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Comparative accounts on proximate and phytochemical compositions and antioxidant properties of *Garcinia quaesita* and *Garcinia zeylanica.*

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ABSTRACT

Garcinia quaesita and Garcinia zeylanica are endemic to Sri Lanka and have been used in wide array of applications with respect to healthcare, food additives, food preservation, etc. nevertheless, the studies on phytochemical, proximate compositions, and antioxidant activity of their leaves have not been documented enough so far. Therefore, this study was aimed on those studies of both plants for the purpose of producing a scientific repository and to be used as support in transforming leaves into functional foods. Plant extraction was done using two techniques i.e., maceration and ultrasound-assisted-extraction and phytochemical screening and proximate composition were done following standard methods. Standard spectrophotometric methods were used for quantification of phytochemicals. DPPH and FRAP assays were used to measure the antioxidant capacity. The results of ultrasound-assisted-extraction showed as an efficient method for extraction of potential phytochemicals. Phytochemical screening reveals that most of the important phytochemicals are available in both garcinia varieties. The higher antioxidant capacity and acceptable proximate composition contain with both varieties showing that each has unique characteristic. The statistical analysis studies showed that G. quaesita is the best than G. zeylanica, showing the highest total antioxidant capacity of 225.63 ± 2.01mg Trolox Eq/g in FRAP assay and highest polyphenolic content (202.14 ± 2.27mg GAE/ g extract) and appreciable proximate composition. As a conclusion, this study provided the detailed analysis of phytochemical, proximate and antioxidant properties of the leaves of both garcinia varieties grown in Sri Lanka to be used by scientific community for further chemical analysis and applications in pharmacological aspects and value addition.

Keywords: Antioxidant, garcinia varieties, maceration, phytochemicals, proximate**,** sonication,

INTRODUCTION

Sri Lanka is one of the focused hot spots for its biodiversity in tropical rainforests where endemic terrestrial evergreen plant is recorded to be approximately 70 % (Gunatilleke *et al*., 2017). However, the potential use of Sri Lankan endemic plants in scientific world has not been in practice enough. In our previous study, we have reported on scientific studies of an endemic plant, *Dialiumovoideum thwaites* for its phytochemical compositions and antioxidant properties (Bulugahapitiya *et al*., 2020). *Garceinia quaesita* (Clusiaceae family) is a plant, endemic to Sri Lanka and locally referred as "Rath Goraka (Red-Goraka, Red fruited)" and commonly called as "Goraka" and Brindle berry in English. *Garcenia zeylanica* is also an endemic plant to Sri Lanka, which also belongs to the same family of *G. quaesita* and locally known as Ëla Goraka/Kaha Goraka (Yellow-Goraka, yellow fruited). The fruits of *G. quaesita* and *G. zeylanica* are used as a condiments or spice and importantly in folk/indigenous medicine. As a major-primary compound is known as hydroxycitric acid (HCA) in its fruits and rinds. Normally fruits are prescribed for ailments in Indian folk tradition and rinds are used as Sri Lankan curry ingredients and condiments (Farzana *et al*., 2010).

As literature on phytochemical, proximate compositions, and antioxidant activity of leaves are not available respect to above two endemic species, this study was aimed on comparative account on phytochemicals and proximate composition, quantification of total polyphenolic, flavonoid, tannin and terpenoid contents and antioxidant capacity of two garcinia varieties; *G. quaesita* and *G. zeylanica* for the purpose of producing a repository to be used for the scientific community and public and intension to replace the demand of Garcinia fruits with Garcinia leaves in pharmaceutical industry.

MATERIAL AND METHODOLOGY

Materials: Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$ 98%), Aluminum chloride anhydrous ($AlCl_3$,98%), Dimethyl sulfoxide (DMSO,99.5%), Folin-Ciocalteu reagent, Sodium carbonate monohydrate ($Na_2CO_3 \cdot H_2O$,99.5%), Gallic acid monohydrate (98%), Tannic acid, Phosphomolybdic acid, 2, 22 -Diphenyl-1-Picrylhydrazyl Radical (DPPH*), 2,4,6-Tripyridyl-S-triazine (TPTZ,99 %), Linalool, Trolox (97%) are come under AR grade, Methanol (MeOH, 99.85%) GC grade and Quercetin (95%) HPLC grade.

Sample collection: Fresh leaves of *G. quaesita* and *G. zeylanica* were plucked (Sample size-03) around Matara, Sri Lanka which were authenticated in Peradeniya botanical garden, Sri Lanka**.** Healthy leaves were washed with tap water followed by distilled water and open-air dried for 3 days. Dried leaves were ground into powder by using grinder to be used in the extraction process.

Extraction: Two extraction techniques; maceration and sonication were used to optimize the extraction method to extract important phytochemicals. Ground powder (20.00 g) was macerated with MeOH (300 ml) for 48 hours at room temperature (RT) (Tachakittirungrod *et al*., 2007) and other part of ground powder (20.00 g) was sonicated by using ultrasound-assisted-extractor (ROCKER Ultrasonic cleaner, Model: SONER 202H) with MeOH (200 ml) for one hour at RT (Musa *et al*., 2011, Nguyen *et al*., 2013). The extracts were filtered through cotton plug and after concentration at 45^0C using a rotary evaporator (HAHNVAPOR, Model No: HS-2005S) crude extracts were obtained and stored at 4 ^{0}C till use.

Phytochemical qualitative analysis: Screening tests for phytochemicals namely alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, carbohydrates, phenol, phlobatannins, protein, coumarins, anthocyanins, chalcones, phytosterol, soluble starch, anthracene derivatives, anthraquinones, betacyanin and quinones were carried out in triplicates for the each methanolic extracts of leaves of two varieties, following the standard procedures described in the literatures with slight changes (Wadood *et al*., 2013,Gayathri *et al*., 2014).

Phytochemical quantitative analysis: The ground powder of methanolic extract (0.10 g) was dissolved in small amount of DMSO and diluted with methanol (100 ml) to make 1000 ppm concentration and used for quantification of phenolics, tannins, flavonoids and terpenoids using spectrophotometric methods.

Total Phenolic contents (TPC) and Total Tannin contents (TTC): The TPC and TTC were determined by Folin and Ciocalteu method which is described in the literature (Abeysuriya *et al*., 2020, Ekwueme *et al*., 2015, Prabhavathi*et al*., 2016). TPC is expressed in Gallic acid equivalents (mg GAE/g of methanolic extract) while TTC is expressed in Tannic acid equivalents (mg TAE/g of methanolic extract).

Total Flavonoid contents (TFC): The TFC was determined by spectrophotometric method with $AlCl_3$ described by Pêkal *et al* (2014) and Fernandes *et al.* (2014).The TFC is expressed in Quercetin equivalents (mg QE/g of methanolic extract).

Terpenoid contents (TC): The TC was determined by spectrophotometric method with phosphomolybdic acid (Ekwueme *et al*., 2015).TheTC is expressed in Linalool equivalents (mg LE/g of methanolic extract).

Antioxidant analysis

DPPH radical scavenging assay: The free radical scavenging activity of methanolic extracts was determined by the method discriped by Mosquera *et al.* (2009) with some slight changes. The DPPH solution in methanol (0.06 mM, 3.0 mL) was mixed with 1.5 mL of methanolic extract at different concentrations. The samples were kept in the dark for 30 minutes, and absorbance was measured at 517 nm (Ascorbic acid was used as the standard).

Ferric Reducing Antioxidant Power (FRAP) Assay: The ferric reducing power of the methanoilic extracts were determined using a standard method described in the literarure (Firuzi *et al*., 2005, Gliszczyñska-Świgło, 2006, Biglari *et al*., 2008). About 3 ml of freshly prepared FRAP reagent (300 mM acetate buffer-pH 3.6: 10 mM TPTZ in 40 mM HCl: 20 mM $FeCl_3$ in 10 : 1 : 1 ratio) was mixed with 100 µL of diluted sample and absorbance at 593nm was recorded after 30 min incubation at 37 °C. Trolox (0–400 ppm) was used for calibration.

Proximate analysis: Standard protocols of Association of Official Analytical Chemists (AOAC) were followed for the estimation of proximate composition such as moisture, ash, crude fiber, crude fat, crude protien, carbohydrate, total solids and energy (Nielsen 2010, Ilodibia *et al*., 2014, Maisarah *et al*., 2014)

Statistical analysis: Different statistics such as analysis of variance (ANOVA), T-test (LSD) (LSD-Least Significant Difference) and Non-parametric Cochran's Q test were carried out for analyzing the data obtained and comparisions. SAS, R-studio and Excel were used to perform the statistical analysis. Data were reported as means ± standard deviation.

RESULTS AND DISCUSSION

Extraction: An efficient extraction method is necessary to get almost all phytochemicals seperated from the plant.Out of many methods, solvent extraction techniques have been widely used. In this study, two different solvent extraction techniques, *i.e.;*maceration (conventional) and ultrasound-assisted-extraction (non-conventional) were compared to obtain extract rich in bioactive compounds. Comparison has been evaluated by using extraction yield and phytochemical qualitative analysis. The Fig.1 indicates about the comparison of both macerated and sonicated extraction yields. Sonicated extraction yields are comparatively higher in both garcinia varieties than macerated one while yield of *G. quaesita* has shown the highest percentage in both macerated and sonicated extraction.

Phytochemical qualitative analysis: The phytochemicals in the methanolic extracts of garcinia varieties is tabulated in Table 1. It showed the presence of highly important secondary metabolites in the leaves of both garcinia varieties. Out of them, flavonoids, saponins, alkaloids, glycosides, phenol, tannins, terpenoids, phytosterol, carbohydrates, proteins, soluble starch, coumarins, anthracene derivatives, betacyanin, anthraquinones and quinones are remarkable in the leaves of both garcinia varieties under two different extraction methods while some phytochemicals such as anthocyanins, phlobatanins and chalcones are absent in methanolic extracts.

Non-parametric Cochran's Q test was analyzed to make sure the presence and the absence of the phytochemical's availability in each plant samples statistically. Non-parametric Cochran's Q test has proofed that both *G. quaesita* and *G. zeylanica* leaves methanolic extracts contain these revealed phytochemicals and there is no significant

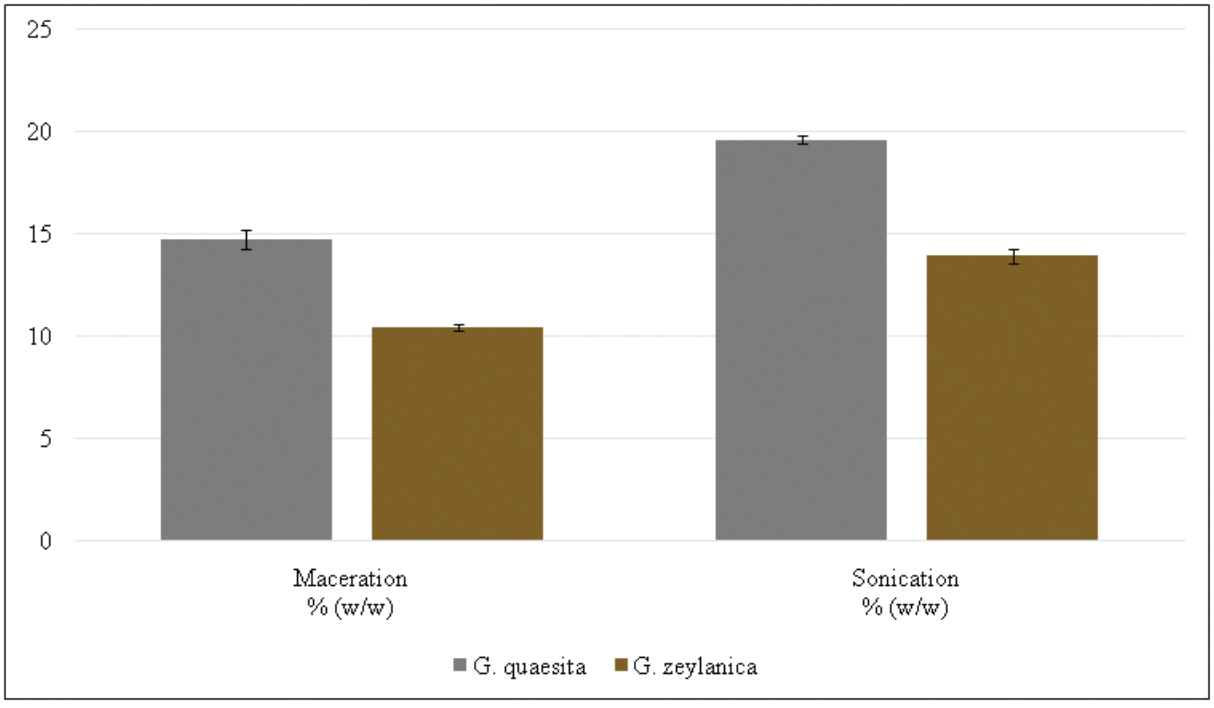

Fig. 1: Comparison of extraction yield by maceration and sonication of two garcinia varieties

Table 1: Statistically analyzed phytochemical screening results of methanolic extracts of leaves of two garcinia varieties under two extraction conditions i.e., sonication (S) and maceration (M).P: Present, A: Absent, 1: *G. quaesita*, 2: *G. zeylanica*.

Phytochemicals	Test method	1		2	
		M	S	M	S
Alkaloids	1). Mayer's Test	P	P	P	P
	2). Wagner's Test	P	P	P	P
	3). Dragendroff's Test	P	P	P	P
Glycosides	1). Keller-kilani Test	P	P	P	P
	2). Modified Borntrager's Test	A	A	A	A
	3). Legal's Test	P	P	P	P
Flavonoids	1). Alkaline reagent Test	P	P	P	P
	2). Shinoda Test/ Mg turning Test	P	A	P	A
	3). Lead acetate Test	P	P	P	P
	4). $AlCl_3$ Test	P	P	P	P
	5). NH_4OH Test	P	P	P	P
Saponins	1). Froth Test	P	P	P	P
	2). Olive Oil Test	P	P	P	P
Tannins	1). Bramer's Test	P	P	P	P
	2). Lead Acetate Test	P	P	P	P
Terpenoids	1). Salkowski's Test	P	P	P	P
	2). Liebermann- Burchardt Test	P	P	P	P
	3). Copper acetate Test	P	P	P	P
Carbohydrate	1). Fehling's Test	P	P	P	P
	2). Benedict's Test	P	P	P	P
	3). Molisch's Test	P	P	P	P
Phenols	1). Ferric Chloride Test	P	P	P	P
Phlobatannins	1). HCl Test	A	A	A	A
Protein	1). Xanthoproteic Test	P	P	P	P
	2). Biuret Test	A	A	A	A
	3). Ninhydrin Test	P	P	P	P
Coumarins	1). UV light Test	A	A	A	A
	2). NaOH Test	P	P	P	P
Anthocyanins	1). HCl & NH3 Test	A	A	A	A
Chalcones	1). NaOH Test	A	A	A	A
Phytosterol	1). Salkowski's Test	P	P	P	P
Soluble Starch	1). KOH Test	P	P	P	P
Anthracene Derivatives	1). Chloroform Test	P	P	P	P
Anthraquinones	1). Borntrager's Test	P	P	P	P
	2). Borntrager's Test 02	P	P	P	P
Betacyanin	1). NaOH Test	P	P	P	P
Quinones	1). H_2SO_4 Test	P	P	P	P

difference in presence chemicals constituents in both macerated and sonicated methanolic extracts of both varieties.

According to the yield percentage, ultrasound-assisted-extraction has shown the highest values in both garcinia varieties whereas phytochemical qualitative analysis has revealed that there is no

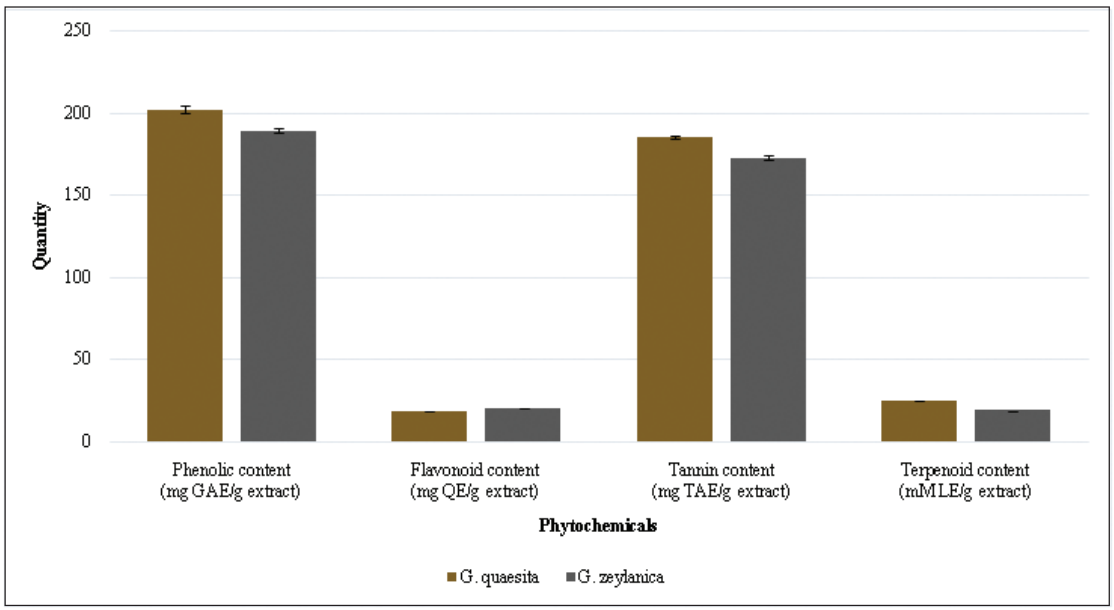

Fig. 2: comparison of phytochemical quantitative analysis data for macerated methanolic extracts of two garcinia varieties.

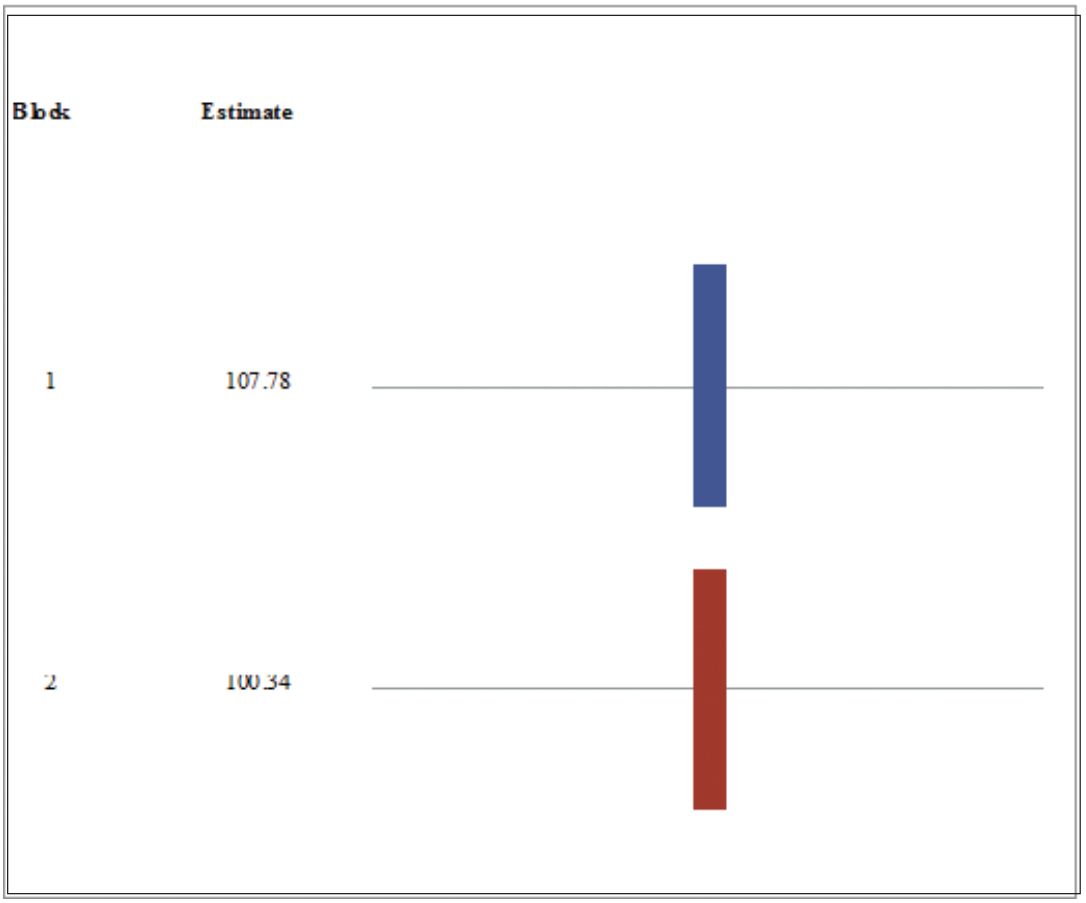

Fig. 3:T-test (LSD) for overall phytochemicals quantification of two garcinia varieties (Alpha = 0.05) (1: *G. quaesita*, 2: *G. zeylanica*, means covered by the same bar are not significantly different).

significant difference in presence of compounds in both macerated and sonicated methanolic extracts of two varieties. As sonication time is shorter (1 hour) than maceration (48 hours) and the consumption of solvent in sonication is also less than maceration, sonication would be recommended to extract active chemical constituents. This result supports the outstanding pharmacological activities associated with garcinia, which have been reported by various researchers and the applications of garcinia in traditional medicine. Even though, qualitative phytochemical data of both garcinia varieties revealed no significant differences between them, raising a question whether there will be a significant difference in the quantity of them.

Phytochemical quantitative analysis

Quantitative analysis of some important phytochemicals such as polyphenol, tannin, flavonoid, and terpenoid in the leaves indicated that both *G. quaesita* and *G. zeylanica* contain in varying amounts and the data is represented in Fig. 2. Interestingly, polyphenolic, tannin and terpenoid contents were high in *G. quaesita*; 202.14 ± 2.27 mg GAE/g extract, 185.45 ± 0.86 mg TAE/g extract and 25.23 ± 0.03 mM LE/g extract, respectively. Contrastingly, flavonoid content was high in *G. zeylanica* (20.29 ± 0.10 mg QE/g extract).

Total phytochemicals quantity was statistically analyzed by using T-test (LSD) and which is shown in Fig. 3. The statistical analysis clearly concludes that there is a significant difference in between both garcinia varieties and interestingly *G. quaesita* shows the highest phytochemical contents.This phytochemical quantification strongly revealed about the importance of each variety of garcinia, and the statistical analysis indicated that the uniqueness of both garcinia varieties in phytochemical quantity.

Antioxidant analysis

DPPH and FRAP assays were used. The results of DPPH assay are expressed in IC_{50} values while the results of FRAP assay are expressed in mg Trolox Equivalents/g extract and the results of both assays have been presented in Table 2.

Table 2: Antioxidant analysis data of methanolic leaf extracts of two garcinia varieties. Values represent mean ± standard deviation of triplicate sample (TE: Trolox Equivalents)

Antioxidant Assay	*G. quaesita*	*G. zeylanica*	Ascorbic acid
FRAP assay (mg TE/g extract)	225.63 ± 2.01	154.91 ± 6.96	N/A
DPPH assay IC_{50} value (ppm)	47.45 ± 0.03	39.59 ± 0.02	4.06 ± 0.01

According to the results of radical scavenging assay, the highest radical scavenging activity was observed in *G. zeylanica*. The statistical analysis with T-test (LSD) of DPPH assay perfectly revealed that both *G. quaesita* and *G. zeylanica* are statistically significance while *G. zeylanica* has shown the highest radical scavenging activity out of selected a garcinia variety which is shown in Fig.4. As phytochemical qualitative and quantitative analysis have revealed about the availability of vast array of bioactive compound present in both *G. quaesita* and *G. zeylanica* methanolic leaves extracts, further studies needed to reveal the pharmacological properties of these two Sri Lankan endemic species.

According to the FRAP assay, both *G. quaesita* and *G. zeylanica* extracts had reducing power but not at the same level. The result clearly indicated that the macerated methanolic extract of *G. quaesita* leaves had higher reducing power than *G. zeylanica*. Fig. 5 explains about the statistical analysis of FRAP assay for garcinia varieties. According to the T-test (LSD), FRAP assay also clearly revealed that there are significant differences between the varieties and interestingly, FRAP assay clearly revealed *G. quaesita* having the highest potential of reducing power.

Proximate analysis

Table 3 presents the proximate composition of leaves of selected garcinia varieties. 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) statistic, there are variation between the varieties.

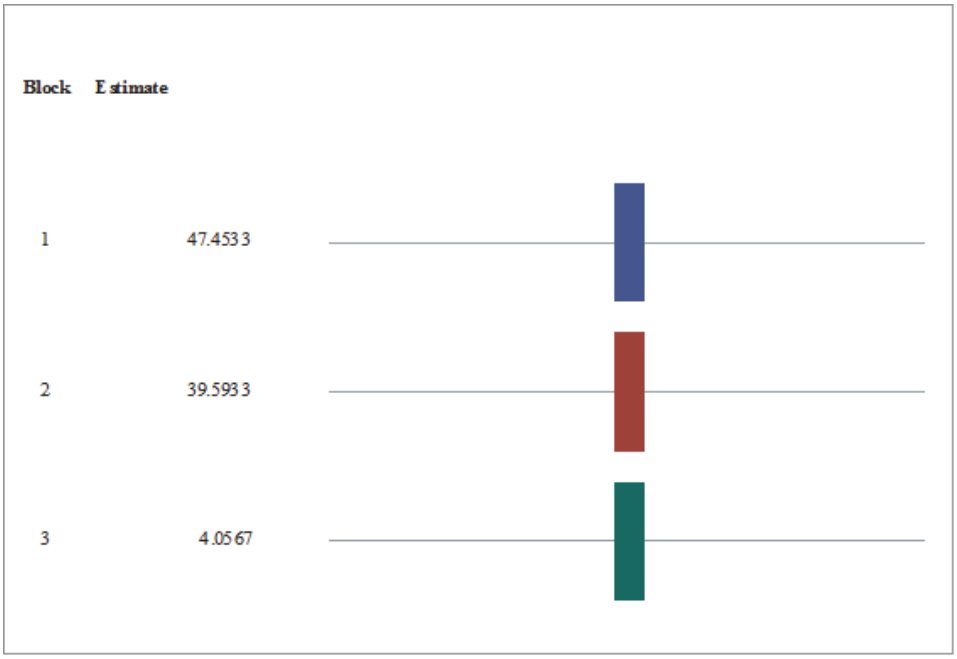

Fig. 4: T-test (LSD) for DPPH radical scavenging activity assay of methanolic extract of two garcinia varieties (Alpha = 0.05) (1: *G. quaesita*, 2: *G. zeylanica*, 3: Ascorbic acid, means covered by the same bar are not significantly different).

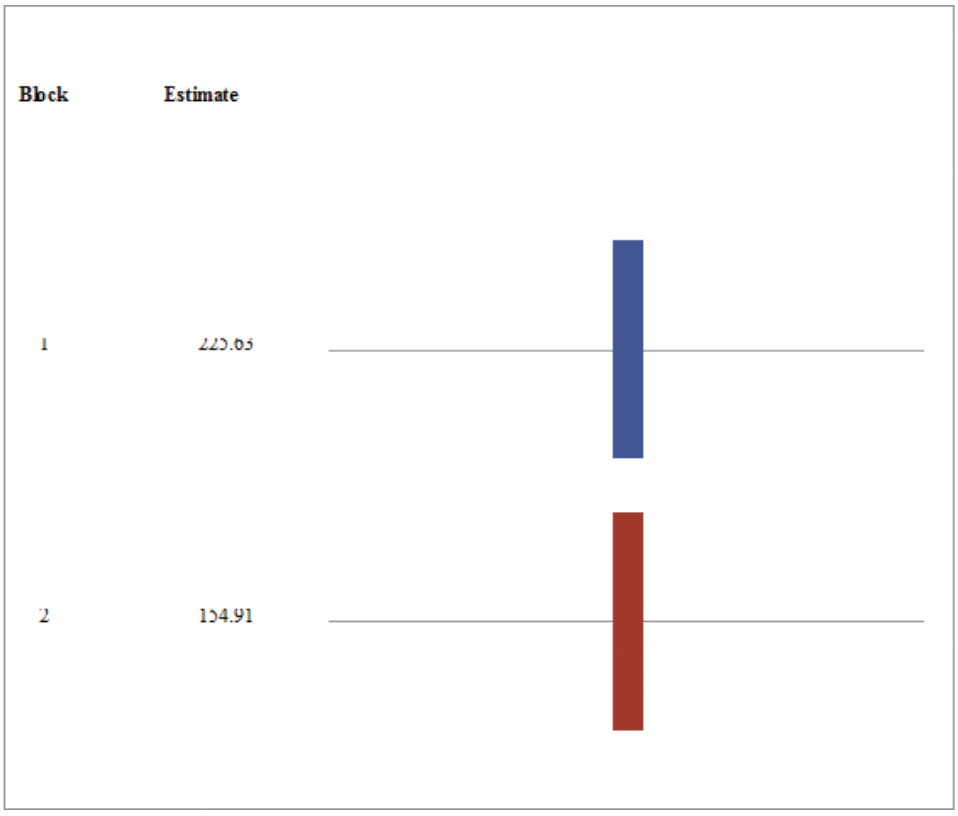

Fig. 5: T-test (LSD) for FRAP analysis of methanolic extract of two garcinia varieties (Alpha = 0.05)(1: *G. quaesita*, 2: *G. zeylanica,* means covered by the same bar are not significantly different).

Table 3: Proximate analysis data of dried leaves of two garcinia 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)
G. quaesita	17.32 ± 0.03	82.68 ± 0.03	4.95 ± 0.60	2.47 ± 0.13	27.83 ± 1.08	8.75 ± 0.00	66.51 ± 0.74	323.24 ± 1.82
G. zeylanica	22.16 ± 0.06	77.84 ± 0.06	4.39 ± 0.10	2.34 ± 0.22	26.23 ± 0.05	9.64 ± 0.14	61.47 ± 0.30	305.49 ± 0.85

According to the T-test (LSD) statistic, moisture content was significantly different between two varieties yet highest was observed in *G. zeylanica*. In contrast to moisture content the highest total solid percentage was observed in *G. quaesita* whereas ash, fat and fiber contents were significantly no differences between two varieties. Protein and carbohydrate contents of garcinia leaves revealed that there is significant difference between the two varieties and protein content of *G. zeylanica* was relatively higher than *G. quaesita*. In contrast, carbohydrate content was higher in *G. quaesita* than *G. zeylanica*. Finally, energy level in garcinia leaves have shown significant differences each other but out of both varieties *G. quaesita* has revealed the highest energy level. Therefore, this proximate analysis of garcinia leaves clearly indicated that each variety having proximate composition at different levels.

CONCLUSION

This is the first detailed study on phytochemical, proximate composition, and antioxidant properties of leaves of two Sri Lankan endemic Garcinia varieties *viz*., *G. quaesita* and *G. zeylanica*. All the varieties contain diverse of pharmacologically important bioactive chemical compositions but no significant difference in phytochemical profile of leaves among both garcinia varieties selected in this study. Moreover ultrasound-assisted extraction, an accelerated extraction method would be recommended for extraction of bioactive compounds efficiently. Both garcinia varieties show antioxidant capacity and *G. quaesita* is placed at the top. Proximate composition reveals each variety has unique characteristic in most of the parameters. Therefore, this study will be a repository for the scientific community to be used in further studies and give overview on suitability on value addition via functional foods and nutraceuticals to be used in health promotion aspects.

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