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SIMULTANEOUS DETERMINATION OF PHENOLIC COMPOUNDS IN *CATHARANTHUS ROSEUS* LEAVES BY HPLC METHOD

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ABSTRACT: In *Catharanthus roseus*, Rutin, Quercetin and Kaempferol are the most important phenolic compounds with antioxidant activity. For simultaneous determination of Rutin, Quercetin and Kaempferol in *Catharanthus roseus* a simple and rapid HPLC method was developed. Phosphate buffer (pH=5.8) and solvent Acetonitrile are used in ratio of 55: 45 as mobile phase. Rutin, Quercetin and Kaempferol were determined by HPLC by using Athena C18 column and UV absorbance were measured at 254 nm. Retention time (Rt) of standards, Rutin, Quercetin and Kaempferol was found to be 2.357, 6.093 and 9.373 respectively, while in *Catharanthus roseus* the Retention times of Rutin, Quercetin and Kaempferol are 2.403, 6.143 and 8.903 are found to be matching with standards Retention time values. Thus for quantitative estimation of phenolic compounds, this HPLC method was found to be simple and convenient.

INTRODUCTION: Antioxidants always play important roles in delaying the development of chronic diseases such as cardiovascular diseases (CVD), cancer, atherosclerosis, inflammatory bowel syndrome and Alzheimer's diseases [1].

Oxidative stress caused by an unbalance between pro-oxidants and antioxidants. Reactive oxygen species (ROS) are divided in two main classes consisted of non-radical species (H_2O_2) or free radical forms (O_2^-, OH^-, and OH_2.).

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Accumulation of high concentrations of ROS is potentially detrimental to plants cells causing damage to valuable biomolecules like DNA, proteins, lipids, chlorophyll, membrane etc. [2].

Antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias [3, 4].

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 [5]. The importance of antioxidants in health and disease is now recognized by every branch of medicine and biological science [6].

Catharanthus roseus or *Vinca rosea* belongs to the family Apocynaceae; *Catharanthus roseus* has a variety of medicinal properties, such as antibacterial [7], antifungal [8] and antiviral [9]. A variety of different alkaloids is present in C. roseus: more than 130 different compounds have been reported including about 100 monoterpenoid indole Alkaloids [10]. The flower petals, seeds and other parts of the C. roseus exhibit antioxidant properties [11, 12].

Phenolic compounds play important role in plant driven antioxidant activity which is due to their redox properties, permit them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. Phenolics belong to a large heterogeneous group of secondary plant metabolites that are widespread in the plant kingdom and have multiple applications in food, cosmetic and pharmaceutical industries [13, 14].

The present study was designed to for simultaneous determination of Rutin, Quercetin and Kaempferol in ethanolic leaves extract of *Catharanthus roseus* by high-performance liquid chromatography.

MATERIALS AND METHOD:

Reagents and Materials: All chemicals and solvents used were of analytical grade. The standard Rutin and Quercetin were purchased from Yucca Enterprises,MumbI (purity >97%).The standard Kaempferol MP Biomedicals, Mumb(purity >97%). Phosphate buffer (pH=5.8) and solvent Acetonitrile used as mobile phase were obtained from S.D. Fine Chem Limited, Mumbai. The column type was, Athena C18 250X 4.6 (CNW Technology).

Plant material: The basic plant material of *Catharanthus roseus* leaves was obtained from Mount Opera Garden, near Ramoji Film City, Hyderabad. The plant was 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 desiccator and it was used for subsequent experiments. Preliminary photochemical screening revealed the presence of Polyphenols, flavonoids and glycosides.

Preparation of standard and sample solutions: Phosphate buffer (pH=5.8) and solvent Acetonitrile used as mobile phase.10 mg of Standard Rutin and Quercetin were dissolved in 25ml of mobile phase, while 15mg of kaempferol were dissolved in 25ml of mobile phase and 10 mg of Sample solution of extract of *Catharanthus roseus* were dissolved in 25ml of mobile phase as above as standard preparation.

Chromatographic conditions:

Flow rate	: 1 ml/min
Detection	: 254 nm
Injection quantity	: 0.02ml
Column used	: Athena C18 250X 4.6
Column temperature	: 35°C
Mobile phase ration	: 55: 45 % v/v
Mobile phase and Acetonitrile	: Phosphate buffer (pH=5.8)

The operating temperature was maintained at room temperature. Identification of the compounds was achieved by comparison with retention times of standards with the samples.

Assay formula:

$$\frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Standard weight}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight}} \times \text{Standard Purity}$$

RESULTS AND DISCUSSION:

Quantification of Rutin, Quercetin and Kaempferol in *Catharanthus roseus* (C1): The retention time (Rt) of standards Rutin, Quercetin and Kaempferol were found to be 2.357, 6.093 and 9.373 with 100% area (**Fig. 1-3 and Table 1**). While the retention time (Rt) of Rutin, Quercetin and Kaempferol in *Catharanthus roseus* (C1) extract, was found to be 2.420, 6.160 and 9.463 respectively (**Fig. 4 and Table 2**), which are matching with standards Rt values respectively. The amount of rutin, quercetin and kaempferol in *Catharanthus roseus* (C1) was found to be 21.59%, 0.12 % and 0.028 % w/v respectively. The mobile phase include acetonitrile and Phosphate buffer (pH=5.8) were tested and the results showed the good resolution and good peaks shape.

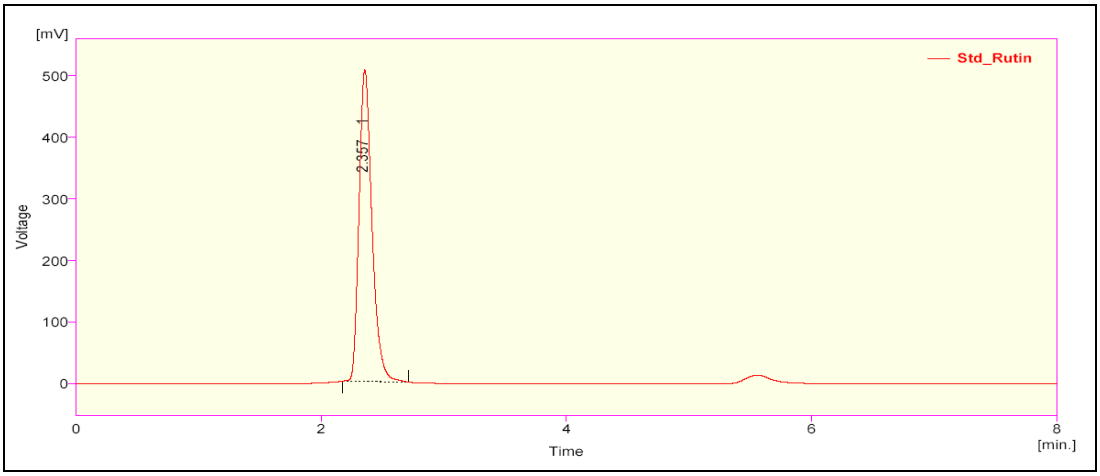

FIG: 1 HPLC CHROMATOGRAM OF STANDARD RUTIN

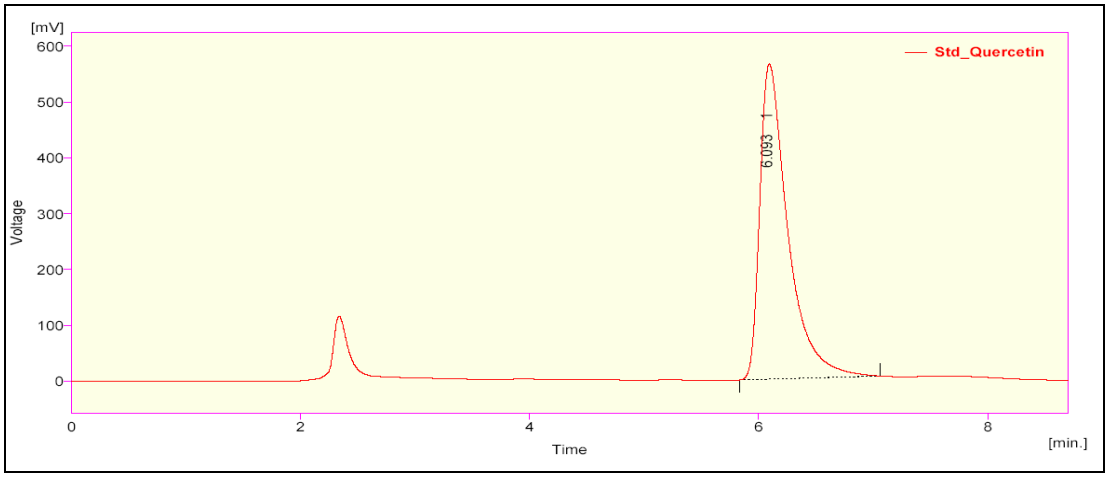

FIG. 2: HPLC CHROMATOGRAM OF STANDARD QUERCETIN

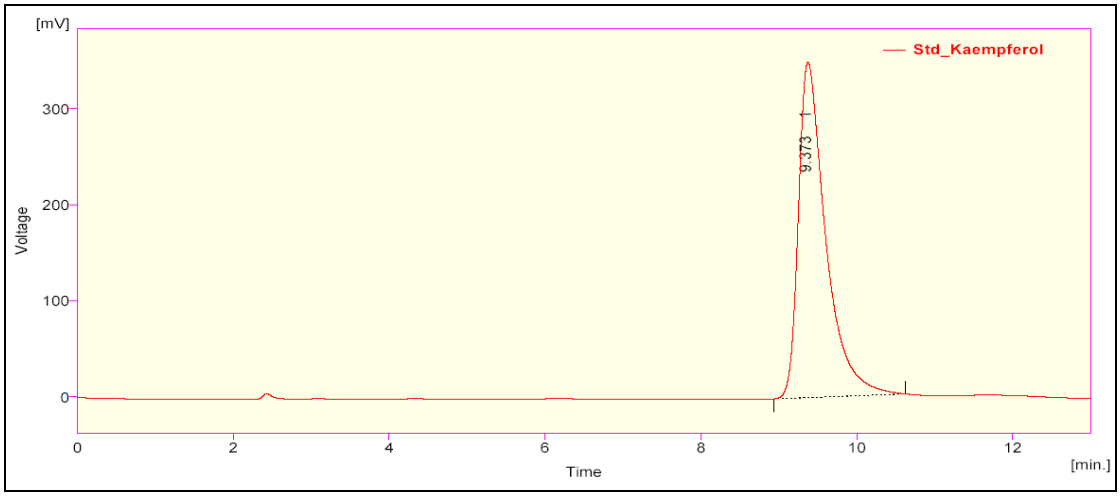

FIG. 3: HPLC CHROMATOGRAM OF STANDARD KAEMPFEROL

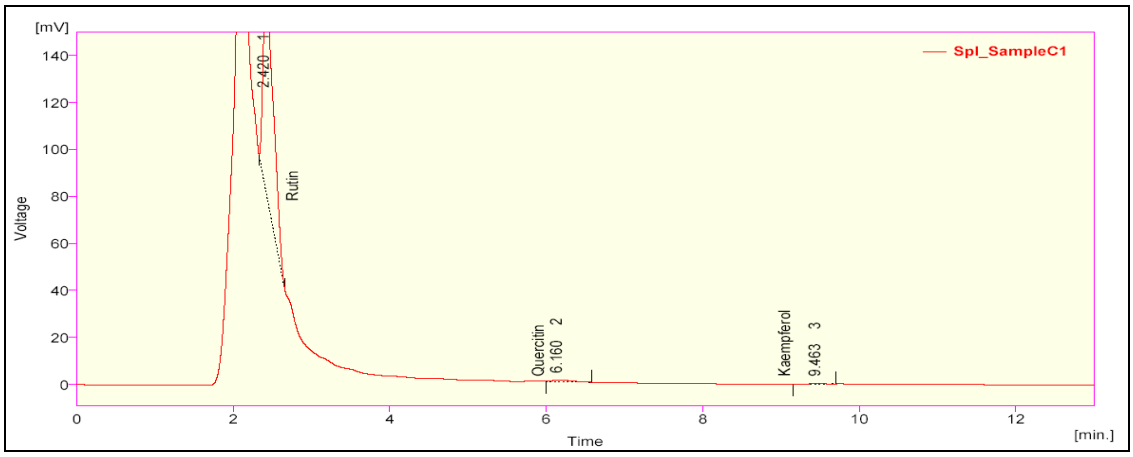

FIG. 4: HPLC CHROMATOGRAM OF EXTRACT *CATHARANTHUS ROSEUS* (C1)

TABLE 1: RETENTION TIME, HEIGHT AND % AREA OF STANDARDS RUTIN, QUERCETIN AND KAEMPFEROL

Standards	Retention time (min)	Area (mV.s)	Height (mV)	Area (%)
Rutin	2.357	3700.301	505.494	100
Quercetin	6.093	9594.659	564.435	100
Kaempferol	9.373	8468.410	348.791	100

TABLE: 2 RETENTION TIME, HEIGHT AND % AREA OF RUTIN, QUERCETIN AND KAEMPFEROL IN EXTRACT OF *CATHARANTHUS ROSEUS* (C1):

Standards	Retention time (min)	Area (mV.s)	Height (mV)	Area (%)
Rutin	2.420	822.531	89.473	98.41
Quercetin	6.160	11.680	0.736	1.40
Kaempferol	9.463	1.643	0.111	0.20
Total		835.854	90.319	100

CONCLUSION: The HPLC in-house analytical methods were developed, which was found to be excellent technique for simultaneous determination of rutin, Quercetin and Kaempferol in ethanolic leaves extract of *Catharanthus roseus.* The cost and Running time per analysis are found to be low relatively in comparison with other methods. Hence this method can be applied for the Quantitative analysis of rutin, Quercetin and Kaempferol. Furthermore, the method can be used as quality control for phenolic compounds and was found to be simple, rapid and efficient.

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