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Embelin: an HPTLC method for quantitative estimation in five species of genus *Embelia* Burm. f.

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Abstract

Background: The plants belonging to the genus *Embelia*, a significant tropical genus with many biological activities, are benefiting because of their robust medicinal properties. Embelin is one of the principal bioactive molecules responsible for the medicinal properties of the genus *Embelia*. The quantification of the embelin compound among different species in this genus has not yet been investigated, so still uncertain which species and which part should be accepted. The present study was intended to establish a speedy and precise high-performance thin-layer chromatographic (HPTLC) method for quantitative study of embelin in various plant parts of *Embelia ribes*, *Embelia tsjeriam-cottam*, *Embelia basaal*, *Embelia adnata*, and *Embelia gardneriana*.

Result: This research confirmed the method as per the International Conference on Harmonization (ICH) guidelines. We achieved separation on silica gel 60 F254 HPTLC plates using propanol: butanol: ammonia (7:3:7 v/v/v) as a mobile phase. Densitometry scanning performed for detection and quantification at 254 nm and 366 nm. Among the species investigated, the highest amount of embelin was found in *E. ribes* fruits.

Conclusion: *Embelia ribes* fruits are the best source of embelin. Embelin was first described in the endemic species, such as *E. adnata* and *E. gardneriana*. The method illustrated in this research may be applied for quantification of embelin and fingerprint analysis of other species within *Embelia* genus or described genera and chemo taxonomic studies of this genus.

Keywords: Embelin, HPTLC, *Embelia ribes*, *Embelia tsjeriam-cottam*, *Embelia adnata*, *Embelia gardneriana*

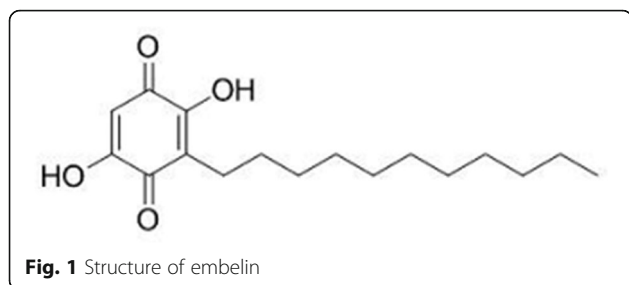
Background

The genus *Embelia* (fam Primulaceae) offers a considerable position among the pharmaceutical field as a number of medicinal properties have been noted. The species under this genus are commonly used in the traditional practices of medicines like Ayurveda, Chinese medicine, and Siddha for a number of decades. In Ayurveda, dried berries of this genus is noted as “vidanga” which carry various biological activities like anthelmintic, carminative, antibacterial, antibiotic, and hypoglycemic properties and is an ingredient in about 75 traditional Ayurvedic drug formulations [1, 2]. New studies revealed

the medicinal potential of this genus by reporting biological properties, like hepatoprotective, analgesic, amylase inhibitory, trypsin inhibition, antibacterial, anticonvulsant, adaptogenic, antifertility, anticancer, antihyperlipidemic, antifungal [3], anti-diabetic [4], antimicrobial [5], antioxidant, and α -glucosidase inhibitory activities [6]. Variety of medicinal properties implies the presence of different potent effective compounds of this genus [6–13].

Embelin (2,5 dihydroxy-3-undecyl-1,4 benzoquinone) (Fig. 1) is a significant active compound present in this genus. Pharmacotherapeutics of embelin are neurodegenerative disorders, hepatoprotective, anthelmintic, anti-inflammatory, nanomedicine, antioxidant, antimicrobial, radio protective, anticancer, respiratory disorders,

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contraceptive, antispermatogenic, anti-infective, antihyperlipidemic, antihyperglycemic, analgesic, antipyretic, gastrointestinal disorders, and wound healing activity [14–17]. The presence of embelin was published in many species of *Embelia* in its fruits, but from the other parts is very minimal. Quantification of embelin from the different parts as well as different species of the *Embelia* is very important, since the new source of this important compound should be identified and explored. The objective of this work was, hence, to determine a simple, solid, cost effective, and rapid HPTLC method for quantification of embelin in different parts of *Embelia ribes*, *E. tsjeriam-cottam*, *E. basaal*, *E. adnata*, and *E. gardneriana*. Marker compounds are being employed for evaluation, standardization, and characterization of medicinal plants [18], and HPTLC is generally accepted applied analytical technique for this [19–21]. The developed method was verified as per the International Conference on Harmonization (ICH) guidelines [22].

Method

Plant material collection

We collected the plant parts of different species of *Embelia* for the study from different wild regions of Kerala and Tamil Nadu states of India (Table 1) with approval solicited from the Forest Department. The material was identified and verified by Dr. Udayan P. S., Head, Department of Botany and Research Centre, Sreekrishna College, Kerala, India, and deposited at CALI herbarium. Based on our previous experience in similar studies, the best season for collection of plants material for phytochemical studies is spring/summer season, so samples were collected during the months of February and March. Collected samples were separated into distinct parts, such as stem, leaves, and bark. In all

the species except *E. basaal*, roots were exempted from the collection because of the limited population. The samples were washed in running tap water, followed by double distilled water and dried at room temperature (28–33 °C). We kept the powdered dry samples in air-tight bottles at room temperature until analysis.

Preparation of sample

Different parts like leaf, stem, bark, root, and fruits of the selected species were shade dried and pulverized. We extracted 10 g each sample in 100 ml of n-Hexane for 6 h in a soxhlet apparatus. Extracts were concentrated by evaporating the solvent and dissolved in methanol (10 ml) and exposed to different analyses for the detection and further quantification of embelin.

Chemicals

All the solvents used for the study were of analytical grade and were procured from Merck (Mumbai, India). The standard embelin was purchased from Sigma-Aldrich, St. Louis, MO, USA.

Chemical method

For the qualitative description of embelin, the extract was dissolved in petroleum ether and diluted ammonia solution was added to it. Formation of bluish violet precipitate shows the presence of embelin.

Development of optimum mobile phase

For the development of optimum mobile phase, the standard embelin was dissolved in methanol and loaded on an activated pre-coated TLC plate using a capillary tube and was allowed to develop in TLC chamber saturated with various mobile phases with various combinations and concentrations of solvents.

Preparation of stock solution

The stock solution of standard embelin (1 mg/ml) was drawn up by dissolving 10 mg of embelin standard in 10 ml of methanol. From the stock solution, using standard solution of embelin was prepared by further dilution of 1 ml of ready solution into 10 ml with methanol (100 µg/ml). For quantification purpose, various volumes of stock solution (1, 2, 3, 4, 5 µl) were spotted on the TLC plate along with sample solution.

Table 1 Collection details of different species of *Embelia*

Sl. no.	Species name	Locality	Voucher specimen no.
1	<i>Embelia basaal</i>	Idukki, Kerala	17691, 17692, 17693, 17694
2	<i>Embelia adnata</i>	Mathikettan Shola National Park, Idukki, Kerala	17695, 17696
3	<i>Embelia gardneriana</i>	Sisparah, Silent Valley, Kerala	17690
4	<i>Embelia ribes</i>	Valparai, Tamilnadu	5795
5	<i>Embelia tsjeriam-cottam</i>	Thrissur, Kerala	5176

Chromatographic instruments and conditions

A CAMAG automatic TLC sampler 4 (CAMAG, Muttenz, Switzerland) and a CAMAG 25 μ l sample syringe with CAMAG ATS 4 applicator were used for sample application. Pre-coated silica gel 60 F254 HPTLC plates (E. Merck, Darmstadt, Germany) of 0.2 mm thickness was used as the stationary phase. After the spotting of samples, the plates were developed in a twin trough chamber pre saturated with mobile phase and run to solvent front 90 mm under room temperature. Photographs of the developed plates were performed employing a CAMAG TLC visualizer at 254 nm and 366 nm. Densitometry scanning of the developed plates were performed using CAMAG TLC scanner in 366 nm and 254 nm with a slit dimension of 6 \times 0.30 mm and better quantification, and data analysis was worked out by the CAMAG winCATS integrated software.

Preparation of calibration curve of embelin and quantification of embelin

Samples and the standard embelin were spotted on a pre-coated and activated silica plates. The different concentration of embelin (1-5 μ l) corresponding to 100, 200, 300, 400, and 500 ng embelin were employed on the plates along with samples with a bandwidth of 8 mm and a distance between tracks of 13.5 mm. Before the development of the plates, the chamber was saturated with the mobile phase. Then the plates were developed to a height of 9 cm at room temperature. After

development, the plates were dried under air current. The photographs of the plates were taken and scanning was performed under 366 nm and 254 nm. The peak areas were noted and the calibration curve was plotted with concentration vs peak areas (Fig. 2).

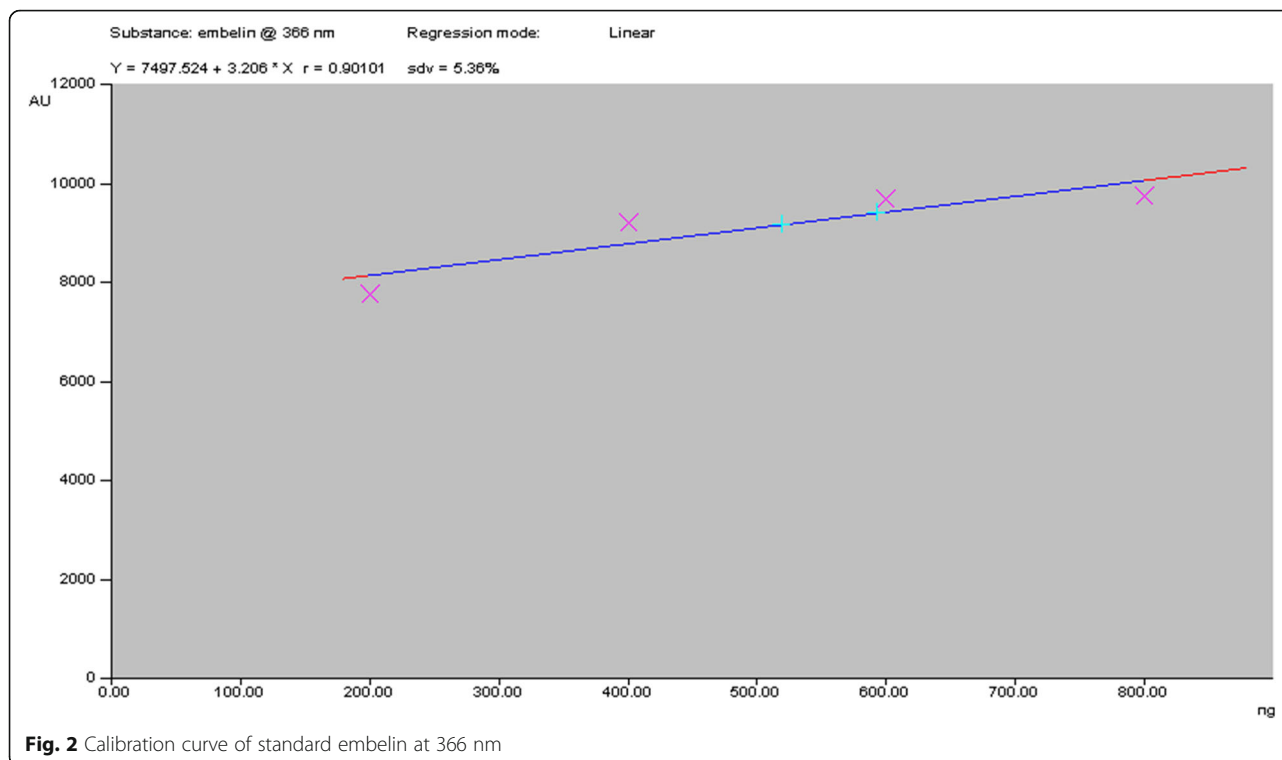
Results

Optimization of HPTLC separation

The optimal mobile phase used for the effective identification and quantification of embelin was propanol:butanol: ammonia in 7:3: 7 v/v/v. This combination resulted in formation of an intact band specifically for embelin (Figs. 3, 4, 5, 6, and 7). The bands were visible with a pink coloration. The R_f value was calculated as 0.6. The standard compound was too scanned, and its spectrum was recorded in the range of 100 to 800 ng. The HPTLC chromatogram of standard embelin is presented in Fig. 8. Of all the 18 investigated samples, except leaves showed a positive result for the presence of embelin. The concentration of embelin in different extracts varied significantly between samples. The proposed HPTLC method was validated in terms of linearity, precision, accuracy, LOD, and LOQ. The summary of the HPTLC validation parameters and R_f value was given in Table 2.

Quantification of embelin in different extracts

The results of the quantification of embelin in different samples studied are presented in Table 3. From the details of the quantification study, it can be noticed that



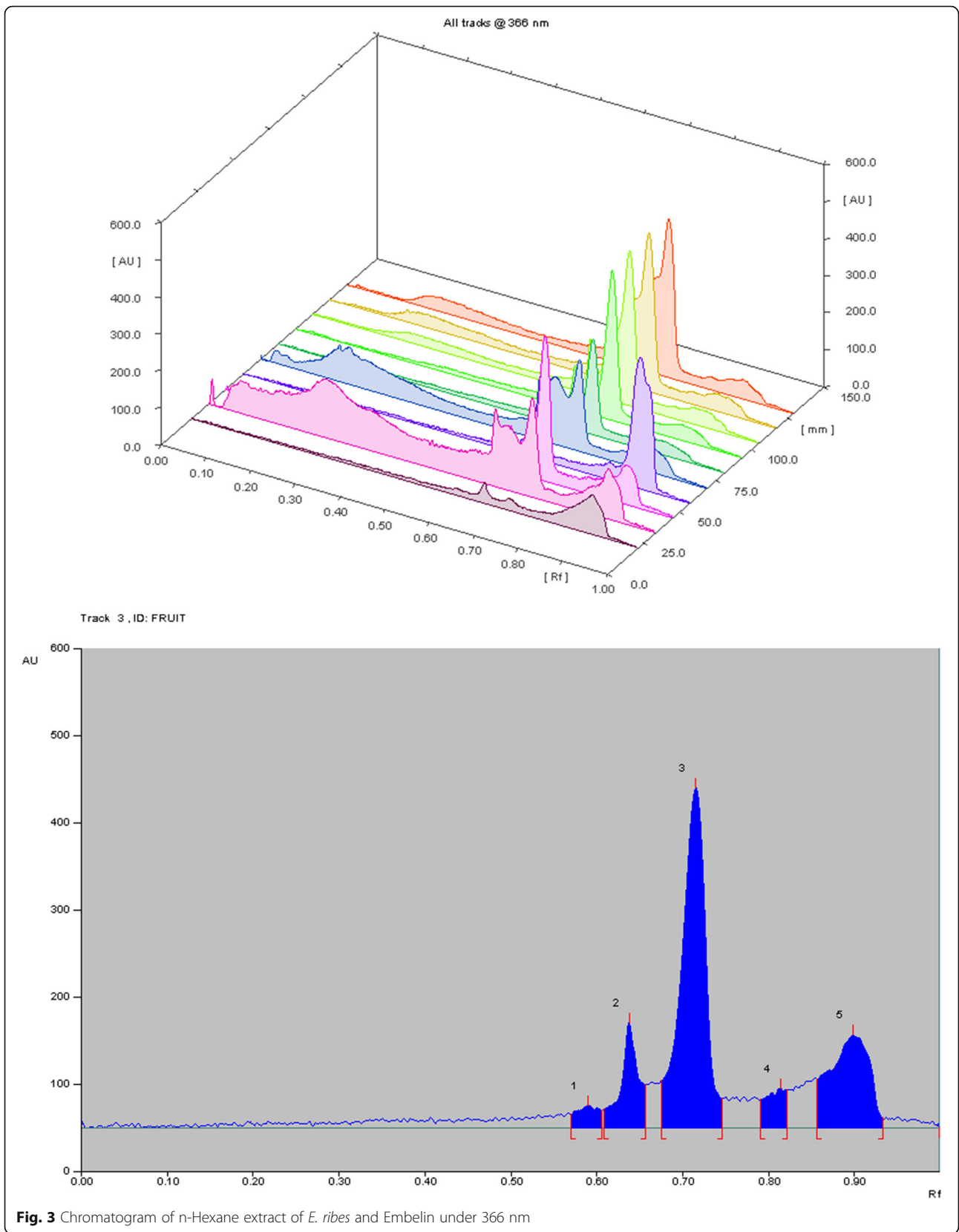


Fig. 3 Chromatogram of n-Hexane extract of *E. ribes* and Embelin under 366 nm

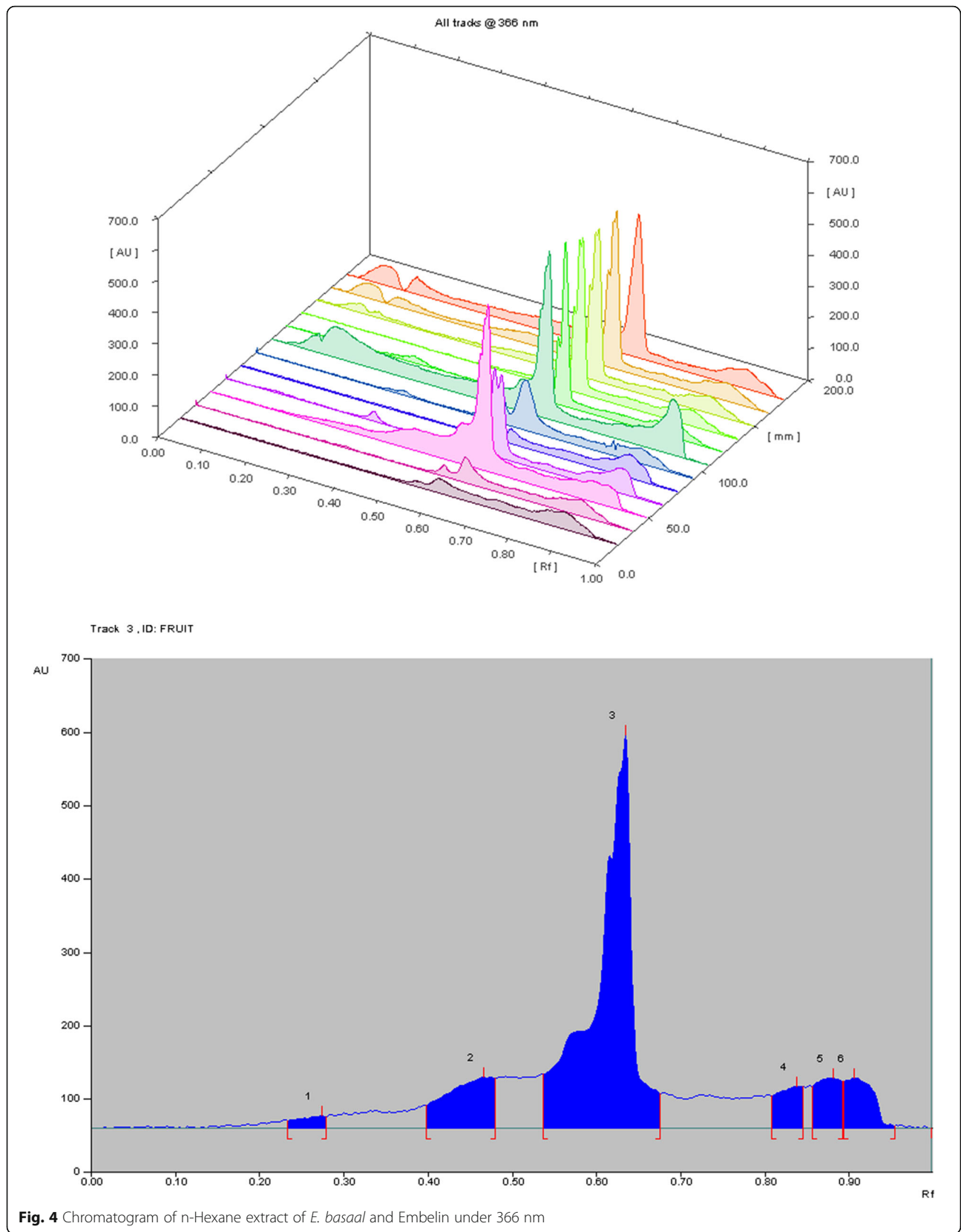


Fig. 4 Chromatogram of n-Hexane extract of *E. basaal* and Embelin under 366 nm

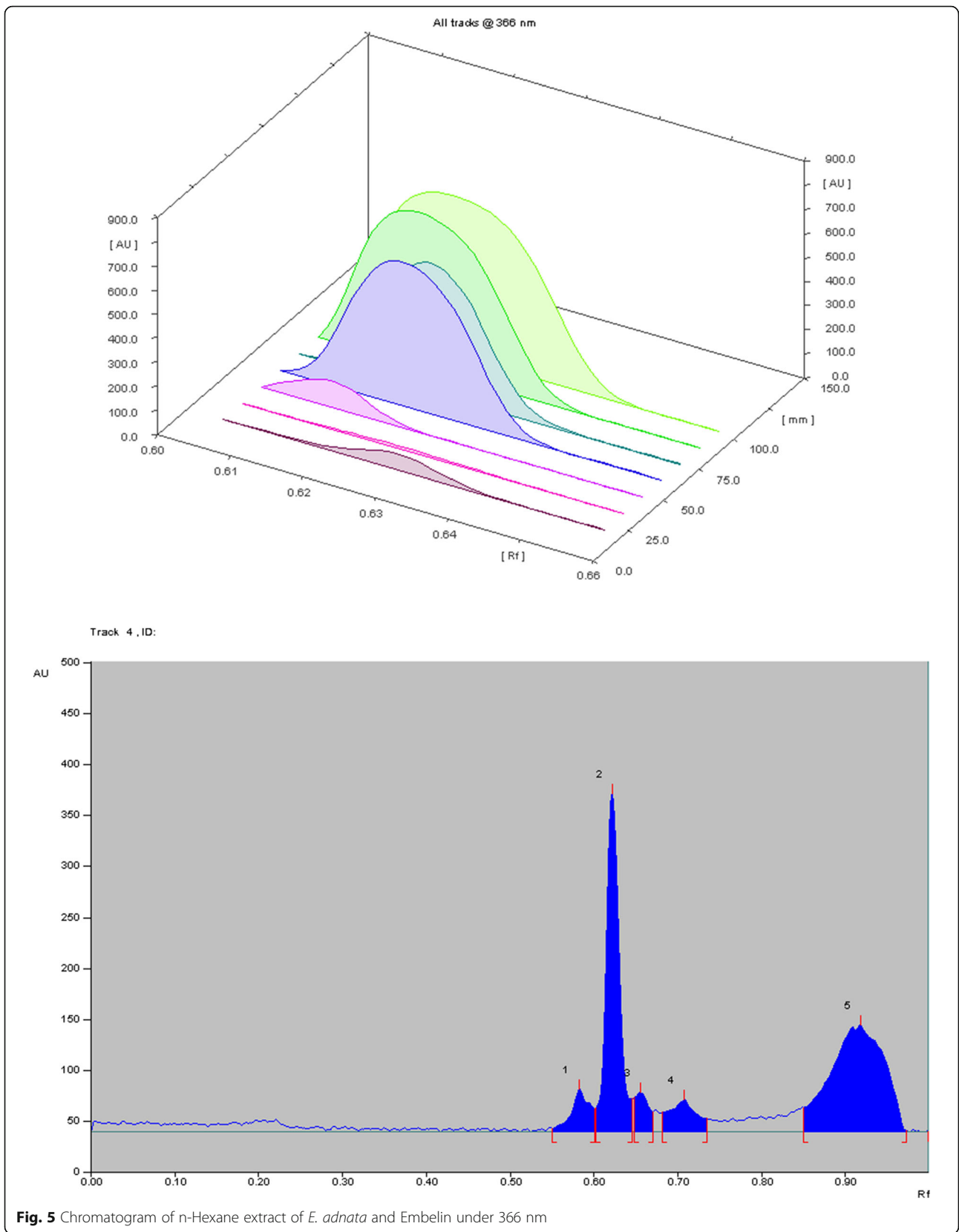


Fig. 5 Chromatogram of n-Hexane extract of *E. adnata* and Embelin under 366 nm

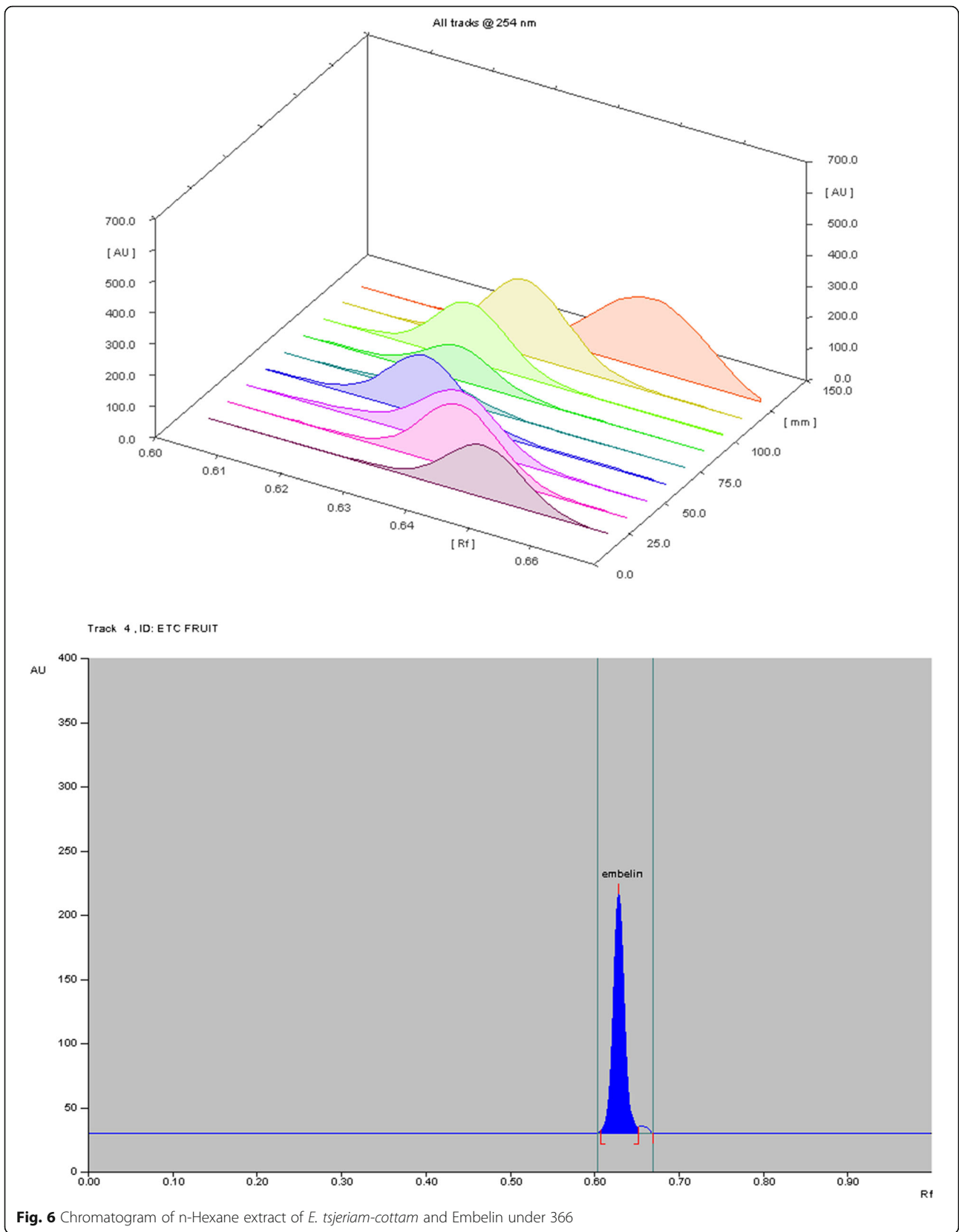
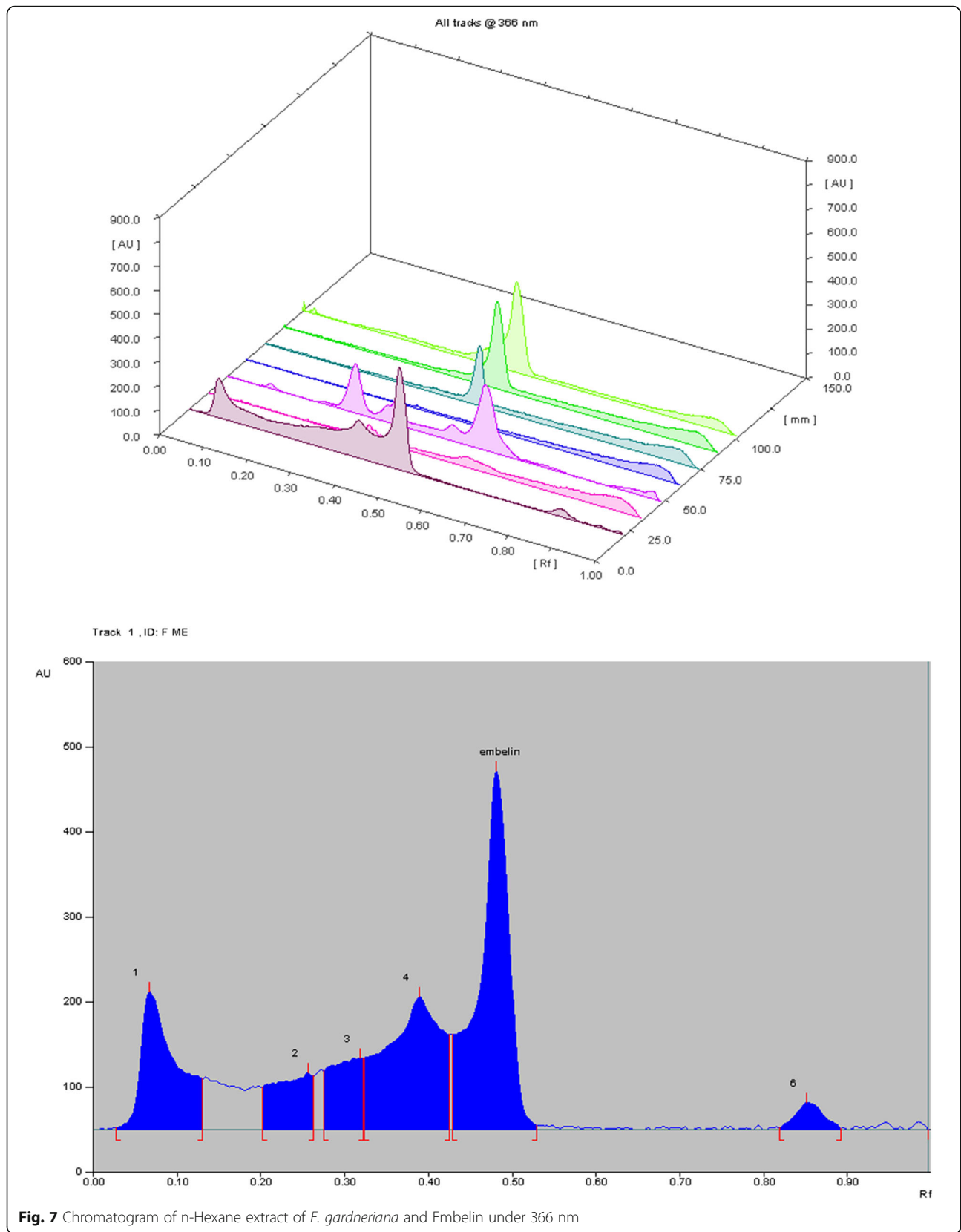


Fig. 6 Chromatogram of n-Hexane extract of *E. tsjeriam-cottam* and Embelin under 366



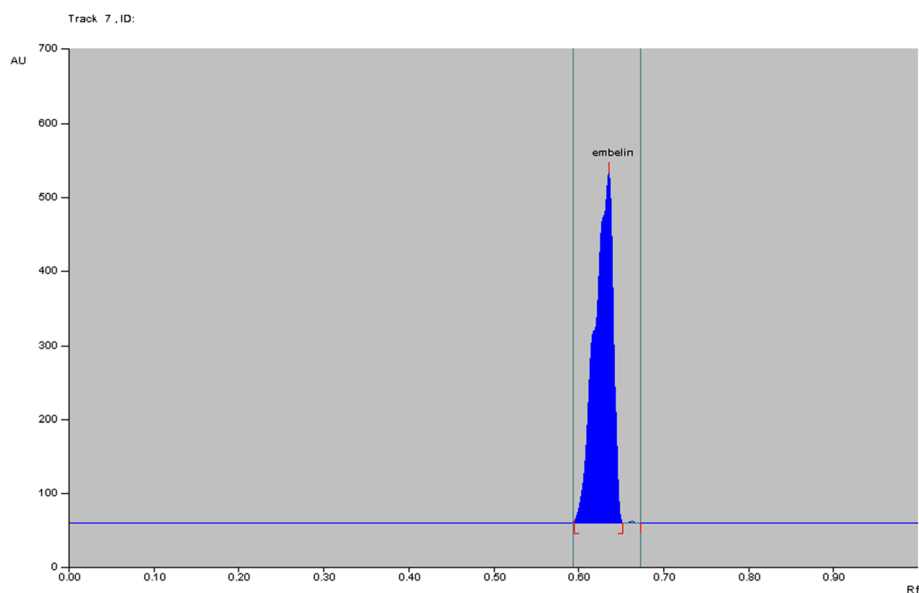


Fig. 8 HPTLC chromatogram of standard embelin at 366 nm

the embelin concentration from different samples ranges from 100 to 900 ppm. The concentration of embelin showed considerable variation among samples. The highest concentration of embelin was established in fruits of *E. ribes* which was followed by *E. basaal* fruits. The lowest concentration was reported in *E. gardneriana* fruits. In all other samples, including stem and bark, even though the presence of the compound was noted, the quantification was challenging due to the very low concentration. It is a very important discovery that the embelin is not detected in the leaves of species we studied.

Discussion

Nowadays, phytochemicals have been receiving recognition and are belonging to an important part of drug design. So identification and quantification of promising compounds from plants have significant value in research. Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) is an alkyl substituted hydroxy quinone isolated from numerous medicinal plants chiefly from

the genus *Embelia* and have various medicinal properties [14, 23]. There are several papers available for the quantification of embelin in different *Embelia* species. Most of the studies were restricted to *E. ribes* and *E. tsjeriamcottam* [24–26]. A good mobile phase equally plays an absolutely vital part in chromatographic methods. As much as assessment of embelin by chromatographic methods is concerned, hardly a few number of HPTLC solvent systems have been described [24, 27]. In our research, HPTLC method was developed with a view in developing an assay method for simultaneous estimation of embelin in a single solvent system. The mobile phase consisting mixture of propanol: butanol: ammonia (7: 3: 7 v/v/v) produced sharp and symmetrical peaks with the Rf value of 0.6. The limit of determination (LOD) is referring to the smallest amount or concentration of an analyte that can be detected but not necessarily quantified as an exact value [28, 29], whereas the LOQ is referring to the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used principally for the determination of impurities and or degradation products [28]. Moreover, it is the indicator of the extraction [29]. The calculated LOD and LOQ in this investigation were identified to be inadequate, and this implies the sensitivity of the method.

The quantification and comparison of embelin within the species of *Embelia* have considerable importance. As well as it is too important to notice out new plant sources of the embelin and to identify in which plant

Table 2 Rf value and linear regression data for HPTLC analysis of embelin

Linearity range (ng)	100-500 ng
Regression equation	7497.524+3.206x
Correlation coefficient (r^2)	0.8118
LOD (ng band ⁻¹)	98 ng per band
LOQ (ng band ⁻¹)	323 ng per band
Rf	0.6

Table 3 Estimation of embelin in different *Embelia* species

Species	Parts used	Quantity of extract	Peak area	Embelin content
<i>Embelia ribes</i>	Bark	5.0 µl	Out of permitted range	Not quantifiable
	Leaf	5.0 µl	Not detected	
	Fruit	5.0 µl	1946.78	0.09%/900 ppm
	Stem	10.0 µl	Out of permitted range	Not quantifiable
<i>Embelia tsjeriam-cottam</i>	Bark	5.0 µl	Out of permitted range	Not quantifiable
	Leaf	5.0 µl	Not detected	
	Fruit	5.0 µl	6449.61	0.0133%/133 ppm
	Stem	10.0 µl	Out of permitted range	Not quantifiable
<i>Embelia basaal</i>	Bark	5.0 µl	Not detected	
	Leaf	5.0 µl	Out of permitted range	Not quantifiable
	Fruit	5.0 µl	9163.20	0.06 % / 600 ppm
	Stem	10.0 µl	837.07	Not quantifiable
<i>Embelia adnata</i>	Stem	5.0 µl	Out of permitted range	Not quantifiable
	Leaf	5.0 µl	Not detected	
	Fruit	5.0 µl	8864.42	0.0213 %/213 ppm
<i>Embelia gardneriana</i>	Stem	5.0 µl	Out of permitted range	Not quantifiable
	Leaf	5.0 µl	Not detected	
	Fruit	5.0 µl	9196.55	0.01%/100 ppm

part have this compound in excess. Fruits are the major source of embelin in all the species studied.

Conclusion

We have established an HPTLC method for the study of the embelin content in different morphological parts of five *Embelia* species. The method followed in the research was confirmed for linearity, precision, accuracy, limit of detection and quantification, and specificity by ICH guidelines. This method may be adopted for quantification of embelin and fingerprint study of alternative species within *Embelia* genus or associated genera. Total embelin content was quantified by the developed method and compared in different plant parts of five species for the first time. This research identified embelin in all the species investigated. The study reported the presence of embelin in two endemic species of Western Ghats. The results establish with new findings suggesting embelin may be chemo taxonomic marker of this genus.

Abbreviations

HPTLC: High performance thin layer chromatography; ICH: International Council for Harmonisation; TLC: Thin layer chromatography; LOD: Limit of detection; LOQ: Limit of quantification; Rf: Retention factor

Acknowledgements

The authors gratefully acknowledge Dr. C.T. Sulaiman, Senior Scientist, Phytochemistry Division, Center for Medicinal Plants Research (CMPR), AVS, Kottakkal, Kerala, India, for his critical comments on the manuscript. The help rendered by Dr. P.S. Udayan, Professor in Botany, Sreekrishna College, Guruvayur, Kerala, India, in collection and identification of the plant species are acknowledged with praise. We also thank Dr. Indira Balachandran, Director, CMPR for granting facilities for quantification studies.

Encouragement and support offered by Dr. SyamViswanath, Director, KSCS TE-KFRI, Peechi, Kerala, and financial support extended by KSCSTE are gratefully acknowledged.

Plant authentication

Botanical authentication of the plant parts was carried out at the Department of Botany and Research Centre, Sreekrishna College, Kerala, India, by Dr. P. S. Udayan where voucher specimens of plants have been deposited in the CALI (University of Calicut) herbarium.

Authors' contributions

All authors have read and approved the manuscript. AVR designed and executed the work. RVKP performed experimental works and data compilation.

Funding

The entire study was financially supported by the plan funded by KSCSTE-KFRI.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

Not applicable. This research article does not contain any studies using animals or human.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 15 December 2020 Accepted: 14 February 2021

Published online: 25 February 2021

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