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Identification of volatile oil components from aerial parts of *Trigonella torbatjamensis* Ranjbar by GC-FID and GC-MS methods

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ABSTRACT

***Objective(s)*:** Various species of genus *Trigonella* are important from medical and culinary points of view. The essential oil of *Trigonella torbatejamensis* Ranjbar as an endemic plant in Iran has not been studied previously. The essential oil of this plant was analyzed by different methods for identification of its components.

***Materials and Methods*:** The essential oil composition of aerial parts of *T. torbatjamensis* was obtained by hydro-distillation and analyzed by GC-FID and GC-MS apparatus.

Results: Forty components, representing 98.5 % of the total components, were identified. The pattern of the main grouped components in essential oil was: sesquiterpene hydrocarbons (70.2%), oxygenated sesquitepenes (16.5%), oxygenated monoterpenes (3.5%) and monoterpene hydrocarbons (0.5%). Germacrene –D (33.0%), bicyclogermacrene (26.0%), and viridiflorol (5.3%) were the main components of the essential oil.

Conclusion: The essential oil of *T. torbatjamensis* Ranjbar consisted of forty components with sesquiterpene hydrocarbons as the main group of components.

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Introduction

The genus *Trigonella* L. with nearly 135 species belongs to the tribe Trifolieae and family of Fabaceae. Most of the species are distributed in the dry regions around the east Mediterranean, western Asia, south Europe, and north and South Africa, with only one species being present in South Australia. They are herbaceous and annual or perennial plants with pinnately trifoliate leaves, often emitting an odor; and like other grain legumes, are important in food and medicine production. This genus is represented by ca. 33 species in Iran, 12 of which are endemic (1, 2). *Trigonella torbatjamensis* was recently introduced as an endemic plant in Iran (1). Various species of the genus *Trigonella* have important medical and culinary aspects. Among these, *Trigonella foenum-graecum* L. is commonly grown as a vegetable. It has some medicinal uses. Externally the drug acts as an emollient and internally reduces blood sugar, but the mechanism of its action is unclear. Additionally, a lipid-lowering effect, which is attributed to the saponin fraction, has been proven. Its approved uses by Commition E are loss of appetite and inflammation of the skin. It has been used in Indian medicine as an anti-fever and for treatment of vomiting, anorexia, bronchitis, and colitis. In Chinese medicine, the drug is used to treat impotence and hernia (3). It has been used in Indian folk medicine as an antipyretic, diuretic, and supportive, and for treatment of dropsy, heart disease, chronic cough, and enlargement of the spleen and the liver (3). There are few reports on the essential oil content of members of this genus (4-10). The essential oil of *T. torbatejamensis* Ranjbar, a species of the genus in the eastern part of Iran, has not been studied previously. We report here the composition of the essential oil of *T. torbatejamensis* which is an endemic species growing wild in Iran, for the first time.

Materials and Methods

Aerial parts of *T. torbatejamensis* were collected during the flowering stage from a region between Saghar Cheshmeh and Garmab Olia, (35 ° 45 ′ N, 61 °15′ E), 400 – 500 m., east of Saleh-Abad, near Torbat-e Jam, Razavi Khorasan province, north-east

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Table 1. Percentage of the composition of the essential oil of aerial parts of *Trigonella torbatjamensis* in comparison with other *Trigonella* species

No.	Compound	RI*	Percentage	
			In T. torbatjamensis	In the other Species of *Trigonella*
1	α- pinene	957	0.3	
2	sabinene	993	0.1	
3	*n*-decane	1020	4.6	
4	limonene	1049	0.1	14.6 (A_1); 82.3 (A_2)
5	1,8–cineole	1052	0.1	1.2 (E)
6	α-thujone	1123	0.3	
7	borneol	1182	0.2	1.8 (E)
8	terpinene-4-ol	1191	0.7	
9	α-terpineol	1204	0.2	
10	*n*-dodecane	1209	0.8	
11	carvone	1257	1.8	9.9 (A_1); 13.0 (A_2)
12	bornylacetate	1295	0.2	
13	δ-elemene	1344	0.5	
14	α-copaene	1380	0.3	
15	β-bourbonene	1388	1.1	
16	β-elemene	1395	0.8	
17	*n*-tetradecane	1399	0.3	
18	α-gurjunene	1412	0.1	
19	β-caryophyllene	1422	4.0	
20	β-copaene	1431	0.4	
21	aromadendrene	1441	0.2	
22	*cis*-muurola -3,5 – diene	1446	0.2	
23	allo–aromadendrene	1462	2.2	
24	**germacrene –D**	**1482**	**33.0**	
25	*trans*-β-ionone	1486	0.8	
26	**bicyclogermacrene**	**1496**	**26.0**	
27	γ-cadinene	1511	0.3	1.8 (C)
28	δ-cadinene	1520	1.1	27.6 (B)
29	palustrol	1564	0.4	
30	spathulenol	1575	4.6	17.8 (D)
31	caryophyllene oxide	1580	1.8	7.9 (D)
32	**viridiflorol**	**1587**	**5.3**	
33	ledol	1597	1.6	
34	unknown	1631	1.1	
35	*tau*-muurolol	1634	0.6	
36	α–cadinol	1646	1.3	1.7 (C)
37	valeranone	1663	0.9	
38	heptadecane	1680	0.4	
39	mintsulfide	1722	0.5	
40	octadecane	1774	0.4	
	Major grouped compounds			
	Monoterpene hydrocarbons		0.5	
	Oxygenated monoterpenes		3.5	
	Sespuiterpene hydrocarbons		70.2	
	Oxygented sesquiterpenes		16.5	
	Miscellaneous compounds		7.8	
	Total identified		98.5	

*****RI= The retention Kovats indices that were determined on CP-Sil 8CB capillary column
A_1, Essential oil of *T. foenum-graceum* (Seed) by GC-MS (5); A_2, Essential oil of *T. foenum-graceum* (seed) by head space GC-MS (5); B, Essential oil of *T. foenum-graceum* (aerial parts) (7); C, Essential oil of *T. foenum-graceum* (aerial parts) (8); D, Essential oil of *T. disperma* (aerial parts) (9); E, Essential oil of *T. monantha* (aerial parts) (10)

of Iran in May 2012. The species were identified by Mr MR Joharchi from Herbarium of Ferdowsi University of Mashhad (FUMH) where voucher specimen of the plant was deposited (No.34445).

The air-dried and powdered aerial parts (200 g) of *T. torbatejamensis* were subjected to hydro-distillation using a Clevenger-type apparatus for 3 hr. After decanting and drying over anhydrous sodium sulfate, the slightly yellow-colored oil was recovered in yield of 0.1 % (v/w) and stored in tightly closed dark vials at -20˚C prior to GC-FID and GC/MS analysis.

Gas- chromatography and gas-chromatography-mass spectrometry

Gas chromatography

The GC analysis was performed using a Varian CP-3800 equipped with FID detector, fused-silica column (CP-Sil 8CB, 50 m × 0.25 mm, film thick-

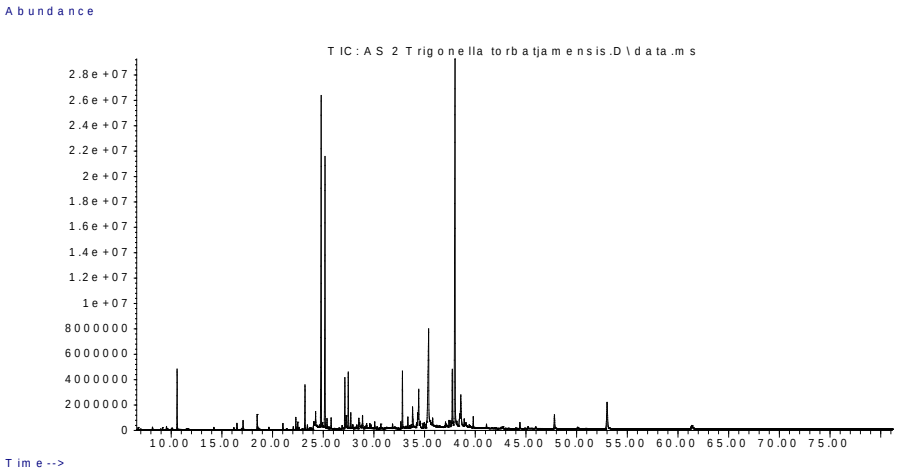

Figure 1. Gas chromatogram of essential oil of the aerial parts of *T. torbatjamensis*

ness 0.12 µm). The operating conditions were: oven temperature 50°C (5 min), 50°C–250°C (3°C /min), 250°C (10 min); injector temperature 260°C, split ratio 1:5, with the carrier gas N2 (2 ml/min); detector temperature 280°C.

Gas chromatography-mass spectrometry

The GC-MS analyses were performed using an Agilent 5975 apparatus with HP-5ms column (30 m x 0.25 mm id, 0.25 µm film thickness) interfaced with a quadruple mass detector and a computer equipped with Wiley 7 n.l library; oven temperature, 50°C (5 min), 50°C–250°C (3°C /min), 250°C (10 min); injector temperature 250°C; injection volume, 0.1 µl; split ration, 1:50; carrier gas was Helium at 1.1 ml min; ionization potential, 70 eV; ionization current, 150 µA; ion source temperature, 250°C; mass range, 35-465 mui.

The constituents of the oil were identified by calculation of their retention indices under programmed temperature conditions for n-alkanes (C8-C20) and the oil on a CP-Sil 8CB column. The individual compounds were identified by comparison of their mass spectra and retention indices (RI) with those of authentic samples and those being given in literatures (11). Quantification of the relative amount of the individual components was performed according to the area percentage method without consideration of calibration factor.

Results

GC and GC–MS analyses showed that 98.5% of the essential oil was comprised of a total of 40 compounds (Figure 1 and Table 1). Considering the quantitative results, the pattern of the main grouped components in essential oil was as follows: sesquiterpene hydrocarbons (70.2%), oxygenated sesquitepenes (16.5%), oxygenated monoterpenes (3.5%) and monoterpene hydrocarbons (0.5%). Germacrene –D (33.0%), bicyclogermacrene (26.0%), and viridiflorol (5.3%) were the main components of the essential oils obtained from the aerial parts of *T. torbatejamensis* .

Discussion

Mazza, *et al* has reported over 50 volatile compounds in *T. foenum- graecum* (4). Another study revealed the presence of the volatile component palmidrol (28.72%), an antiviral, anti-inflammatory and non-steroidal analgesic, was a major bioactive constituent (5).

In this study, headspace dynamic GC-MS claimed the dominance of limonene (82.30%), d-carvone (12.97%) and *n*-caproaldehyde (1.87%), which are reported for the first time in the seeds of *T. foenum-graecum*. Mazza, *et al* reported prominent presence of carbonyl compounds in headspace analysis of this species (6).

The first investigation on compounds of oil obtained from Iranian *Trigonella* species dates back to 2004, when Ahmadiani *et al* examined the composition of the oil of fresh aerial parts of *T. foenum- graecum* L. collected from Kalateh, Ebrahimshah, 25 km from north of Boujnord, North Kohrasan Province (7). The yield of that oil was 0.3 % and had nine components representing 98.5 % of all compounds. Main components of this oil were δ-cadinene (27.6 %), α-cadinol (12.1 %), γ- eudesmol (11.2 %) and α- bisabolol (10.5 %). In a recent study on the same species, aerial parts of the plant were collected from Sari, Mazandaran Province of Iran. Forty components were identified and the main compounds of that oil were α -bisabolol (2.3%), γ- cadinene (1.8%) and α – cadinol (1.7%) (8).

In 2009, the chemical composition of the volatile oil obtained from the leaves of *T. disperma* Bornm.,

an endemic widespread species of the genus *Trigonella* in the western part of Iran, was examined by Ranjbar *et al*. The oil had 18 compounds representing 87.1% of all compounds. Main components of that oil were pentacosane (27.3%), spathulenol (17.3%), and hexahydroxyfarnesyl acetone (6.7%) (9).

Recently, the essential oil from *T. monantha* C. A. Mey., subsp. *Monantha,* has been studied (10). The aerial parts of the plant were harvested from Karaj (Alborz Province). Thirty-eight compounds were identified in the oil of this taxon, representing 88.8% of its composition. The main compounds were dibutyl phthalate (10.3%), hexanal (9.5%), and nonanal (6.6%).

Conclusion

The essential oil of aerial parts of *T. torbatjamensis* Ranjbar is consisted of forty components with sesquiterpene hydrocarbons being the main grouped components.

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