Assessment of total polyphenols, antioxidants and antimicrobial properties of aonla varieties

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Abstract Phenolic content, antioxidant activities and antimicrobial activities of methanolic, ethanolic and ethyl acetate extracts of five different varieties of aonla (*Emblica officinalis*) fruits as well their powders were evaluated. Total polyphenolic content in fresh aonla fruit extracts varied from 70.6 to 159.4 mg GAE/g and their EC 50 (effective concentration) values for antioxidant activity ranged from 46.72 to 359.7 µg/ml. Significant varietal difference were observed in antioxidant activity of the extracts of fresh aonla fruit and powder. Among the variety analyzed, Desi variety exhibited significantly higher TPC (total polyphenol content) and antioxidant activity in fresh as well as dried form in all the extracts. Methanolic extracts of various varieties had maximum TPC and antioxidant activity. Variety NA-7 showed high TPC and antioxidant activity. Almost, similar trend was observed among the extracts of aonla powders for TPC and AOA (antioxidant activity). A high positive correlation coefficient existed between TPC and AOA of different aonla extracts. All the extracts analyzed, exhibited a strong antimicrobial potential against *E. coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans*. This study suggests aonla as potential natural source of antioxidants and antimicrobial agents.

Keywords Polyphenol · Antioxidant property · Aonla · Antimicrobial property

Introduction

Antioxidants are the compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu et al. 1998). Free radicals are reported to be the major cause of some serious pathogenic disorders, such as cardiovascular diseases, atherosclerosis, neurodegenerative disorders, diabetes, cancer, liver cirrhosis, inflammation and cataracts (Aruoma 1998). Due to the presence of the conjugated ring structures with hydroxyl groups, many phenolic compounds by scavenging superoxide anion (Robak and Dryglewski 1988), singlet oxygen (Husain et al. 1987), and lipid peroxy radicals (Torre et al. 1986), and stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species have the potential to function as antioxidants (Shahidi and Wanasudara 1992). Aonla extract reported to have abundant antioxidant and their action prevent LDL oxidation thereby inhibiting endothelial dysfunction and formation of atheromatous plaques (Antony et al. 2006).

Aonla has emerged as excellent nutritional source being a rich source of polyphenols and ascorbic acid which are considered to be responsible for their antioxidant properties. It is believed to increase defense against various diseases viz., diabetes, ulcers, heart troubles, and gastrointestinal disorders. Additionally, it is useful in enhancing memory, lowering cholesterol, combat ophthalmic disorders and antimicrobial agent as well (Khan 2009). Fresh or dry fruit is used in traditional medicines for the treatment of jaundice, diarrhea and inflammations (Deokar 1998).

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Dehydration is considered as important preservation method for the fruits from ancient time, as it reduces the chances of microbial growth and inhibits enzymatic activities (Guine 2008). Antioxidant activity of fresh plant material is higher than their dried form due to degradation during drying process. While, some studies have shown that dried material have higher antioxidant activity and total polyphenols as compared to fresh plant material (Suvarnakuta et al. 2011).

Consumers and food processors have showed a strong desire to reduce the use of synthetic additives for preservation (Liu et al. 2008a, b, c) natural sources including aonla and other fruits exhibiting antioxidants and antibacterial activity can be alternative sources. Therefore, present investigation was planned to evaluate phenolic content, the antioxidant and antimicrobial properties of different aonla extracts and dried powder extracts. Correlation was also assessed between phenolic content and antioxidant activity of different aonla extracts.

Materials and methods

Five aonla (*Emblica officinalis*) varieties namely Banarasi, Chakaiya, Desi, Kanchan and NA-7 were procured at ripened stage from Central State Farm, Hisar.

Methanolic extract

Extraction was done by the method of Liu et al. (2008a, b, c) with some modification. 10 g fresh aonla flesh/2 g aonla powder was extracted with 100 ml methanol in conical flask using shaker at 25 °C and filtered through 0.45 µm filter paper. Residue was extracted twice with methanol. Then combined extract was concentrated at 40 °C in rotary evaporator at low pressure. Residue was lyophilised and stored at 4 °C till further use.

Ethanol extract

Method of Ahmad et al. (1998) was adopted for preparation of extract with some modifications. Two gram powder/10 g fresh aonla fruit was taken in conical flask and 100 ml of 95 % ethanol was used for the extraction. The extract was filtered through 0.45 µm nylon filter paper and filtrate was concentrated in rotary evaporator at 40 °C after that sample was lyophilised and stored at 4 °C until use.

Ethyl acetate extract

Extraction was done using the method of Yokozawa et al. (2007) with modification. Ten gram fresh aonla flesh/2 g

aonla powder was extracted with 100 ml 80 % ethyl acetate in conical flask using shaker at 25 °C and filtered through 0.45 µm filter paper. Residue was extracted twice with 100 ml solvent as mentioned above. Final extract was evaporated at 40 °C using rotary evaporator at low pressure. Concentrate was lyophilised and stored under refrigeration condition till further use.

Total polyphenol content

Total polyphenol was estimated according the method of Anesini et al. (2008). One ml of the extract was taken in test tube and to this 0.5 ml of Folin-Ciocalteu (FC) reagent was added, after 3 min, 1 ml of saturated sodium carbonate solution was added and volume was made 10 ml with distilled water. Absorbance was taken at 760 nm after 30 min. Standard curve was prepared using graded concentration (0–100 ppm) of gallic acid standard and with reference to standard curve concentration of total polyphenols was determined as mg GAE (gallic acid equivalents)/g.

Antioxidant activity by DPPH method

Antioxidant activity of different extracts was assayed by the method of Maizura et al. (2011) with some modifications. One ml of these extracts was diluted to 50 ml with distilled water in volumetric flasks. Then from these solutions 40, 60, 80 and 100 µl and additionally for ethyl acetate extract of fresh aonla varieties 200 and 400 µl sample was taken for analysis. Samples were added from 40 to 120 µl in each test tube containing methanol and 2 ml of 0.1 mM DPPH solution. Mixed thoroughly and kept in dark for 1 h. Absorbance was measured at 517 nm in a UV–Vis spectrophotometer using methanol as blank. From each graded concentration of standard ascorbic acid solution 0.2 ml (50–200 ppm) was taken in test tubes containing methanol and DPPH reagent solution as a positive control. Percentage of DPPH scavenging activity was calculated using the formula.

$$\text{AOA (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Graph was constructed between concentration versus % reduction in absorbance of DPPH by adding ascorbic acid and calculated the IC 50 (Concentration of Ascorbic acid required for 50 % reduction in absorbance). Similarly IC 50 of methanolic solution of sample was determined by adding increased concentration i.e. 40–120 µl in above prepared test tubes containing methanol and DPPH reagent solution.

Antioxidant activity by β -carotene bleaching method

The antioxidant activity of the extracts was determined by β -carotene bleaching method following a modification of the procedure reported by Juntachote and Berghofer (2005). Two mg of β -carotene was dissolved in 20 ml chloroform. Three ml solution was taken into 50 ml beaker and then 40 mg of linoleic acid and 400 mg of tween 20 were added after that chloroform was removed by purging with nitrogen. Hundred ml of oxygenated distilled water, which was prepared by aerating air bubble into distilled water for 1 h was added in above prepared β -carotene emulsion and mixed using vortex mixture. In test tubes each containing 0.12 ml of the extracts 3 ml of above prepared emulsion was added and was mixed thoroughly. Test tubes were immediately placed in water bath at 50 °C. Oxidation of β -carotene emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm. Absorbance was noted at 0, 40 min. A control was prepared by using 0.12 ml of respective solvent like methanol, ethanol and ethyl acetate instead of extract. Degradation rate of the extracts was calculated according to 1st order kinetics.

$$\text{Sample degradation rate} = \frac{\ln a \times 1}{bt}$$

a = initial absorbance at t = 0, b = absorbance at 40 min, t = time in min

$$\text{AOA (\%)} = \frac{\text{Degradation rate of control} - \text{Degradation rate of sample}}{\text{Degradation rate of Control}} \times 100$$

Antimicrobial activity of extract

Antimicrobial activity of the extracts was determined by agar well diffusion method described by Ahmed et al. (1998) with some modifications. Food pathogens: *E. coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans* were obtained from PGI lab, Rohtak. Petriplates containing 20 ml Muller Hinton medium were seeded with culture of bacterial strains for 24 h. Wells were cut and 20 μ l of the extracts (methanol, ethanol and ethyl acetate) was added. Plates were then incubated at 37 °C for 24 h. Antibacterial activity was assayed by measuring diameter of inhibition zone formed around the well. Chloramphenicol was used as a positive control (100 μ g/ml). Percent antimicrobial was calculated as per formula

$$\text{Antimicrobial activity(\%)} = \frac{C - T}{C} \times 100$$

C = inhibition zone of standard, T = inhibition zone of extract

Results and discussion

Total polyphenol content of aonla fruit and powder

Antioxidant properties in aonla fruit exhibit due to the presence of phenolic compounds (Anila and Vijayalakshmi 2002; Sabu and Khuttan 2002; Kumar et al. 2006). In the present study, total polyphenol content of three different extract of five aonla cultivars and their powder were determined. As depicted in Table 1, total polyphenolic content of different aonla varieties fresh fruits ranged from 70.6 to 159.4 mg GAE/g. Significant difference ($p < 0.05$) was observed in total polyphenol content of different varieties. Similarly, total polyphenol content in aonla powders varied from 90.5 to 385 mg/g, showed significant ($p < 0.05$) varietal difference.

Among the varieties, Desi variety was found to have highest polyphenol content followed by variety NA-7, Banarasi and Chakaiya. Similar trend among varieties was also observed for aonla powder. Varietal difference in total polyphenol content could be partially due to the different maturity stages, genetic and agronomic conditions (Hilton and Palmer-Jones 1973; Zheng and Wang 2001). Results of present study showed that solvents act significantly different in extracting polyphenols in aonla varieties. Due to different degree of polarity of solvents and compatibility of compound with the solvents, extraction of total polyphenol varied (Teh et al. 2014; Zhang et al. 2007). Polarity of methanol, ethanol and ethyl acetate is 0.762, 0.654 and 0.228 respectively. Methanol was observed to be efficient solvent for the extraction of phenolic compounds (Table 1). Similarly, study by Chirinos et al. (2007) and Al-Farsi and Lee (2008) reported methanol and ethanol as effective solvents to extract polyphenol linked to polar fibrous matrix. Compatibility of solvent and extracted compound with respect to polarity helps in better and maximum extraction. As given in Table 1, methanol extract of aonla powder had the maximum TPC compared to other solvents. In the study of Chiste et al. (2011) extraction of TPC by ethyl acetate was very less compared to ethanol in annatto. Efficiency of methanol was found more in comparison to ethyl acetate for polyphenol extraction in *Tamarindus indica* L. (Razali et al. 2012). Similar trend was also observed in total phenol in Jambolan fruit extract in study by Singh et al. (2016). Trend of total polyphenol content of aonla powder extract was almost similar among the variety irrespective of solvent used for extraction. Powder of Desi variety showed maximum TPC 385.5, 304 and 188.4 mg/g in methanol, ethanol and in ethyl acetate extract respectively while in variety Chakaiya, TPC was observed lowest i.e. 220.4 mg/g in methanol, 215 mg/g in ethanol and 90.5 mg/g in ethyl

Table 1 Total polyphenols content and antioxidant activities of fresh fruits and powders of different aonla varieties

Solvents	Variety	Total polyphenol content (mg/g)		EC ₅₀ (µg/ml) DPPH		% AOA β-carotene bleaching method	
		Fresh fruit	Powder	Fresh fruit	Powder	Fresh fruit	Powder
Methanol	Desi	159.4 ± 0.2k	385.5 ± 2.1l	46.72 ± 0.8a	10.72 ± 0.7a	75.2 ± 1.2g	79.3 ± 0.7i
	NA-7	156.1 ± 0.3jk	366.9 ± 3.6k	80.1 ± 0.9d	12.93 ± 0.2b	71.8 ± 0.7e	73.9 ± 0.2g
	Banarasi	153.3 ± 0.8j	365.5 ± 2.9k	69.5 ± 0.8c	12.97 ± 0.8b	74.4 ± 1.7g	76.3 ± 0.8h
	Kanchan	133.2 ± 2.1h	287.3 ± 1.2h	113.6 ± 0.2h	12.9 ± 0.6b	69.6 ± 0.3e	68.6 ± 0.6f
	Chakaiya	109.8 ± 1.2e	220.4 ± 2.7f	119.9 ± 0.1i	14.66 ± 0.9c	66.7 ± 0.5d	64.1 ± 0.9e
Ethanol	Desi	139.0 ± 0.6i	304 ± 2.2j	64.18 ± 0.5b	15.8 ± 1.2d	85.6 ± 0.9g	80.9 ± 1.2i
	NA-7	130.8 ± 0.4h	295.4 ± 2.1i	86.8 ± 0.3f	16.1 ± 0.7e	71.9 ± 2.6e	74.3 ± 0.7g
	Banarasi	125.4 ± 6.3g	290.2 ± 0.3hi	83.1 ± 0.2e	16.21 ± 0.7e	70.6 ± 0.9e	74 ± 0.7g
	Kanchan	115.2 ± 4.2f	244.4 ± 1.7g	103.7 ± 0.1g	16.17 ± 0.0e	74.3 ± 0.8f	65.3 ± 0.0e
	Chakaiya	104.1 ± 4.2d	215.0 ± 6.0e	129.1 ± 0.3j	20.78 ± 0.4f	66.0 ± 0.3d	62.3 ± 0.4d
Ethyl acetate	Desi	89.3 ± 0.2c	188.4 ± 1.7d	171.82 ± 0.5k	23.73 ± 0.7g	46.5 ± 0.6c	28.8 ± 0.7c
	NA-7	73.4 ± 2.1ab	163.9 ± 1.1c	359.7 ± 0.8o	27.3 ± 1.6i	40.3 ± 0.4b	24.5 ± 1.6b
	Banarasi	76.8 ± 0.8b	169 ± 1.9c	263.1 ± 0.6l	25.54 ± 0.5h	39.8 ± 0.5b	26.0 ± 0.5b
	Kanchan	73.1 ± 0.5ab	145.8 ± 0.7b	316.45 ± 0.2n	31.08 ± 0.1k	36.9 ± 0.1a	26.6 ± 0.1b
	Chakaiya	70.6 ± 1.9a	90.5 ± 2.1a	277.7 ± 0.4m	27.69 ± 0.0j	35.2 ± 0.4a	22.3 ± 0.0a

The values are mean ± SD of determinations made in duplicates. Mean values followed by different letters within same column differ significantly ($p < 0.05$). EC₅₀: Antioxidant required to reduce DPPH concentration by 50 %

Total polyphenols are expressed as mg GAE (Gallic acid equivalents)/g of material

acetate extract. Total polyphenol content of the extracts was in accordance with the data (81.5–111.1 mg GAE/g) reported by Liu et al. (2008a, b, c) in aonla from six regions of China. Similarly, Mayachiew and Devahastin (2008) reported TPC (130.8 mg/g) in dried sample of aonla.

Antioxidant activity by DPPH method of aonla fruits and powder

It is generalized that most of the diseases are mainly due to homeostatic imbalance in pro-oxidant and anti-oxidant in the body and higher concentration of pro-oxidant may result in tissue injury and subsequent diseases. Dynamic balance of both is required for proper functioning of body (Khan 2009). DPPH assay considered as rapid, economic and widely used method for evaluation of antioxidant activity of different foods using different solvents including methanol, ethanol, water, alcohol, ethyl acetate, benzene (Prakash 2001; Yu 2001; Parry et al. 2005). DPPH is a coloured and stable free radical, get reduced in the presence of antioxidant compound to give yellow colour (Brand-Williams et al. 1995). DPPH test is generally conducted for the evaluation of antioxidant activity of crude extracts (Poli et al. 2003). Owing to wide diversity in chemical nature of antioxidants and it seems unrealistic to isolate these antioxidants and study individually. As depicted in the Table 1, all the varieties of aonla fruit showed appreciable free radical-scavenging activity and data varied significantly ($p > 0.05$). Similarly, data

presented in the Table 1, showed that antioxidant activity among the powders of aonla varieties had significant variation ($p > 0.05$). Among the varieties, Desi variety showed maximum antioxidant activity followed by variety Banarasi, NA-7, Kanchan and Chakaiya. The significantly high activity of variety Desi reflects the higher polyphenolic content of variety. Results indicated that methanol extract of variety Desi possessed the maximum antioxidant activity (EC₅₀ 10.72 µg/ml) while lowest activity was observed in ethyl acetate extract of variety Kanchan (EC₅₀ 31.08 µg/ml). Polarity of the solvent and structure of different phenolic compounds affect the antioxidant activity of samples (Dutta et al. 2012). EC₅₀ value of methanolic extract of dried aonla varied from 11.38 to 45.44 µg/ml selected from six different locations in China (Liu et al. 2008a, b, c). Among varieties and solvents, variety Desi in methanol solvent had the strongest radical scavenging activity (EC₅₀ 46.72 µg/ml) while the lowest value was observed in NA-7 in ethyl acetate (EC₅₀ 359.7 µg/ml), while in powder, ethyl acetate extract was found to have minimum radical scavenging activity (31.08 µg/ml). EC₅₀ value is the concentration of extract required to scavenge 50 % DPPH radical. Similar results were also observed by Dutta et al. (2012) in *Swertia chirata*. This study revealed that solvents of different polarity greatly influence the DPPH radical scavenging activity. Ethyl acetate extract of different parts of *Tamarindus indica* L. showed weaker pattern of radical scavenging capacity compared to methanol extracts (Razali et al. 2012).

Antioxidant activity by β -carotene bleaching method of aonla fruits and powder

Phenolics are responsible for antioxidant properties of various fruits and vegetables (Kaur and Kapoor 2002). Antioxidant activity of different extracts of fresh fruit as well as their powder was also determined by the β -carotene bleaching method based on the oxidation of β -carotene and linoleic acid and results were expressed as percent inhibition relative to the control. Solvent extraction is most common technique conducted for antioxidants extraction, while on the other hand yield and efficacy of the extracted matter strongly depend on solvent polarity and chemical nature of extracted compound (Shabbir et al. 2011).

Various phenolics present in aonla are very effective in preventing auto-oxidation. Redox properties of phenolic compound that summaries free radical scavenging, singlet oxygen quenching and hydrogen donating are basics of antioxidant activity (Mayachiew and Devahastin 2008). Finding by Kaur and Kapoor (2002) showed that total antioxidant activity of ethanolic and aqueous extract of aonla was found to be 86.8 and 84.3 % respectively evaluated by β -carotene bleaching method. Among the varieties, Desi variety showed the highest antioxidant activity followed by NA-7, Banarasi, Kanchan and Chakaiya. Significant varietal difference was observed among the varieties ($p < 0.05$). Antioxidant activity of different extracts of fresh fruit among the varieties varied from 35.2 to 85.6 % (Table 1). Similarly in powder extracts AOA ranged from 22.3 to 80.9 %. Variety Desi in ethanol solvent was found to have the maximum percent antioxidant activity (85.6 %) while the lowest activity was observed in ethyl acetate extract of variety Chakaiya (35.2 %). As depicted from Table 1, in comparison with methanol and ethyl acetate extract ethanol extract exhibited stronger antioxidant activity. Exceptionally in methanol extract less activity was observed compared to ethanol that might be due to better oxidation of β -carotene and linoleic acid by phenolic extracted in ethanol. Similarly, study by Reddy et al. (2005) reported 72 % antioxidant activity in ethanolic extract of aonla powder. Antioxidant activity of powder extracts, are similar to the finding of Mayachiew and Devahastin (2008), who reported activity 86.4 % in ethanolic extract aonla powder.

Effect of drying on total polyphenols and antioxidant activity

In General, drying of various medicinal plants is done under shade and freeze drying is also conducted to retain their quality attributes during processing treatments and their storage is done for long time till further use in product manufacturing (Lin et al. 2011; Sellami et al. 2011; Pinela

et al. 2011). Storage period also significantly affects quality of medicinal plant as it reduces phenolic or other antioxidant compounds and their antioxidant activity (Guimaraes et al. 2011). In present study, effect of cabinet drying was observed on TPC and antioxidant activity of aonla powders extracts. An increase was observed in total polyphenol content as well as in antioxidant activity of dried material compared to fresh fruit. Results reported in present work are in accordance with the data reported by Chang et al. (2006) in tomato after freeze-drying might be due to disruption of cell structure which increased the extraction of phenolic compounds. During heating high temperature deactivates oxidative and hydrolytic enzymes released during cell breakdown and avoid loss of phenolic compound consequently, lead to increase in antioxidant activity. Study by Hossain et al. (2010) reported that fresh sample had lowest TPC and antioxidant activity compared to air-dried, freeze-dried and vacuum oven-dried samples. Chism et al. (1996) has reported that fruits and vegetables have higher phenolic compounds in outer layers and probably, food processes due to cellular breakdown might accelerate the release of bound phenolic compounds. Similar trend of TPC and antioxidant activity in NA-7 and Francis powder was observed higher than their pulp in the study by Kulshreshta et al. (2011). On the contrary, decrease was reported in TPC (37.3 %) and antioxidant activity (38.5 %) for murtilla fruit dried at 65 °C by hot drying method by Alfaro et al. (2014).

Antimicrobial activity

Microbial inhibition zone of aonla fruit and powder

Phenolic present in aonla are credited for antimicrobial activity as these form complex with extracellular and soluble proteins or with bacterial cell walls which cause disruption in membrane of bacteria (Ganguly 2003). In the present study, antimicrobial activity of the different extracts of aonla fruit and powders, extracted with three solvent: methanol, ethanol and ethyl acetate were evaluated for their antimicrobial activity against micro-organisms viz. *E. coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans* using agar well diffusion method with their potency was quantitatively estimated by presence or absence of inhibition zone and by measuring inhibition zone diameter as presented in Tables 2 and 3. Results of present study showed that all of the tested pathogens were sensitive to the different extracts at a specific concentration. Sensitivity of different pathogens was in decreasing order *Salmonella typhi* > *Staphylococcus aureus* > *Candida albicans* > *E. coli* and difference in their sensitivity might be due to variation in the cell wall composition of Gram +ve and Gram –ve bacteria (Grosvenor et al. 1995).

Table 2 Inhibition zone of fresh fruits extracts of different aonla varieties against tested pathogens

Solvents	Variety	Inhibition zone (mm)			
		<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Candida albicans</i>
Methanol	Desi	15.63 ± 0.56j	18.75 ± .050ij	20.93 ± 0.37g	11.36 ± 0.87cde
	NA-7	14.1 ± 0.28i	18.55 ± 0.23i	19.19 ± 0.09f	11.10 ± 0.58cd
	Banarasi	13.61 ± 0.21i	16.60 ± 0.14h	18.22 ± 0.14e	10.27 ± 0.42b
	Kanchan	13.55 ± 0.45i	15.05 ± 0.18g	15.55 ± 0.15c	8.49 ± 0.29a
	Chakaiya	12.35 ± 0.32h	6.90 ± 0.28a	15.00 ± 0.10bc	8.51 ± 0.68a
Ethanol	Desi	11.25 ± 0.42g	21.20 ± 0.21k	28.70 ± 0.40j	27.72 ± 0.21k
	NA-7	10.9 ± 0.40fg	19.05 ± 0.30ij	28.35 ± 0.23j	24.65 ± 0.32j
	Banarasi	8.76 ± 0.61cd	13.41 ± 0.30f	26.25 ± 0.49i	21.75 ± 0.12i
	Kanchan	8.35 ± 0.30bc	12.61 ± 0.28e	23.75 ± 0.21h	20.25 ± 0.53h
	Chakaiya	5.05 ± 0.35a	9.37 ± 0.24b	23.40 ± 0.29h	11.55 ± 0.40e
Ethyl acetate	Desi	10.2 ± 0.30ef	19.23 ± 0.26j	17.20 ± 0.21d	13.69 ± 0.14g
	NA-7	9.45 ± 0.21de	16.25 ± 0.20h	16.75 ± 0.28d	13.65 ± 0.86g
	Banarasi	8.5 ± 0.24bc	15.33 ± 0.15g	15.13 ± 0.23bc	12.54 ± 0.35f
	Kanchan	7.92 ± 0.29bc	11.33 ± 0.30d	14.68 ± 0.17b	11.99 ± 0.29ef
	Chakaiya	7.8 ± 0.10b	9.90 ± 0.14c	13.54 ± 0.15a	10.58 ± 0.41bc

The values are mean ± SD of determinations made in duplicates. Mean values followed by different letters within a same column differ significantly ($p < 0.05$)

Table 3 Inhibition zone of powder extracts of different aonla varieties against tested pathogens

Solvents	Variety	Inhibition zone (mm)			
		<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Candida albicans</i>
Methanol	Desi	13.30 ± 0.46i	20.06 ± 0.67k	27.42 ± 1.04k	14.02 ± 0.49g
	NA-7	11.33 ± 0.10f	15.50 ± 0.45i	14.83 ± 0.54fg	14.02 ± 0.26g
	Banarasi	8.35 ± 0.42d	14.40 ± 0.37h	14.90 ± 0.58g	13.20 ± 0.42g
	Kanchan	8.15 ± 0.78d	11.25 ± 0.14e	12.52 ± 0.76ab	12.35 ± 0.25f
	Chakaiya	7.45 ± 0.23c	12.55 ± 0.24f	14.27 ± 0.32efg	10.38 ± 0.18e
Ethanol	Desi	12.76 ± 0.35gh	20.01 ± 0.12k	18.01 ± 0.14h	17.90 ± 0.24h
	NA-7	12.35 ± 0.67g	19.25 ± 0.48j	14.48 ± 0.54fg	13.98 ± 0.44g
	Banarasi	9.35 ± 0.49e	14.40 ± 0.21h	14.00 ± 0.12def	13.75 ± 0.77g
	Kanchan	8.75 ± 0.08de	13.60 ± 0.32g	12.84 ± 0.32bc	10.15 ± 0.35de
	Chakaiya	8.10 ± 0.29d	7.65 ± 0.14c	13.62 ± 0.24cde	10.35 ± 0.20e
Ethyl acetate	Desi	5.85 ± 0.36b	8.47 ± 0.18d	21.38 ± 0.34j	9.51 ± 0.67d
	NA-7	5.22 ± 0.57a	7.30 ± 1.03c	19.14 ± 0.24i	7.30 ± 0.28c
	Banarasi	5.15 ± 0.81a	7.70 ± 0.58c	18.82 ± 0.69i	6.81 ± 0.36bc
	Kanchan	5.05 ± 0.21a	5.85 ± 0.28a	11.81 ± 0.29a	4.90 ± 0.45a
	Chakaiya	4.85 ± 0.34a	6.50 ± 0.23b	13.38 ± 1.50cd	6.15 ± 0.24b

The values are mean ± SD of determinations made in duplicates. Mean values followed by different letters within a same column differ significantly ($p < 0.05$)

As depicted in Tables 2 and 3, a significant varietal difference ($p < 0.05$) was observed in inhibition zone (mm) against all the tested pathogens. Significant difference ($p < 0.05$) in inhibition zone against all the pathogens was also noted among the different extracts of all the varieties. Methanol extract of Desi variety showed maximum

inhibition zone (15.63 mm) against *Salmonella typhi* while ethanolic extract of variety Chakaiya showed minimum inhibition zone (5.05 mm). Ethanolic extract of variety Desi was observed to have highest inhibition zone against *Staphylococcus aureus* (21.20 mm), *E. coli* (28.70 mm) and *Candida albicans* (27.72 mm) while the methanolic

extract of variety Chakaiya was found to have minimum inhibition zone against pathogen *Staphylococcus aureus* (6.90 mm) and its ethyl acetate extract showed lowest inhibition zone against *E. coli* (13.54 mm).

As presented in Table 2, methanolic extract of variety Kanchan had minimum inhibition against *Candida albicans* (8.49 mm). As depicted from Table 3 methanol extract of variety Desi showed maximum inhibition zone (13.30, 20.06 and 27.42 mm) against *Salmonella typhi*, *Staphylococcus aureus* and *E. coli* respectively while the ethanol extract of same variety showed highest inhibition (17.90 mm) against *Candida albicans*. Ethyl acetate extract of variety Chakaiya was found to have minimum inhibition zone (5.05 mm) against *Salmonella typhi* and ethyl acetate extract of variety Kanchan showed lowest inhibitory zone 5.85, 11.81 and 4.90 mm against *Staphylococcus aureus*, *E. coli* and *Candida albicans* respectively. *E. coli* was the most sensitive out of four test pathogens on the other hand *S. typhi* was found to be least sensitive. Among the solvents, ethanol extract was found most inhibitory against all the tested pathogens except *S. typhi* where methanol extracts were most inhibitory. Plant extracts generally have shown high antimicrobial activity against Gram +ve bacteria compared to Gram –ve bacteria and that might be due to difference in cell wall structure of bacteria. Chemical composition of cell wall is more complex in Gram –ve bacteria compared to Gram +ve bacteria (Agoramoorthy et al. 2007; Fyhrquist et al. 2004; Stainer et al. 1987). Almost same trend was observed in the present study among the solvents. The difference in antimicrobial potency might be due to different method of extraction, solvent types and sensitivity of tested pathogens (Nimri et al. 1999). In the study by Patil et al. (2012) extracts of aonla fruit were evaluated for antimicrobial activity against four pathogens viz. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pasteurella multocida* belonging to both Gram +ve and Gram –ve bacteria. Methanolic extract showed antibacterial activity against *S. aureus* while the aqueous extract showed highest activity against *K. pneumoniae* and the acetone extract was most effective against *E. coli* and *P. multocida*. Results of present finding were in accordance with results of Saeed and Tariq, (2007) study reported that aonla was active against microbes including *Staphylococcus aureus*, *S. haemolyticus*, *S. saprophyticus*, *Micrococcus varians*, *Bacillus subtilis* and *Candida albicans*. Bioactive compound viz. flavonoids, phenols, saponins, tannins present in aonla fruit extract are responsible for their potent antibacterial activity (Javale and Sabnis 2010). Methanolic extract of aonla was reported to be most inhibitory against *Staphylococcus aureus*, *E. coli*, and *Candida species* compared to ethyl acetate and aqueous extract (Vijayalakshmi et al. 2007). Flavonoids in aonla fruits inhibited RNA synthesis in microbes and inhibiting process could be attributed to the presence of B-ring of

flavonoids that play a role in intercalation or hydrogen bonding with the stacking of nucleic acid bases as well as Emblicanin A, B and their derivatives play important role in vitro antimicrobial activity (Tim and Andrew 2005). Study by Nain et al. (2012) reported that crude extract of aonla leaves exhibited highest percentage inhibition against *E. coli* (70.53 %) and lowest percentage against *Salmonella typhi* (33.58 %) among tested pathogen (*Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*). Ethanolic extract of aonla fruit showed inhibition zone (21.87 mm) against *S. aureus* and their MIC and MBC values were 13.97 mg/ml (Mayachiew and Devahastin 2008).

Antimicrobial activity of aonla fruit and powder

Percent antimicrobial activity of methanolic, ethanolic and ethyl acetate extracts of fresh aonla and their powder was calculated using inhibition zone of extracts and standard Chloramphenicol and data pertaining to this is presented in Tables 4 and 5. A varietal significant ($p < 0.05$) difference was observed among percent antimicrobial activity of different aonla varieties. In present study, inhibition zone of standard for *Salmonella typhi*, and *Candida albicans* reported were 33.8, 10.9, 10.8 and 22.7 mm, respectively. Among the varieties strongest antimicrobial potential was observed in variety Desi irrespective of extracts followed by variety NA-7, Banarasi, Kanchan and Chakaiya. Difference in potential activity might be due to difference in sensitivity towards pathogens (Nimri et al. 1999). Antimicrobial activity for methanol extract of Desi variety was found maximum (4.30 %) while activity was observed least in ethanol extract of Chakaiya (1.42 %) against *S. typhi*. On the other hand, antimicrobial activity of ethanolic extract of variety Desi showed maximum activity 5.88, 2.55 and 3.66 % against *S. aureus*, *E. coli* and *C. albicans* respectively and methanolic extract of variety Chakaiya have minimum activity against *S. aureus* (1.91 %) and *C. albicans* (1.12 %). Ethyl acetate extract of variety Chakaiya showed lowest antibacterial activity (1.20 %) against *E. coli*. While, in aonla powder, the highest antimicrobial potential was exhibited by methanolic (3.65, 5.56, 2.43 and 1.83 %) and ethanolic extracts (3.51, 5.56, 1.60 and 2.35 %) of Desi variety against *Salmonella typhi*, *Staphylococcus aureus*, *E. coli* and *Candida albicans* respectively. The reason for higher antibacterial activity of methanol extract might be stronger extraction capacity of active compound accounting for antibacterial potential (Ghosh et al. 2008). The results are in accordance with the study by Ghosh et al. (2008), who reported highest antibacterial activity of methanolic extract of *Terminallia chebulla* compared to hot aqueous extract of respective plant. Overall percent inhibition of different aonla extracts

Table 4 Antimicrobial activity of fresh extracts from different aonla varieties

Solvents	Variety	Antimicrobial activity (%)			
		<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Candida albicans</i>
Methanol	Desi	4.30 ± 0.34j	5.22 ± 0.07ij	1.85 ± 0.09g	1.50 ± 0.09c
	NA-7	3.91 ± 0.22i	5.16 ± 0.21i	1.70 ± 0.21f	1.46 ± 0.07bc
	Banarasi	3.75 ± 0.21i	4.57 ± 0.05h	1.61 ± 0.06e	1.36 ± 0.07b
	Kanchan	3.74 ± 0.14i	4.16 ± 0.23g	1.38 ± 0.17c	1.13 ± 0.13a
	Chakaiya	3.35 ± 0.23h	1.91 ± 0.1a	1.33 ± 0.12bc	1.12 ± 0.12a
Ethanol	Desi	3.10 ± 0.08g	5.88 ± 0.07k	2.55 ± 0.10j	3.66 ± 0.05i
	NA-7	2.99 ± 0.12fg	5.29 ± 0.04ij	2.51 ± 0.13j	3.25 ± 0.08h
	Banarasi	2.41 ± 0.16cd	3.72 ± 0.04f	2.33 ± 0.07i	2.86 ± 0.12g
	Kanchan	2.27 ± 0.1bc	3.49 ± 0.12e	2.10 ± 0.03h	2.66 ± 0.15f
	Chakaiya	1.42 ± 0.06a	2.60 ± 0.08b	2.07 ± 0.09h	1.53 ± 0.10c
Ethyl acetate	Desi	2.81 ± 0.31ef	5.34 ± 0.16j	1.52 ± 0.05d	1.81 ± 0.05e
	NA-7	2.60 ± 0.16de	4.52 ± 0.13h	1.48 ± 0.07d	1.80 ± 0.13e
	Banarasi	2.33 ± 0.13bc	4.26 ± 0.06g	1.34 ± 0.07bc	1.65 ± 0.15d
	Kanchan	2.17 ± 0.43bc	3.14 ± 0.02d	1.30 ± 0.12b	1.57 ± 0.07cd
	Chakaiya	2.14 ± 0.09b	2.74 ± 0.11c	1.20 ± 0.14a	1.39 ± 0.21b

The values are mean ± SD of determinations made in duplicates. Mean values followed by different letters within a same column differ significantly ($p < 0.05$)

Table 5 Antimicrobial activity of powder extracts from different aonla varieties

Solvents	Variety	Antimicrobial activity (%)			
		<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Candida albicans</i>
Methanol	Desi	3.65 ± 0.08h	5.56 ± 0.21k	2.43 ± 0.05i	1.83 ± 0.07f
	NA-7	3.11 ± 0.14f	4.30 ± 0.07i	1.31 ± 0.08e	1.77 ± 0.10f
	Banarasi	2.28 ± 0.12d	3.98 ± 0.16h	1.32 ± 0.14e	1.74 ± 0.09f
	Kanchan	2.24 ± 0.09d	3.10 ± 0.09e	1.11 ± 0.08ab	1.63 ± 0.11e
	Chakaiya	1.95 ± 0.08c	3.48 ± 0.18f	1.26 ± 0.04de	1.37 ± 0.06d
Ethanol	Desi	3.51 ± 0.16gh	5.56 ± 0.04k	1.60 ± 0.03f	2.35 ± 0.08g
	NA-7	3.39 ± 0.09g	5.34 ± 0.12j	1.28 ± 0.12de	1.84 ± 0.04f
	Banarasi	2.58 ± 0.10e	3.98 ± 0.07h	1.24 ± 0.09de	1.81 ± 0.11f
	Kanchan	2.40 ± 0.21d	3.76 ± 0.05g	1.14 ± 0.06bc	1.34 ± 0.06d
	Chakaiya	2.23 ± 0.28d	2.03 ± 0.09c	1.20 ± 0.05cd	1.37 ± 0.09d
Ethyl acetate	Desi	1.61 ± 0.09b	2.35 ± 0.21d	1.89 ± 0.07h	1.26 ± 0.07d
	NA-7	1.43 ± 0.06a	2.02 ± 0.12c	1.70 ± 0.11g	0.96 ± 0.10c
	Banarasi	1.40 ± 0.21a	2.01 ± 0.08c	1.66 ± 0.06fg	0.90 ± 0.17bc
	Kanchan	1.39 ± 0.07a	1.60 ± 0.17a	1.04 ± 0.10a	0.63 ± 0.09a
	Chakaiya	1.33 ± 0.23a	1.79 ± 0.16b	1.14 ± 0.12bc	0.81 ± 0.12b

The values are mean ± SD of determinations made in duplicates. Mean values followed by different letters within a same column differ significantly ($p < 0.05$)

is shown in Tables 4 and 5. Results of the present study showed that sensitivity in terms of % antimicrobial activity of tested pathogens was in decreasing order *S. aureus* > *S. typhi* > *C. albicans* > *E. coli*.

The results of present finding are in agreement with study of Scalbert (1991), who reported that methanol extract showed weak antimicrobial activity against *S. aureus* that

might be due to the presence of tannins, isocorilagin and geraniin. *S. aureus* was found to be most inhibited pathogens by all the extracts of aonla powders (Table 5). No correlation was found between antibiotic resistance of pathogenic strains (*Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans* and *E. coli*) and susceptibility of strains with aonla extracts. Results of present finding are similar to data

reported by Ahmad and Beg (2001) in the study of antimicrobial properties of various medicinal plants. Tannins produced toxicity in microbes by inhibiting extracellular microbial enzyme, deprvation of the substrate required for microbial growth or by action on their metabolism through inhibition of oxidative phosphorylation. Potent antimicrobial activity of aonla was reported against *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *S. paratyphi* A, *S. paratyphi* B and *Serratia marcescens* by Saeed and Tariq (2007). In the study by Javale and Sabins (2010) methanol extract of aonla fruit was found more effective against *S. aureus*, *E. coli* and *Klebsiella pneumoniae* compared to methanolic leaf extract. Results of present study were in accordance with findings of Sukanya et al. (2013) who reported that ethyl acetate extract of leaf and bark extract showed highest antifungal activity while methanolic extract have the lowest activity against *Rhizomucor* species. Promising antibacterial activity of aonla extracts was exhibited due to the presence of alkaloids, cardiac glucosides, saponins, tannins terepenoids and flavonoids.

Correlation analyses

Correlation between phenolic content and antioxidant activities of the extracts of five different varieties was calculated using Pearson's correlation analyses. Results showed high positive correlations of phenolic content with DPPH radical-scavenging capacities ($r = 0.922$; $p < 0.01$) and β -carotene bleaching method ($r = 0.900$; $p < 0.01$) in fresh aonla fruits. Similarly, in aonla powder positive correlation was observed in phenol content and antioxidant activity for DPPH method ($r = 0.936$; $p < 0.01$) and β -carotene bleaching method ($r = 0.895$; $p < 0.01$). Results observed showed the probability that the antioxidant activities were mainly contributed by the phenolic compounds in the extracts. In various studies positive correlations was demonstrated between phenolic content and antioxidant activities of plants (Xu et al. 2014; Anesini et al. 2008; Velioglu et al. 1998; Cai et al. 2004; Kaur and Kapoor 2002; Razab and Abdul Aziz 2010), and results from the present study further strengthen the impact of phenolics as potent antioxidants.

Conclusion

Finding of present study concluded that aonla is a potential source of natural antioxidants and antimicrobial agent. Among the varieties, Desi variety had the highest total polyphenolic content, antioxidant activity and antimicrobial activity. Overall, methanol was the most effective solvent for extraction of antioxidant phenolics from fresh

aonla and its powders while ethanolic and ethyl acetate extracts were potent antibacterial and antifungal agents, respectively. All the extracts of fresh aonla and their powder possessed potent antimicrobial property against tested pathogens. Methanolic extract was found most effective against *S. typhi*, while the ethyl acetate extract was found to have strong antifungal activity against *C. albicans*. The high antioxidant potency of aonla implies it's potential as an alternative source of natural antioxidants. Further studies are needed for isolation of individual phenolic compounds responsible for antioxidant and antimicrobial properties. In vivo studies are also required to further confirm if the observed in vitro activities can be replicated in vivo.

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