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HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) ANALYSIS OF EMBELIN IN DIFFERENT ORGANS / PARTS OF *EMBELIA RIBES* BURM F. A THREATENED MEDICINAL PLANT OF WESTERN GHATS OF MAHARASHTRA.

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ABSTRACT

Embelia ribes Burm F. is woody climbing shrub belonging to family Myrsinaceae, and is an important medicinal plant of Western Ghats of India. It is used as anthelmintic, carminative, stimulant, antifertility, anticancer and in Herbal cosmetics. The present study was carried out for the first time to investigate the principle bioactive constituent, embelin from different parts of the plant by HPTLC method. Mature black, red, green colour fruits, leaves and fruit cover showed characteristic peaks of embelin. However, a lot of variation in the % of embelin content was observed among the samples of different parts. High percentage of embelin content was observed in mature red colour fruits sample. The HPTLC method has given the reliable quantification of embelin from different organs of *Embelia ribes*. This method can be used for analytical work and for selection of plant part for commercial exploitation. It will help to prepared the monograph of *Embelia ribes*.

KEYWORDS : ***Embelia ribes*, different parts, Embelin, HPTLC.**

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INTRODUCTION

Embelia ribes Burm F. belonging to family Myrsinaceae is one of the oldest herbs in Indian traditional systems of medicine[11]. It is commonly known as false black pepper or Vidanga or Baibirang, widely used in several indigenous systems of medicine[14, 20, 23]. This plant is distributed in some patches of Eastern and Western Ghats of India and supposed to be one of the red listed species in India[15]. The plant is a woody climber with slender branches and long internodes. Stem shows presence of red or rusty colour lenticels. The leaves are elliptic, broad and covered with minute glands. The flowers were small, greenish white / yellow racemes arranged in panicle inflorescence at the end of the branches. The fruits are berries, round, green (young), red and black colour and tipped with style[3]. The fruits are astringent, carminative and stimulants. Traditionally the fruits are employed as a remedy for toothache, headache and snakebite. The seeds are mainly used for maintaining healthy skin and to support the digestive function. It is widely used as anthihelmintic and to cure skin diseases[7, 22]. *Embelia ribes* is the most widely used species in Siddha as well as in Unani medicine and is used in more than 75 Ayurvedic commercial formulations. The seed extract is reported to be antidiabetic[2], anti tumour, analgesic, anti-inflammatory[4,8], antispermatogenic[17], free radical scavenging[9], Embelin is the principle chemical compound reported from the seeds. The other chemical constituent isolated from the seeds are quercitol, tannin, christembine an alkaloid, resinoid, volatile oil and Vilangin[5,18]. In the earlier studies, embelin was isolated from the fruits of *Myrsine africana* L (Myrsinaceae) using analytical methods like HPLC and HPTLC[12]. Variation in phenolic content was analyzed among the different market samples of *Embelia ribes* Burm F.[19]. Some studies have been done in isolation of embelin from *E. ribes* and *Embelia robusta* Roxb. (also known as *Embelia tsieriumcottom* Sensu Wight) using plant extract as standard. But there are no studies on quantification of embelin content in different organs of the plant *E. ribes* Burm F. In the present communication embelin content was quantified by HPTLC method from different parts of the plant body of *E. ribes* Burm F. It will help to check whether other organs like leaf, stem and root can be used as a substitute for fruits for the medicinal purpose. Present paper first time reporting quantification of embelin from different stages of development of fruits i.e green , red and black colour fruits of *E. ribes* Burm F. and in different parts of the plant.

MATERIALS AND METHODS

Collection of plant material

Authentic samples of *Embelia ribes* Burm F. were collected from Koyana area in the month of March to June. Mature black, red, green colour fruits, leaves, stem pieces and root pieces were collected. All collected materials were shade dried and then in oven at 38^0 c for a week (Fig.1a – f). Botanical authentification was performed at B.S.I. Pune. The collected plant and fruits of *Embelia ribes* Burm F. were authenticated at Botanical survey of India, Pune and deposited as collection no. VPS02 at BSI Pune. All the chemicals used were of A.R. grade obtained from Merck Chemicals, India.

Figure 1a
***E. ribes* –Dry Black fruits**

Figure 1b
***E. ribes* -Red fruits**

Figure 1c
***E. ribes* - Green fruits**

Figure 1d
***E. ribes* - Leaves**

Figure 1e
E. ribes - Stem pieces

Figure 1f
***E. ribes* - Roots**

Preparation of extract
Different plant parts of *E. ribes* Burm F. were first crushed into course powder and sieved. To prepare stock solutions of samples of plant material, 500 mg of each sample powder mixed with 5 ml of methanol and sonicated for 1 hr. then centrifuged. The filtrate used for quantification of the embelin by HPTLC analysis. Stock solution of standard embelin was prepared by dissolving 2 mg of standard embelin in 2 ml methanol and sonicated for 15 min. Further it is diluted to get 0.1mg/ ml.

Instrument
Analysis was performed on 10 × 10 cm. 250 µm thick precoated with Silica gel 60 F_{254} TLC plates (E. MERCK KG). Samples were applied to the plates by means of CAMAG Linomet 5 automatic sample spotter with the aid of Hamilton 100 µl syringe. The TLC plates were developed in twin trough Chamber. Detection (densitometry) was performed with a CAMAG TLC Scanner IV, Linked to WinCAT S software.

Preparation of Standard solution
2 mg of Embelin (≥ 98% HPLC, powder make: Sigma) was dissolved in 2 ml of methanol and sonicated for 15 min. in ultrasonic bath. Then 1 ml. of sonicated sample was taken and diluted to 10 ml. with methanol to get 0.1 mg / ml.

Preparation of Sample solution
Appropriate aliquots from the stock solutions of samples of plant material were further diluted with same solvent to obtain 150 µg/ ml spot of embelin.

Preparation of Mobile phase
Chloroform: Ethyl acetate: Formic acid (5:4:1 v/v/v) was prepared in a conical flask and poured into 10 × 10 cm CAMAG twin – trough chamber. Saturation period was of 10 minutes[21].

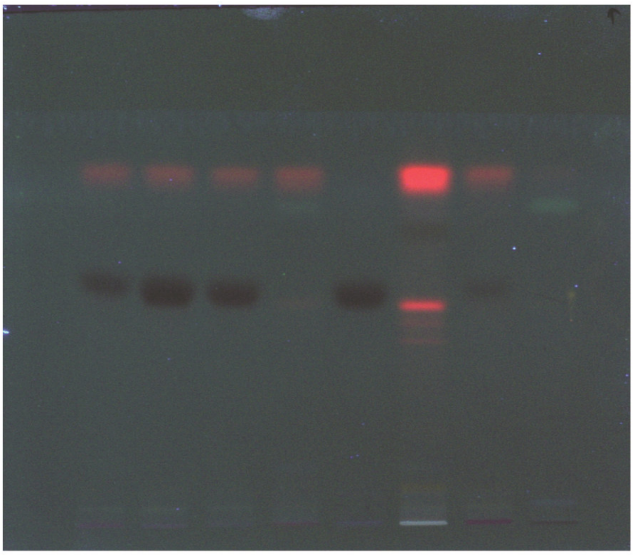
Observations
For quantitative analysis of Embelin in *Embelia ribes* Burm F., extracts of dried powder of each sample were prepared as mentioned in the above section and subjected to optimized HPTLC conditions[16]. Initially many solvent systems were tried. Different system parameters such as composition of mobile phase, method of sample preparation, detection wavelength concentration of sample, amount of sample application were modified to obtain well resolved densitogram.

RESULTS

A solvent system that would give dense and compact band with appropriate RF value was desired for quantification of Embelin in collected samples of *Embelia ribes* Burm F. Various solvent systems like Toluene : ethyl acetate: Formic acid (5:5: 0.5)[13], Chloroform : ethyl acetate: formic acid(5: 4: 0.5) , Chloroform : ethyl acetate: formic acid in different proportions were tried. Chloroform : Ethyl acetate: Formic acid (5: 4: 1 v/v/v) could show a sharp and a symmetrical peak with Rf value 0.58.[21]. HPTLC fingerprint analysis of samples of different parts of *Embelia ribes* Burm F. (fig.2.) showed characteristic peak of Embelin

(fig.3) at Rf value of 0.58. To obtain the fingerprints of authenticated sample, densitogram of samples were overlaid with the densitogram of isolated chemical marker Embelin as shown in (fig.4) which clearly indicated common peak in the sample. It is evident that sample shows peaks at same Rf value as that of Embelin (0.58) and hence can be said to contain same chemical component. After chromatographic development the peak areas of the bands from sample were measured and the amount of embelin was determined from the respective calibration plots. The analytical procedure was repeated three times. Results were shown in table no.1 Samples of different parts of *Embelia ribes* Burm F. showed variation in the amount of embelin content in them. Along with mature black and red colour fruits, even the green fruits showed presence of embelin. Embelin is in traces in leaf. Therefore green fruits and leaves also can be used along with mature black and red colour fruits of *Embelia ribes* Burm F. in preparation of different ayurvedic medicines. For this however different pharmacognostic studies needs to be carried out.

Figure 2
***E. ribes* samples Fingerprint at 366nm wavelength**



[Track 1 – Mature fruit Black colour, 2- Mature fruit Red colour, 3 – Mature fruit Green colour, 4 – Stem powder, 5 – Standard Embelin, 6 – Leaf powder, 7 – Fruit cover powder, 8 – Root powder.]

Figure 3
Standard Embelin peak

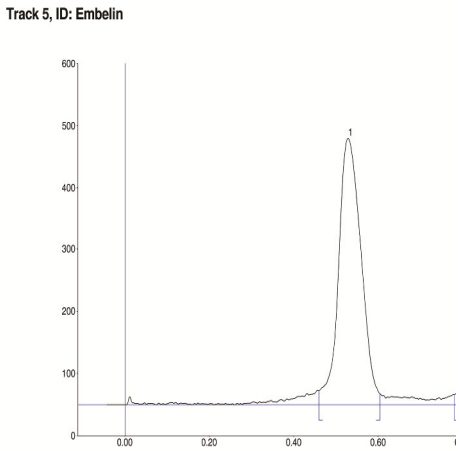
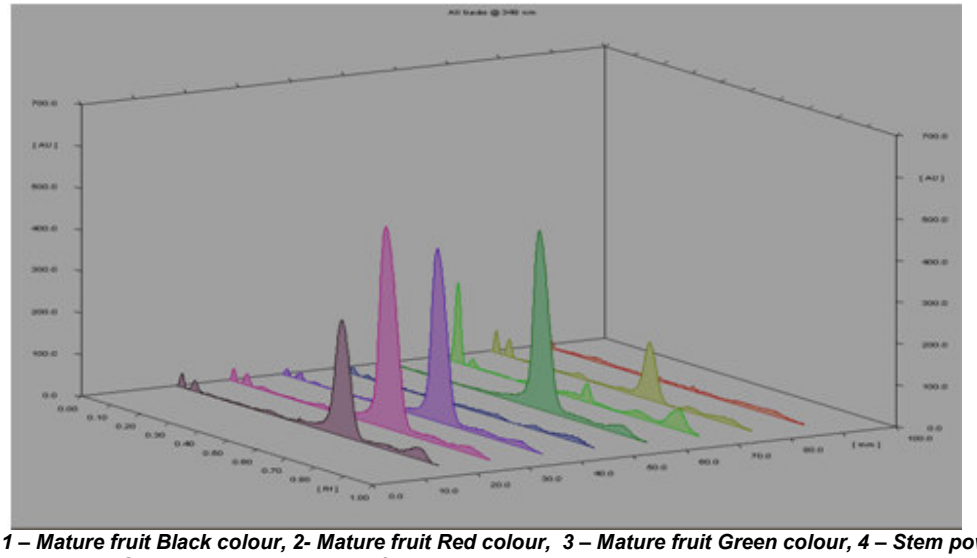


Figure 4
3D densitogram of *Embelia ribes* Burm F. showing Quantity of Embelin in different parts of the plant



Track 1 – Mature fruit Black colour, 2- Mature fruit Red colour, 3 – Mature fruit Green colour, 4 – Stem powder, 5 – Standard Embelin, 6 – Leaf powder, 7 – Fruit cover powder, 8 – Root powder.

Table no.1
Quantitative analysis of Embelin in different parts / organs of *Embelia ribes* Burm F.

Sr no.	Sample*	Concentration	Embelin content – w/w / 100gm
1.	*Embelia ribes* Black fruits.	150 µg / spot	0.43 gm
2.	*E. ribes* Red fruits.	150 µg / spot	0.84gm
3.	*E. ribes* Green fruits.	150 µg / spot	0.64gm
4.	*E. ribes* Stem powder.	150 µg / spot	--
5.	*E. ribes* Leaf powder.	150 µg / spot	0.03gm
6.	*E.ribes* Fruit cover.	150 µg / spot	0.182gm
7.	*E. ribes* Root powder.	150 µg / spot	--

Results are mean of three determinations.

DISCUSSION

H.P.T.L.C. quantitation done at single level. Embelin content in different parts of the plant *Embelia ribes* Burm F. showed a lot of variation. Embelin is present in the cavities on the surface of the seed coat and it is covered by membranous covering of perisperm[6]. Present research shows Fruit cover of *Embelia ribes* Burm F. contain 0.18 % of embelin that is in traces. It confirms that embelin is not present in large quantity in fruit cover but it is located in the cavities present on the seed surface which are covered by the membranous covering called as pericarp. Reported volume of embelin in concentrated extract of fruits of *Embelia ribes* Burm F. is 5.94 % [13] by HPLC analysis of Embelin. Embelin were detected from Market samples of the fruits of *Embelia ribes* Burm F. from Kerala- 4.9 %, Orissa - 4.6 %, M.P - 1.27 % and Maharashtra - 1.20 %.[24]. In fruits of *Embelia ribes* Burm F. embelin was – 2.3 to 3.1 % , *Embelia basaal* (R & S) A.DC embelin was - 1.6 % [10,19]. In fruits of *Embelia ribes* Burm F. it was 4.21 to 4.65 % [1]. Marketed formulations of fruits of *Embelia ribes* Burm F. - 2.19 to 2.20 % (w/w)[21]. In present research embelin content showed variation even in different stages of development of fruits. Red colour mature fruits showed highest content i.e 0.84 gm / 100 gm (0.84 %) embelin then the black mature fruits 0.43 gm / 100 gm (0.43 %) embelin . It shows during maturation or more exposure to light, quantity of embelin decreases. Green colour mature fruits contain 0.64 gm / 100 gm (0.64%) embelin which is higher than black colour

mature fruits. Therefore for medicinal purpose red and green colour mature fruits are more suitable than black colour mature fruits. Embelin is not detected in dried stem and root powder, But embelin is present in dried leaves powder in small quantity i.e 0.03 gm / 100gm (0.03%). And so the leaves can be used in different Ayurvedic medicine.

CONCLUSION

In present research paper first time detected the volume of embelin in different parts of *Embelia ribes* Burm F. collected from the field / forest of Western Ghats of Maharashtra. By dissolved 500 mg powder in 5 ml of methanol and diluted further for quantification of embelin through HPTLC method. Embelin found in powder of *E. ribes* Burm F. mature black fruits is 0.43 %, mature Red fruits. 0.84 %, mature Green fruits 0.64 %, Leaf powder 0.03 %, Fruit cover 0.18 %. In *E. ribes* Burm F. stem and root Powder embelin was not detected so it may be absent or in traces in those two parts of the plant body. The proposed HPTLC method was developed for quantification of Embelin. The method was found to be simple. Hence, the above said method can be successfully applied for routine quality control analysis and quantitative determination of Embelin from *Embelia ribes* Burm F. However different pharmacognostic studies have to be carried out.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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