ORIGINAL ARTICLE

Comparative studies on several Vidanga plants collected from Maharashtra, India, employing phytochemical screening, high-performance thin-layer chromatography analysis, and anti-oxidant potential

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Abstract

Introduction: Vidanga is a well-known herbal treatment that has been used to treat a range of ailments for ages. According to modern literature, Vidanga is most commonly used to treat inflammation especially gut inflammation, namely colitis. Embelin is the major bioactive compound present in Vidanga (*Embelia* spp.) responsible for its therapeutic property. According to Ayurveda, *Embelia ribes* is the indicated botanical as a "Vidanga" but under the name, "Vidanga" four plants are marketed, namely, *Embelia drupacea* (Dennst.) M. R. Almeida and S. M. Almeida, *E. ribes* Burm. f. and *Maesa indica* (Mi) (Roxb.) A. DC., *Embelia tsjeriam-cottam* (Etj) (Roem. and Schult.) A. DC and Mi (Roxb.) A. DC. Hence, it is necessary to identify a therapeutically more effective plant as Vidanga. **Materials and Methods:** We have evaluated more effective species using phytochemical screening; high-performance thin-layer chromatography (HPTLC) characterization; and free radical scavenging potential using bovine serum albumin (BSA) anti-denaturation assay, nitric oxide scavenging (NOS) activity, and 2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay. **Results:** Preliminary phytochemical analysis indicated the presence of quinone, carbohydrates, fixed oils, glycosides, terpenoids, steroids, and tannins in collected Vidanga samples. HPTLC studies revealed that Embelin levels were highest in Etj (1.06 ng/µl), followed by *E. ribes* (Er) (0.92 ng/µl), *E. drupacea* (Ed) (0.5 ng/µl), and absent in *Maesa indica* (Mi). Er exhibited better anti-denaturation activity than Etj, Ed, and Mi. Etj and Er showed a superior ABTS scavenging activity than Ed and Mi. NOS antioxidant activity was also shown to be higher in Er and Etj than in Ed and Mi. **Discussion and Conclusion:** Phytochemicals have been found in all plant species, although, HPTLC screening revealed that Er and Etj have similar quantities of Embelin. The antioxidant properties were strongly linked to the Embelin content, indicating that Er and Etj might be a viable substitution for one another.

Key words: Vidanga, *Embelia* spp., comparative assessment, phytochemical screening, high-performance thin-layer chromatography, embelin, anti-oxidant assays

INTRODUCTION

Herbal medicines (HMs), also known as phytomedicines, have been used to promote health and treat diseases for over a millennium. There has recently been a global preference for HMs due to their minimal toxicity and high therapeutic efficacy.[1,2] Various report suggests that several factors such as a collection of botanically correct plant, the different origin of the raw material, the chemical composition of

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herbal drug, and preparation methods of formulations affect the quality of the herbal products.[3,4] The chemical composition may vary with the concentration of secondary metabolites which is depending on the collection stage, botanicals collected, collection seasons, plant origins, drying processes, and other factors.[5] The Vidanga is one of the most commonly used HMs to treat anti-helminthic and inflammatory conditions.[6,7]

Ayurvedic formulation, like Sunder-Vati (includes *E. ribes* Burm. f. as one of the key factors), exhibited a noteworthy efficiency in treating inflammatory and non-inflammatory lesions in patients having *Acne vulgaris*.[8] Considering all these benefits, most of the time it is challenging to obtain real Vidanga, because four botanicals with almost similar morphology are used as Vidanga, including *Embelia drupacea* (Dennst.) M. R. Almeida and S. M. Almeida, *Embelia ribes* Burm. f., *Embelia tsjeriam-cottam* (Roem. and Schult.) A. DC, and *Maesa indica* (Roxb.) A. DC.[9,10] They belong to the family Myrsinaceae. Locally, they are called with very common names, namely, Ambati, Bhabhiranga, Vidanga, Vavading, Vayavadang, Vayuvidangam, Vayuvidangalu, and Vizhalari.

E. ribes is a climber found in the hilly parts of India from the central and lower Himalayas down.[11] *E. tsjeriam-cottam* (Etj) is a shrub and is found in western and southern states of Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu, and Andhra Pradesh.[12] *E. drupacea* (Ed) is a woody climber growing in semi evergreen to deciduous forests up to an altitude of 1600 m. throughout India.[9] Mi is found in semi-evergreen and deciduous forests of India.[13]

The plant parts used are the fruits, leaves, roots, and seeds, whereas the major used part is fruits.[14] The fruits of Vidanga are bitter and were found to be a good appetizer and cures of ascites, bronchitis, dyspnea, jaundice, mental disorders, diseases of the heart, and scorpion stings, snake bites, tumors, urinary discharges, and toothache.[14-16] It is traditionally used to cure bowel disease and fever. Under the name Vidanga, *E. ribes* is reported to have a high value (Rs. 8000/kg) traded medicinal drug with an estimated annual demand of over 100 Metric[17,18] but most of the time the user receives *Embelia tsjeriam-cottam* instead of *E. ribes*. The therapeutic advantages of HM and their formulations are based on the existence of bioactive phytoconstituents that work in tandem with other phytochemicals. Embelin (2, 5-dihydroxy-3-undecyl-2, 5-cyclohexadiene-1, and four benzoquinones) in berries is credited with the medicinal capabilities of the drug Vidanga under genus *Embelia*.[19,20]

Embelin (Molecular weight: 294.38) is soluble in an organic solvent such as dimethyl sulfoxide, ethyl acetate, methanol, ethanol, and insoluble in water.[21] Embelin is highly acknowledged for its anti-inflammatory, anthelmintic, and anticancer activity, potent oral contraceptive, etc.[22,23]

Recently, advanced analytical methods such as chromatographic techniques developed for the quality determination of phytochemicals in HMs, namely, ultra-performance liquid chromatography.[24] Embelin scavenges the superoxide radical by abstracting its electron and releasing molecular oxygen. The hydroxyl groups in embelin establish strong intramolecular H-bonds with their carbonyl neighbors, making them less scavengeable.[25] Phenolic compounds in herbal medicine show strong antioxidant activity, thus HMs have started to replace synthetic antioxidants.[26] Oxidant radicles such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^·$) formed in the body are very reactive chemical species that lead to oxidative stress.[27]

Hence, the present study was undertaken to determine phytochemical content and ROS scavenging inhibitory activity of Vidanga plants collected from Western Ghats of Maharashtra, India to evaluate their potential as a natural antioxidative source. Here, we have employed the HPLTC technique to qualitatively and quantitatively assess the presence of Embelin, and their comparative antioxidative capacity was measured using various assays to find out the most effective species used as drug Vidanga.

MATERIALS AND METHOD

Collection and Processing of Vidanga Fruits

Mature fruits of Ed, *E. ribes,* Etj and Mi were self-collected from forests of Kemse, Koyna, Satara District of Maharashtra, India. The plants were, identified, collected in 2019 by Taxonomist, Dr. Suresh Jagtap, Associate Professor, Interactive Research School for Health Affairs, Bharati Vidyapeeth Deemed University, Pune, and voucher specimen is submitted at herbaria of MPCC, Pune (MPCC 2743; MPCC: 1526; MPCC 499; MPCC 1917). Prior permission was taken from Maharashtra State Biodiversity Board, Maharashtra.

Chemicals and Reagents

The standard Embelin was purchased from Yucca Enterprises Pvt. Ltd., Mumbai. Solvents and chemicals used for the experimentations were of AR grade level.

Preparation of Herbal Extract

The dry fruits were crushed, and the powder was subjected to hot extraction in Soxhlet using ethyl acetate solvent. After extraction, the solvent was evaporated in a rotary evaporator, and the remaining extract was redissolved in methanol. This was used for preliminary phytochemical screening, high-performance thin-layer chromatography (HPTLC) analysis of active secondary metabolite Embelin.[25]

Phytochemical Screening

Ethyl acetate extracts of Plant Metabolite were subjected to preliminary phytochemical screening. The extracts were

screened for the presence of alkaloids, flavonoids, fixed oils, glycosides, phenols, quinone, and terpenoids.[28]

Quantification of Embelin Content in Vidanga Fruits by HPTLC

A standard solution of 10 mg/ml of extract was prepared, further diluted to prepare the stock solution of 1 mg/ml concentration. Quantitative evaluation of Embelin from fruits of Vidanga was done by following the standard procedure.[29] The test solution was prepared, with the help of 10 mL methanol extract, this solution was further diluted to make the 1 mg/ml concentration test solution. The samples were spotted as bands-width of 8 mm using a 100 µl CAMAG syringe on a 20 × 10 cm aluminum packed TLC plate coated layer of silica gel (0.2 mm) $60F_{254}$ (E. Merck Ltd., Darmstadt, Germany). Linomat V applicator attached to CAMAG HPTLC system, programmed through Win CATS software was used for spotting bands. Different volumes of the standard solution as 2, 2.5, and 3 µl (corresponding to 2, 2.5, and 3 ng, respectively, of Embelin per spot) were applied on the HPTLC plate. The mobile phase (20 ml) was prepared by chloroform: ethyl acetate: formic acid (7:2:1) and used as mobile phase.[30,31] The developed plate was initially air-dried followed by hot air to evaporate solvents from the plate and subjected for TLC scanning. Further photo documentation using UV-chamber under at 336 nm.

In Vitro Antioxidant Assays

Bovine serum albumin (BSA) denaturation assay

The Vidanga extracts were dissolved in methanol to obtain a final concentration of 1000 mg/mL. Different dilutions (20, 40, 60, 80, and 100 mg/ml) of the crude extracts were prepared. The reaction mixtures (0.5 ml) consisted of 0.45 ml BSA (5% aqueous solution) and 0.05 ml of test compound. A small quantity of 1N HCl was used to adjust the pH to 6.3. The samples were incubated for 3 min at 37°C. 2.5 mL phosphate buffer saline (pH 6.3) was added to each tube once the reaction solutions had cooled. Finally, at 660 nm, turbidity was determined spectrophotometrically.[32]

The inhibition of protein denaturation (%) was calculated using the following formula:

$$\text{Inhibition of Denaturation } (\%) = \frac{(A_{Control} - A_{Test}) \times 100}{(A_{Control})}$$

where A control: absorbance of the control; A test: absorbance of the tested compounds. The standard anti-inflammatory medication diclofenac (0.1 mg/mL) was used and processed in the same way as the samples.

ABTS 2, 2'-Azino-Bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity

The ABTS radical cation decolorization method was conducted to test the free radical scavenging activity of Vidanga samples.[33] The interaction between 7 mM ABTS in aqueous form and 2.45 mM potassium persulfate (1:1) produced the ABTS+ cation radical, which was kept in the dark at room temperature for 12–16 h before use. After diluting the ABTS+ solution with methanol, an absorbance of 0.700 at 734 nm was obtained. The absorbance was measured 30 min after the addition of 5 µl of Vidanga extract to 3.995 µl of diluted ABTS+ solution. A standard solution was prepared using Trolox as a standard substance. Percent inhibition of absorbance at 734 nm was calculated using the formula,

$$\text{ABTS scavenging activity } (\%) = \frac{(\text{A Control} - \text{A Test}) \times 100}{(A_{Control})}$$

where A control: absorbance of ABTS radical+methanol; A test: absorbance of ABTS radical + sample extract/standard.

NO Radical Scavenging Assay

At a neutral pH, sodium nitroprusside (SNP) creates NO, which combines with oxygen to create nitrite ion, which is measured using Griess reagent. The samples were incubated at 37°C for 60 min with 2 ml of the Vidanga extract at various concentrations and 50 mM SNP (0.5 ml) in 10 mM Phosphate Buffer Saline.[34] A 0.5 ml aliquot of the incubation solution was pipetted out in the new tube and diluted with 0.5 ml Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride At 540 nm, the absorbance was immediately recorded. The standard curve graph was prepared with the absorbance VS concentrations of sodium nitrite salt. Scavenge NO radicals was calculated by using the following equation:

$$\text{Nitric oxide scavenging activity } (\%) = \frac{(\text{A Control} - \text{A Test}) \times 100}{(A_{Control})}$$

where, A control: the absorbance of the control (the reaction mixture without the extract); A test; the absorbance in the presence of the extract; A control: the absorbance without Griess reagent.

Statistical Analysis

All tests were carried out in triplicate, and the results are given as mean ± standard deviation. The statistical analyses were performed with 95% confidence using the statistical tool Prism 7.0 version. One-way ANOVAs were used to

determine the significance of differences ($P < 0.05$) between mean values obtained from the studies, followed by Tukey's test.

OBSERVATIONS AND RESULTS

Phytochemical Screening

The present study contributes valuable information on bioactive compounds in Vidanga. Qualitative analysis of plant extract was carried out for alkaloids, flavonoids, fixed oils, glycosides, phenols, quinone, and terpenoids. All of the phytochemicals like Alkaloids, Phenols, and quinone were present in the fruit extract of all samples [Table 1]. Flavonoids and terpenoids were present in samples of Er and Etj but absent in Ed and Mi whereas, glycosides were present in all extracts except Ed.

HPTLC

The discrepancy in the Embelin contents between self-collected Vidanga was found to be significant ($P < 0.05$) [Table 2]. Embelin content in Etj (1.06 ng/µl) and *E. ribes* (0.92 ng/µl) was highest. In contrast, there was significantly low content in Ed (0.5 ng/µl) and absent in *Maesa indica*.

Table 1: Qualitative phytochemical screening of selected Myrsinaceae fruit extract

Tests	*Embelia ribes*	*Embelia drupacea*	*Embelia tsjeriam-cottam*	*Maesa indica*
Alkaloids	✓	✓	✓	✓
Flavonoids	✓	×	✓	×
Fixed oils	✓	✓	✓	✓
Glycosides	✓	×	✓	✓
Phenols	✓	✓	✓	✓
Quinone	✓	✓	✓	✓
Terpenoids	✓	×	✓	×

"✓" and "x" denotes the presence and absence of particular phytoconstituents, respectively. *Er: Embelia ribes; Etj: Embelia tsjeriam-cottam; Ed: Embelia drupacea; Mi: Maesa indica*

Table 2: Peak table with Rf values, height, and area of Embelin from different Vidanga sample

Sr. No.	Track (ul)	Rf	Height	Area	ng/µl
1	Ed	0.6±0.01	27.49±1.2	959.14±3.99	0.5
2	Er	0.59±0.03	51.8±2.1	1789.18±6.9	0.92
3	Etj	0.65±0.04	61.55±3.0	2037.56±2.9	1.06
4	Mi	-	-	-	-

All values are mean±SD, *n*=3

Antioxidant Activity

In vitro experiments were used to measure antioxidant activity to compare their antioxidant effects including inhibition of denaturation of protein (BSA), ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfo nic acid) as an oxidant, and NO scavenging (NOS) activity.

Anti-Denaturation Assay

At lower concentrations, Er and Etj had more anti-denaturation activity than ($P < 0.05$). In comparison to Ed and Mi, Er demonstrated superior inhibitory action in BSA at higher concentrations ($P < 0.05$) [Figure 1]. *E. ribes* from the self-collected sample showed a significant IC50 value than other Vidanga extracts ($P < 0.001$). Non-significant results were seen in Ed and Mi extracts [Table 3].

ABTS

At lower concentration, Er exhibited higher anti-oxidant properties than Ed, Mi ($P < 0.05$). Whereas, at higher concentrations Trolox, Er and Etj showed significantly better activity than Ed, Mi ($P < 0.001$) [Figure 2]. The ABTS radical scavenging assay in this study revealed the IC50 of the Vidanga extract where *E. ribes* and Etj showed significant antioxidant activity. The results of the ABTS radical scavenging assay showed that the extract has an antioxidant effect in relatively higher concentrations compared to the standard ($P < 0.05$). Whereas no significant anti-oxidant activity was seen in Ed, Mi extracts.

NOS

The *E. ribes* and Etj extract exhibited significant NOS activity compared to other Vidanga extracts ($P < 0.05$) [Figure 3]. Along with Ed and Mi, extracts did not show significant NOS inhibitory activity.

DISCUSSION

A cursory glance, the list of Ayurvedic pharmaceuticals piques the interest of the scientific community, but ambiguity caused due to local and common names results in loss of trust in herbal medicine. Most herbal drug that is sold on the market is in form of powder, this makes it impossible to identify the authenticity based on morphological structures of a drug. As a result, it is vital to investigate the role of phytomedicine utilizing trans-disciplinary techniques, that is, analytical tools that can provide useful information of attributes present even after the loss of structure.[35] The primary prerequisite for using herbal medicine is authentication, and validation of herbal drugs which can be done by morphological, and biomarker-based identification. Analytical techniques can be the solution in the case where morphological traits are completely lost.[36]

This scientific profiling guarantees the quality assurance of herbal products and helps detect adulterations.[37]

The present study reports the absence of flavonoids and terpenoids in Ed and Mi ethyl acetate extract, Quinones, on the other hand, are electron carriers that play a role in photosynthesis,

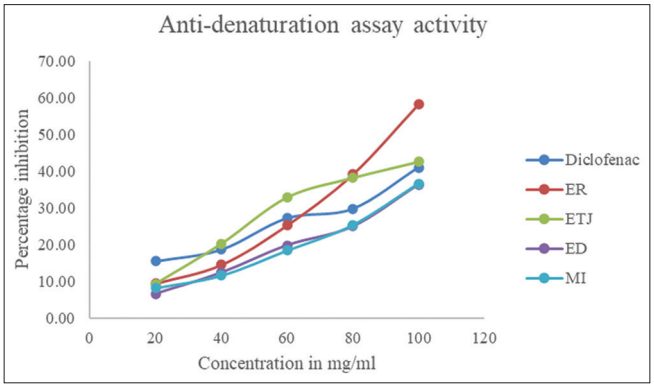

Figure 1: Bovine serum albumin denaturation activity of the Vidanga extract and their IC50 values. *Er*: *Embelia ribes*; *Etj*: *Embelia tsjeriam-cottam*; *Ed*: *Embelia drupacea*; *Mi*: *Maesa indica*.

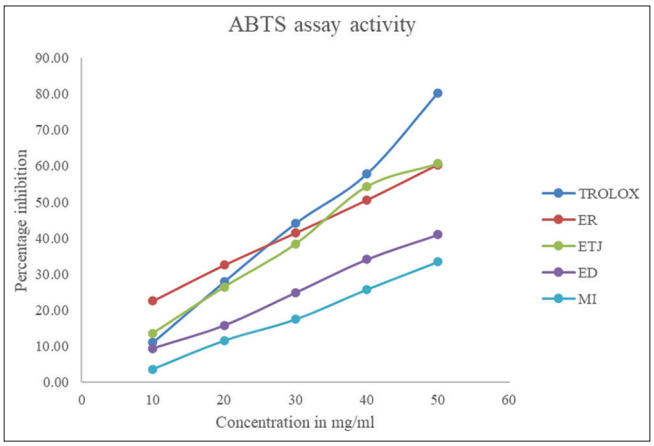

Figure 2: ABTS radical scavenging activity of the Vidanga extract and their IC50 values. *Er*: *Embelia ribes*; *Etj*: *Embelia tsjeriam-cottam*; *Ed*: *Embelia drupacea*; *Mi*: *Maesa indica*

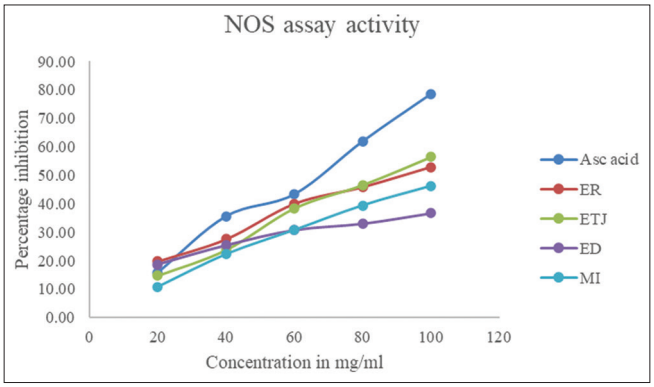

Figure 3: Nitric oxide scavenging activity of the Vidanga extract and their IC50 values. *Er: Embelia ribes*; *Etj: Embelia tsjeriam-cottam*; *Ed: Embelia drupacea*; *Mi: Maesa indica*

as revealed in this study. Quinones boost overall health through their antioxidant activity.[38] Plants produce flavonoids and phenolic chemicals to defend themselves or boost growth in harsh environments. Furthermore, the antioxidant activity of flavonoids, such as radical scavenging activity and/or metal ion chelation capacity, affects functional group organization, configuration, substitution, and the number of hydroxyl groups.[39] Because it has both quinone and phenolic groups on the same ring, Embelin is a fascinating chemical.[40] By reducing its quinone ring and producing molecular oxygen, embelin can scavenge superoxide while also making molecular oxygen. Apart from the π–π interaction, the molecular structure of embelin allows for further reactivity with superoxide.[25] The existence of benzoquinone like embelin was confirmed by qualitative analysis of embelin extracts using HPTLC. Nonetheless, quantitative discrepancies indicate a considerable variance in embelin contents, with the typical embelin peak at 336 nm indicating a major inconsistency in embelin contents [Figure 4]. Nevertheless, in the case of species of the same genus, that is, taxonomically close, a similar phytochemical composition is expected, thus requiring a diverse and more complete approach.[41] The TLC plate displayed bands of embelin present in all the samples except in Mi indicating samples showing significant discriminations between the two genera of the plants [Figure 5]. Mi extract has Kiritiquinone ($C_{28}H_{46}O_4$) a quinonoid structure similar to embelin dissolvable in ethyl acetate. Two additional hydroxyl groups in Kiritiquinone make it soluble in the solvent and assist the separation from Embelin present in Er, Etj, and Ed. HPTLC is more useful in chemotaxonomy for identifying herbs by secondary metabolites and also as a phytoconstituents biomarker.[42] Er and Etj were smaller in size but had a higher concentration of embelin than Ed this may be due to phytochemical compositions of plants being significantly influenced by different agroclimatic conditions, abiotic and biotic environmental factors, edaphic factor, sun-light exposure, and seasonal variations.[43] The outer coat of fruit Vidanga contains a large quantity of Embelin than the inner portion, Ed being large than Er, and Etj has a small surface area to volume ratio this is a major factor contributing to the low concentration of Embelin in Ed.[44] Despite their taxonomic and morphological dissimilarities, the phytochemical profiles of the selected group of herbs were very similar.

Oxidative stress is involved in various acute and chronic disorders such as cardiovascular, kidney, neurodegenerative, and cancer. The high reactivity of these free radicals leads to oxidative stress damaging the cells and ultimately leading to life-threatening disorders.[45] Phytoconstituents of plants are attributed free radical scavenging properties. The ABTS assay showed high free radical scavenging and this may be due to the presence of Embelin, a benzoquinone that has been reported to have ABTS free radical scavenging activity.[46] Endothelial cells, macrophages, neurons, and other cells produce NO, which is involved in the control of a variety of physiological processes. NO levels that are too high are linked to inflammation. Excess NO interacts with

Table 3 : Anti-oxidant potential of the Vidanga extract and their IC50 values (mg/ml)

Sr. No	Anti-oxidant assay	Standard	ER	ETJ	ED	Mi
1.	BSA anti-denaturation activity	135.88±20.30	84.69±14.99	111.04±9.08	149.92±34.38	145.57±15.11
2.	ABTS activity	66.93±1.67	78.27±1.43	78.56±0.92	121.33±5.61	147.67±18.14
3.	NOS activity	63.29±4.10	85.25±20.46	85.13±13.56	252.72±188.62	107.47±15.51

All values are mean±SD, *n*=3

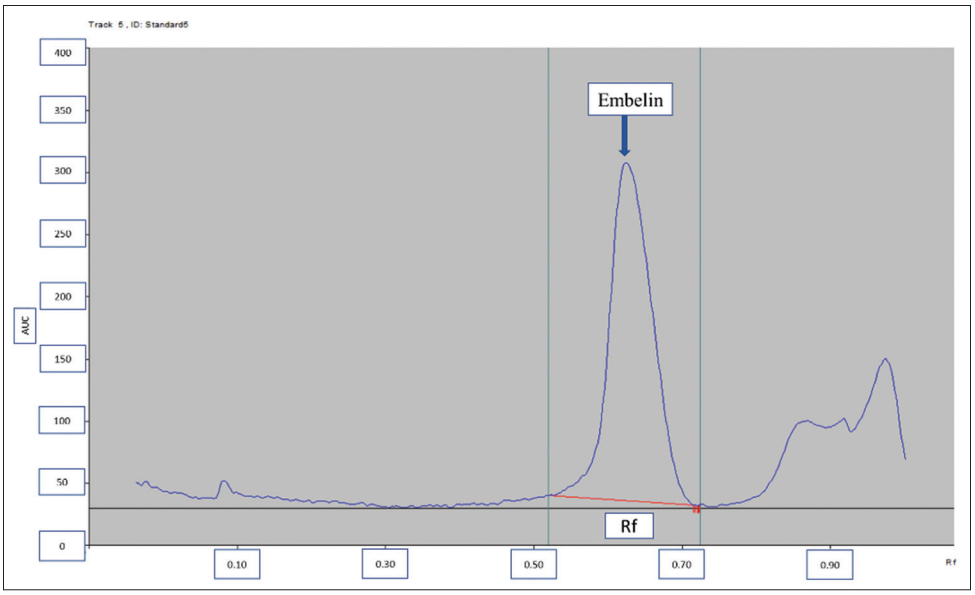

Figure 4: Standard embelin peak from at 336 nm

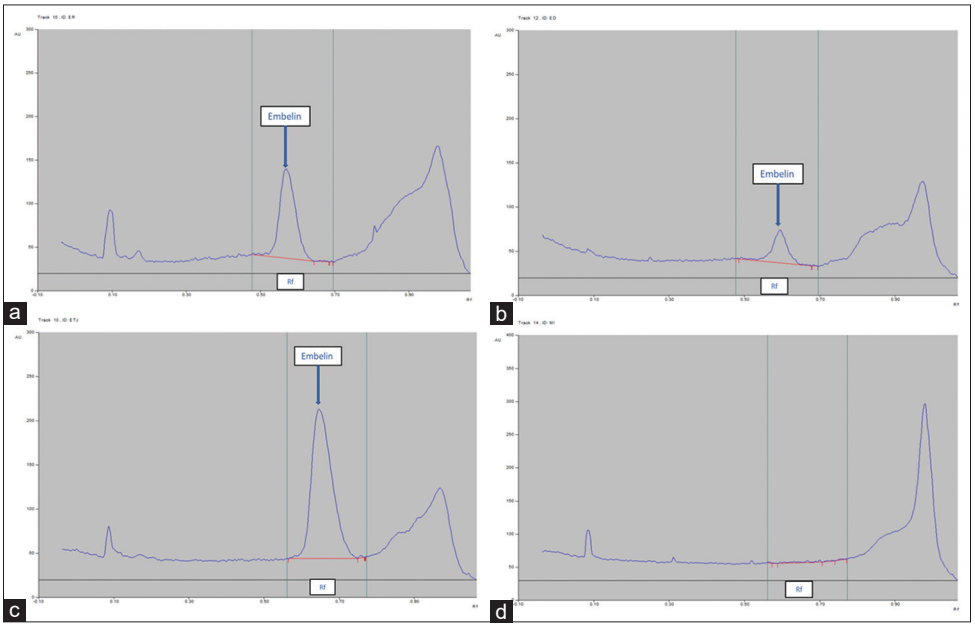

Figure 5: Embelin analysis by HPTLC of Self-collected sample. (a) *Embelia ribes*; (b) *Embelia drupacea*; (c) *Embelia tsjeriam-cottam*; (d) *Maesa indica*

oxygen to form nitrite and peroxynitrite anions that behave as free radicals. In this investigation, the extracts compete with oxygen for the ability to react with NO, preventing the formation of anions.

In plants, high concentration phytochemicals are produced during stress conditions to withstand hostile conditions. Edaphic factors and weather conditions of the semi-arid region usually are appropriate for the growth of most

herbs.[47] Stress-related studies exhibited an elevated number of secondary metabolites and flavonoids.[48] This may be a resulting discrepancy of Embelin concentration in *Embelia* spp. The presence of these medicinal compounds was demonstrated by the HPTLC profiles, confirming the species' ethnobotanical therapeutic applications. The variability concerns GMP in herbal medicine samples that the phyto-complex can exert.

Preliminary phytochemical screening showed the presence of quinone in all Vidanga samples but HPTLC analysis revealed the presence of Embelin (benzoquinone) in all Vidanga extracts except Mi. The concentration of embelin was higher in Er and Etj as compared to Ed and Mi with a similar pattern in the antioxidant potential whereas Er and Etj showed a non-significant difference in embelin concentration and anti-oxidant activity.

CONCLUSION

According to HPTLC analysis, Etj contains a similar amount of Embelin as *E. ribes* and exhibited equal antioxidant properties in *in vitro* biochemical experiments. Hence, only Etj can be substituted as a drug Vidanga. However, more research is required before the herbal products are formulated and standardized using the drug Vidanga.

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ETHICAL APPROVALS

Not applicable.

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