

Phytochemical, Physiochemical and Mineral Contents of Domesticated and Non Domesticated Populations of *Momordica charantia* L. Seeds Harvested at Two Maturity Stages

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Abstract *Momordica charantia* L. is a therapeutically important medicinal plant belonging to family Cucurbitaceae and extensively consumed as a vegetable and used as a treatment for an array of ailments in *Ayurveda* and traditional systems of medicine in Sri Lanka. Seeds of *Momordica charantia* contain an array of bioactive molecules including phenolics, carotenoids and rich source of physiochemical constituents, minerals, conjugated linoleic acid (CLA), conjugated linolenic acid (CLnA) and hence seeds possess anticancer, antitumor, anti-mutagenic, antioxidant, anti-diabetic, anti-inflammatory and anti-atherosclerotic activities. Phyto-constituents and therapeutic activities are depend on plant species or variety, their genetic makeup and maturity stages. Therefore, the present study was undertaken to determine phytochemical physiochemical and mineral composition of six populations of *Momordica charantia* seeds harvested at two different maturity stages. Physiochemical composition was determined according to official AOAC method. Total Antioxidant Capacity (TAC) and Total Phenolic Content (TPC) were determined using Ferric Reducing Antioxidant Power (FRAP) assay and Folin-Ciocalteu method respectively. Mineral content was determined using Atomic Absorbance Spectrophotometric method. There were significant differences ($P < 0.05$) in moisture, dry matter content, ash content, crude fat and crude protein among mature and ripen stages. While maturity progressed crude protein, crude fat, crude fiber and dry matter content were increased and moisture content was decreased. Mineral contents in *Momordica* seeds were varied between maturity stages as well as different populations. The highest mineral content was observed in undomesticated population. TAC and TPC decreased when maturity progressed and the highest TAC and TPC were observed in mature stage of *Momordica* seeds. Therefore, it is suggested to exploit undomesticated *M. charantia* populations with elevated phytonutrient contents for pharmaceutical and nutraceutical industries.

Keywords: antioxidant capacity, mineral content, *Momordica charantia*, phenolics, proximate composition

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1. Introduction

Momordica charantia L. (Bitter gourd) also known as bitter melon belongs to family Cucurbitaceae is mostly cultivated for its use as vegetable but well known for its medicinal properties [9]. According to Nagarani *et al.* [15] different *Momordica* plant parts contain various chemical constituents and bioactive substances. Medicinal value of *Momordica* seeds has received an ample attention in

recent literature due to its potential health benefits such as anticancer, antitumor, anti-mutagenic properties [15]. Bitter gourd seeds contain an array of bioactive compounds including phenolics, carotenoids, cucurbitane, triterpenoids and phytosterols. Further, seeds are rich in catechin, epicatechin, and gallic acid [9,15]. Due to presence of these molecules, *Momordica* seeds exhibit a wide range of therapeutic properties including anti-diabetic, anticancer and antioxidant [15]. When assessing nutritional significance of plant species, plant physiochemical composition plays a major role. Physiochemical composition of bitter gourd

seeds can be varied according to maturity stages and between different populations. According to Anjum *et al.* [5], bitter gourd seeds have been identified as a rich source of fat and protein. Hence, bitter gourd seeds are good source of essential amino acids such as methionine, cysteine, isoleucine, phenylalanine, tyrosine and lysine and could be a good source of protein for functional ingredients in a food system. Bitter gourd seeds are rich in glycoprotein [9] and good source of several minerals such as Ca, Mg, and Na. Due to the presence of these minerals aid in digestion, formation of strong bone and hemoglobin [2]. Bitter gourd is one of a few edible fruits containing conjugated linolenic acid (CLnA) in its seeds. The lipid profiles of seed oil have received ample attention due to its high content of polyunsaturated fatty acids and other bioactive compounds [12, 20]. Due to the presence of high content of CLnA (67.7%) bitter gourd seeds have numerous health benefits such as antioxidant, anti-inflammatory, anti-atherosclerotic, and anti-tumor *in vitro* and *in vivo* serum lipid-lowering activities [12,14,20]. Even though, several domesticated and non-domesticated populations of *Momordica charantia* cultivated in Sri Lanka, information on bioactive molecules present in seeds at different maturity stages are lacking or scattered. Therefore, present study was undertaken to investigate the phytochemical, physicochemical and mineral compositions and antioxidant capacity of seeds of six *Momordica charantia* populations (Domesticated and non-domesticated) harvested at mature and ripening stages.

2. Materials and Methods

2.1. Location

Study was carried out in the laboratories of Faculty of Agriculture and Plantation Management and Faculty of Livestock Fisheries and Nutrition, Wayamba University of Sri Lanka from May to October, 2017.

2.2. Collection of Seed Samples

Six *M. charantia* populations were selected for this study. Thinnaweli White (TW), MC43, Matale Green (MG) populations were considered as domesticated populations whereas, *Kalu Karawila (KK)*, *Geta Karawila (GK)* and SMI were considered as non-domesticated populations. Selected populations were identified and herbarium specimens were prepared and deposited at Institutional Herbarium. Seed samples of above populations were collected from Manikhinna in Kandy district of Central Province and Mahailuppalama in Anuradhapura district of North Central Province of Sri Lanka. All collected seed samples were cultivated in the University Research plots [Low Country Intermediate-Zone (IL1a), at an elevation of 25 m above mean sea level] and seeds were collected from mature and ripened pods at 20 and 30 days after fruit setting respectively.

2.3. Preparation of Samples

Collected seed samples were air dried for three days at room temperature (28±2°C). Then samples were

powdered by 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 15 min. Then it was placed in a water bath at 60°C for 40 min and vortex procedure was repeated at 10 min interval. 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 5mL of 80% methanol. Supernatants were pooled and stored at -20°C prior to analysis.

2.3.1. Determination of Total Antioxidant Capacity (TAC) and Total Phenolic Content (TPC)

Total antioxidant capacity and Total phenolic content were determined using Ferric Reducing Antioxidant Power (FRAP) assay [7] and modified Folin-Ciocalteu method [1] respectively.

2.3.2. Determination of Proximate Composition

The proximate analysis (Moisture, fiber, ash, fat and protein contents) of all the seed samples was determined. The moisture and ash contents were determined using the methods described by AOAC (1990). The fat content of the samples was determined using the Soxhlet extraction method [6]. The crude fiber content was determined using fiber analyzer [4]. The crude protein content was determined using the Kjeldahl method [6].

2.3.3. Determination of Mineral Composition

Mineral contents were determined by using Atomic Absorbance Spectrophotometer (icetm 3000 series Thermo Scientific, U.S.A). Ten milliliters of concentrated HNO₃ was added to 0.5 g of the seed powder sample in a reaction tube. The dispersion was digested in a digestion block MARS 6 Microwave digestion unit (Model-MARS 6 240/50) for 1 hr. After cooling, the digested products were diluted to 50 mL with DI water. The clear solution was taken for mineral determination.

2.4. Statistical Analysis

Mean values were compared by using General Linear Model of ANOVA followed by Tukey Multiple Range Test Using SAS (SAS Institute, 1999). The p values less than 0.05 were adopted as statistically significant.

3. Results and Discussion

In the present study attempts were made to investigate the phytochemical, mineral and physicochemical compositions of seeds of six populations of *M. charantia* cultivated in same soil and climatic conditions.

3.1. Proximate Composition

Results on moisture, dry matter, crude fiber, and crude fat, crude protein and ash contents are presented in Table 1. Significantly, higher moisture content was observed in seeds collected from mature pods and decreased in ripen stage (Table 1). Mature stage and ripening stage moisture contents ranged 43-74% and 33-41% respectively. These results are in agreement with Horax *et al.* [9], who reported that the moisture content of fresh seeds of bitter

gourd ranged from 58.2-72.2%. According to Prashantha *et al.* [18], moisture content of seeds of Sri Lankan bitter gourd MC43; was $53 \pm 13\%$. Further Islam *et al.* [10] reported moisture content of bitter gourd seed ranged from 53-75.9%. However, crude fat and DM contents were significantly increased with the maturity (Table 1). Dry matter (DM) content of mature seeds (25.5-56.5%) significantly lower than the dry matter content of ripen seeds (58-67%). The highest dry matter content in mature stage of seeds was observed in non-domesticated species (*Kalu Karawila*) while in ripening stage highest dry matter content was observed in domesticated species

(MC 43). The ash content of bitter gourd seeds ranged from 2.1-4.2%. These findings are in agreement with Ali *et al.* [3], who reported ash value of three types of *Momordica* seeds varied from 2.29-2.37%. Crude fat content of the bitter gourd plays an important role in therapeutic activity of the seeds. Results of the current study revealed that the fat content of bitter gourd seeds increased at ripened stage (39.2-45.2%) compared to the mature stage (28.9-41.9%). These findings are in agreement with previous findings of Horax *et al.* [9], who reported fat content of mature (18.1%) and ripen (37.6%) bitter gourd seeds.

Table 1. Proximate composition of domesticated and non-domesticated *Momordica charantia* seeds harvested at two maturity stages

Stage	Variety	Moisture (%)	Dry Matter (%)	Crude Ash (%)	Crude Fat (%)	Crude Fiber (%)	Crude Protein (%)
Mature	TW	54.1 ± 0.4^d	45.9 ± 0.4^c	4.2 ± 1.2^a	33.9 ± 0.7^d	18.5 ± 0.3^d	23.6 ± 0.3^d
	MC43	67.5 ± 1.0^b	32.4 ± 1.0^e	3.2 ± 0.3^{abc}	41.9 ± 2.4^b	19.2 ± 0.5^d	22.3 ± 0.2^c
	MG	59.2 ± 3.0^c	40.7 ± 3.0^f	2.9 ± 0.1^{abc}	37.6 ± 0.5^c	21.2 ± 0.7^d	21.4 ± 0.2^c
	KK	43.4 ± 3.0^e	56.5 ± 3.0^d	3.5 ± 0.7^{abc}	28.9 ± 1.4^c	20.4 ± 0.4^d	26.2 ± 0.2^c
	GK	74.5 ± 0.2^a	25.4 ± 0.2^h	3.9 ± 0.0^{ab}	39.1 ± 0.0^{bc}	28.5 ± 0.5^c	15.2 ± 0.2^e
	SM1	57.5 ± 4.0^{dc}	42.4 ± 4.0^{ef}	2.8 ± 0.1^{abc}	39.0 ± 0.4^{bc}	18.6 ± 0.8^d	12.0 ± 0.4^i
Ripe	TW	39.3 ± 0.7^{efg}	60.6 ± 0.7^{bcd}	2.4 ± 0.1^c	40.5 ± 1.7^{bc}	18.0 ± 0.3^d	28.8 ± 0.3^b
	MC43	32.9 ± 0.6^h	67.1 ± 0.6^a	2.7 ± 0.8^{bc}	45.2 ± 2.3^a	20.5 ± 2.0^d	23.4 ± 0.0^d
	MG	41.1 ± 0.9^{ef}	58.9 ± 0.9^{cd}	2.2 ± 0.1^c	41.8 ± 2.2^b	21.6 ± 0.2^d	23.6 ± 0.0^d
	KK	40.4 ± 3.0^{ef}	58.9 ± 0.9^{cd}	2.8 ± 0.2^{abc}	39.2 ± 0.6^{bc}	18.8 ± 0.5^d	31.5 ± 1.0^a
	GK	35.2 ± 0.4^{hg}	64.8 ± 0.4^{ab}	2.6 ± 0.1^{bc}	41.6 ± 1.1^b	34.7 ± 1.8^b	18.6 ± 0.1^f
	SM1	36.4 ± 1.0^{fgh}	63.5 ± 1.0^{abc}	2.1 ± 0.1^c	42.0 ± 1.2^{ab}	38.1 ± 1.2^a	14.4 ± 0.1^h

TW-Thinnaweli White; MG- Matale Green; KK- Kalu Karawila; GK- Geta Karawila; Means denoted by the same letters in a column represent non-significant differences ($p < 0.05$).

Table 2. Total Phenolic Content (TPC) and Total Antioxidant Capacity (TAC) of seeds of different populations of *Momordica* harvested at two different maturity stages

Stage	Variety	TPC (mg GAE/g DW)	TAC (mg TE/gDW)
Mature	TW	4.69 ± 0.3^{bc}	1.84 ± 0.3^{bedc}
	MC43	4.85 ± 0.1^{ab}	1.88 ± 0.0^{bedc}
	MG	4.68 ± 0.2^{bc}	2.38 ± 0.2^{ab}
	KK	5.32 ± 0.1^a	2.08 ± 0.3^{bc}
	GK	5.06 ± 0^{ab}	2.68 ± 0.2^a
	SM1	4.18 ± 0.0^{cd}	1.44 ± 0.2^{cd}
Ripe	TW	4.64 ± 0.2^{bcd}	1.34 ± 0.1^c
	MC43	4.6 ± 0.2^{bcd}	1.87 ± 0.0^{bedc}
	MG	4.22 ± 0.3^{cd}	1.94 ± 0.1^{bdc}
	KK	4.62 ± 0.0^{bcd}	1.65 ± 0.1^{edc}
	GK	4.22 ± 0.1^{cd}	2.20 ± 0.3^{abc}
	SM1	4.4 ± 0.2^{da}	1.32 ± 0.1^c

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.

Table 3. Mineral compositions of seeds of domesticated and non-domesticated populations of *Momordica charantia*

Maturity Stage	Variety	Mineral content(mg/g)					
		Ca	Mg	Na	Cu	Zn	Fe
Mature	TW	0.76 ± 0.0^{bc}	0.58 ± 0^{ab}	0.24 ± 0^{cdef}	0.14 ± 0^{dc}	0.05 ± 0^c	0.01 ± 0^d
	MC43	0.76 ± 0.1^{bc}	0.54 ± 0^{bc}	0.17 ± 0^{ef}	0.23 ± 0.1^c	0.06 ± 0^c	0.04 ± 0^{bc}
	MG	1.18 ± 0.0^{ab}	0.56 ± 0^{bc}	0.17 ± 0^f	0.04 ± 0^{fg}	0.05 ± 0^c	0.06 ± 0^{ab}
	KK	1.30 ± 0.8^a	0.54 ± 0^b	0.28 ± 0.1^{bcdef}	0.05 ± 0^{fg}	0.06 ± 0^c	0.06 ± 0^{ab}
	GK	2.69 ± 0.3^a	0.61 ± 0^a	0.19 ± 0.1^{efid}	0.12 ± 0^{ef}	0.06 ± 0^c	0.03 ± 0^{dc}
	SM1	0.95 ± 0.4^{abc}	0.47 ± 0^e	0.30 ± 0^{bcd}	0.32 ± 0^b	0.10 ± 0^{ab}	0.06 ± 0^{ab}
Ripe	TW	0.65 ± 0.1^c	0.58 ± 0^{ab}	0.29 ± 0^{bcd}	0.07 ± 0^{efg}	0.04 ± 0^c	0.06 ± 0^{ab}
	MC43	0.77 ± 0.0^{bc}	0.55 ± 0^{bc}	0.36 ± 0^{bc}	0.12 ± 0^{ef}	0.07 ± 0^{bc}	0.07 ± 0^a
	MG	0.69 ± 0.1^{bc}	0.54 ± 0^{bcd}	0.16 ± 0^f	0.02 ± 0^g	0.13 ± 0^a	0.06 ± 0^{ab}
	KK	0.90 ± 0.1^{abc}	0.56 ± 0^{bc}	0.51 ± 0.1^a	0.04 ± 0^g	0.05 ± 0^c	0.06 ± 0^{ab}
	GK	1.20 ± 0.0^{abc}	0.51 ± 0^{ed}	0.39 ± 0^{ab}	0.55 ± 0^a	0.07 ± 0^{bc}	0.06 ± 0^{ab}
	SMI	0.99 ± 0.2^{abc}	0.53 ± 0^{cd}	0.31 ± 0.1^{bcd}	0.20 ± 0^{dc}	0.06 ± 0^c	0.06 ± 0^{ab}

TW- Thinnaweli White; MG-Matale Green; KK-Kalu Karawila; GK-Geta Karawila ; Mean values with different letters in the same column are significantly different ($p < 0.05$).

Therefore, seeds especially from ripen bitter gourd, are a good source of oil that is rich in conjugated linoleic acid (CLA) [5,13,17,19]. Therefore, bitter gourd seed can be used as an alternative source of natural rich CLA oil, to replace a synthetic one from other sources. Crude fiber ranged from 18.5-46.1% in bitter gourd seeds among six bitter gourd populations at mature and ripen stages. According to Nyam *et al.* [16] bitter gourd seeds contain $34.8 \pm 1.1\%$ crude fiber and this value is compatible with our results (Table 1). The highest protein content was observed in non-domesticated *Kalu Karawila* seeds at both mature (26.25%) and ripen (31.5%) stages. However, crude fiber content did not show any clear difference with the maturity (Table 1).

3.2. Antioxidant Properties and Phytochemical Composition

Presence of phenolic compounds and antioxidant capacity, of *Momordica charantia* seeds have gained much attention, due to their beneficial implications for human health [8]. As demonstrated in Table 2, There were significant differences in ($P < 0.05$) phenol contents and total antioxidant capacities in mature and ripen stages of bitter gourd seeds of all populations. However, seeds of non-domesticated populations (*Kalu Karawila*, *Geta Karawila* and SM1) exhibited marked phytochemical content in both maturity stages.

3.3. Mineral Composition

Bitter gourd seeds are considered as major source of macro (Ca, Mg) and micro (Cu, Zn, Fe, Na) minerals [9]. All tested seeds of domesticated and non-domesticated populations exhibited presence of macro and micro mineral contents (Table 3). The higher macro nutrients contents were reported in non-domesticated populations (*GK*, *KK* and SM1) when compared to the domesticated populations at both maturity stages. However, no prominent variation was observed between two maturity stages. Our findings are in agreement with Horax *et al.* [9], who revealed that there is no variation of mineral contents in seeds of bitter gourd in mature and ripen stages.

4. Conclusions

Seeds of all tested *Momordica charantia* populations harvested at mature and ripen stages showed presence of physicochemical, phytochemical and mineral constituents. The most of the physicochemical parameters were increased with the maturity but phytochemical and mineral constituents did not exhibit much variation with the maturity. Interestingly, seeds of undomesticated populations exhibited elevated phytochemical, and mineral contents. Therefore, it is suggested to exploit undomesticated *M. charantia* populations with elevated phytonutrient contents for pharmaceutical and nutraceutical industries.

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