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South Khorasan Province, Iran the largest producer of *Berberis vulgaris* in the World: investigation of tannin content in the fruit

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Key words: Tannins, *Berberis vulgaris*, Alkaloids, Berberine, Barberry.

Abstract

South Khorasan province which is located in South East of Iran, possesses a vast variety of plants enriched of pharmacological components. One of these valuable plant is barberry (*Berberis vulgaris*. family Berberidaceae). Considering the importance of plant *Berberis vulgaris* in popular medicine, we have extensively studied fruit of this plant, concentrating on tannins in this article due to biological effects such as anti-bacterial, anti-tumor and inhibitor of HIV replication. Twenty sample of barberry fruit were collected from different regions in South Khorasan Province, Iran. Total amount of tannins, condensed tannins and total phenols of twenty sample were measured. Total tannins, measured by Folin & Ciocalteu method, and the amount of the total phenols were recorded to tannic acid mg/g of extract. Total condensed phenolics content also was analysed by butanol-HCl assay. Then Statistical analysis was performed and all determinations were made in the triplicate and the values were averaged and reported along with the standard deviation. Results showed that the average rainfall has direct correlation with the amount of tannin for the time interval from March 2013 to July 2013. Meanwhile, our results showed negative correlation between average temperatures and the content of tannin for the period.

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Introduction

Medicinal Herbs are in use for thousands of years and are famous for their effectiveness in treatment of many diseases. These natural herbs are very effective in boosting the immune system and are the reservoir of therapeutically bio-active phyto constituents. The amazing structural diversity among them makes a useful source of novel therapeutic compounds (Pradhan *et al.*, 2013).

Berberis vulgaris is a shrub in the family of Berberidaceae. Fruit of the plant is known as barberry. The plant is 1-3 m tall, spiny, with yellow wood, bearing pendulous yellow flowers succeeded by oblong red fruits (barberry).It has leathery and small leaves that are egg-shaped and have little red fruits in the form of clusters hanging from the branches (Zargari, 1990).

Berberis vulgaris is used medicinally in virtually all-traditional medical systems, and have a history of usage in Iranian and Chinese medicine, dating back at least 3,000 years. Studies conducted in Iran and some other countries, show that various parts of the plant have been used as folk medicine for a long time (Shahidi *et al.*, 2008). The plant Berberis vulgaris contains malic acid, resin, fat, albumin, gum, alkaloids, starch, vitamin K and vitamin C, aesculetin, caffeic acid, chlorogenic acid, quercitrin, ursolic acid, delphinidin-3-O-β-D-glucoside, berlambine and tannins (Imanshahidi *et al.*, 2008). Most of these compounds, possess various degrees of antioxidant or free radical scavenging properties. A number of phenolic compounds have medicinal properties and have been used as drugs for a long time (Karou *et al.*, 2006). Among these compounds alkaloids and tannins are very important and substantial.Studies carried out on the chemical composition of this plant, show that the alkaloid constituents with anisoquinolinic nucleus such as berberine, berbamine and palmatine are the most important alkaloids of this plant in regards to pharmacological properties (Imanshahidi *et al.*, 2008). In Table1, structure, family and properties of the main alkaloids in plant of Berberis vulgaris are shown (Okuda *et al.*, 2011). Based on the studies of Iranian researchers the majority of the medical properties of barberries are related to different alkaloids exist in different parts of this plant, including roots, leaves and fruits. Berberine, as a major alkaloid of this plant, has been used for treating diarrhea and gastrointestinal disorders for a long time. Berberine is used in the Orient for the treatment of congestive heart failure. Some studies have shown berberine exerts protective effects against hypertrophy ischemia reperfusion injury of heart and severe congestive heart failure (Javadzadeh *et al.*, 2012; Okuda *et al.*, 2011; Pradhan *et al.*, 2013; Sun *et al.*, 2009; Zhou *et al.*, 2008).

Berberine isolated from many kinds of medicinal herbs, such as Berberis aquifolium, Berberis vulgaris (barberry), Berberis aristata, Hydrastis canadensis (goldenseal), Tinospora cordifolia, Xanthorhiza simplicissima, Phellodendron amurense, Argemone mexicana and Eschscholzia californica (Singh *et al.*, 2010). It is safe, inexpensive and has been extensively used as an antibacterial drug (Okuda *et al.*, 2011). It is proved that berberine has many other pharmacological effects including antimicrobial, antitumor (Imanshahidi *et al.*, 2012), anti-inflammation (Imanshahidi & Hosseinzadeh, 2008), blood glucose lowering (Zhang *et al.*, 2010). Moreover, inhibiting chronic cocaine-induced substances including berberine was beneficial for correcting lipid metabolism disorders (Zhou *et al.*, 2008).

Tannins have diverse effects on biological systems because they are potential metal ion chelators, protein precipitating agent and biological antioxidants. Plant polyphenols, also known as vegetable tannins, are a heterogeneous group of natural products widely distributed in the plant kingdom. Plants containing tannins may contain phenolic compounds ranging from simple molecules such as phenolic acids to highly polymerized molecules such as tannins (Rabbani *et al.*, 1987; Sulaiman *et al.*, 2011). The term tannin was given to

the plant extracts exhibiting astringency, without knowing their chemical structures. The important difference between tannins and plant polyphenols of other types is the binding property of tannin with proteins. Tannins are classified into two groups of pyrogallol type tannins and catechol type (or catechin type) tannins according to the polyphenol groups in their molecules. Progress and developments in tannin chemistry led to the renaming of these two groups to hydrolysable tannins and condensed tannins. In Fig.1 hydrolysable tannins (Acenitannin) and condensed tannins (Epicatechin) are shown. Recent advances in tannin chemistry started with isolation of the ellagitannins led to the additional categorization of tannins and related polyphenols into two types: type A, with constant structures, and type B of variable composition (Alemardan *et al*., 2013; Imanshahidi *et al*., 2008; Karou *et al*., 2006). Although dietary tannins are often perceived as detrimental because of their potential to affect protein digestibility or metal ion availability, it is also possible that tannins are beneficial. Tannins that are potential antioxidants, have biological and pharmacological activities including antimicrobial and antitumor activities, inhibition of tumor promotion and inhibition of the mutagenicity of carcinogens host-mediated antitumor activity. One of the most noticeable antimicrobial activities of tannins is synergistic effects against antibiotic-resistant bacteria (Rabbani *et al*., 1987).

South Khorasan province which is located in South East of Iran, is the main habitat and main producer of this plant. In Iran more than 5,000 tons of barberries are produced each year (Javadzadeh *et al*., 2012). There are many studies performed on fruit, root and leaves of *Berberis vulgaris*. Fruits of the plant have been used as a food additive. In Iran, people drink barberry juice and use its fruit in preparing food, jam, pickles, and syrups, round flat candy, dried barberry concentrate, and Sohan Asali (a traditional Iranian sweet). In order to use barberry fruit in industrial level, many researches have been conducted on preparing different products such as beverages, sauce, jelly, candy, pastilles, colored edible powder etc. in recent years (Alemardan *et al*., 2013). Barberry plant has a great value from the economic perspective for people in South Khorasan Province, Iran. Consequently more research on this plant is beneficial. Tannins and phenolic compounds are some of biological active compounds in barberry fruit.

In this research total phenolic compounds and total tannins are measured based on Makkar method (Makkar *et al*., 1997) and condensed tannins based on Porter method (Porter *et al*., 1986). The object of this study is to compare total phenolic, condensed tannin and hydrolysable tannin of barberry fruit in 20 different regions in South Khorasan Province, Iran and to do the related analytical analysis. Moreover, the correlation between total tannin compounds and total phenolic compounds and rainfall of different regions is studied.

Materials and methods

Materials

Twenty sample of barberry fruit were collected from different regions in South Khorasan Province, Iran. For this purpose mature and healthy trees were selected. Barberry fruits collected and accumulated to dry. The fruits were dried away from sunlight at the room temperature. Then put in oven at 40°C for 7 days and grounded to a fine powder.

Extraction

The material (1 gm) was extracted with 9 ml of 70% acetone in a concealed flask in a shaking condition. The extract was then separated from the residue by filtration through Whatman No.1 filter paper. Barberry solution was centrifuged at 6000 rpm for 10 minutes and total tannins were measured by Folin and Ciocalteu method (Kähkönen *et al*,.1999) . Then, 0.1 ml of the extract was added with 0.5 ml of water, 0.25 ml of Folin reagent (a mixture of phosphomolybdate and phosphotungstate) and 1.25 ml of sodium carbonate solution. The mixture was kept for 40 min at room temperature and absorbance was recorded at 725 nm using a Secomam UV-XE2

spectrophotometer. The amount of the total phenols were recorded to tannic acid mg/g of extract. All materials that were used in this study were prepared with high purity from Merck Company. In order to separate tannin from the tannin containing extract, 0.1 g of the PVP (polyvinylpyrrolidone) was added to 1.0 ml of the extract. Then 1.0 ml of the distilled water was added to the prepared mixture. The tube was vortexed for 5 min and was kept at 4°C for 15 min. The mixture was centrifuged at 3000 rpm for 10 min using a Hettich eba20 centrifuge, supernatant was collected and absorbance was recorded at 725 nm to tannic acid mg/g of extract. Total tannins is calculated by subtracting the values of the total phenols except tannins from the total phenols. In order to determine condensed tannin, 3.0 ml of the butanol-HCl reagent and 0.1 ml of the ferric reagent were added to the 0.5 ml of the above tannin acetone extract . The tubes were vortexed and placed in a boiling water bath for 60 min. The extracts were cooled and absorbance was recorded with UV/Vis at 550 nm.

Statistical analysis

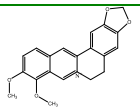
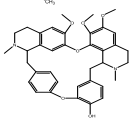
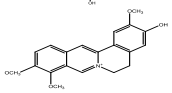
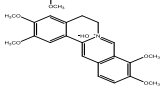
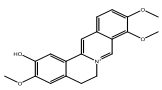
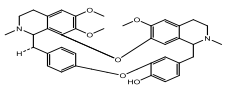
In the statistical analysis, all determinations were made in the triplicate and the values were averaged and reported along with the standard deviation (± S.D.). Statistical analysis was carried out using SPSS software. One of the fundamental class of measures in statistical analysis are correlation coefficients (r) which determined the relationship between investigated variables. Based on normality test (Kolmogorov-Smirnov (K-S) test), all three variables are normally distributed (P>0.01).

Therefore the proper measure of correlation is Pearson correlation test. In Pearson correlation analysis, highly significant linear correlations ($P < 0.01$) existed between CT and TT with $r = 0.73$, TT and TP with $r = 0.80$ and CT and TP with $r=0.83$. It is observed that the correlation between CT and TP is positive and higher than the two other correlations.

Results and discussion

In this study analysis of twenty samples of barberry is achieved. The samples of barberry were collected from the regions that are the main producers of Berberis vulgaris in Iran (Fig. 2). Samples were collected carefully and dried at the room temperature in the absence of direct sunlight.

Table 1. The main alkaloids in plant of Berberis vulgaris.

Alkaloid	Family	Structure	Properties
Berberine	Protopines		Melting point: 140-144°C Yellow
Berbamine	Bisbenzylisoquinolines		Melting point: 168-170°C Yellow
Jatrorrhizine	Protopines		Melting point: 203-206°C White
Palmatine	Protopines		Melting point: 203-205°C White
Columbamine	Protopines		Melting point: 191-194°C Yellow
Oxyacanthine	Bisbenzylisoquinolines		Melting point: 202-214°C White

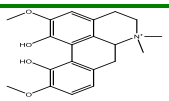
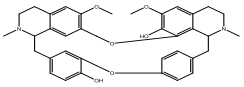
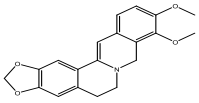
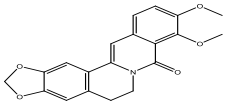
Magnoflorine	Aporphines		Melting point: 250-252°C White
Aromoline	Bisbenzylisoquinolines		Melting point:256-259°C Light Yellow
Lambertine	Protopines		Melting point: 163-164°C Light Yellow
Oxyberberine	Protopines		Melting point: 198-199°C White

Table 2. Total Phenols, tannins and condensed tannins of barberry. Results from 20 different regions in South Khorasan province, Iran.

1: Total Phenols 2: Total Tannins 3: Condensed Tannins

Sample code	Region name	Tp [1] %	TT [2] %	CT [3] %
1	Mahmooee	6.33	2.97	0.21
2	Afzal Abad	7.51	3.58	0.76
3	Rowshanavand	8.43	3.08	1.03
4	Shakhen	5.41	2.84	0.66
5	Asnan	5.51	2.85	0.62
6	Birjand	8.91	3.59	1.11
7	Mood	7.25	3.91	0.69
8	Hosein Abad	6.98	3.94	0.72
9	Estanest	7.53	3.45	1.11
10	Darmian	7.33	4.19	0.95
11	Qaen (1)	7.27	2.89	0.98
12	Qaen (2)	6.72	2.51	0.84
13	Qaen (3)	8.94	3.53	0.94
14	Sehek	7.04	3.67	0.73
15	Bidesk	3.97	1.37	0.04
16	Barkooh	6.21	3.10	0.05
17	Mirik	4.04	1.28	0.05
18	Bimad	5.25	1.58	0.07
19	Esfeden	5.70	2.22	0.03
20	Afriz	5.02	1.85	0.06

Then the sample put in oven at 40°C for 7 days and grounded to a fine powder. The powdered fruit of the plant was extracted with suitable solvent. Choice of solvent is very important, because different solvents have different ability to extract secondary metabolites from plants. So that, material were extracted with 70% acetone. Afterwards, extract was centrifuged and gotten prepared for measurement of total tannins, total phenol and condensed tannin. The amounts of absorbance were recorded with UV/Vis at 550 nm using a Secomam UV-XE2 spectrophotometer.

Table 3. Total phenolic content, total tannins and condensed tannin content.

	Mean±S.D.	
condensed Tannin content	total tannins	total phenolic content
0.58±0.41	2.92±0.87	6.57±1.43

Total phenols, tannins and condensed tannins content of the samples are shown in Table 2. The results in Table 3 are compared with the average rainfall and temperature for the period March 2013 to July 2013. Metrological data was provided by the Islamic Republic of Iran Metrological Organization and the effect of climatic variations in genesis of secondary metabolite in Berberis vulgaris is investigated. We have determined the amount of tannins in barberry produced in twenty different areas of South Khorasan province. As shown in Fig. 3 and 4 the average rainfall has direct correlation with the amount of secondary metabolite for the time interval from March 2013 to July 2013. Meanwhile, our results shown negative correlation between average temperatures and the content of tannin for the period. It has been reported that environmental effects such as rainfall and temperature, can affect the levels of phenolic compounds in plants.

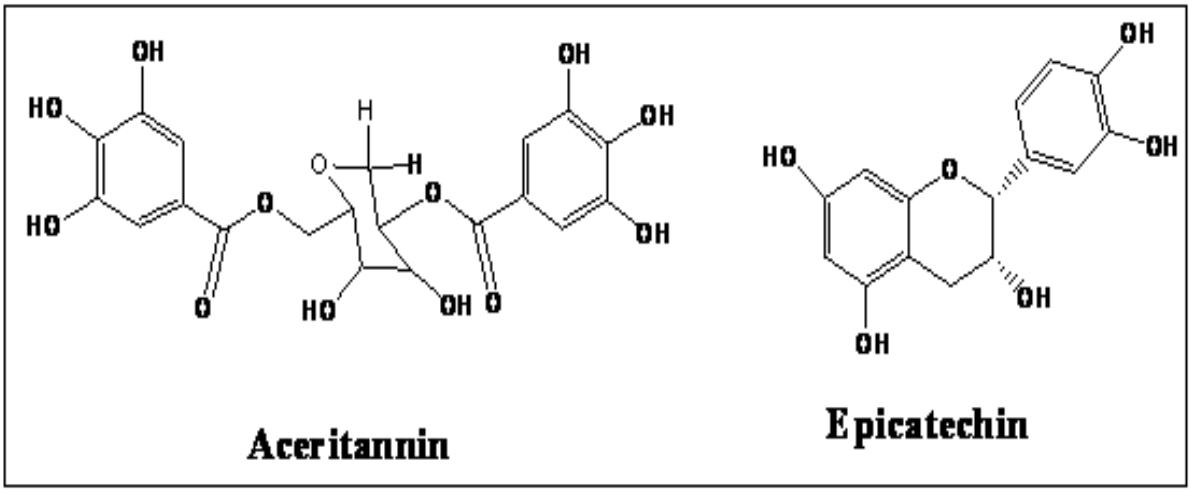


Fig. 1. Hydrolysable tannins (Acenitannin) and condensed tannins (Epicatechin).

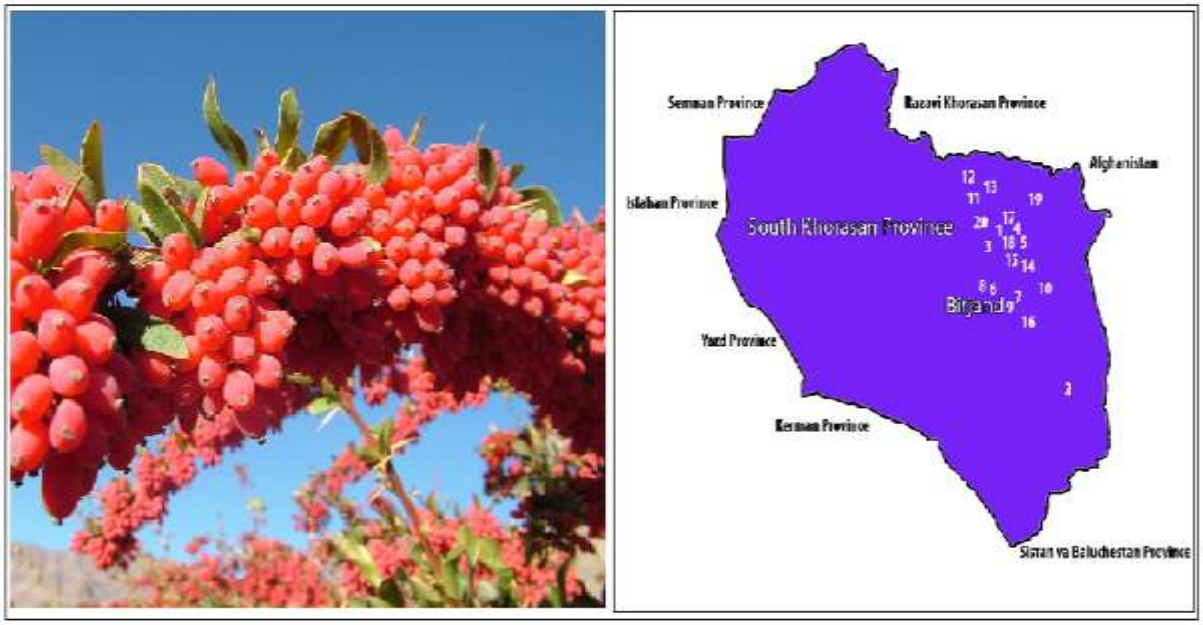


Fig. 2. Barberry plant (Left), Areas from which samples are collected in South Khorasan, Iran (Right).

The results showed that the tannins content in some plants presented negative correlation with temperature. Our results agree with the results of researches in the literature (Rezende *et al.*, 2015). Also some researches showed that average annual temperature and climate have considerable effect on tannin content (Hatano *et al.*, 1986). Similarly, researchers found a close relationship between tannin levels and rainfall (Monteiro and *et al.*, 2006).

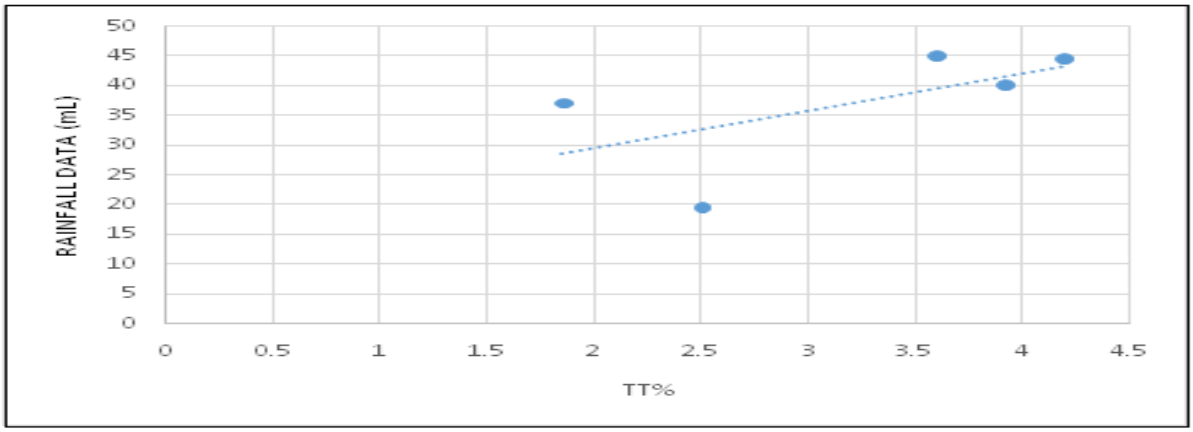


Fig. 3. Variation of TT (total tannins) versus rainfall during July-Sept.

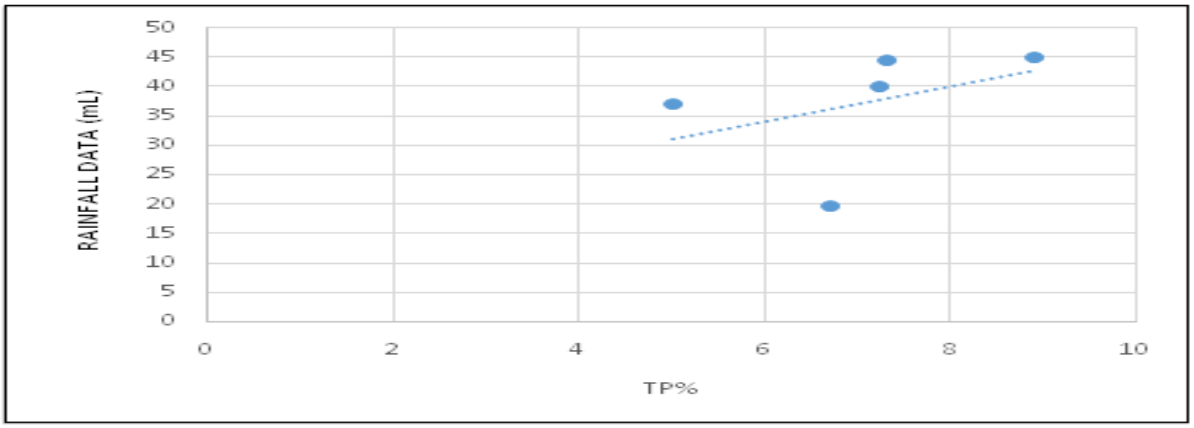


Fig. 4. Variation of TP (total phenolic content) versus rainfall during July-Sept.

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