Effects of altitude on total phenolic and polyphenol content of *Marrubium astracanicum* L. extracts

Firozeh Taremi¹, Vahid Rowshan²*, Mohammad Hosain shirzadi¹

1. Department of Biology, Sciences and Research Branch of Fars Islamic Azad University, Shiraz, Iran.
2. Department of Natural Resources. Fars Research Center for Agriculture and Natural Resources, PO Box 71555-617, Shiraz, Iran.

*Corresponding author email: vahid.rowshan@gmail.com*

**ABSTRACT:** Marrubium astracanicum is a genus of perennial plants which belongs to the family Lamiaeae (Labiatae). Polyphenol compounds were identified by HPLC-DAD. The comparison of polyphenolic in different sites showed that the Cinamic acid from 1269.67 ppm in Dasht-arjand (2050) site, decreased to 433.45 and 537.95 ppm in Abmordi (1832m) and Derak (2500m) sites respectively. The amount of gallic acid in Abmordi was 5.24 ppm that increased to 16.63 ppm in Derak (2500m). The amount of Cachin was 114.20 ppm in Derak (2500m) and changed to 81.84 ppm in Cheshma Anjer (2125m) and 51.58 ppm in Abmordi. The Cholorogenic acid only detected in Cheshma Anjer and Dasht-arjan sites that amount of it was 38.5 ppm in Dasht-arjan and 27.78 ppm in Cheshma Anjer. The results also have been shown that Cinamic acid was the highest amount among phenolic compound in all sites. There was not detected Cumarin. Total phenolic content ranged 9.0- 2.97 mg gallic acid equivalent/g of extracts, Derak (2500m) > Cheshma Anjer (2125m) > Dasht-arjan (2050)> Ab-mordi (1832m).

**Keywords:** Polyphenolic component, Methanolic extract, Marrubium astracanicum, Total phenolic.

**INTRODUCTION**

Medicinal plants (especially those belong to Lamiaeae family) are potential sources of new drugs to improve the treatment of diseases whose treatment is associated to polyphenolic component. Lamiaeae family has been holding a place of value for hundreds of years due to the infusions and tinctures of numerous aromatic species used as components of herbal treatments for a variety of ailments (Naghibi et al., 2005). Marrubium (horehound) a genus of about 40 species of flowering plants in the family Lamiaeae is native to temperate regions of Europe and Asia which has a high reputation in traditional herbal medicine and different cultures with by several known healing attributes (Meyre-Silva and Cechinel-Filho, 2010). Additionally, studies dealing with the composition, antimicrobial and antioxidant activities of essential oils extracted from genus Marrubium have been previously reported (Nagy and Svajdlenka, 1998; Demirci et al., 2004; Javidnia et al., 2007; Sarikurkcu et al., 2008; Laouer et al., 2009; Petrovic et al., 2009; Zarai et al., 2011). Regarding the phytochemical analysis of the plants of this genus, they mostly produce diterpenes, polyphenols, steroids, phenylpropanoids and flavonoids, some of which have important biological properties (Karioti et al., 2003; Alkhatib et al., 2010; Zaabat et al., 2011).

Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts (Wolfe et al., 2003). Measurement of the polyphenols and free radical scavenging activity of herbs has become important tools for the understanding of the relative importance of plant species especially from the health point of view (Chang et al., 2007). Caffeic acid is an organic compound that is classified as hydroxycinnamic acid. This yellow solid consists of both phenolic and acrylic functional groups. It is found in all plants because it is a key intermediate in the biosynthesis of lignin, one of the principal sources of biomass (Boerjan et al., 2003). Catechin is a flavan-3-ol, a type of natural phenol and antioxidant. It is a plant secondary metabolite. It belongs to the group of flavan-3-ols (or simply flavanols). It is often considered to belong to the family of flavonoids. Gallic acid is found both free and as part of hydrolysable tannins. Salts and esters of gallic acid are termed 'gallates'. Despite its name, it does not contain gallium. Gallic acid is commonly used in the pharmaceutical industry (Fiuza, 2004). Gallic acid can also be used as a starting material in the synthesis of the psychedelic alkaloid mescaline (Makepease, 1951). The aim of this research was to determine the total phenolic and polyphenol content of Marrubium astracanicum at several altitudes.
MATERIAL AND METHODS

PLANT MATERIAL

Plant samples were collected from different altitudes (1832m, 2050m, 2125m, and 2500m above sea level) in June 2012 from Fars province. After collecting plant samples washed and then dried them by liquid nitrogen. The experiments were done in agricultural laboratory of Natural Resources Department in Fars province.

Extraction of Polyphenol and HPLC analysis

HPLC analysis was performed by using an agilent 1200 series, equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies), a quaternary pump an online vacuum degasser, an autosampler and a thermostated column compartment, on an agilent Zorbax Eclipse XDB-C18, 5 µm (ID), 4.6x 150 mm (FT) column, at a flow-rate of 1 ml min⁻¹. Solvent gradient was performed by varying the proportion of solvent A (formic acid 1% in deionized water) to solvent B (Methanol (v/v)) as follows: Methanol: formic acid 1%: (10:90), Hold Time: 0min; Methanol: formic acid 1%: (25:75), Hold Time: 10min; Methanol: formic acid 1%: (60:40), Hold Time: 20min, Methanol: formic acid 1%: (70:30), Hold Time: 30min, the total running time was 30 min. The column temperature was 30 ºC. The injected volume of samples and standards was 20µL and it was done automatically using autosampler. Chromatograms were plotted at 280 and 320 nm.

Determination of total phenol content

Total phenolic compound contents were determined by the Folin-Ciocalteau method [12-13], The extract samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteau reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na2CO3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetry at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg ml⁻¹ solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass).

RESULTS AND DISCUSSION

The results showed that the maximum Cinamic acid was detected at 2050m altitude sea level (1269.679) and the altitudes at 2125, 2500 and 1832m were 997.5498, 537.9529 and 433.4516 respectively (Fig. 1). The caffeic and chlorogenic acid only detected in Cheshma Anjer (2125m) and Dasht-arjan (2050) (Table 1). Caffeic acid, which is unrelated to caffeine, is biosynthesized by hydroxylation of coumaroyl ester of quinic ester. This hydroxylation produces the caffeic acid ester of Shikimic acid, which converts to chlorogenic acid. It is the precursor to ferulic acid, coniferyl alcohol, and sinapyl alcohol, all of which are significant building blocks in lignin (Boerjan et al., 2003). The amount of Catchin was 114.20 ppm in Derak (2500m) and changed to 81.84 ppm in Cheshma Anjer (2125m) and 51.58 ppm in Abmordi (Table 1). These results might be seen due to the climate or stress which causes the activities of polyphenols (Gallic acid, Chlorogenic acid and Caffeic acid) to be different at each altitude. There was not detected Cumarin. This group of phenolic compound can be found free in nature or in combined form with sugars as heterosides and glycosides in many dicotyledonous families, including the Asteraceae, Rosaceae, Rubiaceae and Solanaceae. Although mainly synthesised in the leaves, coumarins occur in high levels in fruits, roots and stems. Coumarins, which are also C3-C6 derivatives, belong to a group of compounds known as the benzo-α-pyrene. Quercetin was not detected in Cheshma Anjer (2125m), high amount was seen in 2050m (Dasht-arjan) with 62.24ppm.

The study showed total phenolic content ranged from 9.0- 2.97 mg gallic acid equivalent/g of extracts. There were significant differences (p<0.05) in total phenolic content between four altitudes, the highest level of phenolics was found in Derak (2500m), while the lowest was in Ab-mordi (1832m) (Table 2). Total phenolic content of four altitudes decreased in the following order: Derak (2500m) > Cheshma Anjer (2125m) > Dasht-arjan (2050)> Ab-mordi (1832m)(Table 2).

Some authors(Djeridane et al., 2006; Katsube et al., 2004) have demonstrated a linear correlation between the content of total phenolic compounds and their antioxidant capacity, while others (Czapecka et al., 2005) show poor linear correlation or report total antioxidant activity and phenolic content with no comment. The results obtained in our study show good correlation within one species.
Table 1. Phenolic compounds of Marrubium astracanicum at four altitudes.

<table>
<thead>
<tr>
<th>Altitude</th>
<th>Cumarin (ppm)</th>
<th>Quercetin (ppm)</th>
<th>Caffeic acid (ppm)</th>
<th>Cholorogenic (ppm)</th>
<th>Cetchin (ppm)</th>
<th>Gallic acid (ppm)</th>
<th>Cinamic acid (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab-mordi (1832m)</td>
<td>-</td>
<td>27.858</td>
<td>-</td>
<td>-</td>
<td>51.580</td>
<td>5.241</td>
<td>433.451</td>
</tr>
<tr>
<td>Dasht-arjan (2050m)</td>
<td>-</td>
<td>62.245</td>
<td>22.561</td>
<td>38.530</td>
<td>-</td>
<td>-</td>
<td>1269.679</td>
</tr>
<tr>
<td>Cheshma Anjer (2125m)</td>
<td>-</td>
<td>-</td>
<td>56.406</td>
<td>27.783</td>
<td>81.849</td>
<td>-</td>
<td>997.540</td>
</tr>
<tr>
<td>Derak (2500m)</td>
<td>15.923</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>114.209</td>
<td>16.635</td>
<td>537.952</td>
</tr>
</tbody>
</table>

Figure 1. Cinamic acid of Marrubium astracanicum at four altitudes.

Table 2. Comparison total phenolic content of Marrubium astracanicum at four altitudes.

<table>
<thead>
<tr>
<th>Sample, Altitude</th>
<th>(TPC) Total phenolic content (mg gallic acid equivalent/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab-mordi (1832m)</td>
<td>2.971667± 0.245</td>
</tr>
<tr>
<td>Dasht-arjan (2050m)</td>
<td>3.621667± 0.334</td>
</tr>
<tr>
<td>Cheshma Anjer (2125m)</td>
<td>3.913333± 0.654</td>
</tr>
<tr>
<td>Derak (2500m)</td>
<td>9.005± 0.245</td>
</tr>
</tbody>
</table>

Data are displayed with mean ± SD, and (P<0.05).

REFERENCES


