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Phytochemical and Elemental Profile of *Embelia ribes* Burn. F.

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ABSTRACT

Fruits of *Embelia ribes* Burn. F possesses antifertility activity and is widely used in herbal formulations related to suppress fertility. It is an important component of an ayurvedic contraceptive-Pippalivadi Vati. Medicinal properties of any plant are attributed to the presence of wide array of phytochemicals and minerals. Authentication and identification of plant material for checking adulteration and ensure quality control of plant material is most important problem faced today by herbal industry. In present study preliminary phytochemistry, elemental analysis and chemical fingerprint for fruits of *Embelia ribes* Burn. F was carried out to address these issues. Priliminary phytochemistry revealed presence of wide range of secondary metabolites like Alkaloids, glycosides, flavanoids, phenolics, sterols, steroids, triterpenoids, saponins and tannins. Elements from plants were analysed by ICP-AES technique. 10 elements viz. Copper, Cadmium, Iron, Zinc, Manganese, Sodium, Lead, Nickel, Calcium and Chromium were analysed. Elemental contents of the plant were found to be less than prescribed permissible limits of WHO. The HPTLC fingerprint effectively separates stable chemical constituents and helps us to identify and authenticate plant material. Chloroform: Ethyl acetate: Formic acid (5:4:1 v/v/v) was used as the mobile phase. Derivatisation was carried out with anisaldehyde sulphuric acid and was best observed at 366nm.

Keywords: *Embelia ribes* Burn. F, Phytochemistry, ICP-AES, HPTLC.

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INTRODUCTION

Medicinal properties of plants are attributed to their ability to synthesize wide spectrum of metabolites performing physiological functions and defending pathogenic infections. Natural products from plants are increasingly being identified, isolated and tested for their bioactivity. The bioactivity of plants is also attributed to the presence of minerals. Plants constitutes important source of minerals in human diet. Ancient systems of medicines like Ayurveda and Unani, utilized plants and minerals for therapeutic uses. The traditional Indian medicinal herbs which are responsible for strengthening the body immune system show the presence of many essential and nutritional elements[1]. Increasing demand of medicinal plants by pharmaceuticals industry leads to overharvesting and exploitation thus putting pressure on its natural population. Misidentification of herbals coupled with the use of adulterants and substitute further aggravated the problem. WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [2] . High performance thin layer chromatography (HPTLC) is an effective quality assessment tool for the rapid identification and evaluation of botanical materials. HPTLC based method is being explored as an important tool in routine drug analysis [3].

Embelia ribes Burm.F, is a woody climber, belonging to the family Myrsinaceae. It is popularly known as false black pepper, Vavding or Vidanga. *Embelia ribes* Burm.F grows in semi-evergreen and deciduous forests at an altitude of 1,500 m, throughout India. Aqueous extract of *Embelia ribes* Burn. F*.,* fruit possesses antifertility activity. The fruit powder inhibits 62% fertility in female rats and when administered at the rate of 100mg/day to male bonnet monkeys, it adversely affected quantity and quality of semen. It also led to reduction in testosterone level. Root powder showed 100% antifertility activity in female albino rats for the dosage of 100 mg/kg [4]. Methanolic extract of *Embelia ribes* Burn. F berries along with benzene extract of *Piper longum* lead to inhibition of pregnancy in 80% of animals [5]. Pippaliyadi vati, an ayurvedic contraceptive contains equal parts of powdered fruit berries of *Embelia ribes*, fruit of *Piper longum* and borax powder. It was fed orally to two groups of pregnant rats and humans to study teratogenicity and embryotoxicity . The fetuses of mothers fed with pippaliyadi had low birth weights and were smaller in length with less weight gained during gestation [6].

The present investigation deals with preliminary evaluation of different classes of secondary metabolites, elemental analysis and development of HPTLC fingerprints of medicinal plant *Embelia ribes* Burn. F.

EXPERIMENTAL

Fruits of *Embelia ribes* Burm.F were collected from Western Ghats region of Maharashtra and authenticated at the Department of Botany, RTM University, Nagpur.

Phytochemical Screening

The preliminary phytochemical investigation was carried out with standard protocol for evaluation of secondary metabolites profile of *Embelia ribes* Burm.F. Phytochemicals such as alkaloids (Mayers and Dragendorff's tests), flavonoids (Shinoda test), cardiac glycosides (Keller-Kiliani), saponins (frothing tests), sterols (Liberman-Burchard) and tannins ($FeCl_3$ test) were evaluated [7,8].

Elemental analysis by Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES)

Digestion

Two gram powder of fruit powder was dissolved in nitric acid and it was heated till the reddish brown fumes disappear. Perchloric acid was then added to above solution and heated for 5 min. This was followed by addition of aqua regia and heated then the solution is filtered and the final volume was made up to 25 ml in standard flask by adding deionised water.

Estimation

Estimation was carried out using Inductively Coupled Plasma-Atomic Emission Spectrometer. (Model: ARCOS from M/s. Spectro, Germany)

High Performance Thin Layer Chromatography (HPTLC) Fingerprint

Sample Preparation

Accurately weight 500 mg of fruit powder of *Embelia ribes* Burm.F. was extracted with 10.0 mL of methanol. The mixture was sonicated for 30 min and it was kept overnight for extraction. It was filtered through Whatmann filter paper No. 41 and filtrate was subjected to HPTLC for quantification of Embelin. Plant extracts of the concentration 50 µg/µL was prepared.

Developing Solvent System

Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The desired aim was achieved using Chloroform: Ethyl acetate: Formic acid (5:4:1 v/v/v) was used as the mobile phase and derivatisation was carried out with Anisaldehyde sulphuric acid. The R_f values of these spots were recorded at 254, 366 and 540 nm.

Sample Application

Chromatograph was performed on 20x10 cm aluminium packed TLC plate coated with 0.2 mm layer of silica gel $60F_{254}$ (E. Merck Ltd, Darmstadt, Germany) stored in a dessicator. Extracts of 2µl, 4µl, 6µl, 8µl and 10µl were applied on 8 mm wide band by Hamilton microsyringe (Switzerland), with the nitrogen flow providing a delivery speed of 150nl/s. The syringe was mounted on a Linomat V applicator attached to CAMAG HPTLC system and was programmed through WIN CATS software. Spotting was performed at 25±2^{o}C ascending development of the plate with elution distance of 80 mm (distance to the lower edge was 10 mm).

Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 20 x 10 cm saturated with solvent vapours of respective solvent system for 20 minutes. The linear ascending development was carried out and 25 mL of mobile phase was used per chromatography run.

Detection of spots

The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was dipped in respective derivatizing reagent and dried at 110^{o}C on CAMAG plate heater for 3 min.

Photodocumentation

The plate was kept in photodocumentation chamber (CAMAG TLC VISUALIZER) and captured the images under UV light after derivatization with appropriate reagent at 366 nm and visible light. The R_f values and finger print data were recorded by WIN CATS software version 1.4.6

Densitometric scanning

Finally, the plate was fixed in scanner stage and scanning was done after derivatization at 366 nm and visible light. Densitometric scanning was performed on Camag TLC scanner IV and operated by win CATS software (V 1.4.6).

RESULT AND DISCUSSION

Phytochemical screening revealed that the plant Embelia ribes Burm.F is a good source of secondary metabolites. Preliminary screening shows the presence of secondary metabolites like alkaloids, flavanoids,

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steroids, cardiac-glycosides, saponins, anthraquinones and tannins (Table 1). ICP-AES analysis shows that fruits of Embelia ribes Burm.F are good source of micro and macro elements needed for proper metabolism and development of human body (Table 2). Cadmium and cobalt were found to be negligible with concentration below 0.01 ppm level. Iron, lead, nickel, copper and Zinc levels were found to below the permissible limit set by FAO/WHO. HPTLC fingerprint profile of methanolic extract and densitometric scan (Fig 1, 2) was better visualized at the concentration of 8 and 10 µL after the derivatization at 366 nm.

Figure 1: Fingerprint of fruits of *Embelia ribes* Burm.F. before and after derivatization at 254nm, 366nm and 540nm

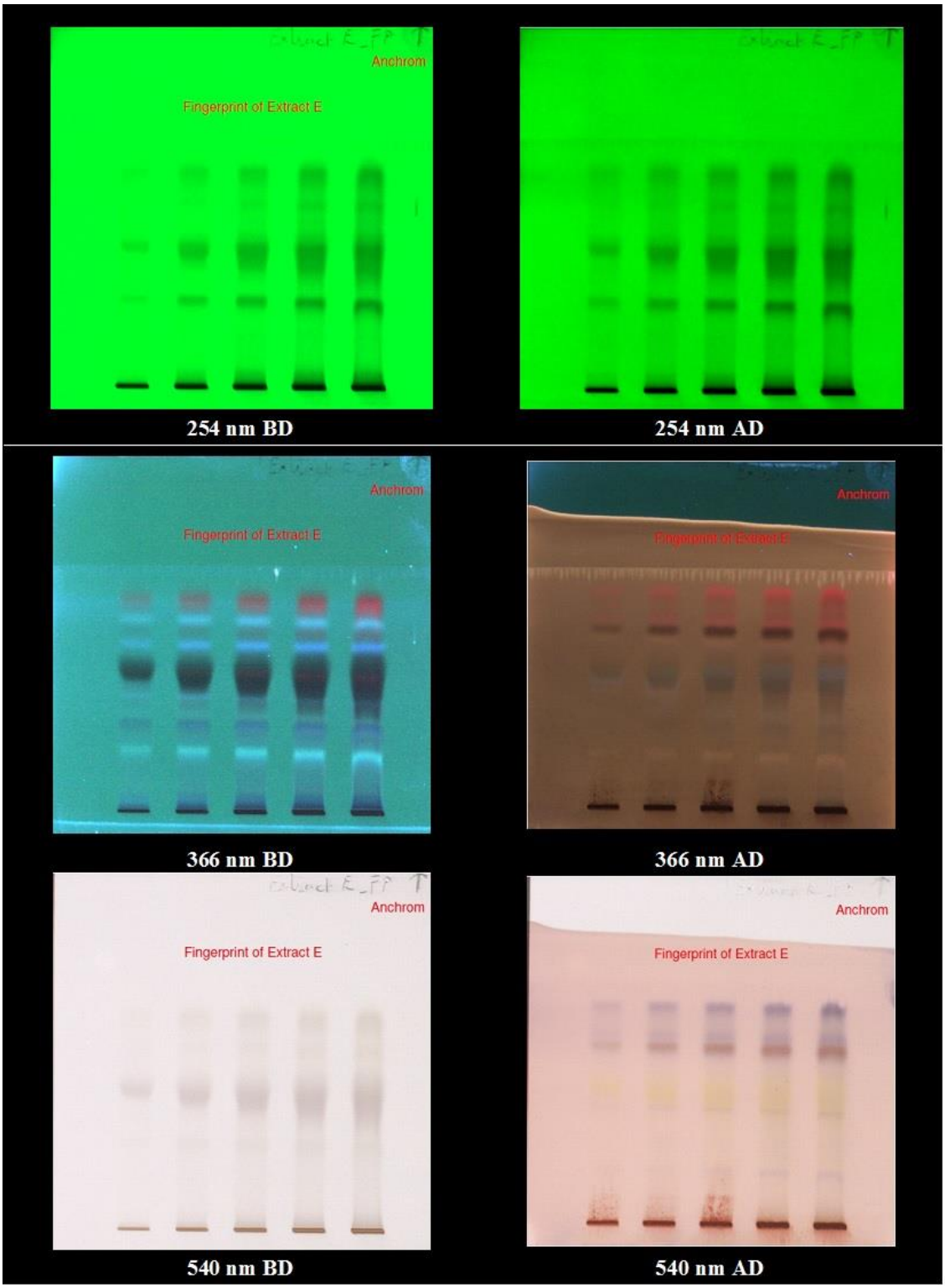

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Figure 2: Densitogram of fingerprint profile of fruits of *Embelia ribes* Burm.F. at 2µL, 4µL, 6µL , 8µL and 10µL concentration.

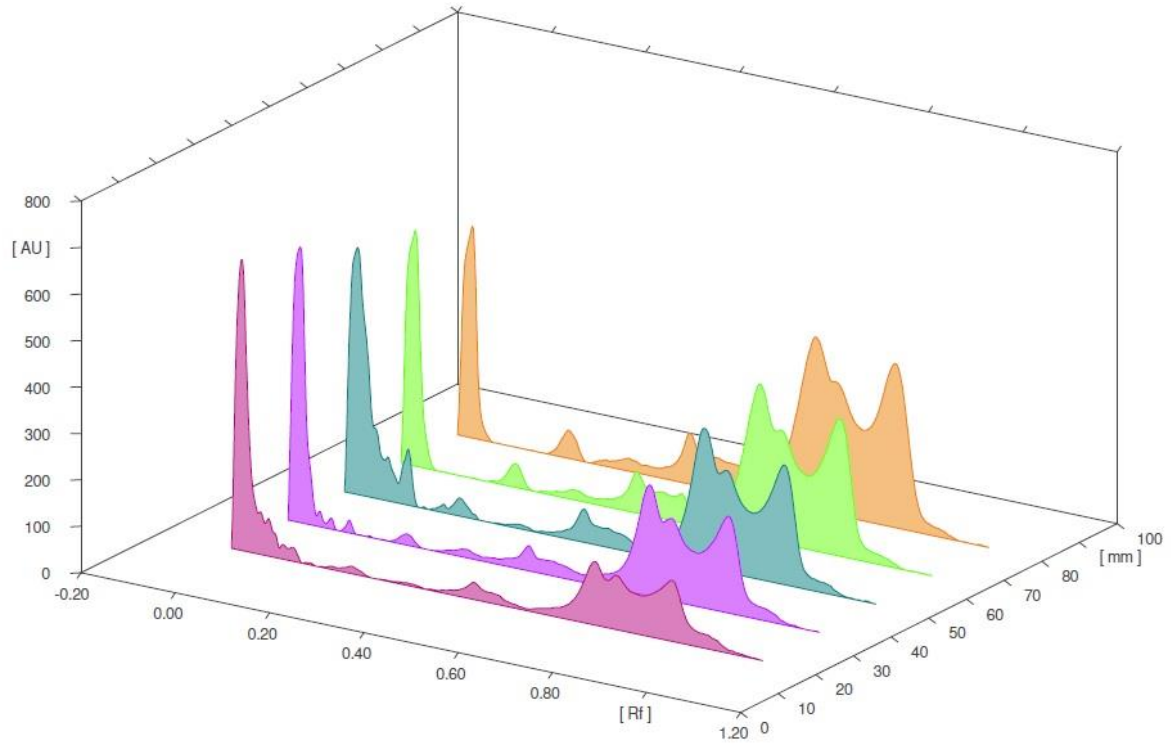

Table 1: Secondary metabolites profile in of *Embelia ribes* Burm.F.

Secondary metabolites	Test	Observation	Inference
Alkaloids	Dragendorff's test Mayer's test	Reddish brown ppt Yellowish ppt	Alkaloids present
Saponins	Frothing test	No frothing	Saponin absent
Steroids	Libermann Buchard test	Blue green colour	Steroids present
Anthraquinones	Borntranger's test:	Pink colour	Anthraquinone present
Cardiac Glycosides	Keller-killani test	Reddish brown junction	Cardiac glycosides present
Tannins	FeCl3 test	Black ppt	Tannins present
Flavanoids	Shinoda test	Reddish colour	Flavanoids present

Table 2: Elemental profile of fruits of *Embelia ribes* Burm.F.

Ca	Cd	Co	Cu	Zn	Fe	Mg	Na	Ni	Pb
168.6	ND	ND	0.716	1.821	5.734	53.779	13.357	0.589	0.123

(Values in ppm; ND means less than 0.01ppm)

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CONCLUSION

The present investigation is a comprehensive study of fruits of *Embelia ribes* Burm.F. It is widely used in herbal medicines and ranks among highly traded species from western Ghats of India, hence elemental and phytochemical standardisation is the need of the hour. A vast array of secondary metabolites was found to be present in the plant under study which is responsible for curative properties of the plant. These natural products are the main sources of bioactive molecules and have played a major role in discovery of lead compounds. Presence of micro and macro elements below the permissible limits of WHO eliminates the danger of mineral toxicity in plant. There is definite correlation between elemental composition and therapeutic capability of the plant, but further research is needed to corroborate the claim. HPTLC fingerprint developed under the above mentioned conditions can be used for authentication and standardisation of the plant material. The fingerprint can serve as a rapid identification tool for correct identification of plant material.

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