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RESEARCH ARTICLE

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

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ABSTRACT

The present investigation is to estimate biologically active flavonoidal compounds, quercetin and rutin in ethanolic leaves extract of *Melia azedarach* Linn by using high-performance thin-layer chromatography (HPTLC). Pre coated silica gel 60 F_{254} used as stationary phase and Toluene: Ethyl Acetate: methanol in ratio of 5: 3: 2 are used as mobile phase. Densitometric estimation and quantification of these compounds was carried out at 254nm. 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 1284.64 and 1037.27 respectively. This HPTLC method was found to be simple and convenient for rapid screening of active compounds and quantification of the investigated flavonoids in *Melia azedarach* Linn.

Keywords: *Melia azedarach*. Linn, HPTLC, Flavonoid compounds, Quercetin and Rutin.

1. INTRODUCTION:

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout 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 activity and 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.

Melia azedarach Linn. (Family: Meliaceae) is a shrub grows in temperate and tropical countries like India, China, and Japan. uman monocytes[10].Leaves and fruits showed antifeedant activity[11,12]. The stem extracts showed larval mortality [13] and insecticidal activity. Meliacine, a peptide isolated from leaves inhibited the multiplication of foot and mouth disease virus in BHK-21 cells[14] and showed antiviral activity against herpes simplex virus type [15]. The plant has also showed antifungal[16], antibacterial[17], cytotoxic[18], antimalarial[19], anthelmintic[20], antilithic[21] and antifertility activity[22].Phytochemical studies have reported the presence of melianin, nimbinene, azaridine, meliacin, quercetin, rutin, kaempferol, rutin, margosine, lupeol, β-sitositosterol[23, 24]

In *Melia azedarach*. 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

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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 [25]. 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 *Melia azedarach* were estimated by HPTLC method.

2. MATERIALS AND METHODS

2.1. Reagents and Materials:

All chemicals and solvents used were of analytical grade and obtained from Desaga Sarstedt Gruppe (Germany). The standard 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).

2.2. Plant material:

The basic plant material of *Melia azedarach* leaves was obtain from Zaheerabab,Medak Dist. The plant was identified and authenticated by Department of Botany and Research office (Botanist) Anwar-ul-loom college of Pharmacy, Hyderabad.

2.3. Extraction of plant material for HPTLC analysis:

The leaves of *Melia azedarach* were dried under shade and powdered in a mechanical grinder. The leaves powders of *Melia azedarach,* 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.

2.4. 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 sample applicator. 100mg of ethanolic extract of *Melia azedarach* was dissolved in 10ml of methanol and filtered. The filtrate (10mg/ml) was used for the HPTLC chemoprofiling.

2.5. 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 10mm wide bands, positioned 10 mm from the bottom of the plate, using an automated TLC applicator Desaga Sarstedt Gruppe, Germany with nitrogen flow providing delivery from the syringe.

2.6. 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 (20 mm × 20 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[26].

3. 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 [27]. 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 [28]. 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 *Melia azedarach* was able to resolve 7 compounds in the developing solvent system. The identity of the bands of quercetin and rutin in the ethanolic extract of *Melia azedarach* 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 *Melia azedarach* showed seven peaks, the third peak Rf value [0.15] was coinciding with standard rutin Rf value and its peak area was 1284.64 (Table.3). The *Rf*

value of second peak of standard quercetin was found to be 0.65 and peak area 1261.24 (Fig.3). The ethonolic extract of *Melia azedarach* showed seven peaks, the sixth peak Rf value [0.64] was coinciding with standard quercetin Rf value and its peak area was 1037.27 (Fig.4).The amount of Rutin was found to be 4µg in the extracts where as the Quercetin was found to be 8.22µg in the sample extracts.

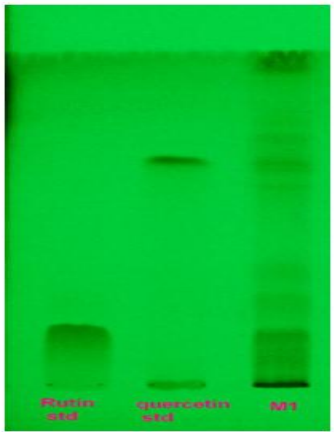

Figure 1: TLC chromatogram of *Melia azedarach* (M1) at UV 254nm in *Visible range*

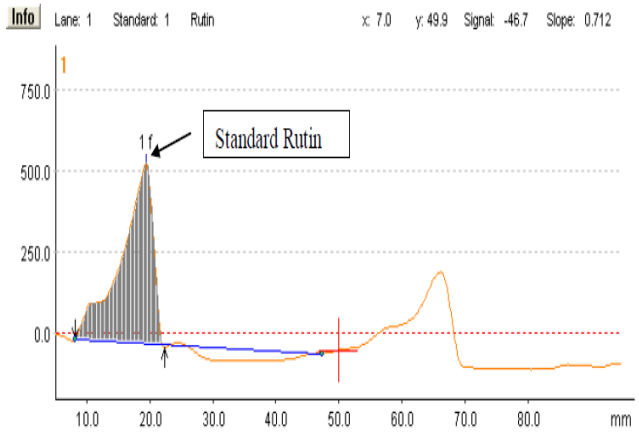

Figure 2: HPTLC chromatogram of rutin, densitogram showing the separation of peaks in rutin

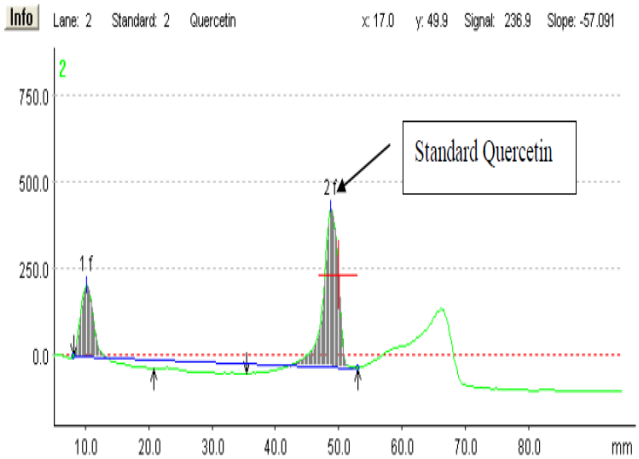

Figure 3: HPTLC chromatogram of Quercetin, densitogram showing the separation of peaks in Quercetin

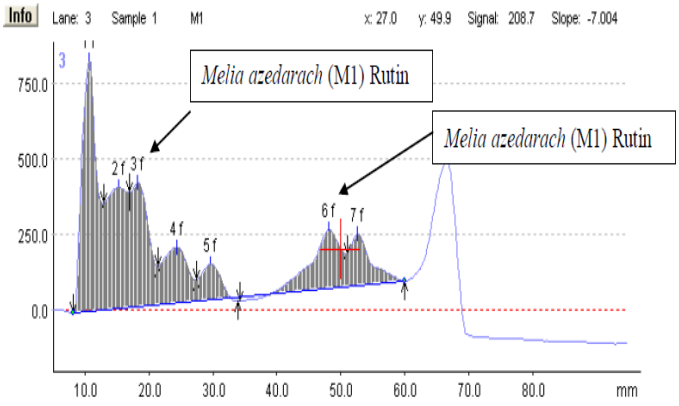

Figure 4: HPTLC chromatogram of *Melia azedarach* (M1) leaves extract.

Peak no	Y-Pos	Area	Area (%)	Height	Rf values
1	19.4	3187.87	100.0	56.53	0.17

Table 1: Peak list & densitogram of Standard Rutin 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 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	10.6	2247.19	28.3	828.14	0.03
2	15.2	1470.35	18.5	402.14	0.10
3	18.2	1284.64	16.2	409.59	0.15
4	24.4	840.73	10.6	184.34	0.25
5	29.6	411.47	5.2	117.12	0.34
6	48.2	1037.27	13.1	194.09	0.64

Table 3: Peak list & densitogram of *Melia azedarach* (M1) at UV 254nm with Rf values of the spots.

4. CONCLUSION

In conclusion densitometric estimation of HPTLC method can be used for the simultaneous quantitative of quercetin and rutin in *Melia azedarach* leaves; HPTLC method is 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 *Melia azedarach* could be a potential source of natural anti-oxidantand.

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Conflict of Interest: None Declared

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