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ANTIHYPERGLYCEMIC ACTIVITY OF CHARANTIN-RICH EXTRACT FROM THE FRUITS OF *Momordica charantia* Linn. IN *in vitro* AND *in vivo* STUDIES

Javed Ahamad[1], Saima Amin[2], and Showkat R. Mir[3,*]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Tishk International University, Erbil - 44001, Kurdistan Region (Iraq)*
[2]*Department of Pharmaceutics,* [3]*Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, Hamdard Nagar, New Delhi - 110 062 (India)*
*****e-mail: *showkatrmir@gmail.com*

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ABSTRACT

Momordica charantia* Linn. (bitter gourd) fruit is one of the common vegetable consumed in India; and is also traditionally used for the management of blood glucose levels (BGL) in diabetes. The present study aimed to evaluate the antihyperglycemic potential of charantin-rich extract from the fruits of bitter gourd in *in vitro* enzyme inhibition assay (α-amylase and α-glucosidase) and *in vivo* using carbohydrate-challenged normal and STZ-induced diabetic rats. The bitter gourd fruits were extracted using an ultrasonicator and the extract was assayed for charantin content using HPTLC method. The standardized extract of *M. charantia* (SMC) contained 0.34 ± 0.15% *w/w* charantin. The charantin-rich extracts of *M. charantia* showed concentration-dependent inhibition of α-amylase and α-glucosidase enzymes with IC_{50} values 1.41 ± 0.17 and 2.29 ± 1.84 mg mL^{-1}, respectively. In starch and sucrose challenged normal rats, pre-treatment with SMC (200 mg kg^{-1}, b.w.) produced 13.04% and 16.32% reduction in peak BGL as compared to the negative control. In starch and sucrose-challenged diabetic rats (induced by a single intraperitoneal injection of streptozotocin, 50 mg kg^{-1} b.w.), pre-treatment with SMC @ 200 mg kg^{-1}, b.w. caused 7.44 and 8.32% reduction in peak BGL when compared with toxic control. The study provided scientific evidence to the fact that bitter gourd fruit is effective in the manangement of BGL in diabetes that can be mainly attributed to charantin.

Keywords: α-Amylase, bitter gourd, charantin, α-glucosidase, HPTLC, *Momordica charantia*.

INTRODUCTION

Momordica genus has about 60 species of annual or perennial herbaceous climbers commonly referred to as bitter gourds belonging to the family Cucurbitaceae. Two varieties of *M. charantia* Linn. are cultivated in India, *M. charantia* var. *charantia* with large fruits that are fusiform in shape and *M. charantia* var. *muricata* that are identified by small, round fruits (Nadkarni *et al.*, 2007). *M. charantia* fruits are commonly consumed as vegetable, which has formed a part of subcontinental diet for centuries. It has traditionally been used to manage blood glucose levels in diabetes across India. The fruits also are used as tonic, stomachic, stimulant, emetic, anti-bilious, and laxative (Ahamad *et al.*, 2017). *M. charantia* contain mainly steroidal glycosides and cucurbitane-type triterpenoids. Charantin is the major bioactive compound present in the fruits of *M. charantia*, and is considered to be a mixture of *β*-sitosterol glucoside and 5,25-stigmastadienol glucoside (Ahamad *et al.*, 2019). *M. charantia* has a long history of human use throughout the world. There is a plethora of reports of experimental and

clinical evidence related to its different uses (Gill *et al.*, 2012; Perumal *et al.*, 2015; Raish *et al.*, 2016; Jia *et al.*, 2017). The antidiabetic activity of *M. charantia* fruit extract, and its constituents such as charantin, vicine, and polypeptides has earlier been reported (Anun *et al.*, 2006; Singh and Gupta, 2007; Nhiem *et al.*, 2010). However, these above studies were performed with either crude extracts or isolated compounds. In case of crude extract, the extracts were not standardized with respect to the bioactive compounds; and in case of isolated compound, the pharmacological activities were found lower than the extracts which may be due to the lack of synergistic activity produced by native extracts (Ahamad et al., 2019). Hence, the present study was aimed to evaluate the antihyper-glycemic activity of charantin-rich extract from the fruits of *M. charantia* using *in vitro* and *in vivo* assays. The prepared extract was quantified with respect to its bioactive compound charantin using the HPTLC method.

MATERIALS AND METHODS

Plant materials and chemicals

Momordica charantia Linn. fruits were purchased from a local vegetable market, Govindpuri, Delhi (India). A voucher specimen (PRL/JH/11/3) was deposited in the Phyto-pharmaceuticals Research Laboratory, Department of Pharmacognosy and Phytochemistry, Jamia Hamdard University, New Delhi, India. Streptozotocin (STZ), α-amylase, α-glucosidase, *p*-nitrophenyl-α-D-glucopyranoside (PNPG), and 3,5-dinitrosalicylic acid (DNS) were purchased from SRL, Bangalore, India. Metformin and acarbose were provided as gift sample by Medley Pharmaceuticals Ltd., Jammu, India. All other solvents and chemicals were of analytical grade.

Preparation of charantin-rich extract

M. charantia fruits were cut into small pieces, and dried in a hot air oven (Toshniwal, India) at 45°C till constant weight was attained. The dried samples were pulverized to a coarse powder in a mixture grinder. The coarse powder of *M. charantia* (50 g) was placed in a stoppered conical flask and extracted in an ultra-sonicator (Toshniwal, India) at 200 W ultrasonic power with methanol-water (80:20 % *v/v*), at 45 ºC for 85 min using her-to-solvent ratio of 1:25 g mL^{-1}. The extracts were filtered and concentrated using a rotary evaporator (Buchi, Switzerland) at 40ºC. The residue was freeze-dried and stored at 2-4ºC till further use (Ahamad *et al.*, 2015). The extract was analysed for charantin content by a validated HPTLC method published elsewhere (Ahamad *et al.*, 2014).

In vitro antidiabetic activity

α-Amylase inhibition assay: *In vitro* αamylase inhibitory activity was assessed as per the method described by Ahamad *et al*. (2020). Briefly, 40 µL SMC in DMSO or acarbose [20 mM sodium phosphate buffer (pH 6.9) with 0.006 M sodium chloride] was premixed with 200 µL α-Amylase solution (1.0 U mL^{-1} in phosphate buffer (pH 6.9), and incubated at 25ºC for 30 min. After pre-incubation, 400 µL of 0.25% starch solution in phosphate buffer (pH 6.9) was added to each tube to initiate the reaction. The reaction was carried out at 37ºC for 5 min and terminated by the adding 1.0 mL DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The test tubes were then kept in a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was then diluted upto 10 mL with distilled water, and absorbance (A) was measured at 540 nm by UV-visible spectrometer (Shimadzu, Japan). Control incubations representing 100% enzyme activity were prepared similarly by replacing the extract with buffer. For blank incubation (absorbance of background), enzyme solution was replaced by buffer solution, and absorbance recorded. The α-amylase inhibitory activity was expressed as percent inhibition and calculated as follows:

$$\% \, Inhibition = \frac{\text{A control} - (\text{A } test - A \; background)}{\text{A control}} \times 100$$

where $A_{control}$, A_{test}, $A_{background}$ represented the absorbance of 100% enzyme activity, test sample with the enzyme and test sample without enzyme, respectively.

α-Glucosidase inhibition assay: *In vitro* α-glucosidase inhibitory activity was assessed by the standard method as described by Ahamad *et al.* (2020). Briefly, 60 mL of SMC or acarbose and 50 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U mL^{-1}) was incubated in 96 well plates at 37ºC for 20 min. After pre-incubation, 50 µL of 5 mM PNPG solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37ºC for another 20 min. The reaction was stopped by adding 160 µL of 0.2 M Na_2CO_3 into each well, and absorbance was recorded at 405 nm by a microplate reader (Agilent, USA) and compared to a control with 60 µL buffer solution in place of the extract.

In vivo antidiabetic activity

***Animals*:** Wistar albino rats (150-200 g) were obtained from Central Animal Facility, Jamia Hamdard, New Delhi (India) and were maintained under controlled conditions of illumination (12 h light/12 h darkness) and temperature (20-25ºC). They were housed under ideal laboratory conditions and maintained on a standard pellet diet (Lipton Rat Feed Ltd., Pune, India) and water ad libitum throughout the experimental period. The animals were acclimatized to the conditions before the start of experiments. The experimental study was approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi, India (vide No. JH/CAHF/173/CPCSEA/28th January/2000, approval No. 2012/926). All the extracts and the standards were administered orally.

Oral carbohydrate tolerance tests in normal rats*:* Wistar albino rats (150-200 g) were fasted overnight. The animals were randomly divided into seven groups, each consisting of six rats (n = 6). Group I served as normal control which received 1 mL kg^{-1} b.w. vehicle (0.5% CMC in distilled water). For oral starch tolerance test, group II served as starch challenge control that received starch (3 g kg^{-1}, b.w.). Group III received acarbose as a standard drug (10 mg kg^{-1}, b.w.) while group IV animals were administered with standardized extracts of *M. charantia* (SMC) at a dose of 200 mg kg^{-1}, p.o. Treatment groups III to IV were fed starch 20 min after the treatment. For the oral sucrose tolerance test, group V served as the sucrose challenge control that received sucrose (4 g kg^{-1}, b.w.). Treatment group VI received acarbose (10 mg kg^{-1}, b.w.), while group VII animals were administered SMC (200 mg kg^{-1}, p.o.), followed by sucrose (4 g kg^{-1}, b.w.) 20 min after treatment. Blood samples were withdrawn from the tail vein at 0, 30, 60, 90, and 120 min after carbohydrate challenge. Blood glucose level (BGL) was measured using a one-touch glucometer (myLife Pura, Switzerland). The AUC was calculated using the trapezoidal method (Purves, 1992).

Oral carbohydrate challenge test in STZ-induced diabetic rat: Wistar albino rats (150-200 g) were fasted overnight. Diabetes was induced in animals by a single intraperitoneal injection of streptozotocin (STZ, 50 mg kg^{-1}, b.w.) prepared freshly in citrate buffer (pH 4.5). Diabetes was confirmed by measuring BGL after 72 h of STZ injection. Animals showing BGL above 200 mg dL^{-1} were considered diabetic and selected for the study. The diabetic animals were randomly divided into seven groups, each consisting of six rats (n = 6). Group I served as diabetic control which received 1 mL kg^{-1} b.w. vehicle (0.5% CMC in distilled water). For the oral starch tolerance test, Group II served as starch challenge control and received starch (3 g kg^{-1}, b.w.). Group III received acarbose as a standard drug (10 mg kg^{-1}, b.w.) while groups IV were administered with SMC (200 mg kg^{-1}, p.o.). Treatment groups III to IV were fed starch 20 min after the treatment. For the oral sucrose tolerance test, group V served as the sucrose challenge control that received sucrose. Treatment group VI received acarbose (10 mg kg^{-1}, b.w.) while VII was administered SMC (200 mg kg^{-1}, p.o.), followed by sucrose (4 g kg^{-1}, b.w.) after 20 min of the treatment. Blood sample was withdrawn from the tail vein at 0, 30, 60, 90, and 120 min after carbohydrate challenge.

Statistical analysis

Values were expressed as mean ± standard deviation. Statistical significance was calculated by using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. The values were considered significantly different when $p < 0.05$.

RESULTS AND DISCUSSION

Standardization of M. charantia extract

The conditions for ultrasonic-assisted extraction for maximizing the charantin content in *M. charantia* extracts were optimized and were reported elsewhere (Ahamad *et al.*, 2015). The optimized conditions for the extraction of charantin from the fruits of *M. charantia* were as: methanol-water (80:20 % *v/v*); 45°C temperature; 85 min of extraction time; and herb-to-solvent ratio of 1:25 g mL^{-1}. *M. charantia* extract was assayed for charantin content by a validated HPTLC method (Ahamad *et al.*, 2014). The charantin content in standardized *M. charantia* (SMC) extract was found 0.34 ± 0.15 % *w/w* on the dry weight basis of the plant material.

Carbohydrate metabolizing enzyme inhibition assay

In vitro α-amylase and α-glucosidase inhibition assays were carried out for charantin-rich extracts of *M. charantia* (SMC) and compared with acarbose. Both were tested at a concentration range of 0.15 to 5 mg mL^{-1} and the results presented in Table 1. SMC and acarbose showed concentration-dependent inhibition of both the enzymes. Acarbose strongly inhibited α-amylase (IC_{50} 0.42 ± 0.02 mg mL^{-1}) in comparison to α-glucosidase (IC_{50} 1.41 ± 0.17 mg mL^{-1}). The *in vitro* enzyme inhibition assay with SMC revealed that it successfully inhibited α-amylase (IC_{50} 1.43 ± 0.27 mg mL^{-1}) and α-glucosidase (IC_{50} 2.29 ± 1.84 mg mL^{-1}) and showed a preference for the latter enzyme.

Table 1: *In vitro* α-amylase and α-glucosidase inhibitory activity of charantin-rich fruit extract *of M. charantia* (SMC)

Conc. (mg mL^{-1})	Percent inhibition			
	α-Amylase		α-Glucosidase	
	Acarbose	SMC	Acarbose	SMC
5.00	81.33 ± 2.31	75.58 ± 1.18	76.82 ± 4.48	68.12 ± 2.10
2.50	74.27 ± 3.02	72.47 ± 2.85	63.82 ± 5.97	55.61 ± 4.99
1.25	61.64 ± 4.21	55.68 ± 3.72	51.24 ± 2.48	45.45 ± 1.20
0.62	54.51 ±2.61	46.19 ± 1.06	39.44 ± 3.32	38.21 ± 2.79
0.31	46.74 ± 4.18	37.33 ± 1.44	28.94 ± 2.64	27.82 ± 3.73
0.15	31.29 ± 4.35	21.72 ± 1.18	16.81 ± 2.68	23.59 ± 1.59
IC_{50}	0.42 ± 0.02	1.43 ± 0.27	1.41 ± 0.17	2.29 ± 1.84

Data presented as mean ± SD, n = 3; IC_{50} = concentration for 50% inhibition; SMC: standardized extracts of *M. charantia*

Oral carbohydrate tolerance test of SMC in normal rats

The oral administration of starch (3 g kg^{-1}, b.w.) and sucrose (4 g kg^{-1}, b.w.) produced a significant increase ($p < 0.01$) in BGL of carbohydrate-challenged control, resulting in carbohydrate-induced hyperglycemia (Table 2). In starch tolerance test, the animals pre-treated with acarbose (10 mg kg^{-1}, b.w.) significantly lowered ($p < 0.01$) peak BGL, with an 18.64% reduction in peak BGL as compared to the starch-challenged control. Pre-treatment with SMC (200 mg kg^{-1}, b.w.) exhibited 13.04% reduction in peak BGL in starch tolerance test. In sucrose tolerance test, pre-treatment with acarbose (10 mg kg^{-1}, b.w.) significantly lowered ($p < 0.01$) peak BGL, with 25.19% reduction in peak BGL as compared to the sucrose-challenged control rats. Pre-treatment with SMC (200 mg kg^{-1}, b.w.) exhibited 16.32% reduction in peak BGL in sucrose tolerance test. Table 2 represents the effect of SMC concerning the area under curve (AUC) in carbohydrate-induced hyperglycemia in normal rats. SMC (200 mg kg^{-1}, b.w.) produced 14.78 and 17.87% reduction in AUC in starch and sucrose-fed rats, while acarbose produced 20.99 and 22.62% reduction in AUC in starch and sucrose-fed normal rats, respectively.

Oral carbohydrate tolerance test of SMC in STZ-induced diabetic rats

The results of oral starch and sucrose tolerance tests with SMC in comparison to acarbose in STZ-

Table 2: Effect of SMC on blood glucose level (mg dL^{-1}) of carbohydrate challenged normal rats

Groups	Blood glucose level (mg dL^{-1})					Increase in PBGL *vs* NC (%)	Reduction in PBGL vs toxic (%)	Reduction in AUC vs toxic (%)
	0 min	30 min	60 min	90 min	120 min			
NC	89.4 ±1.63	89.4±1.83	89.4±1.89	90.2±2.87	90.4±2.73	-	-	-
Starch tolerance test								
StC	87.6±1.29	153.4±2.56^{a**}	147.5±2.01^{a**}	142.8±1.30^{a**}	136.6±2.26^{a**}	71.58	-	-
Acar	83.2±0.86	124.8±2.89^{b*}	114.8±1.88^{b**}	107.8±1.36^{b**}	100.4±1.29^{b**}	39.59	18.64	20.99
SMC	84.4±1.43	133.4±1.63^{b*}	124.2±1.93^{b*}	118.8±2.18^{b*}	110.2±1.74^{b**}	49.22	13.04	14.78
Sucrose tolerance test								
SuC	86.4±2.87	155.6±1.69^{a**}	149.2±0.92^{a**}	142.4±2.06^{a**}	139.4±1.63^{a**}	74.05	-	-
Acar	87.4±2.09	116.4±1.50^{b**}	115.4±1.58^{b**}	109.6±1.25^{b**}	96.6±0.60^{b**}	30.20	25.19	22.62
SMC	89.4±1.21	130.2±0.73^{b*}	120.6±1.63^{b**}	113.6±1.50^{b**}	103.0±2.14^{b**}	45.64	16.32	17.87

Data expressed as mean ± SD, n = 6, NC: Normal control (1 mL kg^{-1} b.w. of 0.5% CMC in distilled water, NC: Normal control (1 mL kg^{-1} b.w. of 0.5% CMC in distilled water); StC: Starch control (3 g kg^{-1}, b.w.); SuC: Sucrose control (4 g kg^{-1}, b.w.); Acar: acarbose (10 mg kg^{-1}, b.w.); PBGL: Peak blood glucose level (30 min); AUC: Area under curve of blood glucose; SMC: Standardized extract of *M. charantia* (200 mg kg^{-1}, b.w., each);
[a]Carbohydrate challenged control (StC and SuC) *vs* normal control;
[b]Treated group *vs* carbohydrate challenged control, $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$.

induced diabetic rats are presented in Table 3. Oral administration of starch (3 g kg^{-1}, b.w.) and sucrose (4 g kg^{-1}, b.w.) produced a significant increase (p<0.01) in BGL of carbohydrate-challenged control animals mimicking PPHG in diabetes. In starch tolerance test, animals pre-treated with acarbose (10 mg kg^{-1}, b.w.) significantly lowered (p<0.01) peak BGL, with a 10.38% reduction in peak BGL as compared to the starch-challenged control rats. Pre-treatment with the SMC (200 mg kg^{-1}, b.w.) exhibited 7.44% reduction in peak BGL in starch tolerance test. In sucrose tolerance test, pre-treatment with acarbose (10 mg kg^{-1}, b.w.) significantly lowered ($p < 0.01$) peak BGL, with 13.22% reduction in peak BGL, as compared to sucrose-challenged control. Pre-treatment with SMC (200 mg kg^{-1}, b.w.) exhibited an 8.32% reduction in peak BGL in sucrose tolerance test (Table 3). Table 3 also represents the effect of SMC concerning AUC in carbohydrate-induced hyperglycemia in diabetic rats. SMC (200 mg kg^{-1}, b.w.) produced 11.35 and 7.12% reduction in AUC in starch and sucrose-fed rats, respectively, while acarbose produced a reduction of 15.21 and 19.73% in AUC in starch and

Table 3: Effect of SMC on blood glucose level (mg dL-1) of carbohydrate challenged in diabetic rats

Groups	Blood glucose level (mg dL^{-1})					Increase in PBGL *vs* DC (%)	reduction in PBGL *vs* toxic (%)	reduction in AUC vs toxic (%)
	0 min	30 min	60 min	90 min	120 min			
DC	204±1.61	206.2±1.53	203.8±1.02	205.4±0.75	205.8±1.06	-	-	-
Starch tolerance test								
StC	206.0±1.61	319.8±2.57^{a**}	315.2±1.74^{a**}	311±1.22^{a**}	305.0±1.14^{a**}	55.09	-	-
Acar	200.8±1.80	286.6±1.69^{b*}	263.4±2.87^{b**}	253.4±2.06^{b**}	229.8±2.57^{b**}	38.99	10.38	15.21
SMC	201.0±2.95	296.0±2.94^{bns}	284.8±3.14^{b*}	263.4±1.98^{b**}	240.8±1.64^{b**}	43.54	7.44	11.35
Sucrose tolerance test								
SuC	203.6±1.69	334.2±3.29^{a**}	324.4±2.82^{a**}	319.8±3.97^{a**}	311.4±0.81^{a**}	62.07	-	-
Acar	202.8±1.59	290.2±3.19^{b*}	246.4±3.14^{b**}	237.4±1.96^{b**}	233.2±1.96^{b**}	40.64	13.22	19.73
SMC	203.1±1.92	306.4±4.11^{bns}	288.4±3.40^{b*}	256±4.07^{b**}	242.8±3.26^{b**}	48.59	8.32	7.12

Data expressed as mean ± SD, n = 6, NC: Normal control (1 mL kg^{-1} b.w. of 0.5% CMC in distilled water); in diabetic rats all animals received STZ (50 mg kg^{-1}, b.w., *i.p.*); DC: Normal control (1 mL kg^{-1} b.w. of 0.5% CMC in distilled water); StC: Starch control (3 g kg^{-1}, b.w.); SuC: Sucrose control (4 g kg^{-1}, b.w.); Acar: Acarbose (10 mg kg^{-1}, b.w.); PBGL: Peak blood glucose level (30 min); AUC: Area under curve of blood glucose; SMC: Standardized extract of *M. charantia* (200 mg kg^{-1}, b.w., each);
[a]Carbohydrate challenged control (StC and SuC) *vs* normal control;
[b]Treated group *vs* carbohydrate challenged control, $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$.

sucrose-fed diabetic rats, respectively.

Conclusion: In this study, a charantin-rich extract from *M. charantia* fruits was prepared, and the extract assayed for anti-hyperglycemic activity. The charantin-rich extract showed a dose dependent inhibition of carbohydrate metabolizing enzymes (α-amylase and α-glucosidase). *In vivo* studies established the efficacy of *M. charantia* extract in lowering the peak blood glucose levels in normal and diabetic rats challenged with starch and sucrose. The present study provides scientific credence to the use of *M. charantia* in maintaining glycemic hemostasis in diabetes.

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Ethical statement: The experimental study was approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi, India (vide No. JH/CAHF/173/CPCSEA/28th January/2000, approval No. 2012/926).

Conflict of interest: All authors declare no conflict of interest.

REFERENCES

Ahamad, J., Alkefai, N.H.A., Amin, S. and Mir, S.R. 2020. Standardized extract from *Enicostemma littorale* ameliorates post-prandial hyperglycaemia in normal and diabetic rats. *Journal of Biologically Active Products from Nature*, **10**(1): 34-43.

Ahamad, J., Amin, S. and Mir, S.R. 2015. Optimization of ultrasound-assisted extraction of charantin from *Momordica charantia* fruits using response surface methodology. *Journal of Pharmacy and Bioallied Sciences*, **7**(4): 304-307.

Ahamad, J., Amin, S. and Mir, S.R. 2014. Simultaneous quantification of gymnemic acid and charantin using validated HPTLC densitometric method. *Journal of Chromatographic Sciences*, **53**(7): 1203-1209.

Ahamad, J., Amin, S. and Mir, S.R. 2019. Antihyperglycemic activity of charantin isolated from fruits of *Momordica charantia* Linn. *International Research Journal of Pharmacy*, **10**(1): 61-64.

Ahamad, J., Amin, S. and Mir, S.R. 2017. *Momordica charantia*: Review on phytochemistry and pharmacology. *Research Journal of Phytochemistry*, **11**: 53-65.

Anun, S.Y., Adisakwattana, S., Yao, C.Y., Sangvanich, P., Roengsumran, S. and Hsu, W.H. 2006. Slow acting protein extract from fruit pulp of *Momordica charantia* with insulin secretagogue and insulinomimetic activities. *Biological and Pharmaceutical Bulletin*, **29**(6): 1126-1131.

Gill, N.S., Rani, P., Arora, R., Dhawan, V. and Bali, M. 2012. Evaluation of antioxidant, antiinflammatory and antiulcer potential of *Momordica charantia* methanolic seed extract. *Research Journal of Phytochemistry*, **6**: 96-104.

Jia, S., Shen, M., Zhang, F. and Xie, J. 2017. Recent advances in *Momordica charantia*: Functional components and biological activities. *International Journal of Molecular Sciences*, **18**(12): 2555 [*doi: 10.3390/ijms18122555*].

Nadkarni, K.M. 2007. *Indian Materia Medica, Volume 1*. Popular Prakashan Pvt. Ltd., Mumbai. India.

Nhiem, N.X., Kiem, P.V., Minh, C.V., Ban, N.K., Cuong, N.X., *et al.* 2010. α-glucosidase inhibition properties of cucurbitane-type triterpene glycosides from the fruits of *Momordica charantia*. *Chemical and Pharmaceutical Bulletin*, **58**(5): 720-724.

Perumal, V., Khoo, W.C., Abdul-Hamid, A., Ismail, A., Shaari, K., *et al.* 2015. Evaluation of antidiabetic properties of *Momordica charantia* in streptozotocin induced diabetic rats using metabolomics approach. *International Food Research Journal*, **22**: 1298-1306.

Purves, R.D. 1992. Optimum numerical integration methods for estimation of area-under-the-curve

(AUC) and area-under-the moment-curve (AUMC). *Journal of Pharmacokinetics and Biopharmaceutics,* **20**: 211-227.

Raish, M., Ahmad, A., Jan, B.L., Alkharfy, K.M., Ansari, M.A., *et al.* 2016. *Momordica charantia* polysaccharides mitigate the progression of STZ induced diabetic nephropathy in rats. *International Journal of Biological Macromolecules,* **91**: 394-399.

Singh, N. and Gupta, M. 2007. Regeneration of *β*-cells in islets of Langerhans of pancreas of alloxan diabetic rats by acetone extract of *Momordica charantia* (Linn.) (bitter gourd) fruits. *Indian Journal of Experimental Biology*, **45**: 1055-1062.