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Comparative Study to Evaluate Ethanol and Ethyl Acetate Extracts of Different 'Vidanga' Species for Antioxidant Efficacy and Phyto-Constituents Screening

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In Ayurveda, 'Vidanga' is one such species high in demand for its various uses. All the species of genus Embelia and Maesa belonging to the family Myrsinaceae are reported as 'Vidanga'. Considering the availability of plant material in the market there is an ambiguity in supplying the authentic species as 'Vidanga'. In the present study, a comparative analysis was carried out to determine the efficacy of different 'Vidanga' spp. in terms of their phyto-constituents, antioxidant potential, and free radical scavenging activity. The highest total phenolic contents (TPCs) and total flavonoid contents (TFCs) were found to be in ethanolic and ethyl acetate extract. Quantitative measurements also showed that abundance of phenolic and flavonoid phytoconstituents was significantly ($P<0.001$) greater in ethanolic extract of all the 'Vidanga' fractions (1.773±0.01 to 137.17±0.19 mg/g GAE and 4.84±0.001 to 302.29±0.07 mg/g of quercetin respectively) than in ethyl acetate extract (1.15±0.003 to 15.12±0.01 mg/g GAE and 7.94±0.05 to 25.20±0.001 mg/g of quercetin respectively). Ethanolic extract of Embelia ribes had significant activity in terms of IC50 than ethyl acetate extracts in the case of 2,2-diphenyl,1-picryl hydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and anti-lipid peroxidation (ALP) (9.53, 9.73 and 6.21 μg/mL respectively) indicates Embelia ribes found to be most effective species as 'Vidanga'. Pearson's correlation (r2) analysis also suggests a significant correlation between different antioxidant parameters and bioactive constituents. This study may helpful to draw attention of researchers to characterize the various bioactive compounds from the Embelia ribes in terms of their antioxidant prospective.

Keywords: Ambiguity; Comparative antioxidant potential; Embelia spp; Phyto-constituents; Vidanga.

Herbal medicines are attainment towards popularization in global health debates due to its natural origin and lesser side effects. However, nowadays world has been gaining importance of Herbal and Ayurveda drugs[1]. 'Vidanga' is one of the medicinal herbs commonly used in Ayurveda, also has a strong traditional as well as an experimental base for its use.

'Vidanga' having species such as *Embelia ribes* Burm. f., *Embelia drupacea* (Dennst.) M.R. Almeida & S.M. Almeida, *Embelia tsjeriam-cottam* (Roem. & Schult) A. DC. and *Maesa*

indica (Roxb.) A. DC. etc. belongs to the family *Myrsinaceae*[2]. These species are known for their medicinal use since thousands of years[3]. *Embelia drupacea* is woody climber growing in semi-evergreen to deciduous forests up to an altitude of 1600 m[4]. *Embelia tsjeriam-cottom* is distributed in the mountains of the Western Ghats of Karnataka, Kerala and Maharashtra[5]. *Maesa indica* is a species belonging to the same family which is distributed in Western Ghats, Eastern Himalayas and North East India[6]. *Embelia ribes* is an Indo Malayan species, distributed in India, Sri Lanka, Singapore, Malaysia, and S. China. *Embelia ribes*, popularly known as 'Vidanga' or 'Vavding' in Ayurveda while it yields embelin, and other highly valued secondary metabolites[7].

Embelia ribes possesses close similarities especially in terms of active ingredient, *viz*., embelin with *Embelia tsjeriam-cottam*. According to a literature survey, the *Embelia tsjeriam-cottam* is used as substitute of *Embelia ribes*[8,9,10,11]. Data show that over 95 percent of the traded species is *Embelia tsjeriam-cottam*[3]. Therefore, there is a timely need to find out potent *Embelia* species as 'Vidanga'. *Embelia ribes* has been used since ages in traditional medicine and it possesses drenching activity in humans. Root of the *Embelia ribes* is effective against chest pains and the fruits also have various properties such as anthelmintic, carminative, antibacterial, hypoglycemic, antifertility etc[2]. Moreover, leaves pasted with honey is consumed to treat mouth ulcers[12]. While with lime, juice roots are grounded to make a paste and taken orally with honey against cough[13]. It is considered to keep the digestive system healthy; also used in skin ailments like acne and pimple, in constipation, in piles, as a brain tonic, etc[14]. *Embelia drupacea* are used to treat toothache, laxative, anthelmintic, carminative, hypoglycemic, antifertility properties, antiseptic etc[4]. *Embelia tsjeriam-cottam* are used for their anti-inflammatory, analgesic, wound healing, antiproliferative, hepatoprotective, antimicrobial, antidiabetic, cardio-protective properties etc[15]. Fruits of *Maesa indica* are consumed raw by Irula tribal. It is good blood purifier and also used in anthelminthic ailments, nutritional, anti-diabetic etc[16].

Oxidative stress has been known to be the basis for development and evolution of many diseases[14]. Hence, the present study is designed to evaluate the potential of authenticate 'Vidanga' species by using different biochemical assays to check their efficacies against reactive oxygen species. Present work is designed to validate the 'Vidanga' species by *in vitro* biochemical, phyto-constituents analysis and comparative investigation among all the four species of 'Vidanga*'*. The total phenolic contents (TPCs) and total flavonoid contents (TFCs) in both the polar and non-polar solvent will be studied and positively correlated with their antioxidant properties.

MATERIALS AND METHODS

Sample collection and extraction

'Vidanga' fruits were collected from different locations Western Ghats in Maharashtra, India. Using mechanical blender, fruits were crushed to fine powders. Both polar and non-polar solvents, *viz*., ethyl acetate and ethanol (90%) were used. Extracts were prepared by Soxhlet apparatus (Rotamantal, Remi) with slight modification at 60-80^{0}C for 16-24 h[19]. Solvents were evaporated under reduced pressure at 45°C in a rotary evaporator (IKA RV 10) leaving small yields of dry plant extracts. The dry extract was dissolved in methanol for obtaining sample of final concentration 1mg/ml for further experiments.

Chemicals and reagents

All the chemicals and reagents used in the experiments were of analytical grades. Ascorbic acid, sodium acetate trihydrate, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), ascorbic acid, gallic acid, Folin–Ciocalteau reagent, methanol purchased from Sigma Aldrich (USA). 2,2- Diphenyl,1-picryl hydrazyl (DPPH), thiobarbituric acid reactive substance (TBARS), KCl, $FeCl_3$, thiobarbituric acid (TBA), trichloro acetic acid (TCA), butylated hydroxy toluene (BHT) was prepared in methanol.

Estimation of TPCs and TFCs

TPCs of different 'Vidanga' samples were determined by the colorimetric Folin-Ciocalteu method. A calibration curve was prepared using standard gallic acid solutions at different concentrations every time analysis was run. TPCs concentrations in the samples were calculated from the standard curve and the results were expressed as gallic acid equivalents per gram (mg GAE/g) dry weight of the extract. TFCs of the different

'Vidanga' samples were also determined using the aluminium chloride colorimetric assay and expressed as quercetin per gram (mg RE/g) of dry weight. All determinations were done in triplicates and averaged.

***In vitro* free radical scavenging and antioxidant potential**

DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate)

The radical scavenging ability of 'Vidanga' plant extract was measured by a method described previously[20] with slight modifications**.** Briefly, 3ml of DPPH (33 mg/L in methanol) solution and diluted plant extracts in the range of (0.05–0.50 mg/mL) along with ascorbic acid as a standard (1 mg/mL). Samples were incubated in the dark for 30 min absorbance measured at 517 nm. The results were expressed as IC_{50} values which required 50% scavenging inhibition of DPPH radical.

Ferric reducing antioxidant power assay (FRAP)

Antioxidant capacity of 'Vidanga' plant extracts were estimated colorimetrically as per Benzie and Strain (1996)[21]. Antioxidant potential was determined on the basis of Fe^{3+} TPTZ complex (colorless complex) reduced to Fe^{2+} -tripyridyltriazine (blue-colored complex) due to the action of electron donating antioxidants at low pH, change in absorbance is measured at 593 nm. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 7.4), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ were prepared and mixed in 10:1:1 proportion respectively. FRAP solution (2850 ìl) was mixed with 150 ìl of plant extract at different concentrations (0.05-0.50 mg/ml) and incubated for 2 h in dark at room temperature and absorbance measured at 593 nm. Ferric ion reducing activity of all the extracts was determined by standard $FeSO_4$ (0.1-1 mM). Results were analyzed from the standard curve of ferrous sulphate and expressed in terms of µM Fe/g of dry mass. The results were expressed as IC_{50} values.

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

$$\% \text{ Scavenging activity} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

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

Anti-lipid peroxidation assay (ALP)

Previously reported protocol was adopted to determine the Fe^{3+}/ascorbic acid-dependent non-enzymatic lipid peroxidation activity of 'Vidanga' plant extract[22]. The lipid peroxidation inhibition potential of extracts was determined by thiobarbituric acid reactive substance (TBARS). The reaction mixture, in the absence and presence of 'Vidanga' extract (0.05-0.50 mg/ml) or standard, containing 0.5 ml of goat liver as lipid source (5 mg/ml), 100 ìl of 1 mM Iron (III) chloride ($FeCl_3$) mixture was incubated at 37°C for 30 min while the reaction was terminated by using 2 ml ice-cold 0.25 N hydrochloric acid (HCl) containing 0.19% thiobarbituric acid (TBA) and 7.5% trichloroacetic acid (TCA). After adding 200 ìl of 0.5% butylated hydroxytoluene (BHT) prepared in methanol mixture were incubated at 80°C in a water bath for 60 min and 1 mM ascorbic acid in 20 mM phosphate buffer used as a standard. 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:

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

where, $A_{induced}$ is absorbance of induced; A_{Sample} is absorbance of sample and A_{Normal} is absorbance of normal.

Preliminary phytochemical screening

Qualitative phytochemical screenings of 'Vidanga' plant extracts to identify the presence of vital phytoconstituents, such as total carbohydrates, alkaloids, tannins, saponins, flavonoids, steroids, terpenes, glycosides, proteins, amino acids, reducing sugars and phenol were carried out using standard biochemical procedures as described previously[23].

Statistical Analysis

A curve was obtained by plotting the percent inhibition values versus extract concentrations and IC_{50} values were determined. Data were analyzed by one-way ANNOVA and expressed as mean ± standard error (SE) (where n>3). *P* value less than *0.05* is considered statistically significant. Karl Pearson's correlation coefficient (r^2) was determined to evaluate the

correlation between the antioxidant activity and the TPCs and TFCs.

RESULTS AND DISCUSSION

Plant material and extraction yield

The fruits of 'Vidanga' species are globose, smooth succulent, tipped with persistent style. The drupes are green when young and become black with wrinkles on the surface (Figure 1).

The extraction yield of different extracts of 'Vidanga' species obtained by ethanolic and ethyl acetate extraction methods are shown in Table 1. Variation in the yield produced by different solvents during extraction indicated that it follows the polarity of solvents. *Embelia tsjeriam-cottam* provided the highest yield of 23% followed by *Embelia ribes* (12.01%), *Maesa indica* (7.3%) in ethanolic extract, and 1.5% in *Embelia drupacea* from ethyl acetate extract.

Phytochemical screening

Many researchers have reported that photochemical such as glycosides, phenols, steroids, saponins, alkaloids and terpenoids have enormous free radical scavenging and antioxidant activities[24,25,26]. Moreover, plants that are rich in phytoconstituents have been shown to exhibit free radical scavenging and antioxidant potentials[27,28,29]. In the present work, a preliminary phytochemical analysis was studied using polar and nonpolar solvents. Glycosides are present in all the fractions except ethyl acetate extracts of *Embelia drupacea* (Table 2). Glycosides, containing either terpenoid or steroids and sugar chains, possess several therapeutic activities like anti-inflamation[30],

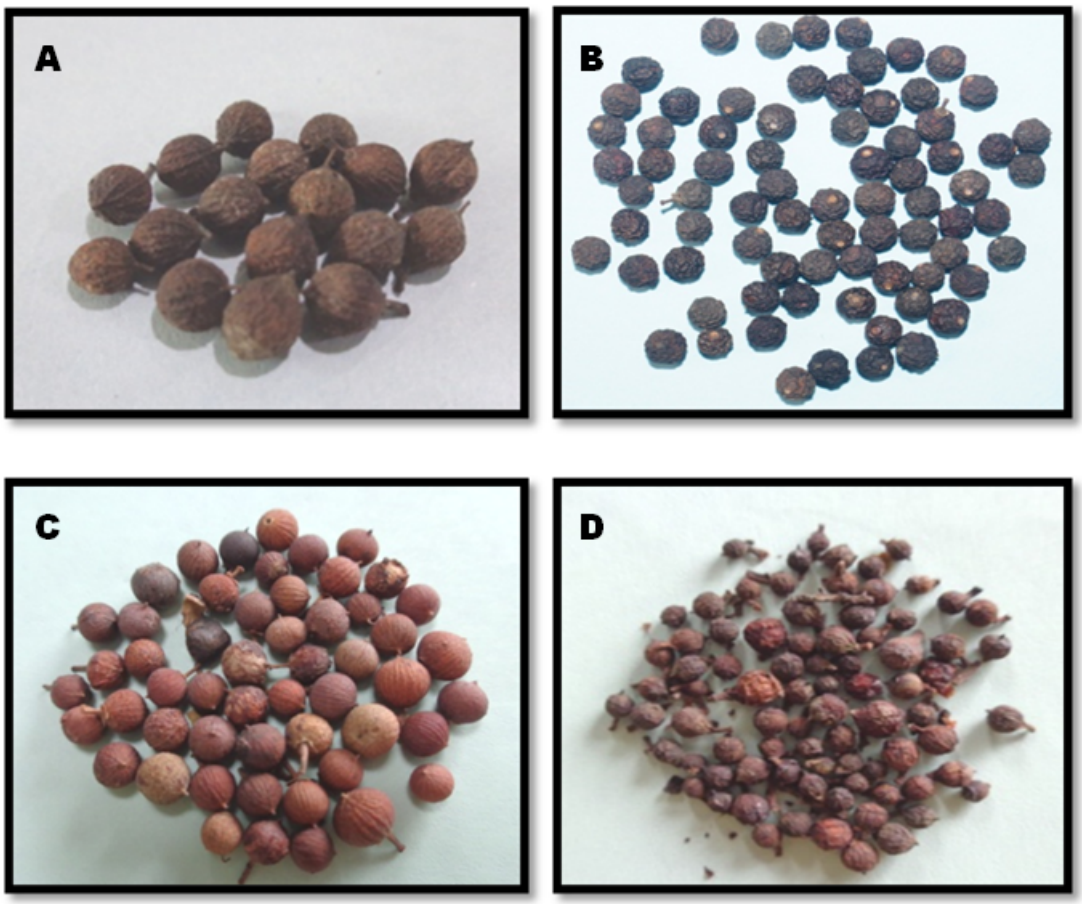


Fig. 1. Fruits of 'Vidanga' species: A. *Embelia ribes*, B. *Embelia tsjeriam-cottam,* C. *Embelia drupacea* and D. *Maesa indica*

immuno-modulatory activity[31] and anti-cancer property[32]. Fixed oils are present in all the fraction of both ethyl acetate and ethanol, while saponins are present only in ethanolic fraction of *Embelia ribes* (Table 2). Saponins are a type of aglycone containing triterpenoids or steroids[33], possesses health promoting properties in functional food[34] and also exerts immune enhancement effect[35,36]. Amino acids play vital role in the synthesis of proteins and precursors in the formation of secondary metabolites[37] that participates in hormone synthesis, protein phosphorylation and antioxidant capacity[38]. Proteins and amino acids are present in all the 'Vidanga' fractions (Table 2). All the constituents showed more appearance in ethanolic fractions. Results shown suggest that phytoconstituents extracted from all the plants are polarity dependent (Table 2).

Quantitative analysis of phytoconstituents

Quantitative results of phytoconstituents in ethyl acetate and ethanolic extracts of 'Vidanga' species showed significant results. TFCs and TPCs present in both the extracts, *i.e.,* ethanol and ethyl acetate showed non-significant amount, but ethanolic extract had higher contents (**302.29**±0.07 and **137.17**±0.19 mg/g respectively) in *Embelia ribes*. According to literature survey, use of ethanol provided much better results for extraction process, as has previously been reported for various *Embelia* spp.[39,40,41,42,43]. According to Xu et al.[44] yield for aqueous extraction is low due to the polyphenols present in the plant tissues are often associated with other molecules also via hydrogen and hydrophobic bond whereas, ethanol is better hydrogen bond donor and acceptor than water[45]. Our results show higher yield of polyphenolic contents in ethanolic extract (Table3). Phyto-constituents, a group of vital secondary metabolites have potent antioxidant properties that contribute to defense mechanisms of the plant[46,47,48]. Polyphenols are good electron donors with the ability to cease the free radical chain reaction by converting them into more stable compounds[42]. Among all the plant extracts *Embelia ribes* and *Mesa indica* showed maximum TFCs and TPCs in ethanolic extract while quite the opposite *Embelia drupacea* and *Embelia tsjeriam-*

Table 1. The extraction yields of two solvent extracts

Sr No.	'Vidanga' species	Ethyl acetate Yield (%)	Ethanol Yield (%)
1	*Embelia ribes*	0.44	12.1
2	*Embelia drupacea*	1.5	1.1
3	*Embelia tsjeriam-cottam*	0.85	23.0
4	*Maesa indica*	5.3	7.3

Table 2. Preliminary phytochemical screening of 'Vidanga' fractions

Tests	Ethyl acetate extract				Ethanolic extract			
	Embelia ribes	*Embelia drupacea*	*Embelia tsjeriam-cottam*	*Maesa indica*	*Embelia ribes*	*Embelia drupacea*	*Embelia tsjeriam-cottam*	*Maesa indica*
Carbohydrates	✓	×	✓	✓	✓	×	✓	✓
Alkaloid's	×	×	×	×	✓	×	✓	×
Test for Fixed oils	✓	✓	✓	✓	✓	✓	✓	✓
Glycosides	✓	×	✓	✓	✓	✓	✓	✓
Proteins and Amino acids	✓	✓	✓	✓	✓	✓	✓	✓
Saponins	×	×	×	×	✓	×	×	×
Gum and Mucilages	×	×	×	×	×	×	×	×
Terpenoids	✓	×	✓	×	✓	×	×	×
Steroids	✓	×	×	×	✓	×	×	×
Tannins	✓	✓	✓	✓	✓	✓	✓	✓

cottam showed significant result in ethyl acetate extract (Table 3). The differences between both groups were statistically significant ($p < 0.05$). The difference between TFCs and TPCs observed may be due to the solvents having different polarities, or may be due to differences in the solubility of these compounds in polar and non-polar solvents.

Antioxidant prospective

DPPH scavenging activity

'Vidanga' species showed considerable antioxidant capacities (Figure 2). A positive linear correlation between DPPH scavenging capacity of 'Vidanga' fruit extracts and quantitative measurements was dependent on phytoconstituents (Table. 4). The highest correlation was observed between DPPH scavenging ability of 'Vidanga' fruit extract and both the phyto-constituents present in extract. Among these, the highest correlation (0.958) observed between phenol content and DPPH indicates that the scavenging ability of 'Vidanga' extract may be due to the presence of phytoconstituents. The report support those of other researchers, who have found positive correlation between polyphenolic contents and antioxidants activities[49,50,51,52].

Maximum % inhibition for DPPH assay was observed in ethanolic fraction of *Embelia ribes* (91.41%) which is significantly higher

Table 3. Polyphenolic content of the 'Vidanga' samples

'Vidanga' species	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
Embelia ribes	15.12±0.01	137.17±0.19	25.20±0.001	302.29±0.07
Embelia drupacea	1.15±0.003	1.773±0.01	7.94±0.05	4.84±0.001
Embelia tsjeriam-cottam	7.88±0.008	4.176±0.01	16.21±0.005	15.64±0.01
Maesa indica	7.13±0.001	1.839±0.03	9.35±0.0005	8.69±0.03

[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).

Table 4. Correlation analysis (r^2) between antioxidant parameters and phytoconstituent of 'Vidanga' species plant extracts

'Vidanga' species extract	Phytoconstituents	r^2 value		
		DPPH assay	ALP assay	FRAP assay
Ethanolic extract	Phenol	0.958	0.859	0.952
	Flavonoids	0.965	0.870	0.958
Ethyl acetate extract	Phenol	0.592	0.311	0.549
	Flavonoids	0.349	0.683	0.458

Table 5. Antioxidant activities in the fractions of 'Vidanga' species by DPPH, ALP and FRAP

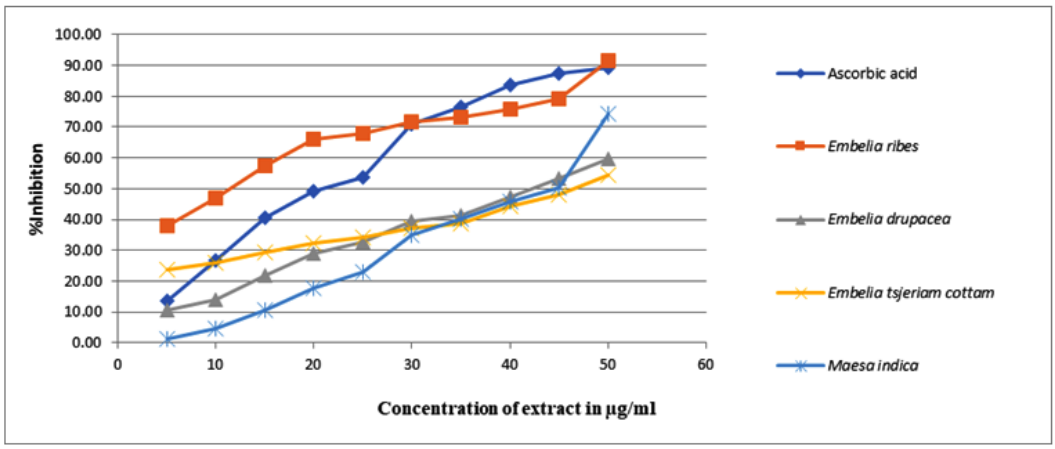
Sr No.	Sample extracts	DPPH (IC_{50} µg/mL)		ALP (IC_{50} µg/mL)		FRAP (IC_{50} µg/mL)	
		Ethanolic	Ethyl acetate	Ethanolic	Ethyl acetate	Ethanolic	Ethyl acetate
1	*Embelia ribes*	9.53	13.47	6.21	7.778	9.73	10.02
2	*Embelia drupacea*	48.15	56.55	23.29	26.80	44.61	47.94
3	*Embelia.tsjeriam-cottam*	29.22	44.81	16.24	17.83	34.28	36.38
4	*Maesa Indica*	40.72	55.06	17.83	21.31	36.78	38.13

*Highlighted numbers indicate highest 50% inhibitory concentration (IC_{50})

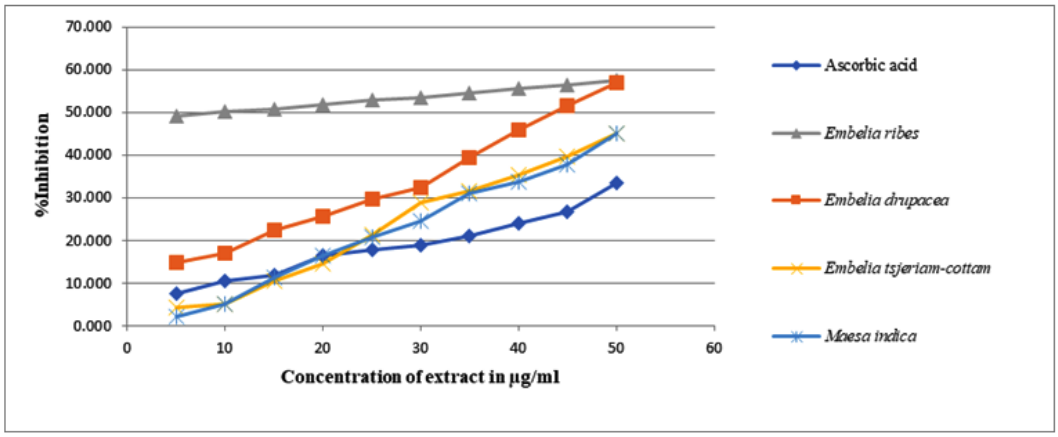
than those of standard ascorbic acid (Figure 2). Various medicinal plant species shown DPPH-free radical scavenging activity[53,54,55.] Zebeaman and Gebeyehu[56] have reported that, *Embelia schimperi* V exhibited 62.4% antioxidant inhibition while the standard vitamin C scored 97% of inhibition. However, ethanolic extract of *Embelia basaal* showed 97% of free radical scavenging activity[52]. Furthermore, the biological system contains several free radical and oxidant sources and they act by multiple mechanisms in a single system[57]. DPPH radical scavenging activities of the different 'Vidanga' sp. fractions showed a large variation of IC_{50} ranging from 9.53 to 56.55 ìg/mL (Table 5).

The highest DPPH scavenging activity in terms of their 50% inhibitory concentration (IC_{50}) exhibited in ethanolic extract of *Embelia ribes* (9.53 µg/mL) whereas the lowest activity exhibited in ethyl acetate extract of *Maesa Indica* (56.55 µg/mL). The result showed that the ethanolic extract of *Embelia ribes* to be the most efficient free radical scavenger.

The order of major DPPH-scavenging activity observed in ethanolic extract of 'Vidanga' species was *Embelia ribes* (9.53µg/mL) followed by *Embelia tsjeriam-cottam* (29.22 µg/mL), *Maesa indica* (40.72 µg/mL) and *Embelia drupacea* (48.15 µg/mL) which was similar to their TFCs.



a.



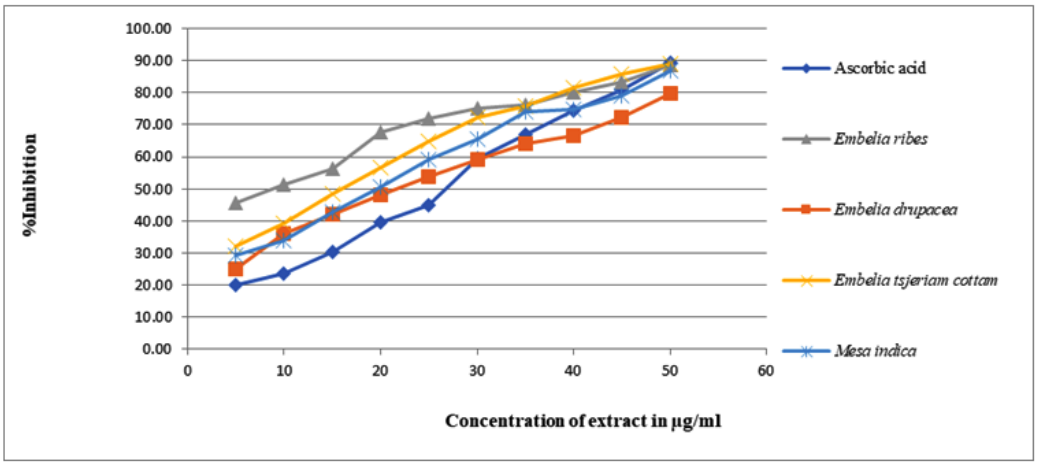
b.

Fig. 2. Comparative DPPH scavenging activity of 'Vidanga' samples a. Ethanolic extract and b. Ethyl acetate extract

Results indicate significant correlation (r^2=0.965) between free radical scavenging activities exhibited by different fractions and their flavonoid contents (Table 4). It is noted that flavonoids possess various biochemical properties. One of the mainly imperative activities is their capacity to serve as antioxidants[58]. Several flavonoids such as baicalein and wogonin are reported for chronic inflammatory disorders,[59] rutin for hepatoprotective activity,[60] butein for adipocyte inflammation activities[61] and quercetin for immunomodulatory activities[32]. 'Vidanga' species are rich in flavonoids, and hence could be used for different pharmacological activities. Among all the 'Vidanga' species, *Embelia ribes* having the highest capacity to scavenge free radicals.

Anti-lipid peroxidation assay (ALP)

The results showed that all the fractions of 'Vidanga' species inhibited lipid peroxidation in dose dependent manner (Figure 3). Significant inhibition was observed in both the fractions (ethanol and ethyl acetate) of 'Vidanga' species. Lipid peroxidation is a process in which, free radicals take electrons from the lipids, resulting in a loss of membrane fluidity with an increase in membrane permeability and a decrease in physiological activity resulting endanger cell viability[43]. The protective effect of 'Vidanga'



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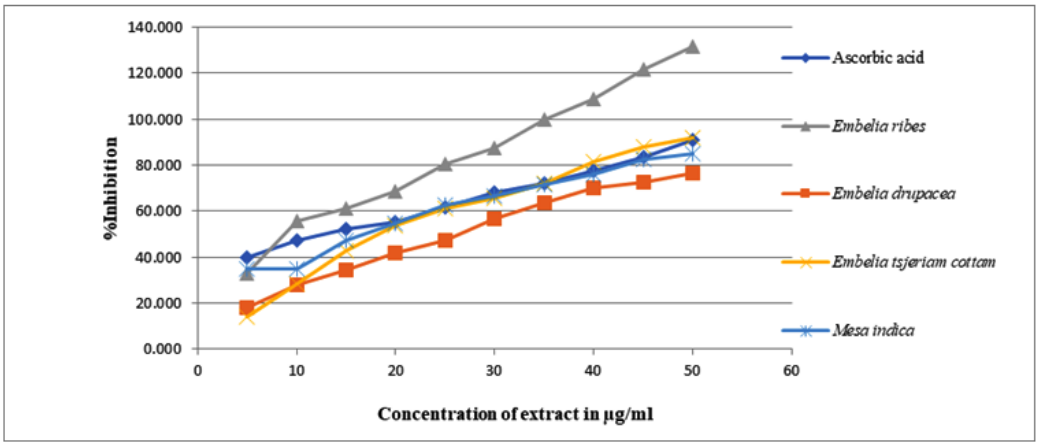


b.

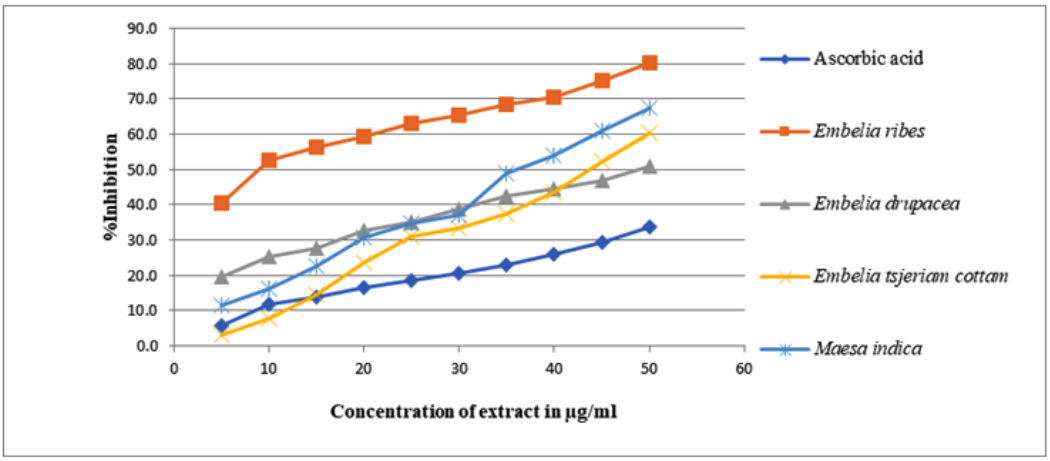
Fig. 3. Anti-lipid peroxidation (ALP) potential of 'Vidanga' samples a. Ethanolic extract and b. Ethyle acetate extract

fractions against $FeSO_4$- induced lipid peroxidation was assessed in the goat liver. During this process, polyunsaturated fatty acids present in the lipid membrane undertake oxidation resulting in formation of the malonaldehyde (MDA) which reacts with molecules of thiobarbituric acid (TBA) to form TBARS[62].

Maximum inhibition in terms of IC_{50} was observed in ethanolic fraction of *Embelia ribes* (6.21µg/mL) whereas lowest activity exhibited in ethyl acetate fraction of *Embelia drupacea* (26.80 µg/mL). Significant lipid lowering ability of *E. ribes* in rats have been reported previously[41,63]. Protection against free radical lipid peroxidation is of great significance for their use against various disorders like inflammation [64] as well as several pathologies like aging, wound healing, oxygen toxicity etc.[65]. Observations indicated a significant correlation (r^2=0.870) between lipid lowering ability of 'Vidanga' species and TFCs content (Table 4). Plant flavonoids have been reported for prevention of lipid peroxidation in microsomes and liposomes[66].

Ferric reducing antioxidant power assay (FRAP)

Antioxidants can be explained as antioxidant inactivators and reductants, measures the reducing potential of the test sample[67]. The antioxidants donating electron or hydrogen atoms to the ferric complex which converts to ferrous complex (Fe^{3+} to Fe^{2+}-TPTZ complex), thus breaking the radical chain reaction[68]. Results



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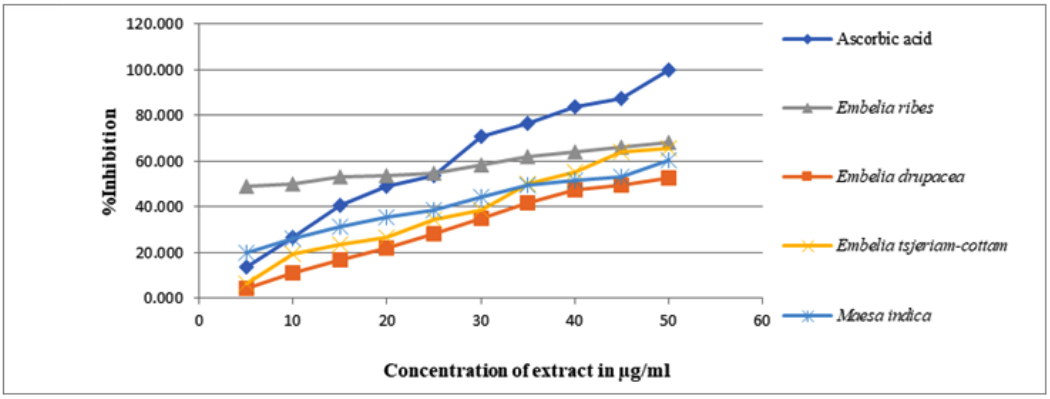

b.

Fig. 4. Ferric Reducing Antioxidant Power (FRAP) potential of 'Vidanga' samples a. Ethanolic extract and b. Ethyl acetate extract

showed lower reducing activity in *Embelia drupacea* extract as compared to the other 'Vidanga' species extract. The reducing power of the plant extracts correlate with their antioxidant activities while this property of antioxidant depends upon its electron donating capacity. In the present study, all the fractions have reducing power and absorbance is increased, due to the formation of the Fe2+-TPTZ complex with an increase in concentration. The highest ferric reducing activity in terms of their 50% inhibitory concentration (IC_{50}) exhibited in ethanolic extract of *Embelia ribes* (9.73µg/mL) whereas the lowest activity exhibited in ethyl acetate extract of *Embelia drupacea* (47.94µg/mL). The ethanolic fractions had slightly higher reducing activity in comparison to ethyl acetate extract.

Maximum % reduction was observed in the ethanolic fraction of *Embelia ribes* (63.8%) which is significantly higher than those of other fractions (Figure 4). A positive linear correlation between Ferric reducing antioxidant power of 'Vidanga' plant extracts and phytoconstituents present in the extract were observed (Table. 4). The significant correlation was observed between Ferric reducing ability of 'Vidanga' plant extract and both the phytoconstituents present in ethanolic extract. While the highest correlation (0.958) was observed between flavonoids and ethanolic 'Vidanga' fractions. Today, antioxidant supplements may provide an important resource to conflict organ-related disorders, accumulation of harmful compounds, and metabolic dysfunctions[69].

Embelia ribes contains different phyto-constituents, *viz*., potassium embelate, 2,5-dihydroxy, 3-undecyl-1,4-benzoquinone, embelin, quercitol, fatty ingredients, vilangin which are previously reported for different activities like antibacterial, antifertility, antiprotozoal, constipation, antifungal, mouth ulcer, sore throat, pneumonia, obesity, analgesic, anti-inflammatory, antioxidant, anthelminthic, antidiabetic, anticonvulsant, anticancer, antihyperlipidemic, wound healing and mollusicidal properties[62]. A comparative study for the presence of phenolic and flavonoid compounds in extracts of different 'Vidanga' species clearly indicates that ethanolic extract had the greatest phenol and flavonoid content than ethyl acetate extracts. The percent yield of the 'Vidanga' fractions was also found to be high in the ethanol extract than ethyl acetate. Among all the species, highest yield was observed in *Embelia ribes* (12.1%). However, the highest activity was also observed in *Embelia ribes* for antioxidant profile. Hence, positive relationship between these methods of antioxidant assays suggesting that *Embelia ribes* is more efficient species among all to assess the DPPH, FRAP and ALP antioxidant activities.

CONCLUSION

This study expanded the current knowledge of phytoconstituents, antioxidant potential, and free radical scavenging activity of both ethanolic and ethyl acetate fractions of 'Vidanga. The results of the present study generally implies that the fruits of *Embelia ribes* could be potential natural source of bioactive compounds and may be greatly utilized as therapeutic agent to reducing or preventing the oxidative stress-related ailments. However, study indicates the affirmative way to researchers to characterize the various bioactive compounds from the *Embelia ribes* in terms of their antioxidant prospective. Further research on *Embelia ribes* may be helpful for the confirmation of most potent species as 'Vidanga' and in treating various health issues.

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Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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