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Original article

Characterization of ascorbic acid and phenolic compounds for identification of nutritionally rich cultivars and accessions of Indian gooseberry

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Summary

Introduction – **Indian gooseberry (*Emblica officinalis* Gaertn.) is a therapeutically important underutilized fruit due to the presence of high amount of two well-known antioxidants, ascorbic acid and polyphenols. The present study aimed at identification and quantification of different phenolic compounds and ascorbic acid in fresh fruits of seven cultivars and two promising accessions of Indian gooseberry.** *Materials and methods* – **Ascorbic acid was estimated by titrimetric method and individual phenolic compounds were identified and quantified by RP-HPLC method with PDA detector. Multivariate method and ANOVA were applied for statistical evaluation.** *Results* – **The content of ascorbic acid varied from 335.24 mg 100 g^{-1} ('NA-7') to 1278.31 mg 100 g^{-1} ('Desi') among the cultivars, whereas it ranged between 418.12 mg 100 g^{-1} (CISH A-31) and 490.13 mg 100 g^{-1} (CISH A-33) among the accessions. Seven phenolic compounds were identified and quantified at various proportions in different aonla cultivars and their variations among the cultivars were assessed. Among the cultivars/accessions studied, 'Desi' aonla possessed maximum amount of total polyphenols (58.207 mg g^{-1}) followed by CISH A-31 (40.708 mg g^{-1}) and 'NA-6' (35.081 mg g^{-1}). Multivariate evaluation revealed that gallic acid, *p*-coumaric acid, total polyphenols and ascorbic acid were the major variables in aonla and 'Desi' and 'NA-6' were the most important cultivars.** *Conclusion* – **'Desi' aonla fruits were the richest source of ascorbic acid and phenolic compounds among the cultivars. Two accessions along with cultivars 'NA-6', 'Krishna' and 'Lakshmi 52' were also rich in both the nutraceuticals. These cultivars and accessions are not only beneficial for human health but can also be used in processing and phyto-pharmaceutical industries.**

Keywords

'Desi' aonla, gallic acid, *p*-coumaric acid, ascorbic acid, HPLC characterization, multivariate evaluation

Significance of this study

What is already known on this subject?

- Indian gooseberry (aonla) is a fruit well-known for its therapeutic value and traditionally used in Ayurvedic medicine system in India. It is very rich in ascorbic acid and polyphenols which have antioxidant potential against many human ailments.

What are the new findings?

- The present study provides an original description of the profiling of seven phenolic compounds and ascorbic acid in fruits of seven Indian aonla cultivars and two promising accessions identified by this institute at edible stage. 'Desi' aonla and two accessions possessed very good amounts of ascorbic acid and phenolic compounds.

What is the expected impact on horticulture?

- Being a therapeutically important but underutilized fruit crop in the world, it is important to identify and quantify various phenolic compounds in different aonla cultivars and accessions. Characterization of polyphenols will be useful for the future breeding activities also. A deep knowledge of useful phenolic compounds in fruit can also have impact on processing and phyto-pharmaceutical industries as aonla is extensively used for these purposes. Aonla accessions with good antioxidant potential can be released as varieties so that their health benefit can be availed by the consumers.

Introduction

Polyphenols or phenolic compounds are secondary metabolites of plants and are widely distributed in plant-based food products. Phenolic compounds have the capacities to quench lipid peroxidation, prevent DNA oxidative damage, scavenge free radicals and prevent inhibition of cell communication (Cao and Cao, 1999) which are all indication of antioxidant properties. The antioxidant activity of phenolic compounds is due to the reactivity of phenol moiety (hydroxyl group) on aromatic ring, which has the ability to scavenge free radicals via hydrogen or electron donation (Shahidi and Wanasundara, 1992). Gallic acid has anti-inflammatory, antimicrobial, antimutagenic, anticancer and radical scavenging activities (Madsen and Bertelsen, 1995); ellagic acid exhibits antimutagenic, anticancer and antiviral abilities and stimulates the activities of detoxifying enzymes (Mandal *et al*., 1988); catechin and epicatechin protect against congestive heart failure, myoglobinuric acute renal failure and myocardial ischemia, have anticancer and anti-aging properties (Augustyniak *et al*., 2005); ellagic acid, chlorogenic acid and catechin also show α-glucosidase inhibitory activity (Čakar

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et al., 2018b). Ascorbic acid (vitamin C) is known to be a potent antioxidant having capacity to eliminate reactive oxygen species, keep the membrane-bound antioxidant α–tocopherol in the reduced state, act as a cofactor maintaining the activity of a number of enzymes (by keeping metal ions in the reduced state), appears to be substrate for oxalate and tartarate biosynthesis and has a role in stress resistance (Davey *et al*., 2000; Arrigoni and De Tullio, 2002). Many fruits are rich source of phenolic compounds and ascorbic acid. Phenolic compounds are the main cause of astringency in many fruits and also contribute to the taste, colour, odour and preservation of fruits and their processed products.

Indian gooseberry or aonla (*Emblica officinalis* Gaertn.) belongs to the family Euphorbiaceae and is grown in many countries like Indonesia, India, Philippines, Brazil, Sri Lanka, Mexico, Thailand, Malaysia, Vietnam, Ghana, Nigeria, *etc*. A single aonla fruit contains almost 20 times more ascorbic acid than two oranges (Yadav *et al*., 2011). Due to the presence of high amount of phenolic compounds, fruits are astringent in taste and difficult to consume fresh, resulting in its main use in processing and Ayurvedic medicine. The value-added products prepared from aonla are juice, preserve (murabba), candy, pickle, segments-in-syrup, powder, laddoo, *etc*. (Rakesh *et al*., 2004). Many popular aonla cultivars are grown in central and northern India. Information on the content of ascorbic acid and total polyphenols in various aonla cultivars is plenty and few attempts have also been made to identify and quantify individual phenolic compounds in some aonla cultivars (Liu *et al*., 2008; Kumar and Rao, 2010; Parveen *et al*., 2019; Parveen and Khatkar, 2019). As the proportion of ascorbic acid and polyphenols varies as per variety as well as location, the present study was, therefore, undertaken to identify and quantify various phenolic compounds and ascorbic acid present in fresh fruits of seven Indian aonla cultivars and two promising accessions conserved at ICAR-CISH, Lucknow, India, using reverse phase HPLC technique and multivariate evaluation methods.

Materials and methods

Plant materials

Panna district of Madhya Pradesh, India, is rich in aonla diversity and many accessions are collected from Panna and grown at the institute as accessions. Freshly harvested mature aonla fruits of seven cultivars ('Krishna', 'Kanchan', 'NA-6', 'NA-7', 'Chakaiya', 'Lakshmi-52' and 'Desi') and two promising accessions (CISH A-31 and CISH A-33) were collected from the Institute farm at Rehmankhera, Lucknow. After washing of fruits in water to remove any dirt and air drying to remove surface water, segments were separated and macerated in a mixer-grinder. One gram pulp sample (in triplicate) was collected for the analysis of both phenolic compounds and ascorbic acid.

Chemicals

Reference standards of various phenolic compounds like gallic acid, chlorogenic acid, catechin, epicatechin, caffeic acid, ellagic acid and *p*-coumaric acid along with L-ascorbic acid were purchased from Sigma-Aldrich, India Branch, Mumbai. HPLC grade solvents (methanol and water) were procured locally. Stock solutions of 1,000 µg mL^{-1} were prepared by dissolving 25 mg of each reference standard in 25 mL of HPLC grade methanol. Working solutions of 5, 10 and 50 µg mL^{-1} for each reference standard (phenolic compounds) were prepared by subsequent dilution in methanol. Chemical structures of ascorbic acid and phenolic compounds are presented in Figure 1.

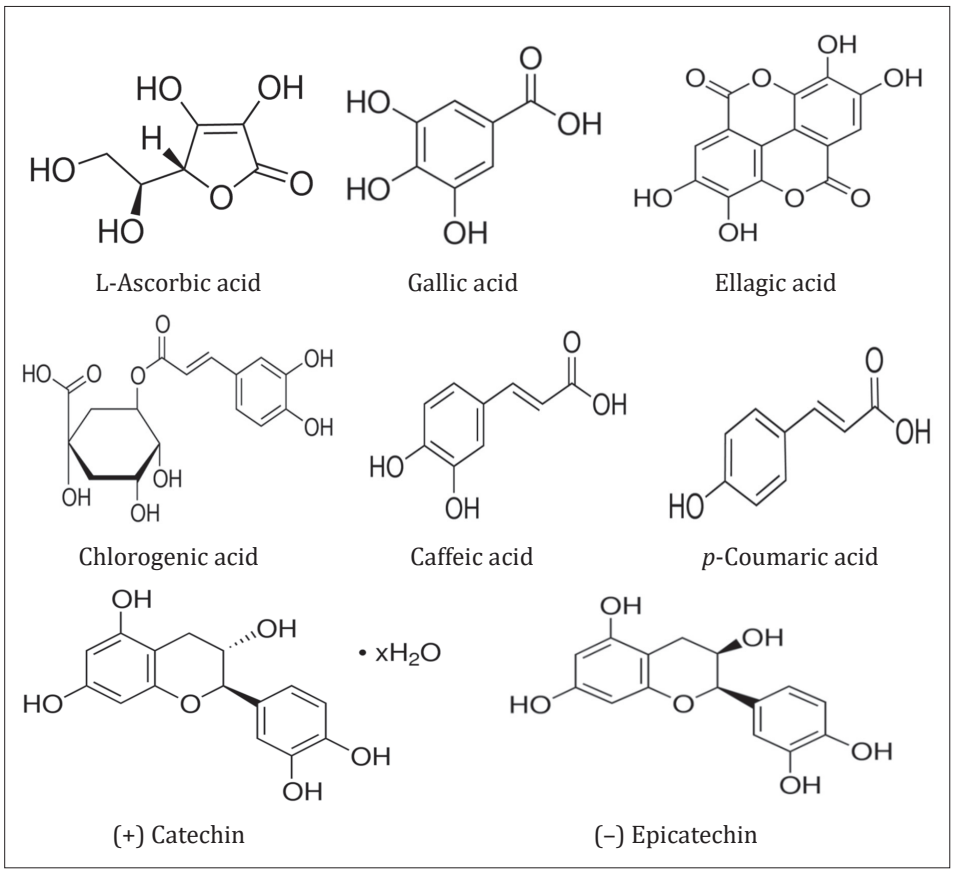

Figure 1. Chemical structures of ascorbic acid and seven phenolic compounds identified in aonla cultivars/accessions.

Table 1. The contents of quantified phenolic compounds in different cultivars/accessions of Indian gooseberry (fresh weight basis).

Cultivars/ accessions	Gallic acid (mg g^{-1} fw)	Ellagic acid (mg g^{-1} fw)	Chlorogenic acid (mg g^{-1} fw)	Caffeic acid (mg g^{-1} fw)	*p*-Coumaric acid (mg g^{-1} fw)	Catechin (mg g^{-1} fw)	Epicatechin (mg g^{-1} fw)	Total of selected quantified phenolic compounds (mg g^{-1} fw)
Kanchan	1.41 ± 0.088^{e}	0.75 ± 0.121^{c}	1.41 ± 0.065^{f}	0.61 ± 0.039^{e}	0.09 ± 0.001^{f}	6.67 ± 0.329^{e}	1.30 ± 0.130^{g}	12.25 ± 0.467^{g}
Krishna	5.21 ± 0.083^{cd}	1.16 ± 0.033^{b}	9.01 ± 0.216^{b}	0.80 ± 0.041^{d}	0.16 ± 0.021^{d}	11.12 ± 0.489^{c}	4.19 ± 0.257^{e}	31.65 ± 0.950^{d}
NA-6	3.59 ± 0.486^{d}	0.64 ± 0.021^{cd}	2.56 ± 0.305^{e}	8.28 ± 0.077^{a}	0.24 ± 0.029^{c}	10.88 ± 0.394^{c}	8.88 ± 0.339^{a}	35.08 ± 0.620^{c}
NA-7	1.78 ± 0.228^{e}	0.19 ± 0.008^{e}	3.28 ± 0.149^{d}	1.41 ± 0.069^{c}	0.18 ± 0.008^{d}	0.89 ± 0.109^{f}	7.27 ± 0.620^{b}	15.00 ± 0.860^{f}
Chakaiya	1.50 ± 0.156^{e}	1.34 ± 0.144^{b}	2.65 ± 0.431^{e}	1.35 ± 0.291^{c}	0.11 ± 0.013^{ef}	6.62 ± 0.412^{e}	4.53 ± 0.199^{e}	18.09 ± 0.318^{e}
Lakshmi-52	5.18 ± 0.214^{cd}	1.15 ± 0.193^{b}	8.54 ± 0.387^{b}	0.78 ± 0.009^{de}	0.13 ± 0.003^{e}	11.78 ± 0.930^{c}	3.42 ± 0.167^{f}	30.97 ± 0.521^{d}
Desi aonla	24.05 ± 2.445^{b}	0.37 ± 0.029^{de}	ND	4.47 ± 0.041^{b}	1.51 ± 0.007^{a}	18.54 ± 1.537^{a}	9.28 ± 0.048^{a}	58.21 ± 3.973^{a}
CISH A-31	7.96 ± 0.752^{bc}	0.24 ± 0.024^{e}	9.77 ± 0.332^{a}	1.39 ± 0.064^{c}	0.40 ± 0.025^{b}	14.22 ± 0.592^{b}	6.73 ± 0.302^{c}	40.71 ± 1.558^{b}
CISH A-33	6.83 ± 1.343^{a}	1.93 ± 0.404^{a}	7.41 ± 0.250^{c}	0.96 ± 0.040^{d}	0.25 ± 0.034^{c}	8.67 ± 0.493^{d}	5.97 ± 0.303^{d}	31.85 ± 0.469^{d}

The values are expressed as mean ± SD; ND = Not detected.
Data with different number in column as superscript are statistically significant.

Extraction

One gram pulp sample was extracted with 20 mL of 80 per cent methanol by centrifugation at 10,000 rpm for 10 min for phenolic compounds. The process was repeated twice and pooled supernatant were collected, methanol was evaporated completely in a rotary vacuum evaporator and residue was dissolved in 5 mL of HPLC grade methanol. Final volume was made up to 10 mL with the same solvent.

HPLC analysis

High performance liquid chromatography (HPLC) is the most favourable tool to estimate and quantify phenolic compounds in aonla fruits, juice and spray-dried powder (Kumar and Rao, 2010; Bhattacherjee *et al*., 2011a; Bhattacherjee *et al*., 2011b; Parveen and Khatkar, 2019; Parveen *et al*., 2019). Here also phenolic compounds were estimated with the help of a Shimadzu, Japan make HPLC (model SCL 10 AVP) coupled with rheodyne manual injector and photodiode array (PDA) detector. The method suggested by Bhattacherjee *et al*. (2011b) has been employed with slight modifications in mobile phase combination and its flow-rate. The linear gradient of mobile phase [potassium dihydrogen orthophosphate solution (0.01M) (A) : acetonitrile-water (75:25, v/v) (B) = 80:20] was carried out at a flow-rate of 1.0 mL min^{-1} at 280 nm. The stationary phase consisted of reverse phase Phenomenex® Luna C-18 column (250 × 4.6 mm i.d., 100 Å, 5 µ film thickness). Before injecting to HPLC, all the samples were filtered through a sample clarification kit with nylon membrane filter (Axiva, 13 mm diameter, 0.45 µ thickness). The content of ascorbic acid was estimated by titrimetric method using 2,6-dichlorophenol indophenol dye solution (Ranganna, 2000). Total amount of selected quantified phenolic compounds were calculated by adding the amounts of individual polyphenols detected by HPLC.

Statistical analysis

The statistical analysis was carried out for analysis of variance (ANOVA) using completely randomized design with three replications by Web Agri Stat Package (WASP) v. 2.0 software through student's *t*-test (LSD) at p≤0.05. Coefficient of variation (CV) was also presented to show the variation among the cultivars. The multivariate methods – principal component analysis and canonical discriminant function analysis – were performed using SPSS v. 16 and SAS 9.3 software.

Results

Seven phenolic compounds (gallic acid, chlorogenic acid, catechin, epicatechin, caffeic acid, ellagic acid and *p*-coumaric acid) were simultaneously detected and quantified in seven cultivars and two accessions of aonla by RP-HPLC. The data are presented in Table 1. Ascorbic acid was also estimated in these cultivars and accessions.

Ascorbic acid

Ascorbic acid is the predominant vitamin present in aonla fruit and the main reason for its therapeutic activity. Both the accessions and cultivars were found very good source of ascorbic acid. Accession CISH A-33 possessed maximum amount of ascorbic acid (490.13 mg 100 g^{-1}) among the accessions followed by CISH A-31 (418.12 mg 100 g^{-1}). However, among the cultivars 'Desi' aonla was the richest source of ascorbic acid. Though it was smaller in size (14–18 g fruit weight), it contained 1278.31 mg 100 g^{-1} of ascorbic acid, which was quite high compared to other cultivars and accessions (Figure 2). Variation in ascorbic acid content in

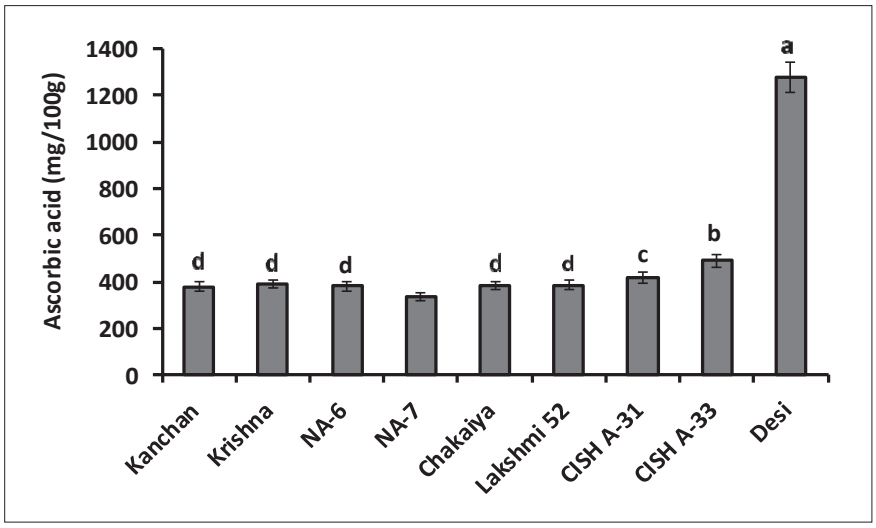

Figure 2. Ascorbic acid content (fresh weight basis) in different aonla cultivars/accessions.

other aonla cultivars was also prominent (CV = 2.158) where 'Krishna' contained 390.34 mg 100 g^{-1} of ascorbic acid and 'NA-7' the lowest (335.24 mg 100 g^{-1}). Two accessions were found slightly richer in ascorbic acid content than popular cultivars except 'Desi' aonla. A highly significant difference (LSD = 18.287) was noticed in ascorbic acid content among the cultivars and accessions.

Gallic acid

Gallic acid is one of the most important phenolic compounds present in aonla fruits. Maximum gallic acid content (24.05 mg g^{-1}) was recorded in 'Desi' aonla which was distantly seconded by CISH A-31 (7.96 mg g^{-1}) and CISH A-33 (6.83 mg g^{-1}). Lower gallic acid content was observed in three cultivars, *i.e.*, 'Kanchan' (1.41 mg g^{-1}), 'Chakaiya' (1.50 mg g^{-1}) and 'NA-7' (1.77 mg g^{-1}) (Table 1). A highly significant difference (LSD = 1.689 and CV = 15.405) was observed in gallic acid content between cultivars and accessions.

Chlorogenic acid

The content of chlorogenic acid varied significantly among the cultivars and accessions tested (LSD = 0.503 and CV = 5.207). Accession CISH A-31 contained maximum amount of chlorogenic acid (9.77 mg g^{-1}) closely followed by 'Krishna' (9.01 mg g^{-1}), whereas 'Kanchan' contained minimum amount of this phenolic acid (1.41 mg g^{-1}) (Table 1). Cultivar 'Lakshmi-52' and accession CISH A-33 also possessed a good amount of chlorogenic acid (8.54 and 7.41 mg g^{-1}, respectively). Interestingly, chlorogenic acid was not detected in 'Desi' aonla.

Catechin

Catechin belongs to flavans group of phenolic compounds and is the major phenolic compound detected in all aonla cultivars and accessions except 'NA-7', where epicatechin was recorded as major phenolic compound. Maximum amount of catechin (18.54 mg g^{-1}) was detected in 'Desi' aonla (Table 1). Other cultivars like 'Lakshmi-52' (11.78 mg g^{-1}), 'Krishna' (11.12 mg g^{-1}) and 'NA-6' (10.88 mg g^{-1}) as well as accessions CISH A-31 (14.22 mg g^{-1}) and CISH A-33 (8.67 mg g^{-1}) were the other very good source of this antioxidant. However, 'NA-7' contained minimum amount of catechin (0.89 mg g^{-1}), which is quite low compared to other cultivars. Here also a highly significant difference (LSD = 1.214 and CV = 7.124) was noticed among the cultivars studied.

Epicatechin

It also belongs to flavans category of phenolic compounds from flavonoids group. Variation in the content of epicatechin was significant between the cultivars (LSD = 0.523 and CV = 5.336). HPLC data revealed that Desi aonla fruits were the richest source of this compound (9.28 mg g^{-1}) closely followed by NA-6 (8.88 mg g^{-1}) (Table 1). Other cultivars and accessions rich in epicatechin were NA-7 (7.26 mg g^{-1}), CISH A-31 (6.73 mg g^{-1}) and CISH A-33 (5.79 mg g^{-1}). Minimum amount of epicatechin was noticed in Kanchan (1.30 mg g^{-1}).

Ellagic acid

Ellagic acid is another important phenolic compound identified in aonla fruits which is being popular because of its anti-cancer activity. The content of ellagic acid varied significantly between 0.19 mg g^{-1} ('NA-7') and 1.93 mg g^{-1} (CISH A-33). Cultivars 'Chakaiya' (1.34 mg g^{-1}), 'Krishna' (1.16 mg g^{-1}) and 'Lakshmi-52' (1.15 mg g^{-1}) were the other good sources of ellagic acid (Table 1). The LSD value for this compound is 0.28 and CV value is 18.88, indicating highly significant variation among the cultivars tested.

Caffeic acid

A significantly higher amount of caffeic acid (8.28 mg g^{-1}) was quantified in 'NA-6' by HPLC analysis (Table 1). 'Desi' aonla contained next best amount of this phenolic acid (4.47 mg g^{-1}), while minimum amount of caffeic acid was detected in 'Kanchan' (0.61 mg g^{-1}). Other cultivars and accessions, which were good source of caffeic acid, were 'NA-7' (1.41 mg g^{-1}), 'Chakaiya' (1.35 mg g^{-1}) and CISH A-31 (1.39 mg g^{-1}). A highly significant difference (LSD = 0.187 and CV = 4.888) was observed between the cultivars and accessions regarding the content of caffeic acid.

***p*-Coumaric acid**

Among the phenolic compounds detected, *p*-coumaric acid was quantified least in aonla cultivars. The content of *p*-coumaric acid ranged between 0.085 mg g^{-1} (minimum in 'Kanchan') to 1.51 mg g^{-1} (maximum in 'Desi') among the cultivars (Table 1). Other cultivars/accessions with good to moderate amount of *p*-coumaric acid were CISH A-31 (0.40 mg g^{-1}), CISH A-33 (0.25 mg g^{-1}) and 'NA-6' (0.24 mg g^{-1}). A significant variation was noticed in the content of *p*-coumaric acid among the cultivars (LSD = 0.033 and CV = 5.681).

Total of selected quantified phenolic compounds

After adding all the individual phenolic compounds detected, it was found that 'Desi' aonla contained maximum amount of total quantified phenolic compounds (58.21 mg g^{-1}), distantly followed by accession CISH A-31 (40.71 mg g^{-1}). Fruits of cultivars 'NA-6', 'Krishna', 'Lakshmi-52' and accession CISH A-33 also contained good amount of total quantified phenolic compounds (35.08, 31.65, 30.97 and 31.85 mg g^{-1}, respectively) (Table 1). Other three cultivars contained less than 20 mg g^{-1} of total quantified phenolic compounds with Kanchan having the minimum amount (12.25 mg g^{-1}). Here also a significant variation (LSD = 2.624 and CV = 5.027) in total quantified phenolic compounds content was recorded between the cultivars.

Multivariate evaluation

Seven aonla cultivars ('Kanchan', 'Krishna', 'NA-6', 'NA-7', 'Chakaiya', 'Lakshmi-52', 'Desi') and two accessions (CISH A-31 and CISH A-33) were evaluated over nine variables as seven phenolic compounds, total of selected quantified phenolic compounds and ascorbic acid through multivariate analysis – principal component analysis (PCA) and canonical discriminant function analysis. Principal component analysis suggested that *p*-coumaric acid (0.96), gallic acid (0.94), total polyphenols (0.91) and ascorbic acid (0.91) had higher contribution in controlling variation through correlation with principal component one (PC1) which accounted for highest (57.8%) variation in 27 × 9 data matrix as observed from highly significant Eigen value (5.20) (Table 2) and also had a significant role in cultivar identification. The result of canonical discriminant function (CDF) analysis revealed five significant functions controlling 99.92 per cent of total variation in data matrix 27 × 9 (Table 3). Regardless of positive or negative values, CDF indicated that 'Desi' and 'NA-6' were the most important cultivars in terms of nutraceuticals composition, followed by 'Krishna', 'Lakshmi-52' and CISH A-31.

Discussion

Ascorbic acid is the most important antioxidant found in aonla fruits. 'Desi' aonla contained maximum amount of ascorbic acid and phenolic compounds in the present investigation. 'Desi' aonla fruits are smaller in size (14–20 g) with high surface area, which might be the reason for the presence of maximum ascorbic acid and total polyphenols in it. Several other literatures have also highlighted high vitamin C content in 'Desi' aonla fruits. Parveen and Khatkar (2015) have reported that 'Desi'' aonla had minimum fruit weight (14.27 g) but maximum ascorbic acid content (3.29 g 100 g^{-1} dry weight basis) among the five varieties studied. Mishra *et al*. (2009) have reported higher ascorbic acid content (560 mg 100 g^{-1}) in 'Desi'/wild aonla variety as compared to 'Chakiya' variety (463 mg 100 g^{-1}). Ascorbic acid content in aonla is varietal as well as location-specific trait which is evident in various literatures. Among the five cultivars grown under semi-arid conditions of Faizabad, UP, India, ascorbic acid varied from 613.64 mg 100 g^{-1} in 'Kanchan' to 730.63 mg 100 g^{-1} in 'NA-7' (Singh *et al*., 2016) which is quite high compared to present findings. High ascorbic acid content in four aonla cultivars grown at Faizabad, UP, India has also been reported by Mishra *et al*. (2018). Similarly, higher ascorbic acid content (>700 mg 100 g^{-1} of pulp) has been recorded in eight aonla cultivars cultivated in Raipur, Chattisgarh, India (Singh *et al*., 2009). Slightly higher amount of ascorbic acid (451.2 to 564.4 mg 100 g^{-1}) was noticed in six aonla varieties grown under Bengaluru, Karnataka, India conditions where fluctuation in temperature during winter season is minimal (Chiranjeevi *et al*., 2018). Kumar *et al*. (2009) have mentioned a significant variation in ascorbic acid content (690.9 to 881.8 mg 100 g^{-1}) among the eight aonla cultivars studied by them. In the present study, two accessions collected from Panna, Madhya Pradesh, India contained >400 mg 100 g^{-1} of ascorbic acid and was found very promising.

Phenolic compounds are another important antioxidant present in aonla fruits. Aonla is very rich in phenolic compounds which also vary from cultivar to cultivar. Even the amount of individual phenolic compounds also varied significantly from cultivar to cultivar. In the present investigation, catechin was found as the most dominant phenolic compound in eight out of nine cultivars followed by epicatechin, chlorogenic acid, and gallic acid. Like ascorbic acid, 'Desi' aonla was also found as a rich source of phenolic compounds with maximum amounts of gallic acid, catechin, epicatechin, *p*-coumaric acid and total polyphenols. Accessions CISH A-31 and CISH A-33 and cultivars 'NA-6', 'Krishna' and 'Lakshmi-52' were also a very good source of phenolic compounds. Parveen *et al*. (2019) have identified four phenolic compounds, *viz*. gallic acid, ellagic acid, ethyl gallate, and quercetin in aonla extract by RP-HPLC at 270 nm. They have also observed significant variations in the amount of individual phenolic compounds among the five aonla varieties where 'Desi' variety contained the highest level of phenolic compounds. Gallic acid and ellagic acid were the most abundant phenolic compounds in aonla varieties as mentioned by the authors. Six phenolic compounds namely geraniin, quercetin 3-β-d-glucopyranoside, kaempferol 3-β-d-glucopyranoside, quercetin, kaempferol and isocorilagin were identified in Chinese emblica fruit by chromatographic and spectroscopic methods (Liu *et al*., 2008). Ethyl gallate, methyl gallate, ellagic acid, quercetin 3-*O*-rhamnoside, isocorilagin, mucic acid-1, 4-lactone-3-*O*-gallate and hamamelitannin were identified in another Chinese emblica fruit using HPLC coupled with mass spectroscopy after microwave-assisted extraction (Li *et al*., 2019). Singh *et al*. (2016) have also noticed a significant variation in total polyphenols content in five aonla varieties grown under semi-arid conditions at Faizabad, India. Kumar and Rao (2010) have identified nine phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic

Table 2. The principal component loading of nine variables (phenolic compounds and ascorbic acid) in fruits of nine aonla cultivars.

Variables	Principal components (PC) with loading of each variable		
	PC1	PC2	PC3
Gallic acid	0.94	0.23	-0.2
Chlorogenic acid	-0.32	0.73	0.53
Catechin	0.78	0.47	0.14
Epicatechin	0.72	0.34	0.42
Caffeic acid	0.56	-0.53	0.45
Ellagic acid	-0.38	0.52	0.13
p-Coumaric acid	0.96	0.02	0.24
Total polyphenols	0.91	0.34	0.23
Ascorbic acid	0.91	0.07	0.38
Eigen value	5.2	1.62	1.01
Per cent of variation	57.8	18.1	11.22
Cumulative per cent	57.8	75.9	87.12

Table 3. The canonical discriminant functions (unstandardized score) evaluated at group means of nine aonla cultivars with nine variables.

Cultivars	Functions at group centroids				
	1	2	3	4	5
Kanchan	-14.55	0.63	-17.44	11.39	-5.28
Krishna	-31.08	-17.40	8.82	2.03	1.02
NA-6	17.97	78.70	12.06	1.01	-0.14
NA-7	-8.05	10.23	-18.71	-15.29	0.32
Chakaiya	-9.22	6.78	-11.69	3.77	2.09
Lakshmi-52	-32.33	-15.87	8.26	5.57	0.32
CISH A-31	-24.17	-20.85	13.92	-8.50	-7.22
CISH A-33	-10.91	-15.61	3.15	-0.77	9.60
Desi	112.34	-26.60	1.63	0.79	-0.71
Per cent of variance	60.97	31.53	4.90	1.85	0.67
Cumulative variance	60.97	92.50	97.40	99.25	99.92
Canonical correlation	0.99	0.99	0.99	0.99	0.98

acid, syringic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, and T-cinnamic acid) by HPLC in juice of seven aonla genotypes at varying proportions. Gallic acid, protocatechuic acid and vanillic acid were the major phenolic acids found in aonla juice. Gallic acid was one of the most significant polyphenols present in aonla juice and spray-dried aonla powder as reported earlier (Bhattacherjee *et al*., 2011b). Bhattacherjee *et al*. (2011a) have identified gallic acid, caffeic acid, kaempferol, chlorogenic acid, catechin, epicatechin and *p*-coumaric acid in aonla juice (cv. 'Chakaiya') by HPLC and reported that gallic acid might play a significant role in browning of juice during storage. The multivariate statistical methods can be used to study fruit crops for understanding the role of morphological characters associated to yield, assessment and evaluation of varieties and understanding of characters playing a significant role in controlling variation as reported in evaluation of variability in jamun varieties (Singh *et al*., 2007), bio-chemical profiling of Indian gooseberry (Pandey *et al*., 2016) and differentiation of wines made from berry and drupe fruits (Čakar *et al*., 2018a).

Conclusion

Seven phenolic compounds were identified and quantified in seven cultivars and two accessions of aonla along with ascorbic acid. 'Desi' aonla was found as the richest source of both ascorbic acid (1278.31 mg 100 g^{-1}) and phenolic compounds (58.21 mg g^{-1} total quantified phenolic compounds) in spite of chlorogenic acid not detected in it. Accessions CISH A-31 and CISH A-33 along with cultivars 'NA-6', 'Krishna' and 'Lakshmi-52' were also a very good source of these two antioxidants. All these aonla cultivars and accessions could be a cheap and readily available source of dietary polyphenols and vitamin C with great antioxidative potential. These four aonla cultivars can be suggested for use in medicinal purposes and rest of the cultivars for processing purposes. Two accessions can be developed as varieties and can also be used for breeding purposes to develop nutraceutically rich varieties.

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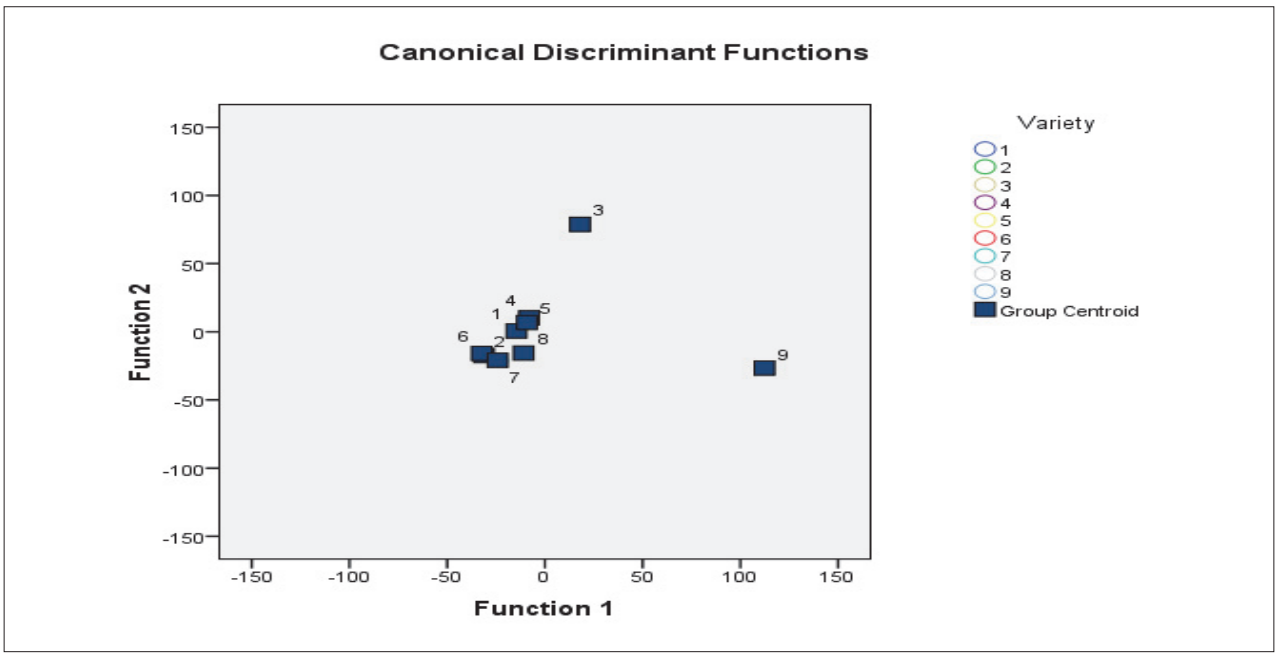

Supplemental Information – Figure S1. Canonical discriminant function (Function-1 and Function-2) for evaluation of nine varieties: 1: 'Kanchan', 2: 'Krishna', 3: 'NA-6', 4: 'NA-7', 5: 'Chakaiya', 6: 'Lakshmi-52', 7: CISH A-31, 8: CISH A-33, 9: 'Desi'. These nine centroids for nine varieties have performance (score) over nine variables as gallic acid, catechin, epicatechin, caffeic acid, ellagic acid, chlorogenic acid, *p*-coumaric acid, total polyphenols and ascorbic acid.