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HPLC Estimation of Flavanoid (quercetin) of leaves and stem extracts of *Ocimum sanctum and Tinospora cordifolia*

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ABSTRACT

High performance liquid chromatography method is used for the estimation of flavanoid (quercetin) in leaves and stem extracts of *Ocimum sanctum* and *Tinospora cordifolia*. The dried leaves and stem of both plants were extracted with methanol and ethanol solvent. The concentration of quercetin of methanolic and ethanolic leaves and stem extracts of plants performed by using acetonitrile and methanol 50:50 (V/V) solutions as mobile phase and used a flow rate of 1ml per min and absorbance at 256 nm. We conclude in this study that *Ocimum sanctum* and *Tinospora cordifolia* has significant amount of flavanoid (quercetin), which indicate the high antioxidant activity of both plants and used for further study.

Keywords: Flavanoid (quercetin), HPLC, *Ocimum sanctum*, *Tinospora cordifolia*.

INTRODUCTION

Ocimum sanctum belongs to lamiaceae family because it is the best known family for therapeutic effects and used as a resource of alimentary herbs [1]. This herb is found on tropical and semitropical area of India. The two main species of *Ocimum sanctum* are green-leaved (Sri or Lakshmi Tulsi) and purple-leaved (Krishna Tulsi) cultivated in India and Nepal [2]. *Ocimum sanctum* has a various property such as antioxidant, antibiotic, antiatherogenic, anti-ulcer, anti-inflammatory, analgesic, antipyretic, immunomodulatory, and chemopreventive properties [3]. These all properties of *Ocimum sanctum* is because of the presence of phytochemicals. The stems and leaves of tulsi consist of a various phytoconstituents that may have physiological activity, involving alkaloids, flavonoids, glycosides, saponins, triterpenoids, tannins, carbohydrates, and proteins [4]. *Ocimum sanctum* is the commonly and most occurring reservoirs of all household plants in India. Its leaves, seeds and the complete plant is useful. *Tinospora cordifolia* (*T. cordifolia*) is another medicinal plant, used for this study. It belongs to the Menispermaceae family. It is a particularly accomplished creeper. It is utilized in ayurved medicine system since ancient times. It is commonly known as 'rasayana' drugs in ayurved, which developed the resistance of the body, enhance vitality, relaxing, and as adaptogen [5, 6]. *Tinospora cordifolia* is distributed all over India and in other parts of the world. This plant is valued for medicinal properties in ayurvedic medicine.

Tulsi Plant

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Tinospora cordifolia (Willd.) is commonly known as guduchi, amrita, and giloy. In ayurvedic medicative system, it's used as most divine shrubs for its enormous properties like as medicinal drug, antiarthritis, antidiabetic, inhibitor, antiallergic, antileprotic, antiperiodic, hepatoprotective, antiprotozoal, antineoplastic and immunomodulatory activities [7, 8]. The *Tinospora cordifolia* root has been clinically used to treat jaundice, rheumatoid arthritis, and diabetes [9].

Giloy Plant

Tinospora cordifolia has widely used in ayurvedic, unani, and homoeopathy. The benefits of health observed may be because of the presence of the numerous phytochemicals such as terpenes, phenols, flavonoids, anthocyanins, alkaloids, and glycosides. Flavanoid are the major chemical compound found in *Ocimum sanctum* and *Tinospora cordifolia*. A HPLC method has been used to quantitatively estimate the flavanoid content in the leaves and stem of *Ocimum sanctum* and *Tinospora cordifolia.*

MATERIAL AND METHODS

The leaves and stem of *Ocimum sanctum* and *Tinospora cordifolia* were collected from rural area of chitrakoot. The stem and leaves were shade dried and powdered. Powdered material of both plants extracted with ethanol and methanol solvent [10]. The each solvent extract was filtered and concentrated in rotary evaporator. All extracts of both plants is used for HPLC analysis to quantify the flavanoid (quercetin) content present in *Ocimum sanctum* and *Tinospora cordifolia.*

Qualitative and quantitative hplc analysis: preparation of stock and working standard solution

The HPLC method is used for qualitative and quantitative analysis of flavonoid components (quercetin). It is a secondary metabolites component present in leaves and stem of *Ocimum sanctum and Tinospora cordifolia.* For the preparation of stock solution, weighed 10mg of quercetin component and transferred into a volumetric flask of 10ml, and the volume of the solvent was adjusted with the methanol up to the mark and prepared standard stock solution of 1000ppm. Then 1ml of quercetin was taken from the stock solution and diluted up to 10ml for preparing working standard solution. Different concentration of the solution (0.5, 1.0, 1.5, 2.0, 2.5ml) from standard was transferred into 10ml volumetric flasks and make up the volume up to 10ml with the solvent. Standard drug solutions of 5, 10, 15, 20, 25µg/ml concentration were used.

Extraction of plant samples

10mg plant extracts samples were taken in a volumetric flask of 10ml and diluted with methanol up to marks and this solution was filtered through the help of whatman filter paper and prepared finally volume with the similar solvent and to obtained 1000 µg/ml concentration. Finally, the resulting solution filtered again through whatman filter paper no. 41 and sonicated for 10min.

Determination of λmax

Lambda max (λmax) referred to highest wavelength in the absorption spectrum, where the absorbance is high. Generally, molecules absorb a particular wavelength area around the λmax. It functions as a quantitative parameter to match the absorption differences of various molecules. In the process of spectroscopy individual compounds provide the perticular peaks for the perticular substituents are groups in the combination. We constantly used the transparent solvent in the process of UV- spectrophotometer for the compound otherwise it creates the distortion within the peaks and we get broad peaks then the sharp peaks. To compare different qualities of all type of molecules and substances, lambda max can be used as a parameter.

For the measurement of λmax, taken a 50mg quercetin component and dissolved into 50ml methanol and prepared single aliquots of 10µg/ml in methanol for measuring the λmax **(figure 1)**. By this method, we can calculate the highest absorbance (λmax) of flavonoid (quercetin) in different wavelength on different concentration.

HPLC Unit

HPLC system consisted of a pump, UV- visible detector, C-18 column with 250mm × 4.60mm dimension and data software unit were used for the analysis. The best resolution of flavonoid was obtained at 2.50 ± 0.5min retention time using a column and analytical solvent system contain of acetonitrile and methanol in the ratio 50:50 (v/v). The degassed mobile phase should have passed through a 5µm filter. The flow rates of solvent were kept constant at 1ml/min and detect the absorption in 256nm. For calibration, the standard solution of flavonoids (quercetin) was prepared at concentrations 5, 10, 15, 20, 25µl/min using methanol as solvent. The standard sample was introduced in triplicate and average detector response was measured. The stem and leaves extract also were assayed in triplicate and peak areas corresponding to flavonoids were compare with the calibration curve and quantity of flavonoid (quercetin) was determined.

Chromatographic analysis of flavonoids (Quercetin)

The chromatographic analysis of quercetin being performed at a specific temperature by the use of RP-C18 analytical column filled with a mobile phase consist of Acetonitrile and Methanol (50:50 v/v) and elution process performed at a rate of 1ml/ min. In this method, extracts sample were introduced into the HPLC system in a small volume of 20µl and developed chromatogram. The chromatograms were monitored in the UV detector at 256 nm wavelength. Identification of each sample component was determined by its peak value and retention time under the similar condition with standards. The quantity of the various sample calculated by using the calibration curve through the area of peak valuation. The calibration curve has been plotted between peak area and concentration of respective standard solution (Table 1, figure 2).

RESULT AND DISCUSSION

Identification of marker compound

The solution of plant extracts sample was chromatographed and obtained the concentration of quercetin of both plant extracts sample by using the regression equation. In the presently research, the HPLC process become used to study and quantify the flavanoids (quercetin) component from both selected medicinal plants.

Preparation of the calibration curve of the quercetin

For the preparation of calibration curve, taken standard solutions (quercetin) and injected into the column for 3 times and calculate the mean of peak area of standard drug and plotted the graph against different concentration (5, 10, 15, 20, 25µg/ml) of the drug by using methanol as solvent system. The regression equations were figured out on the basis of the curve. The calibration curve of the standard solution was used for comparing the peak of extracts sample of both plants.

Table 1: Preparation of Calibration curve

S. No.	Concentration	Mean AUC
1	0	0
2	5	410.235
3	10	892.541
4	15	1387.324
5	20	1889.548
6	25	2317.181

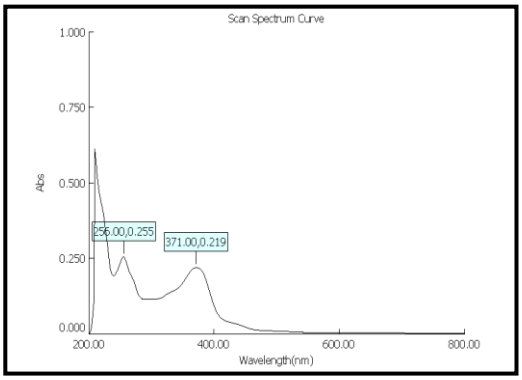

Figure 1: Determination of λmax

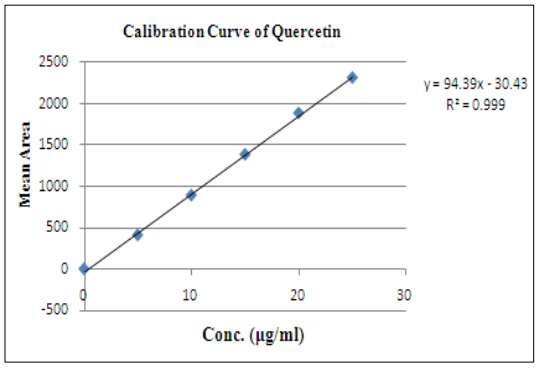

Figure 2: Calibration Curve of the Quercetin

The calibration curve of flavonoids was observed linear relationship over the range of 5, 10, 15, 20, 25µg/ml (R^2=0.99) concentration of standard. The linear regression equation for the curve was Y = 94.39X - 30.43, where y is the ratio of peak area of flavonoids and x is the flavonoid concentration (µg/ml).

Identification of quercetin in two different extracts of the plants

The qualitative and quantitative study of quercetin of selected Phytoextracts was performed by the HPLC method. The HPLC chromatogram of methanol and ethanol extracts of leaves and stem of *Ocimum sanctum* and *Tinospora cordifolia* revealed their highest peaks area for the quercetin as secondary metabolites component. The resulting HPLC chromatogram of standard quercetin and plant extracts sample were recorded and presented in below (T**able 3-10).** The qualitative analysis of phytochemicals was supportive of the quantitative analysis of its. The ongoing study confirmed the presence of flavonoids (quercetin) in both medicinal plants as secondary metabolites.

Table 2: Characteristics of the analytical method derived from the standard calibration curve

Compound	Linearity range (µg/ml)	Correlation co-efficient	Slope	Intercept
	5-25	0.999	94.39	-30.43

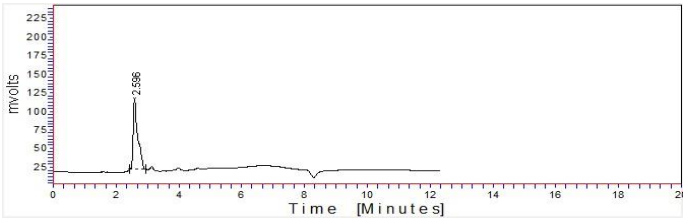

Figure 3: Chromatogram of standard Quercetin

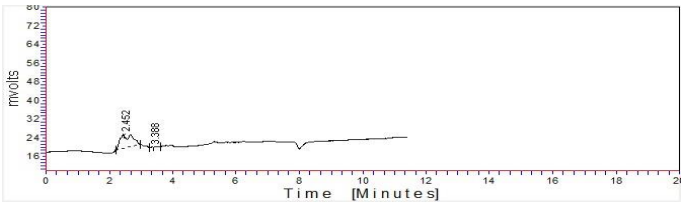

Figure 4: Chromatogram of ethanolic stem extracts of *Ocimum sanctum*

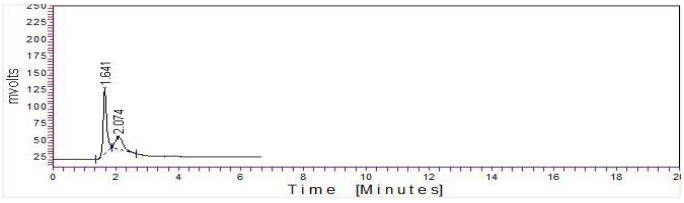

Figure 5: Chromatogram of methanolic leaf extracts of *Ocimum sanctum*

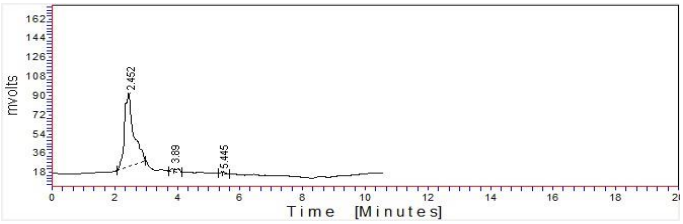

Figure 6: Chromatogram of methanolic stem extracts of *Ocimum sanctum*

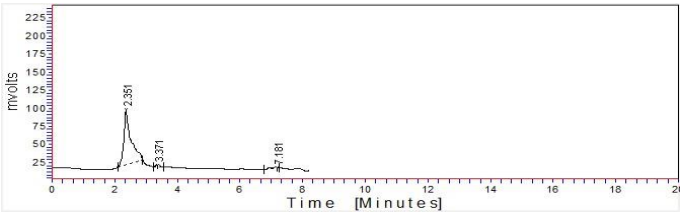

Figure 7: Chromatogram of ethanolic leaf extracts of *Tinospora cordifolia*

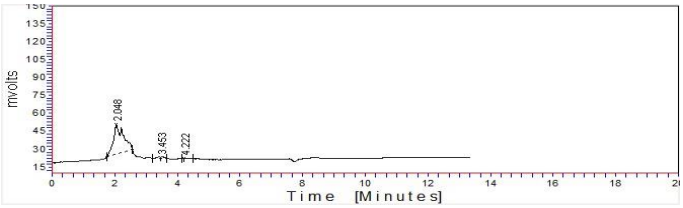

Figure 8: Chromatogram of ethanolic stem extracts of *Tinospora cordifolia*

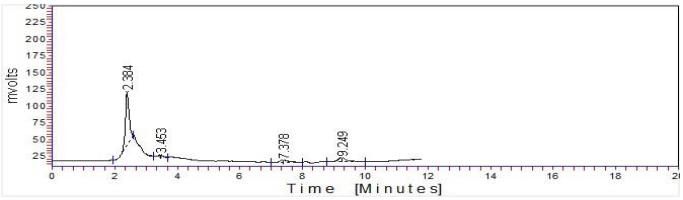

Figure 9: Chromatogram of methanolic leaf extracts of *Tinospora cordifolia*

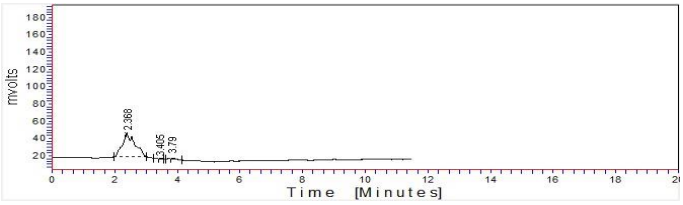

Figure 10: Chromatogram of methanolic stem extracts of *Tinospora cordifolia*

Chromatographic analysis of methanolic and ethanolic leaves and stem extracts of plants performed by using acetonitrile and methanol 50:50 (V/V) solutions as mobile phase and used a flow rate of 1ml per min and absorbance at 256 nm. It gave good separation of quercetin at RT 2.50min. The HPLC chromatogram of methanolic stem extracts of *Ocimum sanctum* revealed higher peak area at RT 2.452 and methanolic leaf extracts of *Ocimum sanctum* revealed higher peak area at RT 2.074 whereas HPLC chromatogram of ethanolic stem extracts of *Ocimum sanctum* revealed higher peak area at RT 2.452 and ethanolic leaves extracts of *Ocimum sanctum* has not shown any type of peak because absence of flavonoid. The HPLC fingerprints of the methanolic stem extracts of *Tinospora cordifolia* has shown major peaks area at the RT 2.368 (min) at a wavelength of 256 nm and methanolic leaves extracts of *Tinospora cordifolia* showed major peak area at the RT 2.38 whereas HPLC chromatogram of the ethanolic stem extracts of *Tinospora cordifolia* showed higher peak area at the RT 2.048 and ethanolic leaves extracts of *Tinospora cordifolia* showed higher peak area at the RT 2.351.

The peak of quercetin in the chromatogram of both plant extracts were compared with the RT values with those obtained by the chromatogram of the standard quercetin under the similar conditions. Standard quercetin gave a strong peak area at the RT 2.59 min and HPLC of methanolic and ethanolic leaves and stem extracts of the plant gave different peaks area at the same retention times as shown in the above figures. Results of the peaks of variety of plant extracts did not show more differences with standard quercetin, which further confirms the occurence of quercetin in the methanolic extracts (leaves and stem) of *Tinospora cordifolia* and *Ocimum sanctum* and ethanolic extracts (leaves and stem) of *Tinospora cordifolia* and only ethanolic stem extracts of *Ocimum sanctum*. Ethanolic leaves extract of *Ocimum sanctum* has not shown in the presence of flavonoids (quercetin). Comparative chromatographic analysis of both plants has been shown the presence of quercetin in both plants. The present study emphasized that both plants showed differences in chemical constituents. The flavonoid (quercetin) component of these two species showed a little amount of variation.

Recently, this technique is achieving popularity within numerous analytical techniques as the primary choice for the study of fingerprinting and for quality control method of herbal plants [11]. Naturally biproducts are frequently isolated ensuring the valuation of a moderately crude extract in a biologicals assay for the purpose of fully characterize its properties. The resolution capability of HPLC is perfectly well suited to the rapid reprocessing of such multi-component samples on both an analytical and preparative scale. Several sponsors explained the use of HPLC for quantification and characterization of secondary metabolites of plant extracts [12, 13].

Quantitative analysis of quercetin in different plant extracts

This research stated the presence of a large quantity of total flavonoids content in *Tinospora cordifolia* and *Ocimum sanctum*. Flavonoids present in methanolic and ethanolic leaves and stem extract of both plants except ethanolic leaves extracts of *Ocimum sanctum*. Flavonoid is mainly occurred in various parts of the plant as glycosides and quercetin and is known to be imparting luxuriant color to the leafy parts, fruits, flowers, etc. It is stated to reveal anti-cancer, anti-histamine, and anti-inflammatory activities which mostly followed its antioxidant features. Quantitative estimation of quercetin in different extracts of *Ocimum sanctum* and *Tinospora cordifolia* were calculated as percentage assay and given in table 3&4, figure 11& 12.

Table 3: Quantitative estimation of Quercetin in different extracts of *Ocimum sanctum*

S. No.	Extract	RT	Area	% Assay
1	OS ethanolic (Stem)	2.452	142.559	0.118793
2	OS methanolic (Leaf)	2.074	286.982	0.271528
3	OS methanolic (Stem)	2.452	1340.61	1.38528

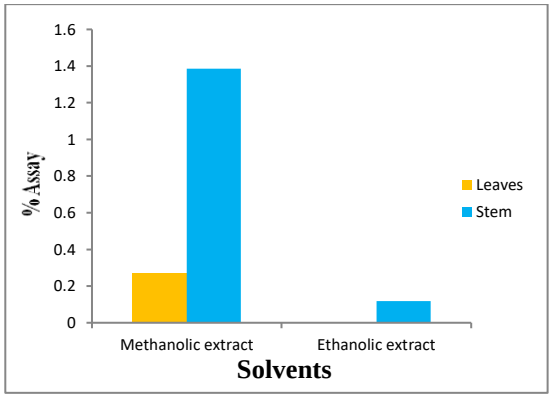

Figure 11: Quantitative estimation of quercetin in different extracts of *Ocimum sanctum*

Table 4: Quantitative estimation of quercetin in different extracts of *Tinospora cordifolia*

S. No.	Extract	RT	Area	% Assay
1	TC ethanolic (Leaf)	2.351	1132.17	1.167218
2	TC ethanolic (Stem)	2.048	527.771	0.526374
3	TC methanolic (Leaf)	2.384	586.435	0.587875
4	TC methanolic (Stem)	2.368	727.053	0.735819

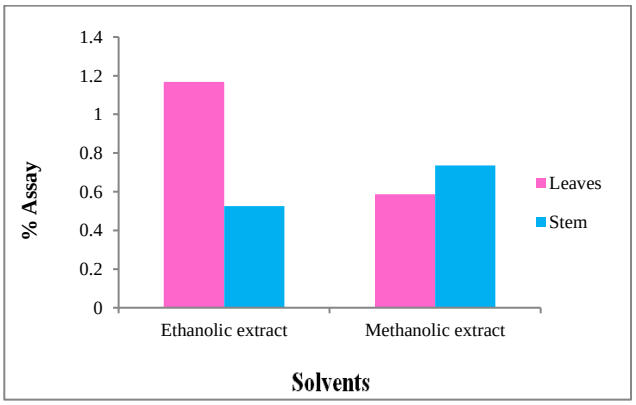

Figure 12: Quantitative estimation of quercetin in different extracts of *Tinospora cordifolia*

CONCLUSION

A significant amount of quercetin was detected in both extracts of *Ocimum sanctum* and *Tinospora cordifolia*. It is reported that flavonoid has good pharmacological, antioxidant and nutraceutical activities. Antioxidant properties of flavonoids are well known to be responsible for human health benefits [14]. The above study has shown that *Tinospora cordifolia* and *Ocimum sanctum* show differences in chemical constituents. The quercetin content of the two plants showed little variation, *Ocimum sanctum* having higher flavonoid concentration in comparison to *Tinospora cordifolia*. The analytical methods presented here are applicable with respect to the identification and quantification of quercetin distinguished the two plant species conveniently.

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