



## Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa

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### Abstract

Recent decades have experienced a sharp increase in the incidence and prevalence of diabetes mellitus. One antidiabetic therapeutic approach is to reduce gastrointestinal glucose production and absorption through the inhibition of carbohydrate-digesting enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase and  $\alpha$ -amylase. The aim of the current study was to screen six medicinal plant species, with alleged antidiabetic properties for  $\alpha$ -glucosidase inhibitory activities. Powdered plant materials were extracted with acetone, and tested for ability to inhibit baker's yeast  $\alpha$ -glucosidase and  $\alpha$ -amylase activities. The largest mass (440 mg from 10 g) of the extract was obtained from *Cassia abbreviata*, while both *Senna italica* and *Mormordica balsamina* yielded the lowest mass of the extracts. Extracts of stem bark of *C. abbreviata* inhibited baker's yeast  $\alpha$ -glucosidase activity with an  $IC_{50}$  of 0.6 mg/ml. This plant species had activity at low concentrations, with 1.0 mg/ml and above resulting in inhibition of over 70%. The other five plant extracts investigated had  $IC_{50}$  values of between 1.8 and 3.0 mg/ml. *Senna italica* only managed to inhibit the activity of enzyme-glucosidase at high concentrations with an  $IC_{50}$  value of 1.8 mg/ml, while *Tinospora fragosa* extracts resulted in about 55% inhibition of the activity of the enzyme at a concentration of 3.5 mg/ml, with an estimated  $IC_{50}$  value of 2.8 mg/ml. The bark extract of *C. abbreviata* was the most active inhibitor of the enzyme, based on the  $IC_{50}$  values (0.6 mg/ml). The bark extract of *C. abbreviata* contains non-competitive inhibitor(s) of  $\alpha$ -glucosidase, reducing  $V_{max}$  value of this enzyme from  $5 \text{ mM}\cdot\text{s}^{-1}$  to  $1.67 \text{ mM}\cdot\text{s}^{-1}$ , while  $K_m$  remained unchanged at 1.43 mM for *para*-nitrophenyl glucopyranoside. Antioxidant activity of the extracts was also investigated. The *C. abbreviata* extract was more active as an antioxidant than the positive control, trolox. The extracts did not inhibit  $\alpha$ -amylase activity more than about 20% at the highest concentration tested.

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**Keywords:** Antioxidant;  $\alpha$ -Amylase;  $\alpha$ -Glucosidase activity; *Cassia abbreviata*; Diabetes mellitus

### 1. Introduction

The South African black population is in a lifestyle-changing phase that is characterized by an increase in cases of diseases such as diabetes mellitus, obesity and hypertension (Walker, 1995). Diabetes mellitus is responsible for about 5% of global

deaths (World Health Organization, 2010). It is characterized by chronic hyperglycaemia and abnormal fat and protein metabolism caused by defects in insulin production or action. Type 1 diabetes mellitus result from failure of pancreatic  $\beta$ -cells to produce insulin while the most common type 2 diabetes mellitus is caused by a decreased sensitivity of target cells to insulin (Burcelin et al., 1999).

One of the strategies adopted to treat diabetes mellitus involves inhibition of carbohydrate-digesting enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase in the gastrointestinal tract, with associated retardation of intestinal glucose absorption and lowering of

**Abbreviations:** ABTS, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); DMSO, dimethylsulphoxide.

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postprandial blood glucose levels (Rhabasa-Lhoret and Chiasson, 2004). Alpha glucosidase cleaves glycosidic bonds in complex carbohydrate to release absorbable monosaccharides. Inhibitors of  $\alpha$ -glucosidase display useful anti-hyperglycaemic effects (Stuart et al., 2004). Acarbose and miglitol are examples of competitive inhibitors of  $\alpha$ -glucosidases (Davis and Granner, 1996). In a country like South Africa, where a great proportion of the population relies on plant-derived remedies to treat diseases, such inhibitors may come in the form of medicinal plant preparations.

Many medicinal plant species have  $\alpha$ -glucosidase inhibitory activity. Andrade-Cetto et al. (2008) reported that *Cecropia obtusifolia*, *Malmea depressa* and *Acosmium panamense* have  $\alpha$ -glucosidase inhibitory activity with  $IC_{50}$  values (concentration that results in half of the enzyme's activity inhibited) lower than that resulting from acarbose (128  $\mu$ g/ml). Jung et al. (2006) attributes  $\alpha$ -glucosidase inhibition by some plant species to active principles such as polyphenols, flavonoids and glycosides. In this study we investigated the inhibition of yeast  $\alpha$ -glucosidase with acetone extracts of six medicinal plants used in Ga-Mashishimale village in Limpopo Province. Ga-Mashishimale village is situated about 20 km west of Phalaborwa.

*Mormodica balsamina* L. (Cucurbitaceae) ("nku" in Northern Sotho, the local language of the village) is widely used as a vegetable in this area. The leaves may also be boiled in combination with prickly-pear to treat diabetes mellitus, a claim consistent with literature records (Lewis and Elvin-Lewis, 1977). The hypoglycaemic effects of leaves and fruits of *M. balsamina* have been demonstrated in diabetic rabbit model (Karumi and Bobboi, 1999). This climber is found growing on fences surrounding homesteads at Ga-Mashishimale village. The healers in this area use *Senna italica* Mill. (Fabaceae) (*Setlommana* in the local language) root extract to treat sexually-transmitted infections. *Cassia abbreviata* Oliv. (Fabaceae) (*Molomanama* in the local language) stem bark extract is used by healers in this area to treat stomach complaints and sexually-transmitted infections. *Tinospora fragosa*, also called "makgonatsohle" in the local language, is used to treat diabetes mellitus and sexually-transmitted infections (STIs). The local name of the plant species indicates that this species is effective against all diseases known in this area. *Waltheria indica* L. (Malvaceae) (*motayabannyana* in the local language) is indicated for STIs and to a small extent, diabetes mellitus. *Gymnosporia buxifolia* (*sephatwa*) is not indicated for diabetes mellitus. It is used to stop vomiting. The aim of this study was to determine the  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory capabilities of acetone extracts of six widely-used medicinal plants from Ga-Mashishimale village.

## 2. Materials and methods

### 2.1. Plant collection and extraction

Plant materials were collected at Ga-Mashishimale village, Limpopo Province (South Africa) in woven bags and allowed to dry at room temperature. Voucher specimens were deposited at the H.G.W.J. Schweickerdt Herbarium at the University of

Pretoria where they were identified. Each deposited herbarium specimen was allocated a number (PRU number). The plant species used in this study were *W. indica* L. (aerial parts) (family Malvaceae) (PRU 113813.0), *S. italica* Mill. (aerial parts) (family Fabaceae) (PRU 113814.0), *C. abbreviata* (stem bark) (family Fabaceae) (PRU 113819.0), *M. balsamina* L. (aerial parts) (Cucurbitaceae) (PRU 113790.0) and *T. fragosa* (aerial parts) (PRU not yet allocated).

The dried materials were then powdered using iron pestle and mortar. Five grams of each plant material was extracted with 25 ml acetone on an orbital shaker for 30 min. Acetone was used as an extractant following reports elsewhere that it extracts a near full complement of both polar and non-polar compounds (Eloff, 1998).

The resulting extracts were filtered using Whatman No. 1 filter paper to remove plant debris, and the filtrate was allowed to dry under a stream of air at room temperature. Dried extracts were weighed and dissolved in dimethylsulphoxide (DMSO) to yield a stock solution concentration ranging from 34 mg/ml to 88 mg/ml. The solutions were stored 4 °C and used within 5 days.

### 2.2. $\alpha$ -Glucosidase inhibition assay

The  $\alpha$ -glucosidase inhibition was determined using the modified version of the method according to Matsui et al. (1996). The  $\alpha$ -glucosidase reaction mixture contained 2.9 mM *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) (Sigma-Aldrich), 0.25 ml of extract (varying concentrations) in DMSO and 0.6 U/ml baker's yeast  $\alpha$ -glucosidase (Sigma-Aldrich, South Africa) in sodium phosphate buffer, pH 6.9. Control tubes contained only DMSO, enzyme and substrate, while in positive controls acarbose replaced the plant extracts. Mixtures without enzyme, plant extract and acarbose served as blanks. The reaction mixtures were incubated at 25 °C for 5 min, after which the reaction was stopped by boiling for 2 min. Absorbance of the resulting *p*-nitrophenol (pNP) was determined at 405 nm using Helios  $\beta$  spectrophotometer (Thermo Electron Corporation) and was considered directly proportional to the activity of the enzyme. Glucosidase activity inhibition was determined as percentage of control as follows:

$$\begin{aligned} \% \text{Glucosidase inhibition} &= 100\% \\ &\quad - \% \text{activity of test as percentage of control} \\ \% \text{Activity of test} &= \frac{\text{corrected } A_{405} \text{ of test} \times 100\%}{A_{405} \text{ of controls}} \end{aligned}$$

In order to eliminate background readings, the absorbance of the extract without substrate and enzyme was subtracted from absorbance of the extract and substrate mixture as follows:

$$\text{Corrected } A_{405} \text{ test samples} = A_{405} \text{ extract and substrate mixture} - A_{405} \text{ extract alone (background)}.$$

The activity in controls (with  $\alpha$ -glucosidase but without inhibitor) was considered to be 100%. Concentrations of extracts resulting in 50% inhibition of enzyme activity ( $IC_{50}$  values) were determined graphically. Different plant species

were compared on the basis of their  $IC_{50}$  values estimated from the dose response curves.

### 2.3. Kinetics of $\alpha$ -glucosidase activity

The acetone extract of the most active plant, *C. abbreviata*, was tested in experiments to determine the type of inhibition exerted on  $\alpha$ -glucosidase. The reaction mixture was as described above, except that the concentration of the substrate varied from 0.15 to 5 mM, and that the concentration of the extract was kept constant at 0.4 mg/ml. The reaction was started by addition of enzyme, and the reaction was monitored at 405 nm, at 30 seconds intervals for 5 min. The initial rates of reaction were determined using calibration curves constructed using varying concentrations of *p*-nitrophenol. The results were used to construct Lineweaver–Burk plots for determination type of inhibition,  $K_m$  and  $V_{max}$  values.

### 2.4. $\alpha$ -Amylase activity inhibition

Dimethylsulphoxide-dissolved plant extracts (250  $\mu$ g/ml) were included in a reaction mixture containing 0.25% starch in 100 mM sodium phosphate buffer (pH 6.8) and 2 U  $\alpha$ -amylase (Sigma-Aldrich, South Africa). The reaction mixture was incubated for 5 min. at 37 °C. Dinitrosalicylic acid (100  $\mu$ l) was added to 200  $\mu$ l aliquots of the reaction mixture. The resulting mixture was boiled for 15 min, and then diluted with 900  $\mu$ l of water. Absorbance at 540 nm was determined using Helios  $\beta$  spectrophotometer. Absorbance readings resulting from mixtures of plant extract with DMSO, water and starch represented background readings. To correct the absorbance readings in reaction mixture, background readings were subtracted from reaction mixture readings. Inhibition of activity was calculated as a percentage of the control. Controls without plant extracts were considered to contain 100% enzyme activity, and thus 100% hydrolysis of pNPG to pNP and glucose.

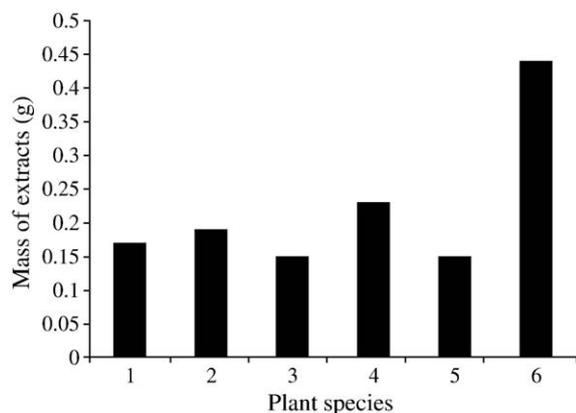


Fig. 1. Mass of the extracts (g) from 10 g plant material. 1, *W. indica*; 2, *G. buxifolia*; 3, *M. balsamina*; 4, *T. fragosa*; 5, *S. italica*; and 6, *C. abbreviata*.

### 2.5. Antioxidant activity

Antioxidant activity was based on the decolourization of the preformed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS<sup>•+</sup>) through reduction in the presence of hydrogen-donating antioxidants in plant extracts (Re et al., 1999). Plant extracts were dissolved in DMSO to a concentration of 1 mg/ml. The plant extracts were diluted to yield concentration range from 7.8 to 1000  $\mu$ g/ml in a total volume of 100  $\mu$ l. ABTS<sup>•+</sup> (300  $\mu$ l) was added to these extracts and the mixture incubated for 6 min. at room temperature. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a positive control. Absorbance at 734 nm was determined using a microplate reader. Background (absorbance of plant extracts without ABTS<sup>•+</sup>) was subtracted from reaction mixture readings. Antioxidant activity was calculated as a percentage of the negative controls. Controls without plant extracts and trolox were assumed to contain 100% oxidized ABTS. The readings were calculated based on the amount of ABTS reduced as obtained by comparing with the controls. %ABTS oxidized =  $A_{734}$  of test  $\times$  100/ $A_{734}$  of negative controls. Antioxidant activity (amount of ABTS reduced) % = 100% – amount of ABTS oxidized.

## 3. Results and discussion

### 3.1. Inhibition of glucosidase activity

Extracts of varying concentrations were obtained from materials from different plant species under investigation. *Cassia abbreviata* yielded the highest mass (440 mg) of acetone extract from the 10 g extracted. All the other plant material resulted in mass of extracts of between 150 and 250 mg (Fig. 1).

The  $\alpha$ -glucosidase inhibitor effectiveness of extracts of the different plant species were compared on the basis of their resulting  $IC_{50}$  values. *Cassia abbreviata* inhibited the activity of  $\alpha$ -glucosidase with an  $IC_{50}$  of 0.6 mg/ml (Fig. 2A; Table 1). The highest concentration (6.4 mg/ml) of *C. abbreviata* permitted the release of 12% of pNP from pNPG, amounting to 88% inhibition of  $\alpha$ -glucosidase activity. *Senna italica* extract, with an  $IC_{50}$  value of 1.8 mg/ml was the second most active of the species tested (Fig. 2B; Table 1). Only 60% inhibition of  $\alpha$ -glucosidase activity was achieved with the highest tested concentration (2.2 mg/ml). *Tinospora fragosa* with an  $IC_{50}$  value of 2.80 mg/ml, *W. indica* ( $IC_{50}$  = 2.1 mg/ml), *M. balsamina* ( $IC_{50}$  = 2.1 mg/ml) and *G. buxifolia* ( $IC_{50}$  = 2.4 mg/ml) were less active (Fig. 2; Table 1). Acarbose, the positive control used in this study, inhibited the activity of  $\alpha$ -glucosidase with an  $IC_{50}$  value estimated at 17 mg/ml. This finding is consistent with literature reports, where acarbose was found to exert little inhibition on  $\alpha$ -glucosidase activity. Anam et al. (2009) as well as Oki et al. (1999) have reported absence of  $\alpha$ -glucosidase inhibition by acarbose. *Cassia abbreviata* was the most active of the plant species tested. The results of *C. abbreviata* are in support of traditional uses of the species to reduce blood glucose levels. It is highly likely that long term treatment may achieve the desired results with diabetes mellitus

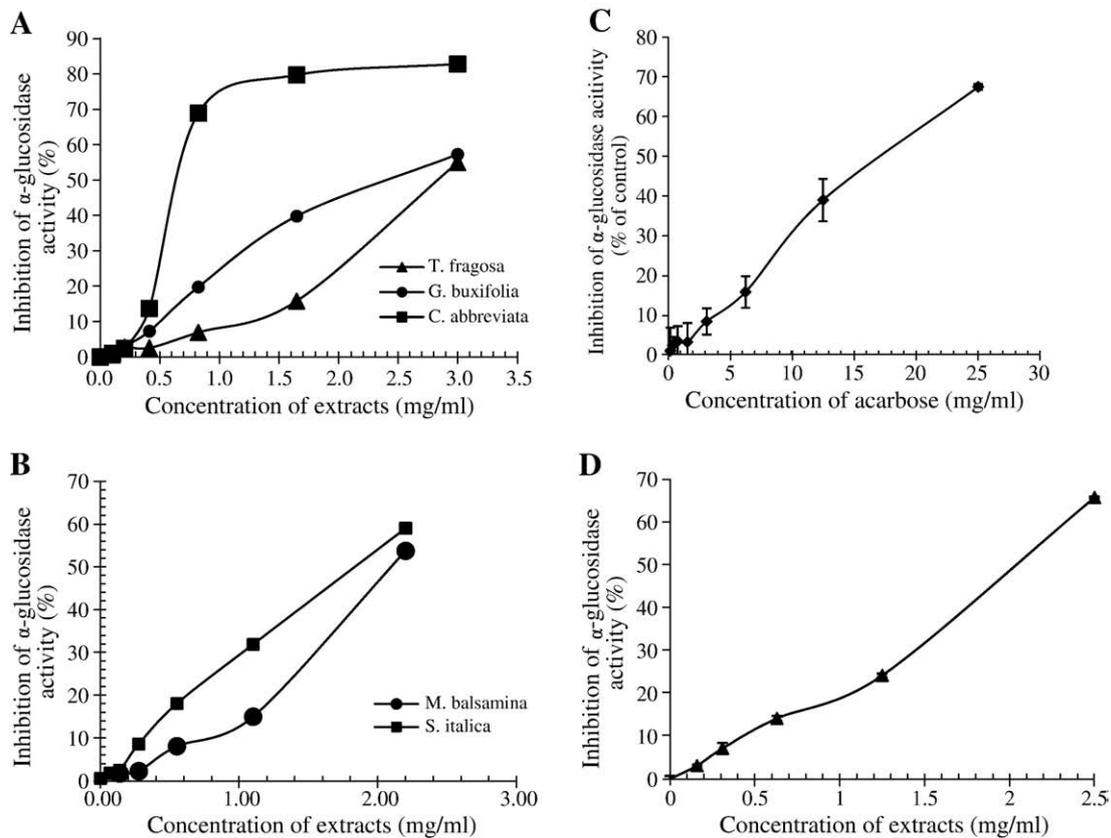


Fig. 2. The inhibition of  $\alpha$ -glucosidase activity of extracts at increasing concentrations. The extracts were *T. fragosa*, *G. buxifolia*, *C. abbreviata* (A), *M. balsamina*, *S. italica* (B) and *W. indica* (D). Acarbose (C) was used as a positive control. The result represents the means of two independent triplicate experiments.

patients. However, long term usage may be questionable considering that some investigators found that the toxicity of *C. abbreviata* was high, with an  $LC_{50}$  of 39  $\mu$ g/ml (Moshi et al., 2006). The stem bark of this plant is used by traditional healers of Ga-Mashishimale village to treat diabetes mellitus. In Kahama District in Tanzania, the leaves and stem bark are also used to treat diabetes mellitus, among other diseases treated (Dery et al., 1999).

Despite a high  $IC_{50}$  value of *M. balsamina* extract on  $\alpha$ -glucosidase activity in this study, it was reported elsewhere that this plant species stimulates glucose utilization by hepatocytes, though with slightly advanced toxicity (Van de Venter et al., 2008). The leaves and fruits have hypoglycaemic effects in rats (Van de Venter et al., 2008). Our results suggest that the

hypoglycaemic effects of *M. balsamina* do not involve carbohydrate-hydrolysing enzymes of the gastrointestinal tract, but are rather mediated via a different mechanism. *Momordica balsamina* contains alkaloids, volatile oils, carotene and fixed oils (Karumi et al., 2004), and it is not yet confirmed if these molecules are responsible for part of the activity in this study.

*Senna italica* was the second most active species in this study (Fig. 2B; Table 1). This plant is not listed as a treatment for diabetes mellitus in the village investigated. This plant was

Table 1

The inhibition of yeast  $\alpha$ -glucosidase by extracts of six medicinal plants collected from Ga-Mashishimale village. The results represent the mean and standard deviation of two independent triplicate experiments. Acarbose was not active at the concentrations (0.02–1.00 mg/ml) tested.

Plant extract	$IC_{50}$ (mg/ml)
<i>Cassia abbreviata</i>	0.6 $\pm$ 0.2
<i>Waltheria indica</i>	2.1 $\pm$ 0.1
<i>Senna italica</i>	1.8 $\pm$ 0.2
<i>Gymnospora buxifolia</i>	2.4 $\pm$ 0.2
<i>Momordica balsamina</i>	2.1 $\pm$ 0.1
<i>Tinospora fragosa</i>	2.8 $\pm$ 0.2
Acarbose	17.1 $\pm$ 1.4

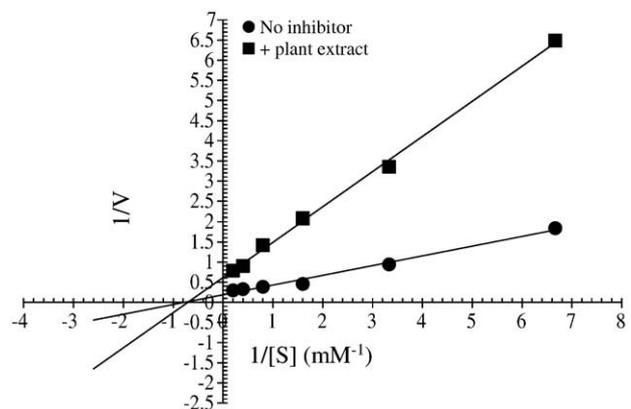


Fig. 3. Type of inhibition of  $\alpha$ -glucosidase with *Cassia abbreviata* extracts. The results represent the means of three independent triplicate experiments.

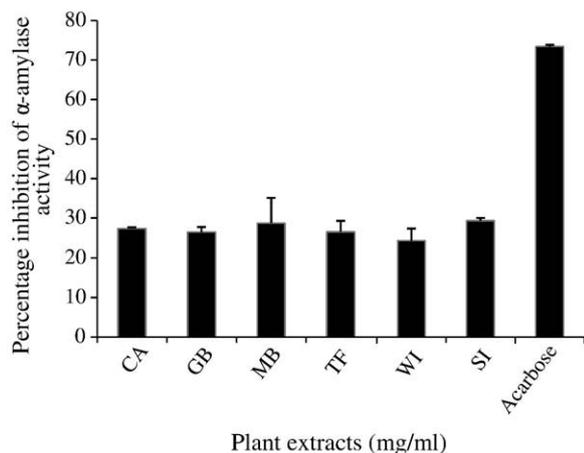


Fig. 4. Alpha-amylase inhibitory activity of extracts of plants collected from Ga-Mashishimale village. The results represent the mean and standard deviations of two independent duplicate experiments.

selected randomly for screening purposes, prompted by its long history of usage in traditional medicine. It is used in mixtures to treat sexually-transmitted infections. Medicines containing this plant species are usually prepared through boiling in water and administered in 2 l bottles. The dosage or concentrations of ingredients and chemical components remains unknown as proportions of each plant species' material are estimated. In other studies, a variety of toxic effects resulting from treatment with seed extracts of *S. italica* were observed in rats (Al-Araidh et al., 2004).

*Tinospora fragosa* was active only at high concentrations with an  $IC_{50}$  of 2.8 mg/ml. In other studies, it has been reported that a plant species closely related to *T. fragosa*, namely, *Tinospora cordifolia* had an antioxidant activity in alloxan-induced diabetes mellitus rats (Prince and Menon, 1999). The leaves of *T. cordifolia* exhibit antidiabetic effects in alloxan-induced diabetes mellitus in rats (Noreen et al., 1992). It would seem important to investigate the antidiabetic activity of *T.*

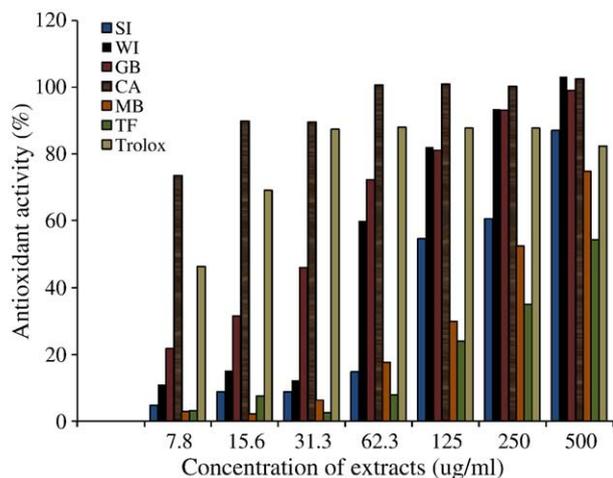


Fig. 5. Antioxidant activity of six medicinal plants collected from Ga-Mashishimale village. The activity was calculated as a percentage of the control (without plant extract). The results represent the means of two independent triplicate experiments.

Table 2

Antioxidant activity extracts of various plants collected from Ga-Mashishimale village. The results represent the mean and standard deviation of two independent triplicate experiments. The  $IC_{50}$  values were estimated using the graphical method.

Plant extract	$IC_{50}$ ( $\mu$ g/ml)
<i>Cassia abbreviata</i>	<7.8
<i>Waltheria indica</i>	80 $\pm$ 3.5
<i>Senna italica</i>	120 $\pm$ 5.2
<i>Gymnospora buxifolia</i>	40 $\pm$ 1.0
<i>Momordica balsamica</i>	200 $\pm$ 3.1
<i>Tinospora fragosa</i>	430 $\pm$ 10.4
Trolox (positive control)	11 $\pm$ 0.9

*fragosa* through determining antioxidant activity and testing efficacy in an animal model. The extracts may be exerting their antidiabetic activity through a separate mechanism other than the inhibition of carbohydrate hydrolyzing enzymes of the gastrointestinal tract.

### 3.2. Type of inhibition, $K_m$ and $V_{max}$ values new addition

The acetone extract of *C. abbreviata* displayed patterns of non-competitive inhibition, with  $K_m$  remaining unchanged at 1.43 mM. The value of  $V_{max}$  was reduced from 5  $mM \cdot s^{-1}$  in the absence of extract to 1.67  $mM \cdot s^{-1}$  in the presence of 0.4 mM of *C. abbreviata* extract (Fig. 3). These results suggest that the active components in the extract do not compete with the substrate for binding to the active site, rather the inhibitor(s) bind to a separate site on the enzyme to retard the conversion of substrate to product.

### 3.3. Inhibition of $\alpha$ -amylase activity and antioxidant activity

Plant extracts were also investigated for inhibition of  $\alpha$ -amylase, and plant extracts with appreciable inhibition (about 70–100% inhibition) at 1 mg/ml were to be selected for further studies, including determination of  $IC_{50}$ , type of inhibition and isolation of active compounds. The plant extracts had similar levels of inhibition of  $\alpha$ -amylase activity (new paragraph to explain). The inhibition of the enzyme was estimated at about 20% for all the extracts tested. Acarbose resulted in around 70% inhibition at the concentration tested (Fig. 4). The extracts were tested at a concentration of 1 mg/ml for inhibition of  $\alpha$ -amylase.

Antioxidant activity plays a role in the treatment of diabetes mellitus (Raphael et al., 2002). Some plant species have been reported to possess both antidiabetic activity as well as antioxidant activity. Tea phenolics, which possess antioxidant activity, have also been found to inhibit both  $\alpha$ -amylase and sucrase (Matsumoto et al., 1993). Tchinda et al. (2008) also demonstrated that some compounds isolated from *Pycnanthus angolensis* have both  $\alpha$ -glucosidase inhibitory and antioxidant properties. Furthermore, it is also reported that the concentrations of antioxidants are reduced in diabetic patients (Valabhji et al., 2001). Mai et al. (2007) demonstrated positive correlation between  $\alpha$ -glucosidase inhibition and antioxidant activity. Therefore, antioxidant activity of the six plant species was investigated using the ABTS reduction method. *Cassia*

*abbreviata* had pronounced antioxidant activity which was in most cases more than that resulting from the positive control, trolox. The lowest concentration of *C. abbreviata* tested, 7.8 µg/ml led to about 70% reduction of ABTS. The other plant extracts started reducing appreciable levels of ABTS at higher concentrations (62 µg/ml and above) (Fig. 5; Table 2). It is not yet established if the antioxidant activity of *C. abbreviata* is important in the medicinal value of the plant species, including the inhibition of α-glucosidase activity.

In conclusion, *C. abbreviata* possesses compounds that inhibit the glycosidic bond cleavage by α-glucosidase. The type of inhibition by *C. abbreviata* was confirmed as non-competitive. We are currently investigating the effects of these plant species on the mammalian version of the enzyme, α-glucosidase, and the effects of varying concentration of extracts on inhibition of α-amylase. Attempts to isolate the active compounds from *C. abbreviata* are already underway.

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