Identification of Compounds Nonpolar
Extract *Ziziphora persica* Growing in Iran By GC-MS

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Abstract: The aerial parts of the plant *Ziziphora persica* were collected on Jun 2011 from Darkesh (North Khorassan Province of Iran). The chemical compounds of the plant were isolated by solvent extraction (solvent: n-hexane) . A total of 57 constituents, representing more than 77.5% of the oil were identified by gas chromatograph/mass spectrometry (GC/MS). The most presented compounds of the extract for aerial parts of *Z. Persica* were Bis (2-ethylhexyl) 1,2-benzenedicarboxylate (12.88%), Dodecane (12.47%), Decane (7.8%), Tetradecane (5.93%), Ethyl palmitate (4.12%) and Hexadecane (2.99%).

Key words: Chemical composition · n-Hexane extraction · Midicinal plant · GC-MS · *Ziziphora persica*

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Thus study of plant species that traditionally have been used as pain killer should still be seen as a strategy in research for new analgesic drugs. *Ziziphora persica* is an edible medicinal plant [1], which is widely distributed in Iran and leaves, flowers and stems are frequently used as wild vegetable or additive in foods to offer aroma and flavor. In Iranian folk medicine, *Ziziphora* species has been also used as infusions for various purposes such as sedative,stomachic and carminative among others [2]. The genus *Ziziphora* L. belongs to the Lamiaceae family consists of four species (*Z. clinopodioides*, *Z. capitata*, *Z. persica* and *Z. tenuior*) that widespread all over Iran [3]. *Ziziphora* with the common Persian name "*kakuti-e kuhi*" comprised nine subspecies native to Iran. The composition and antibacterial and antioxidant activity of the essential oil and various extracts of *Ziziphora* were reported [4].

Keeping this in view, the present study has been done to identify nonpolar compounds of n-hexane extract of this plant.

MATERIALS AND METHODS

The experimental protocol used in this study was approved by the research committee of North khorasan payame Noor University, Bojnourd, Iran.

Plant Material: The plant *Ziziphora persica* were collected at the flowering stage from the Bojnourd, Iran, In Jun 2011, and identified at the Research Center for Plant Sciences, Ferdowsi University of Mashhad, Iran. A voucher specimen has been deposited in the Enveriomental Department of Bojnourd Herbarium (EDBH:00113).

Isolation of Chemical Compounds: The arial parts of plant *Ziziphora persica* were air-dried for 3 days before isolation of chemical composition. The plant material (100 g) was cut into small pieces .The plant powdered was macerated in 95% pure n-hexane as a solvent for 48 hours, filtered through a Whatman paper, then evaporated off the solvent in vacuum by rotary Evaporator to yield light yellow oil and dried over by adding anhydrous Na_2SO_4. In absolute oil recovery, concentrate oil was dissolved in minimum volume of absolute alcohol to

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remove the natural waxes present in the essential oil. It was kept at -14°C for 48 hrs and then it was filtered through a filter paper. Alcohol was removed by distillation and traces of alcohol were removed by passing nitrogen gas through it [5].

Gas Chromatography and Mass Spectrometry: Gas chromatographic analysis was performed on an Hewlett-Packard (HP) 6890 A instrument equipped with a flame ionization detector and Rtx-5MS (15 m × 0.25 mm × 0.25 μm) capillary column, while the essential oil components were identified on an Agilent Technologies 5973N mass spectrometer. The GC settings were as follows: the initial oven temperature was held at 35 °C for 6 min and ramped at 5 °C min^{-1} to 15° °C for 0 min and then ramped at 10 °C min^{-1} to 280 °C for 3 min. The injector temperature was maintained at 250 °C. The samples (1 μL) were injected, with a split ratio of 1:10. The carrier gas was helium at flow rate of 1.0 mL min^{-1}. Spectra were scanned from 20 to 550 m/z at 2 scans s^{-1}. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available

in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [6-9].

Component relative percentages were calculated based on GC peak areas without using correction factors.

RESULTS AND DISCUSSION

Solvent extraction plant (SEP) method from aerial parts of *ziziphora pesica* was analyzed by GC–MS and resulted in the identification of 56 compounds representing 77.5% of the oil. The average yield of chemical composition of the plant *Ziziphora persica* was about 0.6%. The extracts contained *n*-alkanes, carboxilic acid, triterpenoids, esers, cycloalkane, alcohol, ketone, sesquiterpens, aldehyde, alkene, emine, alkyl halids, carbohydrates and some unidentified compounds.

Table 1: Percentage composition of the chemical compounds isolated from aerial parts of *ziziphora persica*

No.	Compound	Retention Index (RIª)	Percentage	Method of Identification
1	Dimethyl sulfoxide	772	0.54	MS-GC
2	Diacetone alcohol	843	0.34	MS-GC
3	3-Methylnonane	976	0.39	MS-GC
4	Decane	1000	7.8	MS-GC
5	Undecane	1090	0.69	MS-GC
6	5-Ethyldecane	1144	0.12	MS-GC
7	2,6-Dimethyldecane	1158.6	0.6	MS-GC
8	2-Methylundecane	1168.5	0.18	MS-GC
9	3,8-Dimethyldecane	1177	1.01	MS-GC
10	2,6-Dimethyldecalin	1186	0.85	MS-GC
11	1,6-Dimethyldecalin	1196	1.91	MS-GC
12	1-Hexyl-3-methyl-cyclopentane	1198	0.81	MS-GC
13	Dodecane	1200	12.47	MS-GC
14	2,6-Dimethylundecane	1202	0.29	MS-GC
15	decahydro-1,5-dimethyl- Naphthalene	1216.4	1.72	MS-GC
16	Ethyl nonanoate	1284	0.18	MS-GC
17	Tridecane	1289	1.82	MS-GC
18	5-methyl tridecane	1343	0.3	MS-GC
19	3-methyl-tridecane	1359.5	0.41	MS-GC
20	Tetradecene	1380.15	0.16	MS-GC
21	Tetradecane	1389.5	5.93	MS-GC
22	Trans-caryophyllene	1411	0.15	MS-GC

Table 1: Continued

No.	Compound	Retention Index (RIª)	Percentage	Method of Identification
23	Germacrane –D	1473.8	0.33	MS-GC
24	Pentadecane	1488.6	0.15	MS-GC
25	2,4-Di-tert-butylphenol	1499	0.52	MS-GC
26	(2,6,6-Trimethyl-2-hydroxycyclohexylidene)acetic acid lactone	1522	0.27	MS-GC
27	3-Methylpentadecane	1573	0.25	MS-GC
28	Ethyl laurinate	1599	0.27	MS-GC
29	Hexadecane	1606.4	2.99	MS-GC
30	3-Methylheptadecane	1754.2	0.16	MS-GC
31	Ethyl myristate	1776	0.33	MS-GC
32	n-Octadecane	1782.4	1.04	MS-GC
33	Neophytadiene	1821.5	0.17	MS-GC
34	6,1°,14-Trimethyl-2-pentadecanone	1826.7	0.4	MS-GC
35	Diisobutyl phthalate	1852	1.48	MS-GC
36	Ethyl pentadecanoate	1874	0.12	MS-GC
37	Hexadecanoic acid	1941.5	1.56	MS-GC
38	Ethyl hexadec-9-enoate	1967	0.31	MS-GC
39	Ethyl palmitate	1973.6	4.12	MS-GC
40	Eicosane	1980	0.42	MS-GC
41	Ethyl heptadecanoate	2071	0.3	MS-GC
42	n-Heneicosane	2077	0.09	MS-GC
43	Phytol	2098	0.65	MS-GC
44	Linoleic acid	2110	0.76	MS-GC
45	Methyl linolenate	2117	2	MS-GC
46	Ethyl linoleate	2140	1.37	MS-GC
47	Ethyl linolenate	2147	2.12	MS-GC
48	n-Butyl palmitate	2162	0.46	MS-GC
49	n-Docosane	2175	0.14	MS-GC
50	1-Chloro nonadecane	2274	0.09	MS-GC
51	3,3′-Dichlrobenzylideneazine	2348	0.69	MS-GC
52	Ethyl stearate	2368	0.69	MS-GC
53	n-Pentacosane	2471	0.14	MS-GC
54	Bis(2-ethylhexyl) 1,2-benzenedicarboxylate	2525	12.88	MS-GC
55	28-Norolean-17-en-3-one	2704	1.27	MS-GC
56	Vitamin E	3100	0.29	MS-GC
Total			77.5	

a: Retention Indices on RTX-5MS

The most representative compounds of the essential oil were hydrocarbons and esters and among them the main constituents were Bis (2-ethylhexyl) 1,2-benzenedicarboxylate (12.88%), Dodecane (12.47%), Decane (7.8%), Tetradecane (5.93%), Ethyl palmitate (4.12%) and Hexadecane (2.99%).

To the best of our knowledge, the nonpolar composition of *ziziphora persica* has not been reported before and therefore our results could be considered as the first report about the nonpolar composition of this plant. Table 1 reports the chemical composition of the phytochemical components under study.

The various compounds were identified by comparison of their Kováts retention indexes, determined utilizing a non-logarithmic scale on non-polar (Rtx-5MS) columns and by comparison of the mass spectra of each GC component with those of standards and with reported data [10,11].

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