A New Methodology for the Quantitative Estimation of Rutin

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ABSTRACT

The new calorimetric methodology of rutin estimation developed using 2% $FeCl_3$ at the wavelength of 575 nm. The solvents, butanol: acetic acid: water (4:1:5) and isopropanol : ammonia : water (10:1:1) were used for the isolation of rutin. The two dimensional paper chromatography spotted and noticed Rf 0.45 and Rf 0.22 in the butanol : acetic acid : water (4:1:5) and isopropanol : ammonia : water (10:1:1), respectively. The reaction mixture of rutin, methanol and 2% $FeCl_3$ developed a deep orange colour. The optical density of reaction mixture measured after 15 minutes at 575 nm for rutin estimation.

INTRODUCTION

The researches of plant extraction for the phytochemical evaluation require the methods of easy and quick analysis. The taxonomically unsolved taxa can be classified using the qualitative and quantitative estimation of the different natural products. Many researches published for the estimation of rutin (Chu, 1998; Tsuchiyal, 1988; Brolis, 1998; Kurtic, 1998) but this attempt can make a quick and easy estimation of rutin.

The secondary metabolite, rutin, is the principle active constituents in some of the medicinal plants of Sikkim. In this context, the new approach of rutin estimation could be able to establish the economic value of medicinal plants at cultivation or field area.

MATERIALS AND METHODS

To standardize the methodology, the plant samples containing rutin as secondary metabolites were collected for the estimation of rutin. The standard curve developed using 1 mg authentic rutin , 1 ml methanol and 1 ml 2% $FeCl_3$. The reaction mixture kept to develop the deep orange coloration at the room temperature. The optical densities were measured after fifteen minutes at absorption maxima 575 nm in Spectrophotometer.

10 mg of the powdered leaves sample was refluxed with 50 cc of methanol for 30 minutes and filtered. The filtrate was concentrated under reduced pressure in the water bath. The purification of rutin performed by the paper chromatography in the solvents butanol: acetic acid: water(4:1:5) or isopropanol : ammonia : water (10:1:1). The two dimensional paper chromatography was also spotted with the pure rutin to locate the position on the Whatman paper (3mm) . The strips containing the rutin at Rf 0.45 in the butanol: acetic acid: water(4:1:5) or Rf 0.22 in the isopropanol: ammonia: water(10:1:1) were dissolved in the hot methanol to make 10 ml. Out of 10 ml, 1 ml of filtrate mixed with 1 ml 2% $FeCl_3$ to develop the deep orange colour.

After fifteen minutes, the optical density was measured at 575 nm to determine amount of rutin from the standard curve.

RESULTS AND DISCUSSION

A new methodology was developed for the quick and easy estimation of the rutin from the medicinal plants. The earlier methods performed by the number of workers were based on spectrophotometer, calorimeter and others. But with the help of this method, 10 mg of the dry sample found to be sufficient for the estimation of rutin. This method is found economical and less time consuming.

The numbers of the works have been reported in connection with the estimation of rutin. Bakh(1954) used direct absorptiometry of paper chromatograms for quantitative estimation of flavonoids. The absorptiometric method was used by Troyer (1956) to estimate rutin and four other flavonoids. Rutin and quercetin were also estimated by Skamoto and Takamura (1978) by using $SnCl_2$ $2H_2O$. The quantitative determination by chromatoscpectrophotometric method was also developed by Balandiria(1980). To determine the quantitative measure of the medicinal plants, this method shall be easy, economical and technically fit. Other calorimetric method for quantitative estimation of rutin was based on reaction with diazotized sulfanilic acid (Brejcha, 1958) .

Rutin estimation with the reaction mixture containing 0.1 M aluminium chloride, 1M potassium acetate and 1 N HCl had also been used (Sethi, 1997) . Some of the recent advances in other plants for the quantitative estimations of polyhydroxyflavones by high performance liquid chromatographic method was performed by Tsuchiya (1998); Chu, (1998).

The flavonoids such as rutin, hypericin, quercetin were separated by an aqueous phosphoric acid, acetonitrile menthol gradient within 50 min (Brolis, 1998) and the spectrophotometric determination of rutin was investigated by Kurtic et al. (1998).

However, this newly developed methodology is more effective and economically viable compared to other earlier reports.

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