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RESEARCH ARTICLE

QUANTITATIVE DETERMINATION AND OPTIMIZATION OF EXTRACTION CONDITIONS FOR EMBELIN IN EMBELIA SCHIMPERI BY UV-VIS SPECTROMETRY

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ABSTRACT:

Embelin is a unique chemical compound found in nature, composed of quinone moiety resembling Coq10 (Ubiquinones), having ketone and hydroxy groups with an aliphatic chain. An Optimum solvent for extracting Embelin from *Embelia schimperi* and its simultaneous determination of the content of Embelin in the extract has been performed using UV-VIS spectrometry. The identification of Embelin spot obtained from hot ethyl acetate extract of the seed is confirmed by Nuclear Magnetic Spectroscopy (NMR). A plot of peak absorbance versus concentration of Embelin was found to be linear over the range of 3-12 µg/ml. The limit of detection was 0.11µg/ml and the limit of quantitation was 0.37 µg/ml. Extraction conditions were also optimized for the best possible extraction of Embelin from the fruits of *Embelia schimperi* in different solvent polarity like n-hexane, carbon tetrachloride, diethyl ether, chloroform, ethyl acetate, propanol and ethanol. The determination of Embelin in various solvent extract exhibited a mean content of 0.66-5.79 % w/w. Carbon tetrachloride and ethyl acetate was found to be best for the highest possible recovery of the analyte, Embelin. The developed UV method was validated in terms of precision, accuracy, stability, LOD and LOQ

Keywords: Embelia schimperi, Embelin, spectrometry, extraction

INTRODUCTION

Parasitic helminthics affect animals and man, causing considerable hardship and stunted growth. Most diseases caused by helminthics are of chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The high cost of modern anti-helminthics has limited the effective control of parasitic helminthics [1].

In some cases widespread intensive use of low quality anti-helminthics has led to development of resistance and hence a reduction in the usefulness of available anti-helminthics [2-3]. The use of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of helminthic parasites, and as a means of reducing the cost of controlling helminthic diseases [3-4]. The emergence of resistant strains of pathogenic helminthics has stimulated the desire to search for additional chemotherapeutic agents that might allow more efficient control of helminthic parasites [5].

The use of plants for the treatment of various diseases is universal and has been practiced by many people for many years. During the last decade, the use of traditional medicine has expanded globally and has gained popularity.

It has not only continued to be used for primary health care of the poor in developing countries, but has also been used in countries where conventional medicine is predominant in the national health care system (WHO, 2000). *Embelia schimperi* vatke belonging to the family *Myrisinaceae*, is the most widely used plant. Literature revealed an overflow of reports on the pharmacological efficacy of the extracts of the *Myrisinaceae* species as anti-helminthics and other activities. *Embelia schimperi* (E*nkoko in Amharic*) is scandent or climbing shrub which reaches the height of 2-13 meters [6]. It has branches with prominent lenticels. Fruit decoction is useful in fevers and diseases of chest and skin. Infusion of roots is used for cough and diarrhea. Aqueous extract of the fruits showed antibacterial and anti-fertility activities [7]. Seeds were found to possess antibiotic and anti-tubercular properties. A gum obtained from the plant is used as a warming remedy in the treatment of dysmenorrheal.

Decoction of the leaves is used as a blood purifier. The fruit, 5-8mm in diameter, is orange yellow, reddish–green to red in color when ripen. Each fruit often has one seed that has a diameter of 4.5-7mm. It is brown in color with irregular orange markings when ripen [8].

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The chief constituent of the plant is embelin, which is chemically known as 2, 5-Dihydroxy-3-undecyl-1, 4-benzoquinone. As herbal drugs are getting popularized due to their fewer 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.

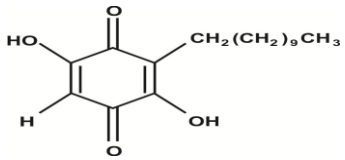

Figure 1: Structure of Embelin

In the present study, an attempt has been made to develop a UV Spectrophotometric method for estimation of Embelin in *E. schimperia*. In addition to this, extraction solvent optimization for the analyte, Embelin was carried out using various solvent polarities. The developed method were validated in terms of accuracy, specificity, precision, linearity range, robustness, limit of detection (LOD) and limit of quantization (LOQ).

MATERIALS AND METHODS

Plant material

Embelia schimperi fruits were purchased from local medicinal market in Gonder, North of Ethiopia. Fruits were pulverized to a fine powder in a mechanical blender. This fine powder was utilized for further experimental purposes.

Instrument

HPLC experiments were performed using a waters alliance system equipped with a degasser, quaternary solvent mixer, waters 600 pump, and a waters UV detector. The experiments performed in 200-400 nm range and extracted at 291 nm. The mobile phases were 0.1% ortho phosphoric acid in water (Solvent A) and methanol (Solvent B) as organic modifier, the peak shape found good. The absorbance was measured using UV-VIS double beam spectro photometer (Lab Shimadzu, Japan). Drying and concentration steps were performed using rotavapor (Buchi, Switzerland).

Chemicals

All the solvents used in this study were purchased from Merck Chemicals, USA, of analytical grade.

Isolation of Embelin from *E. schimperia*

The reference compound Embelin working standard was isolated from 1.1 kg of powdered seeds of *E. schimperi* by extraction with hot ethyl-acetate for 25 minutes and then transferred to a shaker for 4 h and filtered with whatman paper no 41. The marc was extracted one more time with the same solvent. Solvent evaporated under vacou and the resulting extract 119 g was successively washed using cold hexane which yielded 5 g highly purified an orange crystal of embelin having a melting point 142-143 ^{O}C whose identity is fully elucidated by ^{1}H, ^{13}C, DEPT-135, HSQC, HMBC, HPLC, and UV spectra.

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 schimperi* fruit. Extraction conditions were also optimized for best possible extraction of embelin from the fruits of *Embelia schimperi* in different solvents like n-hexane, carbon tetrachloride, diethyl ether, chloroform, ethyl acetate, propanol and ethanol. Carbon tetrachloride and ethyl acetate was found to be best for the 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.

Analytical wave Length selection

Embelin was dissolved in ethanol and this solution was scanned in the UV range 200- 400 nm. It showed maximum absorbance at 291 nm as shown in figure 2.

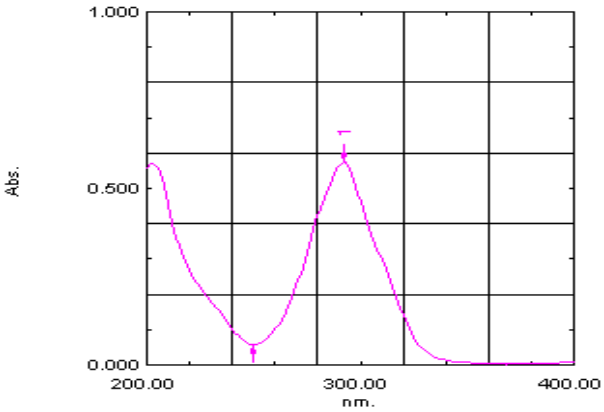

Figure 2: UV spectrum of isolated reference Embelin

Determination of extractable matter and evaluation of various Extract

WHO, protocol was followed with slight modification. 4.0 g of accurately weighed coarsely powdered air-dried material, was placed in a glass-stoppered conical flask and macerated with 100ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, and then allowed to stand for 2 hours. The resulting extract was filtered rapidly taking care not to lose any solvent; 25 ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporate to dryness on a water-bath. An aliquot from the same filtrate was used for the quantitative analysis by making appropriate dilution. Then, dried at 105°C for 6 hours, cool in desiccators for 30 minutes and weighed without delay. The extractable matter was calculated in mg per g of air-dried material [10]. The embelin content in each extracts was determined by the method and is reported in Table 2.

Preparation of Standard solution

Embelin, working isolated standard, in the range of 25 mg was accurately weighed or transferred into a 50 ml volumetric flask and dissolved in ethanol to obtain solution with 0.5 mg/ml concentration of Embelin. This solution was then further diluted to obtain the concentration 0.05 mg/ml of embelin stock solution and stored at −50°C and brought to room temperature before use.

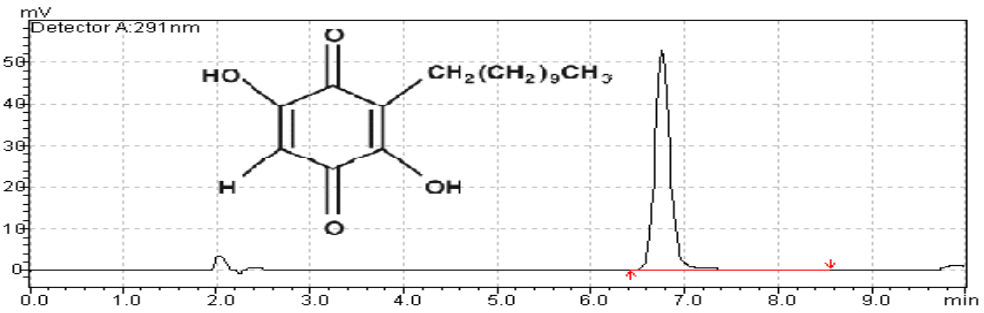

Figure 3: HPLC of the isolated reference embelin

Method Validation

The proposed method has been validated to show some specific parameters like Limit of Detection (LOD), Limit of Quantification (LOQ), Precision, Linearity and Robustness. The Accuracy at LOQ level, shown here in terms of percent recovery of the known quantity of Embelin added to extract sample and its recovery calculated.

Precision

The system precision has been verified by observing the %RSD of replicate injections of the isolated reference standard. It was verified with different day analysis to show the repeatability. The results are reported in table 1.

Limit of Quantification and Limit of Detection

There are several terms that have been used to define LOD and LOQ. In general, the LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test. The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test[11]. To determine the LOD and LOQ of the proposed method, a concentration which is showing signal to noise ratio 3 for LOD and ration 10 for LOQ was used. Signal to noise ratio has been scanned and calculated using kSD/m, where k is the constant for LOD and LOQ, SD is the standard deviation of the analytical signal, and m is the slope of the concentration/response graph. The results are reported in Table 1.

Linearity

Linearity was determined by using Embelin standard stock solution 0.05 mg / ml in ethanol. The solution further diluted to get 2.5 to 15 µg /mL of the standard solution was prepared (n = 3). The calibration graphs were obtained by plotting the absorbance versus concentration of the standard solutions and its correlation coefficient has been verified in Figure 4 and Table 1.

Stability

Solutions of embelin in ethanol were studied for their stability at ambient temperature for 24 h. The method showed linearity covering the range of 3-12 µg/ml with a correlation coefficient value of 0.997. The equation of the straight line was Y = 0.878x + 0.0735. 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 % recovery. The lowest detection limit was calculated as 0.11µg µg/mL and the lowest quantitation limit was calculated as 0.37 µg/ml.

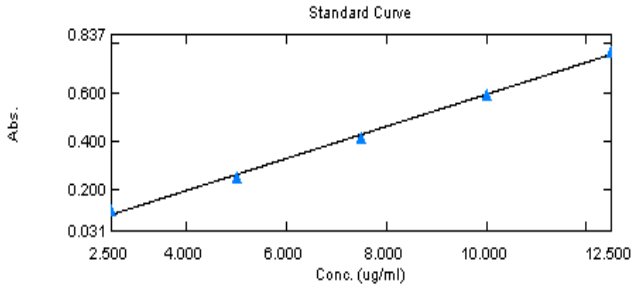

Figure 4: Calibration curve of the isolated reference embelin

Table1: Summary of validation parameters of embelin

Parameters	Results
Linearity	
Range	3-12 µg/ml
Linear equation	Y = mx + C
Slope (m)	0.878
Intercept (C)	0.0735
Correlation coefficient (r)	0.997
Residual Standard deviation (SD)	0.033
Standard error of estimate	0.037
Precision (% RSD)	
Intraday precision (n=3)	% RSD = 0.23
Interday precision (n=3)	% RSD = 0.06
Accuracy (% Recovery)	98%
Limit of Detection (LOD)	0.11µg/ml
Limit of Quantification (LOQ)	0.37 µg/ml

Table 2: Optimization of extraction solvent by UV Spectrophotometry

S.No	Solvent	Extractive Weight* (mg/g)	Embelin Content* in (%)
1	n-Hexane	20.00	0.657
2	Carbon tetrachloride	60.53	5.79
3	Ethyl acetate	56.33	5.46
4	Diethyl ether	74.57	4.43
5	Chloroform	77.53	4.41
6	Propanol	86.87	3.49
7	Ethanol	128.80	2.63

**Results are mean of three determinations*

CONCLUSION

The need for quality assurance, including confirmation of the label strength and content uniformity has long been recognized even for herbal medicinal products. 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 and semi-purified extract as well as for formulated herbal products. As the best possible extraction for Embelin was made with different solvent the same can be employed for the extraction of Embelin in crude and semi-purified extract as well as for formulated herbal products. The relative standard deviation for the investigated *Embelia schimperi* extracts indicates that the method is precise and reproducible. Hence, the above said method could provide an important reference to establish the quality control method for other *Embelia schimperi* medicinal preparations for manufacturers as well as for different regulatory agencies.

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