RESEARCH ARTICLE

Comparative account on antioxidant properties, proximate and phytochemical compositions of seven guava varieties grown in Sri Lanka

S. Kokilananthan[a], Vajira P. Bulugahapitiya[a]*, C.S. Gangabadage[a], H. Manawadu[a]

[a]*Department of Chemistry, Faculty of Science, University of Ruhuna, Wellamadama, Matara, Sri Lanka*

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*Correspondence: vajira@chem.ruh.ac.lk

ABSTRACT

Sri Lanka is rich with many guava cultivars/varieties. But, less scientific data are available especially on guava leaves. Therefore, this study was aimed on comparative account of phytochemical and proximate compositions, and antioxidant properties of leaves of seven guava varieties for the purpose of selecting the best variety to be transformed into functional foods and value additions. Extraction of phytochemicals in leaves was undertaken using two techniques i.e. maceration and sonication. Screening for phytochemicals and investigation of proximate composition were carried out following standard protocols. Spectrophotometric and gravimetric methods were used in quantification of phytochemicals. DPPH free radical scavenging assay and FRAP reducing power assay were used to measure the antioxidant capacity. Out of two extractions methods applied, sonication showed more efficient. The results showed that the important phytochemicals are available in all the guava varieties with a slight difference among them. High anti-oxidant capacity and acceptable proximate composition were noted with all varieties showing that each has unique characteristic. The statistical analysis and comparison studies showed that common guava is the best variety out of all, showing the highest antioxidant capacity of 722.44±6.58 mg Trolox Eq/g in FRAP assay, IC_{50} value of 192.89±0.07 ppm in DPPH assay, highest polyphenolic content (479.29±2.16 mg GAE/g) and appreciable proximate composition. As a conclusion, this study provided a detailed analysis of phytochemical, proximate and anti-oxidant properties of the leaves of guava varieties grown in Sri Lanka to be used by scientific community for further analysis as well as for applications them into novel functional foods to be used in healthcare purposes.

Keywords: *Guava varieties, Maceration, Sonication, Phytochemicals, Antioxidant, proximate*

INTRODUCTION

Herbs have played a prominent role in human sustainability throughout the history. Being the backbone of the pharmacological world, endless research is being conducted continuously to increase its feasibility further and to aid human society. *Psidium guajava* is a medicinal tree which is commonly known as guava of the family *Myrtaceae*, and it is a native plant of tropical America but cultivated throughout the tropics (Chahal *et al*., 2011; Barbalho *et al.,* 2012). *P. guajava* is used traditionally for several ailments since a long time in history. Despite the fruit, some studies on guava leaves have revealed its chemical composition that has to be known to correlate with important

pharmacological activities. For example, some flavonoids extracted and identified from guava leaves namely, morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin and quercetin-3-O-arabinoside and those are known to possess strong antibacterial activity (Arima and Danno, 2002); Cytotoxic compounds; guavinoside C, D, E and F have been isolated from the leaves by Feng *et al.* (2015) and new terpenoids; lanost-7-en-3β-ol-26-oic acid, lanost-7-en-3β, 12β-diol-26-oic acid, lanost-7-en-3β, 12β, 29-triol-26-oic acid and lanost-7-en-3β-ol-26-oic acid-3β-D-glucopyranoside were identified from guava leaves by Bagri *et al*. (2016) and that were exhibited significant antidiabetic activity (Díaz-de-Cerio *et al*., 2016).

In addition more pharmacological activities have been reported by various researchers; antioxidant activity (Fernandes *et al*., 2014), antimicrobial activities (Biswas *et al*., 2013), antidiabetic activity (Oh *et al*., 2005), antitumor activities and anticancer effect (Ashraf *et al*., 2016), hepatoprotective activity (Taju *et al*., 2011) and anti-allergic effects (Han *et al*., 2011) etc.

As non-communicable diseases have become global health threat (Islam *et al*., 2014), despite all the advanced technologies, medicines and treatment methods available around the globe, preventive measures through consuming healthy foods, food supplements and value-added products based on plants with medicinal value has attracted interest by general public. The information disseminated through natural product research and food-based research has changed mindset of people to move towards consuming plant-based products or their value-added products as preventive causes of non-communicable diseases and health promoting aspects.

Sri Lanka is rich with many guava cultivars/varieties including commonly grown wild and introduced varieties; *E.g.* common-guava (*Psidium guajava:* red, pink and white flesh fruits and small, middle and large size fruits), strawberry-guava, apple-guava, sour-guava and the introduced varieties such as *Kanthi*, *Pubudu*, *Costorican*, *Horana red*, *Horana white*, etc. Common-guava is available in all over the area in Sri Lanka wheras strawberry-guava mostly can be found in southern province in Sri Lanka; introduced varieties can be collected mainly from Fruit Crops Research and Development Centre (FCRDC), Horana, Sri Lanka. As noted above, guava has become multi-functional therapeutic plant, and its fruits and leaves seem equally contribute to it. Though Sri Lanka is the host for such a vast variety of guava, its utilization for human wellbeing and health protective purposes have not been attended enough. Therefore, the research on Sri Lankan guava varieties needs to be strengthen in order to be transformed them to more consumable status among public as a very few studies is available so far (Kariawasam *et al*., 2017).

Therefore, this study was aimed on comparative account on phytochemical screening, proximate composition, quantification of total polyphenolic,

flavonoid, tannin, terpenoid, saponin and alkaloid contents and antioxidant analysis of guava varieties namely, *Psidium pomiferum* (Apple-guava), *Psidium guineense* (*Embul-pera*), *Psidium guajava* (*Getta-pera* and Common guava) and introduced varieties of *Psidium guajava* (*Kanthi* and *Pubudu*) and *Psidium guineense* (*Costorican*) for the purpose of producing a repository to be used for the scientific community and general public and to transform them into value added products as the extension of this study.

METHODOLOGY

Sample collection

Fresh leaves of *Kanthi*, *Pubudu* and *Costorican* guava were collected from FCRDC, Horana, Sri Lanka, Apple-guava was collected from Lake Serenity Resort & Spa, Gonapitiya Road, Ratnapura, Sri Lanka, *Getta-pera*, Common guava (middle size fruit) and *Embul-pera* were plucked from home gardens around Janarajamawatha, Matara, Sri Lanka. Plant materials have been authenticated from Department of Botany, University of Ruhuna. After plant collection, healthy guava leaves were washed several times and open-air dried for 3 d. Dried plant leaves were ground into powder by using a grinder to be used in the extraction process as well as some analysis in its powder form.

Extraction

One part of the dried powder of each variety was extracted using maceration with methanol for 48 h at room temperature (Batubara *et al*., 2017)). Other part was extracted by ultrasound assisted extractor (ROCKER Ultrasonic cleaner, Model: SONER 202H) at room temperature with methanol for one hour (Batubara *et al*., 2017). After filtration, the excess solvent was evaporated under vacuum at 45 °C using a rotary evaporator (HAHNVAPOR Rotary evaporator, Model No: HS-2005S).

Phytochemical qualitative analysis

Screening tests for phytochemicals were carried out in triplicates for the methanolic extracts of leaves of each variety, by the standard procedures described in the literatures (Arya *et al*., 2012; Biswas *et al.,* 2014). Accordingly, tests for alkaloids, glycosides, flavonoids, saponins, tannin, terpenoids, carbohydrates, proteins, soluble starch, polyphenols, phlobatannins, coumarins, anthocyanins, chalcones, betacyanin, anthraquinones, phytosterol, anthracene and quinones were carried out.

Phytochemical quantitative analysis (Gravimetric method)

Total alkaloid and saponin content were determined using standard procedure described by Ajuru *et al*. (2017) with some modification.

Phytochemical quantitative analysis (Colorimetric method)

Total phenolic content, total tannin content, total flavonoid content and total terpenoid content were determined by slightly modified spectrophotometric method as described in the literature (Ekwueme *et al.*, 2015; Abeysuriya *et al.*, 2020; Pękal and Pyrzynska, 2014).

Antioxidant analysis

DPPH* (2,2′-Diphenyl-1-Picrylhydrazyl Radical) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay were carried out by standard procedure discribed (Gangwar *et al.*, 2014; Biglari *et al.*, 2008) with slight modification.

Proximate analysis

Proximate analysis was carried out in triplicates for the dried powder of leaves of each variety by the standard protocols of AOAC described in the literatures (Busuttil-Griffin *et al.*, 2015; Maisarah *et al.*, 2014; Shahnawaz *et al.*, 2009; Kumari *et al.*, 2017; Ocran, 2012). Accordingly, moisture content, ash content, fiber content, fat content, protien content, carbohydrate content, total solids and energy were determined.

Statistical analysis

Different statistical techniques such as analysis of variance (ANOVA), T-test (LSD), Tukey's Studentized Range (HSD) Test and Dunnett's t-Tests and Non-parametric Cochran's Q test were carried out for analyzing the data obtained from different types of dates, and to study the relationship between phytochemicals, antioxidant analysis and proximate composition. Each parameter was measured twice and double checked. SAS, R-studio and Excel were used to perform the statistical analysis and graphical representation of the data. Data were reported as means±standard deviation of the mean. Differences at $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Extraction

In this study, two different extraction techniques, namely maceration and ultrasound-assisted extraction, were compared in order to obtain an extract rich with bioactive compounds (Figure 1). Extraction yields obtained by sonication are higher in all the seven guava varieties than in extraction yields by maceration. Nevertheless, yield of common guava has shown the highest yield in both macerated and sonicated extracts.

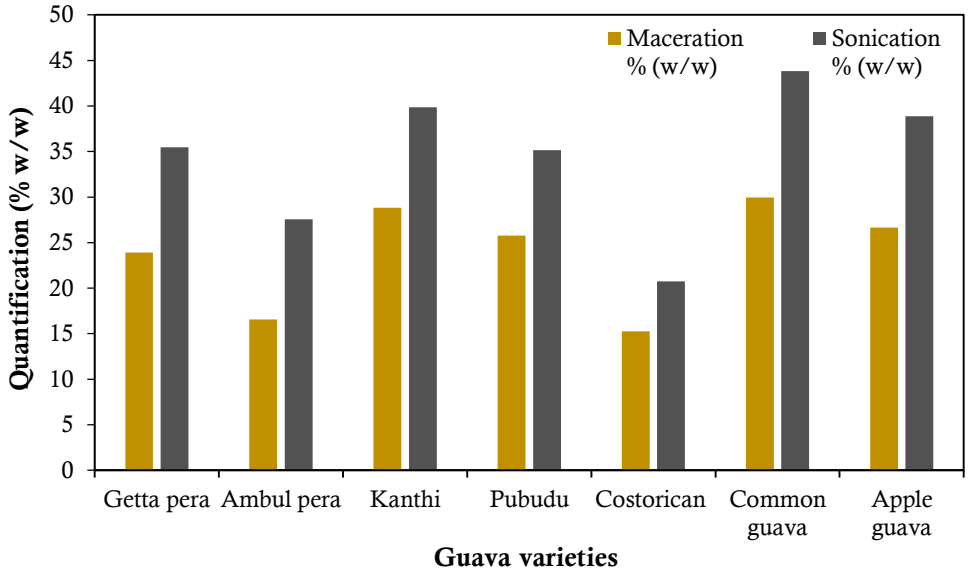

Figure 1: Comparison of extraction yield by maceration and sonication for seven guava varieties.

Phytochemical qualitative screening

The methanolic extracts of common-guava, *Getta-pera*, *Embul-pera*, Apple-guava, *Kanthi*, *Pubudu* and *Costorican* obtained by both sonication and maceration have subjected to phytochemical qualitative analysis and the corresponding results are tabulated in Table 1.

As shown in the Table 1, the study revealed that the entire guava is rich with vast array of important secondary metabolites. Interestingly, chalcones is present only in three introduced guava varieties; *Kanthi*, *Pubudu* and *Costorican*. Non-parametric Cochran's Q test was used to confirm the presence and the absence of the phytochemical in each plant samples. It reveals that there is no significant (P>0.05) difference in phytochemicals in both macerated and sonicated methanolic extracts. Qualitative phytochemical data of guava varieties revealed no differences between the varieties raising the question whether there will be a significant difference in the quantity of them.

Table 1: Results of methanolic extracts of leaves of seven Guava varieties in two extraction conditions, i.e. sonication and maceration. M: Maceration, S: Sonication, P: Present, A: Absent.
1: Common-guava, 2: *Getta-pera*, 3: *Empul-pera*, 4: Apple-pera, 5: *Kanthi*, 6: *Pubudu*, 7: *Costorican*.

Phytochemicals	Test method	1		2		3		4		5		6		7	
		M	S	M	S	M	S	M	S	M	S	M	S	M	S
Alkaloids	1). Mayer's Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	2). Wagner's Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3). Dragendroff's Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Glycosides	1). Keller-kilani Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	2). Modified Borntrager's Test	A	A	A	A	P	P	P	P	P	P	P	P	P	P
	3). Legal's Test	P	P	P	P	P	P	P	P	A	A	A	A	A	A
Flavonoids	1). Alkaline reagent Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	2). Shinoda Test/ Mg turning Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3). Lead acetate Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4). $AlCl_3$ Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	5). NH_4OH Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Saponins	1). Froth Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	2). Olive Oil Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Tannins	1). Bramer's Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	2). Lead Acetate Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Terpenoids	1). Salkowski's Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	2). Liebermann-Burckardt Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3). Copper acetate Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Carbohydrate	1). Fehling's Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	2). Benedict's Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3). Molisch's Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Phenols	1). Ferric Chloride Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Phlobatannins	1). HCl Test	A	A	P	P	A	A	A	A	A	A	A	A	A	A
Protein	1). Xanthoproteic Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	2). Biuret Test	P	P	P	P	A	A	A	A	A	A	A	A	A	A
	3). Ninhydrin Test	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Coumarins	1). UV light Test	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	2). NaOH Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P

Anthocyanins	1). HCl & NH3 Test	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Chalcones	1). NaOH Test	A	A	A	A	A	A	A	A	P	P	P	P	P	P
Phytosterol	1). Salkowski's Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Soluble Starch	1). KOH Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Anthracene Derivatives	1). Chloroform Test	P	P	A	A	P	P	A	A	P	P	P	P	P	P
Anthraquinones	1). Borntrager's Test	P	P	A	A	P	P	A	A	P	P	P	P	P	P
	2). Borntrager's Test 02	A	A	P	P	P	P	A	A	P	P	P	P	P	P
Betacyanin	1). NaOH Test	A	A	P	P	A	A	P	P	P	P	P	P	P	P
Quinones	1). H_2SO_4 Test	P	P	P	P	P	P	P	P	P	P	P	P	P	P

Phytochemical quantification

Quantitative analysis of the pharmacologically important phytochemicals in the selected plants indicated that all the varieties of guava contain polyphenol, flavonoid, tannin, terpenoid, saponin and alkaloid in varying amounts in the leaves as represented in Figure 2.

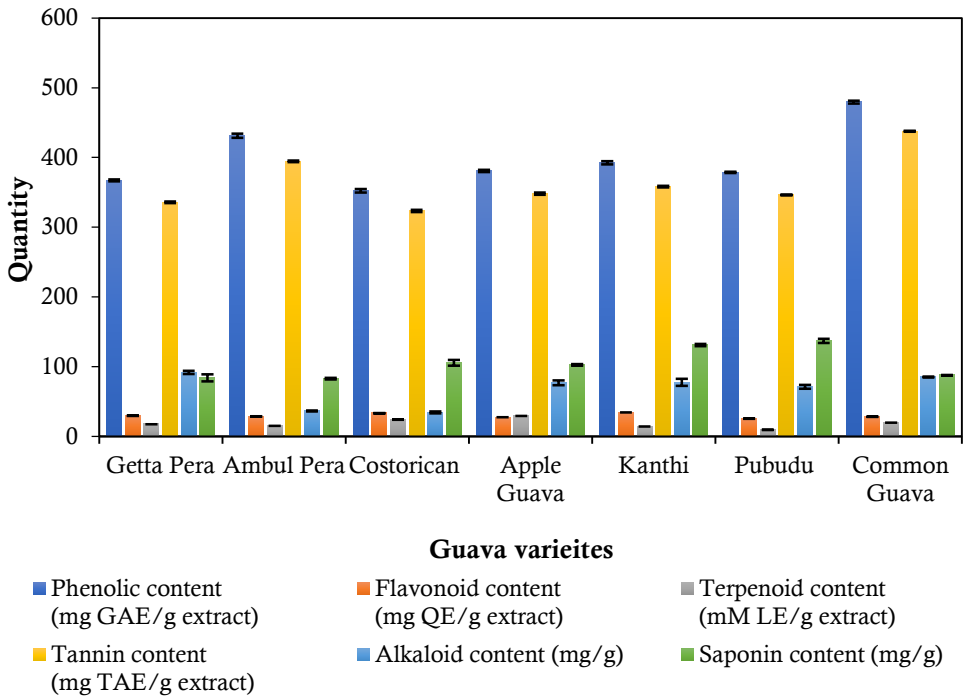

Figure 2: Comparison of phytochemical quantitative analysis data for methanolic extracts of seven guava varieties. (GAE: Garlic acid equivalent, QE: Quercetin equivalent, LE: Linalool equivalent, TAE: Tannic acid equivalent). Each value is the mean of at least three independent experiments ± SD.

As given in Figure 2, the polyphenolic content was found in all the guava varieties between the range of 479.29±2.16 - 352.21±2.72 mg GAE/g. All the guava varieties showed high polyphenolic content and tannin content, compared to other quantified phytochemicals with the highest polyphenolic content in common-guava and the lowest in *Costorican*. Tannin content also showed a similar trend to polyphenol content but with a lower range in quantity: 437.54±0.57 - 323.27±1.71 mg TAE/g. The quantity of flavonoid was particularly high in *Kanthi* and low in *Pubudu*. Total flavanoids, terpenoids, saponins and alkaloids content were quantified but obtained in lower range with respect to phenolic and tannin content. The lowest was observed in terpenoid content in all seven varieties. The range of flavonoid has found in all the guava varieties in between 34.23±0.05 - 25.25±0.13 mg QE/g. Terpenoid showed the range of 29.29±0.09 - 9.26±0.08 mM LE/g.

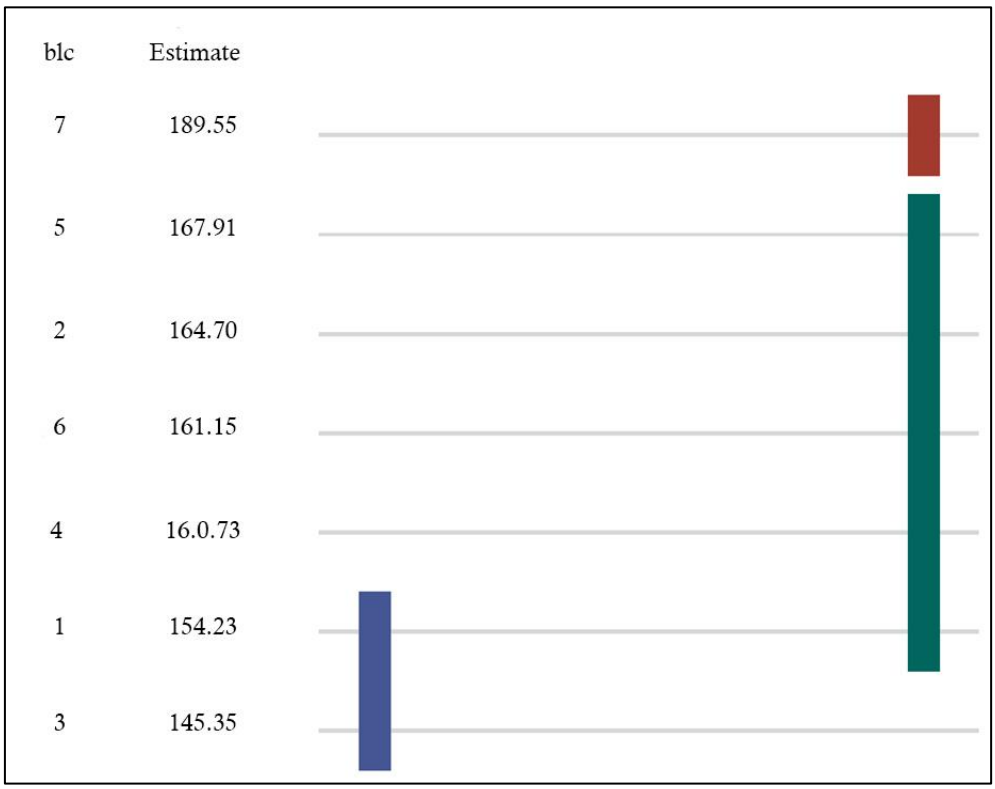

Figure 3: T-test (LSD) for overall phytochemicals quantification of seven guava varieties (Alpha = 0.05). blc: block (guava varieties: 1: *Getta-pera*, 2: *Embul-pera*, 3: *Costorican*, 4: Apple-guava, 5: *Kanthi*, 6: *Pubudu* and 7: Common guava), Estimate: mean value of total phytochemicals (means covered by the same bar are not significantly different).

The quantity of saponin was particularly high in pubudu (136.77±2.90 mg/g) and less in *Embul-pera* (82.60±1.25 mg/g). The alkaloid content was revealed

the range of 91.73±2.41 - 34.23±1.22 mg/g where *Getta-pera* has shown the highest alkaloid content and *Costorican* has shown the lowest content. Statistical analysis (T-test (LSD)) of phytochemical quantification has strongly proved that there is a significant (P<0.05) difference between common-guava and other selected guava varieties. Interestingly, T-test (LSD) clearly indicates (Figure 3) that the common guava shows higher quantity of phytochemicals than other guava varieties while there is a significant difference with all other guava varieties. Also, T-test (LSD) clearly explained, *Kanthi*, *Embul-pera*, *Pubudu*, Apple-guava and *Getta-pera* are comparatively same in quantity of phytochemicals whereas *Costorican* and *Getta-pera* showed no significant (P>0.05) difference between each other. Common guava is different from other selected guava varieties. The statistical analysis strongly indicated that the uniqueness of common-guava in phytochemical quantity.

Antioxidant analysis

The results of DPPH assay are expressed in IC_{50} values (concentration required to inhibit 50% of the oxidative reaction). The results of DPPH assay are shown in Figure 4 and Trolox and Ascorbic acid were used as standards to compare with methanolic extracts of leaves of guava varieties.

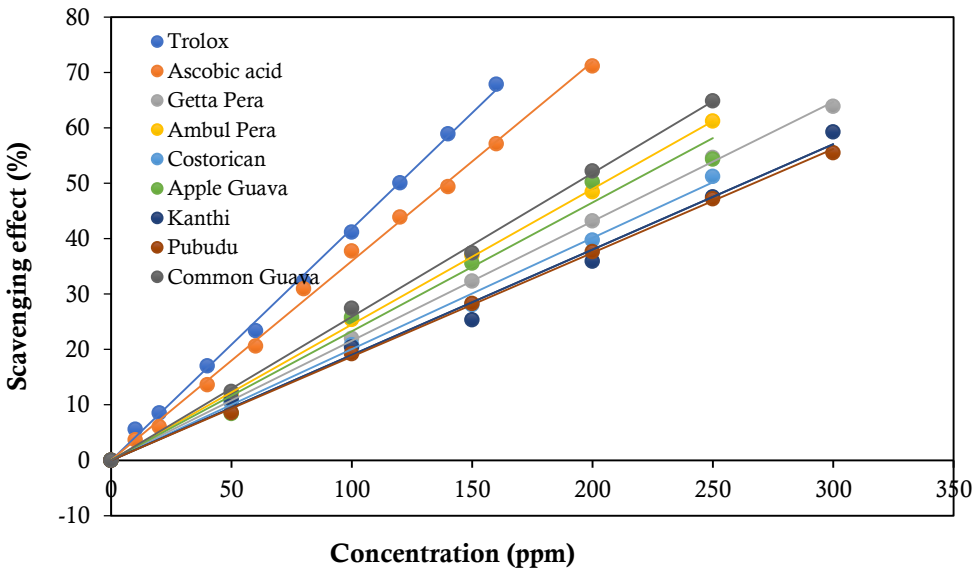

Figure 4: DPPH scavenging activity of standards and methanolic leaves extracts of seven Guava varieties.

According to the results (Figure 4), the highest radical scavenging activity (as characterized by the lowest IC_{50}) was observed in common guava (IC_{50} value: 192.89±0.07 ppm) followed by *Embul-pera*, Apple-guava, *Getta-pera*, *Costorican*, *Kanthi* and *Pubudu*. Hence, the lowest radical scavenging activity (as characterized by the highest IC_{50}) was observed in *Pubudu* (IC_{50} value:

267.10±0.28 ppm). Statistical analysis; T-test (LSD) of DPPH radical scavenging activity perfectly revealed that all the guava varieties were significantly (P<0.05) different (Figure 5). This shows that each guava has their own characteristic feature in radical scavenging activity. Interestingly, common guava has shown the highest radical scavenging activity out seven guava varieties.

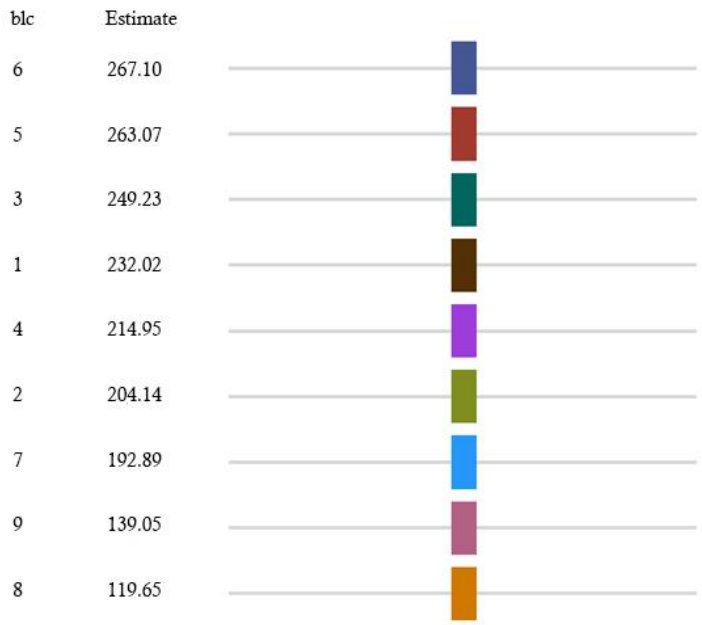

Figure 5: T-test (LSD) for DPPH Radical Scavenging Activity assay (Alpha = 0.05). blc: block (guava varieties: 1: *Getta-pera*, 2: *Embul-pera*, 3: *Costorican*, 4: Apple-guava, 5: *Kanthi*, 6: *Pubudu*, 7: Common guava, 8: Trolox and 9: Ascorbic acid), Estimate: mean of IC_{50} value (means covered by the same bar are not significantly different).

Ferric Reducing Antioxidant Power (FRAP) assay:

Figure 6 shows the ferric reducing power of methanolic leaves extracts of selected guava varieties. These extracts showed variable reducing power indicating that the methanol extract of common-guava leaves has the highest reducing power (722.44±6.58 mg Trolox Eq/g), whereas the least reducing power has indicated by *Costorican* (453.46±3.62 mg Trolox Eq/g).

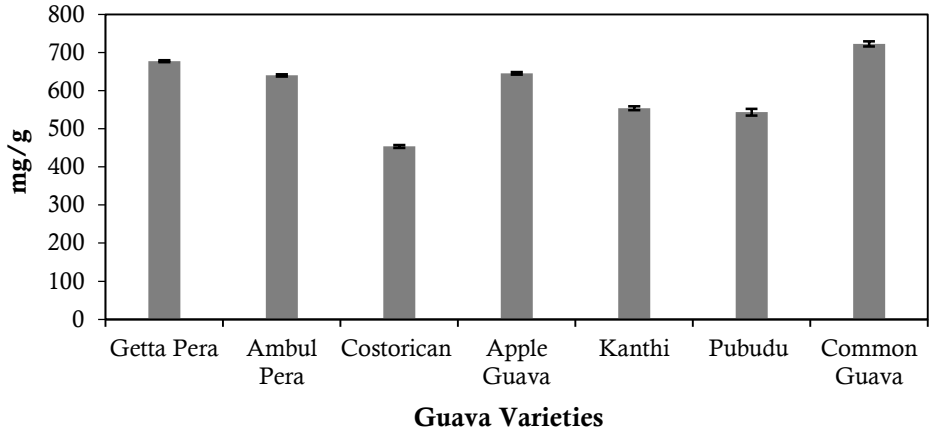

Figure 6: Total antioxidant capacity (in mg Trolox Eq/g) of seven Guava varieties based on FRAP assay.

Even though, Figure 6 shows variation in each variety, statistical analysis; T-test (LSD) has revealed that there are some similarities in reducing power of some guava varieties, but common-guava and it shows a significant (P<0.05) difference between all the guava varieties having the highest potential of reducing power (Figure 7). Table 2 presents the proximate composition of leaves of selected guava varieties.

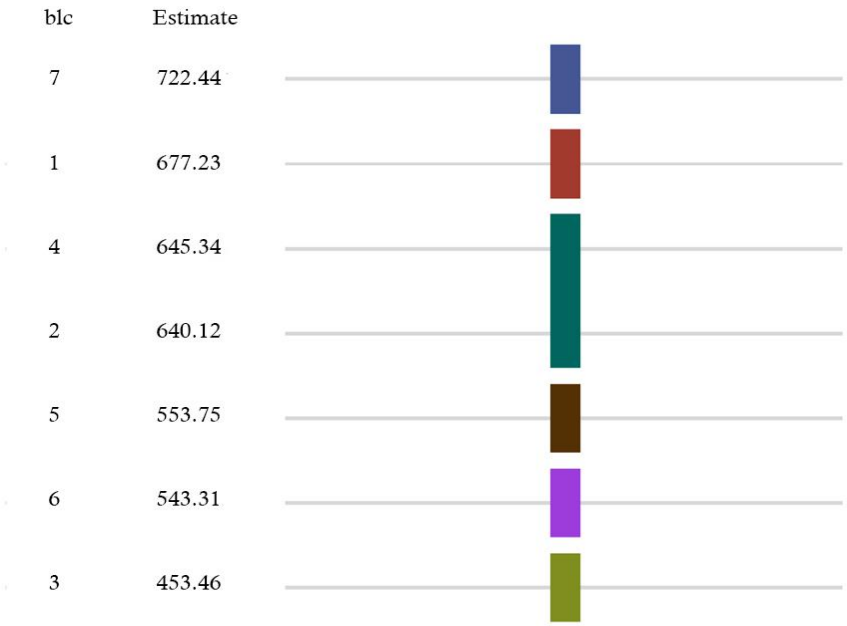

Figure 7: T-test (LSD) for FRAP analysis (Alpha = 0.05). blc: block (guava varieties: 1: *Getta-pera*, 2: *Embul-pera*, 3: *Costorican*, 4: Apple-guava, 5: *Kanthi*, 6: *Pubudu* and 7: Common guava), Estimate: mean of FRAP value (means covered by the same bar are not significantly different).

Proximate analysis

Table 2: Proximate analysis data of leaves of seven Guava varieties. Values represent mean ± standard deviation of triplicate sample.

Plant Name	Moisture (%)	Total solid (%)	Ash (%)	Crude Fat (%)	Crude Fibre (%)	Crude Protein (%)	Carbohydrate (%)	Energy (kcal/100g)
Getta Pera	14.11 ± 0.16	85.89 ± 0.16	4.62 ± 0.06	2.72 ± 0.30	20.96 ± 0.34	9.32 ± 0.14	69.23 ± 0.47	338.62 ± 1.79
Embul Pera	15.02 ± 0.10	84.98 ± 0.10	3.90 ± 0.05	1.80 ± 0.06	26.87 ± 0.52	7.45 ± 0.14	71.83 ± 0.06	333.29 ± 0.40
Common Pera	13.89 ± 0.15	86.11 ± 0.15	5.49 ± 0.08	1.81 ± 0.15	18.05 ± 0.32	7.70 ± 0.14	71.11 ± 0.38	331.54 ± 0.36
Apple Pera	11.22 ± 0.13	88.78 ± 0.13	5.13 ± 0.11	2.14 ± 0.10	18.55 ± 0.78	9.08 ± 0.14	72.43 ± 0.45	345.30 ± 0.70
Kanthi	10.80 ± 0.00	89.20 ± 0.00	3.90 ± 0.03	1.67 ± 0.19	20.48 ± 0.07	9.16 ± 0.14	74.47 ± 0.30	349.52 ± 0.85
Pubudu	11.10 ± 0.06	88.90 ± 0.06	3.70 ± 0.34	1.90 ± 0.08	11.93 ± 0.26	8.67 ± 0.14	74.63 ± 0.34	350.29 ± 1.45
Costorican	10.31 ± 1.40	89.69 ± 1.40	6.51 ± 0.43	0.93 ± 0.03	24.54 ± 0.04	6.89 ± 0.14	75.37 ± 1.09	337.35 ± 4.10

Moisture, total solid, ash, fat, fiber, protein and carbohydrate contents were recorded in percentage of dry material whereas energy was recorded in kcal/100 g. According to the T-test (LSD) statistics, there was a variation between the varieties. Moisture content was significantly similar yet the highest was observed in *Embul-pera* whereas the lowest was recorded in *Costorican*. In contrast to moisture content the highest total solid percentage was observed in *Costorican*. Ash content was significantly (P<0.05) high in *Costorican* whereas less in *Pubudu*. *Getta-pera* showed a significantly (P<0.05) high value in fat determination whereas the least value was observed in *Costorican*. *Embul-pera* has shown the highest fiber content whereas least value was observed in *Pubudu*. Protein content of guava leaves revealed that the *Getta-pera*, *Kanthi* and Apple-guava were relatively same and higher than other varieties whereas the least value was observed in *Costorican*. Carbohydrate was high and the same in *Costorican*, *Pubudu* and *Kanthi* whereas the least carbohydrate content was observed in *Getta-pera*. *Pubudu* and *Kanthi* have shown no significant (P>0.05) difference in energy content and higher than other guava varieties whereas *Embul-pera* and common-guava have revealed no significant (P>0.05) difference and lower than other guava varieties.

CONCLUSIONS

This is the first detailed study on phytochemical composition, proximate and anti-oxidant properties of different guava varieties available in Sri Lanka. All the varieties contain diverse of pharmacologically important phytochemicals but no significant difference in phytochemical profile of leaves among guava varieties selected in this study. Moreover sonication, an accelerated extraction method would be recommended for extraction of phytochemicals efficiently. All the guava varieties possess higher anti-oxidant capacity and common guava is placed at the top out of all. Proximate composition reveals each guava variety has unique characteristic in all the parameters. Therefore, this study will be a repository for the scientific community and general public to get to know about guava varieties and their proximate composition, phytochemical data and antioxidant capacity. Importantly, the information provided in the study will be immensely useful for transforming the leaves into novel value added products and nutraceuticals to be used in heath promoting purposes.

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