

ESTIMATION OF BIO-ACTIVE CONSTITUENTS BY RP-HPLC**Chaitanya Sarathe *¹, Suparna Ghosh¹ Shweta Sharma¹, Prabhat Jain²**1. **Career College, Bhopal (M.P.) Pin- 462023, India**2. **Scan Research Laboratories, Bhopal (M.P.)***Corresponding Author's E mail: Chaitanyasarathe@gmail.com

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ABSTRACT

Qualitative phytochemical analysis was done ariel part of plant of *Eclipta alba*. The test confirms the presence of various phytochemicals like Alkaloids, Glycoside, flavonoids, saponins, and terpenoids in hydrolcoholic extract. Quantitative analysis of bioactive constituent rutin also estimated by RP-HPLC method. The results suggest that the hydroalcoholic extract shows the presence of rutin during screening. The developed method for the estimation of rutin method has the following advantages: rapid extraction, easy sample preparation and short time injections.

Keywords: Qualitative, Quantitative, Bioactive, *Eclipta alba*.**INTRODUCTION**

Eclipta alba (L.) Hassk. (Asteraceae), a small, branched annual herb with white flower heads, is native to the tropical and subtropical regions of the world. It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases ¹. The plant is commonly used in hair oil all over India for healthy black and long hair. The fresh juice of leaves is used for increasing appetite, improving digestion and as a mild bowel regulator. The plant has a reputation as an antiageing agent in Ayurveda. *Eclipta alba* is used as a general tonic for debility. Externally it is used for inflammation, minor cuts and burns and the fresh leaf juice is considered very effective in stopping bleeding. *Eclipta alba* is widely used in India as a cholagogue and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder ².

Rutin is one of the bioactive flavonoid compounds which are present in substantial amount in plants. Some related investigations showed that rutin has a broad range of physiological activities ³. Rutin is also an antioxidant ⁴ and may help prevent the oxidation of vitamin C and have some positive lipid effects⁵. Rutin shows anti-inflammatory activity in some animal and *in vitro* models ⁶. Rutin has a veterinary use in the management of chylothorax in dogs and cats ⁷. Rutin inhibits platelet aggregation ⁸.

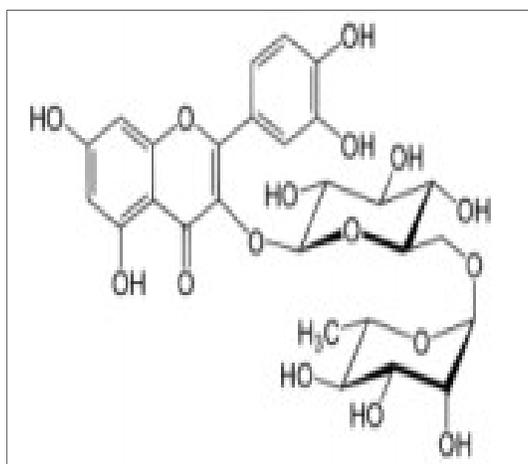


Figure 1: Chemical structure of rutin

Materials and methods

Collection of plant material

The aerial part of plant of *Eclipta alba* was collected from the local surroundings at Bhopal city of M.P, during the month of January to February 2017.

Preparation of extract

The dried powdered plant part of *Eclipta alba* was extracted using Petroleum ether, and Ethanol (70%) for 72 hours. The extracts were dried under reduced pressure using rotator evaporator to get the crude and were stored below 4°C until further used. When needed, the extract was suspended/dissolved in desired solvent and used.

Phytochemical screening

Phytochemical screening of the extracts was carried out according to the standard procedures of Trease and Ewan, 1989)⁹. The Petroleum ether, and hydroalcoholic extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. Alkaloids, Terpenoids, Glycosides, Steroids, Flavonoids, Carbohydrates, Saponins and Tannins^{10,11}.

Identification of marker compound rutin by HPLC

A reverse phase C-18 column equilibrated with mobile phase methanol: acetonitrile (50:50, v/v) was used. Mobile phase was filtered through Whatmann filter paper and degassed. Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 256 nm. The sample was injected using a

20 μ l fixed loop, and the total run time was 10 min. The sample solution was chromatographed and a concentration of rutin in Extract sample was found out using regression equation.

Preparation of the calibration curve of the rutin

Each of the standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Table no. 1 Preparation of Calibration curve of Rutin

S. No.	Conc.	Mean AUC
1.	0	0
2.	5	110.469
3.	10	222.814
4.	15	323.334
5.	20	440.478
6.	25	562.082

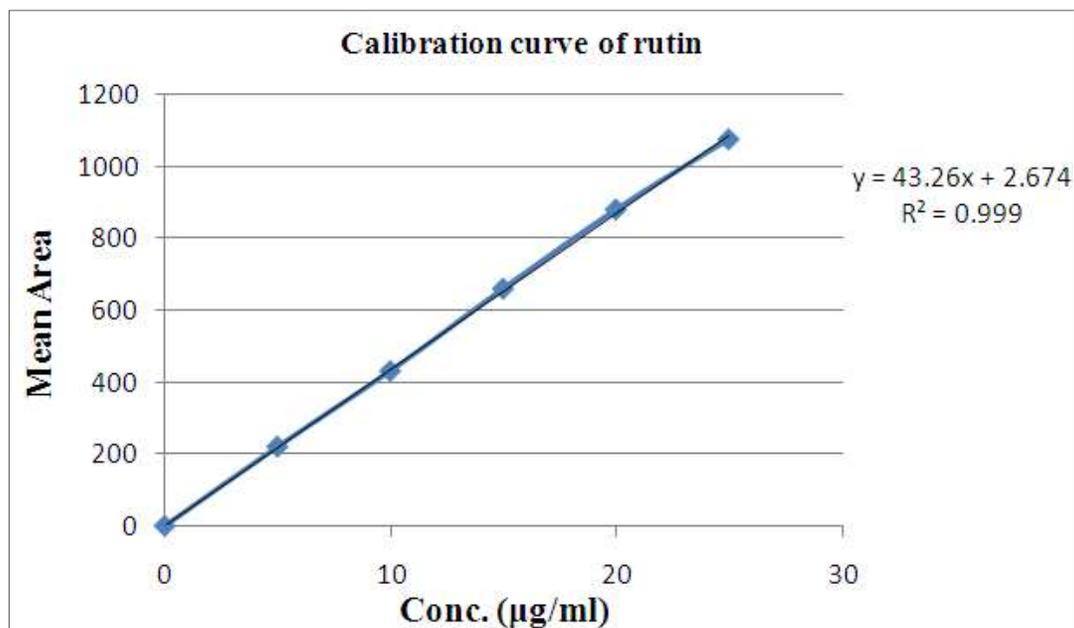


Figure 2: Calibration Curve of the rutin

Table no. 2: Characteristics of the analytical method derived from the standard calibration curve

Compound	Linearity range µg/ml	Correlation co-efficient	Slope	Intercept
rutin	5-25	0.999	43.26	2.674

Chromatogram of standard rutin

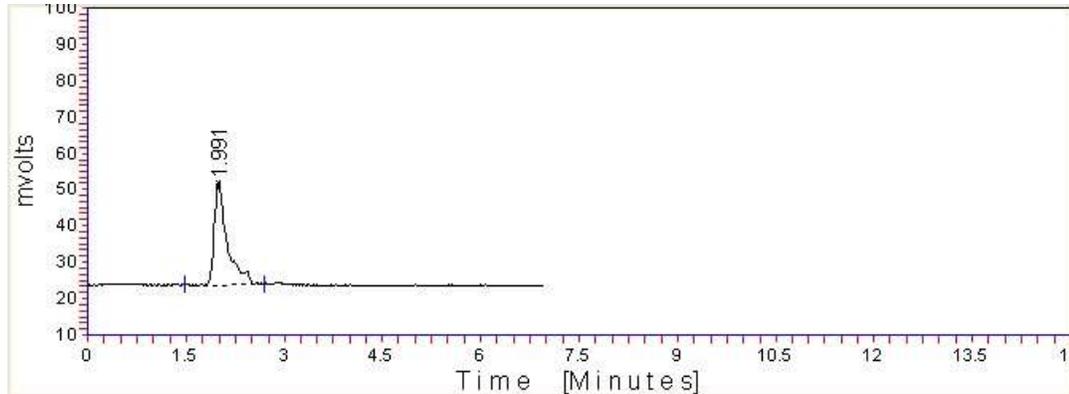


Figure 3: Chromatogram of standard rutin

Chromatogram of extract

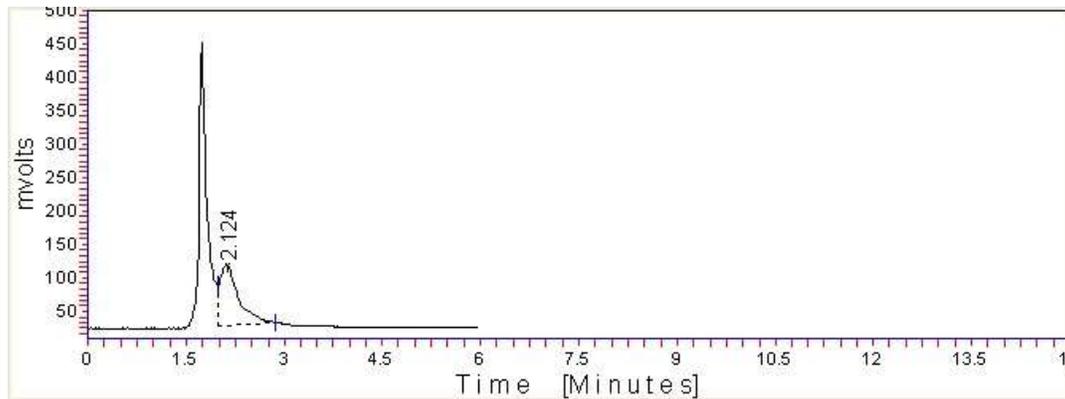


Figure 4: Chromatogram of hydroalcoholic extract

Table no. 3 % assay of different extract

S. No.	Extract	% Assay
1.	Hydroalcoholic extract	0.120%

Discussion and Conclusion

From the results obtained it is clear that the ariel part of plant *Eclipta alba* shows the presence of alkaloids, glycosides, saponins, tannins, flavonoids, amino acid terpenoids, were found present in ariel parts when extracted with ethanol (70%) using soxhlet extraction procedure. Flavanoids are the phytochemicals that are present in hydroalcoholic extracts of *Eclipta alba*.

The quantitative method developed here was successfully applied in the analysis of rutin in a crude Hydroalcoholic extract of *Eclipta alba*. Taking into account the results obtained in this study, the proposed method can be conveniently used for the analysis of rutin in crude Hydrolcoholic extract of *Eclipta alba*. The proposed method demonstrated high specificity at 256 nm. The developed method for the estimation of rutin method has the following advantages: rapid extraction, easy sample preparation and short time injections. In summary, the method above can be considered specific, exact, precise, linear, robust and easy to perform.

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