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Assessment of Embelin in Fruits of *Embelia tsjeriam-cottam* A. DC., A threatened medicinal Plant of Odisha, India

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ABSTRACT

Embelin is the principal phytoconstituent of *Embelia tsjeriam-cottam* A. DC., commonly known as Baibidanga, belonging to the family Myrsinaceae. Embelin, obtained from *E. tsjeriam-cottam* (an alternative source to *E. ribes*), is having diverse biological activities and used as anti-inflammatory, anti-diabetic, antimicrobial, anticancer and antioxidant. Fruit-samples of *E. tsjeriam-cottam* were collected from five different geographical locations of Odisha. These fruits were extracted and assessed for embelin content through spectrophotometric and high performance liquid chromatographic (HPLC) methods utilizing different solvent systems. Significant variation in embelin content was observed among the samples. Embelin content in fruits of *E. tsjeriam-cottam* was ranged from 4.93 to 2.1% (dry wt.), when extracted with chloroform and 4.88 to 1.03% (dry wt.), when extracted with methanol. However, in case of HPLC analysis, embelin content in the selected fruits were varied from 2.72 to 1.32% (dry wt.), when extracted with chloroform and 1.75 to 0.5% (dry wt.), when extracted with methanol. In general, embelin content was found higher in the fruits collected from Chura Reserve Forest (AG₃) followed by Sulia Reserve Forest (AG₄), Bargarh (AG₁), Ghana Reserve Forest (AG₅) and Jajpur (AG₂). Among the selected solvent systems, chloroform prevailed to be the excellent solvent for extraction of embelin.

Keywords: Baibidanga, *Embelia tsjeriam-cottam*, Embelin, Geographical Regions, HPLC, Spectrophotometry

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INTRODUCTION

Embelia tsjeriam-cottam A. DC., is a vulnerable (under RET category) medicinal plant, commonly known as Baibedanga, belonging to the family Myrsinaceae. It is a climbing shrub distributed in the mountains of the Western Ghats, Kerala, Malabar, Meghalaya, Assam and sporadically in Odisha¹. In Odisha, it is distributed throughout Sambalpur, Puri, Ganjam, Phulbani, Deogarh, Nayagarh, Khurda, Gupteswar RF, Gobindpalli and Dhenkanal^{2,3}. Embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone), a naturally occurring alkyl benzoquinone, is the active principle compound in the fruits (berries) of *E. tsjeriam-cottam*⁴. The fruits of *E. tsjeriam-cottam* are used extensively in Ayurvedic system of medicine for the treatment of various diseases such as gastrointestinal disorders, dyspepsia, bronchitis, asthma, anaemia and skin diseases^{1,5}. Polyherbal formulations containing fruits of *Embelia* have been reported for treatment of various liver diseases like jaundice⁶. Embelin has been shown to possess antihelminthic, analgesic, anti-inflammatory, antipyretic, antidiabetic, antimicrobial, anticancer, antioxidant, antiulcer, wound healing, chemopreventive and contraceptive properties^{7, 8, 9, 10, 11}. It also strengthens the nervous system, improves the brain functioning and normalizes the digestive activities. It helps in purifying the blood¹².

Though major source of embelin is fruits of *Embelia ribes*, yet this plant is hardly found in Odisha and in a verge of depletion not only in Odisha but also in other parts of India. Alternatively *E. tsjeriam-cottam*, another threatened species, acts as an alternate source for embelin production. Since it is found in a sporadic manner in few geographic locations of Odisha, embelin content in various fruits of *E. tsjeriam-cottam* required to be quantified to find out the most suitable geographic location for its conservation, growth & development and sustainable utilization. With this background, the present piece of work was undertaken to report embelin content in various fruits of *E. tsjeriam-cottam* obtained from different geographic locations in Odisha through comparative analysis following different methods and solvent systems.

MATERIALS AND METHODS

Materials

Wild fruits of *Embelia tsjeriam-cottam* were collected from five geographic locations of Odisha viz. Bargarh (AG₁) in Bargarh Dist., Jajpur (AG₂) in Jajpur Dist., Chura Reserve Forest (AG₃), Sulia Reserve Forest (AG₄) and Ghana Reserve Forest (AG₅) in Kalahandi Dist. The fruits were shed-dried and powdered for further use.

Standard Preparation

Methanolic stock solution of 1 mg/ml was prepared with embelin (SIGMA) as standard and kept the same in amber vials at 4°C and brought to room temperature before use.

Sample Extraction

Finely powdered *Embelia tsjeriam-cottam* fruit-samples were extracted using Soxhlet method. Powdered sample (10 g) was extracted through Soxhlet apparatus for 16-18 hrs with methanol and chloroform solvent systems separately. The total extract was condensed in dry bath and kept as embelin sample stock solution¹³

Estimation of Embelin through Spectrophotometric Method

By making suitable dilutions to the standard stock solutions, concentrations ranging from 100-1000 µg/ml were prepared for developing standard curve. The absorbance of the prepared solutions was measured at 291 nm as the detection wavelength. A standard curve was prepared with the measured absorbance vs known standard solutions. The absorption of the sample extracts were also measured at the same wavelength & embelin content was quantified referring the standard curve.^{14,15}

ESTIMATION OF EMBELIN THROUGH HPLC METHOD

Identification & Isolation through Thin Layer Chromatography (TLC) Procedure

Crude embelin extract (10 µl each) solution was used for the chromatographic method to identify and isolate the pure embelin. The mobile phase selected for use was n-Propanol: n-Butanol: Ammonia in a ratio of 7:1:2^{16,17} Spots were detected by using 1% solution of vanillin in methanolic sulfuric acid as chromatogenic reagents and viewed under ultraviolet light at 365 nm wavelength for identification of the separated compounds. The R_f value of sample was determined to ensure presence of embelin in the extracted sample against the standard^{16,18,19}.

Evaluation of Embelin through HPLC

HPLC analysis was performed in a Waters make HPLC system equipped with a binary pump (Model-1525) and porous Silica with 5 µm diameter C₁₈ 4.6 × 150 mm column. The mobile phase consisted of a mixture of methanol: 0.1 % TFA in a ratio of 88:12, at a flow rate of 1 ml/min. The peaks eluted were detected at 291 nm wavelengths and identified with authentic standard embelin sample²⁰. The reproducibility of the analysis was verified by ten replicate injections of the standard and three replicate injections of each extract. The HPLC method was validated by defining the linearity, peak purity, retention time, correlation coefficient, limit of quantification and detection, relative standard deviation, accuracy and specificity.

Statistical analysis

All the values are expressed as Mean±SD. The results were analyzed statistically through Two-

way RM ANOVA using GRAPH PAD PRISM 6.0 and variations in both Spectrophotometric and HPLC results were observed at 99% significant level.

RESULTS AND DISCUSSION

Embelin Content estimated by spectrophotometer

Embelin content was found to be ranged from 0.5 to 4.93% (dry wt.) in the wild fruits collected from different geographic zones. Chloroform extracted fruit samples collected from AG₃ region yielded maximum embelin content (4.93% dry wt.), whereas fruits from AG₂ gave minimum amount of embelin (2.1% dry wt.). The results showed significant variation (P value < 0.0001). Methanol extracted fruits collected from AG₃ region produced maximum embelin (4.88% dry wt.) and fruits from AG₂ yielded minimum amount of embelin (1.03% dry wt.). The results showed significant variation at P value = 0.0018 (Table-1, Figure-1).

Table- 1: Embelin content in various fruits of *Embelia tsjeriam-cottam* estimated through Spectrophotometer

Solvent Used	Fruit Source (Geographic zones)	Embelin Content (% dry wt.)
Methanol	AG ₁	1.65 ± 0.208
	AG ₂	1.03 ± 0.169
	AG ₃	4.88 ± 0.601
	AG ₄	3.79 ± 0.645
	AG ₅	1.15 ± 0.054
Chloroform	AG ₁	2.14 ± 0.211
	AG ₂	2.1 ± 0.222
	AG ₃	4.93 ± 0.239
	AG ₄	4.09 ± 0.105
	AG ₅	4.05 ± 0.167

Abbreviations: AG₁-Bargarh, AG₂-Jajpur, AG₃-Chura Reserve Forest, AG₄-Sulia Reserve Forest, AG₅-Ghana Reserve Forest

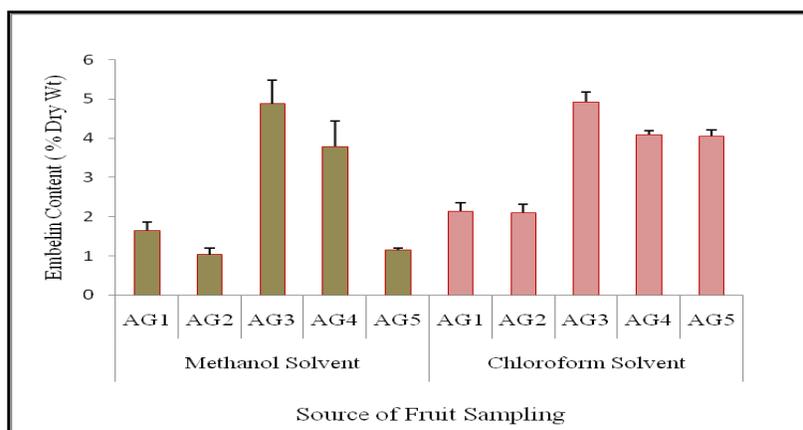


Figure-1: Embelin content in various fruits of *Embelia tsjeriam-cottam* estimated through Spectrophotometer

Embelin content estimated through HPLC

During the course of isolation of pure embelin for HPLC, the R_f values of the standard and the extracted samples were found to be 0.35 through TLC (Figure-3). Amongst chloroform extracted fruit samples, the highest amount of embelin was found in the samples belonging to AG₃ (2.72% dry wt.) whereas fruits sampled from AG₂ region yielded minimum amount of embelin (1.32% dry wt.). The results showed significant variation at P value < 0.0001. However, methanol extracted fruit samples collected from AG₃ produced the maximum embelin (1.75% dry wt.) and fruits collected from AG₂ showed minimum amount of embelin content (0.5% dry wt.). The results showed significant variation at P value = 0.0110 (Table-2, Figure-2).

Table- 2: Embelin content in various fruits of *Embelia tsjeriam-cottam* estimated through HPLC

Solvent Used	Fruit Source (Geographic zones)	Embelin Content (% dry wt.)
Methanol	AG ₁	1.15 ± 0.592
	AG ₂	0.5 ± 0.755
	AG ₃	1.75 ± 0.229
	AG ₄	1.28 ± 0.456
	AG ₅	1.11 ± 0.023
Chloroform	AG ₁	1.65 ± 0.535
	AG ₂	1.32 ± 0.095
	AG ₃	2.72 ± 1.12
	AG ₄	1.75 ± 0.765
	AG ₅	1.51 ± 0.023

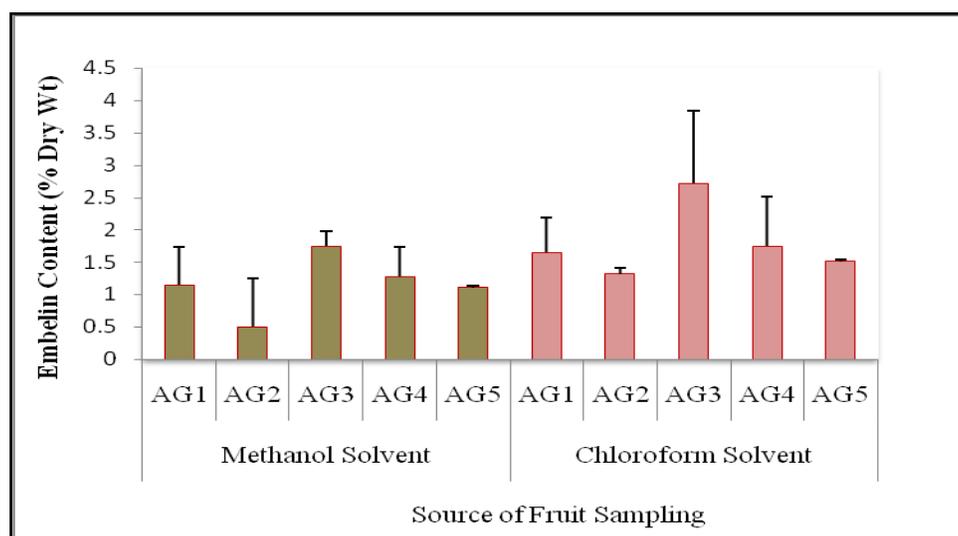


Figure-2: Embelin content in various fruits of *Embelia tsjeriam-cottam* estimated through HPLC

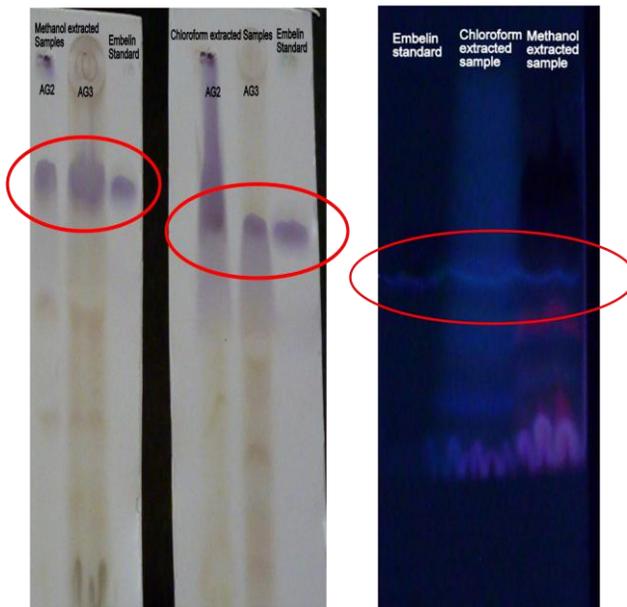
Abbreviations: AG₁-Bargarh, AG₂-Jajpur, AG₃-Chura Reserve Forest, AG₄-Sulia Reserve Forest, AG₅-Ghana Reserve Forest

Plants growing in different climatic and environment conditions, usually vary in their morphological characterization and biochemical constitution. The quality and quantity of active ingredients in medicinal plants, growing in varying geographical locations, also vary considerably. In this piece of work, embelin, i.e. the active principle of *E. tsjeriam-cottam*, collected from various locations were estimated through Spectrophotometric and HPLC method to validate the plant as an immediate alternative to *E. ribes* (due to its' rarity) for embelin yield. Five different geographical locations were considered i.e. Bargarh (AG₁), Jajpur (AG₁), Chura Reserve Forest (AG₃), Sulia Reserve Forest (AG₄) and Ghana Reserve Forest (AG₅).

The Chura reserve forest (AG₃) showed higher yield of embelin in fruit samples followed by Sulia Reserve Forest, Bargarh, Ghana Reserve Forest and finally by Jajpur. Comparative account on embelin content from fruit samples of *E. tsjeriam-cottam* collected from various geographical locations was not reported so far with special reference to Odisha, India. However, similar type of zonal comparison in terms of embelin content was carried out with fruits of *E. ribes* through HPLC analysis by Nagamani et al., 2013. In their study, embelin content was found to be 4.6% (dry wt) within samples collected from Odisha²¹.

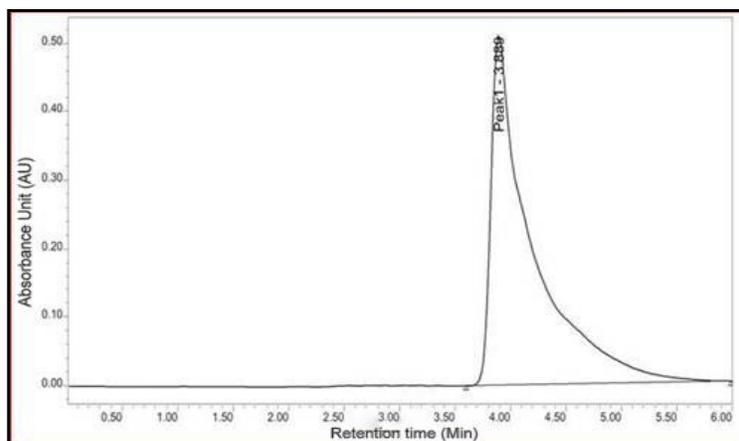
In spectrophotometric analysis, chloroform extracted fruit samples of *E. tsjeriam-cottam* collected from AG₃ region yielded maximum embelin content (4.93% dry wt.). However Methanol extracted fruit samples collected from AG₃ with region produced maximum embelin (4.88% dry wt.). Exact trend is being maintained in both the selected solvent systems, used for extraction process. Moreover when the purified and isolated embelin compounds were analyzed through HPLC procedure, Chloroform extracted fruit samples belonging to AG₃ yielded the highest amount of embelin (2.72% dry wt.) and Methanol extracted fruit samples collected from AG₃ produced the maximum embelin (1.75% dry wt.).

Among the selected solvent systems, chloroform showed promising result in extraction process of embelin than the methanol. A similar type of observation was carried out in which Chloroform extracted fruit samples of *E. ribes* showed the highest yield of embelin (33.34% w/w) whereas Methanol extracted fruit samples yielded 14.31% w/w²⁰. In another study Chloroform extracted fruit samples of *E. ribes* yielded 8.3% dry wt. of embelin content, whereas Methanol extracted fruit sample yielded 3.6 % dry wt. of embelin content. In both the studies chloroform extracted fruit samples showed a prominent higher margin of difference in its embelin content than that of methanol extracted fruit samples, which strongly favours our observations²².

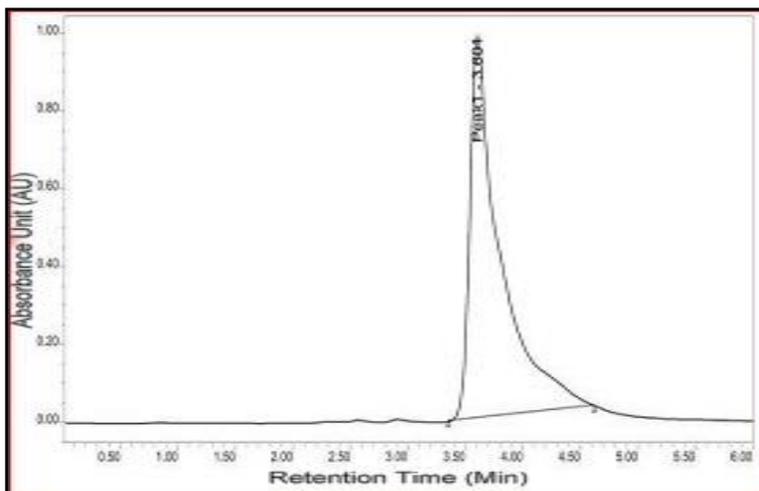


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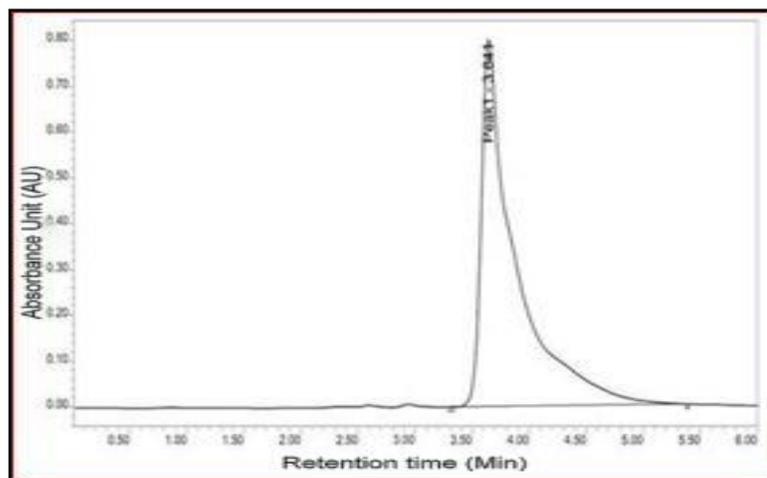
Figure-3: Presence of Embelin in Sample Extracts against Standard on TLC Sheets



Chromatogram of Embelin Standard



Chromatogram of Chloroform extracted embelin sample



Chromatogram of Methanol extracted embelin sample

Figure-4: HPLC Chromatograms of Embelin Standard and Sample

CONCLUSION

Embelin content was assessed from the fruits of *E. tsjeriam-cottam*, collected from different geographic zones, both through Spectrophotometric and HPLC method. From this experiment *E. tsjeriam-cottam* was found to be the suitable alternative for embelin content to that of *E. ribes* and Chura reserve Forest of Odisha is appeared to be the rich source of embelin content plant *E. tsjeriam-cottam*. The *E. tsjeriam-cottam* plants of this geographic zone may be mass multiplied and domesticated properly to help this vital plant, as the source of embelin content, from being extinct entirely. Furthermore chloroform prevailed to be the most effective solvent system for extraction of higher amount of embelin content from *E. tsjeriam-cottam*.

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