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Inhibitory effect on key enzymes relevant to acute type-2 diabetes and antioxidative activity of ethanolic extract of *Artocarpus heterophyllus* stem bark

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ABSTRACT

Objective: To investigate the *in vitro* antioxidant activity of ethanolic extract of *Artocarpus heterophyllus* (*A. heterophyllus*) stem bark and its inhibitory effect on α-amylase and α-glucosidase.
Methods: The *A. heterophyllus* stem bark was extracted using methanol and tested for antioxidative activity.
Results: The results revealed that the ethanolic extract has polyphenolics and free radical scavenging compounds which were significantly higher ($P < 0.05$) than their respective standard, at concentration dependent manner. The ethanolic extract of *A. heterophyllus* stem bark was observed to show inhibitory activities on α-amylase and α-glucosidase with IC_{50} of (4.18 ± 0.01) and (3.53 ± 0.03) mg/mL, respectively. The Lineweaver-Burk plot revealed that ethanolic extract of *A. heterophyllus* stem bark exhibited non-competitive inhibition for α-amylase and uncompetitive inhibition for α-glucosidase activities. Also, gas chromatography–mass spectrometry showed the presence of different bioactive compounds in extract.
Conclusions: Therefore, it can be inferred from this study that ethanolic extract of *A. heterophyllus* stem bark may be useful in the management of diabetes mellitus probably due to bioactive compounds observed in the extract.

1. Introduction

Diabetes mellitus is one of the major global public health problems of the 21st century affecting about 347 million people worldwide[1]. This number is expected to double by the year 2030, if necessary action were not taken[2]. Mainly there are two types of diabetes, that is type I and II. Type II is the most common and accounts for more than 95%, characterized by pancreatic beta cells dysfunction leading to hyperglycaemia. This may be attributed to oxidative stress[3]. The management of diabetes mellitus is a global problem and successful treatment is yet to be discovered[4].

In addition, the available drugs (insulin, sulphonylureas, biguanides, *etc.*) used in the management of diabetes mellitus has been characterized with side effects[5]. In view of this there is a need to search for alternative remedy. Ojo *et al.*[6] reported that several plants species has been ascribed with normoglycaemia with no minimal side effect.

An example of plant that may be useful in this category is *Artocarpus heterophyllus* (*A. heterophyllus*) (jack fruit). It belongs to a family of Moraceae, an exotic fruit grown in tropical climates. Its fruit has an average weight of 10 000 g. *A. heterophyllus* has been considered a rich source of carbohydrates, minerals, dietary fiber and vitamins amongst others[7]. Also its various parts have been used in traditional medicines (jack fruit honey, jack fruit powders, *etc.*)[8]. All the medicinal plants used in the management of diabetes mellitus must have the abilities of inhibiting α-amylase and α-glucosidase.

α-Amylase inhibitors are also called starch blockers. This is because they contain substances that prevent dietary starch from being absorbed into the body system, which may be useful in the management of diabetes. α-Amylase may exert blood glucose lowering effect through inhibition of salivary and pancreatic amylase[4,9]. In other vein, α-glucosidase inhibitors are suppressor of postprandial hyperglycaemia in diabetic mellitus patients by inhibiting the activity of α-glucosidase in the intestine, this reduces glucose absorption by delaying carbohydrate digestion and increases digestion time[10].

Therefore, the aim of this study was to evaluate the *in vitro* antioxidative as well as α-amylase and α-glucosidase inhibitory activities in stem bark of *A. heterophyllus* couple with identification of active compounds in the plant parts as well as

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mechanism of action of α-amylase and α-glucosidase inhibitions.

2. Materials and methods

2.1. Chemical and reagents

α-Amylase, α-glucosidase, para-nitrophenyl-α-D-glucopyrano-side (pNPG), *p*-nitrophenol, gallic acid, ascorbic acid, rutin, 1,1diphenyl-2-picrylhydrazyl radical (DPPH), starch, maltose, dinitrosalicylic acid, ferric chloride, Griess reagent, Folin-Ciocalteu reagent and aluminum chloride were obtained from Sigma–Aldrich, Germany. All other chemicals and reagents were of analytical grades.

2.2. Sample collection and identification

The fresh peeled stem bark of *A. heterophyllus* were collected on 10th September, 2015 at a farm in Ibadan, Oyo State, Nigeria and washed with distilled water. It was then identified and authenticated at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

2.3. Sample preparation

The stem bark of *A. heterophyllus* was shade-dried [at ambient temperature of (30 ± 2) °C] for four weeks to constant mass. The dried sample was pounded into fine power with the aid of kitchen blender and stored in air-tight containers. Thereafter, 100 g of powdered sample was extracted with 1 L of 70% ethanol for 48 h. The extract was filtered with Whatman filter paper and the filtrate was evaporated to dryness using a freeze dryer. Then, the extract was reconstituted in distilled water and used for subsequent analysis.

2.4. Measurement of polyphenolic contents

2.4.1. Total phenol

This was determined by the modified Folin-Ciocalteu method[11]. The ethanolic extract of *A. heterophyllus* stem bark (1–5 mg/mL) was mixed in a separate test tube with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Thereafter, the absorbance was then measured at 765 nm using the AJ-1C03 spectrophotometer (Anjue Co. Ltd., Anqing, China). Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 10.44x$, $R^2 = 0.9466$, where x is the absorbance and y is the tannic acid equivalent (mg/g).

2.4.2. Total flavonoid

This was carried out using the method described by Khan *et al.*[12]. A volume of 0.5 mL of 2% AlCl in ethanol solution was added to 0.5 mL of sample solution at different concentrations of 1–5 mg/mL. After 1 h at room temperature, the absorbance was measured at 420 nm using the AJ-1C03 spectrophotometer. A yellow colour indicated the presence of flavonoid. Total flavonoid content was calculated as quercetin (mg/g), using the following equation based on the calibration curve: $y = 1.030x$, $R^2 = 0.8842$, where x is the absorbance and y is the quercetin equivalent (mg/g).

2.4.3. Total proanthocyanidin

The method described by Sun *et al.*[13] was employed. A volume of 0.5 mL of the extracts solution at different concentrations of 1–5 mg/mL was mixed with 3 mL of 4% vanillin-methanol and 1.5 mL hydrochloric acid. The mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm using the AJ-1C03 spectrophotometer. Total proanthocyanidin was expressed as catechin equivalents (mg/g) using the equation based on the calibration curve: $y = 0.5516x$, $R^2 = 0.9185$, where x was the absorbance and y is the catechin equivalent (mg/g).

2.5. Evaluation of antioxidants activities

2.5.1. DPPH radical scavenging ability

The method described by Liyana-Pathiranan and Shahidi[14] was employed in this determination. A solution of DPPH (0.135 mmol/L) in methanol was prepared and 1 mL of this solution was mixed with 1 mL of varying concentrations of the extract (1–5 mg/mL). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm with AJ-1C03 spectrophotometer using vitamin C as the standards. The ability to scavenge DPPH radical was calculated as:

DPPH radical scavenging ability (%)

$$= \frac{\text{Abs (control)} - \text{Abs}\left(\frac{\text{sample}}{\text{standard}}\right)}{\text{Abs (control)}} \times 100\%$$

where, Abs (control) = absorbance of the control, Abs (sample/standard) = absorbance of the sample/standard.

2.5.2. Ferric reducing power (FRAP) radical scavenging ability

This was carried out by a method of Duh *et al.*[15]. Different concentrations (1–5 mg/mL) of the extract and the standards (gallic acid) were mixed with phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and potassium ferricyanide (2.5 mL, 1% w/v). The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 mL, 10% w/v) was added to the mixture and then centrifuged for 10 min at 1000 r/min. Thereafter, 2.5 mL of the upper layer of the solution was mixed with 2.5 mL of distilled water and 0.5 mL (0.1% w/v) of ferric chloride. The absorbance was measured at 700 nm using spectrophotometer. The scavenging ability of FRAP was calculated using:

FRAP radical scavenging ability (%)

$$= \frac{\text{Abs (control)} - \text{Abs}\left(\frac{\text{sample}}{\text{standard}}\right)}{\text{Abs (control)}} \times 100\%$$

where, Abs (control) = absorbance of the control, Abs (sample/standard) = absorbance of the sample/standard.

2.5.3. Hydroxyl radical scavenging ability

The method of Halliwell and Gutteridge[16] was used. The extract 0–100 μL (1–5 mg/mL concentrations) was added to a reaction mixture containing 120 μL of 20 mmol/L deoxyribose, 400 μL of 0.1 mol/L phosphate buffer, 40 μL of 500 μmol/L of freshly prepared $FeSO_4$. Thereafter, the volume of the mixture was made up to 800 μL with distilled water, incubated at 37 °C for 30 min and the reaction was stopped by addition of 0.5 mL of 28% trichloroacetic acid. This was then followed by adding 0.4 mL of 0.6% thiobarbituric acid solution. The test tube containing the

mixture was incubated in boiling water for 20 min. The absorbance was measured at 532 nm using spectrophotometer.

Hydroxyl radical scavenging ability (%)

$$= \frac{\text{Abs (control)} - \text{Abs}\left(\frac{\text{sample}}{\text{standard}}\right)}{\text{Abs (control)}} \times 100\%$$

where, Abs (control) = absorbance of the control, Abs (sample/standard) = absorbance of the sample/standard.

2.5.4. Fe^{2+} chelating ability

This was determined by using the modified method of Minotti and Aust[17]. Freshly prepared 500 μmol/L of FeSO$_4$ (150 μL) was added to a reaction mixture containing 168 μL of 0.1 mol/L Tris–HCl (pH 7.4), 218 μL saline and the extracts (0–25 μL) (of 1–5 mg/mL concentrations). Thereafter, the reaction mixture was incubated for 5 min, before the addition of 13 μL of 0.25% 1,10phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer.

Fe^{2+} radical scavenging ability (%)

$$= \frac{\text{Abs (control)} - \text{Abs}\left(\frac{\text{sample}}{\text{standard}}\right)}{\text{Abs (control)}} \times 100\%$$

where, Abs (control) = absorbance of the control, Abs (sample/standard) = absorbance of the sample/standard.

2.6. Enzyme assays

2.6.1. Determination of α-amylase

The inhibitory action of α-amylase activity was determined according to the method of Shai et al.[18]. A volume of 250 μL of the extract (at different concentrations of 1–5 mg/mL) was incubated at 25 °C for 10 min with 500 μL of hog pancreatic amylase (2 IU/mL) in 100 mmol/L phosphate buffer (pH 6.8). Then 250 μL of 1% starch dissolved in 100 mmol/L phosphate buffer (pH 6.8) was then added to the reaction mixture and incubated at 25 °C for 10 min. Also 1 mL of dinitrosalicylic acid (color reagent) was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm and the inhibitory activity was expressed as percentage of a control sample without inhibitors.

$$\alpha\text{-Amylase inhibition (\%)} = \frac{A_{540} \text{ control} - A_{540} \text{ sample}}{A_{540} \text{ control}} \times 100\%$$

2.6.2. Determination of α-glucosidase

The α-glucosidase inhibitory activity was determined according to the method described by Ademiluyi and Oboh[19], with slight modifications. Briefly, 250 μL of the extract, at different concentrations of 1–5 mg/mL, was incubated with 500 μL of 1.0 IU/mL α-glucosidase solution in 100 mmol/L phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, 250 μL of pNPG solution (5 mmol/L) in 100 mmol/L phosphate buffer (pH 6.8) was added and the mixture was further incubated at 37 °C for 20 min. Then the absorbance of the released p-nitrophenol was measured at 405 nm using UV–visible spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibitors.

$$\alpha\text{-Glucosidase inhibition (\%)} = \frac{A_{540} \text{ control} - A_{540} \text{ sample}}{A_{540} \text{ control}} \times 100\%$$

2.6.3. Mechanism of α-glucosidase and α-amylase inhibitions

The extract was subjected to kinetic experiments to determine the type of inhibition exerted on α-glucosidase and α-amylase. The experiment was conducted according to the protocols described in the determination of α-glucosidase and α-amylase with different extract concentrations of 1–5 mg/mL with a variable concentration of substrate. For the α-glucosidase inhibition assay, 0.625–5 mmol/L of pNPG was used and 0.125%–1% of starch was used for the α-amylase inhibition assay. The initial rates of reactions were determined from calibration curves constructed using varying concentrations of p-nitrophenol and maltose for the α-glucosidase and α-amylase inhibition assays respectively. The initial velocity data obtained were used to construct Lineweaver-Burk plot to determine the Km (Michaelis constant) and Vmax (maximum velocity) of the enzyme as well as the Ki (inhibition binding constant as a measure of affinity of the inhibitor to the enzyme) and the type of inhibition for both enzymes[20].

$$\text{Reaction rate (v) (mg/mL)} = \frac{\text{Amount of product liberated (mg/mL)}}{1200 \text{ (s)}}$$

2.7. Gas chromatography–mass spectrometric (GC–MS) analysis

The method described by Kawamura-Konishi et al.[20] was employed for this determination. Briefly, the extract was subjected to GC–MS analysis, which was conducted with an Agilent Technologies 6890 GC coupled with an Agilent 5973 mass selective detector and driven by Agilent Chemstation software (Agilent Technologies, Santa Clara, USA). A DB-5SIL MS capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness) was used. The carrier gas was ultra-pure helium at a flow rate of 0.7 mL/min and a linear velocity of 37 cm/s. The injector temperature was set at 250 °C. The initial oven temperature was 60 °C, which was programmed to 280 °C at the rate of 10 °C/min with a hold time of 3 min. Injections of 2 μL were made in the splitless mode with a manual split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were ion source temperature (230 °C), quadrupole temperature (150 °C), solvent delayed 4 min and scan ranged 50–700 amu. Compounds were identified by direct comparison of the retention times and mass fragmentation pattern with those from the National Institute of Standards and Technology library.

2.8. Statistical analysis

Data were analyzed by using SPSS version 20 and One-way ANOVA using Duncan multiple range post-hoc test. Values were considered significantly different at $P < 0.05$.

3. Results

The polyphenol contents of the ethanolic extract of A. heterophyllus stem bark was characterized by the presence of

considerable amount of phenolic compounds (Table 1). The plant extract had a high level of total phenols (12.31 mg/g tannic acid equivalent) followed by flavonoids (10.49 mg/g quercetin equivalent) and proanthocyanidins (8.34 mg/g catechin equivalent) at a concentration of 5 mg/mL. The increase observed in the extract was in concentration dependent from 1 to 5 mg/mL.

Table 1
Polyphenolic contents of ethanolic extract of *A. heterophyllus* stem bark.

| Concentration (mg/mL)	Total phenol (Tannic acid mg/g)	Total flavonoid (Quercetin mg/g)	Total proanthocyanidin (catechin mg/g)
1	6.63 ± 0.02	3.95 ± 0.01	2.98 ± 0.12
2	8.65 ± 0.11	5.32 ± 0.03	3.48 ± 0.02
3	9.91 ± 0.12	6.49 ± 0.01	4.48 ± 0.04
4	11.79 ± 0.01	8.99 ± 0.20	6.01 ± 0.02
5	12.31 ± 0.03	10.49 ± 1.10	8.34 ± 0.01

Values were represented as mean ± SEM in triplicate experiment.

In addition, Figures 1–4 showed the percentage *in vitro* free radical scavenging ability of DPPH, FRAP, hydroxyl and Fe^{2+} chelating respectively. There was significant increase ($P < 0.05$) in concentration dependent manner of 1–5 mg/mL in the *in vitro* antioxidants determined. The ethanolic extract of *A. heterophyllus* stem bark showed significant increase ($P < 0.05$) of all the *in vitro* antioxidants determined when compared with their respective standards.
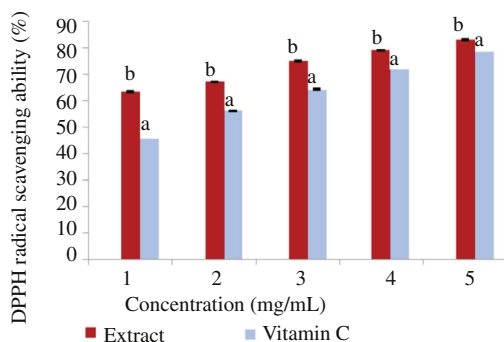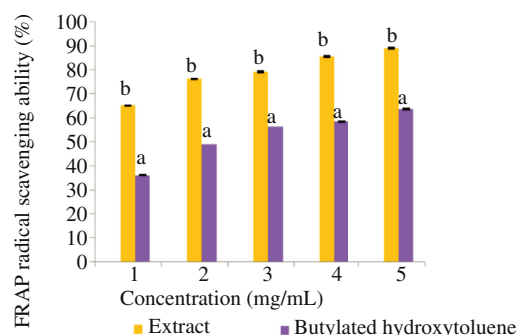

Figure 1. DPPH free radical scavenging ability of ethanolic extract of *A. heterophyllus* stem bark.
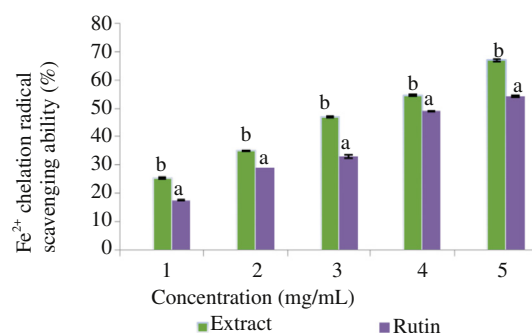Values were represented as mean ± SEM of triplicate experiment.
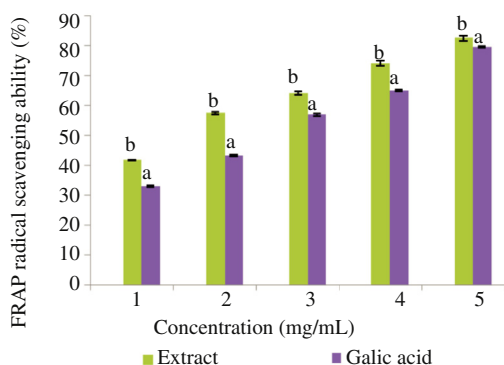
Figure 2. FRAP free radical scavenging ability of ethanolic extract of *A. heterophyllus* stem bark.
Values were represented as mean ± SEM of triplicate experiment.

Figure 3. Hydroxyl free radical scavenging ability of ethanolic extract of *A. heterophyllus* stem bark.
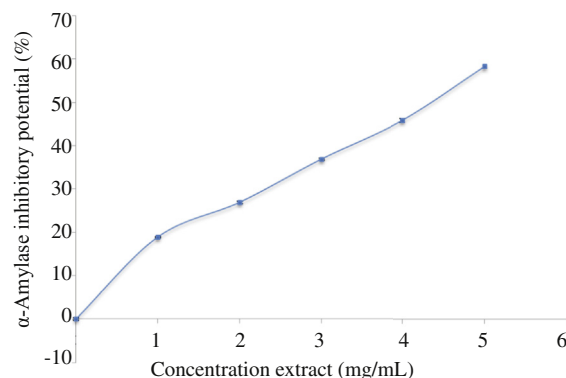Values were represented as mean ± SEM of triplicate experiment.

Figure 4. Fe^{2+} chelating free radical scavenging ability of ethanolic extract of *A. heterophyllus* stem bark.
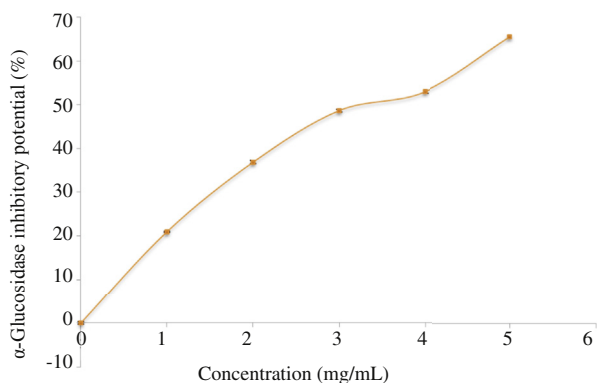Values were represented as mean ± SEM of triplicate experiment.

Figures 5 and 6 showed the percentage inhibition of α-amylase and α-glucosidase ethanolic extract of *A. heterophyllus* stem bark. The extract inhibited α-amylase and α-glucosidase activities in a concentration dependent manner of 1–5 mg/mL. The concentration of the extract required to cause IC_{50} against α-amylase and α-glucosidase were (4.18 ± 0.01) and (3.53 ± 0.03) mg/mL (Table 2). Also, Figure 7 depicts the Lineweaver-Burk plot for α-amylase, which shows that ethanolic extract of *A. heterophyllus* stem bark inhibited α-amylase in a competitive manner while mode of inhibition of α-glucosidase by the extract was a non competitive type of inhibition (Figure 8).

Figure 5. Inhibitory activities of ethanolic extract of *A. heterophyllus* stem bark against α-amylase.
Values were represented as mean ± SEM of triplicate experiment.

Figure 6. Inhibitory activities of ethanolic extract of *A. heterophyllus* stem bark against α-glucosidase.
Values were represented as mean ± SEM of triplicate experiment.

Figure 7. Mode of action of α-amylase by ethanolic extract of *A. heterophyllus* stem bark.

Table 2

IC$_{50}$ of enzymes inhibitory abilities of ethanolic extract of *A. heterophyllus* stem bark.

Enzymes	IC$_{50}$ values
α-Amylase	4.18 ± 0.01
α-Glucosidase	3.53 ± 0.03

Values were represented as mean ± SEM in triplicate reading.

Figure 8. Mode of action of α-glucosidase by ethanolic extract of *A. heterophyllus* stem bark.

The GC–MS chromatogram of the ethanolic extract of *A. heterophyllus* stem bark was presented in Table 3. Thirteen peaks were observed to be visible in the chromatogram and the various chemical constituents at those peaks were identified

Table 3

Bioactive compounds in ethanolic extract of *A. heterophyllus* stem bark by GC–MS.

Name of compound	Molecular formula	Molecular weight	Peak area %	Retention time	Nature of compound
Phenol, 3,4,5-trimethoxy-	C$_9$H$_{12}$O$_4$	184	9.17	8.990	Phenolic compound
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C$_{10}$H$_{12}$O$_3$	180	7.93	10.748	Phenolic compound
Scopoletin	C$_{10}$H$_8$O$_4$	192	3.05	13.056	Phenolic compound
n-Hexadecanoic acid	C$_{16}$H$_{32}$O$_2$	256	3.34	13.164	Fatty acid
3,5-Dimethoxy-4-hydroxycinnamaldehyde	C$_{11}$H$_{12}$O$_4$	208	2.29	13.234	Aldehyde compound
Hexadecanoic acid, ethyl ester	C$_{18}$H$_{36}$O$_2$	284	1.24	13.327	Fatty acid ethyl ester
Ethyl 9,12-hexadecadieno-ate	C$_{18}$H$_{32}$O$_2$	280	2.99	14.612	Fatty acid
Ethyl oleate	C$_{20}$H$_{38}$O$_2$	310	1.99	14.652	Fatty acid
Hexadecanami-de	C$_{16}$H$_{33}$NO	255	3.19	14.718	Amino compound
1*H*-Pyrido[3,4-b]indol-1-one, 2,3,4,9-tetrahydro-	C$_{11}$H$_{10}$N$_2$O	186	2.19	14.783	Amino compound
9-Octadecenamide, (*Z*)-	C$_{18}$H$_{35}$NO	281	23.10	15.849	Amino compound
Decanamide-	C$_{10}$H$_{21}$NO	171	0.62	15.989	Amino compound
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C$_{19}$H$_{38}$O$_4$	330	3.63	16.662	Fatty acid ethyl ester
Bis(2-ethylhexyl) phthalate	C$_{24}$H$_{38}$0$_4$	390	5.20	16.881	Fatty acid compound
Pyrazolo[3,4-b]thiopyrano[4,3-d]pyridin-1-1-amine, 3,6,8,9-tetrahydro-8,8-dimethyl-5-phenyl-	C$_{17}$H$_{18}$N$_4$S	310	5.05	17.292	Amino compound
10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenz(*b,f*) oxepin	C$_{16}$H$_{16}$O$_4$	272	1.42	17.454	Phenolic compound
Butyl 9,12-octadecadienoate	C$_{22}$H$_{40}$O$_2$	336	5.25	17.600	Fatty acid
Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C$_{21}$H$_{42}$O$_4$	358	2.44	17.745	Fatty acid
Stigmast-4-en-3-one	C$_{29}$H$_{48}$O	412	8.30	18.090	Phytosterol
Squalene	C$_{30}$H$_5$O	410	1.35	18.451	Phytosterol
Methanone, (5-hydroxy-3-benzofuryl)(2,5-dimethoxyphenyl)	C$_{17}$H$_{14}$O$_5$	298	0.87	18.825	Ketone compound
2,4a,8,8-Tetramethyldecahydrocyclopropa[*d*]naphthalene	C$_{15}$H$_{26}$	206	1.87	19.663	Phenolic compound
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	C$_{30}$H$_{52}$O	428	1.32	19.879	Phenolic compound
Vitamin E	C$_{29}$H$_{50}$O$_2$	430	2.18	21.107	Phenolic compound
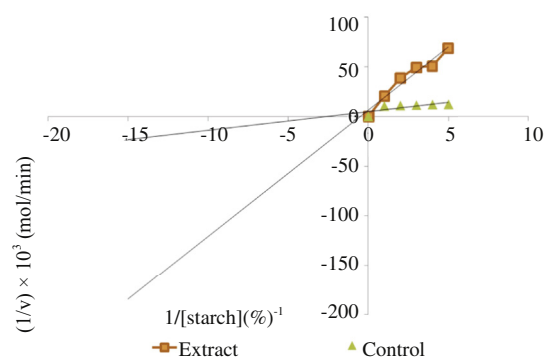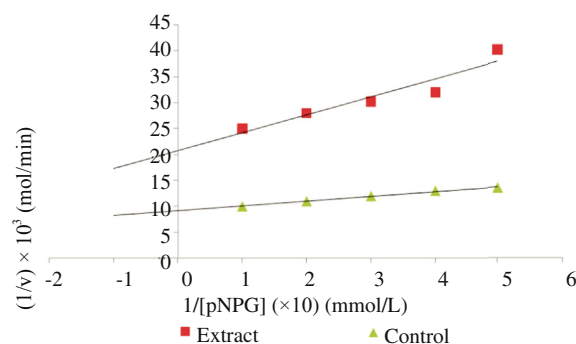
from the National Institute of Standards and Technology library. The retention time and molecular mass of the detected compounds were also provided in Table 3. The most abundant phytochemicals (> 80%) identified by the library was phenolics.

4. Discussion

There is increasing evidence that oxidative stress plays a vital role in the pathogenesis of many diseases and antioxidants have been considered as useful tools in its management/treatments[21]. The present study demonstrated that ethanolic extract of *A. heterophyllus* stem bark has significant antioxidant activity. Tepe *et al.*[22] reported that polyphenolics (Table 1) in ethanolic extract of *A. heterophyllus* stem bark are the major plant compounds with antioxidant activity and probably the activity of the extract is due to the redox properties of these compounds. Zheng and Wang[23] reported that the properties may play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen/decomposing peroxides. For example the hydroxyl groups attached to the aromatic ring structure of flavonoid, enabled them to undergo redox reaction and thus scavenge free radicals[21]. The phenolics in *A. heterophyllus* stem bark (Table 1) may delay the onset of lipid oxidation and decomposition of hydroperoxides in food products as well as in living tissues[24]. This may make the extract useful in ameliorating oxidative stress link diseases (*e.g.* diabetes mellitus, cancer amongst others).

Moreover, one of the antioxidant indices studied was DPPH free radical scavenging ability (Figure 1). This attracts hydrogen or electron from stable molecules, thereby turning them into free radicals with itself becoming a stable molecule[25]. Thus, the observed DPPH radical scavenging ability of ethanolic extract of *A. heterophyllus* stem bark may be attributed to the presence of polyphenolics in the plant material.

Sunmonu and Afolayan[21] reported that reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of its potential antioxidant activity. Also, Luximon-Ramma *et al.*[26] reported that FRAP is widely used in the evaluation of the antioxidant component of dietary polyphenols. The ethanolic extract of *A. heterophyllus* stem bark showed very good reducing capacity which served as a significant indicator of its antioxidant activity (Figure 2)[27]. This property may enable the ethanolic extract of *A. heterophyllus* stem bark to mop up noxious toxic metabolites released during pathological states and confer protection on the affected organs. The extract may as well exert its effect by preventing chain initiation or decomposition of peroxides[28]. The reductive capability of the ethanolic extract of *A. heterophyllus* stem bark was concentration dependent which may indicate that its antioxidant activity is likely to elevate with increasing in concentrations.

Hydroxyl radical (Figure 3) is a very reactive free radical that is capable of damaging all types of biomolecules. This can cause peroxidation of membrane lipids and damage to DNA[29]. This result also correlates with other *in vitro* antioxidant discussed earlier which may be attributed to polyphenolic in the ethanolic extract of *A. heterophyllus* stem bark.

In other vein, iron chelating may prevent generation of hydroxyl radical (Figure 4)[29]. Iron acts as a metal catalyst in producing hydroxyl radicals from hydrogen peroxide[30]. The

ability of the ethanolic extract of *A. heterophyllus* stem bark to chelat Fe^{2+}, implies that generation of hydroxyl radicals in Fenton reaction may be attenuated and prevent possible damage of hydroxyl radical to biomolecules. This is because accumulation of iron may lead to development of oxidative stress[29]. Adefegha and Oboh[29] reported that minimizing postprandial hyperglycaemia is an effective way of managing diabetes mellitus most especially type II diabetes mellitus. This can be accomplished by inhibiting carbohydrate hydrolyzing α-amylase and α-glucosidase (Figures 5 and 6) in the gastrointestinal tract[25]. The ability of the ethanolic extract of *A. heterophyllus* stem bark to inhibit α-amylase and α-glucosidase in a concentration dependent manner may be attributed to polyphenolics in the extract, which correlate with the report of Adefegha and Oboh[31]. The inhibitory properties of α-amylase and α-glucosidase by the extract may be useful in minimizing the side effects associated (*e.g.* flatulence, diarrhea, abdominal distention, *etc.*) with synthetic drugs.

In this study, it can be deduced that ethanolic extract of *A. heterophyllus* stem bark inhibited the activity of α-amylase and α-glucosidase (Figures 7 and 8) in a non-competitive and uncompetitive manners respectively. This may be attributed to polyphenolic in the extract.

The possible bioactive compounds in the ethanolic extract of *A. heterophyllus* stem bark were detected using GC–MS analysis. The compounds identified were presented in Table 3. However, the antidiabetic activity of the ethanolic extract of *A. heterophyllus* stem bark may be attributed to the presence of these compounds.

In conclusion, this study indicated that the ethanolic extract of *A. heterophyllus* stem bark contains polyphenolic compounds, possesses free radical scavenging ability and has inhibitory potential of α-amylase and α-glucosidase which were mediated by non-competitive and uncompetitive respectively. Furthermore, the identified bioactive agents in this study may be responsible for its antidiabetic ability and further studies may investigate its antidiabetic potentials.

Conflict of interest statement

The authors report no conflict of interest.

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