Network for Research and Development in Africa
International Journal of Pure and Applied Science Research
ISSN: 2384-5918, Volume 11, Issue 11
PP 98-118 (April, 2024)
DOI: 4572771111117
arcnjournals@gmail.com
https://arcnjournals.org

Antidiabetic and Antioxidant Effects of Fixed Dose Recipe of Aqueous Seeds Extracts of Acacia Nilotic and whole Plants of Anisopus Manni in Streptozocine induced Diabetic Rats

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Abstract: *The study was conducted to evaluate the antidiabetic and antioxidant effects of fixed dose recipes of aqueous seed extracts of Acacia nilotica and whole plants of Anisopus manni in streptozocin-induced diabetic rats. The methodology involved preparation of the extracts, phytochemical screening, and quantitative determination of phytochemicals. Hypoglycemic and anti-hyperglycemic activities were assessed using different experimental groups. The rats were treated with various doses of the extracts and monitored for changes in fasting blood glucose levels, body weight, and biochemical parameters. The results revealed significant reductions in fasting blood glucose levels and improvements in body weight in rats administered with the fixed-dose recipe. Additionally, the recipe showed promising effects on biochemical parameters related to liver and kidney functions. These findings suggest the potential of the fixed-dose recipe as a natural remedy for managing diabetes and associated complications.*

Keywords: *Antidiabetic, Antioxidant, Aqueous Extracts, Phytochemical Screening, Hypoglycemic Activity, Body Weight, Biochemical Parameters.*

Introduction

Hyperglycemia, glucosuria, and hyperlipaemia are the hallmarks of diabetes mellitus (DM), a chronic metabolic disorder of the endocrine system (Medhavi and Muthuirulappan, 2015). Numerous factors, including the body's resistance to the effects of insulin or insufficient insulin secretion by the pancreas, may contribute to high blood glucose levels (Ozkum *et al*., 2013). Retinopathy, nephropathy, and neuropathy are examples of microvascular problems that can arise from uncontrolled blood glucose levels. Additionally, according to Ghosh and Collier (2012), it may result in vascular problems including coronary artery disease, which can cause myocardial infarcts and strokes. Because of the underutilization of glucose, diabetes mellitus (DM) causes hyperlipidemia, which is related to the excessive mobilization of fat from adipose tissue (Krishnakumar *et al*., 2000).

The illness is linked to higher risk factors for mortality and morbidity as well as a lower quality of life. Diabetes mellitus, or DM, was originally discovered in antiquity as a condition linked to "sweet urine" and significant muscle loss. Sweet pee gets its name from the spilling of glucose into the urine caused by elevated blood glucose levels, or hyperglycemia. Normally, the pancreas secretes the hormone insulin, which carefully regulates blood glucose levels. Insulin is secreted by the pancreas to normalize the blood glucose level when

it rises (for example, after eating) (Liu *et al*., 2007). When insulin is not produced, persons with diabetes experience hyperglycemia. Diabetes is a chronic illness, which means that even with lifelong management, it cannot be cured. Increased oxidative glucose metabolism in diabetes mellitus (DM) boosts O2− production in the mitochondria, which is subsequently transformed into HO- and H2O2. Free fatty acids (FFAs) also enhance the generation of reactive oxygen species (ROS) through direct actions on mitochondria, in addition to glucose. According to some theories, diabetes-related increases in superoxide production are caused by overexpression and activation of mitochondrial inner membrane uncoupling proteins (UCPs) (Rolo and Palmeira, 2006). According to research on diabetic experimental animal models, oxidative stress results in chronic and persistent hyperglycemia, which weakens the antioxidant defense system's functions and encourages the production of free radicals in different ways (Frode and Medeiros, 2008).

Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), and Type 2 diabetes, also known as non-insulin-dependent diabetes mellitus (NIDDM), are the two main forms of diabetes. Type 2 diabetes is caused by a combination of resistance to insulin action and an insufficient compensatory insulin-secretary response. Type 1 diabetes is caused by an absolute lack of insulin secretion (American Diabetes Association, 2005). Diabetes is treated in a variety of ways. For example, insulin treatment is used for type 1 diabetes. Sulphonyl ureas release insulin from the pancreas by blocking ATP-sensitive potassium channels; biguanides reduce insulin resistance; thizaolidinediones enhance insulin sensitivity; alpha-glucodase inhibitors, such as acarbose, reduce intestinal glucose absorption, hence lowering postprandial hyperglycemia; and metiglinides, such as repaglimide and nateglamide, are insulin secretogogues (Kadarian *et al*., 2002; Andrade-Cetto and Wiedenfeld, 2004).

Herbal remedies that are traditionally used are a significant component of diabetic treatment. Many researches have reported the antidiabetic benefits of numerous plants, and numerous herbs have been suggested for the treatment of diabetes (Bordoloi and Dutta, 2014). The hypoglycemic impact of plant extracts has also been shown in a number of pharmacological investigations. Examples of these research include cashew gum (Arduino and Soares, 1951), mango in Nigeria (Aderibigbe and Emudianughe, 2001), and periwinkle of Madagascar (Som *et al*., 2001), which support its application in conventional treatment. According to Ghorbani (2013), plants have long been a valuable resource for developing novel treatments for illnesses affecting humans. Just a small portion of the hundreds of plants that have been investigated for diabetes have undergone clinical trials and animal testing.

Acacia nilotica (L.), subfamily Mimosoideae of the Fabaceae family, is a popular name for babul or kikar. The Acacia nilotica (L.) tree has a dome shape and grows to a height of 15–18 meters. It has a single stem. Yellow globular heads are the flowers (Malviya *et al*., 2011). The herb Acacia nilotica has therapeutic properties. A reddish gum that the stem exudes is employed in a variety of culinary dishes (Kumari and Jain 2013). According to Mohanty *et al*., (1996), leaves can be used to treat dysentery, diarrhea, eczema, and abscesses (Siddiqui *et al*., 1989). The leaf of Acacia nilotica (L.) has been found to have chemo-preventive, antimutagenic, antibacterial, anticancer, astringent, and antimicrobial properties. Its medicinal qualities have been assessed. Tender leaves have anti-inflammatory, aphrodisiac, ulcer-dressing, and Alzheimer's disease properties (Kalaivani *et al*., 2010; Kalaivani *et al*., 2011).

In alloxan-induced diabetic rats, Medhavi and Muthuirulappan (2015) examined the antidiabetic and antioxidant properties of an ethanolic leaf extract from Acacia nilotica. According to their findings, the extract from Acacia nilotica leaves has a strong hypoglycemic, antioxidant, and hypolipidemic impact. Compared to the usual medication glibenclamide, the extract from the leaves of Acacia nilotica shown a better reaction. One species of flowering plant in the Asclepiadaceae family is Anisopus manni (N.E. Br). It is indigenous to the West Indians and other tropical Americas. Although it is rarely grown, it is widely distributed throughout northern parts of Nigeria. In folk medicine, it is frequently utilised. According to Oludaretemitope et al.

(2016), the plant is a perennial herb with widely spaced leaves and a petiole that is 1.3 to 2.0 cm long. It is a glabrous twining shrub that is strong to climb, and it has greenish flowers in globose, lateral umbelliform cymes and horizontally opposite follicles that are 6 to 8 inches long and roughly half an inch thick, tapering to a slightly hooked point at the apex. Among the Hausa people of northern Nigeria, it is referred to as "Sakayau" or "Kashezaki" (meaning "sweet killer"), and a cold decoction of the stem is traditionally used as a treatment for diabetes (Sani *et al*., 2009). It is a well-known herb in traditional northern Nigerian medicinal preparations, where a decoction of the entire plant is used as a treatment for piles, diabetes, diarrhoea, and bacteria (Oludaretemitope *et al*., 2016).

3.1 Materials

3.1.1 Chemicals and Reagents

Streptozoctocin was purchased from Sigma Chemicals Company, St. Louis, USA while Glibenclamide, was obtained from May and Baker Limited, United Kingdom and Insulin from Biochin IU Mumbai, India. All other chemicals and reagents used were of analytical grade.

3.1.2 Equipment

Glucometer “one call plus” (MDDS Gmbh schiffgraben 41 Hannover Germany, Spectrophotometer (JENWAY 6305), Water bath (HH 420PEC MEDICAL USA), Centrifuge 80-2, Magnetic stirrer hot plate (78-1 PEC MEDICAL USA).

3.1.3 Plants Samples Collection and Identification

Acacia nilotica and *Anisopus manni* were collected from Jimtilo, outskirts of Maiduguri, Borno State Nigeria in June, 2019. Identification with vernacular names of the plants were done before validation by a taxonomist. Voucher specimen number 7019A and AAUA 2132 were allocated respectively for the two plants and deposited at the herbarium unit of Biochemistry Department, Faculty of science.

3.1.4 Experimental Animals

Seventy (95) adult Wister strain albino rats of both sexes, raised in the Animal House of the Department of Biochemistry, University of Maiduguri weighing between 180-200g were used in the study. The rats were housed in standard cages, maintained under standard laboratory conditions and were fed with standard rats’ pellets and water *ad libitum*.

3.2 Methodology

3.2.1 Preparation of Aqueous Extracts

One-hundred-gram (100 g) powder of each individual plant and recipe (50g each of the two plants) were boiled in 1 liter of water for ten (10) minutes. The extracts were decanted into clean dry conical flasks and then filtered. The filtrates were evaporated to dryness at a reduce temperature (45°C) and pressured using a rotary evaporator (CS501-3C LABMAN-UK).

3.3 Phytochemical Screening

The extracts were screened for presence of secondary metabolites using standard phytochemical methods (Edeoga *et al*., 2005, 2009).

3.3.1 Test for Tannins

Two millitre (2 ml) of 5 % Fecl3 was added to 2 ml aqueous extract of each sample yellow brown precipitate indicates the presence of tannins (Edeoga *et al*., 2005).

3.3.2 Test for Alkaloids

One millitre (1) ml of 1% Hydrochloric acid was added to 2 ml methanolic filtrate samples. The solutions were heated and six drops of dragendroff reagent was added. Orange precipitate confirms the presence of alkaloids (Edeoga *et al*., 2005).

3.3.3 Test for Saponins (Froth test)

Two grams (2g) powder was made and subjected to frothing test. Frothing persistence indicated presence of saponins. Later the froth was mixed with few drops of olive oil. Formation of emulsion further confirms the presence of saponins (Edeoga *et al*., 2005).

3.3.4 Test for Flavonoids (Shimoda's Test)

Two grams (2g) of extract was extracted in 10 ml H_2O few drops of HCl followed by 0.5 g of Zinc turnings were added. Tubes were boiled for 5 minutes. Formation of pink Colour indicates the presence of flavonoids (Jassal, 2013).

3.3.5 Test for Phenolics

To two millitre (2 ml) of aqueous extract, 1 ml of 1% ferric chloride solution was added Blue colour indicates the presence of phenols (Masod, 2010).

3.3.6 Test for Anthraquinones

One millilitre (1ml) of the filtrate was shaken with 10 ml of benzene; the mixture filtered and one 5ml of 10% (v/v) ammonia was added, then shaken and observed. A pinkish solution indicates a positive test for anthraquinones (Edeoga *et al*., 2005).

3.4 Quantitative Determination of Phytochemicals

3.4.1 Alkaloids

Two and a half grams (2.5g) of the powder was extracted using 100 ml of 20 % acetic acid in ethanol. The solution was covered for 4 hours; the filtrate was concentrated to 25 ml. Concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitate settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed (Masood, 2010).

3.4.2 Saponins

Ten grams (10g) of each sample (seeds of *Acacia nilotica*, whole of plant of *Anisopus manni* and their recipe) was mixed with 100 ml of 20% aqueous ethanol. The mixture was kept for 4 hours on water bath shaker at 55°C and then filtered. The combined extract was concentrated to 40 ml over water bath at 90°C. Concentrate

obtained was transferred into separating funnel and 10 ml of diethyl ether added to it, after shaking vigorously aqueous layer recovered and ether layer discarded. The process was repeated to the aqueous layer n-butanol was added. The whole mixture was washed in separating funnel twice with 10 ml of 5% aqueous NaCl. Upper part was retained and heated in water bath until evaporation. Later it was dried in oven to a constant weight (Akbar, 2010).

3.4.3 Tannins

Two gram (2 g) of plant powder from each sample (seeds of *Acacia nilotica*, whole of plant of *Anisopus manni* and their recipe) was extracted thrice in 70% acetone. Samples were centrifuged and supernatant collected. Different aliquots were prepared and final volume was made to 3 ml by distilled water and vortexed, 1 ml of 0.0016M K_3 (Fe (CN_6) 1ml of 0.02M $Fecl_3$ in 0.1M HCl were added. Tubes were shaken and then kept as such for 15 minutes, 5 ml of stabilizer (3:1:1 ratio of water H_3PO4 and 1% gum Arabic) was added and tubes again revortexed. Absorbance was taken at 700nm, standard curve was plotted using different concentrations of 1.9 mg % garlic acid (Akbar, 2010).

3.4.4 Flavonoids

Ten grams (10g) of the samples (seeds of *Acacia nilotica*, whole of plant of *Anisopus manni* and their recipe) was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper No 42. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to constant weight (Sawhney, 2013).

3.5 Screening for Hypoglycaemic and Anti-hyper glycaemic Activity

3.5.1 Hypoglycaemic Studies

- Twenty-five (25) wistar strain albino rats divided into the following five (5) groups were used for this study:
- Group 1 – Normal control
- Group 2 – Experimental group administered 100mg/kg body weight aqueous extract of the seeds of *Acacia nilotica*
- Group 3 – Experimental group administered 100mg/kg body weight aqueous extract of the whole plant of A*nisopus manni*
- Group 4 – Experimental group administered 100mg/kg body weight of the recipe
- Group 5 – Positive control group administered 1.0 mg/kg body weight glibenclamide.

3.5.2 Anti –hyperglycaemic studies

Induction of Diabetics Mellitus

Diabetes was induced by a single intrapretoneal injection of 50mg/kg body weight of streptozoctocin dissolved in phosphate buffered saline after overnight fast. After 72hours surviving rats with blood glucose of more than 200mg/dl were considered as streptozoctocin-induced diabetic rats (Al-Shamaony *et al*., 1994).

The Experimental design for the antidiabetic screening of the extracts of the STZ-induced diabetic rats is as follows;

Group 1: Normal control

Group 2: Negative control

Group 3: Diabetic rats administered 100mg/kg body weight of *A. nilotica* seeds aqeous extract

Group 4: Diabetic rats administered 100mg/kg body weight of *A. manni* aqeous extract

Group 5: Diabetic rats administered 100mg/kg body weight of the aqeous extract of the recipe

Group 6: Diabetic rats administered 1mg/kg body weight of Glibenclamide.

Fasting blood glucose was first assayed in 18 hour fasted rats after which distilled water, extracts and the glibenclamide was administered to the respective groups. Blood glucose values were then estimated at 30, 60, 120 and 180 minutes (Gidado *et al*., 2005).

3.5.3 Estimation of Blood Glucose

Drops of blood from tip of rat's tail was collected and glucose concentration assayed using Accu-check glucometer (Active 333).

3.6 Anti Diabetic Effect of 28 Days Oral Administration of the Extracts

3.6.1 Experimental Design

Fourty five (45) wistar strain albino rats of both sexes were used for the evaluation of 28 days antidiabetic activity of the extracts and recipe. The rats were divided into the following nine (9) groups of five (5) rats each.

Group 1: normal control (administered distilled water).

Group 2: negative control (induce with diabetic but not treated).

Groups 3: diabetic, administered aqueous extract of *Acacia nilotical* (100mg/kg)

Groups 4: diabetic, administered aqueous extract of *Anisopus manni* (100mg/kg)

Group 5: diabetic, administered extract of recipe (100mg/kg)

Group 6: diabetic, administered extract of recipe (200mg/kg)

Groups 7: diabetic, administered extract of recipe (400mg/kg)

Group 8: positive control diabetic, administered standard drugs (glibenclamide) 1mg/kg

Group 9: positive control diabetic, administered standard drugs (insulin) 1mg/kg

Distilled water, aqueous extracts and glibenclamide were administered to the different groups for 28 days. Fasting blood glucose and body weights were monitored weekly. At the end of 28 days, 24hrs after the last treatment, the rats were sacrificed by humane decapitation, blood, liver and kidney were collected. The blood was collected in plain tubes and serum harvested. Serum harvested was used to assay for some biochemical parameters, liver and kidney homogenates were prepared and used for the assay of catalase and lipid peroxidation as antioxidants.

3.6.2 Weekly Body Weight

The body weight of each rat was measured using a measuring balance (digital scale kerro BL 20001) during the acclimatization period, a day before commencement of the administration, once weekly during the study period and on the day of sacrifice.

3.7 Biochemical Parameters

The serum sample was used to assay for some biochemical parameters including parameters for liver and kidney functions using standard biochemical methods.

3.7.1 Measurement of Aspartate Aminotransferase (AST) Activity (Retma and Franklin, 1957)

Principle

The enzyme, aspartate aminotransferase, reversibly transfers an amino group from aspartate to a-ketoglutarate. The AST was measured by monitoring the concentration of Oxaloacetate hydrazone formed with 2, 4-dinitrophenyldydrazine.

Aspartate + a-ketoglutarate ——————AST glutamate + oxaloacetate

Procedure:

Test tubes were prepared for blank and tests. In the tube for test 100 ul of sample was placed. For the blank, it was 100 ul of distilled water. Five hundred micro liters (500 ul) of the AST reagent was added to both the blank and the test. The tubes were mixed and incubated for exactly 30 minutes at 37c. Five hundred micro liters (500 ul) of 2, 4-dinitrophenyl hydrazine was added to the test tubes; they were mixed and allowed to stand for exactly 20 minutes at 25°C. Sodium hydroxide (5ml) was added to the tubes; the contents of the tubes were mixed and allowed to stand at room temperature for 5 minutes. Absorbance of the sample was measured against blank at 564nm wavelength. Activities of AST in the serum obtain from a table of absorbance and concentration in IU/L.

3.7.2 Measurement of Alanine Aminotransferase Activity ALT (Jendrassik and Grof, 1938)

Principle:

Alanine + α-ketoglutarate ALT Alanine aminotransferase reversibly transfer the amino group from Alanine to α-ketoglutarate, forming Pyruvate and glutamate. The ALT is measured by monitoring the concentration of Pyruvate Hydrozone formed with 2,4-monitoring dinitrophenylhydrazine.

Procedure:

Test tubes were prepared for blank and test in the tube for test 100ul of sample was placed. For the blank it was 100 ul of distilled water. Five hundred micro liters (500 ul) of the ALT reagent was added to both the blank and the test. The tubes were mixed and incubated for exactly 30 minutes at 37^{o}C. Five hundred micro litres (500ul) of 2, 4-dinitrophenydrazıne was added to both tubes. They were mixed and allowed to stand for exactly 20 minutes at 20 to 25°C.

Sodium hydroxide (5m) was added to the tubes. The contents were mixed and allowed to stand at room temperature for 5 minutes. Absorbance of the test sample was measured against the reagent black at 546 nm wavelength. Activity ALT in the serum was obtained from a table of absorbance and concentration in IU/L.

3.7.3. Measurement off Alkaline Phosphatase Activity ALP (McComb and Browers, 1972)

Principle:

Serum alkaline phosphate (ALT) catalyses the hydrolysis of p-Nitrophenylphosphate to P-nitronitrophenylate ion and phosphate. The substrate is colourless, but the p-nitrophenylate resonates to a quinoid form in alkaline solution and strongly absorbs light at 404nm.

Procedure:

Into a cuvet, 10ul of sample was place at room temperature (25°c). The reaction was initiated by adding 500ul of ALP reagent which was previously warmed to room temperature (25°c). It was mixed and the initial absorbance was read at 404nm and a timer was started. The absorbance was read again after 1,2, and 3 minutes.

Calculation

AIkaline phosphatase activity (ul) was calculated as follows: -

U/1 = 405nm/minx 2760

3.7.4 Measurement of Serum total protein Concentration (Biuret Method) (Tietz, 1995)

Principle:

In alkaline solution cu^{2}+ react with peptide linkage of protein to form a violet- coloured complex. The intensity of the colour produced is proportional to protein concentration.

Procedure:

Test tubes were prepared for blank, test and standard. In each tube, 50ul of distilled Water and standard solution was added to blank, test and standard respectively. Buret reagent (2.5ml) was added to each of the tubes. The tubes were incubated for 10minutes at 37°c. The absorbance was measured against the reagent blank at 540nm wavelength.

Serum total protein was calculated from the formulae:

$$C_{sample} = \frac{A_{Sample} \times C_{standard}}{A_{standard}}$$

$$= \frac{A_{Sample} \times 60 = g/l}{A_{standard}}$$

Where A = absorbance, C = concentration, 60g/l = concentration of protein standard.

3.7.5 Measurement of Serum Albumin Concentration (Tietz, 1995)

Principle:

Bromocresol green, an anionic dye, binds tightly to albumin when added to serum, and the complex absorbs light much more intensely at PH4.20 and 628nm, than does the unbound dye. The increase in light absorption is directly proportional to the albumin concentration.

Procedure:

Test tubes labeled blank, tests and standard, 5ml of working bromocresol green solution was placed. Working bromocresol green solution was placed. Twenty-five micro litres (25ul) of water, sample and standard were respectively added. The contents were mixed and allowed to stand for 10 minutes at room temperature. The absorbance was read at 630nm against reagent blank.

Serum Albumin was calculated as follows: -

$$\text{Serum albumin} = \frac{A_{\text{sample}} \times C_{\text{sample}}}{A_{\text{standard}}}$$

$$= \frac{A_{\text{sample}} \times 60 = g/l}{A_{\text{standard}}}$$

Where A = absorbance, c = Concentration, 60g/1 =Concentration of Albumin standard soluion.

3.7.6 Measurement of Total Bilirubin (TB) and Direct Bilirubin (DB) (Koch and Doumas 1982)

Principle:

Direct (conjugate) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Procedure:

Total Bilirubin (TB)

Into a test tube labeled "sample blank" 0,20ml of reagent 1 (sulphanilic acid) and 1.00ml of reagent (caffeine) 3 was placed, in addition, I drop of reagent 2 (sodium nitrite) was added. A 0.20ml of sample was added to both sample blank and sample test tubes. They were mixed and allowed to stand for 10mins at 20-25^{o}c reagent 4 (Tartrate) 1.00ml was added to tubes.

Mixed and allow to stand for 5-30 minute at 20-25^{0}C and absorbance was read at 578nm wavelength against the blank.

Direct (conjugated) Bilirubin (DB)

Test tubes marked "sample blank" and "sample" into each tube 0.2ml of reagent 1 was placed. Then a drop of reagent 2 was added to the tube marked sample" was added to each of the tubes. The contents were mixed and allowed to stand for exactly 5mins at 20 - 25C. Absorbance was read at 564nm wavelength against "the sample blank.

Calculation

Total Bilirubin (Umol/L) = 185 x ATB (578nm)

Direct Bilirubin (Umol/L) = 246 x ADB (546nm)

Where A_{TB} = absorbance for total Bilirubin, 185 Umol/L = Concentration of Total bilirubin A_{DB} = Absorbance for direct Bilirubin, 246 Umol/L = Concentration of direct bilirubin.

3.7.7 Measurement of Serum Total Cholesterol (TC) (Allain *et al.,* 1974)

Principle:

Total serum cholesterol was estimated after enzymatic hydrolysis and oxidation.

Cholesterol ester was hydrolysed to free cholesterol (FC) and free fatty acid (FFA) by cholesterol esterase (CHE) enzymes. The FC formed is oxidized by cholesterol oxidase (CHO) to cholestene -3- one hydrogen peroxide which react with 4-aminophenazone and phynol by phynol oxidoreductase (POD) to produce quinoneimine (cloured compound), the intensity of which is proportional to the concentration of TC present in the sample.

Cholesterol ester + H_20 CHE ——cholesterol + fatty acid

Cholesterol + 0_2 CH0 ——cholesterol -3- one H_2O_2

$2H_20_2$ + aminophenazone + phenol POD ——quinoneimini + 4 H_20.

Procedure

Test tube prepared for blank, test and standard respectively. In each tube, 100ul cholesterol liquicolour reagent was place and 10ul distilled water, serum and cholesterol standard solution was added to blank, test and standard respectively. The content of the tubes was mixed and incubated at room temperature for 10mintes. The absorbance was red against the reagent blank at 500nm wavelength.

Serum TC concentration was calculated from the formular:

$$\text{Sample} = \frac{\text{A sample x 200mg/dl}}{\text{A standard}}$$

Where A = absorbance, C = Concentration, 200mg/d1 = Concentration of cholesterol standard.

3.7.8 Measurement of Serum Triacylglycerol

Principle

The Triacylglycerol (TG) is determined after enzymatic hydrolysis with lipasis Triacylglycerol are hydrolysed by lipases to glucerol and FFA. The glycerol reacts with adenosine triphophate (ATP) to form glycerol -3-phophate and adenosine di-phosphate (ADP) under the influence of glycerol kinases (CK). Glycerol-3-phophate is oxidized by glecerol phosphate oxidase (GPO) to dilydroxyacetone and hydrogen peroxide. Hydrogen peroxide then combined with 4-anminoantipyTine to form quinoneimine (coloured compound). The colored was red at 500nm wavelength.

Triacylglycerols lipases glycerol + fatty acid

Glycerol+ATP GK glycerol -3- phosphate + ADP

Glycerol-3 - phosphate +O_2 GPO dihydroxyacetone phosphate + H_20_2

H_2O_2 +4 aminoantipyrine POD quinoneimine +HCl + H_20 chlorophenol.

Procedure:

Test tubes for blank, test and standard. In each tube, 1000ul TGs reagent solution was placed and 10ul distilled water, serum and TGs and standard solution was added to blank, test and standard respectively. The contents of the tubes were mixed and incubated for 10 minutes at room temperature, the absorbance was measured against the reagent blank at 500nm wavelength.

Serum TG concentration was obtained as follows:

$$\text{Sample} = \frac{\text{A Sample}}{\text{A Standard}} \times 200\text{mg/dl}$$

Where A = Absorbance, C = Concentration, 200mg/dl = Concentration of TG standard.

3.8 Antioxidant studies

3.8.1 Determination of Catalase (CAT)

Catalase activity was determined in erythrocyte lysate using Aebi's method (Aebi, 1984). Fitty microliter of the lysate was added to a cuvette containing 2 ml of phosphate buffer (pH 7.0) and 1 mL of 30-mM H_20_2. Catalase activity was measured at 240 nm for 1 min using spectrophotometer. The molar extinction coefficient of H_2O_2, 43.6 M cm^{-1} was used to determine catalase activity. One unit of activity is equal to 1 mmol of H_2O_2 degraded per minute and expressed as minute per milligram of protein.

3.8.2 Determination of Lipid Peroxidation (LPO)

This method described by Okhawa (1979) is as follows: The tissues are homogenized in 0.1 M buffer pH 7.4 with a Teflon-glass homogenizer. LPO in this homogenate is determined by measuring the amounts of malondialdehyde (MDA) produced primarily. Tissue homogenate (0.2mL), 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5ml of 20% acetic acid and 1.5 mL of 8% TBA are added. The volume of the mixture is made up to 4 mL with distilled water and then heated at 95°C on a water bath for 60 min using glass balls as condenser. After the incubation the tubes are cooled to room temperature and final volume was made to 5 mL in each tube. Five mL of butanol: pyridine (15:1) mixture is added and the contents are vortexed thoroughly for 2 min. After centrifugation at 300 rpm for 10 min, the upper organic layer is taken and its OD is taken at 532 nm against an appropriate black without the sample. The levels of lipid peroxides can be express as a mole of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of 1.56 x 10 ML cm.

3.8.3 DPPH radical scavenging activity of the samples

DPPH radical scavenging activity of the samples was estimated as described by Burits and Bucar (2000). An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (0.0001 mg/ml, 0.001mg/ml, 0.01mg/ml, 0.1mg/ml and 1mg/ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 min the increase in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the expression: % inhibition = $[(A_0-A_1)/A_0] \times 100$

Where A_0 is the absorption of the blank sample and A_1 is the absorption of the extract

3.9 Statistical Analysis

Result are presented as mean ± SSEM. Data collected was subjected to analysis of variance (ANOVA) using SPSS statistical package version 21.0, and Duncan Multiple Range was used to compare the means. The results were considered statistically significant at $p<0.05$

4.0 RESULTS

4.1 Screening (Qualitative) of the extracts for Phytochemical constituents

Table 4.1 showed the results of the phytochemical (qualitative) screening of the aqeous extract of seed of *Acacia nilotica,* the whole plant of *Anisopus manni* and the recipe. The result indicated the presence of tannins, saponins, and flavonoids in all the extracts. Alkaloids, and free anthraquinones were not detected.

4.2 Phytochemical (Quantitative) results of the Plant

Table 4.2 presents the quantitative phytochemical analysis for the aqeous extracts of *Acacia nilotica* and *Anisopus manni* for the secondary metabolite's alkaloids, saponins and flavanoids. The results of the alkaloids obtained were 0.0275±0.02 mg/100g, 0.0420± 0.01mg/100 g and 0.98± 0.03mg/100g for *Acacia Nilotica, Anisopus manni* and the recipe respectively. There was significant ($P<0.05$) difference in the saponins and the flavonoid contents of the plants and the recipe. In all cases, the recipe had high values for the alkaloids, saponins and flavonoids.

Table 4.1: Preliminary Phytochemical (Qualitative) Screening of the plant extracts and Recipe

Test	Method	Results		
		A. nilotica	*A. manni*	Recipe
Alkaloids	Dragendoff's Reagent	-	-	-
Tannins	Mayer's reagent	+	-	+
	Ferric chloride	+	+	+
	Lead acetate	-	+	+
Cardiac glycoside S	Salkowski's test	-	+	+
Saponnins	Frothing test	+	+	+
Free anthraquinones	Bornrager's test	-	-	-
Flavonoids	Shinoda's test	+	+	+
	Ferric chloride	+	+	+
	Lead acetate	+	+	+
	Sodium hydroxide test	-	-	-
Terpenoids	Keller-killianis test	+	+	+

Key = + present

= - absent

Table 4.2: **Phytochemical constituents of the Different extracts**

		Quantitative Phytochemical (mg/100g)		
S/NO	Extract	Alkaloids	Saponins	Flavanoids
1	*Acacia nilitica*	0.0275±0.02	6.744±0.03	4.385±0.02
2	*Anisopus manni*	0.0420±0.01	1.606±0.01	0.818±0.02
3	Recipe	0.980±0.03	9.360±0.02	6.21±0.10

Values are mean ± SEM, n = 3

4.3 Screening for Hypoglyceamic Activities of the Aqeous extracts

Oral administration of the individual plant's extracts did not significantly increase or decrease the fasting blood glucose of the experimental rats. The recipe, however, significantly ($P<0.05$) reduced the glucose level of the groups of rats. The reduction amounts to 17.69% after 180 minutes of the recipe extract administration as shown in figure 4.1.

4.4 Screening for Anti-diabetic Activity of the Aqeous Extracts

Oral administration of the individual plant extracts significantly ($P<0.05$) affected the fasting

Blood glucose (negative group) of the experimental rats, a group administered *Acacia nilotica* indicated a reduction level from 0mins to 180 mins with the value of 230.40± 17.59 to 197.00± 23.30 and A group administered *Anisopus manni* showed a reduction level with the values of 207.00±10.32 to 182.00±15.21. Also, the recipe group significantly ($P<0.05$) reduced the fasting blood glucose of 1 to 5 rats from 245.70±2.56 to 165.80±5.29 with a reduction percentage of 32.52 % after 180 minutes as shown in figure 4.2

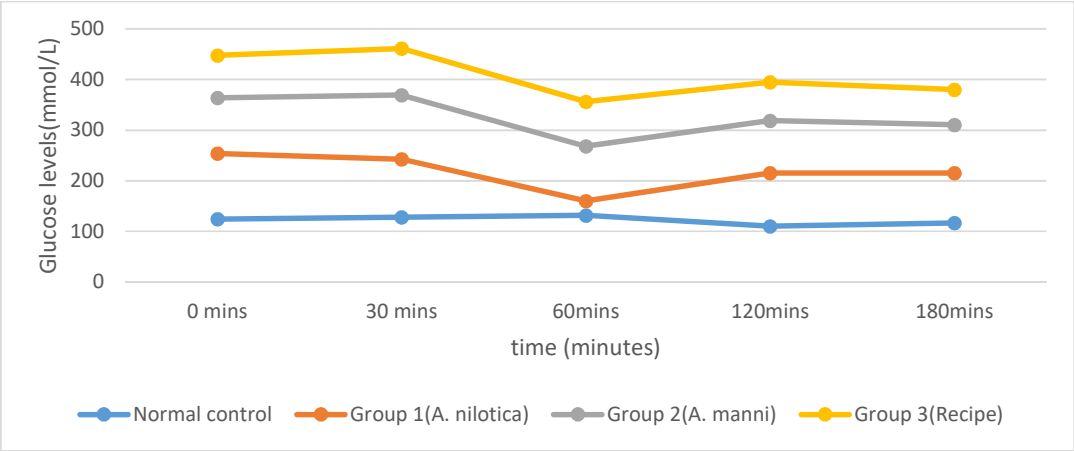

Figure 4.1: Screening for Hypoglycaemic Activity of the Aqueous Extract plants and their recipe in Normalglycaemic rats.

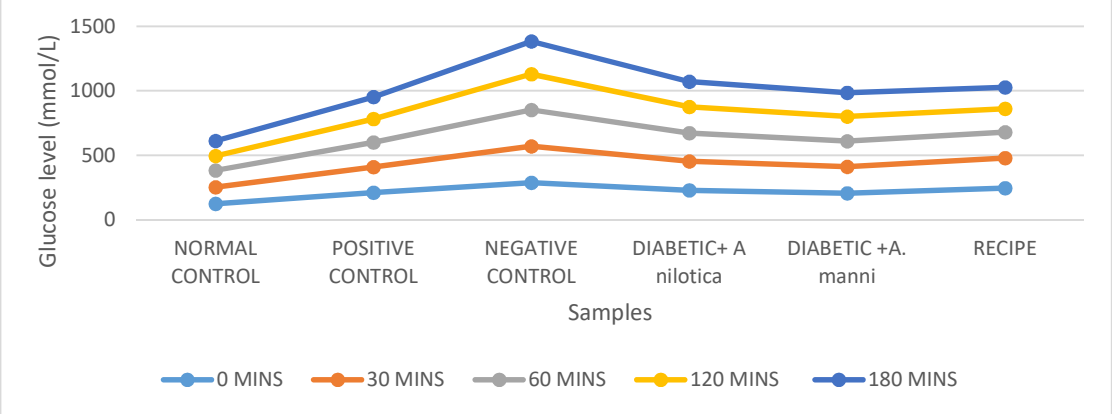

Figure 4. 2: Screening for hypoglycaemic activity of the Aqueous Extract and their recipe in Diabetic rats

4.5: Effects of 28-days oral administration of the different extracts

Table 4.3 present the 28-days oral administration of extracts. All extract of *Acacia nilotica* and *Anisopus manni* showed a significant ($p<0.05$) reduction in fasting blood sugar, at 100mg/kg dosage of each extract showed a percentage reduction of 4.70% and 3.65% respectively. Recipe with dosage of 100ml/kg showed 7.44% and for 400mg/kg dosage of the recipe 15.61%. The highest reduction showed was the group with the standard drugs glibenclamide and insulin with 16.16% and 28.18% respectively.

Table 4.3. 28-days of oral administration of aqueous extract and their recipe

Treatment	Initial BG(mg/dl)	Final (mg/dl)	Percentage (%) Reduction
Normal control + Water	122.20±1.36^{a}	113.24±2.05^{c}	7.33%
Negative control	239.38±1.63b^{c}	282.42±3.89^{a}	
Diabetic rats+ AN (100mg/kg)	237.04±1.06^{a}	225.88±0.49^{d}	4.70%
Diabetic rats + AM (100mg/kg)	237.04±1.06^{a}	228.38±0.53^{e}	3.65%
Diabetic rats + RCP1 (100mg/kg)	235.14±0.48^{a}	217.64±0.79^{e}	7.44%
Diabetic rats + RCP2 (200mg/kg)	230.58±0.35^{a}	209.22±1.07^{e}	9.26%
Diabetic rats + RCP3 (400mg/kg)	225.60±1.33^{a}	190.00±0.97^{e}	15.61%
Diabetic rat + GLB	221.22±0.77^{a}	185.46±1.08^{e}	16.16%
Diabetic rats + insulin	215.54±1.52^{a}	154.78±1.69^{e}	28.18%

Values are mean ± SEM, n=5

Values with different superscript along the row are significantly different (P<0.05)

Key=

AN =*Acacia nilotica*

AM= *Anisopus manni*

RCP= Recipe

GLB= Glibenclamide

BG= Blood glucose

4.6: Body weight change and percentage body weight reduction of the experimental rats

Table 4.4. Below presented the body weight change and percentage body weight reduction of the experimental rats. A significant (P<0.05) increase was observed in the final body weight of all the treatment groups except for the negative control group, where a significant (P<0.05) reduction was observed. For the percentage body weight change, a significant (P<0.05) difference was observed between normal control and negative control group, and also with the other control groups. No significant (P>0.05) difference was observed between the percentage reduction of the group administered glibenclamide and diabetic group administered *Acacia nilotica* and *Anisopus manni.* However, groups of recipes of different doses (100, 200 and 400ml/kg showed an increase body weight simultaneously.

Table 4.4: Body weight change and percentage body weight reduction of the experimental rats

Treatment	Initial Body weight(g)	Final Body weight (g)	% change in body weight
Normal Control	153.90± 1.91	196.00±1.40	28.06
Negative control	246.40±19.20	128.00±14.98	-48
Positive control(Glibenclamide)	183.36±6.48	199.06±8.61	8.45
Streptozotocin induced diabetic rats+ AN+100mg/kg	232.36±18.08	241.52±18.64	3.94
Streptozotocin induced diabetic rats+ AM+100mg/kg	234.36±12.02	242.18±12.17	3.37
Recipe 100mg/kg	195.34±12.63	215.76±14.09	10.45
Recipe 200mg/kg	198.24±10.02	209.15±11.12	10.01
Recipe 400mg/kg	232.62±12.02	254.52±23.18	9.10

Values are mean ± SEM, n = 5

Values with different superscripts along the row under the same heading, are significantly different (P<0.05)

Key:

AN =*Acacia nilotica*

AM= *Anisopus manni*

4.7 Biochemical Parameter

4.7.1 Effect of Oral Administration of Aqueous Extract of Seeds of Acacia nilotica, Whole Plant of *Anisopus manni* and their Recipe on Some Biochemical Parameter in Streptozoctocine Induce Diabetic Rats (*in vivo*).

Table 4.5: present the effect of oral administration of aqueous extract and their recipe on some biochemical parameter in experimental rats. The result showed that the AST and ALT level a reduction significant ($p<0.05$) was observed after administered the extract and recipe compared to negative groups. The groups administered the standard drugs (glibenclimide and insulin) showed no significant ($p<0.05$) different was observed with the lowest values of *acacia nilotica* and *anisopus manni* respectively for ASAT. The result of alkaline phosphate and total bilirubin showed a significant ($p<0.05$) reduction compared with both the normal and negative groups. Total protein, Direct bilirubin and Albumin levels of negative control also showed a significant increased from the normal control group down to the group administered standard drug (glibenclimide and insulin). Triglycerides shows a significant ($p<0.05$) decrease was observed with the group administered 400mg/kg of recipe compared with the negative groups.

Result of triglycerides and cholesterol levels of negative groups control showed a significant ($p<0.05$) increase when compared to the normal groups, the group administered with the standard drug also showed a decreased in cholesterol and triglycerides level. The groups administered extract of each plant and their recipe showed a significant ($p<0.05$) a decreased in cholesterol and triglycerides levels with groups administered 400mg/kg recipe.

Table 4.5: Effect of Oral Administration of Aqueous Extracts of *Acacia nilotica* seeds, *Anisopus manni* (whole plant), Recipe comprising *Acacia nilotica* (seeds), *Anisopus manni* (whole plant) on some biochemical parameters in Streptozotocin induced diabetic rats

Treatment Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TP(mg/dL)	TBIL (µmol/L)	D.BIL (µmol/L	CHOL (mmol/L)	TG (mmol/L)	ALBUMEN
Normal control	4.05±0.00^{a}	3.22±0.29^{a}	0.54±0.00^{a}	0.52±0.00^{c}	1.32±0.01^{d}	4.11±0.53^{g}	4.23±0.11^{b}	2.19±0.07^{c}	22.11±2.11^{c}
Negative control	25.00±0.07^{a}	25.06±0.07^{a}	45.52±0.09^{a}	12.90±0.07^{a}	20.00±0.07^{b}	13.16±0.05^{a}	52.20±0.86^{bc}	6.84±0.07^{b}	347.20±0.086^{a}
Streptozotocin induced diabetic rats+ AN (100mg/kg)	0.03±0.01^{e}	9.60±0.20^{c}	0.65±0.01^{b}	0.88±0.00^{b}	1.73±0.01^{b}	7.90±0.03^{b}	4.39±0.25^{a}	2.22±0.03^{a}	44.41±1.14^{ab}
Streptozotocin induced diabetic rats+ AM (100mg/kg)	4.6±0.30^{b}	10.50±0.37^{b}	0.52±0.00^{c}	0.86±0.02^{b}	1.76±0.01^{b}	7.91±0.06^{b}	3.55±0.17^{b}	2.21±0.02^{a}	42.90±1.39^{b}
Streptozotocin induced diabetic rats+ RCP (100mg/kg)	3.96±0.14^{b}	9.36±0.24^{c}	0.58±0.02^{c}	0.72±0.01^{d}	1.76±0.02^{b}	7.41±0.03^{c}	3.17±0.07^{b}	2.07±0.05^{a}	31.85±2.09^{cd}
RCP+DB+200mg/k Streptozotocin induced diabetic rats+ RCP (200mg/kg)	2.20±0.23^{c}	8.38±0.14^{d}	0.54±0.01^{c}	0.70±0.00^{b}	1.74±0.02^{b}	7.34±0.03^{c}	3.59±0.15^{b}	1.81±0.16^{b}	27.76±0.67^{d}
Streptozotocin induced diabetic rats+ RCP (400mg/kg)	1.12±0.37^{d}	7.68±0.17^{e}	0.52±0.01^{c}	0.65±0.01^{d}	1.73±0.01^{b}	7.31±0.02^{c}	3.32±0.20^{b}	1.18±0.03	20.16±0.89^{ef}
Streptozotocin induced diabetic rats + GLB	0.04±0.00^{e}	7..48±0.38^{e}	0.64±0.03^{b}	0.81±0.01^{c}	1.74±0.01^{b}	8.09±0.06^{b}	3.42±0.13^{b}	1.99±0.03^{dc}	33.30±2.02^{c}
Streptozotocin induced diabetic rats + insulin	0.03±0.00^{e}	7.38±0.38^{e}	0.61±0.01^{b}	0.80±0.01^{c}	1.76±0.01^{b}	7.94±0.03^{b}	3.52±0.21^{b}	1.99±0.02^{c}	32.70±2.95^{c}

Values are mean ± SEM, n = 5

Values with different superscripts along the row under the same heading, are significantly different (P<0.05)

4.8 Effect of Oral Administration of Seed of Acacia nilotica, Whole Plant of Anisopus Manni and Their Recipe on Liver and Kidney Homogenates Antioxidant in Experimental Rats (invitro)

The results of the effect of oral Administration of the Extracts plants and their recipe and the antioxidant capacity of experimental rat were presented in table 8. The catalase level in the liver (0.90±0.02) and kidney (0.090±0.01) of negative control group were significantly ($p<0.05$) higher than that of the normal control group with liver catalase (0.28±0.018). Standard drugs glibenclamide and insulin, a significant ($p<0.05$) reduction in the catalase level both in the liver and kidney was observed. Group administrated extract and their recipe, a significant ($p<0.05$) reduction was observed in both the liver and the kidney catalase. However, the reduction was also showed in group administered 200mg/kg and 400mg/kg for liver and kidney catalase.

The result of the liver LPO and kidney LPO of the negative control groups was significantly ($p<0.05$) no different with the normal groups. The group administered with standard drugs (glibenclamide and insulin showed a significant reduction. Oral administration of the extract and recipe causes significant ($p<0.05$) reduction in the liver LPO and kidney LPO as shown in table 4.6.

Table 4.6: Effect of Oral Administration of Aqueous Extracts of *Acacia nilotica* seeds, *Anisopus manni* (whole plant), Recipe comprising *Acacia nilotica* (seeds), *Anisopus manni* (whole plant) on some liver and kidney antioxidant capacities in Streptozotocin induced diabetic rats

GROUPS	CAT(Umg?/protein) KIDNEY	CAT(um/protein) LIVER	LPO (µmol/L) KIDNEY	LPO(µmol/L) LIVER
Normal control	0.28 ± 0.18^{a}	0.28 ± 0.18^{a}	0.06 ± 0.01^{c}	0.06 ± 0.09^{d}
Streptozotocin induced diabetic rats+ H_20	0.90 ± 0.01^{a}	0.90 ± 0.02^{a}	0.06 ± 0.00^{j}	0.06 ± 0.00^{g}
Streptozotocin induced diabetic rats+ AN (100mg/kg)	0.11 ± 0.00^{c}	0.11 ± 0.01^{c}	0.10 ± 0.00^{g}	0.10 ± 0.00^{e}
Streptozotocin induced diabetic rats+ AM (100mg/kg)	0.12 ± 0.00^{b}	0.12 ± 0.01^{c}	0.10 ± 0.00^{g}	0.10 ± 0.00^{e}
Streptozotocin induced diabetic rats+ RCP (100mg/kg)	0.16 ± 0.01^{bc}	0.16 ± 0.01^{c}	0.16 ± 0.00^{e}	0.17 ± 0.00^{c}
Streptozotocin induced diabetic rats+ RCP (200mg/kg)	0.17 ± 0.01^{bc}	0.17 ± 0.00^{c}	0.18 ± 0.00^{d}	0.18 ± 0.00^{b}
Streptozotocin induced diabetic rats+ RCP (400mg/kg)	0.18 ± 0.00^{bc}	0.18 ± 0.01^{c}	0.20 ± 0.01^{b}	0.20 ± 0.01^{a}

Streptozotocin induced diabetic rats + GLB	0.85±0.01[a]	0.61±0.21[b]	0.07±0.01[i]	0.07±0.00[f]
Streptozotocin induced diabetic rats + insulin	0.86±0.01[a]	0.87±0.01[a]	0.08±0.00[h]	0.07±0.00[f]

Values are mean ± SEM, n = 5

Values with different superscripts along the row under the same heading, are significantly different (P<0.05)

Key; LPO= Lipid peroxidation AN= *Acacia nilotica* AM= *Anisopus manni* RCP= Recipe GBL =Glibenclamide

4.9 Scavenging Activity towards DPPH free radicals

The results of the radical scavenging activity of samples towards table 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical as analyzed against the reference antioxidant ascorbic acid are shown in figure 4. 3. The result shows that the concentrations (mg) increases, the scavenging activities (%) of extract plants and recipe also increases. However, the groups administered the recipe had high scavenging activities compare to the group administrated the extract. The group administrated the recipe compute favourably in term of scavenging activities with the activity of vitamin C.

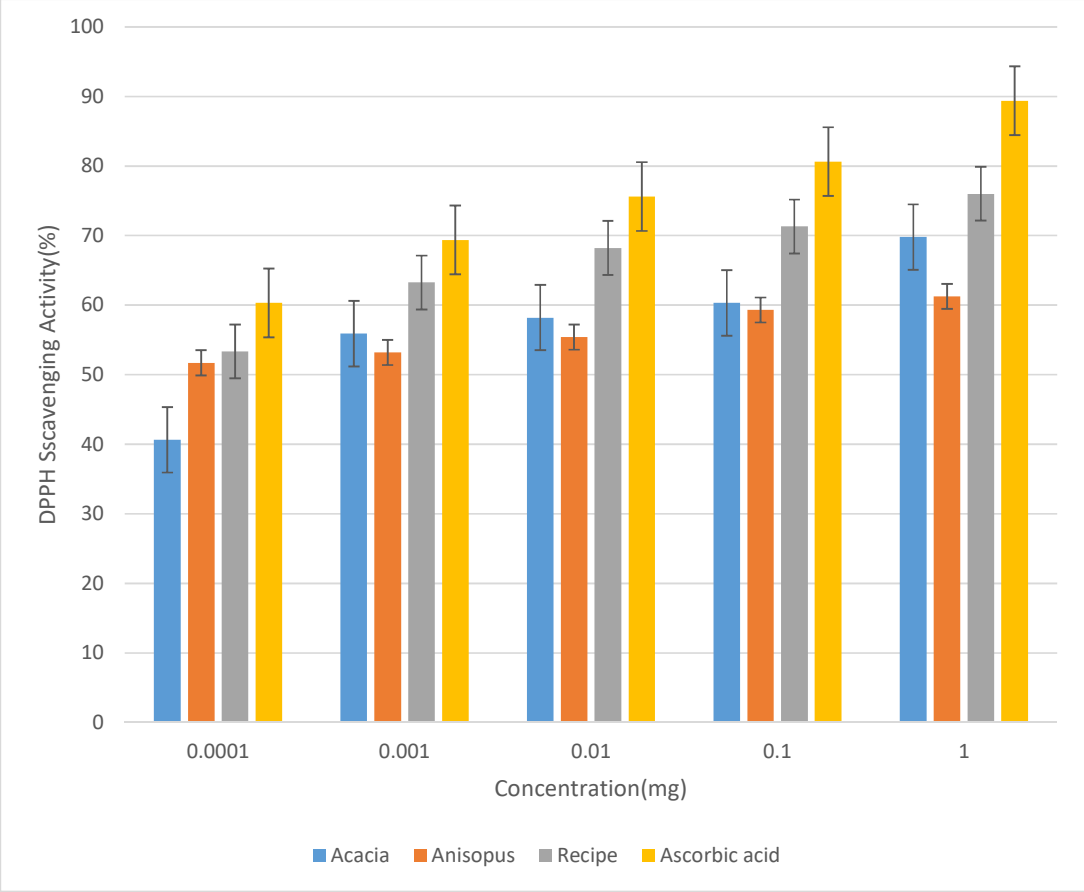

Figure 4.3: DPPH scavenging activities of plant extracts their recipe

Conclusion

In conclusion, the study underscores the significant medicinal potential of plants, particularly Acacia nilotica and Anisopus manni, in managing diabetes mellitus. The evaluation of phytochemical constituents revealed the presence of various bioactive compounds such as alkaloids, saponins, flavonoids, cardiac glycosides, and steroids, which are known for their antidiabetic activities. The observed hypoglycemic effects of the plant extracts and their recipe suggest potential mechanisms including increased glucose utilization, stimulation of insulin secretion, and enhancement of insulin sensitivity. Moreover, the study highlights the importance of considering synergistic effects when combining plant extracts in traditional recipes. Furthermore, the extracts demonstrated favorable effects on lipid profiles and antioxidant enzyme levels, suggesting potential protective effects against diabetic complications. Overall, this research provides scientific validation for the traditional use of these plants in diabetes management and underscores their potential as sources of novel antidiabetic agents.

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