



CODEN [USA]: IAJPBB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.1197078>Available online at: <http://www.iajps.com>

Research Article

**COMPARATIVE AND QUANTITATIVE DETERMINATION OF
QUERCETIN AND RUTIN IN *TRIBULUS TERRESTRIS L.*
FRUITS FROM DIFFERENT SEASONAL AND
GEOGRAPHICAL POPULATIONS OF SOUTH INDIA****Suresh Reddy Yanala* and D. Sathyanarayana**

Department of Pharmacy, Annamalai University, Chidambaram, Tamil Nadu - 608002, India

Abstract:

The objective of the present research work was comparative and quantitative determination of quercetin and rutin in *Tribulus terrestris* fruits from different geographical regions and seasons by using HPLC. The *Tribulus terrestris* fruit samples collected from three different geographical regions of south India in June and December months. In the current HPLC analysis flavonoids in *Tribulus terrestris* Linn fruits were quantified at 360 nm with help of peak area by comparing to a calibration curve of standard samples of quercetin and rutin. The study concluded that *Tribulus terrestris* fruit samples collected in June season showed maximum amount of rutin and quercetin content and fruit samples collected in December season shown lesser percentage of flavonoid compounds. This research also conforms fruits collected from various geographical regions showed variation in the concentration of flavonoid compounds of rutin and quercetin. The HPLC results also showed that the methanolic extracts of *Tribulus terrestris* fruits collected in June and December months showed remarkable variation in elution of diverse types of chemical constituents with differences in the retention time.

Key Words: *Tribulus terrestris*, South India, Seasonal and Geographical variations, Quercetin and Rutin**Corresponding author:****Suresh Reddy Yanala,**
Department of Pharmacy,
Annamalai University, Chidambaram,
Tamil Nadu - 608002,
India

QR code



Please cite this article in press as Suresh Reddy Y and D. Sathyanarayana., *Comparative and Quantitative Determination of Quercetin and Rutin in Tribulus Terrestris L. Fruits from Different Seasonal and Geographical Populations of South India*, *Indo Am. J. P. Sci*, 2018; 05(03).

INTRODUCTION:

Tribulus terrestris Linn is generally known as puncture vine, goat head, caltrop and devil's thron. *Tribulus terrestris* belongs to a member of Zygophyllaceae family and it is widely distributed in temperate and tropical regions [1]. *Tribulus terrestris* fruits and seeds are used as a dietary supplement with great importance in Ayurveda, Unani, and other medicinal systems in India [2]. *Tribulus terrestris*, which was also used by traditional, ancient medicine in Greece, China. It was recommended as a remedy for erectile dysfunction, impotence, infertility, and low libido [3]. The HPLC method has been employed for quantification of quercetin and rutin in *Tribulus terrestris* L. fruits. The earlier research shows some variation of the content of biologically active constituents in *Tribulus terrestris* L. fruits. The samples from Turkey, Greece, Bulgaria, Macedonia, Serbia, Iran and Georgia shown identical chemical profile and some quantitative differences in the content of prototribestin, protodioscin, tribulosin, dioscin and the flavonoids quercetin and rutin [4].

The current researches on *Tribulus terrestris* published mainly with techniques of isolation and structural explanation of the major biologically active constituents, principally the steroidal saponins, as they are utilized as an industrial source for the development and production of food supplements and medicinal products [5]. Analysis on the content of biologically active constituents in the large-scale populations of this species in south India, as well on the geographical and seasonal factors on the process of their aggregation in the plant, are in an initial phase in our country. The objective of the present research is to determine phytochemical variation and identify quantitative variation of quercetin and rutin

content in the *Tribulus terrestris* fruit samples from different geographical regions and seasons.

MATERIALS AND METHODS:

All the chemicals and reagents were analytical grade. Quercetin and Rutin procured from Xcelris Labs, Ahmedabad. Solvents used for extraction and chromatographic analysis were procured from Merk, Germany. Phytochemical analysis profile was conducted on the fruits of *Tribulus terrestris* L. collected from three different geographical locations of south India (Nakrekal – Telangana, Bellary – Karnataka, Kattumannarkoil – Tamil Nadu) in June and December seasons, when the fruits were fully ripening stage. The fruit samples were immediately washed and dried in shade. The fully dried fruit samples were powdered and stored in a well closed glass container [6].

Tribulus terrestris fruit powdered samples (each of 10 g) were homogenized with 80 % analytical grade methanol (50 ml of each). The whole content was sonicated for 30 min, and then centrifugated at 14,000 rpm followed by filtration. Prepare 5 mg/mL solutions with HPLC grade methanol [7]. The chromatographic analysis was achieved with HPLC system (SHIMADZU), Germany on a 250 mm x 4.36 mm i.d, C -18 column with acetonitrile 80%, water 15.5% and formic acid 4.5% as mobile phase. Flow rate was 1 ml /min, UV detection at 360 nm. Determinations were performed by injecting each extract was in triplicate (n = 3). Prior injection, all extracted samples were filtered through a 0.45µm Chromafil syringe filter (Chromafil PA 40/25). Each sample solution was injected in triplicate with injection volume of 20µl.

RESULTS AND DISCUSSION:

Table 1: HPLC based Quantitative analysis of quercetin from fruits of *Tribulus terrestris* methanolic extract.

Samples	Compounds and injection Volume	retention Time (min)	Sample Concentration (mg/ml)	Quercetin amount (µg/g)
Sample - 1a (June)	Quercetin -20µL	34.21	5	45.67
Sample - 1b (December)	Quercetin -20µL	35.02	5	24.89
Sample - 2a (June)	Quercetin -20µL	36.19	5	38.21
Sample - 2b (December)	Quercetin -20µL	31.24	5	18.9
Sample - 3a (June)	Quercetin -20µL	35.54	5	35.54
Sample - 3b (December)	Quercetin -20µL	37.86	5	27.89

Table 2: HPLC based Quantitative analysis of rutin from fruits of *Tribulus terrestris* methanolic extract.

Samples	Compounds and injection Volume	retention Time (min)	Sample Concentration (mg/ml)	Rutin amount (µg/g)
Sample - 1a (June)	Rutin -20µL	27.89	5	38.9
Sample - 1b (December)	Rutin -20µL	26.76	5	35.54
Sample - 2a (June)	Rutin -20µL	23.89	5	46.42
Sample - 2b (December)	Rutin -20µL	26.56	5	31.21
Sample - 3a (June)	Rutin -20µL	24.67	5	48.9
Sample - 3b (December)	Rutin -20µL	26.86	5	24.43

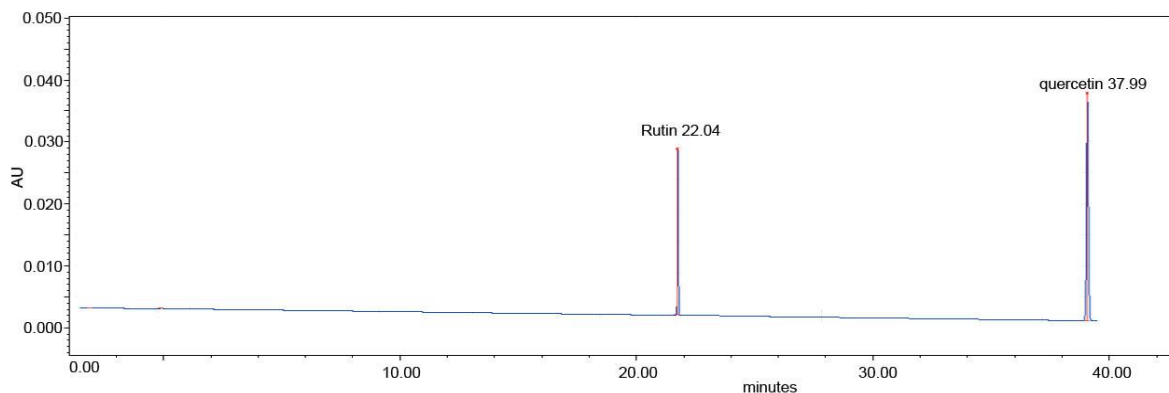


Fig. 1: HPLC chromatogram of standard samples of Rutin and Quercetin

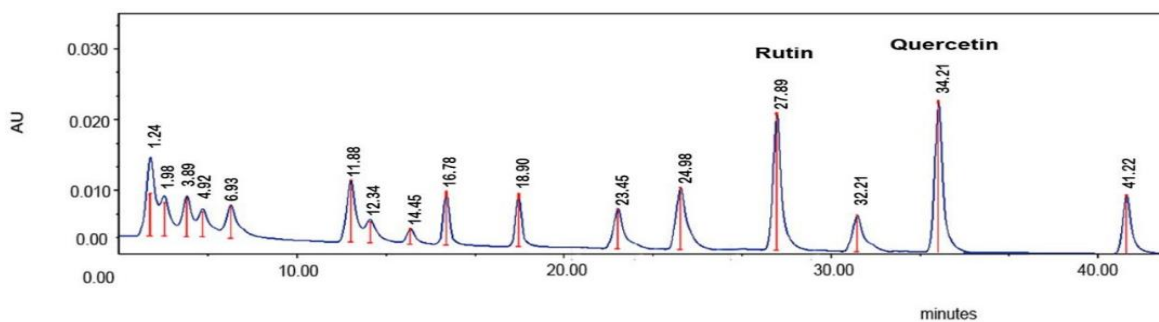


Fig. 2: HPLC chromatogram of sample 1a collected from Telangana in June

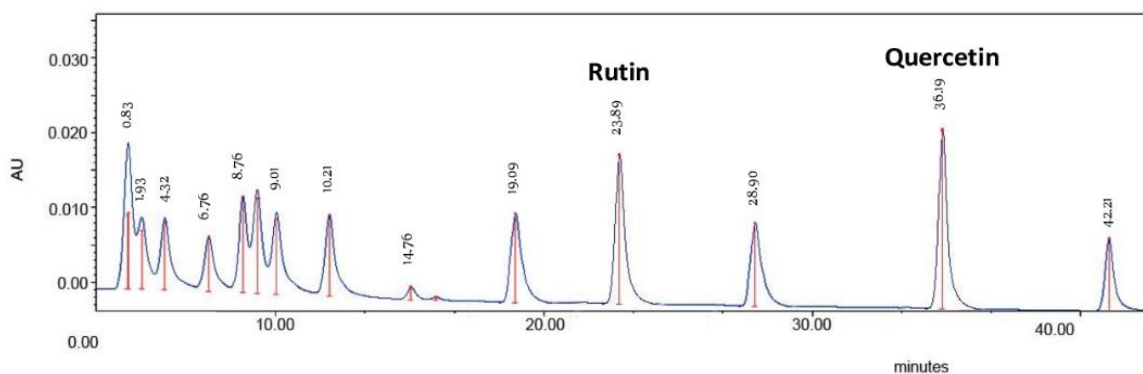


Fig. 3: HPLC chromatogram of sample 2a collected from Karnataka in June

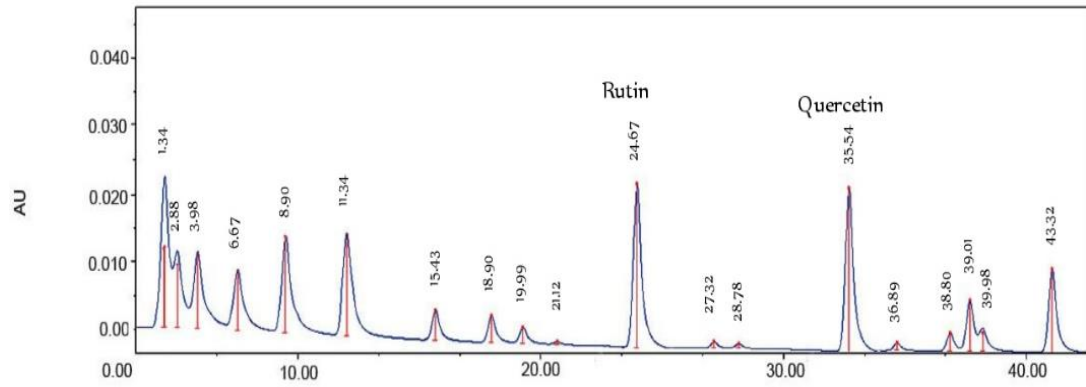


Fig. 4: HPLC chromatogram of sample 3a collected from Tamil Nadu in June

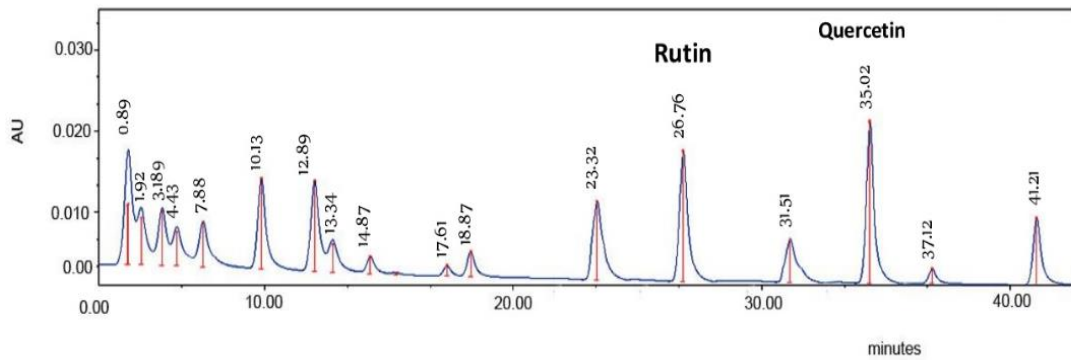


Fig. 5: HPLC chromatogram of sample 1b collected from Telangana in December

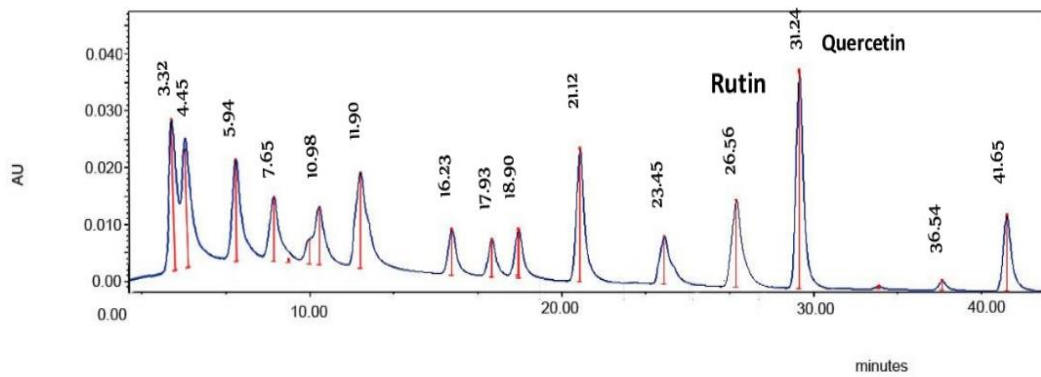


Fig. 6: HPLC chromatogram of sample 2b collected from Karnataka in December

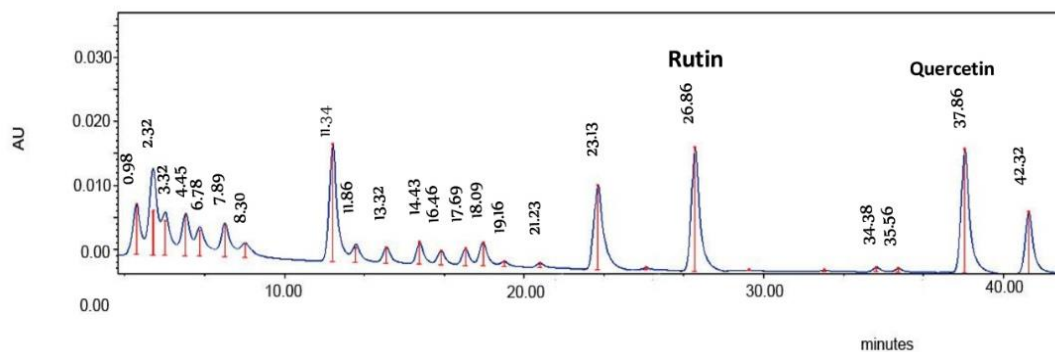


Fig. 7: HPLC chromatogram of sample 3b collected from Tamil Nadu in December

The HPLC, data revealed that there are several flavonoids present in *Tribulus terrestris* Linn fruits, but rutin and quercetin are two principle flavonoids, which were identified and quantified with HPLC. The HPLC results showed that *Tribulus terrestris* fruits collected in June, showed maximum rutin and quercetin content, compared with *Tribulus terrestris* fruits collected in December month.

In June month, sample 1a collected from Nakrekal (Telangana) showed maximum quercetin content (45.67 $\mu\text{g/g}$), sample 2a collected from Bellary (Karnataka) have moderate percentage of quercetin (38.21 $\mu\text{g/g}$). The minimum percentage of quercetin content (35.54 $\mu\text{g/g}$) was found with *Tribulus terrestris* fruit sample 3a collected from Kattumannarkoil (Tamil Nadu). While in case of rutin, sample 3a collected from Kattumannarkoil (Tamil Nadu) in June showed maximum concentration (48.09 $\mu\text{g/g}$), sample 2a from Bellary (Karnataka) showed moderate content (46.42 $\mu\text{g/g}$) and sample 1a from Nakrekal (Telangana) showed minimum rutin concentration (38.9 $\mu\text{g/g}$).

All *Tribulus terrestris* fruit samples collected in December month showed lesser quercetin and rutin concentration with compared to fruits collected in June month. TT fruit sample 3b from Kattumannarkoil (Tamil Nadu) showed maximum quercetin (27.89 $\mu\text{g/g}$) and minimum rutin content (24.43 $\mu\text{g/g}$). Sample 1b from Nakrekal (Telangana) showed moderate quercetin content (24.89 $\mu\text{g/g}$) and maximum rutin content (35.54 $\mu\text{g/g}$). Sample 2b from Bellary (Karnataka) showed minimum quercetin content (18.9 $\mu\text{g/g}$) and moderate rutin content (31.21 $\mu\text{g/g}$).

The HPLC results showed that the methanolic extracts of *Tribulus terrestris* fruits collected in June showed remarkable variation in elution of diverse types of chemical constituents with differences in retention time. Sample 1a (Telangana, June) showed

nearly 16 chromatographic peaks, sample 2a (Karnataka, June) showed 14 chromatographic peaks and sample 3a (Tamil Nadu, June) showed 19 chromatographic peaks with different retention time.

Tribulus terrestris fruit samples collected in December also showed clear differences in chromatographic elution of various chemical constituents. Sample 1b (Telangana, December) showed 17 HPLC peaks, sample 2b (Karnataka, December) resulted elution of 15 peaks and sample 3b (Tamil Nadu, December) showed nearly 22 chromatographic peaks. The number of peaks with different retention time showing chemical constituent variation in the fruits of *Tribulus terrestris* collected from different geographical regions and seasons.

CONCLUSION:

The study concluded that the *Tribulus terrestris* fruit samples collected in June month showed maximum amount of rutin and quercetin content and fruit samples collected in December month shown minimum percentage of flavonoid compounds. This research also confirms fruits collected from various geographical regions shown variation in the concentration of flavonoid compounds of rutin and quercetin.

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