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Comparison of antioxidant capacity and α-glucosidase inhibitory activity between bitter melon (*Momordica charanti*) fruit and leaf extract

Eun-Sun Hwang✉

Department of Nutrition and Culinary Science, Hankyong National University, 327 Chungang-ro, Anseong-si, Gyeonggi-do, 17579, Korea

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ABSTRACT

Objective: To compare the physiologically active substances, antioxidant and antidiabetic activities *in vitro* of bitter melon fruit and leaf extract. **Methods:** Total polyphenol and flavonoid contents were measured using spectrophotometrically by gallic acid and catechin standard curves, respectively. The radical-scavenging potential of bitter melon fruit and leaf extract were measured by DPPH, ABTS and hydroxyl radical scavenging ability and reducing power and anti-diabetic ability was evaluated by α-glucosidase activity. **Results:** It was confirmed that the bitter melon leaf contained more total polyphenols and flavonoids than bitter melon fruits. Bitter melon leaf extract contained 2.8-fold and 4.9-fold higher total polyphenols and flavonoids than bitter melon fruits, respectively. The DPPH radical scavenging activity of bitter melon leaf was 5.81- and 5.70-fold higher than that of the bitter melon frui, based on 200 μg/mL and 400 μg/mL of the extract, respectively. In ABTS, hydroxyl radical scavenging ability and reducing power, the bitter melon leaf extract all showed higher antioxidant capacity than the bitter melon fruit. Bitter melon fruit showed 2.52- and 2.63-fold higher α-glucosidase inhibitory activity than bitter melon leaf extract at 200 μg/mL and 400 μg/mL, respectively. **Conclusions**: Based on our results, bitter melon may improve antidiabetic effects by upregulating α-glycosidase activity. Even, bitter melon leaf extract shows higher antioxidant potential than its fruit but bitter melon leaf extract does not show higher α-glucosidase inhibitory potential than bitter melon fruit. The overall results support that bitter melon fruit and leaf may have specific target effects on antidiabetic and antioxidant, respectively.

1. Introduction

Bitter melon (*Momordica charantia*) is cultivated in Middle East, Africa, India and China as a tropical or semi-tropical plant beloinging to the Cucurbitaceae family. It is apparently similar to cucumber, immature fruit is emerald green and orange-yellow color changes with maturity. It has a very bitter taste and is consumed as a vegetable in an immature state, while it is used as condiments when it is mature[1]. Bitter melon has been widely used as a natural or folk remedy to treat diabetes by the general public[2]. It has also been used to alleviate symptoms of kidney-stone, piles, galactagogue,

✉First and corresponding author: Eun-Sun Hwang, Department of Nutrition and Culinary Science, Hankyong National University, Gyeonggi-do, Republic of Korea.
Tel: +82 31 670 5182
Fax: +82 31 670 5189
E-mail: ehwang@hknu.ac.kr

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contraceptive, abortifacient, anthelminitic, gout, and pneumonia[2-5].

Bitter melon has large amoutns of physiologically active substances and various vitamins. It is rich in minerals, such as zinc, iron, magnesium, calcium and is an excellent source of fiber[6]. Especially, bitter melon contains a special triterpenoid called charantin, which has been reported to have excellent antidiabetic properties[7]. Bitter melon has high amount of momorcharins, momordins. These compounds inactivate ribosomes, and momordins alter gastrointestinal traisit time and lower the blood glucose[3,8].

Traditionally, the wild bitter leaves have been used to treat burns, insect bites, rashes, bee strings by juicing[9]. It is reported that drinking tea with boiled bitter melon leaves and its fruits are good for preventing or treating hypertentsion, betes, stomachache, toothache, liver diseases[10].

Fruit leaves are agricultural by-product of the tree, and contain more antioxidants of natural source, such as polyphenols, flavonoids, lignin and semi-secoirdoids than those of fruits[11,12]. Previous studies have indicated that fruit leaves such as blackcurrent, bilberry, sea buckthorn and quince leaves exhibit numerous health-promoting biological activites inculidng antioxidant activity, lipid peroxidation inhibition and anti-mutation activitie[12-14].

At present, several studies have been reported on antioxidant activity of bitter melon fruit and there is little information available on bitter melon leaf. The knowledge about active compounds of bitter melon leaf are mostly unknown. This study was conducted to investigate the physiologically active substances in bitter melon fruit and leaf extract, and to measure their antioxidant and antidiabetic activities *in vitro*.

2. Material and methods

2.1. Chemicals

Gallic acid, catechin, Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), nitro blue tetrazolium chloride, nicotinamide adenine dinucleotide, Tris-HCl, para-methyl styrene, sodium phosphate monobasic, and α-glucosidase were purchased from Sigma Chemical (Sigma-Aldrich Co., St. Louis, MO, USA). Hydrogen peroxide, trichloroacetic acid, ethylendiaminetetraacetic acid, and sodium phosphate dibasic anhydrous were purchased from SamChun Chemical (Pyeongtaek city, Korea). 2-thiobarbituric acid was purchased from Wako chemical (Tokyo, Japan) and all chemicals used were of analytical grade.

2.2. Sample preparation

Bittere melon fruit and leaf grown in Cheonan, Korea in October 2016 were purchased. Water-washed bitter melon fruit was cut to a thickness of 0.5 mm and freeze-dried in a freeze-drier (Ilshin BioBase, Dongducheon, Korea) after freezing at -80 ℃. Bitter melon leaf was also washed and freeze-dried after freezing at -80 ℃. The lyophilized bitter melon fruit and leaf were finely ground using a blender (Tefal, Seoul, Korea). Lyophilized powder sample (5 g) was taken in an Erlenmeyer flask, then 125 mL of 80% ethanol was added and extracted for 2 h at 60 ℃.

The extracts were filtered on a vacuum connected device using Whatman #2 filter paper (Whatman International Ltd., Maidstone, UK), and this extraction procedure was repeated 3 times. The filtered liquid was evaporated using a rotary vacuum concentrator (EYELA, Tokyo, Japan) at 40 ℃. The concentrated extract was dried with a freeze-drier and pulverized so that the particle sizs was less than 1.0 mm. The samples were air-sealed, placed in a plastic bag and stored at -20 ℃ for the next experiment.

2.3. Measurement of total polyphenol and flavonoid contents

Total polyphenol contents were determined by referring to the color measurement method of Folin-Ciocalteu[15]. The total flavonoid content in the samples was determined with reference to Woisky and Salatino[16].

2.4. Measurment of antioxidant capacity

The DPPH radical scavenging ability of the sample was measured with the method of Cheung *et al*[17]. The ABTS radical scavenging ability of the sample was measured by Re *et al*[18]. The hydroxyl scavenging ability of the sample was measured by Chung *et al*[19]. The reducing power of the sample was measured by Oyaizu[20].

2.5. Measurement of α-glucosidase activity

The α-glucosidase activity of the sample was measured by reference to the method of Kim *et al*[21]. α-Glucosidase was dissolved in 100 mM sodium phosphate buffer (pH 7.0) at a concentration of 0.2 unit/mL. A total of 2 μL of the sample and 100 μL of the enzyme solution were mixed well. Mixed samples were transferred to 96-well plate and absorbance at 405 nm was measured. Then, 2 mM *p*-nitrophenyl α-D-glucopyranoside (100 μL) was rapidly added into 96-well plate and the absorbance at 405 nm was further measured. Acabose (100 μg/mL) which was used to proven the α-glucosidase inhibitory activity was used as a positive control, and the α-glucosidase activity was calculated according to the following formula.

Inhibitory activity (%) = (Absorbane$_{sample}$ / Absorbance$_{control}$) ×100

2.6. Statistical analysis

Statistical analysis was performed using the SPSS software package, version 17.0 (Statistical Package for the Social Sciences,

Spss Inc., Chicago, IL, USA), and the significance was verified by one-way analysis of variance. $P<0.05$ was considered significant.

3. Results

3.1. Total polyphenol and flavonoid contents

The content of total polyphenol measured by gallic acid equivalent was (16.15±0.31) mg/g in bitter melon fruit and (45.55±0.34) mg/g in bitter melon leaf. The total flavonoid content was measured on the basis of catechin, (9.70±0.27) mg/g in bitter melon fruit and (47.25±1.48) mg/g in bitter melon leaf. According to the results, total polyphenol and flavonoid contents were higher in bitter melon leaf extract than bitter melon fruit extract.

3.2. Antioxidant activity

Antioxidant activities of bitter melon fruit and leaf extract were measured by *in vitro* method for inhibtion of DPPH, ABTS and hydroxyl radical formation and reducing power. The DPPH radical-scavenging ability depends on the concentration (25–400 μg/mL) of bitter melon fruit and leaf extract, and the higher the extract concentration, the higher the DPPH redical formation was suppressed to a higher percentage (Table 1). The 100 μg/mL ethanol extract of bitter melon fruit inhibited the production of DPPH radicals by 2.50% on average, and the DPPH radical production was suppressed up to 5.38% at 200 μg/mL and 10.63% at 400 μg/mL of bitter melon fruit extract. The inhibition of DPPH radical formation at 100 μg/mL of ethanol extract of bitter melon leaf was 16.23%, and the in inhibition rate was increased to 31.28% and 60.63% as the concentration of extract increased to 200 μg/mL and 400 μg/mL, respectively. The DPPH radical scavenging activity of the bitter melon leaf extract was 5.81- and 5.70 times higher than that of bitter melon fruit at 200 μg/mL and 400 μg/mL, respectively.

Table 1

DPPH radical-scavenging activity (%) of bitter melon fruit and leaf (mean ± SD).

Concentration (μg/mL)	Bitter melon fruit	Bitter melon leaf
25	$0.93 \pm 0.10^{a***}$	$3.08 \pm 0.22^{a***}$
50	$1.38 \pm 0.10^{a***}$	$6.30 \pm 0.37^{b***}$
100	$2.50 \pm 0.18^{b***}$	$16.23 \pm 0.69^{c***}$
200	$5.38 \pm 0.17^{c***}$	$31.28 \pm 0.55^{d***}$
400	$10.63 \pm 0.87^{d***}$	$60.63 \pm 0.75^{e***}$

[a-e]values with different superscript letters within the same column are significantly different at $P<0.05$. [***]$P<0.001$ by paired t-test.

As shown in Table 2, as the concentration of bitter melon fruit and leaf extract increased from 25 μg/mL to 400 μg/mL, the ABTS radical scavenging ability was also increased. The bitter melon fruit extract inhibited ABTS radical formation up to 3.45% at 100 μg/mL, 6.38% at 200 μg/mL and 11.75% at 400 μg/mL. In the case of bitter melon leaf extract, inhibition of ABTS radical formation was 10.01% at 100 μg/mL, 20.28% at 200 μg/mL, and 45.30% at 400 μg/mL, which showed a higher inhibition rate than the fruit extract of bitter melon. The extract of bitter melon leaf showed ABTS radical scavenging activities of 3.24- and 3.86 times higher than those of bitter melon fruit at 200 μg/mL and 400 μg/mL, respectively.

Table 2

ABTS radical-scavenging activity (%) of bitter melon fruit and leaf (mean ± SD).

Concentration (μg/mL)	Bitter melon fruit	Bitter melon leaf
25	$0.25 \pm 0.06^{a***}$	$0.43 \pm 0.03^{a***}$
50	$1.58 \pm 0.05^{b***}$	$3.65 \pm 0.37^{b***}$
100	$3.45 \pm 0.34^{c***}$	$10.01 \pm 0.89^{c***}$
200	$6.38 \pm 0.43^{d***}$	$20.68 \pm 1.93^{d***}$
400	$11.75 \pm 1.10^{e***}$	$45.30 \pm 2.64^{e***}$

[a-e]values with different superscript letters within the same column are significantly different at $P<0.05$. [***]$P<0.001$ by paired t-test.

When compared based on the same concentration, the bitter melon leaf extract had a higher hydroxyl radical-scavenging power than the bitter melon fruit (Table 3). At the same concentration, the extracts of bitter melon leaf showed stronger hydroxyl radicals-scavenging activity at 400 μg/mL than those of bitter melon fruit at the same concentration. The inhibition of hydroxyl radical formation at 100 μg/mL of ethanol extract of bitter melon leaf was also higher than that of bitter melon fruit. The ethanol extract of bitter melon leaves showed stronger radical-scavenging power than bitter melon fruit. Bitter melon leaf extract showed 1.48- and 1.30 times higher hydroxyl radical scavenging power at 100 μg/mL and 400 μg/mL, respectively, than the bitter melon fruit.

Table 3

Hydroxyl radical-scavenging activity (%) of bitter melon fruit and leaf (mean ± SD).

Concentration (μg/mL)	Bitter melon fruit	Bitter melon leaf
25	$4.38 \pm 0.31^{a***}$	$21.65 \pm 0.99^{a***}$
50	$5.48 \pm 0.32^{b***}$	$22.30 \pm 1.25^{a***}$
100	$15.75 \pm 0.73^{c**}$	$23.38 \pm 1.56^{ab**}$
200	$17.88 \pm 1.10^{d**}$	$25.18 \pm 1.30^{b**}$
400	$19.70 \pm 0.52^{e**}$	$25.55 \pm 1.72^{b**}$

[a-e]values with different superscript letters within the same column are significantly different at $P<0.05$. [**]$P<0.01$ and [***]$P<0.001$ by paired t-test.

The reducing power of ethanol extracts in bitter melon fruit and leaf is shown in Table 4, and numerically higher absorbance means that the reducing power is high. The absorbance values of bitter melon fruit and leaf extracts were 0.19 and 0.23 at 100 μg/mL, respectively. The highest absorbance was observed in bitter melon leaf extracts at 400 μg/mL ($OD_{700\ nm}$ 0.43) which is 1.48-fold higher reducing power than bitter melon fruit extract at the same concentration.

Table 4

Reducing power activity (absorbance at 700 nm) of bitter melon fruit and leaf (mean ± SD).

Concentration (μg/mL)	Bitter melon fruit	Bitter melon leaf
25	0.17 ± 0.01^{a}	0.18 ± 0.01^{a}
50	0.18 ± 0.01^{a}	0.20 ± 0.01^{b}
100	$0.19 \pm 0.01^{b*}$	$0.23 \pm 0.01^{c*}$
200	$0.22 \pm 0.01^{c***}$	$0.29 \pm 0.01^{d***}$
400	$0.29 \pm 0.00^{d***}$	$0.43 \pm 0.00^{e***}$

[a-e]values with different superscript letters within the same column are significantly different at $P<0.05$. [*]$P<0.05$ and [***]$P<0.001$ by paired t-test.

3.3. Determination of α-glucosidase activity

Table 5 shows that the higher the concentration of bitter melon fruit and leaf extracts, the greater the inhibitory activity of α-glucosidase. The α-glucosidase inhibitory effect of bitter melon fruit was higher than its leaf extracts at the same concentration. The average α-glucosidase inhibitory activity at 50 μg/mL in bitter melon fruit was 23.97%, but lowered in leaf extracts as -5.13%. At 100 μg/mL of bitter melon extract, α-glucosidase inhibitory activity of bitter melon fruit was 30.00, which was higher than those in the bitter melon leaf extracts and acarbose, 2.59% and 22.66%, respectively. Bitter melon fruit showed 2.52- and 2.63-fold higher α-glucosidase inhibitory activity than bitter melon leaf extracts at 200 μg/mL and 400 μg/mL, respectively. Bitter melon fruit showed better inhibitory effects of α-glucosidase than acarbose which is commercially used antidiabetic drug to treat diabetes mellitus type 2 in some countries. Based on our results, bitter melon fruit may improves antidiabetic effects by upreguating α-glycosidase activity. Even, bitter melon leaf extracts showed higher antioxidant potential than its fruit but bitter melon leaf extracts did not show α-glucosidase inhibitory potential than bitter melon fruit.

Table 5

α-Glucosidase inhibitory activity of bitter melon fruit and leaf (%) (mean ± SD).

Concentration (μg/mL)	Acarbose	Bitter melon fruit	Bitter melon leaf
25	3.53 ± 1.37^{bA}	13.09 ± 2.92^{cA}	-12.33 ± 0.88^{aA}
50	18.20 ± 1.41^{bB}	23.97 ± 1.41^{cB}	-5.13 ± 0.24^{aB}
100	22.66 ± 3.71^{bBC}	30.00 ± 2.44^{cC}	2.59 ± 1.23^{aC}
200	26.29 ± 3.46^{bC}	34.19 ± 1.70^{cC}	13.58 ± 1.60^{aD}
400	32.26 ± 2.97^{bD}	43.38 ± 3.30^{bD}	16.52 ± 1.81^{aE}

[a-c]values with different superscript letters within the same row are significantly different at $P<0.05$.

[A-E]values with different superscript letters within the same column are significantly different at $P<0.05$.

4. Discussion

The bitter melon contains various substances such as catechin flavonoids, caffeic acid, *p*-coumarc acid, ferrulic acid, isoflavones, terpenes, glucosinolates, and these substances cause bitter taste[3,22,23]. Teleszko and Wojdylo[12] have determined the polyphenol compounds from the 7 selected edible fruits (apple, quince, Japanese quince, chokeberry, blackcurrant, cranberry and bilberry) and their leaves. They found that fruit leaves contained higher amount of polyphenol compounds than those of fruits. According to the study, the highest content was in the order of quice leaves, cranberry, apple, chokeberry, Japanese quince, bilberry, and blackcurrant[12]. In the leaves of cranberry, the difference was 20-fold, in the case of Japanese quince 23-fold, in bilberry and quince 13-fold, in blackcurrant 9-fold, and 7-fold in the case of chokeberry leaves and fruits[12].

The antioxidant activity of bitter melon leaf extracts was higher than that of fruit. Bitter melon fruit showed 2.52- and 2.63-fold higher α-glucosidase inhibitory activity than bitter melon leaf extracts at 200 μg/mL and 400 μg/mL, respectively. Bitter melon leaf extract showed higher antioxidant potential than its fruit but did not show α-glucosidase inhibitory potential compared to its fruit. Overall, the results of this experiment indicate that bitter melon fruits have an effect on diabetes and bitter melon leaves are effective in preventing or delaying oxidation.

Accorind to the results of Wu and Ng[9], the inhibitory activity of bitter melon on DPPH radical formation was highe in water extracts than in ethanol extracts, suggesting that water-soluble oxidation inhibitors were dissolved in water extract. Teleszko and Wojdylo[12] reported that antioxidant activity of leaves were higher than their fruits. The strongest antioxidant potential determined by ABTS assay was observed by qunce leaves and these values were 15- and 12-fold higher compared to the fruits. The higher content of total polyphenols and flavonoids in lentil leaves is known to have the ability to inhibit the formation of DPPH and ABTS radicals[12].

α-Glucosidase (EC 3.2.1.20), which is distributed in small intestinal epithelial cells, play a role in liberating glucose molecules by decomposing 1,4-α-glucopyranoside bonds in carbohydrates[24]. Thus, α-glucosidase plays an important role in regulating blood glucose levels after meals and keeping blood glucose levels within normal ranges. The inhibition of α-glucosidase may delay the hydrolysis of carbohydrates and induce glucose uptake in the small intestine[25]. Therefore, the substance that inhibits the secretion of α-glucosidase can be used as a treatment for type 2 diabetes, which has the highest blood glucose level after ingesting carbohydrates, and can inhibit the digestion of carbohydrates including starch and table sugar in the small intestine[25]. In general, carbohydrates are converted to simple sugars and absorbed from the intestine. Thus, α-glucosidase inhbitors reduce blood sugar by inhibiting the conversion of carbohydrates to simple sugar[25]. Matsuura *et al*[26] reported that administration of bitter melon juice after inducing diabetes by injecting streptozotocin into rats suppressed α-glucosidase activity and decreased blood glucose levels. Several studies have reported that blood glucose levels are reduced by injecting bitter melon juice or whole powder into people with type 2 diabetes or diabetes-induced rodents[27-29].

Conflict of interest statement

We declare that we have no conflict of interest.

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