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Variation of Phytochemical Content and Antioxidant Capacity of Domesticated and Non-Domesticated *Momordica Charantia* L. Populations in Different Maturity Stages

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Abstract *Momordica charantia* Linn. commonly known as bitter melon or bitter gourd is an annual plant, belongs to family Cucurbitaceae. Bitter gourd possesses antidiabetic, anticancer, anti-inflammatory, antivirus, and cholesterol lowering effects. The content and composition of bioactive molecules are varied according to the plant parts and maturity levels of the plant. However, phytochemical distribution of leaves and fruits at different maturity stages of domesticated and non-domesticated populations of M. charantia populations cultivated in Sri Lanka is scattered or lacking. Therefore, the present study was undertaken to determine the phytochemical distribution of leaves and fruits of domesticated and non-domesticated populations of M. charantia at different maturity stages. Fruits were harvested at three different maturity stages viz. 10 days (immature), 20 days (mature) and 30 days (ripen) after fruit set. Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were determined using Ferric Reducing Antioxidant Power (FRAP) assay, modified Folin-Ciocalteu colourimetric method and the colourimetric method respectively. Results revealed that TPC and TAC were higher in immature stages and decreased with the maturity. However, values were slightly increased at ripening stage. Significantly higher TPC, TFC and TAC were reported in leaves than fruits. In conclusion, since most of the tested phytochemicals were high in immature fruits and leaves of domesticated and non-domesticated populations of *Momordica charantia*, immature fruits and leaves can be recommended for the production of pharmaceuticals and nutraceuticals with elevated therapeutic activity.

Keywords: *antioxidant capacity, Bitter gourd, flavonoids, Momordica charantia, phenolics*

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1. Introduction

Momordica charantia Linn. commonly known as bitter gourd belonging to the family Cucurbitaceae is a herbal climber grown in tropical and subtropical regions [1]. It is indigenous to Asia, South America and widely distributed in China, Malaysia, India, Tropical Africa and America [2]. In Sri Lanka, it can be grown in all over the country during both *Yala* and *Maha* seasons [3]. It is an important vegetable containing the high amount of ascorbic acid, vitamin A and C, iron and other minerals [4,5]. Bioactive phytochemical constituents of bitter gourd produce definite physiological effects on human body and protect them from various diseases [6]. It is used for the treatments for various diseases in *Ayurveda* and traditional systems of medicine in Sri Lanka. However, scientific information on the variation of phytochemical content and antioxidant capacity of leaves and fruits of different *M. charantia* populations and different stages of maturity are scattered. Therefore, there is an urgent necessity of investigation of phytochemical content and antioxidant capacity of existing domesticated and non-domesticated

populations *Momordica charantia* at different maturity stages.

2. Materials and Methods

2.1. Establishment of Research Plots

Collected seeds of seven populations, *M. charantia* through a systematic survey in Sri Lanka, Thinnaweli White (TW), MC-43, Matale Green (MG), Hybrid, *Kalu Karavila (KK*), *Geta Karavila (GK)* and Small population (SM1) were planted in 5 m x 1.6 m x 0.15 m planting beds with 1.5 m x 1 m spacing. All the agronomic practices were carried out according to the recommendations of Department of Agriculture.

2.2. Preparation of Samples

For evaluation of phytochemical distribution, fruits were harvested at 10 days (10D-immature), 20 days (20D-mature) and 30 days (30D-ripen) after fruit set. Leaf samples were also randomly collected from the top, middle and lower part of the vine.

Both fruits and leaves were cut into small pieces and air dried for three days at room temperature (28 ± 2°C). Then samples were powdered using motor and pestle and sieved with 0.25 mm mesh. Powdered sample (0.1 g) was mixed with 5 mL of 80% methanol vortexed for 15min. Then it was placed in a water bath at 60°C for 40min and the vortex procedure was repeated at 10 minutes intervals. After centrifugation at 4,000 rpm for 5 min, the supernatant was decanted into a 15 mL centrifuge tube and the remaining was re-extracted with 5 mL of 80% methanol. Supernatants were pooled and stored at-20°C prior to analysis.

2.3. Total Phenolic Content (TPC)

Total phenolic content was quantified using a modified Folin-Ciocalteu method [7]. Briefly, 4 mL of distilled water and 0.5mL of properly diluted sample extract were mixed with 0.5mL of 0.5 N Folin-Ciocalteu reagent (FCR) and allowed to react for 3 min. Then 1mL saturated sodium carbonate solution was mixed and incubated in a water bath for 2 hr at 30°C. The absorbance were measured at 760 nm using UV visible spectrophotometer (Shimadzu, UV Mini 1240, and Japan). Gallic acid was used as the standard and data were expressed as mg of Gallic Acid Equivalent (GAE) per gram of dry weights.

2.4. Quantification of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined by a colorimetric method, with slight modifications [8]. Briefly, 0.5mL of the plant extract was diluted with 3.5mL of distilled water. Then 0.3mL of 5% $NaNO_2$ solution was added to the mixture. After 6min, 0.3mL of a 10% $Al(NO_3)_3$. $6H_2O$ solution was added, and the mixture was allowed to stand for another 6min. Then 2mL of 2 M NaOH was added, and top up to 8mL with distilled water. After thoroughly mixing, the absorbance was measured at 510 nm using UV visible spectrophotometer (Shimadzu UV-160, Japan). Rutin was used as the standard and data were expressed as mg of Rutin Equivalent (RE)/g DW.

2.5. Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using Ferric Reducing Antioxidant Power (FRAP) assay [9], with slight modifications. Methanolic extract of sample (100µL) was mixed with 900 µL of freshly prepared FRAP reagent of pH 3.6 containing 2.5 mL of 10mol/L, 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution in 40 mmol/L, HCl plus 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 300 mol/L acetate buffer. Absorbance was measured at 593 nm using the spectrophotometer (Shimadzu, UV Mini 1240, Japan) after incubating for 4min. Trolox was used as the standard solution and TAC was expressed as mg Trolox Equivalents (TE)/g DW.

2.6. Statistical Analysis

To verify the statistical significance of all parameters, the values of means and ± SD were calculated. Statistical comparison of mean values was performed by General Linear Model (GLM) of ANOVA followed by Tukey Multiple Range Test using SAS (SAS institute, 1999).

3. Results and Discussion

In the present study, fruits and leaves of 7 populations of domesticated and non-domesticated *M. charantia* were collected from the plants established in same soil and climatic conditions in a same growing season. Therefore, obtained results reflect the variations of phytochemical content due to true populational and maturity levels. *M. charantia* is generally harvested at immature or mature stages for consumption and at ripen stage for seed collection. However, the therapeutic activity of plant materials may change on the phytochemical content and antioxidant capacity of different plant parts and during the growth and maturity [10,11].

3.1. Variation of TAC and Phytochemicals in Fruits of *M. Charantia*

All tested maturity stages of *M. charantia* fruits exhibited the marked content of TPC and TAC and significantly varied among different populations. Greater content of TPC and TAC were observed in fruits harvested at the immature stage and decreased with maturity (Table 1). These findings are in agreement with Horax *et al*, who reported that the TPC changed significantly during fruit maturation in pericarp and seeds of *M. charantia.* Moreover, Siriamornpun and Kaewseejan [12] reported that TPC was superior in green fruits and clearly decreased with ripening. Further, Kubola and Siriamornpun [13], who investigated green fruit extract of *M.charantia* possessed the highest value of antioxidant activity meanwhile, slightly increment of TPC and TAC in ripening stage. This may be due to the significant

increment of total anthocyanin content during the fruit ripening stage [14] The slight increment of TPC and TAC during ripening are in agreement with Olaniyi *et al*. [15], who observed an increase of TSS, sugar, and TPC with advancing maturity of pomegranate (cv. Ruby) fruit. Total phenolic content of Non-domesticated populations at the immature stage of fruit was varied between 3.09 ± 0.12 to 5.96 ± 0.05mg Gallic acid equivalent/g DW whereas, TPC of domesticated populations at the immature stage of fruit varied between 2.45 ± 0.08 to 5.18 ± 0.14mg Gallic acid equivalent/g DW. Moreover, TAC of non-domesticated populations at the immature stage of fruit was ranged 2.52 ± 0.13 to 4.41 ± 0.06 mg Trolox equivalent/g DW while TAC of domesticated populations at the immature stage of fruit was ranged 2.63 ± 0.23 to 4.31 ± 0.26 mg Trolox equivalent/g DW. The reduction in antioxidant activities during bitter gourd fruit development may be associated with an apparent decrease in the quantity of polyphenols in the fruit [16,17]. Anthocyanin is known as antioxidant compounds and their accumulation have been observed during fruit development. A significant ($p < 0.05$) increase in antioxidant capacity in fully ripened fruits could be due to the further significant accumulation of anthocyanin in the fully ripened fruits (Olaniyi *et al*.,).

3.2. Variation of TAC and Phytochemicals in Leaves of *M. Charantia*

As shown in Table 2, marked TAC and TPC were observed in leaf extract of all seven populations of bitter gourd tested. These results are in agreement with Kubola and Siriamornpun, who reported the highest value of antioxidant activity in leaf extract of *M. charantia* compared to the fruits. The highest TPC and TAC were recorded in leaf extract of population *Geta Karavila* (10.22 ± 0.33 mg GAE/g DW and 7.80 ± 0.25mg TE/g DW respectively). The lowest TPC and TAC were recorded in leaf extracts of Hybrid and SM1 (7.59 ± 0.15 mg GAE/g DW and 4.98 ± 0.29 mg TE/g DW respectively). The highest TFC was recorded in leaf extract of the Hybrid population (14.67 ± 0.54 mg RE/g DW). The lowest TFC was recorded in leaf extract of *Kalu Karavila* (8.43 ± 0.74 mg RE/g DW).

Table 1. Total Phenolic Content (TPC) and Total Antioxidant Capacity (TAC) of domesticated and non-domesticated populations of *Momordica charantia* harvested at different maturity stages

Nature of Population	Population	Maturity Stage	TPC (mg GAE/g DW)	TAC (mg TE/g DW)
Domesticated	TW	Immature	5.18 ± 0.14^{b}	3.83 ± 0.33^{cd}
		Mature	2.94 ± 0.83^{ef}	2.21 ± 0.08^{hi}
		Ripen	5.11 ± 0.40^{b}	3.40 ± 0.15^{de}
	MC43	Immature	3.47 ± 0.15^{cde}	4.31 ± 0.26^{ab}
		Mature	3.29 ± 0.26^{cde}	3.88 ± 0.35^{bc}
		Ripen	3.50 ± 0.24^{cde}	3.98 ± 0.26^{abc}
	MG	Immature	3.23 ± 0.12^{cde}	3.23 ± 0.13^{e}
		Mature	2.08 ± 0.15^{gh}	2.29 ± 0.13^{ghi}
		Ripen	3.74 ± 0.23^{cd}	3.11 ± 0.08^{ef}
	Hybrid	Immature	2.45 ± 0.08^{fg}	2.63 ± 0.23^{gh}
		Mature	1.16 ± 0.24^{i}	1.34 ± 0.05^{k}
		Ripen	3.10 ± 0.02^{cdef}	1.58 ± 0.16^{jk}
Non-domesticated	KK	Immature	3.09 ± 0.12^{ef}	3.70 ± 0.09^{cd}
		Mature	2.95 ± 0.09^{ef}	3.19 ± 0.12^{ef}
		Ripen	5.36 ± 0.30^{ab}	3.41 ± 0.18^{de}
	GK	Immature	3.41 ± 0.15^{cde}	2.52 ± 0.13^{gh}
		Mature	1.48 ± 0.18^{hi}	1.89 ± 0.22^{ij}
		Ripen	3.55 ± 0.06^{cde}	2.47 ± 0.07^{gh}
	SM1	Immature	5.96 ± 0.05^{a}	4.41 ± 0.06^{a}
		Mature	3.79 ± 0.14^{c}	2.20 ± 0.06^{hi}
		Ripen	4.88 ± 0.14^{b}	2.75 ± 0.05^{fg}

Means denoted by the same letters in a column represent non-significant differences($p<0.05$); TE-Trolox Equivalent; GAE-Gallic Acid Equivalent; DW-Dry Weight; TW-Thinnaweli White; MG-Matale Green; KK-Kalu Karavila; GK-Geta Karavila; SM1-Small population.

Table 2. Phytochemical contents and antioxidant capacity of leaves of domesticated and non-domesticated *Momordica charantia* populations

Nature of Population	Population	TPC (mg GAE/g DW)	TFC (mg RE/g DW)	TAC (mg TE/g DW)
Domesticated	TW	8.39 ± 0.12^{abc}	12.29 ± 0.89^{ab}	5.21 ± 0.29^{d}
	MC 43	10.16 ± 0.53^{a}	11.05 ± 0.21^{bc}	6.64 ± 0.36^{b}
	Hybrid	7.59 ± 0.15^{bc}	14.67 ± 0.54^{a}	5.81 ± 0.09^{cd}
	MG	9.95 ± 0.03^{ab}	13.19 ± 0.23^{ab}	6.10 ± 0.34^{bc}
Non-domesticated	*GK*	10.22 ± 0.33^{c}	13.14 ± 0.25^{ab}	7.80 ± 0.25^{a}
	KK	9.75 ± 0.15^{ab}	8.43 ± 0.74^{c}	6.05 ± 0.36^{bc}
	SM1	9.00 ± 0.60^{abc}	13.38 ± 0.16^{ab}	4.98 ± 0.29^{d}

Means denoted by the same letters in a column represent non-significant differences($p<0.05$); TE-Trolox Equivalent; RE-Rutin Equivalent; GAE-Gallic Acid Equivalent; DW-Dry Weight; TW-Thinnaweli White; MG-Matale Green; KK-Kalu Karavila; GK-Geta Karavila; SM1-Small population.

4. Conclusions

Variation in phytochemicals in different populations of *Momordica charantia* at different maturity stages was investigated in order to provide useful information regarding quality changes during fruit development. Results obtained showed that immature fruits and leaves contain higher levels of TPC and TAC and TFC and hence it could be concluded that immature fruits and leaves could be effectively incorporated into the production of value-added nutraceuticals for better therapeutic activity.

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