Volatile Oil Constituents of *Vicia variabilis* Frein & Sint from Iran

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The water distillation of aerial parts of *Vicia variabilis* have been analyzed by a combination of GC-FID and GC/MS methods. Ninety compounds representing 92.7 % of the total oil were characterized. The main components were hexahydrofarnesyl acetone (6.6 %), β -ionone (6.4 %), myrtenol (3.7 %), (Z)-phytol (3.3 %) and 1-octen-3-ol (3.1 %). Oxygenated monoterpenes constitute the major fraction of the oil (26.3 %), followed by aromatic compounds (11.9 %), sesquiterpenes oxygenated (10.7 %) and hydrocarbon aldehydes (9.9 %) while sesquiterpene hydrocarbons was not observed.

Key Words: *Vicia variabilis*, Papilionaceae, Essential oil composition, Hexahydrofarnesyl acetone, β -Ionone, Myrtenol, (Z)-phytol, 1-Octen-3-ol.

INTRODUCTION

The genus *Vicia* L. (Papilionaceae) comprises 166 species in the world and its diversity centre is the Mediterranean region¹. Fourty five or 46 species of *Vicia* has been reported from Iran^{2,3}. Some researches from the genus *Vicia* have shown biological activity. The methanolic and ethanolic extracts of *Vicia faba* was tested for their antimicrobial, antioxidant and cytotoxic activities⁴. The methanolic extract of *Vicia faba* showed highest antimicrobial activity and high free radical scavenging activity but it had not remarkable cytotoxic activity against FL-cells. The hepatoprotective activity of flavonol glycosides from extract of the aerial parts of *V. calcarata*, was evaluated in a rat model with a liver injury induced by daily oral administration of CCl₄ for four weeks⁵. The data obtained from this study revealed that the flavonol glycosides protect the rat liver from hepatic damage induced by CCl₄ through inhibition of lipid peroxidation caused by CCl₄ reactive free radicals. Also, antiherbivory defense of *V. sativa* and *V. hirsuta* with and without extrafloral nectarines was investigated⁶. Indirect ant defense of *V. sativa* was not consistently reliable. In addition, *V. sativa* was more vulnerable to attack by herbivores than *V. hirsuta*. The estimated total amount of sugars secreted by EFNs of *V. sativa* corresponded to 0.5 % of total leaf biomass and 0.07 % of total plant biomass, indicating a low investment to the production of extrafloral nectar. *V. sativa* plants grew more rapidly than *V. hirsuta* plants during the reproductive stage. The antioxidant activity of the polyphenols extracted from the seeds of 28 *Vicia* species collected

in southern Spain was studied⁷. The highest specific polyphenols antioxidant activity was observed in the extracts from *V. parviflora* and *V. tenuifolia*. On the contrary, the highest total antioxidant activity was observed in *V. sativa*. The results showed that the *Vicia* species may represent a source of natural polyphenols with high antioxidant activity. The antimicrobial activity of the isolated essential oils of *V. dadianorum* was investigated⁸ and it showed moderate antimicrobial and antifungal activities against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Mycobacterium smegmatis* and *Candida albicans*. The genus *Vicia* has been studied chemically by some researchers for example tannin⁹, flavonoides^{10,11} and sesquiterpene¹². Although, a few reports appear in the literature on *Vicia* essential oils^{8,13,14}. To the best of our knowledge, no studies have been reported on the oil of *V. variabilis*, hence they decided to investigate the chemical compositions of this oil.

EXPERIMENTAL

The aerial parts of *Vicia variabilis* was collected during the flowering stage in Alamut area, province of Qazvin, Iran, in May 2010. A voucher specimen has been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Isolation of the oil: The air-dried aerial parts *Vicia variabilis* of the plant (in the shade at room temperature, 154 g) was subjected to water distillation using a Clevenger-type apparatus and extracted by diethyl ether for 3 h. After decanting

and drying over anhydrous sodium sulfate, the corresponding yellowish coloured oil was recovered in yields of 0.04 % v/w. The sample were stored in dark glass bottles in a freezer (-5 °C) until further use and analyze.

Gas chromatography-flame ionization detector analysis was performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless (ratio 1:30), injector (250 °C) and a flame ionization detector (250 °C). N₂ was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60 °C for 3 min and then heated to 220 °C with a 5 °C/min rate and kept constant at 220 °C for 5 min. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

Gas chromatography-mass spectroscopy analysis was performed using a Hewlett-Packard 5973 with a HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60 °C for 3 min and programmed to 220 °C at a rate of 5 °C/min and kept constant at 220 °C for 5 min. The flow rate of helium as carrier gas was (1 mL/min). MS were taken at 70 eV, mass range, 30 to 350 amu and scan time 2 scans/sec.

Identification of components: The compounds were identified by comparison of their mass spectra with the Wiley library or with published mass spectra¹⁵. The identifications were confirmed by comparison of KI from HP-5MS column with those reported in the NIST library and the Pherobase database^{16,17}. The Kovats indices for all the components were determined according to the Van Den Dool method, using *n*-alkanes as standards¹⁸.

RESULTS AND DISCUSSION

The volatile constituents obtained from *Vicia variabilis* in the flowering stage are listed (Table-1). In this table, the percentage and kovats indices of the components are given. Ninety compounds were obtained that representing 92.7 % of the total constituents in the essential oil. The main components were characterized by hexahydrofarnesyl acetone (6.6 %), β-ionone (6.4 %), myrtenol (3.7 %), (Z)-phytol (3.3 %) and 1-octen-3-ol (3.1 %). Oxygenated monoterpenes constitute the major fraction of the oil (26.3 %), followed by aromatic compounds (11.9 %), sesquiterpenes oxygenated (10.7 %) and hydrocarbon aldehydes (9.9 %) while sesquiterpene hydrocarbons was not observed.

TABLE-1
COMPOSITION OF *Vicia variabilis* ESSENTIAL OIL

Compound	RI ^a	RI ^b	(%) ^c
1 (E)-2- Hexanal	853	855	2.7
2 (E)-3-Hexen-1-ol	858	860	2.0
3 <i>n</i> -Heptanal	904	898	0.4
4 α-Pinene	937	939	1.7
5 Acetonyl acetone	932	933	0.7
6 (E)-2-Heptenal	956	960	0.3
7 Benzaldehyde	957	961	0.7
8 Isoamyl propionate	972	969	0.8
9 1-Octen-3-ol	978	979	3.1
10 6-Methyl-5-hepten-2-one	984	986	0.5
11 2-Pentyl-furan	989	994	0.9

Compound	RI ^a	RI ^b	(%) ^c
12 2-(1-Pentenyl) furan	998	1000	0.5
13 <i>n</i> -Octanal	999	1001	0.4
14 (E,E)-2,4-Heptadienal	1003	1010	0.9
15 Limonene	1025	1031	1.1
16 1,8-Cineole	1033	1039	1.1
17 Benzeneacetaldehyde	1037	1042	1.8
18 2-Octenal	1054	1056	0.4
19 (E,Z)-3,5-Octadien-2-one	1066	1068	0.9
20 1-Octanol	1068	1068	0.7
21 (E,E)-3,5-Octadien-2-one	1099	1107	1.0
22 Linalool	1106	1112	1.0
23 <i>n</i> -Nonanal	1108	1114	2.4
24 Phenethyl alcohol	1118	1116	0.6
25 <i>cis</i> - <i>p</i> -Menth-2-en-1-ol	1123	1122	0.7
26 <i>trans</i> - <i>p</i> -Menth-2-en-1-ol	1142	1140	0.6
27 Camphor	1146	1143	2.0
28 (E,Z)-2,6-Nonadienal	1158	1156	0.3
29 <i>p</i> -Menth-3-one	1168	1166	0.3
30 Borneol	1171	1169	0.5
31 Terpinene-4-ol	1177	1182	0.4
32 α-Terpineol	1185	1189	0.5
33 Myrtenol	1199	1196	3.7
34 Decanal	1207	1206	1.1
35 Safranal	1209	1212	0.6
36 2,5-Thiophenedicarboxaldehyde	1224	1220	0.5
37 Piperitol	1226	1223	0.7
38 β-Cyclocitral	1228	1226	0.8
39 Nerol	1224	1228	2.6
40 (Z)-Citral	1235	1238	0.7
41 Geraniol	1253	1255	1.7
42 β-Homocyclocitral	1262	1261	0.2
43 (E)-Citral	1265	1267	0.8
44 3-Methyl dodecane	1278	1270	0.5
45 Dehydroedilane IIA	1287	1284	1.2
46 Dehydroedilane IA	1291	NR	2.2
47 2-Methoxy-4-vinyl phenol	1309	1313	0.6
48 (E,E)-2,4-Decandienal	1313	1317	0.5
49 Myrtenyl acetate	1325	1327	1.4
50 Citronellyl acetate	1356	1354	0.4
51 Neryl acetate	1365	1362	0.5
52 <i>n</i> -Tetradecane	1399	1400	0.4
53 Methyl eugenol	1401	1401	0.3
54 2-Methyl-5-(1,1,5-trimethyl) furan	1447	1440	1.2
55 Geranyl acetone	1454	1453	1.7
56 3-Methyl tetradecane	1465	1462	1.1
57 BHT-quinol	1480	1473	0.4
58 β-Ionone	1486	1485	6.4
59 Geranyl propionate	1489	1486	0.5
60 1-Pentadecene	1495	1490	0.4
61 <i>n</i> -pentadecane	1498	1500	0.7
62 2,4- <i>bis</i> (1,1-dimethylethyl) phenol	1512	1512	0.5
63 Dihydro-actinidiolide	1525	1522	0.4
64 7-Methyl pentacosane	1545	1548	0.6
65 <i>trans</i> -Nerolidol	1564	1564	0.3
66 <i>cis</i> -3-Hexenyl benzoate	1571	1570	0.2
67 Spathulenol	1582	1578	0.7
68 Caryophyllene oxide	1589	1583	0.4
69 <i>n</i> -Hexadecane	1589	1600	0.5
70 Miristolaldehyde	1610	1613	0.5
71 Bezophenone	1628	1628	0.7
72 epi-α-Murolol	1646	1642	0.3
73 <i>n</i> -Hexyl salicylate	1678	1675	0.3
74 <i>n</i> -Octadecane	1798	1800	0.3
75 Shiromool	1812	1810	1.6
76 Hexahydrofarnesyl acetone	1847	1845	6.6

Compound	RI ^a	RI ^b	(%) ^c
77 Diisobutyl phthalate	1872	1874	1.9
78 (<i>E,E</i>)-Farnesyl acetone	1922	1921	0.8
79 Methyl palmitate	1930	1927	0.4
80 Palmitic acid	1966	1964	1.5
81 Dibutyl phthalate	1970	1968	1.3
82 <i>n</i> -Eicosane	1999	2000	0.3
83 <i>n</i> -Heneicosane	1198	2100	0.4
84 (<i>Z</i>)-Phytol	2116	2114	3.3
85 Linoleic acid	2148	2146	0.2
86 <i>n</i> -Tricosane	2298	2300	0.8
87 <i>n</i> -Pentacosane	2498	2500	0.5
88 <i>bis</i> -(2-Ethylhexyl) phthalate	2555	2550	0.3
89 <i>n</i> -Heptacosane	2699	2700	0.8
90 <i>n</i> -Octacosane	2797	2900	0.6
Group components			
Hydrocarbons			
Alkanes, alkenes			7.9
Alcohols			5.8
Aldehyde			9.9
Ketones			3.1
Terpenoids			
Monoterpene hydrocarbons			2.8
Oxygenated monoterpenes			30.1
Sesquiterpene hydrocarbons			0.0
Sesquiterpenes oxygenated			10.7
Diterpenoids			3.3
Aromatic compounds			11.9
Fatty acids and fatty acid esters			2.9
Other compounds			4.3
Total identified			92.7

^aKovats indices determined on a HP-5MS column. ^bKovats indices obtained from references, ^cPercentages obtained by FID peak-area normalization, NR: Not reported

In other research on *Vicia* genus, the volatile oil of flowers of *V. faba* was shown to be a complex mixture of some 27 identifiable compounds¹³. In addition to the ubiquitous mono and sesquiterpenes, the porous-polymer entrained volatiles included a diverse range of phenylpropenoids which together accounted for over 7 % of the total. Cinnamyl alcohol was also found to be the most abundant free alcohol in the epicuticular wax of *V. faba* flowers. Two new classes of epicuticular wax esters consisting of saturated C16, C18, C20, C22 and C24 fatty acids esterified with the phenylpropenoid, cinnamyl alcohol and with the diterpene, phytol have been identified. In another report, the leaf volatile constituents of *V. sativa* L. have been studied using HS/PME-GC/MS and GC-FID¹⁴. Forty-three components, aliphatic hydrocarbons, alcohols, aldehydes and ketones, aromatic aldehydes, esters and alcohols, monoterpenes and sesquiterpenes were fully characterized by mass spectra, linear retention indices and injection of standards; the average composition as single components and classes of substances was reported. Leaf volatiles in *V. sativa* were characterized by a high amount of aldehydes, with the (*Z*)-2-hexenal the main component. In recent study, chemical composition of the essential oil of *V. dadianorum* have been isolated by hydro and microwave distillations (HD and

MD)⁸. The compositions of the essential oils were characterized by GC-FID and GC/MS. A total of seventy-six and fifty-six compounds were identified, constituting over 90.9 % and 80.1 % of oil composition respectively. Sesquiterpene hydrocarbons were shown to be the main group of volatiles (HD: 26.2 % and MD: 15.9 %). The major terpene constituent of the oils was γ -elemene (HD, 13.7 % and MD, 8.4 %). In our research, some similar compounds such as fatty acid and esters, phytol and aldehydes were obtained.

Conclusion

In this research, 90 major and minor constituents of essential oils from *V. variabilis* were identified. The main components were characterized by hexahydrofarnesyl acetone, β -ionone, myrtenol, (*Z*)-phytol and 1-octen-3-ol. Oxygenated monoterpenes constitute the major fraction of the oil followed by aromatic compounds, sesquiterpenes oxygenated and hydrocarbon aldehydes. This result may be used in biological activities of *V. variabilis*. Considering to the major constituents, *V. variabilis* herb can be utilized as medicinal plant.

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