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Chemical Composition, Antioxidative and Anti-Hyperglycemic Activity of Combined Seed Extracts of *Xylopia aethiopica* and *Trichilia emetica* in Alloxan Induced Diabetic Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AD developed the study design and performed statistical analysis. Author YGN managed the study and developed the first draft of the manuscript. Author HAO conducted the literature review. All authors read and approved the final manuscript.

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ABSTRACT

The chemical composition, in vivo antioxidative and antihyperglycemic activities of aqueous and ethanol seed extracts of combined *Xylopia aethiopica and Trichilia emetica in* a ratio of 1:1 mixture was studied in alloxan- induced diabetic wistar rats. Twenty-five wistar rats weighing between 100-150g were used. They were divided into five (5) groups, Normal control (received 1ml of distilled water), Negative control (received 100 mg/kg body weight of alloxan only), Positive control (2.5 mg/kg body weight of glibenclamide) and extracts treated groups which received 300mg/kg body weight of aqueous extract of combined *Trichilia emetica* and *Xylopia aethiopica* seeds (ATX) and ethanol extract of combined *Trichilia emetica* and *Xylopia aethiopica* seeds. Diabetes was induced by intraperitoneal injection of 100 mg/kg body weight of alloxan for 21 days. The phytochemical composition conducted using a GC (Buck Scientific-GC M910, USA) of the combined seeds extracts of *Xylopia aethiopica* and *Trichilia emetica* had varying composition of saponins, flavonoids,

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alkaloids, glycosides, phenols, terpenoids, tannins and steroids. Acute toxicity studies showed no sign of toxicity and death recorded of the relatively high doses of 3000mg/kg body weight of aqueous and ethanol seed extracts administered. Blood glucose concentration showed significant ($p<0.05$) decrease when compared to the alloxan treated group. There was significant ($p<0.05$) reduction in malondialdehyde (MDA) and significant ($p<0.05$) increase in enzymic (superoxide dismutase (SOD), catalase (CAT) and non-enzymic glutathione (GSH) antioxidants compared to negative control groups. From the findings of this study, it can be concluded that 300mg/kg body weight of aqueous and ethanol extracts of combined *Trichilia emetica* and *Xylopia aethiopica* seeds exhibited antioxidant and anti-hyperglycemic activities in alloxan induced diabetic rats. Combination of the various bioactive components found in the seed extracts may have contributed significantly to these activities and could be considered as potential therapeutic agent in the management of hyperglycemia.

Keywords: Chemical composition; hyperglycemia; antioxidant; alloxan; glucose.

1. INTRODUCTION

Hyperglycemia causes damage to many organs in the body and contribute to impairment in insulin secretion and resistance and consequently, diabetes mellitus, a prominent endocrine disorder. The defense mechanism of the body is often impaired in this condition resulting to inefficient free radical scavenging capability [1]. Currently, oral drugs like sulfonylureas, biguanides, and α-glucosidase inhibitors are effectively used to control hyperglycemia [2,3]. However, these substances are associated with unwanted site effects such as gastro intestinal disorders, increased body size, skin rash, polyuria and others [4,5,6,7]. In order to decrease side effects associate with their use, many natural products from plant origin and their derivatives have been used in combination with these drugs to achieve remarkable glycemic control [8,9].

Xylopia aethiopica and *Trichilia emetica* are two plants that have been widely studied for their therapeutic properties. *Xylopia aethiopica* commonly known as Ethiopian pepper or *uda* in igbo belongs to the Annonaceae family that can grow up to 20m high. It is a native to the lowland rainforest in the Savanna [10]. Medicinally, *Xylopia aethiopica* is utilized in the reduction of intraocular pressure, stomach ache, bronchitis, and dysentery [11]. *Trichilia emetica* belongs to the family of Meliaceae. It is commonly known as the Natal mahogany and found in riverine vegetation and open woodland in Tropical Africa. It has glossy dark green leaves and sweet-scented flower that attract bees and birds to it. *Trichilia emetica* grows up to 25m high, with separate male and female plants. *Trichilia emetica* has been studied to exhibit anti-inflammatory, antiplasmodial, anticonvulsant, anti-oxidant and hepatoprotective properties [12,13]. It has been used for its emetic, diuretic and purgative properties [14]. Plants are rich source of phytochemicals which have been known to influence various physiological processes. Medicinal plants are widely used worldwide to address a variety of health problems. In recent times many pharmaceutical products are derived from plants [15]. The World Health Organization (WHO) has recommended the evaluation of medicinal plants treatments for diabetes [16]. Herbal treatments in combination with conventional drugs have been used in patients with diabetic conditions [17,18]. The use of some plants and its products still lack scientific rationale supporting their inclusion in the management of diseases like diabetes. The present study was conducted to evaluate the chemical composition and invivo anti-oxidative and anti-hyperglycemic activity of combined seed extracts of *Xylopia aethiopica* and *Trichilia emetica* in alloxan induced diabetes in wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant materials used for this study were seeds of *Trichilia emetica* and *Xylopia aethiopica.* They were obtained from mile 3 market in Port Harcourt and authenticated by a plant taxonomist at the Department of Plant Science and Biotechnology, University of Port Harcourt with specimen sample number, UPH C-049 and UPH C- 050 for future reference.

2.2 Preparation of Plant Extracts

Trichilia emetica (T.emetica) and *Xylopia aethiopica (X.aethiopica)* were sorted, cleaned

and ground into powdered form. 65.5 g of the powdered seeds of *T. emetica* and *X. aethiopica* were combined and macerated with 600 ml of distilled water to yield the aqueous extract and 90% ethanol to yield the ethanol extract for 72 hours at room temperature. The mixtures were filtered through 5 layers of muslin cloth and the filtrate obtained was subjected to dryness in an oven at a temperature of 50^0C.

Percentage Yield of the Aqueous and Ethanol Seed Extracts of *T. emetica and X. aethiopica:*

The percentage yield was calculated as follows:

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of powder}} \times 100$$

The % yield of the aqueous (1.02) and ethanol (0.96) extracts were reconstituted separately in distilled water to give a stock solution of concentration 0.1g/ml.

2.3 Chemicals and Reagents

Ellmans reagent, Alloxan monohydrate, Thiobarbituric acid, Hydrogen peroxide and Pyrogallol were procured from Inqaba BIOtec, West African LTD. Glibenclamide (Daonil) 5mg, Sanofi aventis Pharma India was obtained from Ebus Pharmacy Port Harcourt .All other reagents and Chemical were of analytical grade and supplied by the Department of Biochemistry, Rivers State University, Nigeria.

2.4 Analysis of Phytochemical Compounds

The Phytochemical components of the combined seeds of *Xylopia aethiopica* and *Trichilia emetica* was conducted using a GC (Buck Scientific-GC M910, USA) equipped with flame ionization detector. A RESTEK 15-meter MXT-1 column (15 m x 250 µm x 0.15 µm). The injector temperature was 280^oC with splitless injection of 2 µl of sample and a linear velocity of $30cms^{-1}$, Helium 5.0 Pas was the carrier gas with a flow rate of 40 ml/min. The oven operated initially at 200^oC, it was heated to 330^oC at a rate of 3^oC/min and was kept at the temperature for 5 minutes. 1g of the crushed samples were weighed and transferred into a test tube containing 15 ml of ethanol and 10 ml of 50% w/v potassium hydroxide. The contents of the test tubes were allowed to stand in a water bath at a temperature of 60^oC for 60 minutes after which they were carefully transferred into a separating funnel and rinsed with 10 ml of cold water, 10 ml of hot water, 20ml of ethanol and 3ml of hexane. The extract in each test tube was washed three times with 10ml of 10% v/v ethanol solution and dried with anhydrous sodium sulphate and the solvent evaporated. A sample of the extract was then made soluble in 1000 µl of pyridine of which 200 µl was transferred into a vial on the Gas Chromatography machine for the analyses of bioactive components. The Linearity of the dependence of response factor on concentration of daily standards was verified by regression analysis. The identification was based on comparison of retention times, spectral data with daily standards.

2.5 Experimental Animals

The experimental animals used for this study were wistar rats of both sexes weighing between 100-150 g. They were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt and kept in the animal house of the Department of Biochemistry, Rivers State University, Port Harcourt, Nigeria. They were allowed to acclimatize for two weeks under laboratory conditions. Feed and water were provided for the animals ad *libitum* prior to the conduct of experiment.

2.6 Determination of LD_{50} and Acute Toxicity of the Seed Extracts

The LD_{50} / acute toxicity study of aqueous and ethanol extracts of combined seeds of T. emetica and X aethiopica were assessed using normal healthy albino rats. The rats were divided into three groups A, B and C (n=5). Group A served as control and was not administered with the extracts. Groups B and C were allowed to fast for a period of 16 hours and were administered with the extracts. Group B was administered with relatively high dose of 3000mg/kg body weight of aqueous extract of combined *T. emetica* and *X aethiopica* seeds while group C was administered with 3000 mg/kg body weight of ethanol extract combined *T. emetica and X aethiopica* seeds by oral route for a period of 14 days. The rats were continuously observed for different toxicological responses such as behavioral, neurological, autonomic responses as well death of any of the rats [19].

2.7 Working Volume of Stock Extracts

The volume of stock to be administered based on the body weights of the animals were calculated according to the formula given by Nwafor et al., [20].

Volume=(D×P)/C
D= dose to be administered
P= body weight of animals in Kg
C= concentration of stock solution

2.8 Preparation of Standard Drug

Glibenclamide tablets (oral hypoglycaemic drug) were used as reference drug. It was freshly prepared in distilled water and administered at a dose of 2.5mg/kg body weight orally [21].

2.9 Induction of Diabetes

Diabetes was induced in the rats by injecting 100 mg/kg body weight of alloxan monohydrate in 0.9% normal saline solution to rats that were fasted overnight intraperitoneally (i.p) using insulin syringes. The rats were kept for 24 hours on 10% glucose solution bottles to prevent hypoglycemia. Following intraperitoneal injection of alloxan, animals were observed for 24-48 hours for behavioral changes and fatal- induced hypoglycemia associated with initial alloxan injection. After 48 hours of i.p injection, blood glucose was collected at the tail end for the determination of glucose level using Accu check Active glucometer. Rats with blood glucose levels equal to 200 mg/dl or > 200 mg/dl were considered diabetic and used for this study [22] The Experimental design and protocol for treatment is presented in Table 1. After the last dose, the rats were fasted overnight and sacrificed under chloroform anesthesia. Samples were collected for analyses after 21 days of continuous treatment.

2.10 Collection of Samples

The Liver samples were excised from the rats and washed in 0.05M ice-cold phosphate buffer saline (PBS) (pH 7.4) to remove excess blood and weighed. (1: 10 w/v) was homogenized in cold phosphate buffer solution. The homogenates were centrifuged at 10000 g for 10 mins and the supernatant collected for determination of oxidative stress markers.

2.11 Determination of Biochemical Parameters

Assay of fasting blood glucose concentration: The glucose in plasma was assayed using the (Accu check glucometer), containing a glucose strips and glucometer. The principle here involves the sample glucose reacting with available glucose oxidase enzyme in strips which serves as electrode through which oxidation occurs and which resulted into gluconic acid and the temporary transfer of two electrons from glucose to the enzyme. The reduced enzyme mediates the transfer of a single electron to each of the two mediator ions, thus returning to its original state. At the electrode surface, the reduced mediator was re-oxidized providing amperometry signal whose magnitude was equivalent to glucose concentration in sample.

2.12 Determination of Oxidative stress Markers

SOD was determined by the auto-oxidation method of pyrogallol described by Marklund and Marklund [23]. Pyrogallol undergoes rapid autoxidation in an alkaline solution generating superoxide ions, SOD inhibits its autooxidation by dismutation and allow the superoxide ions formed to be converted to hydrogen and molecular oxygen. The activity of SOD is measured spectrophotometrically at 420 nm. Catalase determined by the method of Shina [24]. Catalase in the crude enzyme source splits hydrogen peroxide in the presence of heat and dichromate / acetic acid medium to form chromic acetate and unstable perchromic complexes. Hydrogen peroxide not consumed was determined by the absorption of colored chromic acetate and measured spectrophotometrically at a wavelength of 610 nm. GSH was determined by the method of Ellman's reagent [25]. The method involves the oxidation of GSH by 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). DTNB and glutathione (GSH) react to generate 2-nitro-5- thiobenzoic acid (TNB) which has yellow color. The absorbance of the color developed after reaction is read at 412 nm and concentration of GSH calculated using the formula, $A = \Sigma CL$, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length. The results were expressed in nmol/mg of protein. MDA a product of lipid peroxidation was as assayed according to the method described by Devasagayam et al. [26]. In this method, the samples are heated with thiobarbituric acid (TBA) reagent for 20 mins in a boiling water bath to give a pink-coloured complex that is read at 535 nm wavelength. The concentration of MDA was calculated using the molar extinction coefficient of malondialdehyde (1.56×10^5 mol/L/cm) using the formula, $A = \Sigma CL$, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length. The results were expressed in nmol/mg of protein.

Table 1. Treatment protocol for the experimental rats

S/N	Group Code	Group Identity	Treatment
1	No C	Normal control	Received 1 ml of distilled water only
2	Ne C	Negative control	100mg/kg body weight of alloxan only
3	Po C	(Positive control)	2.5 mg/kg body weight of Glibenclamide
4	AXT	Aqueous extract of *T. emetica*+ *X. aethiopica* (ATX)	300mg/kg body weight of ATX + 100mg/kg body of alloxan
5	EXT	Ethanol extract of *T. emetica* + *X. aethiopica* (ETX)	300mg/kg body weight of ETX + 100mg/kg body of alloxan

Table 2. Percentage (%) composition of some bioactive components in the aqueous and ethanol extracts of combined X. *aethiopica* and *T. emetica* seeds

Phytochemical	Aqueous extract	Ethanol extract
Saponins	39.92 ± 0.08	56.16 ± 0.37
Flavonoids	61.79 ± 1.23	71.34 ± 0.68
Alkaloids	49.19 ± 2.22	60.13 ±2.05
Glycosides	37.71 ± 3.16	34.12 ± 1.04
Phenols	53.33 ± 0.09	58.74 ± 1.11
Terpenoids	27.38 ± 1.04	20.56 ±1.51
Tannins	19.33 ± 0.77	28.67 ± ± 0.56
Steroids	4.57 ± 1.03	13.93 ± 0.89

Values are expressed as mean± S.E.M (n=3).

2.13 Statistical Analysis of Data

Statistical analysis of the data obtained in this study was conducted using SPSS version 20.0. Results are expressed as mean + S.E.M. They were subjected to one way analysis of the variance (ANOVA) followed by Tukey post hoc test for multiple comparison. Values with $p< 0.05$ were considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

Acute toxicity study of seed extracts of combined *T. emetica and X. aethiopica:* The result obtained from *acute* toxicity study/LD_{50} showed that there was no sign of toxicity and no death recorded for the animals after oral administration of relatively high doses of 3000 mg/kg body weight (B.W) of aqueous and ethanol seed extracts of combined *T. emetica* and *X. aethiopica.* Thus, one tenth of 3000 mg/kg B.W (300 mg/kg B.W) was selected and used as fixed dose for this study.

The aqueous and ethanol extracts of combined *T. emetica* and *X. aethiopica* seeds had varying percentage composition of bioactive components such as saponins, flavonoids, alkaloids, glycosides, phenols, terpenoids, tannins and steroids as presented in Table 2.

Effect of treatment on blood glucose concentration: The basal glucose concentration before administration of alloxan showed no significant ($p>0.05$) change in all the experimental animals. However, significant ($p<0.05$) increase in blood glucose concentration was recorded for all the groups after administration of alloxan compared to the normal control group presented in Fig. 1.

The effect of oral administration of aqueous and ethanol extracts of combined *T. emetica* and *X. aethiopica* seeds in alloxan induced hyperglycemic rats is shown in Fig. 2. There was significant ($p<0.05$) decrease in glucose concentration in animals that were treated with the extracts and the positive control group treated with reference drug compare to the negative control groups which received only alloxan. The percentage change or decrease for the ethanol extract treated group (43.90%) was higher than the positive control group (38.54%) and aqueous extract treated group (37.90 %).

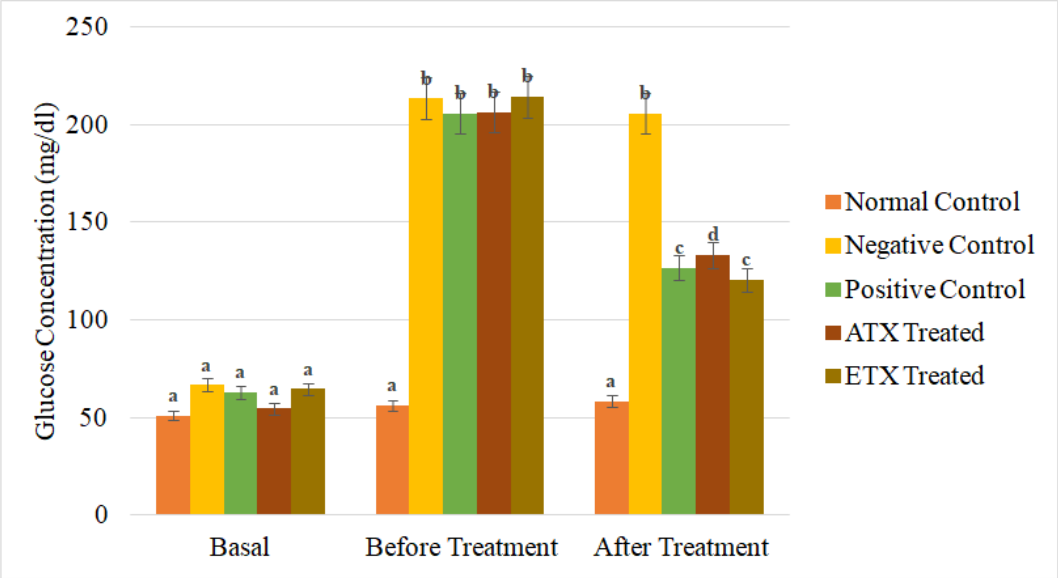

Fig. 1. Effects on Glucose Concentration (mg/dl) of rats treated with aqueous and ethanol extracts of combined T. emetica and *X. aethiopica* seeds.

Values are expressed as mean± S.E.M (n=3). Means with different superscripts (a-c) are significantly different (Turkey HSD, p<0.05). ATX= Aqueous extract of combined T.emetica and X. aethiopica seeds, ETX= Ethanol extract of combined T.emetica and X. aethiopica seeds

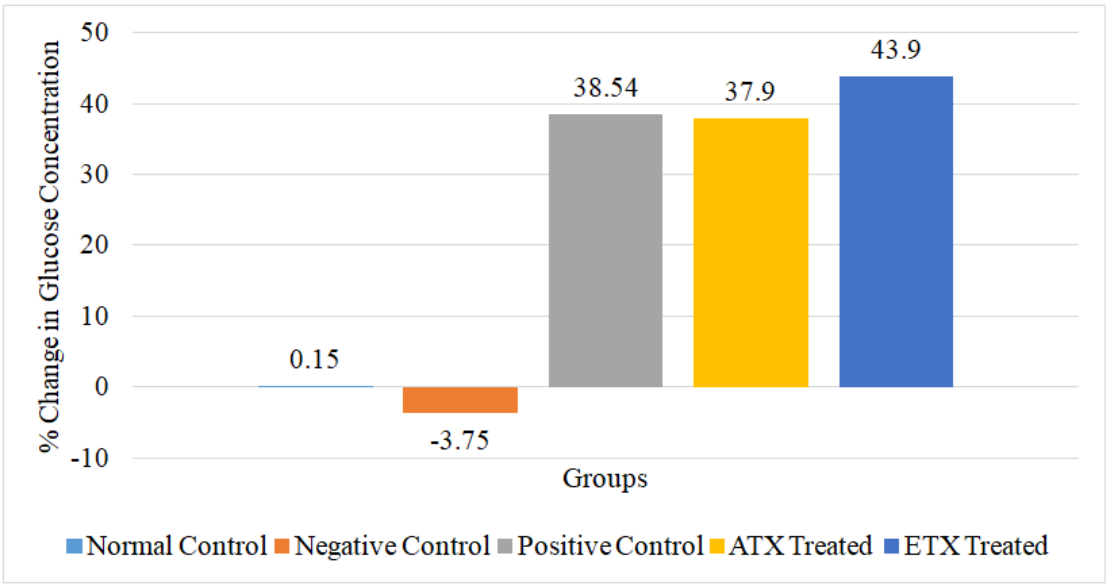

Fig. 2. Reduction of glucose concentration in Rats Treated with Aqueous and Ethanol Extracts of Combined *T. emetica* and *X. aethiopica* seeds

Values are expressed as mean± S.E.M (n=3). ATX= Aqueous extract of combined T.emetica and X. aethiopica seeds, ETX= Ethanol extract of combined T.emetica and X. aethiopica seeds

Fig. 3 showed the results for oxidative stress markers. The negative control group showed significant ($p<0.05$) decrease in CAT, SOD and GSH while MDA showed significant ($p<0.05$) increase compared to the normal control group 1. In the extract treated groups (ETX and ATX) there was significant ($p<0.05$) decrease in MDA concentration compared to the negative control group2 while CAT, SOD and GSH showed significant ($p<0.05$) increased. control group 1. The result obtained for the positive control group were not significant compared to normal control.

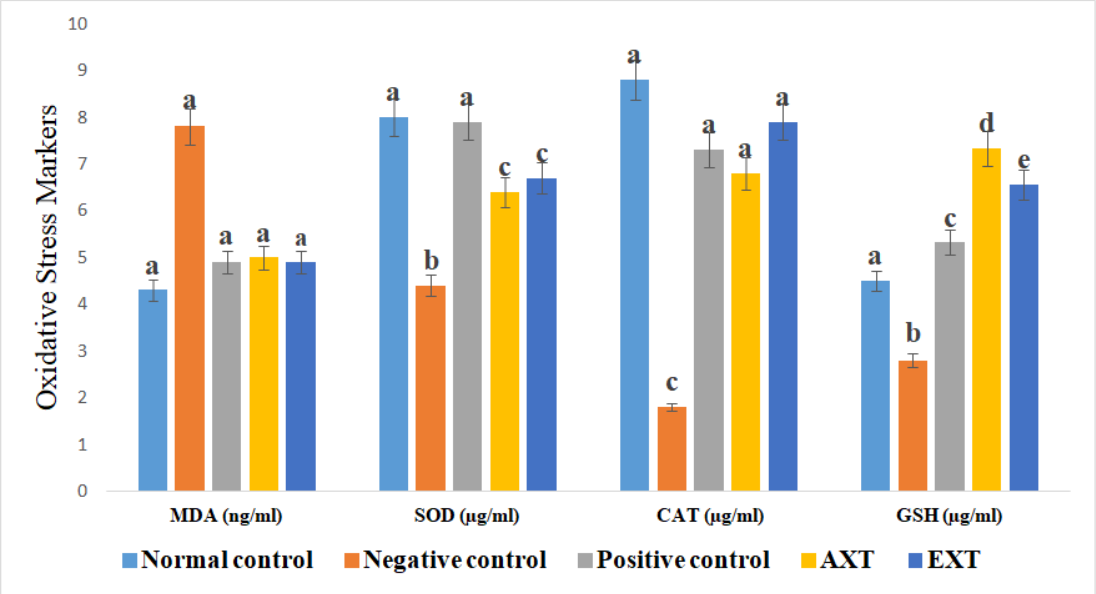

Fig. 3. Effect on oxidative stress markers in rats treated with aqueous and ethanol extracts of combined *T. emetica* and *X. aethiopica Seeds*

Values are expressed as mean± S.E.M (n=3). Means with different superscripts (a-e) are significantly different (Turkey HSD, p<0.05). ATX= Aqueous extract of combined T.emetica and X. aethiopica seeds, ETX= Ethanol extract of combined T.emetica and X. aethiopica seeds

3.2 Discussion

Plants contain wide range of bioactive components that have been reported to exhibit varying therapeutic and pharmacological properties [27,28,29,68,69]. Several studies have been conducted to established the hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory, anti-infectivity, antimicrobial, antiviral, hepatoprotective, nephroprotective and cardio protective potentials of these components found in plant and its products [30,31,32,33]. In the present study, the aqueous and ethanol extracts of combined *and Trichilia emetica* and *Xylopia aethiopica* seeds showed different bioactive components in varying compositions with flavonoids having the highest percentage composition in the aqueous and ethanol extracts. Flavonoids are polyphenols with two aromatic ring structure linked together by three (3) carbon atoms which form an oxygenated heterocyclic compound [34,35]. Polyphenols are also known antioxidants that scavenge free radicals and prevent oxidative damage in biological systems by reactive oxygen and nitrogen species [36,37]. Terpenoids, alkaloids, flavonoids, phenolics, glycosides, carotenoids found in plant extracts have been studied to exhibit antidiabetic potentials in different animal models [17,33,38,39,40,41].

Molecular derangements in glucose metabolism can result to hyperglycemia. This is a condition in which glucose accumulate in the blood stream and cannot be converted to glycogen for storage by the liver even at normal or increased concentrations of circulating insulin [42,43]. In this study, all rats treated with alloxan had significant increase in glucose concentration. This is an indication of hyperglycemia in the experimental animals. Numerous biochemical pathways and mechanisms have been suggested for glucose toxicity. Alloxan is a well appreciated chemical agent used in inducing hyperglycemia in many animal studies [44,45,46]. It is a toxic analog of glucose and works by selective destruction of the pancreatic β- cells and generation of reactive oxygen species (ROS) which affect insulin biosynthesis and secretion and in turn disrupt glucose homeostasis [47]. Deficiency in glucose homeostasis can confer glucose toxicity on key organs like the liver and other peripheral abnormalities [48,49]. From this study, animals administered with aqueous and ethanol extracts of *T. emetica* and *X. aethiopica* seeds had decrease in glucose concentration. This is an indication of the antihyperglycemic potentials of the seed extracts. The Results obtained for the percentage in glucose reduction were comparable to levels of the control groups (normal and positive). The antihyperglycemic

properties may be attributed to enhanced utilization of glucose by tissues via glycolysis, tricarboxylic acid cycle and the shunt pathway [42,43].

Alloxan is also very unstable and have the capacity to inhibit the activities of many functionally important sulfhydryl (-SH) groups present in proteins via its redox cycle reactions [45,46]. Continuous redox cycle reactions subsequently lead to the production of highly reactive oxygen species such as superoxide radical anion ($O2.^-$) and hydroxyl (OH) radical. These species have contributed to significant increase in glucose toxicity and organ dysfunction like the liver which depends on insulin for glucose hemostasis [48,50,51,52]. Various studies have shown that chronic hyperglycemia is linked to increased oxidative stress, increased production of major reactive oxygen species such as superoxide radical ($O_2•^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•) [53,54,55,56,57,47].

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) help to catalyze reactions that prevent the accumulation of ROS and damage to different organs. The superoxide radical anion can undergo dismutation reaction to H_2O_2 by SOD an enzymic antioxidant that is found in all tissues [58,59]. CAT prevents the accumulation of H_2O_2 and subsequent generation of highly reactive hydroxyl (OH) radical by decomposing the hydrogen peroxide to water and molecular oxygen [60,61] (Ray et al., 2007). There was significant decrease in the non-enzymic (GSH) and enzymic (CAT and SOD) antioxidants while MDA a measure for lipid peroxidation also showed significant increase in the hepatic tissues of animals treated with alloxan only. Decrease in the concentration of these enzymes may be attributed to the quick response in combating free radicals generated by alloxan. Low activity of antioxidant enzymes can lead to increased production of ROS and subsequent disruption on glucose homeostasis [62,63]. Ability of alloxan to attack sulfhydryl groups has been established by many studies to be linked the depletion of GSH and increased production of its toxic products via autoxidation [64,65,52]. In the extract treated groups, there was marked elevation of these antioxidant enzymes and low MDA. This is an indication of improved antioxidant capacity exhibited by the extracts to ameliorate toxicity associated with alloxan. Several studies have shown the capacity of plant extracts to stimulate the activity antioxidant enzymes to prevent or reverse toxic effects of chronic hyperglycemia [54,66,67]. The antioxidant activity exhibited by aqueous and ethanol extract of combined *T. emetica* and *X. aethiopica* can also can also attributed to inhibition of toxic products formation and autoxidation and is considered as a possible mechanism offered by extracts to protect liver.

4. CONCLUSION

In the study, it can be concluded that combination of various bioactive components found in the aqueous and ethanol extracts of *T. emetica* and *X. aethiopica* seeds exhibited antioxidant and antihyperglycemic activity in alloxan induced hyperglycemic rats and could be considered as potential therapeutic target in the management of hyperglycemia and diabetes.

CONSENT

It's not applicable.

ETHICAL APPROVAL

The procedures of this work were conducted according to the University's ethical guidelines of the use of laboratory animals and approved by the Institutional Animal Ethical Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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