GC/Mass analysis of the volatile compounds of \textit{P. hyrcanicum} diethyl ether extract and GC profiling of some Iranian \textit{Polygonum} species

S. Saeidnia\textsuperscript{1}, P. Sarkhail\textsuperscript{2}, F. Moradi-Afrapoli\textsuperscript{3}, A.R. Gohari\textsuperscript{1}*\textsuperscript{,} M. Nikan\textsuperscript{1}, N. Mokhber-Dezfuli\textsuperscript{1}, G.R. Amin\textsuperscript{4}, A. Hadjiakhoondi\textsuperscript{1,4}

\textsuperscript{1}Medicinal Plants Research Center, Tehran University of Medical Sciences, Tehran, Iran.\textsuperscript{,} 
\textsuperscript{2}Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran.\textsuperscript{,} 
\textsuperscript{3}Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.\textsuperscript{,} 
\textsuperscript{4}Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Abstract
In this study, the relationship among four species of \textit{Polygonum} (including \textit{P. hyrcanicum} (three samples), \textit{P. persicaria}, \textit{P. avicular}, and \textit{P. hydropiper}) was investigated by GC profiling. Furthermore, the major compounds of the ethyl ether extract of \textit{P. hyrcanicum} were identified by GC/MS as: \textit{\textalpha}-bisabolol (17.5\%), cedrol (15.9\%), sesquisabinene hydrate (13.0\%), \textit{\textalpha}-elemol (10.5\%) and trans-longipinocarveol (10.1\%). All the identified compounds were sesquiterpenes and no monoterpene, fatty acid and/or hydrocarbone were detected in the extract. Chemical distances among the mentioned species were calculated in order to construct the dendrogram of closely related samples. Results indicated that the distance between two samples of \textit{P. hyrcanicum} was considered to be short and their GC profiles were quite similar to each other and also there was a close relationship between the two samples of \textit{Polygonum} with \textit{P. avicular}. \textit{P. hydropiper} was observed far from the two samples of \textit{P. hyrcanicum} in comparison to other samples. Interestingly, \textit{P. hyrcanicum}, gathered from Veresk, had no close relationship with other pairs of \textit{P. hyrcanicum}. The results of this study support the phylogenetic relationships among these \textit{Polygonum} species which was previously reported.

Keywords: \textit{P. avicular}, \textit{P. hydropiper}, \textit{P. hyrcanicum}, \textit{P. persicaria}, volatile compounds

Introduction
The genus \textit{Polygonum} L. (Polygonaceae), named “Alafe-Haftband” in Persian language, grows as a wild weed in diverse parts of Iran. \textit{P. hyrcanicum} and \textit{P. persicaria} are two examples of its nine endemic species [1,2]. It is reported that \textit{Polygonum} (especially their rhizomes) were used for the treatment of atherosclerosis, hypertension and dermatitis [3].

Bibliography data revealed that among the isolated compounds of this genus, flavonoids have been applied as the chemotaxonomic markers in the systematic studies of Polygonaceae [4]. Furthermore, there are some reports about the chemical analysis of the volatile oils of these plants. It was reported that the essential oil of \textit{P. hydropiper} contained (E)-\textit{\textbeta}-farnesene (44.1\%), phytol
(10.8%), (E)-caryophyllene (9.3%) and (E)-nerolidol (6.9%), [5]. In another study, the major compounds of the volatile oil of *P. hydropiper* were identified as dodecanal (3–40%), (E)-2-hexenal (20–35%), decanal (4–22%), (Z)-3-hexen-1-ol (4–31%), hexanal (1.7–5.1%) and β-caryophyllene (1.7–2.3%) [6].

Literature review showed that chemotaxonomic (using volatile constituents as chemical markers) investigation of the *Polygonum* species have not been reported until now. Recently, we have reported the phylogenetic comparison and genetic diversity of some wild species of this genus [7]. In the present study, we have focused on comparing some species (growing in Iran) via GC profiling and UPGMA analysis of the diethyl ether extracts together with the GC/Mass analysis of the volatile ether extract of *P. hyrcanicum* which has not been published elsewhere.

**Experimental**

**Plant material**

Plant samples of four *Polygonum* species including *P. hyrcanicum* Rech. f. (three samples), *P. persicaria* Boiss & Bushe, *P. avicularia* L., and *P. hydropiper* L. subsp. *hydropiper* were collected during the flowering period from diverse growing areas in North of Iran (July 2010). The plant materials were dried in shade. The voucher specimens (*P. hyrcanicum*, 6729-TEH; *P. persicaria*, 6733-TEH; *P. avicularia*, 6731-TEH, and *P. hydropiper* L. subsp. *hydropiper*, 6735-TEH) have been deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

**Detection of the main volatile components**

Leaves of the plants (30 g) were dried, cut into small pieces and extracted by ethyllic ether (10 mL) at 4 ºC for 24 h. A silica gel cartridge was used for removing the chlorophyll [8]. The extracts were concentrated using nitrogen gas to the approximate volume of 0.3 mL. Each concentrated extract was injected to the Gas Chromatograph.

GC was carried out using a Dani Master GC (Fast Gas Chromatograph) with OV1 (SE54CB, 25 m×0.25 mm i.d., 0.25 μm film thickness); carrier gas, N2; split ratio, 1:20, and flame ionization detector (FID). Temperature programming was performed from 75 ºC (4 min) to 250 ºC (14 min) at 15 ºC/min, injector temperature 250 ºC and detector temperature 260 ºC.

GC/Mass was performed on a cross-linked 5% methyl phenyl siloxane (HP-5, 30 m×0.25 mm i.d., 0.25 μm film thickness), carrier gas, He; split ratio, 1:15; quadruple mass spectrometer (Hewlett-Packard 5973) operating at 70 eV ionization energy. The retention indices for all the components were calculated by using retention times of n-alkanes (C8-C25) that were injected at the same temperature and conditions, after the essential oil. The components were identified by comparing the retention indices (RI, DB-5 or DB-1) with those reported in the literatures [9, 10] and also by comparing their mass spectra with the published mass spectra [11] or Wiley library. The percentage of each component was calculated on the basis of the peak area.

**Results and Discussion**

Pair-wise comparison of GC profiles revealed a similar matrix. Simple matching coefficients (Sm) and chemical distances (d), derived from GC profiles, are shown in table 1. The distance between the two samples of *P. hyrcanicum* originated from Sari and Goharbaran regions, was considered to be short (0.790) and their GC fingerprint patterns were quite similar to each other; also there was a close relationship between these two samples of *Polygonum* with *P. avicularia* (0.826). The cladogram and rectangular phylogram, constructed on the basis of chemical distances derived from GC analyzing, are shown in figure 1. The GC chromatograms for the diethyl ether extracts of four *Polygonum* species are shown as a sample in figure 2. Clustering analysis was carried out on the basis of UPGMA (unweighted pair-group method arithmetic average) [12,13]. The cladograms were designed by the software Dendroscope which is freely available from www.dendroscope.org [14].

As it is shown in the dendrogram, *P. avicularia* represented the closest relationship with two samples of *P. hyrcanicum*. Not only *P. hydropiper* was far from the two samples of *P.
Figure 1. Cladogram and rectangular phylogram of the Polygonum samples obtained from GC profiling and on the basis of UPGMA

Figure 2. GC chromatograms of the diethyl ether extracts of four Polygonum species (P. hyrcanicum was gathered from Sari).
hyrcanicum, but it also showed different patterns of GC fingerprint compared to the other samples. It was also interesting that *P. hyrcanicum*, gathered from Veresk, had no close relationship to the other pairs of *P. hyrcanicum* (from Sari and Goharbaran).

A survey on the main morphological characters of the Polygonum species [15] indicated that *P. hydropiper* was morphologically different from *P. hyrcanicum*, specially, in case of the leaves shape and color and size of the flowers. Moreover, oval lance shaped leaves of *P. avicular* and *P. hydropiper* showed similarities to each other which were in agreement with the results of GC profiling and genetic diversity [6]. There is no report about the phytochemical investigation of two endemic species of Iran, *P. hyrcanicum* and *P. persicaria*. One of the interesting points obtained from this study is that *P. hyrcanicum* (gathered from Veresk village near the top of Gadook Mountain) showed different GC profile and RAPD/ISSR pattern from other samples even *P. hydropiper* (Goharbaran and Sari near the Caspian Sea).

Table 1. Simple matching coefficient (Ssm, above the diagonal) and distances (d, below the diagonal) between pairs of *Polygonum* samples resulted from GC profiling

<table>
<thead>
<tr>
<th>NO</th>
<th>Name</th>
<th>Retention Indices</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ar-curcumene</td>
<td>1479</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>α-zingiberene</td>
<td>1493</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>trans-α-farnesene</td>
<td>1505</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>β-sesquiphellandrene</td>
<td>1521</td>
<td>3.8</td>
</tr>
<tr>
<td>5</td>
<td>α-elemol</td>
<td>1548</td>
<td>10.6</td>
</tr>
<tr>
<td>6</td>
<td>trans-nerolid</td>
<td>1561</td>
<td>2.7</td>
</tr>
<tr>
<td>7</td>
<td>sesquisabinen hydrate</td>
<td>1577</td>
<td>13.0</td>
</tr>
<tr>
<td>8</td>
<td>cedrol</td>
<td>1600</td>
<td>15.9</td>
</tr>
<tr>
<td>9</td>
<td>trans-longipinocarveol</td>
<td>1634</td>
<td>10.1</td>
</tr>
<tr>
<td>10</td>
<td>β-eudesmol</td>
<td>1644</td>
<td>9.6</td>
</tr>
<tr>
<td>11</td>
<td>α-bisabolol</td>
<td>1685</td>
<td>17.5</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>-</td>
<td>89.4</td>
</tr>
</tbody>
</table>

The samples used in this analysis were: *P. hyrcanicum* (three samples including Goharbaran (4), Sari (5) and Veresk (2)), *P. persicaria* (1), *P. avicular* (3), and *P. hydropiper* (6).

For this reason the ethylic ether extract of this sample was selected for GC/Mass analysis. The results of GC/Mass analysis has been summarized in table 2. As it is shown, the major compounds of the ethylic ether extract of *P. hyrcanicum* were α-bisabolol (17.5%), cedrol (15.9%), sesquisabinen hydrate (13.0%), α-elemol (10.6%) and trans-longipinocarveol (10.1%). All the identified compounds were sesquiterpenes (C15) and no monoterpene, fatty acid and /or hydrocarbon were detected in the extract which made this sample different from other samples of *P. hyrcanicum*. On the basis of our unpublished data, the volatile oils of *P. hyrcanicum* and *P. persicaria* extracted by hydro-distillation have completely different main compounds and are commonly enriched of fatty acids.

**Acknowledgements**

This research has been supported by Tehran University of Medical Sciences and Health Services for plant gathering and identification.

**References**


[7] Saeidnia S, Faraji H, Sarkheil P, Moradi-Afrapoli F, Amin G. Genetic diversity and relationships detected by intersimple sequence repeat (ISSR) and randomly amplified polymorphic DNA (RAPD) analysis among
**GC/Mass analysis of the volatile compounds of P. hyrcanicum diethyl ether extract**


