

Bioactive Properties of Fruit Juice of Pomegranate (*Punica granatum*) Grown in Dry Regions of Sri Lanka

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ABSTRACT. Pomegranate (*Punica granatum*) is a popular fruit in Sri Lanka, rich with medicinal properties and extensively used in traditional Ayurvedic medicines. Total phenolic content, vitamin C and antioxidant activity of the fruit (aril) juice were determined in fruits harvested from three locations in the southern dry zone (DL 1a). The total phenolic content and vitamin C, as determined by the Folin-Ciocalteu method, were 22.2 ± 6.5 and 8.7 ± 2.3 g/L, respectively, but significantly lower than that of pomegranate collected from Chilaw, a popular pomegranate growing area in the north-west (DL 3). However, the antioxidant capacity of the fruit juice of pomegranate from DL 1a was comparable with the same of DL 3. Antioxidant capacity of pomegranate fruit juice was positively correlated with total phenol content and vitamin C content. The results revealed the bioactive properties of fruit juice extracted from local pomegranate trees grown in home gardens of the southern dry region of Sri Lanka, emphasizing potential exploitations of these properties of pomegranate in the fruit juice industry.

Keywords: Pomegranate, total phenolic count, antioxidant capacity, Vitamin C

INTRODUCTION

Epidemiological studies suggest that a high intake of food rich in natural antioxidants increases the antioxidant capacity of the plasma and reduces the risk of some, but not all, cancers, heart diseases, and stroke (Wang *et al.*, 1996). According to The World Health Report 2002, low fruit and vegetable intake is estimated to cause about 31% of ischaemic heart disease and 11% of stroke worldwide. As a consequence, diet related chronic diseases caused 18.3% of all deaths in Sri Lanka in the mid-1990s. Since 1998 ischaemic heart disease has taken the first place for hospital deaths in Sri Lanka (Annual Health Statistics, 2007).

Pomegranate fruit (*Punica granatum* L.) is one of the most popular fruits in Sri Lankan home gardens; especially in dry and intermediate zones. The edible parts of pomegranate fruits can be consumed fresh or in processed form. It has valuable compounds in different parts of the fruit whose functional and medicinal effects such as antioxidant, anticancer and anti-atherosclerotic effects have been confirmed (Mertens-Talcott *et al.*, 2006; Perze-Vincent *et al.*, 2002). Many scientists in the world have investigated the phenolic compounds and

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antioxidant activity in pomegranate juices of many varieties. The present studies was undertaken to investigate the bioactive properties of pomegranate juice extracted from the local varieties found in Sri Lankan home gardens.

MATERIALS AND METHODS

Pomegranate fruits at the edible stage were collected from dry zone home gardens in the Southern dry zone (Hambantota district) to determine their bioactive properties. Samples were collected from Angunukola palassa (A'palassa), Sooriyawewa and Weerawila (DL 1b) and compared with the same from a popular pomegranate growing area in the North-west in Arachchikattuwa (A'kattuwa) (DL 3) during the *Yala* season. Fruits were harvested from three to four plants from each location separately into perforated polyethylene bags. Fruits with defects (sun-burns, cracks, cuts, and bruises in husk) were discarded as practiced by Alighourchi *et al.* (2008). Properly cleaned fruits were peeled out and seeds and juicy rind of pomegranate were collected to a cotton cloth for extraction of juice by squeezing. Filtrates were stored in labeled plastic bottles under -20°C after sealing with Parafilm. All measurements were conducted in triplicates as reported by Ahmed and Seerat (2009). Half of frozen samples were centrifuged for 20 min at 2500 rpm and 2 ml of the supernatant was filtered for 3 min through a 0.45 µm syringe filter and collected into labeled eppendorf tubes. Then 20 µl of filtrate was mixed with 980 ml distilled water and vortexed for 5 sec in high velocity (Vergara-Infante, 2008). Three dilutions were prepared for each sample.

Determination of total phenolic content (TPC): 100 µl of diluted juice sample was measured in to an eppendorf tube and 500 µl of 10% Folin–Ciocalteu reagent was mixed with it by shaking for 3 min at room temperature. Then 500µl of 6% Na₂CO₃ was added and shook for 15 min at room temperature. Spectrophotometer reading was taken at 600 nm promptly. A calibration curve was prepared with gallic acid and the results were expressed as mg of gallic acid equivalents (GAE) per liter of fresh juice (Velioglu *et al.*, 1998).

Determination of Vitamin C: The sample extraction prepared for total phenolics analysis was used to measure the vitamin C content too. Differently, the spectrophotometer reading was taken at 600 nm promptly after adding Folin–Ciocalteu reagent and shaking for 3 min. Gallic acid was used to prepare the standard curve (Velioglu *et al.*, 1998).

Determination of antioxidant capacity (AOC): FRAP reagent was prepared by mixing 30 ml acetate buffer (300 mmol/L; pH 3.6), and ferric chloride (20 mmol/L) with 3ml of 2,4,6-tripyridil-s-triazin (10 mmol/L) solution. 40 µl of diluted samples were mixed with 120 µl of distilled water and 800 µl of FRAP reagent in labeled eppendorf tubes and shook for 10-15 sec at room temperature. After 4 min of reaction period spectrophotometer reading was taken at 600 nm wave length. A range of iron sulfate concentrations from 500 to 2500 µmol/L was used to prepare the calibration curve (Benzie & Strain, 1996).

Simple Linear Regression procedure was followed for calibration of standard curves. Statistical analysis of data were done through one-way analysis of variance (ANOVA) and was followed by Duncan's Multiple Range Test, using statistical software, SAS.

RESULTS AND DISCUSSION

Total phenolic content and Vitamin C

The TPC of pomegranate juice was significantly lower (at $p=0.01$) in the locations in the Southern dry zone (7.7-13.7 mg/L), compared to Arachchikattuwa; DL 3 (21.7 mg/L). TPC of the fruit juices taken from fruits harvested from three sampling locations in the Southern dry zone (DL 1b) was not significantly different among each other (Fig. 1). The TPC found were towards the higher side of the ranges reported for exotic varieties (Weerakkody *et al.*, 2009).

Vitamin C content of pomegranate juice was in the range of 6.3-8.4 mg/L among the sampling locations within the agro-ecological region (DL 1b). These were significantly lower than the vitamin C content found in the samples from Arachchikattuwa (11.02 mg/L of juice) (Fig. 1).

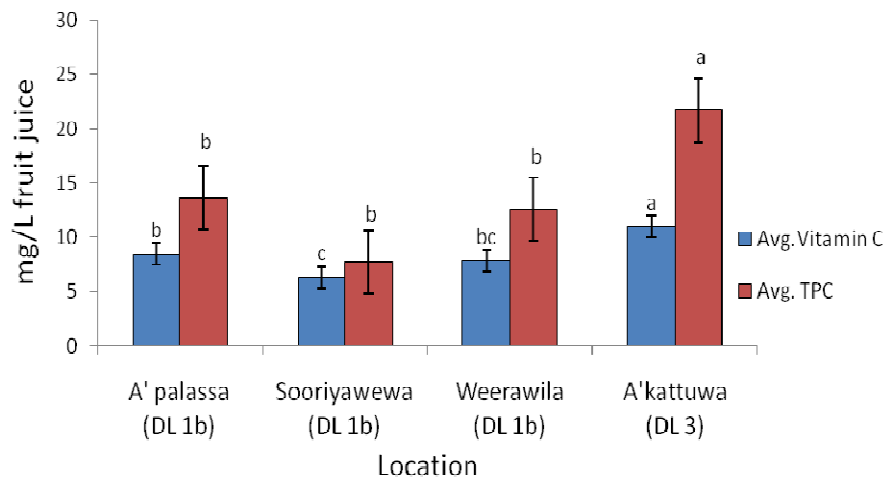


Fig. 1. Total phenolic content and Vitamin C of pomegranate juice

(Means followed by the same letter are not significantly different at $p=0.05$)

Antioxidant capacity

The AOC was not significantly different among the locations (17-33 mmol/L). Similar to the trend shown by the two juice parameters discussed above, the AOC of the fruit juice from DL 3 (87.6 mmol/L juice) was apparently higher than the same from DL 1b (Fig. 2).

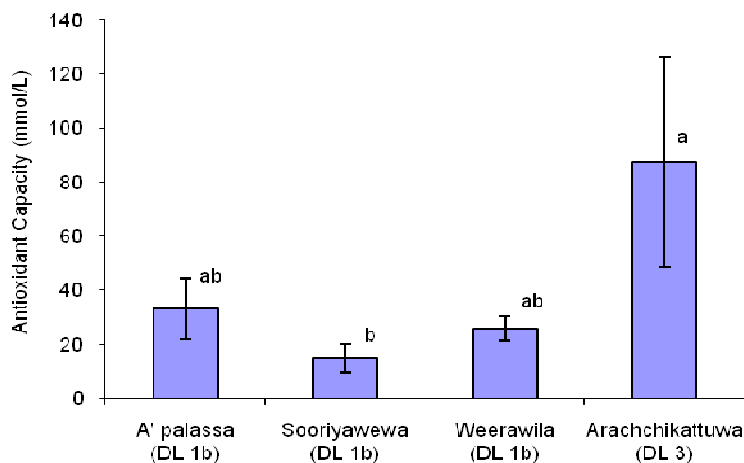


Fig. 2. Antioxidant capacity of pomegranate juice in four locations

Means followed by the same letter are not significantly different at $p=0.05$

Correlations between different bioactive measures of pomegranate juice

It was evident that there is a significant correlation between vitamin C content and AOC ($R^2=0.943$). A positive relationship ($R^2=0.944$) was also observed between AOC and TPC of pomegranate fruit juice.

CONCLUSIONS

Preliminary tests for bioactive properties of pomegranate fruit (aril) juice revealed that total phenolics and vitamin C contents are found within 7.7-13.6 mg/L and 6.3-8.5 mg/L, respectively in the Southern dry zone (DL 1b) and are significantly lower than the reference location, Arachchikattuwa (in agro-climatic region, DL 3). However, this difference was not revealed with respect to the AOC of pomegranate juice (40.4 ± 16.2 mmol/L) despite the fact that AOC is positively correlated with the total phenolic and vitamin C contents. Further research can be suggested to examine the influence of region-based agro-climatic differences on the bioactive properties of pomegranate fruit juice.

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