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Bio-active compounds of bitter melon genotypes (*Momordica charantia* L.) in relation to their physiological functions

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Abstract

Background: Bitter Melon (*Momordica charantia* L) is one of the most popular cooked vegetables in many Asian countries. Its experimental use in mice has indicated improvement in glucose tolerance against Type II diabetes and reduction in blood cholesterol. However, it has not been proven which alkaloids, polypeptides, or their combinations in the Bitter Melon extract are responsible for the medicinal effects. Green and white varieties of Bitter Melon differ strikingly in their bitter tastes, green being much more bitter than white. It is not yet known whether they are different in their special nutritional and hypoglycemic properties. Nutritional qualities of Bitter Melons such as protein, amino acids, minerals, and polyphenolics contents were determined using four selected varieties such as Indian Green [IG], Indian White [IW], Chinese Green [CG], and Chinese White [CW] grown at the University of Arkansas at Pine Bluff [UAPB] Agricultural Research Center. Results indicated that protein levels of IW were significantly higher than IG in both flesh and seed.

Methods: Four Bitter Melon varieties, Indian Green [IG], Indian White [IW], Chinese Green [CG] and Chinese White [CW] were used for phytochemical analyses to determine protein contents, protein hydrolysis, amino acids contents, and their antioxidant and antimutagenic activities. All analyses were conducted following standard methods. Statistical analyses were

conducted using JMP 5 software package [SAS]. The Tukey's HSD procedure was used for the significance of differences at the 5% level.

Results: Moisture contents across the four varieties of Bitter Melon flesh ranged between 92.4 and 93.5%, and that of seed ranged between 53.3 and 75.9%. Protein contents of the flesh were highest in IW [9.8%] and lowest in CG [8.4%]. Seed protein contents were the highest in IW [31.3%] and lowest in IG [27.0%]. Overall, white varieties had higher protein contents than the green varieties. Compared with soy protein, most of the essential amino acid contents of Bitter Melon were similar as in soy proteins. Some amino acids such as Alanine, Glycine, and Valanine were relatively higher in Bitter Melon flesh than in soy protein. Phenolics contents of the flesh, seed, and seed coat tissue [SCT] were significantly different [$p<0.05$] among the four varieties. The four varieties were similar in their antioxidant activities of the flesh tissues; however, they were significantly different in their antioxidant activities in the seed and seed coat tissues [SCT]. Bitter melon varieties IW and CG, tested for antimutagenic effects, both flesh and seed had considerably high activities against benzo[a]pyrene with *Salmonella* TA98 [92-100% inhibition] and S*almonella* TA100 [79-86% inhibition].

Conclusion: Based on these studies, Bitter Melon is a good source of phenolic compounds. All four varieties tested showed considerably high antioxidant and antimutagenic activities. Therefore, these natural plant phenolics can be a good source of biologically active compounds that may be applied in many food systems to enhance food values and special nutritional qualities. Further studies will be needed using more genetically diverse varieties to pin point the bioactive and functional compounds and their physiological properties.

Key words: *Momordica charantia*, protein, polyphenolics, antioxidant, antimutagenicity

Background

Nutrition-related health problems such as hypertension, diabetes, ageing, obesity, arthritis, and cardiovascular diseases are prevalent among disadvantaged rural and urban populations, especially minorities in the Lower Mississippi Delta Region [1, 2]. Food consumption habits, dietary intakes, and meal preparation methods are believed to contribute to these problems. Dietary intervention through the consumption of specialty foods may alleviate the nutrition related disorders. In addition to the commonly used native herbs and vegetables in the US, many exotic herbs and vegetables are known for their special nutritional and medicinal properties [5]. During the past decade or two, Americans have realized that they could control their own health problems and reduce risks of many chronic illnesses by changing certain dietary behaviors [6]. The popular belief of Bitter Melons to improve glucose tolerance in Type II diabetes and lower the blood cholesterol. However, it has not been ascertained if or which alkaloids, polypeptides, or combination of these chemicals, are responsible for the medicinal effect.

Certain vegetables contain an abundance of polyphenolics, terpenoids, isoflavones, anthocyanins, amino acids, minerals, vitamins, and other antioxidants that are associated with protection from cancer, cardiovascular diseases, diabetes and hypertension [4, 7-12]. The most popular and useful group of the vegetables belong to the family *Cucurbitaceae* [13]. One of the members of this family is Bitter Melon [*Momordica charantia* L.], also known as Karela or balsam pear, an annual fruity vegetable. It is a delicacy for the East and Southeast Asian people, especially the people of India, China, Japan, Taiwan, Bangladesh, Pakistan, Thailand, Malaysia, Indonesia, Philippines, Nepal, Bhutan and Sri Lanka. Bitter Melon is also a popular vegetable in the West Indies, Brazil, Colombia, Cuba, Mexico, Panama, Peru, and some European countries. The bitter taste, for which the fruit is named, is perhaps due to the alkaloid momordicine, sometimes generalized as cucurbitacine. The green warty fruits of this plant are used as a vegetable, rich in vitamin A, vitamin C, and iron [14]. Fruits vary in size [1.0"- 9.8" long and 1.0 - 5.9" wide]. Fruits may be of oval, round, oblong, club, etc., in shape. Fruit colors at harvest vary from dark green to creamy white. Fruits mature in 45 to 80 days and turn red when ripe.

The fruits, leaves, and roots of Bitter Melon are traditionally believed to have medicinal value in reducing blood sugar levels for diabetic patients [15, 16]. The various medicinal properties of bitter melon are well known in eastern Asia [17]. Sofowora [18] reported several uses of *M. charantia* in traditional medicines in Africa. Pharmacologically, the hypoglycemic properties of the plant organs were established by Lotlikar et al. [19]. The polypeptides from the seeds and fruits of *M. charantia* were considered as antidiabetic agents [20-24]. Cucurbitacines isolated from several species of the family *Cucurbitaceae* showed antitumor effects [25-27]. Lin et al. [28] isolated two lectins from seeds of *M. charantia*, momordin and agglutinin, showing momordin to inhibit protein synthesis by Ehrlich Ascites cells. Licastro et al. [29] announced that two proteins isolated from the seeds of *M. charantia* inhibited protein synthesis and subsequent DNA synthesis in normal and leukaemic human peripheral blood lymphocytes. Spreafico et al. [30] reported a protein inhibitor from *M. charantia* showing immunomodulatory activity in mice.

Bitter Melons are used as cooked vegetables by the ethnic people from Asia, Africa and Europe, but American consumers have not yet developed a taste and appreciation for it. The crop could be introduced in the US as an additional food source possessing special nutritional properties. Therefore the functional compounds contained in these foods and their medicinal effect demand for thorough investigation followed by precise clinical studies. Research is also required for developing suitable varieties of this vegetable for their production potential and specific nutritional and medicinal qualities. In our studies during the past seven years, we have established that Bitter Melons can be successfully produced in Arkansas and the southern states. Also, we have some data generated on the recipes and their acceptability to the local consumers.

Methods

Four selected varieties of Bitter Melon were tested in randomized complete block

design with four replications on Calloway silt loam soil at the UAPB Agricultural Research Center. Plants were grown on upright trellises with 10 ft. between rows and 12 ft. within rows. Two four-week old seedlings were transplanted in each hole at the trellises. N, P, and K fertilizers were applied pre-plant, using 200 lbs per acre of 13-13-13. Irrigation was provided by drip irrigation system. Weeds were controlled by covering the beds with black plastic. Roundup [Glyphosate] was used in controlling weeds around the beds and allies. Melons were harvested twice a week beginning mid-June and ending mid-September. Fruit samples were stored in sealed plastic bags in ice chests for phytochemical analyses. Phytochemical analyses were conducted at the Department of Food Science, University of Arkansas, Fayetteville, AR.

Bitter Melon Sample Preparation: The fruits were washed with deionized water, drained at ambient temperature and cut in halves, lengthwise. The seeds were removed and flesh was thinly sliced and collected separately from the seeds. Flesh was divided into two halves. One half was freeze dried and the other half was oven dried. Seeds and flesh was oven dried at 80 ^{O}C for 3 days. Dried flesh and seeds were then ground to flour and passed through a 60-mesh U.S.A. standard testing sieve [W.S. Tyler Incorporated, Mentor, OH].

Protein Extraction and Determination: Bitter Melon protein isolates were prepared following a standard procedure for soy protein isolation [31]. Ten grams of bitter melon flour were extracted with 100 mL of water at pH 8.0 by stirring at ambient temperature for 2 h. The suspension was then centrifuged at 1500 g for 30 min at 10 ^{O}C to separate the supernatant from the solid phase. The supernatant was then adjusted to pH 4.5, held at 4 ^{O}C for 2 h and centrifuged again at 1500 g for 30 min at 10 ^{O}C. The resulting precipitate was dissolved in 20 mL deionized water, adjusted to pH 8.0 and freeze-dried. Soy proteins were isolated using the same procedure. Protein content of bitter melon flour and its protein isolates were determined using a Kjeldahl Unit [Kjeltec Analyzer Unit, Foss Tecator AB, Hoganas, Sweden]. Approximately 50 mg of each sample was digested with concentrated sulfuric acid in the presence of 1 Kjeltab Unit with 0.1 N HCl. Kjet-Sorb was used as the receiver during titration. Protein contents were calculated using a factor of 6.25.

Protein Hydrolysis: Protein hydrolysis was done using a protocol described by Eveleigh and Winter [32]. Ten mg of proteins were dispersed in 5 mL of 6 N HCl. Two hundred microliters of the suspension were taken into a vacuum hydrolysis tube [Pierce Chemical Co., Rockford, IL]. The tube was connected to a vacuum pump for 5 min. The tube was then sealed and placed in a Reacti Heating Module [Pierce Chemical Company] at 150 ^{O}C overnight [12 h]. After dialysis, the tube was cooled to room temperature before the aliquot was transferred to a 2.5 mL amber vial. The hydrolyzate was then evaporated to dryness under a stream of nitrogen at 60 ^{O}C. To the dried amino acids was then added 0.5 mL sample diluting buffer [Beckman Coulter Inc., Fullerton, CA] and the aliquot was filtered through a 0.2 µm filter prior to amino acid analysis.

Amino Acid Composition and Analysis: Amino acid composition was analyzed using an Amino Acid Analyzer 126 Beckman HPLC system with a post column derivatization reactor [Beckman Instruments, Inc. Palo Alto, CA]. Bitter melon flour and protein isolates were hydrolyzed with 6 M HCl to produce amino acids and then analyzed with the Amino Acid Analyzer. Chromatographic equipment consisted of a Beckman liquid chromatograph model 126 HPLC equipped with a System Gold Nouveau software [Beckman Instruments, Inc, Palo Alto, CA] was used. The absorbance of the effluent was monitored at 570 nm. The mobile phase consisted of sodium buffer Na-E [pH 3.3], Na-F [pH 4.3] and Na-D [pH 6.3] [Beckman Coulter Inc., Fullerton, CA]. A Hi-performance regeneration solution [Na-R] [Beckman Coulter Inc., Fullerton, CA] was used to regenerate the column after each run. Flow rate was set at 0.44 mL/min and column temperature was set an initial temperature of 50 ^{O}C for 7 min and 75 ^{O}C for the rest of the test. The initial solvent condition was buffer Na-E. After 19.5 min the buffer was switched to Na-F and at 30 min the buffer was switched to Na-D until 65 min. Then the column was washed with solvent Na-R for 3 min before the solvent was brought to the original condition. The flow rate of the ninhydrin [Nin-RX, Beckman Coulter Inc., Fullerton, CA] was set at 0.23 ml/min. A sample size of 20-100 L was injected during HPLC analysis. The amino acid composition was calculated from standard curves calibrated using the 17 standard amino acids.

Determination of Phenolics: Total phenolics of fine ground flesh, seed and SCT of bitter melons were determined by Folin-Ciocalteu method. One hundred milligrams of each sample was weighed into a screw-cap test tube and vortexed with 10 mL of methanol. The dispersion was heated in a water bath of 65 ^{O}C for 2h and allowed to cool at room temperature. One (1) mL of deionized water was added to 1 mL of the clear solution in a screw-cap test tube. The tubes were vortexed and allowed to stand for 2h at room temperature. Absorption of the solution at 726 nm was measured using a spectrophotometer. The total phenolic content was expressed as chlorogenic acid equivalents in mg/g dry material. The phenolic acid constituents were analyzed by HPLC [33].

Antioxidant activity determination: Antioxidant activity was carried out by oxidizing linoleic acid methyl ester [MeLo] in the presence of phenolic extracts as antioxidants [34]. Two mg of the extracts were dissolved in 10 mL of methanol. Five hundred microliters of the extracts solution were added into 0.2 g of MeLo [500 ppm extract in MeLo], and the methanol was evaporated under a stream of nitrogen at ambient temperature. Five hundred microliters of methanol were added into 0.2 g of MeLo for blank as a reference. Oxidation of MeLo in the present of extract was carried out in at 40 °C for 72h. Two mg of sample aliquots were taken at the starting point [zero time] and after 72h of oxidation [at 40 °C] and dissolved in 10 mL of 2,2,4-trimethylpentane [isooctane]. The conjugated diene absorption of the aliquots was read using a spectrophotometer [Shimadzu Model UV-1601, Kyoto, Japan] at a wave length of 234

nm. The antioxidant activities were expressed as percentage inhibition of conjugated diene hydroperoxides formation of MeLo after 72h of oxidation comparing with blank from MeLo as a reference antioxidant as follows: % inhibition= [(AB(72h) - AB(0h))- (AE(72h) – AE(0h)]/ [AB(72h) - AB(0h))] x 100; where A= absorbance, E= extract, and B= blank.

Antimutagenic activity determination: The antimutagenic activity of the methanolic extract from freeze-dried bitter melon flesh and seed from varieties India white and China green was determined by the method of Ames et al. [35]. The histidine requiring stains of *Salmonella typhimurium* TA98 and TA 100 were used for this test. Benzo[a]pyrene and sodium azide were used as mutagens for mutagenic and antimutagenic tests. The percentage inhibition of mutagenesis was calculated using the following equation: inhibition% = [1-(number of revertants in the presence of fraction/number of revertants in the absence of fraction)] X 100.

Statistical analysis: All phytochemical analyses values were reported as means of three determinations. Completely randomized design was conducted using JMP 5 software package [SAS 2002] and Tukey HSD procedure was performed for the significance of differences at the 5% level.

Results and Discussion

Nutritive, Protein, and Moisture Content of Bitter Melon: The Bitter Melon fruit has considerable amount of potassium, calcium, magnesium, vitamin C, protein, and dietary fiber [**Table 1**] as compared with other commercial vegetables [14]. It also has niacin, thiamin, riboflavin, vitamin A, protein, organic acids and other nutrients. Physical separation of bitter melons resulted in flesh and seed fractions. Samples of Bitter Melon flesh (edible portion) in general contained about 93% moisture over the four varieties; whereas, the moisture content of melon seeds varied from about 53% in Indian Green (IG) to 75% in Indian White (IW) [**Table 2**]. Bitter melon flesh contained 8.4% to 9.8% protein; whereas, seed contained 27% to 31% protein.

Table 1. Nutritive composition of per 100g edible portion of Bitter Melon [*Momordica charantia* L.] Fruits [Wills et al., 1984].

Component		Minerals [mg]		Vitamins/Protein/Fat	
Edible portion [%]	84.0	Ca	22.0	Vitamin A [mg]	0.04
Water [%]	93.8	K	260.0	Thiamin [mg]	0.05
Energy [KJ]	20.0	Mg	16.0	Riboflavin [mg]	0.03
Carbohydrate [g]	0.20	Fe	0.9	Niacin [mg]	0.40
Dietary fiber [g]	3.30	Na	3.0	Vitamin C [mg]	50.00
Organic acids [g]	0.11	Zn	0.1	Protein [g]	0.90
Ash [g]	0.60			Fat [g]	0.10

Table 2. Protein and moisture content of flesh and seeds of different varieties of bitter melons grown in University of Arkansas at Pine Bluff [UAPB] Agricultural Research Farm

Name of the variety	Protein content [%][b]	Moisture content [%]
Indian Green [F]	8.9 ± 0.1b	93.5 ± 0.2a
Indian White [F]	9.8 ± 0.2a	92.7 ± 0.3a
Chinese Green [F]	8.4 ± 0.2c	92.4 ± 0.2a
Chinese White [F]	8.9 ± 0.1b	92.9 ± 0.1a
Indian Green [S]	27.0 ± 0.1c	53.3 ± 0.5d
Indian White [S]	31.3 ± 0.1a	75.9 ± 2.2a
Chinese Green [S]	29.0 ± 0.3b	67.1 ± 0.6b
Chinese White [S]	30.2 ± 0.2b	71.8 ± 0.3c

Values were means ± SD. F= Flesh, S= Seed. Mean values in a column within the same fractions [F or S] with the same letters were not significantly different at $p \geq 0.05$. [b]Values were on dry basis.

Amino Acid Composition of Bitter Melon: Freeze dried melon flesh was high in lysine [mole percentage] compared to soy protein isolate. Flesh was relative lower in glutamic acid and arginine [**Table 3**]. Essential amino acids, including threonine, valine, methionine, isoleucine, leucine, and phenylalanine are comparable in amount to soy proteins and other legume proteins. On the other hand, oven dried flesh had a much lower percentage of lysine and a lower percentage of arginine while other amino acids were almost similar. Amino acid compositions of seeds are given in **Table 4**. Seed protein was higher in glutamic acid and arginine but lower in lysine compared to flesh proteins. Glycine was also higher in bitter melon compared to soy proteins.

Quality Criteria: The general quality criterion of Bitter Melon is a fresh appearance with uniform coloration and firmness without excessive seed development. The bitter melons are harvested, selected for size and uniformity of fruit surface characteristics and commonly packed in carton or wood boxes containing 5, 10, or 20 kg of fruit. It is a chilling sensitive vegetable, and may be air-cooled to 10 ^{O}C–12 ^{O}C [36, 37]. Bitter melons are intermediate in perishability. The common postharvest defects are in seed development, softening and ripening with internal /external color change. The recommended temperature and relative humidity for postharvest handling of bitter melon are 10–12.5 ^{O}C and 85-90%, respectively, with estimated shelf-life 7 to 14 days [36, 37].

Phenolics contents: Overall phenolics content in oven-dried samples were significantly higher than freeze-dried samples [**Table 5**]. Phenolic contents of the oven dried and freeze-dried tissues ranged from 5.39-8.94 mg of chlorogenic acid equivalent [CAE]/g dry matter and 4.64-8.90

mg/CAE.g dry matter, respectively. Phenolic contents of seed, SCT, and flesh ranged from 4.67-8.02, 4.64-8.94, and 5.36-8.90 mg/CAE dry matter, respectively. Phenolic contents of the flesh were significantly higher than those of the SCT and seed; phenolic contents of the seed were the lowest among those of all the tissues. The total phenolic contents of four varieties were significantly different with the highest in IW, followed by CW, CG, and IG.

Table 3. Amino acid composition [mole %] of flesh from four varieties of bitter melon Grown on UAPB Agricultural Research Farm at Pine Bluff.

Acids	Freeze-Dried Flesh				Oven-Dried Flesh				
	IGF	IWF	CGF	CWF	IGF	IWF	CGF	CWF	SPI
ASP	9.4	8.9	7.9	7.7	8.6	8.5	8.9	9.0	10.7
THR*	4.0	4.2	3.9	3.8	3.8	3.4	4.1	4.0	3.8
SER	4.7	6.2	5.2	4.8	5.3	4.9	6.1	4.9	5.0
GLU	10.8	12.0	9.6	10.2	10.4	13.8	11.5	10.5	15.2
PRO	5.2	5.5	3.2	4.8	6.8	5.3	5.5	6.1	6.0
GLY	8.1	7.8	8.9	8.1	9.1	9.5	9.9	9.7	8.5
ALA	7.5	8.4	9.0	8.8	9.9	7.9	8.7	8.8	7.2
CYS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.5
VAL*	6.0	6.4	7.0	6.9	7.3	7.8	6.9	7.6	6.3
MET*	1.0	0.9	1.1	1.0	0.8	1.2	1.2	1.3	1.4
ILU*	4.5	4.2	4.7	4.5	4.8	4.7	4.8	4.9	4.6
LEU*	6.9	6.9	7.2	6.8	7.6	7.1	7.3	7.1	7.1
TRY*	2.8	2.1	2.8	2.5	1.8	2.3	2.1	2.3	2.8
PHE*	4.4	4.0	4.5	4.5	4.1	4.6	4.1	4.3	4.6
HIS*	3.3	3.2	3.2	4.0	2.3	2.8	2.0	2.4	2.7
LYS*	7.7	7.5	6.9	7.7	5.8	4.4	4.5	4.9	6.6
ARG*	5.7	4.4	5.3	5.5	4.2	3.9	3.7	4.2	6.4

IGF=Indian Green Flesh; IWF=Indian White Flesh; CGF= Chinese Green Flesh; CWF=Chinese White Flesh, SPI=Soy Protein Isolate.*=Essential amino acid, ASP= Asparagine; THR= Threonine, SER=Serine, GLU=Glutamine, PRO=Proline, GLY=Glycine, ALA=Alanine, CYS=Cysteine, VAL=Valine, MET=Methionine, ILU=Isoleucine, LEU= Leucine, TRY=Tryptophan, PHE=Phenylalanine, HIS=Histidine, LYS=Lysine, ARG=Arginine.

Table 4. Amino acid composition [mole %] of oven-dried seeds from different varieties of bitter melon grown on UAPB Agricultural Research Farm at Pine Bluff

Amino acids	IGS	IWS	CGS	CWS	SPI
ASP	8.5	8.6	8.6	8.6	10.7
THR*	3.0	3.1	3.0	3.3	3.8
SER	3.9	4.0	4.1	4.8	5.0
GLU	14.2	13.9	14.0	13.6	15.2
PRO	5.4	5.0	5.1	5.2	6.0
GLY	10.6	10.3	10.6	10.3	8.5
ALA	8.7	8.9	9.1	10.0	7.2
CYS	0.0	0.1	0.2	0.2	0.5
VAL*	7.0	6.9	6.7	7.0	6.3
MET*	1.5	1.5	1.4	1.4	1.4
ILU*	4.8	4.9	4.6	4.2	4.6
LEU*	7.7	7.7	7.6	6.9	7.1
TRY*	2.7	2.9	2.9	2.6	2.8
PHE*	4.5	4.5	4.3	4.4	4.6
HIS*	3.4	3.1	3.2	3.1	2.7
LYS*	4.6	5.1	4.7	4.9	6.6
ARG*	8.4	8.6	8.6	8.4	6.4

IGF=Indian Green Seed; IWF=Indian White Seed; CGF= Chinese Green Seed; CWF=Chinese White Seed, SPI=Soy Protein Isolate. *=Essential amino acid. ASP= Asparagine; THR= Threonine, SER=Serine, GLU=Glutamine, PRO=Proline, GLY=Glycine, ALA=Alanine, CYS=Cysteine, VAL=Valine, MET=Methionine, ILU=Isoleucine, LEU= Leucine, TRY=Tryptophan, PHE=Phenylalanine, HIS=Histidine, LYS=Lysine, ARG=Arginine.

Table 5. Total phenolic contents of bitter melon tissues [mg/g dry matter]

Varieties	Oven-dried			Freeze-dried		
	Flesh	SCT*	Seed	Flesh	SCT*	Seed
Indian Green	6.5 ±0.06c	6.8 ±0.12b	6.7 ±0.08b	6.4 ±0.06d	4.6 ±0.10d	4.7 ±0.02c
Indian white	7.1 ±0.12b	8.9 ±0.36a	8.0 ±0.02a	7.2 ±0.04c	7.9 ±0.09a	6.0 ±0.05b
China Green	5.4 ±0.06d	7.0 ±0.07b	7.7 ±0.05a	7.8 ±0.14b	5.7 ±0.25c	6.2 ±0.02b
China White	7.8 ±0.07a	6.1 ±0.16c	6.9 ±0.31b	8.9 ±0.09a	6.6 ±0.23b	6.7 ±0.19a

*SCT= Seed coat tissue; Mean with different letters in the same column are significantly different [$p<0.05$].

Antioxidant activities of Bitter Melon extract: There was no significant difference in the antioxidant activities [% inhibition] of the methanolic extracts from bitter melons among varieties and drying methods [oven and freeze-dried] [**Table 6**]. The antioxidant activities of Indian green, Indian white, China green and China white ranged from 79-88, 79-87, 80-86, and 79-87% inhibition, respectively. The antioxidant activities of the oven-dried samples and the freeze-dried samples were 79-88 and 79-86% inhibition, respectively. The antioxidant activities of the methanolic extracts of flesh and SCT were not significantly different, while they were significantly higher than that of seeds. Shu-Jing and Lean-Teik [38] reported that bitter melon extracts possess potent antioxidant and free radical scavenging activities. These antioxidant activities could have contributed, at least partly, to the therapeutic benefits of certain traditional claims of wild bitter melons.

Table 6. Antioxidant activities [% inhibition] of melon tissues

Varieties	Oven-dried			Freeze-dried		
	Flesh	SCT*	Seed	Flesh	SCT*	Seed
Indian Green	82 ±3.8a	88 ±0.4a	85 ±2.7a	84 ±1.6a	86 ±0.8a	79 ±1.6b
Indian white	83 ±2.7a	87 ±1.1ab	79 ±1.2b	83 ±2.0a	86 ±0.5a	83 ±1.0ab
China Green	84 ±2.0a	81 ±1.4c	85 ±0.7a	84 ±0.8a	86 ±1.2a	80 ±1.0ab
China White	87 ±2.0a	84 ±2.6bc	79 ±2.6b	84 ±0.1a	83 ±1.0ab	84 ±0.9a

*SCT= Seed coat tissue; Mean with different letters in the same column are significantly different [$p<0.05$].

Antimutagenicity of bitter melon extract: Bitter melon varieties IW and CG showed higher antimutagenic effects against benzo[a]pyrene with *Salmonella* TA98 [92-100% inhibition] and S*almonella* TA100 [79-86% inhibition] [Table 7] but lower antimutagenic effects against sodium azide (data not shown). Similar finding was reported by several authors [39, 40]. Wattenberg [41] discussed chemopreventive agents as blocking agents that prevent carcinogens from reaching or reacting with critical target sites, and as suppressing agents that prevent evolution of the neoplastic process in cells that otherwise would become malignant. Bitter melon extract contains both agents [40]. Although the exact mechanism of the chemopreventive effects of bitter melon is not yet known, these findings suggest that bitter melon is a possible chemopreventive agent against carcinogenesis.

This piece of research had a limitation of amount of field samples and the data for the chemical analyses. The scopes and resources for elaborate statistical analyses and testing were limited. However, efforts will be made to change the situations in the follow up research.

Table 7. Antimutagenic activities [% inhibition] of methanolic extracts from bitter melon against benzo[a]pyrene with *Salmonella* stains TA98 and TA100

Bitter Melon extracts	TA98	TA100
China Green flesh	99.3	86.2
China Green flesh	91.7	81.5
India White seed	100	78.7
India White seed	100	80.9

Concentration of extract: 500 µg/plate; benzo[a]pyrene and sodium azide [10 µg/plate].

Conclusions

Bitter Melon is an excellent source of phenolic compounds, antioxidants, and antimutagen. This can find application in food products and dietary supplements. The phenolic extracts showed high inhibition effect to prevent lipid oxidation. These natural plant phenolics can be a good antioxidant which may be applied in many food systems to enhance and maintain food quality. More detailed investigations on bitter melon phenolics, peptides, and proteins are needed to provide information for their nutraceutical values. Bitter melon has high demand in the ethnic markets, especially Asian, African, and certain South American countries. To the best of our knowledge in normal vegetable usage of Bitter Melon, no toxicity has been reported. However, high dose of bitter melon capsule which is now available in the US as well as international markets may cause some kind of toxic effect, like an overdose of any other polyphenolics. More detailed investigations on Bitter Melon phenolics, peptides, and proteins are needed to provide information for their nutraceutical values. There are scopes of varietal improvement for special nutritional and medicinal qualities. Genetics and breeding research evaluating new germplasm and developing some unique varieties possessing special quality characteristics is the demand of the day.

Abbreviations:

SCT= Seed coat tissue, IGF=Indian Green Seed; IWF=Indian White Seed; CGF= Chinese Green Seed; CWF=Chinese White Seed, SPI=Soy Protein Isolate.

Competing interests

No competing interests.

Authors Contributions

All the authors contributed equally. The principal author is writing a grant proposal and manuscript. E-mail: islams@uapb.edu

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