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Phenolic Acids and Antioxidants Profile, Antioxidative Enzymes and Antioxidant Activity of Mango

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ABSTRACT

In an effort to evaluate the antioxidative property in mango, a laboratory study was conducted at BCKV, Mohanpur during 2019 with ten varieties of mango selected on the basis of popularity and area coverage. The pulp of the fruit samples harvested at the firm ripe stage showed wide genotypic variability in the contents of antioxidants and activity of the antioxidative enzymes tested. Ascorbic acid and total phenol ranged from 16.20 to 44.26 mg 100 g^{-1} (around 55% of the RDA of ascorbic acid, on average) and 12.753 to 31.265 mg TE g^{-1} whereas CAT, PAL, POD and SOD were in the ranges of 15–101, 0.039–0.440, 0.230–2.333 and 3.194–6.695 corresponding units respectively. Antioxidant activity ascertained by DPPH and FRAP assays averaged 5.065 and 14.837 mg TE g^{-1} respectively. Five phenolic acids, one belonging to hydroxybenzoate (gallic acid) and four to hydroxycinnamates (chlorogenic acid, caffeic acid, *p*-coumaric acid and ferulic acid) along with *t*-cinnamic acid were analysed for their contents and ranged from 1282.02–12150.82, 210.34–1189.97, 2.31–56.82, 2.84–317.82, 9.68–41.59 and 3.52–18.59 µg g^{-1} respectively. The phenolic acids were ranked on the basis of antioxidant activity as gallic acid>ferulic acid>caffeic acid>chlorogenic acid>*p*–coumaric acid. There were important correlations between paired variables. Principal component analyses done with all the variables revealed that 'Akhil gooti' may be selected as the best variety with respect to antioxidative properties followed by 'Himsagar' and can be used for the improvement of the varieties and of the human diet.

KEYWORDS: Antioxidant, DPPH, mango, phenol, PAL, PCA, SOD

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1. INTRODUCTION

Mango (*Mangifera indica* L.; family: Anacardiaceae), one of the most popular and commercially important tropical fruits (Loeillet, 1994), possesses medium calorific and high nutrient values (Salunkhe and Desai, 1984). All the edible cultivars of mango represent the species *indica* originated in the Indian sub-continent (Patil et al., 2019). In 2020 India produced 2.6 million tons of mangoes, mangosteens and guavas altogether, which was around 47% of the world production in comparison to 2.6, 4.3 and 0.36% as shared by Bangladesh, Pakistan and Srilanka respectively (Anonymous, 2020). Mango fruit develops rapidly after fruit set and becomes ready for harvesting within 13–20 weeks, depending upon the variety and climate (Hada and Singh, 2017).

Mango is adored for its sensory properties and for being the source of important vitamins and phytochemicals e.g., ascorbic acid, carotenoids, tocopherols and a great variety of phenolic compounds *esp*., hydroxybenzoic acids including gallic acid, gallo- and ellagitannins; cinnammic acid and its derivatives *viz*., caffeic and ferulic acids; flavonol quercetin and importantly, a polyphenolic glucosyl xanthone, mangiferin (Schieber et al., 2000; Ribeiro et al., 2007; Masibo and He, 2008). A variety of phenolic compounds are responsible for imparting the characteristic aroma and flavour of the fruit and also providing protection against various infections (Dixon and Harrison, 1990; Maher et al., 1994; Dixon and Pavia, 1995; Agatonovic-Kustrin et al., 2018) and providing important health benefits by exhibiting antioxidative, anti-inflammatory, anti-microbial, and anti-proliferative effects (Bravo, 1998; Masibo and He, 2008; Pascual-Teresa et al., 2010; Quideaeu et al., 2011). All such important health promoting attributes of mango have categorized it as a medicinal plant from time immemorial (Shah et al., 2010).

A lower probability of degenerative diseases has been found to be associated with high consumption of fruits and vegetables and partially ascribed to the antioxidants as essential components, the majority of which belong to the class of phenolics (Kaur and Kapoor, 2001) which are more effective antioxidants as compared to others including vitamins C and E (Dai and Mumper, 2010). Phenolics exert their antioxidative property either by acting as reducing agents, hydrogen donors and free radical scavengers and/or by chelating metal ions like iron that decreases the catalytic generation of free radicals (Almeida et al., 2011). Consumption of fruits and vegetables benefits human health due to possessing antioxidants and also the additive and synergistic effects of the complex mixture of phytochemicals which cannot be achieved through micronutrient supplementation (Liu, 2003). Hydroxybenzoic acid and derivatives (HBAs) are potent free radical- and singlet oxygen scavengers probably interfering with DNA damage and tumour promotion (Soobrattee et al., 2005). Hydroxycinnamic acids (HCAs) on the other hand, largely benefit human health with antioxidant properties due to better resonance stabilization of the phenoxyl radical followed by hydrogen donation (Mathew et al., 2015). Phenolic acids are the predominant compounds in the mango pulp (Robles-Sanchez et al., 2009) with higher content when ripe, playing a significant role in neutralizing free radicals (Rosa et al., 2010; Paraflox-Carlos et al., 2012).

Besides phenolics and other antioxidant phytochemicals, all plants *esp*., fruits and vegetables possess enzymatic antioxidants *viz*., superoxide dismutases (SOD), catalases (CAT), peroxidases (POD) that essentially inhibit the formation and/or scavenge a wide variety of reactive oxygen/nitrogen species (ROS/RNS) including free radicals. Phenylalanine ammonia-lyase (PAL), an important enzyme of phenolics biosynthesis pathway, also contributes substantially to the ROS detoxification process by diverting the path of carbons from primary to secondary metabolism.

Although India is a prominent producer as well as consumer of mango, little is known about the antioxidant property and the extent of health-promoting effects exhibited by the fruit. Therefore, the present project was undertaken to ascertain the antioxidants contents and quality in the edible pulp of ten selected mango varieties grown under West Bengal conditions for selecting the best performing one(s) aiming at improvement of the varieties and human diet through future breeding programmes.

2. MATERIALS AND METHODS

A laboratory study was conducted at BCKV during May–June 2019 with ten prominent mango varieties including cvs. Akhil gooti, Ashwina, Bharati, Fajli, Gooti asha, Himsagar, Lakshmanbhog, Lakshmibhog, Mohanbhog and Rakhalbhog selected for their general acceptability as choice varieties as per quality as well as large quantities of production that impact commercial processing. Properly matured fruits were harvested from different orchards in Malda district of West Bengal and brought to the laboratory at BCKV, cleaned, washed, dried with a soft tissue and kept under ambient conditions until the attainment of the 'just ripe' stage. The fruits were peeled and the edible pulps were cut and chopped with a sharp knife and immediately analysed for the contents of ascorbic acid and the activities of the antioxidative enzymes *viz*., SOD, POD, CAT and PAL. A portion of the fresh, chopped pulp from each sample was air-dried followed by drying in an air-oven at 45–50°, then ground and sieved to a uniform particle size and analysed for phenol content and the antioxidant activity under different assay systems.

2.1. Analysis of ascorbic acid

One gram of finely chopped pulp tissue was extracted with 20 ml of 4% oxalic acid following maceration in a pestle and mortar and the material was centrifuged for 30 min at 10,000 *g*. Ascorbic acid content was determined by reading absorbance at 518 nm of a reaction mixture containing 1 ml supernatant and 2 ml 1.72 mM 2,6-dichlorophenolindophenol dye immediately after mixing and expressed as mg 100 g^{-1} of the fresh sample (Davis and Masten, 1991).

2.2. Analysis of total phenols

Total phenols were extracted in 50% methanolic 1.2 N HCl by boiling 1 g of powdered dried pulp tissue for 1.0 h at 80–90° following the method of Vinson et al., 1998 and subsequent analysis was with the Folin-Ciocalteau reagent using trolox as standard.

2.3. SOD activity

One gram finely chopped pulp tissue extracted with 10 ml extraction buffer containing 0.1 M potassium phosphate buffer, pH 7.5, 2% polyvinylpyrrolidone and 0.25% Triton-X detergent following centrifugation at 10,000 *g* for 30 min, to inhibit photochemical reduction of nitroblue tetrazolium (NBT) in riboflavin light NBT system (Beauchamp and Fridovich, 1971) was used to determine SOD activity. Absorbance at 560 nm was recorded and % inhibitory activity was calculated as $[(A_0-A_e)A_0^{-1}]\times100$ where A_0 = absorbance without extract and A_e=absorbance with the extract.

2.4. POD activity

One gram of fresh finely chopped pulp tissue was macerated in a pestle and mortar and extracted with 10 ml 0.1 M phosphate buffer (pH 7.5) containing 2% PVP and 0.2% Triton-X to determine POD activity. Triturated samples were centrifuged at 10,000 *g* for 30 min at 4° and supernatants were assessed for enzyme activity according the method followed by Lin and Kao, 2001. The POD activity was calculated by using the absorption coefficient (26.6 $mM^{-1}\,cm^{-1}$) at 470 nm for tetraguaiacol and expressed as µmoles of guaiacol oxidized min^{-1} $gram^{-1}$ fresh wait (Unit g^{-1} FW) of a sample.

2.5. CAT activity

CAT activity was determined by monitoring the rate of disappearance of H_2O_2 at 240 nm (ε=40 mM^{-1} cm^{-1}) following the protocol of Aebi, 1984. 3 Ml of reaction mixture comprised of 2.8 ml of 0.1M sodium phosphate buffer (pH 7.5), 0.1ml of H_2O_2 (1%) and 0.1ml of plant extract. Absorbance was taken at 240 nm. One unit of CAT activity was defined as the amount of enzyme that destroyed 1 µmol of hydrogen peroxide min^{-1} g^{-1} of fresh tissue.

2.6. Phenylalanine ammonia lyase (PAL)

PAL activity was assayed by using a method of D'Cunha et al., 1996 with slight modification. The enzyme was extracted by macerating 2 g finely chopped fresh pulp tissue in a prechilled pestle and mortar with 0.1 M Tris-HCl buffer (pH 8.8) containing 2% PVP and 0.25% Triton-X detergent. 0.1 Ml enzyme extract taken from the saved supernatant obtained from centrifugation of the triturated extract at 10,000 *g* for 30 min at 4°, was added to the reaction mixture comprised of 1.9 ml of 0.1M Tris-HCl buffer (pH 8.8), 1 ml of 10 mM L-phenylalanine (prepared in 0.1 M Tris-HCL buffer, pH 8.8) to initiate the reaction and was incubated at 30° for 15 min, then terminated by the addition of 6 M HCl following the recording of absorbance at 290 nm. One unit represented the amount of enzyme that produced 1 µmol of *t*-cinnamic acid (ε=9630 $M^{-1}\,cm^{-1}$) per hour and expressed as a Unit g^{-1} fresh sample.

2.7. 2,2-Diphenyl-1-picrylhydrazyl Radical Scavenging Activity

The total phenol extracts of mango pulp samples were used to monitor the scavenging effect for stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the method of Brand-Williams et al., 1995. A 0.15-ml extract was added to a 0.004% methanolic solution of DPPH making up a volume of 3 ml. Absorbance at 517 nm was recorded after 30 min and % inhibitory activity was calculated as $[(A_0-A_e)\ A_0^{-1}]\times100$ where A_0=absorbance without extract and A_e=absorbance with the extract.

2.8. Ferric reducing antioxidant power

The FRAP, based on the reduction of Fe (III) by the sample extract, was determined following the method described by Benzie and Strain, 1996. In FRAP assay, the change in absorbance at 593 nm due to the formation of a blue coloured Fe(II)-tripyridyltriazine compound from colourless oxidized Fe(III) form in presence of a particular concentration of sample extract is measured. FRAP reagent was made by mixing 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tri (2-pyridyl)-*s*-triazine (TPTZ) and 20 mM ferric chloride (10:1:1, v v^{-1} v^{-1}). The absorbance of the reaction mixture consisting of 0.15 ml of suitably diluted sample extract and 2.85 ml of FRAP reagent solution was read at 593 nm. The results were expressed as mg TE g^{-1} DW of sample calculated from a standard curve prepared using trolox instead of sample extract.

2.9. High performance liquid chromatographic (HPLC) estimation of phenolic acids

Reverse phase HPLC of was done using a 20 l phenol extract adjusted to pH 2–3 was separated with Shimadzu HPLC system equipped with a Shimadzu-SPD-M20A Prominence photodiode array detector set at 280 nm

wavelength, on a 150×4.6 mm² i.d., packed with adsorbent of 5 particle size in an Agilent Zorbox Ecclipac XDB–C18 column at ambient temperature. The mobile phase consisted of solvent A (0.1% acetic acid in acetonitrile) and solvent B (1.0% acetic acid), and gradient elution was performed with 85% A for initial 0–12 min, 75% A for 12–16 min, 15% A for 16–25 min and 85% A for 25–60 min.

2.10. Statistical analyses

Data were subjected to ANOVA of a randomized complete block design and means separated by Duncan's multiple range test. Principal component analysis (PCA), as the method of identifying the factor dimension of the data, was used to summarize varietal information in a reduced number of factors for the selection of the best performing cultivar(s). Statistical analyses were done using SPSS Professional Statistics ver. 7.5 (SPSS Inc., Irvine, CA).

3. RESULTS AND DISCUSSION

3.1. Antioxidant constituents

A wide genetic variability ranging from medium to high contents of ascorbic acid, a nonenzymatic water soluble antioxidant and vitamin, was observed in the mango pulp samples from ten different cultivars tested (Figure 1). The values obtained in Mohanbhog and Lakshmibhog were almost double compared to other varieties. The ascorbic acid content in horticultural crops might differ owing to a difference in species (Davey et al., 2000), variety or cultivar (Cruz-Rus et al., 2010, Mellidou et al., 2012), its differential oxidative degradation during ripening (Aina, 1990), ripening stage (Appaiah et al., 2011), ambient storage temperature (Thomas and Oke, 1980), storage period (Azad et al., 2009), fruit tissue, climate, cultural practice and post–harvest factors (Lee and Kader, 2000). Our values were comparable to those of other authors (Youssef et al., 2002, Rebeiro et al., 2007, Hossain et al., 2014, Vinci et al., 1995) indicating that around 55% of the recommended dietary intake of ascorbic acid could be supplied from the pulp samples of the cultivars investigated.

Although non-phenolic antioxidant principles in plant extracts play a considerable role to inhibit the harmful oxidative effects of different ROS at a cellular level (Hassimoto et al., 2005, Harrish and Shivanandappa, 2006), the large family of phenolics has been attributed to play the major antioxidative role in inhibiting lipid peroxidation and scavenging dreadful superoxide and hydroxyl free radicals and other ROS (Hall, 1997). The cultivar Akhil gooti produced the highest phenol content (31.27 mg TE g^{-1}, Figure 1) along with values (mg TE g^{-1}) from all other cultivars varying widely among themselves from 12.75 in Rakhalbhog to 23.42 in Lakshmanbhog. The average (19.28 mg TE g^{-1}) and other values obtained could not be compared to those of other authors due to the use of different methodologies and units of measurement. However, the concentration of phenol decreases during ripening (Selvaraj and Kumar, 1989) and cannot unequivocally picturise the true antioxidative property unless the quality of phenols in terms of structure-activity relationship (Grace, 2005) is considered.

3.2. Antioxidative enzymes

SOD activity (IC_{50}: mg ml^{-1}) of the mango cultivars (Figure 2) ranged from the lowest in Fajli (6.70) to the highest in Akhil gooti (3.19) with an average of 4.31, exhibiting significant genetic variability. Ashwina and Fajli produced substantially low activity values than others, pulling down the average activity across the cultivars by 14%. SOD activity, implied for membrane integrity, is usually higher in epicarp than in tissues at higher depths and gradually declines with the advancement of the ripening process leading to enhanced perishability (Reddy and Srivastava, 2003) and membrane permeability (Zhaoqi et al., 1997). Our results, therefore, point to the differential

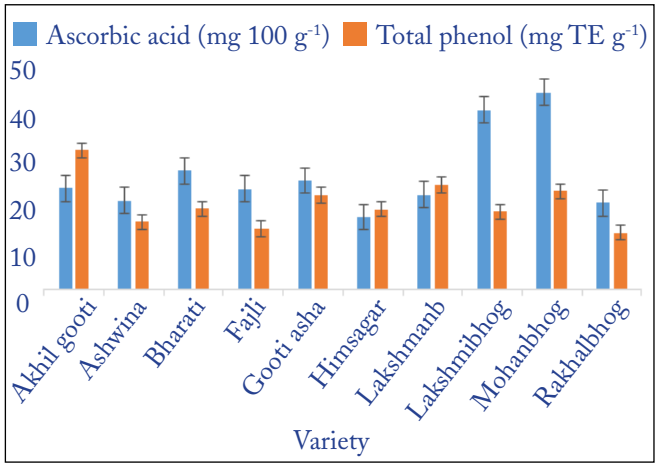

Figure 1: Antioxidants contents in mango

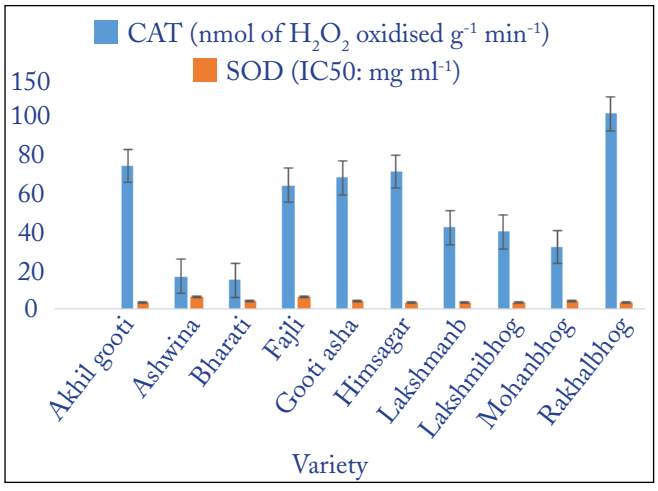

Figure 2: CAT and SOD activities in mango

elaboration of the SOD activity by different cultivars even at the same ripening stage. CAT activity (Figure 2) also showed a wide significant variation amongst the cultivars with low (Ashwina and Bharati), medium (Mohanbhog, Lakshmibhog and Lakshmanbhog), high (Fajli, Gooti asha, Himsagar and Akhil gooti) and very high (Rakhalbhog) indicating respective potentials to neutralize H_2O_2 *via* a disproportionation reaction. Peroxidases also catalyze disproportionation of H_2O_2 exerting antioxidative function besides their role in lignin synthesis *via* coupling of phenols. The highest level of POD activity was exhibited by Himsagar and Lakshmanbhog and the lowest by Ashwina and Fajli showing only around 10% activity compared to the formers (Figure 3). Gooti asha, Akhil gooti and Bharati produced around 42, 30 and 25% POD activity respectively as compared to Lakshmanbhog. The highest PAL activity (Figure 3) was shown by Gooti asha and Lakshmanbhog, the lowest. PAL is responsible for diverting the path of carbon from primary to secondary metabolism towards the synthesis of phenylpropanoids from which a number of phenolics originate (Rosler et al., 1997, Rinaldo et al., 2010). The process of ripening fruits and the biosynthesis of phenolic compounds though has a complex relationship (Rinaldo et al., 2010), yet evidence that the ripening process directly affecting the phenylpropanoid pathway (Singh et al., 2004, Palafox-Carlos et al., 2012) are common. From these considerations, Gooti asha and Lakshmanbhog were found important in possessing increased potentiality to produce phenylpropanoids.

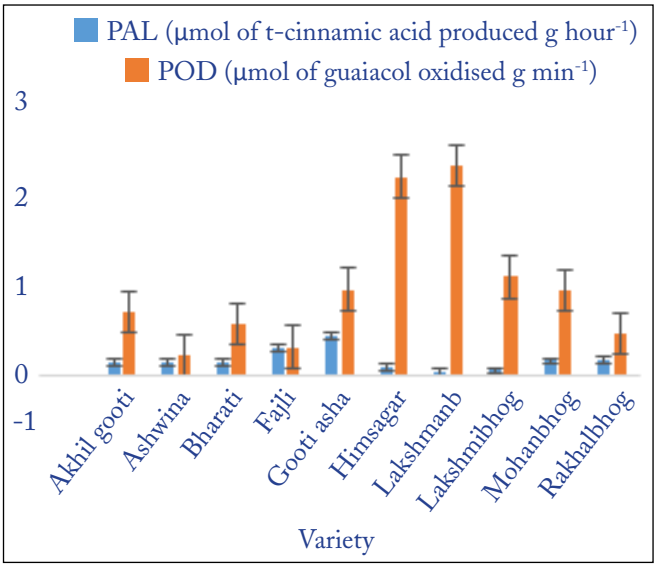

Figure 3: PAL and POD activities in mango

3.3. *Antioxidant activity*

Antioxidant activity was measured separately under two different systems of the assay (Figure 4) to have a comprehensive idea of the potentiality of the substrate

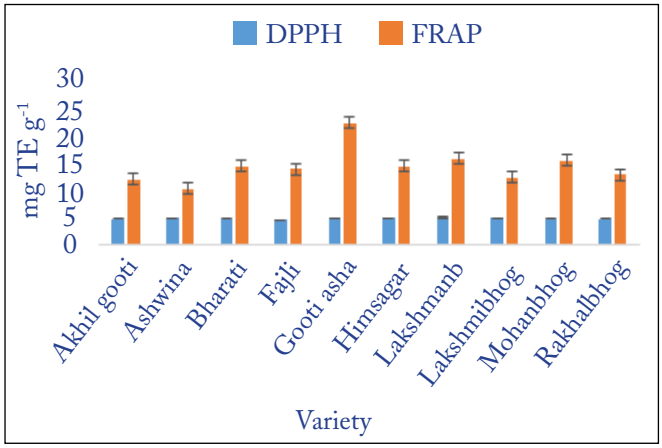

Figure 4: Antioxidant activities in mango

to fight stress. Mango varieties produced DPPH activity values differing significantly with only 7.6% variation in Fajli (lowest value) from Lakshmanbhog (highest value). DPPH and FRAP are two important assays that were followed to assess the antioxidative potential of mango varieties tested, in totality. While DPPH assay follows a sequential electron and proton transfer, or hydrogen atom transfer mechanism, the FRAP assay is based solely on single electron transfer to DPPH$^{\cdot}$ radical and Fe (III) ion respectively by the antioxidant during the experiment to develop a colour which is measured spectrophotometrically. FRAP of mango variety Gooti asha was remarkably high compared with others.

3.4. *Phenolic acids*

The antioxidative potential of any plant extract is largely due to its constituent phenolics, a large family of compounds of secondary metabolism, with diverse classes and functions. Results of the determination of individual content of phenolic acids (Figures 5 and 6) predominantly occurring in mango *viz.*, *t*-cinnamate and four of its hydroxy derivatives (HCAs) including chlorogenic acid, caffeic acid, *p*-coumaric acid and ferulic acid as well as gallic acid, a hydroxybenzoic acid (HBA) derivative, revealed the occurrence of the last

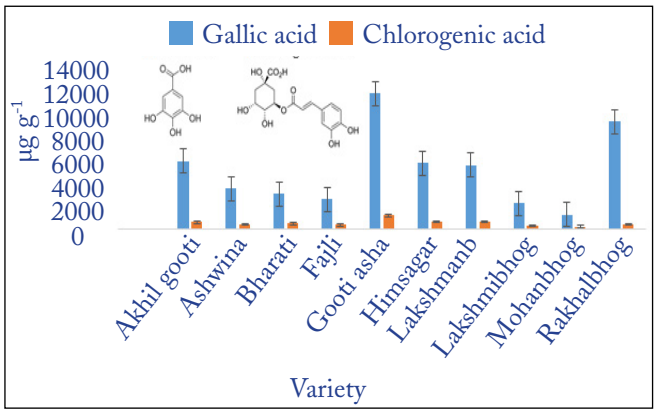

Figure 5: Gallic and chlorogenic acid contents in mango

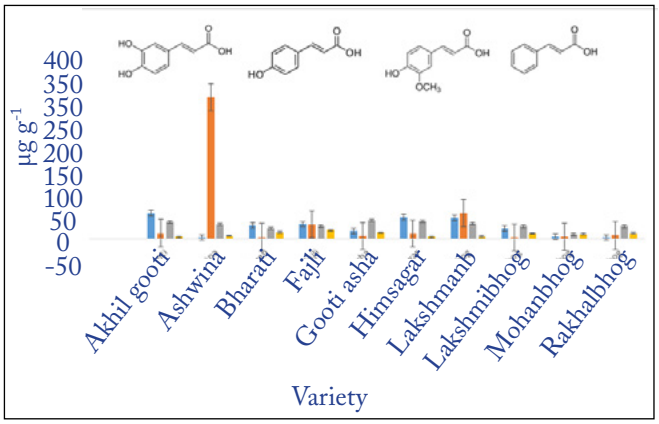

Figure 6: Caffeic-, p-coumaric-, ferulic- and t-cinnamic acid contents in mango

compound with roughly 10 times more abundant than its successor chlorogenic acid, the hydroxycinnamate derivative leading in the mango varieties.

HCAs contribute largely to antioxidant properties (Siger et al., 2008, Shahidi and Chandrasekara, 2010) by imparting resistance to lipid peroxidation (Rice-Evans et al., 1996, Nardini et al., 1995), decreasing protein oxidation (Zhu et al., 2006), chelating transition metals that promote oxidative reactions (Gasper et al., 2009), scavenging ROS (Firuzi et al., 2003, Wu et al., 2007, Foley et al., 1999, Gasper et al., 2010), and inhibiting oxidative stress enzymes (Sud'lna et al., 1993, de la Puerta et al., 1999). Gallic acid, the predominant HBA, has also strong antioxidative property manifested by its radical-and singlet oxygen scavenging activity. Gooti asha was found to be rich in almost all the phenolic acids analysed producing the highest content of gallic, chlorogenic and ferulic acids including a high content of *t*-cinnamic acid. *p*-Coumaric acid was most abundant in Ashwina whereas *t*-cinnamate, in Fajli. Schieber et al., 2000 and Abbasi et al., 2015 identified gallic acid to be the major phenolic acid component in mango compared to protocatechuic, caffeic and *p*-coumaric acid whereas Agratonovic-Kustrin et al., 2018 observed around a 3-fold concentration of chlorogenic acid compared to that of gallic acid. The authors indicated the HCAs as more potent free radical scavengers due to their -CH=CH-COO^- group ensuring greater hydrogen donating ability and subsequent radical stabilization arising from the conjugation of ring *pi*-electrons with the *pi*-bond in the side chain, than the HBAs having -COO^-, instead. The remarkably high value of gallic acid observed in our experiment might originate from *in vivo* hydrolysis of hydrolysable tannins or due to acidified methanolic extraction (Scalbert and Williamson, 2000). *t*-Cinnamic acid failed to show any response to the three antioxidant activity assays studied (Table 1).

Table 1: Antioxidant activity of phenolic acid standards

Phenolic acid standards	FRAP	ABTS	DPPH
Gallic acid	0.8352	-12.857	-0.3846
Chlorogenic acid	0.0097	-0.0698	-0.0031
Caffeic acid	0.0084	-0.1424	-0.0011
p-Coumaric acid	0.008	-0.0035	-0.001
Ferulic acid	0.0692	-0.0064	-0.0239

3.5. *Correlation analysis*

Important correlations were found between paired variables. Gallic acid and ferulic acid were strongly correlated (R^2=0.804** and 0.740** respectively) with chlorogenic acid. Ferulic acid was also correlated with gallic acid (R^2=0.649**) and caffeic acid (R^2=0.511**) exhibiting mutual dependence of the phenolic acids. Caffeic acid, however, was in a negative association (R^2=-0.388**) with the parent compound *t*–cinnamic acid. Though lower, the negative correlation helps to establish the conversion of *t*–cinnamic acid to caffeic acid. The activity of the most important H_2O_2 neutralizing enzyme CAT was associated in a low correlation (R^2=-0.364) exhibiting replacement of one with another in terms of antioxidative function. PAL, the key enzyme of phenol metabolism, was correlated with its products *t*-cinnamic acid (R^2=0.597**) and chlorogenic acid (R^2=0.521**). Moderate correlation of PAL to gallic acid (R^2=0.465**) might indicate the biosynthesis of the latter from the activated HCA or the hydroxycinnamyl coAs, involving hydroxylation of HCcoAs at ß carbon of the side chain double bond followed by oxidation and deacetylation; in addition to its biosynthesis *via* enolization and dehydrogenation of 3-dehydroshikimate. The negative correlation of SOD (R^2=-0.462*) and t-cinnamic acid (R^2=-0.524**) with total phenol might suggest a mutual exclusion in both. However, a strong positive correlation between total phenol and caffeic acid (R^2=0.554**) was quite expected because an increase of any individual phenolic acid might be reflected by a positive change in the total phenol content. So far as antioxidant activity assays are concerned, *t*-cinnamic acid was detected to be in a negative association with DPPH activity (R^2=-0.401*) implying that the aromatic ring of *t*-cinnamic acid without hydroxyl substitution cannot take part in electron or hydrogen donation. Strong correlation of FRAP with chlorogenic acid (R^2=0.760**) and gallic acid (R^2=0.554**) spoke in favour of their one-electron donating capacity; whereas that with PAL (R^2=0.600**) was indicative of higher one-electron donation potential of the phenolic products.

3.6. *Principal component analysis*

3.6.1. *Based on the antioxidative parameters*

PCA was used to obtain a simplified view of the relationship

between variables, and the PCA component loadings for the first four factors are presented in Table 2. These factors were chosen because their eigenvalues exceeded 1.0, which explained 85.17% of the total variance. The first factor explained 32.65% of total accounted for variance and was highly positively loaded with POD, DPPH and total phenol and slightly with FRAP and Ascorbic acid whereas highly negatively loaded with SOD, PAL and slightly with CAT. Considering the first-factor cvs. Himsagar, Lakshmibhog, Akhil gooti, Mohanbhog, Bharati, Gooti asha, Rakhalbhog and Ashwina (Figure 7) can be selected as having optimum values of POD, DPPH, total phenol as well as SOD and PAL. The second factor explaining an additional 22.31% of total variance showed high positive loadings of FRAP, CAT, PAL and POD as opposed to the small negative loadings of ascorbic acid and SOD. The cvs. Gooti asha, Rakhalbhog, Himsagar, Akhil gooti, Lakshmanbhog, Fajli and Bharati can be selected to be important. Considering both the first and second factors the cvs. Himsagar, Rakhalbhog, Akhil gooti, Mohanbhog and Bharati were selected for possessing optimum values of all antioxidative parameters studied. The third factor explained another 16.68% of total variance where a higher increases of ascorbic acid and FRAP along with moderate PAL were associated with a considerable and mild decrease in CAT and POD respectively. Considering the third factor (Figure 8), cvs. Mohanbhog, Gooti asha, Bharati, Lakshmibhog, Ashwina, Akhil gooti and Himsagar may be selected as performers. The fourth factor explaining an additional 13.54% of the total variance was considerably positively loaded with DPPH, SOD, POD and FRAP which were associated with a similar decrease in total phenol, ascorbic acid and CAT. Considering the fourth factor (Figure 9), cvs. Himsagar, Lakshmibhog, Mohanbhog, Bharati, Rakhalbhog, Gooti asha and Ashwina may be selected. Now considering all the four factors and the average values of all the antioxidant components the cv. Akhil gooti is selected as the best performing mango variety with the highest value of total phenol and optimum values of all other components, followed by Himsagar, Mohanbhog and Rakhalbhog and represent improved materials for breeding.

Table 2: Results of PC analysis of antioxidative parameters of mango varieties

Principal component	Eigen value	% variance	% Cumulative variance	
Eigen values and variance accounted for (%) by PCA based on correlation matrix				
PC1	2.612	32.649	32.649	
PC2	1.785	22.308	54.956	
PC3	1.334	16.680	71.636	
PC4	1.083	13.535	85.171	
Variables	PC1	PC2	PC3	PC4
Factor loadings due to PCs with eigenvalues greater than 1				
CAT	-.125	-.688	-.548	-.216
PAL	-.659	.630	.382	.049
POD	.821	.224	-.139	.331
SOD	-.773	-.257	.120	.394
AA	.175	-.350	.719	-.361
TP	.561	.238	.156	-.554
DPPH	.729	-.064	.154	.527
FRAP	.118	.784	.539	.235

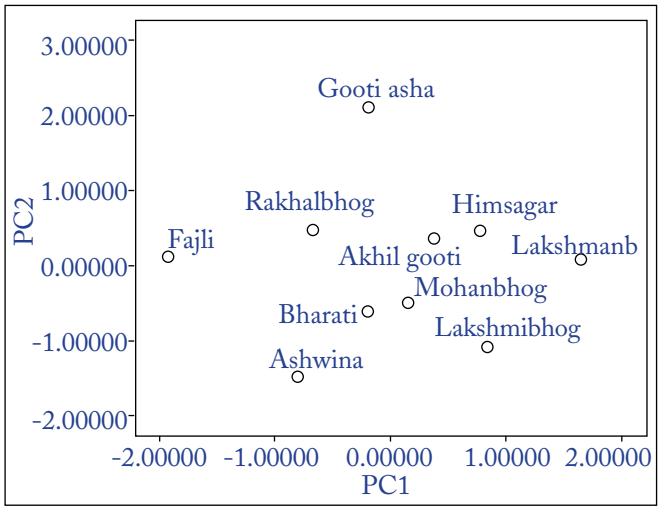

Figure 7: Scattergram of regression factor scores produced by PC1 and PC2 for antioxidants in mango

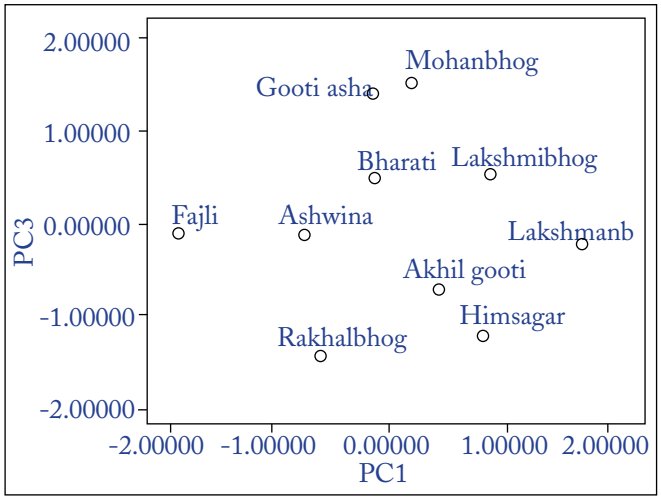

Figure 8: Scattergram of regression factor scores produced by PC1 and PC3 for antioxidants in mango

3.6.2. *Ranking of phenolic acids*

In vitro antioxidant activity of the five phenolic acids frequently found in mango was analysed under three

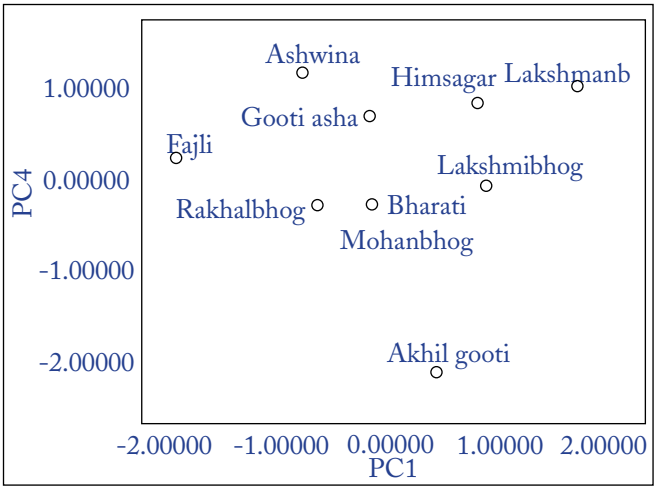

Figure 9: Scattergram of regression factor scores produced by PC1 and PC4 for antioxidants in mango

different antioxidant activity assay systems *viz*., DPPH, ABTS and FRAP (Table 2). On the basis of the analysis, the individual phenolic acids were ranked indicating their contribution to the antioxidant activity following application of the 'technique for order of preference by similarity to ideal solution' (TOPSIS) method (Filar et al., 2003) in the light of 'multiple criteria decision making' (MCDM) approach (Hwang and Yoon, 1981) as follows: gallic acid>ferulic acid>caffeic acid>chlorogenic acid>*p*-coumaric acid.

3.6.3. PCA based on the phenolic acid concentration

PCA was done with the mango varieties to select the best performing one having the optimum combination of individual phenolic acids providing the highest antioxidant property regardless of other parameters studied. This was an effort to find out the variety with the best combination of the phenolic acids tested correlating with the antioxidative activity. The first three factors were chosen as having eigenvalue greater than 1 and PCA component loadings for these were presented (Table 3). The first factor explained 44.91% of the total variance and a large increase in the loading of gallic, chlorogenic and ferulic acid and medium increase of caffeic acid were associated with a small decrease of *p*-coumaric acid. According to the first factor cvs. Gooti asha, Akhil gooti, Himsagar, Lakshmanbhog, Rakhalbhog, Ashwina and Bharati can be selected in order of preference (Figure 10). The second factor explaining an additional 23.15% of the total variance was loaded with little to moderate positive values of gallic and chlorogenic acid and was associated with similar negative loadings of ferulic, *p*-coumaric and caffeic acid. Based on the second factor cvs. Bharati, Lakshmibhog, Mohanbhog, Himsagar, Lakshmanbhog, Akhil gooti and Ashwina can be selected as performers. On the basis of the first and second factors the cvs. Akhil gooti, Himsagar, Lakshmanbhog, Bharati and Lakshmibhog were selected. The third factor explained an

Table 3: Results of PC analysis of phenolic acid contents of mango varieties

Principal component	Eigen value	% variance	% Cumulative variance
Eigen values and variance accounted for (%) by PCA based on correlation matrix			
PC1	2.695	44.913	44.913
PC2	1.389	23.153	68.066
PC3	1.229	20.478	88.544
Variables	PC1	PC2	PC3
Factor loadings due to PCs with eigenvalues greater than 1			
Gallic acid	0.801	0.395	0.315
Chlorogenic acid	0.890	0.272	0.165
Caffeic acid	0.523	-0.346	-0.732
p-Coumaric acid	-0.136	-0.595	0.743
Ferulic acid	0.917	-0.185	0.113
t-Cinnamic acid	-0.358	0.807	0.050

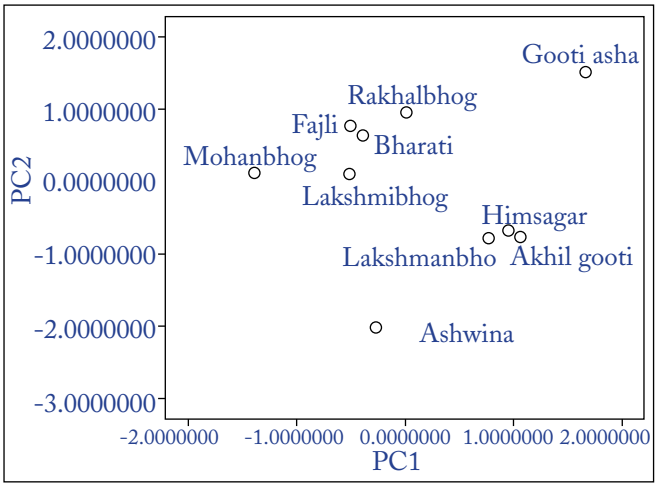

Figure 10: Scattergram of regression factor scores produced by PC1 and PC2 for phenolic acids in mango

additional 20.48% of the total variance and was positively loaded with a high value of *p*-coumaric acid along with a moderate value of gallic acid and little values of chlorogenic and ferulic acid. All these were associated with a large negative loading of caffeic acid. Based on the third factor the cvs. Akhil gooti, Himsagar, Lakshmanbhog, Bharati, Fajli, Lakshmibhog and Mohanbhog may be selected (Figure 11). Now, considering all the three factors and the average value of all the ranked phenolic acids contributing to antioxidant activity concerning to their individual level, cv. Akhil gooti was selected as the best performing variety on the basis of the phenolic acids tested, followed by Himsagar and Lakshmanbhog.

Remarkably, results of the PC analysis involving phenolic

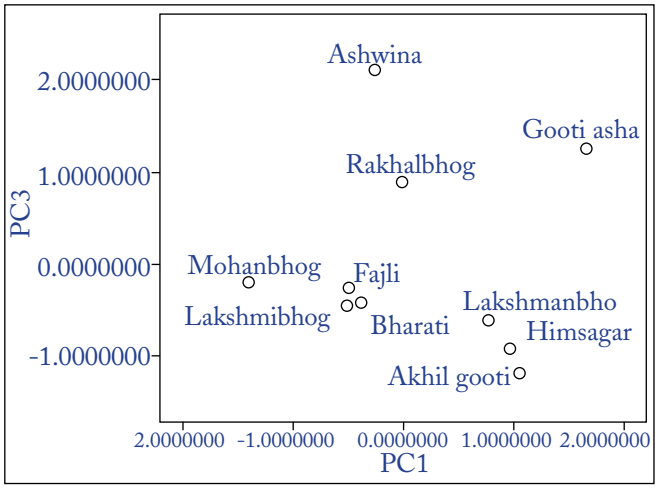

Figure 11: Scattergram of regression factor scores produced by PC1 and PC3 for phenolic acids in mango

acids as variables reinforced that of PCA with all other variables tested. Therefore, cvs. Akhil gooti and Himsagar may be the improved varieties and can be useful in a breeding programme.

4. CONCLUSION

The mango varieties furnished variable levels of antioxidants and enzyme activities along with more than double antioxidant activities under the FRAP assay compared to the DPPH assay. The average values of all the parameters tested and the results of PCA done both separately with phenolic acids contents and other antioxidative parameters, together pointed towards the selection of 'Akhil gooti' and 'Himsagar' as the improved varieties followed by others to be used as breeding materials to improve the quality of germplasm and human diet.

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