BIOACTIVE COMPOUND AND NUTRITIOUS CHARACTERISTIC OF BITTER MELON FRUIT (MOMORDICA CHARANTIA L.)

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ABSTRACT

The bitter melon nutritious profile which was full of nutrition all contributed to flexibility towards curing some kind of diseases. The purpose of the research is to investigate nutritious composition of bitter melon (*Momordica charantia* L.). The parameter used to detect was as follow: ash content, water content, crude protein, crude fat, total carbohydrate, pectin, dietary fiber, calcium, vitamin C, β -carotene, diosgenin, stigmasterol, campesterol and β -sitosterol. The research finding showed that the carbohydrate content by different method in the bitter melon was about 60.7-70.90 %. The most dominant dietary fiber content was soluble dietary fiber including 19.2-21% pectin as well as the most dominant phytochemical compound was β - sitosterol. Furthermore, crude protein content, crude fat, and ash from fresh bitter melon each was 12.8-15.4%; 0.2-0.4%; 11.6-13.4%. Moreover, micro nutritious content such as calcium, vitamin C, and β -carotene each was 676.2-713.4 ppm; 119.2-126.2 ppm and 1.5-1.7 ppm. The existence of indigenous components made the bitter melon having high potential as one of the functional food sources.

KEY WORDS

Fresh bitter melon, phytochemical compound, nutrition, functional food.

The bitter melon nutritious profile which is full of nutrient consists of sample and beneficial complex phytochemical compound and all contribute to effective flexibility in curing some kind of disease. The use of bitter melon is not only used as a vegetable in Indonesia, but it is also used traditionally as phlegm emetic, lowering fever and increasing appetite. Bitter melon leaves is utilized as menstruation emetic, burning wound, skin, and worm medicine (Pramono *et al.*, 1998). The utility of bitter melon for southern Japanese society is to cure constipation, laxative and worm disease. Moreover, in India extract bitter melon is used to cure diabetic, rheumatic, *liver, and limfa disease* (Kumar and Bhowmik, 2010).

The phytochemical compounds in bitter melon are diosgenin and sterol compounds, such as stigmasterol, campesterol and β -sitosterol. Diosgenin and sterol compound were reported having hypocholesterolemic effect by inhibiting cholesterol absorption and increasing cholesterol secretion (Son *et al.*, 2007). Diosgenin was also reported as biologically active phytochemical that was used to cure some diseases such as leukemia, inflammation, hypercholesterolemia and cancers (Patel and Thompson, 2006). In addition, sterol compounds in bitter melon are sterol compound having similar structure with cholesterol that can inhibit intestinal cholesterol absorption and fat so that they increase bile acid secretion on lumen intestine (Lefebvre *et al.*, 2009).

Bitter melon has been known as vitamin source especially vitamin C as well as mineral, such as calcium, magnesium, iron, zinc, phosphorus, manganese, folic acid, and having high dietary fiber content (Krawinkel and Keding, 2006). Dietary fiber available in bitter melon

consists of soluble dietary fiber and insoluble dietary fiber. Dietary fiber has an important role to decrease body's weight because of some factors, that is, soluble dietary fiber is fermented in gastro intestine producing two gut hormone, namely, glucagon-like peptide (GLP-1) and peptide YY (PYY) having effect satiety so that dietary fiber can decrease energy intake significantly (Keenan *et al.*, 2006, Tucker and Thomas, 2009). Second, soluble dietary fiber affects the delay of gastro intestinal emptiness and decrease macronutrient absorption so that it decreases postprandial blood glucose content and insulin (Jenkins *et al.*, 1978).

Besides, the studies of soluble dietary fiber in bitter melon, characteristic of β -carotene content in bitter melon was also much reported. One of them was the research conducted by Abo *et al.* 1. They stated that β -carotene content of bitter melon was doubled compared with broccoli; calcium of bitter melon was doubled higher than spinach, and its total potassium content was doubled higher than banana. The dominant nutritious composition in bitter melon is vitamin C (570 – 36444) ppm, Calcium (130 – 433) ppm, diosgenin (1.69 mg/gm), and protein (9000 – 181000) ppm. Bitter melon consists of macronutrient such as protein, fat, and carbohydrate as well as micronutrient such as mineral and higher vitamin needed by human body (Ali *et al.*, 2008). In addition, bitter melon (*Momordica charantia*, L) is one of food material sources having potential to be developed in functional culinary involving saponin steroid compounds possessing good role to biological activities such as *antidiabetic*, *antihypercholesterolemic*, *antiobesity*, *antitumor*, *antiinflammatory*, *analgesic* and *antidepressant* (Kumar and Bhowmik, 2010). However, study on nutritious component inside the bitter melon in East Java, Indonesia, is never done yet. By doing so, the research was focused on the nutritious characteristic of fresh bitter melon in Kediri area, East Java, Indonesia. Based on the characteristic result done, information on comprehensive composition of bitter melon is expected to give accurate information on the content of ash, water, crude protein and fat, total carbohydrate, pectin, calcium, vitamin C, β -carotene, diosgenin, stigmasterol, campesterol and β -sitosterol from bitter melon fruit in Kediri area.

MATERIALS AND METHODS OF RESEARCH

Material of bitter melon was harvested in Kediri residence. Bitter melon fruit from harvesting was moved to Food Industrial Laboratory, Department of Industrial Technology, Engineering Faculty, Universitas Negeri Malang using basket. The other materials used to analyze nutritious component as well as bioactive component analysis of bitter melon were attained from *Sigma Chemical Co.* and other chemical store in Malang city.

Bitter melon from farmer could be harvested 15 to 20 times every three days. On dry season, the frequency of harvesting was many more if it was supported with good maintenance of plant. The repetition of three times research was done by taking the bitter melon of second, third, and fourth harvesting. Bitter melon picked was packaged with thin plastic and put into fruit basket with maximum two piles that was arranged alternately. To avoid fruit crashing, piece of paper was interspersed between it. Preparation of bitter melon material that would be conducted to analyze nutritious component was done in Universitas Negeri Malang, Food Industrial Laboratory, Industrial Technology Department, Engineering Faculty. Bitter melon fruit having been harvested from the garden was prepared for the analyses of nutritious composition and bioactive compound involving: 1) ash, 2) protein, 3) fat, 4) total carbohydrate, 5) total sugar, 6) pectin, 7) calcium, 8) vitamin C, 9) diosgenin, 10) stigmasterol, 11) β -sitosterol, and 12) campesterol, 13) β -caroten

Nutritious component analysis consisting of water content, ash content, crude protein, crude fat, total carbohydrate, Total sugar, β – carotene, vitamin C and calcium measurement were done using AOAC method (Horwitz, 1975).

Water content. As much as 1-2-gram sample was put in the flask having been known its weight. Later, the flask and the sample were dried with oven on the temperature of 105°C for 6 hours until the weight was constant. Next, it was cooled in desiccator and weighted. Then, sample was heated in the oven for 30 minutes, cooled, and weighted. Finally, this treatment was repeated until the constant weight was gained. Reduction of material weight is total water content inside the material.

Ash content. As much as 0.5 -1 gram was weighed in dried porcelain cup that had been known its weight. Next, it was blazed in muffle until it was attained the whitish ash. Cup and ash were put in excicator. After it was cold, it was weighed. Finally, ash content was calculated using formula:

$$\% \text{ ash content (wb)} = \frac{\text{cup weight and ash (C)} - \text{cup weight (A)}}{\text{sample weight (B)}} \times 100\%$$

Crude Protein. Protein content analysis was conducted using microkjeldahl method. First, as much as 0.5-gram sample was put in kjeldahl flask. Second, it was added 1 item kjeldahl tablet and concentrated 20 ml H₂SO₄. Third, it was added 2 boiling stone. Destruction was done for 45 minutes (the clear color sample or green light sample). Next, it was cooled for 10 minutes on room temperature. Distillation process was done by adding 50 ml aquades into kjeldahl flask that had been cooled. Then, it was added NaOH 50% as much as 70 ml (brown color sample). Kjeldahl flask was installed in distillation tool. Afterwards, Erlenmeyer consisting of 3% of H₃BO₃ as much as 20 ml and addition of 3 drops metilen blue indicator is prepared to keep the yield. Distillation was executed until the resulted distillate achieved 80 ml. Distillate was done titration using HCL 0.1 N until the color changed to purple. Finally, protein content was calculated using formula:

$$\% \text{ Nitrogen} = \frac{(\text{ml HCl} - \text{ml blank sample}) \times \text{N HCl} \times 14 \times 100}{\text{sample weight (mg)}} \times 100\%$$

$$\% \text{ Protein} = \% \text{ N} \times \text{conversion factor (6.25 for fruit)}$$

Crude fat. Fat analysis was done using extraction soxhlet method. First, refined dried sample was weighed as much as three grams sand packed with non-fat filter paper. Second, filter paper consisting of sample was put in extraction soxhlet tool. Next, condenser tool was installed above it and fat ash having been known its weight was installed below it. Petroleum ether solvent was put in as needed into fat flask and reflux was done for 5 hours until solvent that went down into fat flask had clear color. Solvent inside fat flask was distilled and solvent was kept again. Afterwards, fat flask consisting of fat from extracted result was heated on oven with the temperature of 105°C so that it reached constant weight and it was cooled in excicator. Later, fat flask and its fat were weighed. Finally, fat content can be calculated using formula:

$$\% \text{ fat} = \frac{\text{fat weight (g)} \times 100}{\text{sample weight}}$$

Total carbohydrate. Total carbohydrate was measured using by different method calculated using formula:

$$\% \text{ carbohydrate} = 100\% - \% (\text{water} + \text{ash} + \text{protein} + \text{fat})$$

Total sugar. At first, clear sample solution which was free from impurity was pipette as much as 2 ml. Second, it was put in reaction tube and was added 2 ml sulfate copper. Close reaction tube was placed into 100°C water bath for 10 minutes. Next, it was cooled using flowing water. After it finished, 1ml arseno molibdat reagent was added and mixed evenly. Reaction tube volume was thinned until the determined volume (10-25 ml) depend on the concentration of solution color. The absorbance value was determined on the wavelength (λ) of 500 or 520 nm. The same procedure for blank sample was done by changing sample with aquades. The making of standard curve was done started from concentration of 50, 150 and 300 µg/ml standard glucose solution. The same procedure is used to determine total sugar sample content.

β-carotene. The β-carotene content material that will be measured was refined using porcelain crusher. First, the material was weighed as much as 10 g. β- carotene inside

material was extracted using petroleum ether: acetone (1:1) so that phase ether-carotene was gained. Second, as much as 50 ml sample was washed off for 10 minutes using vortex/*magnetic stirrer*. Third, sample was filtered and separated using separated funnel. Its residue in the glass was rinsed out with the solvent and its extraction was repeated. Finally, 2 ml eluate was taken to observe its absorbance value in spectrophotometer with the wave length of 425 nm.

Vitamin C. Vitamin C analysis was done using Adebayo method (Adebayo, 2010). Indophenol's standard solution was prepared by mixing 0.05 g of 2,6-dichloro Indophenol added with water until 100 ml and filtered. As standard solution, 0.053 g vitamin C (ascorbate acid) was dissolved in 90 ml of 20% metaphosphoric acid and added with water until 100ml. 10 ml of the solution was pipette and put in Erlenmeyer and done titration with indophenol's solution until the color changed into stabile light red for 15 second. Additionally, extracted 2 ml juice from 'calyces' was pipette into Erlenmeyer. 5 ml of 20 % metaphosphoric acid (as agent stabilizer) was added and improved until 10 ml volume sign was achieved. Moreover, the result was done titration with indophenol's solution until stabile light color was composed for 15 second. Finally, vitamin C content in 'calyces' was calculated using:

$$\text{Vit C inside } \mu\text{g}/100\text{ g} = \frac{\text{titration value} \times 0.212 \times 100}{\text{sample weight}}$$

Ca (Calcium). Calcium content was determined using atomic absorption spectrophotometer (AAS) in which dried ashing process of sample was conducted. First, calcium content was determined using percentage or smaller unit measurement (ppm). Ashing process started from weighing ovened porcelain cup which had been identified its weight. Second, 0.5-1 gram sample was added and heated on temperature of 600°C until white ash was composed. Third, ash in the cup was taken, cooled and added with concentrated HCl (6N) of 5 ml and heated in low heating until the result was dry. The heating was done again until it started boiling by adding some what aqueous HCl (3N) as much as 15 ml. The heating to start boiling was repeated again by adding 10 ml of HCl 3N. Afterward, it was cooled and filtered and cup washed with aquades at least three times. Washing water was filtered and filtrate produced was inserted into measurement flask and added with aquades until certain volume. Then, it was filtered and ready to measure its calcium content using atomic absorption spectrofotometer (AAS). Before it was used, AAS tool was set based on manual tool instruction. Later, standard solution, blank sample and sample were measured. During sample determination, standard value was periodically checked whether it was still constant or not. Finally, calcium content could be known from *print out* produced.

Bioactive Compound Analysis. Bioactive compound analyzed was saponin and steroid compound of cultivar aden bitter melon. The analysis done was diosgenin, stigmasterol, β -sitosterol and campesterol compound analysis. Diosgenin, stigmasterol, β -sitosterol and campesterol content were determined with LC-MS/MS instrument with diosgenin extraction procedure using Chapagain and Wiesman 5 method and sterol compound extraction procedure used Pereira *et al.* 15.

RESULTS OF STUDY

Composition of Nutritious Bitter Melon. Bitter melon nutritious component involving water, ash, crude protein and fat, total carbohydrate and β – carotene, vitamin C and calcium on the dry basis were presented in table 1.

Total Carbohydrate ($65.8\% \pm 5.1\%$) was the most dominant component result shown in table 1, namely total carbohydrate with calculation of by different method of bitter melon of about 60.7% to 70.9%, with total sugar content was as much as 4.7% to 7.3%.

Bitter melon phytochemical compound. Phytochemical compound content of bitter melon involving diosgenin, stigmasterol, β - sitosterol and campesterol content were presented in figure 1.

Table 1–Bitter melon nutritious composition

No	Nutritious Composition	Content	Unit (dry basis)
1.	Water content	93.7 + 1.7	%
2.	Ash content	12.5+0.9	%
3.	Crude protein	14.1+1.3	%
4.	Crude fat	0.3+ 0.1	%
5.	Total carbohydrate	65.8+5.1	%
6.	Pectin	20.1+0.9	%
7.	Total sugar	6.0 + 1.3	%
8.	β –Carotene	1.6+0.1	ppm
9.	Vitamin C	122.7+3.5	ppm
10.	Calcium	676.2+37.2	ppm

Figure 1–Bitter melon phytochemical compound content

The dominant bitter melon phytochemical compound content presented on figure 1 was β-sitosterol of about 5275 ± 177.9 ppm, followed by stigmasterol, campesterol and diosgenin compound each respectively of about 2774 ± 38.11 ppm; 1982 ± 239.4 ppm; and 249 ± 5.0 ppm.

DISCUSSION OF RESULTS

Bitter melon ash content is 11.6% to 13.4%. This ash content is approximately to bitter melon's leaves ash content that is in the group of vegetables ($10.4\% \pm 0.1\%$). It is higher than bitter melon's leaves in the group of young category ($9.4\% \pm 0.1\%$) and it is lower than bitter melon's leaves in the group of yellowing ($22.3\% \pm 0.2\%$). The dominant bitter melon micronutrient's component is calcium with the content of 676.2-713.4 ppm. Together with Vitamin D, calcium is the important micronutrient in maintaining the bone and teeth health. Calcium also has a role to alleviate insomnia, arthritis, menopause, premenstruation syndrome and cramps. The role of calcium in preventing obesity, large intestine cancer, hepatic disease and high hypertension are great possible (Wargovich, 2000). The component of dominant micronutrient of bitter melon respectively was calcium, followed by magnesium and iron with the lower content. Component of bitter melon micronutrient from vitamin category was vitamin C with the total of 119.2-126.2 ppm and total carotene content of 1.5%-1.7%. The two components were vitamin and antioxidant on bitter melon with higher content if compared with other vegetables such as cucumber (Abo *et al.*, 2008).

Bitter melon's macronutrient component consists of protein and fat, each with the content of 12.8% to 15.4% and 0.2% to 0.4%. This protein content is almost the same with crude protein content of bitter melon leaves in vegetable's category ($12.2\% \pm 0.3\%$) which is lower than young bitter melon leaves protein and higher than yellow bitter melon leaves on the research result conducted by Zhang *et al.* 22. Meanwhile, for the fat content of bitter

melon in this research result is lower compared with the research conducted. Water content of this vegetable in the research is high enough, that is, $(93.7 \pm 1.7)\%$. The water content value of this fruit was higher than bitter melon's water content in Zhang Min research finding of about $(73.5 \pm 1.2)\%$. The higher water content enables the growth of microorganism causing enzymatic reaction occurred and could alter the component inside (Andress *et al.*, 2006).

Figure 2–Chromatogram of Diosgenin Compound

Bitter melon diosgenin compound content was 243.8 ppm to 253.8 ppm. Diosgenin content of this fruit was higher compared with diosgenin content of this fruit from India, that is, 1690 ppm to 13660 ppm. However, in contrary, sterol content, that is, stigmasterol, β -sitosterol and campesterol of this fruit is still higher than sterol content from India, China, and Thailand. Sterol content of this research respectively each stigmasterol, β -sitosterol and campesterol started from 2735.8 ppm to 2812 ppm, 5097.2 ppm to 5453 ppm and 1742.2 ppm to 2221 ppm. The influence of diosgen into glucose content on the normal mice and diabetic mice was reported by Kato *et al.* (KATO *et al.*, 1995). The research result from Kato *et al.* stated that PO-1 and PO-2 new hypoglycemic drug presented showed that PO-2 drug had extraordinary hypoglycemic activity on normal mice, whereas PO-1 drug decreased little blood glucose. In addition, PO-1 and PO-2 showed significantly hypoglycemic effect on diabetic mice that is streptozotocin induced. However, steroid glycoside (diosgenin) did not show the influence on the blood glucose level both in induced streptozotocin diabetic mice and in normal mice. Anyhow, in Nualkaew *et al.* 13 research result stigmasterol showed significant influence ($p < 0.05$) toward the decrease of diabetic mice blood glucose content.

β -sitosterol and campesterol have influence toward blood glucose content follow Simonen *et al.* 17, stating that the absorption decrease from cholesterol and increase compensation from its synthesis directly could affect insulin resistance.

Figure 3—Chromatogram of Sterol Compound

Cholesterol was absorbed through complex mechanism and plant sterol in this case was β -sitosterol and campesterol that had a role in its process. In practice, plant sterol content, namely, β -sitosterol and campesterol significantly decreased blood glucose content on diabetic mice. Smahelova *et al.* 18 research finding showed the significant relationship between insulin sensitivity and glycemic index in increasing cholesterol synthesis and cholesterol absorption decrease.

CONCLUSION

Phytochemical compounds available in fresh bitter melon in this research were diosgenin in the amount of 243.8-253.8ppm, stigmasterol 2735.8-2812 ppm, campesterol 1742.2-2221 ppm and β -sitosterol 5097.2-5453 ppm. Total carbohydrate by different method was about 60.7-70.9%; crude protein 12.8% -15.4%; crude fat 0.2%-0.4%; ash content 11.6%-13.4%; calcium 676.2-713.4ppm; vitamin C 119.2- 126.2 ppm and total carotene (1.05-1.85) %. Both chemical and phytochemical components of bitter melon that is high enough on the research result revealed its potential as one of functional food sources.

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