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Quantitative estimation of Gallic Acid, Rutin and Quercetin in certain herbal plants by hptlc method

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ABSTRACT

An HPTLC method was developed for the quantitative estimation of gallic acid rutin and quercetin from aqueous and ethanolic extract Eruca sativa, precoated HPTLC silica gel 60 F_{254} as stationary phase and mobile phase for gallic acid Toluene: Ethyl Acetate: Formic Acid [7:5:1 v/v/v/v/v] and mobile phase for quercetin and rutin, ethyl acetate: glacial acetic acid: formic acid: water [100:11:11:25, v/v/v/v/v]. Detection and quantification were performed densitometrically at λ 280nm for gallic acid, 280 nm quercetin and 366nm for rutin. The standard Rf values of gallic acid, quercetin and rutin are 0.35±0.01, 0.98±0.01 and 0.34±0.02 respectively. The total peak areas of the standards [gallic acid, quercetin and rutin] and the corresponding peak areas of extracts were compared and the Gallic acid, quercetin and rutin content were estimated to be 356.1, 4591.0 and 1277.1.

Keywords: *Eruca sativa*, Gallic acid, Rutin, Quercetin, HPTLC, Standardization.

INTRODUCTION

Herbal medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. They are believed to have better compatibility with the human body. Some of the herbal plants traditionally used in formulations as antidiabetic, antioxidant [1-6]. Gallic acid is phenylpropanoid, chemically it is 3, 4, 5,-Trihydroxybenzoic acid, and possess astringent activity. Flavonoids are a group of polyphenolic compounds, which are widely distributed through out the plant kingdom. To date about 300 varieties of flavonoids are known [7]. Many have low toxicity in mammals and some of them are widely used in medicine for maintenance of capillary integrity[8]. Rutin, 5,7,3', 4',tetrahydroxy flavonol -3- rhamnoglucoside and quercetin 5,7,3', 4',- tetrahydroxy flavonol exhibit anti-inflammatory, antihepatotoxic[9], antiulcer[10], antiallergic and antiviral actions and some of them provides protection against cardiovascular mortality[11,12]. Both possess antioxidant activity and antidiabetic reduce low density

lipoproteins [LDL] oxidation[13]. Quercetin in combination with other flavonoids, inhibits a number of enzymes like bradykinin[14], tyrosine kinase[15], and 5'- nucleotidase activity[16]. Rutin and quercetin have shown regulatory activity of hormones, such as transport, metabolism and action of thyroid hormones. High performance thin layer chromatography [HPLC] method is the suitable method for estimation of chemical constituents present in plant materials. Hence *Eruca sativa* contains rutin and quercetin are important active constituents and is estimated by HPLC method. Phytochemical evaluation is one of the tool for the quality assessment, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques. In the last two decades high performance thin layer chromatography [HPTLC] method has emerged as an important toll for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations.This includes TLC fingerprint profiles and estimation of chemical markers and biomarkers[17]. The major advantage of HPTLC is that several samples can be analysed simultaneously using a small quantity of mobile phase. Gallic acid; rutin and quercetin which are important active constituents of *T.chebula* were estimated by HPTLC method[18].

MATERIALS AND METHODS

Preparation of extract

The eight medicinal plants which were used for the preparation of the polyherbal formulation, the plant was identified and authenticated [No.BSI/SC/5/23/07-08/Tech-1219] from Botanical Survey of India [BSA], Tamil Nadu Agriculture University [TNAU] Coimbatore. Fresh leaves were collected for the study whenever required; a voucher specimen was retained in our laboratory. From dried leaf, aerial, seeds were separated and the main part was made into coarse powder. The coarse powder was then extracted with water and ethanol by soxhlet apparatus. The extract was filtered using Whatman filter paper and then concentrated in vacuum and dried.

Reagents and other materials

Rutin, quercetin and gallic acid [Natural remedies, Bangaluru],toluene, acetone, ethyl acetate, dichloromethane, formic acid, glacial acetic acid and methanol [all Reagents of analytical grade, E-Merck] and silica gel $60F_{254}$ precoated TLC aluminium plates [E-Merck].

Preparation of standard and sample solutions

Gallic acid, rutin and quercetin 10mg were accurately weighed into a 10 mL volumetric flask, dissolved in 10mL methanol and the solution was made up to 10 mL with the same solvent [1 mg/mL]. The [100 mg] of extract was extracted by heating 40^{o}C for 10 minutes accurately weighed into a 10 mL volumetric flask, dissolved in methanol and then solution was filtered through Whatman filter paper No. 42 and the filtrate was made up to the mark with methanol.

Development of HPTLC Technique

The samples were spotted in the form of bands with Camag microlitre syringe on a precoated silica gel plates 60F 254 [10 cm X 10 cm with 0.2 mm thickness, E.Merck] using Camag linomat IV applicator. Automatic sample spotter of band width 7 mm. The plates were developed in a solvent system in CAMAG glass twin through chamber previously saturated with the solvent for 30 min. the distance was 8 cm. subsequent to the scanning, TLC plates were air dried and scanning was performed on a Camag TLC Scanner in absorbance at 280 nm and operated by wincats software 4.03 version [Sethi 1996].

Gallic acid estimation in Herbal extracts

Stationary Phase: Silica gel 60 F_{254} plates,Mobile phase Toluene: Ethyl Acetate: Formic Acid [7:5:1 v/v/v/v/v],Standard: Gallic acid 1 mg/ml [5 µL],Sample: Herbal extracts 10 mg/ml [10 µL],Migration distance: 60 mm,Scanning wavelength: 254 nm,Mode of scanning: Absorption [Deuterium]

Rutin and Quercetin estimation in Herbal extracts

Stationary Phase: Silica gel 60 F_{254} plates, Mobile phase ethyl acetate: glacial acetic acid: formicacid:water [100:11:11:25,v/v/v/v/v],Standard:Rutin1mg/ml[5µL],Standard : Quarcetin 1 mg/ml [5 µL],Sample : Herbal extracts 10 mg/ml [10 µL],Migration distance : 60 mm, Detection wavelength : 366nm, Mode of scanning :Absorption [Deuterium]

RESULTS AND DISCUSSION

The *Rf* value of standard gallic acid was found to be 0.39 and peak area 9303.0 [Fig.1]. Aqueous extract *Eruca sativa* showed four peaks, the first peak *Rf* value [0.34] was coinciding with standard *Rf* value and its peak area was 356.1 [Fig.2], The amount of gallic acid was found to be 0.04% w/w. The *Rf* value of standard quercetin was found to be 1.02 and peak area 4591.0 [Fig.3]. The aqueous extract of *Eruca sativa* showed ten peaks, the tenth peak *Rf* value [1.01] was coinciding with standard *Rf* value and its peak area was 15989.2 [Fig.4], The *Rf* value of standard rutin was found to be 0.42 and its peak area was 10585.7 [Fig.5]. *Eruca sativa* showed eight peaks, the third peak *Rf* value [0.39] was coinciding with standard *Rf* value and its peak area was 1277.1 [Fig.6], The amount of quercetin and rutin was found to be 17.94 and 0.24, % w/w respectively.

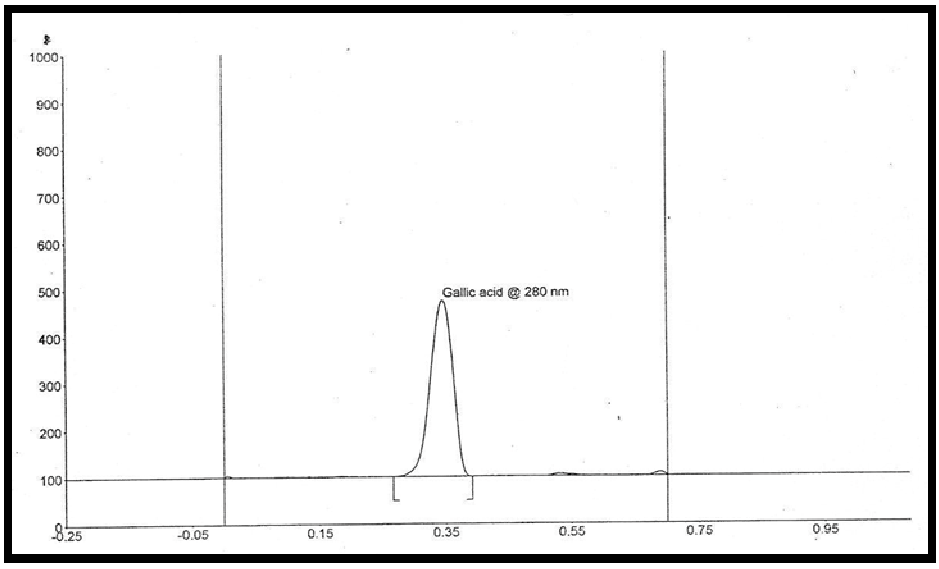

Figure 1. HPTLC Chromatogram of Gallic acid

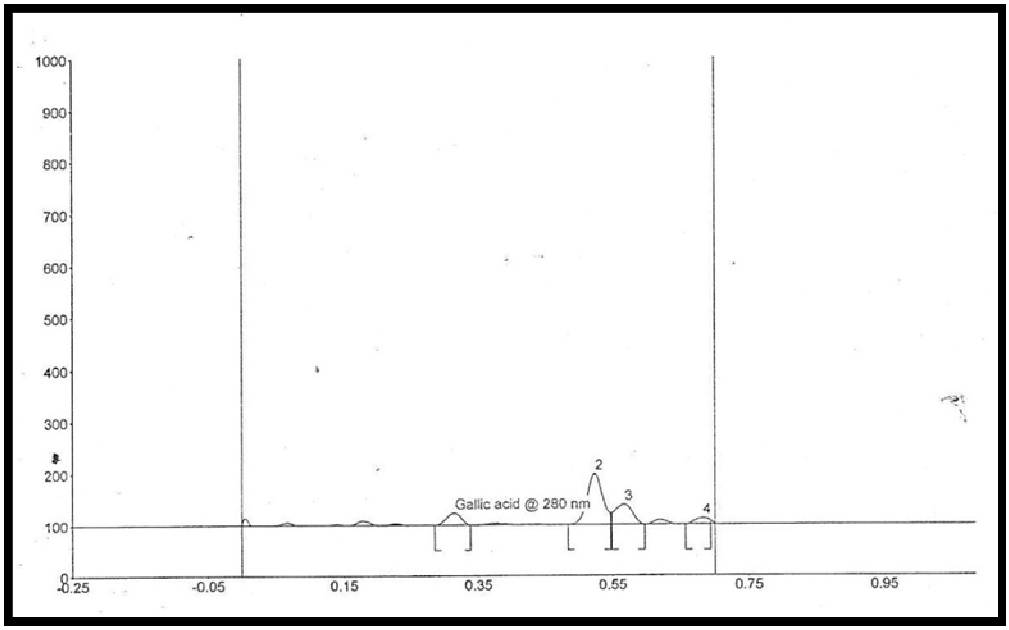

Figure 2. HPTLC Chromatogram of ethanolic extract of *Eruca sativa*

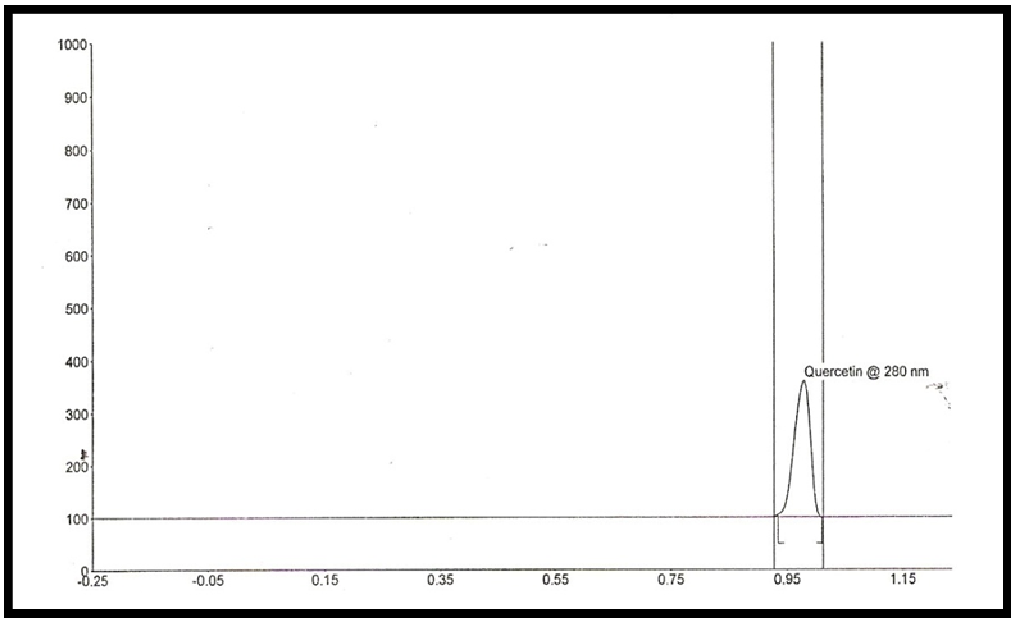

Figure 3. HPTLC Chromatogram of Quercetin

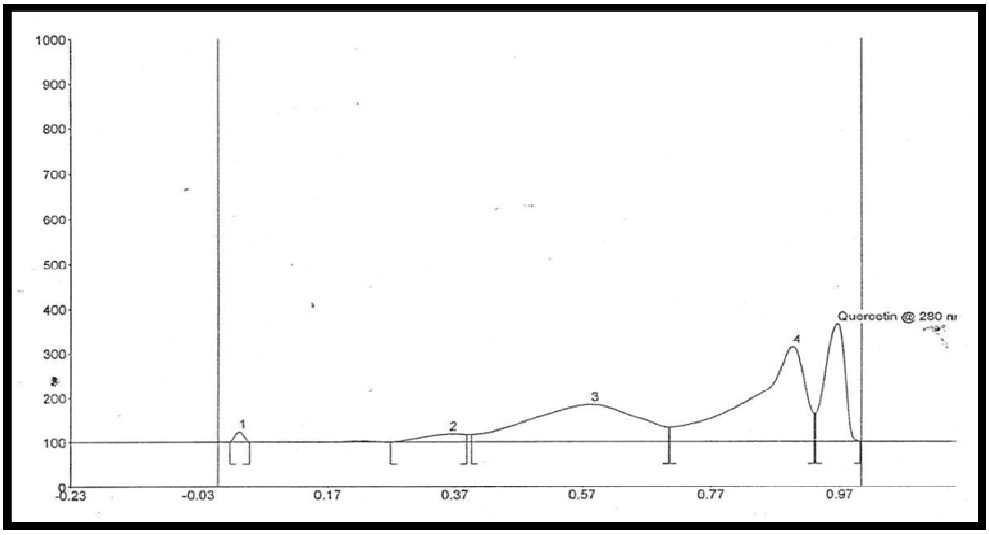

Figure 4. HPTLC Chromatogram of ethanolic extract of *Eruca sativa*

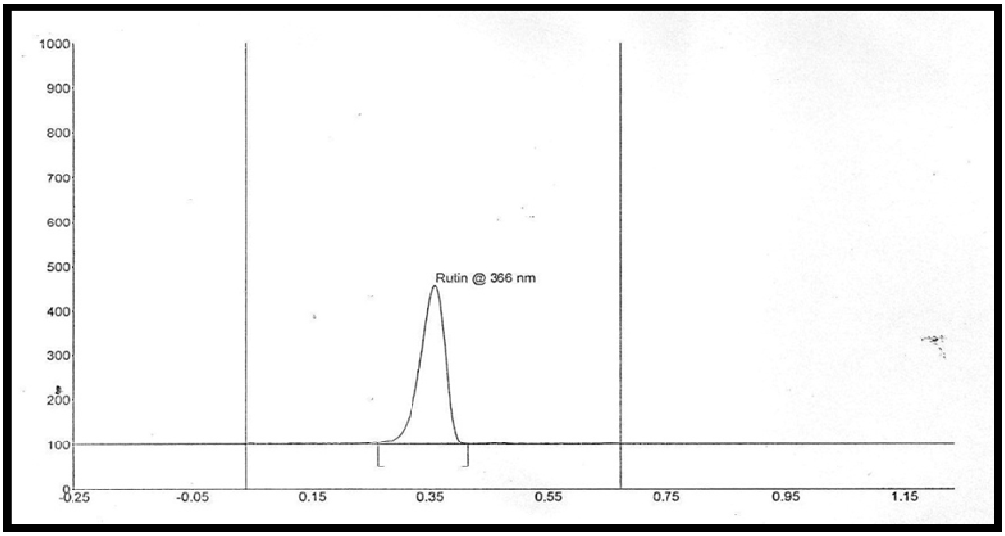

Figure 5. HPTLC Chromatogram of Rutin

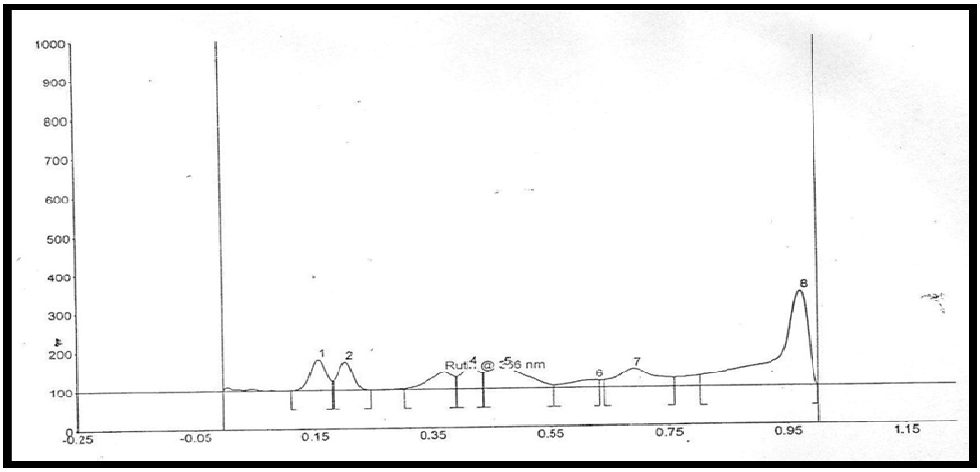

Figure 6. HPTLC Chromatogram of ethanolic extract of *Eruca sativa*

CONCLUSION

Using this analytical method, gallic acid, quercetin and rutin could be determined simultaneously, and the validity of the method was also verified. There was no detectable phenolic compounds in *Eruca sativa* in aqueous extracts from this study. In conclusion, the phytochemicals, the antioxidant and antidiabetic activities of the leaves of *Eruca sativa* aqueous extract were evaluated; the results of the present study support the view that the leaves of *Eruca sativa* could be a potential source of natural antioxidant and antidiabetic drugs.

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