Comparative Study on the α-Amylase and α-Glucosidase Inhibitory Potential of Different Extracts of *Blighia Sapida* Koenig

[1]Kazeem, M. I., [1]Raimi, O. G., [1]Balogun, R. M. and [2]Ogundajo, A. L.

[1]Department of Biochemistry, Lagos State University, P. M. B. 0001, LASU Post-office, Lagos, Nigeria
[2]Department of Chemistry, Lagos State University, P. M. B. 0001, LASU Post-office, Lagos, Nigeria
Corresponding Author: M. I. Kazeem (PhD)
E-mail: mikazeem@gmail.com
Mobile Number: +2348088446672

ABSTRACT

The aim of this study was to investigate the inhibitory effect of *B. sapida* leaf extract on the key enzymes linked to diabetes (α-amylase and α-glucosidase). The inhibitory effect of the leaf extracts on α-amylase and α-glucosidase activities as well as determination of mode of inhibition was performed. The results revealed that the aqueous extract of *B. sapida* was the most potent inhibitor of α-amylase (with IC_{50} 5.80 mg/ml) while ethanolic extract inhibited α-glucosidase (with IC_{50} 4.57 mg/ml) most effectively. The curve of Lineweaver-Burke plot revealed that aqueous extract of *B. sapida* exhibited mixed non-competitive inhibition of α-amylase while ethanolic extract displayed an uncompetitive inhibition of α-glucosidase activities. It can be inferred from this study that the α-amylase and α-glucosidase inhibitory potential of *B. sapida* may be due to the presence of phytochemicals such as saponins and flavonoids. However, further study is required to isolate the enzyme inhibitory component of this plant.

Keywords: Diabetes, *Blighia sapida*, α-amylase, α-glucosidase, enzyme inhibition

{**Citation:** Kazeem, M. I., Raimi, O. G., Balogun, R. M., Ogundajo, A. L. Comparative study on the α-amylase and α-glucosidase inhibitory potential of different extracts of *Blighia sapida* Koenig. American Journal of Research Communication, 2013, 1(7): 178-192} www.usa-journals.com, ISSN: 2325-4076.

1. Introduction

Diabetes mellitus is a metabolic disease which is as old as mankind and its incidence is high (4 – 5%) all over the world (Koyuturk *et al., 2005*). It is also a major cause of disability and hospitalization which results in significant financial burden (Nagappa *et al*., 2003). The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million people likely to be diabetic by the year 2030 as against 191 million estimated in 2000 (Wild *et al*., 2004). The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered.

Besides drugs classically used for the treatment of diabetes such as insulin, sulphonylureas, biguanides and thiazolidinediones, several species of plants have been described in the scientific literature as having hypoglycemic activity (Verspohl, 2002; De Sousa *et al*., 2004; Colca, 2006). Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown (Valiathan, 1998). Some of these plants include *Vernonia amygdalina*, *Zingiber officinale, Ficus exasperata* and *Bligia sapida* (Ogunleye *et al*., 2003; Ojewole, 2006; Ong *et al*., 2011)

Blighia sapida Koenig is a tree plant which belongs to Sapidaceae family. It is found manly in some part of West Africa especially in Nigeria, Ghana and Ivory Coast. The tree is identified with different names like Ackee (English), "Ila" (Nupe), "Isin" (Yoruba), "Gwanja kusa" (Hausa) and "Okpu" (Igbo). It is used in traditional medicine for diabetes mellitus management and hypertension. Its fleshy aril fruit is edible and is known to contain saponins, which are haemolytic (Jimoh *et al*., 2012). The pulp and leaves are used to treat eye conjunctivitis. The seeds are not edible, but the ashes of the dried husks and the seeds are used in

the preparation of soap (Aderinola *et al*., 2007). It is usually planted as a shade tree and its fruit is relished by humans while the leaves are eaten by ruminants. It is well known for its unripe aril that contains hypoglycin A, a water-soluble phytochemical that inhibits gluconeogenesis thus leading to hypoglycemia (Hassall and Reyle, 1955).

α-amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starch from being absorbed by the body. Thus this could be useful in the treatment of obesity and diabetes mellitus. They exert their blood glucose lowering effect through the inhibition of an enzyme such as salivary and pancreatic amylase (Frantz *et al.,* 2005). α-glucosidase inhibitors also has potential to suppress postprandial hyperglycemia in diabetic patients by completely and reversibly inhibiting α-glucosidase in the intestine. This inhibition reduces glucose absorption through delayed carbohydrate digestion and extended digestion time (Shimabukuro *et al.,* 2006). However, a common side effect of enzyme inhibitory drugs like acarbose is the excessive inhibition of pancreatic α-amylase, which can result in abdominal distention, flatulence and diarrhoea (Lebovitz, 1997). These side effects are caused by abnormal fermentation of unhydrolyzed polysaccharides by gut bacteria (Kwon *et al*., 2008).

Therefore the aim of this study was to evaluate α-amylase and α-glucosidase inhibitory potential of different extracts of *Blighia sapida* and assesses the mode(s) of inhibition, as a possible source of hypoglycemic agents in the management of diabetes.

2. Materials and Methods

2.1 *Plant material*

Blighia sapida leaves were collected from its tree in Ibarapa, Ibarapa Local Government Area of Oyo State, Nigeria in the month of May 2012. It was identified and authenticated by Dr.

A. B. Kadiri of the Department of Botany, University of Lagos, Nigeria and voucher specimen (LUH 4726) was deposited in the University herbarium.

2.2 *Chemicals and reagents*

α-amylase from *Aspergillus oryzae*, α-glucosidase from *Saccharomyces cerevisiae* and paranitrophenyl-glucopyranoside were products of Sigma-Adrich Co., St Louis, USA while starch soluble (extra pure) was obtained from J. T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents were of analytical grade and water used was glass-distilled.

2.3 *Preparation of plant extracts*

Fresh leaves of *Blighia sapida* were cut and washed with water to remove all contaminants; they were dried under room temperature and grounded to powder. The powdered leaves were divided into three portions and each portion was dissolved in acetone, ethanol or water. They were all left to steep in covered containers for 24 hours; the resulting infusion was decanted, filtered using muslin cloth and evaporated to dryness in a water bath at 40 ^{o}C. Dried extracts were weighed and dissolved in 10% dimethylsulphoxide to yield a stock solution from which lower concentrations were prepared.

2.4 *Phytochemical screening*

Phytochemical compositions of the leaves were determined using the methods variously described by Trease and Evans (1996) and Sofowara (2006).

2.5 *α-Amylase inhibitory assay*

This assay was carried out using a modified procedure of McCue and Shetty (2004). A total of 250 µL of extract was placed in a tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. This solution was pre-incubated at 25 ^{o}C for 10 mins, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added

at timed intervals and then further incubated at 25 °C for 10 mins. The reaction was terminated after incubation by adding 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 mins and cooled to room temperature. The reaction mixture was diluted with 5 ml distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition.

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{extracts})/Abs_{control}] \times 100.$$

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

2.6 *Mode of α-amylase inhibition*

The mode of inhibition of the leaf extract was conducted using the extract with the lowest IC_{50} according to the modified method described by Ali *et al*. (2006). Briefly, 250 µL of the (5 mg/mL) extract was pre-incubated with 250 µL of α-amylase solution for 10 minutes at 25 ºC in one set of tubes. In another set of tubes α-amylase was pre-incubated with 250 µL of phosphate buffer (pH 6.9). 250 µL of starch solution at increasing concentrations (0.30 – 5.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 mins at 25 °C, and then boiled for 5 mins after addition of 500 µL of DNS to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a maltose standard curve and converted to reaction velocities. A double reciprocal plot (1/V versus 1/[S]) where v is reaction velocity and *[S]* is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on α-amylase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

2.7 *α-Glucosidase inhibitory assay*

The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim *et al*. (2005), using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitropheynyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. Then 100 µL of α-glucosidase (E.C. 3.2.1.20) was pre-incubated with 50 µL of the different concentrations of the extracts (acetone, ethanol and water) for 10 mins. Then 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37 ^{o}C for 20 mins and stopped by adding 2 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow colored para-nitrophenol released from pNPG at 405 nm. The results were expressed as percentage of the blank control.

Percentage inhibition calculated as

$$\% \text{ Inhibition} = [(Abs_{control}-Abs_{extract})/Abs_{control}] \times 100$$

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

2.8 *Mode of α-glucosidase inhibition*

The mode of inhibition of the leaf extract was determined using the extract with the lowest IC_{50} according to the modified method described by Ali *et al*. (2006). Briefly, 50 µL of the (5 mg/mL) extract was pre-incubated with 100 µL of α-glucosidase solution for 10 mins at 25 ºC in one set of tubes. In another set of tubes α-glucosidase was pre-incubated with 50 µL of phosphate buffer (pH 6.9). 50 µL of PNPG at increasing concentrations (0.63 – 2.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 mins at 25 °C, and 500 µL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released

was determined spectrophotometrically using a paranitrophenol standard curve and converted to reaction velocities. A double reciprocal plot (1/V versus 1/[S]) where V is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on α-glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

3.0 Results

Percentage yield of different extracts of *B. sapida* is shown in table 1. Acetone extract has the highest percentage yield when compared to others. From the analysis carried out (Table 2) on the phytochemical screening of different extracts of the plant, it was observed that all the extracts have flavonoids, reducing sugar and tannins. Also terpenoids were detected from aqueous and ethanolic extract while saponin was present in the aqueous extract only.

Table 1: Percentage yield of different extracts of *B. sapida*

Extracts	Initial weight	Final weight	% yield
Acetone	28.31	1.73	6.11
Ethanol	42.82	1.26	2.94
Water	41.82	2.1	5.02

Table 2: Phytochemical composition of different extract of *Blighia Sapida*

Phytochemicals	Extracts inference		
	Acetone	Ethanol	Water
Anthraquinones	-	-	-
Flavonoids	+	+	+
Reducing sugar	-	+	-
Saponins	-	-	+
Steroids	+	-	-
Tannins	+	+	+
Terpenoids	-	+	+

Fig. 1 shows the percentage inhibition of α-amylase by various extracts of *B. sapida*. At low concentration (1.25 – 10 mg/mL) there were no significant differences among all the extracts tested. However, at 10 mg/mL acetone extract significantly reduced ($p < 0.05$) when compared to others. From the IC_{50} extrapolated graphically, it shows that aqueous extract of *B. sapida* has lowest IC_{50} value which gives more inhibitory potential compared to ethanol and acetone extracts (table 3). The Lineweaver-Burke plot was generated to determine the mode of inhibition of the enzyme and the result showed that aqueous extract of *B. sapida* inhibited α-amylase in a mixed non-competitive manner (Figure 2).

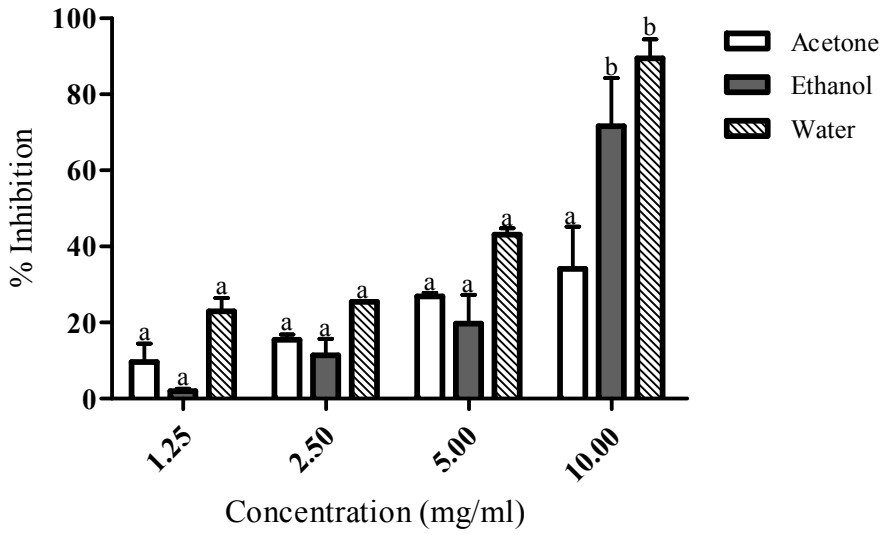

Fig. 1 Percentage inhibition of α-amylase by different extracts of *Blighia Sapida*

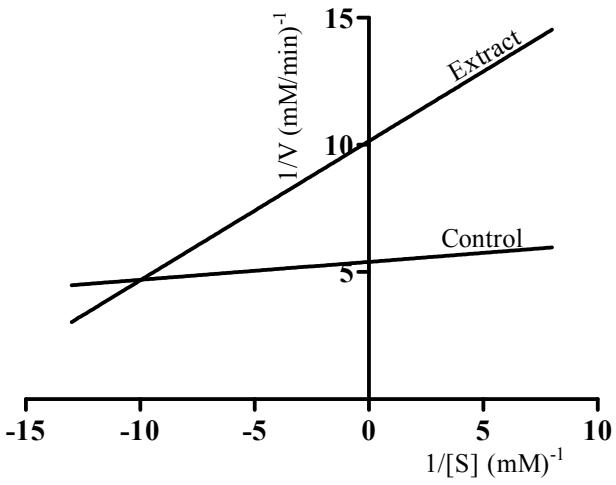

Fig. 2. Mode of inhibition of α-amylase by aqueous extract of *Blighia sapida*

Table 3: IC_{50} value of various extracts of *Blighia sapida* against alpha Amylase

Extracts	IC_{50} (mg/ml)	
	α-amylase	α-glucosidase
Acetone	13.25 ± 2.10	5.15 ± 0.20
Ethanol	8.50 ± 1.31	4.57 ± 0.21
Water	5.80 ± 0.70	5.20 ± 0.41

Figure 3 shows the percentage inhibition of α-glucosidase by different extracts of *B. sapida*. The values from all the extracts were not significantly different ($p > 0.05$) from one another at the same concentration. However, the IC_{50} values obtained from the dose-response curves of the extracts showed that ethanolic extract has the lowest value (Table 3). Figure 4 depicts that the mode of inhibition of α-glucosidase by the ethanolic extract of *B. sapida* is an uncompetitive type.

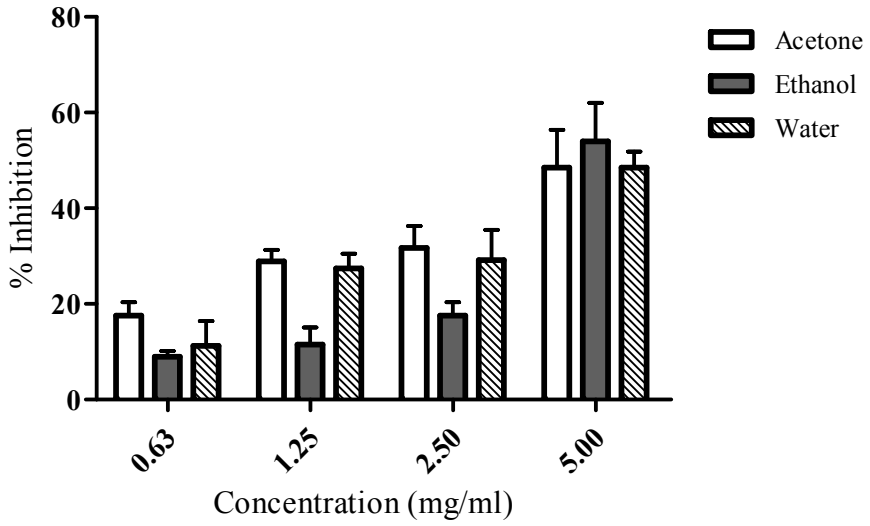

Fig. 3 Percentage inhibition of α-glucosidase by different extracts of *Blighia Sapida*

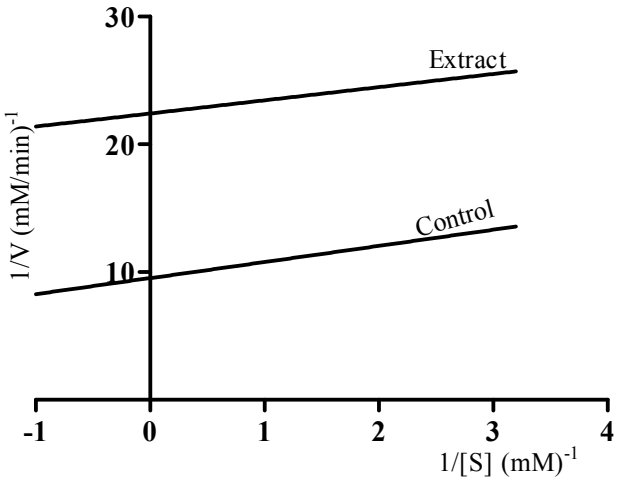

Fig. 4. Mode of inhibition of α-glucosidase by ethanolic extract of *Blighia sapida*

4.0 Discussion

Management of blood glucose level is a critical strategy in the control of diabetes and its complications (Dicarli *et al*., 2003). α-amylase and α-glucosidase inhibitors have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type 2 diabetes mellitus. Inhibition of these enzymes delay carbohydrate digestion and prolong overall carbohydrates digestion time causing a reduction in the rate of glucose absorption and consequently reducing postprandial plasma glucose rise (Kimura *et al*, 2001).

The low percentage inhibition of α-amylase by the extracts of *B. sapida* is a pointer to the fact that the plant is a mild inhibitor of the enzyme, which is desirable in order to prevent some of the side effects produced by synthetic drugs. This is also in agreement with the report of Pinto *et al*. (2009) that dietary management of hyperglycemia linked to diabetes can be targeted through foods or botanical supplements that have moderate α-amylase inhibition. The characteristic mixed non-competitive inhibition by the aqueous extract of *B. sapida* suggest that

the active components in the extract do not compete with the substrate for the active site of the enzyme, rather the inhibitors bind to a separate site on the enzyme to retard the conversion of substrate to product (Mayur *et al*., 2010).

As for α-glucosidase inhibition, the strong inhibition of the enzyme displayed by all the extracts especially ethanoilc extract as shown by the lowest IC_{50} (4.57) suggests that this extract contains potent α-glucosidase inhibitor. This is because any plant or drug which is a strong inhibitor of α-glucosidase could serve as effective therapy for postprandial hyperglycemia with minimal side effects (Kwon *et al*., 2006). Further study to ascertain the mode of inhibition of α-glucosidase by the ethanolic extract of this plant showed the extracts inhibited the enzyme uncompetitively. This suggests that the inhibitor binds exclusively to the enzyme-substrate complex yielding an inactive enzyme-substrate-inhibitor complex (Bacchawat *et al*., 2011).

The inhibitory effects of aqueous and ethanolic extract of *B. sapida* on the α-amlyase and α-glucosidase activities respectively may be attributed to the presence of phytochemicals such flavonoids, tannins and saponins. Previous studies attributed the medicinal property of the aqueous extract of this plant to the presence of saponins (Abolaji *et al*., 2007; Aderinola *et al*., 2007). Moreover, a water-soluble pure compound named hypoglycin was isolated from this plant which also suggests that the plant possesses hypoglycemic properties (Hassall and Reyle, 1955). This also explains the reason behind the effective inhibitory activity displayed by the aqueous extract towards these enzymes when compared to that exhibited by acetone and ethanolic extract. The α-amylase and α-glucosidase potential of this this plant may also be attributed to the presence of flavonoids which have been reported to preserve β-cell integrity and function by mopping up free radicals in the system and therefore protect against the progression of insulin resistance of type 2 diabetics (Sama *et al*., 2012).

It can be concluded that the aqueous extract of *B.sapida* effectively inhibited the activity of α-amylase in a non-competitive manner while ethanolic extract inhibited α-glucosidase in an uncompetitive manner respectively. This inhibitory property of the extract maybe attributed to the presence of phytochemicals such as saponins and flavonoids. However, further study is required to isolate the active enzyme inhibitory component from this plant.

REFERENCES

Abolaji, O. A., Adebayo, A. H. and Odesanmi, O. S. (2007). Nutritional qualities of three medicinal plant parts (*Xylopia aethiopica, Blighia sapida* and *Parinari polyandra*) commonly used by Pregnant Women in the Western Part of Nigeria. Pakistan Journal of Nutrition 6 (6): 665-668.

Aderinola, O. A., Farinu, G. O., Akinlade, J. A., Olayeni, T. B., Ojebiyi, O. O. and Ogunniyi, P. O. (2007). Nutritional potential of *Blighia sapida* Koenig (Ackee) leaves as a dry season feed resource for West Africa dwarf Goats in the derived savanna zone of Nigeria. Livestock Res. & Rural Dev., 19(6): 1-5.

Ali, H., Houghton, P. J. and Soumyanath, A. (2006). Alpha-amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus.* Journal of Ethnopharmacology 107(3): 449-455.

Bachhawat. A. J., Shihabudeen, M. S. and Thirumurugan, K. (2011). Screening of fifteen Indian ayurvedic plants for alpha-glucosidase inhibitory activity and enzyme kinetics. International Journal of Pharmacy and Pharmaceutical Sciences 3(4): 267-274

Colca, J.R. (2006). Insulin sensitizers may prevent metabolic inflammation. Biochem. Pharmacol. 72: 125–131.

De Sousa, E., Zanatta, L., Seifriz, I., Creczynski-Pasa, T. B., Pizzolatti, M. G., Szpoganicz, B. and Silva, F. R. (2004). Hypoglycemic effect and antioxidant potential of kaemp- ferol-3,7-O-(alpha)-dirhamnoside from *Bauhinia forcata* leaves. J. Nat. Prod. 67: 829–832.

Dicarli, M.F., Janisse, J., Grunberger, G. and Ager, J. (2003). Role of chronic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes. J. Am. Coll. Cardiol. 41: 1387–1393.

Frantz, S., Calvillo, L., Tillmanns, J., Elbing, I., Dienesch, C., Bischoff, H., Ertl G. and Bauersachs, J. (2005). Repetitive postprandial hyperglycemia increases cardiac

ischemia/reperfusion injury: prevention by the alpha-glucosidase inhibitor acarbose, FASEB J. 19: 591-593

Hassall, C. H. and Reyle, L. (1955). Hypoglycin A and B, two Biologically Active Polypeptides from *Blighia sapida*. Biochemical Journal., 60: 334-339.

Jimoh, T. O., Buoro, A. T. and Muriana, M. (2012). Utilization of *Blighia sapida* (Akee apple) pod in the removal of lead, cadmium and cobalt ions from aqueous solution. Journal of Environmental Chemistry and Ecotoxicology 4(10): 178-187.

Kavitha, S., Hiranmai Y. R. and Rajeshwari S. (2012). *In vitro* antidiabetic activity of anthocyanin extract of *Asystasia gangetica* (Chinese violet) flower. Asian Pac. J. Trop. Biomed. 1: 1-4

Khang, W. O., Annie H., LiXia, S., De Jian, H., Benny, K. and Huat, T. (2011). Polyphenols-rich *Vernonia amygdalina* shows anti-diabetic effects in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 133: 598–607

Kim, Y. M., Jeong, Y. K., Wang, M. H., Lee, W. Y. and Rhee, H. I. (2005). Inhibitory effects of pine bark extract on alpha-glucosidase activity and postprandial hyperglycemia. Nutrition 21: 756-761.

Kimura, Y., Araki, Y., Takenaka, A and Igarashi, K. (2006). Protective effect of dietary nasunin and parapect induced oxidative stress in rat. Biosci. Biotechnol. Biochem. 63: 799-804.

Koyuturk, M., Ozsoy-Sacan, O., Bolkent, S., Yanardag, R. (2005). Effect of glurenorm on immunohistochemical changes in pancreatic β-cells of rats in experimental diabetes. Indian J. Exp. Biol. 43: 268–271.

Mayur, B., Sandesh, S. Shruti, S. and Sung-Yum, S. (2010). Antioxidant and α-glucosidase inhibitory properties of C*arpesium abrotanoides* L. Journal of Medicinal Plants Research 4(15): 1547-1553.

Mccue, P. and Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. Asia Pac. J. Clin. Nutr 13(1): 101–106.

Nagappa, A, N., Thakurdesai, P. A., Rao, N. V. and Singh, J. (2003). Antidiabetic activity of *Terminalia catappa* Linn. Fruits. J. Ethnopharmacol. 88: 45–50.

Ogunleye, D. S., Adeyemi, A. A. and Sanni, A.M. (2003). Hypoglycaemic activities of the stem bark of *Cola acuminata* Vahl and leaf of *Ficus exasperata* (P. Beauv) Schott and Endl. Nigerian Quarterly J. of Hospital Medicine. 13 (1): 58–60.

Ojewole, J. A. (2006). Analgesic, antiinflammatory and hypoglycaemic effects of ethanol extract of *Zingiber officinale* (Roscoe) rhizomes (Zingiberaceae) in mice and rats. Phytotherapy Research 20: 764–772.

Pinto, M. D., Kwon, Y., Apostolidis, E., Lajolo, F. M., Genovese, M. I. and Shetty, K. (2009). Potential of *Ginkgo biloba* L. leaves in the management of hyperglycemia and hypertension using *in vitro* models. Bioresource Technology 100: 6599–6609

Shimabukuro, M., Higa, N., Chinen, I., Yamakawa, K. and Takasu, N. (2006). Effects of a single administration of acarbose on postprandial glucose excursion and endothelial dysfunction in type 2 diabetic patients: a randomized crossover study, J. Clin. Endocrinol. Metab. 91: 837-842.

Sofowara, A. (2006). Medical plants and traditional medicine in Africa. Reprint edition, Spectrum Books Ltd., Ibadan.

Trease, G. E. and Evans, W. C. (1996). Pharmacognosy. 4th edition, W.B. Saunders, USA.

Valiathan, M. S. (1998). Healing plants. Curr. Sci. 75: 1122–1126.

Verspohl, E. J. (2002). Recommended testing in diabetes research. Planta Med. 68: 581–590.

Wild, S. G., Roglic, A., Green, R., and King, H. (2004). Global prevalence of diabetes. Estimated for the year 2000 and projection for 2030. Diabetes Care 27: 1047–1054.