Nutritional Analysis and Phytochemical Evaluation of Bitter Gourd (*Momordica Charantia*) from Bangladesh

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ABSTRACT---- ***Momordica charantia (M. charantia) is commonly referred to as Bitter Gourd, Karela and balsam-pear, is a very familiar vegetable and widely cultivate in Bangladesh. It has long been used as a traditional medicine for some ailments. Nutritional compositions and phytochemical constituents of two selected hybrid varieties of M. charantia (TIA and GOJNEE) from Bangladesh were analysed using standard analytical methods. The proximate composition like moisture, ash, crude lipid, crude fibre and crude protein contents were showed as percentage. The study results showed that the two varieties of this M. charantia are good source of minerals such as Calcium (Ca), Potassium (K), Magnesium (Mg), Iron (Fe) and Chromium (Cr). Calcium and iron concentrations were higher in both varieties. And their concentrations were 2.35 and 1.04 ppm in GOJNEE, and 2.24 and 0.87 ppm in TIA, respectively. Phytochemicals constituents like alkaloids, tannins, flavonoids and saponins were found in both varieties of M. charantia. Quantity of alkaloids, flavonoids and saponin were 0.05, 2.001 and 5.02 % in seeds of GOJNEE and 0.012, 1.72 and 3.27 % in seed of TIA. Total phenolic content was also determined and found that the highest contents were in ethanol and ethyl acetate extracts in both varieties. Our study result indicates the presence of nutritional and phytochemical constituents which are beneficial for our health along with the medicinal values.***

Keywords---- *Momordica charantia*, Karela, nutritional analysis, phytochemical evaluation

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1. INTRODUCTION

In recent years malnutrition is a common topic in developing countries. Distinct types of evidence suggest that research into nutrition and nutraceuticals have centralized mainly on plant derived products such as leaves, fruits, seeds etc. (Yoshime *et al*., 2016) Among the plant derived products, vegetables play a vital role to meet the nutritional necessity of people in Bangladesh. But for the unawareness of the people, they do not know the nutritive value of vegetables and fruits. So, most of the people in Bangladesh are suffering in malnutrition (Ullah *et al.*,2011).

Herbs and other dietary supplements are used as alternative treatment against different diseases. The medicine involves in this treatment is known as alternative and complementary medicine. From the ancient period, traditional medicinal plants and their derivatives are used as therapeutic agents for human kind. The world health organization (WHO) had listed that one fifty species of medicinal plants are used as commercially for medicinal purpose. Among the 150 species, bitter gourd is most commonly used as therapeutic purpose (Joseph *et al.*, 2013).

Momordica charantia also known as Bitter Melon, Bitter Cucumber, Karela and Balsam-pear, is a tropical vegetable, commonly found in Bangladesh. It is hardly expensive and especially used as a folk medicine. The tree has long leaves, yellow flowers, and elongated fruits that resemble a gourd or cucumber belongs to *Cucurbitaceae* family (Basch *et al*., 2003; Marr *et al*., 2004). Traditionally, the plant extracts of *M. charantia* is used for the treatment of hyperglycaemia, cancer, hypercholesterolemia, heart diseases and other immunological diseases. Because, *M. charantia* contain a hypoglycaemic compound named charantin and different antioxidants such as carotinoid, cryptoxanthin, vitamin C, vitamin E and varieties of phenolic compounds (Behera *et al*., 2007). It has moderate antioxidant and potent cytotoxic activities (Barua *et al*., 2014).

Many of the traditional plants are used with no attention paid to their nutritional values. Till now there are a few data available on the nutrient contents of hybrid bitter gourd varieties cultivated in Bangladesh. In this study, two varieties of cultivated bitter gourds were used. They are locally known as GOJNEE and TIA. Thus, the study was to exploit the nutritional composition as well as biologically important phytochemical profile of two selected hybrid varieties of *M. charantia*.

2. METHODS AND MATERIALS

Collection of samples from two varieties of *M. charantia*

The seeds of TIA variety were collected from the society nursery, and another hybrid variety of *M. charantia*, GOJNEE was collected from the local market of the district of Chittagong, Bangladesh. The sample was collected in the month of March-April, 2011. The study was conducted in 2011 at the Department of Biochemistry and Molecular Biology in the University of Chittagong, Chittagong, Bangladesh. The varieties were identified by researcher at the Department of Botany, University of Chittagong. After washing the samples, the seeds were separated and removed the foreign materials. Then, the fruits (pericarp) were used for experimental analysis. The fruits (pericarp) were air-dried for 5 days and made into powder by using electrical grinder. Dried powder was stored in tight-seal dark containers at 25^0C until needed.

Proximate analysis

The moisture content of *M. charantia* was carried out according to the method of Association of Official Analytical Chemist (AOAC, 1980), and Ash was determined by the standard method of AOAC (1995). The other nutrients like total proteins were determined by Micro-Kjeldahl method of AOAC (1960) using 6.25 factor to calculate protein content from nitrogen content, and crude fibre content was determined by AOAC (1980) while Lipid content by the method of AOAC (1980).

Estimation of Mineral content

The minerals content in the *M. charantia* were analysed by the method as described in analytical methods (Petersen, 2002).

Phytochemical screening

Phytochemical screening of the petroleum ether, ethyl acetate and ethanol extracts of two varieties of *M. charantia* fruits were carried out. The presence of alkaloids, tannins, flavonoids, saponins, and anthraquinones were carried out according to the methods of Sofowora (2006), Harbone (1991), Trease and Evans (2002) and Edeoga *et al*. (2005).

Alkaloid Screening

Determination of alkaloid was performed using the procedure put forward by Harborne (1973) as described by Edeoga *et al*. (2005). Briefly, five grams (5 g) of the powdered sample were weighed into 250 ml beaker. Acetic acid (10%) in ethanol was then added. The mixture was covered and allowed to incubate for 4 h. This was then filtered, and the extract concentrated on a water bath to ¼ of the original volume. Thereafter, concentrated ammonium hydroxide added drop wise until precipitation was completed. The solution was then allowed to settle and the precipitate was collected, then washed with diluted ammonium hydroxide and filtered. The residue was dried and weighed was alkaloid.

Flavonoids Screening

Flavonoids were determined by the methods developed by Boham and Kocipaiabyazan (1994). Briefly, 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was then filtered using Whatman No.42 (125 mm) filter paper. The filtrate was later transferred into crucible and evaporated to dryness over a water bath and weighed to a constant weight. The weighed was flavonoids.

Saponins Screening

Saponins were determined according to the method described by Obadoni and Ochuko (2001). Based on this method, 10 g of the powdered sample for each plant species was transferred into a conical flask, and 50 ml of 20% aqueous ethanol was added. This was heated over a hot water bath for 4 h while stirring continuously at 55° C. Thereafter, the mixture was filtered and the residue re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml using water bath at 90° C. Then, the concentrate was transferred into a 250 ml separatory funnel. Diethyl ether 10 ml was added to the funnel, and the mixture was shaken vigorously. The aqueous layer was recovered after the ether layer was discarded. The purification process was repeated. In addition, 30 ml of n–butanol was added. The combined n-butanol extract was washed twice using 5 ml of 5% aqueous sodium chloride, and the remaining solution was then heated in a water bath.

Phenolic content analysis in different extracts

The total phenolic content of the *M. charantia* extracts was determined using the Folin- Ciocalteu reagent (Mills, 1981). The reaction mixture was contained: 1ml of diluted extract (200mg/ml), 5ml of freshly prepared diluted Folin Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. Mixtures were kept in dark at ambient conditions for 1h to complete the reaction. The absorbance at 765 nm was measured using a spectrophotometer (Shimadzu UV PC-1600). Gallic acid was used as standard, and the results were presented as dry weight basis with µg gallic acid equivalent (GAE)/mg of sample.

3. STATISTICAL ANALYSIS

The data obtained in these investigations were subjected to statistical analysis. The data was analyzed using SPSS. All results are expressed as Mean ± SD.

4. RESULT

This study obtained the results of nutrient analysis, mineral content and the phytochemicals in *M. charantia*. Analysis of proximate content showed that the amount of moisture, ash, total protein, crude fat and crude fibre content were present in these two varieties (GOJNEE and TIA) of *M. Charantia* fruits. It appears that the content of moisture, ash, total protein, crude fat and crude fibre were 98.19, 0.95, 1.02, 0.60 and 2.19 percent in GOJNEE, and 94.05, 0.79, 0.92, 0.81 and 1.25 percent in TIA as shown in Table 1.

Table 1: Selected nutrients in two hybrid varieties of *M. Charantia* (%)*

Sl. No	**Chemical constituents**	***M. charantia* hybrid varieties**	
		GOJNEE	**TIA**
1.	Moisture	93.19±0.04	94.05±0.06
2.	Ash	0.95±0.04	0.79±0.05
3.	Total Protein	1.02±0.03	0.92±0.3
4.	Crude Fat	0.60±0.02	0.81±.02
5.	Crude fibre	2.19±0.04	1.25±0.03

*FWB = Fresh weight basis

The mineral contents of two varieties of *M. Charantia* fruits were analysed and the result was represented in Table 2. The most abundant mineral was calcium as 2.35 and 2.24 ppm in GOJNEE and TIA, respectively. The concentration of other minerals like potassium, magnesium, iron and Chromium was 0.84, 0.57, 1.04 and 0.0037 ppm in GOJNEE, and 0.78, 0.64, 0,87 and 0.0028 ppm in TIA, respectively (Table 2).

Table 2: Selected minerals in two hybrid varieties of *M. charantia* (ppm)

Sl. No	**Minerals**	***M. charantia* hybrid varieties**	
		GOJNEE	**TIA**
1.	Calcium (Ca)	2.35±0.25	2.24±0.18
2.	Potassium (K)	0.84±0.06	0.78±0.07
3.	Magnesium (Mg)	0.57±.08	0.64±0.01
4.	Iron (Fe)	1.04±0.06	0.87±0.08
5.	Chromium (Cr)	0.0037±0.00046	0.0028±0.00056

The phytochemical screening of the two hybrid varieties of *M. Charantia* was analysed, and found that the presence of alkaloids, flavonoids, tannins and saponins were in the extract of ethanol, ethyl acetate and petroleum ether of both GOJNEE and TIA (Table 3). No alkaloid was observed in petroleum ether in both varieties. It was clearly indicated that the two varieties of *M. Charantia* extracts contained almost the similar types of phytochemical constituents. The percentage of alkaloids, flavonoids and saponins were 0.05, 2.001 and 5.02 in GOJNEE, and 0.012, 1.72 and 3.27 in TIA, respectively (Table 4).

Table 3: Phytochemical Screening of two selected hybrid Varieties of *M. charantia*

Sl. No	Phytochemicals	*M. charantia* hybrid varieties					
		GOJNEE			TIA		
		Ethanol Extract	Ethyl acetate Extract	Petroleum ether Extract	Ethanol Extract	Ethyl acetate Extract	Petroleum ether Extract
1.	Alkaloids	+	+	-	+	+	-
2.	Flavonoids	+	+	+	+	+	+
3.	Tannins	+	+	+	+	+	+
4.	Saponins	+	+	+	+	+	+

Table 4: Quantitative (percent) phytochemical evaluation of seeds of both varieties (gm/100gm)

Variety	Alkaloid	Flavonoid	Saponin
GOJNEE	0.05	2.001	5.02
TIA	0.012	1.72	3.27

The total phenolic content (TPC) was expressed as gallic acid equivalents phenol compounds were reported as gallic acid equivalents (GAE) by reference to a standard curve ($y = 0.003x + 0.077$; $r^2 = 0.997$) (data not shown). No significant differences were observed for TPC among the extracts of both varieties. Total phenolic contents of all extracts were in the range of 31.25- 62.60 µg GAE/ mg of dry weight (DW). The ethanol extracts of both varieties showed highest content, and it was 62.60 and 56.65 µg GAE/ mg of dry weight for GOJNEE and TIA, respectively (Table 5). TPC was found in the extract of ethyl acetate and petroleum ether as 45.9 and 39.8 in GOJNEE, and 41.35 and 31.25 µg GAE/ mg of dry weight (DW) in TIA, respectively (Table 5).

Table 5: Phenolic contents of different extracts of two selected variety of *M. charantia*

Variety	Extracts	Total phenolics µg GAE/mg DW
GOJNEE	Ethanol	62.60±1.09
	Ethyl Acetate	45.90±1.49
	Pet. Ether	39.80±2.54
TIA	Ethanol	56.65±1.04
	Ethyl Acetate	41.35± 1.45
	Pet. Ether	31.25±2.69

Values are means (n = 3) ± SD. Here, all values are statistically significant at the 5% level.

5. DISCUSSION

The population of the world is increasing rapidly and creates many serious problems like resources deficiency, food and feed shortage. At present food shortage is a prime challenge for nutritionist to provide more and more protein rich food for growing population. Plants food such as fruits and vegetables are plying a vital role in the diet of human being since centuries, providing enough quantity and quality of fat, protein, carbohydrates, vitamins and minerals (Noomrio, 1956).

Moisture plays an important role in the growth activities of trees. It is also essential for most of the physiological reactions in plant tissue and in its absence, life does not exist (Rangaswami, G; 1976). The moisture content of the hybrid variety TIA (94.06%) was higher than that of GOJNEE (93.2%). The moisture content of karela was reported to be 91.2% (Soomro and Ansari, 2005). The differences in the moisture content between the *M. charantia* hybrid varieties can be attributed to the difference in the genetic composition and also the agro-cultural practices.

The ash content in GOJNEE variety (0.95%) was higher than Tia (0.79%) variety. The low ash content reflects that the mineral contents preserved in the food materials is also low. The result therefore suggests a low deposit of mineral elements in the fruit. (Antia *et al*., 2006).

The crude protein content in GOJNEE variety (1.02%) was slightly higher than TIA variety (0.9%). The proteins and carbohydrates present in the plant may be a conglomerate of bioactive sugars, glycoproteins or proteins which gives the plant its medicinal potency against certain diseases (Srivastava *et al*., 1989; Hokputsa *et al*., 2004). The protein content of hybrid bitter gourd reported to 1.9% by Soomro and Ansari (2005) which is very similar to our result.

Pectin, cellulose, hemicelluloses together with lignin is classified as dietary fibre (Robinson and Lawler, 1980). The crude fibre content between the two varieties revealed that the higher content was in GOJNEE variety (2.19 %) than in TIA variety (1.25 %). Intake of bitter gourd pericarp with high dietary fibre content may have positive health benefits possibly for reduction of colon cancer risk, diabetes, obesity, hypercholesterolaemia, gallstones and constipation (DeVries, 2003; Kritchevsky, 1988; Takeyama *et al*., 2002).

The value of the crude fat for GOJNEE variety (0.6 %) was lower compared with TIA variety (0.81 %). In 2005, Soomro and Ansari reported that hybrid green bitter gourd contained 0.4 % fat which is near about our result. This result suggests that bitter gourd is not good source of lipid. Dietary fats function through increase of palatability of food by absorbing and retaining flavours (Antia *et al*., 2006).

Minerals are called a “spark plugs of life” because they are required to activate hundred of enzyme reactions within the body. Life is dependent upon the body’s ability to maintain balance among the minerals (Watts, 1997). In this study, the elements such as Ca, K and Fe found in reasonable amount while Mg, Cr were relatively low in both varieties. This supports the previous data on *M. charantia* (Wills *et al*., 1984 and Yuwai *et al*., 1991).

The Ca was found at higher amount in GOJNEE variety (2.35 ppm) than TIA variety (2.24 ppm). On the other hand, high amount of Ca was found in bitter gourd as 137.69 mg/100 gm reported by Soomro and Ansari (2005). The actual reason is unknown, and may be the environmental and soil factors are responsible for this difference.

Between the both varieties higher amount of K was recorded from variety GOJNEE 0.84 ppm than the TIA variety (0.78 ppm). potassium helps in release of chemicals which acts as nerve impulses, regulate heart rhythms, deficiency causes nervous irritability mental disorientation, low blood sugar, insomnia and coma (Takeyama et al., 2002).

Iron content of *M. charantia* was 1.04 and 0.87 ppm in GOJNEE and TIA variety, respectively. It plays a significant role in oxygen transport in the body. Iron deficiency causes anaemia, weakness, depression, poor resistance to infection (Trease *et al*., 1989)

The amount of the Mg for GOJNEE variety (0.57 ppm) was lower compared to TIA variety (0.64 ppm). Mg plays important role in formation and function of bones, muscles and prevents high disorders, high blood pressure and depression (Underwood *et al*, 1999) also Mg plays important role in enzyme activity.

The Cr was found least amount in both varieties (0.0037 and 0.0024 ppm for GOJNEE and TIA variety, respectively). Cr is vital element as it works with insulin to stabilize blood sugar level, helps to absorb energy from blood and increase muscle mass reducing fat mass in human body (Verma *et al.*, 1956). Deficiency of Cr results in growth failure, cataract, hyperglycemia, neuropathy, atherosclerosis and leads to diabetes in human (Watts, 1997).

Phytochemical screening of these two varieties of *M. charantia* revealed the presence of alkaloids, flavonoids, saponins and tannins. These results are in correlation with the previous work on M. Charantia (Weight *et al*., 1992). The presence of these secondary metabolites in the fruits of *M. charantia* may contribute to its medicinal value. It has already been reported that the extract of crude bitter gourd is used for different disease such as disease of liver and pancreas, analgesic, anti-inflammatory, reduces cholesterol level, promotes appetite and supports blood sugar managing for diabetes or people with high risk of developing diabetes (Verma and Aggarwal, 1956).

The protective and metabolic role of alkaloids in animals has been documented (Edeoga and Eriata, 2001). It is known that Saponins inhibit Na^+ efflux by blockage of the influx of concentration in the cells, activating a $Na^+ – Ca^{2+}$ antiporter in cardiac muscles. The increase in Ca^{2+} influx through this antiporter strengthens the contraction of heart muscles (Bligh *et al*., 1959). Flavonoids may help to protect against oxidative stress induced diseases through contributing along with other antioxidant vitamins, and enzyme to the total antioxidative defense system of the body. Many studies have attributed that antioxidant properties are due to the presence of flavonoids (DeVries *et al*., 2003).

The present study estimated the phenolic content in the extract of ethanol, ethyl acetate and petroleum ether of *M. charantia*. Our results showed that ethanolic extract contained higher phenolic content (62.60 µg GAE/mg DW) than other two extracts, and GOJNEE variety contained higher phenol levels than TIA. This variation, even within the same variety, depends on many factors including environmental factors, maturity, location, and soil condition. Recent studies have shown that fruit and vegetable`s phenols and polyphenols such as flavonoids are one of the major groups that indicate a large spectrum of biological activities that are principally ascribed to their antioxidant property. They prevent free radical damage and lipid peroxidation (Bernardi *et al*., 2008; Akhila *et al*., 2009). The high content of total phenolic components in the ethanolic extract may have led to the better results found in the total antioxidant activity and free radical scavenging ability when compared with other two extracts.

6. CONCLUSION

Based on the result of our findings, it can be concluded that GOJNEE hybrid variety of *M. Charantia* contains better amount of nutrients and minerals than TIA variety which can contribute to the nutrient and energy requirement of human body. Most of the extracts of the two hybrid varieties contains same types of phytochemicals. Between the extracts of two varieties, the highest total phenolic content was found in ethanolic extract of GOJNEE variety which may have the potential for application in food systems to maintain food quality. Further study is necessary to elucidate detail medicinal values of *M. charantia*, then it would be possible to utilize this vegetable effectively.

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