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Original Article

Essential Oil Constituents of Leaves of the Male and Female Shrubs of *Juniperus chinensis* L. from Isfahan

Suleiman Afsharypuor[a,*], Mohammadreza Rahiminezhad[b], Leili Ghaemmaghami[b], Majid Soleimani[c], Mohammadreza Khanmohammadi[c], Neda Afsharipour[c]

[a]*Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran*
[b]*Faculty of Sciences, Department of Botany, University of Isfahan, Isfahan, Iran*
[c]*Faculty of Sciences, Department of Chemistry, Imam Khomeini International University, Ghazvin, Iran*

Abstract

The composition of essential oil of leaves of the male and female shrubs of *Juniperus chinensis* L. (growing in Isfahan) was analyzed by gas choromatography (GC) and GC-mass spectrometery (MS). The main constituents of leaf oil of the male shrub were: bornyl acetate (26.1%), sabinene (18.3%), *trans*-sabinyl acetate (11.1%), myrcene (9.2%), limonene (6.1%), elemol (5.5%), α-cadinol (2.3%), δ-cadinene (2.2%), *trans*-thujone (1.6%), α-pinene (1.3%) and α-muurolol (1.1%); while the major constituents of leaf oil of the female shrub were: sabinene (18.0%), bornyl acetate (12.3%), carotol (11.4%), elemol (9.9%), myrcene (8.8%), α-pinene (6.4%), δ-cadinol (4.8%), limonene (3.9%), δ-cadinene (3.4%) and α-muurolol (2.7%).

Keywords: Bornyl acetate; Essential oil; *Juniperus chinensis*; Sabinene; *trans*-Sabinyl acetate.
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1. Introduction

Juniperus chinensis L., commonly known as Chinese juniper (Fam. Cupressaceae) is a dioecious or rarely monoecious shrub or tree growing in different parts of China, Japan, Korea, Myanmar and eastern parts of Russia [1]. The ornamental dioecious shrubs of this plant are cultivated widely in different parts of Iran [2]. Leaves of the plant claimed to have antitumour promoting and antitumor activities [3].

*Corresponding author: Prof. Suleiman Afsharypuor, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.
Tel. (+98)311-7922637, Fax (+98)311-6680011
E-mail: afsharypuor@pharm.mui.ac.ir

As the chemical composition of the essential oils of leaves of both the male and female cultivated shrubs of Chinese juniper in Iran has not been investigated previously, and as a part of our long term research programs is to identify new sources of valuable terpenoids in our country, therefore, in the present study the above-mentioned oils were isolated separately, and their active constituents were determined using gas choromatography (GC) and GC-mass spectrometery (MS) methods.

2. Materials and methods

2.1. Plant material

Leaves of the male and female shrubs were collected in May 2004 from populations growing in Isfahan (Iran). The shrubs were identified by the Botany Department of the Faculty of Sciences at the University of Isfahan (Iran). Voucher specimens of the leaves (no.1773) were deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy and Pharmaceutical Sciences at the Isfahan University of Medical Sciences (Iran).

2.2. Isolation of the essential oil

Powdered air-dried leaves of the male and female shrubs were separately hydrodistilled for 4 h. Pale yellow oils (1.29% and 0.66% v/w) were obtained having the refractive indices of 1.4475 and 1.4476 measured at 25 °C.

2.3. GC analysis

Gas chromatographic determinations were run on a Perkin Elmer 8500 instrument using a BP1 capillary column (30 m×0.25 mm; film thickness: 0.25 μm). The carrier gas was nitrogen with a flow rate of 2 ml/min. The oven temperature was programmed from 60-275 °C at 4 °C/min. Injector and detector temperatures were 275 °C and 280 °C, respectively.

2.4. GC/MS analysis

Analysis of the oils was performed on a Hewlett Packard 6890 GC/MS instrument under the following conditions: Injection of 0.1 μl samples, HP-5 MS capillary column (30 m×0.25 mm ; film thickness 0.25 μm); carrier gas He, flow rate 2 ml/min., injector temperature 250 °C, temperature program: 60-275 °C at 4 °C/min.; mass spectra: electronic impact, ionization potential 70 eV, ion source temperature 250 °C, ionization current 1000 μA, resolution 1000, and mass range 30-300.

Identification of the constituents was based on computer matching against the library spectra (Library Database Wiley 275), their retention indices with reference to an n-alkane series in a temperature programmed run, interpreting their fragmentation pattern and comparison of the mass spectra with the literature data [4].

3. Results and discussion

Forty-one constituents in the male shrub leaf oil (representing 96.2% of the total oil) and thirty nine components in the female shrub leaf oil (representing 95.4% of the total oil) were identified (Table 1). Bornyl acetate (26.1%), sabinene (18.3%), *trans*-sabinyl acetate (11.1%), myrcene (9.2%), limonene (6.1%), elemol (5.5%), α-cadinol (2.3%), δ-cadinene (2.2%), α-thujone (1.6%), α-pinene (1.3%) and α-muurolol (1.1%) were the main constituents of the leaf oil of male shrub; while the major constituents of leaf oil of the female shrub were sabinene (18.0%), bornyl acetate (12.3%), carotol (11.4%), elemol (9.9%), myrcene (8.8%), α-pinene (6.4%), α-cadinol (4.8%), limonene (3.9%), δ-cadinene (3.4%) and α-muurolol (2.7%). In a previous work which has been done by other workers, ethyl acetate was reported to be one of the major constituents of leaf oils of *Juniperus chinensis* growing in China (three samples: 20.65%, 12.55% and 19.6%), but it was absent in the leaf oil of *Juniperus chinensis* var. *kaizuca* [5]. The latter mentioned compound could not be detected in our analyzed leaf oils as well. As in case of of *J. chinensis* cv. *pyramidalis* and *J. chinensis* var. *kaizuca* leaf oils, our examined leaf oils did not contain the cedrene/cedrol components [5].

The amount of essential oil obtained from the leaf of the male shrub (1.29% v/w) was about twice the amount of that produced by the leaf of the female shrub (0.66%). As it is obvious from Table 1, the relative percentage production of sabinene and myrcene in both leaf oils is about the same, while the production of bornyl acetate by the leaf of the male shrub (26.1%) is higher than that

Table 1. Percentage composition of leaf essential oils of the male and female shrubs of *Juniperus chinensis* from Isfahan.

No.	Components	Retention indices	Male leaf (%)	Female leaf (%)
1	Tricyclene	927	0.8	0.8
2	α-Thujene	931	0.3	1.2
3	α-Pinene	940	**1.3**	**6.4**
4	Camphene	954	0.6	1.1
5	Sabinene	976	**18.3**	**18.0**
6	β-Pinene	981	t	t
7	Myrcene	991	**9.2**	**8.8**
8	α-Phellandrene	1004	t	0.8
9	α-Terpinene	1018	0.1	0.6
10	Limonene	1031	**6.1**	**3.9**
11	*(E)*-β-Ocimene	1053	0.1	0.3
12	γ-Terpinene	1062	0.4	0.8
13	*cis*-Sabinene Hydrate	1069	0.2	0.5
14	Terpinolene	1089	0.1	0.9
15	*trans*-Thujone	1117	**1.6**	0.1
16	*trans*-Sabinol	1142	0.3	-
17	Camphor	1145	0.2	0.3
18	Camphene hydrate	1150	0.1	0.1
19	Borneol	1168	0.1	0.3
20	Terpinen-4-ol	1177	0.8	1.3
21	Bornyl acetate	1287	**26.1**	**12.3**
22	*trans*-Sabinyl acetate	1293	**11.1**	-
23	δ-Elemene	1341	t	0.1
24	α-Copaene	1379	t	t
25	β-Elemene	1392	t	0.2
26	Germacrene-D	1482	0.4	1.7
27	α-Muurolene	1500	0.2	0.4
28	7-*epi*-α-Selinene	1520	0.3	0.6
29	δ-Cadinene	1525	2.2	3.4
30	Cadina-1,4-diene	1533	t	t
31	α-Cadinene	1539	t	0.1
32	Elemol	1551	5.5	9.9
33	Carotol	1591	5.0	11.4
34	β-Oplopenone	1609	0.4	0.4
35	γ-Eudesmol	1632	0.3	0.3
36	α-Muurolol	1643	1.1	2.7
37	β-Eudesmol	1651	0.2	t
38	α-Cadinol	1654	**2.3**	**4.8**
39	Caryophyllene acetate	1698	t	0.2
40	*epi*-13-Manool	1962	t	0.1
41	Manool	2058	0.5	0.6
	Grouped constituents:			
	Hydrocarbon monoterpenes		37.2	42.7
	Oxygenated monoterpenes		40.6	15.8
	Hydrocarbon sesquiterpenes		3.1	6.5
	Oxygenated sesquiterpenes		14.8	29.7
	Oxygenated diterpenes		0.5	0.7
	Total identified		**96.2**	**95.4**

t = trace (less than 0.1%)

produced by the leaf of the female shrub (12.3%). On the other hand, the leaf of the male shrub produces 11.1% *trans*-sabinyl acetate, while the leaf of the female shrub doesn't produce this monoterpenoid.

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