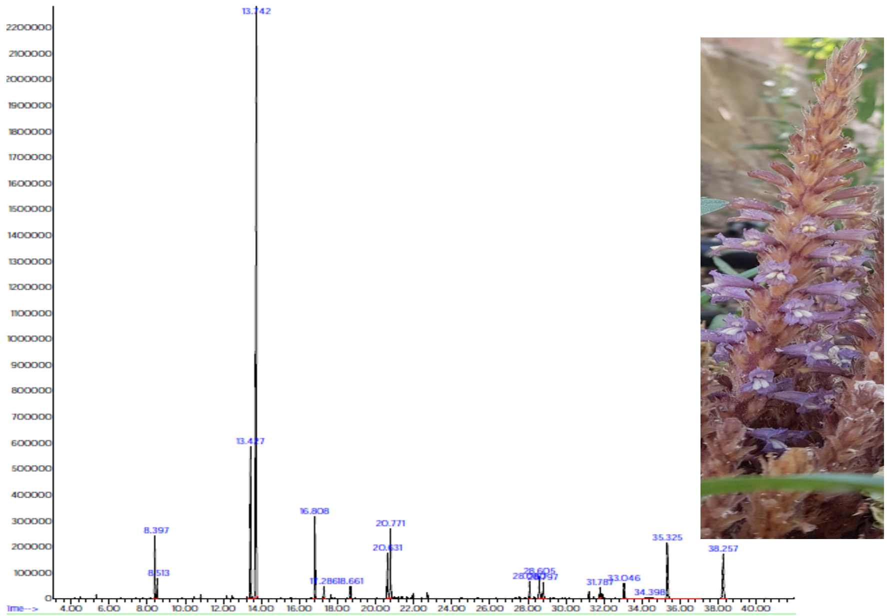

Figure 2. The arial parts of *Orobanche ramosa* and the GC-MS chromatogram of its essential oil.

of the volatile compounds in essential oil were aliphatic hydrocarbons and sesquiterpenes. The main molecules of this essential oil were diethylhexyl adipate (35.34%), 2-methylheptane (12.65 %), α-cadinol (7.25 %), and diethyl phthalate (5.17 %)(14). Another study on *O. alba* essential oil revealed that monoterpenes made up more than 50% of the essential oil content, which is clearly compatible with our samples. Additionally, palmitic acid also constituted a large portion of the composition of this recent species (12.5%)(15). Furthermore, a related investigation on the volatile chemical compounds of four Orbanche species, *O. alba*, *O. flava*, *O. elatior*, and *O. reticulata*, showed significant evidence for the presence of monoterpenes, sesquiterpenes, aliphatic hydrocarbons, and aliphatic ketones. The result of this study demonstrated a strong correlation with previous findings about the presence of monoterpene and sesquiterpene contents in other Orbanche species that it found in the essential oils of *O. laxissima* and *O. ramosa*.

4. Conclusion

This study findings reveal that the *O. laxissima* essential oil of contains a total of 14 identified compounds, constituting 97.15% of the oil's total composition, whereas *O. ramosa* exhibits only 8 identified compounds, amounting to 99.8% of its essential oil profile. Notably, carvacrol was identified as a major component in both species. Furthermore, *O. laxissima* was characterized by the presence of significant amounts of aliphatic hydrocarbons, which were absent in *O. ramosa*. These recognizable differences in essential oil composition suggest that the variations can consider as potential chemotaxonomic markers for future investigations on Orobanche species. Overall, the similarities and differences in the essential oil compositions of these two Orobanche species not only improve our understanding of the chemical diversity within this genus but also provide a fundamental data for future studies.

Conflict of Interest

The authors declare no conflict of interest.

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