

exhibited feeble antibacterial activity but no antifungal activity. From the preliminary phytochemical screening it is revealed that the methanol extract, ethanol extract, chloroform extract showed positive results towards tannins, flavonoids and steroids. So the antimicrobial activity is due to any of these components or all the components. The susceptibility of various microbial agents to these extracts as observed in this preliminary study may suggest some information in developing antimicrobial natural herbal agents which needs further evaluation.

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Determination of embelin in *Embelia ribes* and *Embelia tsjeriam-cottam* by HPLC

S. SUDHAKAR RAJA*, K. P. UNNIKRISHNAN, P. N. RAVINDRAN AND INDIRA BALACHANDRAN
Phytochemistry Division, Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal-676 503

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A simple and reproducible method for the determination of embelin in *Embelia ribes* and *Embelia tsjeriam-cottam* by High Performance Liquid Chromatography was developed. The Embelin content of 4.33 % and 3.96 %w/w was observed in *Embelia ribes* and *Embelia tsjeriam-cottam* respectively. The proposed method being precise and sensitive can be used for quantitative determination of embelin in these plants.

Embelia ribes Burm. f. (Myrsinaceae), commonly known as *Vidangah* is an anthelmintic and is a well-known Ayurvedic drug. Dried berries of the plant are also used in the treatment of constipation, colic, dyspepsia, flatulence and piles^{1,2}. This is a forest species and the availability of the drug is insufficient, so the dried berries of *Embelia tsjeriam-cottam*, closely allied to *E. ribes*, and a common shrub in the plains is often found marketed as *Vidangah*. This plant is reported to have properties more or less similar to that of *E. ribes* and detailed pharmacognosy and pharmacology of both the plants have been carried out³.

Embelin, a dihydroxy benzoquinone, is the main active compound in both *Embelia ribes* and *Embelia tsjeriam-cottam*. The estimation of embelin by reported gravimetric^{4,5} and colorimetric^{6,7} methods involve multiple step extraction, purification, chemical derivatisation etc and thus less precise and time consuming. In the present study, a suitable High Pressure Liquid Chromatographic (HPLC) method for the quantitative determination of Embelin in *Embelia ribes* and *Embelia tsjeriam-cottam* was developed which can be used for the standardization of the plant material.

Dried berries of *Embelia ribes* collected from Kottiyur forest, Kannur district, Kerala and *Embelia tsjeriam-cottam* from Raw Drug Division, Arya Vaidya Sala, Kottakkal, Kerala

*For correspondence

E-mail: sudhakaraja@rediffmail.com

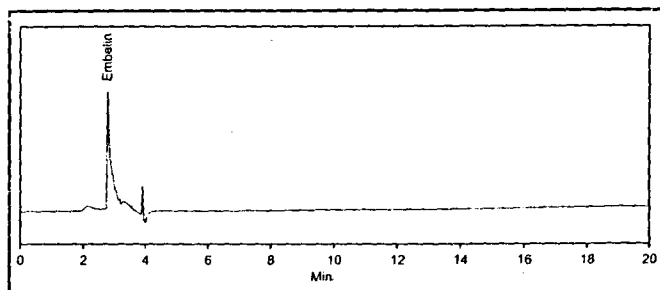


Fig. 1: HPLC Chromatogram of Embelin

were authenticated at the Botany Division, Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala, where the voucher specimens are maintained.

Around 20 g of air-dried berries of *Embelia ribes* and *Embelia tsjeriam-cottam* were powdered and 3 g of the powdered plant material was refluxed with methanol at 60° for 8 h over a water bath. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator below 60°. The concentrated extract was dissolved in 100 ml of methanol and used for the HPLC analysis.

A solution of embelin (1 mg/ml, isolated and purified as per method reported earlier)⁹ was prepared in methanol and filtered using 0.45 µ PVDF filter and used for the HPLC analysis. The identity of embelin was confirmed by UV spectrum.

The Shimadzu HPLC system consisting of LC-10ATVP pump, a rheodyne injector, SPD M10AVP photodiode array detector and CLASS-VP 6.12 SP5 integration software was used for the analysis of embelin. The stationary phase was Phenomenex Luna C 18 (250x4.6mm) column with 5 µ particle size and a guard column. The mobile phase consisting of acetonitrile: water (10:90) was passed through 0.45 µ PVDF filter, degassed and used. The column was equilibrated with the mobile phase for an hour and then pumped at the rate of 1.0 ml/min with a backpressure of 200 kg/sq. cm. The injection volume was 20 µl and the chromatogram was run for 20 min. The injector and the detector were flushed with the mobile phase and the chromatogram was scanned at 237 nm. (Fig. 1)

To validate the method and to know the percentage recovery, a known amount of embelin was added to 3.0 g of previously analyzed powdered samples. These samples were extracted and analyzed by the procedure mentioned above. The embelin content was determined and the percentage recovery was calculated.

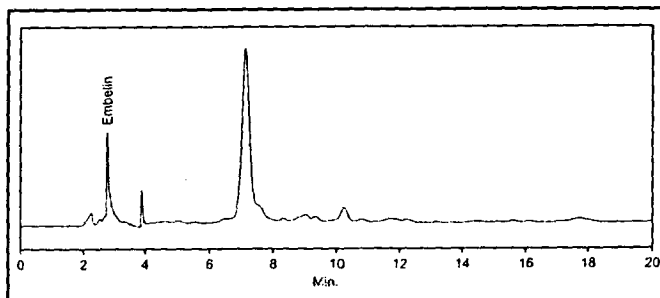


Fig. 2: HPLC Chromatogram of *Embelia ribes*

To select an optimal column for the quantitative determination of embelin, the chromatograms were run using columns of graded polarity. The columns investigated were the octadecylsilyl silica column, columns of intermediate polarity such as the silica column bonded with a monolayer of cyanopropyl moiety and the highly polar unbonded silica column. The best resolution was obtained using the octadecylsilyl silica columns (Phenomenex Luna C 18). The chromatograms were run using different composition of solvents and the optimal mobile phase acetonitrile: water (10:90) with a flow rate of 1.0 ml/min was selected and scanned using photodiode array detector at 237 nm. Using the proposed HPLC method the retention time of embelin was 2.78 min.

The recovery value of embelin by this method is 99.30 ± 0.50 % and 98.94 ± 0.69% w/w for *Embelia ribes* and *Embelia tsjeriam-cottam*, respectively, (Figs. 2 and 3) shows the reliability and reproducibility of the method. The amount of embelin present in *Embelia ribes* and *Embelia tsjeriam-cottam* were 4.33 % and 3.96 % w/w, respectively. The method allows reliable quantification of embelin with a good resolution and separation from the other constituents of the plants. The proposed method is rapid, simple and reproducible for the quantitative determination of embelin in *Embelia ribes* and *Embelia tsjeriam-cottam* and can be used for the routine analytical works.

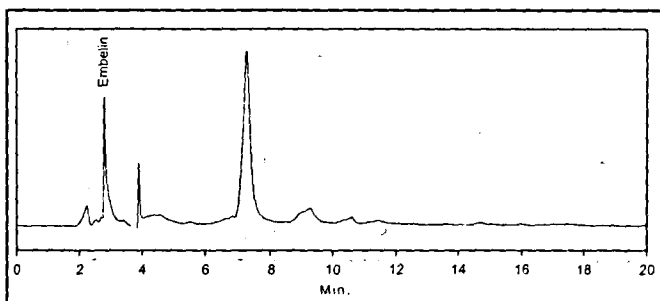


Fig. 3: HPLC Chromatogram of *Embelia tsjeriam-cottam*

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Antioxidant Activity of Ethanolic Extract of *Euphorbia Thymifolia* Linn.

T. PRABHA AND S. K. SINGH*

Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221 005

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The antioxidant activity of the ethanol extract of the whole plant of *Euphorbia thymifolia* Linn. has been evaluated in both *in vivo* and *in vitro* experimental models, by estimating the malondialdehyde content of rat brain, which is one of the products of the lipid peroxidation. The ethanolic extract of the plant showed significant inhibition of lipid peroxidation level comparable to that of Vitamin E used as standard.

Euphorbia thymifolia Linn. (Euphorbiaceae) is a small prostrate, hispidly pubescent, annual weed with numerous horizontally spreading branches occurring in red and green form. It is native of India but also distributed throughout the tropics except North Australia. The leaves, seeds and fresh juice of the whole plant are reported to be used in worm infections, as stimulant and for astringent actions². The plant is reported to contain a large number of phenolics³. As a great deal of interest is being directed towards the bioactivity of the phenolics as dietary source of anti oxidants, it was thought worthwhile to evaluate the antioxidant properties of *E. thymifolia* also, which is rich in phenolics.

The basis of experimental model is the measurement of the amount of malondialdehyde (MDA), which is one of the free radical induced lipid peroxidation products of rat brain phospholipids. MDA forms a colored adduct with thiobarbituric acid (TBA) and are measured colorimetrically. In case of *in vitro* study, FeCl₃ and ascorbic acid has been used for hydroxyl radical (OH[•]) generator while, CCl₄ has been used in *in vivo* study for the generation trichlorocarbon radical (CCl₃[•]).

The plant (*E. thymifolia*) was collected from Tamilnadu and was duly authenticated at the Sugavanam Herbal Center, Pudukkottai. A voucher specimen (No. PCRL 36) has been deposited in the Department of Pharmaceutics, BHU. The air dried whole plant was coarsely powdered

*For correspondence

E-mail: s_ksingh2000@yahoo.com