Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN : 2277 -4572)

Research Article

QUANTITATIVE ASSESSMENT OF EMBELIN CONTENT FROM LEAF, STEM BARK, ROOT OF *EMBELIA TSJERIAM-COTTAM*

Manisha Mohapatra, Uday Chand Basak *

Seed Bank & Seed Biology Division, Regional Plant Resource Centre, R & D Institute of Forest and Environment Department, Govt. of Odisha, Bhubaneswar, Odisha, India

*Corresponding Author Email: uc_basak07@yahoo.co.in

DOI: 10.7897/2277-4572.06251

Received on: 06/04/17 Revised on: 19/04/17 Accepted on: 28/04/17

ABSTRACT

Embelin is a biologically active Phytochemical in benzoquinone group found in fruits of *Embelia ribes* & *Embelia tsjeriam-cottam*. Since *E. ribes* is not available in Odisha, *E. tsjeriam-cottam* is considered as substitute alternate source of Embelin. Embelin elicits diverse ethno-medicinal and pharmacological activities like anticancer, antioxidant, anti-inflammatory, hepatoprotective, antimicrobial properties and many more. Since fruits have been considered as major source of embelin; roots, stem barks and leaves of *E. tsjeriam-cottam* are not yet tested and used properly for preparation of medicinal formulations though are having significant ethno-medicinal documentations. This research paper deals with quantitative assessment of embelin present in roots, leaves and stem barks of *E. tsjeriam-cottam* from Odisha to report usefulness of non-fruit parts for estimation of embelin. Embelin content was assessed through spectrophotometric and HPLC methods of analysis. In case of chloroform extracted samples, embelin content was found to be higher in root parts (2.02%-Spectrophotometric, 0.57%-HPLC) followed by stem bark (1.94%-Spectrophotometric, 0.53%-HPLC) and leaf (1.82%-Spectrophotometric, 0.51%-HPLC). However, in case of methanol extracted samples, embelin content was found to be higher in root parts (1.6%-Spectrophotometric, 0.45%-HPLC) followed by stem bark (1.52%-Spectrophotometric, 0.24%-HPLC) and leaf (1.465%-Spectrophotometric, 0.23%-HPLC). These findings have revealed possible alternative substitute plant part besides fruits as source of embelin, to meet the demand of embelin yield, which will lead to least exploitation of plant species. Though embelin content found in fruit parts is double as compared to non-fruit parts, but it has got a marginable quantity of embelin that can be utilized further.

Key words: *Embelia tsjeriam-cottam*, Embelin, HPLC, Spectrophotometer

INTRODUCTION

Bioactive natural compounds derived from plants are being used in ample amount for use in human health concerns. Natural products play a key role in meeting the global demand for new pharmacologically active substances. To this effect, "Embelin" is an important phytochemical used in copious quantity in Ayurvedic medicinal system for various drug formulations. Embelin is a biologically active benzoquinone derivative of phenolic compound (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone) and is the principle active compound in the fruits of *Embelia tsjeriam-cottam*[1-6]. *E. tsjeriam-cottam* is a medicinal shrub[7] under vulnerable group in the RET list[8]. It is commonly known as Baibidanga and broadly distributed throughout the greater part of India up to an altitude of 5,000 ft[8]. It is distributed in the mountains of the Western Ghats, Kerala, Malabar, Meghalaya, Assam, Odisha etc[9]. In Odisha (20°95'17"N 85°09'85"E) it is distributed throughout Sambalpur, Puri, Ganjam, Kalahandi, Deogarh, Nayagarh, Khurda, Gupteswar RF, Gobindpalli and Dhenkanal[10]. Embelin exhibits diverse pharmacological activities like anticancer, antioxidant[11], anti-inflammatory, antipyretic, hepatoprotective[12], antimicrobial[13] and chemopreventive properties[9, 14-19], analgesic[11], anticancer activity[4]. The seeds of *E. tsjeriam-cottam* are used extensively for the treatment of various diseases such as gastrointestinal disorders, dyspepsia, bronchitis, asthma, anaemia and skin diseases[9, 20]. Besides the importance and utility of fruits of *E. tsjeriam-cottam*, some scarce literatures are available regarding the medicinal uses of other plant parts i.e. roots and leaves of *E. ribes*[9, 16, 21, 22]. These evident literatures have enlightened the path to evaluate embelin content in other non fruit plant parts. This research paper aims to compare the quantitative estimation of embelin content in roots, leaves and stem barks of *E. tsjeriam-cottam* grown in Odisha to address the importance of non-fruit plant parts as source of the naturally occurring phytochemical embelin to find out suitable alternative substitute plant part besides the fruits.

MATERIALS AND METHODS

Materials

Wild leaves, stem bark and roots of *Embelia tsjeriam-cottam* plant were collected from Ghana Reserve Forest of Kalahandi district, Odisha (19°52′15"N 83°53'30"E) in the month of November. The studied plant species was compared with herbarium specimens present in the institutional herbarium (bearing voucher specimen no 4897) and also verified through the reference book "The Flora of Odisha"[10]. Random sampling was done by taking ten numbers of samples from each plant part and three subsequent replicates respectively. All the samples were dried and pulverized into fine powder for analysis process.

Methods

Standard Preparation

For comparison with the unknown extracted samples, the standard stock solution was prepared taking standard Embelin (SIGMA Aldrich, Germany) in HPLC grade methanol (1 mg/mL) and kept at 4°C for use.

Preparation of Extraction

Finely powdered samples (leaf, stem bark and root) were extracted using conventional Soxhlet method. Powdered samples of 10 g weight of each sample were extracted through Soxhlet apparatus for 16-18 hrs with methanol and chloroform solvent systems separately. The total extracts of each were condensed using dry bath and kept as Embelin stock sample[34].

Estimation of Embelin through Spectrophotometric Method

For quantitative estimation of embelin using UV spectrophotometer, a standard calibration curve was prepared. Standard stock solutions of concentrations ranging from 100-1000 µg/mL were prepared by serial dilution method. The absorbance (OD) of the prepared solutions was measured at 291 nm as the detection wavelength and the standard calibration curve was plotted between the measured absorbance (OD) and given concentrations. The sample extracts of each plant parts were also measured in the same wavelength and the embelin content was quantified[35, 36].

Identification & Isolation through Thin Layer Chromatography

Crude extracted embelin extracts of each plant parts (leaf, stem bark and root) were analyzed through the chromatographic method to identify and isolate the pure embelin compound. The selected mobile phase for separation of pure embelin compound was n–Propanol: n–Butanol: Ammonia in a ratio of 7: 1: 2[37, 38]. The desired spots of embelin were detected by using the chromatographic reagent that is 1% solution of vanillin in methanolic sulfuric acid and viewed under ultraviolet light at 365 nm wavelength for the identification of the separated compounds. The Rf value of each samples were determined to check the presence of Embelin; against the standard[37, 39, 40].

Estimation of Embelin through HPLC Method

HPLC was performed in a Dionex make HPLC system equipped with UVD 340 detector and P 680A pump with TCC-100 column compartment controlled by the chromeleon chromatography Management System. The mobile phase, selected for this purpose, consisted of a mixture of methanol: 0.1 % TFA in a ratio of 65:35, at a flow rate of 1 ml/min. The peaks eluted were detected at 291 nm wavelengths and identified with authentic standard Embelin sample[41]. The reproducibility of the analysis was verified by carrying out ten replicate injections of standard and three replicate injections of each extract**.** The HPLC method was validated by defining the linearity, peak purity, retention time, co-relation coefficient, limit of quantification and detection, relative standard deviation, accuracy and specificity[41, 42].

Validation of HPLC Analysis

The optimized HPLC method, selected in this experiment was validated by defining the linearity, peak purity, co-relation coefficient, limit of quantification, limit of detection, relative standard deviation, accuracy, specificity, peak purity, specificity, recovery, sensitivity, selectivity and precision in the retention time (Table 1). The potentiality of chromatographic interference analysis was verified by carrying out ten replicate injections of standard and three replicate injections of each extracted samples. For determining the intra-day accuracy and precision, five replicates of each sample were analyzed thrice on the same day. The inter-day accuracy and precision were assessed by analysis of five replicates of samples on three different days (1st, 3rd and 5th days).

Linearity, Accuracy and Extraction Recovery

A linear calibration curve was developed for the concentration range of 0-1000 µg mL^{-1}. The relative standard deviation (% RSD) values did not exceed 0.013 for any of the concentrations. After linear regression analysis, the slope (±SD of the mean) for the calibration curve of embelin were found to be 16055 (±0.17) with a regression coefficient (r^2) value of 0.994. All the samples showed 98.67% accuracy of the method for the determination of embelin. Percent recoveries of embelin from all the plant parts of *E. tsjeriam-cottam* were between 97-99% (Table 4). Low % RSD values established the extraction efficiency for the selected solvents used in combination for precipitation and also affirmed the robustness of the method.

Statistical Analysis

All the values are expressed as mean ± SD. The results were analyzed statistically through Two-way RM ANOVA along with Sidak's multiple comparisons test using GRAPH PAD PRISM 6.0 and variations in both Spectrophotometric and HPLC results were observed at 99.9% significant level.

RESULTS

Assessment of Embelin Content through Spectrophotometer

Embelin content, when analyzed through spectrophotometer method was found to be ranged from 1.465-2.02% DW. The highest amount of embelin content was found in the root parts of *E tsjeriam-cottam* as compared to other plant parts (stem bark and leaf) in both methanol and chloroform solvent system. In case of chloroform extracted samples, highest amount of embelin was found in the root parts (2.02% DW) followed by stem bark (1.94% DW) and leaf (1.82% DW). In case of methanol extracted samples, highest amount of embelin was found in the root parts (1.6% DW) followed by stem bark (1.52% DW) and leaf (1.465% DW). The results showed significant variation at P value = 0.0013 (Figure 1; Table 2).

Assessment of Embelin Content through HPLC

Isolation of pure Embelin for HPLC analysis through TLC process revealed the Rf values of both the standard and the extracted samples to be 0.35 (Figure 2). Embelin content was found to be ranged from 0.23-0.57% DW in the wild leaves, stem barks and root parts of *E tsjeriam-cottam* using both the solvent systems. Highest amount of embelin content was found in the roots of *E tsjeriam-cottam* as compared to other plant parts (stem bark and leaf) in both methanol and chloroform solvent system. In case of Chloroform extracted samples, highest amount of embelin was found in the roots (0.57% DW) followed by stem barks (0.53% DW) and leaf (0.51% DW). In case of methanol extracted samples, highest amount of embelin was found in the roots (0.45% DW) followed by stem barks (0.24% DW) and then by leaf (0.23% DW). The results showed significant variation at P value < 0.0001 (Figure 3; Table 3). The chromatograms of all the samples were provided in Figure 4).

Accuracy/Recovery Test of Embelin

To check the accuracy of the developed method and to study the interference of samples, recovery experiment was carried out by standard addition method (Table 4). A known amount of sample was taken. To each tube known amount of Embelin was added. Each sample was analyzed by the developed HPLC method and the amount of Embelin recovered for each level, was calculated.

Table 1: Statistical data for validation of HPLC

Parameters	Values
Absorption maxima	291 nm
Correlation coefficient (r^2)	0.994
Regression equation (Y=bx+c)	Y=16055x+0
Intercept (c)	0
Slope (b)	16055
LOD mg/ml	0.671
LOQ mg/ml	2.035
Retention Time	1.25
Precision (% RSD)	0.013
Accuracy (%)	98.67%

Table 2: Embelin content in various plant parts of *Embelia tsjeriam-cottam* estimated through spectrophotometer

Plant Parts used	Solvent system used	Embelin Content (% Dry Wt.)
Leaves	Methanol	1.465 ± 0.008
	Chloroform	1.82 ± 0.03
Stem Bark	Methanol	1.52 ± 0.023
	Chloroform	1.94 ± 0.02 **
Root	Methanol	1.6 ± 0.015
	Chloroform	2.02 ± 0.078 **

NB-All values expressed as Mean ± SD, (n=3). Statistical differences were tested by Two-way RM ANOVA followed by Sidak's multiple comparisons test, where **P=0.0013

Table 3: Embelin content in various fruits of *Embelia tsjeriam-cottam* estimated through HPLC

Plant Parts used	Solvent system	Embelin Content (% Dry Wt.)
Leaf	Methanol	0.23 ± 0.006
	Chloroform	0.51 ± 0.01
Stem Bark	Methanol	0.24 ± 0.025
	Chloroform	0.53 ± 0.015 ****
Root	Methanol	0.45 ± 0.006
	Chloroform	0.57 ± 0.01 ****

NB-All values expressed as Mean ± SD, (n=3). Statistical differences were tested by Two-way RM ANOVA followed by Sidak's multiple comparisons test, where ****P<0.0001

Table 4: Recovery/accuracy test of embelin through HPLC analysis

Sl No.	In Sample (mg/ml)	Added (mg/ml)	Estimated (mg/ml)	% RSD	% of Recovery
1	50	100	146.37±0.007	0.0048	97.58
2	100	150	247.72±0.061	0.025	99.1
3	150	200	347.65±0.032	0.009	99.33

NB-All values expressed as Mean ± SD, (n=3)

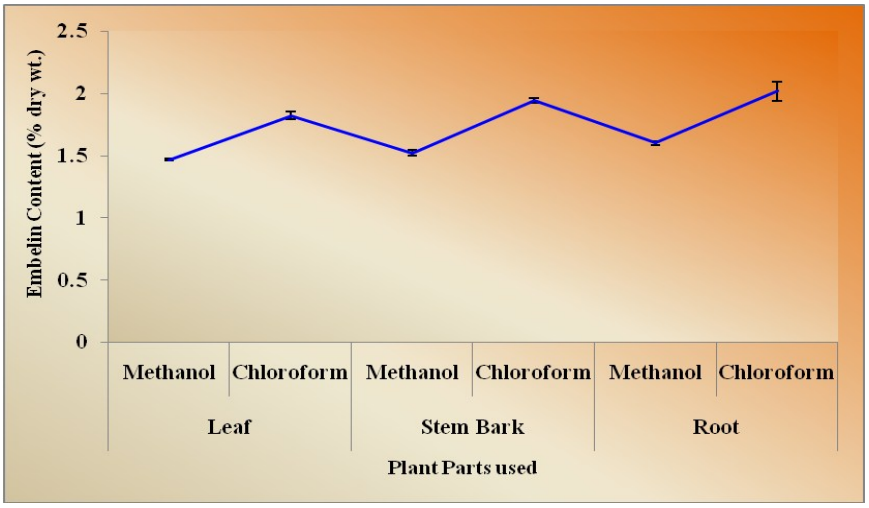

Figure 1: Embelin content in various plant parts of *Embelia tsjeriam-cottam* estimated through Spectrophotometer

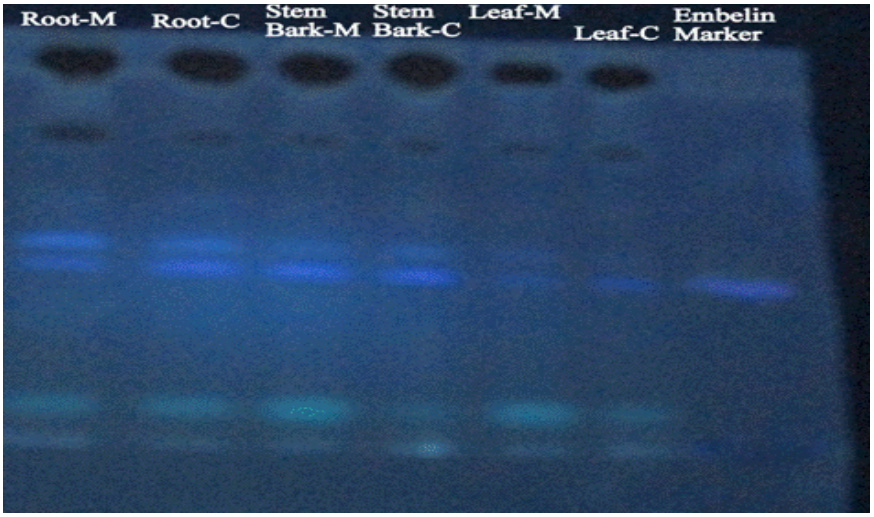

Figure 2: Presence of Embelin in Sample Extracts against Standard on TLC Sheets

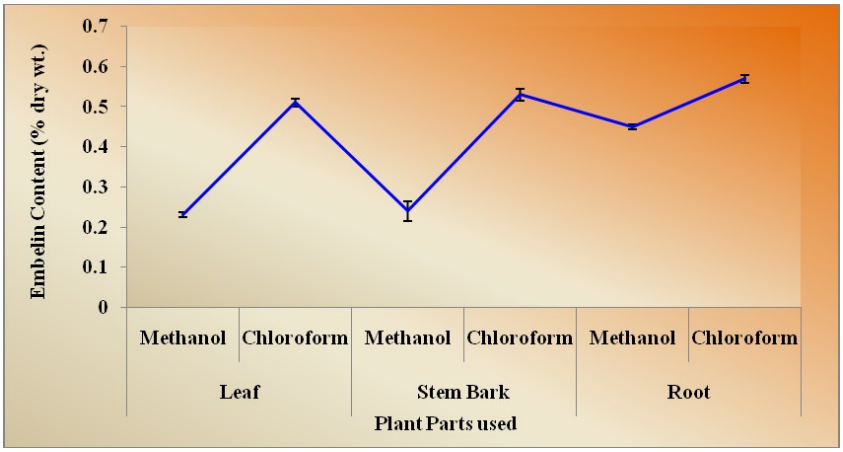

Figure 3: Embelin content in various fruits of *Embelia tsjeriam-cottam* estimated through HPLC

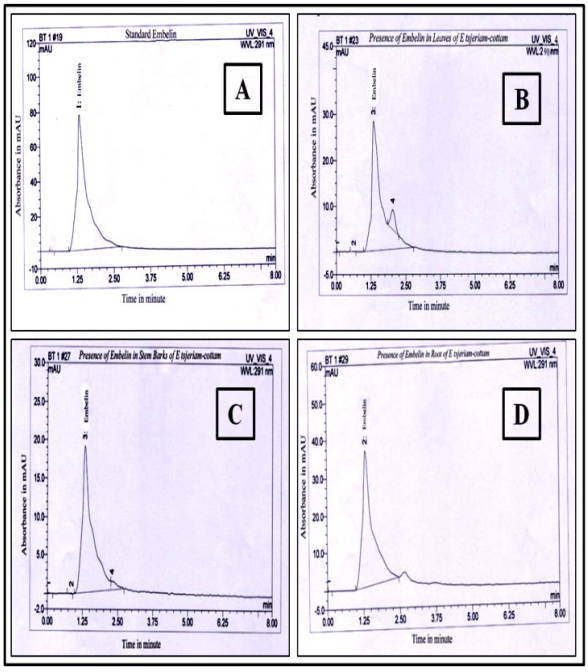

Figure 4: HPLC chromatograms of Embelin standard and samples, where A-Embelin standard; B-leaf samples; C-stem bark samples; D-root samples

DISCUSSION

Quinones are the biologically active natural compounds found to occur in number of medicinal plants which can be divided into four groups including benzoquinones, anthraquinones, naphthoquinones and isoprenoid quinones. They are mainly found in bark, roots and tissues of plants[23]. Embelin is one of the biologically active benzoquinone derivatives that act as the active principle compound in the fruits of *Embelia tsjeriam-cottam* and responsible for its medicinal properties[4]. Several literatures are available regarding presence of embelin in the fruits of *E tsjeriam-cottam*[3-5, 24], but in this case, we have evaluated embelin from other plant parts like leaves, stem barks and the root parts. Several literatures are available regarding the ethno-medicinal use of leaves, stem bark and root parts of *E tsjeriam-cottam*[15, 21, 25-29]. Generally, the plant bioactive compounds, belonging to different groups are extracted through various solvent systems like methanol, ethyl acetate, ethanol, chloroform etc[30]. In many cases, aqueous extracts of *E. ribes* are also being used for several pharmacological activities[31]. But the organic solvents are generally found to be superior to that of the aqueous solvent in case of extracting particularly the secondary metabolites[30].

Extraction is dependent on the solubility as well as the surface permeability of the selected solvent system. Many times the extraction procedure used is not sufficient to extract all the required bioactive compounds due to solubility factor. To counter this problem more than one solvent system with varying polarity index is being used for achievement of complete extraction[32]. Methanol is a readily accepted and frequently used solvent system. Mostly it is used in extraction of polar compounds, but certain group of non-polar compounds are fairly soluble in it[32]. Moreover, its low boiling point is also favourable for extracting and concentrating the bio-active compounds through Soxhlet process. For compounds like hydrophobic nature can be extracted easily through chloroform. Embelin has a long alkyl chain as substitute, which plays a crucial role in its solubility in various solvent systems[33]. However, in some experimentation, embelin content in organogenic and embryogenic callus (from leaf explants) along with in the dried berries of *E. ribes* were being evaluated[21]. Till now no records are available regarding the embelin content in non-fruit parts of *E tsjeriam-cottam* besides the fruit parts in case of Odisha. However, in previous experimentation, we have estimated the embelin content in the fruits of *E tsjeriam-cottam,* collected from various agro-climatic zones of Odisha[24], which ranged in between 1.03-4.93% w/w in case of spectrophotometric method of analysis and 0.5-2.72% w/w in case of HPLC analysis. However, in the current experimentation, embelin content in the non fruit parts (Leaves, Stem Bark & Roots) of *E. tsjeriam-cottam* was found to be half as compared to the fruit parts that has been carried out in our previous experimentation[24]. From this finding it can be opined that the other non fruit parts can act as possible alternative substitutes to that of the fruits as source of embelin.

CONCLUSION

The study revealed the presence of embelin in all other parts of *E. tsjeriam-cottam* leaves, roots and stem barks besides the fruit samples. The non fruit plant parts of *E. tsjeriam-cottam* can also be used for yielding embelin that may mitigate the pressure on fruit and can be used as alternative substitute to that of the fruit samples. This will be helpful for mitigating the demand of embelin yield, which will lead to least exploitation of plant species.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support from Ministry of Forest and Environment Department, Govt. of Odisha.

REFERENCES

1. Nadkarni AK, Nadkarni KM. Indian Materia Medica, 1st ed, Bombay popular prakashan pvt. Ltd. 1976. p. 480
2. Parfitt K, Martindale. The Complete Drug Reference, 3rd ed, The Pharmaceutical Press. London. 2002. p. 105
3. Raja SS, Unnikrishnan KP, Ravindran PN, Balachandran I. Determination of embelin in *Embelia ribes* and *Embelia tsjeriam-cottam* by HPLC. Indian J Pharm Sci 2005; 734-736
4. Stasiuk M, Kozubek A. Embelin-a promising bioactive compound from the Myrsinaceae family. Glob J Biochem 2011; 2: 262-270
5. Pandey AK, Ojha V. Estimation of embelin in *Embelia tsjeriam-cottam* fruits by HPLC to standardize harvesting time. Indian J pharm Sci 2011; 73: 216-219
6. Mohapatra M, Basak UC. Quantitative reckoning of embelin from fruits of *Embelia tsjeriam-cottam* using water bath process as an alternate method of extraction, Indian J. Pharm. Biol. Res 2015; 3: 15-23
7. Khare CP. Indian Medicinal Plants: an illustrated dictionary, Springer-Verlag New York; 2007
8. Ved DK, Kinhal GA, Ravi KK, Sankar VR, Sumathi R, Mahapatra AK, Panda P. Conservation assessment and management prioritization for medicinal plants of Orissa. Regional Plant Resource Centre. Bhubaneswar and Foundation for Revitalization of Local Health Traditions. Bangalore 2008. p. 55-56
9. Kirtikar KR, Basu BD. Indian medicinal plants, 2nd ed, Dehradun. In: Singh B, Singh MP. 1984. p. 1475-1481
10. Saxena HO, Brahmam M. The Flora of Orissa, 3rd ed, Regional Research Laboratory & Orissa Forest Development Corporation Ltd. 1995. p. 1554-1556
11. Mahendran S, Badami S, Ravi S, Thippeswamy BS, Veerapur VP. Synthesis and Evaluation of Analgesic and Anti-inflammatory Activities of Most Active Antioxidant Derivatives of Embelin. British J Pharm Res 2014; 4: 2182-2199
12. Kothavade RJ, Joglekar SN, Barodavalla SA. Protective effect of an indigenous druglivomyn on ketoconazole induced hepatotoxicity. Indian J Pharm Sci 1996; 58: 142-146
13. Poojari R. Phytochemical fingerprinting, cytotoxic, antimicrobial, antitubercular, antimycotic potentials of *Sida rhombifolia* Subsp. *retusa a*nd *Embelia tsjeriam-cottam.* Asia Pac J Life Sci 2011; 4: 201-214.
14. Gupta OP, Ali MM, Ray GBJ, Atal CK. Some pharmacological investigations of embelin and its semisynthetic derivatives. Indian J Physiol Pharmacol 1977; 21:31-39
15. Gupta S, Sanyal S, Kanwar U. Effects of Embelin, a male antifertility agent, on absorptive and digestive functions of rat intestine. J Ethnopharmacol 1991; 33: 203-212
16. Kumara SHM, Krishna V, Shankarmurthy K. Wound-healing activity of Embelin isolated from ethanol extract of leaves of *Embelia ribes* Burm. J Ethnopharmacol 2007; 109:529-534
17. Poojari R, Gupta S, Maru G, Khade B, Bhagwat S. Chemopreventive and Hepatoprotective Effects of Embelin on N-Nitrosodiethylamine and Carbon Tetrachloride Induced Preneoplasia and Toxicity in Rat Liver. Asian Pac J Cancer Prev 2010; 11: 1015-1020

18. Vite MH, Nangude SL, Gorte SM. Anti-inflammatory effect of ethanolic extract of *Embelia tsjeriam-cottam*. Int J Pharm Pharm Sci 2011; 3: 101-102
19. Radhakrishnan N, Gnanamani A. 2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone (Embelin)-A second solid gold of India-A Review. Int J Pharm Pharm Sci 2014; 6: 23-30
20. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda. Central council for research in ayurveda & siddha. Govt. of India. New Delhi. 5th ed. 2002; 478-499
21. Raghu AV, Unnikrishnan K, Geetha SP, Martin G, Balachandran I. Plant regeneration and production of embelin from organogenic and embryogenic callus cultures of *Embelia ribes* Burm. F. - a vulnerable medicinal plant, In Vitro Cell Dev. Biol. Plant 2011; 47: 506-515
22. Souravi K, Rajasekharan PE. A review on the pharmacology of *Embelia ribes* Burm. F.- a threatened medicinal plant. Int J Pharm Biol Sci 2014; 5: 443-456
23. Chansukh K, Charoensup R, Palanuvej C, Ruangrungsi N. Antimicrobial Activities of Selected Thai Medicinal Plants Bearing Quinonoids. Res J Pharm Biol Chem Sci 2014; 5: 425-432
24. Mohapatra M, Basak UC. Assessment of embelin in fruits of *Embelia tsjeriam-cottam* A. DC., a threatened medicinal plant of Odisha, India. Am J Pharmtech Res 2014; 4: 212-221
25. Shankarmurthy K, Krishna V, Maruthi KR, Rahiman BA. Rapid Adventitious Organogenesis from Leaf Segments of *Embelia ribes* Burm. — a Threatened Medicinal Plant. Taiwania Taipei. 2004; 49: 194-200
26. Awino OS, Kiprono PC, Keronei KP, Kaberia F, Obala AA. Antimicrobial Activity of 2, 5-Dihydroxy-3-methyl-1, 4-benzoquinone from *Embelia schimperi*. Z Naturforsch C. 2008; 63 (1-2): 47-50
27. Chandrappa CP, Anitha R, Jyothi P, Rajalakshmi K, Mahammadi HS, Govindappa M, Sharanappa P. Phytochemical analysis and antibacterial activity of endophytes of *Embelia tsjeriam-cottam* Linn. Int J Pharm Biol Sci 2013; 3:467-473
28. Srinath A, Jyothi V, Jyothi AV. Pharmacological, Pharmacognostic and Phytochemical Review of *Embelia ribes*. Int J Pharm Technol 2010; 2: 525-539
29. Gajbhar AV, Kulkarni PS, Nagras MA, Mulgund SV. Fingerprint analysis of *Embelia ribes* churna formulation Using HPLC-PDA and HPTLC methods. J Pharm Assoc India 2013; 1: 31-38
30. Gupta A, Naraniwal M, Kothari V. Modern extraction methods for preparation of bioactive Plant extracts. Int J Appl Nat Sci 2012; 1: 8-26
31. Bhandri U, Ansari MN, Islam F. Cardio protective effect of aqueous extract of *Embelia ribes* Burm fruits against isoproterenol- induced myocardial infraction in albino rats. Indian J Expt Biol. 2008; 46: 35-40
32. Mangal A, Bhadoriya SS, Joshi S, Agrawal G, Gupta A, Mandoria N. Extraction of herbal drugs by using hydrotropy solublization phenomenon. Int. Res J Pharm App Sci 2012; 2: 63-74
33. Maheshwaran S, Sureshkumar C, Mohan KR, Narasimhan S. Reverse Phase HPLC Method for Quantitative Determination of Embelin in Poly Herbal Formulation. Int J pharma Bio Sci 2013; 4: 116-123
34. Patil V, Samuel G, Rai PD, Deokate UA, Khadabadi SS. Standardization and Quality Control Evaluation of *Krimimudgara Rasa* Using Microscopic Studies and HPTLC. Int J Pharm Technol 2011; 3: 1537-1547
35. Ganesan B, Perumal P, Manickam VB, Gotteti SD, Srikakolapu SR, Thirumurthy LS. Optimization of extraction conditions for embelin in *Embelia ribes* by UV spectrophotometry. Arch Appl Sci Res 2010; 2: 49-53
36. Sudani RJ, Vidyasagar G. UV spectrophotometric estimation of embelin and validation of developed method. World J Pharm Res 2012; 1: 379-385
37. Kukkar R, Saluja AK, Shah UD, Kukkar MR. Estimation of Embelin and Strychnine in Krimimudgara Rasa by HPTLC Method. Int J Pharm Qual Assur. 2010; 2: 1-4
38. Rathi SG, Bhaskar VH, Patel PG. *In-Vitro* Anti fungal Screening of *Embelia ribes* Plant Extract through EUCAST Method. Int J Pharm Sci Res 2010; 1: 134-138
39. Chauhan SK, Singh BP, Agarwal S. A TLC Identification and Spectrophotometric Estimation of Embelin in *Embelia ribes*. Ancient Sci Life 1999; XIX: 46-48
40. Vandana, Arora S. Comparison of TLC fingerprint profile of different extracts of *Embelia ribes*. Int J Pharmtech Res 2010; 2: 2438-2440
41. Pandey AK, Shackleton CM. The effect of harvesting approaches on fruit yield, embelin concentration and re growth dynamics of the forest shrub, *Embelia tsjeriam-cottam*, in central India. Forest Ecol Manage 2012; 266: 180-186
42. Rastogi S, Bhatia AK, Kushwaha A, Pandey MK, Sharma A, Singh GN. Development and validation of a liquid chromatography method for determination of embelin in crude extract of *Embelia ribes*. Asian J Biomed Pharm Sci 2014; 4: 9-13

How to cite this article:
Manisha Mohapatra, Uday Chand Basak. Quantitative assessment of embelin content from leaf, stem bark, root of *Embelia tsjeriam-cottam*. J Pharm Sci Innov. 2017;6(2):44-49.

Source of support: Nil, Conflict of interest: None Declared