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# Antioxidant and Anti-diabetic Properties of Bitter Gourd (*Momordica charantia*) Fruit

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**Abstract**

This study aimed to determine the therapeutic values of local bitter gourd relative to the conventional drugs, especially in managing oxidative stress and blood glucose. The therapeutic values of bitter gourd fruit were evaluated using three antioxidant assays (2,2-diphenyl-1-picrylhydrazyl scavenging system [DPPH], the ability of ferric ion reduction in plasma [FRAP], total phenolic content [TPC]) and three hyperglycemic assays (inhibition of α-amylase and α-glucosidase and glucose uptake by human carcinoma liver cell [HepG2]). The result showed that bitter gourd fruit is not only a powerful secondary antioxidant but also a potent anti-diabetic food, which supported the use of bitter gourd fruit in suppressing high blood sugar in traditional medicine.

***Keywords:*** Momordica charantia; antioxidant; glucose uptake; acarbose; insulin.

## 1. Introduction

Type 2 diabetes mellitus (DM) occurs when the pancreas does not produce any or insufficient insulin in monitoring the glucose uptake by body cells. Its occurrence has been reported to be strongly associated with oxidative stress. This is because the insulin deficiency among diabetic patients may promote β-oxidation of fatty acids and results in the generation of hydrogen peroxide [1].

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The production of reactive oxygen species (ROS) such as hydrogen peroxide will in turn reduce the insulin secretion by liver and glucose utilization by peripheral tissues. However, conventional anti-diabetic therapy mainly focuses in maintaining blood glucose level in blood but not oxidative damage. In addition, it also frequently related to the side effect of oral agents, does not effectively maintaining blood sugar level and causes serious complications under long term consumption. This explains why some local patients tend to go for alternative treatment for instance herbal medicines, which treat both illnesses in the same time with lower cost. Momordica charantia (bitter gourd) is an important medicinal vegetable crop [2], mostly found in tropical and subtropical regions of Asia, tropical Africa, Middle East and America. Depending on location, bitter gourd is also known as bitter melon, balsam pear, kugua (China), kerala (India), nigeria uri (Japan), ampalaya (Philippines), Mara (Thailand) and peria (Malaysia). It is a flowering vine from the family Cucurbitaceae. Its fruit is oblong in shape and similar to cucumber with some ridges all over the surface of the fruit. Bitter gourd has received growing attention among all vegetable crops nowadays because it contains an abundance of phytochemicals such as glycoside, saponin, alkaloid, reducing sugar, resin, phenolic compounds, oil and free acids that associated with antioxidants, anti-diabetes, antimicrobial, anticancer, hypertensive properties and others [3-8]. In Malaysia, bitter gourd fruit is mainly used in cooking and as natural remedy among hyperglycemic patients [9]. Unfortunately, the effectiveness of bitter gourd in controlling blood sugar level has been only orally inherited along generations, for which scientific evidence is lacking. Therefore, this study was aimed to experimentally evaluate the therapeutic properties of bitter gourd and find out possible mechanism of the fruit in handling oxidative stress and type 2 DM.

## 2. Material and Methods

### *2.1 Materials*

Apparently fresh mature bitter gourd fruits were harvested from Lekir Agricultural Department, Perak, Malaysia. All fruits were washed cleanly, chopped into small pieces and dried in oven at the temperature of 50°C for 32 hours until the weight reached unchangeable. Dried bitter gourd fruits were grinded into powder and kept in air tight container at chilling temperature.

### *2.2 Extraction of Phytochemicals*

Fruit powder (50 g) was extracted by ethanol 50% with hot reflux extractor at the temperature of 150°C for 6 hours. Extract was then concentrated and dried using rotary vacuum evaporator prior using in the following analysis.

### *2.3Antioxidant Assays*

#### A. *2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging System*

The determination of antioxidant activity through the DPPH scavenging system was carried out according to the method of Musa and his colleagues [10]. Methanolic DPPH solution with the absorbance of 1.00±0.01 unit at 516 nm wavelength was prepared freshly before use. One hundred microliter of bitter gourd fruit extract (50

mg/mL 50% ethanol) with 1.5 mL DPPH solution prepared were kept overnight for scavenging reaction in the dark. An aliquot (200 µL) of samples (bitter gourd fruit extract with methanolic DPPH solution) and blank were then monitored at 516 nm wavelength on the next day with the spectrophotometer. Percentage of DPPH scavenging activity was determined as follow:

$$\text{DPPH scavenging activity (\%)} = \frac{absorbance\ of\ blank - absorbance\ of\ sample}{absorbance\ of\ blank} \text{ x } 100\% \quad (1)$$

***B. Ferric Reducing Ability on Plasma (FRAP)***

The determination of antioxidant activity through FRAP was carried out according to the method of Musa and his colleagues [10]. The FRAP reagent was prepared fresh as using 300 mM acetate buffer (pH 3.6); 10 mM TPTZ (2,4,6- tris (2-pyridyl)-s-triazine), in 40 mM HCl; and 20 mM FeCl3•6H2O in the ratio of 10:1:1 to give the working reagent. About 0.5 mL FRAP reagent was added to 50 µL bitter gourd fruit extract (50 mg/mL 50% ethanol) and the absorbances were taken at 595 nm wavelength with the spectrophotometer after 30 minutes. The calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of dried sample (mg TE/100 g of DW).

***C. Total Phenolic Content***

The determination of antioxidant activity through TPC was carried out according to the method of Musa and his colleagues [10]. About 50 µL bitter gourd fruit extract (50 mg/mL in 50% ethanol) were added with 0.2 mL distilled water and 0.25 mL diluted Folin-Ciocalteu reagent. The samples (bitter gourd fruit extract with Folin-Ciocalteu reagent) were left for 5 minutes before 0.5 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength with the spectrophotometer after 2 hours. The calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of dried sample (mg GAE/100 g of DW).

***2.4 Anti-diabetic Assays***

***A. α-amylase Enzyme Inhibition***

The activity of α-amylase enzyme inhibition was determined through iodine-starch test [11]. The total volume of sample consisted of 120 uL 0.1M sodium phosphate buffer, 40 uL α-amylase (1 U/mL) and 100 uL fruit extract (0.2-1.0 mg/mL). The mixture was incubated for 15 minutes at 37°C. About 100 uL soluble starch (0.2%) was added to the sample and the mixture was reincubated. The enzyme activity was stopped by the addition of 40 uL 1 M hydrochloric acid and hot water bath (5 minutes). Iodine reagent (100 uL) was lastly added to the mixture before the absorbances were taken at 620 nm wavelength with the spectrophotometer. Acarbose and distilled water were used as positive and negative control in the test. Percentage of α-amylase enzyme activity was determined as follow:

$$\text{Relative α-amylase enzyme activity (\%)} = \frac{enzyme\ activity\ of\ sample}{enzyme\ activity\ of\ negative\ control} \text{ x } 100\% \quad (2)$$

### *B. α-glucosidase Enzyme Inhibition*

The activity of α-glucosidase enzyme inhibition was determined using p-nitrophenyl-α-Dglucopyranoside (pNPG) as substrate [12]. About 650 uL phosphate buffer (67 mM, pH 6.9) and 100 uL fruit extract (0.2-1.0 mg/mL) were mixed with 100 uL α-glucosidase (0.5 U/mL). The mixture was incubated for 15 minutes at 37°C. Two hundred and fifty microliter of pNPG (10mM in phosphate buffer) was then added to the sample and the mixture was reincubated. The enzyme activity was stopped by the addition of 250 uL 0.1 M sodium carbonate. Enzyme activity was measured by the absorbance of liberated p-nitrophenol from pNPG at the wavelength of 405 nm using spectrophotometer. Acarbose and distilled water were used as positive and negative control in the test. Percentage of α-glucosidase enzyme activity was determined as follow:

$$\text{α-glucosidase enzyme inhibition (\%)} = \frac{absorbance\ of\ negative\ control - absorbance\ of\ sample}{absorbance\ of\ negative\ control} \text{ x } 100\% \quad (3)$$

### *C. Glucose Uptake Assay*

The glucose uptake assay was carried out according to the method of Abcam's glucose uptake colorimetric assay kit manual by using human carcinoma liver cell (Hep G2). The cell line was cultured in Eagle's Mininum Essential Medium (EMEM), supplemented with 10% fetal bovine serum (FBS) and 2% streptomyxin/penicillin at 37C under 5% $CO_2$ atmosphere. The cell were used for no more than 20 passages after thawing because the increase of number of passages may change the cell characteristics and impair assay result.The Hep G2 cell line was seeded at a density of ~7000 cells per well in a 96 well plate and starved in 100 uL serum free medium overnight. The next day, the cells were washed thrice with phosphate buffer saline (PBS) and starved again by pre incubating them with 100 uL Krebs-Ringer-Phosphate-Hepes buffer containing 2% bovine serum albumin (BSA) for 40 minutes. Later, they were stimulated by fruit extract or 5 uM insulin for 20 minutes to activate the glucose transporter. About 10 uL of 10 mM 2-deoxyglucose (2-DG) were added into every well before the mixture was incubated again for 20 minutes. Exogenous 2-DG was removed by washing the cells with PBS. To degrade endogenous nicotinamide adenine dinucleotide phosphate (NADP) and to denature enzymes, cells were lysed with 10 uL extraction buffer, froze/thawed once and heated at 85C for 10 minutes. The cell lysate was then cooled on ice for 5 minutes, neutralized by adding 10 uL of neutralization buffer, spinned and diluted to the concentration of 1/10. Ten uL Reaction Mix A was added into each well and mixed well before incubated at 37°C for 1 hour. To degrade unused NADP, 90 uL of extraction buffer was added into each well, sealed with aluminium sealing tape and heated at 85C for 40 minutes. Later, 38 uL Reaction Mix B was added into each well and mixed well before incubated at 37°C for 40 minutes. The absorbance of mixture was measured at 412 nm using spectrophotometer. The glucose uptake activity was determined as followed:

$$\text{2-deoxyglucose uptake} = \frac{the\ amount\ of\ 2{-}DG{-}6{-}phosphate\ in\ sample\ well\ calculated\ from\ standard\ curve}{sample\ volume\ added\ into\ the\ sample\ well} \quad (4)$$

### *2.5 Statistical Analysis*

The data were statistically analyzed by student t-test using SPSS version 20. The results were expressed as mean ± standard deviation. $p<0.05$ was considered significant between groups.

## 3. Results and Discussion

### *3.1Antioxidant Properties*

Table 1 showed the antioxidant properties of local bitter gourd fruit. The result confirms the good antioxidant activity of bitter gourd as recognized in alternative treatment. The fruit was proven to be moderate in its radical scavenging ability but very efficient in reducing oxidizing agent and abundance in phenolic compounds. Radical scavenging ability is always related to the primary antioxidative compounds, in which these compounds will suppress the formation of the initiation chain of free radical and destroy the propagation chain by donating hydrogen atom electron so that the free radical can be changed to a more stable form of products [13]. On the other hand, both FRAP and TPC assays measure the efficacy of secondary antioxidants that not only suppress the radical formation but also prevent oxidative damage [14]. In other words, bitter gourd fruit appeared as a weaker primary antioxidant but a powerful secondary antioxidant.

**Table 1:** Antioxidant properties of local bitter gourd (*Momordica charantia*) fruit (n=3)

| Antioxidant Properties | Activities |
|---|---|
| DPPH scavenging system (%) | 51.1±0.5% |
| Ferric reducing ability on plasma (g Trolox equivalent/100 g dried fruit) | 0.63±0.01 |
| Total phenolic content (g gallic acid equivalent/100 g dried fruit) | 2.29±0.01 |

Previous literature review reported that bitter gourd fruit is richer in its antioxidant content than some common vegetables such as cabbage celery, eggplant, onion, green pepper, snap bean, tomato, radish, wild carrot, winter melon and ribbed gourd [15]. Furthermore, Wu and Ng [16] had concluded that bitter gourd fruits exhibited better radical scavenging effect and metal chelating ability than vitamin E in their study while Hamissou and his colleagues [17] revealed that bitter melon is about 13.28 mg gallic acid equivalent (GAE) per gram of fresh weight and 82% as effective as vitamin C in suppressing ROS. This result was in parallel with the study of Leelaprakash and his colleagues [18] in which the inhibition concentration$_{50}$ ($IC_{50}$) of aqueous (66.25 ug/mL) and methanol (73.30 ug/mL) bitter melon extract against free radical were slightly lower than that of the vitamin C (50.00 ug/mL).

### *3.1Anti-diabetic Properties*

Table 2 showed the anti-diabetic properties of local bitter gourd fruit. The anti-diabetic properties were evaluated in relative to conventional drugs for DM, acarbose and insulin. Acarbose is a popular oral anti-diabetic drug that functions as glucosidase inhibitor among type I and II diabetic patients. When the condition of hyperglycemia becomes severe, patients will usually switch to insulin injections, with or without oral drug to improve insulin action. It is noteworthy that the fruit is more powerful than acarbose in inhibiting α-amylase activity, and almost as good as acarbose and insulin in inhibiting α-glucosidase activity and stimulating Hep G2 in glucose uptake assay, respectively. The potent anti-diabetic properties may be contributed by the phytochemicals such as phenolic, flavonoid, tannin, saponin, terpenoid, glucoprotein and polypeptide in the fruit itself.

**Table 2:** Anti-diabetic properties of local bitter gourd (*Momordica charantia*) fruit (n=3)

| Anti-diabetic Properties | Fruit | Standard |
|---|---|---|
| α-amylase activity inhibition ($IC_{50}$, mg/mL)[i] | 0.63±0.02[a] | 0.86±0.01[b] |
| α-glucosidase activity inhibition ($IC_{50}$, mg/mL)[i] | 0.62±0.01[b] | 0.59±0.01[a] |
| Glucose uptake (pmol)[ii] | 17.07±0.04[b] | 19.44±0.05[a] |

[i] n=3, [ii] n=6

Different superscripts letter indicates significant statistical difference in row ($p \leq 0.05$). Standard for enzyme inhibition assay: acarbose; standard for glucose uptake assay: insulin. The carbohydrate-hydrolyzing enzyme inhibition of bitter gourd were also confirmed by a few existing study [19-21]. However, current study exhibited opposite trend where the fruit is more effective in inhibiting activity of α-amylase in current study (Figure 1 and 2) while Ahmad and his colleagues [19] and Wongsa and his colleagues [20] reported that the aqueous extract, oil and poplypeptide obtained from the fruit has stronger ability in α-glucosidase activities. This major difference might be due to variation in terms of species, fruit maturity and geographical location. Bitter gourd fruit is believed to have similar mechanism as acarbose in inhibiting both carbohydrate-hydrolyzing enzymes. They will firstly slow down the enzyme activities at the brush boarder of small intestine, stop the breakdown of both complex and simple carbohydrates, delay the digestion and absorption of carbohydrates in alimentary tract and eventually avoid the rapid rise in blood glucose after meal [22]. In addition, bitter gourd fruit also suggested to possess mimic insulin property, although its effect in stimulating liver cell to take up glucose is slightly weaker than that of the insulin. The stimulation of glucose uptake by Hep G2 indirectly increases the glycogen content in storage and reduces the output of hepatic glucose [23]. Hep G2 was chosen to be used in this glucose uptake assay because it has significant similarities in terms of glucose metabolism pathway as normal hepatic cell from human (Fa2N-4) and mice (FL83B) [24-25]. It preserves most of the functions of the liver, stable through many passages and proven to have great suitability in studying the liver function [26]. Another study carried out by Takanaga and his colleagues [27] also revealed that Hep G2 have all glucose transporter existed in human normal liver cell.

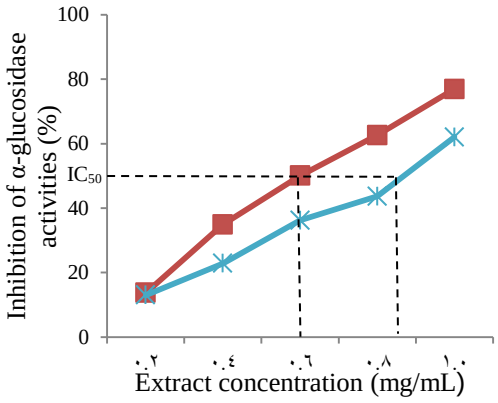


**Figure 1:** Inhibition of α-amylase activities of local bitter gourd (*Momordica charantia*) fruit (■: local bitter gourd fruit; ж: oral anti-diabetic drug, acarbose)

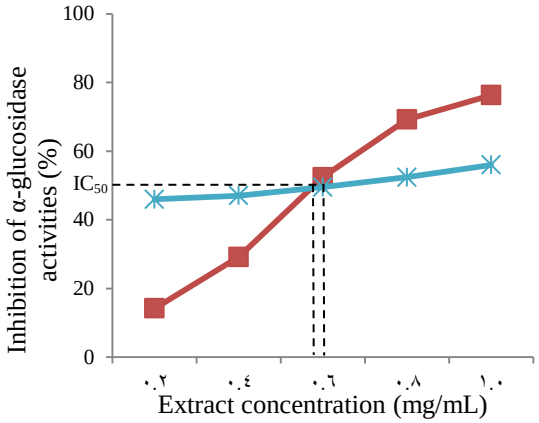


**Figure 2:** Inhibition of α-glucosidase activities of local bitter gourd (*Momordica charantia*) fruit (■: local bitter gourd fruit; ж: oral anti-diabetic drug, acarbose)

Liver plays an important role in glucose homeostasis in which it delivers glucose throughout blood circulation by glycogenesis (process of glucose molecules are added to chains of glycogen to storage in liver), glycogenolisis (process of breakdown of glycogen into glucose) and gluconeogenesis (process of generation of glucose from non-carbohydrate carbon substrate such as lactate, glycerol and glucogenic amino acid) [28]. Insulin and bitter gourd fruit extract are expected to function as allosteric regulator that either inhibiting the activity of glycogen phosphorylase (catalyzes the production of glucose-1-phosphate from glycogen) [29] or promoting the conversion of glucose into glucose-6-phosphate (by glucokinase), glucose-1-phosphate (by phosphoglucomutase), glucose uridine diphosphate (by glucose phosphorylase) and finally glycogen (by glycogen sintase) [30]. Other groups of researchers also proposed that bitter gourd may help to manage DM patients by different pathways. Hossain and his colleagues [31] and Saifi and his colleagues [32] demonstrated the ability of bitter gourd extract in the preservation of beta cells and recovery of the size of pancreatic islets in their *in vivo* studies. Cheng and his colleagues [33] reported the fruit extract was successfully increase the glucose uptake by mouse liver and muscle cells by increasing the sensitivity of secreted insulin while Chordhury and his colleagues [34] outlined the contribution of ethanolic fruit extract in reducing glucose production by suppressing the *in vivo* activities of glucose-6-phosphatase and fructose-1,6-biphosphatase.

## 4. Conclusion

This study validated the effectiveness of bitter gourd in managing oxidative stress and blood sugar among DM patients as perceived in alternative medicines. Bitter gourd has proven to be powerful secondary antioxidant and anti-diabetic fruit by mimicking the mechanism of insulin activity. Thus more extensive research should be focused in the possibility of bitter gourd to complement with and/or replace anti-diabetic conventional drug in future.

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