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Research Article

Determination of Contents of Total Phenol, Flavonoid, Triterpenoid and Peptide and *in Vitro* Angiotensin-Converting Enzyme Inhibitory Activity of Bitter Melon Extract

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Abstract

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Bitter melon (*Momordica charantia* L.) is an important vegetable in the tropical countries. It is claimed to have beneficial effects because of its content of bioactive components. Hypertension has become a common global health problem leading to many chronic diseases, such as cardiovascular disease, renal disease and diabetes. The inhibition of the angiotensin-converting enzyme (ACE) can help to regulate hypertension. In this study, we evaluated the ACE inhibitory activity of eight different lines of bitter melon extracts with the expectation for screening the line(s) with the potential of anti-hypertension. The results showed that the highest ACE inhibition rate in the extract of BM141 was 93.00% with IC_{50} value 15.18 mg mL^{-1}. According to the study, it could be concluded that bitter melon provided inhibition activity against ACE. The bitter melon containing higher total phenols, peptides and triterpenoids had higher ACE inhibition activity and be regarded as indicator components. Further research is needed to validate the antihypertensive ability in bitter melon using experimental animal models of hypertension.

Key words: Bitter melon, Antihypertension, Angiotensin-converting enzyme, Bioactive component.

INTRODUCTION

Bitter melon (*Momordica charantia* L.; BM), a member of the Cucurbitaceae family, is a tropical plant commonly found in Taiwan. Many studies regarding the biological activities of BM have been reported, such as its hypoglycemic, anti-bacterial, anti-viral, anti-tumor, anti-inflammatory, antioxidant, antidiabetes, anthelmintic, antimutagenic, antilipolytic, hepatoprotective and anti-ulcerogenic properties (Jia *et al*. 2017). These beneficial effects are attributed to the various bioactive components of BM, including polysaccharides, peptides, tannins, terpenoids, saponins, flavonoids, sterols, glycosides, fatty acids and phenolic compounds. Furthermore, the most widely known is the effect of improving the condition of type 2 diabetes (Joseph & Jini 2013), and there are related BM health products on the market. Despite the promising biological activities of BM, only a few studies

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explained the antihypertensive effect of BM.

Hypertension has become a common global health problem which leads to many chronic diseases, such as cardiovascular disease, renal disease and diabetes. The human body regulates blood pressure mainly through the renin-angiotensin-aldosterone system to maintain body fluid and blood pressure. The liver releases angiotensinogen which is converted to angiotensin I via the secretion of renin. Then angiotensin I is converted to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II has the effect on causing vasoconstriction and increasing the blood pressure. Therefore, by inhibiting ACE, angiotensin I cannot be converted to angiotensin II to reduce the resistance around the blood vessels and the blood pressure.

According to the National Health Survey of the Ministry of Health and Welfare in 2015–2018, the prevalence of hypertension in Taiwan over the age of 18 is about 25%. The American Heart Association redefined the hypertension standard in 2017. When the blood pressure reaches 130/80 mmHg (systolic pressure of 130 mmHg and diastolic pressure of 80 mmHg), it is called hypertension, and the blood pressure must be controlled below this level. Because the antihypertension drugs may cause some side effects, one appropriate way to improve the condition of hypertension is to find the food material possessing antihypertensive activity and put it into daily meals in combination with the lifestyle change.

It has been reported that polysaccharides in BM have ACE inhibitory effect (Tan & Gan 2016). Previous study indicated that whole-plant aqueous extract of BM dose dependently normalized the hypertension in hypertensive Dahl salt-sensitive rats, followed by acetylcholine mediated pathways (Ojewole *et al*. 2006). Moreover, BM is rich in many phytochemicals, such as polyphenols, flavonoids and triterpenoids, playing a role as an ACE inhibitor in regulating blood pressure (Balasuriya & Rupasinghe 2012). The objectives of this study were to evaluate the ACE inhibition activity of BM extracts and to determine the bioactive components relating to ACE inhibition activity.

MATERIALS AND METHODS

Sample preparation

Freshly matured BMs from 8 breeding lines were provided by the Department of Vegetable Crops of Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute, Kaohsiung City, Taiwan. The inner tissues and seeds were removed and the flesh was chopped into small pieces and pressed into juice using a food blender. The juice was centrifuged at 2,500× *g* for 10 min and the supernatant was collected and dried in an oven at 65℃ for 2 h. The completely dried extract was dissolved in water to 30 mg mL^{-1} for further analysis. The percentage of extraction yield was calculated according to the following equation: (gram of dried extract/gram of supernatant) × 100.

Inhibition assay of *in vitro* ACE

The BM extract and 100 μL of 1 mM hippuryl-L-histidyl-L-leucine (HHL) in a borate buffer were incubated at 37℃ for 5 min. Thereafter, 100 μL of 25 mU ACE solution was added to the reaction mixture and incubated at 37℃ for 60 min. The reaction was stopped by adding 150 μL of 1 N HCl (S). The control group (CK) was conducted to calculate the ACE inhibition activity. Dose-responsive enzyme inhibition activity was determined using different concentrations of each extract. The concentration of the tested extracts which could inhibit 50% of enzyme activity (IC_{50}) was calculated using linear regression analysis plot of % ACE inhibition versus concentrations of tested extract. Three better ACE inhibition activity of BM extracts were chosen for ACE IC_{50} analysis. The reaction mixtures were filtered through 0.45 μm polyvinyl difluoride (PVDF) syringe filters (Millex, Merck, Germany) for high performance liquid chromatography (HPLC)

analysis. The reaction product, hippuric acid (HA), was separated and quantified by a HPLC system (Primaide, Hitachi, Japan). A reverse phased C_{18} column (Purospher STAR, 250 mm × 4.6 mm, particle size 5 μm, Merck, Germany) was used. The mobile phase was comprised of 0.1% trifluoroacetic acid in 50% methanol. The injection volume was 20 μL and flow rate was 0.8 mL min^{-1}. HA was detected by monitoring the absorbance at 228 nm.

The ACE inhibition rate was calculated from the following equation: ACE inhibition rate (%) = (CKarea − Sarea) ÷ (CKarea) × 100. CKarea is the peak area calculated by HPLC without adding sample; Sarea represents the peak area of the sample calculated by HPLC.

Determination of total phenol and total flavonoid contents

The total phenol content of BM extract was determined by the Folin-Ciocalteu method (Singleton *et al*. 1999) with slight modification, and the absorbance was measured at 765 nm in the spectrophotometer. Gallic acid was used as standard phenol and the total phenol content was subsequently calculated as gallic acid equivalent (GAE) per gram.

The total flavonoid content of BM extract was determined using the modified method (Meda *et al*. 2005), and the absorbance was measured at 405 nm. Quercetin was used as standard flavonoid and the total flavonoid content was subsequently calculated as quercetin equivalent (QE) per gram.

Determination of total triterpenoid and peptide content

The total triterpenoid content of BM extract was determined by the following steps. Take 0.1 mL of BM extract and load into micro-centrifuge tube, dry at 85℃, add 0.4 mL of 5% vanillin-acetate reagent and 0.8 mL of 70% perchloric acid, and react at 60℃ for 25 min. After cooling to room temperature (RT), 0.2 mL of acetic acid was added, mixed and reacted at RT for 15 min, then centrifuged (9,500× *g*, 3 min). The absorbance was measured at 548 nm. Oleanolic acid was used as standard triterpenoid and the total triterpenoid content was calculated as oleanolic acid equivalent (OAE) per gram.

The peptide content of BM extract was determined as follows. Mix 0.1 mL of extract with 1 mL *o*-phthalaldehyde colorimetric reagent, centrifuged (9,500× *g*, 1 min), and the absorbance was measured at 340 nm. The peptide content of the sample was calculated from the standard curve of L-leucine.

Statistical analysis

Samples were conducted in triplicate. Data were subjected to analysis of variance (ANOVA) with SAS 7.1 package. Duncan's multiple range test was applied to determine significant differences among observed means ($P < 0.05$). Data are expressed as means ± standard deviation (SD) ($n = 3$). Correlations between the evaluated parameters were obtained using Pearson's correlation coefficient (r).

RESULTS AND DISCUSSION

There are many different types of BM, and the sizes and shapes naturally vary. The morphology of eight different breeding lines of BM selected for the study was shown in Fig. 1. BM141 is the smallest BM with pale green color. BM131, BM135, BM139 and BM142 had gently undulating and warty surface, while BM146, BM152 and BM156 had jagged surface. In order to evaluate the ACE inhibitory activity of BM, extracts of eight different lines of BM were tested using *in vitro* assay. As shown in Fig. 2, most of the BM extracts (30 mg mL^{-1}) showed potent antihypertensive activity. Higher ACE inhibition activities were found in the 30 mg mL^{-1} extracts of BM141, BM152, BM146, with inhibition rates of 93.00, 87.94 and 83.35%, respectively. Line BM139 had the lowest ACE inhibition activity at the inhibition rate of 14.82%. Then, a test to determine the IC_{50} value of BM141, BM152, BM146 and BM156 was

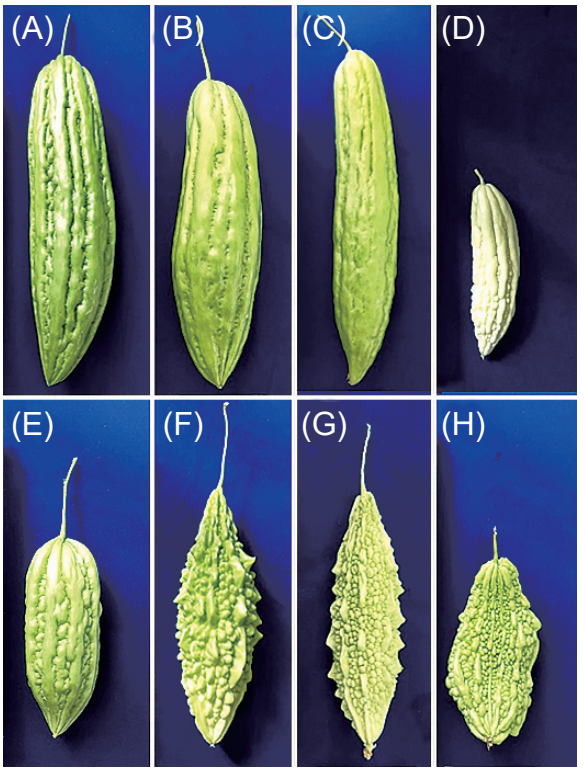

Fig. 1. The morphology of eight different lines of bitter melon fruits. (A) BM131; (B) BM135; (C) BM139; (D) BM141; (E) BM142; (F) BM146; (G) BM152; and (H) BM156.

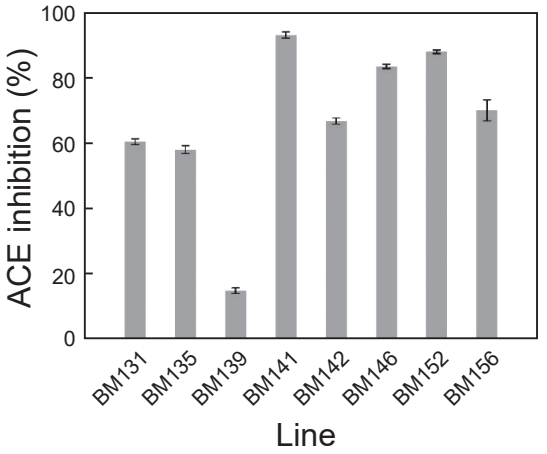

Fig. 2. The percentage of angiotensin-converting enzyme (ACE) inhibition activity in the extracts of different lines of bitter melon. Error bars show standard deviation of the mean (n = 3). The aqueous extracts were 30 mg mL^{-1}.

conducted. Their IC_{50} values are listed in Table 1 (lower IC_{50} values mean stronger ACE inhibition activity). Dose-dependent (6–30 mg mL^{-1}) inhibition of ACE activity is plotted in Fig. 3. The highest inhibition activity was the extract of BM141, with IC_{50} value of 15.18 mg mL^{-1}, followed by the extract of BM152.

The total phenol, flavonoid, triterpenoid,

Table 1. The concentrations (IC_{50}) causing 50% inhibition of angiotensin-converting enzyme (ACE) activity of the extracts from bitter melon lines of BM141, BM146, BM152 and BM156.

Line	ACE IC_{50} (mg mL^{-1})
BM141	15.18 ± 0.18 d[z]
BM146	20.51 ± 0.13 b
BM152	19.08 ± 0.05 c
BM156	25.16 ± 0.59 a

[z] Mean ± standard deviation (n = 3). Means within each column followed by the different letters are significantly different at $P < 0.05$ by Duncan's multiple range test.

peptide content and extraction yield of BM extract were presented in Table 2. The extraction yields of extracts were ranged from 2.5% to 5.6%. Total phenol content of extracts were ranged from 2.44 mg to 7.94 mg GAE g^{-1}, total flavonoid content of extracts were ranged from 0.24 mg to 1.12 mg QE g^{-1}, and total triterpenoid content of extracts were ranged from 5.28 mg to 17.16 mg OAE g^{-1}. The peptide contents of extracts were ranged from 21.04 mg g^{-1} to 98.04 mg g^{-1}. The contents of total phenol, flavonoid, triterpenoid and peptide of BM141 extract were significantly higher ($P < 0.05$) than other BM lines (Table 2). Moreover, ACE inhibition rate exhibited positive correlation with total triterpenoid (r = 0.95), peptide (r = 0.82) and phenol (r = 0.76) contents of the extracts (Table 3).

Hypertension is a worldwide illness with many associated risk factors such as cardiovascular diseases and chronic renal failure. Some of the most effective medications for the treatment of hypertension are ACE inhibitors. ACE is an enzyme which can convert angiotensin I into angiotensin II in plasma, along with the contraction of myocardium and the increase of blood pressure. Therefore, one of the important therapeutic strategies for regulating blood pressure in hypertensive patients is the use of ACE inhibitors, which inhibit the conversion of angiotensin I to angiotensin II (Nyman *et al*. 1998). The inhibition activity of ACE plays an important role in lowering blood pressure (Corvol *et al*. 1995). Recently, it was reported that the phenolic and flavonoid compounds in BM leaves showed potential antihypertensive activity (Lestari *et al*. 2017). However, BM leaves are not traditional food materials in Taiwan, so we chose edible BM flesh as experimental materials instead. We evaluated the ACE inhibition activity in eight different lines of BM extracts and expected to screen the BM with the potential of antihypertension. The results showed that most of the BM extracts displayed *in vitro* ACE inhibitory activity and the highest inhibition activity was exhibited in the extract of BM141.

Previous study underlined the significant

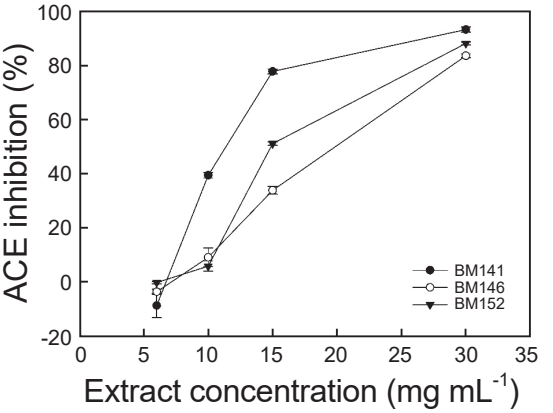

Fig. 3. The dose-response curves of the percentage of angiotensin-converting enzyme (ACE) inhibition activity of the extracts from bitter melon lines of BM141, BM146 and BM152. Error bars show standard deviation of the mean (n = 3).

Table 2. Total phenol, flavonoid, peptide, triterpenoid and extraction yield of the extracts from different lines of bitter melon.

Line	Total phenol (mg GAE g^{-1})[z]	Total flavonoid (mg QE g^{-1})	Peptide (mg g^{-1})	Total triterpenoid (mg OAE g^{-1})	Extraction yield (%)
BM131	5.22 ± 0.10 d[y]	0.24 ± 0.04 f	31.66 ± 0.15 g	13.82 ± 0.01 d	3.5
BM135	3.17 ± 0.00 e	0.56 ± 0.02 d	58.81 ± 0.56 e	12.94 ± 0.21 e	3.8
BM139	2.44 ± 0.10 f	0.47 ± 0.00 e	21.04 ± 0.12 h	5.28 ± 0.07 g	2.5
BM141	7.94 ± 0.10 a	1.12 ± 0.02 a	98.04 ± 0.20 a	17.16 ± 0.17 a	5.6
BM142	7.50 ± 0.17 b	0.71 ± 0.04 c	59.91 ± 0.45 d	10.81 ± 0.19 f	2.7
BM146	6.72 ± 0.10 c	0.83 ± 0.00 b	65.05 ± 0.24 c	16.51 ± 0.17 b	3.1
BM152	5.22 ± 0.10 d	0.83 ± 0.00 b	66.07 ± 0.67 b	16.62 ± 0.20 b	3.2
BM156	6.89 ± 0.19 c	0.68 ± 0.02 c	42.74 ± 0.35 f	15.44 ± 0.25 c	2.6

[z] GAE: gallic acid equivalent; QE: quercetin equivalent; and OAE: oleanolic acid equivalent.
[y] Mean ± standard deviation (n = 3). Means within each column followed by the different letters are significantly different at $P < 0.05$ by Duncan's multiple range test. The aqueous extracts were 30 mg mL^{-1}.

Table 3. Pearson correlation coefficients (r) of total phenol, flavonoid, peptide, triterpenoid and angiotensin-converting enzyme (ACE) inhibition effect of the extracts from different lines of bitter melon.

Item	Total phenol	Total flavonoid	Peptide	Total triterpenoid
ACE inhibition	0.76*	0.69	0.82*	0.95*

*The correlation coefficients are significant at $P < 0.05$.

correlation between ACE inhibitory activity and polyphenols that dietary plants contain (Patten *et al*. 2012). Recent report indicated that phenolic-rich extracts from natural products inhibit ACE activity (Ademiluyi *et al*. 2016). Flavonoid and triterpenoid could be effective ACE inhibitors *in vivo* and *in vitro* (Morigiwa *et al*. 1986; Balasuriya & Rupasinghe 2012). Furthermore, it has been reported that two novel ACEI peptides were successfully screened and characterized from the thermolysin hydrolysate of BM seed proteins (Priyanto *et al*. 2015). Therefore, it would be interesting to study which component in BM is related to the ACE inhibitory activity. Results indicated that the ACE inhibition rate exhibited positive correlation with total phenol, triterpenoid, and peptide content of BM extracts calculated by Pearson correlation coefficients (Table 3). With that, the highest ACE inhibition activity of BM141 could be attributed to its highest total phenol, flavonoid, triterpenoid and peptide contents. Results also suggest that these recognized components as effective ACE inhibitors may contribute to the ACE inhibition activity in BM extracts.

However, even within the same variety, many factors including environmental factors, maturity, location, soil condition and cultivation practices, would affect the bioactive components in BM and their ACE inhibition activity. Further research is necessary to validate the antihypertensive ability in BM using experimental animal models of hypertension in the future.

CONCLUSION

Based on the results from this study, it could be concluded that BM provided inhibition activity against ACE. The highest ACE inhibition rate in the extract of BM141 was 93.00% with IC_{50} value 15.18 mg mL^{-1}. The ACE inhibition activity could be attributed to their total phenol, peptide and triterpenoid contents. Total phenol, peptide and triterpenoid contents play an active role in inhibiting ACE and could be regarded as indicator components

in BM for improving condition of hypertension.

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苦瓜萃取物總酚、類黃酮、三萜類、胜肽含量及體外抑制血管收縮素轉化酶能力之研究

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摘要

洪千雅、楊淑惠、林照能。2020。苦瓜萃取物總酚、類黃酮、三萜類、胜肽含量及體外抑制血管收縮素轉化酶能力之研究。台灣農業研究 69(3):185–192。

苦瓜 (*Momoridica charantia* L.) 是熱帶國家重要的蔬果作物，苦瓜因富含機能性成分而具有許多保健功能，包括降血壓在內。高血壓是全球關切之健康問題，會衍生許多慢性疾病，如心血管疾病、腎臟病和糖尿病。血管收縮素轉化酶 (angiotensin-converting enzyme; ACE) 的抑制有助於高血壓的調控，本研究以 8 種苦瓜品系為原料，測定水萃物體外血管收縮素轉化酶抑制活性，期能協助篩選具調節血壓潛力之苦瓜品系。結果顯示，以 BM141 苦瓜萃取物的 ACE 抑制率最高，抑制率為 93.00%，IC_{50} 為 15.18 mg mL^{-1}。因此，根據試驗結果，苦瓜確實具有抑制血管收縮素轉化酶之活性。總酚、胜肽、三萜類含量相對高的苦瓜品系，其血管收縮素轉化酶抑制活性較高，可以作為調節血壓相關之指標成分。有關運用高血壓動物模式評估苦瓜降血壓能力，則有待進一步探討。

關鍵詞：苦瓜、高血壓、血管收縮素轉化酶、生物活性成分。

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