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Extraction of steroidal glycoside from small-typed bitter gourd (*Momordica charantia* L.)

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ABSTRACT

Extraction of steroidal glycoside from small-typed bitter gourd was optimized by central composite design (CCD) considering two factors: extraction temperature (50°C - 150°C) and ethanol concentration (50% - 100%). The optimum extraction conditions were found to be ethanol concentration of 50% and extraction temperature of 150°C. Under the optimized conditions, the experimental value of steroidal glycoside yield was 10.23 mg/50 g dried bitter gourd, which reasonably close to the predicted value (12.03mg.50 g dried bitter gourd). Previous detection method of steroidal glycoside by high performance liquid chromatography (HPLC) was improved and validated. The quantitative determination of steroidal glycoside was performed on a C18 symmetry column with aqueous methanol as mobile phase at a flow rate of 1 mL/min and injection rate of 200 uL. The eluents were examined at 204 nm using photo diode array (PDA) detector. The improved method was validated for its linearity, repeatability, precision, accuracy, limit of detection and limit of quantification and found to be satisfactorily. Extracted steroidal glycoside showed good DPPH free radical scavenging, equivalent to 2.29 g Trolox/100 g dried bitter gourd and 0.63 g gallic acid/100 g dried bitter gourd respectively while its carbohydrate-hydrolyzing enzyme inhibitory effect was comparable to conventional enzyme inhibitor drug, acarbose.

Keywords: Bitter gourd, Steroidal glycoside, High performance liquid chromatography, Antioxidant, Anti-diabetic

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INTRODUCTION

Momordica charantia, or bitter gourd, is a tropical medicinal plant widely found in Asia, West Africa and North America. 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. Generally, bitter gourd is rich in carbohydrate, protein, mineral, vitamin and other phytochemical such as glycoside, saponin, alkaloid, reducing sugar, resin, phenolic compounds, oil and free acids [1-5]. Out of 45 species bitter gourds reported in Asia and Africa, there are only two species found in Malaysia, namely *peria kambas* (long-typed bitter gourd) and *peria katak* (small-typed bitter gourd). *Peria kambas* is commonly used in culinary while *peria katak* is famous for its natural anti-diabetic property.

Diabetes mellitus (DM) is one of the global health problems. There are two types of DM where the type 1 DM is characterized by the condition without the existence of insulin while the type 2 DM is defined by the condition of insufficient insulin production and resistance of liver cells, peripheral and adipose tissues towards insulin activity [6]. Type 2 DM is more popular compared to type 1 DM and accounted for about 95% of total DM statistic. In Malaysia, prevalence of type 2 DM was reported to be increase by almost two-fold to 22.9% in year 2013, mainly made up of Indians (41.5%), followed by Malays (26.3%), Chinese (20.3%) and other races (11.9%) [7]. Although the conventional treatment is easily accessible, Malaysians tends to go for alternative treatment which involves herb utilization, traditional Chinese medicine and ayuverdic medicine [8].

Bitter gourd has been identified as one of the most famous natural remedy in treating DM because it is cheaper, easier to manage, less side effect and able to provide satisfactory in maintaining blood glucose level for long term effect as compared to conventional medicine. The anti-diabetic property of bitter gourd is always related to phytochemical such as steroidal glycoside, saponin, charantin, visine, polypeptide k, terpenoid and alkaloid. These phytochemicals are able to treat type 2 DM possibly through the reduction of oxidative damage, inhibition of carbohydrate-hydrolyzing enzymes, preservation of beta pancreatic cells and increase in insulin resistance as well as the glucose uptake by liver cells, adipose and peripheral tissues [9,10]. Thus, this study was aimed to determine the optimum extraction condition, validate the method detection by high performance liquid chromatography (HPLC) and find out the possible mechanism of steroidal glycoside from bitter gourd in treating type 2 DM.

EXPERIMENTAL SECTION

Plant material: Local mature small-typed bitter gourd was harvested from Agricultural Department of Lekir, Perak, Malaysia. The fruits were chosen according to the distance of days from flowering and visual observation. Mature fruits were harvested about 3 weeks after flowering while the skin of fruits was green in colour, seeds were fully developed and yellowish white.

Sample preparation: Upon arrival at the laboratory, the mature fruits were further inspected by colorimeter (CR400, Minolta, Japan) and the reading of L* (brightness), a* (redness: +a* represents redness, -a* represents greenish) and b* (yellowish: +b* represent yellowish, -b* represents blueness) was recorded. Fruits were then washed, cleaned, cut into cube 1 cm x 1 cm and dried in drying oven (Beschickung-Loading Modell 100-900, Memmert, Germany) for 32 hours at 50°C. Dried fruits were grinded into fine powder and keep in air tight container at 4°C prior use.

Sample extraction: About 50.0 g dried sample was extracted with 500 mL of ethanol with different concentration at fixed temperature for 6 hours using hot reflux method. Bitter gourd extracts were filtered and evaporated in vacuum at 40°C to obtain viscous crude extract.

Sample purification: The bitter gourd extract were further purified according to the method of [11] with modification. Methanol 50% were added to the viscous crude extract before it was sonicated for 15 minutes and centrifuged at 1000 rpm for 10 minutes to separate the supernatant from the sediment. The supernatant was discarded and the washing process was repeated by using 50% methanol and hexane. The aim of washing the extract with solvent methanol and hexane was to eliminate chlorophyll, sugar and other non polar impurities from the extract. The purified extract was finally evaporated in vacuum at 40°C again before the HPLC analysis.

Experimental design: Extraction of steroidal glycoside from dried bitter gourd was optimized by response surface methodology (RSM). A two-factors (X_1 and X_2) and three level (-1, 0 and 1) central composite design was applied in this study. The complete two blocks-CCD comprised of 14 experiments with four factorial points, four axial points and six center points (Table 1). The independent variables studied were ethanol concentration (X_1, %) and extraction temperature (X_2, °C) while the dependent variable measured was the yield of steroidal glycoside (Y, mg/50 g dried bitter gourd).

HPLC analysis: The yield of steroidal glycoside was determined by HPLC (CBM-20A, Shidmadzu Corporation, Japan) according to the method of [11]. Charantin, steroidal glycoside appeared in bitter gourd was used as standard in the analysis. The stationary and mobile phases were C18 Waters symmetry column (5 um x 3.9 x 150 mm) and methanol: deionized water (100:2, v/v) respectively. The quantitative determination of steroidal glycoside was performed at a flow rate of 1 mL/min and injection rate of 200 uL. The eluents were examined at 204 nm using photo diode array (PDA) detector.

Standard preparation: Charantin standard was prepared by weighing 1 mg of charantin and it was dissolved in 1 mL of solvent chloroform: methanol (1:9, v/v) to obtain concentration of 1 mg/mL. Serial dilution was carried out to prepared standard at different concentration (25 ug/mL, 50 ug/mL, 75 ug/mL, 100 ug/mL, 500 ug/mL and 1000 ug/mL).

Table 1. Central composite design (CCD) and the yield of steroidal glycoside from local mature small-typed bitter gourd

Method	Block	Coded value		Actual value		Yield of steroidal glycoside (mg/50 g dried fruit)
		X_1	X_2	Temperature (°C)	Solvent concentration (%)	
1	1	-1.000	-1.000	50	50	1.66
2	1	1.000	-1.000	150	50	10.94
3	1	-1.000	1.000	50	100	0.59
4	1	1.000	1.000	150	100	1.95
5	1	0.000	0.000	100	75	4.04
6	1	0.000	0.000	100	75	4.12
7	1	0.000	0.000	100	75	4.31
8	2	-1.414	0.000	29.29	75	0.68
9	2	1.414	0.000	170.71	75	7.85
10	2	0.000	-1.414	100	39.65	7.04
11	2	0.000	1.414	100	110.36	2.09
12	2	0.000	0.000	100	75	4.04
13	2	0.000	0.000	100	75	3.45
14	2	0.000	0.000	100	75	4.31

Method validation: The improved extraction and purification methods were validated for its linearity, repeatability, precision, accuracy, limit of detection and limit of quantification. Linearity was determined by the r^2 value obtained from the calibration curve of charantin (25 ug/mL - 1000 ug/mL). Repeatability and precision were determined by intra-day (0, 1 and 2 hours in the same day) and inter-day (day 1, 2 and 3) measurement of standard with different concentration (25 ug/mL - 100 ug/mL) respectively and the results were expressed as percentage of relative standard deviation (% RSD). For accuracy, two standards with known concentration (50 ug/mL and 75 ug/mL) were spiked to the pre-analyzed samples and the recoveries of the sample were calculated. Limit of detection (LOD) and limit of quantification (LOQ) was determined by the formula:

$$\text{Limit of detection (LOD, ug/mL)} = \frac{\text{3.3 x standard deviation of peak area}}{\text{gradient from the plot of sample concentrations against peak area}}$$

$$\text{Limit of quantification (LOQ, ug/mL)} = \frac{\text{10 x standard deviation of peak area}}{\text{gradient from the plot of sample concentrations against peak area}}$$

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 [12]. 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 extracts with 1.5 mL DPPH solution prepared were kept overnight for scavenging reaction in the dark. An aliquot (200 µL) of samples (extracts with methanolic DPPH solution) and blank were then monitored at 516 nm wavelength on the next day with the spectrophotometer (Epoch, Biotek, USA). Percentage of DPPH scavenging activity was determined as follow:

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

Ferric reducing ability on plasma (FRAP): The determination of antioxidant activity through FRAP was carried out according to the method of [12]. 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 extracts 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 fruit (mg TE/100 g of DW).

Total phenolic content (TPC): The determination of antioxidant activity through TPC was carried out according to the method of [12]. About 50 µL extracts were added with 0.2 mL distilled water and 0.25 mL diluted Folin-Ciocalteu reagent. The samples (extracts 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 fruit (mg GAE/100 g of DW).

α-Amylase enzyme inhibition: The activity of α-amylase enzyme inhibition was determined through iodine-starch test [13]. The total volume of sample consisted of 120 uL 0.1M sodium phosphate buffer, 40 uL α-amylase (1 U/mL) and 100 uL 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:

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

α-amylase enzyme inhibition (%) = 100% – Relative α-amilase enzyme activity (%)

The IC_{50} values were determined by the calibration curve of α-amylase enzyme inhibition against concentration of extracts.

α-Glucosidase enzyme inhibition: The activity of α-glucosidase enzyme inhibition was determined using p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate [14]. About 650 uL phosphate buffer (67 mM, pH 6.9) and 100 uL 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:

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

The IC_{50} values were determined by the calibration curve of α-glucosidase enzyme inhibition against concentration of extracts.

Statistical analysis: The Design Expert 9, Stat-Ease Inc. statistical software was employed to design the CCD and the analyze the expeimental data in RSM. Other data were expressed as means ± standard deviation of triplicate measurements. The significance of samples was analyzed using Tukey test in Statistical Package for Social Science (SPSS) 20.0 with p-value < 0.5.

RESULTS AND DISSCUSION

Optimization of extraction process

Steroidal glycoside has been targeted as the desired phytochemical to be extracted from small-typed bitter gourd fruit because it is predicted to possess strong pharmacology properties, especially the antioxidant and anti-diabetic properties. Steroidal glycoside from bitter gourd fruit powder was extracted using 14 methods proposed by CCD. The ranges of ethanol concentration (50%-100%) and extraction temperature (50°C-150°C) were used. Steroidal glycoside was yielded from 0.59% to 10.94% (Table 1). Minimum and maximum yield were obtained from method 3 (50°C and 100% ethanol) and method 2 (150°C and 50% ethanol) respectively. The result of variance analysis, model fitness and adequacy were summarized in Table 2. Factor interaction has been identified as the best model with high adequacy (p<0.0001), insignificant lack of fit (p=0.0989) and high correlation (R^2=0.9766). Extraction temperature exerted stronger effect on the yield as compared to ethanol concentration since it had bigger coefficient. On the other hand, significant but weak interaction effect was also detected in the extraction of steroidal glycoside from mature small-typed bitter gourd. The overall effect of extraction process can be predicted by Equation (1).

Total yield of steroidal glycoside=-
6.6+0.17*temperature+0.07*concentration-(1.58x10^3)*temperature*concentration ------------------------------------(1)

Three dimensional (3D) regression model was plotted to further investigate the effect of individual and interaction factors in the extraction process (Figure 1). The positive coefficient of extraction temperature indicated that the higher the extraction temperature, the higher the yield of steroidal glycoside was obtained. This was proven in the comparison of method 1, 2 and 8, 9 where if the ethanol concentration remained constant (either at 50% or 75%), the increasing of temperature from 50°C to 150°C and 30°C to 171°C recorded a significant increase in extraction yield of 87% and 91% respectively. In contrast, if absolute ethanol was used, the increase of temperature may only resulted in the increased yield of steroidal glycoside until the stationary point between 100°C and 150°C.

Table 2. Variance analysis of factor interaction model fitness in steroidal glycoside extraction of local mature small-typed bitter gourd

Source	Sum of squares	Degree of freedom	Mean of square	F-value	Prob>F
Model	106.04	3	35.35	125.39	<0.0001
A- Temperature	53.98	1	53.98	191.48	<0.0001
B- Solvent concentration	36.38	1	36.38	129.06	<0.0001
AB	15.68	1	15.68	55.63	<0.0001
Residual	2.54	9	0.28		
Lack of fit	2.12	5	0.42	4.08	0.0989
Pure error	0.42	4	0.10		
Cor total	108.58	12			
$R^2 = 0.9783$	$R^2_{adj} = 0.9711$	CV = 12.54%			

Glycoside is made up of two main part that is carbohydrate (glucose) and non carbohydrate (aglycone) [15]. The nature of carbohydrate and non carbohydrate parts in glycoside was hydrophilic and hydrophobic respectively thus the extraction of steroidal glycoside usually involved the mixture of chloroform and ethanol [11]. However the safety of chloroform as extraction solvent was always appeared as a main concern since the World Health Organization (WHO) reported that chloroform may have toxic and carcinogenic effect on liver kidney [16]. Therefore, extraction temperature played an important role in manipulating the polarity of organic solvent used in the extraction of steroidal glycoside.

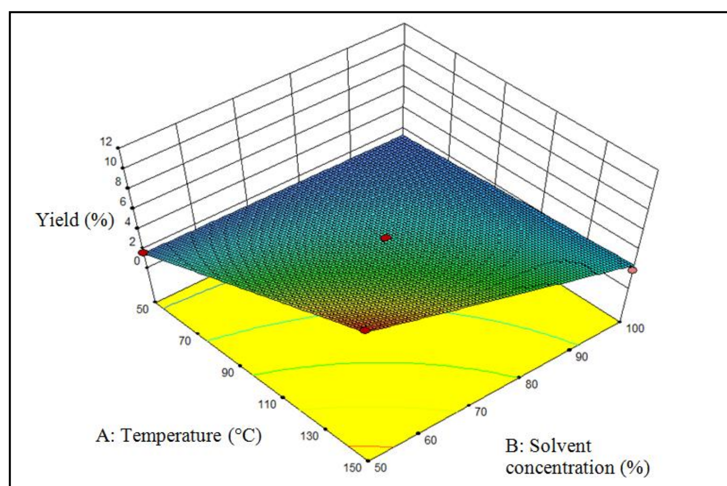

Figure 1. Response surface analysis of the yield of steroidal glycoside from local mature small-typed bitter gourd by hot reflux extraction

At the temperature ≤50°C, the polarity index of ethanol (either absolute or aqueous) was in the range of 5-7 and was too polar to be used in the extraction of steroidal glycoside. When the temperature was increased beyond the boiling point of solvent, dipole-dipole forces and hydrogen bonds in the solvent were broken, thus lowering the polarity index of the solvent [11]. This explains why the increase of yield of steroidal glycoside was observed when higher temperature was applied throughout the extraction process. Besides, high temperature also able to soften the plant tissues, weaken the interaction of phytochemical-protein/polysaccharide in the plant material and speed up the penetration of solvent into the plant matrix [17,18]. For absolute ethanol, the extraction temperature of 100°C-150°C decreased the polarity index to the point where it is unsuitable to dissolve the hydrophilic part of steroidal glycoside this lowering its yield in the extraction process.

The solvent concentration also exerted significant but antagonist effect on the extraction of steroidal glycoside from mature small-typed bitter gourd fruit. Figure 1 showed that the lower the solvent concentration, the higher the yield of steroidal glycoside was obtained. The comparison of method 1, 3 and 2, 4 and 5, 10, 11 exhibited similar trend where if the extraction temperature remained constant, the decreasing of solvent concentration recorded a significant increase in extraction yield of 64%, 82% and 70% respectively. Aqueous organic solvent was more effective in extraction because water mixture in the solvent may accelerate the swelling of plant material and increase the contact surface between solvent and plant matrix [17]. In addition, the existence of water eased the penetration of solvent into the plant matrix and increased the solubility of phytochemical in the solvent [19].

Interaction factor (temperature*solvent concentration) was reported to have similar but weaker effect as solvent concentration on the extraction of steroidal glycoside. In other words, the yield of steroidal glycoside can be maximized in two extraction conditions: (i) lower extraction temperature with higher ethanol concentration and (ii) higher extraction temperature with lower ethanol concentration. Since the effect of extraction temperature was

stronger, the extraction efficacy of higher extraction temperature with lower ethanol concentration is predicted to be better than that of its counterpart.

The main objective of this study was to determine the maximum yield of steroidal glycoside from mature small-typed bitter gourd fruit. CCD forecasted that this objective can be achieved by the extraction temperature of 50°C using 50% ethanol. The maximum yield was predicted to be 12.03 mg/50 g dried bitter gourd according to Equation 1. Extraction was carried out using the optimized method to ensure the predicted value and the reported value was reliable and only 15% lower than the predicted one (10.23 mg/50 g dried bitter gourd). The slightly lower yield was mainly due to the variation of phytochemical content in different bitter gourd fruit samples affected by several environmental factors such as climate, soil and fertilization [20]. The difference in sunlight exposure of leaves and stem and water absorption by root among the fruits from same tree contributed to the different phytochemical content in the fruit itself.

The yield of steroidal glycoside was in the range of previous reported values. [21] reported the yield of steroidal glycoside (charantin) from 10 different cultivars of bitter gourd from Philipines and Japan was about 1.49 - 35.6 mg/50 g dried bitter gourd. Erabu (Japan) and Peacock (Japan) were the two species of bitter gourd with minimum and maximum content of charantin respectively while Sta Monica (Philipines) contained the almost similar content of charantin with the local mature small-typed bitter gourd (≈11.8 mg/50 g dried bitter gourd). On the other hand, [11] obtained 6.3 mg of charantin from 50 g dried bitter gourd using liquid pressurized extraction method. A very low content of charantin (4.1 ug/50 g fresh bitter gourd) was detected in raw fruit and blanching, steaming and microwave (3.6 - 3.8 ug/50 g fresh bitter gourd) were reported to be more effective in retaining the charantin in the fruit than boiling [22]. Besides, the yield of other steroidal glycoside from bitter gourd such as 24(R)-stigmastan-3β, 5α, 6β-triol-25-ene 3-O-glycopyranoside and 25ξ-isoprophenylchole-5,(6)-ene-3-O-β-D-glycopyranoside was about 21.4 ug and 0.3 mg per 50 g fresh fruit respectively [23,24].

Validation of HPLC analysis

Charantin was used as the standard in the detection of steroidal glycoside from bitter gourd fruit extract. It was made up of the mixture of sitosteryl glycoside and stigmasteryl glycoside with the ratio of 1:1. A valid detection method of glycoside steroid by HPLC is vital in ensuring the trueness of the optimization of glycoside steroidal extraction from bitter gourd. The detection method was authenticated by several parameters including linearity, repeatability, precision, accuracy, limit of detection and limit of quantification and found to be satisfactorily. Chromatographic condition was adjusted to obtain a good performance of chromatogram (Figure 2). The detection wavelength at 204 nm was determined by comparing the maximum peak area in chromatogram from the 200 nm - 800 nm. Retention time was recorded at minute 6.9 and the total run was about 10 minit. The major peak at minute 2 was identified as the peak of mobile phase (methanol: deionized water, 100:2).

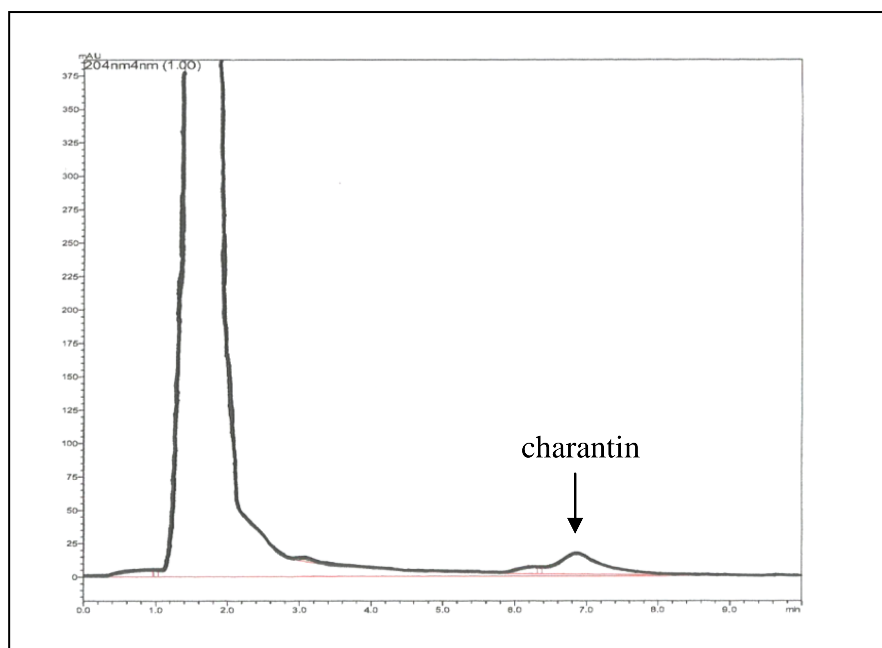

Figure 2. Improved chromatogram of standard charantin (steroidal glycoside)

Table 3 summarized all the tested parameter in the validation of detection method by HPLC. Calibration curve of standard was plotted using 6 different concentrations, ranging from 25 ug/mL - 1000 ug/mL with the correlation coefficient of <0.999. The repeatability and precision that expressed in percentage of relative standard deviation (%RSD) were less than 5% while the accuracy was about 95% - 98%. The limit of detection and limit of quantification were 9.63 ug/mL and 29.17 ug/mL respectively. In other word, the detection method of steroidal glycoside from bitter gourd fruit showed good correlation, satisfactory repeatability, precision and accuracy and high sensitivity thus it is reliable and suitable to be used in routine analysis.

Bioactivity of extracted steroidal glycoside

Steroidal glycoside from local mature small-typed bitter gourd was evaluated for its antioxidant and anti-diabetic properties. The DPPH scavenging percentage of steroidal glycoside was 51.1% while in FRAP and TPC assays, steroidal glycoside from 100 g of dried bitter gourd was equivalent to 0.63 g Trolox and 2.29 g gallic acid respectively (Table 4). The free radical scavenging ability and total phenolic content of steroidal glycoside was similar to the reported values of ripe fruit extract in previous investigations [25,26]. However, [27,28] showed higher values of DPPH, FRAP and TPC assays for crude bitter gourd extracts, most probably due to the absence of heat during extraction.

Table 3. Summary of validation parameters for steroidal glycoside detection method by high performance liquid chromatography

Parameter	Analyte (charantin)
Linearity (25 ug/mL -1000 ug/mL)	
Correlation coefficient	0.9995
Linear equation	y = 3220.4x + 19402
Repeatability (25 ug/mL -100 ug/mL)	
RSD for minit-0	1.84%
RSD for minit-60	1.56%
RSD for minit-120	1.38%
Average RSD	1.60%
Precision	
RSD for day 1	4.46%
RSD for day 2	1.29%
RSD for day 3	4.02%
Average RSD	3.26%
Accuracy	
50 ug/mL spiked with 5 mg extract	95.19%
70 ug/mL spiked with 5 mg extract	97.89%
Limit of detection	9.63 ug/mL
Limit of quantification	29.17 ug/mL

RSD: relative standard deviation; n = 6

Table 4. Antioxidant and anti-diabetic properties of steroidal glycoside from local mature small-typed bitter gourd

Pharmacological properties		
Antioxidant	2,2-Diphenyl-1-picrylhydrazyl scavenging system (%)	51.1
	Ferric reducing ability on plasma (g Trolox equivalent/100 g dried fruit)	0.63
	Total phenolic content (g gallic acid equivalent/100 g dried fruit)	2.29
Anti-diabetic	IC_{50} of α-amylase activity inhibition (mg/mL)	0.63
	IC_{50} of α-glucosidase activity inhibition (mg/mL)	0.62

n = 3; IC_{50}: half maximal inhibitory concentration; IC_{50} of α-amylase activity inhibition for standard acarbose = 0.86 mg/mL; IC_{50} of α-glucosidase activity inhibition for standard acarbose: 0.59 mg/mL

The antioxidant process usually functions in two ways that are chain-breaking (primary antioxidant) or prevention (secondary antioxidant). The good free radical scavenging percentage and high FRAP and TPC values of steroidal glycoside from bitter gourd suggested that it is a strong primary antioxidant. In other words, the antioxidant mechanism of steroidal glycoside is mainly attributed to its scavenging efficiency and redox ability. Besides, bitter gourd also reported to be a valuable secondary antioxidant since it exhibited a metal chelating activity of 75.1%, significantly higher than that of the standard butylated hydroxytoluene (BHT) at 53% [29].

Besides, steroidal glycoside from bitter gourd was able to inhibit the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase (Figure 3). Interestingly, steroidal glycoside (IC_{50}= 0.63 mg/mL) was more effective than acarbose, a conventional drug used in treating diabetes (IC_{50}= 0.86 mg/mL), in inhibiting α-amylase activity. For α-glucosidase activity, steroidal glycoside exhibited stronger enzyme inhibition properties than acarbose from concentration 0.6 mg/mL (data not shown). The enzyme inhibition properties of bitter gourd extracts were also confirmed by a few existing studies [30-32]. Different from other phytochemicals in the fruit extracts, steroidal glycoside was more capable in delaying the breakdown of long chain carbohydrate than the breakdown of oligosaccharides into glucose.

Steroidal glycoside slows down the carbohydrate-hydrolyzing enzymes at the brush boarder of small intestine, stops the breakdown of both complex and simple carbohydrate, delays the digestion and absorption of carbohydrates in alimentary tract and eventually avoid the rapid rise in blood sugar after meal [33]. Other than this, it has been reported to treat diabetes through several mechanisms. It functions to improve blood sugar levels by increasing glucose uptake and glycogen synthesis in the liver, muscles and fat cells [34]. In addition, it also enhances the insulin release from pancreatic beta cells and repairs or promotes new growth of insulin-secreting beta cells [35].

CONCLUSION

This study concluded that the optimum extraction conditions of steroidal glycosides from local mature small-types bitter gourd were found to be ethanol concentration of 50% and extraction temperature of 150°C. The improved detection method of steroidal glycoside showed good correlation, satisfactory repeatability, precision and accuracy with high sensitivity thus it is reliable and suitable to be used in routine analysis. Extracted steroidal glycoside was identified as a potent primary antioxidant and comparable to conventional anti-diabetic drug, acarbose, in inhibiting the activity of carbohydrate-hydrolyzing enzymes. This suggests that the substitution of acarbose by steroidal glycoside from bitter gourd is effective not only in maintaining blood glucose level but also in suppressing free radical formation, reducing diabetes treatment cost and may avoid the side effects of conventional drugs.

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