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Research Paper

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Simultaneous estimation of quercetin and rutin in ethanolic extract of *Catharanthus roseus*. Linn leaves by HPTLC method

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ABSTRACT

The aim of the study is to estimation the two biologically active flavonoidal compounds, quercetin and rutin, in ethanolic leaves extract of Catharanthus roseus. Linn leaves by using high-performance thin-layer chromatography (HPTLC). Pre coated silica gel 60F254 used as stationary phase and Toluene: Ethyl Acetate: methanol in ratio of 5: 3: 2 are used as mobile phase. Densitometric determination and quantification of these compounds was carried out at 254 nm.The standard Rf values of rutin and quercetin are 0.17 and 0.65 respectively. The total peak areas of the standards, quercetin and rutin were compared and the corresponding peak areas of extracts were estimated to be 1914.15 and 552.24 respectively. This HPTLC method was found to be simple and convenient for rapid screening of active compounds and quantification of the investigated flavonoids in Catharanthus roseus. Linn.

KEYWORDS: Catharanthus roseus. Linn , HPTLC, Flavonoidal compounds,Quercetin and Rutin.

INTRODUCTION

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.[1] Herbal medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects.. Flavonoids belong to a group of polyphenolic compounds, which are classified as flavonols, flavonones, flavones, flavanols, flavan-3-ols and isoflavones according to the positions of the substitutes present on the parent molecule.Rutin, 5,7,3′, 4′,tetrahydroxy flavonol -3- rhamnoglucoside and quercetin 5,7,3′, 4′,- tetrahydroxy flavonol exhibit anti-inflammatory, antihepatotoxic[2], antiulcer[3], antiallergic and antiviral actions and some of themprovides protection against cardiovascular mortality.[4,5] Both possess antioxidant activityand antidiabetic reduce low density lipoproteins [LDL] oxidation.[6] Quercetin in combination with other flavonoids, inhibits a number of enzymes like bradykinin[7], tyrosine kinase[8], and 5′- nucleotidase activity.[9] High performance thin layer chromatography [HPLC] method is the suitable method for estimation of chemical constituents present in plant materials.

Catharanthus roseus(L.) G.Don (Madagascar periwinkle) is a terpenoid indole alkaloids (TIAs)producing plant.*Catharanthus Rosea,* which is commonly known as 'Vinca rosea'belongs to family Apocynaceae and is an important source of indole alkaloids, which are present in all plant parts. The important antineoplastic alkaloids namely Vincristine and Vinblastine are mainly present in the leaves whereas antihypertensive alkaloids such as ajmalicine, serpentine, and reserpine are reported to be present in the roots.[10] Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia.[11,12] Medicinal plants also contain a wide variety of natural antioxidants, such as phenolics, flavonoids and tannins which are more potent antioxidant than common dietary plants.[13] C. roseus also contains a significant number of volatile and phenolic compounds including caffeoylquinic acids, flavonol glycosides[14,15,16] which are known to exhibit antioxidant activity . Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful by products generated during normal cell aerobic respiration.[17] The importance of antioxidants in health and disease is now recognized by every branch of medicine and biological science[18]. The flower petals, seeds and other parts of the C. roseus exhibit properties.[19,20]

In *Catharanthus roseus*. Linn 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, chemoprofiling 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.[21] The major advantage of HPTLC is that several samples can be analysed simultaneously using a small quantity of mobile phase. Rutin and quercetin which are important active constituents of *Catharanthus roseus* were estimated by HPTLC method.

MATERIALS AND METHODS

Reagents and Materials:

All chemicals and solvents used were of analytical grade and obtained from Desaga Sarstedt Gruppe (Germany). The standerd Rutin and quercetin were purchased from Lobo Chemie, Mumbai, India (purity >97%). Stock solutions (1 mg/ml) of the standards were prepared daily in methanol immediately before use. TLC aluminum plates pre-coated with silica gel 60 F_{254} (100x 100 mm, 0.2 mm thick) used were obtained from E. Merck Ltd (Mumbai, India).

Plant material:

The basic plant material of *Catharanthus roseus* leaves was obtain from mount opera garden,Near Ramoji Film City, Hyderabad. The plant ware identified and authenticated by Department of Botany and Research office (Botanist) Anwar-ul- loom college of Pharmacy, Hyderabad.

Extraction of plant material for HPTLC analysis:

The leaves of *Catharanthus roseus* were dried under shade and powdered in a mechanical grinder. The leaves powders of *Catharanthus roseus,* weight about (250 g) were individually packed in the thimble of soxhlet apparatus and extracted with ethanol at 55°C for 18 h**.** The extract was concentrated to get dry residue and stored in the dessicator and it was used for subsequent experiments. Preliminary photochemical screening revealed the presence of Polyphenols, flavanoids and glycosides.

Preparation of standard and sample solutions

Standard stock solutions of rutin and quercetin were prepared by dissolving 10mg of rutin and quercetin in 10ml of methanol. From this 10 µl each of these solutions was applied using Linomat applicator. 100mg of ethanolic extract of *Catharanthus roseus* was dissolved in 10ml of methanol and filtered. The filtrate (10mg/ml) was used for the HPTLC chemoprofiling.

Chromatographic conditions:

Chromatography was performed on pre-activated (at 110°C) silica gel 60 F_{254} HPTLC plates. Sample(10µl) and standard (10µl each) compounds were applied to the layer as 10 mm wide bands, positioned 10 mm from the bottom of the plate, using an automated TLC applicator Desaga Sarstedt Gruppe, Germany with nitrogen flow providing deliv-

ery from the syringe.

Detection and quantification of compounds:
TLC was performed with Toluene: Ethyl Acetate : methanol (5: 3: 2, v/v) as mobile phase. Chromatograms were developed at room temperature (24 ± 1°C) in glass twin-trough chambers (100 mm × 100 mm, with metal lids) previously saturated with mobile phase vapor for 30 min. The development distance was 86 mm. Ascending mode was used for development of thin layer chromatography.

Following the development, the TLC plate was dried in a current of air with the help of an air dryer at 110°C for 10min, and immediately scanned at λ = 254 nm and the densitogram were obtained with .Desaga Sarstedt Gruppe having Proquant 1.6 version in absorption reflection scan mode

The presence or absence of the investigated compounds was determined according to their Rf values with the corresponding spot of Standards. Calculations for percentage were done considering standard and sample Rf, AUC and dilution factor**.** For validation of the method, calibration curve was obtained by plotting peak area Vs concentration of rutin and quercetin. Spectra of samples and standard rutin and quercetin were matched. [22]

RESULTS AND DISCUSSION

The use of HPTLC has expanded considerably due to the development of forced flow (FF) and gradient TLC methods, improved stationary and mobile phase selection, as well as new methods of quantitation methods.[23] Recent reviews show that the HPTLC techniques can be used to solve many qualitative and quantitative analytical problems in a wide range of fields, including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology and environmental analysis.[24] The selected mobile phase, Toluene: Ethyl Acetate : methanol (5: 3: 2, v/v) showed good resolution. Well defined spot were obtained after chamber was saturated for 20 min at room temperature. The identity of rutin and quercetin were confirmed by comparing chromatogram of standard rutin and quercetin with that of extract and by comparing retention factor of reference with standard.

The ethanolic extract of *Catharanthus roseus* was able to resolve 8 compounds in the developing solvent system. The identity of the bands of quercetin and rutin in the ethanolic extract of *Catharanthus roseus* was confirmed by comparing the UV-Vis absorption spectra with those of standards.(Fig. 1-4).

The *Rf* value and peak area of standards rutin were found to be 0.17 and 3187.87 (Fig.2 and Table.1). The ethonolic extract of *Catharanthus roseus* showed eight peaks, the second peak Rf value [0.18] was coinciding with standard rutin Rf value and its peak area was 1914.15 (Table.3). The *Rf* value of second peak of standard quercetin was found to be 0.65 and peak area 1261.24 (Fig.3 and Table 2). The ethonolic extract of *Catharanthus roseus* showed eight peaks, the sixth peak Rf value [0.61] was coinciding with standard quercetin Rf value and its peak area was 552.24 (Fig.4). The amount of Rutin was found to be 6µg in the extracts where as the Quercetin was found to be 4.32µg in the sample extracts



Figure 1: TLC chromatogram of *Catharanthus roseus* (C1) at UV 254nm in *Visible range*

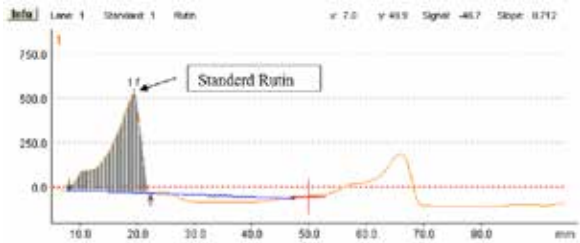


Figure 2: HPTLC chromatogram of rutin, densitogram showing the separation of peaks in rutin

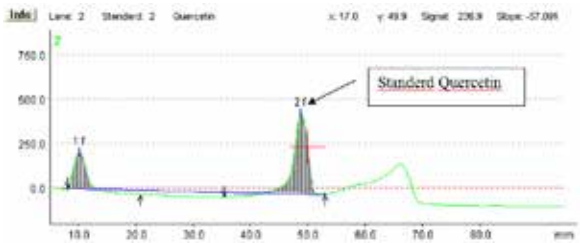


Figure 3: HPTLC chromatogram of Quercetin, densitogram showing the separation of peaks in Quercetin

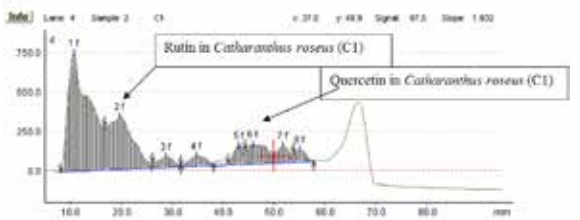


Figure 4: HPTLC chromatogram of *Catharanthus roseus* (C1) leaves extract.

Table 1: Peak list & densitogram of Standerd Rutin at UV 254nm with Rf values of the spots.

Peak no	Y-Pos	Area	Area (%)	Height	Rf values
1	19.4	3187.87	100.0	556.53	0.17

Table 2: Peak list & densitogram of Standerd quercetin at UV 254nm with Rf values of the spots.

Peak no	Y-Pos	Area	Area (%)	Height	Rf values
1	10.2	486.90	27.9	207.40	0.02
2	48.8	1261.24	72.1	455.61	0.65

Table 3: Peak list & densitogram of *Catharanthus roseus* (C1) at UV 254nm with Rf values of the spots.

Peak no	Y-Pos	Area	Area (%)	Height	Rf values
1	10.8	3690.27	49.7	751.58	0.03
2	19.8	1914.15	25.8	337.23	0.18
3	28.8	251.11	3.4	69.19	0.32
4	34.8	267.78	3.6	65.09	0.42
5	43.2	276.34	3.7	120.14	0.56
6	46.0	552.24	7.4	123.03	0.61
7	51.8	289.67	3.9	105.60	0.70
8	55.0	186.43	2.5	77.16	0.75

CONCLUSION

In conclusion densitometric HPTLC method can be used for the simultaneous quantitative determination of quercetin and rutin in *Catharanthus roseus* leaves; mainly because of its simplicity, accuracy, and selectivity. HPTLC method is also the most suitable method for estimation of chemical constituents present in plant materials. The results of the present study also support that the presence of rutin and quercetin in ethonolic leaves extract of *Catharanthus roseus* could be a potential source of natural anti-oxidantand.

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