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In-vitro alpha-amylase and glucosidase inhibitory potential of leaf hexane, ethyl acetate and methanol fractions from *Pterocarpus soyauxii* Taub

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ABSTRACT

Diabetes mellitus is a critical clinical condition characterized by hyperglycemia in which an accelerated amount of glucose circulates in the blood plasma. This work attempts to evaluate the α-amylase and α-glucosidase inhibitory activities of hexane, ethyl acetate and methanol fractions of *Pterocarpus soyauxii* at varying concentrations. 1.0 kg of milled leaf sample was first soaked in 5 L of methanol to obtain a percentage yield of 12%. The assay was carried out using standard procedures. The following IC_{50} values were obtained in the α-amylase inhibition: 0.0395, 0.05995, and 0.0509 mg/mL for hexane, ethyl acetate, and methanol, respectively compared to the standard drug acarbos (0.00812 mg/mL). A dose-dependent increase in percentage inhibition was obtained for α-glucosidase with IC_{50} values of 0.052, 0.059, and 0.065 mg/mL for respective fractions compared to the standard drug used (0.0017 mg/mL). The hexane fraction showed the greatest percentage inhibition for both α-amylase and α-glucosidase, while appreciable inhibition activity was observed in other fractions. The Nigerian *Pterocarpus soyauxii* has been identified for the first time as a very potent anti-diabetic agent useful in management of postprandial hyperglycemia and related therapeutic interventions.

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1. Introduction

Diabetes mellitus (DM), a severe metabolic disorder characterized by high blood glucose levels, continues to be a major medical concern worldwide due to its high prevalence and potential deleterious effects (DeFronzo, 1999; Tanko et al., 2012; Ayat et al., 2015; Hind et al., 2017). In fact, DM is a serious health problem with multifaceted etiologies which is identified by anomalies in carbohydrate, protein and also fat metabolism due to the completion or gross insufficiency of insulin secretion or insulin action (Balkan et al., 2000; Shaw et al., 2010). Postprandial hyperglycemia is a significant risk factor for acute and chronic complications related to diabetes (Hanefeld et al., 1996). It plays an important role in the development of type 2 diabetes mellitus and its associated chronic complications, such as micro- and macro-vascular disorders, e.g. neuropathy, cardiovascular, and cerebrovascular diseases (Boutati and Raptis, 2004). Managing postprandial plasma glucose level is crucial in the early treatment of diabetes mellitus and in decreasing chronic complications (Ortiz-Andrade et al., 2007). An efficient approach for controlling type 2 diabetes is the inhibition of α-amylase and α-glucosidase. Accordingly, inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates in the small intestine and reduce the postprandial blood glucose expedition (Kwon et al., 2007a; Kwon et al., 2007b). There are many assessments of designed enzyme inhibitors and their influence on blood glucose ranges after food uptake (Kim et al., 2005; Shihabudeen et al., 2011). α-Amylase and α-glucosidases inhibitors

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are one type of the anti-diabetic medicine groups, among which acarbose is the most popular one. These types of drugs have a very powerful advantage and are suitable not only for healing diabetes mellitus, but also to stimulate gastrointestinal negative effects that reduce their utility in a preventive strategy (Cheng and Fantus, 2005; de Sales et al., 2012). Currently, several researchers are evaluating and developing nutritional approach to absolutely manage postprandial hyperglycemia, without leading to harmful effects in the digestive tract. Medicinal plants are often considered to be less toxic and free of side effects compared to the synthetic analogues (Santhakumari et al., 2006). Screening of α-amylase and α-glucosidase inhibitors from plants are improving over the years and the inhibitory actions of these enzymes have been recently explored extensively from natural products (Matsuda et al., 2002).

Medicinal plants have been explored from time immemorial due to their efficacy in the prevention and treatment of various disease conditions such as diabetes, oxidative stress, malaria, thiphoid fever, schistosomiasis, onchocerciasis, lymphatic filariasis, African dengue and trypanosomiasis (Pavunraj et al., 2017; Mohammadhosseini, 2017; Mohammadhosseini et al., 2017). Plants occupy a strategic place in the socio-cultural, spiritual and economic definition of societies (Mohammadhosseini et al., 2019). This knowledge was transferred from generation to generation either orally or mystically, and effective plants have been selected by mere trial and error (Wansi et al., 2019). The traditional medicinal system based on herbal therapies has always played a pivotal role in the health systems of many emerging and under developed countries. The significance of the traditional medicine has also gained cognizance in advanced countries of the world (Rai and Nath, 2003). Herbal medicine is spreading widely today because of its biomedical benefits (Savithramma et al., 2016). The plant parts utilized, mode of preparation and administration of these herbal medicines vary significantly from one country to the other one (Saha et al., 2015; Genesan et al., 2017).

The therapeutic effect of some secondary metabolites isolated from medicinal plants with several pharmacological properties such as flavonoids, steroids, alkaloids, terpenes, tannins and lignans has been the subject of incessant phytochemical investigations involving the prospection of new drugs (Mohammadhosseini et al., 2016; Aidi Wannes et al., 2017; Mohammadhosseini, 2017; Nunes and Miguel, 2017). These substances are found as bio-constituents of plant extracts, possessing great activity for different medicinal purposes (Camilo et al., 2017; Ganesan and Xu, 2017; Mohammadhosseini, 2017; Mohammadhosseini et al., 2017; Pavunraj et al., 2017).

Pterocarpus soyauxii Taub (Fabaceae) (Fig. 1), commonly known as African Coral-wood is a popular green vegetable often utilized in the South Eastern part of Nigeria due to its unique taste and high vitamin C content (Lavin and Pennington, 2001; Assanta and Robert, 2011). Crude extract of the leaf has been found to significantly increase red and white blood cells as well as haemoglobin (Dike, 2010). The plant has been used to treat various diseases such as hypertension, intestinal parasites, renal infections, chronic anaemia, skin diseases, and fungal infections (Oteng-Gang and Mbachu, 1990; Bremaud et al., 2011; Saha et al., 2013). Phytochemical investigations of this plant revealed the presence of flavonoids, flavonoid analogs, bioflavonoids, pterostilbene, and ascorbic acid (King et al., 1953; Arnone et al., 1977; Barend and Brandt, 1987; Oteng-Gyang and Mbachu 1987; Tchamadeu et al., 2011). The vitality of most edible vegetables and plants consist basically of nutrients which are required for healthy living of both animals and human beings. These vital nutrients which could be phytochemicals and useful mineral elements are necessary in curing several ailments due to their medicinal potential (Okerulu et al., 2017).

Fig. 1. Fresh leaf of *Pterocarpus soyauxii* plant.

Evaluation of the α-amylase and α-glucosidase inhibitory activities of hexane, ethyl acetate and methanol fractions of *Pterocarpus soyauxii* Taub for antidiabetic therapeutic intervention is reported for the first time in literature.

2. Experimental

2.1. Plant material

Fresh leaf samples of Pterocarpus soyauxii Taub were collected from the Forest Research Institute of Nigeria (FRIN), Ibadan Nigeria and authenticated in the Forest Research Herbarium, where voucher samples were deposited with specimen voucher number FIH-112031. The plant sample was sorted, cut and air-dried for eight weeks.

2.2. Extraction and fractionation of plant material

Extraction of the plant was done using cold maceration. Dried leaf samples of *Pterocarpus soyauxii* were ground using a milling machine. The crude methanolic extract was obtained by soaking 1000 g of dried plant powder in 5 L of methanol for 5 days. Extract was further concentrated using rotary vacuum evaporator at 40 °C and stored at 0-4 °C. Hexane, ethyl acetate and methanol fractions were obtained using successive fractionation in increasing order of polarity.

2.3. Extraction of wheat alpha amylase

500 g of malted whole wheat flour was added slowly with mild stirring to 1 L of calcium acetate solution (0.2%) at room temperature and continuously stirred for 2 hours on a stirrer. The suspension was then centrifuged at 40 °C at 12,000 g for 10 minutes. The resultant clear brown supernatant was stored at 2 °C to 3 °C prior to heat treatment. Since β-amylase interferes with the enzymatic determination of alpha amylase, it was inactivated by heating the extract at 70 °C for 15 minutes. α-Amylase is resistant to inactivation by this treatment at pH between 6.5 and 8.0. The pH of the extract was first adjusted to 6.6 with cold ammonium hydroxide (4.0%). Heat treatment was carried out both at 85 °C to 90 °C and 72 °C to 74 °C using a water bath with continuous stirring. The extract was then cooled to 2 °C to 3 °C until use (Kneen and Sandstedt., 1943).

2.4. Determination of alpha-amylase inhibitor activity

The assay mixture containing 200 µL of sodium phosphate buffer (0.02 M), 20 µL of enzyme and the plant extracts over a concentration range 20-100 µg/mL were incubated for 10 minutes at room temperature followed by addition of 200 µL of starch in all test tubes. The reaction was terminated with the addition of 400 µL of DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 mL of distilled water and the related absorbance was measured at 540 nm. The control samples were prepared without any fraction. The inhibition (%) was calculated according to the formula shown as Eqn. 1.

$$\text{Inhibition (\%)} = \frac{Abs_{540(control)} - Abs_{540(sample)}}{Abs_{540(control)}} \times 100 \quad \text{(Eqn. 1)}$$

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-amylase inhibitor. All tests were performed in triplicate (McCue and Shetty, 2004).

2.5. Determination of yeast alpha-glucosidase inhibitor activity

p-Nitrophenyl-α-D-glucopyranoside, acarbose, baker's yeast alpha-glucosidase were purchased from Sigma (USA). The yeast alpha-glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and used as the enzyme extract. *p*-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Plant extracts were used in the concentration ranging from 0.02 to 0.1 mg/mL. Different concentrations of plant extracts were mixed with 320 µL of 100 mM phosphate buffer (pH = 6.8) at 30 °C for 5 minutes. 3 mL of sodium hydroxide (50 mM) was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without any plant extracts. The inhibition (%) was calculated according to Eqn. 2 as follows.

$$\text{Inhibition (\%)} = \frac{Abs_{410(control)} - Abs_{410(extract)}}{Abs_{410(control)}} \times 100 \quad \text{(Eqn. 2)}$$

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-glucosidase inhibitor and all tests were performed in triplicate (Kim et al., 2005).

3. Results and Discussion

In recent years, the popularity of alternative medicine has increased geometrically since several medicinal plants have been reported to have anti-diabetic activity and hence the use of herbal drugs as complementary and alternative therapy to existing medications for the treatment of diabetes is widely growing (Hasani-Ranjbar et al., 2008; Devesh et al., 2016). Diabetes is increasingly a global health issue; therefore, the development of new therapeutic strategies with fewer side effects is pivotal. The inhibitory activity of three fractions from *Pterocarpus soyauxii* on wheat α-amylase and yeast α-glucosidase was investigated in this study and the results are discussed in the following subsections.

3.1. Percentage yield of leaf extract from *Pterocarpus soyauxii*

1 kg of milled leaf sample was soaked in 5 L of methanol to obtain a percentage yield of 12% (Table 1). The high percentage yield from the leaf extract is higher than other parts, e.g. stem, stem bark, root, root bark of the plant due to the low fiber content of the leaf part.

3.2. Alpha-amylase inhibition of *Pterocarpus soyauxii*

The percentage inhibition obtained for the standard anti-diabetic drug acarbos was relatively high for the concentration range used (0.1-0.02 mg/mL). Maximum percentage inhibition of 95.5% was obtained for acarbos at 0.1 mg/mL and decreased slightly to 93.5% at 0.02 mg/mL. This trend indicates that the percentage inhibition of the standard used in this study is concentration-dependent. All fractions, e.g. hexane, ethyl acetate and methanol fractions exhibited concentration-dependent inhibition similar to acarbos the standard anti-diabetic drug used. Percentage inhibition of the standard was in close range with the hexane fraction with inhibition efficiency of 95.12% at 0.1 mg/mL. Percentage inhibition efficiency of the hexane fraction was higher than the ethyl acetate and methanol fractions at all concentrations as shown in Table 2 and Figs. 2-3..

A graph of percentage inhibition versus concentration (mg/mL) of fractions was plotted from which the IC_{50} values were obtained for each fraction using linear regression analysis in reference to the central standard. An inverse relationship exists between the percentage inhibition efficiency and the IC_{50} values. The higher the IC_{50} value the lower the activity of such fraction/standard

and vice versa. The following IC_{50} values were obtained in the determination of α-amylase inhibition: 0.0395 mg/mL, 0.05995 mg/mL, and 0.0509 mg/mL for hexane, ethyl acetate, and methanol fractions respectively compared to the standard anti-diabetic drug acarbos (0.00812 mg/mL). The standard anti-diabetic drug with the lowest IC_{50} value of 0.00812 mg/mL (Table3) exhibited the highest alpha-amylase inhibition activity followed closely by the hexane fraction (0.0395 mg/mL). The least activity was expressed by the highly polar methanol fraction (0.0509 mg/mL). The high α-amylase inhibition of the hexane fraction in comparison with the standard anti-diabetic drug must be due to the presence of some important bioactive phyto-contituents.

3.3. Alpha-glucosidase inhibition of *Pterocarpus soyauxii*

The percentage inhibition obtained for the standard antidiabetic drug acarbos was relatively high for the concentration range used (0.1-0.02 mg/mL). The optimum percentage inhibition of 90.5% was obtained for acarbos at 0.1 mg/mL and decreased slightly to 83.5% at 0.02 mg/mL. (Fig.s 4-5). This trend indicates that the percentage inhibition of the standard used in this study is concentration-dependent. All fractions, hexane, ethyl acetate and methanol fractions, exhibited concentration- dependent percentage inhibition similar to acarbos the standard anti-diabetic drug used. The highest percentage inhibition was obtained from the hexane fraction with inhibition efficiency of 85.33% at 0.1 mg/mL. The ethyl acetate and methanol fractions exhibited high and concentration dependent α-glucosidase activity as indicated in Table 4.

A graph of percentage inhibition versus concentration (mg/mL) of fractions was plotted for the α-glucosidase inhibition from which the IC_{50} values were obtained for each fraction using linear regression analysis on Microsoft Excel package in reference to the central standard. A dose-dependent increase in percentage inhibition was obtained for the α-glucosidase study with an IC_{50} value of 0.052 mg/mL, 0.059 mg/mL, and 0.065 mg/mL (Table 5) for respective fractions compared to the standard drug acarbos (0.0017 mg/mL). Acarbos, with the lowest IC_{50} value of 0.0017 mg/mL exhibited the highest α-amylase inhibition activity followed closely by the hexane fraction (0.052 mg/mL). The least activity was expressed by the highly polar methanol fraction (0.065 mg/mL). The high α-amylase inhibition of the hexane fraction in comparison with the standard antidiabetic drug acarbos must be due to the presence of some important bioactive phytocontituents in the non-polar fraction of the extract.

3.4. Alpha-amylase and glucocidase inhibition of *Pterocarpus soyauxii*

The hexane fraction showed peak percentage inhibition for both α-amylase and α-alpha-glucosidase , while other fractions showed appreciable inhibition activity. The high inhibition efficiency expressed by the hexane fraction in both the alpha-amylase and alpha glucocidase inhibition studies of the leaf extract of *Pterocarpus soyauxii* must be triggered by the presence of bioactive compounds. The plant extract exhibited a slightly weak alpha-glucosidase enzyme inhibition when compared with alpha-amylase inhibition. Generally, the alpha-amylase and glucocidase inhibition of *Pterocarpus soyauxii* were concentration and polarity dependent.

Table 1
Percentage (%) yield of extraction of *Pterocarpus soyauxii* leaf.

Plant parts	Weight of sample (g)	Weight of extract (g)	Yield (%)
Leaf	1000	120	12

Table 2
Percentage α-amylase inhibition of fractions and standard drug.

Concentration (mg/mL)	ACB (standard)	HF	EF	MF
0.1	95.5	95.12	80.0	92.1
0.08	95.2	78.82	68.8	86.3
0.06	95.0	64.7	52.2	58.3
0.04	94.3	62.94	33.3	33.3
0.02	93.5	49.41	16.6	25.0

ACB: Acarbos, HF: Hexane Fraction, EF: Ethyl acetate Fraction, MF: Methanol Fraction

Table 3
IC_{50} values of acarbos and fractions of *Pterocarpus soyauxii*.

Sample	ACB	HF	EF	MF
Concentration(mg/mL)	0.00812	0.0395	0.05995	0.0509

ACB: Acarbos, HF: Hexane Fraction, EF: Ethyl acetate Fraction, MF: Methanol Fraction

Table 4
Percentage alpha glucocidase inhibition of standard drug and fractions from *Pterocarpus soyauxii*.

Concentration (mg/mL)	ACB (standard)	HF	EF	MF
0.1	90.5	85.33	70.0	71.21
0.08	87.2	68.12	62.8	63.3
0.06	85.8	54.17	52.2	58.3
0.04	84.3	42.54	33.3	43.3
0.02	83.5	39.41	18.6	24.7

ACB: Acarbos, HF: Hexane Fraction, EF: Ethyl acetate Fraction, MF: Methanol Fraction

Table 5
IC_{50} (mg/mL) values of acarbos and fractions of *Pterocarpus soyauxii*.

Sample	ACB	HF	EF	MF
Concentration (mg/mL)	0.0017	0.052	0.059	0.065

ACB: Acarbos, HF: Hexane Fraction, EF: Ethyl acetate Fraction, MF: Methanol Fraction

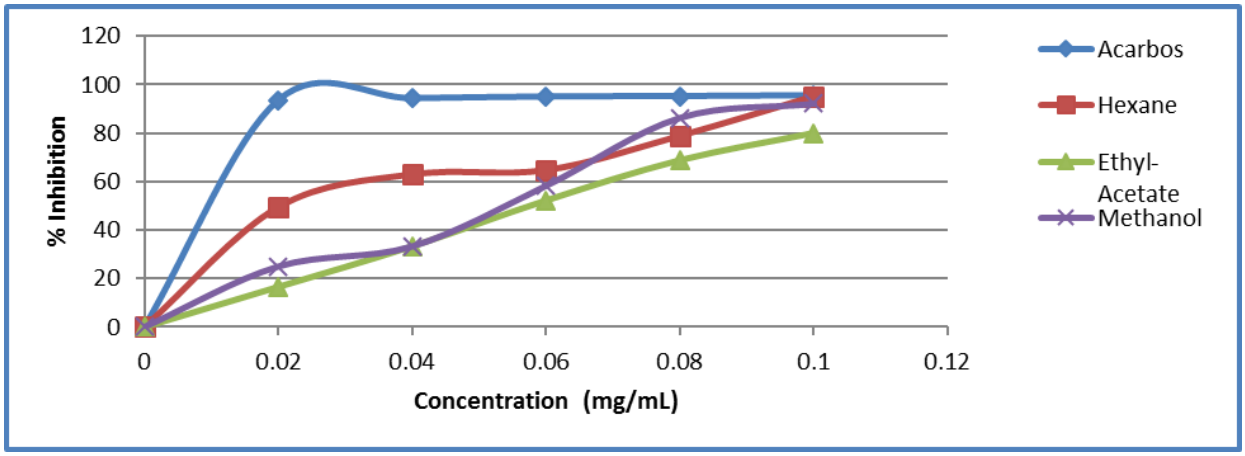

Fig. 2. Graph showing percentage alpha-amylase inhibition versus concentration of fractions and standard drug.

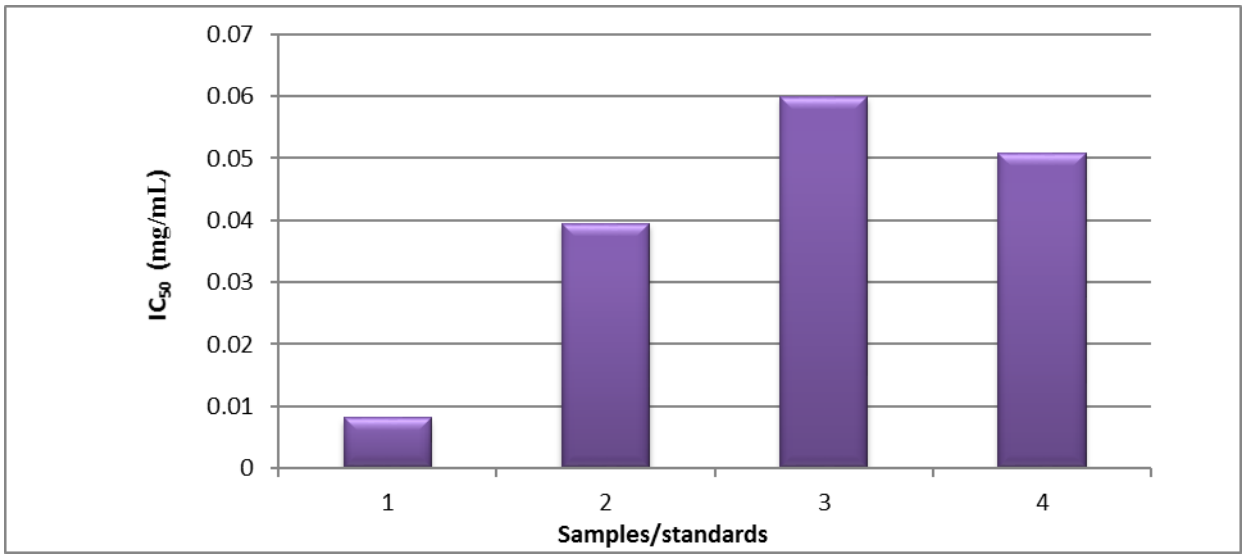

Fig. 3. Bar chart showing IC_{50} (mg/mL) of alpha-amylase inhibition.

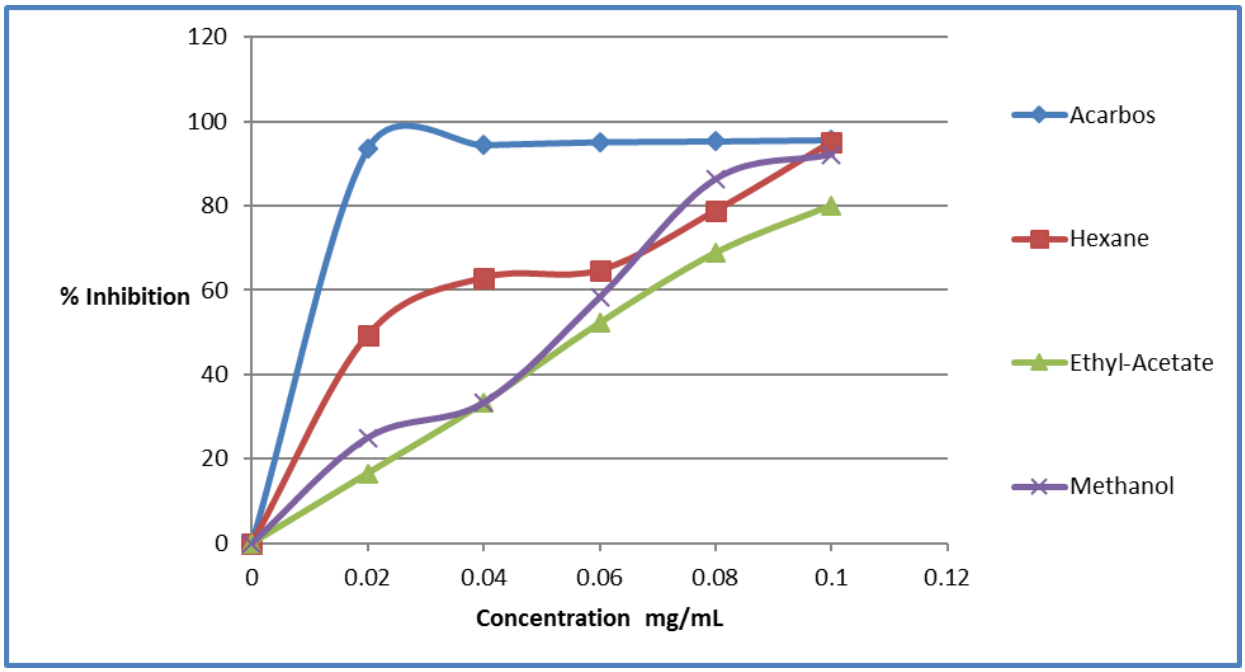

Fig. 4. Graph showing percentage alpha glucocidase inhibition versus concentration of fractions and standard drug.

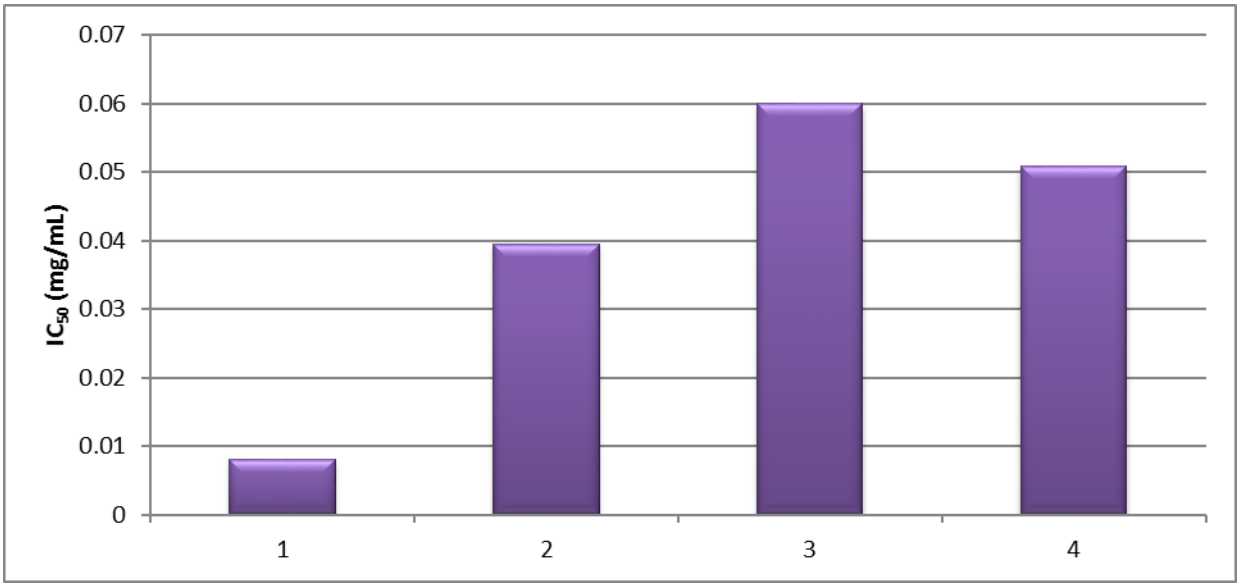

Fig. 5. Bar chart showing IC_{50} (mg/mL) of alpha glucocidase inhibition.

4. Concluding remarks

Based on the high α-amylase and α-glucosidase inhibitory activities expressed by the leaf hexane, ethyl acetate, and methanol fractions of Nigerian *Pterocarpus soyauxii,* the plant has been identified as a very potent anti-diabetic agent useful in the management of postprandial hyperglycemia and related therapeutic interventions.

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Conflict of interest

The authors declare that there is no conflict of interest.

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