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Optimization of extraction conditions for embelin in *Embelia ribes* by UV Spectrophotometry

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Abstract

A simple, precise and accurate UV spectrophotometric method has been established for quantitative determination of embelin. The response of embelin was linear over the range of 5-40 µg/mL. Extraction conditions were also optimized for best possible extraction of embelin from the fruits of Embelia ribes in different solvents like n-hexane, carbon tetrachloride, diethyl ether, chloroform, acetone, ethyl acetate, propanol and methanol. Ethyl acetate was found to be best for highest possible recovery of the analyte, embelin. Quantitative determination of embelin in all solvent extracts was done by the developed method. The developed UV method was validated in terms of precision, accuracy, stability, LOD and LOQ.

Key Words: *Embelia ribes*, Embelin, spectrophotometry, extraction.

INTRODUCTION

Embelia ribes, a plant grown through out India, in area up to 1,500 m in hilly regions. The chief constituent of the plant is embelin, which is chemically known as 2, 5-Dihydroxy-3-undecyl-1,4-benzoquinone [1]. It is used as anthelmintic and oral contraceptive [2-3]. As herbal drugs are getting popularised due to their less side effects, there is a need to develop analytical methods for their quality control. As they are obtained from plants, solvent for their best extraction is to be optimized to get maximum yield. There are few works reported for the determination of embelin by densitometric method [4], HPLC [5] and HPTLC [6], but no UV method has been reported. Hence an attempt has been made to develop a UV spectroscopic method which is a simple, precise and accurate one for the determination of embelin in extract. Best possible extraction of embelin in different solvents like n-hexane, carbon tetrachloride, diethyl ether, chloroform, acetone, ethyl acetate, propanol and methanol was optimized. Among the solvents tried ethyl

acetate was found to be best, for highest possible recovery of embelin. The content of embelin in various solvent extracts obtained was estimated by the proposed method. The developed method will be helpful in the quality control and quantitative determination of embelin in plant material as well as in herbal formulation.

MATERIALS AND METHODS

Plant material

Embelia ribes fruits were purchased from local market. Fruits were pulverized to a fine powder of 14 mesh in a mechanical blender. This fine powder was utilized for further experimental purposes.

Chemicals

All the solvents used in this study were purchased from Merck Chemicals, India, of analytical grade. Reference standard of embelin was purchased from Indofine Chemical Company, Inc, New Jersey.

Apparatus

The absorbance was measured using UV-VIS double beam spectro photometer (Lab India, Mumbai). Drying and concentration steps were performed using rotavapor (Buchi, Switzerland).

Preparation of standard solution and calibration curve

A stock solution of embelin was prepared by dissolving 50 mg of embelin in 50 ml of methanol. By making suitable dilutions, concentrations ranging from 5-40 µg/ml were prepared. The absorbance of the prepared solutions was measured at 291 nm as the detection wavelength and a calibration curve was plotted between the measured absorbance and concentrations. The curve was found to be linear which indicates that it obeys Beer Lambert's law **[8-11]**.

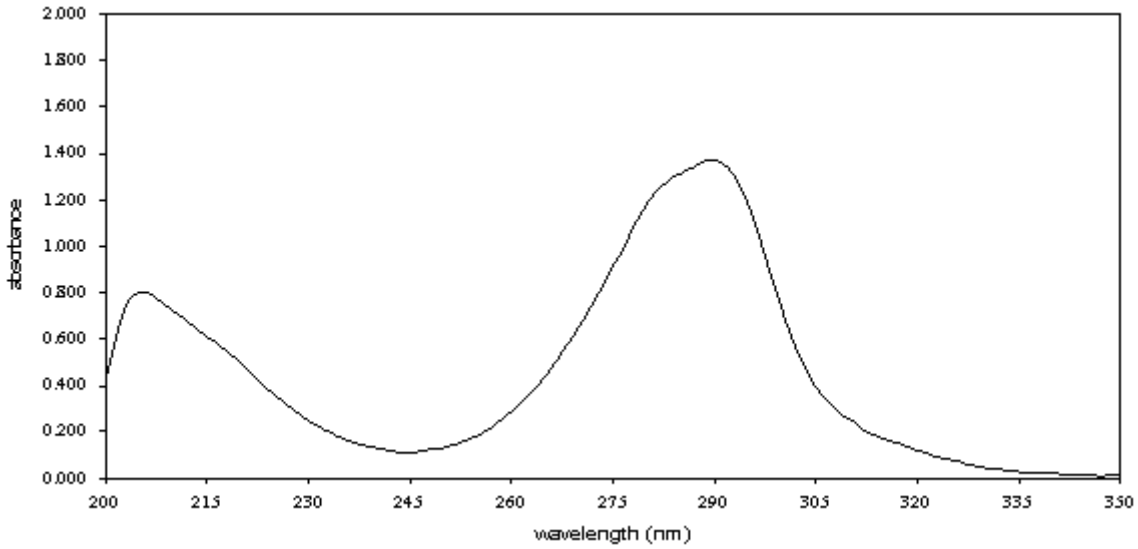

Figure 1 λ_{max} of embelin

Soxhlet extraction and test sample preparation

Soxhlet extraction of 10 g (14 mesh) of dried and powdered fruit was performed for 6 h on a water bath with 200 mL of n-hexane, carbon tetrachloride, diethyl ether, chloroform, acetone, ethyl acetate, methanol and propanol respectively [2]. Extracts thus obtained were concentrated *in vacuo* via rotavapor and re-dissolved in methanol and volume made up to 100 mL. This

solution was taken as test sample for quantification purpose. The embelin content in each extracts was determined by the method and is reported in Table 2.

RESULTS AND DISCUSSION

In the present study, a simple, precise, accurate and rapid UV spectrophotometry method have been developed and validated for the determination of embelin in *Embelia ribes* fruit. Extraction conditions were also optimized for best possible extraction of embelin from the fruits of *Embelia ribes* in different solvents like n-hexane, carbon tetrachloride, diethyl ether, chloroform, acetone, ethyl acetate, propanol and methanol. Ethyl acetate was found to be best for highest possible recovery of the analyte, embelin. Quantitative determination of embelin in all solvent extracts was done by the developed method. The developed method was validated in terms of precision, accuracy, stability, LOD and LOQ.

Linearity and range

For linearity, five different concentrations of embelin were used in a working range of 5-40 µg/ml. Linear regression equations and correlation coefficient (r) values for embelin are presented in Table 1 [7-8].

Precision

The intraday and interday precisions of the proposed method were determined by estimating the corresponding response 3 times on the same day and on 6 different days over a period of 1 week for three different concentrations of 10, 25 and 35 µg/ml of embelin [7-8]. The results are reported in terms of relative standard deviation (RSD) in Table 1.

Accuracy

Accuracy is nearness of a measured value to the true value. It provides an indication of any systematic error or bias in the method [7-8]. The accuracy of the method was determined by calculating recoveries of embelin by the method of standard additions. A known quantity of standard embelin was added to a pre-quantified sample solution. The amount of embelin was estimated by measuring response at appropriate wavelength of 291 nm. The recovery was verified by estimation of the markers in triplicate samples at each specified concentration level.

Sensitivity

Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined by kSD/s where k is a constant (3 for LOD and 10 for LOQ), SD is the standard deviation of the analytical signal, and s is the slope of the concentration /response graph [7-8].

Stability

Solutions of embelin in methanol were studied for their stability at ambient temperature for 24 h. The method showed linearity covering the range of 5-40 µg/ml with a correlation coefficient value of 0.999. The equation of the straight line was Y = 0.056 x + 0.061. The method gave reproducible and precise results during intra-day and inter-day trials. So it is evident that the developed method is precise and the data is supported by relevant statistical analysis. Accuracy of the developed method was satisfactory. The method offered 98.26 % recovery. The lowest detection limit was calculated as 0.0535 µg/mL and the lowest quantitation limit was calculated as 0.1791 µg/ml.

Table 1 Summary of validation parameters of embelin

Parameters	Results
Linearity	
Range	5-40 µg/ml
Linear equation	Y = mx + C
Slope (m)	0.056
Intercept (C)	0.061
Correlation coefficient (r)	0.999
Standard deviation (SD)	0.024
Precision (% RSD)	
Intraday precision (n=3)	% RSD = 0.123
Interday precision (n=3)	% RSD = 0.068
Accuracy (% Recovery)	98.26 %
Limit of Detection (LOD)	0.053 µg/ml
Limit of Quantification (LOQ)	0.179 µg/ml

Table 2 Optimization of extraction solvent by UV spectrophotometry

S. No	Solvent	Extractive Weight* (mg)	Embelin Content* (mg)
1	Hexane	171.67	3.326
2	Carbon tetrachloride	274.67	3.498
3	Diethyl ether	306.33	10.714
4	Chloroform	333.33	4.047
5	Acetone	367.67	6.480
6	Ethyl acetate	307.33	19.160
7	Methanol	305.33	8.768
8	Propanol	324.67	5.539

**Average of three determinations*

CONCLUSION

The proposed method being precise, accurate, sensitive, quick and cheap, it can be used for the determination of embelin in routine quality control analysis of crude drug and formulation. As the best possible extraction for embelin was made with ethyl acetate, the same can be employed for the extraction of embelin in crude drug and formulation.

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