

Simultaneous Determination of Rutin and Quercetin in Different Parts of *Tecomella undulata* (Seem): An Endangered Medicinal Plant.

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ABSTRACT

Simultaneous quantitative determination of two flavonoidal compounds, rutin and quercetin was carried out by using reverse phase -high performance layer chromatography (RP-HPLC) with Uv-visible detector. A simple and rapid chromatographic separation was done by using Zorbax -C18 (5 μ m 4.6 \times 150mm) column by using isocritical flow with the constituted mixture of water and methanol (60: 40) with the flow rate of 1ml/min. Detection was carried out at 370 nm. The linear regression data for the calibration plots showed a good liner relationship with $r= 0.996$, same for both flavanoids. Comparative study of methanolic and ethanolic extract of leaf, bark and flower of *Tecomella undulata* (Seem.) was done. A common trend was observed for both rutin and quercetin in terms of content from the aerial parts, the highest being in flowers followed by leaf and then from bark in both the extracts. Methanol exhibited to be a better solvent for the extraction of both the Flavanoids. However, Quercetin could not be detected in ethanolic extract of leaves while bark exhibited negligible amount of quercetin in both, methanolic and ethanolic extracts. The result of present study supports the view that the aerial parts of *Tecomella undulata* (Seem) could be a potential source of natural antioxidant, anticarcinogenic and other important medicinal drugs.

Key word: HPLC, Quercetin and Rutin, *Tecomella undulata* (Seem).

INTRODUCTION

Over the past decade evidences have been accumulated that plant polyphenols and especially, flavonoids are a most important class of defence anti oxidants. With several endogenous antioxidants they play a role in optimum protection from oxidative stress caused by the increase in the level of reactive oxygen species (ROS) in the human organism. Under oxidative stress conditions ROS (i.e. oxygen-centred free radicals, singlet oxygen, hydrogen peroxide) may be very damaging and play a causative role in aging and several degenerative diseases, for example heart disease, atherosclerosis, cataracts, cognitive dysfunction, hepato-toxicity, inflammation, tumor promotion, and cancer[1].

Flavonoids are a class of Secondary metabolites which are also collectively known as "Vitamin-p" [2]. Flavonoids are polyphenol compounds, widely found in the plant kingdom. Flavonoids, as a major active constituent, display a remarkable role in various pharmacological activities including antiallergic, anti-inflammatory and antioxidant effects. Rutin and Quercetin are two major and most important Flavanoid and Both possesses important medicinal and phytochemical properties [3-6].

Quercetin, (a flavonoid member chemically known as 5, 7, 31, 41 - tetra hydroxy flavonol) possess a lot of therapeutic benefits. It plays an important role in cardiovascular health improvement, cancer reduction, neurodegenerative diseases, aging, osteoporosis, inflammation, hepato

protection, allergies, ulcers and viral diseases [7-10]. Infact rutin is also a member of biflavonoides family chemically known as 5, 7, 31, 41- tetra hydroxy flavanol-3-rhamnoglucoside, it possesses a lot of pharmacological actions including anti-inflammatory, anticarcinogenic, antithrombic, cytoprotective, and vasoprotective activities.[11,12]

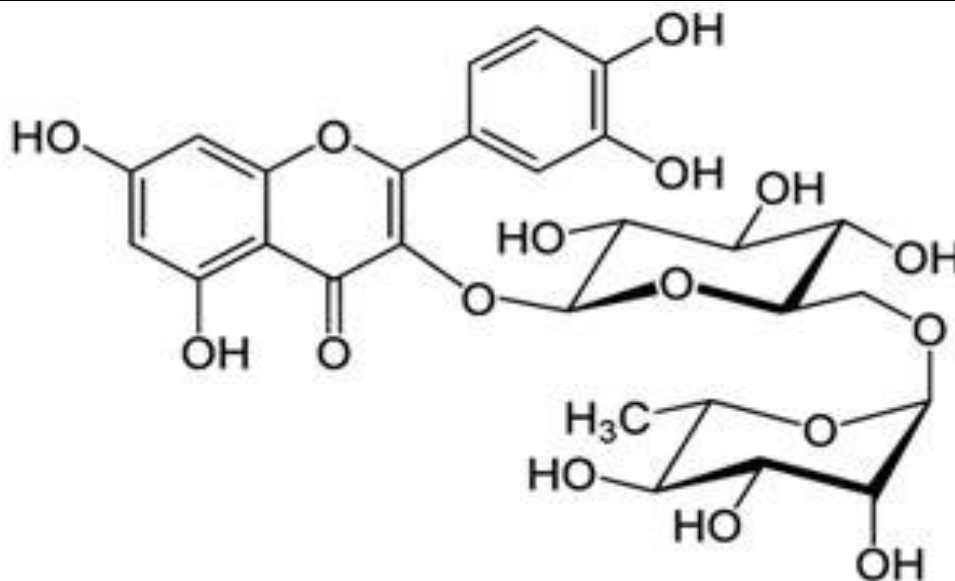
Herbal medicines have stood the test of time for their safety, efficacy cultural acceptability and lesser side effect. They are believed to have better compatibility with the human body. Some of the herbal plants traditionally used in formulation of many medicines [13-18].

Tecomella undulata (Seem) is one of the important medicinal plant. *Tecomella undulata* (Seem) is a deciduous shrub or small tree, found in northwestern India and southern Pakistan. The plant is under endangered category due to its imprudent harvesting from wild. The plant is used to cure leucorrhoea, leucoderma, and enlargement of spleen, traumatic wounds, hepatitis, piles, anorexia, flatulence, tumors, worm infestations and syphilis. Leaves of the plant contain certain compounds which are effective against HIV infection [19].

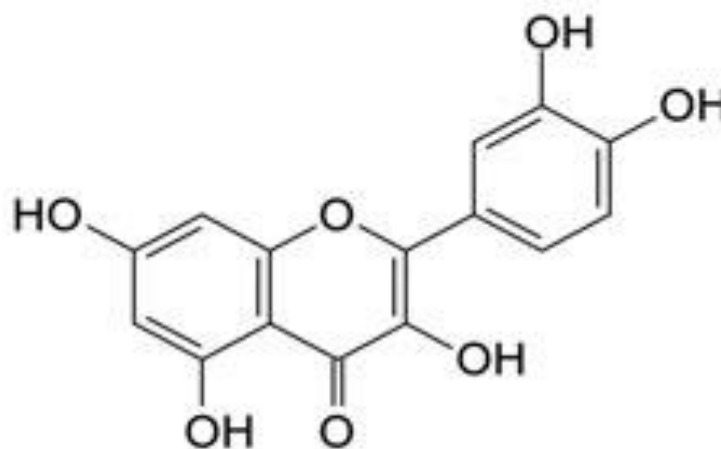
The literature survey shows that no work has been done on simultaneous isolation of quercetin and rutin from aerial parts of *Tecomella undulata* (Seem). The structure of rutin and quercetin exhibits structural similarity (figure 1) and hence a good isolation method has been developed in the current work. The objective of the present study was

Table 1: RU and QU content in different parts of *Tecomella undulata* (Seem)

Solvent	Leaf (ug/ml)		Bark (ug/ml)		Flower (ug/ml)	
	Rutin	Quercetin	Rutin	Quercetin	Rutin	Quercetin
Methanol	125.53	1.39	78.60	Negligible	216.53	5.80
Ethanol	65.13	Nil	40.33	Negligible	79.21	2.21



Rutin



Quercetin

Fig 1: Structure of Rutin and Quercetin

development of method for simultaneous quantitative estimation of quercetin and rutin. It is a comparative study of both the flavanoids in aerial parts of *Tecomella undulata* (Seem) for the first time using HPLC.

MATERIAL AND METHODS

Reagents and materials

Methanol (HPLC grade)

Deionized water (HPLC grade)

Rutin and quercetin (Sigma Aldrich, USA).

Plant material: Plant material was collected from Nagaur and Jalor, (Rajasthan, India). The plant was identified and

authenticated at Blatter's Herbarium, St. Xavier's College, Mumbai. (Accession No 22800).

The plant parts were sorted out and surface contaminants of plant samples were removed by washing with mild detergent followed by rinsing with deionized water. Then the material was air dried and homogenized to fine powder. The powder was stored in air tight glass containers and used for further analysis.

Preparation of sample solution: An amount 0.5 gm of leaf, bark and flower powder was extracted separately in ethanol and methanol by sonication for 10-13 minutes. The solution was first filtered through a filter paper and this

filtrate was again filtered through a micro filter with a regenerated cellulose membrane of the pore size 0.22. This filtrate was applied for HPLC.

Preparation of standard solution: Standard stock solutions of rutin and quercetin were prepared in ethanol and methanol at concentration of 10,20,30,40 and50 ppm. All

Chromatographs

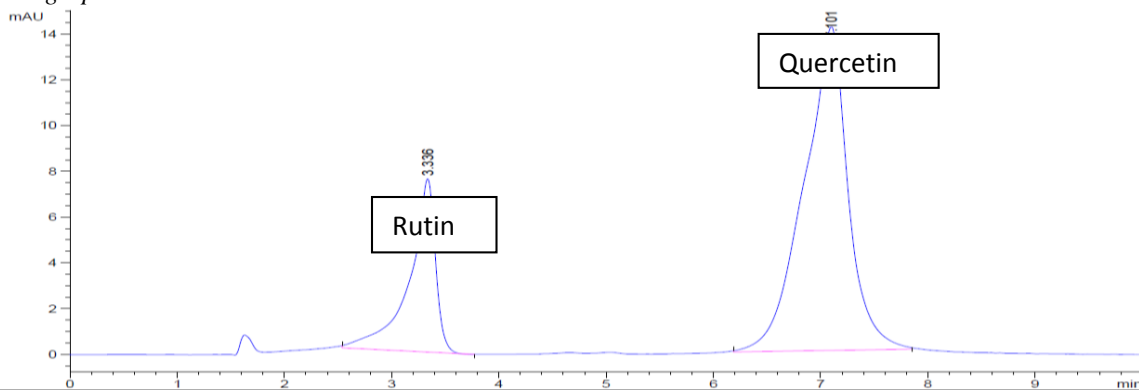


Fig 2: HPLC chromatogram of authentic samples of Rutin and Quercetin

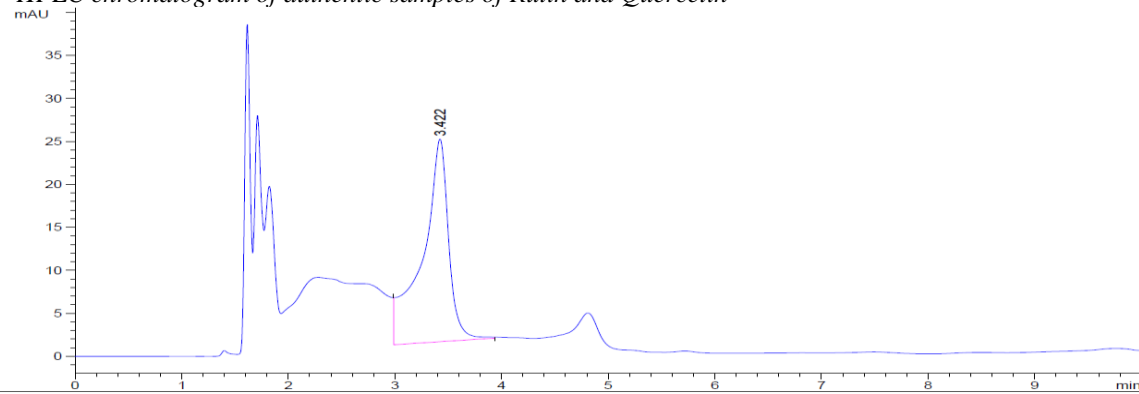


Fig 3: HPLC Chromatogram for leaf-ethanolic extract of Tecomella undulata (Seem)

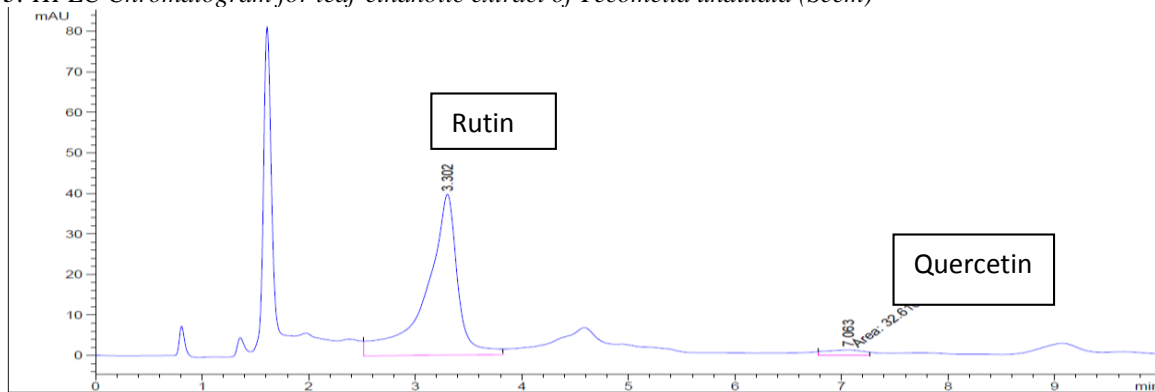


Fig 4: HPLC Chromatogram for leaf- methanolic extract of Tecomella undulata (Seem)

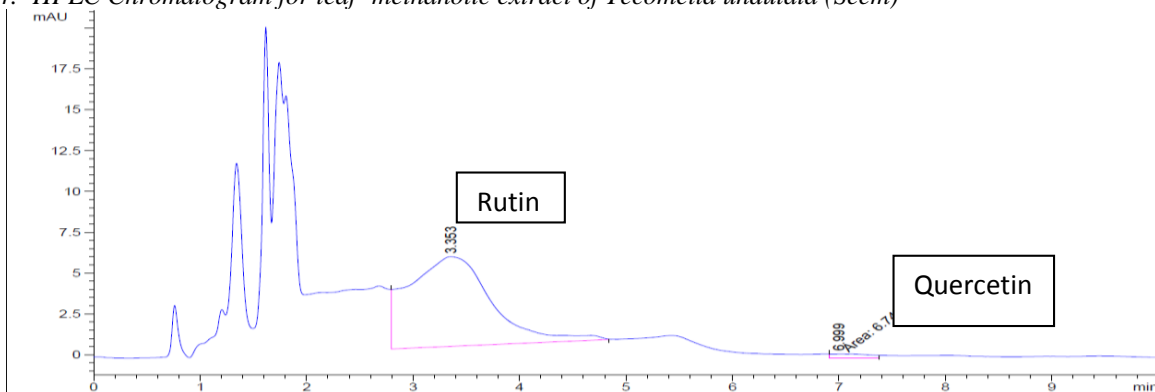


Fig 5: HPLC Chromatogram for Bark- ethanolic extract of Tecomella undulata (Seem)

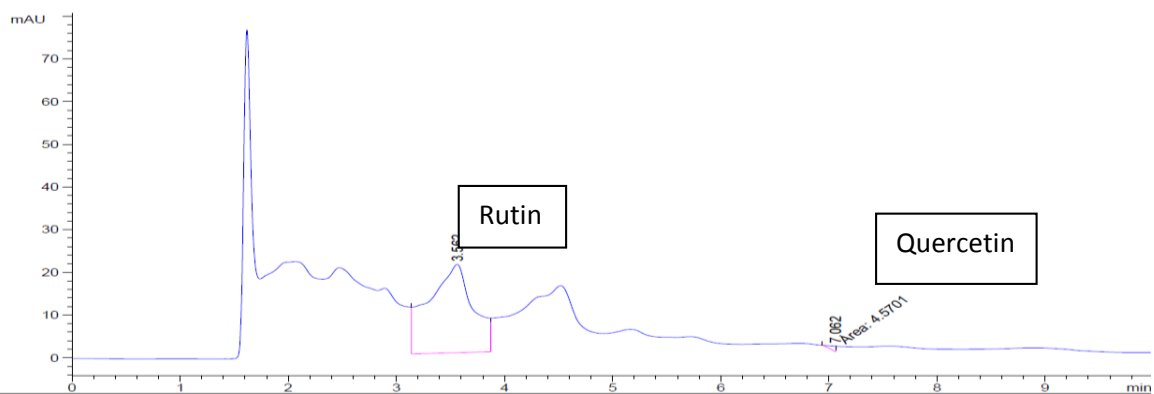


Fig 6: HPLC Chromatogram for bark- methanolic extract of *Tecomella undulata* (Seem)

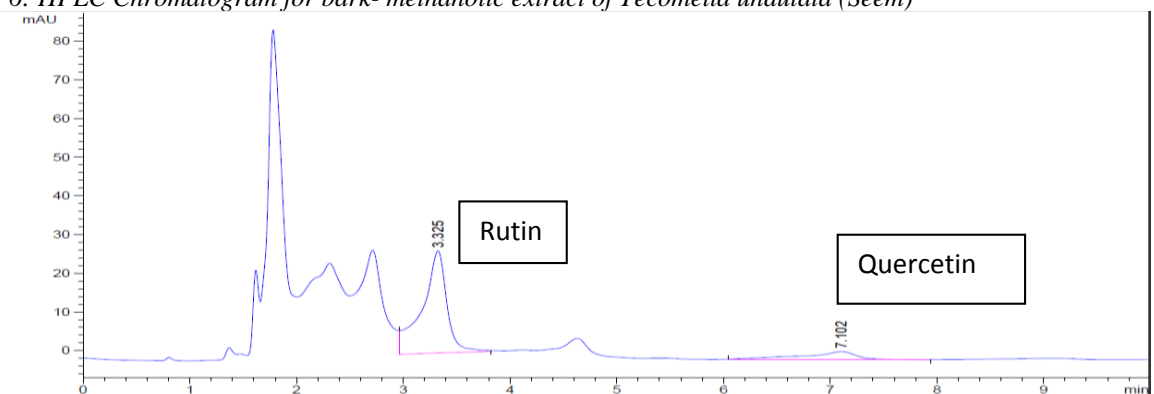


Fig 7: HPLC Chromatogram for flower- ethanolic extract of *Tecomella undulata* (Seem)

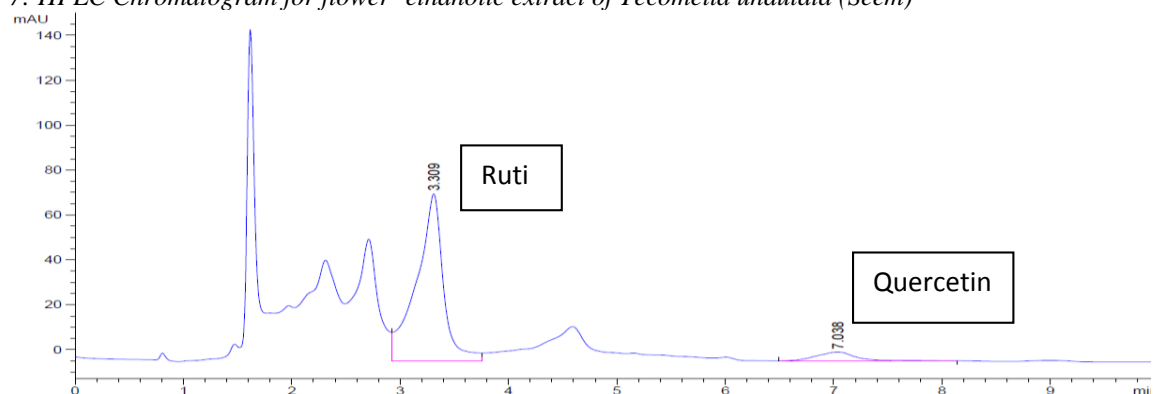


Fig 8: HPLC Chromatogram for flower- methanolic extract of *Tecomella undulata* (Seem)

sample solutions were filtered through 0.22 μm membrane filter and injected directly. Rutin and quercetin 10 mg were accurately weighed into 10 ml volumetric flask, dissolved in 5 ml methanol and the solution was made up to 10 ml with the same solvent (1mg/ml). Rutin (RU) and quercetin (QU) were quantified by HPLC separation at 370 nm.

Chromatographic conditions

Instrument: Agilent (1200 series)

Detector: Uv-visible detector

Flow rate: 1 ml/min

Detection: 370 nm

Injection quantity: 10 μl

Column used: Zorbax C18 (150 mm x4.6 mm i.d., 5 μm)

Column temperature: 30 $^{\circ}\text{C}$

Mobile phase: 60:40 % v/v

Mobile phase: Water: Methanol

RESULT AND DISCUSSION

The method developed for HPLC fingerprinting provided a quick and simultaneous analysis of quercetin and rutin from ethanolic and methanolic extract. However, there is minor structural difference between quercetin and rutin so it was difficult to separate them but the conditions used led to a good separation of peaks which could be identified in the chromatogram (figure no.2), as rutin (RT = 3.333) and quercetin (RT = 7.099 min). The standard response curve for each standard was a linear regression, fitted to triplicate values obtained at each of five concentrations. The linearity relationship between peak areas and concentrations was good and the correlation coefficient (r^2) was 0.996 for both RU and QU. They were identified by comparison with the chromatogram of the 2 reference compounds (Figure2).

Extraction was carried out for rutin and quercetin from leaf flower and bark using two different solvents i.e. ethanol & methanol. The results indicates that the amount of rutin was more in flower as compared to leaf and bark. The

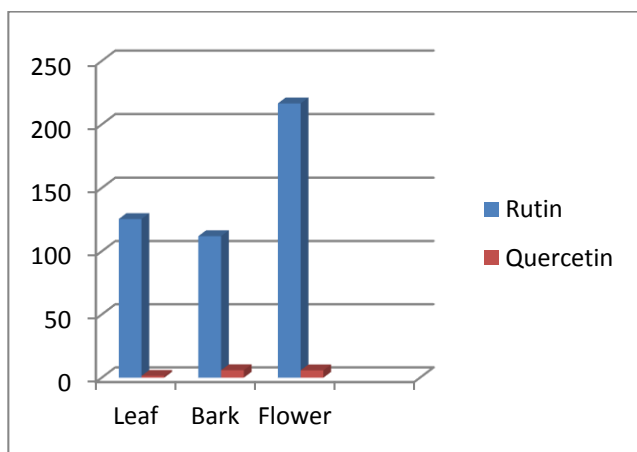


Plate 1: RU and QU content in methanolic extract of different parts of *Tecomella undulata* (Seem). (X-axis – different arial parts and Y-axis amount of RU and QU in ug/ml).

highest amount of rutin was found in methanolic extract of flower. In case of quercetin the highest amount was also found in methanolic extract of flower indicating methanol to be a better solvent for extraction of the Flavanoids. Negligible amount of quercetin was found in both the bark extracts. (Table 1, Figure 3-8)

CONCLUSION

A rapid, simple, accurate and specific HPLC method for quantitative estimation of rutin and quercetin present in *Tecomella undulata* (Seem) has been developed. The developed method can be used for the quantitative and qualitative estimation of rutin and quercetin in herbal formulation. The result of present study supports the view that the arial parts of *Tecomella undulata* (Seem) could be a potential source of natural antioxidant, anticarcinogenic and other important medicinal drugs.

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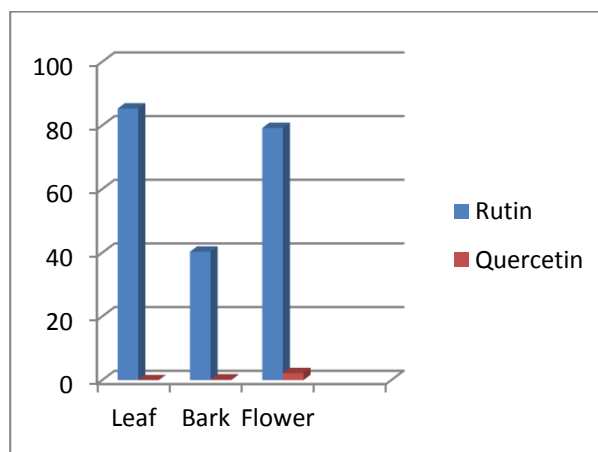


Plate 2: RU and QU content in methanolic extract of different parts of *Tecomella undulata* (Seem). (X-axis – different arial parts and Y-axis amount of RU and QU in ug/ml).

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