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Antioxidative Properties of Bitter Gourd (Momordica charantia) and Zucchini (Cucurbita pepo)

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Antioxidative properties of bitter gourd (*Momordica charantia*) and zucchini (*Cucurbita pepo*)

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Abstract

The enzymatic and non-enzymatic antioxidative capacities of bitter gourd (*Momordica charantia*) and zucchini (*Cucurbita pepo*) were investigated in water extracts and chemical buffer extracts. Bitter gourd and zucchini fruits were purchased from a farmer's market and homogenized separately in water and in a native enzyme extraction buffer. Total phenolic compounds, free radical DPPH scavenging activity, SOD activity and β-glucosidase activity were assayed in the extracts. The average total phenolic compounds recorded in bitter gourd were 13.28 GAE/g fresh weights while in zucchini, the average was 8.67GAE/g fresh weight. This study also found that bitter gourd was 82.05% as effective as ascorbic acid in inhibiting the free radical DPPH while zucchini was 12.19% as effective. The results indicated that bitter gourd was significantly higher in antioxidant content and in β-glucosidase activities than zucchini ($P<0.05$). On the other hand, significantly higher SOD activities were recorded in zucchini than in bitter gourd extracts.

Key words: Phenolic compounds, β-glucosidase, Bitter gourd, Zucchini

Introduction

Bitter gourd (*Momordica charantia* L.), also known as bitter melon, is an important vegetable grown in tropical and sub-tropical regions of Africa, India, China, and several Caribbean countries (Aboa et al., 2008; Wu and Ng, 2008). Bitter gourd belongs to the cucumber family (Cucurbitaceae), a group comprising about 130 genera and 800 species (Radford et al., 1968). Bitter gourd is a herbaceous plant that can grow up to 10 meters tall. The plant bears simple, alternate leaves that are 4-5 cm in width with 3-7 deeply separated lobes. The plant bears oblong fruits with a distinct waxy exterior. The interior of the fruit is somewhat hollow, containing white pith and seeds. When ripe, the fruits become yellow and split into segments that curl back to reveal the seeds. The fruits are eaten while still green and unripe. They have been used for generations by indigenous populations in Africa, India, and Latin America for food and folk medicine (Khan and Anderson, 2003; Dey et al., 2006; Lako et al., 2007; Abo et al., 2008).

Bitter gourd has been the subject of intensive investigations for biologically active compounds and for its medicinal properties (Majekodunmi et al., 1990; Begum et al., 1997). Kubola and Siriamornpun (2008) investigated the phenolic contents and antioxidant properties of leaf, stem, and fruit extracts by analyzing the inhibition of the free radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH). They found that the fruit extract had the highest value of antioxidant activity and that gallic acid was the predominant phenolic compound in the fruit extract. Wu and Ng (2008) compared the antioxidant capacity of bitter gourd extracted in water versus ethanolic extraction and found that both water and ethanol extracts were effective in reducing the stable radical DPPH to the yellow diphenylpicryl hydrazine. They also found that both water and ethanol extracts were effective in reducing the stable radical DPPH to the yellow colored diphenylpicryl hydrazine. The folk medicinal properties associated with bitter gourd includes treatment for various chronic and degenerative diseases, including, diabetes mellitus (Nerurkar et al., 2006; Aboa et al., 2008; Nerurkar et al., 2010) coronary heart disease and cancer (Slater, 1984; Cheng et al., 2003). The seeds of bitter gourd are believed to contain anticancer

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agents and remedies for gastrointestinal diseases (Beloin et al., 2005).

Another vegetable food crop believed to have medicinal values is zucchini (*Cucurbita pepo* L.) The genus *Cucurbita* includes pumpkin, acorn squash, crooked neck squash, and straight neck squash, all of which contain significant amounts of cucurbitane glycosides and triterpenoid compounds. Zucchini is a temperate, small plant that can grow up to 2 meters, having leaves 8-30 cm across, with 3-7 dissected lobes that have notches (Radford et al., 1968). The plant is dioecious and bears orange flowers. The fruits of zucchini are cylindrical and have a thin, smooth, and waxy exterior. The color of the fruit ranges from light green to dark green. The interior of the fruit is white and contains small white seeds. Zucchini is consumed either raw in salads or cooked in soups (Stephens, 2009). The folk medicinal properties associated with zucchini are numerous and include treatment for benign prostatic hyperplasia and leprosy (Dhiman et al., 2012).

Both bitter gourd and zucchini are believed to possess enzymatic and non-enzymatic antioxidant activities against the buildup of reactive oxygen species (ROS) in the cells (Dasgupta and De, 2006; Wu and Ng, 2008; Xanthopoulou et al., 2009; Dhiman et al., 2012). ROS include the singlet oxygen $^1O_2$, the superoxide anion O_2^-, the hydroxyl ion OH^-, the peroxide O_2^{2-}, and the hydrogen peroxide H_2O_2. These free radicals cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes. ROS are also cellular signaling agents in the regulation of plant development, stress responses, hormone regulation and programmed cell death (Palma et al., 2002; Breusegem and Dat, 2006). Organisms defend themselves against the destructive actions of ROS by using enzymatic and non-enzymatic mechanisms. The enzymatic mechanism makes use of the enzyme superoxide dismutase (SOD) and the hydrogen peroxidase enzyme (PO) (Bray, 2000; Breusegem and Dat, 2006; Dasgupta and De, 2006) and the non-enzymatic antioxidant pathway includes the actions of vitamins, phenolic compounds, tannins, and Gallic acid (Breusegem and Dat, 2006; Wu and Ng, 2008; Harr and Ishmail, 2012).

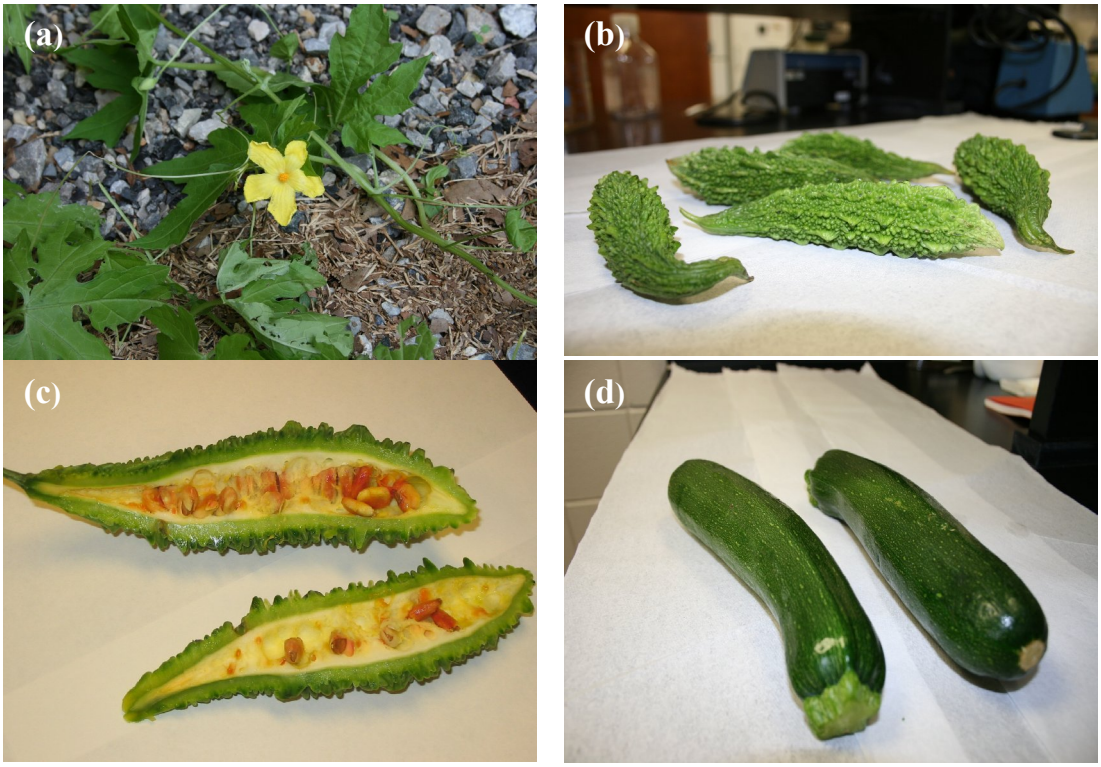

Figure 1. Photographs of bitter gourd (*Momordica charantia*) and zucchini (*Cucurbita pepo*) showing (a) flowering bitter gourd plant, (b) bitter gourd fruit, (c) sliced bitter gourd fruit, and (d) zucchini fruits.

Bitter gourd and zucchini are known to contain a variety of biologically active compounds including the alkaloids momordicine I, momordicine II, and cucurbitacine B (Majekodunmi et al., 1990). Other compounds reported in these vegetables include a cytotoxic ribosome binding terpenoid and several glycosides including charantin, charantoside, and momordicoside (Begum et al., 1997; Harinantenaina et al., 2006; Akihisa et al., 2007; Chang et al., 2008; Dhiman et al., 2012). Glycosides are hydrolyzed by β-glucosidase enzymes (EC 3.2.1.21). β-glucosidase is a member of a large class of enzymes known as glycosidase, which are important for the plant's own defense mechanisms against herbivores, and pathogens. The presence of significant activities of β-glucosidase in plants has been correlated with pharmacological values of the plant (Lako et al., 2007). The USDA Nutrient Database mentioned bitter gourd to be high in carbohydrates and fibers, and to contain large amounts of folate (Vitamin B9) and Vitamin C.

The objectives of this research were to investigate the total phenolic compounds and the effectiveness of bitter gourd and zucchini extracts in inhibiting the free radical DPPH and to investigate the activities of the superoxidase dismutase (SOD) and β-glucosidase enzymes in bitter gourd and zucchini extracts.

Materials and Methods

Plant materials

This research was conducted between 2008 and 2012 in the Department of Biology of Jacksonville State University, Jacksonville, Alabama, USA. Fruits of bitter gourd (*Momordica charantia)* and zucchini (*Cucurbita pepo*) were purchased from the Dekalb Farmer's Market in Atlanta, Georgia (USA) on 2 separate occasions. The fruits were brought to the laboratory where they were weighed and then washed to remove debris as well as residual pesticides. The fruits were separately extracted first in protein extraction buffer for enzymatic analyses and then in distilled water for non-enzymatic antioxidants determination.

Protein extraction

Native enzymes were extracted from each bitter gourd and zucchini fruit as total soluble proteins by grinding the fruits at a ratio of 0.5 g fresh weight per ml extraction buffer (100 mM KPO_4, 1 mM KCl, 1 mM EDTA, 1% PVP, pH 7.5) in a mortar and pestle. The samples were further homogenized in a Teflon glass homogenizer and then sequentially filtered through 1, 2, and finally 4 layers of cheesecloth. The filtrates were centrifuged at 16,000 g for 20 minutes at 4°C. The resulting supernatants were used as total soluble native proteins (enzymes) and used in the determination of superoxide dismutase and β-glucosidase activities. The total protein yield of each extract was determined according to the Bradford (1976) method using bovine serum albumin (BSA) as the standard protein.

Aqueous extraction

For aqueous extraction, the fruits were ground in a Warring blender at a ratio of 0.5 g fresh weight/ml in distilled H_2O. The samples were homogenized in a Teflon glass homogenizer and then sequentially filtered through 1, 2, and finally 4 layers of cheesecloth. The filtrates were centrifuged at 16,000 g for 20 minutes at 4°C and the resulting supernatants were retained as aqueous extracts and used for the non-enzymatic antioxidants determination.

Total phenols assay

The total phenolic compounds of each extract was determined as Gallic acid equivalent (GAE) using the Folin-Ciocalteu method (Zhou and Yu, 2006). The assay consisted of reacting 200 µl of each extract with 1 ml of Folin-Ciocalteu reagent in cuvets and allowing the mixtures to incubate at room temperature for 5 minutes. Following the 5 minute incubation, 1 ml of 0.5 M sodium bicarbonate solution was added to the reaction mixture and incubated at room temperature for an additional 90 minutes. Various concentrations of Gallic acid were made as standards and treated the same way as the experimental cuvets. The absorbances of the cuvets were recorded at 725 nm using a UV/VIS GENESYS spectrophotometer. The absorbances of the samples were compared to those of known concentrations of Gallic acid. The total phenolic content of each extract was expressed as µg of GAE/ml.

DPPH inhibition assay

DPPH free radical scavenging activity was measured spectrophotometrically at 517 nm according to the methods of Cheung et al. (2003) and of Wu and Ng (2008). The procedure consisted of making 3.0 ml dilutions of each sample in spectrophotometer cuvets. The dilutions consisted of 0.1 µg/µl and 0.5 µg/µl total proteins. 3.0 ml extraction buffer was used as a negative control and 3.0 ml of 0.2 µg/µl ascorbic acid was used as a

positive control in 2 separate cuvets. To each cuvet, 3 ml 0.1 mM DPPH were added. The reaction mixtures were incubated at room temperature for 30 minutes and the absorbances were recorded at 517 nm. The percent inhibition of DPPH was calculated as [(Absorbance without extract – Absorbance with extract)/Absorbance without extract] x 100 (Wu and Ng, 2008).

Superoxide dismutase (SOD) activity

The abilities of the extracts to neutralize the superoxide free radical were measured spectrophotometrically according to Beauchamp and Fridovich (1971). Aliquots of 0.1 ml extracts were made in 5 ml cuvets. Three replicates were made for each sample. To each aliquot, 3.0 ml reaction mixture (50 mM sodium phosphate buffer, pH 7.8, 0.2 mM NBT, 10 mM L-methionine, 1 mM EDTA, and 5.0 µM riboflavin) were added. The second set was wrapped in aluminum foil and served as a blank. Both sets were placed in a growth chamber with light intensity of 3,000 mol^{-1} m^{-1} s^{-1} for 10 minutes. The reduction of NBT by the SOD enzymes was measured spectrophotometrically at 560 nm. A standard curve was generated using commercial Chloroplast SOD (Sigma Aldrich, St. Louis, MO, USA) according to Hamissou (2011).

β-Glucosidase assay

β-glucosidase enzyme activity was investigated spectrophotometrically at 420 nm. For each sample, triplicate cuvets containing 2.0 ml each were reacted with 2.0 ml of 8.0 mM p-nitrophenyl glycoside (NPG) in acetate buffer, pH 6.1. β-glucosidase catalyzes the hydrolysis of a wide variety of substrates including p-nitrophenyl- β-glucosides (NPG). This substrate was chosen because one of its reaction products, p-nitrophenol (NP), absorbs strongly in the blue region of the visible spectrum and the reaction can be measured calorimetrically at 420 nm. The reaction mixtures were incubated for 1 hour at room temperature and the reactions were stopped by adding 100 µl of 100 mM sodium bicarbonate to each cuvet. The amount of NP liberated was deduced from a standard curve generated using commercially purchased p-nitrophenol (Sigma Aldrich, St. Louis, MO, USA).

Statistical analyses

The fruits were purchased on two different dates from the Farmers' Markets and subsequently extracted on two different dates. The two different extractions dates were considered blocks. Three bitter gourds and 3 zucchinis were used for each extraction; all biochemical assays were performed 3 times. A two-way ANOVA was performed to compare the data between bitter gourd and zucchini extracts (n = 12).

Results

An average of 4.11 ± 0.67 mg total soluble protein/g fresh weight was obtained in bitter gourd extracts while the average of total soluble protein yield in zucchini extracts was 1.85 ± 0.32 mg/g fresh weight (Table 1).

Table 1. Total soluble proteins recorded in bitter gourd (*Momordica charantia*) and in zucchini (*Cucurbita pepo*) fruits.

	Bitter gourd (mg/g fresh weight)	Zucchini (mg/g fresh weight)
ȳ	4.11^{a}	1.85^{b}
SD	0.67	0.32
SE	0.19	0.09

The means are followed by letters indicating significant differences between bitter gourd and zucchini in total soluble proteins, at 5% probability level (n = 12).

Bitter gourd averaged 13.28 ± 1.71 mg GAE/g fresh weights in total phenolic compounds, while zucchini averaged was 8.67 ± 1.59 mg GAE/g fresh weights (Table 2). On average, bitter gourd was 82.05% ± 7.52 as effective as ascorbic acid (%EAA) in inhibiting the free radical DPPH while zucchini was 12.19% ± 4.20 as effective as ascorbic acid (Table 2).

Table 2. Non-enzymatic antioxidant activities of bitter gourd (*Momordica charantia*) and zucchini (*Cucurbita pepo*) extracts, n = 12.

	Bitter gourd	Zucchini
Total phenolic compounds (GAE/g fresh weight)	13.28^{a} ± 1.71	8.67^{b} ± 1.59
DPPH scavenging (%EAA)	82.05^{a} ± 7.52	12.19^{b} ± 4.20

Numbers in the same row followed by the same letters are not significant at 5% probability level.

The results of the enzymatic antioxidant capacity (SOD) of bitter gourd and zucchini extracts are presented in Table 3. An average of 1.55 ± 0.60 units of SOD activity per µg total proteins (ua/µg total proteins) was recorded for bitter gourd while in zucchini, an average of 1.996 ± 0.88 ua/µg proteins was obtained. The results of β-glucosidase activity are also shown in Table 3. Bitter gourd extracts averaged 0.32 ± 0.14 units of activity per µg total protein (ua/µg total protein while zucchini extracts averaged 0.19 ± 0.02 ua/µg total proteins.

Table 3. Enzymatic antioxidant and β-glucosidase activities of bitter gourd (*Momordica charantia*) and zucchini (*Cucurbita pepo*) extracts, n = 12.

	Bitter gourd	Zucchini
SOD Activities (ua/µg total prot.)	1.55 ± 0.60	2.00 ± 0.88
β-glucosidase activities (ua/µg total prot.)	0.32 ± 0.14	0.19 ±0.02

Discussion

In most plants, total phenolic compounds have been determined to be the main antioxidative compounds. Wu and Ng (2008) and Kubola and Siriamornpun (2008) independently investigated the phenolic compounds of bitter gourd fruits extracted in water and obtained averages of 0.516 mg of GAE/ml per ml and 0.202 mg GAE/ ml respectively. The two independent investigations reported their data in mg GAE/ml extract not specifying the amount of fresh weight in the 1.0 ml extract. In the absence of agreeable expression of units, this study found it more informative to present data in mg of antioxidant/g fresh weight of the fruit or vegetable rather than mg antioxidant/ml extract. Therefore, this research is reporting its findings of total phenolic compounds in mg GAE/g fresh weight. Bitter gourd averaged 13.28 ± 1.71 mg GAE/g fresh weight and zucchini averaged 8.67 ± 1.59 mg GAE/g fresh weight. Although data comparison between the present data and those obtained by Wu and Ng (2008) and by Kubola and Siriamornpun (2008) is not possible, this study found that bitter gourd fruits contain significantly higher amounts of phenolic compounds than zucchini.

Another property useful in determining the non-enzymatic antioxidant values of fruits and vegetable is the capacity of the extracts to inhibit the free radical DPPH. Antioxidants present in the extracts were expressed as the reduction of the purple-colored stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) to the yellow-colored diphenylpicryl hydrazine by donating an electron or a hydrogen. This reduction of the free radical diminishes cellular damage by allowing the neutralization of reactive oxygen species present in the solution (Wu and Ng, 2008). As a result of a buildup of ROS, lipid peroxidation of cell membranes can occur. DPPH scavenging activity is expressed in IC_{50} values or in percent ascorbic acid or Vitamin E equivalents. Ascorbic acid, an antioxidant commonly known as Vitamin C, is known to scavenge 50% of DPPH at 50 µg/ml (Hsu et al., 2007; Arazo et al., 2011; Ramkumar et al., 2012). Wu and Ng (2008) reported IC_{50} values of 129.94 µg/ml in bitter gourd extracted in water. In this research, the DPPH reductions by the zucchini extracts never surpassed the 50% mark, making it impossible to determine IC_{50} values. Since IC_{50} value and percent inhibition indicate the same potential, this research found it necessary to report the present data as percent effectiveness of the extracts to inhibit the free radical DPPH compared to ascorbic acid. Bitter gourd was found to be 82.05% ± 7.52 as effective in scavenging DPPH free radial as ascorbic acid. This is significantly important information to report when considering that zucchini was only 12.19 ± 4.20% as effective as ascorbic acid. Our data are however in agreement with other researchers (Hsu et al., 2007; Wu and Ng, 2008), indicating that bitter gourd is a high antioxidant containing vegetable food.

Enzymatic antioxidants are equally important in protecting organisms against free radical build-up. Enzymatic antioxidants, such as superoxide dismutase, protect cells and tissues from oxidative damage by reactive oxygen species. Superoxide dismutase (SOD), peroxidases (PO) and catalases (Cat) are some of the enzymatic antioxidative defense mechanisms. In this study, only the SOD activity in bitter gourd and zucchini fruits were investigated. In the absence of comparable published data in this area, this research is reporting that zucchini extracts showed higher SOD activity than bitter gourd extracts with values of 1.996 ± 0.30 and 1.55 ± 0.44 u a/ µg total proteins respectively. Although the magnitudes of the values were low, the two-way ANOVA indicated the existence of a significant difference between bitter gourd and zucchini, at 5% probability level. Interestingly, there is evidence suggesting that diabetes and other health problems are complicated by oxidative stress due to generation of free radicals (Garg et al., 1996) and by a decrease in the body's natural antioxidant defenses (Oberly, 1988). In an independent study, Sathishsekar and Subramanian (2005) showed that diabetic rats had lower SOD activity in their kidneys and livers compared to diabetic rats treated with bitter gourd seed extracts.

The presence of β-glucosidase enzyme is another property indicative of potential pharmacological value in plants. The activity of β-glucosidase enzyme does not add to the antioxidant value vegetables and fruits directly, but the products of their reactions may serve as precursors to some non-enzymatic antioxidant compounds (Sanchez-Medina et al., 2001). Zucchini had significantly more β-glucosidase enzyme activity than bitter gourd

with values of 0.19 ± 0.01 units of activity per µg total protein (ua/µg total protein) and 0.32 ± 0.07 ua/µg total proteins, respectively. Plants with high activity of this enzyme have been useful tools in drug discovery due to their importance in generating precursor molecules used in several processes such as hormone regulation, defense mechanisms, and antioxidant production. Some toxic compounds synthesized through the activity of this enzyme include hydrogen cyanide, rotenoids, and quinones. Although minute levels of β-glucosidase activity were detected in both bitter gourd and zucchini extracts, the data establish a foundation for future investigations of the pharmacological properties of bitter gourd and zucchini.

Conclusions

This research found that bitter gourd was 82.05% as effective as ascorbic acid in inhibiting the free radical DPPH while zucchini was 12.19%. Bitter gourd has significantly higher total phenolic compounds than zucchini, indicating that bitter gourd is a high antioxidant containing vegetable food, and that bitter gourd has more β-glucosidase activities than zucchini. On the other hand, zucchini fruits showed higher activities of the enzymes SOD.

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