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In vitro Antioxidant Potential, High-Performance Liquid Chromatography, and Chemometric Analysis of *Embelia ribes* and *Embelia-tsjeriam-cottam* with Respect to their Developmental Stages

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Abstract

The current study sought to quantify Embelin from various ripening phases of *Embelia ribes* (Er) and *Embelia tsjeriam-cottom* (Etj) and differentiate in fruits using the UV Vis spectrophotometric method combined with chemometrics. This study also attempted to determine the antioxidant potential of extracts in vitro and calculate the inhibitory concentration (IC50) value. The mid-ripe stage of Er and Etj had higher anti-denaturation, ABTS scavenging, and NOS antioxidant activity than the unripe stage. The antioxidant potential of fruit extract varied with the ripening stage, as did the concentration of active secondary metabolite. The findings demonstrated a fluctuation in Embelin concentration in three stages, namely unripe, mid-ripe, and ripe. Ripe fruits had more antioxidant activity and Embelin concentration than immature fruits. Fruit antioxidant activities have shown an increasing tendency with fruit growth. The hydroxyl scavenging activity of the fruit decreased with fruit maturity, but the Embelin decreased with fruit maturity. At commercial maturity, Etj had more Embelin, BSA, ABTS, and NOS than Er, which was also a rich source of antioxidant chemicals that could be employed instead of Er. On a C-18 membrane in downhill mode, the reverse phase HPLC was carried out with methanol as the mobile phase. Glacial acetic acid: water: THF. The antioxidant capacity of BSA, NOS, and ABTS was assessed in vitro. Embelin levels were highest in Etj ripe (157.28 µg/ml), followed by Etj mid-ripe (151.12 µg/ml) in HPLC experiments. UV-Vis spectrophotometer was used to differentiate between the different stages of Fruits of Vidanga with help of PCA. To get the most out of herbal medicine that contains multiple entities, harvesting should take place at a precise time and duration of ripening. Initially, antioxidant activity and Embelin content in the fruit increased as the fruit matured. The findings would be beneficial to the nutraceutical business as well as crop enhancement projects.

Keywords: Antioxidant, Embelin, HPLC, In vitro assay.

Introduction

Rugged and precise techniques with a cost-effective biomass and time commitment are needed for bioprospecting or evaluating the optimal medium composition for the intended outcomes. The plant biomass has been extracted using a variety of solvents and extraction methods, and the biochemical activities of phytochemicals in the crude extracts have been determined in order to routinely investigate better possibilities than the ones now available (Altemimi et al. 2017, Tlili et al. 2019, Kamble et al. 2020, Vijayan and Raghu, 2021). Food and medicinal plants are natural reservoirs for a number of physiologically active substances. Numerous pharmacological benefits, including anti-inflammatory, anti-carcinogenic, hypoglycemic, and anti-urolithiatic activities, have been associated with these phytoconstituents (Unuofin et al. 2018). In Ayurvedic remedies, the plant *Embelia ribes* Burm f. (Er) is used. Additionally, it is widely accessible in Kenya, Sri Lanka, Kenya, India, Vietnam, and Madagascar. The fruits are used to cure cancer, diabetes, gastrointestinal disorders, intestinal worms, and analgesia (Qin et al. 2020, Ahn et al. 2007, Sreepriya and Bali, 2005). Er grows in mid to evergreen forests in India between 400 and 1200 meters above sea level. In India's Western Ghats, it is a species that is red-listed as being threatened (Ravikumar and Ved, 2000, Rajashekaran 2001, Mhaskar et al. 2011). Er possesses analgesic, anti-inflammatory, antitumor, antidiabetic, anticancer, antitumor, anti-obesity, and antioxidant properties. Additionally, it aids the digestive system and is used to treat mental health difficulties, sore throats, and fungus infections (Chitra et al. 1994, Bhandari et al. 2002, Xu et al. 2005, Vinutha et al. 2007, Ansari et al. 2008, Ansari and Bhandari, 2008, Choudhary et al. 2012). In Er berries, the marker chemical embelin has been discovered (Soumya et al. 2011). Ayurveda frequently employs *Embelia tsjeriam-cottam* (Roem. & Schult.) A. DC. (Etj) as an anthelmintic. It is a shrub that is found in places like South China, Pakistan, and Sri Lanka. It may be found, among other locations, in Kerala, Karnataka, and Maharashtra (Bohara and Nagalakshmi, 2021). Its fruits are frequently used medicinally as tonics, blood purifiers, antispasmodics, anti-inflammatory agents, and other things. Both Er and Etj have globose berries, however the shapes differ significantly; Er fruits are oval in form. Both Er and Etj are greenish while they are young, but as they mature, the fruits of Er turn black and those of Etj turn brownish red. In contrast to Etj, where the stalk is missing, Er fruit surfaces tend to grow warty as they develop, whereas Etj fruits have parallel striations that run from base to apex. Assuming that there are fruits accessible in the market, Etj is more frequently available than Er.

Embelin is the most abundant bioactive secondary metabolite in Etj and Er. Embelin (MW 294.38) is soluble in organic solvents such as dimethyl sulphoxide (DMSO), ethyl acetate, methanol, and ethanol but insoluble in water (Kaur et al. 2015). Embelin is well known for its anti-inflammatory, anthelmintic, and

anticancer properties, as well as its effectiveness as an oral contraceptive. There is very little evidence available on the antioxidant activity and embelin content of Vidanga in terms of developmental progress. Previous research has discovered phenolic substances and antioxidant activity in many *Embelia* species at full maturity as well as during fruit growth. However, to the best of our knowledge, no papers have evaluated these changes during *Embelia* fruit growth. Information on changes in these bioactive components in the fruit as the fruit matures would be beneficial to both the nutraceutical sector and crop improvement projects. As a result, the current study tried to analyse the dynamics of antioxidant activities and secondary metabolite in two Vidanga species during different stages of fruit development.

Materials and Methods

Plant material

Self-collected fruits of Er and Etj were found in the forests of Kemse, Koyna, and the Satara District of Maharashtra, India. Dr. Suresh Jagtap, a taxonomist and associate professor at the Interactive Research School for Health Affairs (IRSHA), BVDU, Pune, found and collected the plants in 2019, and he has submitted a voucher specimen to the MPCC herbaria in Pune (MPCC: 1526; MPCC: 2743). The Maharashtra State Biodiversity Board (MSBB), in Maharashtra, gave its prior consent.

Chemical and standard for HPLC

While Embelin utilized as standards was acquired from Yucca Enterprises, Mumbai, India, acetic acid and methanol of HPLC quality were obtained by HiMedia Laboratories Ltd., Mumbai, India. In methanol, standard sock solutions containing 100 g/ml were created. The 0.45 m millipore filter was used to filter the herbal extract before it was put into the column.

Preparation of sample extract

Individual extracts of each plant item used in the research were created using a process using ethyl acetate as a solvent (Dandekar and George, 2022). Fruits' ethyl acetate solvent was used for this investigation because it has the greatest Embelin content. As a result, the ethanolic extracts of herbs were employed to assess the in vitro antioxidant capacity and conduct further bioactive component analyses.

HPLC analysis of Er and Etj

The absorption maximum (lambda max) of Embelin was determined using the Shimadzu UV-1800 model. Waters HPLC type with 2487 HPLC UV-Detector and 515 HPLC Pump. The HPLC apparatus and column were kept at room temperature. The experimental Hibar C18 column (250 mm x 2.0 mm, 5 m) was used for reverse phase chromatography, and the mobile phase was a mixture of methanol, water, glacial acetic acid, and THF (85:13.5:1.5:0.1v/v). The sample volume was 20 µL, and the flow velocity was set at 1

ml/min. The HPLC detector was calibrated with an AUFS of 0.1 and a wavelength of 254 nm (Prakash, 1981).

Spectral Acquisition

Using an Epoch multiplate reader, the spectral information of the ethyl acetate extract of the Er and Etj samples was captured. At room temperature (27–28°C), transmittance mode was used to acquire the spectra. With a wavelength precision of 1 nm, spectral data covering the range 190 to 1100 nm was gathered. Blank spaces in the data were filled in during pre-processing with "0."

Data Analysis for Chemometrics

The variables from the spectral data underwent Principal Component Analysis (PCA). Data analysis was performed using Paleontological Statistics (PAST) software, version 4.03.

Evaluation of In vitro antioxidant potential of Extract

Bovine Serum Albumin (BSA) Denaturation Assay

The final concentration of the vidanga extracts was 1 mg/mL. To get a final volume of 1 mL, several quantities (20 µL, 40 µL, 60 µL, 80 µL and 100 µL) were diluted. The reaction solutions (0.5 ml) included 100 and 250 mg/ml of the test chemical and 0.45 ml of bovine serum albumin (5% aqueous solution). pH was changed to 6.3 using a little amount of 1N HCl. The samples were incubated at 37 degrees Celsius for 3 minutes. Each tube was then filled with 2.5 mL of phosphate saline (pH 6.3) once the action liquids had cooled. Turbidity was finally measured spectrophotometrically at 660 nm (Banerjee and Sahu, 2020).

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 test: reflectance of both the tested chemicals; A control: fluorescence of the control. The samples and the common anti-inflammatory drug diclofenac (0.1 mg/mL) were handled similarly.

ABTS 2, 2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulphonic Acid) Radical Scavenging Activity

The scavenging free radicals capacity of Vidanga samples was examined using the ABTS azide decolorization technique (Labiad et al. 2017). The ABTS+ ion radical was created by the reaction of 7 mM ABTS in organic buffer with 2.45 mol l-1 persulfate (1:1), and it was stored at room temperature in the dark for 12 to 16 hours before use. The ABTS+ solution was diluted with methanol to produce an intensity

of 0.700 at 734 nm. 30 minutes after adding 5µl of Vidanga extracts to 3.995 ml of diluted ABTS+ solution, the absorbance was measured. Trolox was used as the standard ingredient to make the standard solution. Using the method, the percentage inhibition of absorption at 734 nm was obtained.

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

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

Nitric Oxide Radical Scavenging Assay

The nitrite ion produced by the reaction of oxygen and sodium nitroprusside (SNP) with a neutral pH is detected using the Griess reagent. The samples were treated with 50 mM SNP (5 mg/ml) on 10 mM Phosphate Buffer Saline and 2 ml of different doses of Vidanga extract for 60 minutes at 37°C (Suluvoy et al. 2017). The Griess agent (1 percent sulfanilamide and 5 percent H_3PO_4 and 0.1 percent N-(1-naphthyl) ethylenediamine dihydrochloride) was added to 0.5 ml of the incubation solution that had been pipetted into the new tube (NED) The absorbance was promptly taken note of at 540 nm. The absorbance VS levels of sodium nitrite salt were used to create the standard curve graph. The calculation of scavenge NO radicals used the equation:

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

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.

RESULTS

HPLC analysis of Vidanga extract

Table 1: Peak table with Rf values, and area of Embelin from different Vidanga sample

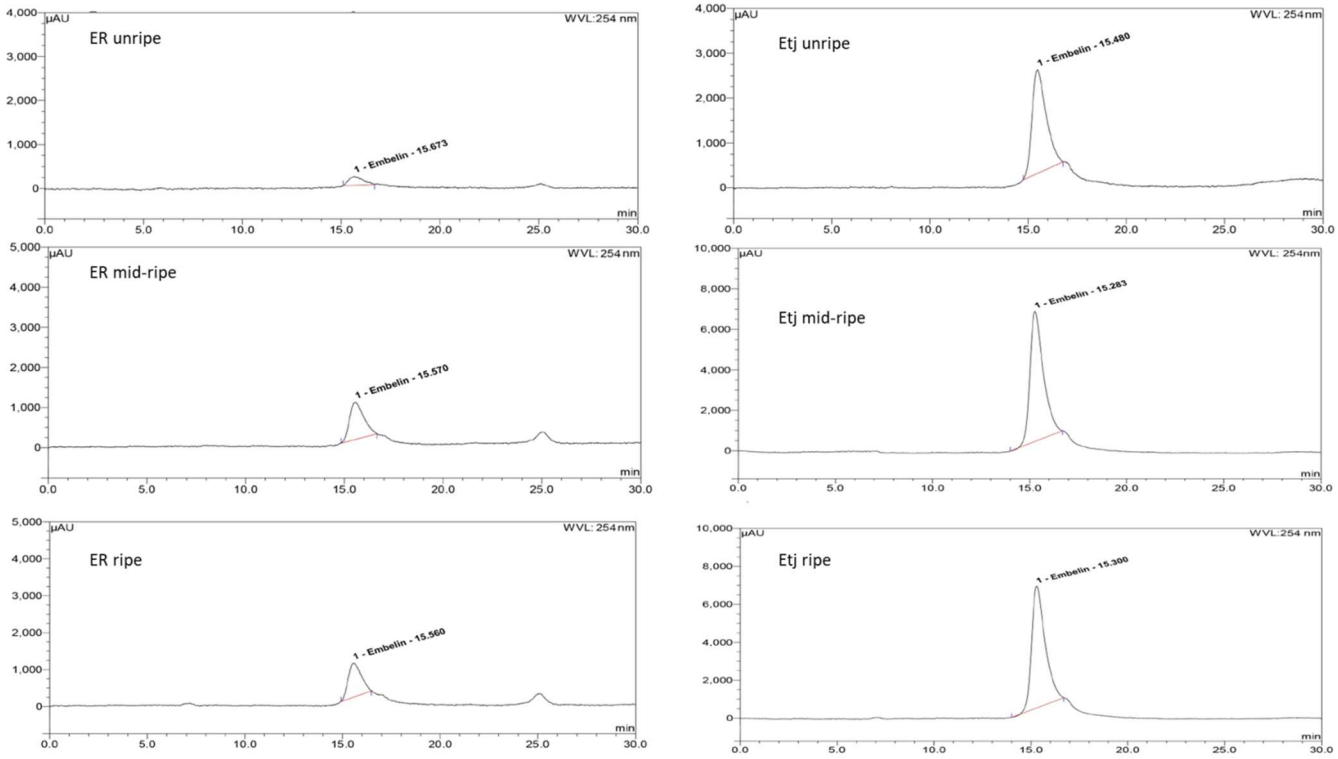
Sr. No.	Peak	Retention time	Area	Concentration (µg/ml)
1	Embelin	15.593	216248	1000

2	Er unripe	15.673	10783	4.98
3	Er mid-ripe	15.570	49691	22.42
4	Er ripe	15.560	45517	21.25
5	Etj unripe	15.480	124926	58.6.32
6	Etj mid-ripe	15.283	330006	151.12
7	Etj ripe	15.300	336788	157.28

Std: Standard; Er unripe: E. ribes unripe fruits; Er mid-ripe: E. ribes mid-ripe fruits; Er ripe: E. ribes ripe fruits; Etj unripe: E. tsjeriam-cottam unripe fruits; Etj mid-ripe: E. tsjeriam-cottam mid-ripe fruits; Etj ripe: E. tsjeriam-cottam ripe fruits.

The concentration of phytocompounds changed significantly during the maturation process of Er and Etj. The varied response was obtained for embelin content removed at various phases of development. The embelin yield ranged from 4.98µg/ml to 157.28µg/ml (Table 1). The Embelin content (Fig 1) in fruits of various stages extract was evaluated in indicated the largest Embelin content in fruits in the mid-ripe stage of Er and Etj and stagnant Embelin content in fruits in the ripened stage.

Fig 1 HPLC study of Embelin fluctuation in Vidanga samples at different stages of ripening

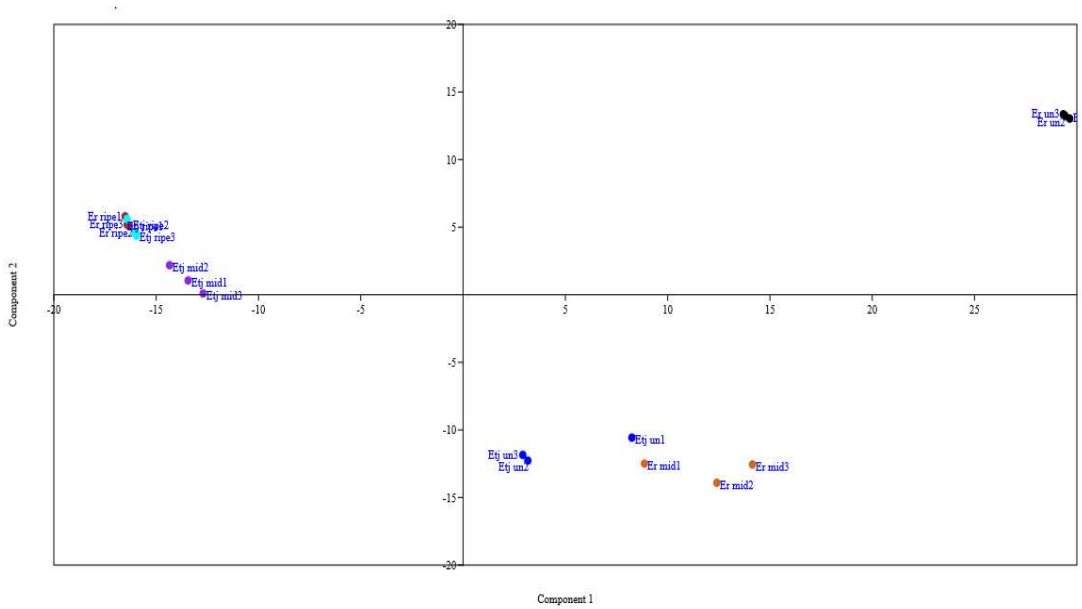


Er unripe: E. ribes unripe fruits; Er mid-ripe: E. ribes mid-ripe fruits; Er ripe: E. ribes ripe fruits; Etj unripe: E. tsjeriam-cottam unripe fruits; Etj mid-ripe: E. tsjeriam-cottam mid-ripe fruits; Etj ripe: E. tsjeriam-cottam ripe fruits.

Principal component analysis

The multidimensional variables of six plants were subjected to principal component analysis (PCA). The PCA reduced the variables to two primary principal components, with components 1 and 2 accounting for 65.9 and 19.8% of the variability, respectively (Fig 2). Both component 1 (PC1) and component 2 (PC3) explained 85.7% of the variance, with PC1 being the most important.

Fig. 2 PCA of different stages of Er and Etj



Er unripe: E. ribes unripe fruits; Er mid-ripe: E. ribes mid-ripe fruits; Er ripe: E. ribes ripe fruits; Etj unripe: E. tsjeriam-cottam unripe fruits; Etj mid-ripe: E. tsjeriam-cottam mid-ripe fruits; Etj ripe: E. tsjeriam-cottam ripe fruits.

In-vitro evaluation of inhibition of antioxidant

Table 2. Anti-oxidant potential of the Vidanga extract and their IC50 values.

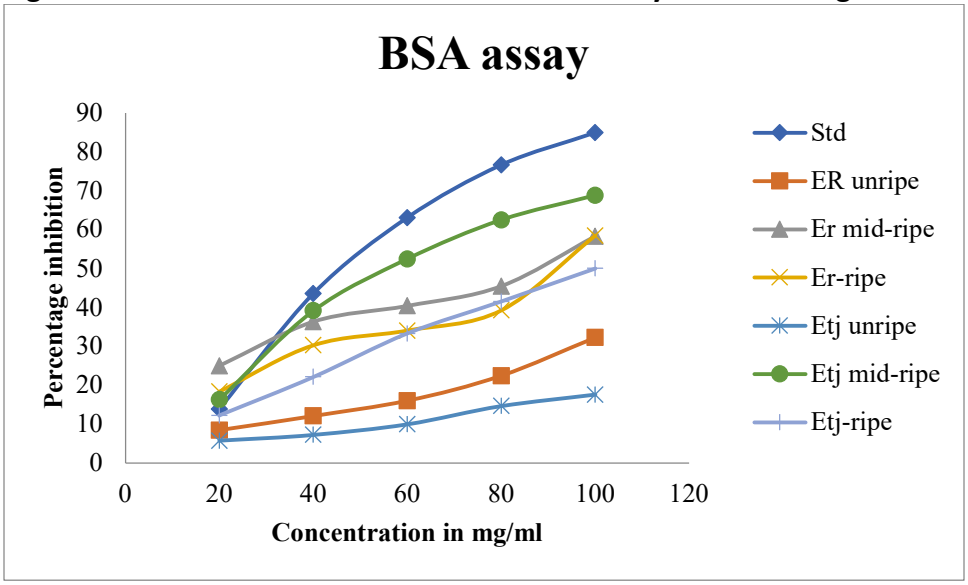
Sr. No	Anti-oxidant assay	Standard	Er unripe	Er mid-ripe	Er ripe	Etj unripe	Etj mid-ripe	Etj ripe
1	BSA anti-denaturation activity	52.64±3.64	170.29±8.55	90.19±25.10	91.91±16.20	310.37±24.97	63.18±5.43	99.25±5.50
2	ABTS activity	24.87±1.54	38.60±3.91	8.34±3.52	13.03±10.93	35.12±5.08	7.09±2.57	11.87±6.19
3	NOS activity	33.93±11.77	117.12±36.83	124.15±30.53	68.66±7.03	155.88±14.58	76.49±9.40	65.43±4.55

Std: Standard; Er unripe: E. ribes unripe fruits; Er mid-ripe: E. ribes mid-ripe fruits; Er ripe: E. ribes ripe fruits; Etj unripe: E. tsjeriam-cottam unripe fruits; Etj mid-ripe: E. tsjeriam-cottam mid-ripe fruits; Etj ripe: E. tsjeriam-cottam ripe fruits. All values are mean±SD, n=3

Anti-Denaturation Assay

Er demonstrated more anti-denaturation action than Etj at unripe stage ($p<0.05$). Er displayed stronger inhibitory activity in BSA at ripe stage as compared to Etj ($p<0.05$) (Fig 3). The IC50 value of Etj was significantly higher than Er ($p<0.001$) in mid-ripe stage of fruit development. Data in Fig. 3 demonstrated a concentration-dependent inhibition of heat-induced BSA denaturation by 20–100 mg/mL Etj mid-ripe or diclofenac sodium. Heat-induced BSA denaturation was substantially ($P<0.05$) more inhibited by Er (IC50 = 91.91±16.20 mg/mL) than by Etj (IC50 = 99.25±5.50 mg/mL). The Er and Etj at ripe stage, had significantly greater antioxidant properties than the Er and Etj at unripe stage, but these differences were insignificant when compared to Er mid-ripe stage ($p<0.001$).

Fig 3. Bovine serum albumin denaturation activity of the Vidanga extract



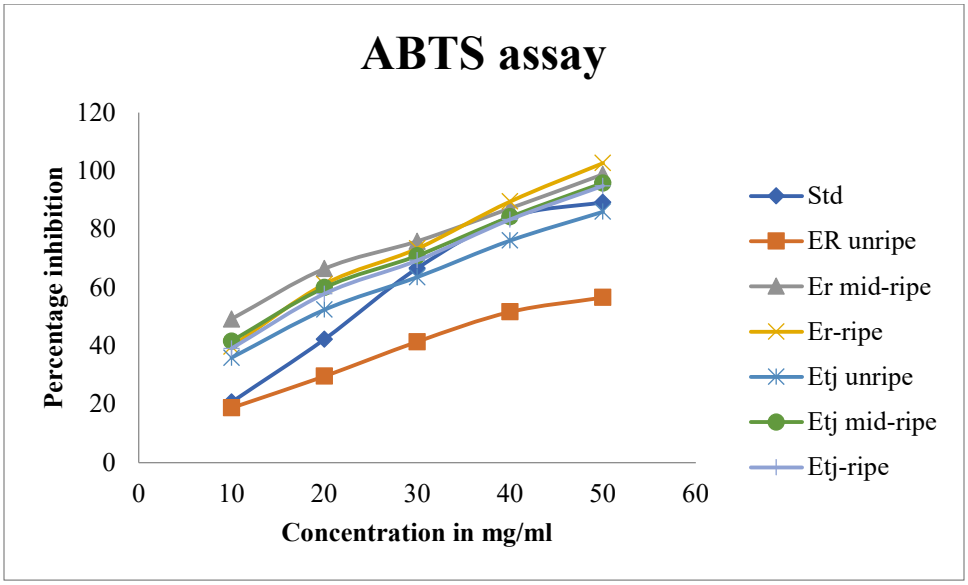
Std: Standard; Er unripe: E. ribes unripe fruits; Er mid-ripe: E. ribes mid-ripe fruits; Er ripe: E. ribes ripe fruits; Etj unripe: E. tsjeriam-cottam unripe fruits; Etj mid-ripe: E. tsjeriam-cottam mid-ripe fruits; Etj ripe: E. tsjeriam-cottam ripe fruits.

ABTS

At lower Er showed better activity than Etj ($p<0.05$) at unripe and ripe stage. While Er substantially outperformed Etj in activity at higher doses ($p<0.001$) (Fig 4). The extract exhibits an antioxidant action at comparatively greater doses than the standard, according to the findings of something like the ABTS radical scavenging experiment ($p<0.05$) (Table 1). In the ABTS assay (Figure 1), the antioxidant activity of the Vidanga showed different, but still statistically significant, results. Er and Etj ripe had the highest

antioxidant activity among the Vidanga samples. There was a statistically significant difference when Er ripe and Etj ripe was compared to Er mid-ripe and Etj mid-ripe ($p<0.05$) (Table 2).

Fig 4. ABTS radical scavenging activity of the Vidanga extract

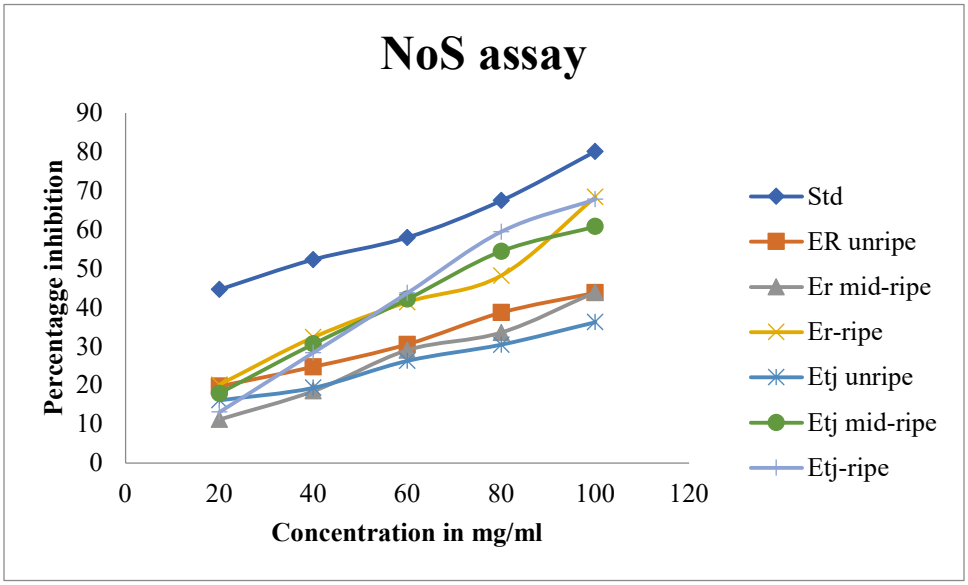


Std: Standard; Er unripe: E. ribes unripe fruits; Er mid-ripe: E. ribes mid-ripe fruits; Er ripe: E. ribes ripe fruits; Etj unripe: E. tsjeriam-cottam unripe fruits; Etj mid-ripe: E. tsjeriam-cottam mid-ripe fruits; Etj ripe: E. tsjeriam-cottam ripe fruits.

Nitric Oxide Scavenging

At ripe stage Etj showed significant activity as compared to Er ripe ($p<0.05$) (Fig 5). The differences between the anti-oxidant means of the Er and Etj were significant (Table 2). The Etj ripe antioxidant properties were significantly greater than the Er ripe and Er mid-ripe and Etj mid-ripe.

Fig 5. Nitric oxide scavenging activity of the Vidanga extract



Std: Standard; Er unripe: E. ribes unripe fruits; Er mid-ripe: E. ribes mid-ripe fruits; Er ripe: E. ribes ripe fruits; Etj unripe: E. tsjeriam-cottam unripe fruits; Etj mid-ripe: E. tsjeriam-cottam mid-ripe fruits; Etj ripe: E. tsjeriam-cottam ripe fruits.

DISCUSSIONS

The genetic makeup of plants heavily influences their ability to synthesize phytochemicals. However, agricultural techniques and other environmental stress factors, such as seasonal fluctuations, geographic location, plant maturity, soil type, and post-harvest processing, to name a few, have a major impact on the concentration of several oxidative phytochemicals. Therefore, only these characteristics may be used to selectively develop plants with high concentrations of phytochemical components and antioxidant capability (Tsao et al. 2006). However, knowing how phenolic chemicals are distributed and change as apples develop might benefit consumers and indeed the food industry by increasing the amount of healthy ingredients. Utilizing a variety of antioxidants may really enhance the protective function of our body's defense systems, defending us from the harmful consequences of free radical buildup. Over the past two decades, there has been an increase in interest in the chemical makeup of medication that is derived from plants. Fruit chemical composition changes depending on the stage of development, the environment in which it grows, the plant's nutrition, the degree of maturity, the time of harvest, and the following storage conditions. As a consequence, both the quality of both the fruits and the quantity of each component vary widely. The phenol content was found to be substantially lower in matured fruits as compared to green fruits. Significant evidence points to the crucial function that antioxidants, which are produced from many herbal and other plant products, play in the prevention of illness. By halting or postponing the development of degenerative diseases, antioxidants may significantly contribute to life

extension. The solitary, scarlet seed within the fragile pericarp of both the dried berries of Er is covered by a thin film and has traces of embelin all over its surface (Sudhakaran et al. 2016). The dried fruit of Etj may readily be distinguished from the dark to black, wrinkling berries of Er by their reddish-black appearance and vertical surface striations (Nayak et al. 2009). With an increase in concentration from overripe to mid-ripe *Embelia* that has the same chemical makeup, the total Embelin pigment concentration changes with maturity stage.

Previous research has discovered phenolic compounds and antioxidant activity in citrus species such as *Citrus limon* at full maturity as well as throughout fruit growth. Sudhakar et al. (2005) reported embelin concentration of 22.42 µg/ml and 157.28 µg/ml percent in Er and Etj, respectively (Sudhakar et al. 2005). When compared to the initial stage of fruit development, final harvest pulp TFC was considerably ($p<0.05$) lower. Even while certain polyphenol concentrations decreased as fruit ripened, all of the cultivars studied and all growth techniques had an overall rise in phenolics overall free radical scavenging activity. About 80–86% of all volatile chemicals are major hydrocarbons, such as limonene, terpinene, camphene, and citral. While sesquiterpenes steadily reduced during the course of fruit growth, the concentration of the aforementioned chemicals peaked near the conclusion of the maturity process. Ledesma-Escobar et al. have also observed that volatile chemicals have a similar pattern of behavior throughout development (2018). 107 metabolites may be found that undergo considerable changes throughout the development phase, which led to obvious, surprising variances during the growth phases of lime, according to research on the fluctuation of volatiles within Persian lime as during maturation process.

The quantity of some metabolites may change throughout the developing and ripening stages depending upon that fruit type and the phytochemicals taken into account. For instance, whereas the amount of certain phenolic compounds remained consistent throughout the ripening of apricots, the quantity of others changed (Dragovic-Uzelac et al. 2007). In addition, three distinct cultivars evaluated at two separate sites had higher levels of carotenoids as the fruit ripened. As a result, the fruit's health benefits may vary depending on when it is harvested. In this regard, research on plums demonstrates how several secondary organic compounds, such as pigments, total phenolics, and anthocyanins, change in concentration as plums mature and ripen on trees of various varieties. However, these studies produced contradicting findings, i.e., Jiang et al. (2019) shown a rise in phenolic compounds whereas Daz-Mula et al. (2008) revealed a reduction in phenolics throughout plum growth. Studying the evolution dietary sugars, organic acids, plus bioactive throughout development, ripening, and overripeness revealed that dilution effects brought on by the fruit's growth affect metabolite levels (Moscatello et al. 2019). These chemicals' concentrations change depending on the plant sections, planting seasons, extraction agents,

and growth stages (Lagnika et al. 2016). Changes in a plant's phytochemistry are the result of a series of intricate reactions that occur throughout its development cycles; for instance, near the conclusion of maturity, a plant's phenolic content may increase or steadily decline (Mahmood et al. 2012). Therefore, it is essential to assess the phytochemical components at various development phases in order to prevent any changes in the medicinal effectiveness of plants. The findings of this study's phytochemical screening demonstrated that Etj's polyphenol content was affected by Etj's growth stages. The net contents per fruit thus rises even when phenolics show a net reduction whether stated in terms of wet or dry weight basis. Therefore, owing to synthesis, there is a net gain in these beneficial chemicals throughout the growth and development of plum fruit.

Along with the embelin concentration the phenolic component concentration varied between the vidanga fruits studied. Fruit ripeness has a significant impact on the amount of phenolic chemicals present. Some phenolic compounds may degrade faster or slower than other phenolic compounds production. According to certain authors, the content of phenolic acids decreases during ripening while the content of flavonoid phenols increases (Manach et al. 2004). Polyphenols and flavonoids levels fall at the conclusion of fruit maturity. These findings imply that phenolic content increases during the earliest stages of maturity, but then decreases as the fruit ripens. The oxidation of polyphenols refers to the decrease in total phenolic content during fruit maturation (Kulkarni and Aradhya, 2005, Fawole and Opara, 2013). During the ripening of tomato fruits, four trends in the changes of individual polyphenol levels were seen: (1) high level in tropical fruit of minor modifications during ripening; (2) consistent increase with maximum level in stone grapes; (3) drop during maturity; and (4) increase with highest extent at half-ripe stage. These differences may be attributed to the distinct functions of different polyphenols throughout plants (Anton et al. 2017, Iijima et al. 2008).

The change in volatile and bioactive ingredients with maturation stages is a complicated phenomenon. Etj contains significant amounts of nutrients and bioactive substances. These concentrations of phytochemicals from UV spectrum can be used to co-relate to the biochemical activity of the vidanga extract. After using correlational studies we can configure the active biomarker from the spectrum having positive co-relation with its in-vitro bioactivity. Therefore, fruit with the highest quantities of health-beneficial chemicals will come from harvesting Etj & Er fruit at the right times. We may infer from the data that among all of the vidanga phases, the ripe stage for both Etj and Er has the greatest embelin content. This investigation supports the existence of quantitative variations in antioxidant capabilities and phytochemical component concentrations at various development stages and seasons. Fruit may be

utilized to make high-quality food items and health-improving supplements because of its outstanding and varied features, but it's important to choose the optimal fruit harvesting stage. Fruit producers and processors will need the information from this research to choose the best time to harvest Vidanga.

Conclusion

The Embelin and antioxidant activity in different fruit development phases of Er and Etj were tested in this study. Significant differences in antioxidant activity and phenolic and flavonoid composition were detected in *Embelia* from immature to commercial maturity. The fruit peel has higher levels of antioxidants and Embelin than the fruit pulp and liquid. Among these extracts, the Er is more suitable for consumption because it has the highest antioxidant activity, including the Embelin in fruit. The Etj were also high in antioxidants and can be used to make industrial products. The current study's findings could be beneficial for evaluating the ideal fruit harvest stage for a given pharmaceutical demand, as well as in crop improvement programmes for developing new kinds with improved medicinal quality.

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Authors' contribution

All the authors have equally contributed.

Conflict of interest

The authors declared that they have no conflicts of interest

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