A TLC Identification and Spectrophotometric Estimation of Embelin in Embelia ribes

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ABSTRACT: A simple, rapid and economical procedure for estimation of Embelin by UV Spectrophotometer in Embelia ribes was developed and is described. The method is based on the identification of Embelin by TLC and on the ultra violet absorbance maxima in chloroform at 285 nm. A value of 4.80% w/w of Embelia was found in test simple.

INTRODUCTION

Embelia ribes Burn f. family Myrsinaceae, commonly known as ‘vidang’ is a well known drug in Ayurvedic system of medicine. The dried fruits mainly constituents the drug which is used as an anthelmintic, carminative stimulant and alternative. Emblein is the main active compound of E.ribes quantitative estimation of which is essential for standardization of E.ribes.

Some methods like gravimetric and calorimetric have been reported in literature but are not very precise and sensitive. The methods involve multiple step extraction, purification chemical dervatisation etc and thus are time consuming. Some methods have also been reported for isolation of Embelin which are also time consuming.

We have developed a precise, sensitive and reproducible method for quantitative estimation of Embelin in E.ribes which can be used to standardize the plant material with reference to Embelin.

EXPERIMENTAL

Instrument: UV – Visible recording spectrophotometer (Beckman DU/64).

STANDARD PREPARATION

A 1.0 mg/ml solution of Embelin reference standard (M/S sigma, USA) was prepared in chloroform. It was further diluted with chloroform to yield the final concentration of 0.005, 0.01 and 0.02 mg/ml.

SAMPLE PREPARATION

A round 25 gm air dried test sample was grinded to pass through 60 mesh ss sieve. 1gm sample from it was accurately weighed and transferred to 100 ml conical flask for extraction. 15ml chloroform was added to the sample in conical flask and extracted by boiling over steam water bath. The sample was boiled for 3 – 4 minutes and then filtered in hot condition using whatman filter paper No. 41. The clear filtrate was transferred to a 100ml volumetric flask. The above extraction was repeated four more times using 15ml chloroform and the clear filtrate was added to the sample of volumetric flask. The filter paper was washed with chloroform (5ml x 2) in hot condition and the washings were added to the extract in volumetric flask. The volume
of volumetric flask was made up to the mark with chloroform. The contents were shaken by stoppering the flask to make it homogenous and 5ml sample was pipetted and transferred to 250 ml volumetric flasks. The volume flask was made up to mark with chloroform and used further for spectrophotometer reading.

IDENTIFICATION BY TLC

For TLC identification the parent sample i.e. 1 gm in 100 ml was used as such without many further dilutions. 10ul of test sample along with 10ul of standard Embelin (0.02 mg/ml) were applied on precoated silicagel G aluminum plate. The chromatogram was developed in the solvent system – Ethylacetata – formic acid: acetic acid: water – 94:1:1:2 up to 80 mm. Under chamber saturation.

A light violet coloured spot appears in test sample at the Rf of standard when observed under visible light (standard also shows similar colour spot). The intensity of the spot increases when the plate is exposed to ammonia vapour.

PROCEDURE

The absorbance of three different concentrations of standard Embelin was measured at 285 nm against chloroform as a blank. The calibration curve was plotted between concentration and absorbance. The linear equation from the calibration curve was plotted which was found to be Y = 0.046 + 50.35 X with a correlation coefficient of 0.999 where Y is the absorbance and x is the concentration in mg/ml. The absorbance of test samples was also measured at 285 n.m. against chloroform as a blank and the concentration was adjusted to give the absorbance between 0.5 to 1.0. The amount of Embelin in test sample was determine using the above equation.

RECOVERY STUDIES

A varying known amount of standard Embelin(1.2 and 5 mg) was added to about 1.0 gm of fine grinded E.ribes in which the content of Embelin had been estimated previously by the proposed method. The sample were extracted, diluted and analysed separately as per the procedure mentioned above. The contents of Embelin were quantified and percentage recovery calculated. The results are mentioned in Table-1.

RESULTS AND DISCUSSIONS

The Under the chromatographic condition mentioned above, the Rf of Embelin was observed at above 0.65. The test sample shows a identical spot at the Rf of standard Embelin, which indicates The presence of Embelin in test sample. the calibration curve was found to be linear between 5.0 to 20.0 ug.The method allows reliable quantification of Embelin from E. ribes Further recovery values were also satisfactory which showed the reliability and suitability of the method. The proposed method is rapid, simple and accurate and hence can be used for standardization and monitoring of Embelin in E. ribes.

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### Table – I

**Method Validation and Recovery of Embelin**

| Sl. No. | Sample of E.ribes | Amount of sample taken (mg) | Amount of Embelin present in A (mg) | Amount of Embelin added to A (mg) | Total Embelin taken B+C (mg) | Total Embelin found (mg) | % of recovery Ex100 D |
|---------|------------------|-----------------------------|-------------------------------------|---------------------------------|-----------------------------|------------------------|---------------------|
| 1       | Sample – 1       | 1010                        | 48.48                               | 1.00                            | 49.48                       | 49.50                  | 100.4               |
| 2       | Sample – 2       | 1000                        | 48.00                               | 2.00                            | 50.00                       | 49.82                  | 99.64               |
| 3       | Sample – 3       | 1020                        | 48.96                               | 5.00                            | 53.96                       | 53.40                  | 98.96               |