

*Full Length Research Paper*

# High-performance liquid chromatography (HPLC) nano analysis of antioxidant compounds of Iranian medicinal plants

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**Four Iranian plants (pomegranate, khakshir, shevid, and chaye-shomal), already known to have various medicinal properties since several decades ago, were evaluated for their antioxidant compounds. Most of these bioactive properties correlating with antioxidant activity of phenolic compounds found in the plants. The high-performance liquid chromatography analysis was used in pomegranate juice and methanol extracts of the rest of the samples. Based on applied reference compounds which are gallic acid, vanilic acid, ascorbic acid, quercetin, caffeic acid, catechin and coumaric acid; khakshir and chaye-shomal demonstrated some high comparable peaks. Both pomegranate and shevid showed very low peaks in this area, indicating another phenolic compounds imposed by these plants.**

**Key words:** High-performance liquid chromatography (HPLC), phenolic, antioxidant, medicinal plants.

## INTRODUCTION

Natural products, such as plant extract, either as pure compounds or as standardized extracts, provided unlimited opportunities for new drug discoveries because of the unmatched chemical diversity they can provide (Cos et al., 2006). According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Iran has various climates with over more than 7500 plant species and claimed that 10 to 15% of them are medicinal plants. These diverse herbs can be found as harvested or farm plants (Khanahmadi and Rezazadeh, 2010).

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family, is a small tree with potential human

health benefits. It is cultivated around the world in California, Turkey, Egypt, Italy, India, Chile and Spain (FAOSTAT, 2011). Edible parts of pomegranate fruit comprises of 78% juice and 22% seed. Pomegranate seeds are rich in sugars, vitamins, polysaccharides, polyphenols and minerals (Kulkarni and Aradhya, 2005). This fruit is widely acknowledged for antioxidant properties, which are higher than most other fruit- related food items that were originally thought to contain the highest amounts of antioxidants (Guo et al., 2003; Gil et al., 2000). Khak shir (*Eragrostis tef*) is an annual grass, a species of lovegrass native to the northern Ethiopian Highlands of Northeast Africa. Nowadays, teff represents the re-discovery of a crop used by ancient civilizations (Seyfu, 1997). Protein level (10 to 12%) of teff grain is similar to other cereal grains. Total phenol levels of millets brown tef reported about 3 mg gallic acid equiv./gram (Guajardo-Flores et al., 2006).

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**Table 1.** Work gradient of HPLC analysis.

Time (min)	A (%)	B (%)
0	100	0
5.25	85	15
10.65	85	15
37.5	60	40
39	60	40
40.5	0	100
43.5	0	100
45	100	0
52.5	100	0

Shevid (*Anethum graveolens* L.) commonly known as dill has been cultivated in Mediterranean region, Europe and central Southern Asia since antiquity. Dill has been reported to pose antioxidant activity (Al-Ismael and Aburjai, 2004; Bahramikia and Yazdanparast, 2009). Tea (*Camelia sinensis* L.) is a widely consumed beverage throughout the world. The leaves of this plant have being reported to have biological properties such as antimutagenic (Halder et al., 2005), anticarcinogenic (Han, 1997) and antioxidant (Sarkar and Bhaduri, 2001). Chaye-shomal is a tea leaves found in north Iran. This tea is quite famous for their golden color and used a lot by Iranian. Oxidation is a natural process in organisms for the production of energy to fuel biological cycles. Conversely, the uninhibited production of oxygen-derived free radicals is involved in the onset of many diseases such as arthritis, atherosclerosis, rheumatoid and cancer as well as in many degenerative diseases related with aging (Halliwell and Gutteridge, 1984). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Phenolic compounds have antioxidant properties and can protect against degenerative diseases. All plants based-foods have phenols, which affect their appearance, odor, taste and oxidative stability (Naczka and Shahidi, 2004). The purpose of this study was to identify four culinary and medicinal plants that were grown in the same location and identify some phenolic compounds by high-performance liquid chromatography (HPLC).

## MATERIALS AND METHODS

### Materials

The Iranian medicinal plants used in these experiments were obtained from some location in Saveh, Markazy province, Iran. These plants are pomegranate (*Punica granatum* L.), khakshir (*Eragrostis tef*), shevid (dill) and chaye-shomal (tea from north Iran). Caffeic acid, vanilic acid, p-coumaric acid, catechin and quercetin were purchased from Sigma Chemical Co. (St. Louis, USA). Gallic acid, ascorbic acid and methanol were purchased from Merck Co. (Germany). All solvents used were the highest grade available.

### Sample preparation

Pomegranate sample prepared by squeezing the seeds and collect the juice. Khakshir (grains), shevid (leaves) and chaye-shomal (leaves) prepared by the same method. First, samples were dried at room temperature for two weeks. Then each of 0.5 g dried sample was added to 10 ml methanol and put in a warm water (40°C) for 24 h. Extracts were collected by centrifugation at 4000 rpm for 5 min then filtered through whatman paper. All of the samples keep in -80°C until used.

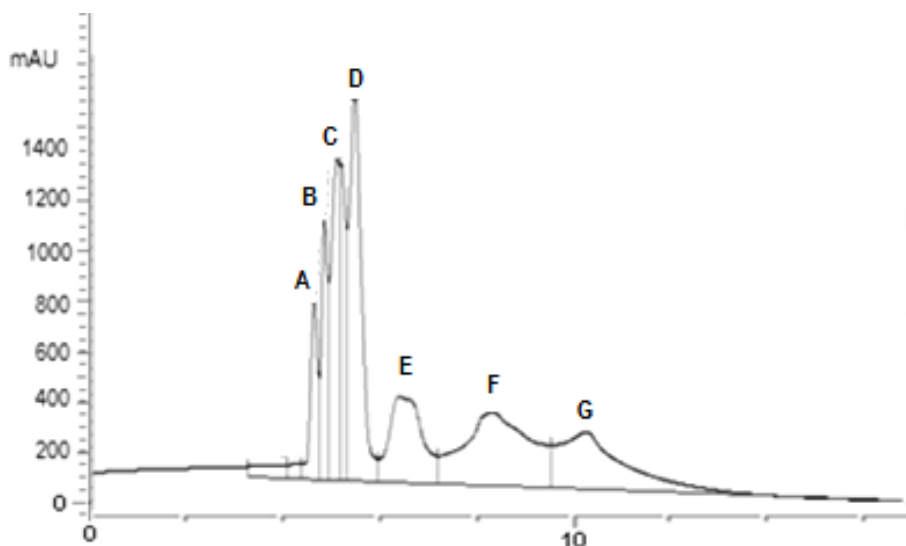
### High-performance liquid chromatography (HPLC) analysis

The separation and identification of polyphenols compounds were made through High performance of liquid chromatography (HPLC). The juice or extracts used for HPLC analysis were passed through a 0.45 µm filter (Millipore, MSI, Westboro, MA) before injection into a HPLC column of 150 mm length (Agilent technologies 1200 series). The mobile phase was acidified water containing 0.1% formic acid (A) and acidified acetonitrile containing 0.1% formic acid (B), eluted in gradient (Table 1). The flow rate was 0.8 mL/min and the wavelengths of detection were set at UV 300 nm, temperature at 30°C, injection volume = 20 µl and analysis time was 60 min. Reference substances is a mixture of gallic acid, vanilic acid, ascorbic acid, quercetin, caffeic acid, catechin and coumaric acid (solutions in methanol, each of them 0.5 mg/ml). Figure 1 presents the mix standard chromatogram used for identification of main compounds.

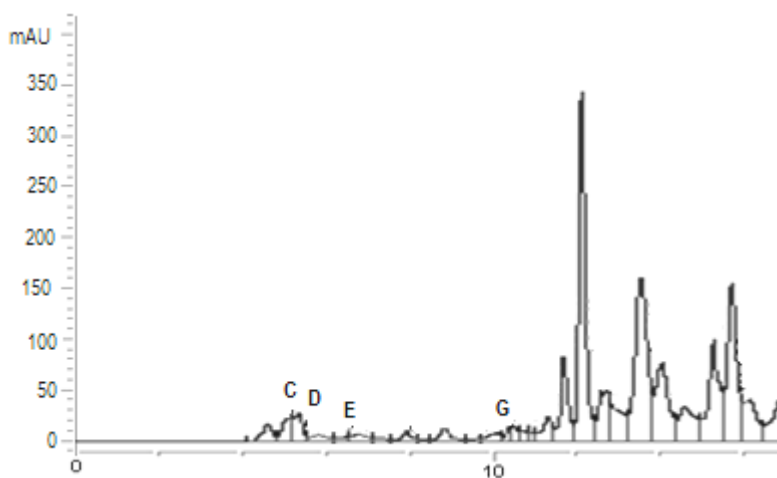
## RESULTS AND DISCUSSION

The HPLC chromatogram of reference substances have peaks that appear in early time (finish at 15 min, Figure 1), while samples have longer time for all of the peaks (data not shown). Considering the reference compound peaks have short retention time, therefore all of chromatogram samples will only be discussed in this area. According to HPLC analysis, we identified gallic acid from all samples and quercetin in three samples except shevid. Ascorbic acid was found in pomegranate and chaye-shomal, while cathecin was only found in pomegranate (Figures 2, 3, 4 and 5). The quercetin and gallic acid content are different relatively to standard. A quite high gallic acid was found in khakshir and chaye-shomal extracts but very low in pomegranate and shevid. Quercetin found in khakshir and chaye-shomal was also high, but low in pomegranate. Based on percent area, gallic acid and quercetin in chaye-shomal are about three times higher than khakshir.

Pomegranate seeds are well known to be rich in polyphenols (Kullkarni and Aradhya, 2005), and therefore has very high antioxidant properties (Gil et al., 2000). The chromatogram of pomegranate juice from Saveh also revealed a lot of peaks that came out until 60 min (data not shown). Part of these peaks can be seen in Figure 2. Peaks that appear until 10 min were very low designated and the little content of phenolic compounds types were compared to references. Pomegranate juice is an important source of anthocyanins, the 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and



**Figure 1.** HPLC chromatogram of standard mixture: caffeic acid (A), vanilic acid (B), ascorbic acid (C), gallic acid (D), quercetin (E), coumaric acid (F) and catechin (G).



**Figure 2.** HPLC chromatogram of pomegranate juice.

pelargonidin, also contains ascorbic acid and hydrolyzable tannins punicalagin and punicalin (Gil et al., 2000). Pomegranate chromatogram showed the peak of ascorbic acid, gallic acid, quercetin and catechin (Figure 2). The existence of ascorbic acid and gallic acid (as hydrolyzable tannin) were compatible with previous study, while another polyphenols like punicalagin, delphinidin, cyanidin and ellagic acid were identified after 10 min (Gil et al., 2000).

According to Dykes and Rooney (2007) teff grain has some phenolic acid such as gentisic, vanilic, ferulic, caffeic, p-coumaric and cinnamic. The HPLC chromatogram of khakshir (teff) revealed the existence of vanilic acid, gallic acid, and quercetin (Figure 3). Since the content of caffeic and coumaric in teff is quite low (Dykes and Rooney, 2007), therefore the peaks could not be

detected or might be overlapped with another high “unknown” peak.

According to Shan et al. (2005), dill showed low antioxidant capacity (between 4 and 11 mmol/100 g of DW) and contained relatively quite low phenolics. The major phenolic compounds found in dill are proto-catechuic acid and catechin, however negative for gallic acid, caffeic acid, p-coumaric acid and quercetin. This report is not really compatible with HPLC chromatogram of shevid, whereas in shevid was found peak for gallic acid but not catechin (Figure 4). This different could be because of some reason such as different types of dill plant or different conditions applied on HPLC analysis. However, both of these results revealed the same information, where shevid (dill) has low antioxidant activity. The low antioxidant activity of shevid showed by

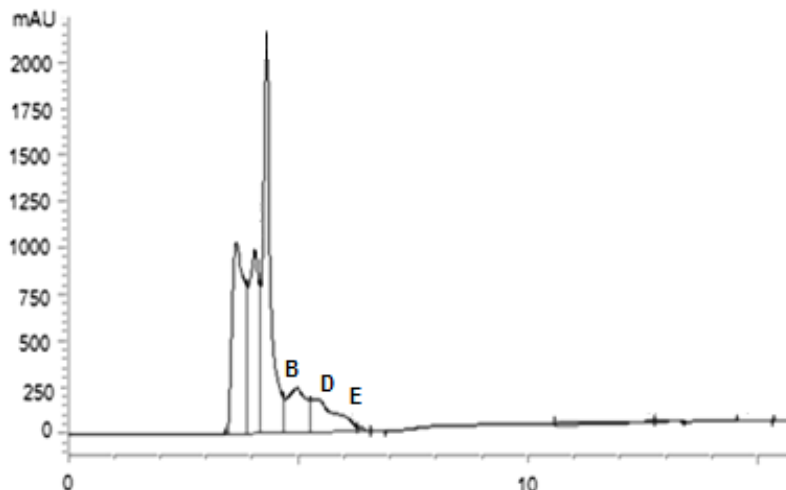


Figure 3. HPLC chromatogram of khakshir (teff) extract.

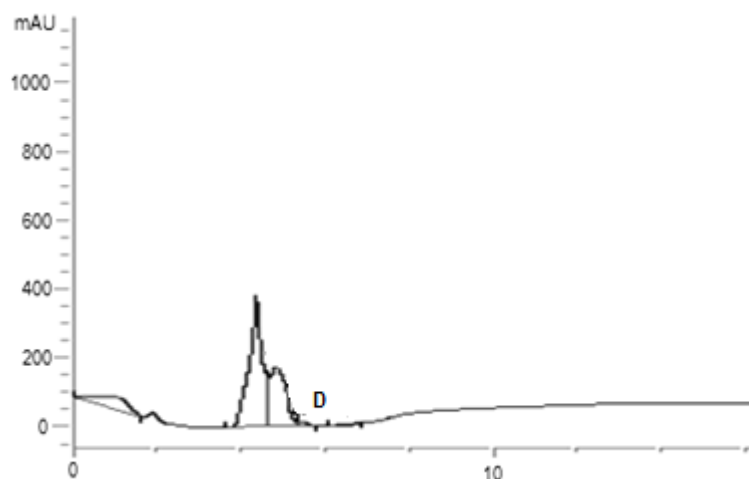


Figure 4. HPLC chromatogram of shevid (dill) extract.

the very low peaks comparing to another peaks of samples, indicating the low content of phenolic compounds.

Phenolics compounds detected in chaye-shomal based on reference compounds are ascorbic acid, gallic acid, and quercetin (Figure 5). Tea polyphenolics have electron-donating antioxidant properties, the relative activity of the different polyphenolic compounds is related to the number and location of the hydroxyl groups and the presence of the galloyl moiety (Lin et al., 1996; Rice-Evans et al., 1996). Green tea contains 30-42% catechins of total dry mass (Graham, 1992). The antioxidant power of all teas correlated strongly with the total phenolics content. Tea antioxidants with a greater number of phenolic hydroxyl groups have greater antioxidant power, that is, epigallocatechin gallate (8 groups) > epicatechin gallate (7groups) > GC (6 groups)

> epicatechin (5 groups) > epigallocatechin (6 groups) (Wiseman et al., 1997). According to HPLC chromatogram of chaye-shomal, catechin was not detected. This is probably because chaye-shomal was extracted using methanol, which is less polar than water, while catechin was found in aqueous solution. Studies confirmed that (+)-catechin adopts a mixture of A- and E-conformers in aqueous solution and their conformational equilibrium has been evaluated to be 33:67 (Kříz et al., 2003).

### Conclusion

Four Iranian medicinal plants showed highly different phenolic content based on HPLC analysis toward seven reference compounds. Khakshir (teff) and chaye-shomal

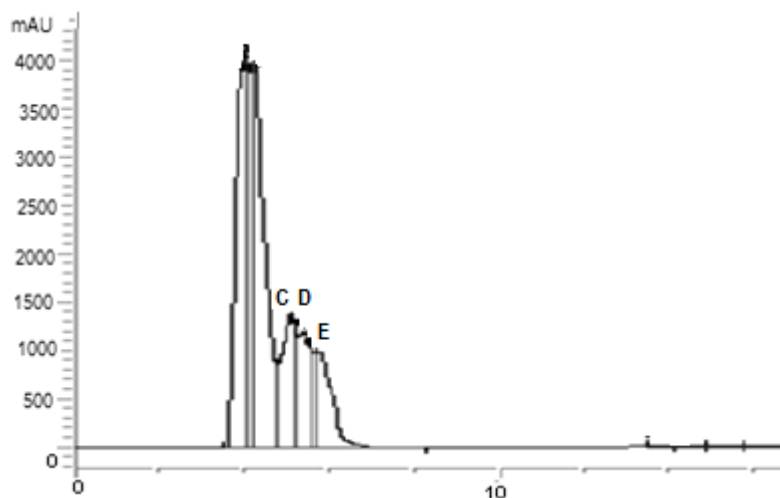


Figure 5. HPLC chromatogram of chaye-shomal (tea) extract.

(tea) demonstrated some high comparable peaks, while pomegranate and shevid (dill) showed very low peaks within references.

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