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Biochemical characterization and antioxidant potential of *Phyllanthus emblica* L.

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Abstract

Phyllanthus Emblica also known as Indian gooseberry is ranked as important fruit crop due its medicinal and nutraceutical properties. Different biochemical characters of eight different varieties viz., Krishna, Kanchan, Banarasi, Chakaiya, Hathijhool, NA-7, NA-10 and wild genotype of *Phyllanthus Emblica* were evaluated during present investigation. The highest Vitamin C content was estimated in the fruits of variety NA-10 (627.11 mg/100 g) and wild fruits were recorded with the lowest (372.57 ± 20.53 mg/100g) vitamin C content. Highest TSS (15.33^0 B) was observed in fruit extract of variety Krishna. However, fruits of wild were recorded with lowest Total Soluble solid (5.4 ± 0.231^0 B). The percent acidity was found to be 5.87% in Kanchan variety with that of Hathijhool and NA-10 (0.66%). Total phenolic content was found to be ranged from 3.653 ± 0.053 mg/g GAE to 8.123 ± 0.053 mg/g of GAE. Variety Banarasi was found to have highest total phenolic content (8.12 mg/g GAE). The total flavanoid content was found to be 6.38 mg/g QE for Kanchan variety. The fruits of wild were observed to have highest tannin content (4.46 mg/g TAE). Aqueous fruit extract for ferrous reducing antioxidant power was reported to be range from 1.978 ± 0.432 to 10.547 ± 1.674 μ mol of FeSo₄ per mg. The varieties analysed during the present study could be explored for multifaceted ethnomedicinal uses.

Keywords: Bio-chemicals, antioxidants, characteristics, *Phyllanthus Emblica*, commercial varieties and wild varieties

Introduction

Phyllanthus Emblica is one of the important indigenous fruit of Indian subcontinent. India ranks first in world for cultivation and production of Aonla (National Horticulture Board). Cultivation of this crop is highly remunerative for the farmers having marginal land. Aonla fruit cultivation is increasing in every part of India and being cultivated in Uttar Pradesh, Maharashtra, Gujarat, Rajasthan, Andhra Pradesh, Karnataka, Tamil Nadu and Himachal Pradesh. Kanchan, Krishna NA6, NA7 and NA10 are commercially cultivated varieties of Aonla all over the country (National Horticulture Board). It has become 21st century's most important fruit crop due to its hardy nature, ability to grow in various agro-climatic conditions, high productivity per unit area, nutritive and therapeutic value and its suitability for processing (Pandey and Mishra, 2007) [26].

Aonla is known for its medicinal and therapeutic properties from an ancient time in India and is considered as a wonder fruit for health-conscious population. Aonla dried fruits are used for treatment of number of diseases like bronchitis, diabetes, fever, diarrhea, anemia, jaundice, and cough including fever, cold, dyspepsia etc. (Luqman and Kumar 2012) [19]. The fruits are used as anti-inflammatory, antipyretic, diuretic, hairtonic, laxative, liver tonic and prevent peptic ulcer (Balgia and Dsouza, 2011) [5]. It is also used for the preparation of various products like hair, oil, dye, shampoo, face cream and tooth powder. The fruits and leaves of plants are used in tanning and dyeing industries (Bakshi *et al.*, 2015) [4].

The fruits are consumed fresh, pickled and processed into a variety of sweet and tasty food products. Several value-added products have been reported from Aonla. It is rich in polyphenols, minerals and is regarded as one of the richest source of ascorbic acid or vitamin C. Fresh aonla fruit had 950/100 gm of ascorbic acid content next to Barbados cherry. The fruit contains tannins, alkaloids, phenolics and flavanoides (Chatterjee *et al.*, 2011) [12]. Polyphenols contribute towards the taste, colour, odour and preservation of fruits as well as processed products (Bhattacharjee and Tandon 2011) [8].

The fruits, bark and leaves are rich source of tannins (Charoenteeraboon, 2010) [11]. Aonla is reported as one of the highly active ailments, free radical scavenging antioxidant (Nampoothiri *et al.*, 2011) [23] and contains high phenol content and super-oxide anion scavenging activity (Kaur and Kapoor 2005) [16].

It is an important plant due to presence of phytochemicals in different parts of plants like fruit, leaves and roots. The main secondary metabolite present in this crop is polyphenols, flavonoids and tannin. The fruits of aonla has a strong antioxidant activity (Anila and Vijayalakshmi, 2003; Bafna and Balaraman, 2004) [1], the antioxidant activity may be partially due to the existence of flavanoids and several gallic acid (Sabu and Kuttan, 2002) [29, 2]. The most abundant antioxidants in aonla fruits are polyphenols and vitamin C (Silva and Sirasa 2018) [30]. keeping in the view of medicinal property of this crop the present study focused upon the comparative phytochemical profiling of different *P. emblica* varieties which could be further explored for the in depth analysis of pharmaceutical properties.

Materials and Methods

Collection of samples: The fruits of *Phyllanthus Emblica* varieties viz., Krishna, Kanchan, Banarasi, Chakaiya and Hathijhool were collected from the Regional Horticulture Research and Training Station (RHR&TS), Jachh, Kangra, Himachal Pradesh. The fruits of varieties NA-7, NA-10 and wild genotype were obtained from College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh. Collection of fruit samples was done in the month of November, 2022. The different biochemicals viz., Vitamin C, Total soluble solid (TSS), percent acidity, total phenolic content, total flavanoid content and total tannin content was estimated in the fruits of eight *Phyllanthus Emblica* varieties.

Estimation of Total soluble solids (TSS) in fruit of *Phyllanthus emblica* L. varieties: Fresh harvested fruits were taken and sliced into small pieces. These pieces were

crushed using pestle mortar and the juice was squeezed physically wrapping the crushed sample in muslin cloth. One drop of extracted juice was poured on the surface of hand refractometer. TSS was measured in °B (Brix). TSS of eight different varieties was measured in triplicates.

Estimation of Vitamin C in fruit of *P.emblica* varieties

Vitamin C content of eight different varieties was determined in the fresh fruit juice as per the method given by Ranganna in 2000 [27]. The fruits of each variety of *Phyllanthus Emblica* were grinded with the help of pestle mortar to extract the juice. To estimate the vitamin C content in the fruit of aonla 10 ml juice was mixed with 90 ml of metaphosphoric acid solution. Further 10 ml of this solution was titrated against 2,6-dichlorophenol indophenol till light pink colour was obtained against standard dye. The experiments were conducted in triplicates.

Total vitamin C content was determined by using formula

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made} \times 100}{\text{Weight of sample} \times \text{aliquot volume}}$$

Assessment of Percent acidity in fruits of *Phyllanthus Emblica* varieties:

The percent acidity was determined in the fruits of *Phyllanthus Emblica* by the standard method using 0.1 N NaOH and phenolphthalein indicator (Ranganna, 1986) [28]. Fresh harvested fruits were sliced into smaller pieces and were crushed with pestle mortar and then squeezed with muslin cloth for extraction of juice. The 10 ml extracted juice was mixed with 90 ml of distilled water. Further 10 ml was taken from this solution and 2-4 drops of phenolphthalein indicator was added into it. Titrate the diluted volume against 0.1 NaOH solution. The solution was titrated until light pink colour was observed.

Percent acidity was determined by using the formula

$$\text{Percent acidity} = \frac{\text{Titre value} \times \text{Normality of NaoH} \times \text{Volume made up} \times \text{molecular weight of standard}}{\text{Weight of sample} \times \text{Aliquot taken} \times 1000} \times 100$$

Preparation of extract for phytochemical analysis

Fresh mature fruits of *Phyllanthus Emblica* varieties were crushed with the help of pestle mortar to make a crude extract. This crude extract was dissolved in 10 ml of distilled water. Aqueous extract of *Phyllanthus Emblica* fruit were screened for estimation of total flavanoid and tannin content. Methanolic extract of fruit was prepared by mixing crude extract (10 mg) in 10 ml of methanol and used to determine the total polyphenol content.

Estimation of total phenolic content of *Phyllanthus Emblica* varieties

The total phenolic content (TPC) in the fruit extract of *Phyllanthus Emblica* was determined as per the protocol given by Singleton *et al.*, 1999 [33]. The reaction mixture was prepared by adding different concentration of methanolic fruit extract, 1 ml of FCR, 2 ml of Na₂CO₃. In case of the blank the composition of reaction mixture was remained same except methanol was added in place of methanolic fruit extract. Then the samples were incubated at room temperature for 30 min. The absorbance was determined at 750 nm using UV-Vis spectrophotometer (Thermo scientific

evolution 201). Gallic acid was taken as standard. The similar procedure was repeated for the standard (Gallic acid) and the standard curve was constructed. The total phenolic content in the fruit extract was prepared as gallic acid equivalent (mg of GA/g of extract).

Estimation of total flavanoid content of *Phyllanthus Emblica* varieties

Flavanoid content of each *Phyllanthus Emblica* variety was determined as per colorimetric aluminium chloride method (Chandrasekara and Sahidi, 2010) [9]. 0.2 ml aqueous fruit extract was taken and 0.4ml of aluminium chloride (10%) was added into it. After 6 min of incubation, the reaction was stopped by adding 1 ml of acetic acid (30%).The total volume was made up to 5.4 ml with distilled water. The absorbance of sample was read after pale yellow colour development on UV-VIS spectrophotometer (Thermo scientific evolution 201) at 415 nm. Quercetin was used as reference standard for making standard curve. The total flavonoid content was determined using linear equation of standard curve prepared with different concentration of quercetin (0.2, 0.4, 0.6, 0.8 and 1.0) The total flavonoid

content (TFC) was expressed as mg of quercetin per gram of fresh fruit.

Estimation of total tannin content of *Phyllanthus Emblica* varieties: Total tannin content (TTC) of *Phyllanthus Emblica* varieties was estimated as per Folin-Ciocalteu method. Total tannin content was determined using linear equation of standard curve prepared with different concentrations of tannic acid. The reaction mixture contained 0.2 ml of aqueous fruit extract and 1 ml of FCR. 2 ml of Na₂CO₃ (35%) was added to it. The reaction was allowed to incubate at room temperature for half an hour. After colour development to dark blue colour reading were observed in UV-Vis spectrophotometer at 760 nm.

Evaluation of antioxidant properties of different *Phyllanthus Emblica* L. varieties

Different antioxidant properties were studied in the fruits of *Phyllanthus Emblica* L. The antioxidant activity of fruits was determined using Ferrous reducing antioxidant power (FRAP) assay.

Preparation of extract for antioxidant estimation: Fresh mature fruits of *Phyllanthus Emblica* varieties were crushed with the help of pestle mortar to make a crude extract. This crude extract was dissolved in 10 ml of distilled water.

$$\text{FRAP value} = \frac{\text{Change in Absorbance of sample from 0-4 min.}}{\text{Change in absorbance of standard from 0-4min.}} \times \text{FRAP value of standard}(100 \text{ mM})$$

Statistical analysis: The experiments were set up in a completely randomized block (CRD) design (Cochran and Cox, 1963 and Gomez and Gomez, 1984). The data was analyzed using a one-way analysis of variance (ANOVA). The differences at $p < 0.05$ were considered significant. The statistical analysis was carried out using MS-Excel and OPSTAT. All the data expressed as mean \pm standard error

Results and Discussion

Total soluble solids (TSS): TSS is an important quality parameter as it has direct impact on the taste of fruit. Total soluble solids of eight varieties of *Phyllanthus Emblica* L. varied from $5.4 \pm 0.231^0\text{B}$ to $15.333 \pm 0.882^0\text{B}$. Among eight, varieties Krishna was found with highest total soluble solid ($15.333 \pm 0.882^0\text{B}$). It was statistically at par with TSS in fruits of Chakaiya ($14.633 \pm 1.02^0\text{B}$) and Banarasi ($14.133 \pm 0.133^0\text{B}$) at 5% level of significance. Fruits of Wild were recorded with lowest Total Soluble solid ($5.4 \pm 0.231^0\text{B}$). The results are shown in Fig. 1. studied fruit morphological characteristics and reported that variety Banarasi had maximum TSS 15.66^0B followed by variety Kanchan 15.50^0B , Krishna 14.16^0B , NA10 and NA7 were found to have 12.50 and 12.33^0B TSS respectively. Their study revealed the lowest TSS in variety Francis 12.16^0B . However, Kumar and Khatkar, (2015) [18] observed fruits of Desi to record the maximum TSS i.e., $15.06 \pm 0.11^0\text{B}$ followed by variety Banarasi $14.43 \pm 0.05^0\text{B}$, Kanchan $14.20 \pm 0.110\text{B}$. Bakshi *et al.*, (2015) [4] evaluated different aonla cultivars under rainfed conditions of lower shivalik foothills of Himalyas and determined highest TSS in fruits of Desi (12.20^0B) and lowest TSS in Chakaiya (9.90^0B).

Percent acidity: Percent acidity among eight varieties of *Phyllanthus Emblica* L. was found to ranged from 0.66 \pm

Aqueous extract of *Phyllanthus Emblica* fruit were screened for estimation of total antioxidant.

Determination of antioxidant activity of *P. emblica* through ferrous reducing antioxidant power (FRAP) assay:

The FRAP antioxidant assay in fresh fruit of *Phyllanthus Emblica* was determined as per the protocol given by Benzie and Strain 1996. This assay measures the antioxidant capacity to reduce the Fe³⁺/ tripyridyl-s-triazine (TPTZ) complex, to the ferrous form (Luqman *et al.*, 2009) [20]. The studies were conducted to estimate the ferric reducing antioxidant power among *Phyllanthus Emblica* varieties. FRAP reagent was prepared freshly by adding 10 ml of 300 mM of acetate buffer along with 1 ml of 10 mM TPTZ and 1 ml of 20 mM FeCl₃. 300 μl of aqueous fruit extract was added to 2700 μl of FRAP reagent. 1 ml of acetic acid (30%) was used to terminate the reaction. The reaction mixture was kept in dark for 5 min. at room temperature. The absorption was measured at 593 nm. in UV-Vis Spectrophotometer (Thermo Evolution 201). Ferrous sulphate (FeSO₄) was used as reference standard. The experiment was done in triplicates. Antioxidant activity of the extract was expressed as μM of FeSO₄.

Ferrous reducing antioxidant power was estimated by using the formula

0.025 to 1.71 ± 0.044 . Fruits of wild were observed to have the highest 1.71 percent acidity. The lowest percent acidity 0.66% was measured in Hathijhool and NA-10. Percent acidity in the fruits of variety of NA-7 (1.65 ± 0.039) was statistically at par with Kanchan (1.46 ± 0.125) at 5% level of significance. The results are represented in Fig. 1. Nayak and Kumar in 2012 [25] studied changes of nutritional and organoleptic quality of flavoured candy prepared from aonla during storage. They studied four cultivars of aonla Krishna, NA7, NA10 and Chakaiya and observed fruits of NA10 to have 1.8% acidity highest among other three cultivars, fruits of NA7 and Chakaiya were recorded with same percent acidity (1.7%). Maholiya *et al.*, (2015) [22] studied 4 cultivars Kanchan, Krishna, Chakaiya and NA 7. Their study reports Kanchan cultivar with highest (2.13%) percent acidity, while variety Chakaiya was recorded with lowest (1.79%) percent acidity. studied physico-chemical properties of Aonla fruit and juice and reported 2.34% acidity present in the fruits of Aonla.

Total Vitamin C estimation

Total vitamin C was estimated in eight different varieties of *Phyllanthus Emblica* L. and significant variation was reported to be range from 3.72 to $6.27 \pm \text{mg/g}$. Variety NA10 was recorded with highest (6.27 mg/g) vitamin C content, which was statistically at par with variety Chakaiya (5.64 mg/ g), Banarasi (5.53 mg/g), NA-7 (5.71 mg/g) and Krishna (5.36 mg/g), wild fruits were recorded with lowest (3.72 mg/g) vitamin C content at 5% level of significance. The data represented in Fig.1. Bakshi *et al.*, (2015) [4] examined six different varieties of aonla (Banarasi, Chakaiya, Neelam, Francis and Desi) and obtained fruits of Banarasi with highest (584.00 mg/100gm) vitamin C content and the fruits of Desi have lowest (480.20 mg/100 gm)

vitamin C content. Tewari *et al.*, (2019) [35] studied physical and chemical characteristics of Indian gooseberry among six cultivars (NA7, NA9, NA10, Balwant and Hathijhool). They reported cv. NA7 recorded a maximum (559.61 ± 0.92 mg/100 g) ascorbic acid content, while cv. Hathijhool was observed to have lowest (528.10 ± 1.08 mg/100 g) ascorbic

acid content. Gocher *et al.*, (2020) [14] evaluated total vitamin C content in *Phyllanthus Emblica* L. of Garhwal Himalaya. They reported maximum (607.05 mg/100 gm) vitamin C content in the fruits of Banarasi whereas, lowest (248.85 mg/100 gm) was observed in the fruits of wild.

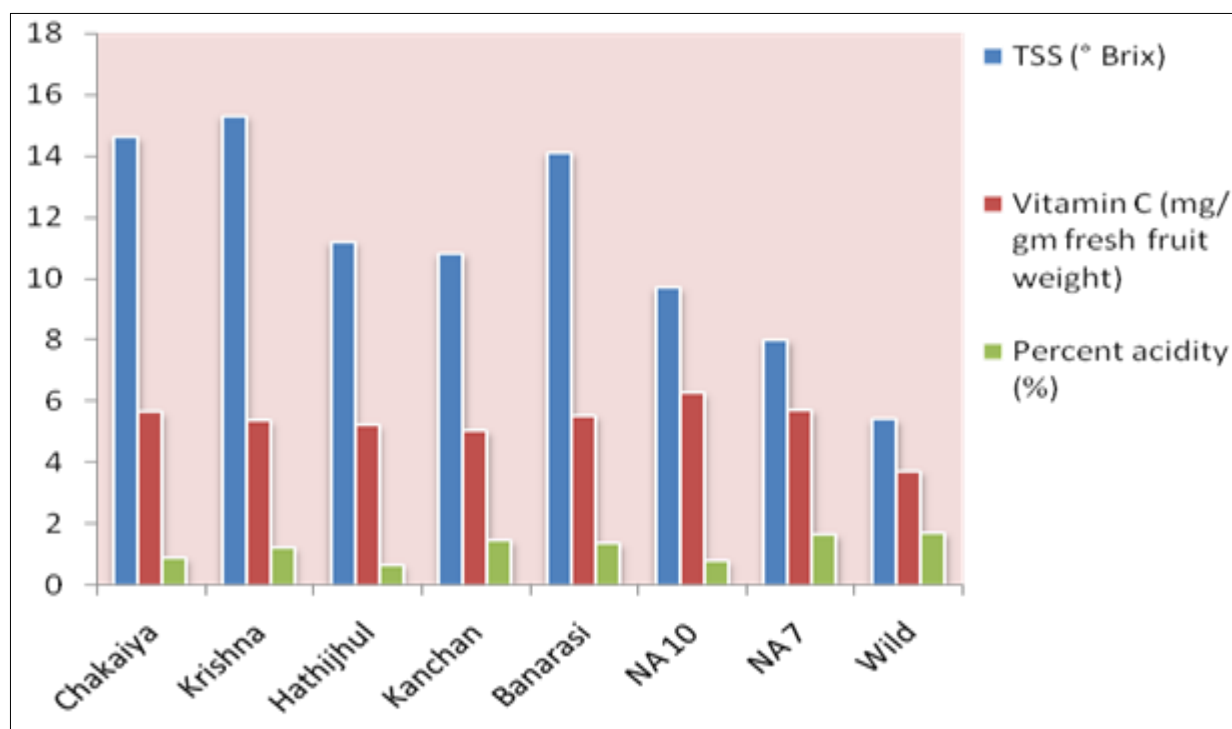


Fig 1: Evaluation of different biochemical's of *Phyllanthus Emblica* L. varieties

Total Phenolic Content (TPC): Phenolic content was estimated in all eight varieties of *Phyllanthus Emblica* L. Total phenolic content was found to be ranged from 3.653 ± 0.053 mg/g GAE to 8.123 ± 0.053 mg/g of GAE (Table 1). Variety Banarasi was observed with highest phenolic content (8.123 ± 0.053 mg/g), whereas, fruits of NA 10 had the lowest phenolic content (3.653 ± 0.053 mg/g). Mishra *et al.*, (2009) [21] studied physico-chemical properties of Chakaiya and wild variety of amla. They observed 3.23 mg/g GAE of total phenolic content in variety wild while Chakaiya variety was observed to have 2.45 mg/g of GAE. Charoenteeraboon *et al.*, (2010) [11] studied antioxidant activities of the standardized water extract from fruit of *Phyllanthus Emblica*. They reported polyphenol content of the water extract of *Phyllanthus Emblica* 3.42 mg per gram of Gallic acid equivalents. Nanasombat *et al.*, (2012) [24] studied antimicrobial and antioxidant activities of Thai local fruits extracts and concluded *Phyllanthus Emblica* fruits containing 4.220 ± 0.121 mg Gallic acid equivalents. Silva and Sirasa (2018) [30] studied antioxidant properties of selected fruit cultivars grown in Sri Lanka. They found highest phenolic content 915.7 ± 27.5 mg/100g GAE in the fruits of aonla.

Total Flavanoid Content (TFC): The experimental examination conducted to study total flavanoid content of eight varieties of amla. During present studies total flavanoid content was found to be ranged from 3.246 ± 0.031 mg/g to 6.383 ± 0.028 mg/g QE (Table 1). Variety Kanchan was observed to have highest flavanoid content

(6.383 ± 0.028 mg/g QE). While variety Krishna was recorded with lowest (3.246 ± 0.031 mg/100 g quercetin equivalents) flavanoid content. observed amla fruits containing 6.40 ± 0.88 mg/g QE. Chaphalkar *et al.*, 2017 [10] study of flavanoid content in amla fruits was found to 3.89 mg/g of quercetin equivalent. Fitriyansyah *et al.*, 2018 studied correlation between total phenolic, flavanoid and carotenoid content of *Phyllanthus Emblica* L. fruit extract. They reported total flavanoid content in *Phyllanthus Emblica* L. extract was found to be range from 2.9 mg/g QE to 3.8 mg/g QE.

Total Tannin Content (TTC): During present studies total tannin content was estimated in *Phyllanthus Emblica* L. varieties. The total tannin content in *Phyllanthus Emblica* L. varieties was varied from 3.27 ± 0.037 to 4.46 ± 0.056 mg/g TAE (Table 1). The highest tannin content was obtained in wild (4.46 ± 0.056 mg/g) and lowest was obtained in NA-10 (3.707 ± 0.046 mg/g). Sriwatcharakul 2019 evaluated bioactivities of *Phyllanthus Emblica* L. seeds and fruits. They reported that the tannin content (52.96 ± 1.04 mg/g TAE) was present in the fruits of *Phyllanthus Emblica*. Tannins in amla mainly contributed for astringency. High tannin content begins cross linking and intermolecular interactions with salivary proteins. This leads to aggregation and precipitation of protein. Thus, later induces the demise of lubrication in mouth, causes contraction of epithelial tissues in the tongue and induces dryness in mouth. Follow paper of Rishika Tewari on thermal and nonthermal processing of underutilized fruit Amla.

Table 1: Total phenolic, flavanoid and tannin content of *Phyllanthus Emblica* L. varieties

Varieties	Total Phenolic content (mg/g GAE)	Total Flavanoid content (mg/g QE)	Total Tannin content (mg/g TAE)
Chakaiya	7.113 ± 0.062	4.470 ± 0.032	3.912 ± 0.049
Krishna	7.293 ± 0.052	3.246 ± 0.031	4.111 ± 0.031
Hathijhul	7.213 ± 0.053	6.347 ± 0.055	3.693 ± 0.031
Kanchan	5.783 ± 0.056	6.383 ± 0.028	4.211 ± 0.049
Banarasi	8.123 ± 0.053	4.632 ± 0.005	3.707 ± 0.046
NA 10	3.653 ± 0.053	3.269 ± 0.037	3.275 ± 0.037
NA 7	4.643 ± 0.07	4.632 ± 0.005	4.259 ± 0.05
wild	5.743 ± 0.036	4.587 ± 0.023	4.465 ± 0.056
CD	0.177	0.088	0.121

Data represents the mean ± standard error of three values.

Estimation of ferrous reducing antioxidant power (FRAP)

During present investigation ferrous reducing antioxidant activity was estimated in aqueous fruit extract of *Phyllanthus Emblica* L. Varieties. Aqueous fruit extract for ferrous reducing antioxidant power was ranged from 1.978 ± 0.432 to 10.547 ± 1.674 µmol of FeSO₄ per mg. Maximum reducing power was found in variety Banarasi (10.547 ± 1.647 µmol of FeSO₄), while the lowest was observed in aqueous fruit extract of Krishna variety (1.978 ± 0.821 µmol of FeSO₄). Results are shown in Table 2. Charoenteeraboon *et al.*, 2010^[11] studied antioxidant activities of aqueous extract of *Phyllanthus Emblica* L. They reported ferric reducing activity for *Phyllanthus Emblica* L. was 7.46 ± 0.56 µmole FeSO₄/mg. Nanasombat *et al.*, 2012^[24] studied antimicrobial and antioxidant activities of Thai local fruit extracts. The reducing ability for *Phyllanthus Emblica* L. was 4.86 µmol/mg. Silva and Sirasa 2016^[30] observed antioxidant properties of six *Phyllanthus Emblica* cultivars grown in Sri Lanka. They reported 10.02 µmol FeSO₄/mg in fruit extract of *Phyllanthus Emblica* L.

Table 2: Estimation of ferrous reducing antioxidant power of *Phyllanthus Emblica* L. varieties

Varieties	FRAP (µmol FeSO ₄ /mg)
Chakaiya	6.027 ± 0.82
Krishna	1.978 ± 0.43
Hathijhul	2.637 ± 0.95
Kanchan	3.484 ± 1.31
Banarasi	10.547 ± 1.67
NA-10	8.004 ± 0.18
NA-7	4.803 ± 0.75
Wild	5.65 ± 0.12
CD (0.05)	2.87

Data represents the mean ± standard error of three values.

Conclusion

The study underscores significant variations in biochemical composition and antioxidant properties among different varieties of *Phyllanthus emblica* L. Notably, Krishna exhibited the highest Total Soluble Solids (TSS), while Wild had the lowest. Percent acidity varied considerably, with Wild showing the highest and Hathijhool and NA-10 the lowest. NA10 exhibited the highest vitamin C content, and Banarasi showcased the highest phenolic content. Kanchan displayed the highest flavonoid content, and Wild had the highest tannin content. Furthermore, Banarasi demonstrated the highest ferrous reducing antioxidant power (FRAP). These findings emphasize the importance of variety selection in harnessing the nutritional and health-promoting properties of *Phyllanthus emblica* L. fruits.

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