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In vitro free radical scavenging activity of aonla (*Emblica officinalis*) varieties at various stages of fruit development

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Abstract

An investigation was undertaken to assess the free radical scavenging activity of aonla (*Emblica officinalis*) varieties *viz*., BSR-1, Chakaiya, Krishna and NA-7 at various stages of fruit development *viz*., initial stage, one-fourth maturity stage, half maturity stage, three-fourth maturity stage and full maturity stage using DPPH assay to identify the variety and stage of fruit development for maximum antioxidant activity. The experimental DPPH assay revealed that the free radical scavenging activity was significantly different among the aonla varieties and also at various stages of fruit development in each variety. It was also found that the DPPH free radical scavenging activities of fresh aonla fruit extracts were found to be significantly higher ($P<0.05$) than the radical scavenging activity of the standard ascorbic acid at varying concentrations. The pattern of total soluble sugars accumulation and free radical scavenging activity at various stages of fruit development in each aonla variety studied were discussed in detail.

Key words: Aonla, free radical scavenging, DPPH, ascorbic acid, total soluble sugars.

Introduction

Oxidative stress has been identified to be the root cause of several chronic degenerative diseases, which occurs as a condition when the formed free radicals are not neutralized within our body system. Studies have proved that free radicals play an important role in pathogenesis of chronic degenerative diseases including cancer, diabetes, autoimmunity, inflammatory, cardiovascular, neurodegenerative diseases and aging (Cantuti-Castelvetri *et al.*, 2000; Surh *et al.*, 2001; Vaya and Aviram 2001; Aruoma, 2003). Antioxidants are known to break the free radical chain reaction and scavenge the free radicals. A great interest has been recently focused on the natural foods, medicinal plants and phyto-constituents due to their well-known abilities to scavenge free radicals (Kukic *et al.*, 2006; Galvez *et al.*, 2005; Uma Nath and Deepak, 2009; Rezaeizadeh *et al.*, 2011).

Aonla **(***Emblica officinalis)* fruit of Euphorbiaceae family often referred to as Indian gooseberry, is one of the richest known sources of vitamin C. The fruits are reported to play an important role in scavenging free radicals (Tewari *et al.*, 1982). High antioxidant activity of the aonla fruit was mainly due to the presence of ascorbic acid in higher percentage (Scartezzini *et al.*, 2006; Ebrahimzadeh *et al.*, 2011). Aonla fruits are a key constituent of many herbal preparations such as *chyvanprash* and *triphala*. The fruits have also been used in traditional medicinal systems, such as Chinese herbal medicine, Tibetan medicine and Indian medicine (Zhang *et al.*, 2000) for centuries. The fruits of aonla are reported to contain hydrolysable tannins like emblicanin-A and emblicanin-B, along with pedunculagin and punigluconin (Ghosal *et al.*, 1996). Recent research and clinical studies indicate that it is these tannoid agents, especially emblicanin-A and emblicanin-B that are responsible for aonla's effectiveness as an antioxidant, anti-diabetic and anti-hyperlipidemic activities (Raghu *et al.*, 2007).

Barring the discovery of ascorbic acid and the presence of large amount of tannins in aonla fuits, there does not seem to have any work done in line to assess its free radical scavenging activity in predominant Indian varieties and at various stages of its fruit development. There are many methods to evaluate the free radical scavenging activity of the tested compounds (Paulova *et al.*, 2004). One of the widely used detection procedures, which facilitate analysis of various antioxidants, is based on 2, 2'-diphenyl-1- picryl hydrazyl radical (DPPH) bleaching (Bondet *et al.*, 1997). Hence the present study was carried out to evaluate the *in vitro* DPPH free radical scavenging activity of the fresh fruit extract collected at various stages of fruit development in the leading varieties of aonla.

Materials and methods

The investigation was carried out at the Micro analytical laboratory of Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Source of aonla fruits: Aonla fruits of five varieties (V), *viz*., BSR-1 (V_1), Chakaiya (V_2), Kanchan (V_3), Krishana (V_4) and NA-7 (V_5) were freshly collected at five different stages of fruit development (S) *viz*., initial stage (S_1), one-fourth maturity stage (S_2), half maturity stage (S_3), three-fourth maturity stage (S_4) and full maturity stage (S_5) from 10 years old aonla trees grown in the orchard of Horticultural College and Research Institute, Coimbatore during July to November.

Chemicals: DPPH (1,1-diphenyl-2-picrylhydrazyl) from M/s. Sigma-Aldrich Chemicals, Bangalore; Ascorbic acid standard from M/s. Merck, Mumbai and analytical grade methanol were used in this study.

Preparation of aonla fruit extracts: 5 g of the fresh aonla fruit was weighed and macerated with 15 mL of methanol using pestle and mortar, which was then centrifuged at 3,000 rpm for

10 min. The supernatant containing the fruit extract was freshly prepared every time in this way for carrying out the experiment immediately after its collection from the orchard.

Preparation of ascorbic acid: 1 mg of the ascorbic acid was dissolved in 1 mL of distilled water and different concentrations of ascorbic acid ranging from 10 µg to 200 µg were prepared accordingly.

Experimental design: The experiment was carried out in Factorial Completely Randomized Design (FCRD) with two factors of Aonla variety (V) and various stages of fruit development (S). The numbers of days taken for fruit development in each stage of aonla with regard to its varieties are depicted in Fig.1.

Determination of total soluble sugars (TSS): A hand held optical refractometer was used to measure the total soluble sugars of aonla varieties at various stages of fruit development.

Evaluation of free radical scavenging activity using DPPH assay: The effect of aonla fruit extracts of major Indian varieties at various stages of fruit development on the DPPH free radical scavenging activity was assayed as per the method described by Hou *et al.* (2001). The scavenging of DPPH free radicals were monitored by recording the decrease in absorbance at 517 nm, which occurs due to the reaction with a radical species or reduction by the antioxidant. Methanol was used as the blank. 1 mL of distilled water along with 2 mL of DPPH served as the control. In this experiment, 50 µL of aonla fruit extracts of five varieties obtained at various stages of fruit development were taken in different test tubes. To this, 950 µL of distilled water and 2 mL of DPPH were added, mixed well and incubated for 20 min at 37 ºC. Absorbance of the reaction mixture was recorded at 517 nm using spectrophotometer (SL159- Elico, UV-VIS). Ascorbic acid was used as the reference compound. The percentage scavenging (or) inhibition was calculated according to the formula,

Percentage scavenging (or) Inhibition = (C- T / C) × 100

Where, C is the absorbance of control and T is the absorbance of test. The experiment was replicated thrice and the data were analysed statistically as per procedure developed by Panse and Sukatme (1985).

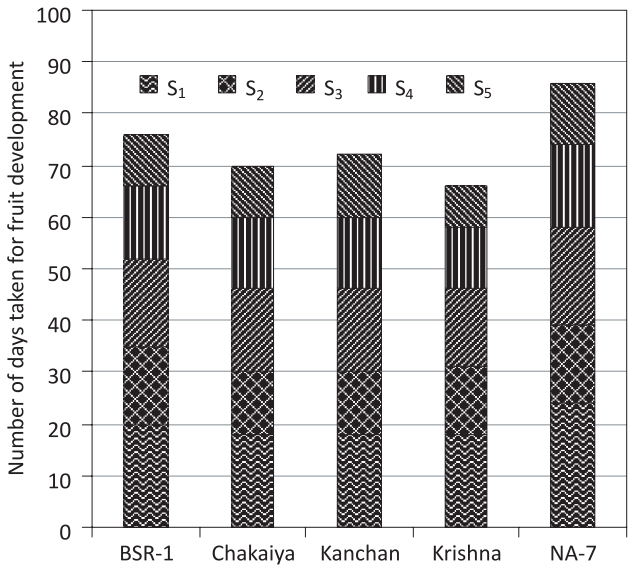

Fig. 1. Aonla varieties and number of days taken for fruit development in each stage. S indicates the various stages of fruit development in the investigated five aonla varieties.

Table 1. Comparison of free radical scavenging activity (%) of aonla varieties (V) at various stages of fruit development (S)

Variety	Free radical scavenging activity (%)					
	S_1	S_2	S_3	S_4	S_5	V-Mean
BSR-1 (V_1)	99.92	99.97	99.79	94.74	99.88	98.86
Chakaiya (V_2)	94.31	94.31	94.31	93.31	91.41	93.53
Kanchan (V_3)	80.88	84.09	84.66	79.48	85.52	82.92
Krishna (V_4)	99.75	99.79	99.89	99.63	99.87	99.78
NA-7 (V_5)	92.11	96.90	96.93	96.29	96.12	95.87
S- Mean	93.39	95.01	95.11	92.69	94.56	

The SE(d) values of V(0.18), S (0.18) and VS(0.40); Similarly the CD (5 %) values of V(0.36), S(0.36) and VS (0.82).

Table 2. DPPH radical scavenging activity (%) of ascorbic acid

Concentration of ascorbic acid (µg)	Free radical scavenging activity (%)
10	20.4±0.4
20	39.8±0.2
40	86.7±0.5
60	92.4±0.1
80	95.7±0.2
100	96.2±0.4
120	96.5±0.3
140	96.6±0.1
160	96.4±0.2
200	96.4±0.2

The values are mean ± SD of the triplicates

Results and discussion

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as a model system to investigate the free radical scavenging activities in several plant extracts. Nitrogen-centred, stable DPPH free radical produces violet colour in methanol solution and gets reduced to a yellow coloured product namely diphenyl picryl hydrazine, on addition of aonla fruit extracts. The experimental results recorded a significant difference (P<0.05) in DPPH free radical scavenging activity among the aonla varieties investigated at various stages of fruit development (Table 1). A difference in DPPH free radical scavenging activity at varying concentration of the standard ascorbic acid (Table 2) was also observed. Among the varieties investigated, variety Krishna recorded significant (P<0.05) DPPH free radical scavenging activity (99.78%) followed by the varieties BSR-1 (98.66%), NA-7 (95.87%), Chakaiya (93.53%) and Kanchan (82.92%). With regard to the stages of fruit development, stages S_2 (95.01%) and S_3 (95.11%) were on par in recording high free radical scavenging activity followed by stages S_5 (94.56%), S_1 (93.39%) and S_4 (92.69%) respectively. Concerning the interaction between aonla varieties and the stages of fruit development, treatments V_1S_1 (99.92 %), V_1S_2 (99.97 %), V_1S_3 (99.79%), V_1S_5 (99.88%), V_4S_1 (99.75%), V_4S_2 (99.79%), V_4S_3 (99.89%) and V_4S_5 (99.87%) were found to be on par in recording high radical scavenging activity, while treatments V_3S_1 (80.88%) and V_3S_4 (79.48%) recorded the least radical scavenging activity.

In the variety BSR-1, radical scavenging activity was found to

be the highest during one-fourth stage of fruit maturity when compared to other stages of fruit development. This may be due to the presence of anthocyanins at early stages of fruit development, which was clearly evident from the dark red pigmentation present on the fruit surfaces and its disappearance at full maturity stage, wherein its activity decreases. This was in agreement with the finding that the concentration of pro-anthocyanins and total flavonols were the highest in early fruit development stages in the craneberry varieties, "Ben Lear" and 'Stevens", during which their antioxidant activity was comparatively higher than the other stages (Vedenskaya and Vorsa, 2004).

Variety Krishna exhibited a significant consistency in its radical scavenging activity at all stages of fruit development compared to other investigated varieties. This may be due to a steady increase in TSS accumulation over the stages of fruit development, which in turn might have attributed to its consistancy in free radical scavenging activity.

Radical scavenging activity gradually increased from initial stage to three-fourth maturity stage of fruit development, where it reached its peak activity and thereafter comparatively decreased at full maturity stage in the variety NA-7. A gradual increase in radical scavenging activity from initial to three-fourth stage of fruit development might have been contributed by a gradual increase in TSS accumulation in the fruit during these stages.

In case of the variety Chakaiya, radical scavenging activity was on par from initial to half maturity stage of fruit development and then started to decrease gradually till it reached the full maturity stage. Amount of polyphenols and tannins accumulated during the early stages of fruit development might be higher, which was evidenced from its astringent taste. Astringency is an indicator for the presence of gallic acid and phyllembellin in higher levels, thereby its potency as free radical scavenger. The early fruit stage radical scavenging activities may be attributed to the "astringency" factor as a result of bitter principles accumulation. At later stages of fruit development, an increase in water content during TSS accumulation could have resulted in dilution of the accumulated phenolic substances, which might have reduced its radical scavenging activity.

Among the varieties investigated, the least radical scavenging activity was observed in variety Kanchan, though its higher free radical scavenging activity was recorded at full maturity stage of fruit development. An average TSS content of 8.90^0 brix, accumulated over the stages of fruit development in the variety Kanchan was found to be relatively less when compared to the other varieties. In this regard, TSS was also found to play a very important role in ascorbic acid and polyphenol accumulations, which in turn might have affected its free radical scavenging activity. At full maturity stage, TSS accumulation in variety Kanchan was found to be comparatively higher effecting its higher radical scavenging activity.

It is evident from Fig. 2 that TSS accumulation was highest in the variety BSR-1 followed by the varieties, Krishna, NA-7, Chakaiya and Kanchan. Peak TSS accumulation for each variety differed with regard to its stage of fruit development *viz*., BSR-1 (one-fourth maturity), Chakaiya (initial to half maturity), Kanchan (full maturity), Krishna (half maturity) and NA-7 (full

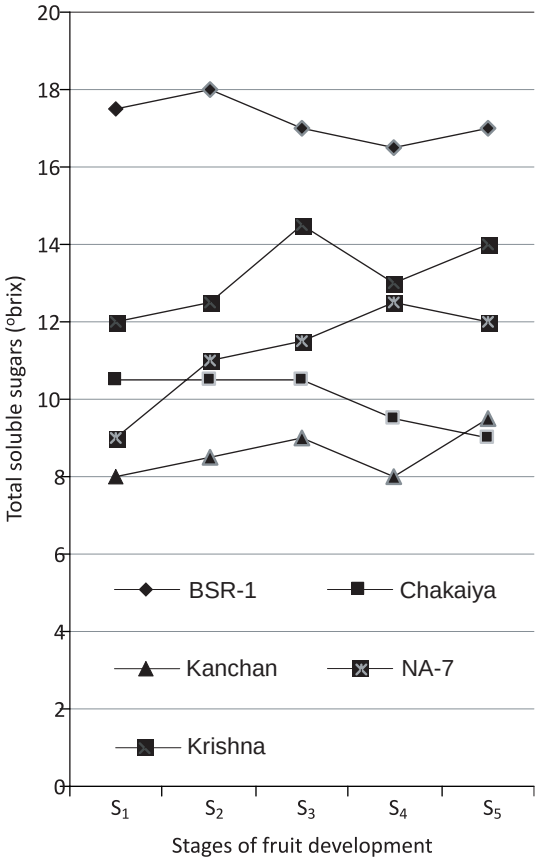

Fig. 2. Comparison of total soluble sugars accumulation in aonla varieties at various stages of fruit development. Data shown are statistically analyzed at 5 % critical difference.

maturity). In all the aonla varieties investigated, a significant decrease ($P<0.05$) in TSS accumulation was noticed in the three-fourth stage of maturity. In general, active sugar transport by means of substrate modification takes place during fruit development transitional stage. In case of aonla, this process might have taken place at three-fourth maturity stage of fruit development, wherein the substrate was chemically phosphorylated during the transport.

While comparing the DPPH free radical scavenging activity of the standard ascorbic acid at varying concentrations with that of the aonla fresh fruit extract obtained from five varieties at various stages of fruit development, it was clearly evident from Table 1 and Table 2 that the overall free radical scavenging activity of aonla was found to be higher than the standard (ascorbic acid) used in this experiment. There are some *in vitro* studies indicating that the antioxidant activities of aonla cannot be attributed to ascorbic acid alone and that the overall effect was also due to the presence of other polyphenols such as ellagic acid, gallic acid, tannins (Kabasakalis *et al.*, 2000; Kim *et al.*, 2005). It has also been found that some compounds in their natural formulations are more active than in their isolated form (Chrousos and Gold, 1992). This was found to be in accordance with the findings of the experiment, while investigating DPPH free radical scavenging activity of predominant aonla varieties at various stages of fruit development.

In aonla, a significant difference in DPPH free radical scavenging activity among the varieties and at various stages of fruit development in each investigated variety was recorded. In

addition, the free radical scavenging activity of fresh fruit aonla extract was found relatively higher than the standard ascorbic acid at varying concentrations. The findings from this investigation could be commercially exploited in choosing the aonla varieties and harvesting fruits at the right stage of fruit development towards formulating natural antioxidant products from aonla fruits effecting high free radical scavenging activity. Future research may be directed towards the study of availability of antioxidants in the aonla varieties subjected to various processing methods.

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