Evaluation of six plant species used traditionally in the treatment and control of diabetes mellitus in South Africa using *in vitro* methods

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Abstract

Context: Numerous plants are used by the local communities of South Africa for the treatment and management of Type II diabetes.

Objectives: For this study we undertook a survey of the plants sold for the management of diabetes in the town of Newcastle, South Africa. Identified plants were subsequently evaluated for their *in vitro* antidiabetic activity

Materials and methods: Plants were identified through an interview with a herbalist at the market. Antidiabetic activity of extracts of purchased plants was evaluated using *in vitro* α-amylase and α-glucosidase activity, as well as islets of Langerhans excretory activity.

Results: *Senna alexandrina* Mill (Fabaceae)., *Cymbopogon citrates* Stapf (Poaceae)., *Cucurbita pepo* L (Cucuribitaceae)., *Nuxia floribunda* Benth (Stilbaceae)., *Hypoxis hemerocallidea* Fisch. & Mey (Hypoxidaceae) and *Cinnamomum cassia* Blume (Lauraceae) were identified. The hexane extract of *S. alexandrina* (EC_{50} = 0.083 mg/ml), ethyl acetate extract of *H. hemerocallidea* (EC_{50} = 0.29 mg/ml) and methanol extracts of *C. citratus* (EC_{50} = 0.31 mg/ml) and *C. cassia* (EC_{50} = 0.12 mg/ml) had the highest α-amylase inhibitory activity, albeit lower than Acarbose (EC_{50} = 0.50 mg/ml). All the plants had good α-glucosidase inhibitory activity (>50%) with the exception of some methanol (*C. cassia*, *N. floribunda* and *C. citratus*) and acetone extracts (*C. pepo*, and *N. floribunda*). Only the *H. hemerocallidea* acetone extract had an insulin stimulatory effect (2.5 U/mI at 8 μg/ml).

Conclusion: All the evaluated plants demonstrated inhibitory activity against the specific GIT enzyme systems evaluated. Only *H. hemerocallidea* had insulin secretory activity,

adding evidence to the traditional use of these purchased plants in the management of the type 2 diabetic post-prandial hyperglycemia.

Keywords: Antidiabetic, islets of Langerhans, α amylase, α glucosidase

Introduction

Diabetes mellitus, one of the most important non-communicable diseases of the 21st century, causes morbidity and mortality in both young and old (Zimmet et al., 2001). It is a metabolic disease characterized by hyperglycemia, polyuria and polydipsia and results from defects in insulin secretion and/or action. This disease once considered to be minor a few decades ago is now a major threat to human health (Zimmet et al., 2001). In the last three decades the number of people diagnosed with diabetes has increased to the current incidence of 150 million to 220 million afflicted persons (Eastman et al., 1996). According to WHO statistics and a study conducted by Danaei *et al* in 2012 there were 347 million people living with diabetes. While the disease predominates in first world countries, the disease is expected to increase in the prevalence from 3.8% to 4.3% in Africa by 2030 (Unwin, 2012).

Several forms of diabetes mellitus have been identified, of which two predominant: type I diabetes, the autoimmune-mediated form, is characterized by pancreatic β cell islets destruction while type II diabetes is characterized by insulin resistance or the abnormal secretion of insulin. People inflicted with type I diabetes are wholly dependent on exogenous insulin for survival while people with type II diabetes produce insufficient amounts of endogenous insulin to regulate the blood glucose concentrations (Shafrir, 1997). To manage diabetes, numerous treatments have been developed. Treatment for type I diabetes typically requires exogenous insulin supplementation while a combination of drug therapy, diet modification and physical exercise is usually recommended for type II diabetes (Zimmet et al., 2001). For Type II, drug therapy is aimed at decreasing blood glucose concentrations by either enhancing endogenous insulin release, increasing insulin effect, or modulating glucose absorption. For the most part, treatment involves the use of commercial antidiabetic agents. However, crude botanical extracts are also commonly used in the treatment and control diabetes (Hanefeld, 1998). The use of herbal remedies for diabetes management is also an important treatment modality

available in South Africa. This study evaluates plant species being sold at a local community market as a treatment aid in diabetic people, for their ability to modulate blood glucose using dedicated diabetic laboratory models.

Materials and methods

Purchase and preparation of plant material

To facilitate the study plant parts recommended by a herbalist, Mr. Radebe who practices his trade at the local market in the Newcastle region of Kwa-zulu (South Africa), were used. Mr Radebe was able to provide the names and samples of the 6 plants that he prescribes for the management of high blood sugar. These specific plants were recommended to patients who either requested treatment after being diagnosed with diabetes by their medical doctor or following on his own diagnosis based on the ability of a patient's urine sample to attract ants. Mr Radebe also indicated that his knowledge was passed down from his grandfather. The local names, plant parts used and the methods of preparation are presented in Table 1.

The scientific names were obtained from literature and species of the plant to which the material belonged was provided by the curator of the Pretoria National Herbarium of SANBI (South African National Biodiversity Institute) in Pretoria. Voucher specimen numbers were only assigned by SANBI to only those plants that were indigenous to South Africa (Table 1). The plant material (3 g) were subsequently finely ground and extracted with 30 ml of acetone, methanol hexane or ethyl acetate on a shaker platform for 30 min, in order to best identify activity within the specified plant (Eloff, 1998b). The extracts were then dried on a rotatory evaporator (Buchi) and stored in a refrigerator. All extracts were re-constituted in acetone prior to testing.

α Amylase inhibitory assay

Alpha amylase inhibitory activity was determined by modification of the methods of Hansawasdi et al., (2000). Starch (0.5%) azure was dissolved in 75 ml of sodium phosphate buffer (pH 6.9) at 60-70˚C in a water bath. Predetermined concentrations of the plant extracts (0.2, 0.4, 0.6, 0.8 and 1 mg/ml) made up in 0.2 ml 50% dimethyl sulfoxide (DMSO), distilled water and porcine pancreatic solution rich in amylase (10 U/ml) were incubated for 5 min at 25˚C. For the control blank (0.2 ml) 50% DMSO replaced the extract. Following incubation, a starch (0.5%) solution was added to each test tube, vortexed and incubated at 37˚C for 3 min. The amylase activity was indirectly determined by concentration of maltose formation. Maltose was quantified calorimetrically, by reacting 1 ml of the solution with 1 ml of a 3, 5-dinitrosalicylic acid (DNS) stock solution in a water bath at 85˚C for 15 min. After this incubation 900 µl of distilled water was added to each tube to dilute the mixture. The absorbance for each sample was measured by a spectrophotometer (Helios Beta, Thermo Electron Cooperation) at 690 nm (corrected to a 1cm path length). Absorbance was converted to actual concentration using a maltose calibration curve which was linear between 0 and 1.2 µg/ml (R^2= 0.9844)(Ali *et al.*, 2006). The EC_{50} was determined using an Emax inhibitory model with the exception of acarbose in hexane, which was fitted using Hills equation (Kinetica 4.4, Thermo Scientific).

α Glucosidase inhibitory assay

The α glucosidase activity was determined by a modification of the methods of Nishioka et al., (1998) and Bhandari et al., (2008). For the assay, 0.2 ml of 56 mM sucrose (Sigma-Aldrich) was made up in 0.1M potassium phosphate buffer, pH 7, (0.2 ml), and mixed with 0.1 ml of the plant extract in 50% dimethyl suphoxide (DMSO) in solution (0.2 ml). This mixture was incubated at 37˚C for 5 min. A solution of α-glucosidase (0.125 g in 25 ml of buffer) was prepared from rat intestinal acetone powder (Sigma-Aldrich) and 0.2 ml of this enzyme solution was added to the pre-incubated mixture of sucrose (56 mM in 0.1 M potassium phosphate buffer, pH 7) and plant extract. The reaction was stopped after

30 min by adding 0.75 ml of 2M Tris-HCL buffer at pH 6.9. The solution was centrifuged for 20 min at 3000 rpm and the supernatant removed and filtered using a micro-pore filter (0.20 µm).

The percentage conversion of sucrose to glucose was determined by using a commercial glucose oxidase test kit (GAGO-20, Sigma). The negative control contained 0.1 ml of 50% DMSO. The glucose calibration curve was prepared using the components supplied within the commercial glucose test kit (GAGO-20, Sigma-Aldrich). Absorbance at 540 nm was converted to actual concentration using a glucose calibration curve which was linear between 0 and 0.8 µg/ml (R^2= 0.9979).

Insulin stimulatory activity

This was determined by a modification of the method of Shewade et al., (1999). The procedure for the isolation of islets of Langerhans from two Sprague Dawley rats (approximately 8 weeks old) was approved by the Animal Use and Care Committee of the University of Pretoria. The animals were euthanized by an isoflurane overdose; their pancreases aseptically removed, macerated and washed three times with 20 ml of Hank's balanced salt solution (HBSS) prior to being digested in 0.1% collagenase (Type V, Sigma-Aldrich) for 30 min. The digest was suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mg/ml soybean tyrosine inhibitor (STI) and 2% bovine serum albumin (BSA) with 0.5 mg/ml of collagenase for another 30 min. The cells were then pelleted at 300xG and seeded in culture flasks (25 cm^2) with DMEM supplemented with 10% BSA with additions of RPMI-1640, HEPES and Krebs-Ringer bicarbonate solution (KPBS) buffer (pH 7.4) and incubated at 37˚C in 5% CO_2 in the air for 48 h under high humidity.

Following incubation, the cells were transferred into tubes containing 1 ml of 10 mM KPBS supplemented with HEPES at pH 7.4, 10% BSA and 50 mg of glucose and incubated for one hour at 37˚C on a shaker at 30 rpm (Heindolph polymax 1040). The islets were then re-incubated for 60 min at

37˚C with 2, 4, 6, 8 or 10 µg/ml of the plant extract; the concentrations were selected to preclude a toxicity effect as drugs seldom reach tissue concentrations of 10 µg/ml *in vivo* clinically. After incubation the mixture was centrifuged and the supernatant was stored at -20˚C for insulin assay. Insulin concentration in the supernatant was analyzed by a commercial chemical pathology laboratory (AMPATH, South Africa) using a chemoluminescence method. The positive controls were acarbose (1 mg/ml) and glibenclamide (1 mg/ml).

Results

Plants material

Six plant species were identified as being effective against diabetes at the specific market. According to the traditional healers, the plants have been in use in the area for a substantial period of time. The plant names, scientific and local name and method of preparation are presented in Table 1. Published information on the use of the plants in ethno-medical practices are listed in Table 2. Of the six plants identified *Cymbopogon citratus* stapf (Poaceae)., *Cucurbita pepo* L (Cucuribitaceae)., *Hypoxis hemerocallidae* Fisch. & Mey (Hypoxidaceae)., *Cinnamomum cassia* Blume (Lauraceae) *and Senna alexandrina* Mill (Fabaceae)., all have a history of medicinal use in either Africa or Asia. Of these plants, *C. citratus, C. pepo, H. hemerocallidae* and *C. cassia* have all been studied to some extent as antidiabetic agents under either *in vitro* or *ex vivo* conditions. *C. cassia* is known to contain an insulin-like peptide, while *C. pepo* is reported to contain a protein that promotes the secretion of insulin at very high doses. This is however, the first report of *N. floribunda* being used medicinally to treat diabetes.

α Amylase Inhibitory activity

Twenty-four extracts were prepared from six plants using the four different solvents. The concentration versus percentage amylase inhibitory activity of each plant extract and the acarbose control is presented the Figure 1, with the EC_{50} (mg/ml) and Emax presented in Table 3. Three of the plants

extracted with the acetone, methanol and ethyl acetate demonstrated α-amylase enzyme inhibitory activity, while all six hexane extracts were effective. The acetone extracts of *C. pepo* and *H. hemerocallidea* (EC_{50}=1.82 and 0.92 mg/ml respectively), the methanol extracts of *C. citratus* and *C. cassia* (EC_{50}= 0.29 and 0.12 mg/ml respectively) and the ethyl acetate extracts of *H. hemerocallidea, N. floribunda* and *C. citratus* had greater enzyme inhibitory activity than acarbose (EC_{50}= 0.56 mg/ml). The lowest activity was observed for *N. floribunda* extract (EC_{50} = 10.00 mg/ml). The highest observed enzyme inhibitory activity was in *C. cassia* and *C. pepo* (EC_{50} = 0.23 and 0.50 mg/ml respectively).The best activity was observed in the ethyl acetate extracts.

α Glucosidase inhibitory activity

The α glucosidase inhibitory activity (Table 4) of the plants was in general much higher than that for the α-amylase activity (> 75%). The hexane crude extracts of *N. floribunda* and *C. citratus* showed very high inhibitory activity (99%) at 1 mg/ml. The ethyl acetate extracts of all the plant species used in this study had an inhibitory activity above 90% against α-glucosidase at 1 mg/ml. When compared to acarbose all the plant species used in this screening study showed good activity against the α-glucosidase enzyme with the exception of the methanol extract of *C. cassia*.

Insulin stimulatory activity

Only the *H. hemerocallidea* acetone extract elicited the secretion of insulin of 2.5 U/ml at dosages of 8 µg/ml and 10 µg/ml. The insulin concentrations produced by all other extracts were less than 0.2 U/ml. The positive control of acarbose and glibenclamide induced an insulin release of 11.5 and 19.8 U/ml respectively at 1 mg/ml.

Discussion

The treatment goal of type II diabetes is to maintain near normal levels of glycemic control in both the fasting and post-prandial states. In modern medical science, this is best achieved through control of the diet and the control of plasma insulin levels. The former can be achieved by prescribing a specific low calorie diet plan, or through the use of diet modifying agents. While the latter may be facilitated through the use of insulin secretory agents, insulin or insulinomimics, insulin secretogouges and α-amylase/ α-glycosidase inhibitors predominate.

Many natural products have been investigated with respect to the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestines (Bhandari et al., 2008). α Amylase catalyses the hydrolysis of α-1.4-glucosidic linkages of starch, glycogen and various oligosaccharides and α-glucosidase further breaks down the disaccharides into simpler sugars, readily available for intestinal absorption (Bhandari et al., 2008). The inhibition of their activity in the digestive tract is considered to be effective to control diabetes by diminishing the absorption of glucose produced from the starch by these enzymes (Bhandari et al., 2008). The ability of all the plant extracts to inhibit alpha-amylase and alpha-glucosidase supports the use of these plants in the management of type-II diabetes via dietary modification, where these plants are incorporated into patient diet as supplements or spices. This is also the first evidence that the listed plants may be used as oral agents to manage hyperglycemia i.e. they have inhibitor activity on a GIT enzyme system. *Hypoxis hemerocallidea* and *C. citratus* were the only plants that possessed inhibitory activity against both enzyme systems almost to the same extent as acarbose. However, if the activity of the different extracts is considered, the activity

was predominantly within the relatively non-polar ethyl-acetate fraction, this questions the traditional use of water extracts from these plants.

The exact mechanism involved in the activity shown remains unknown at this stage. In previous studies, it has been argued that plants high in polyphenolic compounds may produce an artificial α-amylase activity *in vitro*, as a result of enzyme precipitation instead of enzyme inactivation (Ali et al., 2006). The absence of significant polyphenols in the ethyl acetate extracts (results not shown) tends to suggest that the activity may be in part due to the presence of yet to be identified compounds in the hexane and ethyl acetate extracts.

The ability of the extract to stimulate the release of insulin is the other common mechanism through which anti diabetic medication work. The isolated islet cells, according to Bhonde et al., (2007) provides a handy model system for ex vivo determination of insulin secretory activity of beta cells, due to their independence from the somatic and nervous system i.e. secretion of insulin is a self-regulatory process. Only *H. hemerocallidea* acetone extract led to a stimulation of insulin production of 2.5 U/ml by the islet cells. From the published literature, *C. pepo* has been previously reported to contain an insulin secretory compound, albeit at higher exposure levels (Quanhong et al., 2005). The failure of a plant extract to demonstrate activity in this assay may be due to a sub-optimal dose of exposure. The doses used in this study were tested at low concentrations, to prevent toxicity against the pancreatic beta, as toxicity of the extracts were not known i.e. lower concentrations were used to prevent overt cell toxicity. This is also the first time that a mechanism for *H. hemerocallidae* anti diabetic activity has been described. When the activity of the *H. hemerocallidae* extract is compared to that for acarbose and glibenclamide, it is noticed that these pure products only produced a 5-8 fold greater increase in insulin secretion despite the exposure being 100 fold higher in concentration. This would indicate that the *H.*

hemerocallidea acetone crude extract may possess potent secretagogue compounds therein, which requires further investigation.

Conclusion

All the plant species in this study had activity in at least one of the chosen assays. The majority of the plant species had significant α-amylase and α-glucosidase inhibitory activity. From the *in vitro* results, it can be concluded that all the tested plants may have some merit in the management of diabetes mellitus type II, as suggested by the ethnomedicinal lead.

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Declaration on interest

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Table 1: Plant species used to treat diabetes mellitus in the Newcastle region (Kwa-Zulu Natal).

Table 2: Published information on the six identified plants

Table 3: The inhibition of α Amylase activity (%) and EC_{50} (mg/ml) for each of the solvents used in this study at the minimum and maximum dose.

Table 4: The glucose quantity (mg) present after treatment of intestinal rat acetone powder and the α Glucosidase inhibitory activity (%) of different extracts of six plants used to treat and manage diabetes

Table 1: Plant species used to treat diabetes mellitus in the Newcastle region (Kwa-Zulu Natal).

Family	Scientific name	Local name	Parts used and mode of preparation
Caesalpiniaceae	*Senna alexandrina* Mill. *(Cassia angustifolia* Vahl.)	Senna leaves	Leaves are boiled in water and taken orally
Poaceae/Gramineae	*Cymbopogon citratus* Stapf.	Isiqunga (lemon grass)	An infusion of the whole plant in water
Curcuribitaceae	*Cucurbita pepo* L.	Intshunga (pumpkin leaves)	Infusion of the upper parts (leaves and stem) in water taken orally
Loganiaceae	*Nuxia floribunda* Benth.	Umlulama (forest elder)	Infusion of bark and roots in water taken orally
Hypoxidaceae	*Hypoxis hemerocallidea* Fisch. & Mey.	Inongwe (African potato)	Infusion of the corms boiled in water and taken orally
Lauraceae	*Cinnamomum cassia* Blume.	Cinnamon	Infusion of the bark in water taken orally

Table 2: Published information on the six identified plants

Plant	Description	Ethnomedical use	Use in diabetes	Describe mechanism
S. alexandrina	Shrub, about 1 m. Has yellow flowers and papery pods containing six to eight seeds (Watt & Breyer-Brandwijk, 1962). Indigenous to hot barren regions and cultivated commercially in the Middle East.	The plant is used medicinally in Mozambique as a purgative (Watt & Breyer-Brandwijk, 1962)	Although the plant is believed to have anti-diabetic activity, no specific activity has been quantified (Watt & Breyer-Brandwijk, 1962)	Non applicable
C. citratus	An aromatic tropical grass with clumped, bulbous stems that gives origin to the leaf blades. This plant is native to Asia (Sri Lanka, Indochina, etc.) (Watt & Breyer-Brandwijk, 1962).	Used to treat athlete's foot, and is helpful in treating coughs, cuts, asthma, bladder disorders and headaches (Watt & Breyer-Brandwijk, 1962). Extract also have the ability to reduce serum cholesterol levels *in vivo* (Agbafor & Akubugwo, 2007).	Aqueous leaf extracts in animal models had the ability to lower fasting plasma glucose levels dose-dependently (Adeneye & Agbaje, 2007). The most significant hypoglycaemic effect is seen at the oral dose of 500 mg/kg/ per day of the extract (Adeneye & Agbaje, 2007).	The fresh leaf aqueous extract is able to lower fasting plasma glucose dose-dependently (Adeneye & Agbaje, 2007) and Leite *et al.*, 1986).
C. pepo	*H*as a long prickly stem bears large downy leaves and deep yellow	The seeds are used to treat the prostate gland and the oil from the	Pumpkin seed and fruit reduces blood glucose levels in type II	The protein bound polysaccharide isolated from

	coloured funnel-shaped flowers and it bears orange fruit containing numerous flat white seeds (Watt & Breyer-Brandwijk, 1962). This plant is endemic to Americas (Mexico) (Smith, 1997).	seeds is used in the treatment of urinary ailments (Watt & Breyer-Brandwijk, 1962).	diabetic patients at 30 g of pumpkin powder/person (Caili & Quanhong, 2006; Quanhong *et al.*, 2005). The fruit and seeds of pumpkin have hypoglycaemic activity in normal and alloxan-induced rats and rabbits (Zhang, 2001; Peng, 2002). The activity of pumpkin leaves is yet to be investigated.	pumpkin increases levels of serum insulin, reduces glucose levels and improve glucose tolerance *in vivo* albeit at very high dose (Quanhong *et al.*, 2005).
N. floribunda	A small to medium sized tree 3 to 10 m tall and has sweetly scented cream-white flowers small in size, around 3 mm long-cymose panicles.. This plant is endemic to southern and central tropical Africa (Von Breitenbach, 1965).	The forest elder has a number of uses as traditional Zulu medicine. Several parts of the plant are used to an array of ailments from coughs to colds and influenza (Hutchings *et al.*, 1996).	no literature	Not applicable
H.hemerocallidea	This plant is a tuberous perennial plant with strap like leaves and bright yellow star-shaped flowers.	It is used by the Botswana people as a charm against thunder and storms (Watt & Breyer-Brandwijk,	I*n vivo* rodent studies showed the aqueous plant extract to posess anti-diabetic agent (Drewes *et al.*, 1984;	This plant's aqueous extract causes a significant reduction in blood glucose levels

	The leaves are up to 400 mm long. The lower surface of leaves is densely hairy. It is endemic to southern Africa (Eastern cape, Free state, KwaZulu-natal, Mpumalanga, Gauteng and Limpopo), occurs in open grassland and woodlands (Watt & Breyer-Brandwijk, 1962).	1962). Traditional western ailments treated include urinary infections, hypertension, testicular tumours and HIV/AIDS.	Ojewole, 2002; Mahomed & Ojewole, 2003; Ojewola 2006).	through the stimulation of insulin which enhances the cellular uptake and utlization of glucose (Drewes *et al.*, 1984; Ojewole, 2002; Mahomed & Ojewole, 2003; Ojewola 2006).
C. cassia	A slender evergreen tree that grows up to 20 metres high. Young branches are smooth and brown. It has small white flowers and green fleshly fruits with one seed and turns dark purple or black when mature. This plant is endemic to China (Barceloux, 2009).	Used as an astringent, germicide, chronic bronchitis and many other ailments (Barceloux, 2009) (Table 1).	The efficacy of *C. cassia* has been proven in several clinical trails suggesting its effectiveness in lowering plasma glucose levels in patients (Khan *et al.*, 2003; Mang *et al.*, 2006; Dugoua *et al.*, 2007; Pham *et al.*, 2007).	It has been reported that bark extracts of *C. cassia* contains an insulin-like peptide, cinnanaldehyde known to inhibit aldose reductase and is more active than other derivatives of the compound (Lees, 2002).

Table 3: The inhibition of α Amylase activity (%) and EC_{50} (mg/ml) for each of the solvents used in this study at the minimum and maximum dose.

Solvents	Acetone		Methanol		Ethyl acetate		Hexane	
Concentrations (mg/ml)	Emax	EC_{50} (mg/ml)	Emax	EC_{50} (mg/ml)	Emax	EC_{50} (mg/ml)	Emax	EC_{50} (mg/ml)
Acarbose in water	84.06	0.56	84.06	0.56	84.06	0.56	84.06	0.50
H. hemerocallidea	59.62	0.92	52.33	1.32	87.82	0.29	54.00	0.960
C. citratus	88.21	0.65	88.07	0.31	44.81	1.2	34.99	1.30
C. cassia	0	>1	100.88	0.12	0	>1	99.93	0.72
C. pepo	35.10	1.82	0	>1	0	>1	72.29	0.70
N. floribunda	0	>1	0	>1	37.91	1.6	55.11	0.88
S. alexandrina	0	>1	0	>1	0	>1	97.10	0.083

EC_{50}: Concentration that produces 50% of the maximum response. Emax: Maximum inhibitory activity (100% is maximum inhibition)

Table 4: The glucose quantity (mg) present after treatment of intestinal rat acetone powder and the α Glucosidase inhibitory activity (%) of different extracts of six plants used to treat and manage diabetes

Plant species	Glucose quantity (%)*				Inhibition (%) **			
	Ethyl acetate	Hexane	Methanol	Acetone	Ethyl acetate	Hexane	Methanol	Acetone
Acarbose	<0.0001	<0.0001	<0.0001	<0.0001	100	100	100	100
C. pepo	0.004	0.018	0.297	0.654	99.56	98.24	70.30	34.58
C. cassia	0.004	0.126	1.066	0.624	99.64	87.38	NA*	61.27
S. alexandrina	0.009	0.021	0.387	0.350	99.09	97.93	61.27	65.04
N. floribunda	0.005	<0.00001	0.871	0.659	99.53	100	12.85	34.11
H. hemerocallidea	0.052	0.010	0.414	0.156	94.83	99.01	58.61	84.35
C. citratus	0.050	<0.0001	0.546	0.294	95.02	100	45.42	70.55

*amount of glucose produced was calculated as: Glucose (mg): y = (17.75x + 0.0162)*10 (y=OD; x= concentration (mg); 10= dilution factor made in sample preparation). Values are means of *n*=3. *NA-not detectable (active). **Percentage inhibition was calculated as follows: Inhibitory activity (%) = (100- G_a), G_a is the percentage amount of glucose (table 6) that was left over after the reaction was stopped. NA: No activity