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# Inhibitory effect of banana (*Musa* sp. var. Nanjangud rasa bale) flower extract and its constituents Umbelliferone and Lupeol on α-glucosidase, aldose reductase and glycation at multiple stages

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ABSTRACT

Postprandial hyperglycaemia is characterized as the earliest symptom of diabetes and its management attenuates several of the associated secondary complications. In this context, we investigated the role of ethanol extract of banana flower (EF) for its antihyperglycaemic effects. The EF showed a strong inhibition towards α-glucosidase and pancreatic amylase which play a vital role in clinical management of postprandial hyperglycaemia. The major active compounds present in EF were identified as Umbelliferone (C1) and Lupeol (C2) using various spectroscopic methods. C1 ($IC_{50}$: 7.08 ± 0.17 μg/ml) and C2 ($IC_{50}$: 7.18 ± 0.14 μg/ml) were found to inhibit α-glucosidase in a non-competitive mode of inhibition, with low $K_i$ values. Further, *in vitro* glycation assays showed that EF and its compounds prevented each stage of protein glycation and formation of its intermediary compounds. EF, C1 and C2 also exhibited a potent inhibition on aldose reductase with $IC_{50}$ values of 2.25 ± 0.29, 1.32 ± 0.22 & 1.53 ± 0.29 μg/ml respectively. Our results suggest that, the observed potential of EF in antihyperglycaemic activity *via* inhibition of α-glucosidase and in antidiabetogenic effect by inhibition of polyol pathway and protein glycation is more likely to be attributed to the presence of C1 and C2.

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## 1. Introduction

Diabetes mellitus, a chronic metabolic condition is attributed to an impaired regulation of carbohydrate and lipid metabolism by inappropriate functioning of pancreatic hormone, insulin, which is characterized by increased blood glucose levels. According to the International Diabetes Federation (IDF), the number of diabetic patients is likely to reach 592 million by the year 2035 which will reflect on the cost for treating it which is expected to reach US $548 billion, annually (International Diabetes Federation, 2013). Rapid absorption of dietary carbohydrates aided by glycoside hydrolases (α-glucosidase, α-amylase) converts them into simpler monosaccharide units, which results in elevated blood glucose levels characterized as postprandial hyperglycaemia. This is distinguished as the earliest symptom of diabetes, and the use of glycoside inhibitors is widely accepted as an efficient method in restraining postprandial hyperglycaemia by inhibiting the release of free glucose units facilitating a smooth glucose profile (Ramith et al., 2014). Prolonged postprandial hyperglycaemia leads to diverse secondary complications all of which result in a series of diabetic complications *viz.*, neuropathy, retinopathy and nephropathy. High blood glucose levels cause a significant flux of glucose into the polyol pathway to convert them into sorbitol by the action of aldose reductase (Kador et al., 1985). Diabetic state leads to overproduction of sorbitol, which in turn leads to its accumulation in tissues *viz.*, nerves, kidney, retina and lens due to impaired membrane permeability or reduced metabolism by sorbitol dehydrogenase. Sorbitol accumulation is associated with various microvascular complications and cardiovascular diseases which can be efficiently averted by inhibition of the key enzyme aldose reductase (Peter et al., 1985). Other major concern in diabetes is glycation of proteins resulting in partial loss of its activity as a consequence of perpetual hyperglycaemia. A non-enzymatic nucleophilic addition reaction of the carbonyl residue of sugar with the free amino group of proteins forming a reversible Schiff base, which further forms a more stable Amadori product constitutes the primary step in protein glycation. The Amadori products subsequently undergo a series of reactions *via* the dicarbonyl intermediates resulting in less characterized compounds known as the advanced glycation end products (AGEs) which subsequently accumulate in the tissues and are responsible for the aetiology of diabetic micro- and macro-vascular complications (Nessar, 2005). Consequently, the identification of compounds that inhibit each stage of glycation as well as the formation of intermediary products in the pathway proves useful in

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generation of novel therapeutic interventions to delay and prevent these complications (Samuel and James, 2003). Also, the glycated proteins and AGEs by interfering in the signal transduction cascade induce the generation of reactive oxygen species (ROS) concomitantly resulting in oxidative stress, which constitutes key factors in the progression of several vascular complications in diabetes (Su-Yen and Mark, 2008). The paramount method of protection from ROS mediated damage is provided by the antioxidants which neutralize the generated free radicals. Collectively, a therapy that involves an integrated antioxidant and antiglycation properties with a potent α-glucosidase inhibition and lower α-amylase inhibition can prove optimal in control of diabetes and its complications (Prathapan et al., 2012).

In this regard, owing to the harmful effects of the long known synthetic antidiabetic drugs, recently there has been increasing focus on natural substances which have inhibitory potential on the development of hyperglycaemia and its associated complications. Banana fruit consumed worldwide is known for several beneficial properties. Banana flower, one of the secondary products of banana cultivation, has been consumed as vegetable in many countries (Joshi, 2000). It is a part of traditional medicine, used in treatment of various disorders like kidney stone and ulcers (Ivan, 2003). Its antihyperglycaemic effects have also been accounted and included in some of the Ayurvedic formulations in the treatment of diabetes (Bhaskar et al., 2011; Joshi, 2000). However, the mechanism underlying and the active principles responsible for the beneficial role are less exploited. Hence, the aim of the present study is to evaluate the potential of ethanol extract of banana flower (EF) in inhibiting α-glucosidase and α-amylase that are responsible for hyperglycaemia as well as inhibition of aldose reductase and protein glycation that are responsible for various diabetic complications. Glycation markers at each stage of glycation *viz.*, fructosamines (early stage), protein carbonyls (intermediate stage) and AGEs (late stage) were also evaluated. Besides, isolation of active compounds responsible for the beneficial properties was carried out. The findings could become a rationale to include EF as an effective therapeutic adjunct for management of hyperglycaemia and associated complications.

## 2. Materials and methods

### 2.1. Chemicals

Butylated hydroxyl anisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzthiazoline-6-sulfonate (ABTS), phenazine methosulfate (PMS), nitro blue tetrazolium (NBT), gallic acid, dinitro phenyl hydrazine (DNPH), L-cysteine, porcine pancreatic α-amylase (EC 3.2.1.1), α-glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae*, *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), β-NADPH and aminoguanidine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Thiobarbituric acid (TBA), trichloro acetic acid (TCA), sodium carbonate and nicotinamide adenine dinucleotide-reduced (NADH) were obtained from Sisco Research laboratories (Mumbai, India). Acarbose (Glucobay, 50 mg) was obtained from Bayer India (Thane, India). Reagents and solvents used for extraction and silica-gel for column chromatography were procured from Merck (Mumbai, India). All of the other reagents were of analytical grade.

### 2.2. Plant material

Immaculate inflorescences of *Musa* sp. cv. Nanjangud rasa bale were collected from banana cultivating farms of Nanjangud, Karnataka, India. The specimen was identified by the Department of Horticulture, Government of Karnataka, Mysore, India. Flowers were separated from the inflorescence followed by discarding the spathe. The isolated flowers were cleaned, cut into small pieces and dried at 40 °C in an oven. This was powdered using a homogenizer and further stored at 4 °C until use.

### 2.3. Extraction

The coarse powder was subjected to hot extraction using ethanol in a Soxhlet apparatus. Extraction was performed twice with 95% ethanol (500 ml) and filtered. The resulting filtrate was concentrated under vacuum using rotary evaporator (Rotavapor R-200, Buchi, Switzerland) and the yield of ethanol extract was recorded. Subsequently, ethanol extract of banana flower (EF) was subjected to preliminary phytochemical screening to identify the phytoconstituents present based on standard protocols (Harbone, 1973). The total phenol content (TPC) for the EF was estimated as per Shuxia et al. (2013).

### 2.4. α-Amylase inhibition assay

The α-amylase (EC 3.2.1.1, categorised as type-VI B porcine pancreatic α-amylase) inhibition was assayed according to the procedure described by Worthington (1993) with slight modifications. The α-amylase activity was determined, using soluble starch (1%) as a substrate in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The enzyme (0.5 mg/ml) dissolved in 500 μl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) and 500 μl (0–1000 μg/ml) of EF (dissolved in DMSO) were pre-incubated for 10 min at 25 °C. The residual α-amylase activity was evaluated by adding 1% starch solution (500 μl) and incubation at 25 °C for 15 min. The reaction was stopped by adding 1000 μl of DNS reagent and kept in a boiling water bath (85 °C) for 5 min. The resulting mixture was diluted by 10 ml distilled water and absorbance (A) was measured at 540 nm in a spectrophotometer (Shimadzu UV-1800). Subsequently, the absorbance of blank (without enzyme) was subtracted from each test sample (EF) and the results were compared with the control (without analyte). Acarbose was used as a positive control. The α-amylase inhibitory activity was expressed in percent inhibition.

$$\text{Inhibition}\ (\%) = \left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}} \times 100$$

$IC_{50}$ values were determined from a curve relating the % inhibition of each sample to the concentration of sample.

### 2.5. Yeast α-glucosidase inhibition assay

The yeast α-glucosidase (EC 3.2.1.20, categorised as type-1 α-glucosidase) inhibition was assayed using the substrate *p*NPG according to the modified method described by Worawalai et al. (2012). In short, 700 μl phosphate buffer (50 mM, pH 6.8) and 100 μl EF with varying concentrations dissolved in dimethyl sulfoxide (DMSO) were mixed prior to the addition of 100 μl of yeast α-glucosidase (0.4 U/ml). One unit of α-glucosidase is defined as the amount of enzyme liberating 1 μmol of *p*-nitrophenol formed from *p*NPG per minute under the assay conditions specified. The mixture was pre-incubated for 10 min at 37 °C. After incubation, 100 μl of 0.5 mM *p*NPG solution in 50 mM phosphate buffer (pH 6.8) was added and the reaction was maintained at 37 °C for 20 min. The reaction was terminated by adding 250 μl 0.1 M $Na_2CO_3$. Enzyme activity was determined by measuring the absorbance of the liberated *p*-nitrophenol from *p*NPG at 405 nm using micro-plate reader (Spectramax 340, Molecular Devices, Sunnyvale, USA). The absorbance was compared with the control, containing buffer instead of test sample. Acarbose was used as a positive control. The results were expressed as percent α-glucosidase inhibition obtained using the formula given below:

$$\text{Inhibition}\ (\%) = \left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}} \times 100.$$

Each experiment was performed in triplicates, along with appropriate blanks. The concentration required to inhibit 50% of the α-

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