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Full Length Research Paper

Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase

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The mammalian and yeast α-glucosidase inhibitory activities of *Senna italica, Tinospora fragosa, Manilkara mochisia, Xanthocercis zambesiaca, Ozoroa cf. albicans, Peltophorum africanum, Cassia abbreviata* were investigated using *p*-nitrophenyl glucopyranoside as a substrate. All the plant species tested had low inhibitory activity against rat intestinal α-glucosidase. The highest concentration of *C. abbreviata* resulted in no observable loss of rat intestinal α-glucosidase activity. At 2.5 mg/ml, *X. zambesiaca* managed only 25% inhibition of rat intestinal glucosidase activity. *M. mochisia* led to about 60% loss of activity at 5 mg/ml. On the other hand, all plant species tested had good inhibitory activity against Baker's yeast α-glucosidase. These findings suggest that a chemical substance that inhibits yeast α-glucosidase activity will not necessarily inhibit mammalian α-glucosidase. The validatation of the antidiabetic activity of medicinal plant extracts, with the focus on the inhibition of carbohydrate-digesting enzymes, will require, in addition, investigation of mammalian enzymes. The antioxidant activity of these plant species was also investigated, with *C. abbreviata* and ascorbic acid as the positive controls. The IC_{50} values were estimated. *P. africanum* had good antioxidant activity with an IC_{50} value of 0.030 mg/ml, similar to the IC_{50} value resulting from the positive control, ascorbic acid.

Key words: Diabetes mellitus, α-glucosidase activity, antioxidant activity, plant extracts.

INTRODUCTION

Inhibition of polysaccharide-cleaving enzymes has been shown to control blood glucose concentration in patients afflicted with type II diabetes mellitus (Davis and Granner, 1996). Drugs such as acarbose, miglitol and voglibose reduce post-prandial blood glucose levels through the inhibition of carbohydrate-digesting enzymes of the gastrointestinal tract and the retardation of the rate of absorption of monosaccharides (Tiwari and Rao, 2002). Extracts of various plant species have been investigated for potential to inhibit α-glucosidase and α-amylase activities. Several reports have focused on yeast α-glucosidase (Subramanian et al., 2008; Lee and Lee, 2001; Andrade-Cetto et al., 2008), rat intestinal α-glucosidase (Bhandari et al., 2008) or both (Babu et al., 2004). Some plants contain α-amylase and α-glucosidase inhibitors (Ortiz-Andrade et al., 2007; Shirwaikar et al., 2005), and it is hardly surprising that researchers focus on this natural source for potential antidiabetic drugs.

In our earlier study, acetone extract of the stem bark of *Cassia abbreviata* was found to non-competitively inhibit yeast α-glucosidase (Shai et al., 2010). This finding alone was not enough to declare the beneficial effects of this plant species in lowering post-prandial blood glucose

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concentration. It is extremely essential to also study the ability of this plant extract to inhibit mammalian α-glucosidase, and furthermore, to study its effects in an *in vivo* model. Interestingly, methanol extract of dried flowers of *C. auriculata* exert strong antihyperglycemic effects in rats (Abesundara et al., 2004).

A plant species that is able to inhibit mammalian glucosidase and amylase to the same extent as commercial α-glucosidase inhibitors, with less side effects and low toxicity, would be a good candidate for production of good phyto-formulations in the fight against diabetes mellitus (Gallaher and Schneeman, 1986; Heacock et al., 2005).

The aim of this study was to investigate the *in vitro* inhibitory effects of *C. abbreviata* acetone extract against both rat intestinal and yeast α-glucosidase. In addition, we also investigated the α-glucosidase inhibitory activity and antioxidant activity of several randomly-selected plant species used in ethnomedical practices at Mashishimale village, Phalaborwa, South Africa. The selected plants were *Ozoroa cf. albicans, Xanthocercis zambesiaca, Peltophorum africanum,* and *Manilkara mochisia.* We have previously reported yeast α-glucosidase inhibitory activity of *Senna italica* and *Tinospora fragosa* crude extracts (Shai et al., 2010).*O. cf. albicans* is used by rural people of Mashishimale village to treat a variety of diseases.

The stem bark is soaked in water and the resulting extract administered to patients for stomach complaints. It is believed that stomach problems are associated with "*sejeso*" (organism introduced into the stomach through witchcraft) or "*makgoma*", a condition perceived to be caused by uncleanliness resulting from death of relative or immoral sexual practices. The bark of this plant species is used to treat these conditions which are generally perceived to manifest in the form of stomachache. Interestingly, the human immunodeficiency virus and associated onset of the acquired immunodeficiency syndrome are believed, especially in the older generations, to be in the "*makgoma*" group.

X. zambesiaca is widely used at Mashishimale village to treat stomach complaints and "*nyoko*", a disease perceived to be characterised by high concentration of acids in the body that allegedly impairs the functioning of the gall bladder. The root or stem bark is boiled in water and the juice is drunk, resulting in diarrhoea that is thought to reduce acid concentration in the body. Furthermore, Nojima et al. (1998) reported that, aqueous methanol extracts of *X. zambesiaca*, at a concentration of 50 mg/kg, reduced blood glucose concentration in streptozotocin-induced diabetic mice. *P. africanum* is widely used by the villagers of Ga-Mashishimale to treat stomach complaints.

The leaves are crushed, immersed in warm water and the extract is administered to patients. Bizimenyera et al. (2005) reported antioxidant properties of this plant species.

MATERIALS AND METHODS

Plant collection and extraction

Stem bark *C. abbreviata, S. italica, Ozoroa cf. albicans* (PRU 115079), *X. zambesiaca* (PRU 115078), *M. mochisia* (PRU 115080), as well as the leaves *P. africanum* (leaves) (PRU 113816) were collected from Mashishimale village in Limpopo, South Africa in February 2010. The grid references were recorded. Herbarium specimen were deposited and identified at H.G.W.J. Schweickerdt Herbarium at the University of Pretoria, South Africa. The plant materials were ground to powder using iron pestle and mortar. Two grams of powdered plant material was extracted with 10 ml of acetone for at least 4 h at room temperature. The resulting extract was filtered through Whatman no. 1 filter paper, and filtrate evaporated to dryness under a stream of air. The extract was dissolved in dimethylsulphoxide to a concentration of 20 mg/ml.

The determination of mammalian α-glucosidase activity

The inhibitory activity of plant extracts against rat intestinal α-glucosidase was determined by the method of Yamaki and Mori (2006). Two-fold serial dilutions of the extracts were performed on microtiter plates in 100 µl 0.5 M phosphate buffer, pH 6.9. Then, 100 µl of 0.90 mg/ml *p*-nitrophenyl-α-D-glucopyranoside (pNPG) was added into each well. Rat intestinal acetone powder (30 mg/ml powder in buffer, pH 6.9) was sonicated for 3 min. The suspension was centrifuged at 4 000 rpm for 10 min to remove particulate matter. The resulting supernatant was used as α-glucosidase. The reaction was initiated by addition of 50 µl of the enzyme to the reaction mixture in microtiter plates. The plates were incubated for 60 min at 37°C. The amount of p-nitrophenol (pNP) released was estimated by reading the absorbance at 405 nm. Absorbance of plant extract dilutions alone was subtracted from the absorbance of reaction mixture in order to eliminate background. The inhibitory activity was determined as a percentage of the negative controls as follows:

% Glucosidase inhibition = 100% – % glucosidase activity of test as percentage of control

$$\% \text{ glucosidase activity of test} = \frac{\text{Corrected } A_{405} \text{ of test}}{A_{405} \text{ of control}} \times 100$$

Yeast α-glucosidase activity determination

The acetone extracts (dissolved in DMSO to 20 mg/ml) of plants were serially diluted in 96-well microtitre plates, resulting in concentration from 0.08 to 5 mg/ml. To the dilutions of plant extracts, 100 µl of pNPG (0.9 mg/ml) was added, including the controls (blank, negative and positive controls). The reaction was started by addition of 20 µl of yeast α-glucosidase (0.5 mg/ml, 0.04 U). The reaction mixture was incubated for 5 min at room temperature (±25°C). The amount of p-nitrophenol released was determined by measuring absorbance at 405 nm. The calculations were performed as described for the mammalian version of the enzyme.

Antioxidant activity

The antioxidant activity of plant extracts was based on the extent to which they reduce 2,2-diphenylpicryl hydrazyl (DPPH). Plant extracts were two-fold serially diluted in DMSO in microtitre plates to a total volume of 100 µl. Then, 100 µl of 0.025% DPPH was added into each well except the blank and colour controls. Radical scavenging activity was determined by measuring absorbance at

Table 1. The IC50 (mg/ml) values of various plant species against yeast α-glucosidase activity.

Plant species	IC_{50} (mg/ml)
X. zambesiaca	ND
P. africanum	0.04
M. mochisia	0.05
O. cf. albicans	0.05
C. abbreviata	0.01
Acarbose	1.50

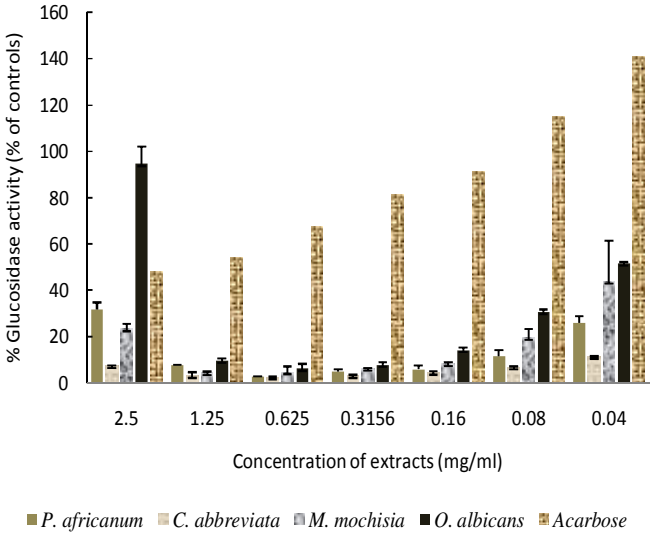


Figure 1. Effects o acetone extracts of selected plant species against baker's yeast α-glucosidase, using para-nitrophenylglucopyranoside as a substrate. We have presented the inhibitory activities of *T. fragosa* and *S.italica* previously (Shai et al., 2010)

492 nm using a microplate reader. *C. abbreviata* was used as a comparative positive control as its antioxidant activity was reported previously (Shai et al., 2010). Furthermore a well known antioxidant, ascorbic acid, was used as the main positive control. Negative controls were assumed to have 100% oxidized DPPH. Antioxidant activity was expressed as a percentage of the control as follows:

% Radical scavenging activity = 100% - (A_{492} of Test x 100%) / A_{492} of control

The A_{492} of test was corrected by subtracting A_{492} of plant extract dilution only.

RESULTS AND DISCUSSION

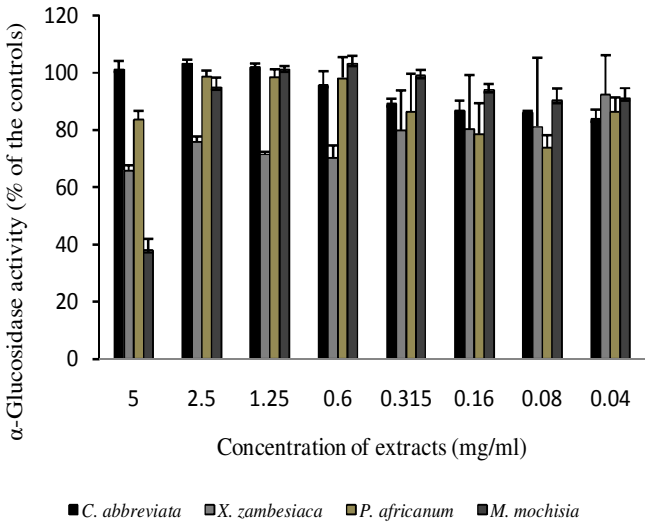
α-Glucosidase inhibition

All the plants tested have considerably high yeast α-glucosidase inhibitory activity. The IC_{50} values indicated that all the extracts tested were more active than acarbose, the positive control. The IC_{50} values resulting from plant extracts ranged from 0.01 to 0.05 mg/ml. The IC_{50} value resulting from acarbose, was about 1.5 mg/ml. We have previously reported an IC_{50} value of 17 mg/ml for acarbose against yeast α-glucosidase at 0.6 U in reaction mixture (Shai et al., 2010). This disparity is as a result of different concentrations of the enzymes (0.04 U in this study). *C. abbreviata* exerted the most inhibition on the activity of yeast α-glucosidase, with an IC_{50} value of 0.01 mg/ml (Table 1 and Figure 1).

However, it was observed that acetone extracts of the plant species used in this study slightly inhibited the catalytic activity of the mammalian version of α-glucosidase (Figure 2). At 5 mg/ml, *M. mochisia* inhibited about 60% activity of mammalian α-glucosidase activity (Table 2 and Figure 2). The estimated IC_{50} value for *M. mochisia* against mammalian α-glucosidase activity was 4.5 mg/ml, almost a 100-fold higher than its IC_{50} value against yeast α-glucosidase. Acarbose resulted in an estimated IC_{50} value of 0.4 mg/ml. It would appear that inhibition of yeast α-glucosidase does not translate into comparable inhibition of the mammalian version of the enzyme. This is supported by the finding that plant extracts that exerted inhibition on yeast enzyme failed to inhibit mammalian enzyme from rat intestinal acetone powders. These findings are in agreement with a study conducted by Babu et al. (2004), who observed that crude methanol extracts of several plants exerted superior inhibition on yeast α-glucosidase than the mammalian equivalent. The claim that a plant extract may inhibit digestion of complex carbohydrates to monosaccharides in the human gastrointestinal tract may be validated by inhibition of mammalian enzyme, not only the microbial version of the enzyme. The discrepancy might be as a result of structural differences (Lee and Lee, 2001). Babu et al. (2004) reported varying inhibition pattern by extracts of some medicinal plants on yeast and mammalian α-glucosidase. *Cassia auriculata* extracts exhibited potential antihyperglycemic effects in Sprague-Dawley rats (Abesundara et al., 2004). The *in vivo* antihyperglycemic activity of *C. abbreviata* is currently being investigated in streptozotocin-induced diabetic rats.

Antioxidant activity

Reactive oxygen species are associated with diabetes mellitus. Damage to the pancreas, resulting in impaired insulin secretion can be attributed to an increase in the concentration of free radicals or compromised antioxidant defenses (Ceriello, 2000; Baynes and Thorpe, 1999; Baynes, 1991). Antioxidants have been reported to reverse some of the damaging effects of free radicals on the pancreas, resulting in the restoration of insulin functioning and subsequent lowering of blood glucose concentration (Maritim et al., 2003), and may retard associated complications (Packer et al., 2000). In addition, Mai et al. (2007) reported positive correlation



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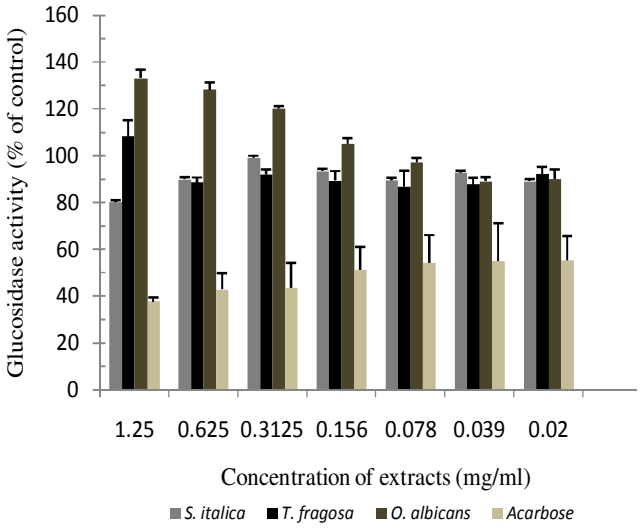

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Figure 2. The effects of *C. abbreviata, X. zambesiaca, M. mochisia* (A), *S. italica, T. fragosa* and *O. albicans* (B) on the catalytic activity of α-glucosidase from rat intestinal acetone powder. The activity assay was based on the catabolism of p-nitropheyl-glucopyranoside into glucose and nitrophenol, and was expressed as a percentage of the control (without treatment).

Table 2. The inhibition of the activity of α-glucosidase from rat intestinal acetone powders. The results are expressed as IC_{50} values (mg/ml).

Plant species	IC_{50} (mg/ml)
X. zambesiaca	>2.5
P. africanum	>2.5
M. mochisia	>2.5
O. cf. albicans	>2.5
C. abbreviata	>2.5
Acarbose	0.4

Table 3. Antioxidant activity of plant extracts (expressed as IC_{50} values). ND, not done.

Plant species	IC_{50} (mg/ml)
X. zambesiaca	3.5
P. africanum	0.03
M. mochisia	0.04
O. cf. albicans	0.04

between mammalian α-glucosidase inhibition and antioxidant activity. In this study, all the acetone extracts, with the exception of *X. zambesiaca* had high antioxidant activity, with IC_{50} values as low as 0.03 mg/ml for *P. africanum* (Table 3). Bizimenyera et al. (2005) also reported antioxidant activity of *P. africanum* in a paper outlining the rationale for continued traditional utilization of the species. The antioxidant activity of *P. africanum* was comparable to that of the positive control, ascorbic acid. Ascorbic acid had the highest antioxidant activity with an estimated IC_{50} of 0.030 mg/ml. However, it would seem that inhibition of mammalian α-glucosidase activity is not related to antioxidant activity, since extracts that had high antioxidant activity did not necessarily exert high inhibition on the mammalian enzyme. Further work to investigate the effects of these extracts on blood glucose concentration in diabetic rats may shed light on the hypoglycemic effects of the extracts.

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REFERENCES

Abesundara KJM, Matsui T, Matsumoto K (2004). α-Glucosidase Inhibitory Activity of Some Sri Lanka Plant Extracts, One of Which, *Cassia auriculata*, Exerts a Strong Antihyperglycemic Effect in Rats Comparable to the Therapeutic Drug Acarbose. J. Agric. Food Chem., 52: 2541-2545.

Andrade CA, Becerra JJ, C´ardenas VR (2008). Alfa-glucosidase-inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes. J. Ethnopharmacol., 116: 27-32.

Babu KS, Tiwari AK, Srinivas PV, Ali AZ, China RB, Rao JM (2004). Yeast and mammalian a-glucosidase inhibitory constituents from Himalayan rhubarb Rheum emodi Wall.ex Meisson. Bioorg. Med. Chem. Lett., 14: 3841-3845.

Baynes JW, Thorpe SR (1999). Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes, 48: 1-9.

Baynes JW (1991). Role of oxidative stress in development of complications in diabetes. Diabetes, 40: 405-412.

Bizimenyera ES, Swan GE, Chikoto H, Eloff JN (2005). Rationale for using *Peltophorum africanum* (Fabaceae) extracts in veterinary medicine. J. S. Afr. Vet. Assoc., 76: 54–58.

Davis SN, Granner DK (1996). Insulin. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th ed, pp. 1487-1517.

Heacock PM, Hertzler SR, Williams JA, Wolf BW (2005). Effects of a medical food containing an herbal a-glucosidase inhibitor on postprandial glycemia and insulinemia in healthy adults. J. Am. Diet. Assoc., 105: 65-71.

Ceriello A (2000). Oxidative stress and glycemic regulation. Metabol. 49: 27-29.

Lee DS, Lee SH (2001). Genistein, a soy iso£avone, is a potent K-glucosidase inhibitor. FEBS Lett., 501: 84-86.

Mai TT, Thu NN, Tien PG, Chuyen NV (2007). Alpha-Glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. J. Nutr. Sci. Vitaminol., 53: 267-276.

Maritim AC, Sanders RA, Watkins III JB (2003). Diabetes, Oxidative

Stress, and Antioxidants: A Review. J. Biochem. Mol. Toxicol., 17: 24-37.

Ortiz ARR, Garc´ıa JS, Castillo EP, Ram´ırez AG, Villalobos MR, Estrada SS (2007). α-Glucosidase inhibitory activity of the methanolic extract from *Tournefortia hartwegiana*: An anti-hyperglycemic agent. J. Ethnopharmacol., 109: 48-53.

Packer L, Rfsen P, Tritschler HJ, King GL, Azzi A (2000). Antioxidants in Diabetes Management. Marcel Dekker, New York, pp. 1-338.

Subramanian R, Asmawi MZ, Sadikun A (2008). *In vitro* α-glucosidase and α-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. Acta Biochimica Polonica, 55: 391-398.

Shai LJ, Masoko P, Mokgotho MP, Magano SR, Mogale AM, Boaduo N, Eloff JN (2010). Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. S. Afr. J. Bot., 76: 465-470.

Shirwaikar A, Rajendran K, Punitha ISR (2005). Antidiabetic activity of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin-nicotinamide induced type 2 diabetic rats J. Ethnopharmacol., 97: 369-374.

Tiwari AK, Rao JM (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr. Sci., 83: 30-38.

Yamaki K, Mori Y (2006). Evaluation of alpha-glucosidase inhibitory activity in Colored Foods: A trial using slope factors of regression curves. Nippon Shokuhin Kagaku Kogaku Kaishi in Japanese, 53: 229-231.