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# 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 region[1]. 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 activities[4]. 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 $CCl_4$ for four weeks[5]. The data obtained from this study revealed that the flavonol glycosides protect the rat liver from hepatic damage induced by $CCl_4$ through inhibition of lipid peroxidation caused by $CCl_4$ reactive free radicals. Also, antiherbivory defense of *V. sativa* and *V. hirsuta* with and without extrafloral nectarines was investigated[6]. 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 studied[7]. 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 investigated[8] 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 tannin[9], flavonoides[10,11] and sesquiterpene[12]. 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 ºC) 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 ºC) and a flame ionization detector (250 ºC). $N_2$ 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 ºC for 3 min and then heated to 220 ºC with a 5 ºC/min rate and kept constant at 220 ºC 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 ºC for 3 min and programmed to 220 ºC at a rate of 5 ºC/min and kept constant at 220 ºC 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 spectra[15]. The identifications were confirmed by comparison of KI from HP-5MS column with those reported in the NIST liberary 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 standards[18].

## 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 | *n*-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 |
| 12 | 2-(1-Pentenyl) furan | 998 | 1000 | 0.5 |
| 13 | *n*-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 | *n*-Nonanal | 1108 | 1114 | 2.4 |
| 24 | Phenethyl alcohol | 1118 | 1116 | 0.6 |
| 25 | *cis-p*-Menth-2-en-1-ol | 1123 | 1122 | 0.7 |
| 26 | *trans-p*-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 | *p*-Menthan-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 | Dehydroedilan IIA | 1287 | 1284 | 1.2 |
| 46 | Dehydroedilan 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 | *n*-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 | *n*-pentadecane | 1498 | 1500 | 0.7 |
| 62 | 2,4-*bis* (1,1-dimethylethyl) phenol | 1512 | 1512 | 0.5 |
| 63 | Dihydro-actinidiolide | 1525 | 1522 | 0.4 |
| 64 | 7-Methyl pentacosane | 1545 | 1548 | 0.6 |
| 65 | *trans*-Nerolidol | 1564 | 1564 | 0.3 |
| 66 | *cis*-3-Hexenyl benzoate | 1571 | 1570 | 0.2 |
| 67 | Spathulenol | 1582 | 1578 | 0.7 |
| 68 | Caryophyllene oxide | 1589 | 1583 | 0.4 |
| 69 | *n*-Hexadecane | 1589 | 1600 | 0.5 |
| 70 | Miristaldehyde | 1610 | 1613 | 0.5 |
| 71 | Bezophenone | 1628 | 1628 | 0.7 |
| 72 | epi-α-Muurolol | 1646 | 1642 | 0.3 |
| 73 | *n*-Hexyl salicylate | 1678 | 1675 | 0.3 |
| 74 | *n*-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 | (*E,E*)-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 | *n*-Eicosane | 1999 | 2000 | 0.3 |
| 83 | *n*-Heneicosane | 1198 | 2100 | 0.4 |
| 84 | (*Z*)-Phytol | 2116 | 2114 | 3.3 |
| 85 | Leinoleic acid | 2148 | 2146 | 0.2 |
| 86 | *n*-Tricosane | 2298 | 2300 | 0.8 |
| 87 | *n*-Pentocosane | 2498 | 2500 | 0.5 |
| 88 | *bis*-(2-Ethylhexyl) phthalate | 2555 | 2550 | 0.3 |
| 89 | *n*-Heptacosane | 2699 | 2700 | 0.8 |
| 90 | *n*-Octacosane | 2797 | 2900 | 0.6 |
| | **Group components** | | | |
| | **Hydrocarbons** | | | |
| | Alkanes, alkenes | | | 7.9 |
| | Alcohols | | | 5.8 |
| | Aldehyde | | | 9.9 |
| | Ketones | | | 3.1 |
| | **Terpenoids** | | | |
| | Monoterpene hydrocarbones | | | 2.8 |
| | Oxygenated monoterpenes | | | 30.1 |
| | Sesquiterpene hydrocarbones | | | 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 |

[a]Kovats indices determined on a HP-5MS column. [b]Kovats indices obtained from references, [c]Percentages 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 compounds[13]. 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-FID[14]. 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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