RESEARCH ARTICLE

inventi Spreading Knowledge

Assessment of Antidiabetic Potential of Flowers of *Musa sapientum* and Development of Tablet Dosage Form

S A Choudhari[1*], P F Khatwani[1], S R Kulkarni[1]

Abstracts: The flowers of *Musa sapientum* L. (MS) commonly known as 'Banana Flowers' are reported to be used in Indian folk medicine for the treatment of diabetes mellitus. But the probable mechanisms of antidiabetic activity are yet to be studied. The present study was designed to evaluate the antihyperglycemic activity of banana (*Musa sapientum*) flowers and development of suitable dosage form. The air dried powder sample of flower of *Musa sapientum* was extracted with ethanol and water by maceration method and subjected for phytochemical analysis. The extracts were analyzed by TLC which shown the presence of gallic acid. The quantitative estimation of gallic acid from extract was done by High Performance Thin Layer Chromatography (HPTLC). The antidiabetic potential of the extracts was tested using Oral Glucose Tolerance Test (OGTT) in normal rats. In OGTT, there was a significant reduction in glucose level of animals treated with aqueous and ethanolic extract at the dose of 200 mg/kg. *In-vitro* assays were also performed for estimation of inhibition of α-glucosidase and α-amylase. IC50 values were calculated and inhibition efficiencies of aqueous and ethanolic extract were compared. The results have shown that both the extracts have good potential in management of diabetes. The human equivalent dose was calculated from OGTT results and tablet of 750 mg was formulated each containing 500mg aqueous extract of MS with optimized formula of excipients. The formulation was characterized and evaluated for physicochemical stability, and the gallic acid content in the table was assayed by HPTLC.

INTRODUCTION

The prevalence of diabetes is rising all over the world due to aging, urbanization, an increase of obesity and physical inactivity. Studies in India indicate that more than 50% of people with diabetes have poor glycemic control. [1] The synthetic hypoglycemic agents can produce serious side effects including hematological, coma and disturbances of liver and kidney. In addition they are not suitable for use during pregnancy. Research is going on to seek more effective treatment of diabetes with minimal side effects. In the indigenous system of medicine many herbs have been claimed to be useful in the treatment of diabetes mellitus. Amongst them, banana (*Musa sapientum*) is one of the plants which flowers reported to have good antihyperglycemic effect when tested in Alloxan induced diabetic rats. The juice of the *Musa sapientum* flowers is claimed to have beneficial effects in reducing the blood sugar. [2]

The present investigation was undertaken to study the effect of extracts of *Musa sapientum* flowers on blood glucose level in rats. The attempts were further made to study *in-vitro* activity of extracts on of α-glucosidase and α-amylase enzymes. The tablet dosage form from aqueous extract was developed and evaluated for stability.

MATERIALS AND METHODS

Inflorescences of *Musa sapientum* were obtained from local market, Dadar, Mumbai and authenticated (Voucher specimen no. #SAC/070312) at G. N. Khalsa College, Mumbai, India. Then samples were air-dried at 50°C in a hot air oven. Dried samples were ground to powder using a mechanical grinder, and stored in a sealed plastic container. Microcrystalline cellulose, Methyl paraben, Talc, Cross povidone, Sodium starch glycolate and Magnesium stearate were supplied by BASF Pvt Ltd, Mumbai, India.

[1]Department of Pharmacognosy and Phytochemistry, Bombay College of Pharmacy, Kalina, Mumbai-98, Maharashtra, India.
E-mail: sagarashok2008@gmail.com
*Corresponding author

Extraction

The dried powder of flowers was extracted by maceration using solvents were water and ethanol (Herb to menstrum ratio 1:10). [3] Dried powder of inflorescence was kept for maceration in stoppered container with frequent agitation for 18 h. The filtrate was separated and solvent was removed by distillation. Brownish colored dried aqueous and ethanolic extracts were obtained. Alcoholic & aqueous extracts were subjected to phytochemical investigation.

Evaluation of Extracts for Presence of Gallic Acid by Chromatography [4]

A thousand parts per million solutions of aqueous and ethanolic extract were prepared and sonicated on bath sonicator (Expo High-Tech). Each extract solution was applied on TLC plate (silica gel 60F $_{254,}$ E. Merck) of thickness 0.2 mm. Then plate was conditioned for 15 min in a pre-saturated chamber in solvent system Chloroform: Methanol: Formic acid (9:1:0.075). After development a plate were dried and observed under 254 and 366. The visualization of gallic acid was achieved by spraying the plate with Methanolic $FeCl_3$.

Quantitative Estimation of Gallic Acid in Aqueous and Ethanolic Extract by HPTLC (High Performance Thin Layer Chromatography) [5]

The stock solution of sample was prepared of 50mg/ml. Each extract was applied (application system LINOMAT –V) in 5µl quantity on aluminium (10x 20 cm^2) TLC Plate (Silica gel 60F $_{254}$ E. MERCK) of thickness 0.2 mm. The plates were then conditioned for 20 min in pre-saturated twin-trough chamber in solvent system Chloroform: Methanol: Formic acid (9:1:0.075) and developed to a distance of 8.5 cm. After development, plates were air dried and densitometric scanning was performed at wavelength 254 nm and 366 nm with CAMAG TLC scanner III operated in reflectance-absorbance mode controlled by WinCATS software (v1.2.1). The gallic acid in the extracts was confirmed by comparing the R_f, 3-D spectrum and spectral overlay of the spot of standard gallic acid and extracts shown in Figure 1 and 2.

Table 1: Quantity of Reagents Taken for Measurement of Blood Glucose Level

S. No.	Quantity of Reagents into Tubes	Blank (µl)	Standard (µl)	Test (µl)
1	Working reagent	1000	1000	1000
2	Distilled water	10	—	—
3	Standard	—	10	—
4	Test (serum)	—	—	10

Table 2: Composition of Tablet of Aqueous Extract of *Musa sapientum*

S. No.	Ingredients	mg/tablet
1	Aq. Extracts	500
2	Microcrystalline cellulose	218
3	Cross povidone	12.6
4	Methyl paraben	1.4
5	Talc	12
6	Magnesium stearate	6
	Total weight	750

Table 3: Serum Glucose Level (mg/dl) (Mean± SD) at Various Time Points in Control, Aqueous Extract and Ethanolic Extract Fed Group and (*) Indicates P < 0.05

S. No.	Time (min)	Control	Et. extract	Aq. extract
1	0	106.09±13.2	102.80±1.8	103.92±2.7
2	30	147.20±16.14	*114.46±9.43	*115.58±9.91
3	60	147.42±9.99	*115.45±4.15	119.7±3.5
4	90	142.84±17.33	118.96±17.76	122.42±8.1
5	120	126.35±22.68	110.14±19.13	120.95±9
6	180	111.84±12.29	107.18±15.87	115.94±11.6

Table 4: % Increase in Serum Glucose Level (mg/dl)

S. No.	Time	Control	Aq. extract	Et. extract
1	0	0	0	0
2	30	41.11	11.66	11.66
3	60	41.33	15.78	7.65
4	90	6.75	18.5	6.16
5	120	20.26	17.03	16.34
6	180	5.75	12.02	14.38
Total increase in serum sugar level		115.2 (100%)	74.99 (65.1%)	56.19 (48.8%)

Table 5: Inhibition of α-Glucosidase by Standard Acarbose, Aqueous Extract and Ethanolic Extract

S. No.	Conc. (ppm)	% Inhibition by Std. Acarbose	% Inhibition by Aq. extract	% Inhibition by Et. extract
1	25	37.98±1.72	3.87±0.92	0.95±1.48
2	50	43.22±1.38	7.89±0.50	2.82±1.77
3	100	46.17±1.01	12.33±0.59	5.40±2.45
4	150	49.47±1.63	24.01±0.85	19.44±3.43
5	200	49.40±0.65	29.71±0.53	27.35±2.66
6	250	53.04±0.98	34.32±1.39	37.02±6.82
7	500	59.80±3.47	43.12±1.26	48.51±4.36
8	1000	70.46±3.52	52.94±1.45	56.37±2.81

Table 6: Inhibition of α-Amylase by Standard Acarbose, Aqueous Extract and Ethanolic Extract

S. No.	Conc. (ppm)	% Inhibition by Std. Acarbose	% Inhibition by Aq. Extract	% Inhibition by Et. Extract
1	25	28.21±2.22	9.11±11.84	2.78±1.67
2	50	44.37±4.54	18.99±8.08	24.91±9.98
3	75	53.44±1.56	23.43±9.76	37.78±7.63
4	100	59.40±1.09	31.61±11.76	44.38±10.5
5	150	61.09±1.93	39.14±14.64	46.29±3.38
6	200	64.06±1.66	41.46±12.18	48.85±0.92
7	250	65.21±0.85	46.74±15.36	57.58±5.84
8	500	73.06±7.06	58.90±7.41	64.51±3.35
9	1000	76.96±4.92	64.69±7.22	67.87±3.79

Table 7: Evaluation of Pre-compression Parameters for Tablets

S. No.	Parameters	Units
1	Bulk density	0.60 gm/cc
2	Tapped density	0.66 gm/cc
3	Compressibility Index	9.09 %
4	Hausner's ratio	1.1
5	Angle of repose	28.58 ± 022
6	Particle size (moderately coarse)	180 to 335 µm

Table 8: Evaluation of Post-compression Parameters for Anti-Diabetic Tablets [10]

S. No.	Parameters	Units
1	Diameter	12.5 ± 0.5 mm
2	Thickness	3.5 ± 1 mm
3	Friability (100 rotation)	0.25 %
4	Hardness	3.2 ± 2 kg/cm^2
5	Weight variation	750 ± 13.84 %
6	Disintegration time	14min±10 sec

Table 9: Assay for Content of Gallic Acid Present in the Formulation

Assay for Stability of Tablets	At 0 months	At 3 Months		
		2-8°C	25°±2°C/60±5%RH	40°±2°C/75±5%RH
Gallic acid(%w/w)	0.560 (%w/w)	0.542 (%w/w)	0.496 (%w/w)	0.473 (%w/w)

OGTT (Oral Glucose Tolerance Test)

Animals

Healthy adult Sprague Drawly rats (180-250 g) were used for the study. Housed in polypropylene cages, maintained at standard conditions, the animals fed with standard rat pellet, diet and water *ad libitum.* The study was approved by the Institutional Animal Ethical Committee.

Experimental Procedure

The OGTT was performed on overnight fasted rats. Rats were divided in control group administered drinking water and drug treated groups (n=3) administered crude extracts. Aqueous and ethanolic extracts were tested at dose 200mg/kg. [2] Glucose was fed after the administration of extract. The blood was withdrawn from the retro orbital plexus, collected just prior to glucose administration (0 min) & 30, 60, 90, 120 & 180 min after glucose loading. [6-7] An unhaemolyzed serum was separated. The blood glucose level in serum sample was analysed spectropohtometrically using diagnostic kit procured from Erba Mannhelm as per the procedure in the Table (1). The mixture was shaken well and incubated at 37°C for 15 min. Samples were analyzed spectrophotometrically at 505 nm against blank. [8]

In-vitro Antidiabetic Activity Studies

Requirements

- Acarbose solution: 50 mg of acarbose in 50 ml of distilled water.
- Extract solution: 100 mg aqueous and ethanolic extract were dissolved in distilled water and ethanolic extract of flowers was dissolved in DMSO and volume was made up to 100 ml with water. Then concentration range from 25 to 1000 ppm was prepared.
- 1 % w/v Starch solution:
- 3, 5-dinitosalicylic acid solution (DNS)

α-Glucosidase Inhibition Assay [9]

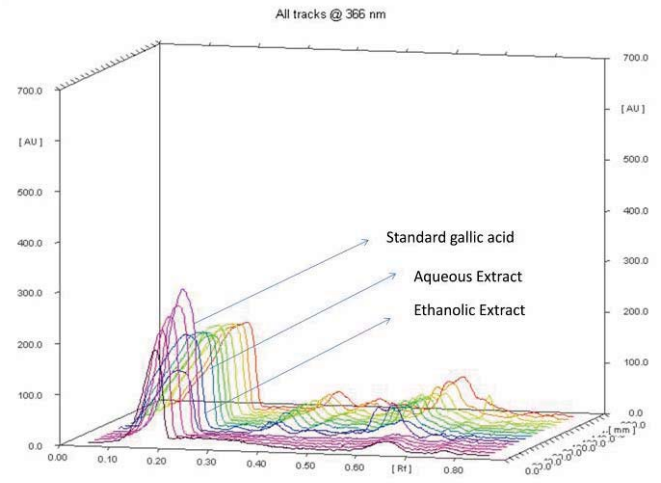
Intestinal α-glucosidase is the enzyme which hydrolyses oligosaccharides, trisaccharides and disaccharides into glucose and other monosaccharides in the small intestine. α- glucosidase inhibitors are saccharides that acts as competitive inhibitors of enzyme needed to digest carbohydrate. Inhibition of this enzyme reduces the rate of digestion of carbohydrates. Therefore less glucose is absorbed.

Isolation of α-Glucosidase

SD rats were kept for fasting for 18 hrs. After fasting, each animal (n = 3) was sacrificed by cervical dislocation. The small intestine was isolated and flushed several times with ice-cold 0.9 % NaCl, sodium phosphate buffer (pH 7.0) and small intestine between duodenum and cecum was cut, rinsed with ice cold saline and homogenized with phosphate buffer (pH 7.4), this small intestine homogenate was used as an enzyme source.

Procedure

To 100 µl of extract solution, 200 µl enzyme solutions were added and the mixture was preincubated for 15 min at 37°C. Then, 100 µl of starch solution was added in microcentrifuge tubes and incubated for 5 min at 37°C. It was centrifuged at 4000 rpm. Then, 100 µl of supernatant was pipette from all microcentrifuge tubes separately. To each tube, 1ml of glucose reagent from glucose kit was added and was incubated for 15 min at 37°C. The absorbance was measured at 505 nm. Individual's blanks were prepared for correcting background absorbance (enzyme replaced by buffers). In the same way control incubation was conducted (extract replaced by distilled water and DMSO). Acarbose solution was used as standard for comparing activity of extract with respect to standard.



(Tracks 1-5: standard gallic acid, Track 6: aqueous extract, Track 7: ethanolic extract, other tracks represent stability testing of tablet formulation at various temperature and humidity)

Figure 1: 3-Dimentional view of standard gallic acid, aqueous extract and ethanolic extract of *Musa sapientum*

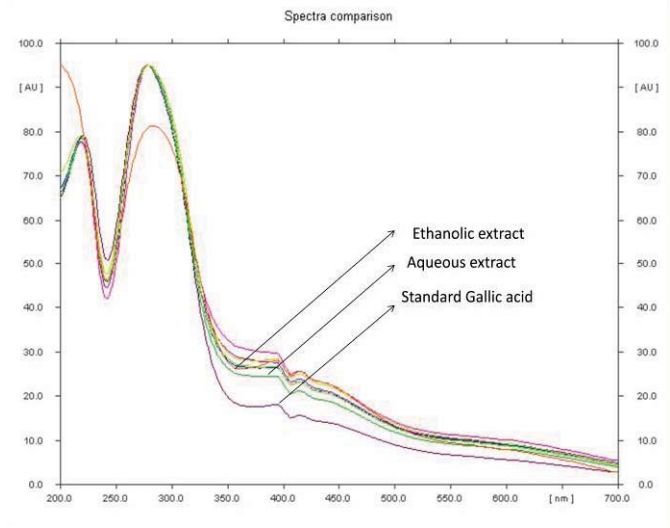


Figure 2: Spectral overlay of standard gallic acid, aqueous extract and ethanolic extract of *Musa sapientum*

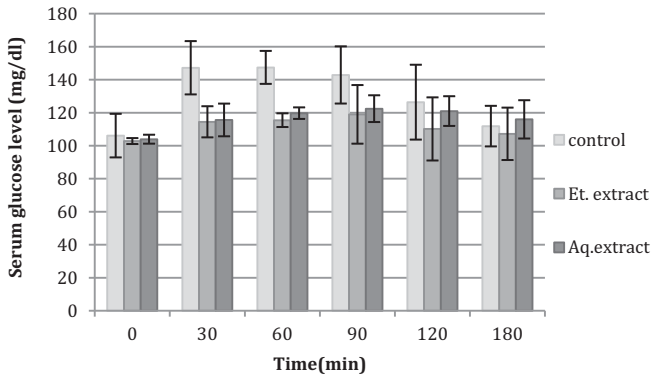


Figure 3: Serum glucose level (mg/dl) (Mean± SD) at various time points in control, aqueous extract and ethanolic extract fed group

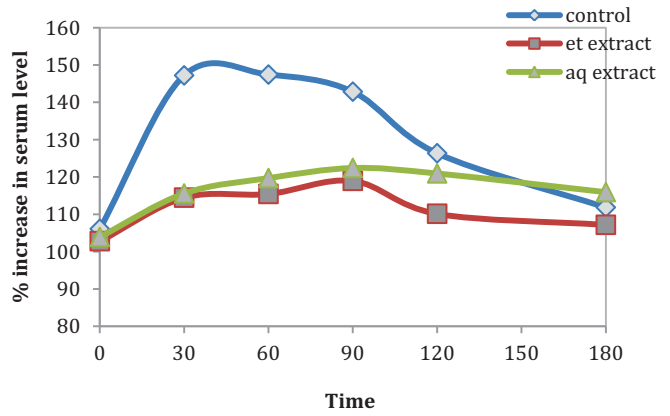


Figure 4: % Increase in serum glucose level (mg/dl)

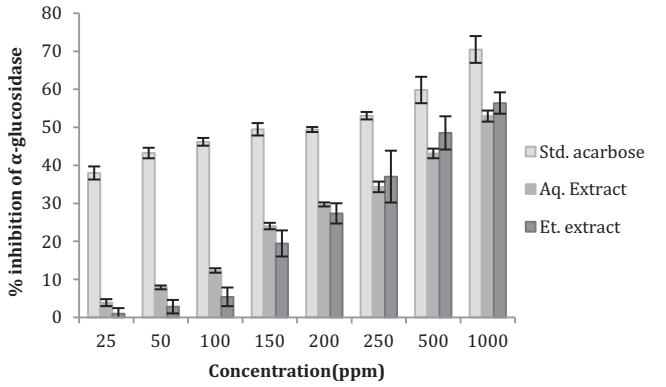


Figure 5: % Inhibition of α-glucosidase by standard acarbose, aqueous extract and ethanolic extract

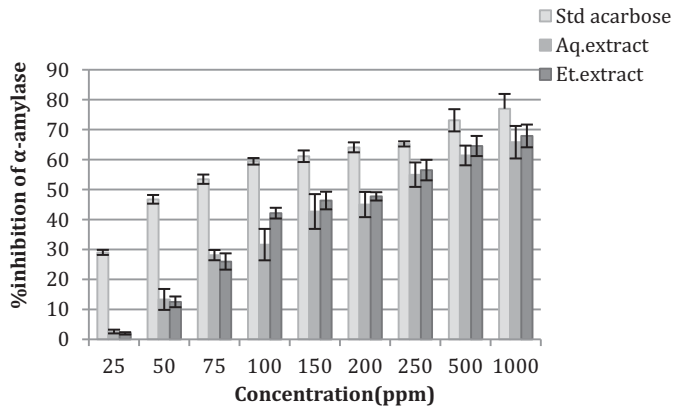


Figure 6: % Inhibition of α-amylase by standard acarbose, aqueous extract and ethanolic extract

α-Amylase Inhibition Assay [10-11]

Pancreatic α-amylase is the enzyme which hydrolyses starch into maltose and dextrin. The generation of maltose was quantified by the reduction of 3, 5-dinitrosalicylic acid which changes color from orange yellow to red is detectable at 540 nm.

Isolation of α-Amylase

SD rats were killed under light diethyl ether anesthesia, and the pancreases were removed, trimmed free of fat, weighed and kept at -70°C until used. A 10 % homogenate of the tissues was prepared in saline sodium phosphate buffer (pH 7.4) using tissue homogenizer. Homogenates were

centrifuged at 5000 rpm for 30 min at 4°C. The supernatant was collected and used as the source of α-amylase.

Procedure

To 0.5 ml of extract solution, 0.5 ml of enzyme was added and the mixture was preincubated at 37°C for 15 min. To this, 0.5ml of starch solution was added and test tubes incubated at 37°C for 15 min. Then 0.5 ml of DNS was added and the tubes placed into boiling water bath. The reaction mixture was removed from the water bath, cooled and diluted with 4.5 ml distilled water. The absorbance value was recorded at 540 nm. Individual blanks were prepared (enzyme replaced by buffer solution) for correcting the background absorbance. Control incubation conducted in a similar way by replacing extract with water and DMSO. Acarbose solution was used as standard for comparing activity of extract with respect to standard. The experiments were performed in triplicate and averaged. The concentration of the extract required to inhibit the activity of the enzyme to 50 % was calculated. The inhibition was calculated by formula

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Development of Tablet Formulation

The aqueous extract of flower had shown significant antihyperglycemic activity at dose 200 mg/kg in oral glucose tolerance test. The Human Equivalent Dose was calculated based on following formula. [12] The Human dose calculation was done by considering at given dose 200 mg/kg of body weight of rats. The calculated dose was 1.945 g ≅ 2 gm, which was divided into four units such that each will contain 500 mg of aqueous extract.

Human Equivalent Dose (mg/kg) = Animal Dose (mg/kg) × (Animal km/ Human km)

Human Adult weight= 60 kg, Human Adult km=37, Rat km=6

The aqueous and ethanolic extracts were tried for preparation of tablet dosage form. The ethanolic extract was restricted to formulate it into tablet dosage form because of more stickiness, poor flow characteristics of powder blend and lesser yield. So, the tablet dosage form of aqueous extract of flower was formulated using standard excipients. The aqueous extract and microcrystalline cellulose were mixed in geometric proportion. The granules formed were air dried and mixed with talc, magnesium stearate, cross povidone, and methyl paraben in geometric proportion. The attempt involved mechanism of direct compression. [13] Single station tablet punching machine and punches of size 12.5 mm diameters was used. By changing the concentrations of various excipients the final tablet formula was optimized for 750 mg of tablet containing 500 mg of aqueous extract. The composition of tablets given in Table 2. The pre-compression and post-compression parameters of tablet were evaluated as per standard procedures [15-16] and stability testing was done as per ICH guidelines. [17]

RESULTS

The yield of the aqueous and ethanolic extract was 13.54 ± 0.66 and 4 ± 0.8 (% w/w). The preliminary phytochemical screening of both extracts of flower showed the presence of steroids, flavonoids, alkaloids, tannins and phenolic compounds.

TLC and HPTLC Analysis of Extracts

The HPTLC chromatogram showed the zone of gallic acid at R_f 0.18 in all tracks. Peak area & peak height of standard gallic acid, aqueous and ethanolic extracts and formulation were compared by densitometric scanning of spots. The 3-D view, spectral overlay of standard gallic acid at wavelength 366 nm confirmed the presence of gallic acid in the aqueous and ethanolic extract as shown in Figure 1 & 2. The amount of gallic acid in the aqueous and ethanolic extracts of flower was calculated from calibration curve of standard gallic acid. The aqueous and ethanolic extracts were found to contain 2.793 % w/w and 4.124 % w/w gallic acid, respectively.

***In-vivo* Activity (Oral Glucose Tolerance Test)**

It was reported that Chloroform extract of flowers was tested at doses 150, 200, 250 mg/kg. The dose 200 mg/kg showed significant blood glucose lowering activity. [2] So, we tested aqueous and ethanolic extracts at 200 mg/kg dose which have shown significant glucose lowering activity on rats. The blood glucose data obtained in OGTT on normal rats (Table 3) clearly shows that the aqueous and ethanolic extract of flower of *Musa sapientum* can produce the significant antihyperglycemic effects. The results were expressed as means ± SD. The significance of various treatments was calculated using student t-test & considered statistically significant when $P < 0.05$.

By considering initial serum glucose level 0 and subtracting serum glucose level at all time points from initial value, the % increase glucose level was calculated. The % increase in glucose level for aqueous extract and ethanolic extract at dose of 200 mg/kg were found to be 65 % and 50 % respectively, while considering 100 % for vehicle control group as shown in Table 4.

***In-vitro* Studies**

These studies provide information of possible mechanism of action of the drug. α-Glucosidase and α-amylase inhibition activity of aqueous and ethanolic extracts of flower of *Musa sapientum* was studied. Inhibition of α-glucosidase and α-amylase by standard acarbose, aqueous extract and ethanolic extract was shown in Table 5 & 6. Minimum concentration of extract required to inhibit the enzyme by 50% was calculated for α- glucosidase and α-amylase. IC_{50} of acarbose, aqueous extract and ethanolic extract for α- glucosidase was found to be 201.086 ± 1.536, 875.322 ± 69.028 and 607 ± 98.957 ppm, respectively from Figure 5. IC_{50} of acarbose, aqueous extract and ethanolic extract for α-amylase was found to be 64.344 ± 2.99, 225.789 ± 21.685 and 214.433 ± 8.806 ppm, respectively from Figure 6.

From the observation it was found that the aqueous extracts of flower of *Musa sapientum* have good α-glucosidase and α-amylase inhibitory activity. But as compared to standard acarbose and ethanolic extract it was having less activity.

Formulation of Tablet and Evaluation

The tablet was formulated and evaluated for pre-compression and post-compression parameters shown in Table 7 & 8. The tablets were kept for stability study as per ICH stability guidelines. The content of gallic acid in tablet was assayed by HPTLC as shown in Table 9.

DISCUSSION

The flowers of *Musa sapientum* L. were taken up for the present study. Exhaustive extraction of the plant material was done with water and ethanol. It was subjected for the phytochemical screening, HPTLC analysis, and antihyperglycemic activity testing by *in-vivo* and *in-vitro* methods. Moreover, the extracts were formulated into a tablet using different polymers. Tablet formulated from aqueous extract was of good quality with regards to characteristics like hardness, friability, weight variation and disintegration time. Finally, the anti diabetic activity was found to be significant by *in-vivo* OGTT and *in-vitro* enzyme inhibitory activity testing.

Hence, these investigations have supported the selected medicinal plant which ascertains its pharmacology lead to rapid development in diabetes treatment. In addition to this, these studies also provide information of possible mechanism of action of the drug. Thus, this holds great promise for future research for the formulation of potent antidiabetic drug.

REFERENCES AND NOTES

1. Ramachandran A, Das A. Current status of diabetes in India and need for novel therapeutic agents. Journal of Association of Physician of India, 58: 7-9, 2010.
2. Pari L, Uma Maheswari J. Hypoglycaemic effect of *Musa sapientum* L. in alloxan-induced diabetic rats. Journal of ethnopharmacology, 68 (1): 321-325, 1999.
3. Sheng Z W, Wei H M, Jin H G, Yang B, Wei M Z. Antioxidant properties of banana flower of two cultivars in China using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reducing power, 2,2'-azinobis-(3-ethylbenzthiazoline-6- sulphonate (ABTS) and inhibition of lipid peroxidation assays. African Journal of Biotechnology, 10: 4470-4477, 2011.
4. Borde V U, Pangrikar P P, Tekale S U. Gallic acid in Ayurvedic herbs and formulations. Recent Research in Science and Technology, 3(7): 51-54, 2011.
5. Kondawar M S, Kamble K G, Mali D S. Quantitative estimation of gallic acid in a marketed herbal medicine: Triphala churna by High Performance Thin layer Chromatography, 3(3): 1593-1599, 2011.
6. Barik R, Jain S, Owatra D, Joshi A, Tripathi S G. Antidiabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin-nicotinamide induced type-II diabetes in rats. Indian Journal Pharmacol, 40(1): 19–22, 2008.
7. Nelson I O, Chioli P C, Samuel I G. Effects of aqueous leaf extract of *Ocimum gratissimum* on oral glucose tolerance test in type-2 model diabetic. African Journal of Pharmacy and Pharmacology, 6(9): 630-635, 2012.
8. Ali L, Kalam A K, Iqbal R M, Mosihuzzaman M, Nahar N. Studies on Hypoglycemic effects of fruit pulp, seed and whole plant of *momordica charantia* on normal diabetic model rats. Planta Med, 59: 408-412, 1993.
9. Ortiz-Andrade R R, Garcia-Jimnez S, Castillo-Espana P, Ramirez-Avila G, Villalobos-Molina R, Estrada-Soto S. Glucosidase inhibitory activity of the Methanolic extract from *Tournefolia hartwegiana*: An antihyperglycemic agent. Journal of Ethnopharmacology, 109: 48-53, 2007.
10. Reddy M K, Heda G D, Reddy J K. Purification and characterization of α-amylase from rat pancreatic acinar carcinoma comparison with pancreatic α-amylase. Journal of Biochem, 242: 681-687, 1987.
11. Nickavar B, Yousefian N. Inhibitory effects of six Allium species on? Amylase activity. Iranian Journal of Pharmaceutical Reasearch, 8(1): 53-57, 2009.
12. Shaw R S, Nihal M, Nihal A. Dose translation from animal to human studies Revisited. FASEB Journal, 22: 659-661, 2007.
13. Lachman L, Lieberman H A, Kanig J K. The theory and practice of industrial pharmacy. 3rd ed. Mumbai, Varghese Publishing House, 296-311, 1968.
14. USP 30/NF 25. United State Pharmacopoeia Convention, Vol (I), 242-243, 2007.
15. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Walfare. The Indian Pharmacopoeia commission, Gaziabad, 632-634, Vol(II), 2007.
16. ICH Guidelines, Stability Testing of New Drug Substances and Products Q1A (R2). In Proceedings of the International Conference on Harmonization; Geneva, 1-22, 2003.

Acknowledgments

Authors are thankful to UGC for providing financial assistance.

Cite this article as: S A Choudhari, P F Khatwani, S R Kulkarni. Assessment of Antidiabetic Potential of Flowers of *Musa sapientum* and Development of Tablet Dosage Form. Inventi Impact: Ethnopharmacology, 2013(1): 45-50, 2013.