Imperial Journal of Interdisciplinary Research (IJIR)
Vol-3, Issue-1, 2017
ISSN: 2454-1362, http://www.onlinejournal.in

Comparison of phytochemicals present in locally available Sri Lankan and imported (Indian) fruits of *Punica granatum* (Lythracea)

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Abstract: *Plants have been extensively used as medicine and for disease management since early days, due to presence of valuable chemical identities. Present study was carried out to compare the phytochemicals present in local (Sri Lankan) and imported (Indian) fruits of Punica granatum. Juice, peel and seed extracts of imported (Indian) and thirteen Sri Lankan P. granatum fruits collected from different localities were analyzed to determine the total anthocyanin (TA), total flavonoid (TF) and total phenolic (TP) content and were compared. Phytochemical analysis revealed that TA content is high in Indian fruits and TF and TP contents are high in Sri Lankan fruits. Peel and seed extracts were subjected to GC-MS analysis and peaks were obtained. Among them 27 peaks were selected and their medicinal properties were identified. It was revealed that peel and seeds have antimicrobial, antioxidant, anti-inflammatory and anticancer activity.*

Keywords: *Anthocyanin, Flavonoids, Phenolic compounds, Punica granatum, Pomegranate, GC-MS*

1. Introduction

Punica granatum L. (Pomegranate) is a medicinal plant with immense properties of importance not only in indigenous medicine but also in Western medicine. This is one of the popular fruit species in Sri Lankan home gardens in all climatic zones. People are not aware about the medicinal value of the peel and aril, although these parts are rich in antioxidants, antimicrobial and anti-cancer properties and many more. Due to lack of information and studies on the benefits of seeds and peels, they are still underutilized and only the juice is being used. There are some studies which reported the presence of higher antioxidant, antimicrobial and anti-cancer compounds in the peel or seed than the pulp itself. Other parts of the *P. granatum* plant such as bark, leaves, fruit peels and roots are also being exploited extensively for their medicinal properties [1].

Presences of secondary metabolites vary with the microclimatic conditions and stress conditions. Present study was designed to analyze, screen and compare the phytochemicals present in juice, peel and seed extracts of *P. granatum* grown in different geographical areas of Sri Lanka. As imported *P. granatum* becoming more attractive to the general public, due to dark red skin and juicy nature, phytochemical constituents in juice, peel and seed of them were also analyzed and compared with those of local varieties.

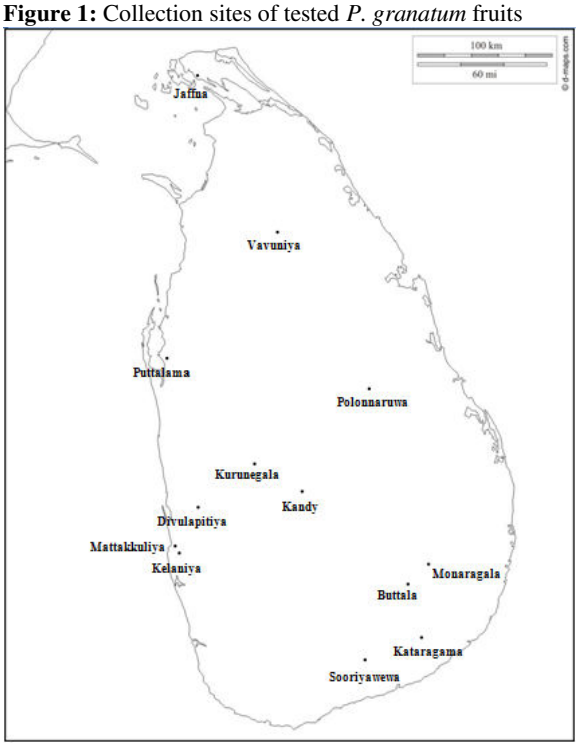
2. Materials and Methods

P. granatum fruits at the edible/ripening stage were collected from home gardens in thirteen different localities, Mattakkuliya, Kelaniya, Sooriyawewa, Buttala, Jaffna, Polonnaruwa, Kandy, Diwlapitiya, Vawniya, Kurunegala, Monaragala, Kataragama, Puttlam in Sri Lanka. (Figure 1) and market available Indian varieties were obtained from the supermarket. Collected fruits were initially washed with tap water, peeled and edible portion (arils) was carefully separated. Separated arils were manually pressed to obtain the juice and samples were centrifuged (SiGMA 1-15) at 3000 rpm separately for five minutes. The supernatant from each sample were collected and stored at -20 °C for further analysis.

Peel and seeds of each sample were dried separately in hot air oven at 40°C for nearly five days then size reduced and absolute ethanol (100.0 ml) was added to 10.0 g of powdered samples separately. Phytochemicals were extracted in each sample over a period of 8 hours using a Soxhlet extractor with the temperature range of 60-70 °C. Resultant crude extracts were evaporated in a rotary evaporator at 40 °C with pressure reduction starting from 300 mbar.

Exactly 20.0 mg of concentrated extract was dissolved in 10.0 ml of absolute ethanol then centrifuged (SiGMA 1-15) at 3000 rpm for five minutes and the supernatant was collected and used for preliminary screening and analysis of phytochemicals.

Figure 1: Collection sites of tested *P. granatum* fruits



2.1. Determination of total Anthocyanin (TA) content

TA content was determined by pH differential method using two buffer systems - potassium chloride (KCl) buffer [pH 1.0 (25 mM)] and sodium acetate buffer [pH 4.5 (0.4 M)] [1]. Reaction mixtures were prepared using 0.4 ml of sample with 3.6 ml of corresponding buffers separately and absorbance (A) was measured by a UV–Visible spectrophotometer (UV Chrome TECH CT-8200) at 510 nm and 700 nm. Water was used as the blank for juice samples and absolute ethanol was used as the blank for peel and seed extracts.

$(A) = (A_{510\ nm} - A_{700\ nm})$ pH 1.0 - $(A_{510\ nm} - A_{700\ nm})$ pH 4.5
pH 1.0 = Potassium chloride buffer
pH 4.5 = Sodium acetate buffer

$TA = [A \times MW \times DF \times 100] \times 1/MA$

Where:
TA: Total anthocyanin content; A: Absorbance; MW: Molecular weight of cyanidin-3-glucoside (449.2 g/moL); DF: Dilution factor; MA: Molar absorptivity coefficient of cyanidin-3-glucoside (26.900)

2.2. Determination of total Flavonoid (TF) content

Spectrophotometry method was used in order to determine the TF content in samples [2].
A 2.0 ml aliquot of extracts was mixed with 2.0 ml of 2% methanolic aluminum chloride and allowed to form flavonoid-aluminium complex by incubating at room temperature for 15 minutes. Absorbance of the reaction mixtures were measured at 430 nm with a UV–Visible spectrophotometer (UV Chrome TECH CT-8200).
A standard calibration plot for absorbance of Rutin was measured at different concentrations (25.0mg/l, 50.0mg/l, 100.0mg/l, and 150.0mg/l). Water was used as the blank for juice samples and absolute ethanol was used as the blank for peel and seed extracts and the assay was carried out in triplicates. Results were expressed as mg Rutin equivalent in a liter of sample (mg RTN/L of sample).

2.3. Determination of Total Phenolic content (TPC assay)

The total phenolic content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu assay [3]. An aliquot of 0.5 ml of extracted sample was added to 1.5 ml of Folin-Ciocalteu's reagent (1:10 v/v). After mixing 3.5 ml of 7.5% aqueous sodium bicarbonate was added and the mixture was allowed to stand for 90 min with intermittent shaking at room temperature. Absorbance was measured at 760 nm using a spectrophotometer (UV Chrome TECH CT-8200). A standard calibration plot of absorbance of Gallic acid was measured at different concentrations (5.0mg/l, 10.0mg/l, 15.0mg/l, and 20.0mg/l). Water was used as the blank for juice samples and absolute ethanol was used as the blank for peel and seed extracts and the assay was carried out in triplicates. Results were expressed as mg Gallic acid equivalent in a liter of sample (mg GAE/l of sample).

2.4. Screening of phytochemicals present in peel and seeds of *P. granatum* using Gas Chromatography- Mass Spectrometry (GC-MS)

The sample (1.0 µl aliquot) was subjected to Agilent Technologies 7890A GC system coupled with (an Agilent) 5975C Mass Selective detector (Shimadzu GCMS – QP2010). A HP-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness) was used. Helium was the carrier gas. Injector temperature was set at 260˚C. The initial oven temperature was at 70˚C which was programmed to increase to 280˚C at the rate of

10˚C/min with a hold time of 4 min at each increment. Injections of 1.0 µl were made in split mode with a split ratio of 100:1. The mass spectrometer was operated in the electron ionization mode at 70 eV. Compounds were identified by direct comparison of the mass spectrum of the analyte at a particular retention time to that of a reference standard library. At least 80% similarity index was considered significant. Total GC-MS running time was 35 minutes. The process was repeated with each and every sample separately. Chromatograms obtained were used to determine and compare phytochemicals present in each sample.

3. Results and Discussion

TA, TF and TP content of juice, peel and seed extracts of *Punica granatum* fruits collected from fourteen different localities (1:Imported- Indian, 2:Mattakkuliya, 3:Kelaniya, 4:Sooriyawewa, 5:Buttala, 6:Jaffna, 7:Polonnaruwa, 8:Kandy, 9:Diwlapitiya, 10:Vawniya, 11:Kurunegala, 12:Monaragala, 13:Katharagama, 14:puttlam) were compared. (Table 1, Tble2 and Table3)

3.1. Determination of Total Anthocyanin (TA) content

There was a significant difference in TA content of juice extracts among samples. TA content in samples vary from 1.00 ± 0.03 to 29.56 ± 0.06mg/l for local samples. It was significantly higher in Indian variety (34.74 ± 0.07 mg/l). (Table 1).
Among local samples highest TA content was recorded in samples collected from Jaffna (29.56 ± 0.06 mg/l) followed by Vawniya (19.37 ± 0.03 mg/l), Monaragala (15.53 ± 0.03 mg/l), Sooriyawewa (12.69 ± 0.02 mg/ l) and Puttlam (10.86 ±0.02mg/l). A significantly low TA was reported from samples collected from Kurunegala, Polonnaruwa, Kandy and Diwlapitiya which ranged from 1.0 – 2.5 mg/l. There was a significant difference in TA content of peel extracts among tested Sri Lankan samples and the values ranged from 0.94 ± 0.03 (Kurunegala)to 9.84 ± 0.02 mg/l (Jaffna). For imported Indian sample TA content in peel was significantly higher and was 13.59 ± 0.02mg/l.

TA content in peels of *P. granatum* collected from 15 different locations showed a significant difference between samples. The values ranged from 0.94 ± 0.03 to 9.84 ± 0.02 mg/l of local samples and peel from imported sample was 13.59 ± 0.02 mg/l. However, the peel colour of imported samples was more reddish than local *P. granatum* fruits indicating high levels of anthocyanin in there. Sri Lankan fruits vary from pink to yellow. The highest composition of TA in local samples were observed in the samples from Jaffna (90.84 ± 0.04 mg/l) followed by Vawniya (9.02 ± 0.04 mg/l), Sooriyawewa (8.02 ± 0.06 mg/l), Monaragala (7.68 ± 0.07 mg/l) and Puttalam (7.35 ± 0.06 mg/l).

Table 1: Total Anthocyanin content in juice, peel and seeds of *P. granatum* fruits collected from different locations

Location	Total Anthocyanin content (mg/l) ± SE		
	Juice	Peel	Seed
1	34.74 ± 0.06	13.59 ± 0.02	10.02 ± 0.03
2	9.85 ± 0.05	5.01 ± 0.06	4.51 ± 0.05
3	4.68 ± 0.04	4.18 ± 0.03	3.84 ± 0.05
4	12.69 ± 0.02	8.02 ± 0.06	8.68 ± 0.04
5	8.02 ± 0.04	7.18 ± 0.03	6.85 ± 0.10
6	29.56 ± 0.07	9.84 ± 0.02	9.18 ± 0.07
7	2.17 ± 0.02	1.54 ± 0.03	1.00 ± 0.08
8	2.54 ± 0.06	1.32 ± 0.03	1.50 ± 0.07
9	2.51 ± 0.08	1.87 ± 0.03	1.34 ± 0.04
10	19.37 ± 0.03	9.02 ± 0.04	7.68 ± 0.10
11	1.00 ± 0.03	0.94 ± 0.03	0.84 ± 0.05
12	15.53 ± 0.03	7.68 ± 0.07	7.01 ± 0.19
13	3.34 ± 0.05	2.98 ± 0.03	2.67 ± 0.09
14	10.86 ±0.02	7.35 ± 0.06	6.85 ± 0.06
LSD 5%	0.01	0.01	0.01

TA content in seeds of *P. granatum* collected from 15 different locations showed a significant difference between samples. The values ranged from 0.84 ± 0.05 to 9.18 ± 0.07 mg/l of local samples and seeds from imported sample was 10.02 ± 0.03 mg/l where the difference was non-significant comparing to those Jaffna sample. TA content of seed extracts of imported sample was greater than all the tested local samples. The highest composition of TA in local samples was observed in the samples collected from Jaffna (9.18 ± 0.07 mg/l) followed by Sooriyawewa (8.68 ± 0.04 mg/l), Vawniya (87.68 ± 0.10 mg/l), Monaragala (7.01 ± 0.19 mg/l) and Putlam (6.85 ± 0.06 mg/l).

Among three different tested extracts juice showed high TA content compare to the peel and seed extracts, and in all three cases Indian sample showed higher TA content than local samples. *P. granatum* fruit samples collected from Jaffna, Vawniya, Sooriyawewa, Butthala and Monaragala showed higher TA content. Fruits collected from Kurunegala had the lowest anthocyanin content in all three tested parts.
Relatively high TA content in *P. granatum* juice extract compared to the peel and seed extracts has been reported [4] and the results of the present study confirmed those findings. Another study [5] revealed

that environmental factors have a considerable effect on TA content in fruits and vegetables. However, presence of high TA content in imported *P. garantum* than the local fruits could be either due to the effect of environmental factors or differences in genetic makeup. Present study revealed that TA content of local fruits collected from dry zone (Jaffna, Vawnya, Sooriyawewa) is higher than the fruits collected from wet zone (Kandy, Kurunegala, Kelaniya) indicating that the climatic conditions have great influence on TA content in fruits of *P. granatum.*

Anthocyanins are the major pigments responsible for the colour of fruits and seeds of *P. granatum* which has a vast range of health benefits. Prevention of non-communal diseases such as cancer, cardiovascular diseases, diabetic, antimicrobial and anti-inflammatory properties, control of obesity and improvement of visual and brain functions are recorded as potential health benefits of plant derived compounds no only that anthocyanins also contribute to the fruit's colour significantly effects market demand [6]. Present study revealed that not only juice which is commonly used, but also peel and seeds of *P. granatum* also can be used for pharmaceutical purposes as it is considered as a waste in general.

3.2. Determination of Total Flavonoid (TF) content

TF content of juice of *P. granatum* from 15 different localities used in the present study found to be significantly different and vary from 75.27 ± 0.54 to 177.76 ± 0.96 mg RTN/l and in Indian sample showed a comparably lower TF content (109.80 ± 1.19 mg RTN/l). Highest composition of TF was observed in the samples from Polonnaruwa (177.76 ± 0.96 mg RTN/l) followed by Diwlapitiya (152.46 ± 0.54 mg RTN/l), Jaffna (134.56 ±0.65 mg RTN/l), Imp Pa (122.99 ± 0.48 mg RTN/ L) and Kandy (118.65 ± 0.48 mg RTN/ L). (Table 2)

Peels also showed a significant difference among locations where the fruits were collected. TF content of peel extracts varied from 308.64 ± 1.10 to 668.36 ± 0.65 mg RTN/l in local samples and Indian sample showed the lowest (286.22 ± 0.72 mg RTN/l). Highest composition of TF was observed in the samples collected from Polonnaruwa (668.36 ± 0.65 mg RTN/l) followed by Vawniya (516.16 ± 0.48 mg RTN/l). TF content of peel extracts of all the local samples were greater than that of Indian sample.

TF of seeds also showed a significant difference among localities where fruits were collected. Flavonoid content in seeds ranged from 25.92 ± 0.65 to 65.87 ± 0.65 mg RTN/l in locally collected samples and in Indian sample it was only 40.38 ± 0.65 mg RTN /l. Highest composition of TF in seeds was also observed in samples from Polonnaruwa (65.87 ± 0.65 mg RTN/l) indicating that fruits collected from Polonnaruwa are rich in flavonoids than any other local or imported Indian fruits from the market. Highest TF content was observed in peels followed by juice and seeds.

Table 2: Total Flavonoid content of *P. granatum* fruits collected from different locations

Location	Total Flavonoid content (mg RTN/L) ± SD		
	Juice	Peel	Seed
1	109.80 ± 1.19	224.94 ± 0.83	40.38 ± 0.65
2	77.26 ± 1.13	515.26 ± 0.18	50.14 ± 1.19
3	78.52 ± 0.54	416.03 ± 0.83	25.92 ± 0.65
4	78.52 ± 0.63	422.52 ± 0.63	30.80 ± 0.31
5	92.44 ± 0.96	453.98 ± 0.63	42.37 ±0.48
6	134.56 ±0.65	380.58 ± 0.54	30.98 ± 0.96
7	177.76 ± 0.96	668.36 ± 0.65	65.87 ± 0.65
8	118.65 ± 0.48	308.64 ± 1.10	36.22 ± 1.01
9	152.46 ± 0.54	312.80 ± 0.31	49.60 ± 0.65
10	92.62 ± 0.94	516.16 ± 0.48	62.80 ± 0.31
11	88.28 ± 1.54	368.47 ± 0.54	37.49 ± 0.65
12	75.27 ± 0.54	482.72 ± 0.48	47.79 ± 1.13
13	101.30 ± 1.54	333.22 ± 1.13	35.68 ± 0.65
14	110.16 ± 0.36	354.73 ± 1.01	38.57 ± 0.72
LSD 5%	0.01	0.01	0.01

Flavonoid is one of the important chemical found in plants which possess antimicrobial, anti-allergic and anti-inflammatory activity. Previous research [7] revealed that plants rich in flavonoids and phenolic compounds are potential candidates for natural antioxidants. Another study [6] revealed that the peel extracts were more potent than the juice, indicating that peels of *P. granatum* has more phytochemicals that could be used as effective compounds for health benefits. In the present study it was observed that the TF content is higher in peel extract than juice and seed extracts confirming the observations of [4] also reported that, TF contents in peel of *P. granatum* are higher than those of juice and seed extract. The reasons for the differences in TF content in tested samples might be due to climate, temperature and other microclimatic conditions of the localities where the plants are growing. However, it could be suggested that not only *P. granatum* juice, but also peel and seed can be used as sources of natural antioxidants.

3.3. Determination of Total Phenolic (TP) content

There was no significant difference in TP content in juice, peel or seed extracts of *P. granatum* samples collected from different localities. The TP content varies from 27.41 ± 0.06 to 29.29 ± 0.08 mg GAE/l for juice and peel samples in all local and Indian fruits. However, the TP content in seeds were significantly lower than juice and of all tested samples.

Highest composition of TP was observed for the samples from Polonnaruwa for juice (28.17 ± 0.06 mg GAE/l) for peels (28.96 ± 0.03 mg GAE/l) (Table 3). It is a well-known fact that local *P. granatum* is more pucker than imported fruits found in the market. However the present study revealed that there is no significant difference in local or Indian samples for the presence of phenolic compounds.

Table 3: Total Phenolic content of imported and local variety of *P. granatum* collected from different locations

Location	Total Phenolic content (mg GAE/l) ± SE		
	Juice	Peel	Seed
1	27.75 ± 0.05	28.20 ± 0.06	11.09 ± 0.06
2	28.13 ± 0.89	29.03 ± 0.09	11.33 ± 0.11
3	28.02 ± 0.05	29.09 ± 0.01	11.30 ± 0.03
4	28.15 ±0.06	29.29 ± 0.08	8.98 ± 0.08
5	28.03 ± 0.05	28.93 ± 0.08	16.89 ± 0.09
6	27.98 ± 0.03	29.09 ± 0.09	9.96 ± 0.08
7	28.17 ± 0.06	28.96 ± 0.03	13.31 ± 0.09
8	27.94 ± 0.08	28.87 ± 0.90	12.37 ±0.05
9	28.01 ± 0.03	28.67 ± 0.01	11.01 ± 0.02
10	28.05 ± 0.03	28.64 ± 0.10	18.36 ±0.10
11	27.96 ± 0.05	28.68 ± 0.02	13.47 ± 0.07
12	27.84 ± 0.12	28.70 ± 0.07	12.11 ± 0.06
13	28.09 ± 0.01	28.56 ± 0.03	11.305 ±0.01
14	27.92 ± 0.06	28.37 ± 0.13	12.14 ±0.11
LSD 5%	0.41	0.01	0.01

3.4. Screening of phytochemicals present in peel and seeds of *P. granatum* using Gas Chromatography- Mass Spectrometry (GC-MS)

GC-MS chromatogram of the ethanolic extract of peel and seeds of *P. granatum* obtained from different locations in Sri Lanka indicates the presence of 27 different phytochemicals with high medicinal values.

Out of these phytochemicals, Siloxane derivatives identified as 2-Dimethyl(prop-2-enyl)silyloxypropane, cycloheptasiloxane-tetradecamethyl, 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane, hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl, cyclononasiloxane-octadecamethyl, octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-dodecamethyl have antibacterial, antifungal and antiviral activity [8].

Decanoic acid derivatives such as tridecane, 3-methyl, hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid, methyl ester, 9-Octadecanoic acid(Z)-, methyl ester, 7-tetradecyne, 1-hexadecanol have antioxidant, antimicrobial, anti-inflamatory activity [9].

According to the literature Eicosanoic acid, methyl ester has abilty to cure bronchitis and pneumonia and alpha-D-glucopyranoside has anti-inflamatory activity. Gamma tocopherol is identified as a type of vitamin E which has antioxidant and anti-inflamatory activity [10]. Literature said that (all-E)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene involved in steroid biosynthesis in body [10] and Bicycloheptane,2,6,6-trimethyl has antimicrobial activity [8]. Furan,2,5-dimethyl, tetracosane, Methyl beta-d-galactopyranoside have anticanser activity.

4. Conclusions

The results of the present study revealed that not only juice but also peel and seeds of Sri Lankan *P. granatum* fruits have higher beneficial phytochemical content than Indian samples commonly found in Sri Lankan supermarkets. From locally available *P. granatum*, fruits obtained from Jaffna, Vawniya, Sooriyawewa, Butthala and Monaragala contained relatively high phytochemical content compare to fruits obtained from other locations. It revealed *P. granatum* grow in dry zone accumulate more phytochemicals than fruits grow in wet zone. It confirmed the phytochemical content in *Punica granatum* fruits vary due to climate, temperature and other microclimatic conditions. And also revealed that inedible portion of *P. granatum* contains higher amount of medicinal value. Therefore not only juice, but also peel and seeds can be used for medicinal purposes.

5. Acknowledgements

This research was supported by University of Sri Jayewardenepura (USJP), Sri Lanka.

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