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Antioxidant and antidiabetic properties of Chinese and Indian bitter melons (*Momordica charantia* L.)

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Abstract

Bitter melon (*Momordica charantia* L.) has been used for anti-diabetes treatment for decades. Indian and Chinese bitter melons (BM) are two commonly produced cultivars in the US market. This study has comparatively evaluated the effects of two processing methods (fresh and freeze-drying) on Chinese and Indian BM by measuring their bioactivity in terms of total phenolic content (TPC), total triterpene content (TTC), antioxidant activity, and antidiabetic properties using the DPPH free radical scavenging and reducing power assays, and the α-amylase and α-glucosidase inhibition assays. The TPC (GAE mg/g dw) in freeze-dried BM were 6.03 and 6.09, and in fresh BM were 4.81 and 4.83 for Indian and Chinese BM, respectively. The TTC (OAE mg/g dw) in Indian BM were 7.25 and 5.63, and in Chinese BM were 5.88 and 3.87 for fresh and freeze-dried samples, respectively. TPC and TTC in the freeze-dried BM samples were significantly higher than that in the fresh ones ($p < 0.05$). The DPPH IC_{50} of India BM was significantly lower than that of Chinese BM ($p < 0.05$). All BM samples ranged from 9.18 to 18.6 mg/ml. The reducing power was significantly different between Indian and Chinese BM ($p < 0.01$) for fresh samples, but after freeze-drying, there was no detectable difference in reducing power ($p \geq 0.05$). The Indian BM showed a significantly stronger α-glucosidase inhibition effect as compared to the Chinese BM. TTC was positively correlated with reducing power ($p < 0.05$). TPC was negatively correlated with α-amylase inhibition efficiency ($p < 0.05$).

Keywords: *Momordica charantia*; Chinese bitter melon; Indian bitter melon;

Antidiabetic; Phenolics; Triterpene.

1. Introduction

Many consumers look for healthy food choices to prevent chronic health issues such as diabetes. As a result, a lot of conventional medicinal vegetables have received renewed attention (Krishnaiah *et al*., 2011). Bitter melon (*Momordica Charantia* L.) is a medical vegetable widely used in many Asian countries (El Batran *et al*., 2006; Islam *et al*., 2011).

Bitter melon (BM) are grown in tropical and sub-tropical regions, including Africa, USA, Australia, Brazil, China, India, Iran, Malaysia, Thailand, and Turkey (Krishnaiah *et al.,* 2011; El Batran *et al.,* 2006). BM species vary in shape, color, and strumose surface (Huang, 2010). The flesh can be sliced and used with salad, cooked alone as a vegetable, stir-fried with shrimp or meat along with garlic and chili, or fried as BM chips. Sometimes the flesh is pre-boiled with a little bit of salt or spice added to reduce the bitter flavor. A combination of BM juice and tea is very popular (Subratty *et al.*, 2005).

The moisture content of BM flesh ranges from 92.9 to 93.8% (Wills *et al*., 1984; El Batran *et al*., 2006). The flesh contains higher lysine, lower glutamic acid/glutamine, and arginine compared with soy protein isolates. Essential amino acids in BM, such as threonine, valine, methionine, and isoleucine are similar to soy proteins and other legume proteins (Islam *et al*., 2011). BM contains no fructose, sucrose, or fat. Glucose, starch, and dietary fiber are ~0.1, ~0.1, and ~3.3 g/100 g, respectively (Wills *et al*., 1984). BM has fewer calories than common vegetables and

58 fruits (Wills *et al*., 1984; Rebello *et al*., 2013), and has lower levels of sodium and
59 higher levels of magnesium and iron than most vegetables (Wills *et al*., 1984). El
60 Batran *et al*. (2006) had found that BM contained significantly higher α-carotene than
61 other vegetables. In addition, BM is high in phenolic compounds, including gallic acid,
62 tannic acid, catechin, caffeic acid, ρ-coumaric acid, ferulic acid, and benzoic acid
63 (Krishnaiah *et al*., 2011; Wang *et al*., 2014). Phenolic compounds, such as quinic acid,
64 catechin, caffeic acid, protocatechic acid, syringic acid, and 4-coumaric acid in BM
65 have been shown to have DPPH free radical scavenging ability and ferric reducing
66 antioxidant power (Kenny *et al.*, 2013).

67 Triterpenoids are the major contributors to the antidiabetic effects of BM (Tan *et*
68 *al*., 2008). Cucurbitane triterpenoids increase synthesis of insulin-responsive glucose
69 transporter GLUT4 in muscle and fat cells by increasing the activity of
70 AMP-activated protein kinase (AMPK) (Tan *et al*., 2008). Triterpene extracted from
71 BM lowered blood sugar using *in vivo* and *in vitro* studies (Chang *et al*., 2011).
72 Triterpene has been shown to have antioxidant activities (Liu *et al*., 2010). Chung *et*
73 *al*. (2017) measured three new compounds: triterpene esters, triterpenoids, and
74 triterpene glycosides, which were isolated from *Panax ginseng* Meyer. The DPPH
75 IC_{50} of the triterpenoid and triterpene glycoside ranged from 30 to 60 μg/ml, and the
76 antioxidant value of the triterpene ester was much weaker than the former two.
77 Saponins are traditionally categorized as triterpenoid (Vincken *et al*., 2007). Charantin,
78 a mixture of two saponin compounds, is one of BM's major bioactive components. It
79 was reported that a charantin-rich extract of BM led to a significant decline in blood

glucose, plasma glucose intolerance, and insulin resistance in a mouse model (Wang *et al*., 2014).

BM has been used for anti-diabetes treatment for decades (Islam *et al*., 2011). Many studies have shown that BM extracts can lower blood sugar in diabetics by stimulating pancreatic secretion, decreasing hepatic gluconeogenesis, increasing hepatic glycogen synthesis, and increasing peripheral glucose oxidation (Wang *et al*., 2014).

Indian and Chinese BM are two commonly produced cultivars. They are different in color, shape, texture, and taste. The Chinese BM has a creamy white or light green pericarp and is oval in shape. The flesh is relatively loose with smooth strumae. The bitter taste is relatively lower than that of the Indian ones, which make it the most popular for consumers. Indian BM is dark green. The shape varies from ovals to clubs. The flesh is tight. The strumae surface is relatively rough, or even sharp (Huang, 2010).

BM flesh is mainly consumed as a vegetable, which means that multiple phytochemicals may function at the same time. It would be beneficial to understand the physiological properties of bitter melon flesh as a whole. In addition, a comparison of the antioxidant and anti-diabetes activities between these two cultivars of bitter melon has not been done. These are the most available cultivars in the USA, Fresh BM is normally difficult to store for a long period of time with refrigeration. Freeze-drying can preserve the function of most bioactive compounds during processing (Nunes *et al.,* 2016). It could be an option for extent shelf life and

preventing antioxidant degradation. The objectives of this study were to compare the total phenolic content, total triterpene content, antioxidant activities, and antidiabetic properties of fresh and freeze-dried Chinese and Indian BM cultivars. *In vitro* methods were used to evaluate antioxidant and antidiabetic properties.

2. Materials and methods

2.1 Materials

All the chemicals, solvents, standards, and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The BM were grown in the greenhouse at the Tennessee State University experimental farm in Nashville, TN (latitude 36°10' N, longitude 86°49' W, elevation 127 m). Seeds of two bitter melon varieties were used in the experiment. Seeds of Chinese bitter melon (#365 hybrid) and Indian bitter melon (#318 India hybrid) were purchased from Kitazawa Seed Company (Oakland, CA, USA). Seeds were started in germination trays with Miracle-Gro potting soil (Scotts Miracle-Gro Co., Marysville, OH, USA), once seedlings were 3" tall, they were transferred to 12" pots (2 seedlings/pot), grown, trellised and continued in these pots until the experiment terminated (6 months). All the plants were given the same amount of Osmocote 14-14-14 NPK (Greenhouse Megastore, Danville, IL, USA) slow release fertilizer (2 tablespoons/pot, twice during the growing season), pH 6.8. The temperature in the greenhouse was maintained at 25 ± 2˚C and the summer shade was closed during the entire growing season.

A randomized block design (RBD) was used to grow the BM with three blocks for each variety. There were 10 pots in every block. Once blooming, all pistils were pollinated manually using stamens of the same variety. All the fruits were harvested at their maturity dates of 63 and 55 days for Chinese and Indian BM, respectively, following the instructions provided by the seed company (https://www.kitazawaseed.com). Eight fresh whole fruits 11-12 inches long (Chinese BM) and 6-7 inches long (Indian BM) were selected from every replicate for the experiment. All the fruits in each replicate were rinsed with deionized water in the laboratory (Milli-Q® Integral Water Purification System, MilliporeSigma, St. Louis, MO, USA), dried thoroughly with a clean paper towel, and kept in a 4 ℃ refrigerator for analysis within 3 days.

2.2. Methods

Fruits were prepared with the following procedure: BM fruits were washed and seeds were removed, and the flesh (including skin) was homogenized using a Polytron (PT 2100, Kinematica AG, Lucerne, Switzerland) at power 6 for 1 min and adjusted to an expected concentration with distilled water. Due to potential effects of thermal processing described by Nunes *et al.*, (2016), extraction was done at room temperature (22 ℃) based on the preliminary studies, which indicated that the 12 h overnight extraction showed the best extraction yield for the fresh BM. Dry matter content was determined based on weight difference after drying the samples at 103 ˚C for over 12 h to reach a consistent weight in a forced air oven (SM01, SHEL LAB,

VWR, Atlanta, GA, USA). The dry matter of fresh Chinese and Indian BM were 7.04 and 7.16%, respectively. Part of the flesh was stored at -20 ºC overnight followed by freeze-drying (FreeZone 6 L Console Freeze Dryer with Stoppering Tray Dryer, Labconco Corp., Kansas City, MO, USA) for 76 h at -40 ºC. The dried samples were ground into fine powder using an IKA Heavy-Duty analytical mill (IKA 1600360, Cole-Palmer, Vernon Hills, IL, USA), and kept in a 4 ºC refrigerator for a maximum of 4 wk for further analysis.

2.3. Total phenolic compounds

The total phenolic content (TPC) was measured using the method described by Zhang *et al*. (2014) with modifications. Briefly, 0.1 g of finely ground freeze-dried BM and 0.1 g dry mater equivalent of fresh BM were extracted with 20 ml of 70% methanol in 12 mM HCl for 24 h at room temperature. After centrifugation at 4000 g for 10 min (Z206A Hermle, Labnet International Inc., Edison, NJ, USA), supernatant was filtered through a 0.45 µm PTFE syringe filter (Nalgene® Syringe Filters, PTFE, 25 mm, VWR). The residue was re-extracted two more times following the same procedures with 10 ml of solvent each time, and the supernatants were combined. TPC was determined spectrophotometrically (G10S UV-Vis, Thermo Fisher, Waltham, MA, USA) using the Folin-Ciocalteu procedure as described by Singleton *et al.* (1999) with modifications. In brief, 250 µl of extract was mixed with 1.25 ml of 0.04 N Folin-Ciocalteu reagent, and 1 ml of sodium carbonate solution (4% w/v). After 120 min of incubation at room temperature in the dark, the absorbance was measured at

740 nm. TPC was calibrated using a standard curve of gallic acid. The total phenolic content of each solvent extract was expressed as mg gallic acid equivalents (GAE) /g dry weight (dw) BM.

2.4. Total triterpene content (TTC)

Triterpenes were extracted from 0.05 g freeze-dried BM and 0.05 g dry matter equivalent of fresh BM, according to the methods of Huang *et al*. (2014) and Ren *et al*. (2012) with modifications. In brief, dry samples were extracted at room temperature overnight using methanol at a dry mater to solvent ratio of 0.05 g/5 ml. After adding 10 ml distilled water, the pH of the solution was adjusted to 13-14 using a NaOH granule. The mixture was centrifuged at 4000 g for 10 min. The pH of the supernatant was adjusted to 2-3 using concentrated sulfuric acid followed by keeping the solution at room temperature for 48 h. The residues were rinsed with distilled water while filtering through a filter paper (Whatman® filter paper, WHA5230090, Sigma-Aldrich, Corp.) until the pH of the filtrate reached 7. Then, the residues on the filter paper were rinsed with 95% ethanol to reach a final filtrate volume of 25 ml. The ethanol mixture was centrifuged at 4000 g for 10 min. Then 1 ml supernatant was evaporated to dryness in a 60 ℃ water bath, followed by adding 0.3 ml of 5% (w/w) vanillin-acetic acid solution, and 0.9 ml perchloric acid into the tube. The test tube was capped, and the sample was kept in a 60 ℃ water bath for 20 min. After the samples cooled down to room temperature, the absorbance was measured at 540 nm. TTC was calibrated using a standard curve of oleanolic acid. The total triterpine content of each solvent

extract was expressed as mg oleanolic acid equivalent (OAE)/g dw BM.

2.5. DPPH free radical scavenging ability

The radical scavenging ability was measured according to the method described by Paiva-Martins and Gordon (2001), and Wu *et al.* (2016) with modifications. The 400 µl BM aqueous solution (concentration range from 1.0 to 5.0 mg/ml) was mixed with 800 µl of metholic (100%) DPPH (400 µM). The mixtures were kept in the dark for 30 min. The absorbance was measured at 517 nm. A lower absorbance of the reaction mixture indicates a higher free radical scavenging activity. Ascorbic acid was used as a control. The capacity to scavenge the DPPH free radicals was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [A_0 - (A - A_b)/A_0] \times 100\%$$

Where A_0 is the absorbance of DPPH solution without sample; A is the absorbance of the test sample mixed with DPPH solution, and A_b is the absorbance of the sample without DPPH solution.

2.6. Reducing power assay

The reducing power was determined according to Oyaizu (1980) with slight modification. BM solutions (0.5 ml) with various concentrations (1.0 to 5.0 mg/ml) were mixed with 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.0) and 0.5 ml of 1% (w/v) potassium ferricyanide. The mixtures were incubated at 50 ºC for 20 min, followed by adding 1 ml of 10% (w/v) trichloroacetic acid, 1 ml of deionized water,

and 0.1 ml of 0.1% ferric chloride, sequentially. The absorbance was measured at 700 nm against deionized water as a blank. A higher absorbance indicates a higher reducing power.

2.7. In vitro *antidiabetic properties*

Both fresh and dried samples (2% dw) were extracted using water for 12 h at room temperature. The extracts were centrifuged (8000 g for 10 min), and the supernatants were stored at -80 °C for <24 h prior to the assay of α-amylase and α-glucosidase inhibition.

2.7.1. Inhibition assay for α-amylase activity (AMY)

The α-amylase inhibitory activity was measured according to the procedure described by Mahendran *et al*. (2015), Kim *et al*. (2004) and Wang *et al*. (2010). Azure starch (0.5% w/w) (S7629-5G, Sigma) was added to the Na_2HPO_4 buffer (0.05 M, pH 6.9, containing 1 mM $CaCl_2$) to a substrate concentration of 0.5%. Substrate solutions were kept in boiled water for 5 min and were transferred to a 37 °C water bath for 10 min. The sample solution (500 µl) was mixed with 100 µl of α-amylase (20 U/ml) (A3176-2.5MU, Sigma) and pre-incubated in a 37 °C water bath for 10 min. Distilled water was used to replace the samples for the control measurement. Azure potato starch (1 ml) was added into the sample-enzyme mixture, followed by incubation at 37 °C water bath for 10 min. The reaction was terminated by adding 0.1 ml 50% acetic acid. The mixture was diluted with 2 ml buffer and centrifuged at 8000 g for 10 min. The

α-amylase inhibition activity was measured at 595 nm against the buffer as a blank. The α-amylase inhibition activity was calculated as:

$$\alpha\text{-amylase inhibitory activity (\%)} = (OD_{control} - OD_{test\ sample} / OD_{control}) \times 100\%$$

2.7.2. Inhibition assay for α-glucosidase activity (GLU)

The α-glucosidase inhibitory activity was measured according to the procedure described by Mahendran *et al*. (2015) and Kim *et al*. (2004). α-Glucosidase (50 µl, 10 U/ml) was mixed with 0.75 ml of sample extract. The same amount of KH_2PO_4 buffer (0.05 M, pH 6.8) was used as the control. The sample-enzyme mixture was pre-incubated for 10 min at 37 °C. The mixture was added into glass tubes containing 1 ml p-NPG (30 mM in buffer) (N1377-5G, Sigma) in each. The mixture was incubated for 30 min at 37 °C and terminated by adding 2 ml Na_2CO_3 (0.1 M) solution. The solution was diluted 10 times using distilled H_2O. Absorbance was measured at 405 nm against the distilled H_2O as a blank. The α-glucosidase inhibition activity was calculated as:

$$\alpha\text{-glucosidase inhibitory activity (\%)} = (1 - OD_{test\ sample} / OD_{control}) \times 100\%$$

2.8. Statistical analysis

Statistical analysis was done using the Statistical Analysis System (SAS Version 9.4, SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was done to test the effect of variety and processing method on TPC, TTC, antioxidant activities, and antidiabetic properties using the GLM procedure. Tukey's method was used for

multiple comparisons if an effect was found significant. All data were expressed as the means ± SD. $P < 0.05$ was generally used for significant difference. $P < 0.01$ was also used for some of the data to indicate the greater significance of the differences. Linear regression was used to calculate DPPH IC_{50} values. The correlation analysis of TTC, TPC, DPPH, reducing power, and α-amylase and α-glucosidase inhibition activity was done using the CORR procedure, and the results were evaluated using the method of Evans (1996) based on the absolute value of r: 0.00-0.19 as "very weak"; 0.20-0.39 as "weak"; 0.40-0.59 as "moderate"; 0.60-0.79 as "strong"; and 0.80-1.0 as "very strong". All experiments were run in triplicate.

3. Results and discussion

3.1. Total phenolic content (TPC)

The TPC in all samples ranged from 4.81 to 6.09 GAE mg/g dw. As shown in Fig. 1, freeze-dried samples had higher TPC than the fresh samples in both varieties ($p < 0.05$). Although TPC in Chinese BM were slightly higher than that in Indian BM, no significant difference ($p \geq 0.05$) was observed.

It was reported that TPC in BM was affected by the extraction method. Wu and Ng (2008) obtained a much higher TPC value (51.6 mg/g dw) extracting with boiling water for 1 h, while Tan *et al*. (2014b) reported a lower value (around 10.6 GAE mg/g dw) using water with various time-temperature combinations. In the current study, a lower TPC was obtained after extraction at room temperature, which was consistent with the results at similar extraction conditions reported by Tan *et al*. (2014a).

278 Furthermore, a significant difference in TPC between freeze-dried and fresh BM was
279 observed. The higher TPC in freeze-dried samples might be caused by the increased
280 tissue porosity after freeze-drying, which promoted phenolic compounds release
281 during extraction (Chang *et al*., 2006).
282
283 *3.2. Total triterpene content (TTC)*
284 The comparison of TTC between fresh and freeze-dried samples from the two
285 varieties is shown in Fig. 2. TTC ranged from 3.87 to 7.25 OAE mg/g dw. Acton
286 (2013) showed that the total triterpene contents ranged from 0.03 to 6.93 mg/g in BM
287 fruit extracts. Moreover, saponin, an indicator of triterpene compounds, was reported
288 in the range of 0.01 to 0.21 mg/g dw in BM (Zhang, 2009; Vincken *et al*., 2007). The
289 results showed that TTC in Indian BM was significantly higher than that in Chinese
290 BM for both fresh and freeze-dried samples as shown in Fig. 2 ($p < 0.01$). These
291 results were consistent with Zhang *et al*. (2009), who found that freeze-dried BM had
292 significantly higher TTC for the same variety.
293
294 *3.3. DPPH free radical scavenging ability*
295 The IC_{50} value is commonly used to evaluate the total antioxidant efficiency of
296 samples. The results of DPPH free radical scavenging ability are shown in Fig. 3. The
297 IC_{50} values of all BM samples ranged from 9.18 to 18.6 mg/ml. These results were
298 similar to the IC_{50} values from leaf, stem, green fruit, and ripe fruit of BM, which
299 were 9.7, 17.8, 11.0 and 27.6 mg/ml, respectively (Kubola & Siriamornpun, 2008;

300 Ghous *et al*., 2015). Kenny *et al*. (2013) also showed an IC_{50} value of 38.2 mg/ml
301 after using different extraction methods with other BM varieties.

302 As shown in Fig. 3, there was no significant difference in DPPH scavenging
303 ability between fresh and freeze-dried samples ($p \geq 0.05$). However, a significant
304 difference ($p < 0.05$) between Chinese and India BM was observed. The DPPH free
305 radical scavenging ability of Indian BM was higher ($p < 0.05$) than that of the Chinese
306 BM in both fresh and freeze-dried samples (Fig. 3). Both content and composition of
307 TPC and TTC contribute to the antioxidant activities. To neutralize the free radical
308 status of DPPH, the antioxidants transfer either electrons or hydrogen atoms to DPPH
309 (Kubola and Siriamornpun, 2008). For total polyphenols, it was the hydrogen on the
310 carboxyl groups of phenolic acid that helped reduce DPPH free radicals (Kubola and
311 Siriamornpun, 2008). Such phenolic acids include tannic acid, catechin acid, ferulic
312 acid, and benzoic acid in BM (Krishnaiah *et al*., 2011), as well as quinic acid,
313 chlorogenic acid, and protocatechic acid (Kenny *et al*., 2013). Specifically, the caffeic
314 acid and gallic acid might contribute the most to the DPPH free radical scavenging
315 ability (Kubola and Siriamornpun, 2008; Kenny *et al*., 2013). Meanwhile, catechol
316 and resorcinol contributed antioxidant activity as well (Janeiro and Oliveira Brett,
317 2004). For phenolic compounds in BM, higher contents of quinic acid (145 ng/mg),
318 caffeic acid (33.0 ng/mg) and protocatechic acid (25.3 ng/mg) were reported with
319 higher scavenging ability because the DPPH free radical was reduced by H^+ donated
320 by phenolic acids (Kenny *et al.,* 2013). This was also supported by Zheng and Wang
321 (2003) who investigated the antioxidant effect of the critical composition of phenolics,

and concluded that antioxidant activity was enhanced with more phenolic hydroxyl groups and primary alcohols.

Another factor contributing to DPPH radical scavenging abilities was the TTC. India BM had a higher TTC and showed a stronger DPPH scavenging ability in both freeze-dried and fresh samples (Fig. 3). It was reported that BM with higher saponin content gave increased antioxidant capacities (Tan *et al*., 2014c). Chung *et al*. (2017) measured three new isolated triterpene compounds, among which the triterpene glycosides showed the strongest DPPH free radical scavenging ability and reducing power, followed by triterpenoid, and triterpene ester. This was consistent with the content of hydroxyl groups, which donated hydrogens according to their structures.

In addition, the correlation and regression relationships of phytochemical content and antioxidant activities were also evaluated using Pearson correlation coefficients among TPC, TTC and DPPH IC_{50} values (Table 1). TPC was not correlated with the IC_{50} value, but significantly correlated with TTC. It was reported that the DPPH free radical scavenging ability had a significant correlation with TPC (Kubola & Siriamornpun, 2008). Shan *et al*. (2012) showed that with the increase of flavonoids concentration, the antioxidant activity and free radical scavenging power increased. However, their study showed no significant difference in the antioxidant activities of the BM methanol extracts among species and preparation methods, even though the antioxidant activities were significantly influenced by TPC (Islam *et al*., 2011).

344 *3.4. Reducing power assay*

345 Fig. 4 shows a significant difference in reducing power between Indian and
346 Chinese BM at all concentrations ($p < 0.01$) for fresh BM samples. No detectable
347 difference ($p \geq 0.05$) was observed between the two varieties after freeze-drying. At a
348 higher concentration range (3.0-5.0 mg/ml), the reducing power of freeze-dried BM
349 was stronger than that of the fresh samples, and no significant difference was detected
350 ($p \geq 0.05$) at lower concentrations (1.0-3.0 mg/ml). Tan *et al*. (2014b) also measured
351 antioxidant activities of BM using the ferric reducing power assay, and reported that
352 the Indian BM showed stronger antioxidant activity than that of the other 5 BM
353 varieties. It was reported that different parts of BM showed significant different
354 antioxidant activities, and found that the antioxidant activity in fresh BM fruit was
355 higher than that in ripened fruit, but lower than that in BM stem and leaves (Kubola &
356 Siriamornpun, 2008). Similarly, Krishnaiah *et al.* (2011) reported that green BM fruit
357 showed lower antioxidant activity than BM leaves, but higher than stem and ripe fruit.

358 Furthermore, the reducing potential was related to the ratio of compounds which
359 donate hydrogen atoms to break the free radical chain (Kubola & Siriamornpun, 2008).
360 Liu *et al*. (2010) isolated new multiflorane and cucurbitane triterpenoids from the
361 stems of BM. The three newly discovered triterpenoids were found with carboxyl and
362 hydroxyl groups. The hydrogens ionized from carboxyl and hydroxyl groups donated
363 by triterpenes were oxidized by Fe^{3+} in the ferricyanide complex. The reducing power
364 of BM also varied according to different fractions based on molecular weight cutoff
365 (MWCO). Fractions with smaller MWCO showed superior antioxidant activities

366 compared to the other fractions (Kenny *et al.,* 2013). In the current study, fresh Indian
367 BM has a stronger reducing power than Chinese BM, which may relate to the change
368 in TTC levels in BM of different varieties (Table 1).

369 Other than the phytochemical compounds investigated in the current study, many
370 other chemical compounds in BM may also contribute to the antioxidant activities. In
371 the study of Kenny *et al*. (2013), hexane extract of BM showed good antioxidant
372 activity. It should be noted that the solvent was highly non-polar. Thus, the antioxidant
373 ability might be contributed by fatty acids and essential oils rather than phenolic based
374 compounds. This assumption was supported by the report that the carboxyl and alkene
375 groups on fatty acids and essential oils also acted as reducers in the DPPH free radical
376 scavenging assay (Kubola &Siriamornpun, 2008).

377

378 *3.5. Alpha-amylase inhibition effect*

379 BM has been used for anti-diabetes treatment for decades. Many compounds in
380 BM contribute to antidiabetic properties. Studies have shown that BM extracts
381 showed hypoglycemic activity in diabetes by stimulating pancreatic secretion,
382 decreasing hepatic gluconeogenesis, increasing hepatic glycogen synthesis and
383 increasing peripheral glucose oxidation (Wang *et al*., 2014). In the current study,
384 antidiabetic activities were evaluated using α-amylase and α-glucosidase inhibition
385 effects.

386 The α-amylase inhibition effect of the fresh BM was stronger than that of the
387 freeze-dried BM (Fig. 5), and the difference was significant ($p < 0.05$) for Indian BM,

388 but not significant for Chinese BM ($p \geq 0.05$). No significant difference was detected
389 among varieties for both fresh and freeze-dried BM. Rather than TTC, the α-amylase
390 inhibition effect was significantly influenced by TPC (Table 1). It was also reported
391 that lupenone, a triterpene, showed strong α-amylase inhibition, and different
392 triterpenes would show varied degrees of inhibition on α-amylase (Yonemoto *et al*.,
393 2014).

394 Ali *et al*. (2006) suggested that the inhibition effect on α-amylase could be
395 attributed only to triterpene acids, and not the other triterpenoids. This was agreed by
396 Hou *et al*. (2009), who reported 6 triterpenes extracted from leaves of *Lagerstroemia*
397 *speciosa* that showed no or weak inhibitory activity against α-amylase. Some phenolic
398 compounds also show α-amylase inhibition activity, and the synergy of
399 phytochemicals was also relevant to enzyme inhibition (Sousa and Correia, 2012;
400 Apostolidis *et al*., 2007; Cheplik *et al*., 2010). Therefore, the inhibition effect on
401 α-amylase might come from a group of phytochemicals like some specific triterpenes,
402 and phenolic compounds.

403 TPC and TTC were measured instead of specific compounds. Further
404 investigation is needed to understand structure-functionality relationships of the
405 individual compounds in terms of the mechanism of enzyme inhibition effects.

406

407 *3.6. Alpha-glucosidase inhibition effect*

408 The α-glucosidase inhibition effect was determined *in vitro* and the results are
409 shown in Fig. 5. A significant difference was detected between the two varieties for

410 both fresh and freeze-dried BM (Fig. 5). The inhibition effect of the Indian BM was
411 stronger ($p < 0.05$) than that of the Chinese BM in both freeze-dried and fresh samples.
412 There was no significant difference ($p \geq 0.05$) between freeze-dried and fresh samples
413 for the same varieties.

414 Pearson correlation analysis showed that the α-glucosidase inhibitory effect was
415 not significantly correlated with TPC (Table 1). The negative correlation was
416 supported by Djeridane *et al*. (2015), who reported that α-glucosidase inhibition
417 activity is inversely proportional to the contribution of phenolic compounds. However,
418 Ali *et al.* (2006) implied that flavanone glycosides and luteolin (a flavonoid) had
419 inhibitory effects on α-glycosidase. This suggested that the effect of α-glucosidase
420 inhibition was a comprehensive result contributed by many phytochemicals
421 (Djeridane *et al*., 2015).

422 A number of compounds have been reported with antidiabetic effects. Charantin in
423 BM is recognized as one of the signature constituents for antidiabetic effects
424 (Pitipanapong *et al.,* 2007). Charantin is a mixture of two saponin compounds, and is
425 one of BM's major bioactive contents. It is reported that the charantin-rich extract of
426 BM led to a significant decline in blood glucose, plasma glucose intolerance, and
427 insulin resistance in the mice model (Wang *et al*., 2014). BM seeds showed an
428 inhibition of 38% for α-amylase and an inhibition of 79% for α-glucosidase. This was
429 done using polypeptide aqueous extracts of BM, which also showed the hypoglycemic
430 function (Virdi *et al*., 2003). BM polysaccharides were reported with
431 anti-hyperglycemia effect by defending pancreatic islet tissue, raising glucose

tolerance and promoting metabolism of glucose (Wu *et al*., 2006; Xu *et al*., 2015; Xu *et al*., 2006). TTC did not significantly contribute to either the α-amylase or α-glucosidase inhibitory effect. This was agreed by Hou *et al*. (2009), who have reported that there was no or weak inhibitory activity of 6 triterpene compounds against α-amylase, while they implied different inhibitory activities against α-glucosidase. Nhiem *et al*. (2010) compared α-glucosidase inhibitory activities of 14 triterpenes extracted from BM using methanol. Two of them showed moderate α-glucosidase inhibitory, 9 of them showed weak inhibitory activity, and three of them were inactive. According to Zeng *et al.* (2014) and Chang *et al*. (2011), the -OMe groups in triterpene might contribute to the antidiabetic activities due to their specific location in the molecule's structure. In the current study the Indian BM had significantly higher TTC content that that of Chinese BM. Therefore, the increased TTC in Indian BM may contribute to its higher α-glucosidase inhibition effect.

4. Conclusion

Phytochemical compounds, antioxidant activities, and antidiabetic properties of fresh and freeze-dried BM between Indian and Chinese varieties were compared. The results showed that the Indian BM had higher phytochemical contents, stronger antioxidant activities, and antidiabetic properties. The fresh BM sample mostly showed better antioxidant and antidiabetic activities. TPC and TTC in the freeze-dried BM samples were significantly higher than the fresh ones ($p < 0.05$). TTC in the Indian BM was significantly higher than that in the Chinese BM for both the fresh and

freeze-dried samples ($p < 0.01$). The DPPH IC_{50} values of the Indian BM were significantly lower than that of the Chinese BM for both fresh and freeze-dried samples ($p < 0.05$), which indicated that the Indian BM showed stronger DPPH scavenging activities. The reducing power of the fresh Indian BM was significantly stronger than that of the fresh Chinese BM ($p < 0.05$). The antidiabetic property assays showed that the fresh Indian BM had significantly stronger anti-α-amylase inhibition effects than the freeze-dried BM, but no significant differences were detected between the two varieties. For the anti-glucosidase inhibition effect, the Indian BM showed significantly stronger effect than the Chinese BM. The correlation study showed that TPC was not correlated with the reducing power or the DPPH IC_{50} value. TTC was positively correlated to reducing power ($p < 0.01$). TPC was significantly negatively correlated with α-amylase inhibition efficiency ($p < 0.05$). In summary, fresh Indian BM showed the best antioxidant and antidiabetic properties. Freeze-drying increased the TPC, TTC, and reducing power in BM, but not the DPPH scavenging activity and antidiabetic properties.

Conflict of interest

All authors have declared that there is no conflict of interest for publishing this research.

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649

650 Table 1. Correlation coefficients of TPC and TTC with antioxidant activities and
651 antidiabetic properties

	TPC	TTC	DPPH	RP	AMY	GLU
TPC	1.000					
TTC	0.680*	1.000				
DPPH	0.177	-0.285	1.000			
RP	0.557	0.797*	-0.123	1.000		
AMY	-0.676*	-0.423	-0.164	-0.019	1.000	
GLU	-0.317	0.364	-0.500	0.319	0.270	1.000

652 ** Significantly correlated at $p < 0.01$; * Significantly correlated at $p < 0.05$; DPPH:
653 IC_{50} value of DPPH free radical scavenging ability; RP: reducing power; AMY:
654 α-amylase inhibition; GLU: α-glucosidase inhibition;
655

656 **Figures**

657 Fig. 1 Total phenolic content of freeze-dried and fresh bitter melon samples. Values
658 are shown as mean ± standard deviation (n=3). Values with the different
659 superscript letters a and b, are significantly different at $p < 0.05$.

660 Fig. 2 Total triterpene content of freeze-dried and fresh bitter melon samples. Values
661 are shown as mean ± standard deviation (n=3). Values with the different
662 superscript letters (a, b and c) are significantly different at $p < 0.05$.

663 Fig. 3 IC_{50} value of DPPH free radical-scavenging ability of bitter melon samples.
664 Values are shown as mean ± standard deviation (n=3). Values with the different
665 superscript letters (a, b and c) are significantly different at $p < 0.05$.

666 Fig. 4 Reducing power of fresh and freeze-dried bitter melon samples. Values are
667 shown as mean ± standard deviation (n=3).

668 Fig. 5 A–amylase inhibition effects and α–glucosidase inhibition effects of fresh and
669 freeze-dried bitter melon samples. Values are shown as mean ± standard deviation
670 (n=3). Values with the different superscript letters (a and b for α–amylase
671 inhibition effect; x, y and z for α–glucosidase inhibition effect) are significantly
672 different within each effect at $p < 0.05$.

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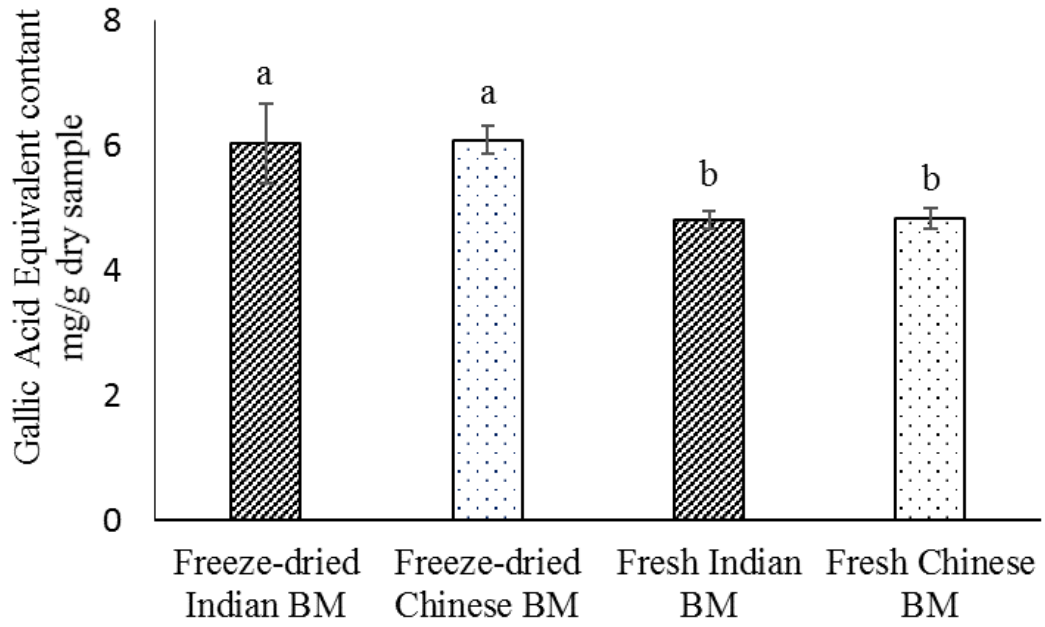

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679 Fig. 1

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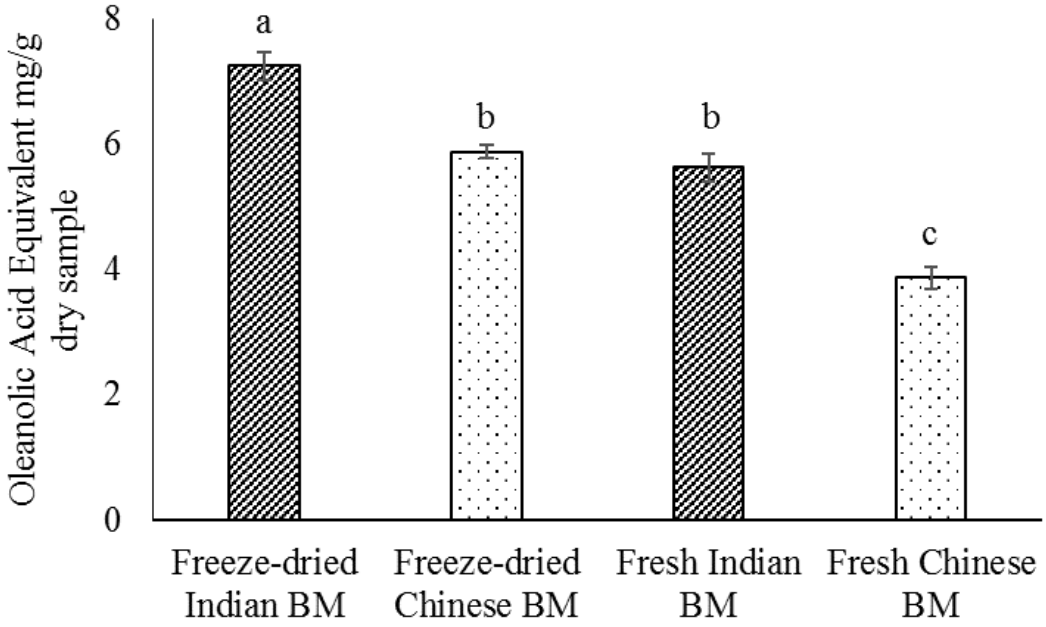

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683 Fig. 2

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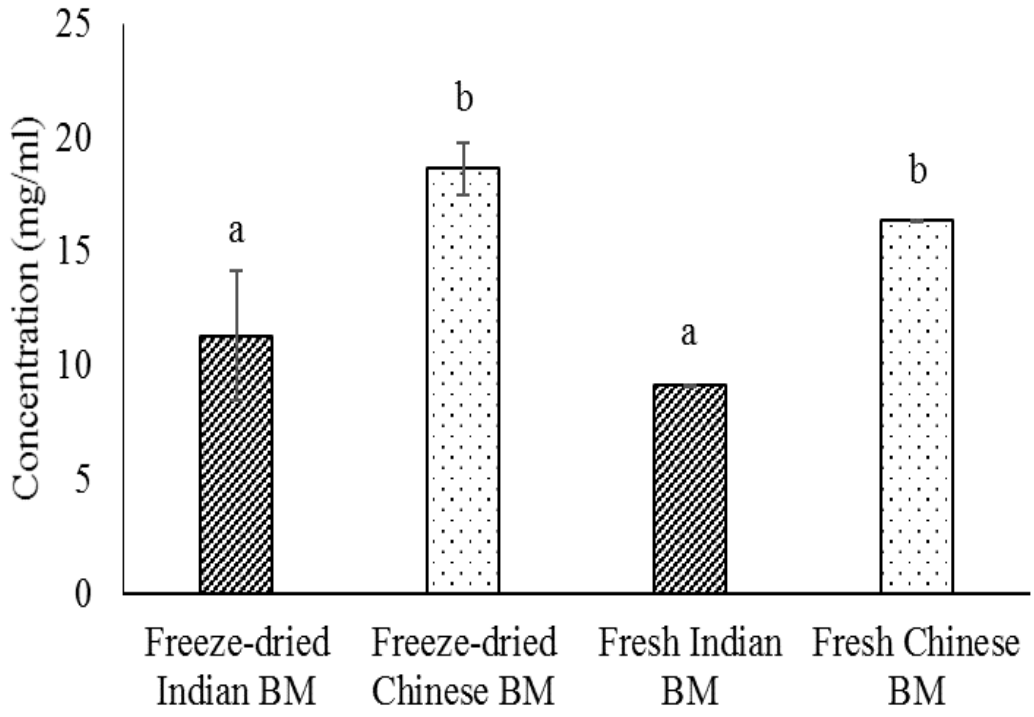

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687 Fig. 3

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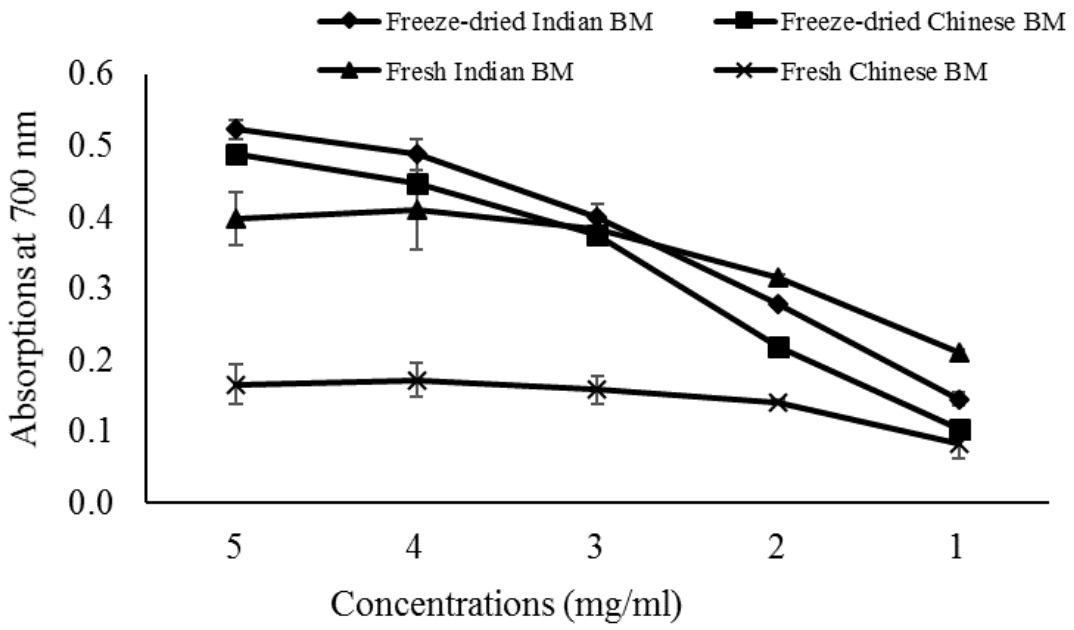

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691 Fig. 4

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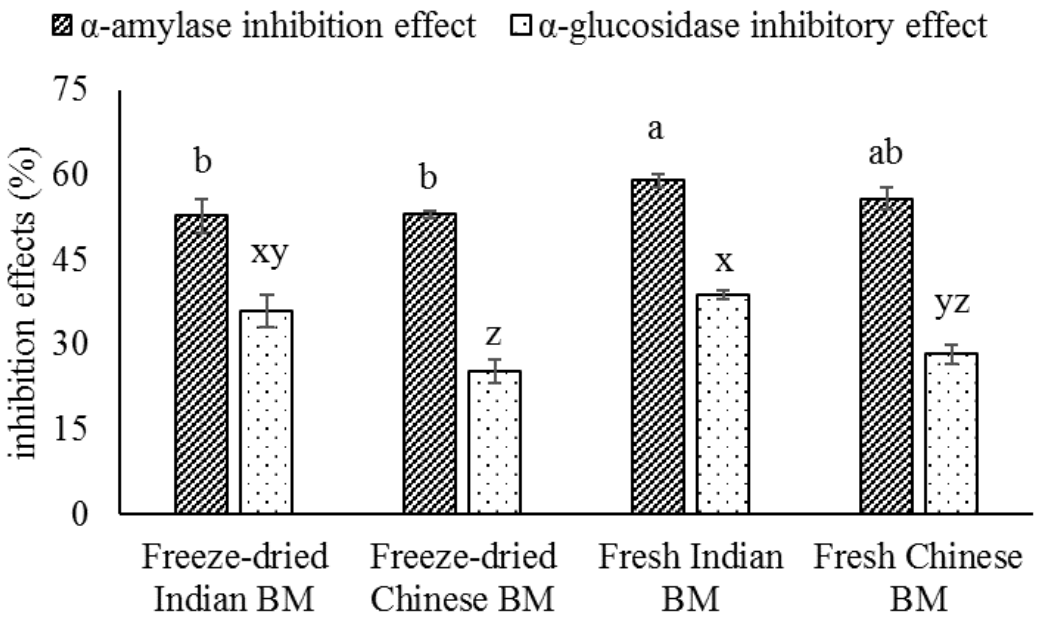

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695 Fig. 5.