A COMPARATIVE IN VITRO ANTIHYPERGLYCEMIC ACTIVITIES OF EXTRACTS OF MUSA PARADISIACA STEM

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ABSTRACT

Alpha- amylase and alpha glucosidase enzymes present in the small intestines are vital in the digestion of carbohydrates. Glucose absorption is delayed by inhibiting the two enzymes thereby preventing a sharp increase of blood glucose. This has been a different therapeutic approach in the management of patients with diabetes. In this study, acetone and ethanol extracts from stem of plantain (Musa paradisiaca) were estimated for their in vitro antihyperglycemic activity and their total phenolic content using standard protocols. The study was informed following lead by traditional herbal healers and paucity of information on the antidiabetic property of this part of the plantain in literature. Significantly ($p < 0.05$) higher values were noticed in the phenolic composition of acetone extract when compared with ethanol extract at the least concentration of extracts used (0.25 mg/mL). Also, lower concentration of phenolic content was detected in the ethanol extract relative to the higher concentration of acetone extract used (1.00 mg/mL). Both extracts (ethanol and acetone) significantly inhibited the carbohydrate-hydrolyzing enzymes activities in a manner that was dose dependent. Comparatively, acetone extract gave better inhibitory activity in relation to the extract of ethanol and the total phenolic composition was also higher in this extract (acetone). Thus, in this study, extracts of M. paradisiaca showed high inhibitory activity of alpha glucosidase and moderate alpha amylase inhibitory activity hence suggesting to be potential antihyperglycemic agents.

Keywords: Antihyperglycemic activity, Alpha- amylase , Musa paradisiaca, and Alpha glucosidase enzymes

INTRODUCTION

Musa paradisiaca (family-Musaceae) is a medicinal plant with medicinal properties. Parts of it like the flowers, leaves, stem and roots have medicinal values. Juice of the leaf for instance is used in wounds treatment and cuts. Also, the leaves are used for arborrification while its sap is used for dysentery, diarrhea and hysteria treatment (Salawu et al., 2010) M. paradisiaca, a tropical plant is known also as plantain. The plant is native to India and it is a widely cultivated staple crop found in the tropics of sub-Saharan Africa. It is valuable because of its carbohydrate content. Also, the fruit can be consumed when ripe or unripe (Abenkora et al., 1997). M. paradisiaca has been said to have some pharmacological activities like antilithiatic, antibacterial, antioxidant, antiulcer, antidiabetic, antidiarrhoeal, hepatoprotective, hypocholesterolaemic, anti-snake-venom, antimenorrhagic and antifungal activities (Lavanya et al., 2016). Extract (chloroform) of Musa sapientum flowers given orally had been described to cause a substantial reduction in blood glucose level and glycosylated haemoglobin (Pari and Uma- Maheswari, 1999). Diabetic mellitus (DM) is a dangerous metabolic disorder associated with macro and micro vascular problems leading to morbidity and mortality (George, 2000).

Abnormalities of glucose homeostasis is usually linked to DM and several experimental observations have indicated that DM associated hyperglycemia contribute to oxidative stress (Fagbohun and Odufunwa, 2010). Oral hypoglycemic agents like biguanides, sulphonylureas are used for DM treatment but severe side effects have been associated with their

usage (Wadkar et al., 2008).Thus necessitating the continuous search for new and better antidiabetic agents (Fagbohun and Odufunwa, 2010).

One therapeutic strategy for treating type 2 DM is to cause a reduction of the levels of post-prandial glucose. This could be achieve by inhibiting carbohydrates-hydrolysing enzymes like α-glucosidase and α-amylase thereby causing delay in the absorption of glucose (Laar et al., 2008; Inzucchi , 2002).α-Amylase (E.C.3.2.1.1) is a calcium metalloenzyme that catalyses the hydrolysis of internal α-1, 4-glycosidic linkages in starch to produce glucose and maltose (Sundarram et al., 2014). While α-glucosidase (EC 3.2.1.20) is a glucosidase that acts on 1,4-α bonds thereby breaking down starch ultimately into glucose. Though the substrate specificity of α-glucosidase differs depending on the source (Kimura et al., 2004) .Earlier studies have revealed that the amount of polyphenols in plants and their antioxidant activities is dependent on biological factors, edaphic and environmental conditions (Bano et al., 2003). Solvent type (polarity), extent of polymerization and interaction are said to be factors that influence the phenolic compounds solubility (Djeridane et al., 2006). The total phenol composition of acetone, ethanol and petroleum ether extracts of banana leaf had been estimated (Meenashree et al., 2014). Also evaluated and reported are the antioxidant properties, phytochemicals, proximate and vitamins composition of the leaves of M. paradisiaca using ethanol extract (Enechi et al., 2014); effects of the stem extrude of M. paradisiaca on haematological indices in rats (Onyenekwe et al., 2013); the inhibitory activity of α-glucosidase fractions from ethanol extracts of banana flowers (Sheng et al., 2014). Still, there is paucity of information as regards the antihyperglycemic properties of parts of the plant particularly the stem.

Therefore, following claim from herbal medicine practitioner about the efficacy of plantain stem in the treatment of diabetes, this study was undertaken to compare the in vitro antihyperglycemic properties of two different solvents extracts of the stem of M. paradisiaca.

MATERIAL AND METHODS

Collection and Treatment of Plant Materia

The stems of plantain (M. paradisiaca) w collected from site III of the Delta St University, Abraka and were authenticated in herbarium unit of the Botany Department of De State University, Abraka, Nigeria. The method Arokiyaraj et al. (2009) as cited by Okoro et a (2014) was used with slight modification treatment of the stems. In brief, outer layers of stems were removed until the innermost layer w obtained and cut to pieces. They were washed a air-dried at room temperature and pulveriz using pestle and mortar. Thereafter, the air-dri samples were sequentially extracted with solve of increasing polarity (acetone and ethanol).

In vitro α-amylase inhibitory assay

This assay was done by the modified procedure McCue et al., (20004) as cited by Okoro et a (2015). Calculation of the α-amylase inhibit activity was done using the equation:

$$\% \text{ Inhibition} = \{(Ac - Ae)/Ac\}\ 100$$

Where: Ac= absorbance of control; Ae absorbance of sample.

In vitro α-glucosidase inhibitory assay

The inhibitory effect of the extracts on glucosidase activity was evaluated following protocol described by Kwon et al.(2006) a adopted by Okoro et al., (2015). The results expressed as percentage as in the equation belo

$$\% \text{ Inhibition} = \{(Ac - Ae)/Ac\}\ 100.$$

Where: Ac= absorbance of control; Ae absorbance of extract.

Determination of Total Phenolic Content

The Folin–Ciocalteau reagent method described by Singleton et al. (1999) was used the determination of the extract's phen content. The reaction was started by mixing 1 of 0.3 mg/mL of methanolic solution of extrac mL of Folin-Ciocalteu,s reagent, 9 mL of dist water and 10 mL of 7% sodium carbonate and absorbance was taken at 765 nm after 90 min incubation at room temperature. The total phe content was afterwards calculated as gallic equivalent.

Statistical Analysis

All assays were performed in triplicates and expressed as mean ± standard deviation (SD). Statistical analysis of all the experimental data was performed using a one-way analysis of variance (ANOVA) followed by Sidak's test in Graphpad Prism, version 6.0 0 (Graph Pad Software, San Diego, CA, USA). The confidence limits used for this study was based on 95% ($p \leq 0.05$).

Shown in Figure 2 are the results for a-Amylase inhibitory activity of M. paradisiaca stem. There is significant increase ($p<0.05$) of a-Amylase inhibitory activity displayed by both extracts in a dose dependent fashion. Thus, a trend similar to that of the α-Glucosidase inhibitory activity is also seen in this enzyme's (α-Amylase) inhibitory activity by the two extracts. The least and highest percentage inhibitory activities of 21.20±1.98 and 81.85±0.49 by ethanol extract and 28.50±0.99 and 88.55±0.64 by acetone extract are observed at the lowest (0.02 mg/mL) and highest (1.00 mg/mL)

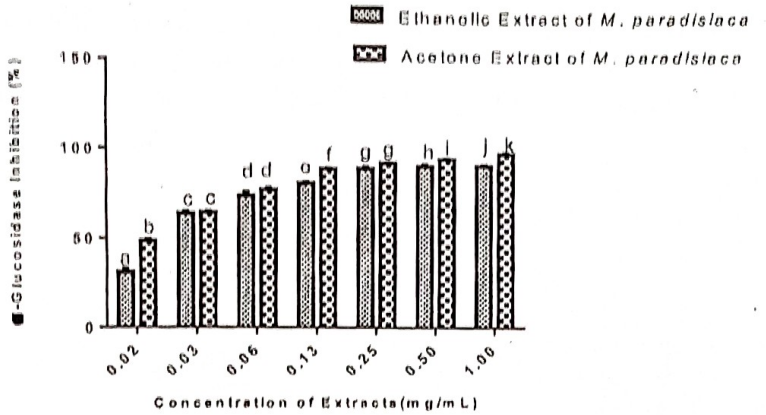

Figure 1: α-Glucosidase inhibitory activity of *M. paradisiaca* stem
*Values represent mean ±standard deviation, n = 3
*Bars with different letters differ significantly ($p<0.05$)

RESULTS AND DISCUSSION

Results for the α-Glucosidase inhibitory activity of M. paradisiaca stem are presented in Figure 1. There is significant ($p<0.05$) increase in the α-Glucosidase inhibitory activities exhibited by the two extracts of M. paradisiaca stem in a dose dependent manner with the lowest percentage inhibition of 31.20±1.98 and 48.50±0.99 shown by the ethanol and acetone extracts respectively at the least concentration of 0.02 mg/mL while the greatest inhibitory activities by the extracts (91.85±0.49 by ethanol extract and 98.55±0.64 by acetone extract) are seen at the highest concentration of 1.00 mg/mL. Significantly ($p<0.05$) higher percentage of inhibitions are observe with the acetone extract at concentrations of 0.02, 0.13, 0.50 and 1.00 mg/mL when compared with the ethanol extract.

concentrations respectively. Also, significantly ($p<0.05$) higher percentage inhibition is shown by the acetone extract of M. paradisiaca relative to the ethanol extract at all concentrations except at 0.03 and 0.06 mg/mL.

Results for the total phenolic contents of M. paradisiaca stem extracts are shown in Figure 3. Significant ($p<0.05$) difference is observed when the acetone extract is compared with ethanol extract at 0.25 mg/mL (least concentration) while no difference is observed (significantly) between the two extracts at the concentration of 0.50 mg/mL.

However, a significantly higher value is observed with the acetone extract relative to the ethanol extract at 1.00 mg/mL concentration. Thus the extracts exhibit increase in total phenolic content in a manner that is concentration dependent.

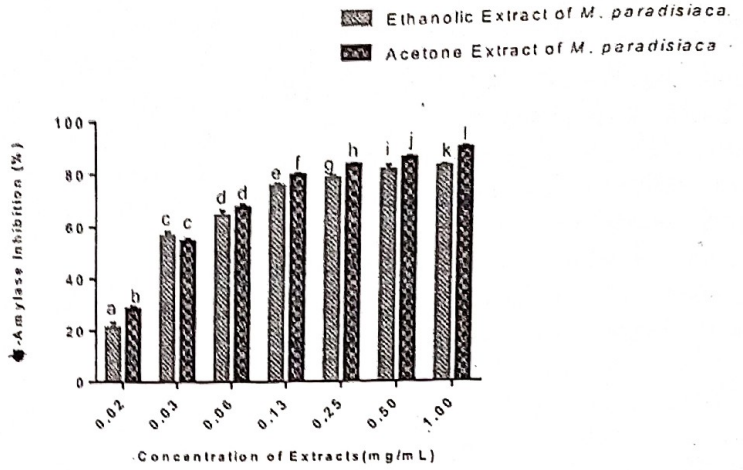

Figure 2: α-Amylase inhibitory activity of *M. paradisiaca* stem
*Values represent mean ±standard deviation, n = 3
*Bars with different letters differ significantly ($p < 0.05$)

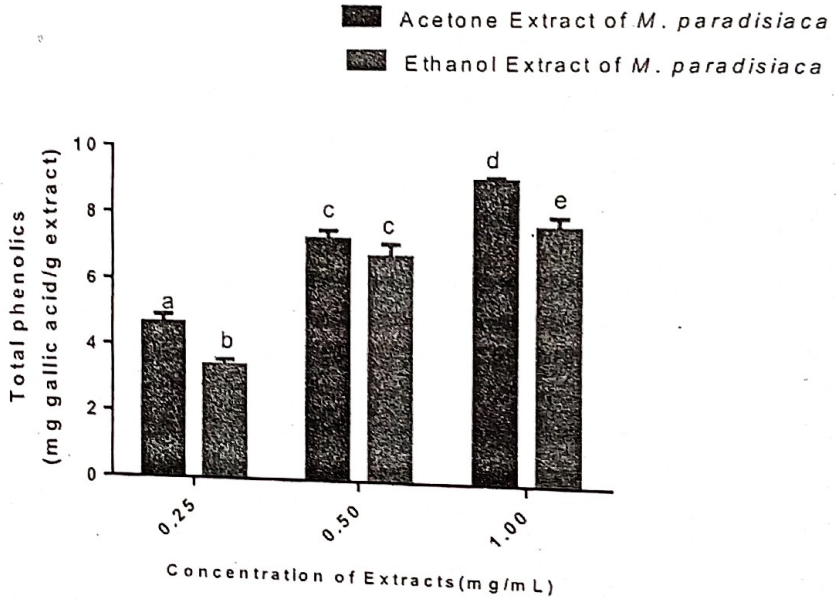

Figure 3: Total phenolic content of two extracts of *M. paradisiaca* stem
*Values represent mean ±standard deviation, n = 3
*Bars with different letters differ significantly ($p < 0.05$)

Diabetic mellitus (DM) is a disorder arising as a result of abnormal metabolism of carbohydrate and it is stimulated by factors, namely, insulin resistance and/or insulin deficiency. DM prevails worldwide at a startling rate globally. This metabolic derangement is associated with various complications affecting all vital organs of the body (Wadkar et al., 2008; Ashok et al., 2011). The increasing percentage of the aging population, sedentary lifestyle, consumption of diet rich in calorie, and obesity have contribu worldwide to the incredible increase in incidences of diabetics (George, 2000).

We have determined the in vitro antidiabe property and total phenolic content of two extra (acetone and ethanol) of the stem of M. paradisiaca in this study. Significantly hig values of total phenolic content were observe the acetone extract when compared with eth

extract at the least concentration (0.25 mg/mL) of extracts used.

Also, lower concentration of the phenolic content was noticed in the ethanol extract relative to the acetone extract at 1.00 mg/mL (highest concentration of extract used). Consequently, the two extracts displayed increase in total phenolic content in a concentration dependent manner with the acetone extract showing higher overall values amongst the extracts. This observation agrees with the report of Meenashree et al. (2014) in which they reported that acetone gave better extraction of phenol compounds than ethanol. Similarly, Alothman et al. (2009) stated that polar solvents are employed for hydrophilic phenols and the least polar solvents are largely considered to be better choice solvents for extracting lipophilic phenols, except with the use of very high pressure. Thus, the phenol content of an extract is greatly influenced by the solvent used and its polarity.

In previous studies, ethanol, acetone, methanol, propanol, dimethylformamide and ethyl acetate have been used for extraction of phenolic content from banana fruits (Tan et al., 2012). Likewise, the recovery of polyphenols from plant has been said to be influenced by factors like the type of solvent, solubility, extent of polymerization of phenols, insoluble complexes formation and the interaction between phenols and other plant constituents (Galvez et al., 2005). Also, according to Fratianni et al. (2007), the phenol content of a said plant depends on intrinsic (extracting solvent, genetic) and extrinsic (environmental, development stage and handling) factors. Besides, the content of polyphenols as well as biological activity of plant is also influenced by its physiological stage. Retardation of the cleavage of glucose from disaccharide by inhibition of a-glucosidase present in the digestive organs is one therapeutic method being used to treat postprandial hyperglycemia (Tewari et al., 2003). Results of this study on the in vitro antidiabetic property of the extracts of M. paradisiaca revealed that both extracts of the plant indicated a potent inhibition of a-amylase and a-glucosidase enzymes. The acetone and ethanol extracts significantly hindered α-glucosidase and α-amylase activities in a dose dependent manner. The least and highest percent inhibitory activities by ethanol and acetone extract were observed at the lowest (0.02 mg/mL) and highest (1.00 mg/mL) concentrations respectively. Also, significantly higher inhibition (percent) was shown by the acetone extract of M. paradisiaca relative to the ethanol extract at most concentrations of extracts. The findings from this study are in agreement with some previous reports where saliva a-amylase activity was inhibited by plant phytochemicals from black and green tea (Zhang and Kashket, 1998), the report of Nickavar and Yousefian (2009) on the inhibitory effects of Allium spp. on α-amylase activity and the report of Kwon et al., (2006) that aqueous extracts of the clonal herbs of Lamiaceae species inhibited a-glucosidase activity in vitro. Similarly, Sheng et al. (2014) has reported the presence of five a-glucosidase inhibitors (vanillic acid, ß-sitosterol, daucosterol, ferulic acid, and 9-(4-hydroxyphenyl)-2-methoxyphenalen-1-one from the ethanol extract and fractions of banana flowers.

Previously, the inhibitory activities of medicinal plant extracts on the α-amylase and α-glucosidase activities has been recorded in a number of reports (Ashok Kumar et al., 2011; Shodehinde and Oboh, 2012; Nair et al., 2013; Marikkar et al., 2016). The amount of total phenolic and flavonoids in plant has been reported to influence their a-amylase inhibitory activity. Studies have also shown that phenolic phytochemicals exhibit anti-diabetic effect by inhibiting carbohydrate-hydrolyzing enzymes. Furthermore, many phenolic compounds such as flavonoids have been stated as potential antidiabetic agents largely because they exert potent inhibitory activity against a-amylase and could possibly be useful as part of a dietary strategy for prevention of diabetes mellitus (Cazarolli et al., 2008). Several findings have also indicated that phenolic synergies may have a role in mediating amylase inhibition and thus have the potential to add to type 2 diabetes management (Kwon et al., 2006).

CONCLUSION

Findings from this study on the in vitro antidiabetic property of the extracts of M. paradisiaca show that both extracts used displayed a potent inhibition of α-amylase and α-glucosidase enzymes. The ethanol and acetone extracts

inhibited significantly the carbohydrate-hydrolyzing enzymes activities, in a dose related manner. Extract of M. paradisiaca in acetone is found more effective than that of ethanol. In this study, the two extracts of M. paradisiaca showed high alpha glucosidase inhibitory activity and moderate alpha amylase inhibitory activity.

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