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α-Glucosidase inhibitory potential of selected anti-diabetic plants used in North-Western Nigeria

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α-Glucosidase inhibitory potential of selected anti-diabetic plants has been studied. The study evaluated α-glucosidase inhibition using α-glucosidase from *Saccharomyces cerevisiae* and *p*-nitrophenyl α-D-glucopyranoside as substrate. The result showed that the extract of *Albizzia chevalieri* leaf, *Khaya senegalensis* stem bark, *Zizypus spina-christi* stem bark, *Arachis hypogea* seed varieties and *Mangifera indica* leaf had significant ($P < 0.05$) α-glucosidase inhibitory effect in a concentration dependant manner as compared to acarbose. Cardiac glycosides, alkaloids, saponins, flavonoids and tannins were identified in the extracts. The study concludes that the plant extract contain bioactive compound that may be source(s) of lead compounds with α-glucosidase inhibitory potentials and may explain their hypoglycaemic effects.

Key words: Postprandial hyperglycaemia, α-glucosidase inhibitor, antidiabetic plants, hypoglycaemic potential.

INTRODUCTION

Type 2 diabetes mellitus is one of the most common chronic diseases in most countries. The prevalence of the disease is estimated to double by 2030 with 69% increase in developing countries and 20% increase among adults in developed countries (Shaw et al., 2010). Conventional treatments for the management of diabetes mellitus include: enhancement of the action of insulin at the target tissues, with the use of sensitizers (biguanides, thiozolidinediones); stimulation of endogenous insulin secretion, with the use of sulfurnylureas (glibenclamide, glimipiride), and reduction of the demand for insulin using specific enzyme inhibitors (acarbose, meglitol) (Groop et al., 1997). However, there is a burden of unwanted side-effects that may among others, include; hypoglycemia, diarrhea, nausea, dyspepsia, myocardial infarction, peripheral edema and dizziness, with the use of these drugs. Also, the incalculable costs as well as unavailability of these drugs are also deterrent factors to drug adherence. These challenges calls for concern and therefore underscores the need for appropriate and effective therapies in the management of the disease and its complications. Different medicinal plants in North-Western Nigeria have been explored for their anti-diabetic properties, and several scientific research has collaborated these claims (Etuk and Mohammed, 2009; Saidu et al., 2007a, 2010).

Postprandial hyperglycaemia contributes to the development of macro and micro vascular complication associated with diabetes (Baron, 1998). Therefore one of the therapeutic approaches in type 2 diabetes is to reduce

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the demand for insulin by lowering the corresponding postprandial hyperglycemic levels (Adams et al., 2010). Research has shown that Inhibition of α-glucosidase enzyme located at the intestinal brush border of the intestine may play a role in the lowering of postprandial hyperglycemia (Franco et al., 2002; Notkins, 2002). Available α-glucosidase inhibitors include; acarbose, voglibose and meglitol. Other probable sources of inhibitors or lead compounds with potential α-glucosidase inhibition are medicinal plant preparations with significant hypoglycaemic effect (Adolfo et al., 2008; Prabhakar and Doble, 2008). Attempt at understanding the pharmacological features of these medicinal plants with their different chemical compound that may hitherto poses hypoglycemic potentials necessitated this study. The current study therefore reports the α-glucosidase inhibitory potentials of some medicinal plants used in North-Western Nigeria for treatment of diabetes mellitus.

To achieve the stated objective as above, the α-glucosidase inhibitory potentials of 10 indigenous medicinal plants used in the study area were screened. The plants are listed subsequently.

Albizzia chevalieri

Studies on *Albizia* species have indicated the presence of phenolic compounds from *Albizzia amara* with significant antioxidant activity (Muchuweti et al., 2006), and *Albizzia lebbeck* was reported to contain 3, 5 dihydroxy 4, 7 dimethoxy flavone and N-benzoyl-lphenyl alaninol (Rashid et al., 2003). *A. chevalieri* was reported for effective hypoglycaemic agent (Saidu et al., 2007a).

Khaya senegalensis A. Juss

This is one of the most popular medicinal meliaceous plants used in traditional African remedies (Iwu, 1993). The plant extracts of *K. senegalensis* have been reported to exhibit anti-diabetic effects (Etuk et al., 2009) as well as anti-bacterial (Koné et al., 2004), anti-tumor, anti-oxidant (Androulakis et al., 2006), and anti-plasmodial activities (El-Tahir et al., 1998).

Solanum incanum Linnaeus

The presence of pharmacologically active compounds in *Solanaceae* species has been known for centuries, solanocapsine of *Solanum pseudocapsicum* is antibacterial, the drug solanine, found in potatoes (*Solanum tubersum*) is antifungal. Beta-solamarine isolated from *Solanum dulcamara* inhibits sarcoma in mice, and until recently, the only therapeutic agents (antispasmodic agents) for Parkinsonism were obtained from this family.

Zizypus spina-christi Willd

This plant has versed pharmacological and nutritional values (Eden Foundation, 1992; Burkill, 1997; Al-Khalifa and Al-Arify, 1999). It was reported to demonstrate antidiabetic, antidiarrhoeal, antibacterial, anticancer, anti-nociceptive, antihypertensive and central nervous system (CNS) effect (Ali et al., 2001; Shahat et al., 2001; Adzu et al., 2001, 2002a, 2003; Abdel-Wahhab et al., 2007).

Arachis hypogea (peanut)

Is native to the study area. Some therapeutic effects have been reported for peanut seed extracts, such as antioxidative, antidiabetic, antibacterial, antifungal, and anti-inflammatory activities. *A. hypogea* are a potent producer of stilbene-derived phytoalexins (Subba and Strange, 1995; Sobolev et al., 2006, 2009). Stilbenoids have been considered the major sustaining factor of the plant's resistance to diseases (Subba and Strange, 1995).

Vernonia amygdalina Del

The hypoglycaemic effect of the aqueous extract of the leaves of *vernonia amygdalina* has been reported (Akah and Okafor, 1992). This was strengthened by the observation that the aqueous extract produced significant hypoglycaemic effect in diabetic and normal rats when compared to the effect of the standard drug – chlorpropamide (Osinubi, 1996). Further studies by Uhuegbu and Ogbechi (2004) and Nwanjo and Nwokoro (2004) on the effects of the aqueous extracts of the plant corroborate these claims.

Calotropis procera leaf

Is known to possess multifarious medicinal properties. The blood glucose reducing property of *C. procera* was assessed by an oral glucose tolerance test (OGTT) in STZ-diabetics (Uddin et al., 2008). The root of *C. procera* is used as a carminative in the treatment of dyspepsia (Kumar and Arya, 2006). The root bark and leaves of *C. procera* are used by various tribes of central India as a curative agent for jaundice (Samvatsar and Diwanji, 2000). The chloroform extract of the root has been shown to exhibit protective activity against carbon tetrachloride induced liver damage (Basu et al., 1992).

Azadirachta indica leaf De Jussieu (neem)

The possible mechanisms underlying the hypoglycaemic activity of the aqueous leaf extract have been discussed (Dubey, 1994). Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia (Manickam et al., 1997). Aqueous leaf extract also reduces hyperglycaemia in streptozotocin diabetes and the effect is possibly due to presence of a flavonoid or quercetin (Gomes, 1995; Chattopadhyay, 1996). A significant hypoglycaemic effect was also observed by feeding neem oil to fasting rabbits (Dubey, 1994).

Mangifera indica leaf Lin

The phytochemical contents of the different parts of *M. indica* are reviewed in Ross (1999). Muruganandan et al. (2002, 2005) investigated the effects of mangiferin on hyperglycaemia, atherogenicity and oxidative damage to cardiac and renal tissues in streptozotocin-induced diabetic rats. They reported that the antidiabetic activity of mangiferin could involve mechanisms other than pancreatic β-cell insulin secretion. In glucose-loaded normal rats, mangiferin induces a significant improvement in oral glucose tolerance but without alteration of basal plasma glucose levels (Muruganandan et al., 2005).

Moringa oleifera leaf

Moringa leaves are rich source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, phenolics, flavonoids, and carotenoids (Makkar and Becker, 1996; Anwar et al., 2005). The plant has also been reported to exhibit other varied activities. Aqueous leaf extracts can be used to treat hyperthyroidism and exhibit an antioxidant effect; they also regulate thyroid hormone (Pal et al., 1995a, b; Tahiliani and Kar, 2000).

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used for this study were of analytical grade. α-Glucosidase (G0660) (from *Saccharomyces cerevisiae*), 4-nitrophenyl α-D-glucopyranoside (N1377), arcabose (A8980) and L-Glutathione (G4251) were obtained from Sigma-Aldrich, Germany.

Plants

All plants were collected within the study area (130° 21' 16" N and 50° 5' 37" E), most plant materials were collected in and around the main campus of Usmanu Danfodio University, Sokoto, Nigeria. *A. chevalieri* was obtained from Sanyinna village, 50 km south of Sokoto, Nigeria while *A. hypogea* seed varieties (bahausa, madina and kampala) were purchased from Sokoto Central market. The plant materials were identified and authenticated by a Taxonomist, Dr Umar Abdullahi, from the Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto (UDUS). Voucher specimens were deposited at the herbarium of UDUS and voucher numbers assigned: *A. chevalieri* leaf (UDUS/VS/2004/09), *K. senegalensis* stem bark (UDUS/VS/2011/21), *S. incanum* leaf (UDUS/VS/2011/22), *Z. spina-christi* stem bark (UDUS/VS/2011/23), *A. hypogea* seed varieties; bahausa (UDUS/VS/2011/24), *madina* (UDUS/VS/2011/25), kampala (UDUS/VS/2011/26), *V. amygdalina* leaf (UDUS/VS/2011/27), *C. procera* leaf (UDUS/VS/2011/28), *A. indica* leaf (UDUS/VS/2011/29), *M. indica* leaf (UDUS/VS/2011/30), *M. oleifera* leaf (UDUS/VS/2011/31).

Preparation of plant materials

The leaves and stem bark of corresponding plants were sun dried and ground to fine powder and then stored in plastic bags until required. Cold aqueous extracts of the plant material were prepared by soaking the plant powder in distilled water for 24 h. A 5% w/v of all the plants material with the exception of *A. hypogea* varieties in which a 40% w/v of the seed with its seed coat intact were prepared. The mixtures were subsequently filtered through a muslin cloth to remove debris and then filtered through a Whatman no.1 filter paper and evaporated in a drying cabinet set at 40°C (Harborne, 1973). The corresponding percentage yield was calculated. Dry weight of each crude extract was further reconstituted (10% w/v) in distilled water and used for screening for phytochemical and α-glucosidase inhibitors.

Phytochemical screening

The methods described by Trease and Evans (1989) and Abalaka et al. (2011) were used for phytochemical screening of the extracts.

1) Alkaloids: 1 ml of 1% HCl was added to 3 ml of the extract in a test tube. The mixture was then heated for 20 min, cooled and filtered. About 2 drops of Mayer's reagent were added to 1 ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.
2) Tannins: 1 ml of freshly prepared 10% KOH was added to 1 ml of the extract. A dirty white precipitate showed the presence of tannins.
3) Cardiac glycoside (Keller-Killani test): 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of cardiac glycoside.
4) Saponins-frothing test: 2 ml of the extract was vigorously shaken in the test tube for 2 min. Persistent frothing indicated the presence of saponins.
5) Phlobatanins: 1 ml of the extract was added to 1% HCl. Red precipitate indicated the presence of phlobatanins.
6) Flavonoids: 1 ml of 10% NaOH was added to 3 ml of the extract. A yellow colouration was indicative of the presence of flavonoids.

Determination of α-glucosidase inhibition

α-Glucosidase inhibition was determined by the method of Adams

Table 1. Percentage yield of the aqueous plant extracts of antidiabetic plants used in North-Western Nigeria.

Plant	Parts used	Yield (%)
A. chevalieri	Leaf	10.3
A. hypogea (bahausa)	Seed	0.7
A. hypogea (madina)	Seed	0.6
A. hypogea (kampala)	Seed	0.4
A. indica	Leaf	17.3
C. procera	Leaf	21.6
K. senegalensis	Stem bark	13.3
M. indica	Leaf	12.3
M. oleifera	Leaf	21.3
S. incanum	Leaf	18.3
V. amygdalina	Leaf	22.3
Z. spina-Christi	Stem bark	8.0

et al. (2010) with modifications. The reaction mixture contained 5 ml, 67 mM potassium phosphate buffer, pH 6.8, 0.2 ml 3 mM glutathione (GSH) and 0.2 ml α-glucosidase (0.15 U/ml) from *Saccharomyces cerevisiae*. The mixture was equilibrated to 37°C for 5 min. The reaction mixture was activated by the addition of 0.5 ml 10 mM *p*-nitrophenyl-α-glucoside in the absence or presence of 50 µg of the different plant extract for 20 min at 37°C. Into a test tube containing 8 ml of 100 mM Na_2CO_3 was added 2 ml of the reaction mixture to terminate the reaction. The enzyme activity was monitored by taking the spectrophotometric absorbance of *p*-nitrophenol at 400 nm using optima sp-300 spectrophotometer. One unit of α-glucosidase was taken as the amount of enzyme liberating 1.0 µmol of *p*-nitrophenyl from *p*-nitrophenyl-α-glucoside per minute at pH 6.8 and 37°C. Arcabose was used as the positive control.

Median inhibitory concentration IC_{50}

The IC_{50} of the plant extract which had α-glucosidase inhibition above arcabose were determined using the procedure for determination of α-glucosidase inhibition as described above but with increasing concentration of 01.25 to 50 µg/ml of plant extract. The IC_{50} value of the test substance was determined through a nonlinear regression analysis of the dose response curve.

Statistical analysis

Results are presented as mean ± standard deviation. Significant differences between the mean values were determined by analysis of variance (ANOVA) followed by Dunnett's test, and $P < 0.05$ was considered statistically significant.

RESULTS

Percentage yields

The percentage yields of the extracts are presented in Table 1. The percentage yield ranged from 0.4 to 22.3%.

Phytochemical screening

Table 2 shows phytochemical content of the extracts. The phytochemical detected include tannins, saponins, flavonoids, cardiac glycosides and phlobatannins. Based on the phytochemical study conducted for these plants, the result shows that three of the plants; *A. hypogea* (kampala), *Z. spina-christi,* and *K. senegalensis* had similar phytochemical distribution, four of the plants; *M. indica, M. oleifera, A. chevalieri* and *S. incanum* also had similar phytochemical distribution while two plants; *A. hypogea* variety (bahausa and madina), *V. amygdalina* and *C. procera* had distinct phytochemical distribution from any other. Saponin was the major phytochemical found in all the extract while alkaloids was completely absent, the other phytochemicals were either present or absent.

Determination of α-glucosidase inhibition

Preliminary evaluation of the percentage inhibition of α-glucosidase activity in the presence of 50 µg/ml of all extracts/acarbose is presented in Figure 1. The result indicated that not all the extract posses α-glucosidase activity. The result revealed that while *K. senegalensis* stem bark (82.2 ± 1.0)*, Z. spina-christi* stem bark (92.3 ± 4.3), *A. chevalieri* leaf (82.2 ± 1.0)*, M. indica* leaf (57.1 ± 6.0) and *A. hypogea* seed varieties: madina (93.5 ± 1.9), kampala (88.9 ± 2.4) and bahausa (93.2 ± 2.1) showed significant ($P < 0.05$) inhibitory activity on α-glucosidase compared to control drug arcabose (32.9 ± 3.8), *V. amygdalina* (11.8 ± 3.8) leaf, *M. oleifera* leaf, *C. procera* leaf and *S. incanum* leaf were not significant ($P > 0.05$) compared with control. It was shown that the extract of *M. oleifera* (-8.6 ± 5.6) leaf and *S. incanum* leaf (-24.8 ± 7.1) enhanced α-glucosidase activity.

Median inhibitory concentration IC_{50}

IC_{50} value of extracts that were significantly ($P < 0.05$) higher than control (Figure 1) were further determined. Dose response evaluation and determination of IC_{50} values of plant extract against α-glucosidase (*S. cerevisiae)* activity is presented in Figures 2 and 3, respectively. The extracts exhibited a hyperbolic, sigmoidal and negative sigmoidal dose dependent inhibitory effect. The *K. senegalensis* stem bark (IC_{50} = 7.7 ± 0.02 µg /ml) had the most effective inhibitory potential closely followed by *Z. spina-christi* stem bark (IC_{50} = 7.9 ± 0.02 µg/ml), *A. hypogea* seed (bahausa) (IC_{50} = 8.2 ± 0.02 µg/ml), *A. hypogea* seed (danmadani) (IC_{50} = 8.7 ± 0.05 µg/ml), *A. hypogea* seed (kampala) (IC_{50} = 10.5 ± 0.01 µg/ml) and *A. chevalieri* leaf (IC_{50} =

Table 2. Phytochemical screening of aqueous extracts of anti-diabetic plants used in north-western Nigeria

Plant	Tannin	Saponin	Flavonoid	Alkaloid	Cardiac glycoside	Phlobotanin
A. chevalieri	-	+	+	-	-	-
A. hypogea (bahausa)	-	+	+	-	+	-
A. hypogea (madina)	+	+	-	-	-	-
A. hypogea (kampala)	+	+	-	-	+	-
A. indica	+	+	+	-	-	-
C. procera	+	+	+	-	+	-
K. senegalensis	+	+	-	-	+	-
M. indica	-	+	+	-	-	-
M. oleifera	-	+	+	-	-	-
S. incanum	-	+	+	-	-	-
V. amygdalina	-	+	+	-	+	+
Z. spina-Christi	+	+	-	-	+	-

+ = Present, - = Not detected.

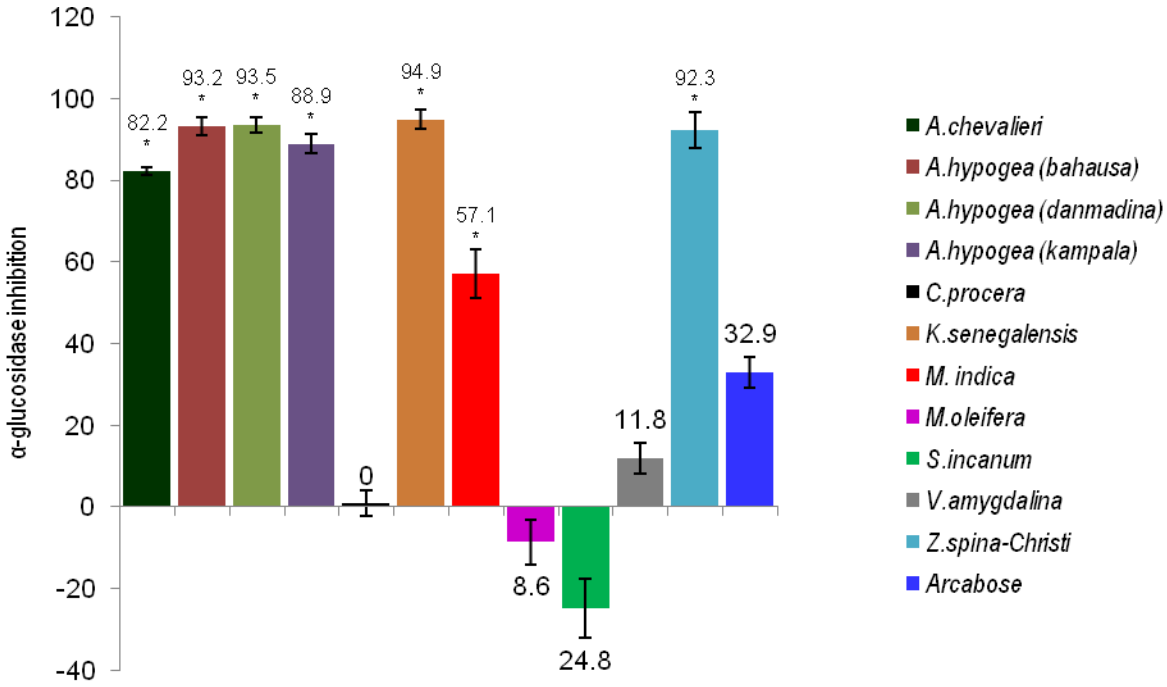

Figure 1. Percentage α-glucosidase inhibition in the presence of 50 µg/ml of extract/acarbose. Data is indicated as mean ± SD of n = 3. Inhibition% ($P < 0.05$) are significantly higher than control (acarbose) determined by ANOVA.

28.2 ± 0.05 µg/ml), the least effective was observed in *M. indica* leaf (IC_{50} = 59.0 ± 0.17 µg /ml).

DISCUSSION

Treatment of type II diabetes is complicated by several factors inherent to the disease, and elevated post prandial hyperglycaemia (PPHG) is one of the risk factors (Gin and Rigalleau, 2000). PPHG is elevated by the action of glucosidases, a class of enzymes that helps in the breakdown of complex carbohydrates into simple sugars such as glucose. α-Glucosidase inhibitors play a major role in managing PPHG in diabetic patients by

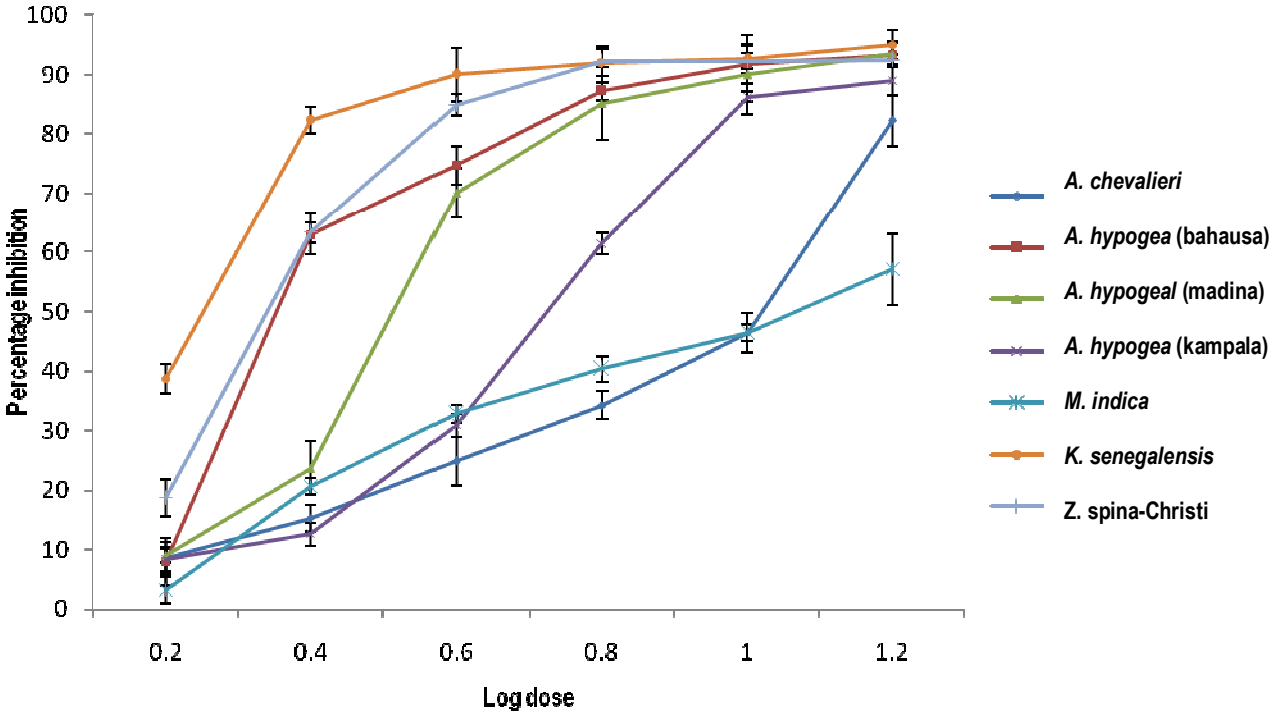

Figure 2. Percentage inhibition and dose response evaluation of extracts of *K. senegalensis, M. indica, A. chevalier, A. hypogea (bahausa,) A. hypogea (madina), A. hypogea (kampala), Z. spina-christy* at concentrations of 50, 20, 10, 5, 2.5, and 1.25 µg/ml *on S. cerevisiae* α-glucosidase activity. Data is indicated as mean ± SD for n = 3.

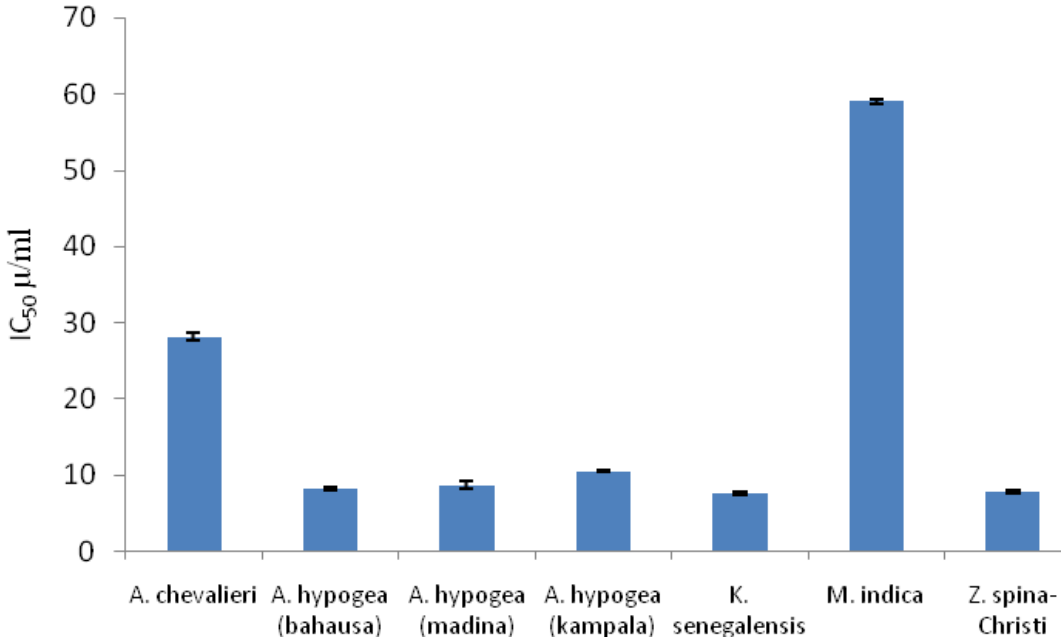

Figure 3. IC_{50} values of extracts of *K. senegalensis, M. indica, A. chevalier, A. hypogea (bahausa,) A. hypogea (madina), A. hypogea (kampala), Z. spina-christy on S. cerevisiae* α-glucosidase. Data is indicated as mean ± SD for n = 3.

reducing starch hydrolysis which shows beneficial effects on glycemic index control in patients (Notkins, 2002).

Plants are natural reservoir of bioactive compounds that may be source(s) of lead compounds with α-glucosidase inhibitory potentials. Some of these compounds have been shown to inhibit α-glucosidase activity (Funke and Melzig, 2005). Herbal extracts used in the study area has been reported for their anti-diabetic activities (Etuk and Mohammed, 2009). The hypoglycemic, toxicity and hypolipidemic effect of the aqueous leaf extract of *A.chevalieri* has been reported extensively by Saidu et al. (2007a, b, c, 2009, 2010). Bilbis et al. (2002) also reported the hypoglycemic and hypolipidemic effect of *A. hypogea* seed in alloxan induced diabetic rats. *V. amygdalina, C. procera,* and *M. indica* aqueous extracts were also reported to possess significant hypoglycaemic effects (Etuk and Mohammed, 2009).

The present research reports plant extracts under study to contained at least two or more secondary metabolite which were differently distributed in the extract (Table 2). It was shown that *K. senegalensis* and *Z. spina-Christi* had similar qualitative composition (tannin, saponin and cardiac glycoside) of compound present while the composition of secondary metabolite in the other extracts varied considerably (Table 2). From the result of the IC_{50} values (Figure 3), the α-glucosidase activity of the extract was ranked. This result is consistent with previous studies on α-glucosidase activity of closely related enzyme (Dineshkumar et al., 2010); it however does not correlate to the ranking based on 'Informant Consensus Informant Selection' study of some of these plants by Etuk and Mohammed (2009) in the study area. Possible explanation for this disparity may be that, even though presence of different secondary metabolites in plant is suggested to be responsible for its hypoglycemic activity, each of this metabolite may have more than one mode of action of antidiabetic activity. For instance, tannins in addition to their α-glucosidase inhibitory activity also inhibit insulin degradation and improve glucose utilization (Peungvicha et al., 1998; Mohamadin et al., 2003). Tannins have antioxidative effect, oxidative stress is one of the important factors in tissue injury in diabetes mellitus and hence potent antioxidants may protect beta cells and increase insulin secretion (Feillet-Coudray et al., 1999). Saponin present in some medicinal plants has been described to demonstrate glucagon decreasing effect which may enhance glucose utilization and lower blood glucose. It was equally reported that saponins stimulates insulin release from pancreas (Norberg et al., 2004). The study reports extract of *A. chevalieri* leaf (IC_{50} = 28.2 ± 0.05 µg/ml), *K. senegalensis* stem bark (IC_{50} = 7.7 ± 0.02 µg /ml), *Z. spina-christi* stem bark (IC_{50} = 7.9 ± 0.02 µg/ml), *A. hypogea* seed varieties; bahausa (IC_{50} = 8.2 ± 0.02 µg/ml), kampala (IC_{50} = 10.5 ± 0.01 µg/ml), madina (IC_{50} = 8.7 ± 0.05 µg/ml), and *M. indica* leaf (IC_{50} = 59.0 ± 0.17 µg /ml) as a good glucosidase inhibitor showing inhibition against α-glucosidase from *S. cerevisiae*. Their inhibitions were significantly ($P < 0.05$) higher than acarbose. The presence of phenolic compounds may suggest their α-glucosidase inhibitory activity. Phenolic compounds have an electron donor capability and are readily oxidized to form phenolate ion or quinone, which is an electron acceptor (Michalak, 2006) thus, they have the ability to block or enhance specific enzymes responsible for digestion of carbohydrates. Tannins are the oligomeric higher molecular weight polyphenolic compounds occurring naturally in plants (Reed, 1995). Hagerman et al. (1992) reports that due to their binding ability with protein and carbohydrates, tannins can inhibit digestive enzymes and reduce the bioavailability of different proteins.

The present study also observed that *M. oleifera* (-8.6 ± 5.6%) leaf and *S. incanum* leaf (-24.8 ± 7.1%) possess α-glucosidase activator/enhancer. Previous reports by Marugan et al. (2010) isolated pulicarside from *Pulicaria undulate* with a strong α-glucosidase promoter activity. The activation of α-glucosidase enzyme is related to prolongation of its stability, this may be either shelf stability or operational stability (Marugan et al., 2010). This observation however, does not contradict other findings of *M. oleifera* leaf and *S. incanum* leaf as hypoglycaemic agent since they may posses other mechanism of hypoglycaemia.

Dose-dependent α-glucosidase inhibitory activity was also observed among the afore-mentioned plants extracts and they were significantly ($P < 0.05$) higher than acarbose. The inhibitory activity became more significant with increasing concentration of the extract. There is possibility to suggest that the bioactive compounds present in the plant extract may be responsible for their α-glucosidase inhibitory activity. However further studies would be required to isolate the bioactive compound and determine their individual IC_{50}. The rich phytochemical constituent and high α-glucosidase inhibitory activity of selected plant extracts under study supports local claims on the efficacy of these plants and provides possible lead for isolation of active compounds.

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