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Short communication

Essential oil composition of *Hypericum triquetrifolium* Turra growing wild in Iran

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Abstract

The chemical composition of the volatile oil from aerial parts of *Hypericum triquetrifolium* Turra was studied by GC-MS. Fifty components (97.1% of the total composition) were detected in the volatile oil. Germacrene-D (21.7%), β-caryophyllene (18.3%), δ-cadinene (6.4%), *trans*-β-farnesene (4.3%), α-humulene (3.8%), β-selinene (3.7%), γ-cadinene (3.3%) and *trans*-phytol (3.2%) were found to be the major constituents of the oil. The oil of *H. triquetrifolium* consisted of five monoterpene hydrocarbons (3.4%), two oxygenated monoterpenes (0.4%), twenty-two sesquiterpene hydrocarbons (77.1%), eight oxygenated sesquiterpenes (7.9%) and one oxygenated diterpene (3.2%). Twelve nonterpenic compounds were also consisted 5.1% of the oil. In conclusion, the oil of *H. triquetrifolium* was characterized by a high content of sesquiterpenes (85.0%), whereas monoterpenes contained only 3.8% of the essential oil.

Keywords: *Hypericum triquetrifolium*; Essential oil composition; Germacrene-D

INTRODUCTION

The genus *Hypericum*, belonging to the Hypericaceae family, consists of about 380 species (1). The genus represented in the flora of Iran by seventeen species including three endemics (2). *Hypericum perforatum* L. (St. John's wort), the most famous species of the genus, is well known as a medicinal plant for the treatment of moderate depression (1).

Previous phytochemical study of *Hypericum* has led to the isolation naphthodianthrones of hypericin and pseudohypericin (3), phloroglucinol derivatives (4), flavonoids (5), xanthones (6), tannins (7) and essential oils (8).

Hypericum triquetrifolium Turra is an herbaceous perennial plant and one of the Iranian native species of Hypericaceae which is distributed in the south-west of the country. According to the literature, *H. triquetrifolium* contains hypericin (9), flavonoids, phenolic compounds such as chlorogenic acid (10) and essential oil (11).Volatile oil constituents of *H. triquetrifolium* from Italy have previously been reported and n-nonane (15%), germacrene-D (13%), caryophyllene oxide (12%), β-caryophyllene (11%), α-pinene (10%), myrcene (5%), β-pinene (4%) and sabinene (3%) were recognized as main components of the oil (12). 1-Hexanal (18.8%), 3-methylnonane (12.5%), α-pinene (12.3%), caryophyllene oxide (4.7%), 2-methyldecane (4.5%) and α-amorphene (4.2%) are predominant constituents of the essential oil of the aerial parts of *H. triquetrifolium* grown in Turkey (13) and α-humulene, *cis*-calamenene, δ-cadinene, bi-cyclogermacrene, eremophilene, β-caryo-phyllene, (E)-γ-bisabolene and α-pinene were also found as the major components of the Tunisian *H. triquetrifolium* oil (14).

There are some reports on the antioxidant (15), antibacterial (16), antiinflammatory (17), antinociceptive (18) and cytotoxic activities (19) of this plant. From ethyl acetate extract of the aerial parts of *H. triquetrifolium* four compounds including one biflavonoid, one flavonol, one flavonol-glycoside and one phenolic acid, namely, 3,8" biapigenin, quercetin, rutin and chlorogenic acid are

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reported. Study of the antioxidant activity of isolated compounds indicated that 3,8" biapigenin had an activity similar to α-tocopherol, while rutin, quercetin and chlorogenic acid exhibited a slightly weaker activity than α-tocopherol (20).

In another study, the antioxidant activity of ethanol extract of *H. triquetrifolium* was investigated. The extract was highly active in the DPPH radical scavenging assay with IC_{50} value of 39.0 μg/ml. It means that ethanol extracts of the plant is a potential source of natural antioxidants (21). Potential new antioxidant agents are interested for their role in the maintenance of the antioxidant system and prevention of aging, atherosclerotic and inflammatory diseases (22).

Inhibition of mono amino oxidase (MAO) activity of bioactive constituents of hypericin is the most important factor of antidepressive effect of *Hypericum* extracts (23). Only few species of *Hypericum* contain hypericin and *H. triquetrifolium* is one of them (9). Different biological activities of the medicinal plants reported in the literature candidate them as an interesting medicinal source. In this direction and as a part of our research on the aromatic flora of Iran, the constituents of essential oil of *H. triquetrifolium* growing wild in Iran was investigated.

MATERIALS AND METHODS

Plant material

The aerial parts of *H. triquetrifolium* were collected during May 2012 from the Fars province in the south-west of Iran at an altitude of ca. 1260 m above the sea level. The plant was identified by Department of Biology, Science and Research Branch, Tehran Islamic Azad University of Iran and a voucher specimen of the plant numbered as 2819 is deposited in the Herbarium of the School of Pharmacy and Pharmaceutical Sciences of Isfahan University of Medical Sciences, Isfahan, Iran.

Isolation of the oil

The essential oil of the aerial parts of *H. triquetrifolium* was obtained by hydro-distillation using a Clevenger-type apparatus for 3h according to the method recommended in the British Pharmacopoeia (24). The volatile oil was dried over anhydrous sodium sulfate and stored in a sealed vial at 4 °C until analysis.

Analysis of the oil

Gas chromatography combined with mass spectrometry was used for identification of the oil components. The analysis was performed on an Agilent 5975C mass selective detector coupled with an Agilent 7890A GC, equipped with an HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The oven temperature was programmed from 60-280 °C at the rate of 4 °C per min.

Helium was used as the carrier gas at a flow rate of 2 mL/min. Injector and detector temperatures were set at 280 °C. The MS operating parameters were as follows: ionization voltage, 70 eV; ion source temperature, 230 °C (25). The MSD ChemStation was used as operating software.

Retention indices were calculated by using retention times (RT) of *n*-alkanes (C_8-C_{24}) that were injected following oil injection under the same conditions. Components of the oil were identified by comparison of their retention indices (RI) with those reported in the literature (26) and computer matching with NIST and Wiley275.L libraries. The fragmentation patterns of the mass spectra were also compared with those reported in the literature (26,27).

RESULTS

The aerial parts of *H. triquetrifolium* yielded 0.1% (v/w) of a pale yellowish essential oil. Fifty components were detected in the volatile oil. The identified components and their percentage are given in Table 1, where the components are listed in order of their elution on the HP-5MS column. Germacrene-D (21.7%), β-caryophyllene (18.3%), δ-cadinene (6.4%), *trans*-β-farnesene (4.3%), α-humulene (3.8%), β-selinene (3.7%), γ-cadinene (3.3%) and *trans*-phytol (3.2%) were found to be the major constituents of the oil.

The structure of major constituents of essential oil of *H. triquetrifolium* could be seen in Fig. 1.

The oil of *H. triquetrifolium* consisted of five monoterpene hydrocarbons (3.4%), two oxygenated monoterpenes (0.4%), twenty-two sesquiterpene hydrocarbons (77.1%), eight oxygenated sesquiterpenes (7.9%) and one oxygenated diterpene (3.2%). Twelve nonterpenic compounds were also consisted 5.1% of the oil. In conclusion, the oil of *H. triquetrifolium* was characterized by a high content of sesquiterpenes (85.0%), whereas monoterpenes contained only 3.8% of the essential oil.

Table 1. Percentage composition of the essential oil of *Hypericum triquetrifolium* Turra.

No.	RT	Compound	%	RI
1	2.61	*trans*-2-hexenal	0.4	853
2	3.19	nonane	0.3	899
3	3.79	α-pinene	0.1	936
4	4.43	3-methylnonane	0.1	970
5	4.61	β-pinene	0.1	978
6	4.87	myrcene	0.1	990
7	5.65	p-cymene	0.5	1025
8	6.53	γ-terpinene	2.6	1061
9	7.60	undecane	0.7	1098
10	7.73	nonanal	0.2	1102
11	10.30	α-terpineol	0.1	1189
12	13.48	thymol	0.3	1290
13	13.74	tridecane	2.1	1297
14	15.22	α-cubebene	0.9	1348
15	15.70	cyclosativene	0.2	1368
16	15.88	α-ylangene	0.5	1372
17	16.04	α-copaene	2.3	1374
18	16.30	β-bourbonene	0.9	1384
19	16.42	β-elemene	0.8	1387
20	**17.57**	**β-caryophyllene**	**18.3**	**1422**
21	17.72	β-copaene	1.6	1428
22	17.97	aromadendrene	0.4	1437
23	18.22	*cis*-muurola-3,5-diene	0.6	1446
24	**18.43**	**α-humulene**	**3.8**	**1451**
25	**18.68**	***trans*-β-farnesene**	**4.3**	**1459**
26	**19.47**	**germacrene-D**	**21.7**	**1485**
27	**19.64**	**β-selinene**	**3.7**	**1488**
28	19.75	δ-selinene	2.1	1491
29	20.17	*E*-*E*-α-farnesene	2.9	1508
30	**20.29**	**γ-cadinene**	**3.3**	**1512**
31	**20.61**	**δ-cadinene**	**6.4**	**1523**
32	20.79	*trans*-cadina-1(2),4-diene	0.7	1532
33	20.93	α-cadinene	1.0	1536
34	21.06	α-calacorene	0.2	1542
35	21.51	germacrene-B	0.5	1556
36	21.87	*cis*-3-hexenyl benzoate	0.3	1567
37	22.20	caryophyllene oxide	2.9	1580
38	22.48	salvial-4(14)-en-1-one	0.7	1589
39	22.59	β-copaen-4-α-ol	0.5	1591
40	22.78	juniperol	0.5	1597
41	23.44	1,10-di-epi-cubenol	0.3	1622
42	23.97	*epi*-α-cadinol	0.5	1641
43	24.20	α-cadinol	1.9	1651
44	25.03	khusinol	0.6	1679
45	32.15	hexadecanoic acid	0.4	1969
46	**35.59**	***trans*-phytol**	**3.2**	**2113**
47	36.15	ethyl linoleolate	0.2	2144
48	39.55	n-tricosane	0.1	2298
49	43.52	n-tetracosane	0.2	-
50	47.21	n-pentacosane	0.1	-

RI; Retention indices on HP-5MS capillary column, %; Percentages calculated from TIC data.

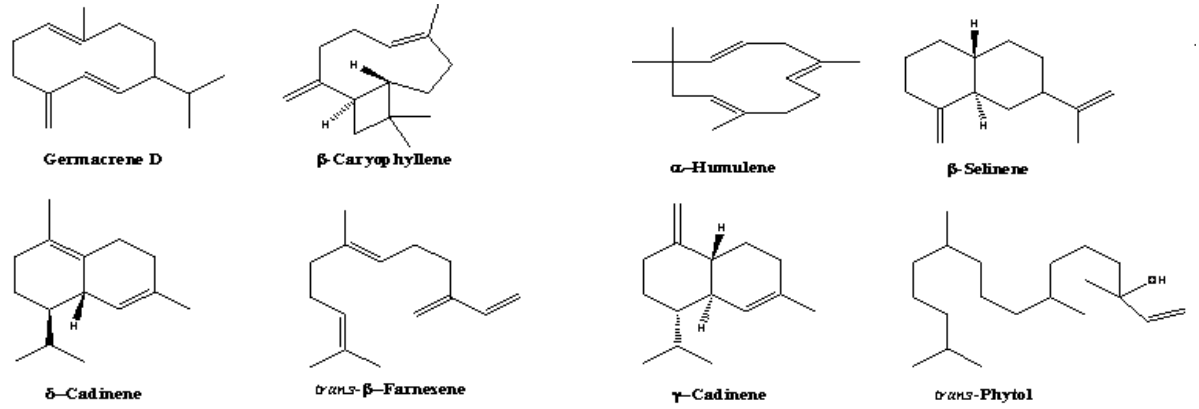

Fig. 1. Structure of major components of essential oil of *H. triquetrifolium*

DISCUSSION

Volatile oil of the leaf of *H. triquetrifolium* from Calabria (Italy) have previously been reported to contain *n*-nonane (15%), germacrene-D (13%), caryophyllene oxide (12%), β-caryophyllene (11%), α-pinene (10%), myrcene (5%), β-pinene (4%) and sabinene (3%) (12). Evidently nonane, caryophyllene oxide and α-pinene are the major components of the oil of the leaf of *H. triquetrifolium* from Calabria (Italy), while these components was found to be present in trace amounts in the volatile oil examined in the present study.

While sesquiterpenes are the dominant fractions of essential oil of the aerial parts of Tunisian *H. triquetrifolium,* α-humulene, *cis*-calamenene, δ-cadinene, bicyclogermacrene, eremophilene, β-caryophyllene and (E)-γ-bisabolene were found as the main sesquiterpenes components presented in this oil. α-Pinene was also reported as the main monoterpene of the oil (14).

In contrast to Iranian and also Tunisian sample of *H. triquetrifolium*, study of volatile oil constituents of aerial parts of *H. triquetrifolium* grown in Turkey showed that monoterpene concentrations were higher than sesquiterpene levels. 1-Hexanal (18.8%), 3-methylnonane (12.5%), α-pinene (12.3%), caryophyllene oxide (4.7%), 2-methyldecane (4.5%) and α-amorphene (4.2%) are three predominant constituents of the oil of *H. triquetrifolium* samples grown in Iran and Tunisia (13). Essential oils composition of the plants could be affected by many parameters such as seasonal variation (28), phonological cycle (29) and geographic distribution. Study of volatile constituents of five populations of Tunisian *H. triquetrifolium* indicates that the essential oil compositions are variable and four chemotype groups could be recognized (30).

CONCLUSION

It is concluded from this study that in spite of some similaritIes in essential oil composition of *H. triquetrifolium* growing in Iran to one growing in Italy, the effect of geographic distribution in variety of components and their percentages could completely be observed.

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