



Polyphenolic constituents, antioxidant and hypoglycaemic potential of leaf extracts of *Acalypha godseffiana* from Eastern Nigeria: *In vitro* study

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Background: *Acalypha godseffiana* is an important plant used as an ornamental and herbs; its leaves are used in the management of diseases like diabetes in Eastern Nigeria.

Aim: The study aims at linking the hypoglycemic activity extracts of leaves of *A. godseffiana* to their polyphenolic contents.

Setting: Fresh leaves of *A. godseffiana* were obtained from Imo State, Nigeria, identified in University of Lagos Herbarium, air-dried, pulverized and kept for investigations.

Method: The phytochemical compositions and antioxidant potentials of acetone, aqueous, ethanol and methanol extracts of *A. godseffiana* were determined using adopted methods. An *in-vitro* approach was used to evaluate the hypoglycemic potentials of the extracts on α -amylase and α -glucosidase enzymes. The mechanism of inhibitions was studied using the Lineweaver-Burk plot.

Results: Antioxidant results revealed that total antioxidant capacity of the acetone extract (IC₅₀: 0.34 mg/mL) showed better activity compared to the standards (silymarin 0.52 mg/mL; gallic acid 0.51 mg/mL). The hypoglycemic findings confirmed that acetone extract demonstrated strong and mild inhibitory potential against α -amylase and α -glucosidase respectively, showing concentration dependent with IC₅₀ values of 2.33 mg/mL and 0.13 mg/mL. The observed hypoglycemic and antioxidant potentials of acetone extract of *A. godseffiana* correlate to its high polyphenolic contents which include phenols (133.20 mg gallic acid g⁻¹), flavonoid (350.60 mg quercetin g⁻¹) and tannins (264.67 mg catechin g⁻¹). The mechanisms of action exhibited by the acetone extract were uncompetitive and mixed non-competitive which can be attributed to its inhibitory properties on α -glucosidase and α -amylase respectively.

Conclusion: The results obtained from this study validate the acetone leaves extract of *A. godseffiana* as potential agent in management of sugar related disorder.

Introduction

Non-communicable diseases such as diabetes mellitus (DM) are among the five leading causes of death in the world (Joseph & Jini 2011). In sub-Saharan regions of Africa, such as Nigeria and South Africa, it was reported by World Health Organization that the burden of diabetic epidemic of all age groups in Africa was approximately 7.1 million at the end of 2000, and is estimated to increase to 18.6 million (Shaw, Sicree & Zimmet 2010) by 2030. Worst still, Nigeria and South Africa are at Africa were reported to be at higher risk countries in Africa, with an incidence rate of 4.50% and 8.30%, respectively (Abdullah et al. 2014). Diabetes mellitus is a chronic metabolic disease characterised by high blood sugar resulting from the inability of the body to produce insulin or utilise the insulin produced, or both. This metabolic disease is initiated by either the failure of the cells to utilise the produced insulin or the incapability of the pancreas to produce enough insulin, or both (Gardner & Greenspan 2011). The defective responsiveness of body tissues to DM is categorised into three major types: type 1 DM, type 2 DM and gestational diabetes. Type 1 DM (also known as insulin-dependent DM) is caused by autoimmune destruction of the pancreatic beta cells, leading to loss or insufficient production of insulin; it is prevalent among juveniles. Type 2 DM (also known as adult onset DM) is caused by the inability of the cells to utilise insulin properly. Gestational diabetes occurs during pregnancy because of high blood glucose level as a result of the inability of the system to produce enough insulin for cells (Maori, Ezekiel & Bilal 2012). The DM pandemic is associated with unhealthy life style, civilisation, ageing and effects of reactive oxygen species (ROS)

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(Wild et al. 2004). If uncontrolled, it results in serious long-term complications such as cardiovascular disease, stroke, chronic kidney failure, foot ulcers and damage to the eyes, which eventually cause death of sufferers (Levitt 2008). Recent evidence suggests that besides obesity and physical inactivity known to be major risk factors for type 2 diabetes (T2DM), oxidative stress may contribute to the pathogenesis of T2DM by increasing insulin resistance or impairing insulin secretion (Montonen et al. 2004). The advancement of diabetes and its hitches are worsened by oxidative stress by generation of highly reactive free radicals mainly as a result of hyperglycaemia. Besides, excess generation and/or insufficient removal of these free radicals leads to vascular dysfunction and damage to cellular proteins, membrane lipids and nucleic acids (Johansen et al. 2005). The ability to inhibit the formation of ROS or scavenge free radicals via antioxidants is a therapeutic strategy to prevent oxidative stress and the related diabetic vascular complications (Bajaj & Khan 2012). Plant polyphenols such as phenols, phenolic acids and flavonoids (Zhou & Ibrahim 2010) have been reported as potential and viable source of antioxidants. *Acalypha godseffiana* (Euphorbiaceae family) belongs to the genus *Acalypha*, comprising about 570 species (Ogundaini 2005). It is commonly called copperleaf, Joseph's coat, fire dragon and match-me-if-you-can (Ikewuchi et al. 2011). It originated in Pacific Island, a fast-growing bushy shrub, an ever-green plant with green leaf blade and creamy-white margins. The young developing leaf has pinkish tinge. In traditional medicine, the roots are used as cathartic and leaves are reported to have laxative and vulnerary properties (Sahoo et al. 2014). The expressed juice or boiled decoction of the leaves of *A. godseffiana* has been used in traditional health care practice in Eastern Nigeria, for the management of gastrointestinal disorders, fungal skin infections, hypertension and DM. The leaf of species of *Acalypha* has also been reported to possess antimicrobial (Akinyemi et al. 2005; Ogundaini 2005; Oladunmoye 2006), hypolipidaemic, hypoglycaemic (Quds et al. 2012), diuretic and anti-hypertensive (Seebaluck, Gurib-Fakim & Mahomoodally 2015) properties. In this study, the polyphenolic constituents, antioxidant and hypoglycaemic potentials of *A. godseffiana* leaves were investigated with respect to acetone, ethanol, methanol and aqueous extracts.

Materials and methods

Chemicals and reagents

The chemicals used in the study were the following: gallic acid, silymarin, quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), iron III chloride (FeCl_3), potassium ferricyanide [$\text{KFe}(\text{CN})_6$], sodium hydroxide (NaOH), sodium nitrite (NaNO_2), aluminium chloride (AlCl_3), Folin and Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS^+), sodium persulphate solution (PPS), ammonium molybdate, sodium phosphate, ferrocene, phosphate buffer trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrogen peroxide (H_2O_2) (Merck Chemical Supplies, Darmstadt, Germany) pancreatic α -amylase, α -glucosidase and p-nitrophenyl- α -D-glucopyranoside (pNPG) from Sigma Chemical Co. (St. Louis, MO, USA). Starch, dinitrosalicylic acid

(DNS), maltose and sucrose were products of J. T. Baker Inc., Phillipsburg, NJ, USA, while acarbose was obtained from Bayer Medical Co. Leverkusen Germany. The additional reagents and solvents used in the study are of standard grades.

Collection, authentication and preparation of plant sample

The leaves of *A. godseffiana* were obtained from Imo State, Eastern Nigeria, from October to December 2015. The plant was authenticated (LUH 2753) and deposited at the herbarium, Department of Botany, Faculty of Science, University of Lagos, Akoka. The plants were air-dried for 5 days and pulverised to coarse powder. An amount of 100 g of the coarse sample was separately macerated with acetone, ethanol and methanol for 72 h and lukewarm distilled water with continuous agitation (to enhance extraction of metabolites) for 12 h. The solvent extracts were concentrated to dryness using rotary evaporator (BUCHI Rotavapor R-300, New Castle, Delaware, USA) at 37°C , while water extracts were freeze-dried (Lyo Lab 3000, Apollo Scientific CC, Port Elizabeth, South Africa). The extracts were kept at 4°C until use.

Phytochemical analysis

Qualitative phytochemical analysis

The qualitative phytochemical components of the leaf extracts were determined using standard protocols (Harborne 1984; Trease & Evans 2002).

Analysis of total phenolic composition

Total phenol contents: The total phenol in the extracts was calculated using Folin and Ciocalteu's phenol reagent (Adedapo et al. 2009; Nabavi et al. 2008). The volume of 2.5 mL of 10% Folin-Ciocalteu reagent (v/v) and 2 mL (75 g/L) Na_2CO_3 solution was added to a test tube containing the extract at different concentrations. The tubes were agitated for 15 s and let to stand at 40°C for 30 min for the reaction to be completely observable with colour development. Absorbance was then measured at 760 nm using UV-Vis spectrophotometer (DU 7400, Beckman Coulter Inc, USA). Gallic acid was used as a standard and the total phenolic content of the samples was expressed as equivalent gallic acid (mg/g) using the calibration curve equation: $Y = 13.417x + 0.1919$, $R^2 = 0.9593$ of gallic acid.

Total flavonoids determination: The method of Samarth et al. (2008) was employed to determine total flavonoids in the extracts. The sample at different concentrations in a test tube was added 2 mL distilled water and 0.15 mL of 5% of NaNO_2 and allowed to stand at 25°C for 5–6 min. A volume of 0.15 mL of 10% AlCl_3 was added and allowed to stand for another 6 min, 1 mL of 4% NaOH was then added to the solution along with 5 mL of distilled water, vortexed for 15 min and colour change was observed. The spectrophotometer at 420 nm was used to obtain the absorbance. The flavonoids in the samples were calculated using quercetin (mg/g) as a standard using the following equation: $Y = 1.755x + 0.0237$, $R^2 = 0.977$.

Total proanthocyanidins content: Determination of proanthocyanidin was done using the methods of Sun et al. (1998). A volume of 0.5 mL of 1 mg/mL of the extracts at different concentrations was added with 4% vanillin-methanol reagent (3 mL) and 1.5 mL hydrochloric acid; the solution was kept for 15 min on rest. The spectrophotometer at 500 nm was used to obtain the absorbance. The total proanthocyanidin in the extracts was calculated as catechin equivalents (mg/g) using the following equation: $Y = 1.0733x + 0.0226$, $R^2 = 0.9956$.

Determination of antioxidant activities

Scavenging activity using 1,1-diphenyl-2-picrylhydrazyl assay

The scavenging properties of the extracts on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) radicals were assessed by adopting the protocols described by Liyana-Pathiranan and Shahidi (2005). A volume of 1.0 mL of 0.135 mM DPPH in methanol was added to a test tube containing the extracts (0.125–0.1 mg/mL). The reaction mixture was shaken thoroughly and kept in dark for 30 min at 25 °C for colour change from purple to yellow. Then the degree of colour change of the solution was estimated at 517 nm using spectrophotometer. Silymarin and gallic acid were used as standard antioxidants. The ability to scavenge DPPH radical was calculated using Equation 1:

$$\% \text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100. \quad [\text{Eqn 1}]$$

where A_0 = absorption of blank sample and A_1 = absorption of test extract solution.

Total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity (TAC) of the extracts was evaluated by the method adopted by Silici, Sagdic and Ekici (2010). The significant aspect of this assay is the ability of the extracts to reduce Mo (VI) to Mo (V) with the formation of a green phosphate/Mo (V) complex at acidic pH. A volume of 30 μ L extracts of varying concentrations (0.125–0.1 mg/mL) was mixed with 300 μ L of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in 96-well plates. For the blank, 30 μ L methanol was mixed with 300 μ L of the reagent. Gallic acid and silymarin were used as control. The 96-well plates containing the mixture were kept in oven at 95 °C for 90 min, and the degree of reduction from Mo (VI) to Mo (V) of the extracts was obtained at 695 nm using microplate reader. The percentage inhibition of the extracts and standards was calculated using Equation 2:

$$\% \text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100. \quad [\text{Eqn 2}]$$

where A_0 = absorption of blank sample and A_1 = absorption of test extract solution.

Ferric ion reducing antioxidant power assay

Ferric ion reducing antioxidant power (FRAP) of the extracts was determined using the method adopted by Müller,

Fröhlich and Böhm (2011). Extracts at different concentrations (0.125–0.1 mg/mL) were mixed with 2.5 mL of 20 mM phosphate buffer and 2.5 mL potassium ferricyanide (1%, w/v $\text{KFe}(\text{CN})_6$), and the solution was then kept in an oven at 50 °C for 30 min. A solution of 2.5 mL trichloroacetic acid (10% w/v) and 0.5 mL ferric chloride (0.1% w/v) was added to the mixture in 96-well plates and kept for 10 min. The ability of the extract to convert brown ferric ion (Fe^{3+}) to blue ferrous ion (Fe^{2+}) was measured at 700 nm using a spectrophotometer; gallic acid and silymarin were used as standard antioxidants. The percentage inhibition of the extracts and standards was calculated using Equation 3:

$$\% \text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100. \quad [\text{Eqn 3}]$$

where A_0 = absorption of blank sample and A_1 = absorption of test extract solution.

Scavenging activity on 2,2'-Azino-bis (3-ethylbenzothiazoline-6-acid) radical

The 2,2'-Azino-bis (3-ethylbenzothiazoline-6-acid) (ABTS^+) scavenging activities of the extracts were assessed by the modified method proposed by Floegel et al. (2011). The stock solution of the ABTS^+ was prepared by dissolving 38.4 mg of 7 mM ABTS^+ in 10 cm^3 of distilled water and 2.45 mM $\text{K}_2\text{S}_2\text{O}_8$ (sodium persulphate solution) prepared by dissolving 6.623 mg $\text{K}_2\text{S}_2\text{O}_8$ in 10 cm^3 of distilled water. 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and $\text{K}_2\text{S}_2\text{O}_8$ were mixed in a 1:1 ratio and the mixture was kept in dark for 12 h. The stock solution of the ABTS^+ was diluted with methanol to an absorbance of 0.7 ± 0.005 at 734 nm to generate the ABTS reagent employed in this analysis, and methanol solvent was used as blank. A volume of 200 μ L of the extract of varying concentrations (0.125–0.1 mg/mL) was added to the diluted ABTS^+ solution (200 μ L). The solution was vortexed and allowed to react for 7 min; absorbance was recorded at 734 nm using a 96-well microplate reader (BIORAD, Model 680, Japan). The mixture of methanol solvent and ABTS solution alone was used as control; gallic acid and silymarin were used as standard antioxidants (see Equation 4):

$$\% \text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100. \quad [\text{Eqn 4}]$$

where A_0 = absorption of blank sample and A_1 = absorption of test extract solution.

The half-maximal inhibitory concentration (IC_{50}) value was calculated from the linear regression equation using $y = mx + c$, where y is the percentage activity and equals 50, m is the slope, c is the intercept and x is the IC_{50} value.

Antidiabetic assays: *In vitro*

Alpha glucosidase inhibition and kinetic methods

The inhibitory capacity of the extracts on α -glucosidase enzyme was evaluated using the protocol of Sabiu, O'Neill and Ashafa (2016). Distilled water was used for preparing 0.25–8.0 mg/mL concentrations of extracts of *A. godseffiana* and acarbose. To 96-well plates containing 50 μ L of each

concentration was added 0.1 M phosphate buffer of 1.0 M of α -glucosidase solution (100 mL, pH 6.9). The solution was kept in an oven at room temperature for 10 min. A volume of 50 mL of p-nitrophenyl- α -D-glucopyranoside (5 mM p-NPG) solution in phosphate buffer (0.1 M, pH 6.9) was added to each well at timed intervals. The solutions were kept in an oven at 25 °C for 5 min. The microplate reader (BIORAD, Model 680, Kumamoto, Japan) at 405 nm was used to measure the absorbance. The experiments were carried out in triplicate and the inhibitory activity of the extract was obtained as percentage inhibition using Equation 5:

$$\% \text{ Inhibition} = \frac{\Delta A \text{ control} - \Delta A \text{ extract}}{\Delta A} \times 100. \quad [\text{Eqn 5}]$$

where ΔA control and ΔA extract are the differences in absorbance of the control and extract, respectively. The inhibitory concentration (IC_{50}) of the extracts' enzyme activity was determined graphically.

Alpha-amylase inhibitory assay

The modified method of McCue and Shetty (2004) was adopted; 250 μ L of sodium phosphate buffer (0.02 M, pH 6.9) containing a solution of α -amylase (0.5 mg/mL) was added to 250 μ L of the extract in a test tube. The mixture was initially heated in an oven at 25 °C for 10 min, followed by an addition of 250 μ L of starch solution (1%) in sodium phosphate buffer (0.02 M, pH 6.9) at a calculated time and heated again for another 10 min at room temperature. The dinitrosalicylic acid (DNS) reagent (500 μ L) was added to stop the reaction. The reaction tubes were boiled in water for five min and left to cool to 25 °C. About 5 mL of distilled water was added to dilute the solution and the absorbance was recorded using a microplate reader (BIORAD, Model 680, Kumamoto, Japan) at 540 nm. Distilled water was used as the control and acarbose as the standard drug. ΔA control and ΔA extract are the differences in absorbance of the control and extract, respectively. The inhibitory concentration (IC_{50}) of the extracts' enzyme activity was determined graphically.

Mechanism of inhibitions

Mechanism of alpha-glucosidase inhibition: The mechanism underlying the inhibition of acetone extract of *A. godseffiana* on α -glucosidase enzyme was studied using the protocol of Dnyaneshwar and Archana (2013) with modification. In a set of test tubes, 50 μ L of 5 mg/mL acetone extract and 100 μ L of α -glucosidase solution were added and pre-incubated for 10 min at room temperature. In another set of test tubes, α -glucosidase and 50 μ L of phosphate buffer (pH 6.9) were added and pre-incubated at the same temperature. An amount of 50 mL of 0.63–2.0 mg/mL concentrations of p-nitrophenyl- α -D-glucopyranoside (pNPG) was added to both sets of tubes to initiate the reactions. The tubes were kept in oven at room temperature for 10 min; the reaction was terminated by adding 500 mL of Na_2CO_3 . The concentration of reducing sugars was determined colourimetrically using

p-nitrophenol standard curve. The Lineweaver and Burk method was employed to study the nature of inhibition (Lineweaver & Burk 1934).

Mechanism of alpha-amylase inhibition: The protocol of Ali, Houghton and Soumyanath (2006) was employed in studying the mode of inhibition of α -amylase by the acetone extract of *A. godseffiana* (the most potent extract). In a set of test tubes containing 250 μ L (5 mg/mL) of the extract was added an equal volume of α -amylase solution (250 μ L, 0.5 mg/mL). The tubes were kept in oven at 25 °C for 10 min. Alpha-amylase in phosphate buffer (250 μ L, pH 6.9) was also heated in another set of tubes. Starch solution (250 μ L) was added to each set of reaction mixtures to initiate the reaction at an increasing concentration of 0.3–5.0 mg/mL. Both sets of test tubes were then kept in the oven at 25 °C for 10 min. They were then removed from the oven, and 500 μ L of DNS was added to each tube and reheated for five min to terminate the reaction. The total concentration of reducing sugars were spectrophotometrically evaluated using a maltose standard curve. The Lineweaver and Burk method was employed for studying the nature of inhibition (Lineweaver & Burk 1934).

Statistical analysis

Antioxidant activities were expressed in percentage. Quantification of phytochemicals was expressed as mean \pm standard deviation (SD), while GraphPad Prism 5.0 statistical package was employed for determining the percentage inhibition of α -glucosidase and α -amylase. One-way analysis of variance (ANOVA), followed by the Bonferroni test, was used for data analysis, and the results were expressed as mean \pm standard error of the mean (SEM) for triplicate determinations.

Results

Phytochemical analysis

The qualitative phytochemical analysis of acetone, ethanol, methanol and water extracts of *A. godseffiana* revealed the presence alkaloids, saponins, flavonoids, steroids, tannins, phenolic compounds, terpenoids and cardiac glycosides in varying concentrations with respect to the extracting solvent (Table 1). The results of quantification of phenolic compounds (Table 2) revealed that acetone extracted more phenolic compounds followed by methanol, ethanol and water the least.

TABLE 1: Phytochemical components of leaves of *Acalypha godseffiana*.

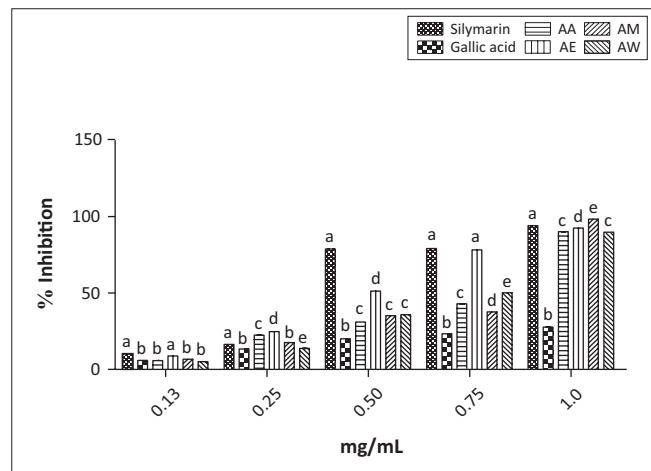
Phytochemical	AA	AE	AM	AW
Alkaloids	+	++	++	++
Flavonoids	++	++	++	+
Tannins	++	+	+	+
Saponins	-	-	++	+
Terpenoids	-	+	++	-
Phenolics	++	++	++	+
Cardiac glycoside	+	+	++	++

+, Present; ++, abundant; -, absent; AA, acetone extract; AE, ethanol extract; AM, methanol extract; AW, water extract.

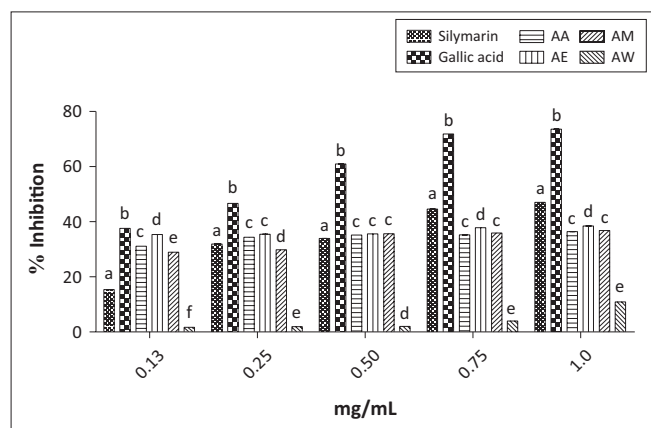
TABLE 2: Total phenolic composition of extracts of *Acalypha godseffiana* (leaves).

Phytochemicals	AA	AE	AM	AW
Total phenols (mg gallic acid g ⁻¹)	133.20 ± 0.001	208.03 ± 0.003	189.17 ± 0.002	53.52 ± 0.002
Total flavonoid (mg quercetin g ⁻¹)	350.60 ± 0.002	304.25 ± 0.001	379.66 ± 0.001	156.11 ± 0.002
Total proanthocyanidin (mg catechin g ⁻¹)	264.67 ± 0.015	45.09 ± 0.002	75.84 ± 0.001	13.73 ± 0.001

AA, acetone extract; AE, ethanol extract; AM, methanol extract; AW, water extract.



Key: The values are expressed as means ± SEM of triplicate determinations. Bars not sharing common letter at the same concentration are significantly different ($p < 0.05$). AA, acalypha acetone; AE, acalypha ethanol; AM, acalypha methanol; AW, acalypha water

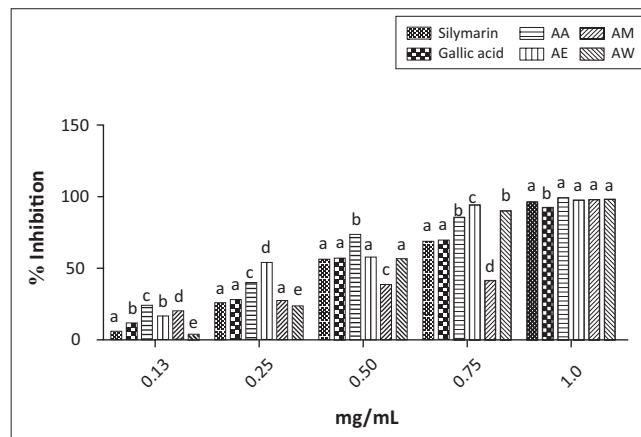
FIGURE 1: 1,1-Diphenyl-2-picryl-hydrazyl scavenging potential of leaf extracts of *Acalypha godseffiana*.

Key: The values are expressed as means ± SEM of triplicate determinations. Bars not sharing common letter at the same concentration are significantly different ($p < 0.05$). AA, acalypha acetone; AE, acalypha ethanol; AM, acalypha methanol; AW, acalypha water.

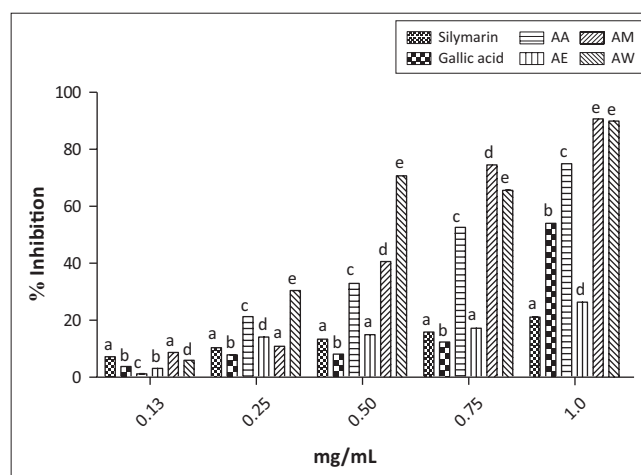
FIGURE 2: Ferric reducing antioxidant power of leaf extracts of *Acalypha godseffiana*.

Antioxidant activity

Figures 1–5 show the scavenging activities, metal chelating and total antioxidant potentials of acetone, ethanol, methanol and aqueous extracts from the leaves of *A. godseffiana*. The extracts exhibited varied scavenging and reducing potentials on free radicals. The acetone extract exhibited the highest antioxidant potential compared to other extracts and standard, with IC_{50} of 0.340 mg/mL for TAC assay (Table 3). The remarkable antioxidant potential can be attributed to its high contents of phenolic compounds such as phenols (133.20 g gallic acid g⁻¹), flavonoid (350.60 g quercetin g⁻¹) and tannins (264.67 g catechin g⁻¹) (Table 2) which are reported as natural antioxidants.



Key: The values are expressed as means ± SEM of triplicate determinations. Bars not sharing common letter at the same concentration are significantly different ($p < 0.05$). AA, acalypha acetone; AE, acalypha ethanol; AM, acalypha methanol; AW, acalypha water.

FIGURE 3: Total antioxidant capacity by phosphomolybdenum of *Acalypha godseffiana* (leaves).

Key: The values are expressed as means ± SEM of triplicate determinations. Bars not sharing common letter at the same concentration are significantly different ($p < 0.05$). AA, acalypha acetone; AE, acalypha ethanol; AM, acalypha methanol; AW, acalypha water.

FIGURE 4: Percentage inhibition of metal chelating potentials of extracts *Acalypha godseffiana* (leaves).

In vitro enzyme inhibitory potentials of extracts

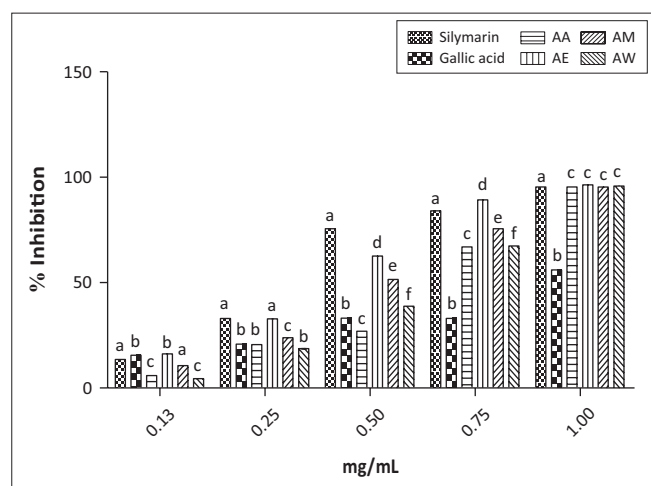
The results of inhibitory study of the extracts on α -glucosidase enzyme revealed a dose-dependent inhibition of the α -glucosidases by the extracts (acetone, ethanol, methanol and aqueous) of *A. godseffiana* (Figure 6). There were no significant differences between the extracts at low concentrations (0.125–0.1 mg/mL). However, as the concentration increased, the acetone extracts exhibited significant inhibition of the enzyme compared with other extracts and acarbose. This is evident in their IC_{50} values represented (Table 4). The mechanism of inhibition of the enzyme using the Lineweaver-Burk plot showed that the acetone extract exhibited independent inhibition for

α -glucosidase (Figure 6). In the same manner, the percentage inhibition of the extracts against α -amylase activity produced a concentration dependent effect (Figure 7). There were no significant differences between the extracts at lower concentrations (0.125–0.1 mg/mL). The methanol extract inhibited the α -glucosidases enzyme most followed by acetone extracts as shown in Table 4. However, the Lineweaver-Burk plot of the mechanism of inhibition showed that acetone extract inhibited α -glucosidase by mixed uncompetitive manner (Figure 8) and α -amylase by mixed non-competitive manner (Figure 9).

Discussion

Medicinal plants contain secondary metabolites which are responsible for their exhibited therapeutic or toxicological properties (Khan et al. 2008). Plants have been used in crude form in the treatment and management of diseases (Pistolato, Giampieri & Battino 2015). The acetone extract exhibited the highest phenolic compounds when compared with methanol, ethanol and water extracts, which is in line with the findings of Ashafa, Grierson and Afolayan (2010). The strong antioxidant capacity of plant extracts could be linked to high phenolic compounds (Afolayan et al. 2007). Phenolic compounds have been reported to terminate the oxidative stress initiated by free radical reaction in the pathogenesis of diabetes mellitus. Oxidative stress has been reported to cause cell damage or even cell death, thereby accelerating

ageing and causing a variety of degenerative diseases such as DM, and ROS are one of the factors that trigger insulin resistance. Hence, there is the need of antioxidants in the management of DM (Guichard et al. 2008; Spadiene et al. 2014). This study revealed that the scavenging properties of ABTS⁺ by ethanol extracts (IC₅₀, AE = 0.43) were found to be higher than that of DPPH (IC₅₀, AE = 0.51), supporting the findings of Ashafa et al. (2010) and Jiang et al. (2015). Similarly, Wang et al. (1998) also found that some compounds that are ABTS⁺ scavengers are also moderate DPPH scavengers, as observed in this study (IC₅₀; AM; ABTS⁺ = 0.5, DPPH = 0.64). This buttresses the fact that the extract has different free radical scavenging potentials, which is a useful mechanism for treating radical-related pathologic damage (Mohdaly et al. 2011). In the assessment of antioxidant activities of the extracts, a FRAP assay was also used, and it was reported that the majority of secondary metabolites that are redox-active compounds can be detected by the FRAP assay (Halvorsen et al. 2006). The results revealed that all the extracts exhibited moderate reducing capacity, with methanol extract exhibiting the best activity (IC₅₀ 2.25). The result was in line with the reports of Sowndhararajan and Kang (2013). The TAC of the extracts was measured using the phosphomolybdenum method, which is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate Mo (V) complex. In this method, a higher TAC value corresponds to a higher antioxidant activity (Prieto, Pineda & Aguilar 1999; Sofidiya & Familoni 2012). The results of this study showed that acetone and ethanol extracts showed higher phosphomolybdenum reduction capacity. The TAC result of this study agrees with the findings of Sofidiya and Familoni (2012), where ethanol extract exhibited the highest activity. The observed antioxidant activities of the extracts were related to the redox properties of the extracts in absorbing and neutralising free radicals and quenching ravaging chain reactions of ROS, with an inhibitory effect on carcinogenesis (Uttara et al. 2009). There is also a positive relationship between the phenolic content and observed antioxidant activity of the extracts of *A. godseffiana* as observed in this study, supporting the findings of Ashafa et al. (2010). Polyphenolic compounds are very important plant metabolites which defend the body from diverse types of oxidative damage (Shukla et al. 2009). The strong antioxidant activity exhibited by the extracts from leaves of *A. godseffiana* could be attributed to the high flavonoids, proanthocyanidins and phenols present in the plant leaves. Flavonoids are the most diverse and widespread group of natural polyphenolic compounds known for



Key: Values are expressed as means \pm SEM of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($p < 0.05$). AA, acalypha acetone; AE, acalypha ethanol; AM, acalypha methanol; AW, acalypha water

FIGURE 5: Percentage inhibition of 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) potentials of *Acalypha godseffiana* (leaves) extracts.

TABLE 3: IC₅₀ (mg/mL) of *Acalypha godseffiana* (leaves) in different anti-oxidative models.

Assay	SLY	GAL	ACE	ETE	MEE	AQE
DPPH	0.47 \pm 0.01 ^a	1.90 \pm 0.03 ^b	0.66 \pm 0.03 ^c	0.51 \pm 0.02 ^a	0.64 \pm 0.05 ^a	0.69 \pm 0.01 ^c
FRAP	0.33 \pm 0.03 ^a	1.00 \pm 0.05 ^b	3.79 \pm 0.01 ^c	3.92 \pm 0.01 ^d	2.25 \pm 0.03 ^e	5.38 \pm 0.02 ^f
TAC	0.52 \pm 0.01 ^a	0.51 \pm 0.03 ^a	0.34 \pm 0.01 ^b	0.37 \pm 0.04 ^b	0.59 \pm 0.02 ^c	0.41 \pm 0.05 ^d
MCH	3.01 \pm 0.01 ^a	1.21 \pm 0.05 ^b	0.68 \pm 0.02 ^c	2.18 \pm 0.05 ^d	0.56 \pm 0.01 ^e	0.51 \pm 0.02 ^e
ABTS ⁺	0.42 \pm 0.01 ^a	0.97 \pm 0.03 ^b	0.60 \pm 0.01 ^c	0.43 \pm 0.05 ^a	0.50 \pm 0.02 ^d	0.58 \pm 0.02 ^c

Note: Each value of IC₅₀ obtained by linear regression equation is presented as mean \pm SEM (where $n = 3$). Values with different superscripts in the same row for each parameter are significant ($p < 0.05$) to each other.

DPPH, 1,1-diphenyl-2-picrylhydrazyl; TAC, total antioxidant capacity by phosphomolybdenum; FRAP, ferric reducing ability of plasma; MCH, metal chelating ability; ABTS⁺, 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation; OH, hydroxyl radical scavenging capacity; SLY, silymarin; GAL, gallic acid; AA, acetone extract; AE, ethanol extract; AM, methanol extract; AW, water extract.

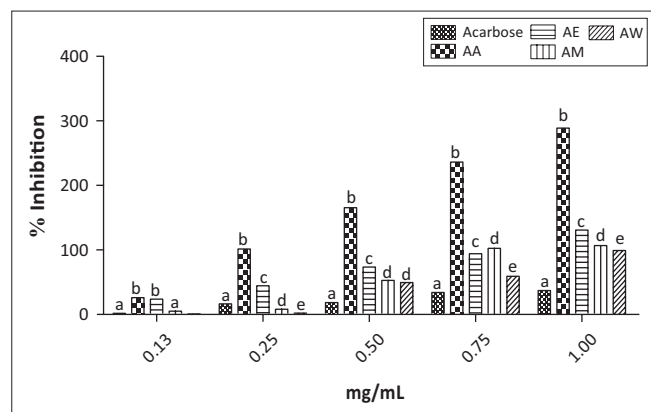
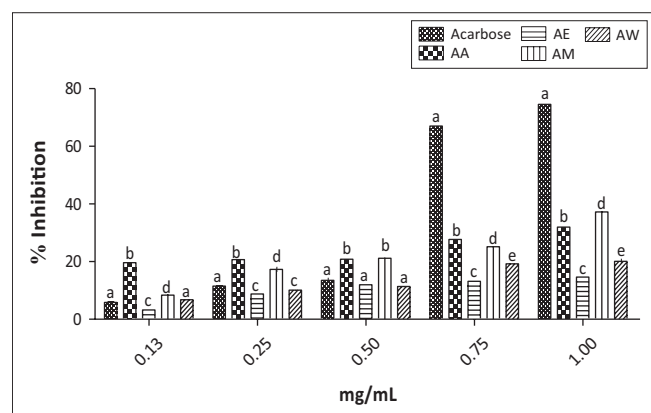


FIGURE 6: Inhibitory potential of *Acalypha godseffiana* leaf extracts against α -glucosidase activity. Key: Values are expressed as means \pm SEM of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($p < 0.05$). AA, acalypha acetone; AE, acalypha ethanol; AM, acalypha methanol; AW, acalypha water.

TABLE 4: IC_{50} values of α -amylase and α -glucosidase inhibition by *Acalypha godseffiana* (leaves) extracts.

Sample	α -Amylase	α -Glucosidase
Acarbose	0.71 ± 0.02^a	1.28 ± 0.04^a
Acetone extract	2.33 ± 0.01^b	0.13 ± 0.01^b
Ethanol extract	3.97 ± 0.01^c	0.31 ± 0.05^c
Methanol extract	1.52 ± 0.04^d	0.50 ± 0.04^d
Water extract	2.85 ± 0.03^e	0.30 ± 0.02^e

Note: Each value of IC_{50} obtained by linear regression equation is presented as mean \pm SEM (where $n = 3$). Values with different superscripts in the same column for each parameter are significant ($p < 0.05$) to each other.



Key: Values are expressed as means \pm SEM of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($p < 0.05$). AA, acalypha acetone; AE, acalypha; AM, acalypha methanol; AW, acalypha water.

FIGURE 7: Inhibitory potential of *Acalypha godseffiana* different leaf extracts against α -amylase activity.

their antioxidant, anti-inflammatory and anticarcinogenic properties (Samatha et al. 2012). Proanthocyanidin, known as condensed tannin, is a polyphenolic compound with powerful antioxidant activity (Lee et al. 2007) and has been reported by Yokozawa et al. (2012) to ameliorate the diabetic condition by significantly reducing serum glucose, glycosylated protein and serum urea nitrogen. It also decreases the urinary protein and renal-agenesis in streptozocin-induced diabetic rats and serum glucose with substantial decrease of glycosylated protein in type 2 diabetic mice. It was also reported by Jiao et al. (2013) that proanthocyanidin being abundant in the acetone leaf extract (264.67 mg catechin g^{-1} compared with methanol extract

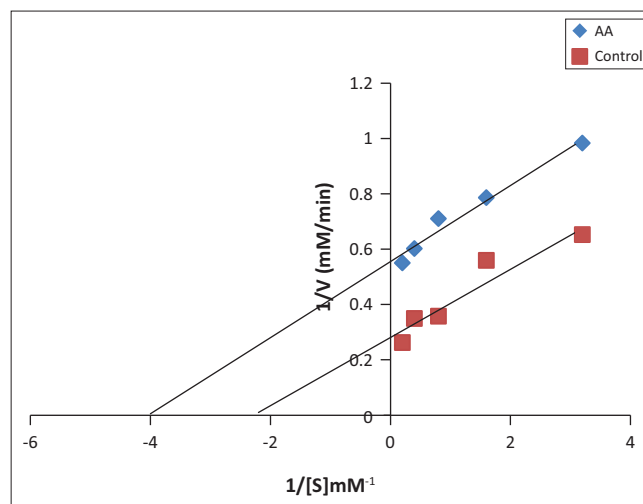


FIGURE 8: Lineweaver-Burk plot of acetone extract of leaves of *Acalypha godseffiana* eliciting uncompetitive inhibition on α -glucosidase activity. Results = mean \pm SEM ($n = 3$); ($p < 0.05$). AA, *A. godseffiana* acetone leaf extracts.

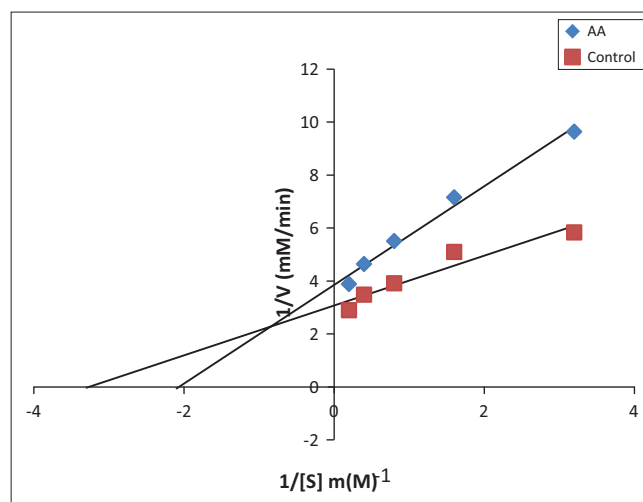


FIGURE 9: Lineweaver-Burk plot of acetone extract of leaves of *Acalypha godseffiana* eliciting mixed non-competitive inhibition on α -amylase activity. Results = mean \pm SEM ($n = 3$); ($p < 0.05$). AA, *A. godseffiana* acetone leaf extracts.

75.84 mg catechin g^{-1} , ethanol extract 45.09 mg catechin g^{-1} , water extract 13.73 mg catechin g^{-1}) of *A. godseffiana* in this study, is a potential antidiabetic drug candidate.

The outcome of hypoglycaemic analysis from this investigation showed that acetone extract of *A. godseffiana* exhibited a remarkably strong inhibitory activity on α -glucosidase and mild α -amylase at IC_{50} 0.13 mg/mL and 2.33 mg/mL, respectively, when compared with the standard acarbose at IC_{50} 1.28 mg/mL and 0.71 mg/mL, respectively. Kwon, Vattem and Shetty (2005) also reported that any prospective antidiabetic agent should be a mild inhibitor of α -amylase and a potent inhibitor of α -glucosidase so as to prevent the drawbacks of synthetic drugs (like acarbose), which occur because of the excessive inhibition of the enzyme resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon. The Lineweaver-Burk plot showed that the acetone extract of *A. godseffiana* inhibited α -glucosidase and α -amylase in an uncompetitive and mixed non-competitive mode, respectively. The uncompetitive

nature of the acetone extract to α -glucosidase proposes that there is no structural relationship between the inhibitor and the substrate; hence, the inhibitor completely forms bonds with the enzyme-substrate complex, forming an enzyme-substrate inhibitor complex which is inert in nature (King et al. 2009). The mixed non-competitive mode of inhibition obtained from the Lineweaver-Burk plot infers that there is no competition between the bioactive compounds in the extracts and the substrate for binding active site, but the inhibitors form bonds with a distinct spot on the enzyme so as to delay the conversion of disaccharides into monosaccharides (Kazeem, Adamson & Ogunwande 2013). The extracts may therefore be useful in the development of neutraceuticals and management of diabetes. The results agreed with the findings of Ashafa et al. (2010) and Kwon, Apostolidis and Shetty (2008), which reported that moderate α -amylase inhibition with potent α -glucosidase inhibitory activity exhibits better therapeutic approach in delaying the availability of dietary carbohydrate substrate for glucose production in the alimentary canal. The antidiabetic capability of alkaloids, phenols, tannins, flavonoids, saponins and sterols to regenerate pancreatic β -cell and inhibit activity of carbohydrate metabolising enzymes has been documented (Patel et al. 2011). This may justify the hypoglycaemic potential of acetone extract of leaves of *A. godseffiana* as observed in this study.

Conclusion

The data obtained from this study indicated that *A. godseffiana* exhibited promising antidiabetic effect. Our findings also suggested that *A. godseffiana* contains active phytochemicals with hypoglycaemic and antioxidant potentials which should be explored in detail because it may serve as a future lead drug in the management of diabetes.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors' contributions

O.T.A. and O.B.F. designed the research project, supervised the experimental work and assisted in the preparation of the manuscript. A.K.A. carried out the experiments and wrote the manuscripts. A.O.T.A. supervised the antioxidant and hypoglycaemic experiments. O.O. supervised the phytochemical experiments.

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