



Simultaneous Estimation of Plumbagin and Embelin by Reverse Phase-High Performance Liquid Chromatographic method

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Abstract

A simple, rapid, precise RP-HPLC method was developed for simultaneous estimation of plumbagin and embelin containing different extract. The maximum resolution was achieved by using mobile phase acetonitrile: 50 mM potassium dihydrogen phosphate buffer: acetonitrile in the ratio (45:55) at pH 3.5. This mixture was found to be appropriate allowing good separation of both the components at a flow rate of 1 ml/min and detection wavelength 290 nm. Qualitative and quantitative results obtained using an HPLC method are described, which will be useful for developing highly effective herbal drug.

Keywords: *Plumbago zeylanica*; *Embelia ribes*; Extracts; HPLC/UV

Introduction

Plumbago zeylanica L. is a semi-climbing shrub that grows throughout Asia and Africa. This plant is distributed in thickets and grasslands at low elevations in Taiwan [1]. The whole plant and its roots have been used as a folk medicine for the treatment of rheumatic pain, dysmenorrhea, carbuncles, and contusion of the extremities, ulcers and elimination of intestinal parasites [2]. In traditional Indian medicine, *P. zeylanica* L. has been assigned medicinal properties and is used in formulations for a number of ayurvedic compounds [3]. Skin disorders are among the major diseases for which traditional medicine is utilized at larger scale. There is hardly any location in the world where there is nobody who knows a local plant suitable for application to an early burn or wound. For instance, the traditional practice of topically treating dermatological conditions with plant-derived medicines predates the cultures of ancient Egypt and remains vital today in both civilized and uncivilized cultures [4,5]. A number of plants are used as traditional medicines in Ethiopia as well for the treatment of several skin diseases, which include, among others, eczema, psoriasis, and fungal infections. *Plumbago zeylanica* (*Plumbaginaceae*), locally known as Amera (in Amharic) is a shrub widely distributed in the West and Northwest parts of Ethiopia at 1500–2200m above sea level. It is also widely spread in tropical and subtropical regions of Asia, Australia and Africa [6]. In Ethiopia, it is traditionally used for the treatment of wound, eczema, scabies, leishmania, leprosy and rheumatoid pain [7]. The roots of *Plumbago* species have been demonstrated

to possess immunosuppressive and antitumor activities. Moreover, root extracts of *Plumbago zeylanica* are used by many of the population in South Africa as an oral treatment for complaints related to infections of the urinary tract.

Pharmacological screening of *Plumbago zeylanica* has revealed that the alcoholic extract possesses antimicrobial activity [8]. Phytochemical screening of extracts of *Plumbago zeylanica* revealed the presence of several constituents, including plumbagin, lineoleic acid, palmitic acid, nonylnonanoate, stigmasterylacetate, lupeol acetate, friedelinol, lupeol, lupanone, sitosterone and stigmasteryl [9-12]. Plumbagin is the major constituent of the root of this plant and reported to possess antimicrobial, antiprotozoal, antifertility, pesticidal activities [13]. In addition, plumbagin is also shown to have a glucogenic effect [14,15]. *Embelia ribes* Burm. is a threatened woody shrub belongs to the family Myrsinaceae, which is sparsely distributed in the moist deciduous forests of the Western Ghats, India, Sri Lanka, Malaysia and South China [16]. In Indian system of medicine 'Ayurveda', the plant is popularly known as Vidanga or Bashmak or Krimigna (Sanskrit); Baberang or Wawrung (Hindi); Vayuvilanga (Kannada) and it is used as one of the adjuvant in most of the drug preparations. The whole plant is used in the treatment of anti-inflammatory to relieve rheumatism and fever [17]. The fruit is bitter in taste, good appetizer, cures tumors, ascites, bronchitis, jaundice and mental disorders [18]. Seeds are used as antibiotic, anthelmintic, antituberculosis, alterative and stimulative [19]. Leaves are astringent, demulcent, depurative and useful in pruritus, sore throat, ulcers of mouth, indolent, skin diseases and leprosy [20]. The traditional medical practitioners residing in the vicinity of the Lakkinakoppa forest range of Bhadra Wild life Sanctuary, are being used the tender leaf paste of this species to cure cut wounds and leprosy. In the present paper, we report the HPLC/UV analyses of different extracts of the aerial parts of two samples of *Plumbago zeylanica* L and *Embelia ribes*. We have developed a chromatographic method to detect and quantify plumbagin and embelin from various extracts simultaneously. This analytical method was used to evaluate the content of constituents in petroleum ether, chloroform, ethyl acetate and methanol extracts obtained by cold and hot maceration of the herbal drugs.

Material and methods

Plants material and Chemicals

Standards were obtained from Captain srinivasa murthi drug research institute. *Plumbago zeylanica* L and *Embelia ribes* were obtained from Govt siddha research centre at Chennai in southern India, belonging to Tamil Nadu state. The plant was identified and authenticated by Medicinal Plants Survey and Collection Unit, Ootacamund, Tamil Nadu, India. All the solvents used for the extraction and HPLC analysis (petroleum ether, chloroform, ethyl acetate, methanol and acetonitrile) were HPLC grade from Merck. Water was purified using a Milli-Q system (Milford, MA, USA).

Standard and sample solutions

10 mg of each drug containing (plumbagin and embelin) was dissolved in 5 ml of acetonitrile in a 10 ml volumetric flask and the volume was made up to 10 ml with the same solvent (stock solution). Various concentrations were prepared from the stock

solution. 10 mg each of different extracts of *Plumbago zeylanica L* and *Embelia ribes* was dissolved in 5 ml of acetonitrile in a 10 ml volumetric flask and the volume was made up to 10 ml with the same solvent (stock solution). Various concentrations were prepared from the stock solution and used for HPLC analysis and stored between 2 – 8°C until use.

HPLC

Separations were performed on a reversed-phase column Princeton SPHER C-18, (250×4.6) with a particle size of 5µ. The eluents were acetonitrile: 50 mM potassium dihydrogen phosphate buffer: acetonitrile in the ratio (45:55) at pH 3.5 at a flow rate of 1.0 ml/min. The system was operated with oven temperature at 26°C; the injection volume was 50 µl. Before HPLC analysis, each sample was filtered through a membrane (0.45 µm,) and injected immediately. Chromatograms were recorded at 290nm to detect embelin and plumbagin.

Results and discussion

Chromatography

Standardization of plumbagin and embelin in different parts of *Plumbago zeylanica L* and *Embelia ribes* by RP-HPLC method was carried out using the optimized chromatographic conditions. Plumbagin and embelin were extracted by cold and hot maceration techniques. The extract was chromatographed. The typical chromatogram is presented in Fig. 1-2. The results are shown in Table .1

Fig.1 Typical chromatogram of standard solution

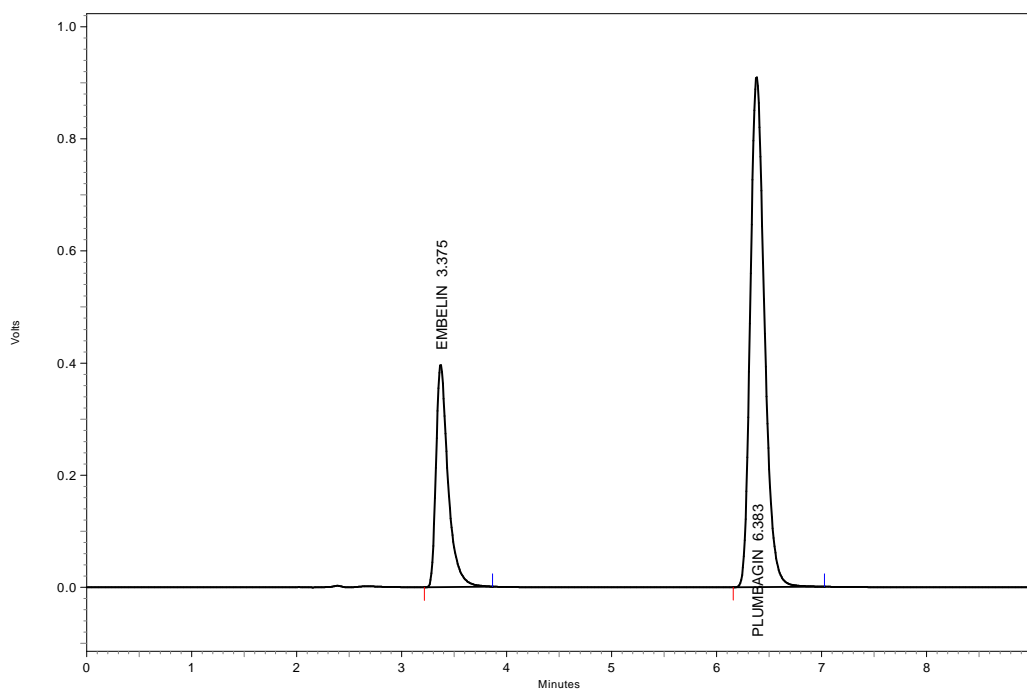


Fig. 2 Typical chromatogram of sample solution

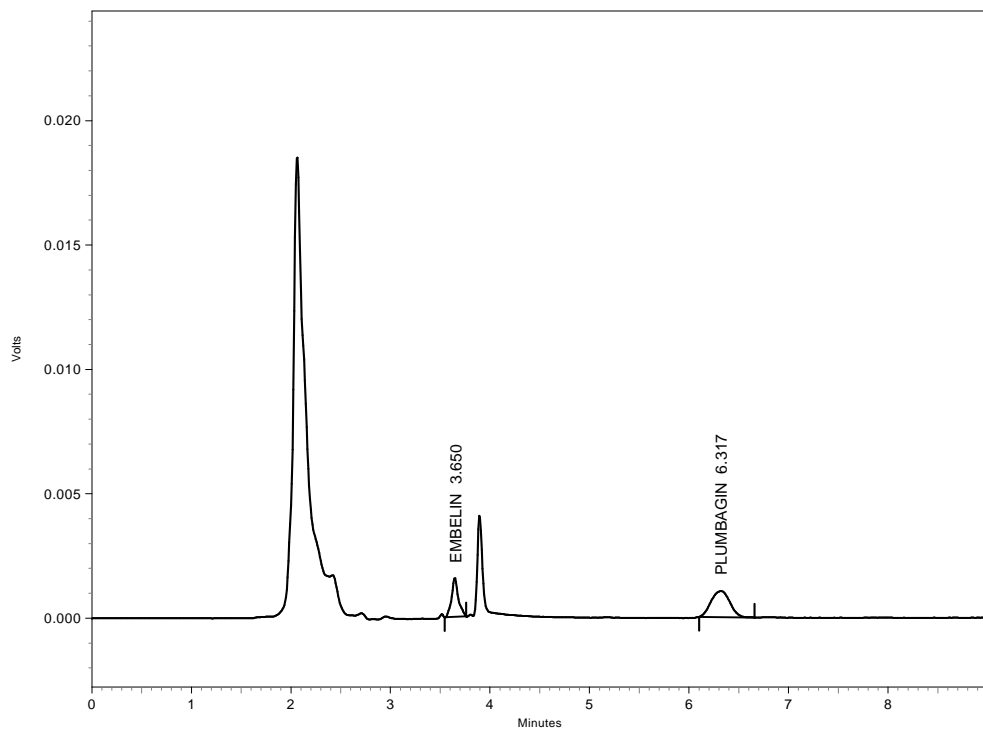


Table 1 Results of HPLC analysis of Plumbagin and Embelin in the extracts

Plant extract Cold maceration		Amount found by proposed Method (% w/w)	RSD (%) (n=5)
Petroleum ether	Embelin	0.0167	1.46
Methanol		1.1203	2.13
Ethyl acetate		0.0456	0.38
Chloroform		1.0721	1.39
Soxhlet extract methanol		1.3514	1.06
Petroleum ether	Plumbagin	0.2158	0.23
Methanol		1.5634	1.26
Ethyl acetate		0.2947	2.57
Chloroform		0.5029	1.31
Soxhlet extract methanol		2.3791	0.97

Method validation

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and is presented in Table 2a and 2b. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra day studies, six repeated injections of validation samples (10, 60, 100 µg/ml for plumbagin and 0.5, 1.50, 3.0 for embelin) were made and the peak area and percentage co-efficient of variation (CV) were calculated. In the inter day variation studies, six repeated injections of validation samples (10, 60, 100 µg/ml for plumbagin and 0.5, 1.50, 3.0 for embelin) were made for three consecutive days and peak area and percentage CV were calculated. The results shown in Table 3a and 3b. From the data obtained, the developed RP-HPLC method was found to be precise.

Linearity and Range

The linearity of the method was determined at seven concentration levels ranging from 10 to 100 µg/ml for plumbagin and 0.5 to 3.0 µg/ml for embelin. The calibration curve was constructed by plotting peak area against concentration of drugs. The slope and intercept value for calibration curve was calculated.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C₁₈, Phenomenex LUNA C₁₈, Princeton SPHER C₁₈ and Hichrom C₁₈. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there are no marked changes in the chromatograms thus demonstrating that the developed RP-HPLC method is rugged and robust.

In conclusion, this study reports the development and validation of a rapid, sensitive and selective assay for the quantitation of Plumbagin and embelin, in *Plumbago zeylanica L* and *Embelia ribes* by reversed phase high performance liquid chromatography (HPLC).

Conclusion

The proposed RP- HPLC method for standardization of plumbagin and embelin in different extracts *Plumbago zeylanica L* and *Embelia ribes* is simple, rapid, accurate, precise, linear, rugged and robust.

Table 2a Results of Recovery analysis

Plant extract	Amount of plumbagin Present in (ng)	Amount of plumbagin added to A (ng)	Plumbagin taken (A+B) (ng)	Total plumbagin plant extract found (ng)	% Recovery (D/C) × 100 (Mean)
	A	B	C	D	
Cold maceration					
Petroleum ether	215.00	500.00	715.00	706.00	98.74
Methanol	1563.00	500.00	2063.00	2015.00	97.67
Ethyl acetate	294.00	500.00	794.00	787.00	99.11
Chloroform	502.00	500.00	1002.00	997.00	99.50
Soxhlet extraction					
Methanol	2379.00	500.00	2879.00	2858.00	99.27

Table 2b Results of Recovery analysis

Plant extract	Amount of embelin Present in (ng)	Amount of embelin added to A (ng)	Embelin taken (A+B) (ng)	Total embelin plant extract found (ng)	% Recovery (D/C) × 100 (Mean)
	A	B	C	D	
Cold maceration					
Petroleum ether	16.00	250.00	266.00	253.00	95.11
Methanol	1120.00	250.00	1370.00	1263.00	92.18
Ethyl acetate	45.00	250.00	295.00	258.00	87.45
Chloroform	1072.00	250.00	1322.00	1274.00	96.36
Soxhlet extraction					
Methanol	1351.00	250.00	1601.00	1486.00	92.81

Table 3a Results of intra and inter-day variability for Plumbagin

Nominal Concentration ($\mu\text{g/mL}$)	Intra -day			Inter- Day (N=5)		
	Mean S.D (\pm)	Precision	% Accuracy	Mean S.D (\pm)	Precision	% Accuracy
10.00	9.45 \pm 0.80	8.52	94.52	9.17 \pm 0.95	10.41	91.72
60.00	55.59 \pm 3.17	5.70	92.66	56.99 \pm 2.20	3.86	94.99
100.00	95.09 \pm 3.86	4.07	95.10	93.49 \pm 6.72	7.19	93.50

Table 3b Results of intra and inter-day variability for Embelin

Nominal Concentration ($\mu\text{g/mL}$)	Intra -day			Inter- Day (N=5)		
	Mean S.D (\pm)	Precision	% Accuracy	Mean S.D (\pm)	Precision	% Accuracy
0.50	0.44 \pm 0.02	6.46	89.20	0.41 \pm 0.01	2.75	82.80
1.50	1.43 \pm 0.04	3.40	95.60	1.42 \pm 0.05	3.53	95.07
3.00	2.77 \pm 0.22	8.08	92.33	2.63 \pm 0.25	9.78	87.67

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