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UV SPECTROPHOTOMETRIC ESTIMATION OF EMBELIN AND VALIDATION OF DEVELOPED METHOD

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ABSTRACT

Embelia ribes burm f., also known as Vidanga, is one of the oldest herbs in Indian traditional medicine. Embelia ribes have a long history of use in ayurvedic system of medicine in various forms like churna, asava, aristha, lauha and taila. It is used mainly as an anthelmintic, carminative and stimulants. A simple and reproducible UV Spectrophotometric method has been developed for the estimation of Embelin in Vidanga churna formulations. Methanol was used as a solvent. Scanning wascarried out in the UV range of 200-400 nm. Embelin showed maximum absorbance at 291 nm.Developed method showed good regression (r^2= 0.9982± 0.0003)and the recovery of Embelin was in the range of 98.64 – 102.38%. The limit of detection and limit of quantitation were found to be 0.05µg/ml and 0.1571µg/ml respectively. The method was validated for precision, recovery, limit of detection and limit of quantitation. The proposed method being precise and sensitive can be used for detection, monitoring and quantification of Embelin in E. ribes.

KEY WORDS: Vidanga, Embelin, UV Spectrophotometric

INTRODUCTION

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. In olden times, vaidyas used to treat patients on individual basis, and

prepare drug according to the requirement of the patient. But the scene has changed now; herbal medicines are being manufactured on a large scale in pharmaceutical units [1].Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants [2-4]. Over the past decade, interest in drugs derived from higher plants, especially the phytotherapeutic ones, has increased expressively. In some particular cases, such as antitumoral and antimicrobial drugs, about 60% of the medicines currently available on the market and most of those in the late stages of clinical trials are derived from natural products, mainly from higher plants [3].

Embelia ribes burm f. also known as Vidanga [5], is one of the oldest herbs in Indian traditional medicine. Embelia ribes have a long history of use in ayurvedic system of medicine in various forms like churna, asava, aristha, lauha and taila. It is an Indo-Malaysian species, mainly found in India, SriLanka, Singapore, and Malaysia. In Indiait is majority found in central and lower Himalayas, Arunachal Pradesh, Assam, Bengal, Orissa, Andhra Pradesh and Madhya Pradesh [6]. It is available throughout India up to an altitude of 5000 feet [7].The main active component is Embelin, chemically 2,5-dihydroxy-3-undecyl-1,4-benzoquinone [Fig.1]. Embelin is occur in golden yellow needles and is insoluble in water but soluble in alcohol, chloroform and benzene. Other components are christembine, qercitol, vilangin and resinoid[8]. It is mainly used as an anthelmintic, carminative and stimulant. It is also used in treatment of abdominal disorders, lung diseases, constipation, indigestion, fungus infections, mouth ulcer, sore throat, pneumonia, heart disease and obesity [9].

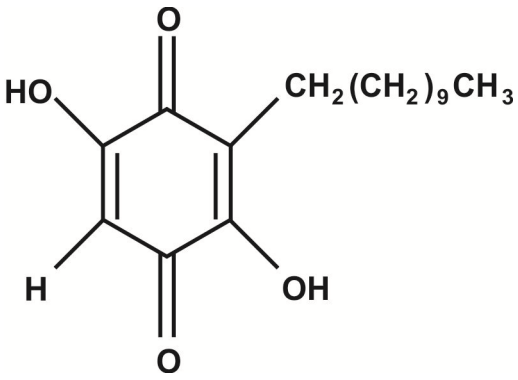

Figure 1: Structure of Embelin

In the present study, UV Spectrophotometricmethod has been developed for estimation of Embelin in E. ribes. The developedmethod were validated in terms of accuracy, specificity, precision, linearity range, robustness, limit of detection (LOD) and limit of quantitation (LOQ) as per ICH guidelines. The developed method can also be used as a quality control tool.

MATERIALS AND METHODS

A Shimadzu UV Spectrophotometer (model-1800) with 1cm matched quartz cells was used for all spectral measurement. Dried ripe fruits of Embelia ribes Burm. f., family-Myrsinaceae were procured from local market of Guwahati, Assam in the month of November. The collected fruits of Embelia ribes were authenticated at Botanical survey of India, Pune. All the chemicals used were of A.R. grade obtained from Merck chemicals, India.

Preparation of extracts of marketed formulations

Marketed formulations (F-I, F-II and F-III) were macerated with chloroform at room temperature for 48 hrs in dark. The extract was filtered and concentrated over water bath till a dry residue was obtained. This extract was further used for UV analysis.

Selection of Analytical wavelength

Embelin was dissolved in methanol and this solution was scanned in the UV range 200-400 nm. It showed maximum absorbance at 291 nm as shown in figure 2. Similarly to confirm the applicability of the analytical wavelength to the solution of extract of F-I, F-II and F-III, The solutions of the extracts of concentration equivalent to 10 µg/ml of embelin were also scanned in the same range which showed absorption maxima at 291 nm. Hence the wavelength 291 nm was selected for the further studies.

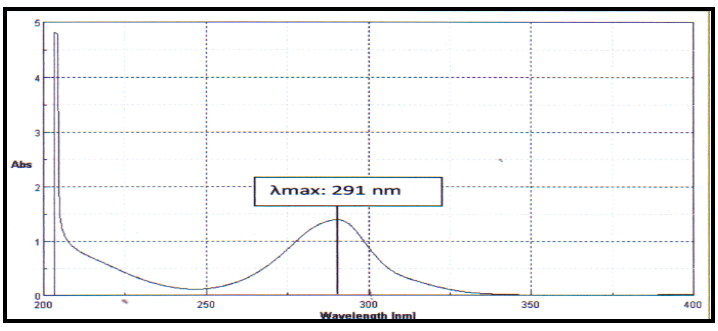

Figure 2: UV spectrum of Embelin

Analysis of marketed formulations

The extracts of F-I, F-II and F-III respectively were weighed equivalent to 10 mg of Embelin, dissolved it in sufficient volume of Methanol and sonicated for 10 min. The volume was made up to 10 ml with methanol to get the concentration of 1000 µg/ml. Further dilutions were made to get 10 mg/ml solution from the above stock solution.

Validation

The developed UV spectrophotometric method was validated in terms of linearity, precision, specificity and accuracy as per ICH guidelines [10]

Linearity and range

The stock solution 1000 µg/ml of Embelin was prepared in methanol and this stock solution was diluted with methanol to obtain final concentrations of 2-12 µg/ml of Embelin for UV analysis.

Precision

Precision of the method was verified by repeatability (intra-day) and intermediate (inter-day) precision studies. Intra-day and inter-day precision were performed by analysis of 10 µg/ml of Embelin for UV analysis on the same day and three different days respectively. Absorbance of active compound was expressed in terms of % relative standard deviation (% R.S.D.) and standard error (S.E).

Limit of detection and Limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using following formulae.

$LOD = 3.3(SD)/S$

$LOQ = 10 (SD)/S$

Where, SD = Standard Deviation of response

S = avg. of the slope of the calibration curve

Accuracy

Accuracy of the method was tested by carrying out recovery studies at different spiked level by standard addition method. Standard Embelin solution was added at three different levels (80,100 and 120%). At each level three determinations were performed and results were calculated by the difference between the spiked and un-spiked sample analyzed under the same conditions.

RESULT AND DISCUSSION

Validation of method

Linearity plots and detection limits

The linearity for embelin was established by plotting the peak area versus concentration. The linearity of calibration curves was verified by correlation study and the correlation coefficients were found to be 0.9982. The linearity of calibration graphs and adherence of the system to Beer's law validated by determining correlation coefficients and SD values which were found to be well within the accepted limit. The LOD and LOQ for embelin were found to be 0.05µg/ml and 0.1571µg/ml respectively as shown in Table 1.

Table1: Calibration curves, Limit of detection and Limit of quantitation of Embelin

Linear range*	Correlation coefficient	LOD*	LOQ*
2-12 µg/ml	0.9982	0.05µg/ml	0.1571 µg/ml

** Results are mean of three determinations*

Precision

The % RSD value for inter-day and intra-day precision study of Embelin was found to less than 2 (n=6), hence both the methods were found to be precise. The results of precision study of three formulations drugs are shown in Table 2.

Accuracy

The average recoveries of the embelin were in the range of 98.64-102.38%. Satisfactory recoveries with small % relative standard deviations (less than 2) were obtained, which

indicate the accuracy of the method. The results of precision study of three formulations drugs are shown in Table 2.

Table 2: Precision and Accuracy for Embelin

Formulation	Precision(%RSD)		Recovery*					
			80%		100%		120%	
	Interday	Intraday	Recovery	%RSD	Recovery	%RSD	Recovery	%RSD
F-I	0.699	0.249	98.98%	1.9	100.08%	0.84	102.38%	1.12
F-II	0.168	1.14	99.75%	0.14	98.64%	0.27	100.38%	0.26
F-III	0.637	1.33	100.4%	0.38	99.62%	0.74	99.78%	0.74

** Results are mean of three determinations*

Quantitative analysis of embelin in marketed formulations

For assay of marketed formulations F-I, F-II and F-III, extracts were prepared as mentioned in the above section and subject to optimized UV spectrophotometric analysis. The results of embelin content in marketed formulations were shown in Table 3.

Table3: Quantitative analysis of Embelin from Marketed formulations

Formulation	Concentration	% Purity*	Embelin content* (w/w)
F-I	10µg/ml	96.34	2.11%
F-II	10 µg/ml	99.27	2.18%
F-III	10 µg/ml	104.06	2.30%

** Results are mean of three determinations*

CONCLUSION

The proposed UV spectrophotometric method was developed and validated for estimation of Embelin. The method was found to be simple, sensitive, accurate, rapid and precise. Hence, the above said method can be successfully applied for routine quality control analysis and estimation of Embelin.

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