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Antioxidant Potential of Pomegranate (*Punica granatum* L.) Cultivars Grown in Sri Lanka

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ABSTRACT. *The present study was carried out to determine the total phenolic content (TPC) and antioxidant potential of extracts obtained from aril of Sri Lankan cultivars Nayana, Daya, Nimali and Indian cultivar of pomegranate (Punica granatum L.). Total phenolic content was determined using Folin-Ciocalteu colorimetric method and expressed as mg gallic acid equivalents (GAE) per L of extract. Antioxidant potential of the fruit extracts was measured using DPPH radical scavenging and FRAP assays and β-carotene/linoleate model system. The total antioxidant capacity (TAC) was measured using ABTS radical. The TPC varied widely among cultivars from 1199 mg GAE/L of extract in Indian cultivar to 2390 mg GAE/L of extract in Daya. The Daya cultivar possessed the highest TPC, followed by Nayana, Nimali, and Indian cultivars. Despite the moderate TPC, Nayana showed the highest antioxidant potential as determined by all four methods (IC_{50} value of 0.182 mg/mL, TAC of 93%, FRAP of 6.33 mol Fe (II)/L and 91% inhibition of oxidation of β-carotene). The TPC and antioxidant activity were not well correlated* ($0.21 \leq r \leq 0.68$). *The poor correlation may be attributable to the differential behaviour of phenolic constituents. Results revealed that the pomegranate can be categorized as a fruit with extremely high antioxidant potential.*

Keywords*: Antioxidants, IC_{50} value, pomegranate (Punica granatum L.), total antioxidant capacity, total phenolic content*

INTRODUCTION

Epidemiological studies indicate that frequent consumption of fruits and vegetables is associated with low risk of chronic diseases such as diabetes, cardiovascular diseases and cancers (Kriengask *et al*., 2006, Onder *et al*., 2009 and Safaa, *et al*., *2010*). The increased intake of natural antioxidants, particularly the antioxidative compounds present in fruits and vegetables contributes to the antioxidant capacity of plasma and these constituents are reported to mitigate the damage caused by the oxidative stress (Lie *et al*., 2005, Oviasogie *et al*., 2009 and Vidhan *et al*., 2010). Recent studies reveal that the defensive effect of fruits and vegetables is at least partially attributable to the phytochemicals such as vitamins A, C and E, flavonoids, phenolic acids, lignans and carotenoids (Marja *et al*., 1999, Namjooyan *et al*., 2010 and Vinay *et al*., 2010). These constituents serve as free radical scavengers, hydrogen donating compounds, singlet oxygen quenchers and metal chelators. Therefore, it

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is imperative to evaluate the commonly consumed fruits and vegetables for their antioxidative efficacy.

Pomegranate (*Punica granatum* L.) is considered as a medicinal and nutritional fruit due mainly to its antioxidant, antitumor, antihepatotoxic, antilipoperoxidative and antibacterial properties. It is popularly consumed as a fresh fruit, a beverage (juice and wine) and in processed forms such as jam and jelly (Wenjuan *et al*., 2010 and Vidhan *et al*., 2010). Research work carried out using animal and human models indicate that pomegranate juice has strong antioxidant activity, which is attributable to a diverse group of polyphenols including ellagitannins, gallotannins, ellagic acid, and flavonoids such as anthocyanins (Maria *et al*., 2000, Wenjuan *et al*., 2010, and Vidhan *et al*., 2010). Furthermore, some studies have exhibited that pomegranate possesses strong antioxidant activity and polyphenolic content superior to those of red wine, grapes, cranberry, orange, and apple while equal to or better than that of green tea (Maria *et al*., 2000, Kriengask *et al*., 2006 and Navindra *et al*., 2008).

More detailed information on health promoting components of pomegranate may provide a better insight into the neutraceutical, pharmaceutical and medicinal values of pomegranate which will invariably increase the consumption of pomegranate by the general public. The antioxidant activity of pomegranate juice has been found to vary considerably depending upon the cultivar, geoclimatic factors, harvesting, processing, and storage conditions. However, detailed investigations of the antioxidant activity of pomegranate varieties cultivated in Sri Lanka are very limited. In this backdrop, the objective of this study was to determine the total phenolic content and antioxidant capacity of most abundant pomegranate cultivars namely, *Nayana*, *Daya*, *Nimali*, and to compare the activities of Sri Lankan cultivars with a common Indian cultivar.

METHODOLOGY

2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), Folin-Ciocalteu's phenol reagent, gallic acid, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), linoleic acid, 2, 4, 6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), β-carotene, and Tween 20 were purchased from Sigma chemicals company (MO,USA). All other chemicals used were of analytical grade and obtained from either Sigma Chemicals or Himedia.

Matured healthy pomegranate fruits (with firm texture and colour fully developed) of three cultivars, namely *Nayana, Daya*, and *Nimali* were obtained from the Regional Research Station of Department of Agriculture, Makandura, Sri Lanka. Fruits belonging to a commonly available Indian cultivar were purchased from the local market. Fig. 1 illustrates the external features of the four cultivars of pomegranate used for the study.

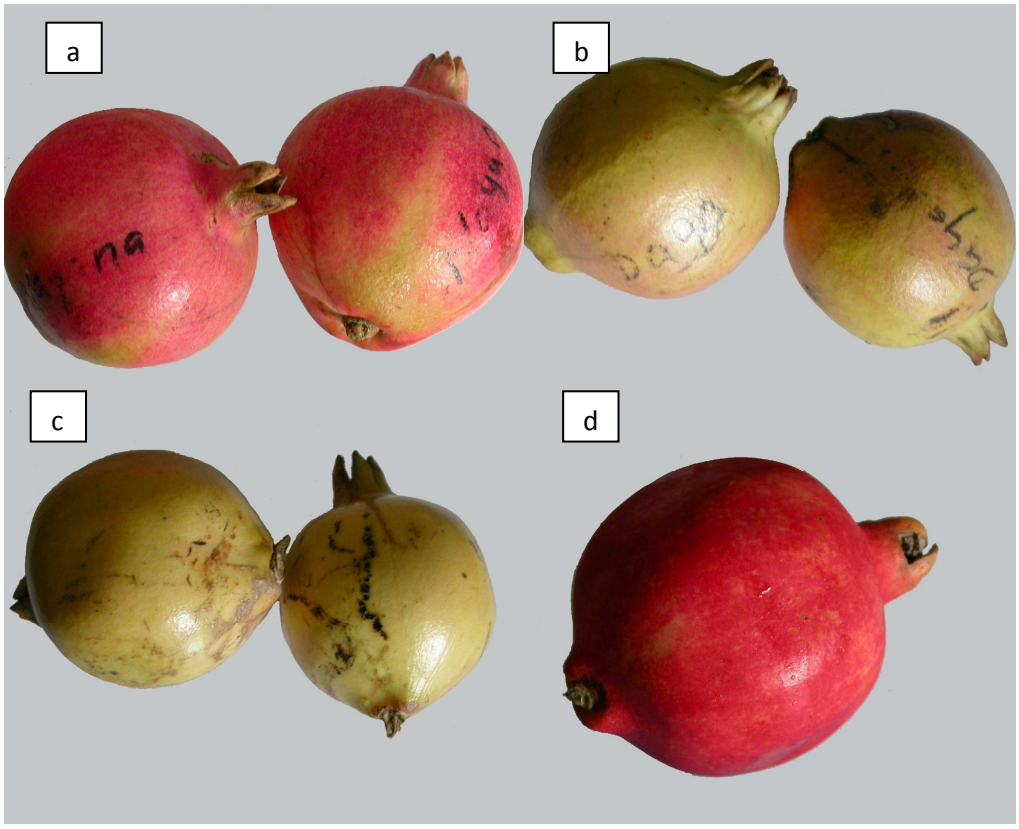

Fig. 1. External features of (a) *Nayana* (b) *Daya* (c) *Nimali* and (d) Indian pomegranate cultivars used for the study.

Preparation of aril extract

Pomegranate fruits were washed with pure water, peeled and edible portion (arils) was carefully separated. The so separated arils were manually pressed to obtain fruit extract, which was then filtered through a cotton mesh and subsequently through a filter paper (Whatman No. 01) and the resulting extracts were stored at -20 °C until further analysis. The extracts were appropriately diluted with distilled water and used for chemical analysis.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of the extracts was determined colorimetrically using Folin–Ciocalteu's reagent as described by Kriengsak *et al.* (2006) with some minor modifications. Fruit extracts (20 µL) were mixed with 100 µL of Folin-Ciocalteu's reagent, left for 3 minutes and subsequently 300 µL of sodium carbonate (0.7 M) was added and vortexed. The absorbance of the resulting mixture was measured at 725 nm using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan) after leaving for 30 minutes at the room temperature (25 °C). The results were expressed as mg gallic acid equivalents (GAE) per litre of extract using a gallic acid (50 – 500 mg/L) standard curve.

Determination of DPPH radical scavenging capacity

Antioxidant capacity of the fruit extracts was assessed using 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH). The DPPH radical scavenging activity of pomegranate extracts was quantified according to the method reported by Kai Marxen *et al.,* (2007) with minor modifications.

For DPPH radical scavenging assay, five different concentrations ranging between 0.1 and 1.0 mg/mL of each extract (0.5 mL) was mixed with 2.5 mL of methanolic DPPH radical (0.1 mM). After leaving for 20 minutes in the dark at room temperature, the absorbance was recorded at 517 nm using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan). The radical scavenging activity (RSA) was calculated as percentage DPPH discoloration using the following equation.

$$RSA\ (\%) = [(A_{DPPH} - A_{Sample}) / A_{DPPH}] \times 100$$

where, A_{Sample} is the absorbance of the solution containing the extract after 20 minutes and A_{DPPH} is the absorbance of the DPPH solution devoid of extract. Results were expressed as IC_{50} value that denotes the concentration of the sample required to scavenge 50% of DPPH radicals.

Determination of ABTS radical scavenging capacity

The total antioxidant capacity of the extracts was determined using ABTS radical. The $ABTS^{\bullet+}$ was generated by reacting 2,2'-azobis (2-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) with 2,2'-azobis (2-methylpropanimidamide) dihydrochloride (AAPH), which acts as the radical generator. Each extract (40 µL) was mixed with 1.96 mL of ABTS radical solution and absorbance was measured over 6 minutes at 1 minute interval at 765 nm using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan). The radical scavenging activity (RSA) after lapse of 1 minute was calculated as percentage of $ABTS^{\bullet+}$ discoloration (Robereta *et al*., 1999).

Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) assay was performed according to the method explained by Kriengask *et al*. (2006) with some modifications. The FRAP reagent included 10 mM 2, 4, 6-tris (2-pyridyl)-1, 3, 5-triazine (TPTZ), 20 mM $FeCl_3$, and 0.3 M acetate buffer at 1:1:10 (v/v) ratios. Three millilitres of FRAP reagent at 37 °C were mixed with 50 µL of the extract. After a lapse of 4 minutes, the absorbance was read at 593 nm against a reagent blank. The results were expressed as mol of Fe (II) per litre of extract using a Fe (II) (0.2 – 1.0 M) standard curve.

Determination of antioxidant efficacy using β-carotene/linoleate model system

The ability of pomegranate extracts to inhibit β-carotene bleaching was estimated according to the method explained by Maria *et al.* (2010) with minor modifications. β-Carotene/linoleate emulsion was prepared by mixing 10 mg of β-carotene, 40 mg of linoleic acid, 600 mg of Tween 20 emulsifier and 100 mL of oxygenated deionized water. The so prepared emulsion (2 mL) was mixed with 200 µL of extracts and incubated at 50^{o}C. The oxidative loss of emulsion was monitored using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan) at 470 nm wave length at every 15 minute for 120 minutes. The

antioxidant activity (AA %) was calculated according to the following equation and expressed as percentage value:

$$AA\ (\%) = \{[(AC_{120} - AC_0) - (AA_{120} - AA_0)] / (AC_{120} - AC_0)\} \times 100$$

where, AA_{120} is the absorbance of the antioxidant at 120 minutes, AC_{120} is the absorbance of the control at 120 minutes, AA_0 is the absorbance of the antioxidant at 0 minute, and AC_0 is the absorbance of the control at 0 minute.

Analysis of data

All experiments were conducted in triplicates and completely randomized design was used. The statistical analysis was carried out using MS Office (Excel) and MINITAB version 16 software.

RESULTS AND DISCUSSION

Total Phenolic Content

Phenolic constituents are one of the major group of compounds serving as primary antioxidants, especially as free radical terminators (Marja *et al*., 1999 and Oviasogie *et al*., 2009). The total phenolic content of the selected pomegranate fruit extracts tested is illustrated in Fig 2. The Total phenolic content varied widely among the cultivars tested from 1199 mg GAE/L (Indian cultivar) to 2390 mg GAE/L (*Daya* cultivar). The *Daya* cultivar possessed the highest TPC followed by *Nayana, Nimali*, and Indian cultivars. These results confirm that the amount of TPC is cultivar-dependant. Similar results have been reported in fruits such as strawberry, guava and blueberry (Kriengask *et al*., 2006 and Onder *et al*., 2009).

Based on the TPC, pomegranate aril juice can be identified as a fruit with potential antioxidant capacity. Fruits that show TPC of 1 – 5 g GAE/L are considered fruits with medium TPC (Maria do *et al*., 2010). Pomegranate contained high TPC compared to most of the commonly available fruits. The TPC of pineapple, mango, papaya, apple, and berries are 47.9, 56.0, 57.6, 11.9, and 28.7 mg/100g, respectively (Kriengsak *et al*., 2006). Moreover, pomegranate is superior even to green tea (1029 mg GAE/L) and equal or better to red wine (2036 mg GAE/L) (Maria do *et al.,* 2010). Maria *et al.* (2000) have also reported similar findings indicating that fresh pomegranate from Africa contains 1800 – 2100 mg GAE/L. Further, the results of the present study tally with that of Mariela *et al*. (2010) which reported TPC of 1303 – 2270 mg GAE/L.

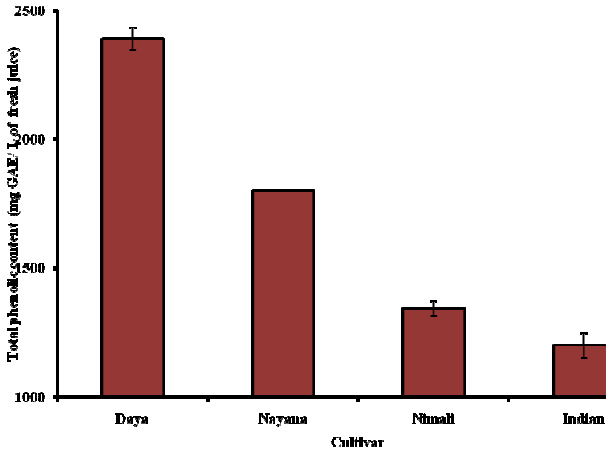

Fig. 2. Total Phenolic Content (TPC) of pomegranate extracts.

DPPH radical scavenging activity and total antioxidant capacity

DPPH and ABTS radical scavenging activities of the tested extracts are summarized in Table1. The DPPH radical scavenging activity was expressed as IC_{50} value which is inversely proportional to the antioxidant activity (Kriengask *et al*., 2006 and Onder *et al*., 2009). Results shown in Table1 indicate that the IC_{50} value of pomegranate extracts ranged between 0.182 mg/mL to 0.446 mg/mL with *Nayana* cultivar showing the highest antioxidant activity followed by the Indian cultivar, *Nimali,* and *Daya.*

Fruits showing an IC_{50} value of less than 1 mg/ mL is categorized as fruits with extremely high antioxidant potential (Safaa *et al*., *2010*). According to the IC_{50} value, pomegranate can be categorized as a fruit with high antioxidant potential. The antioxidant activity measured by DPPH assay was comparable to that of red grapes 0.43 mg/mL which contained an exceptional antioxidant activity (Kriengsak *et al*., 2006). Therefore, pomegranate can be considered a fruit that has an exceptionally high antioxidant activity. Furthermore, Kriengsak *et al.* (2006) reported that the IC_{50} value of selected fresh fruits, namely banana, tomato, orange, apple, dates, strawberry, kiwi, and pear range from 1.65 – 15.93 mg/mL. Safaa *et al. (*2010) also reported similar results for pomegranate (0.53 mg/mL) and results of these studies support the findings of the present study.

Table 1. DPPH and ABTS radical scavenging activity of pomegranate extracts

Cultivar	IC_{50} value (mg/mL)	ABTS (%)
Daya	0.446 ± 0.05^{c}	72.73 ± 1.9^{f}
Nayana	0.182 ± 0.02^{b}	93.1 ± 2.0^{d}
Nimali	0.368 ± 0.05^{a}	91.2 ± 1.5^{d}
Indian	0.362 ± 0.04^{a}	89.7 ± 2.03^{e}

±SD values with different superscripts are significantly different ($p \leq 0.05$)

Total antioxidant capacity of pomegranate extracts as measured by ABTS radical varied between 93.1% and 72.73% with the highest being observed in *Nayana* cultivar (Table 1). The reaction between the extracts and $ABTS^{\bullet+}$ was almost completed within the first minute

of the reaction (0.60 to 0.059) (Fig. 3). The extract of *Nayana* cultivar was the most active (the absorbance after 6 minutes. was 0.010). The activity of *Nayana*, *Nimali,* and the Indian cultivar was clearly stronger than that of *Daya* (Table 1 and Fig. 3). Based on the fast scavenging activity, pomegranate extracts revealed an extremely high antioxidant activity. Fruits at 10% level showing an absorbance less than 0.5 after 1 minute of reaction time are categorized as fruits with extremely high antioxidant capacity (Zhang *et al*., 2009).

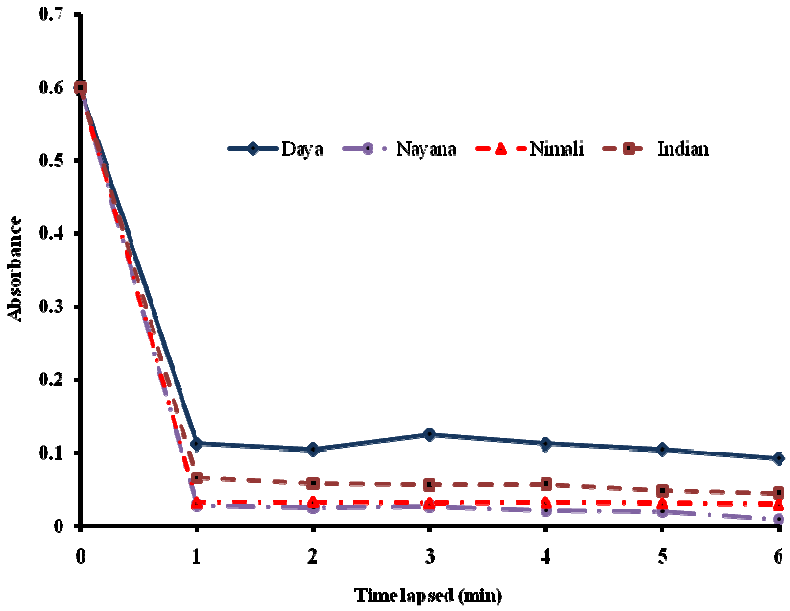

Fig. 3. Reduction of the concentration of $ABTS^{\bullet+}$ reflected by the reduction of absorbance in the presence of different pomegranate extracts over six minutes.

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) assay is based on the reduction of Fe^{+3}-TPTZ complex into Fe^{+2} -TPTZ form in the presence of antioxidants (Kriengsak *et al.,* 2006). It quantifies the reducing power of antioxidative extract which is an integral attribute of such compounds. FRAP values obtained from the regression equation of a calibration curve ($y = -0.311x + 0.986$, $R^2 = - 0.89$) is illustrated in Fig. 4. The FRAP value of Indian and *Nayana* cultivars ranged from 2.12 to 6.33 mol of Fe (II) / L. The highest reducing power was observed in *Nayana* cultivar extract while the lowest effect was observed in *Daya* cultivar.

FRAP value of all pomegranate cultivars tested lied above 1000 µmol Fe (II)/ g of extract. Thus, pomegranate can be categorized as fruit with high antioxidant activity (Vinay *et al*., 2010). Furthermore, pomegranate extracts showed high FRAP value compared to foods containing high level of antioxidant activity such as green tea and red wine (Maria *et al.*, 2000).

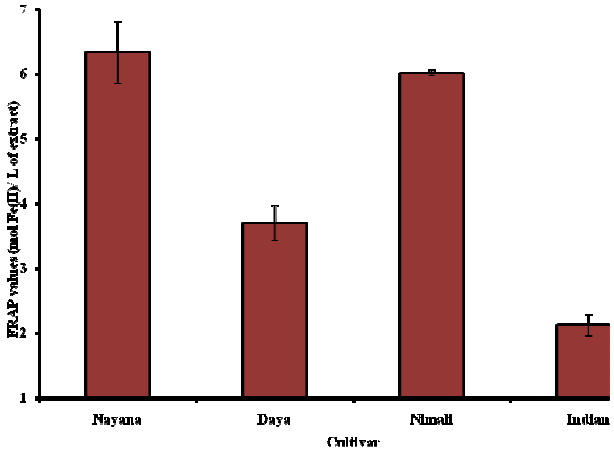

Fig. 4. FRAP values exhibited by different pomegranate extracts.

β-Carotene-linoleate model system

β-Carotene-linoleate model system is based on the inhibition of generation of volatile organic compounds, conjugated dienes, and hydroperoxides arising from linoleic acid oxidation (Onder *et al*., 2009). The antioxidant capacity of pomegranate extracts tested as measured by percentage of oxidation inhibition is illustrated in Fig. 5. The highest antioxidant capacity was observed in *Nayana* (91.02%), followed by *Nimali* (86.02%), Indian (84.88%), and *Daya* (79.94%) cultivars.

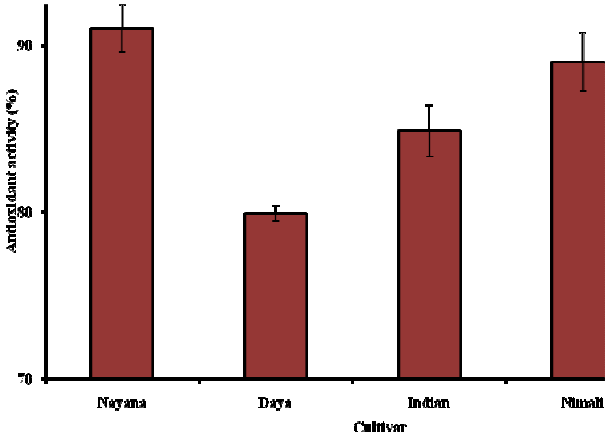

Fig. 5. The efficacy of different pomegranate extracts in inhibiting bleaching of β-carotene.

All pomegranate extracts tested effectively retarded the oxidation of β-carotene in the emulsion. Maria do *et al.* (2010) classified antioxidant capacity of fruits as high (>70%), intermediate (40–70%) or low (<40%) levels depend on oxidation inhibition. Therefore, pomegranate can be categorized as a fruit with high antioxidant capacity.

Antioxidant activity as measured by ABTS, DPPH, FRAP assays and using β-carotene/linoleate model system were not well correlated with TPC ($0.21 \leq r \leq 0.68$). Poor

correlation between TPC and these four parameters is reported by some authors worked on fruits. Kriengsak *et al.* (2006) reported no correlation between TPC and antioxidant capacity as determined by DPPH, FRAP, and ABTS assays in peaches, plums and nectarines. Moreover, Onder *et al.* (2009) also reported a poor correlation for *jujube* fruit.

Despite the moderate TPC, *Nayana* cultivar showed the highest antioxidant activity as measured by DPPH, ABTS, FRAP assays, and β-carotene/linoleic model system. On the other hand, *Daya* cultivar showed a relatively less antioxidant activity though it contains high level of TPC.

The poor correlation may be attributable to differential behaviour of phenolic constituents. The presence of other phytochemicals such as ascorbic acid, tocopherol and pigments, which also contribute to the total antioxidant capacity may also be attributable for the poor correlation.

Use of a single method to determine antioxidant capacity is insufficient, thus adoption of different assays and model systems provide a better insight into the actual activity of the extracts. The correlation among TAC, DPPH radical scavenging activity, FRAP and the protective activity as measured by β-carotene-linoleate model system was strong and ranged between 0.68 and 0.98 with the strongest correlation observed between DPPH radical scavenging activity and the efficacy as measured by the β-carotene-linoleate model system ($r = 0.96$) while the lowest correlation was observed between TAC and FRAP assays ($r = 0.68$).

Based on the chemical assays carried out in this study, *Nayana* and *Nimali* cultivars exhibited strong antioxidant activity than the Indian cultivar while *Daya* showed a relatively lower activity.

CONCLUSIONS

Pomegranate can be categorized as a fruit with high antioxidant potential. *Nayana* cultivar possessed the strongest antioxidant activities followed by *Nimali*, Indian and *Daya* cultivars.

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