

## Analysis of Phenolic acids in Different Market Samples of *Vidanga* (False black pepper)

SHRADDHA U. NAYAK \* V. K. JOSHI \*\* S. MAURYA \*\*\* U. P. SINGH \*\*\*

Dr. D. Y. Patil College of Ayurveda and Research, Navi Mumbai & Institute of Medical Sciences, B.H.U., Varanasi.

**ABSTRACT :** *Vidanga* (*Embelia ribes* Linn, Myrsinaceae) is a promising medicinal plant of high medicinal value documented in Ayurvedic classics, traditional Indian medicine. It is said to be the best against human pathogens. It is observed that in the crude drug market, substitutes and adulterants are commonly seen because of non-availability of genuine drug. Therefore, a market survey of *Vidanga* was done for the available species. Astonishingly it was seen that *Embelia robusta* Roxb. was found instead of *E. ribes* which is the botanically accepted species. Samples of *Vidanga* were collected from different parts of India and Pakistan, which were then subjected to HPLC analysis for the presence of phenolic acids. The analysis revealed higher level of phenolic acids in *E.ribes* than *E.robusta*, particularly cinnamic acid which is highly anthelmintic.

**Key words :** *Vidanga*, *Embelia ribes*, *E.robusta*, Myrsinaceae, Adulteration, phenolic acids, HPLC analysis.

### INTRODUCTION

India is a large country with wide biodiversity. The flora varies from region to region and the plants growing under different climatic conditions possess different medicinal properties. *Vidanga* (*Embelia ribes*, Burm. F.) or False black pepper is one such promising medicinal plant mentioned in Ayurveda (the traditional medicine of India), with potent actions. It is a shrub whose leaves are 7.5 by 3 cm., in size, obtusely acuminate, base cuneate or rhomboid, nerve slender; flowers scarcely 0.2 cm. long, white ovary ovoid, not conic upwards. Fruits globular, varying in colour from dull red to nearly black, warty or wrinkled<sup>1</sup>.

The fruit of *E. Ribes* has been described as best krimighna (wormicidal in Ayurvedic classics) against pathogens<sup>2</sup>. Its efficacy has been claimed against pathogens affecting eyes, nose, head etc. and also against skin diseases, spleenomegaly, wounds, fistula, piles, anaemia, epilepsy. Additionally it is a good rejuvenator. Berries are recommended for use after one year of plucking<sup>3</sup>. The plants contain potassium embelate, 2,5-dihydroxy, 3-undecyl-1, 4-benzoquinone while the fruits are rich in embelin, quercitol, tannin, christembine, embelic acid, fatty ingredients, resinoid, volatile oil and vilangin<sup>4</sup>.

Many pharmacological studies have been carried out, to see the efficacy of this drug against several

diseases, for instance, alcoholic extract cured 80% of the cases infected by *Ascaris lumbricoides*<sup>5</sup> and embelin in the dose of 120 mg/kg body wt. prevented implantation by disturbing oestrogen and progesterone levels<sup>6</sup>. At a dose of 100 mg/day orally the drug was found to have contraceptive activity in male bonnet monkeys<sup>7</sup>. The extracts did not possess any toxicity at the cellular level and did not alter the functional integrity of the male reproductive organs<sup>8</sup>. Aqueous extracts of the fruit showed anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli*<sup>9</sup>.

The fruits of false black pepper resemble black pepper and therefore the drug is adulterated with cubebs, black pepper and exported in large quantities. Due to great decline in the export of cubebs and black pepper, the collection of *Vidanga* also decreased and rather the crude drug market received the supply of *Vidanga* from another species i.e. *Embelia robusta* Roxb. Also the collection of *E. robusta* is much easier than *E. ribes* as former is a large shrub and bears more fruits than *E.ribes* and is also distributed throughout India, while *E.ribes* has restricted distribution<sup>10</sup>. There is gradual disappearance of *E.ribes* due to inherent sterility of the seeds<sup>11</sup>. Also there is similarity in the structure of endosperm i.e. variegated spots. On account of these reasons *E. ribes* is adulterated with *E.robusta* though the active constituent (embelin) of the fruit is 2.3-3.1 % in *ribes* and only 1.6 % in *E.robusta*<sup>12</sup>.

Distinguishing characters of *Embelia ribes* and *Embelia robusta* have been reported by Shah and Khanna<sup>13</sup>. The fruits of *E.ribes* are small, 2.5 to 4 mm in diameter, usually greyish black with warty surface and that of *E. robusta*, 3.5 to 5 mm in diameter, usually reddish longitudinal striations. Also *E. robusta* fruits can be

\* Lecturer, Faculty of Ayurveda, Dr. D.Y. Patil College of Ayurveda & Research, Navi Mumbai.  
Email : shradhaayu1@rediffmail.com

\*\* Dean, Faculty of Ayurveda, I.M.S., Banaras Hindu University, Varanasi.

\*\*\* Plant Pathology Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

distinguished by the presence of characteristic oil glands which are not found in *E. ribes*.

Both practitioners and industries depend on crude drug market for collection of drugs. The samples of Vidanga were therefore collected from various crude drug markets to standardize the samples through qualitative analysis i.e. HPLC with phenolic acids as reference standards.

## MATERIALS AND METHODS

The samples collected were examined macroscopically. All samples had striations on fruits except the 1st sample from Meghalaya and Jaipur which had warty surface similar in appearance as *Piper nigrum*.

### Extraction of phenolic compounds :

The phenolic compounds were extracted as per the method of Singh et al.<sup>14</sup>. Twenty two samples of false black pepper were collected from different crude drug markets throughout India and one from Pakistan based on availability. One gram of each sample was macerated and suspended in 5 ml ethanol-water (50:20; v/v). The collected samples were subjected to ultrasonication (Branson Sonifier, Danbury, CT, supernatant) was pooled prior to evaporation under vacuum (Buchi rotavapor Re Type, Labco, India, Ambala Cantt, India). Dried samples were resuspended in 1.0 ml high performance liquid chromatography (HPLC)-grade methanol by vortexing and filtered through ultra membrane filter (pore size 0.45  $\mu$ m: Millipore) before HPLC analysis.

### HPLC analysis :

Quantitative analysis of the samples was performed according to the method of Singh et al. (2002). The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable Shimadzu

SPD-10 AVP UV-VIS detector and a Rheodyne Model 7725 injector with a loop size of 20  $\mu$ l. The peak area was calculated with a Winchrom integrator. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250 x 4.6 mm i. d., particle size 5  $\mu$ m, Luna 5  $\mu$ C-18 (2); phenomenex, Torrance, CA, USA) at 25°C. Running conditions included: injection volume, 5  $\mu$ l; mobile phase, methanol: 0.4% acetic acid (80:20 v/v); flow rate, 1 ml/min; and detection at 290 nm. Samples were filtered through an ultra membrane filter (pore size 0.45  $\mu$ m; B-Merck, Darmstadt, Germany) prior to injection in the same loop. Tannic, gallic, vanillic, caffeic, chlorogenic and cinnamic acids were used as internal and external standards. Phenolic acids present in each samples were identified by comparing chromatographic peaks with the retention time (Rt) of individual standards and further confirmation by co-injection with isolated standards. The amount of each phenolic acid is expressed as micrograms per gram of fresh weight unless otherwise stated.

## RESULTS

HPLC (High Performance Liquid Chromatography) analysis of various market samples of false black pepper collected from different crude drug markets all over India was done including one sample from Pakistan. Fig 1a, a shows the peaks of reference phenolic compounds. On analysis of these samples it was found that in Meghalaya sample no. 1 (Fig. 1a, b) five phenolic acids caffeic, vanillic, chlorogenic, cinnamic and o-coumaric acids were detected in which caffeic acid (448.163  $\mu$ g/g), was observed maximum followed by cinnamic (20.59), chlorogenic (8.888  $\mu$ g/g), vanillic (4.086  $\mu$ g/g). O-coumaric acid (0.878  $\mu$ g/g) was detected in trace amount. The phenolic acid content of other samples is tabulated in Table 1 and the peaks are depicted in Fig. 1a and Fig. 1b.

TABLE NO. 1 : PHENOLIC ACIDS IN FRUITS OF VIDANGA :

Plant		Place of Collection						Phenolic acid (mg/g dry wt)			
		Tannic acid	Gallic acid	Caffeic acid	Vanillic acid	Ferulic acid	Chlorogenic acid	Cinnamic acid	O-coumaric acid	Oxalic acid	Salicylic acid
Embelia ribes	Meghalaya	UDL	UDL	448.163 ± 0.06	4.086 ± 0.12	UDL	8.888 ± 0.08	40.59 ± 0.12	0.878 ± 0.13	UDL	UDL
E. robusta	Meghalaya	52.593 ± 0.09	0.423 ± 0.09	10.859 ± 0.119	UDL	UDL	1.169 ± 0.06	0.091 ± 0.384	UDL	UDL	UDL
E. robusta	Delhi-1	87.169 ± 0.09	UDL	UDL	UDL	UDL	19.565 ± 0.06	3.376 ± 0.10	UDL	285.26 ± 0.12	UDL
E. robusta	Delhi-2	UDL	1.614 ± 0.128	0.765 ± 0.12	0.359 ± 0.06	0.439 ± 0.15	UDL	0.065 ± 0.01	0.058 ± 0.009	UDL	2.305 ± 0.096
E. robusta	Delhi-3	UDL	2.814 ± 0.118	1.534 ± 0.14	UDL	0.374 ± 0.10	UDL	0.034 ± 0.009	0.0969 ± 0.108	UDL	2.778 ± 0.154

E. robusta	Delhi-4	UDL	14.275 ± 0.13	UDL	2.191 ± 0.04	4.298 ± 0.126	UDL	0.184 ± 0.06	UDL	UDL	3.668 ± 0.100
E. robusta	Delhi-5	0.654 ± 0.06	4.055 ± 0.009	UDL	0.882 ± 0.13	UDL	UDL	0.195 ± 0.024	2.234 ± 0.06	UDL	3.212 ± 0.135
E. robusta	Delhi-6	0.572 ± 0.09	1.366 ± 0.058	UDL	UDL	1.491 ± 0.09	UDL	0.011 ± 0.006	0.278 ± 0.059	UDL	1.604 ± 0.129
E. robusta	Dehradun	0.261 ± 0.059	0.527 ± 0.125	1.641 ± 0.101	UDL	0.335 ± 0.067	UDL	0.045 ± 0.005	0.883 ± 0.046	UDL	2.108 ± 0.009
E. robusta	Haridwar	0.294 ± 0.06	0.981 ± 0.04	0.612 ± 0.09	3.4478 ± 0.08	0.501 ± 0.038	UDL	UDL	1.18 ± 0.044	UDL	5.865 ± 0.06
E. robusta	Gauhati	UDL	6.076 ± 0.01	4.472 ± 0.102	UDL	2.249 ± 0.059	UDL	0.434 ± 0.099	UDL	UDL	1.038 ± 0.008
E. robusta	Lucknow 1	UDL	7.466 ± 0.07	4.632 ± 0.09	UDL	3.401 ± 0.05	UDL	UDL	0.238 ± 0.006	UDL	0.241 ± 0.008
E. robusta	Lucknow 2	0.949 ± 0.01	4.534 ± 0.11	2.654 ± 0.047	2.923 ± 0.048	UDL	UDL	0.067 ± 0.006	1.17 ± 0.024	UDL	6.563 ± 0.006
E. robusta	Kolkatta-1	UDL	3.393 ± 0.05	0.365 ± 0.005	0.419 ± 0.008	UDL	UDL	0.025 ± 0.003	0.189 ± 0.003	UDL	1.913 ± 0.058
E. robusta	Kolkatta-2	UDL	0.689 ± 0.005	0.378 ± 0.006	0.241 ± 0.003	0.237 ± 0.002	UDL	0.001 ± 0.0003	UDL	UDL	0.001 ± 0.0001
E. robusta	Jaipur	UDL	5.016 ± 0.08	1.361 ± 0.06	UDL	0.186 ± 0.005	UDL	14.071 ± 0.03	0.303 ± 0.01	UDL	3.861 ± 0.06
E. robusta	Mumbai	UDL	13.151 ± 0.13	1.279 ± 0.06	UDL	4.174 ± 0.03	UDL	0.08 ± 0.003	UDL	UDL	1.251 ± 0.03
E. robusta	Bangalore-1	UDL	UDL	0.909 ± 0.004	UDL	UDL	UDL	UDL	0.176 ± 0.005	17.08 ± 0.455	UDL
E. robusta	Bangalore-2	UDL	UDL	UDL	UDL	UDL	UDL	UDL	UDL	30.156 ± 0.397	UDL
E. robusta	Udupi	UDL	UDL	0.97 ± 0.02	UDL	UDL	UDL	1.74 ± 0.06	UDL	14.22 ± 0.15	UDL
E. robusta	Kerala-1	UDL	UDL	0.171 ± 0.005	UDL	UDL	UDL	UDL	0.458 ± 0.008	17.819 ± 0.62	UDL
E. robusta	Kerala-2	UDL	7.2295 ± 0.045	0.269 ± 0.01	0.244 ± 0.003	0.182 ± 0.01	UDL	0.005 ± 0.0005	0.420 ± 0.01	UDL	UDL
E. robusta	Pakistan	0.509 ± 0.009	2.983 ± 0.05	2.26 ± 0.02	UDL	0.559 ± 0.006	UDL	UDL	0.214 ± 0.0006	UDL	9.299 ± 0.28

UDL = Under detection limit.

**TABLE NO. 2 : THERAPEUTIC USES OF PHENOLIC ACIDS :**

Phenolic acid	Biological activity
Phenolic compounds	Antioxidant <sup>15</sup>
Gallic acid	
Ferulic acid	
Cinnamic acid	
4-propoxy cinnamic acid	See Singh <i>et al.</i> <sup>16</sup>
Chlorogenic acid	
Caffeic acid	
Vanillic acid	Anti-sicking property in sickle cell anaemia <sup>17</sup>
Cinnamic acid	Anthelmintic <sup>18</sup> , Antiviral <sup>19</sup>
Oxalic acid	Haemostatic agent (Vet) <sup>18</sup>
Salicylic acid	Antipyretic <sup>20</sup> Anti-inflammatory Topical keratolytic <sup>18</sup> Externally as antiseptic, antifungal agent for various skin conditions <sup>18</sup> .

## DISCUSSION

Phenols comprise the largest group of plant secondary metabolites. Phenolic acids are a special class of simple phenols and mainly include benzoic and cinnamic acid derivatives such as gallic acid, salicylic acid, caffeic

acid, vanillic acid, ferulic acid, chlorogenic acid etc<sup>18</sup>. All these phenolic acids have potent pharmacological activities, such as antioxidant<sup>15</sup>, anti-inflammatory<sup>19</sup>, antiviral<sup>19</sup>, antibacterial and antifungal<sup>16</sup>, antisickling<sup>7</sup>; anthelmintic<sup>8</sup> (Table 2).

FIG : 1 A : HPLC OF VARIOUS MARKET SAMPLES OF EMBELIA SPECIES :

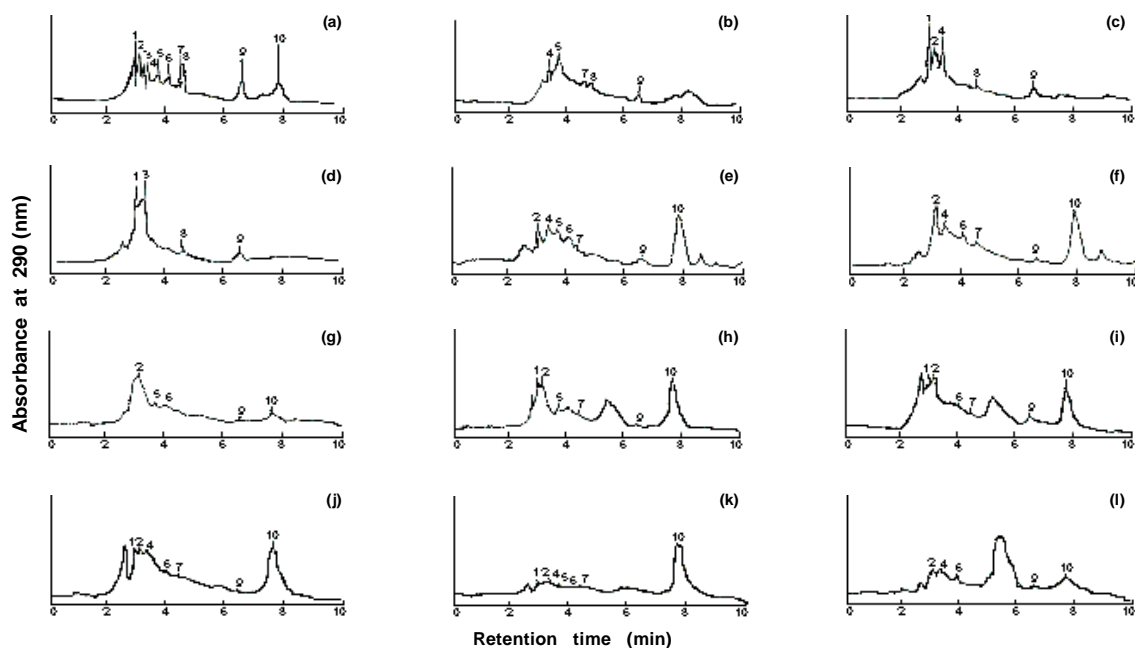


Fig. 1 a. HPLC of various market samples of Embelia species (a) Standard phenolic acids, (b) *Embelia ribes* (Meghalaya-1), (c) *Embelia robusta* (Meghalaya-2), (d) *Embelia robusta* (Delhi-1), (e) *Embelia robusta* (Delhi-2), (f) *Embelia robusta* (Delhi-3), (g) *Embelia robusta* (Delhi-4), (h) *Embelia robusta* (Delhi-5), (i) *Embelia robusta* (Delhi-6), (j) *Embelia robusta* (Dehradun), (k) *Embelia robusta* (Haridwar), (l) *Embelia robusta* (Gauhati). Peak Nos. 1. taniic, 2. gallic, 3. oxalic, 4. caffeic, 5. vanillic, 6. ferulic, 7. o-coumaric, 8. chlorogenic, 9. cinnamic, 10. salicylic acids.

FIG : 1 B : HPLC OF VARIOUS MARKET SAMPLES OF EMBELIA SPECIES :

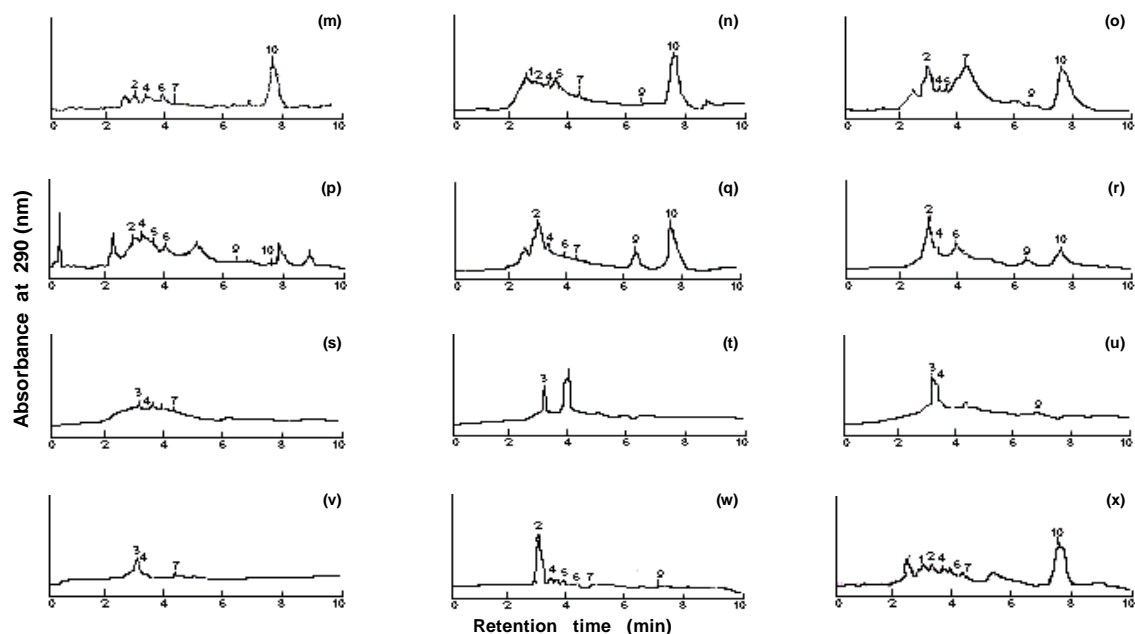
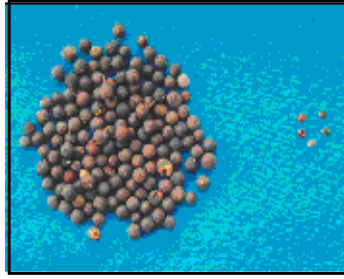
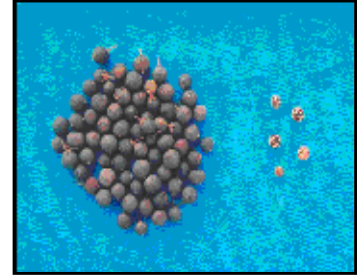
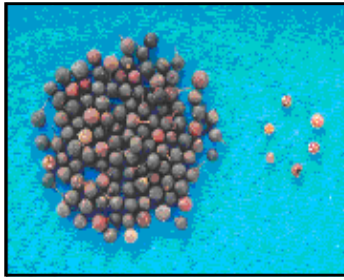
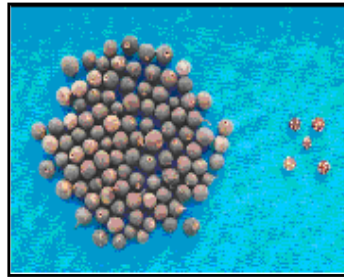
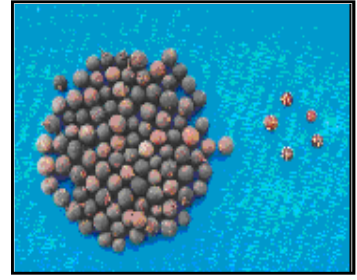
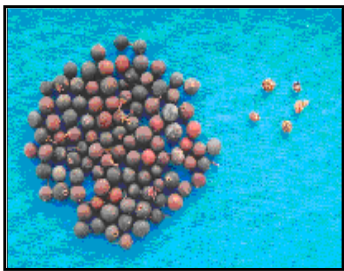
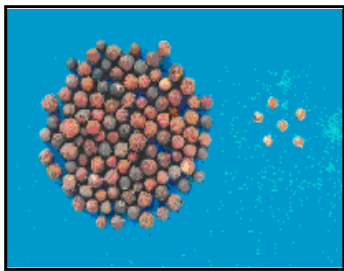
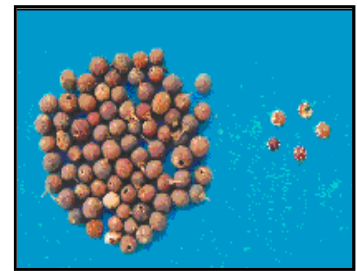
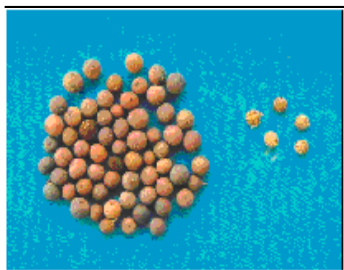
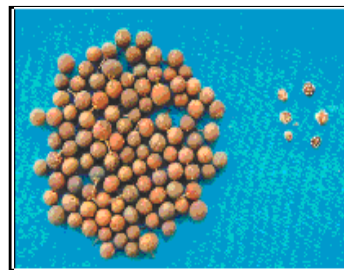
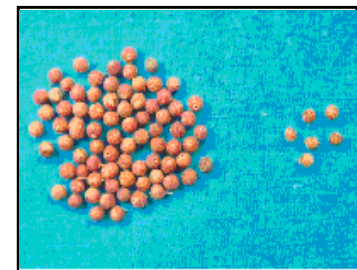


Fig. 1 b. HPLC of various market samples of Embelia species (m) *Embelia robusta* (Lucknow-1), (n) *Embelia robusta* (Lucknow-2), (o) *Embelia robusta* (Calcutta-1), (p) *Embelia robusta* (Calcutta-2), (q) *Embelia robusta* (Jaipur), (r) *Embelia robusta* (Mumbai), (s) *Embelia robusta* (Bangalore-1), (t) *Embelia robusta* (Bangalore-2), (u) *Embelia robusta* (Udupi), (v) *Embelia robusta* (Kerala), (w) *Embelia robusta* (Kerala), (x) *Embelia robusta* (Pakistan). Peak Nos. 1. taniic, 2. gallic, 3. oxalic, 4. caffeic, 5. vanillic, 6. ferulic, 7. o-coumaric, 8. chlorogenic, 9. cinnamic, 10. salicylic acids.

## PLATE 3. MARKET SAMPLES OF FRUITS OF VIDANGA :

*Embelia ribes* (Meghalaya)*Embelia robusta* (Meghalaya)*Embelia robusta* (Delhi-1)*Embelia robusta* (Dehradun)*Embelia robusta* (Haridwar)*Embelia robusta* (Gauhati)

## PLATE 4. MARKET SAMPLES OF FRUITS OF VIDANGA :

*Embelia robusta* (Lucknow -2)*Embelia robusta* (Jaipur)*Embelia robusta* (Mumbai)*Embelia robusta* (Bangalore)*Embelia robusta* (Kerala-2)*Embelia robusta* (Pakistan)

*Embelia ribes* is the botanically accepted species of vidanga. It is rather observed that *E. robusta* is commonly sold as vidanga instead of *E. ribes* because of the restricted distribution, inherent sterile seeds and less yield of fruits of the latter. A survey of crude drug market was conducted to know the status of adulteration. Twenty-two samples from various places in India and one sample from Pakistan were collected based on availability. From certain places like Delhi samples were collected from different crude drug markets of the same place. Morphological examination revealed that only one sample from Meghalaya was *E. ribes* and that from Jaipur was a mixture of *E. ribes* and *E. robusta*. All other samples were of *E. robusta*. The samples which were blackish brown in colour with anastomosing wrinkled surface, beak like projection were identified as *E. ribes*. The others which were reddish black, globose having striations from apex to base of the drupe were identified as *E. robusta*.

The samples were then subjected to HPLC analysis with special reference to phenolic acids. Phenolic acid profile revealed interesting results. Cinnamic acid (reported to have anthelmintic, anti-viral, antioxidant and antifungal property) was found to be maximum in *E. ribes* sample of Meghalaya followed by Jaipur sample. Caffeic acid known to have antioxidant, antiviral, and anti-inflammatory action, was also seen to be in higher amounts in *E. ribes*. Vanillic acid was also found more in the same sample. Among the samples of *E. robusta* Delhi sample no. 1 had cinnamic acid content in higher amount.

The exact reason behind the difference in Phenolic acid content amongst different samples could be because of the soil, climatic conditions or most importantly the difference in time duration of collection and storage of samples. The findings obtained through HPLC and literary study corroborate therapeutic uses as found in Charak samhita and Sushruta samhita, the original scriptures of Ayurveda. Preparations from both the species, i.e., *E. ribes* and *E. robusta* can be used as vidanga as both have similar therapeutic activities. However, classical as well as our findings prove *E. ribes* as the best antipathogenic (Krimighma) drug. This is the first report of phenolic acids i.e. tannic, gallic, caffeic, vanillic, ferulic, chlorogenic, cinnamic, oxalic and salicylic acids in *Embelia ribes* and *Embelia robusta*.

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## हिन्दी सारांश

### विडंग के बाजार नमूनों में फेनोलिक एसिड का गुण संबंधी और प्रमाण संबंधी विश्लेषण

श्रद्धा यु. नायक, वी. के. जोशी, एस. मौर्य एवं यु. पी. सिंह

विडंग आयुर्वेद शास्त्रो में वर्णित एक महत्वपूर्ण औषधी है, जिसे कृमि में सर्वश्रेष्ठ माना गया है। औषधि निर्माता और वैद्य अपने आसपास के बाजारों से औषधियों को खरीदते हैं लेकिन ज्यादातर ये पाया गया है कि इन बाजारों में असली औषधियों के कुछ संमिश्रित या कुछ और ही मिलते हैं। इसीलिए विविध प्रांतों के अलग अलग बाजारों से विडंग के नमूने संग्रहीत किये गए। ये देखने में आया कि एंबेलिया राईब्स के बदले एंबेलिया रोबुस्टा ही ज्यादातर मिलता है। इन नमूनों का एच.पी.एल.सी. विश्लेषण द्वारा फेनोलिक एसिड की मात्रा देखी गयी इससे साबित हुआ कि एंबेलिया राईब्स में फेनोलिक एसिड की मात्रा एंबेलिया रोबुस्टा से ज्यादा है।

