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Antioxidant Capacity and Nutritional Value of Peels and Seeds of Selected Pomegranate (*Punica granatum* L.) Cultivars from Sri Lanka

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ABSTRACT

Purpose: *Nonedible portion of Pomegranate is reported to be rich with a diverse range of phytochemicals which embrace with health promotive features. Though antioxidant power and nutritive value of fruit juice are well known, knowledge of nonedible fraction is very poor. Hence, evaluation of antioxidant power and nutritional value of fruit peel and seed of pomegranates was targeted.*

Research Method: *IC_{50}, Ferric Reducing Antioxidant Power (FRAP), Total Phenolic (TPC), Total Flavonoid (TFC), Total Anthocyanin and pro-anthocyanidin Contents (ProAC) were determined. Nutritional value was studied by proximate analysis.*

Findings: *IC_{50}, TPC, FRAP and TFC values of peels were ranged from 4.6 to 41.1µg/mL, 318±1.77 to 478±5.62 mg Gallic acid equivalent/g, 4.270±0.83 to 6.690±0.15 mM Fe^{2+}/g and 52.64±0.24 to 75.99±0.849 Rutin equivalent mg/g respectively. Antioxidant power and TFC of all the peel extracts were well above as compared with juice and seed samples, the highest IC_{50} and TPC in Daya peel whereas the highest FRAP and TFC in Nimali and Kalpitiya red peels respectively. Kalpitiya red juice and peel had the highest TAC and ProAc.*

Proximate analysis revealed that Protein, lipid and fiber contents were higher in seeds than peels. Carbohydrate content of all the peels was higher than the seeds.

Research Limitations: *There were some practical limitations such as long dry spells and also finding suitable fields for the experiment, due to farmers' hesitation on possible yield reduction.*

Originality/ Value: *Findings reveal that selected cultivars of pomegranate peel possess exceptionally high antioxidant power and could be applied as an excellent source of natural antioxidant in future therapeutic and medicine and as a safer natural antioxidant in food industries. High nutrient contents in pomegranate by-products facilitate to develop nutritionally valuable components such as functional food ingredients and nutraceuticals.*

Keywords: *antioxidant activity, peel and seeds, Pomegranate, total flavonoid, total phenol, nutritional value*

INTRODUCTION

Pomegranate (*Punica granatum L.*, family Punicaceae), is used in folklore medicine for the treatment of various diseases. Because of the high nutritional and nutraceutical value of the juice, pomegranate has been a very popular fruit crop among the growers and consumers worldwide (Singh *et al.,* 2002). Pomegranate fruit juices rich in ellagtannins (ETs) proved their efficacy as antioxidant and anticancer agents, especially against breast and colon cancer (Moneim and Dkhil, 2011).

Pomegranate peels (pericarp, rind or hull) that amount to approximately 60% of the fruit weight

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is some valuable waste rich with diverse range of bioactive compounds such as phenolics, flavonoids, proanthocyanidin and ellagitannin and its isomers which have been shown to bear antimicrobial, antioxidant and anticancer properties. In addition to its health promotive properties, this waste fraction of the fruit holds up relatively high amount of minerals and fibers for a wide range of dietary requirements. (Ismail *et al.,* 2014; Jalal *et al.,* 2018).

Several scientific studies have confirmed biological activities and medicinal effects of the edible part of the fruit, but very few data exist about the bioactivity of non-edible part. Apart from that, antioxidant activity of pomegranate has been found to vary considerably depending upon the cultivar, geoclimatic factors. Bopitiya and Madujith (2012) reported antioxidant activity of fruit juice of the pomegranate growing in Sri Lanka, but no work is reported so far on antioxidant activity of waste part of the fruit. In this backdrop we aimed to assess the antioxidant power of fruit peel and seed of pomegranate cultivars grown in Sri Lanka.

Further, findings of the study will facilitate to develop nutritionally valuable, healthy and eco-friendly products that could find several applications in the food, pharmaceutical, herbal and cosmetic industries.

MATERIALS AND METHODS

Plant material

Matured healthy pomegranate fruits of *Kalpitiya red, Nimali, Daya* and *Nayana* (with firm texture and fully developed color) varieties were collected at harvesting time from Agricultural Research Station, Kalpitiya, Sri Lanka.

Chemicals

Gallic acid, 2,2' -diphenyl-1 picryhydrazyl (DPPH), Folin–Ciocalteu's reagent, catechin, 2, 4, 6-tris (2-pyridyl)-1, 3, 5-triazine (TPTZ), rutin hydrate, vanilin, sodium carbonate and methanol were obtained from Merck (Germany) and Sigma-Aldrich (Germany).

Preparation of fruit juice extract

Fruits were washed with distilled water, edible portions (arils), peels and seeds were separated and juice obtained were filtered through a cotton mesh and subsequently through a filter paper (Whatman No. 01).

Preparation of peel extract

Pomegranate peels were cut into small pieces, air dried and finely powdered using a blender. Seeds were also cleaned, dried and powdered. Both powdered samples packed in an airtight plastic bottle and kept in refrigerator at -23 °C for further analysis. Phenolic compounds in the peel and seed powder were extracted with methanol (10g of powder in methanol/water 70:30 v/v, 125.00 mL) by continuous stirring for 5 h at room temperature. Extracts were filtered, filtrate was concentrated in a rotary evaporator at 40 °C and dried residue stored at 4 °C for further analysis.

Determination of total phenols content (TPC)

The total phenols in the extracts were determined spectrophotometrically following the Folin Ciocalteu method (Bakour *et al.,* 2017). TPC value of the compounds was quantified as mg of Gallic acid equivalent, using a calibration curve. TPC value was expressed as gallic acid equivalents mg GAE/per gram.

DPPH radical-scavenging activity

The free radical scavenging activity was measured using the stable DPPH radical method as described in Kai Marxen *et al.* (2007). The scavenging activity was expressed as a percentage using the following equation.

scavenging activity (%) = [(Abs Control – Abs Sample)]/ (Abs Control)] × 100

Abs Control - the absorbance of the control

Abs Sample - the absorbance of the sample. The concentration required for 50% inhibition (IC_{50}) was calculated from the graph by plotting

inhibition percentage against concentration of the extract.

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power was determined according to the method adopted by Benzie and Strain (1996) with some modifications. The results were expressed as mM of Fe(II)/g.

Determination of Total Flavonoid content (TFC)

The total flavonoids content (TFC) was determined as described by Kong *et al*. (2012). The result was expressed as mg of Rutin equivalent /g. (mg RE/g)

Determination of Proanthocyanidin content

Proanthocyanidins content in pomegranate peel and juice extracts was estimated by the method described by Li *et al*. (2006). Results were expressed as mg catechin equivalents/gram (mg CE/g).

Evaluation of Proximate Composition

Finely powdered samples were analyzed following methods of AOAC (2000). Kjeldahl method was used to determine nitrogen content and protein content was calculated by multiplying nitrogen content by nitrogen conversion factor (6.25).

Moisture was determined by heating a 5.0 g of powdered sample in a hot air oven at 105 ± 5^{o}C until constant weight was obtained. Total Ash content was determined by incinerating the sample at 550°C-600°C for 5-6 h.

Crude fat was determined by extraction with hexane in a Soxhlet apparatus followed by evaporating all the traces of solvent. Crude fiber content was determined by acid and alkali digestion followed by incineration at 550^{o}C for 6h to acquire ash. Loss in weight on ignition was used to calculate crude fiber content.

Crude carbohydrate was estimated using the following formula.

100 – (moisture% + protein% +fiber%+ fat% +ash%)

Statistical analysis

All data were analyzed in triplicate and reported as mean ± standard error to compare the results between peel, seed and cultivars. Statistical analysis of the mean values was performed using one-way ANOVA followed by turkey pairwise comparison test at 95% significance level using Minitab 17 statistical software (Minitab Inc., IL, USA, State College, PA, USA).

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

TPC values observed for the peel extracts were in the range of 318±1.77^{b} to 478±5.62^{a} mgGAE/g. Cultivar *Daya* had the highest whereas *Kalpitya red* had the lowest. TPC values of *Nimali* and *Nayana* were very close to each other and they were significantly different ($P < 0.05$). TPC of all peel samples were very high with comparison to TPC of juice and seed samples. TPC values observed for the juice in the current study were well agreed with the values reported in Bopitiya and Madujith (2012).

As observed in this study, TPC of *Nimali, Nayana* and *Daya peels* were well compatible with the values reported for the pomegranate peel of Indian variety (Kumar and Neeraj 2018) and Tunisian varieties (Abid *et al.,* 2017) and Thailand variety reported in Manestan *et al.* (2012*)*. TPC of *Kalpitya red* peel was very close to TPC of Yemen variety peel (Shiban *et al.,* 2012). However, TPC of all other peel samples in the current study was very much higher than that of Yemen variety. TPC of the peels of Chinese (Yan *et al.,* 2017), Oman (Al-Rawahi, 2014), Syrian (Zam *et al.,* 2012), Tunisian (Elfalleh *et al.,* 2012) and Egytian (Abid *et al.,* 2017) varieties was also very much lower than that of TPC of peels in the current study.

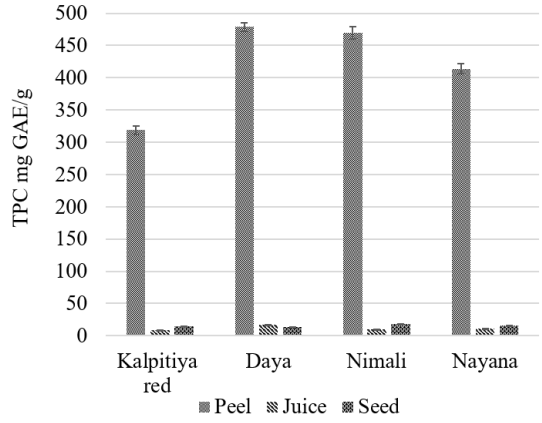


Figure 01: Total Phenolic Content of fruit peel, seed and juice of pomegranate cultivars (Error Bars ±SE)

All these findings imply that antioxidant power of pomegranate peels of Sri Lankan varieties are well above with respect to the peels of Tunisian (Boutheina *et al.,* 2018), Egyptian (Abid *et al.* 2017), Chinese (Yan *et al.,* 2017), Yemen (Shiban *et al.,* 2012), Oman (Al-Rawahi, 2014) and Syrian varieties (Zam *et al.,* 2012).

DPPH radical scavenging activity

IC_{50} of *Daya* and *Nimali* peel were observed as 4.6 and 7.8 µg/mL respectively, (Table 01) showing very high antioxidant activity. IC_{50} of *Nayana* and *Kalpitiya red* peels were found as 20.6 and 41.1µg/mL respectively which could also be considered as higher antioxidants. IC_{50} of Juice and seed samples indicate poor antioxidant activity with respect to all peel samples as IC_{50} inversely proportional to antioxidant activity. This implies that antioxidant power of all peel extracts was well above as compared with that of juice and seed samples. IC_{50} values obtained for the juice in the current study also well compatible with values reported in Bopitya and Madujith (2012). All these data reveal that pomegranate peel in the current study exhibit exceptionally high antioxidant power and these IC_{50} values were in consistent with the values reported for peel of pomegranate reported in Ali *et al.* (2014).

IC_{50} of *Garcinia.mangostana* peel (Zarena and Sanka 2009) which was reported to be very effective against cancers was recorded as 23.0 µg/mL. IC_{50} of peel samples, observed in the current study were also well compatible with the value reported for *Garcinia mangostana* peel. This implies that antioxidant potential of the peel of four pomegranate varieties was very high and almost the same as mangosteen peel. Nevertheless, IC_{50} of Egyptian (Abid *et al.,* 2017) and Thailand pomegranate peels (Manestan *et al.,* 2012) were very much lesser than the values observed in the current study. This reveals that DPPH radical scavenging activity of the pomegranate peel of Sri Lankan varieties is very much higher than the peels of Egyptian (Abid *et al.,* 2017) and Thailand varieties (Manestan *et al.,* 2012).

Table 01: DPPH radical scavenging activity of fruit peel, seed and juice of pomegranate cultivars

	IC_{50} mg /mL		
	Peel	Juice	Seed
Kalpitiya red	0.0411 ± 0.00153^{a}	0.357 ± 0.0013^{d}	0.324 ± 0.0023^{a}
Daya	0.004623 ± 0.000813^{b}	0.416 ± 0.0016^{a}	0.152 ± 0.0053^{c}
Nimali	0.007807 ± 0.00014^{b}	0.382 ± 0.009^{c}	0.130 ± 0.009^{b}
Nayana	0.0206 ± 0.00053^{c}	0.178 ± 0.0041^{a}	0.248 ± 0.0031^{d}

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

FRAP assay is based on reduction of Fe^{3+} TPTZ complex into Fe^{2+} TPTZ form in the presence of antioxidant which is used to quantify reducing power of antioxidative extract. FRAP value of the peel and the juice extracts was shown in Figure 02. FRAP values of the peel extracts were ranged from 4.270 ± 0.83 to 6.690± 0.15 mM/g and they were very much higher than all juice and seed samples. Of the peel samples, *Nimali* and *Nayana* peels had the highest FRAP values. *Kalpitiya red* had the lowest value.

FRAP values for *Psidium guajava, Mangifera indica* and *Citrus sinensis* peel were very much lower (Shen *et al.,* 2012) when compared with FRAP values of Sri Lankan pomegranate peels. All these data reveal that pomegranate peel possesses an exceptionally high antioxidant power compared to other fruit peels.

Total flavonoid content (TFC)

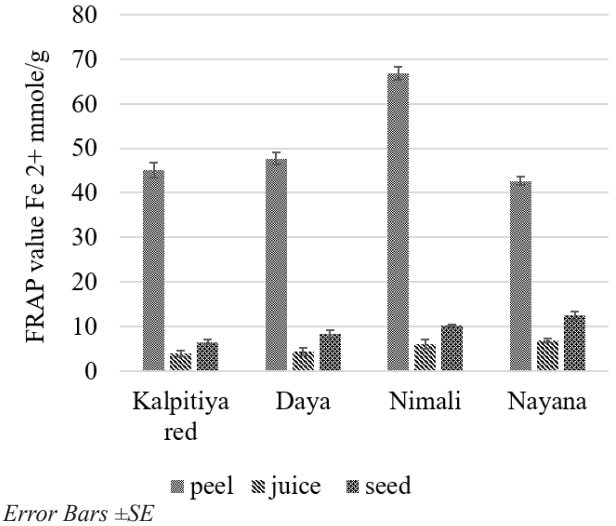
As presented in Figure 03 TFC of Nimali, *Nayana, Daya* and *Kalpitya red p*eel was found to be 67.672 ±0.594, 52.64±0.241, 59.575 ±0.327 and 75.99 ±0.849 RE mg/g respectively. TFC of the peels of each pomegranate cultivar was very much high with respect to that of its juice and seeds. TFC observed for peels in current study was concomitant with the values reported of the peels of Yemen (Shiban *et al.* 2012), Chinese (Yan *et al.,* 2017) and Tunisian (Elfalleh *et al.,* 2012) pomegranate varieties.

TFC of *Daya* and *Nimali* peel was the highest. *Kalpitiya red* peel had the lowest. TFC of all juice and seed samples was lower than all peel samples. Seed samples had the lowest value. However, TFC of the peel of pomegranate Indian varieties (Kumar and Neeraj, 2018) was very much less in comparison with the values of current study. TFC of pomegranate peel of Egyptian variety (Abid *et al.,* 2017) was very close to the values of Sri Lankan cultivars. TFC of pomegranate peel described in Yan *et al*. (2017) and Al-Rawahi *et al*. (2014) was very much lower than TFC of the current study.

Pro-anthocyanidin content (ProAC)

Pro-anthocyanidin contents of the peels in the current study are summarized in Figure 04. ProAc is higher in *Kalpitiya red* and *Nayana* peels, lower in *Daya* and *Nimali* peel. Of the juice samples *Kalpitiya red* juice had the highest ProAc whereas all other juice and seed samples showed a very low value. Difference in these values could be attributed with skin color of the fruit of each pomegranate cultivar.

ProAc of the peel observed in the current study was higher than that in pomegranate peels reported in Li *et al*. (2006), Yan *et al*. (2017) and Zam *et al*. (2012) Overall results showed that Pro-Anthocyanidin Content of the peel of Sri Lankan cultivars is higher than that of China, Oman and Syrian varieties.



Error Bars ±SE

Figure 02: FRAP values of fruit peel, seed and juice of 4 pomegranate cultivars

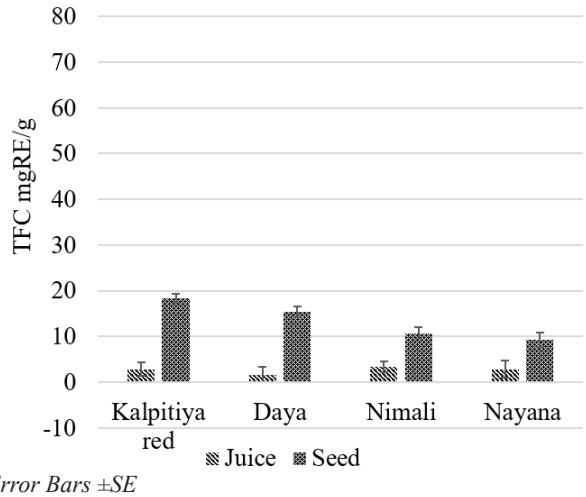


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Figure 03: Total Flavonoid Content in fruit peel, seed and juice of pomegranate cultivars

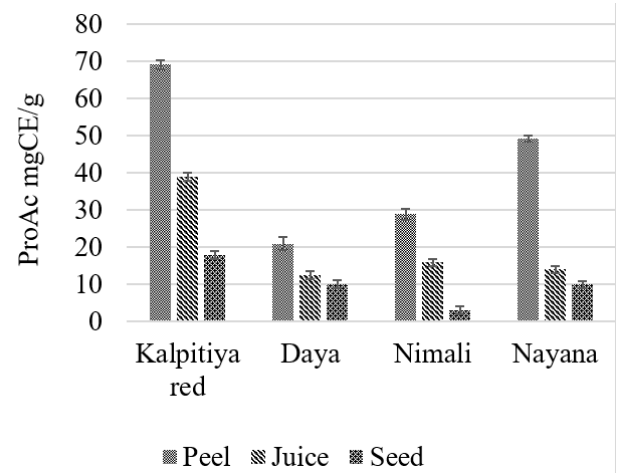

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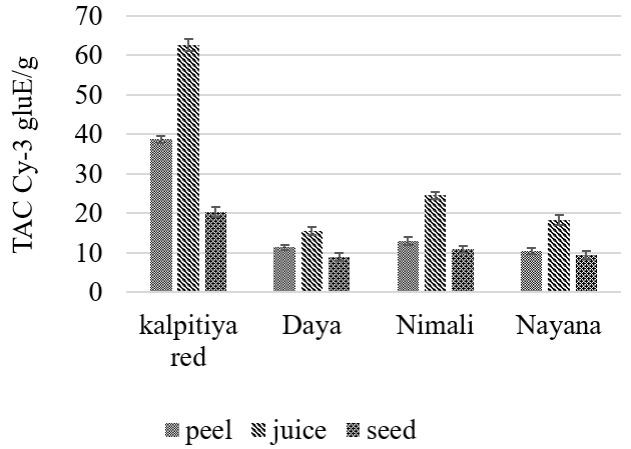
Figure 04: Pro-Anthocyanidin Content in fruit peel, seed and juice of pomegranate cultivars



Error Bars ±SE

Figure 05: Total Anthocyanin Content in fruit peel, seed and juice of pomegranate cultivars

Total Anthocyanin (TAC)

TAC of *Kalpitiya red* juice was very much higher than all other juice, peel and seed samples. Of the peel samples also *Kalpitiya red* showed the highest value. Here also higher TAC level in *Kalpitya red* Juice and peel was reflected by color of juice and skin color of the peel.

Proximate analysis

As summarized in Table 02, Fiber, lipid and protein content was higher in seed than peels. All the seed sample had a ~30-40% fiber content. Lipid content in the seeds was also nearly 20%. *Kalpitiya red* seed has the highest protein content.

Carbohydrate content of all the peels was in the range of 50- 70% and higher than that in seeds (Table 2). Carbohydrate content of *Kalpitiya red* and *Nayana* peel was almost similar and higher than that in *Daya* and *Nimali* peels. However, other nutrients were lower in peels with respect to seeds.

Table 02: Proximate composition of peel and seed samples of pomegranate cultivars

		Kalpitiya red	*Daya*	*Nimali*	*Nayana*
Moisture	peel	15.19±0.21^{a}	12.606±0.067^{b}	11.3± 0.10^{a}	10.32± 0.32^{c}
	seed	8.18± 0.01^{c}	8.22± 0.04^{c}	7.73± 0.07^{a}	8.63± 0.02^{b}
Ash	peel	3.58± 0.07^{ab}	1.263± 0.04^{b}	1.555± 0.02^{a}	3.74± 0.12^{c}
	seed	4.008± 0.29^{b}	1.853± 0.08^{c}	1.352± 0.01^{b}	3.015± 0.06^{a}
Fiber	peel	15.23± 0.21^{c}	14.02± 0.60^{a}	12.66± 0.97^{c}	15.03± 0.10^{a}
	seed	28.05± 0.17^{a}	32.06± 0.01^{b}	30.8± 0.06^{c}	29.61± 0.70^{ab}
Lipid	peel	1.41± 0.08^{c}	1.21± 0.05^{b}	0.91± 0.04^{a}	0.82± 0.03^{c}
	seed	21.5± 0.10^{a}	16.24± 0.31^{c}	18.47± 0.62^{b}	17.82± 0.32^{ab}
Protein	peel	19.6± 0.37^{ab}	11.73± 0.10^{a}	18.81± 0.23^{c}	4.73± 0.42^{b}
	seed	33.3± 0.61^{b}	23.83± 0.41^{a}	16.24± 0.52^{b}	20.91± 0.54^{c}
Carbohydrate	peel	44.79± 4.37^{a}	58.05± 3.46^{b}	50.82± 3.6^{a}	69.6± 2.76^{c}
	seed	7.227± 0.37^{c}	17.56± 1.02^{ab}	26.54± 2.37^{b}	25.61± 1.37^{a}

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

Statistical analysis showed that there is a significant difference between the moisture, lipid, ash, crude fiber, crude protein and carbohydrate content (p (0. 000) < 0.05) in seed and peel powder.

TPC, TFC, TAC, ProAc and FRAP values were significantly different (P < 0.05) among the cultivars and organs (peel, seed and juice).

CONCLUSION

Based on the results of the current study, the peels of four pomegranate cultivars, *Kalpitiya red, Nimali, Daya* and *Nayana* proved to be an excellent source of natural antioxidant. This high antioxidant activity of the peel extract appeared to be attributed to its high phenolic content. Ingestion of natural antioxidants could lower the risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing. Hence, it is worthwhile to explore the possibility of applying this natural source in future therapeutics and medicine as bioactive compounds as well as developing **a** safer natural antioxidant which can replace the synthetic ones.

Higher protein, fiber and lipid content in seeds and high carbohydrate content in the peel imply that pomegranate by-products could be used as a substrate for the production of nutritionally valuable components that could find several applications as functional food ingredients, food additives and nutraceuticals

Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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