ISSN 2250-3153

Comparative evaluation of the antidiabetic and hypoglycaemic potentials of the parts *Musa paradisiaca* plant extracts

Jayarama Reddy[1] and Jeldi Hemachandran[2]

[1] Centre for Molecular and Computational Biology, St. Joseph's College, 36, Lalbagh Road, Bangalore, Karnataka, India. 560027.
[2]Department of Physiol¬ogy, Faculty of Medi¬cine, Jazan University, Jazan, Saudi Arabia

Abstract- A comparative evaluation of the antidiabetic and hypoglycaemic potential of the extracts from different plant parts of *Musa paradisiaca* (MP) was carried out *in vitro*. MP extracts were prepared sequentially with methanol, chloroform and petroleum ether. A comparison was made between the action of extracts from different parts. Different concentrations of each extract were made by using phosphate buffer and subjected to α-amylase and α-glucosidase inhibitory assays using maltose and 2-Chloro-4-Nitrophenyl-α-Maltotrioside as substrates respectively. Using this method, the percentage of α-amylase inhibitory activities of each extract were calculated. The enzymes were extracted from the sheep intestines. The absorbencies ware read at 595 nm and 405nm respectively using spectrophotometer. Methanol extracts were found to show higher inhibition. It was found that fruit and stem extract showed high rate of inhibition. At 100 µg/ml concentration of the stem extract a high of 83% inhibition of α-amylase was recorded. A high of 80% inhibition was recorded for α-glucosidase activity when 100 µg/ml of stem extrats were used. Leaf and flower extracts were also good in the inhibition of both the enzymes but the rate of inhibition was vey less and at 100 µg/ml concentration the rate of inhibition was 18%. The results conclusively provide proof that MP extracts are andidiabetic by being hypoglycaemic. The results strongly recommend the use of banana extracts in the control and the treatment of type-2 diabetic mellitus.

Index Terms- Musa paradisiaca, antidiabetic, diabetic mellitus, hypoglycaemic

I. INTRODUCTION

Diabetes is a serious metabolic disorder affecting the metabolism of not only carbohydrate but also protein and fat. A number of studies have shown that diabetes mellitus is associated with oxidative stress, leading to an increased production of reactive oxygen species. It is a metabolic disease characterized by hyperglycemia and disturbances in fat and protein metabolism that results from defects in both insulin secretion and/or insulin action (Teixeira et al, 2000). Type 2 is more associated with an adulthood and elderly people, which are mainly due to insulin resistance or abnormal insulin secretion. The exact causes of pancreatic failure and insulin resistance not clearly known, but they are associated with disease state, environmental impact and food habit. Diabetic patients are more susceptible to various type of infections such as skin diseases and carbuncles (Warjeet Singh, 2011). The prevalence of diabetes mellitus is increasing worldwide, associated with the aging population and the epidemiological transition (Yusuf, et al, 2001). It is estimated that by 2030, 366 million individuals will be affected by the disease worldwide, being 11.3 million living in Brazil where this would represent an increase of almost three times the number of individuals with the disease today (Wild et al, 2000). India accounts for the largest number of people (50.8 million) suffering from diabetes in the world, followed by China (43.2 million) and the United States (26.8 million). This is based the figures released by the International Diabetes Federation (IDF). The onset and progression of long-term complications in diabetes mellitus appear to be related to the degree of hyperglycemia and the overall metabolic control. It is highly beneficial to identify the traditional herbal drugs which might be useful in controlling diabetes and play a role preventing or mitigating cellular damages related to diabetes. It is advisable to use plant based inhibitors of α-amylase and α-glucosidase enzymes that participate in the breakdown of polysaccharides into disaccharides and monosaccharides in the intestine. Which in turn lead to hyperglycemia, one of the major complication of type-2 diabetic mellitus. It has been shown that activity of pancreatic α-amylase in the small intestine correlates to an increase in postprandial glucose levels, the control of which is therefore an important aspect in treatment of type-2 diabetes (Kim, et al, 2005 and Matsui et al, 1996). Inhibitors of pancreatic α-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the postprandial serum glucose levels. Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose, and a number of α-(l-6) and α-(1-4) oligoglucans. These are then acted on by alpha glucosidases and further degraded to glucose that on absorption enters the blood stream. Plantains are a good source of vitamin A (carotene), vitamin B complex (thiamin b, niacin riboflavin and B6) and vitamin C (ascorbic acid). *Musa paradisiaca* (Musaceae) is widely distributed throughout the tropical regions. It is a plantain and a tall herb with aerial pseudostem dying after flowering. Fruits are produced several clusters and are golden yellow colour on ripening. Plantains provide a better source of vitamin A than most other staples (Aurand, 1987). Plantain is employed in the folklore management of diseases such as diabetes, ulcer and wound healing due to its hypoglycaemic, anti-ulcerogenic and analgesic properties (Bischoff, 1994). Earlier report on the role of green plantain products in the control of hyperglycaemia had

been discussed (Oboh and Erema, 20 10, Willet et al., 2002). The stem juices of *Musa paradisiaca* have been used in treatment of diabetes mellitus as claimed in literature (Vedamurthy et al, 1997). Hence the present study was focused on investigating the antidiabetic potential of plantain. The study was also aimed at investigating the exact part the plant which is having high potential in controlling diabetes. These studies will provide a more in depth picture on the potential of this interesting traditional and widely consumed tropical fruit.

II. MATERIALS AND METHODS

Preparation of plant extract: Plantain variety of *Musa paradisiaca* (MP) which is a commercially cultivated was used in the present study. The plant materials (Fig.1) were procured from the local growers. Extraction was carried using flowers, leaves, pseudostems and unripe fruits. The samples were minced into fine paste using manual grinder sterilized with 77% ethanol. The extraction was done using soxhlet extraction method as described in AOAC (1980). Extraction set-up left for twelve hours after which the extract was recovered by using different solvents such as, chloroform, methanol and petroleum ether separately. The extracts were glass evaporated by rotary evaporator and a thick paste was obtained as homogenate It was used as the sample.

Fig-1: *Musa paradisiaca* flowering and pseudostem used in the experiment

Preparation of enzymes: Small intestines of sheep were procured from the local market freshly and stored in cold condition. The fresh intestine was cut into small pieces and macerated in chill conditions using phosphate buffer and then finely grinded into smooth paste (homogenate). The paste was centrifuged in refrigerated centrifuge at 10,000rpm. The clear solution obtained as the supernatant was used as the source of α-amylase and α-glucosidase.

Inhibition of α-amylase:

The fresh intestine was cut into small pieces and macerated in chill conditions using phosphate buffer and then finely grinded it into smooth homogenate. The homogenate prepared from small intestine of sheep was used as enzyme source. Final volume of supernatant was maintained to 20% (w/v). Here, 40 µl tissue homogenate was mixed with 80 µl of test standard plant extract and incubated for 15 min at 37°C. Then, 280 µl maltose (37 mM) was added as the substrate for the enzymatic activity. The reaction mixture was incubated for 30 min. ultimately, the enzymatic reaction was stopped by putting the tubes in boiling water for 10 min. Later, the tubes were centrifuged and glucose concentration was assessed in the supernatant by glucose oxidase/presence of peroxidase method based kit. Readings (OD) were taken in the spectrophotometer. Standard spectrophotometric assay method with slight modification (Kajaria, et al, 2011) was used. The absorbencies were read at 595 to record the rate of inhibition of α-amylase.

Inhibition of α-glucosidase:

Readings (OD) were taken in the spectrophotometer. Standard spectrophotometric assay method (Kumar et al, 2011) was used by slightly modifying the original procedure. The 90 µl of homogenate-supernatant was mixed with 180 µl of 40 mM phosphate buffer (pH 6.9), plant extract, and positive control of various concentrations and incubated at 37°C for 15 min. To this reaction mixture, 360 µl of substrate, 2-Chloro-4-Nitrophenyl-α-Maltotrioside (CNPG $_{3}$- 0.5 mg/ml) was added, mixed and for incubated 37°C for 10 min. Finally, absorbance was measured at 405 nm against blank in spectrophotometer. A control reaction was carried out without the test sample.The enzyme inhibitory rates of samples were calculated as follows:

$$\text{Inhibition}\% = \frac{\text{control absoption} - \text{sample absorption}}{\text{Control absorption} \times 100}$$

III. RESULTS

There are a number of plants that show antidiabetic property but in this study MP was used mainly because it is widely consumed as a vegetable in tropical regions. MP extracts were prepared sequentially with methanol, chloroform and petroleum ether. Out of these four extracts only the methanol extracts obtained through were found to give good rate of inhibition. Hence all the further studies and analyses were carried out using only the methanol extracts. Four different MP extracts were tested for the inhibition of the α-amylase (Table-1, Figs-2 and 4) and α-glucosidase (Table-2, Figs-3 and 5) enzymes. 10 µg/ml to 100 µg/ml range of MP extracts were only used for final analysis as after 100 µg/ml concentration there was decline in the inhibition rate. It was found that fruit and stem extract showed high rate of inhibition. At 100 µg/ml concentration of the stem extract a high of 83% inhibition of α-amylase was recorded. A high of 80% inhibition was recorded for α-glucosidase activity when 100 µg/ml of stem extrats were used. Leaf and flower extracts were also good in the inhibition of both the enzymes but the rate of inhibition was very less. At 100 µg/ml concentration the rate of inhibition was 18%. The results conclusively proved that MP extracts are andidiabetic by being hypoglycaemic.

	10 µg/ml	**20** µg/ml	**30** µg/ml	**40** µg/ml	**50** µg/ml	**60** µg/ml	**70** µg/ml	**80** µg/ml	**90** µg/ml	**100** µg/ml
STEM	**2**	**8**	**24**	**34**	**45**	**62**	**71**	**73**	**81**	**83**
FRUIT	**1**	**9**	**27**	**45**	**53**	**60**	**68**	**72**	**74**	**76**
LEAF	**0**	**0**	**05**	**09**	**15**	**16**	**16**	**17**	**18**	**18**
FLOWER	**0**	**0**	**0**	**0**	**7**	**9**	**12**	**12**	**13**	**14**

Table-1: Percentage of inhibition by MP extracts. Different concentrations of methanol extracts were used to inhibit the activity of α-amylase enzyme obtained from the small intestine of sheep.

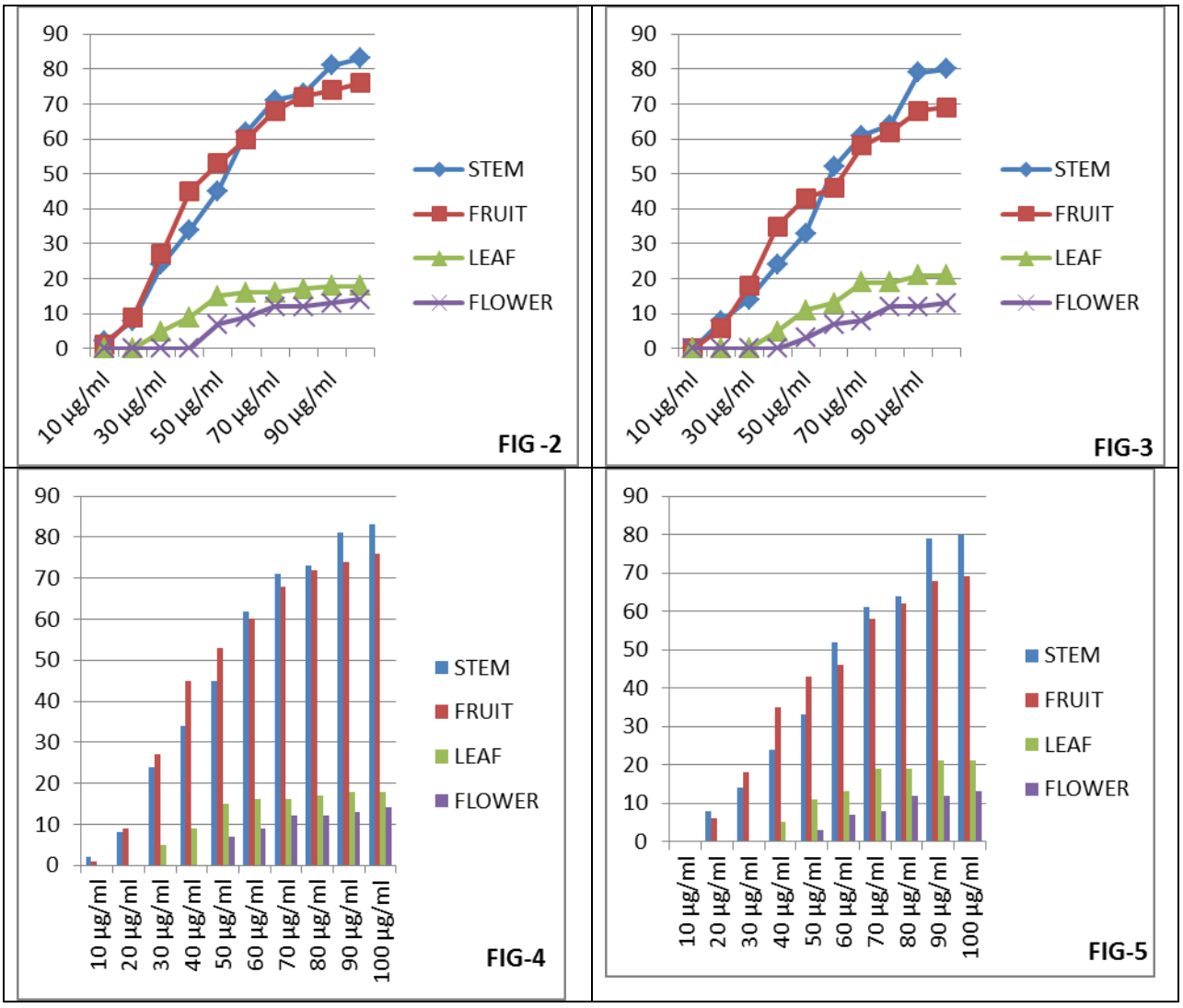
	10 µg/ml	**20** µg/ml	**30** µg/ml	**40** µg/ml	**50** µg/ml	**60** µg/ml	**70** µg/ml	**80** µg/ml	**90** µg/ml	**100** µg/ml
STEM	**0**	**8**	**14**	**24**	**33**	**52**	**61**	**64**	**79**	**80**
FRUIT	**0**	**6**	**18**	**35**	**43**	**46**	**58**	**62**	**68**	**69**
LEAF	**0**	**0**	**0**	**5**	**11**	**13**	**19**	**19**	**21**	**21**
FLOWER	**0**	**0**	**0**	**0**	**3**	**7**	**8**	**12**	**12**	**13**

Table-2: Percentage of inhibition by MP extracts. Different concentrations of methanol extracts were used to inhibit the activity of α-glucosidase enzyme obtained from the small intestine of sheep.

IV. DISCUSSION

Diabetes mellitus, a common heterogeneous metabolic syndrome, is prevalent throughout the world and has been projected to become one of the world's main disablers and killers within the next 25 years. Blood glucose level, urine sugar and body weight have been commonly measured to monitor the glycemic control mechanism. Several authors reported flavonoids, sterols, alkaloids and polyphenols as bioactive antidiabetic principles (Ojewole and Adewunmi, 2003). The result of phytochemical screening on the stem juice of *M. paradisiaca* reveals that the extract contained various pharmacologically active compounds such as tannins and alkaloids. There are several studies reported positive correlation between phenolic content of plants and their respective antidiabetic activities (Anam et al, 2009 and Momo et al, 2009). Currently available oral therapies for treatment of diabetes mellitus are sulfonylureas, biguanides, α-glucosidase inhibitors, and glinides, which can be used alone or combined with other drugs to achieve better effect. Many of these oral antidiabetic agents have a number of serious adverse effects, thus, the management of diabetes without any side effects is still a challenge (Sharma et al, 2009). Plants like *Musa paradisiaca* containing natural antioxidants such as tannins, flavonoids, vitamin C and E can preserve β-cell function and prevent diabetes induced ROS formation. Polyphenols, which are classified into many groups such as flavonoids, tannins and stilbenes, have been known as health-beneficial properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action and antidiabetogenic potentiality. Natural anti-diabetic drugs from medicinal plants, are the other available therapy for the treatment of diabetse mellitus due to their well-known biological activity. Substances extracted from fruiting bodies, cultured mycelia, and culture media have exhibited promising *in vitro* and *in vivo* biological activity including anti-diabetes (Lu et al, 2010). The ethnobotanical information reports a huge number of plants that may possess anti-diabetic potential, of which *Momordica charantia* (*M. charantia*), *Pterocarpus marsupium* (*P. marsupium*), and *Trigonella foenum* (*T. foenum*) greacum have been reported to be beneficial for treatment of type 2 diabetes. Herbal treatments for diabetes have been used in patients with insulin dependent and non-insulin dependent diabetes, diabetic retinopathy, diabetic neuropathy etc. The families of plants with the most potent hypoglycaemic effects include Leguminoseae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Euphorbiaceae and Araliaceae (Bnouham et al, 2006). This indicates that there are many plants that can be used as drug to control diabetes. Moreover it is good to use the plant product

than the synthetic drugs which will have severe side effects. There are a number of plants that show antidiabetic property but in this study MP was used mainly because it is widely consumed as a vegetable in tropical regions. In the current investigation we found that some parts of plantain, like fruit and stem were extremely good in inhibiting the enzymes. They showed 80% inhibition at certain concentrations. Thus these finding support the fact that *Musa paradisiacal* is a source of potential drug against diabetes. This is the reason probably in India traditionally people have been eating food on plantain leaf. Our results show that the all most all part of the plantain were successful in inhibiting the two most important intestinal enzymes that regulate blood sugar level.



Figs. 2,3,4,5: Percentage of inhibition by MP extracts. Different concentrations of methanol extracts were used to inhibit the activity of enzymes obtained from the small intestine of sheep. Fig-2 and 4 indicate the inhibition of α-amylase and Fig-3 and 5 indicate the inhibition of α Glucosidase.

REFERENCES

[1] Anam K, Widharna RM, Kusrini D 2009: α-glucosidase inhibitor activity of Terminalia species. Int J Pharmacol, 5:277-280.

[2] Aurand WL. 1987. Food Composition and Analysis of Food , Von Nostrand Reinhold, New York, pp. 135 -138.

[3] Bischoff H. 1994. Pharmacology of alpha - glucosidase inhibition. Eur J Clin Invest., 24 :3 - 10.

[4] Bnouham, Mohamed, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. 2006: Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990–2000) Int J Diabetes Metab.;14:1–25.

[5] Kajaria D, Tripathi JS, Tiwari SK. 2011. Comparative evaluation of phenol and flavonoid content of polyherbal drugs. Pharmacol Online;3:1365-73.

[6] Kim Y, Jeong Y, Wang M, Lee W, Rhee H. 2005. Inhibitory effect of pine extract on α-glucosidase activity and postprandial hyperglycemia. Nutrition;21:756-61.

[7] Lu Y, Lu Z, Lv F, Wang Y, and Bie Y, 2010. Hypoglycaemic effect of comatin, an antidiabetic substance separated from Coprinus comatus broth, on alloxan-induced-diabetic rats. Food Chem.;121:39–43.

[8] Matsui T, Yoshimoto C, Osajima K, Oki T, Osajima Y. 1996. In vitro survey of α-glucosidase inhibitory food components. Bio Biotechnol Biochem;60:2019-22.

[9] Momo CEN, Fuh Ngwa A, Dongmo GIF, Oben JE. 2009. Antioxidant properties and α amylase inhibition of Terminalia superba Albizia sp., Cola

nitida, Cola odorata and Harungana madagascarensis used in the management of diabetes in Cameroon. J Health Sci, 55:732-738.

[10] Oboh HA and Erema VG. 2010. Glycemic indices of processed unripe plantain (Musa paradisiaca) meals. Afr J Food Sci., 4 :514 - 521.

[11] Ojewole JA, Adewunmi CO. 2003. Hypoglycemic effect of methanolic extract of Musa paradisiaca (Musaceae) green fruits in normal and diabetic mice. Methods Find Exp Clin Pharmacol. Jul-Aug;25(6):453-6.

[12] Sharma S, Khajja BS, Jain K, Jain GC. 2009. Evaluation of hypoglycemic and anti hyperglycemic potential of Tridax procumbens (Linn.) BMC Complement Altern Med.;9:48.

[13] Teixeira CC, Rava CA, Da-Silva PM, Melchior R, Argenta R, Anselmi F, Almeida CR, Fuchs FD: 2000. Absence of antihyperglycemic effect of jambolan in experimental and clinical models. J Ethnopharmacol, 71:343-347.

[14] Vedavathy S, Mrudala V. and Sudhakar. 1997. A. Tribal Medicine of Chittoor District.: pp 56-58.

[15] Warjeet Singh L. 2011. Traditional medicinal plants of Manipur as anti-diabetics. J Med Plants Res.;5:677–687.

[16] Wild S, Roglic G, and Green A, 2004: Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030. Diabetes Care, 27:1047-1053.

[17] Willett W, Manson J and Liu S. 2002. Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutrition , 76 , 274S - 80S.

[18] Yusuf S, Reddy S, and Ôunpuu S. 2001: Global burden of cardiovascular diseases: Part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. Circulation, 104:2746-2753.

AUTHORS

First Author – Jayarama Reddy, Centre for Molecular and Computational Biology, St. Joseph's College, 36, Lalbagh Road, Bangalore, Karnataka, India. 560027., Email: drjayaramreddy@gmail.com

Second Author – Jeldi Hemachandran, Department of Physiol¬ogy, Faculty of Medi¬cine, Jazan University, Jazan, Saudi Arabia