ORIGINAL ARTICLE

Validation of pharmacopoeial characters of marketed samples of *Embelia* species (*Vidanga*)

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Abstract

Introduction: *Embelia ribes* Burm. f., Myrsinaceae is a promising medicinal herb valued for its digestive, carminative, anthelmintic, and laxative property since Vedic period. Due to its excessive harvesting, it is reported in red list data book as vulnerable. Hence, in markets, *E. ribes* is generally found adulterated with *E. robusta* due to its close morphological similarity with *ribes*. Thus, the present study attempts to validate and compare physicochemical parameters and chromatographic profile of fruits of two species of *Vidanga,* that is, *E. ribes* and *E. robusta* for proper identification of the drug in dry form and to check adulteration. **Materials and Methods:** Evaluation of macroscopic characters, physicochemical parameters such as organoleptic, loss on drying, total ash, acid insoluble ash, water soluble ash, alcohol, and water soluble extractives as well as high performance thin-layer chromatography (HPTLC) analysis were performed for fruits of both the species of *Vidanga,* namely, *E. ribes and E. robusta* as per standard guidelines. **Results:** The macroscopic study section revealed that the fruits of both the species vary in color, size, and presence of beak such as projection and membrane over the pericarp. The distinct character of *E. ribes* is the presence of whitish spots on its seeds (*Chitratandula*) which is found to be absent in *robusta* seeds. All the physicochemical parameters of *E. ribes* were found in compliance with the official standards laid down by Ayurvedic Pharmacopeia of India. These findings accomplished the chief objective of validating the pharmacopoeial characters. Moreover, it was observed that there are no significant differences in the physicochemical constants of both the species except one parameter, that is, water soluble extractives. Apart, HPTLC proffered some identical as well as some dissimilar R_f. **Discussion:** As no remarkable differences were observed in conventional quality control assays for possible phyto and physicochemical variations between the samples, it is concluded that the spotless *vidanga,* that is*, E. robusta* can also be utilized in pharmaceutics. Moreover, extended studies with multiple samples from different market sources can be carried out to generate further evidences on analytical and therapeutic ground.

Key words: *Embelia ribes*, *Embelia robusta*, Chromatography, Physicochemical, Standards, *Vidanga*

INTRODUCTION

Quality and safety are the cardinal aspects in current scenario for the acceptance of any substance especially drugs. Desired efficacy of a drug always depends on authenticity of raw material. Hence, identification of crude material through accessible parameters is significant. The process of identification and standardization can be accomplished by 3P's – pharmacognostic, physicochemical, and phytochemical analysis in stepwise manner. Proper identification and quality assurance of the raw materials are prerequisite and vital to ensure reproducibility of herbal medicine which will ensure its safety and efficacy.[1] Morphological characters accompanying therapeutic effectiveness of the Ayurvedic drugs have been established and well documented by the great seers in the form of classics attributed to them. Even so, just relying on the ancient treatises, appropriate recognization of a drug has become an arduous task due to its variable vernacular names and numerous synonyms bemusing

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different plants for one another. Incorrect identification of many rare, endangered plants along with deforestation and extinction of various species caused an adverse impact on the availability and supply of these drugs resulting in the practice of adulteration and substitution. Therefore, various quality control parameters were codified into national pharmacopoeias. The word pharmacopoeia was first used as a general term in *Pharmacopoeae Jacobi Sylvii libri tres* of French physician Jacques[2] in 1548. The *Pharmacopoeia Augustana* published in 1601 was the first officially recognized work having used the word pharmacopoeia.[3] Later on, *British Pharmacopoeia*[4] introduced identity and quality standards for individual herbal drugs. It incorporated information pertaining to identifying morphological characters, origin, good collection practices, etc. Besides, organoleptic characteristics and application of magnifiers were introduced by another pharmacopoeia, *Dispensatorium Lippiacum*.[5] Further, chemical analysis of drugs was first inducted by *Pharmacopoea Wirtenbergica*[6] in 1741. India has a plethora of medicinal plants which are being used on a large scale by the AYUSH as well as conventional system of medicine. In such scenario, it is imperative to have strict guidelines and measures for quality assurance. Therefore, Government of India has presented pharmacognostic characters and quality control measures of these medicinal plants in its official pharmacopoeia, The *Ayurvedic Pharmacopoeia of India* to ascertain adequate standards. The need of the moment is to validate them.

Vidanga is a wonderful drug in Indian system of medicine, Ayurveda. Modern botanists have studied this plant as *Embelia ribes* Burm. F., family Myrsinaceae. It is a large woody tropical forest scandent shrub with slender branches and gland dotted leaves, sparsely distributed in the moist deciduous forests of the Western Ghats of South India, Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Assam, and Maharashtra, Sri Lanka, Singapore, Malaysia, and South China.[7,8] It is commonly known as False Black Pepper. This indigenous medicinal plant is mainly recommended as best *krimighna*[9] (wormicidal) in ancient treatises. It is a promising medicinal herb reputed for its antihyperlipidemic,[10] antifungal,[11] antioxidant,[12,13] wound healing,[14] antitumor, and anti-inflammatory[15] property. Its fruits are extensively utilized in about 75 Ayurvedic formulations videlicet *Vidangarista*, *Vidanga Lauha*, *Abhayarishtam*, *Ayaskriti*, *Pippallyasavam*, *Anu tailam*, *Chandraprabha vati, Kaishore guggulu, Vyoshadi guggulu, Kachuradi tailam,* etc. Its fruits contain a quinone derivative embelin (3-undecyl 2, 5- dihydroxy, 1,4-benzoquinone), an alkaloid christembine and a volatile oil vilangin; its chemical constituent is 2,5-dihydroxy-4-undecyl-3,6-benzoquinone.[16] Several pharmacological studies have been executed, to see the efficacy of this drug against various ailments, for instance, its alcoholic extract cured 80% of the cases infected by *Ascaris lumbricoides*.[17] On oral administrating at a dosage of 100 mg/day, the drug was found to have contraceptive activity in male bonnet monkeys.[18] Even, the extracts neither showed any toxicity at the cellular level nor altered the functional integrity of the male reproductive organs.[19]

E. ribes is one of the 32 therapeutic plant species distinguished by the Medicinal Board, Govt. of India, New Delhi, as being significant for large-scale cultivation due to its commercial use.[20] It is reported in red list data book as vulnerable due to its excessive harvesting. There is gradual disappearance of *E. ribes* due to inherent sterility of the seeds.[21] Besides, it is a lot simpler to collect *Embelia robusta* in comparison to *E. ribes* as former is a large shrub and bears more fruits than *E. ribes*. In addition, it is also distributed throughout India, while *E. ribes* has restricted distribution.[22] Hence, in markets, *E. ribes,* are generally found adulterated with *E. robusta* (*Embelia tsjeriam cottam* syn.) on account of these reasons along with its close morphological similarity with *ribes.* In 1972, Singh *et al.* and Sareen in 1996 described the distinguishing morphological features of *E. ribes*, *E. robusta,* and *Myrsine africana*. In spite of these kinds of studies, issues in identifying the species persist. Thus, the present study was undertaken with the aim to validate and compare physicochemical parameters and chromatographic profile of two species of fruits of *Vidanga,* that is, *E. ribes* and *E. robusta* for proper identification of the drug in dry form and to check adulteration.

MATERIALS AND METHODS

Procurement of Raw Materials

The dried berries of *E. ribes* Burm. F *and* were procured from Shri ram herbals, Jaipur. Besides, other sample of *vidanga,* that is, *E. robusta* auct.Non roxb was purchased from Khari Baoli Market, New Delhi. Authentication was done by Pharmacognosy Lab, All India Institute of Ayurveda, New Delhi. Fine powder of the samples was used for determination of physicochemical and chromatographic profile. The dried fruits of both the species were grinded in mixer grinder individually and passed through sieve # 80 to obtain fine powder.

Equipment and Apparatus Used

Digital analytical balance, grinder, digital oven, silica crucible, evaporating dish, electric muffle furnace, tongs, desicator, sonicator, thin-layer chromatography (TLC) plate, Twin trough development chamber, hot plate, digital pH meter, Whatman filter paper no.1, common glass ware, microscope with camera, and ultraviolet (UV) chamber.

Chemicals Used

Distilled water, methanol, dilute HCl, chloroform, ethyl acetate, and formic acid.

Macroscopic Study

The fruits of both species were spread on a table and then examined for size, shape, color, and touch.

Physicochemical Analysis

Physicochemical study of both the samples was carried out in accordance with standard procedures of various physiochemical parameters as mentioned in Ayurvedic Pharmacopoeia of India.[23] This study involves determination of loss on drying (LOD), total ash value, acid insoluble ash, water soluble ash, water, and alcohol soluble extractive value for both the samples.

High-Performance TLC (HPTLC)

HPTLC fingerprinting is proved to be a reliable, accurate, and precise technique for herbal identification and authentication. Thus, the developed chromatogram and Rf values will be specific with selected solvent system, and serve the better tool for standardization of the test drugs. The principle remains the same as of TLC, that is, adsorption.

Steps involved in HPTLC were as followed:-

1. Preparation of test solutions
2. Preparation of solvent system
3. Sample application
4. Plate development
5. Visualization
6. Scanning and documentation.

Preparation of test solutions

One gram powder of each sample was accurately weighed and added in 10 ml of distilled water individually to prepare aqueous extracts. In the same manner, methanol extracts of both the samples were also prepared. The solutions were kept for 24 h. Next morning, the solutions were sonicated for 20 min and then filtered through Whatman filter paper no.1. The filtrates were collected, coded as Ri-M, Ro-M, Ri-A, and Ro-A. They all were further subjected to chromatographic analysis.

Preparation of Solvent System

Various solvent systems were checked to separate the maximum number of active chemical constituents in the drug. Finally, the solvent system chloroform:ethylacetate:formic acid (5:4:1 v/v/v) was selected.

Sample Application

A volume of 5.0 µl of all the extracts was applied in the form of a band on TLC aluminum plate pre-coated with silica gel 60 F_{254} of 20 cm × 10 cm size and 0.2 mm thickness with the help of CAMAG Linomat V applicator under nitrogen stream.

Development of Plate

The linear ascending development of plate was carried out over a distance of 70 mm in a CAMAG Twin trough chamber having SS lid (20 cm ×10 cm) previously saturated with mobile phase for 30 min.

Visualization

After development, the plate was dried at TLC Plate Heater at 60°C for 5 min and visualized at λ 254 nm (short U.V.) and λ 366 nm (long U.V.) in UV chamber. The photographs were taken with high resolution camera.

Detection

The plate was detected in CAMAG TLC scanner III and densitometric scan was performed using slit dimension of 6.00 mm × 3.00 mm, micro in the absorbance mode at multiple wavelengths, namely, at 254nm, at 291nm, and at 366nm.

OBSERVATIONS AND RESULTS

The morphological characters of fruits of both the species are depicted in Table 1.

Chromatographic Fingerprinting

Several bands [Figures 1 and 2] were observed on visualization under UV chamber. Densitogram analysis also showed a number of peaks for all the tracks at different wavelengths [Figures 3-5]. The Rf values of bands for various tracks are given in Table 2.

DISCUSSION

The structural concept of pharmacopeias can be understood as a compilation of pharmaceutical specifications that are intended to maintain uniformity in the composition, quality, and therapeutic activity of drug and that are declared essential and obligatory in a country by legally competent authority.[3] Organoleptic parameters, which include evaluation of the taste, aroma, texture, and sensation, are the principal assessment tools for determining the relative quality of a raw drug. Ancient classics have developed a very sophisticated system of sensory evaluation to understand pharmacology of the plant material. Seers have correlated these sensory assessments such as hot, cold,

dry, and bitter with the physiological effect of drug in the body system. In Ayurveda, these parametric analysis is specifically described in *Dravyaguna* which primarily deals with the six *Rasa* (tastes), *Guna* (physical properties), *Veerya* (potency), *Vipaka* (post-digestive effect), and *Prabhava* (specific or unique action). *Avicenna* in his Canon mentioned that taste is the most precise indication of the nature of the drug.[24]

The macroscopic study section of present study revealed that there is very minute difference in the morphological characters of fruits of *E. ribes* and *robusta*. The distinct character of *E. ribes* is the presence of whitish spots on its seeds which is described as *Chitratandula* in Ayurveda. It is considered as cardinal sign of desired *Vidanga* species but was found to be absent in *robusta* seeds. In addition, the fruits of both the species vary in color, size, and presence of beak such as projection and membrane over the pericarp. Apart, Table 3 showed the difference in color, aroma, and taste of powders of both the species. These observations will be helpful in identification of desired species of the herb.

The physicochemical constants of drugs are also chief measures in detecting adulteration or improper handling of

Table 1: Macroscopic characters of fruits of *Embelia ribes and Embelia robusta*

S. No.	Characters	*Embelia ribes*	*Embelia robusta*
1.	Color	Reddish black	Brownish black
2.	Shape	Globular	Globular
3.	Size	2–4 mm in diameter (smaller)	3–5 mm in diameter
4.	Surface	Warty with a beak like projection at apex	Smooth and do not have any projection
5.	Pericarp	Brittle enclosing a single seed covered by a thin membrane.	Brittle enclosing a single seed only. Membrane was absent
6.	Seed	Brown and have whitish spots (*Chitra tandula*)	Brown but do not have white spots

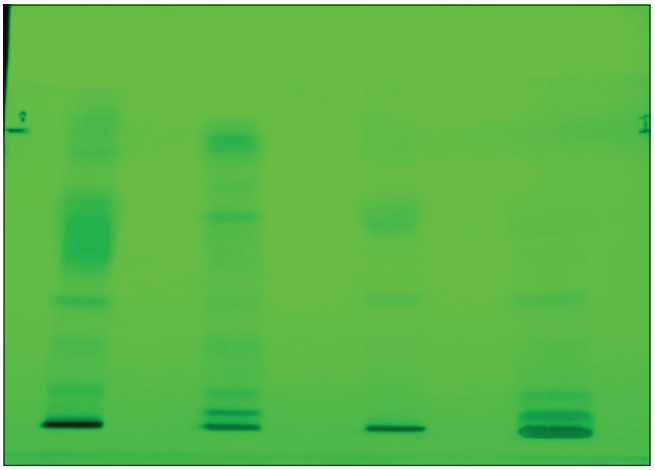

Figure 1: Bands observed in different extracts of ribes and robusta @254 nm

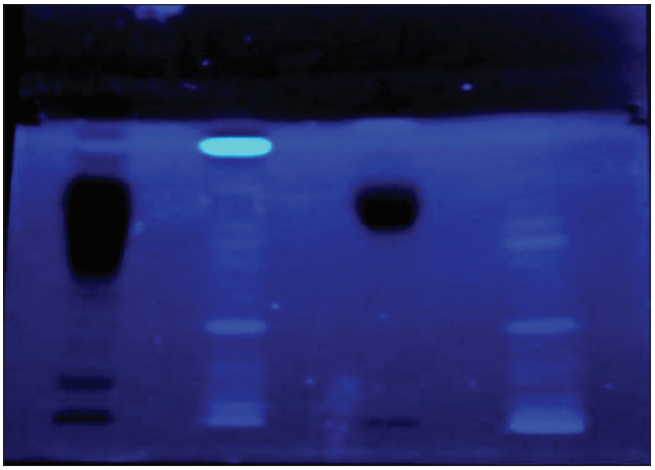

Figure 2: Bands observed in different extracts of ribes and robusta @366nm

Table 2: Rf values obtained in different extracts of *ribes* and *robusta* at various wavelengths

Position of band	Name of sample	No. of Spots@			Rf values@		
		254	291	366	254	291	366
1	Ribes- Methanol (Ri-M)	8	9	4	0.13, 0.28, 0.36, 0.42, 0.60, 0.68, 0.92, 0.98	0.13, 0.28, 0.42, 0.60, 0.63, 0.89, 0.92, 0.99, 1.02	0.13, 0.28, 0.60, 1.00
2	Robusta- Methanol (Ro-M)	10	8	10	0.12, 0.19, 0.27, 0.42, 0.49, 0.55, 0.63, 0.70, 0.80, 0.97	0.12, 0.19, 0.27, 0.42, 0.58, 0.70, 0.80, 0.97	0.11, 0.20, 0.28, 0.41, 0.52, 0.58, 0.68, 0.75, 0.81, 0.94
3	Ribes- Aqueous (Ri-A)	7	7	7	0.16, 0.21, 0.28, 0.42, 0.68, 0.92, 0.98	0.12, 0.15, 0.28, 0.42, 0.67, 0.92, 0.98	0.11, 0.15, 0.28, 0.42, 0.67, 0.88, 0.99
4	Robusta- Aqueous (Ro-A)	7	8	6	0.12, 0.26, 0.42, 0.55, 0.59, 0.65, 0.98	0.12, 0.20, 0.28, 0.42, 0.49, 0.59, 0.68, 0.98	0.12, 0.19, 0.28, 0.42, 0.59, 0.96

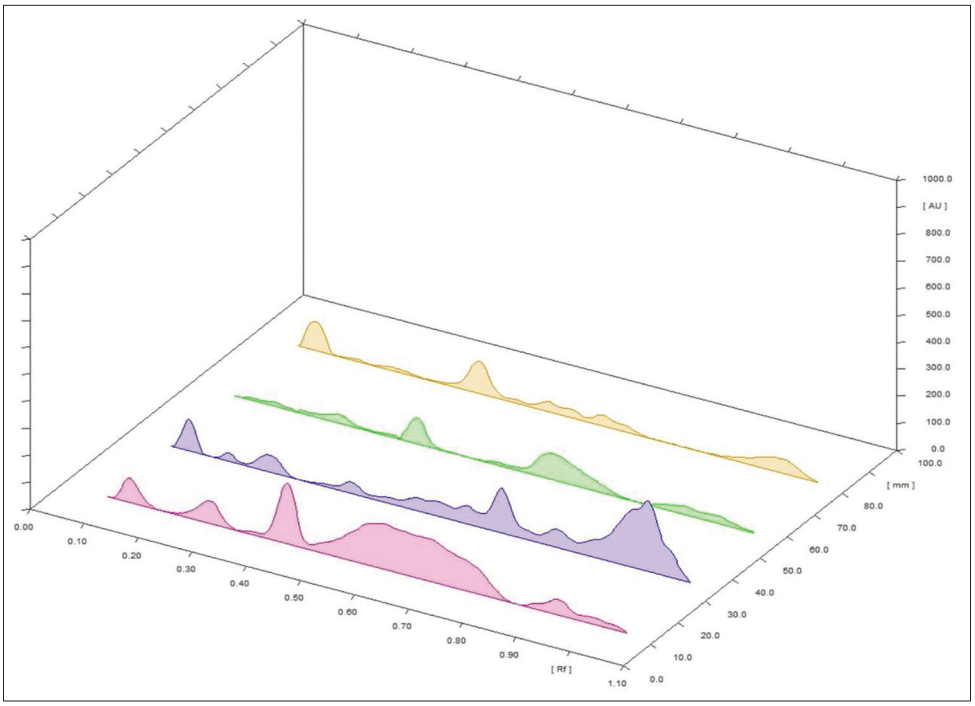

Figure 3: 3D overlay Chromatogram at @254nm

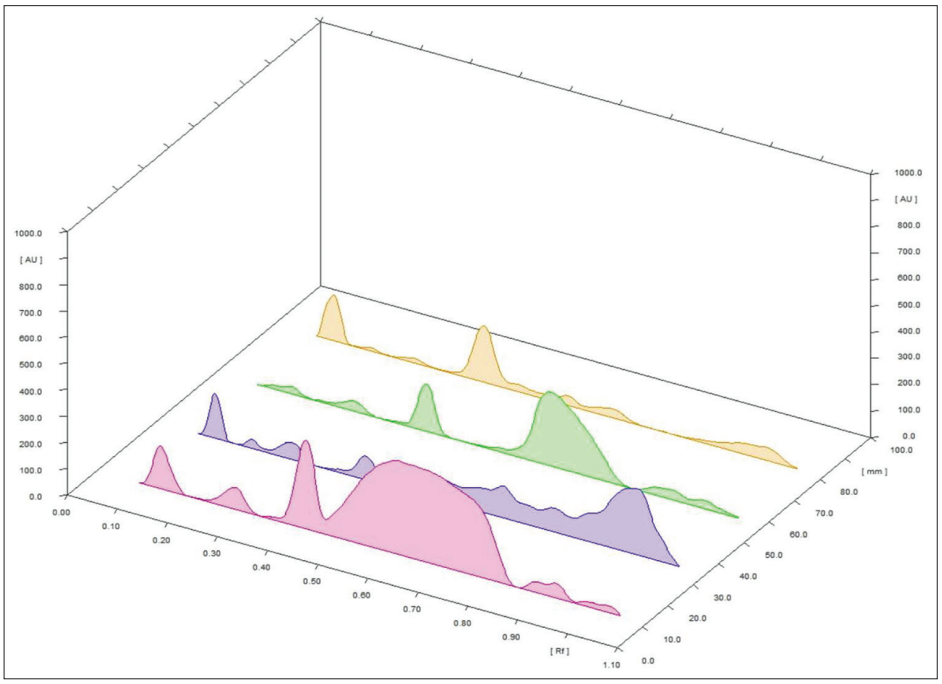

Figure 4: 3D overlay Chromatogram at @291nm

Table 3: Organoleptic characters of powder

S. No.	Characters	*Embelia ribes*	*Embelia robusta*
1.	Color	Brown	Grey
2.	Touch	Smooth	Smooth
3.	Odor	Mild, characteristic	---
4.	Taste	*Tikta* (Bitter), *Katu*	*Kashaya* (Astringent)

drugs. LOD is the loss of weight expressed in percentage (w/w) resulting from water and volatile matter of any kind that can be driven off under specific conditions. Moisture is an important factor that plays a key role in stability of any drug. At instances, presence of water/moisture will facilitate microbial growth in the product. More moisture % may also lead to hydrolysis of hydrolysable constituents. Thus, no or minimum moisture content is advisable in most of the drug materials. In general, there are two types of

ash, namely, physiological which is obtained from the plant tissue, for example, carbonates, and phosphates and another is non-physiological which is obtained from the externus matter such as silicates and silica. Total ash is combination of both. It is the residue remaining after incineration of material. This value denotes purity of the drugs, that is, the presence or absence of foreign inorganic matter. Acid insoluble ash is the part of total ash, which is insoluble when treated with diluted hydrochloric acid. Therefore, it indicates only the silica and earthen material. Water and alcohol soluble extractives indicate a particular amount of principle contents which is readily soluble in aqueous and alcohol media. Every drug has a particular amount of principal components soluble in different media; this value plays a significant role in evaluation of drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. Taking Table 4 into consideration, it was found that all the physicochemical parameters of fruits of *E. ribes* were found in compliance with the prescribed limit as per Ayurvedic Pharmacopoeia of India. These findings accomplished the chief objective of validating the pharmacopoeial standards. In addition, it was also observed that there are no remarkable differences in the physicochemical parameters of fruits of both the species except one parameter, that is, water soluble extractives. This value was observed very low in *robusta* when compared with *ribes*.

HPTLC fingerprinting proffers a layout of the active constituents present in crude plant drugs. It is considered as a sophisticated parameter for quality and purity of a drug material. As a chemical analytical tool, HPTLC is extremely versatile tool equipped with a high degree of sensitivity, especially useful in the detection of adulterations. The Rf values obtained from qualitative evaluation of chromatographic analysis reflect the phyto constituents of the respective plant which may establish the identification, quality control of the drug, and ensure therapeutic efficacy. Densitogram scanning revealed that methanol extracts of both the species showed common Rf values 0.13, 0.28, and 0.42 at 254 nm. Similarly, Bands

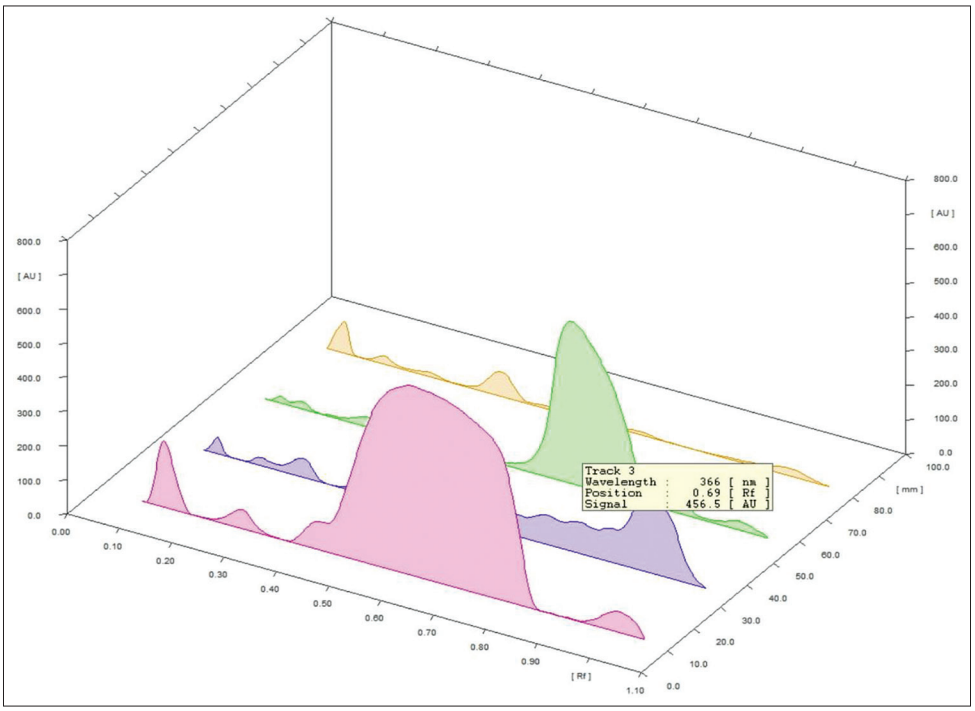

Figure 5: 3D overlay Chromatogram at @366nm

Table 4: Physicochemical parameters of *Embelia ribes and Embelia robusta*

S. No.	Characters	*Embelia ribes* (%)	*Embelia robusta* (%)	API*
1.	Loss on drying at 105°C	8.23	7.89	Not mentioned
2.	Total Ash	2.89	3.34	Not more than 6%
3.	Acid insoluble ash	0.58	0.72	Not more than 1.5%
4.	Water soluble ash	2.40	2.15	
5.	Alcohol soluble extractives	12.04	10.2	Not <10%
6.	Water soluble extractives	10. 49	3.87	Not <9%

*mentioned only for *Embelia ribes*

of Rf values 0.42, 0.98 were found in aqueous extracts of both species. On account of Table 2, the same observation can also be seen at other wavelengths too. Moreover, extra bands were found to be separated in methanol extract of *robusta* at 254 and 366nm. It indicates the presence of additional active constituents in *robusta* in comparison of *ribes*. As *Vidanga* possess a broad spectra of medicinal properties, which leads to its huge demand. Earlier studies have also reported that the *ribes* species are scarcely available. In the present work, it has been observed that there is no significant difference in the physicochemical parameters of both of these species of *vidanga*. Therefore, standards for *E. robusta* may be included in API for its authenticated use in pharmaceutics.

CONCLUSION

Vidanga is reputed to have tremendous pharmacological potential with wide clinical applications. It is one of the important ingredients in a range of Ayurvedic formulations indicated in various ailments. These factors leads to its over harvesting and counterfeiting. Poor germination ability and tedious artificial germination process superimposes its paucity. Hence, it is generally substituted with *E. robusta* or adulterated with *M. africana L.*, *Maesa indica,* etc. On analysis of morphology, it has been discovered that *E. robusta* does not have the spotted impressions on their seeds which are described as the distinct characteristic of desired quality of *vidanga*. Conventional quality control assays for possible phyto and physicochemical variations between the samples revealed minor differences excluding water extractives. Apart, other parameters were found as per the standards laid down in official pharmacopeia. Hence, it is concluded that the spotless *vidanga,* that is, *E. robusta* can also be utilized in pharmaceutics. Moreover, extended studies with multiple samples from different market sources can be carried out to generate further evidences on analytical ground. In addition, various experimental as well as clinical trials can also be conducted to evaluate differences in therapeutic efficacy.

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