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Quantitative HPLC Analysis of Phenolic Compounds in *Prosopis farcta* from Two Different Ecological Zones of Iran

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Abstract

Variation in content of polyphenols has been correlated with a diverse set of ecological factors. *Prosopis farcta* is one of the important medicinal plants known in Iran. The present study was carried out to evaluate the phytochemicals present in this plant roots and branches collected from wild populations of *P. farcta a* shrubs developed in two distinct localities of Iran. The collected plant material extracted with acetone and methanol. Methanol extracts of roots and branches of *P. farcta a* from Darreh Shahr showed a higher amount of total phenolic content compared to Ilam. The HPLC analysis of *P. farcta a* extracts obtained from these two geographic regions revealed the presence of various amounts of several phenolic compounds. The extracted phenolic compounds were such as Apigenin, Myricetin, Vitexin, Luteolin, Isovitexin, Quercetin and Luteolin. Vitexin in branches and caffeic acid derivative in roots were found to be in highest amounts in plants collected from Ilam while 5-deoxy luteolin showed the highest amount in roots of plant obtained from Darreh Shahr. Our results conclusively showed that there were quantitative differences of polyphenols due to different environmental conditions that allowed us to justify the occurrence of two varieties for *P. farcta a* in Ilam Province, Iran.

Keywords:* *Prosopis farcta; Phenolic compounds; High-performance liquid chromatography; Apigenin; Myricetin; Vitexin

Introduction

Plants, herbs and spices have been used as remedies in traditional medicine and attained a wide recognition as sources of bioactive phytochemicals with medicinal benefits. A distinctive property of these active principles is their antioxidant capacity which is common in many secondary metabolites from the plant kingdom [1]. Plant phenols, as named in the industry, are used as the active ingredients in many actual formulations [2]. They are also important parts of the human diet. It has been found that some biological activities can be especially attributed to the flavonoids, e.g., antioxidant, anti-inflammatory [3] and anti-angiogenic for quercetin [4], cardioprotective [5] and neuroprotective [6] for luteolin and anti-

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carcinogenic [7] and anxiolytic for apigenin [8]. The antioxidant content of plant might be influenced by genotype × environment (G × E) interactions. Prosopis spp. is an endemic tree that belongs to the Leguminosae family and Mimosaceae subfamily and comprises 44 species distributed in arid and semiarid regions over one-third of the earth surface [9]. *Prosopis farcta* is a small, prickly shrub, 30 cm to 80 cm tall or "shrub-tree" 2 m to 3 m or taller and is native to North Africa (Algeria, Egypt and Tunisia) and much of Southwest Asia, from South Kazakhstan to the Indian subcontinent and west to the Middle East and Asia Minor. It is also found in the United States [10]. The plant extract may be used as wound healing [11] and anti-diabetic agent [12]. Moreover, the plant extract has shown endothelium-dependent vasorelaxant effects [13]. Roots of *P. farcta a* are being used for management of angina pectoris and hypertension by Iranian tribal peoples, particularly in Ilam Province. Some of the compounds existing in *Prosopis* plant are Quercetin, Apigenin [14] Tryptamine, L-arabinose and Lectin [15]. This plant is a shrub that grows naturally in two distinct biogeographical regions of Ilam Province. This species is also well adapted to drought and high temperatures and exhibits a high degree of salt tolerance [16]. Pods and seeds of this plant, collected at different localities, differ in form, size and color, though those collected at each locality show considerable uniformity. Study of the root stems and pollen morphology has been reported to allow distinguishing also important differences between distinct populations of *P. farcta a* [17]. Variation in phenolic compounds has been correlated with a diverse set of ecological factors, such as UV radiation [18], excess photochemical energy [19], resource availability [20] and ecosystem function [21]. In this study, we posed the following questions: 1) Do differences in phenolic levels occur among sites and, if so, does this variation from a latitudinal cline and; 2) Do phenolic levels in vegetative tissues vary in a site-specific manner, suggesting localized environmental control of levels of these compounds? To address these questions, we measured the quantity of phenolic compounds and composition of the total extract in *P. farcta a* collected from two distinct regions in Ilam Province, Iran.

Materials and Methods

Sample collection

Plant material (branches and roots) used in this investigation was collected from wild populations of *P. farcta a* shrubs occurring in two distinct localities, i.e., south of Darreh Shahr and north of Ilam Counties in Ilam Province, Iran (FIG. 1), lying approximately 630 m and 1337 m, respectively, above sea level. The climate in Ilam Province is semiarid. The mean annual rainfall (mm) varies from 39.9 for Darreh Shahr to 49.09 for Ilam. The mean annual temperature (°C) is 21.01 in Darreh Shahr and 16.75 in Ilam Counties. Botanical samples were authenticated by Dr. Gholamreza Amin at the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. A voucher specimen (No. 6719 THE) was deposited at the Herbarium of the Department of Pharmacognosy. Plant material was collected in July. The collected plant material was washed in water. 500 g of adult branches and roots were air dried at slightly above room temperature (21°C), grounded with a mortar and weighed.

Extraction and analysis of phenolic compounds

Sample powders were extensively dewaxed with petroleum ether at room temperature. The powders were then packed into soxhlet apparatus. The plant material was re-extracted successively with acetone and methanol. All extracts were concentrated by rotary evaporation at 40°C. After the extraction process, the percent yield of the dried crude extract was 4.4%. The concentrated extracts were resuspended in 80% aq. acetone or methanol to a total volume of 10 ml and a sample of

each of the extracts was analyzed for flavonoid content by High-Performance Liquid Chromatography (HPLC). Individual components were identified by comparison of their UV spectra with those indicated in the literature [22].

HPLC analysis

Sample analysis was carried out using HPLC (Waters 2487, Autosampler Waters 717, a controller with gradient Pumps and Diode Array (PDA) Detector). HPLC was fitted with a Li Christopher (LC) 100 RP-18 (250 mm × 4.6 mm, 5 µm particle size) Column using a 20 min linear gradient of 25% to 100% MeOH in 1% aq. AcOH at 1 mL/min. Eluting solvents were 2% aq. AcOH and MeOH, AcOH, H_2O in the proportion of 18:1:1. The sample injection was set at 10 µL and the flow rate kept constant at 1.0 mL/min. The HPLC system was operated at ambient temperature (28 ± 1°C).

Results and Discussion

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [23]. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total [24]. Phenolic composition of plant extracts is affected by different factors such as plant variety, climate, storage, processing, etc. Solvents such as methanol, ethanol, acetone, propanol and ethyl acetate have been commonly used for the extraction of phenolics from fresh product [25]. The extraction of phenolic compounds of the plant is influenced by their chemical nature, the extraction method, the sample size, time and storage conditions as well as the presence of interfering substances [26]. The phenolic extracts of plants are always a mixture of different classes of phenols that are selectively soluble in the solvents. Using an aqueous methanol extract has been demonstrated to provide more satisfactory results regarding total phenolic content compared to aqueous ethanol or acetone extracts [27]. In the present study, the methanol extracts of all samples showed the maximum yield of phytochemicals while a number of phenolic compounds were lesser when the extraction process was carried out using acetone. Using a mixture of alcohol and water not only modulates the polarity of alcohol solvents but also increases the solubility of polyphenolic compounds which are mainly dependent on the hydroxyl groups, the molecular size and the length of hydrocarbons [27]. While some authors have found a correlation between the total phenolic content and the antioxidant activity [28], no such relationship has been observed by others [29]. It would be possible that the phenolic compounds existing in acetone extract possess an ideal structure for the scavenging of free radicals since they present a number of hydroxyl groups as hydrogen donators which make them important and very powerful antioxidant agents [30]. A number of the phenolic compounds extracted from plant roots was higher, compared with those obtained from branches, when methanol extracts were employed. Roots of *P. farcta a* from Darreh Shahr plant population showed the highest amount of phenolic compounds when a methanol extract was used (26.4%). A number of phenolic compounds from branches was higher than that obtained from roots in acetone extract from Ilam plant population (TABLE 1). It has been reported that the methanol extracts of *P. farcta a* [14] and other plant species such as *Grevillea superb* (Schultes et al. 2012) showed some good results regarding their phytochemical contents like phenolics, alkaloids and tannins. The HPLC analysis of *P. farcta a* extracts detected at least ten flavonoid derivatives including Apigenin C-glycoside, Myricetin 3-*O*-glucoside, Vitexin, Luteolin 7-*O*-glucoside, Isovitexin, Quercetin 3-*O*-glucoside, 5-deoxy luteolin, caffeic acid derivative, luteolin and quercetin 3-methyl ether (TABLE 2). Results from quantitative analysis of five major phenolic compounds within roots and branches of *P. farcta a* are summarized in TABLE 3. Four phenolic compounds, including isovitexin, 5-deoxy luteolin, caffeic acid derivative and luteolin were found to be distributed diversely

in plant roots. Vitexin in branches and caffeic acid derivative in roots were found to be in highest amounts in plants collected from Ilam. However, the roots of the Darreh Shahr plant populations showed the highest amounts of 5-deoxy luteolin (TABLE 3). Luteolin was abundant in roots and moderate in branches from both localities.

TABLE 1. **Total phenolic content (%) of *Prosopis farcta* root and branch extracts obtained from two distinct localities, Ilam and Darreh Shahr counties, in Ilam Province, Iran.**

Acetone extracts (%)		
Organs	Darreh Shahr locality	Ilam locality
Roots	1.2	0.7
Branches	0.8	1.6
Methanol extracts (%)		
Organs	Darreh Shahr locality	Ilam locality
Roots	26.4	16.5
Branches	9.2	6.4

TABLE 2. **Phenolic compounds and their retention time (RT) identified in *Prosopis farcta* root and branch extracts from two distinct localities in Ilam Province, Iran.**

Phenolic compounds in branches	**RT (min)**
Tannins	2.56
Apigenin C-glycoside	9.38
Myricetin 3-*O*-glucoside	10.85
Vitexin	11.54
Luteolin 7-*O*-glucoside	12.11
Isovitexin	12.83
Quercetin 3-*O*-galactoside	13.35
5-Deoxyluteolin	14.69
Caffeic acid derivative	15.29
Luteolin	16.79
Quercetin 3-methyl ether	17.87
Phenolic compounds in roots	**RT (min)**
Tannins	2.49
Isovitexin	12.81
5-Deoxyluteolin	14.69
Caffeic acid derivative	15.28
Luteolin	16.88

TABLE 3. **Major phenolic compounds of *Prosopis farcta* root and branch extracts obtained from two distinct localities, Ilam and Darreh Shahr counties, in Ilam Province, Iran.**

Phenolic compounds	Branches		Roots	
	Ilam	Darreh Shahr	Ilam	Darreh Shahr
Vitexin	(+++)	(+)	(-)	(-)
Isovitexin	(++)	(++)	(+)	(+)
5-Deoxyluteolin	(+)	(+)	(+)	(+++)
Caffeic acid derivative	(++)	(++)	(+++)	(+)
Luteolin	(++)	(++)	(+++)	(+++)
(-) absent; (+) minor; (++) moderate; (+++) abundant				

Geographical data and seasonal climatic conditions of Ilam and Darre Shahr regions (FIG. 1) are summarized in TABLE 4. Darreh Shahr and Ilam Counties lie approximately 630 m and 1337 m, respectively, above sea level [31]. Ilam region has a mountainous climate while Darre Shahris semi-arid. The mean annual rainfall (mm) varies from 39.9 in Darreh Shahr to 49.09 in Ilam. The mean annual temperature (°C) is 21.01 in Darreh Shahr and 16.75 in Ilam. The synthesis and release of phenolics are induced by various biotic and abiotic factors [32]. Therefore, factors mentioned in TABLE 4 may influence the content, composition and activity of the phenolic compounds in plants. Among these factors, the geographical origin and the nature of the cultivar are certainly those that have a pronounced influence on the phenolic composition. Some studies have already published concerning the influence of these factors on some French [33], Spanish [34] and Italian [35] cultivars. The samples that were the objects of this study had different geographical origins and various maturation indices. Some studies have demonstrated that temperature stress can affect the production of secondary metabolites and other compounds [36] which are usually the basis for the medicinal activity of the plants. The cold acclimation usually can increase the accumulation of total phenolic yields which is positively related to antioxidant capacity [37] but the results of the current study are not in line with this scenario probably due to some other interfering factors that could not be evaluated in this study and need more attention by some other comprehensive and analytical studies. As an evidence for confirming the above statement, the water availability alone [38], or in combination with enhanced UV-B radiation may also influence the production of phenolics even irrespective of the climate alone. In addition, as clouds attenuate the UV light reaching the earth surface, cloud covering may also affect the phenolics yield as well [39]. In the present study, the roots and branches differed significantly with regard to the accumulation of phenolic compounds (TABLES 1 and 3). The highest levels of vitexin and caffeic acid derivatives were detected in branches and roots of Ilam plant population, respectively, while 5-Deoxyluteolin was accumulated mainly in roots of Darreh Shahr population. The regional distribution of this medicinal plant may be an important source of chemical variability and should be considered while optimizing the processing methodology of wild-harvested plant material is important too. Such data could also be useful for the elucidation of the chemotaxonomic purpose of the corresponding compounds and the phytochemical evaluation of the plant. Three phenolic compounds have previously been identified in pods and seeds of *P. farcta a* [40] and 16 phenolic compounds in its roots, pods, seeds, branches and leaves [14].

TABLE 4. **Geographical data and seasonal climatic conditions of *P. farcta* growing localities in Ilam Province, in Western Iran.**

Sites	Latitude (N)	Longitude (E)	Elevation (m)	Mean temperature (°C)	Mean annual rainfall (mm)
Darreh Shahr	33°08′	47°22′	630	21.01	39.9
Ilam	33°38´	46° 26´	1337	16.75	49.09

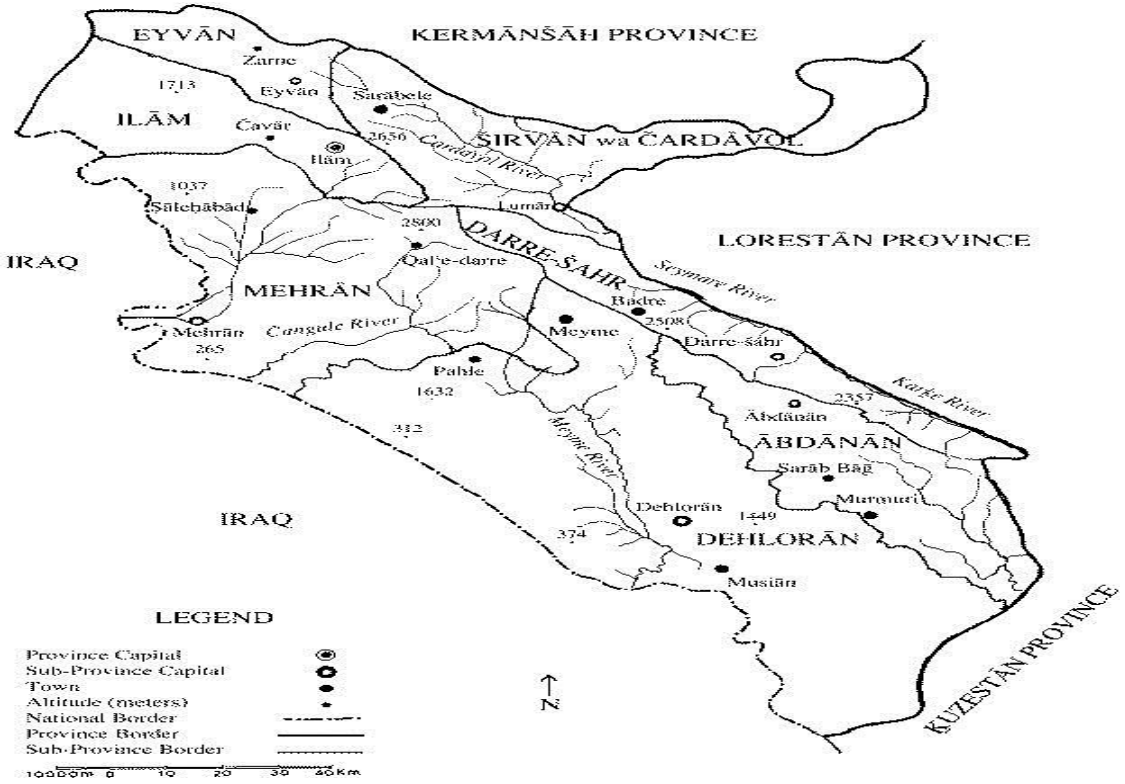

FIG. 1. **Two distinct localities where *P. farcta* were collected, i.e., south of Darreh Shahr and north of Ilam Counties in Ilam Province, Iran.**

In the present study, ten phenolic compounds were identified from *P. farcta a* occurring in Ilam Province, Iran. Seventeen flavonoids and nine major ones have also been identified in several populations of *Prosopis reptans* collected along the Texas Gulf coast [41]. Different data from prior studies indicate the different distribution of phenolic compounds in this plant species. The chemical variability in the content of phenolic compounds evaluated among the plant populations may be attributed to the different environmental conditions of the sampling sites. For example, the two plant populations are separated by a distance of 180 km and have a substantially different environment. However, the present findings also indicate

a significant similarity among the plant populations of these regions. For example, luteolin was abundant in roots and moderate in branches and vitexin was absent in roots from both localities.

Conclusion

Our results have conclusively shown that variability in the content of phenolic compounds evaluated among the plant populations may be attributed to the different environmental conditions of the sampling sites and allow us to justify the occurrence of two varieties for *P. farcta a* in Ilam Province in Western Iran.

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Conflict of Interest

The authors state no conflicts of interest in the manuscript.

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