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ESTIMATION OF RUTIN IN HYDROALCOHOLIC AND ITS FRACTIONS OF *TEPHROSIA PURPUREA* LEAVES

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ABSTRACT

The present investigation was designed to evaluate the chromatographic separation of Rutin in hydroalcoholic extract and its different fractions such as chloroform (CHTP) , ethyl acetate (EATP), butanol soluble (BSTP), butanol insoluble fraction (BISTP). A simple, rapid HPTLC method had been developed for estimation of flavonoid rutin on aluminium baked plates coated with silica gel 60F254 as the stationary phase and mobile phase consist of ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:27) for rutin . Detection and quantification were performed densitometrically at λmax 254 nm for rutin . The standard R_f value of rutin was found to be 0.52. The total peak areas of the standard rutin and the corresponding peak areas of extracts were compared and the highest rutin content was estimated to be 26.86 mg in butanol soluble (BSTP) fraction.

Keywords: HPTLC, Rutin, *Tephrosia Purpurea.*

1. INTRODUCTION

Ayurveda is originated in india long back in prevedic period. Ayurveda means 'science of life' as people are more concern about their future complications people now refer ayurvedic treatment, medicines. Many herbal drugs are shifts from fringe to main stream to use herbal remedies for the treatment of the disease [1]. Plants contains various phytoconstituents which are responsible for exhibiting their pharmacological activities. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time [2].*Tephrosia purpurea* Linn. (Leguminoseae), commonly known in Sanskrit as Sharapunkha is a highly branched, sub-erect, herbaceous perennial herb. In Ayurvedic literature this plant has also given the name of "Sarwa wranvishapaka" which means that it has the property of healing all types of wounds [3] It is an important component of some preparations such as "Tephroli" and "Yakrifit" used for liver disorders [3,4]. The roots and seeds are reported to have insecticidal and piscicidal properties and also used as vermifuge. The roots are also reported to be effective in leprosy wound and their juice, to the eruption on skin [5]. The aqueous extract of seeds has shown significant in vivo hypoglycaemic activity in diabetic rabbits [6]. The ethanolic extracts of *Tephrosia purpurea* possessed potential antibacterial activity. The total flavanoids were extracted from plant found to have antimicrobial activity [7]. The whole plant may be used for its rich flavonoid. The phytochemical investigations on

Tephrosia purpurea have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols [8,9] The plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries . In this present study, the HPTLC quantification of biomarker rutin in various fractions of *Tephrosia purpurea* was studied which may be used as marker for quality evaluation and standardization of the drug.

2. MATERIALS AND METHODS

2.1 Plant material

Plant *Tephrosia purpurea* was collected locally in the month of October from Nagpur. The plant was identified and authenticated by Dr. Dongarwar, Department of Botany, Rahtrasant Tukadoji Maharaj Nagpur University Campus, Nagpur. The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

2.2 Preparation and extraction of plant material

The coarse powder of *Tephrosia purpurea* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using petroleum ether for defatting . The extract was then transferred into the previously weighed empty beaker and evaporated on water bath to get a semisolid mass of petroleum ether extract. The defatted plant material is air dried to remove traces of solvent and then it is subjected to maceration with hydroalcoholic solvent (50:50) The extract was kept on water bath for evaporation to yield semisolid mass of hydroalcoholic extract.. This extract was further fractionated with different solvents like chloroform, ethyl acetate, n-butanol to get chloroform soluble, ethyl acetate soluble, n-butanol soluble and n- butanol insoluble fraction which was then placed in air tight labeled container for further use.

3. HPTLC PROFILE (HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY)

3.1 Chemicals

Standard rutin was purchased from Sigma Mumbai. All solvents were used of AR grade.

3.2 Sample preparation

3.2.1 *Preparation of standard solution.*

Accurately weighed 10 mg Rutin was placed into a 10 mL volumetric flask, dissolved in 5 mL methanol and the solution was made up to 10 mL with the same solvent to get final concentration (1 mg/mL).

3.2.2 *Preparation of test solution*

Accurately weighed about 100 mg of extracts and dissolve in 20 ml solvent to get final concentration 5 mg/ml.

3.3 Developing Solvent System

A number of solvent systems were tried for standard and extracts, but the satisfactory resolution was obtained in the solvent system ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:27).

3.4 Instrumentation

A Camag HPTLC instrument was used for the complete analysis. The TLC plates were 10x 10cm, Precoated with silica gel F254 TLC plates (E.Merck) (0.2mm thickness); spotting device was Camag Linomat 5 sample applicator, syringe was a 100μl (Hamilton); developing chamber was a CAMAG glass twin trough chamber (20x 10); densitometer was CAMAG TLC Scanner 3 having winCATS software for execution of instrument

3.4.1 *Hptlc procedure:*

A Camag Linomat HPTLC system equipped with an automatic TLC sampler, TLC scanner, and integrated software was used for the analysis. The separation was carried out by applying the sample solutions as 10μl of sample solution along with the reference sample on a pre-coated TLC plate silica gel 60 F254 plate of 0.2mm layer thickness. Chromatographic development was carried as mobile phase ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:27). The samples were applied on the plate as 6mm wide bands with a semiautomatic TLC sampler under flow of inert gas, application speed 150nl/sec. The length of development distance was

fixed at 80.0mm from the base of the TLC plate. After that TLC plates were dried in a current of air, followed by heating on Camag HPTLC plate heater III at 60°C for about 5mintues. The scanning was carried out at 254nm. The identity of the separated band of rutin in the sample track was confirmed by overlying their 3D spectra with those of the respective bands of reference sample using a Camag TLC Scanner 3 with win CATS software. The spectral scanning was performed over the range of 200 to 700 nm. The sources of radiations utilized were tungsten and deuterium.

3.4.2 Estimation of rutin in sample

The peak height and area of sample were calculated and content of Rutin was quantified using linear regression equation from the standard curve.

4. RESULT AND DISCUSSION

Recent years have seen tremendous increase in research antioxidant properties of medicinal plants. Flavonoid like rutin and quercetin attributes to antioxidant potential of plants and thus has potential therapeutics uses in prevention of many diseases related to brain, heart, liver and kidney. [10] High performance thin layer chromatography (HPTLC) is very simple, sensitive, accurate and precise technique for qualitative and quantitative analysis of active constituent in plant extract. It can be used as tool for standardization of medicinal plants.

In the present study, HPTLC analysis of the hydroalcohol, chloroform, ethyl acetate, n-butanol soluble, n-butanol insoluble fraction of *Tephrosia purpurea* were carried out along with the standard flavonoid rutin in ethyl acetate: formic acid: glacial acetic acid (100:11:11:27) as mobile phase The Rf value for standard rutin was reported to be 0.52 . A good linear relationship was observed between the concentration ranges of 5.0-25.0 μg with respective to peak area and height (r^2 = 0.9575 and 0.9627) respectively. The regression equation was found to be Y= 62.93+13.34X with respective to height and Y=1103+341.5X with respect to area, where Y is the peak height / area and X is concentration of rutin. The highest content of rutin was found to be 26.86 mg in butanol soluble fraction (BSTP) using the present HPTLC method.
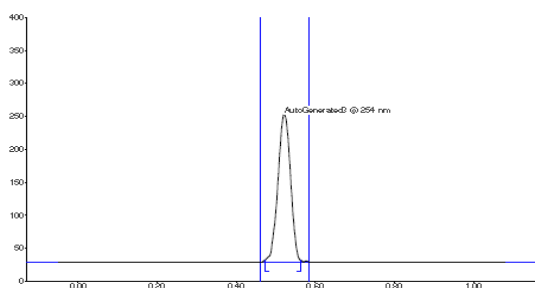

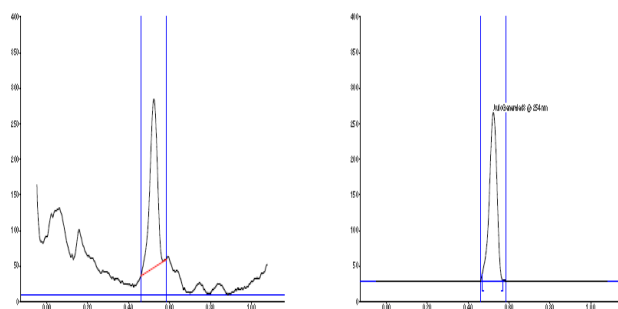**Figure 1: Chromatogram of standard Rutin**

Figure 2: Chromatogram of hydroalcoholic extract (HATP)

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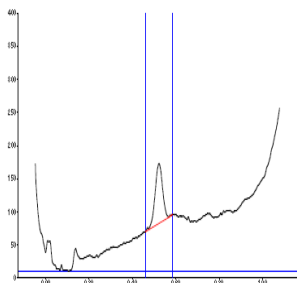*(Scholarly Peer Review Publishing System)*

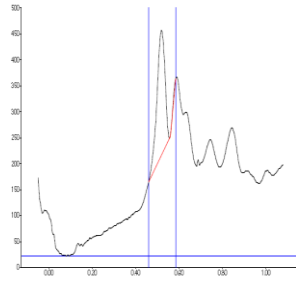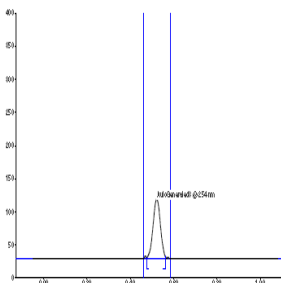**Figure 3: Chromatogram of chloroform extract (CHTP)**

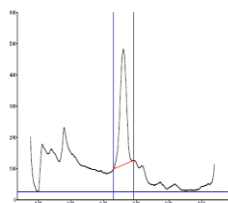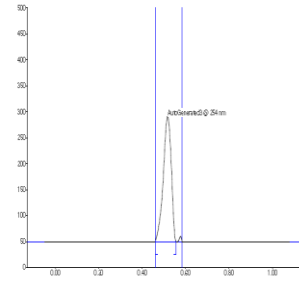**Figure 4: Chromatogram of ethyl acetate extract (EATP)**
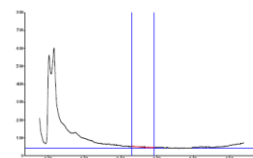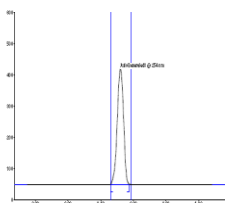

Figure 5: Chromatogram of butanol soluble extract (BSTP)

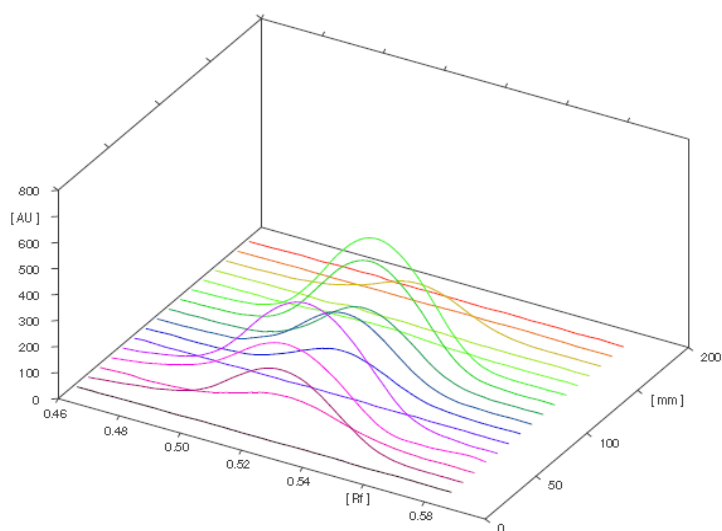**Figure 6: Chromatogram of butanol insoluble extract (BISTP)**
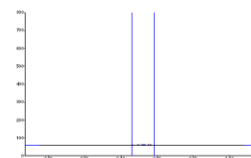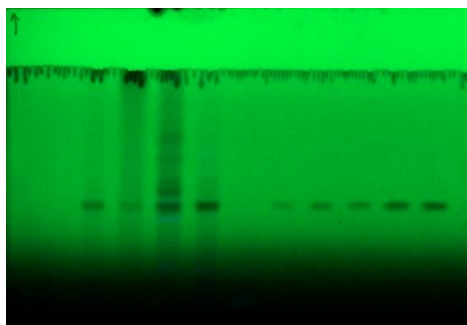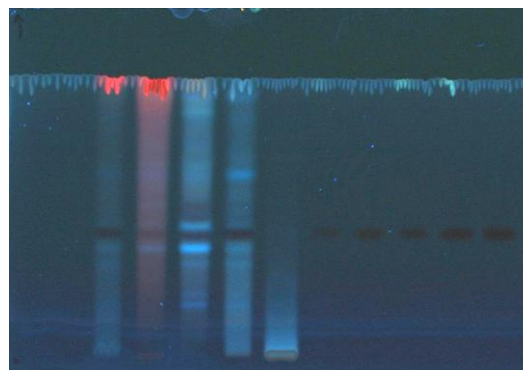
.

Figure 7: 3D chromatogram of standard and extracts.

HA CH EA BS BIS   Rutin

Figure 8: Plate scanned at 254 nm.

HA CH EA BS BIS   Rutin

Figure 9 : Plate scanned at 366 nm.

5. CONCLUSION

In the present study HPTLC quantification of rutin was performed in hydroalcohol (HATP), chloroform (CHTP), ethyl acetate (EATP), butanol soluble (BSTP) and Butanol insoluble (BISTP) fractions. It was reported that butanol soluble fraction (BSTP) contains highest amount of rutin. so study support the view that the leaves of *Tephrosia purpurea* could be a potential source of natural antioxidant, antidiabetic, antiepileptic, anticonvulsant drug.

6. ACKNOWLEDGMENTS

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