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The measurement of the quercetin of different parts of *Tribulus terrestris* by HPLC

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ABSTRACT

Background and aims: *Tribulus terrestris* fruit, leaf, and root have medical effects in the treatment of cancer, viral infections and prevention of cardiovascular diseases. The present study was aimed to evaluate the quercetin flavonoid levels from different parts of the *Tribulus terrestris* collected from different regions of Khuzestan in 2014.

Methods: In this experimental study, four parts of the *Tribulus terrestris* including; fruits, leaves, stems and roots were collected from different regions of Khuzestan including Shushtar, Mollasani and Andimeshk. The analysis was carried out to compare the chemical profile of the different extracts of *Tribulus terrorists* using reverse phase HPLC with UV detector. The mobile phase that consisted of phosphoric acid buffer with pH=3 and acetonitrile was used for isocratic elution. The flow rate was adjusted to 1.0 ml/min. The detection wavelength was at 203 nm. All separations were performed at ambient temperature.

Results: The results reported that the quercetin flavonoid level were highest in the Andimeshk leaves samples (69.57427 ppm). However, the Andimeshk fruits samples (4.141953 ppm) have the lowest levels of the quercetin flavonoid.

Conclusion: Considering the cost effectiveness in extracting compounds from medicinal plants, it is recommended to identify the highest level of the quercetin flavonoid in each region and in each part of the plant.

Keywords: Experimental study, Medicinal plant, Quercetin flavonoid.

Original article

INTRODUCTION

The genus Tribulus, is belonging to Zygophyllaceae family. There are 20 species of Tribulus in the world. *Tribulus terrestris* is a small prostrate with 10-60 cm height, hirsute or silky hairy shrub. The leaves are often unequal and opposite.[1,2]

Fig 1: *Tribulus terrestris*

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Tribulus terrestris flowers are yellow in color. Its carpel fruits are of characteristic, stellate shape, somewhat round-shaped, compressed, five cornered, and covered with princkles of yellow color very light.[3] There are several seeds in each crocus with transverse partitions between them. The seeds are oily in nature. *Tribulus terrestris* is a well-patronized medicinal herb.[4] The *Tribulus terrestris* fruit, leaf, and root are used as medicine for a wide-ranging complaints. *Tribulus terrestris* is used for kidney problems, skin disorders, male sexual problems, heart and circulatory system problems, high cholesterol, anemia, digestion problems, cancer, liver disease, inflammation, and chronic fatigue syndrome (CFS). The *Tribulus* root and fruits are used for male virility and general vitality, respectively. The roots enhance libido and sexual wellbeing without affecting testosterone while the fruits appear to be potently protective of organ function.[5-7]

The active components of *Tribulus terrestris* are including saponins, flavonoids, glycosides, alkaloids, and tannins.[8] Based on the results of studies, the saponin composition and the saponin content of *Tribulus terrestris* is different in various geographic regions.[9] The quantity of main flavonoids is about 1.5 times that of main saponins, therefore, the *Tribulus terrestris* flavonoid contents should be evaluated, developed, and further used.[10] Louveaux et al evaluated flavonoids in *Tribulus terrestris* using liquid chromatography (HPLC) with high-performance and detected 18 flavonoids including; caffeoyl derivatives, quercetin glycosides, including rutin and kaempferol glycosides in four *Tribulus* species leaf extracts.[11] In other study, isolated three flavonoid glycosides, viz. quercetin 3-*O*-glycoside, quercetin 3-*O*-rutinoside, and kaempferol 3-*O*-glycoside from the aerial parts of *Tribulus terrestris* in Iran.[12] Quercetin has multi medical effects and it is used in the treatment of cancer and viral infections.[13] Free radicals in the body cause diseases such as cancer and atherosclerosis and quercetin eliminates or chelates the free radical. Therefore, quercetin is beneficial in the prevention of cardiovascular diseases.[14] The present study was aimed to evaluate the quercetin flavonoid levels from different parts of the *Tribulus terrestris* collection from different regions of Khuzestan in 2014.

METHODS

In this experimental study, four parts of the *Tribulus terrestris* including; fruits, leaves, stems and roots were collected from different regions of Khuzestan including Shushtar, Mollasani and Andimeshk. Quercetin is one of the flavonoids found in the *Tribulus terrestris*. The following method was used to measure the active substance of *Tribulus terrestris.*

The analysis was carried out to compare the chemical profile of the different extracts of *Tribulus terrorists* using high Performance Liquid Chromatography (RP- HPLC) equipped with a UV detector (Model K-2600 Knuer). The high performance of liquid chromatoghraphic column was a C18 Nucleosil C8 (25 cm × 4.6 mm) analytical column. The mobile phase that consisted of phosphoric acid buffer with pH-3 and acetonitrile was used for isocratic elution. The flow rate was adjusted to 1.0 ml/min. The detected wavelength was at 203 nm. All separations were performed at ambient temperature. The plant material, 0.5g was extracted two times with 5 ml of 50% aqueous acetonitrile by sonication for 15 min. The samples were centrifuged at 4900 rpm for 10 min. The supernatant was lyophilized for 15 min. The extract was dissolved in 50% aqueous acetonitrile.[15] The line equation and correlation coefficient (r^2) were calculated from the standard Quercetin. The results reported r^2= 0.99 (Fig 2).

Prior injection, all samples were filtered through a 0.45µm membrane. Each sample solution was injected in duplicate with injection volume of 20µl.

RESULTS

We extracted the quercetin flavonoid levels from four parts of the *Tribulus terrestris* including; fruits, leaves, stems and roots. The results demonstrated that in collected samples from Shushtaŕs, the quercetin flavonoid levels were similar in stems (39.35797) and roots (39.72033). In Mollasani, the stems (55.27314) and fruits (29.63946) have the highest and the lowest levels of the quercetin flavonoid, respectively. However, the quercetin flavonoid levels were highest in the leaves samples of Andimeshks (69.57427 ppm). Also, the Andimeshk fruits samples (4.141953 ppm) have the lowest levels of the quercetin flavonoid. The quercetin levels were calculated for each sample and presented in Table 1.

Table 1: Quercetin levels for each sample in different location

Location	Parts of extraction	Quercetin (ppm)
Shushtar	Fruits	56.38523
	leaves	58.83139
	Stems	39.35797
	Roots	39.72033
Mollasani	Fruits	29.63946
	leaves	47.7845
	Stems	55.27314
	Roots	53.52593
Andimeshk	Fruits	4.141953
	leaves	69.57427
	Stems	38.98975
	Roots	40.23218

Analysis and validation of different concentrations 0.01, 0.02, 0.04 (ppm) were used. Calibration curve analysis is presented on the Fig 2, and Quercetin chromatogram is presented in Fig 3.

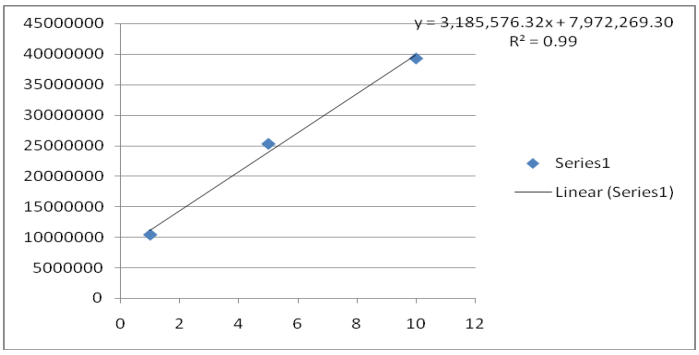

Fig 2: Calibration curve to the area under the curve concentration of quercetin

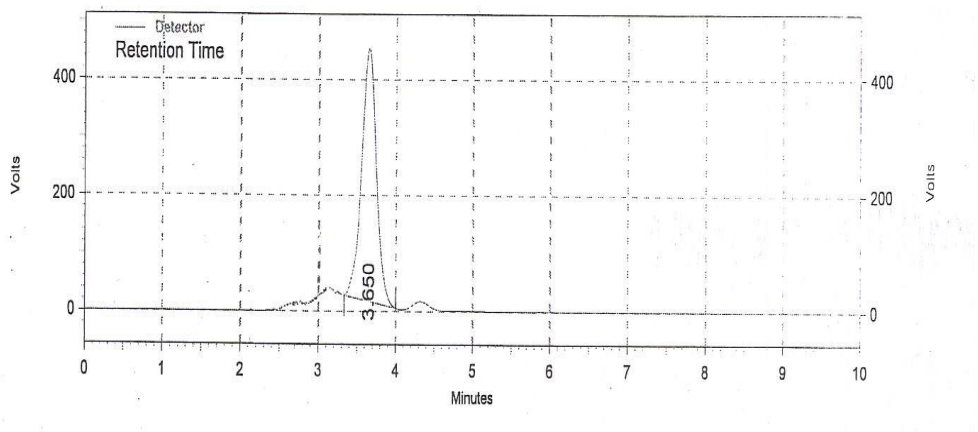

Fig 3: Quercetin chromatogram

DISCUSSION

Environmental factors play an important role in the production and accumulation of secondary metabolites in medicinal plants. Temperature, precipitation, light intensity, and the above height of sea level are the most important environmental factors affecting the accumulation of secondary metabolites.[16] On the other hand, the accumulation and distribution of secondary metabolites in the plant is not the same. In a conducted study on Crataegus monogyna, the level of the quercetin flavonoid was higher in the flowers than the leaves or fruit.[17] The present study, the quercetin flavonoid levels from different parts of the *Tribulus terrestris* collection evaluated from different regions of Khuzestan in 2014. The quercetin flavonoids have several beneficial health effects. This flavonoid is ubiquitously present in foods such as the vegetables, fruit, and countless food supplements.

The results of the present study showed that the quercetin flavonoid level were highest in the Andimeshks leaves samples. However the Andimeshk fruits samples have the lowest levels of the quercetin flavonoid.

Considering the medical effects of quercetin in the treatment of cancer, viral infections[13] and the prevention of cardiovascular diseases,[14] this plant is favorite for researcher in different parts of the worldwide.

Other medical effects of quercetin flavonoid are including protection against osteoporosis, pulmonary, cardiovascular diseases and aging. It is well known that the ability of quercetin to scavenge is highly relative to reactive species such as peroxynitrite and the hydroxyl radical.[17]

A study evaluated the safety of the flavonol in the typical diet. The result of this study confirmed the safety of Quercetin as a food addition.[18]

A study reported the Quercetin as a plant polyphenols to reduce inflammation or insulin resistance associated with obesity. This study demonstrated that Quercetin has equally or more effective than trans-resveratrol in attenuating TNF-α-mediated inflammation and insulin resistance in primary human adipocytes.[19]

Always achieving the best results with the least expenditure of interest to researchers is the cost.[13,20] Therefore, it is attempted to recognize the regions that have the highest level of the Quercetin flavonoid. The identification of the highest level of plant Quercetin flavonoids will help to achieve higher levels of Quercetin flavonoids while researchers have spent less cost.

CONCLUSION

Considering the cost effectiveness in extracting compounds from medicinal plants, it is recommended to identify the highest level of the quercetin flavonoid in every region and every part of the plant.

CONFLICT OF INTEREST

There is no conflict of interest associated with this study.

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