Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2009; 1(3): 146-149

Research Article ISSN 0975 1556

Embelin - An HPLC Method for Quantitative Estimation in *Embelia ribes* Burm. F.

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ABSTRACT
An RP-HPLC method with photodiode array detection was established for the determination of major constituent, Embelin in *Embelia ribes* samples. The Embelin was separated by using isocratic mode consisting of 0.1 % trifluoroacetic acid in water and methanol (in proportion of 88:12) at a flow rate of 1.0 mL/min. Under these conditions, a plot of integrated peak area versus concentration of Embelin was found to be linear over the range of 5.0-75.0 µg/mL, with a relative standard deviation of 0.61-0.96 %. The limit of detection was 20 ng on column and the limit of quantitation was 50 ng on column. The determination of the Embelin content in various solvent extracts exhibited a mean content of 0.44-33.0 % w / w. Recovery experiments led to a mean recovery rate of 96.49 ± 2.42 %. The proposed method is less time-consuming, sensitive and reproducible and is therefore suitable for routine analysis of Embelin in various extracts of *E. ribes*.

Keywords: *Embelia ribes*; Vidang; Pharmaceutical analysis; Embelin; High performance liquid chromatography.

INTRODUCTION

The family *Myrsinaceae* consists of nearly 1000 species of trees and shrubs spread over 33 genera including four genera namely *Myrsine*, *Maesa*, *Rapanea* and *Embelia*, which are widely used in herbal medicines. [1] *Embelia ribes* Burm. F. is the most correlated species of *Vidanga* (Sanskrit), a drug used in *Ayurveda*, *Siddha* as well as in *Unani* medicine system as anthelmintic and to cure skin diseases. [2-3] *Embelia ribes*, a natural source of Embelin has restricted and sporadic distribution mainly in the Western Ghats and Eastern Himalayas. [4] It is also a critically endangered species of conservation importance in India. [5-6]

Embelia ribes (Embelia fruit) is used in India and in the Eastern Colonies for numerous traditional medicinal uses. The fruit are used as an anthelmintic, diuretic, carminative, contraceptive, anti-bacterial, anti-inflammatory and anti-astringent as reported in various literatures. [7] Also fruit decoction is useful in fevers and diseases of chest and skin. Infusion of roots is used for cough and diarrhoea. Aqueous extract of the fruits showed antibacterial and antifertility activities. Seeds were found to possess antibiotic and antitubercular properties. A gum obtained from the plant is used as a warming remedy in the treatment of dysmenorrhoea. Decoction of the leaves is used as a blood purifier. [8]

Embelin (embelic acid / 2, 5-dihydroxy-3-undecyl-2, 5-cyclohexadiene-1, 4- benzoquinone) has been isolated in *Embelia ribes* Burm. f. (Fig. I) and other species of *Myrsinaceae* family. [9] Embelin revealed antifertility, analgesic, anti-inflammatory, antioxidant and antitumour properties. [10-11] Embelin as such evaluated against *Heligmosomoides polygyrus* in mice significantly reduced the total worm counts. [12] Embelin reported to be a potent oral contraceptive. [13] Embelin inhibited pregnancy and also possessed anti-estrogenic and weak progestational activity. [14] There are no chromatographic methods available for quantitation of Embelin in *Embelia ribes Burms*. Hence, the present work focuses on development and validation of high-performance liquid chromatographic method with photodiode array detection for the determination of this major constituent, Embelin, in *Embelia ribes* extract.

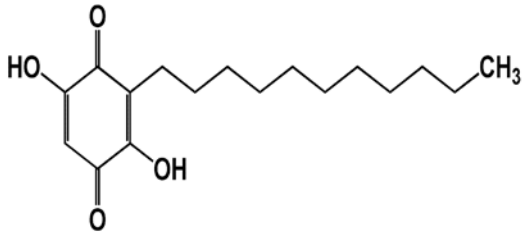

Fig. 1: Structure of Embelin

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MATERIAL AND METHODS

Embelin was isolated, purified and structural confirmation was carried out. [15] The working standard purity was determined to be more than 98.0 % by and hence, was considered as working standard for the analysis purpose.

Chemicals

HPLC grade Methanol from Merck Specialty Private Ltd (Mumbai, India) and Tri-fluoro Acetic acid from Spectrochem Private Ltd (Mumbai, India). Deionized water was obtained with an in-house Milli-Q Nanopure (Millipore, Bedford, MA, USA).

Collection and authentication of plant

The fruit of *Embelia ribes* was purchased from local market and botanical authentication was performed at the Botany Department of Piramal Life Sciences Ltd, Mumbai. Collected plant material (fruits) were dried under shade and grounded in to # 18 powder.

Preparation of the extract

The 100 g of *Embelia ribes* fruit dried powder was extracted with 500 ml solvent (Hexane, Chloroform, Methanol and Water) by stirring at 50°C for 1 h. The filtered extract was concentrated under reduced pressure to remove the solvent. The extraction carried out for two times with the above-mentioned protocol. The extract was obtained by drying the concentrated pooled extract under vacuum. These extracts were used for estimation of Embelin content.

Equipment

The HPLC system consisted of an Agilent 1200 series liquid chromatograph equipped with an autosampler, a photodiode array detector and column (Unisphere Aqua C18, 3 µm, 4.6 × 150 mm, Agela Technologies, USA). The absorption was measured in a full spectrum (200-400 nm) or at 288 nm for Embelin. The chromatographic data was recorded and processed with EZChrom Elite software.

Chromatography

Analyses were carried out at 30°C on a Aqua C_{18} column (3 µm, 100 Aº 4.6 × 150 mm, Cat No UA315059-0, Serial No M9310515BI0019) (Agela Technologies, USA). The mobile phases consisted of Methanol (A) and 0.1 % TFA (B) (in proportion of 88:12 v/v) was degassed before used. The flow-rate was kept at 1.0 ml/min, temperature of column was set at 30°C±2°C and the injection volume was 10µl. Quantification of Embelin was carried out at 288 nm. The peak in the HPLC chromatogram of *Embelia ribes* extract was identified by comparing the retention time and UV spectra of Embelin in the samples with working standard of Embelin. The peak purity was checked by PDA software.

Standard solution

Embelin, working standard, in the range of 5 mg was accurately weighed or transferred into a 25 ml volumetric flask and dissolved in methanol to obtain solution with 0.2 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 −20°C and brought to room temperature before use. In the same way, three sets of control for Embelin was prepared from a separate stock, so as to lie in the lowest, middle and highest regions of the calibration curves. Further standard solutions are prepared freshly each day by appropriate dilution of stock solution with methanol for intraday as well as interday analysis.

Sample solutions (Evaluation of various extracts)

Test sample preparation

Different *Embelia ribes* extracts (25 mg) were exactly weighed into a 25-ml volumetric flask fitted with a glass stopper. (Borosil cat. # 14-962-26F) and volume is made by methanol and extracted using a sonicator 5 min and allowed to stand for 5 min. The mixture was then filtered through Whatmann no.42 filter paper and the desired concentration (0.5 mg/ml) is obtained. Then 10 µl of the resulting solution was subjected to HPLC analysis and the concentration of the major constituent, Embelin, in different *Embelia ribes* extracts were calculated based on the equations for the calibration curves.

Linearity and limit of quantitation

For a long-term use of the analytical method a rigorous validation is indicated and requires the following procedures. For the preparation of calibration curve the stock solution was diluted freshly with methanol to obtain a set of 6 calibration standards. These standards were measured and the integrated peak areas were plotted against the corresponding concentrations of the injected standards. The complete procedure was repeated on three consecutive days. The so obtained three calibration curves were used to calculate a mean calibration graph. The limit of quantification was defined as that lowest concentration where accuracy better than 20.0% was achieved. [16]

Intraday and interday analysis using Embelin

Three different concentrations using a different stock solution of Embelin were prepared (25.0; 50.0 and 75.0 µg/mL). For the determination of the intraday precision and accuracy three replicates of the standard solution were analyzed at the same day. The precision and the accuracy of the interday analysis were determined by analyzing the standard solution on 3 different days.

Stability

Embelin (500 µg) and *Embelia ribes* methanolic extract (5 mg) was transferred into a 10-ml volumetric flask and made up to volume with methanol. The sample solutions were put at 30°C and analyzed on 16 and 24 h to observe the stability of sample solutions.

Robustness and Ruggedness studies

Robustness and ruggedness parameters were applied by making small deliberate changes of the conditions (mobile phase composition, column temperature, different lot of stationary phase, analyst and equipment) to validate the method.

RESULTS AND DISCUSSION

Chromatography

Under the current conditions, Embelin along with other phytoconstituents of *E. ribes* extract were eluted within 10 min. Fig. I & II shows the typical LC chromatograms of working standard of Embelin and various extracts of *E. ribes* samples at 288 nm respectively. Fig. 7 shows the UV spectrum of working standard Embelin along with its UV maxima at 288 nm and peak purity at three different levels of peak. Various extracts (Heaxane, Chloroform, Methanl and Water extract) of *Embelia ribes* were analysed by the proposed method and the data are recorded in Table I.

Limit of detection and limit of quantitation

The limit of detection (LOD) was obtained by successively decreasing the concentration of Embelin as long as a signal-to-noise ratio of 3:1 appeared. The LOD was found to be 20 ng on column (volume of injection is 10 µL; corresponding to a concentration of 2 µg / ml). The limit of quantitation (LOQ) was found to be 50 ng on column (volume of

injection is 10 µL; corresponding to a concentration of 5 µg/ml) of Embelin.

Linearity and reproducibility

The calibration was based on the duplicate analysis of calibration working solutions at six concentration levels on 3 consecutive days for Embelin (5-75 µg/ml) with regression (r^2) more than 0.9998. (Fig. III) The reproducibility of the method was evaluated by analyzing three sets of controls (n=3) on 3 separate days (n=3) and calculating the relative standard deviation (RSD). As shown in Table II, the RSD (%) were founded in the range of 0.49 – 3.61 %.

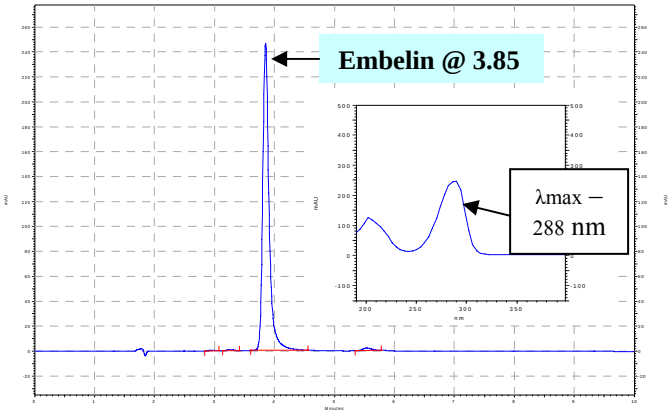

Fig. I: LC Chromatogram of working standard Embelin along with its UV spectrum

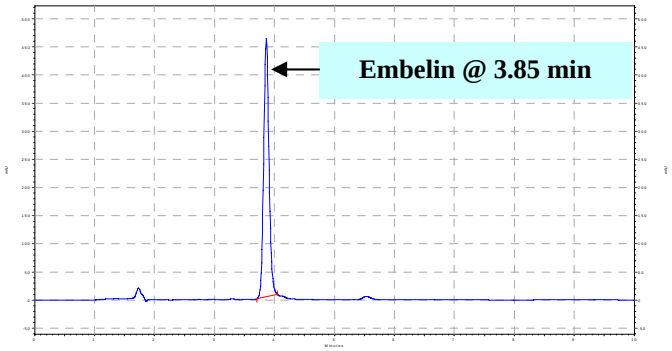

Fig. II: LC Chromatogram of Methanolic extract of *E. ribes*

Table I: Percentage of Embelin in different samples of *Embelia ribes* extracts

Sr.	Sample name	Concentration of test sample	Embelin content (% w/w)
1	Hexane extract	100 µg / ml	10.10
2	Chloroform extract	50 µg / ml	33.34
3	Methanol extract	100 µg / ml	14.31
4	Water extract	500 µg / ml	0.46

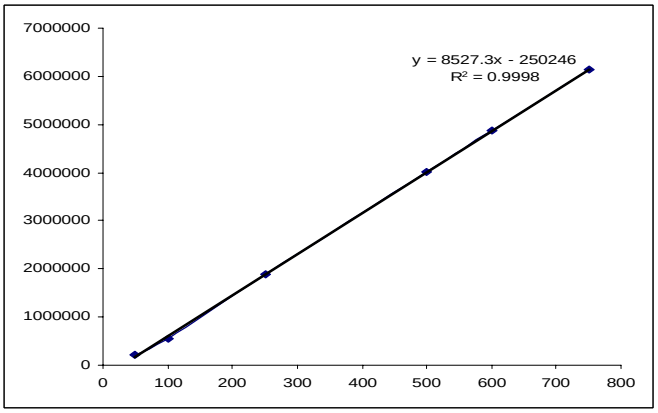

Fig. III: Calibration curve of Embelin with respect to the area under curve at various concentration

Table II: Precision and accuracy of the recalculated calibration samples

Given (µg/mL)	Found, mean ± S.D. (µg/mL)	Precision (R.S.D., %)	Accuracy (% Deviation)
25	24.730 ± 0.89	3.6114	- 1.078126
50	48.249 ± 0.23	0.4911	- 3.501832
75	71.046 ± 1.27	1.7930	- 5.270758

Intraday and interday analysis using Embelin

Furthermore the precision and accuracy of the intraday and interday analysis were investigated on the basis of a set of standard solution. The results given in Table III stands for a quite good trueness of the proposed method particularly considering interday and intraday analysis.

Table III: Intraday and interday precision and accuracy of Embelin

Given (µg/ml)	Intraday		Interday	
	Precision (R.S.D., %)	Accuracy (Percent deviation)	Precision (R.S.D., %)	Accuracy (Percent deviation)
25	2.569	- 1.98	2.803	- 2.09
50	1.096	- 2.60	1.540	- 2.72
75	1.643	- 3.78	1.884	- 4.06

Stability

In the current assay, analyses of stability samples in methanol on regular interval (16 and 24 h) revealed that the Embelin, a major constituents in the methanolic extract of Embelia ribes is stable in solution form with relative standard deviation (RSD (%) 1.85 (n = 3) for Embelin at 30°C respectively.

Robustness and Ruggedness studies

The method was found to be re-producible from one analyst to another. The low values of R.S.D. (1.873 % - 3.219 %) obtained after small deliberate changes of the conditions (mobile phase composition, column temperature, different lot of stationary phase, analyst and equipment) used for the method indicated its robustness.

CONCLUSION

The need for quality assurance, including confirmation of the label strength and content uniformity has long been recognized even for herbal medicinal products. A high-performance liquid chromatography method has been developed for the detection and quantitation of major constituents of *Embelia ribes* extract using a photodiode array detector. Analysis of *Embelia ribes* extract samples with the proposed method does assure prolong life of column and system due to lower percentage of acid in mobile phase and Embelin can be quantitated successfully, using standard calibration curve. The method was found to be specific and suitable for routine analysis because of its simplicity, and reproducibility. The relative standard deviation for the investigated *Embelia ribes* extracts indicates that the method is precise and reproducible.

REFERENCES

1. Januaro AH, Fatima DM, De Silva F, Viera PC, Fernandes JB, Dammarane and cycloartane triterpenoids from three Rapanea species. *Phytochemistry* 1992; 4: 1251-1253.
2. Githiori JB, Höglund J, Waller PJ, Baker LR, *Vet. Parasitol.* 2003; 118 (3-4): 215-226.
3. Swamy KHM, Krishna V, Shankarmurthy K, Rahiman AB, Mankani KL, Mahadevan KM, Harish BG, Naika RH, Wound healing activity of Embelin isolated from the ethanol extract of leaves of *Embelia ribes*. *J. Ethnopharmacol.* 2007; 109 (3): 529-534.

4. Ma OSW, Saunders RMK, Comparative floral ontogeny of Maesa (Maesaceae), Aegiceras (Myrsinaceae) and Embelia (Myrsinaceae): taxonomic and phylogenetic implications *Plant Syst. Evol.* 2003; 243: 39-58.
5. Ravikumar K, Ved DK, Illustrated field guide: 100 Red Listed Medicinal Plants of Conservation Concern in Southern India, Foundation for Revitalisation of Local Health Traditions, Bangalore 2000, 467
6. Warrier PK, Nambiar VPK, Ganapati PM, Some Important Medicinal Plants of the Western Ghats, India: a Profile, Blackwell Publishers, New Delhi 2001, 141-156
7. Seth SD, Johri N and Sundaram KR, Antispermatogenic effect of Embelin from *Embelia ribes. Ind. J. Pharmac.* 1982; 14 (2), 207-211.
8. Anonymous, Wealth of India: Raw Materials, Vol III, CSIR, New Delhi, 1952, 167 – 168.
9. Chauhan SK, Singh BP, Agrawal S, *Indian Drugs* 1999; 36: 41-43.
10. Krishnaswamy M, Purushothaman KK. Antifertility properties of *Embelia ribes*: (Embelin). *Indian J Exp Biol* 1980; 18: 1359.
11. Chitra M, Sukumar E, Suja V, Shyamala Devi CS. Antitumor, anti-inflammatory and analgesic property of Embelin, a Plant Product. *Chemotherapy* 1994; 40: 109.
12. Githiori JB, Hoglund J, Waller PJ, Baker LR, "Evaluation of anthelmintic properties of some plants used as livestock dewormers against *Haemonchus contortus* infections in sheep. *Parasitol.* 2004; 129 (2): 245-253.
13. Rathinam K, Santhakumari, Ramiah N, *J. Res. Indian Med. Yoga & Homeopathy* 1976; 11 (4): 84-90.
14. Li XH, McLaughlin JL, Bioactive Compounds from the Root of Myrsine Africana. *J Nat. Prod.* 1989; 52 (3): 660-662.
15. Indian Herbal Pharmacopeia Revised edition Published by Indian Drug Manufacturer's Association, Mumbai. 2002, 500 – 501.
16. Draves AH, Walker SE Determination of Hypericin and Pseudohypericin in pharmaceutical preparations by liquid chromatography with florescence detection. *Journal of Chromatography B Biomed. Sci. Appl.* 2000; 749 (1): 57-66.