

Extraction and measurement of the Quercetin flavonoid of *Prosopis farcta* in Khuzestan climatic condition

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ABSTRACT

Background and aims: Medicinal plants are a valuable resource for flavonoids extraction. *Prosopis fracta* is one of the plants with medicinal properties. *Prosopis fracta* was found in abundance in southern regions in Iran. Coersetin is medicinal substance found in the fruit of this plant. Quercetin is used in treatment of cancer and viral infections. This study was conducted to determine the Quercetin flavonoid in *Prosopis fracta* samples in different regions of Khuzestan.

Methods: The *Prosopis fracta* fruit collected from different regions of Khuzestan (Susangerd, Ahvaz, Abadan, Mollasani, Behbahan and Ramhormoz). The beads were isolated from the fruit. Shell and the flesh were dried in an Oven. The dried materials were mixed and flavonoids extracted with a suitable solvent. The extract was injected into the High Performance Liquid Chromatography (HPLC) system. Then, the compound, Quercetin quantity and standard peak in each sample have been determined.

Results: Based on the results, the Susangerd samples (0.0033 mg/ml) and Abadan (0.0008 mg/ml) have maximum and minimum levels of Quercetin flavonoid, respectively.

Conclusions: Quercetin flavonoid extracted from *Prosopis farcta* fruits of Susangerd is richer than other regions of Khuzestan province. Therefore, it is recommended to use the *Prosopis fracta* fruit grown in Susangerd for extraction of the Quercetin flavonoid.

Keywords: Gastric ulcers, Medicinal plant, HPLC, Second metabolism.

INTRODUCTION

Prosopis farcta is from Leguminosae and sub-family of Mimosoideae, the indigenous of dry and semi-dry areas of America, Asia, and Africa.^{1,2}

Three species of *Prosopis* in Iran were founded, including *Prosopis cineraria*, *Prosopis koelziana* and *Prosopis farcta*. *Prosopis fracta* is a plant of the Leguminosae family and sub-family of Mimosoideae. *Prosopis fracta* is native to

arid and semi-arid tropics of America, Africa and Asia.²

Prosopis fracta is a shrub with semi-white, hairy, thin and sharp spine branches. *Prosopis fracta* be found in the provinces of Khuzestan, Gilan, Fars, Hormozgan, Baluchestan, Khorasan and Tehran.³

Prosopis fracta (Fig. 1) has multi medical effects and consider as a main

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family of medicinal plants.⁴ Some of medicinal properties of this herb are including the treatment of gastric ulcers, miscarriage, dysentery, rheumatism, laryngitis, dyspnea and chest pain.⁵ Fleshy parts of the *Prosopis farcta* fruit have food and drug uses. It is used also for the treatment of bronchitis, asthma, skin lesions, rheumatism, and scorpion sting.³

The active components of *Prosopis farcta* are included; Lectin, L-arabinose, 5-hydroxyl, Quercetin (Flavonoid), Tryptamine (Alkaloid), Apigenin, Tryptamin and toxin. The combination of Quercetin namely 2-(3, 4-Dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one has C₁₅H₁₀O₇ molecular composition (Fig 2). Its molecular weight is 302.24. C₁₅H₁₀O₇ is dehydrated to form yellow needles from alcohol concentrations at 95 to 97 degrees. One gram of this combination of Quercetin dissolves in 290 ml grain alcohol and 23 ml of boiling alcohol. It is soluble in acetic acid glasyal and yellow in liquid alkaline solution. This compound is insoluble in water and their alcohol test is very bitter.⁶



Fig 1: *Prosopis farcta*

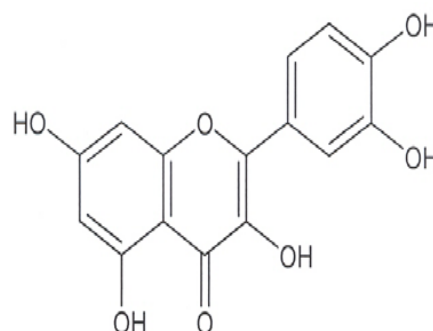


Fig 2: C₁₅H₁₀O₇ molecular composition

Quercetin has multi medical affects and it is used in the treatment of cancer and viral infections.⁷ Free radicals in the body cause diseases such as cancer and atherosclerosis and Quercetin clears free radicals. Therefore, Quercetin is beneficial in the prevention of cardiovascular diseases.^{8,9}

METHODS

The *Prosopis farcta* fruit collected from different regions of Khuzestan including Susangerd, Ahvaz, Abadan, Mollasani, Behbahan and Ramhormoz (Table 1). Details of soil sampling location were shown (Table 2). The beads were isolated from the fruit. The shell and the flesh were dried in an oven at 35°C during 48 hours. The dried materials were mixed and flavonoids extracted with a suitable solvent.

Table 1: Geographical location of the sampling

Location	Latitude	Longitude	Altitude (m)	The mean maximum annual temperature (C°)	The mean minimum annual temperature (C°)
Susangerd	40' 31"	6' 48"	130	32.5	16.3
Mollasani	36' 31"	53' 48"	20	36	19.5
Ramhormoz	16' 31"	36' 49"	150.5	32.7	19.5
Ahvaz	15' 31"	33' 48"	22.5	32.9	17.6
Behbahan	36' 30"	14' 50"	313	32.3	17
Abadan	22' 30"	15' 48"	6.6	32.9	17.9

Table 2: Details of soil sampling location

Location	Soil texture	EC (ds/m)	pH	Soil organic carbon (%)	Potassium (ppm)	Phosphorus (ppm)
Susangerd	Cilty clay	6.9	7.6	0.86	274	6.8
Mollasani	Clay loam	7.1	7.6	0.45	172	6.5
Ramhormoz	Loam	6.8	7.8	0.86	182	6.3
Ahvaz	Cilty clay	8.7	7.6	0.39	167	6.4
Behbahan	Loam	6.8	7.8	0.88	180	6.2
Abadan	Cilty clay	10.8	7.8	0.32	207	6.2

The flesh and shell of each sample were grounded separately. Then the powders were used to prepare the test solution.

One gram of sample powder was poured into balloons of 250cc. It was added to 50 ml of methanol, 20 ml sterile water and 8 ml 1 normal hydrochloric acid. The sample was placed inside a bath water under reflux and the duration 135 minutes. At this stage, the red color solution was not necessarily representing the reaction. After cooling, the supernatant liquid was poured into 100 ml volumetric flask, and 20 ml methanol was added to the balloons. Then, Reflux was done for 10 minutes. The resulted liquid was passed through the filter paper, and then was transferred into the previous solution 100 ml volumetric flask and the volume was completed with methanol.

HPLC is suitable for extracting plant flavonoid Quercetin in *Prosopis fracta* because of greater sensitivity and better selectivity method HPLC with UV detector. This method most commonly used in comparing to other methods for the analysis of flavonoids.

Device specifications of High Performance Liquid Chromatography

Extracts were analyzed by HPLC Knauer model. The flow rate was 1 ml per

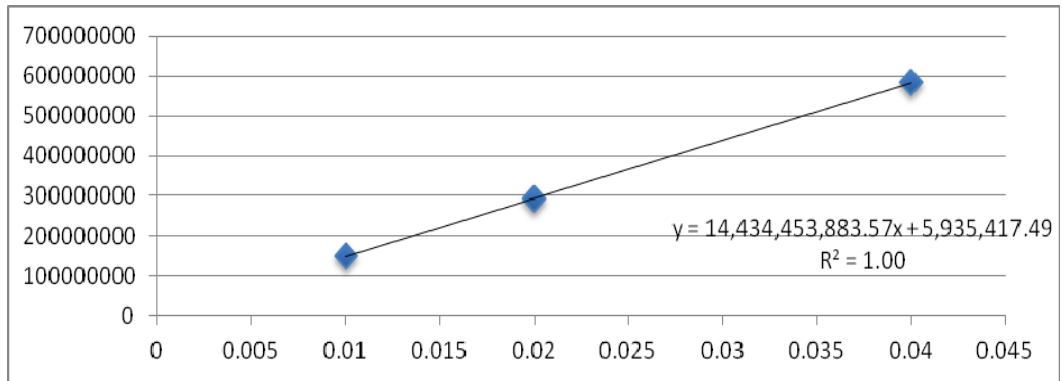
minute. Composition of the mobile phase used methanol, 1% phosphoric acid/ water (40:60). The Filling materials were C8, column length was 25 cm, and the particle size was 5 micrometers. Wavelength NM 270 UV detector was used and the injection volume was 20 ml. The lab temperature was 25 °C and Isocratic forming system was used.

Different concentrations 0.01, 0.02, 0.04 (mg/ml) of Quercetin standard in methanol were prepared. Each concentration was injected three times with HPLC, and area under the curve was plotted versus the injected amount. Then, the line equation and correlation coefficient (r^2) were calculated from the standard Quercetin. The results reported $r^2= 1$.

Then, the *Prosopis fracta* Shell and the flesh dried extract of the samples of different location were separately using USP method ¹⁰, and injected into the HPLC system to identify and specify the amount of Quercetin. The total amount of Quercetin in the samples of different locations was calculated separately.

Analysis and validation

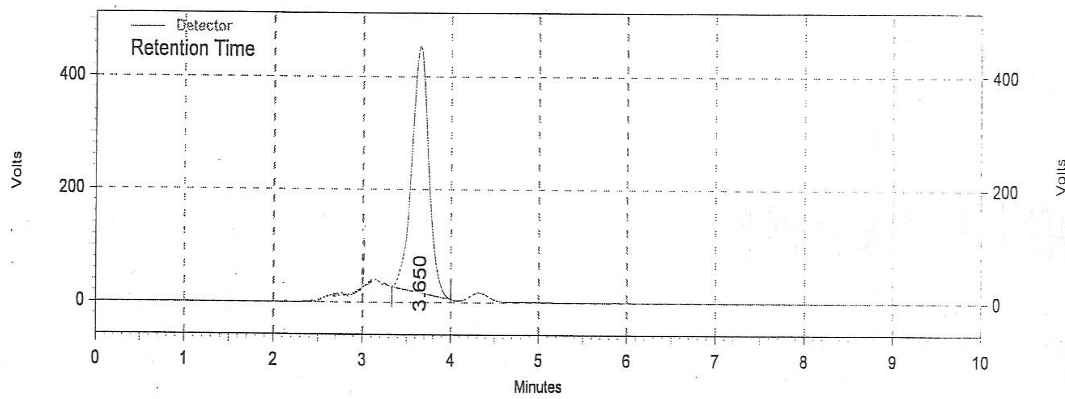
Different concentrations 0.01, 0.02, 0.04 (mg/ml) were used. Calibration curve analysis is presented (Graph 1).



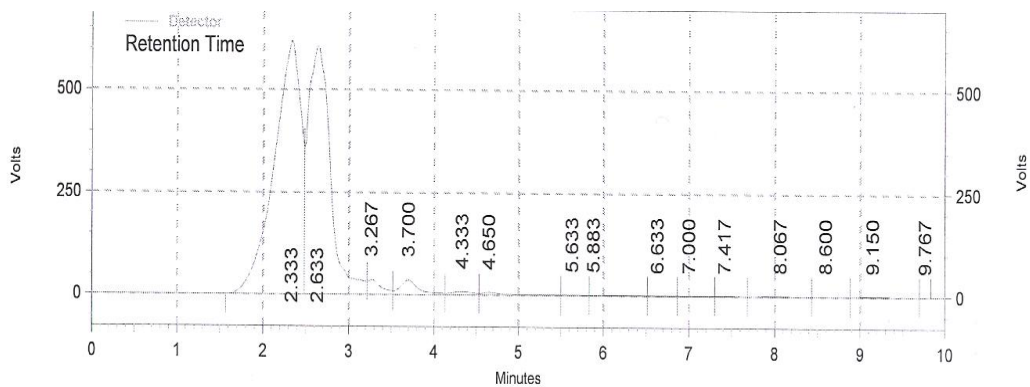
Graph 1: Calibration curve to the area under the curve concentration of Quercetin

Quercetin chromatogram is presented in (Graph 2). The isolation of Quercetin peak

chromatogram of the flavonoids Quercetin in extract samples is presented (Graph 3).



Graph 2: Quercetin chromatogram



Graph 3: Typical chromatogram analysis *Prosopis fracta* (Quercetin 3.7 minutes)

RESULTS

The Quercetin flavonoid levels were higher in samples with electrical conductivity of the soil, almost neutral or acidic soil and more organic matter. The level of Quercetin flavonoid extracted from *Prosopis farcta* fruits of the Susangerd, was higher than other regions of Khuzestan province. Therefore, soil quality is likely one of the affecting factors on the Quercetin flavonoid levels in *Prosopis farcta* fruit. In addition to the climatic conditions of the region, the edaphic condition has an important impact of the changes of the Quercetin flavonoid levels in *Prosopis farcta* fruit. The Quercetin levels were calculated for each sample and presented in Table 3.

Table 3: Quercetin levels for each sample in different location

Location	Quercetin (mg/ml)
Susangerd	0.0033
Mollasani	0.0016
Ramhormoz	0.0015
Ahvaz	0.0011
Behbahan	0.0014
Abadan	0.0008

DISCUSSION

The *Prosopis* plant has some of the compounds including: Toxin, Quercetin (Flavonoids), Tryptamine, Apigenin, 5-hydroxytryptamine (Alkaloids), L-arabinose, Lectin.^{11, 12} Studies showed that compounds like flavonoids have been very valuable for obtaining information about relationships and the hybrid origin of taxa.³⁻¹⁷

Because flavonoids are routinely used for systematic studies, in the present study, we investigated the flavonoid Quercetin levels of different samples obtained from Khuzestan province.

The results showed that this concentration is different in samples collected from different locations of

Khuzestan province. Based on the results the Susangerd samples (0.0033 mg/ml) and Abadan (0.0008 mg/ml) have maximum and minimum levels of Quercetin flavonoid respectively. As far as, we could not find any Iranian study in the determination of the flavonoid Quercetin levels.

A study evaluated the chromatographic characterization of different *Prosopis* species.¹⁸

In another study evaluated the chromatographic variation within the *P. juliflora* complex,¹⁹ and morphology and flavonoid Chromatographies of *Prosopis* species and hybrids of the Argentinian Chaquena region.²⁰ In other study, Harzallah et al evaluated the patterns of *P. farcta* flavonoids in branches, leaves, roots, flowers and pods without seeds of two plant populations belonging to this species occurring in the northeast and the southeast of Tunisia. Based the results of this study, a relative similarity was found in the compounds of plants from the two populations.¹¹

Prosopis farcta is widely used by people traditionally for curing lesions in Iran. An Iranian researcher examined the local effect of the fruit dusk powder and root extract of *Prosopis farcta* on diabetic healing. The results of this study reported the anti-diabetic, anti-inflammatory and healing effects of this plant.²¹ Other studies have also confirmed the anti-diabetic and anti-inflammatory effects of the *Prosopis farcta*^{22, 23}

CONCLUSIONS

Quercetin flavonoid extracted from *Prosopis farcta* fruits of Susangerd is richer than other regions of Khuzestan province. Therefore, it is recommended to use the *Prosopis farcta* fruit grown in Susangerd for extraction of the Quercetin flavonoid.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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