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The Bitter Gourd *Momordica charantia* L.: Morphological Aspects, Charantin and Vitamin C Contents.

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Abstract: *Bitter gourd (Momordica charantia L.) is an important vegetable crop of several countries in the tropics. Bitter gourd has recently received growing attention due to its anti-diabetic compound in its immature fruits. This study was conducted to analyze the morphological, charantin as anti-diabetic compound and Vitamin C contents in bitter gourd fruits. Eight accessions of Bitter gourd germplasm -2 from Indonesia and 6 from India- were grown and fruits were collected for further charantin and Vitamin C isolation and analysis. The charantin and Vitamin C content of bitter gourd fruits were investigated in 5,10,15, 20 days after fruit setting among 8 accessions of bitter gourd by HPLC. Result of the study shows that the content of charantin and Vitamin C in fruit was higher than leaf. The high level of vitamin C was found at D10 (2343.869mg/100g DW). Three accessions, KSI 2, KSI 7, KSI 8 showed highest charantin contents as 0.73 (D10), 0.98(D15), 0.77(D5)mg/100g.*

Keywords - *Bitter gourd, Charantin, Vitamin C, healthy vegetables*

I. Introduction

Bitter gourd (*Momordica charantia* L), an indigenous vegetable to tropical regions of Asia. Taxonomically, bitter gourd is belonging to the Cucurbitaceae. Locally, bitter gourd was known as bitter melon (Eng.), balsam-pear (Eng.), balsam-apple (Eng.), concombre africain and margose (French), Balsambirne (German), balsamito (Spanish), peria (Malay), Paria, pare (Indonesian), karalla (India). These reflect that Bitter gourd globally was recognized as vegetables [1].

Plants contains an active phytochemicals compounds which important for human, such as triterpenes, proteins and steroids. Scholars point out that *M. charantia* rich in term of minerals such as Cu, Fe, Mg, Zn, and Ca. There are also fatty acid were identified in *M. charantia* includes lauric, myriaatic, palmitic, stearic, and linolaic [2]. Charantin is a typical cucurbitane-type triterpenoid in *M. charantia*. Structurally, Charantin is the mixture of two compounds, namely, sitosteryl glucoside and stigmasteryl glucoside. Scholars point out that Charantin is a potential substance with anti diabeticproperties characteristics [3] and could be used to treat diabetes [4].

Momordicin is the functional components of bitter gourd. Momordicin was found at fruit, leaves, roots. Further components of charantin is reported to be highly effective for controlling the blood glucose in relation to insulin resistance. In addition, there is a study on the improvement of the functionality, such as obesity, gourd, colon cancer prevention in progress [5]. In spite of modern medical science and the development of diabetes, hypertension, heart disease, and the occurrence of cancer increases steadily. Bitter gourd has been known for its hypoglycemic effects. The methanolic extract of *M. charantia* fruit has ability to increases healing of gastric ulcer [6]. This extract also identified contributes to prevents development of gastric ulcers and duodenal ulcers in rats. Fruit and leaves, seeds of bitter melon are traditionally used as medicinal herbs as, anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial, anti-diabetic, and anti-tumor, to name a few [1]. Unripe fruits of bitter gourd have been found to have blood sugar lowering capacity, similar to that of insulin and can be used to treat patients with diabetes.

Since 2000, bitter gourd is recognized as a healthy vegetable. Bitter gourd becomes one of the important commodities. There are however, problems related to the production of bitter gourd. Many farmers are experiencing many difficulties cultivation technology to supply stock of stable bitter gourd yearly. Therefore, are also lack information of the important chemical content of bitter gourd and its relationship with plant morphological variety an origins. There are potential diversity of *M. charantia* in which it is potential to explore the diversity level of diversity of charantin and vitamin C contents. As far, there are lack of research on the basic bitter gourd [7][8]. There is no information whether morphological characteristics can be used as a key characteristics to assess charantin and vitamin C contents. This information especially important for further germplasm development to find the best cultivar with is environmental conditions to product optimum level of

charantin and vitamin C contents. Strategically, this information also contributes to the information and utilization of bitter gourd as a functional food and for pharmaceutical purposes in community.

II. Methodology

Material

Bitter Gourd were collected from Indonesia and India. The lines were in the nursery on April 1, 2014 and transplanted May 10. The plant material for charantin and vitamin C contents evaluation was performed by harvesting individuals growth shows has more than 30 internodes. For theses analysis, fruits and leaves more than 5days after fruit setting (5D), 10days after fruit setting (10D), 15days after fruit setting (15D), and 20days after fruit setting (20D) were harvested to evaluate the characteristics.

Methods

Seedling and planting techniques

The seedling was done in the soil with plastic mulching. The distance of planting holes was about 60×55cm. Plants were cultured using bamboo frame. A week after transplanting planting, main stem need to topping to vigorous side branches. There is no compost application during plant vegetative growth. Bitter gourd has been identified as plant with high resistance to drought and water stress. Thus, irrigation typically is applied weekly. Flowering begins about 4-5weeks after transplanting. In generative phase, the nitrogen fertilizer was applied to enhance efficient pollen-mediated insect which are important to increase the quality of fruit. GA application was done to increase the number of female flowers, fruiting, fruit development.

Morphological data analysis

Morphological analysis was performed in individual with has15days ages after fruit setting and about 30^{th} internodes. Horticultural characteristics such as leaf size and color, size, weight, tubercular of unripe fruit were evaluated. About 10 samples of each accession were subjected to morphological evaluation.

Isolation of Charantin (β-sitosterol β-D-glucoside)

The isolation method was modified from the protocol as described in Pitipanapong [4]. Ripe or unripe fruits and leaves of bitter gourd were cut into small pieces, dried and grounded into fine powder in a grinder (HR2860, Philips, Netherland). Approximately 0.5 g samples were soaked in 5 mL of 1:1(v/v) dichloromethane:methanol mixture. The mixture was then sonicated in an ultrasonic cleaner (Ultrasonic cleaner (5510 EDTH, BRANSON, U.S.A.) for 4 hours and centrifuged at 1,763×g for 10 min to separate the supernatant from the precipitate. The supernatant was evaporated under a concentrator (miVac Modular Concentrator Series, Genevac Inc., U.S.A.) to remove the solvent, and then suspended in an ultrasonic cleaner with 3 mL hexane. The suspended sample was centrifuged at 1,763 g for 10 min. The precipitate from this step was re-suspended in 1 mL hexane and centrifuged at 1,763×g for 10 min. Hexane was removed and the precipitate was dried in a concentrator. The pellet was dissolved in an ultrasonic cleaner with 0.5 mL of 1:1(v/v) chloroform:methanol mixture. The re-suspended solution was centrifuged at 12,400×g for 3 min before analyzed by an HPLC.

Isolation of vitamin C from Momordica charantia and HPLC analysis

The isolation method was done using standard the protocol [9]. The fruits of bitter melon were cut into small pieces, dried and ground into fine powder in a grinder (HR2860, Philips, Netherland). Approximately 0.1 g samples were soaked in 4 mL of 5% meta-phosphoric acid in water solution. The mixture was then sonicated in an ultrasonic cleaner (Ultrasonic cleaner (5510 EDTH, BRANSON, USA.) for 5 minutes and centrifuged at 1,763 g for 5 min to separate the supernatant from the precipitate. The supernatant was re-centrifuged at 12,400 g for 5 minutes to remove the particles.

HPLC Analysis

HPLC analysis was performed with a ZORBAX SB-C18 column (5 μm particle, 4.6 x 250 mm ID) and equipped with UV detector (Agilent 1290 Infinity LC with ISET, agilent technologies Ltd., U.S.A.). The mobile phases consisted of solvent A (water, HPLC Grade, J.T. Baker, U.S.A.) and solvent B (acetonitrile, HPLC Grade, J.T. Baker, U.S.A.). The initial solvent composition was 13% solvent A and 87% solvent B for 5 min. The solvent B was then increased from 87% to 95% within 20 min. This solvent composition was maintained for 10 min and returned to the initial composition in 10 min. The UV detection wavelength was monitored at 205 nm, flow rate was set at 1.2 mL/min, column temperature was maintained at 40º, and the sample injection volume was 30 μL. The concentrations of β-sitosterol β-D-glucoside (Fluka, Switzerland) in the sample were calculated from standard curve showing a plot of peak areas depending on concentrations for a series of standard solution.

HPLC analyses to evaluate vitamin C was performed as follow. The 5µL re-centrifuged supernatant was injected into the HPLC. HPLC analysis was performed with a ZORBAX SB-C18 column (5 µm particle, 4.6 x 150 mmID) and equipped with UV detector (Agilent 1290 Infinity LC with ISET, agilent technologies Ltd., U.S.A.). The mobile phases consisted of solvent A (0.1% phosphoric acid in water solution) and solvent B (80% acetonitrile in water solution). The initial solvent composition was 100% solvent A for 2 min. The solvent B was then increased from 0% to 80% within 0.1 minute. This solvent composition was maintained for 1 min and returned to the initial composition in 10 min. The UV detection wavelength was monitored at 254 nm, flow rate was set at 1.0 mL/min and column temperature was maintained at 35℃. The concentrations of vitamin C in the sample were calculated from standard curve showing a plot of peak areas depending on concentrations for a series of standard(L-Ascorbic acid, Sigma-Aldrich) solution.

III. Result and Discussion

Morphological Characteristics

The diversity of morphological characters represent the diversity of genetics aspect of populations. It is especially important among breeders to identify the superior's strains of plant based on morphological characters. The morphological characters represent the genetic diversity potentials of plants which are important for further superiors strain development [10] [11]. Morphological evaluation of bitter gourd 15 days after fruit setting was given in Table 1.

Table 1. Characteristics of genetic resources in Bitter Gourd

Codes	Origin	Leaf length (cm)	Leaf width (cm)	Leaf depth (cm)	Stem Diameter (cm)	External Color	Fruits Shape	Size of tubercular	Number of tubercular	Fruits Length (cm)	Fruits Width (cm)	Fruits weight (g)
KSI 1	India	20.5	25.1	15.5	2.2	Green	Spindle	Medium	Many	20.5	4.6	200.0
KSI 2	India	18.9	21.9	15.2	2.0	Dark Green	Spindle	Large	Many	29.7	4.6	280.0
KSI 3	India	18.8	22.3	12.9	2.1	Green	Spindle	Large	Many	32.0	3.4	230.0
KSI 4	India	21.4	27.3	13.5	2.2	Dark Green	Spindle	Large	Many	36.5	4.2	300.0
KSI 5	India	17.4	22.1	13.2	2.0	Dark Green	Spindle	Large	Many	23.4	4.2	220.0
KSI 6	India	17.8	25.9	12.9	2.0	Dark Green	Spindle	Large	Many	40.0	3.3	230.0
KSI 7	Indonesia	18.7	21.3	13.2	2.3	Light Green	Cylindrical	Medium	Few	35.0	4.3	240.0
KSI 8	Indonesia	17.9	22.7	11.3	2.0	Light Green	Cylindrical	Medium	Few	27.5	4.5	230.0

The leaf length of bitter gourd leaves are ranging from 17.4 to 21.4cm. Leaf width is longer than the leaf width (21.3 - 27.3 cm). All stem diameters are about 2 cm. The size of fruit is proportional to the size of leaves likes as KSI 4, KSI 6 (Table 1). KSI 4 has 36.5 × 4.2cm and KSI 6 has 40.0 × 3.3cm. Morphologically, India lines KSI 1~6 have spindle fruit shape but Indonesia line KSI 7, KSI 8 has cylindrical fruit shape. India lines fruit are only as strong lots tubercular. Indonesia type has soft and weak tubercular. Compared to the Indian line, Indonesia lines have light green color. Generally, a little bigger fruit and strong plant vigor cause later maturing. India lines are genetically late maturing. There are no further information regarding environmental impact to leaf, steam and fruit of bitter gourd. But there are possibility environment aspect contributes to the plant morphological diversity [12]. Geographically, India and Indonesia has different physical aspect which are able to provide impact to plant organs.

Charantin and Vitamin C Contents

The comparison of charantin (β-sitosterol β-D-glucoside) and vitamin C (Ascorbic acid) were given in table 2. The vitamin C in the leaf is lower than the fruit; whereas the content of charantin between the leaves with the same fruit (Table 2 and 3). The content charantin leaf which is higher than the average contents which are obtained in the three genotypes, namely KSI 3 (0.50), KSI 7 (0.59) and KSI 8 (0.58). The leaf of KSI 1 and KSI 5-leaf showed higher content of charantin than fruits. Scholar point out that Vitamin C is one of the abundant component in plants. It found in plant tissues with different levels. Some plant species and its tissues has abundance vitamin C, such as citrus [13]. The availability of nutrient influence biosynthesis of vitamin C, but there are also contribution of genetics [14].

Table 2. The content of charantin and Vitamin C in fruit and leaf of Bitter Gourd origins from India and Indonesia.

Sample	Vitamin C (mg/100 g DW)	Charantin (mg/100 g DW)	Parts	Origin
KSI 1-D10	2836.82	0.44	fruit	
KSI 1-D15	2096.67	0.29	fruit	
KSI 1-D20	1851.82	0.33	fruit	
KSI 1-leaf	327.86	0.46	leaf	
KSI 2-D5	2133.94	0.54	fruit	India
KSI 2-D10	2301.31	0.73	fruit	
KSI 2-D15	1588.04	0.48	fruit	
KSI 2-D20	1667.44	0.33	fruit	
KSI-2-leaf	385.97	0.33	leaf	

KSI 3-D5	1510.05	0.33	fruit	India
KSI 3-D10	2098.90	0.32	fruit	
KSI 3-D15	2003.68	0.31	fruit	
KSI 3-D20	1841.96	0.52	fruit	
KSI 3-leaf	40.26	0.50	leaf	
KSI 4-D5	2062.15	0.49	fruit	India
KSI 4-D10	2444.75	0.32	fruit	
KSI 4-D15	1928.67	0.27	fruit	
KSI 4-D20	1332.49	0.22	fruit	
KSI 4-leaf	119.22	0.34	leaf	
KSI 5-D5	1475.68	0.32	fruit	India
KSI 5-D10	1974.79	0.27	fruit	
KSI 5-D15	1447.59	0.42	fruit	
KSI 5-D20	1515.41	0.36	fruit	
KSI 5-leaf	301.38	0.42	leaf	
KSI 6-D5	1558.98	0.29	fruit	India
KSI 6-D10	1730.91	0.31	fruit	
KSI 6-D15	1517.66	0.29	fruit	
KSI 6-D20	1479.08	0.46	fruit	
KSI 6-leaf	232.67	0.35	leaf	
KSI 7-D5	2466.73	0.47	fruit	Indonesia
KSI 7-D10	2557.71	0.57	fruit	
KSI 7-D15	2083.03	**0.98**	fruit	
KSI 7-D20	1994.24	0.39	fruit	
KSI 7-leaf	156.41	0.59	leaf	
KSI 8-D5	2903.09	**0.77**	fruit	Indonesia
KSI 8-D10	2805.76	0.56	fruit	
KSI 8-D15	2421.41	0.53	fruit	
KSI 8-D20	2456.06	0.42	fruit	
KSI 8-leaf	79.33	0.58	leaf	

Table 3. Comparison of charantin and Vitamin C in fruit and leaf of Bitter Gourd.

Plant organs	Vitamin C (mg/100g DW)	Charantin(mg/100g DW)
Leaf	205.388 a	0.446 a
Fruit	2022.563 b	0.425 a

*Mean separation within columns of each lines by Duncan`s multiple range test at 5% level.

Table 4. The comparison of charantin and Vitamin C in different maturity stage.

Ages of fruit	Vitamin C (mg/100g DW)	Charantin (mg/100g DW)
D5	2093.228 a	0.438 a
D10	2343.869 b	0.439 a
D15	1937.799 ac	0.424 a
D20	1767.313 c	0.379 a

*Mean separation within coloums of each lines by Duncan`s multiple range test at 5% level.

Generally, the content of Vitamin C and charantin was expressed higher in young stage fruits (Table 4). Fruits is one of the significant plant organs with large amount of vitamin C [15]. In this research, highest vitamin C content was found at the age of 10D (2343.869mg/100g DW). The content charantin among all four age is the same, but the charantin content much higher than the average. It can be observed at KSI 2-D10, KSI 7-D15 and at KSI 8-D5. Indonesia lines showed higher genetic than India.

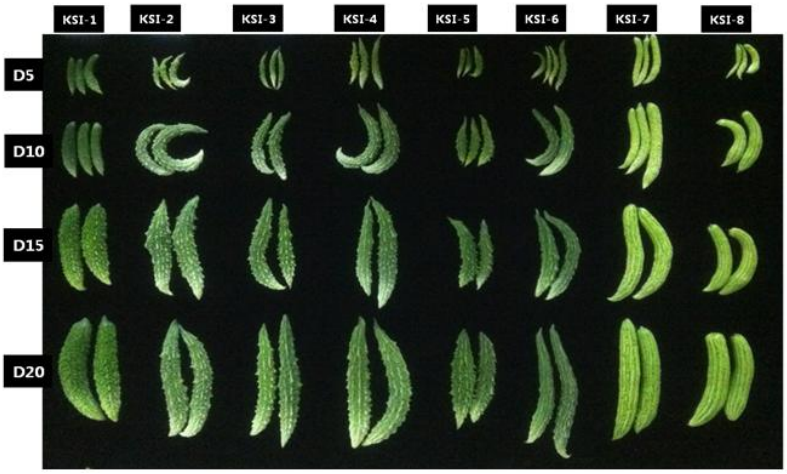

Figure 3. Growth stages fruit shape of lines 5 Day after ftuit setting (D5), 10 Day after ftuit setting (D10), 15 Day after ftuit setting (D15), and 20Day after fruit setting (D20).

Unripe fruit, seeds and aerial parts of *Momordica charantia* have been used in various parts of the world to treat numerous health problems, such as diabetes. Oral administration of the fruit juice or seed powder causes a reduction in fasting blood glucose and improves glucose tolerance in normal and diabetic animals and in humans. Although a wide range of compounds have been isolated from *Momordica charantia*, notably steroidal compounds and proteins, the orally active antidiabetic principle has not been adequately identified. A polypeptide, p-insulin, produces hypoglycaemic effects in humans and animals on subcutaneous injection, but oral activity is questionable. Other reported hypoglycaemic principles from *Momordica charantia* include the sterol glucoside mixture charantin (fruit) and the pyrimidine nucleoside vicine (seeds) [16] [17] [18] [19]. These aspect shows that *Momordica charantia* has prospective biomaterial as healthy vegetables.

IV. Conclusion

Morphologically, bitter gourd from Indonesia has external color light green with cylindrical fruit shape. The bitter gourd origin from India has green to dark green with c Spindle fruit shape. The morphological characters of bitter gourd from Indonesia seems to be preferred by local market and therefore it is crucial to conserve these morphological performance. The content of charantin and Vitamin C in fruit was higher than leaf. The high level of vitamin C was found at D10 (2343.869mg/100g DW). Three accessions, KSI 2, KSI 7, KSI 8 showed highest charantin contents as 0.73 (D10), 0.98(D15), 0.77(D5)mg/100g.

Future breeding and genetic emphases in bitter gourd improvement should be placed on the development of nutritious, high-yielding cultivars with superior resistance to major diseases and exceptional fruit quality. This study can be used for preliminary data in stable, high quality bitter gourd production and selection. Further studies are needed to understand underlying mechanism determining charantin content variations in different fruit maturation stages in bitter gourd.

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