ORIGINAL ARTICLE

Ambiguity in the authenticity of selected "Vidanga" market samples with respect to their biochemical and chromatographic evaluation

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Abstract

Objective: There are various factors responsible for variations in the traded herbal composition on the basis of species variation, fruit immaturity, climatic conditions, substitutability, and uncertain trade practices. There is timely need to resolve this ambiguity. The present study designed to resolve the variation in traded fruits of "Vidanga." **Materials and Methods:** "Vidanga" fruits were collected from markets of different locations in India. The antioxidant activity was evaluated by 2,2-diphenyl-1-picryl-hydrazyl-hydrate, anti-lipid peroxidation (ALP), and ferric reducing antioxidant power (FRAP) using two different solvents, namely, ethanol and ethyl acetate. Total phenolic and flavonoid contents were studied by standard methods, namely, Folin-Ciocalteu and aluminum chloride respectively. Phytoconstituents investigation was also observed and chemical constituents were identified by liquid chromatography-mass spectrometry (LC-MS). **Results and Discussion:** Ethanolic and ethyl acetate extracts of "Vidanga" market samples exhibited highest total phenolic content in Pune sample (0.860 ± 0.003 mg/g gallic acid equivalent [GAE] equivalent**)** and in Nagpur (0.179 ± 0.015 mg/g quercetin equivalent**),** respectively. All the samples showed 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging activity in the order, highest activity in samples of Delhi followed by Nagpur, Mumbai, Bengaluru, and Pune. The ethyl acetate extract obtained from Nagpur sample had the strongest free radical-scavenging activity with IC_{50} value of 89.54 µg/mL ± 0.001. ALP shows the order as lowest in Bengaluru sample and with slight increasing order followed by Nagpur, Delhi, Pune, and Mumbai samples which shows the high activity. Ethyl acetate extract of Pune sample showed the highest (40.47 ± 0.004 µg/mL) IC_{50}. FRAP activity showed maximum inhibition in ethyl acetate extract of the Pune sample (67.76%) while the lowest activity showed in Delhi sample (33.64%). FRAP showed the lowest activity in Pune followed by Nagpur, Mumbai, Delhi, and high activity in Bengaluru sample. Phytochemical investigation shows the presence of alkaloids, carbohydrate, tannins, proteins, amino acids, fixed oils, gum and mucilage, glycosides, terpenoids, and steroids. LC-MS revealed phytoconstituent variations among all the samples. **Conclusion:** By evaluating commercial "Vidanga" samples from major markets, namely, Bengaluru, Delhi, Mumbai, Nagpur, and Pune, it is concluded that significant variation exists in the traded samples regarding their morphology, identity and medicinal attributes as studied from a string of biochemical assays as well as mass spectrum. Hence, the study suggests awareness in all scientific community, traders, practitioners, manufacturing units, pharmacies, etc., for an urgent need for the selection of authentic raw materials.

Key words: "Vidanga", Antioxidant assays, Liquid chromatography-mass spectrometry fingerprints, Total flavonoid content, Total phenolic content

INTRODUCTION

India is a rich chest of medicinal plant biodiversity. Recently, more than 1.5 million medicinal plants are been used for preventive, promotional, curative, etc., purposes in India. Ayurveda uses about 700 species, Unani 700, Siddha 600, Amchi 600, and modern medicine around 30 species. Plant based medicines are the root of the modern medicinal health system; however traditional

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medicinal knowledge and plants play a central role in biological activity research and drug discovery.[1] About 80% of the population in the world still relies on the traditional system of medicine for their primary healthcare.[2]

Herbal plants contain antioxidant compounds which protects cells against degenerative effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl, radicals, hydroxy radicals, which are free radicals. The balance between ROS production and antioxidant defense is lost due to "oxidative stress," functioning a series of events, de-regulating the cellular function and all these leads to various diseases such as aging, arthritis, asthma, carcinogenesis, diabetes, rheumatism, inflammation, and neurodegenerative disease.[3,4] Over the centuries, traditional medicines or folk medicines includes natural substances derived from plants for the treatment of many diseases. Secondary metabolites present in traditional medicinal plants in the form of free radicals having various health benefits.[5]

"Vidanga" is a group of traditionally used medicinal plants, which are used by the tribal people in India as a medicine. "Vidanga" plants belong to the family Myrsinaceae and this family consists of nearly 1000 species of trees and shrubs spread over 33 genera. Among these, four genera namely *Myrsine, Maesa, Rapanea* and *Embelia*, which are widely used in herbal medicines.[6] It is meagrely distributed in the evergreen to moist ephemeral forests of the Western Ghats. National Medicinal Plant Board and The Maharashtra State Horticulture and Medicinal Plant Board having a "Species List" which also mentions *Embelia ribes* as a priority plant.[6] Species used as "Vidanga" have various uses in Ayurveda such as *E. ribes* used as anthelmintic, carminative, antibacterial, antibiotic, hypoglycemic, antifertility medicine etc.;[7] *Embelia drupacea* used for the treatment of laxative, antiseptic, antihelmintic, toothache etc.;[8] *Embelia tsjeriam-cottam* uses are as hepatoprotective, anti-inflammatory, analgesic, antidiabetic, wound healing etc.;[9] *Maesa indica is* used for blood purification and as an anthelmintic.[10]

Species under the name "Vidanga" are known by different local names in many regional languages in India as well as other countries. In market, drug under the name "Vidanga," the samples supplied contains either fruits of *Embelia tsjeriam cottam* (Roem. and Schult) A. DC. and *Embelia ribes* Burm. f. or mixture of different species like *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. Fruits of these species are morphologically alike and create the ambiguity in identification and use of authentic species as "Vidanga." Certain ambiguities in the use of exact plant due to challenging disarrangement with reference to their different vernacular names for same species or different plant species is the major concern.[11, 12] For example, *E. ribes* is being adulterate/substituted with *E. tsjeriam-cottam*, *E. drupacea* and *M. indica* and also various other drugs in the market, so genuineness of drug need to be assessed before use, as this may lead to the decrease in therapeutic efficacy. Hence, the availability of genuine sample of "Vidanga" needs immediate attention to maintain healthy therapeutic value.

In the present work, we have studied antioxidant activity by adopting various *in vitro* methods viz. DPPH radical scavenging activity, Ferric reduction potential assay, anti-lipid peroxidation (ALP) assay, estimation of total phenol content and estimation of total flavonoid content. Also compound profile of selected market samples was analyzed using liquid chromatography–mass spectrometry (*LC-MS*) to study ambiguity with respect to their biological activity, antioxidant activity, and compound profile of the "Vidanga" samples.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and reagents used in the experiments were analytical grade. DPPH, thiobarbituric acid reactive substance (TBARS), KCl, $FeCl_3$, thiobarbituric acid, trichloro acetic acid, and butylated hydroxy toluene were prepared in methanol, ascorbic acid, sodium acetate trihydrate, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), ascorbic acid, gallic acid, and Folin–Ciocalteu reagent obtained from Sigma-Aldrich, USA.

Market Sample Collection and Extraction

"Vidanga" fruits samples were purchased from major markets of different locations in India, that is, Bengaluru, Delhi, Mumbai, Nagpur, and Pune. Fruits were pulverized to fine powder in a mechanical blender. These powders were utilized for further experimental purposes. Extracts were prepared by Soxhlet apparatus (ROTAMANTAL, REMI)[13] using solvents, namely, ethyl acetate and ethanol at 60–80°C for 24 h.[14] These extracts were consequently evaporated under reduced pressure at 45°C in a rotary evaporator (IKA RV 10).

Polyphenolic Content Assays

Polyphenolic content of extracts was determined using the Folin-Ciocalteu method. It is demonstrated as mg of GAEs/gram of dry weight of the extract. Total flavonoid content was assessed using aluminum chloride colorimetric method and it is expressed as mg of quercetin per gram of dry weight.[5] All the experiments were carried out in triplicates.

Free Radical Scavenging Assays

DPPH

DPPH is nitrogen-centered, stable free radical, which can get readily destroyed by free radical quencher and shows a strong

absorption band at 517 nm.[15] *In vitro* DPPH-free radical scavenging assay was determined as per Brand-Williams *et al*.[16] Ascorbic acid was used as standard. The results were expressed as IC_{50} values, which required 50% scavenging inhibition of DPPH radical.

Ferric Reducing Antioxidant Power (FRAP) Assay

Hydrophilic antioxidants present in plant extracts were determined as per Benzie and Strain.[17] Antioxidant potential was estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). Ferric ion reducing activity of all the extracts was determined by standard $FeSO_4$ (0.1–1 mM). Results were analyzed from standard curve of ferrous sulfate and expressed in terms of µM Fe/g of dry mass.

The percentage free radical scavenging activity was calculated by the formula:

$$\%\,scavenging\ activity = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100 \quad (1)$$

Where $A_{Control}$ is absorbance of the control and A_{Sample} is absorbance of samples.

ALP Assay

The lipid peroxidation inhibition potential of extracts was determined by TBARS using goat liver as lipid source is described by Wade *et al*.[18] Percent inhibition of free radicals was calculated and IC_{50} values were compared. All the experiments were carried out in triplicates.

Percentage lipid peroxidation was calculated using formula:

$$\%\ \text{Lipid peroxidation} = \frac{A_{Induced} - A_{Sample}}{A_{Induced} - A_{Normal}} \times 100 \quad (2)$$

Where, $A_{Induced}$ is absorbance of Induced; A_{Sample} is absorbance of sample; and A_{Normal} is absorbance of normal.

Preliminary Phytochemical Screening

The ethyl acetate and ethanolic extracts were subjected to qualitative chemical examination for the identification of various plant constituents, namely, carbohydrates, alkaloids, fixed oils, glycosides, proteins and amino acids, saponin, mucilages, terpenoids, steroids, and tannins and were determined as per Sahira and Cathrine.[19]

LC-MS

The samples were loaded onto Infinity Lab Poroshell 120 SB-C18 column of 2.7 µm particle size and dimensions 3.0 mm × 100 mm (Agilent Technologies) maintained at 40°C. Aqueous solvent phase was 90% water + 9.9% acetonitrile and 0.1% formic acid. The organic phase consisted of 90% acetonitrile + 9.9% water and 0.1% formic acid. The flow rate was 0.3ml/min and runtime were 44 min.

After achieving separation through column, it was passed onto mass spectrometer that was operated in negative ion polarity mode. The MS was storing data in profile mode with mass range (MS) 100–1700; MS/MS range 50–1700 m/z and 100–1700. The fragment or voltage was set at 175V, specific collision energies were kept with respect to charge content (2, 3, 1, and >3) of proteins. Other important parameters were isotope model small molecules, gas temp 325°C, and capillary voltage 3500V.

Statistical Analysis

Data were analyzed by one-way ANNOVA and expressed as mean±standard deviation. $P < 0.05$ considered statistically significant.

RESULTS

Polyphenolic Content

Polyphenols are the secondary metabolites; hydroxyl groups present in their structure are responsible for scavenging ability of that extract. Among polyphenolic content, phenolic and flavonoids are the major group of compounds which are believed to be responsible for antioxidant activity of the extract. In the present study, both ethanolic and ethyl acetate extracts were studied [Table 1]. The ethanolic and ethyl acetate extracts of "Vidanga" presented the highest total phenolic content in market sample collected from Pune (0.860 ± 0.003) and in Nagpur (0.179 ± 0.015), respectively [Table 2].

Free Radical Scavenging Activity

In the present study, extracts of all the market samples showed some free radical scavenging activity, namely DPPH-scavenging activity, ALP, and FRAP activity in terms of % inhibition [Figures 1-3 respectively]. Results revealed that, all extracts were able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH-H, based on electron and H atom transfer. We also studied comparison among all the market samples in terms of %

Table 1: The extraction yield of two solvent extracts

Market samples	Ethyl acetate	Ethanol
Bengaluru	5.167	13.82
Delhi	3.977	17.59
Mumbai	5.097	10.81
Nagpur	5.431	14.334
Pune	6.462	1.64

Table 2: Polyphenolic content of the "Vidanga" market samples

Market samples	Total phenolic content (50µg/ml) (mg/g GAE equivalant[a, b])		Total flavonoid content (1mg/ml conc.) (mg/g quercetin equivalant[a, c])	
	Ethyl acetate extract	Ethanolic extract	Ethyl acetate extract	Ethanolic extract
Bengaluru	0.221±0.023	0.111±0.002	0.135±0.023	0.098±0.002
Delhi	0.246±0.002	0.104±0.003	0.138±0.010	0.091±0.022
Mumbai	0.178±0.012	0.102±0.004	0.120±0.012	0.115±0.016
Nagpur	0.229±0.015	0.094±0.003	0.179±0.015	0.093±0.008
Pune	0.207±0.015	0.860±0.003	0.122±0.015	1.122±0.151

[a]All values are mean±SE, n=3, [b]Values are expressed as equivalent to gallic acid (mg/g of GAE), [c]Values are expressed as equivalent to quercetin (mg/g of quercetin)

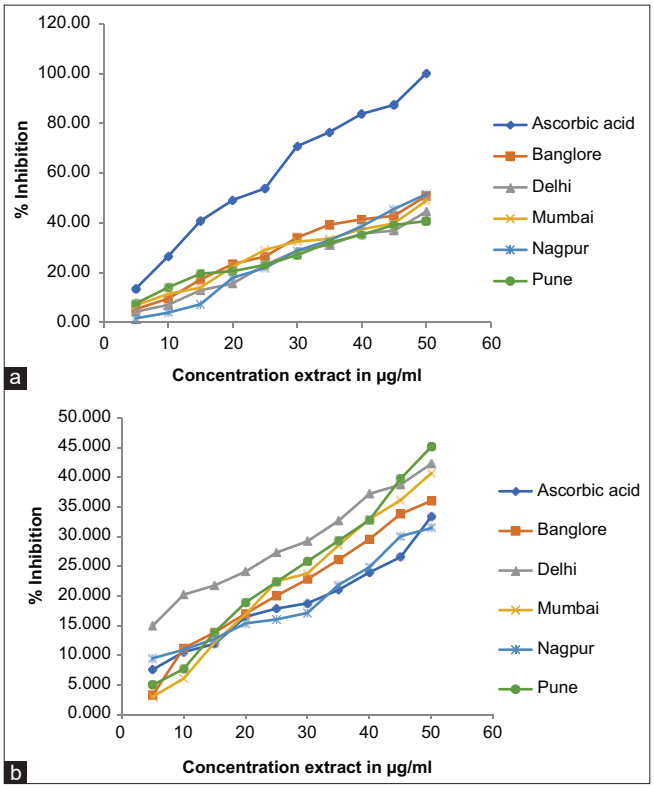

Figure 1: Comparative DPPH scavenging activity of "Vidanga" market samples (a) ethanolic extract and (b) ethyl acetate extract

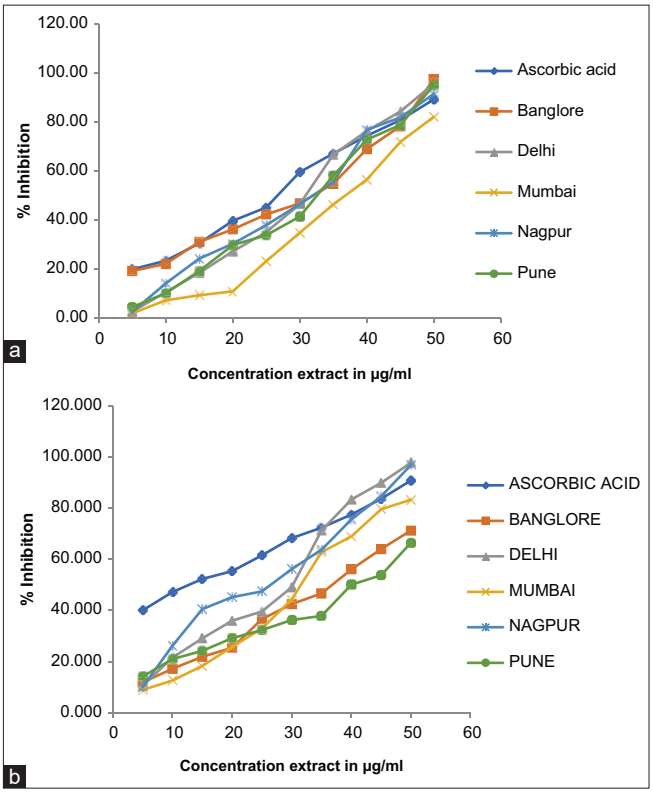

Figure 2: Anti-lipid peroxidation potential of "Vidanga" market samples (a) ethanolic extract and (b) ethyl acetate extract

inhibition [Figures 1-3]. Standard used was ascorbic acid (IC_{50} 25.69 ± 0.13 µg/ml). The ethyl acetate extract obtained from Nagpur market sample had showed highest IC_{50} value of 89.54 µg/mL ± 0.001. While, lowest activity was observed in ethanolic extract of Pune market sample showed strongest free radical scavenging activity (IC_{50} value of 41.57 µg/mL ± 0.003) [Table 2]. On the contrary, all the market samples showed lower DPPH scavenging activity as compare to the authenticate plant extract (unpublished observation). This might be due to some unknown adulterant present in the sample, which may be contributed in activity.

FRAP assay is based on electron transfer reaction.[20,21] FRAP abilities of these extracts in terms of % inhibition is also shown in Figure 3. Results suggest that, maximum inhibition observed in ethyl acetate extract of the sample collected from Pune market (67.76%) while the lowest activity showed in Delhi market sample (33.64%).

ALP utilized as a direct measure of oxidative damage, lipid-peroxidation is one of the major injuries in living cells or system due to increased concentration of cellular ROS. The IC_{50} values of ALP activity ranged from 23.88 ± 0.004 to 40.47 ± 0.004 µg/mL [Table 3]. Again, ethyl acetate extract of Delhi market sample has the lowest IC_{50} values. While, the highest IC_{50} observed in ethyl acetate extract of Pune market sample.

Phytochemical Screening

The detailed methods are described above and the results are also given in [Table 4]. Saponins were not detected in

Table 3: Antioxidant results of extracts by DPPH, ALP, and FRAP

Samples	DPPH (IC_{50} µg/mL)		ALP (IC_{50} µg/mL)		FRAP (IC_{50} µg/mL)	
	Ethanolic	Ethyl acetate	Ethanolic	Ethyl acetate	Ethanolic	Ethyl acetate
Pune	41.57±0.003	66.76±0.002	30.32±0.003	40.47±0.004	48.92±0.02	48.79±0.003
Nagpur	49.26±0.002	89.54±0.001	29.17±0.002	24.83±0.006	51.39±0.002	50.28±0.005
Mumbai	48.97±0.01	60.93±0.001	35.92±0.01	30.94±0.003	67.93±0.003	60.79±0.005
Delhi	56.67±0.002	63.86±0.004	29.22±0.005	23.88±0.004	55.09±0.01	68.59±0.003
Bengaluru	48.70±0.002	69.56±0.003	27.68±0.001	35.51±0.005	66.08±0.001	69.17±0.001

Highlight indicates the highest free radical scavenging capacity. DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate, ALP: Anti lipid peroxidation, FRAP: Ferric reducing antioxidant power

Table 4: Preliminary phytochemical screening of "Vidanga" market samples

Tests	Ethyl acetate extract					Ethanolic extract				
	Bengaluru	Delhi	Mumbai	Nagpur	Pune	Bengaluru	Delhi	Mumbai	Nagpur	Pune
Carbohydrates	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Alkaloid's	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fixed oils	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Glycosides	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Proteins and Amino acids	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Saponins	×	×	×	×	×	×	×	×	×	×
Gum and Mucilages	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Terpenoids	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Steroids	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tannins	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

"✓" and "×" denotes the presence and absence of particular phytoconstituents, respectively

all market samples while terpenoids were present only in Mumbai market sample.

Chemical Composition of Fractions

Due to high sensitivity and selectivity, liquid chromatography coupled with mass spectroscopy is widely-used for screening of secondary metabolites present in herbal products, which is critical for natural product samples to analyzing complex matrices. In the LC-MS method, in CI, ion molecule reactions occur between ionized reagent gas molecules (G) and volatile analyte neutral molecules (M) to produce analyte ions. Pseudo-molecular ion MH+ (positive ion mode) or [M-H]- (negative ion mode) are often observed. Unlike molecular ions obtained in EI method, MH+ and [M-H]- detection occurs in high yield and less fragment ions are observed.

Positive ion mode:

GH+ + M ------> MH+ + G

In the present work, full mass spectrum obtained from LC-MS analysis of all the market samples is presented in Figure 4. The spectrum showed highest broad peak observed in the range of 43.86–44.02 in all the samples. Peak observed at 43.88 indicates the presence of Embelin while peak area was very negligible in all the samples. The spectrum showed high area peaks in Bengaluru market sample at 45.10, 42.17, 39.23, 36.61, 33.44, 35.02, etc., while Delhi market sample showed peaks at 40.40, 37.66, 36.34, 34.80, 33.15, etc., whereas Mumbai, Nagpur and Pune market sample showed the high peaks at 46.36, 45.16, 40.60, 37.98, 34.90, 33.25, etc., 45.14, 41.64, 37.63, 36.42, 35.21, etc., 45.13, 41.75, 40.67, 39.58, 38.15, 35.09 etc., respectively.

Broad peak indicates presence of number of constituents. Apart from this, some other peaks were observed in all the samples which were showing variation in peak values at same count at different interval. Other than spectrum analysis, there have been variations observed in phytochemical constituent studied by different biochemical analysis. Previously, Vijayan and Raghu *et al*.[22] reported first time qualitative profiling of important classes of secondary metabolites in the genus *Embelia*.

Fruits of *E. ribes* bear a resemblance to black pepper and therefore the drug is adulterated with cubebs, black pepper

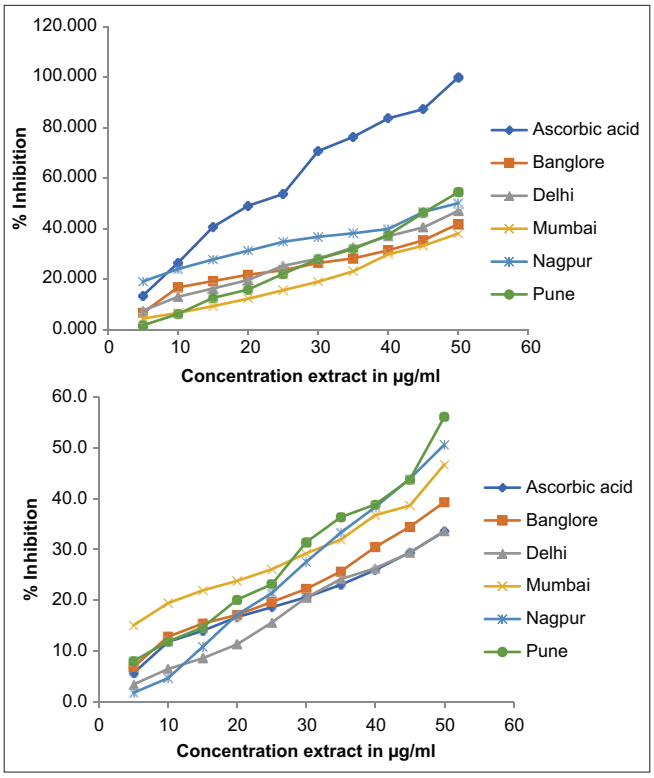

Figure 3: Ferric reducing antioxidant power potential of "Vidanga" market samples

etc. Nayak *et al*.[23] The present study observed compound 228 in Bangalore market sample, 218 in Mumbai market sample, 190, 199, and 208 in Nagpur market sample were recorded at RT 15.92, 16.49, 15.44, 15.93, and 16.50 mn, respectively. They produced [M-H] - ion at *m/z* 555.24 in Bengaluru and Mumbai market samples while in Nagpur market sample it was observed at *m/z* 555.25. These fragment ions of bisalkaloids which are tentatively characterized as Dipiperamide C while the contents were reported in commercial Black, White, Green, and Red whole and ground peppercorns.[24,25]

These results suggest variation in peak area which indicates the presence of different constituent along with Embelin in all the market samples. Hence, this might be due to the ambiguity in market samples.

DISCUSSION

The main traditional system of medicine "Ayurveda," the science of life, has been successfully practiced in India since ages with disease prevention remained its main objective, achieved through natural products derived from various plants, minerals, and animal-based products as dietary supplements and medications. Although, in this system some plant species are morphologically alike and creates the ambiguity in identification from market samples.[26,27] Therefore, there is a growing trend in seeking

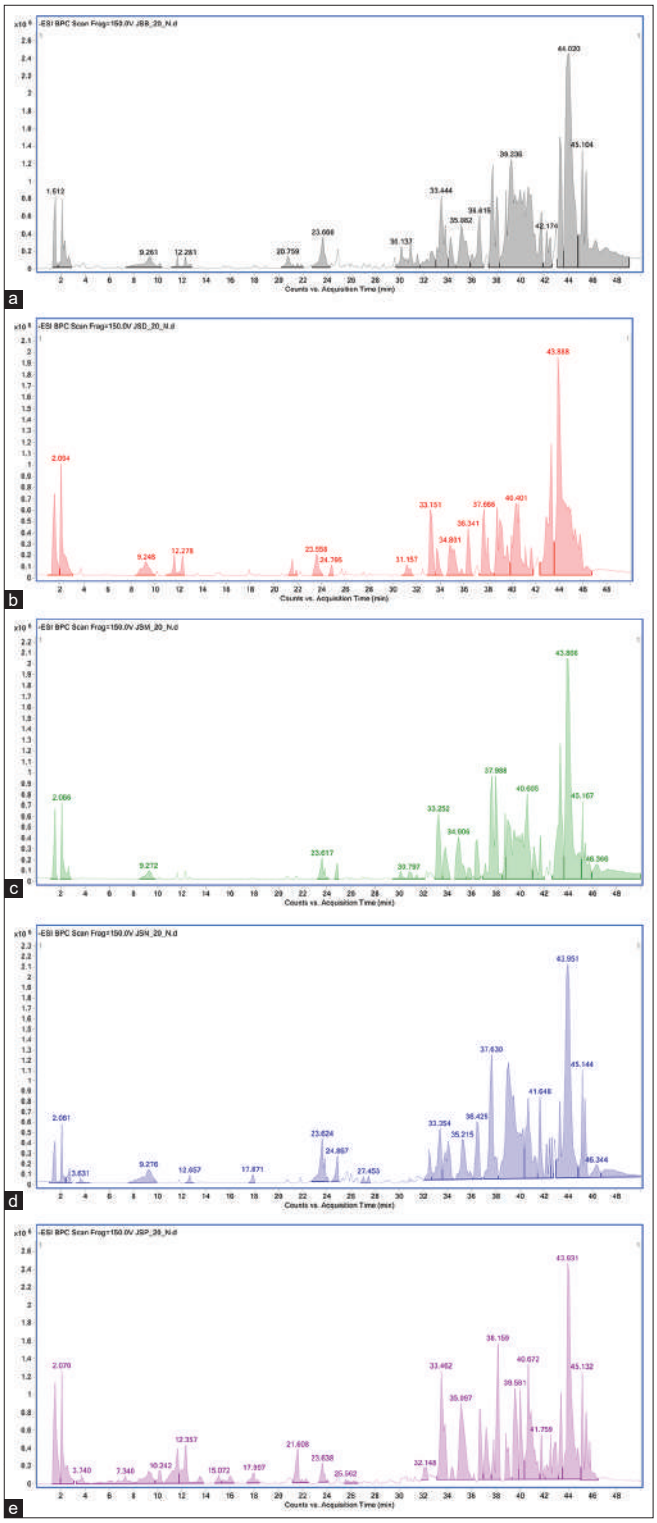

Figure 4: Base peak chromatograms of plant extracts collected from (a) Bengaluru, (b) Delhi, (c) Mumbai, (d) Nagpur, and (e) Pune

evidence-based validation for traditional medicine. According to Ayurveda the *E. ribes* is the botanically accepted species as a "Vidanga" on the basis of description given.[23,27] It is rather observed that *E. tsjeriam-cottam*, *E. drupacea, M. indica* and also various other drugs in market is commonly sold as "Vidanga." Previously, Nayak

et al.[23] reported ambiguity in "Vidanga" market samples from various region of India and Pakistan. For present work, five samples were collected from major markets of various places in India such as Bengaluru, Delhi, Mumbai, Nagpur and Pune.

Raghu *et al*.,[28] reported *E. ribes* is a red listed species, popularly known as "Vidanga" or "Vavding" in Ayurveda. Bharat and Neeraj[29] distinguishing characters of *E. ribes* and *E. robusta*. The fruits of *E. ribes* are small, 2.5–4 mm in diameter, usually greyish black with warty surface. As per the literature survey, "Vidanga" fruits are used as an adulterant to black pepper because of their resemblance in appearance.[23] Morphological examinations revealed variation in the surface morphology of each market sample [Figure 5] suggest ambiguity. In the present work, we investigated the DPPH, anti-lipidperoxidation and FRAP activities of five market samples within India along with identification of polyphenols using mass spectrum. The DPPH radical-scavenging activity was measured by reducing the stable, violet DPPH radicals to the yellow DPPH-H. The degree of color change depends on the free radical-scavenging activity of the tested samples through their hydrogen-donating ability. The sample with the strong antioxidant activity will have the high percentage of radical-scavenging activity.[30]

In the present work, the order of DPPH antioxidant activities was observed highest in market samples of Delhi followed by Nagpur, Mumbai, Bengaluru, and Pune. "Vidanga" market samples were able to reduce the stable DPPH radical to 50% reduction which termed as IC_{50} value. Standard used was ascorbic acid (IC_{50} 25.69 ± 0.13 µg/ml). The ethyl acetate extract obtained from Nagpur market sample showed IC_{50} value of 89.54 µg/mL ± 0.001. While, the lowest activity was observed in the ethanolic extract of Pune market sample (IC_{50} value of 41.57 µg/mL ± 0.003) had strongest free radical scavenging capacity [Table 3]. A similar trend in anti-oxidant capacity was observed by Kulkarni *et al*.[31] in case of *Nardostachys jatamansi* market samples. As mentioned above, all the market samples showed lower DPPH scavenging activity as compared to the authenticate plant extract (unpublished observations). This might be due to some unknown adulterant present in the sample, which may be contributed in activity. While in case of ALP the results showed highest IC_{50} values in Mumbai market samples while the lowest observed in in Bengaluru market sample. Ethyl acetate extract of Delhi market sample had the lowest (23.88 ± 0.004 µg/mL) IC_{50} values. While the highest (40.47 ± 0.004 µg/mL) IC_{50} observed in ethyl acetate extract of Pune sample. Ferrous reducing antioxidant capacity suggest maximum inhibition observed in ethyl acetate extract of the Pune market sample (67.76%) while lowest activity showed in Delhi market sample (33.64%). The results of all the market samples are ordered as lowest in Pune and increases in Nagpur, Mumbai, and Delhi accordingly. Although comparison between all the market samples clearly shows the variation in the peak values [Figure 6], which indicates the presence of various other constituents present in all the market samples.

The study undertaken evidently demonstrates, there is ambiguity in "Vidanga" samples distributed in market[23] also revealed variation in phenolic acid content of "Vidanga" samples collected from various regions from India by using high-performance liquid chromatography technique. The samples were subjected to LC-MS analysis revealed interesting results. There are several chemical components such as sitosterol, embeliol, rapanone, embelinol, daucosterol present in *E. ribes*, but the most important and active compound is Embelin.[32] In the present work, the spectrum showed highest broad peak observed in the range of 43.86–44.02 in all the samples. While there is phyto-constituent variation observed among all the market samples sold as "Vidanga." The standard Embelin spectrum showed peak at 43.93 retention time. The present work indicates lack of awareness in the traders or seller about ambiguity. The unintentional adulteration in herbal plants was addressed by many researchers in various market samples.[23,29,33]

Figure 5: Fruits of "Vidanga" market Samples (a) Bengaluru, (b) Delhi, (c) Mumbai, (d) Nagpur, and (e) Pune

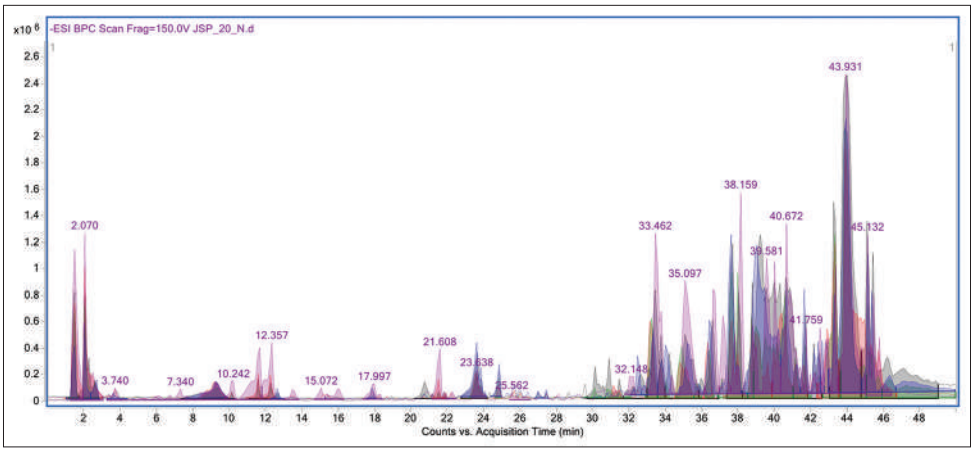

Figure 6: An overlapped base peak chromatogram of plant samples collected from various locations

CONCLUSION

After evaluating five commercial samples of "Vidanga" from different market locations from India viz. Bengaluru, Delhi, Mumbai, Nagpur, and Pune, it is concluded that distinct variation exists in the traded samples regarding their morphology, identity and medicinal attributes as studied from a string of biochemical assays as well as mass spectrum. Although traders deal with copious "Vidanga," authentic samples may be scarcely available in the market and appear morphologically almost similar. This study thus gives awareness in all scientific community, traders, practitioners, manufacturing units, pharmacies, etc., for an urgent need for the selection of authentic raw materials in the market, exclusively on the basis of location specific correct botanical identification and or well-defined biochemical criteria.

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