Data Article

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

Abstract

The chemical composition and invivo antioxidative and antihyperglycemic activity 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, Negative control (received 100mg/kg body weight of alloxan only), Positive control (2.5mg/kg body weight of glibenclimide) and extracts treated groups which received 300mg/kg body weight of the combined aqueous (ATX) and ethanol (ETX) seed extracts. Diabetes was induced by intraperitoneal injection of 100mg/kg body weight of alloxan for 21 days. The phytochemical composition was conducted using a GC (Buck Scientific-GC M910, USA). The combined seed extracts of Xylopia *aethiopica* and *Trichilia emetica* had varying composition of saponins, flavonoids, alkaloids, glycosides, phenols, terpenoids, tannins and steroids. 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) antioxidant compared to control groups. From the findings of this study, it can be concluded that 300mg/kg body weight of combined seeds of *Xylopia aethiopica and Trichilia emetica* exhibited antioxidant and anti-hyperglycemic activity in alloxan induced diabetic rats. Combination of the various bioactive components found in the seed extracts have contributed to these activities observed and could be considered as potential therapeutic target in the management of hyperglycemia.

Key Words: Chemical composition, Hyperglycemia, Antioxidant, alloxan, glucose,

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 (Kawahito *et al*., 2009). Currently, oral drugs like

sulfonylureas, biguanides, and α-glucosidase inhibitors are effectively used to control hyperglycemia (Krentz *et al*., 2005; Bolen *et al*., 2007). However, these substances are associated with unwanted site effects (Codario, 2005; Syiem and Wariri, 2015). 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 (Prabhakar *et al*., 2013; Semkoff, 2015).

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 (Burkill, 1985). Medicinally, *Xylopia aethiopica* is utilized in the reduction of intraocular pressure, stomach ache, bronchitis, and dysentery (Uzodike and Onuoha, 2010). *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 (Dharani *et al*., 2010; Baatile *et al*., 2011). It has been used for its emetic, diuretic and purgative properties (Komane *et al*., 2011). 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 (Chen *et al*., 2016). The World Health Organization (WHO) has recommended the evaluation of medicinal plants treatments for diabetes (Rupueshkumar *et al*., 2014). Herbal treatments in combination with conventional drugs have been used in patients with diabetes conditions (Patel *et al.,* 2012; Xu *et al.,* 2018). 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.

Materials and Methods

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.

Preparation of Plant Extracts

Combined seeds of *Trichilia emetica (T.emetica)* and *Xylopia aethiopica(X.aethiopica)* were sorted, cleaned and ground into powdered form. 65.5 g of the powdered seeds were macerated with 600ml of 90% ethanol and distilled water, for 72 hours at room temperature and then filtered through 5 layers of muslin cloth. The filtrate obtained was subjected to dryness in an oven at a temperature of 50^0C to determine percentage yield. The dried extracts were then re-dissolved in distilled water in a ration of 1: 1 and dose of extract were administered according to the body weight of animals.

Chemicals and Reagents

Ellmans reagent, Alloxan monohydrate, Thiobaruric acid and pyrogallol were procured from Inquaba, Africa. All other reagents and Chemical were of analytical grade supplied by the Department of Biochemistry, Rivers State University, Nigeria and procured from reputable firms.

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 (15m x 250µm x 0.15µm). The injector temperature was 280^{o}C 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 40ml/min. The oven operated initially at 200^{o}C, it was heated to 330^{o}C at a rate of 3^{o}C/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^{o}C for 60 minutes after which they were carefully transferred into a separating funnel and rinsed with 10ml of cold water, 10ml 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 1000µl of pyridine of which 200µl 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.

Experimental Animals

Wister albino rats weighing 100 – 150 g were used for this study. They were purchased from the animal house of University of Port Harcourt and kept in the animal house of the Department of Biochemistry, Rivers State University, Port Harcourt, Nigeria for two weeks (14) days with 12

hours light-darkness cycles. They were fed with standard animal feeds. Water was given ad *libitum* prior to the conduct of the experiment. The experiment was conducted according to the University's ethical guidelines of the use of laboratory animals.

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*., (2009).

Volume=(D×P)/C

D= dose to be administered

P= body weight of animals in Kg

C= concentration of stock solution

Preparation of Standard Drug

Glibenclamide tablets, an 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 (Warri *et al*., 2021).

Induction of Diabetes

Diabetes was induced in the rats by injecting 100mg/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 200mg/dl or > 200mg/dl were considered diabetic and used for this study (Bamidele *et al*., 2014) 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.

Table 1: Treatment Protocol for the Experimental Rats

S/N	Group Code	Group Identity	Treatment
1	Normal control	Normal control	Received 1 ml distilled water
2	Negative control	Negative control	100mg/kg body weight of alloxan only
3	Positive control	(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

Collection of Samples

Livers were excised from rats, washed in 0.05M ice-cold phosphate buffer saline (PBS) (pH 7.4) to remove excess blood and weighed. (1: 10w/v) was homogenized in cold phosphate buffer

solution. The homogenates were centrifuged at 10000g for 10 mins. The supernatant of this fraction was collected for determination of oxidative stress markers

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.

Determination of Oxidative stress Markers

SOD was determined by the auto-oxidation method of pyrogallol described by Marklund and Marklund (1974), Catalase determined by the method of Shina (1972), GSH was determined by the method of Ellman's reagent (Ellman, 1959) and (MDA) a product of lipid peroxidation was as assayed according to the method described by Devasagam *et al*., (2003).

Statistical Analysis of Data

Data obtained from this study were expressed as mean ± SEM followed by one-way analysis of the variance (ANOVA) and turkey post hoc test for the establishment of significance differences set at ($p<0.05$).

Results and Discussion

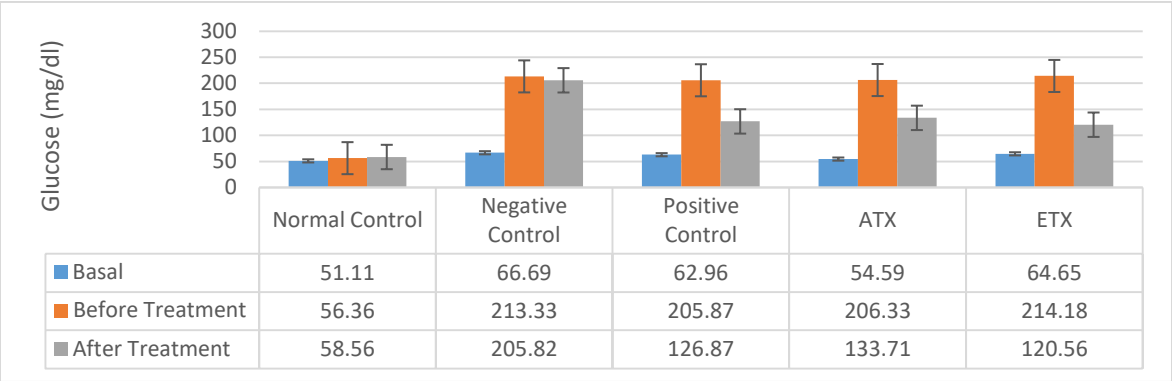
Results

The percentage composition of bioactive components investigated is shown in Table2. The result showed that aqueous and ethanol extracts of combined seeds of Xylopia *aethiopica* and *Trichilia emetica* had varying percentage composition of bioactive components such as saponins, flavonoids, alkaloids, glycosides, phenols, terpenoids, tannins and steroids.

Table2: Percentage (%) Composition of Some Bioactive Components in the Aqueous and Ethanol Extracts of Combined seeds of *Xylopia aethiopica* and *Trichilia emetica*

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

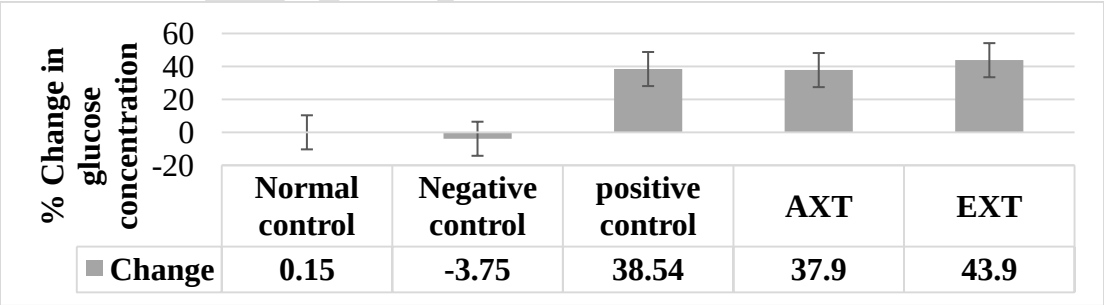
The blood glucose concentration before administration of alloxan had no significant ($p>0.05$) change. However, significant ($p<0.05$) increase in blood glucose concentration was recorded for groups after administration of alloxan as presented in Figure1. This is an indication of hyperglycemia induced by alloxan.



	Normal Control	Negative Control	Positive Control	ATX	ETX
Basal	51.11	66.69	62.96	54.59	64.65
Before Treatment	56.36	213.33	205.87	206.33	214.18
After Treatment	58.56	205.82	126.87	133.71	120.56

Figure1: Effects on fasting Glucose Concentration (mg/dl) of rats treated with aqueous and ethanol extracts of combined seeds of *Xylopia aethiopica* and *Trichilia emetica*

The effect of oral administration of aqueous and ethanol extracts of combined *Xylopia aethiopica* and *Trichilia emetica* in the alloxan induced hyperglycemic rats is shown in Figure2. There was significant ($p<0.05$) reduction in glucose concentration of animals that were treated with the combined seed extracts and positive control compared to the negative control groups. The reduction obtained for the ethanol treated group was not significant ($p>0.05$) compared to the result obtained for the positive and normal control groups.



	Normal control	Negative control	positive control	AXT	EXT
Change	0.15	-3.75	38.54	37.9	43.9

Figure2: Reduction of glucose concentration in rats treated with aqueous and ethanol extracts of combined seeds of *Xylopia aethiopica* and *Trichilia emetica*

The lipid peroxidation marker TBARS and oxidative stress markers (CAT, SOD and GSH) in group 2 were observed to show significant ($p<0.05$) decrease in CAT, SOD and GSH and

significant ($p<0.05$) increase in TBARS. In all the extracts treated groups, significant reduction in TBARS concentration was observed comparable to the concentration obtained in positive control group while the concentration of CAT, SOD and GSH showed significant ($p<0.05$) increased compared to normal control group.

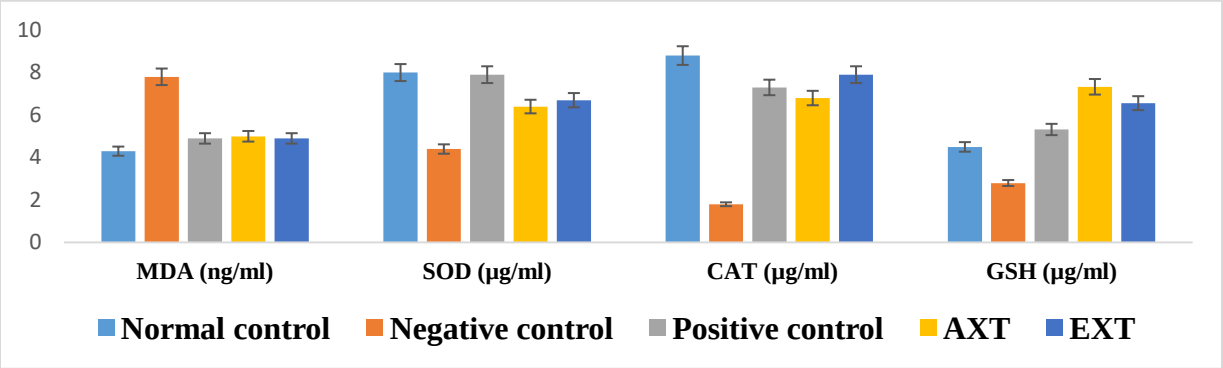


Figure3: Effect on Oxidative Stress Marker and antioxidant enzymes in rats treated with aqueous and ethanol extracts of combined leaves of *Xylopia aethiopica* and *Trichilia emetica*

Discussion

Plant materials contain wide range of bioactive components that have been reported to exhibit varying therapeutic and pharmacological properties (Rochfort *et al*., 2008; Sasidharan *et al*., 2011; Azmir *et al*., 2013). Several studies have been conducted to established the hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory, anti-infectivity, antimicrobial, antiviral, hepatoprotective, nephroprotective and cardio protective potentials of bioactive components found in plant and its products (Bagalkotar *et al*., 2006; Perumal and Gopalakrishnakone, 2010; Dokubo *et al*., 2017; Obomanu *et al*., 2017). In the present study, the aqueous and ethanol extracts of combined seeds of Xylopia *aethiopica and Trichilia emetica* showed different classes of bioactive components in varying compositions with flavonoids having the highest

composition in both aqueous and ethanol extracts. Flavonoids are polyphenols which two aromatic rings structured linked together by three (3) carbon atoms that form an oxygenated heterocyclic compound (Lo Piparo *et al*., 2008; Agrawal, 2013). Polyphenols are known antioxidants that scavenge free radicals thus exerting a protective effect in biological systems towards attack by reactive species (Aruoma, 1994; Srinivasan *et al*., 2007).

Bioactive compounds also known as phytochemicals such as terpenoids, alkaloids, flavonoids, phenolics, glycosides, carotenoids and others found in plants different plant extracts have shown antidiabetic potential (Patel *et al.*, 2012). The mechanism of action of bioactive components are often elucidated by matching bioactives with molecular targets and assaying for specific biomarkers (Foito and Stewart, 2018; Heilker *et al*., 2019). For glucose, Numerous biochemical pathways and mechanisms have been suggested. Decline in beta cell function have achieved wide acceptability to induce glucotoxicity, decrease insulin secretion and concentration (Kahn, 2003; Martens and Casteele, 2007). Molecular derangements in glucose metabolism often leads to glucose toxicity and chronic hyperglycemia subsequently. This is a condition in which glucose accumulate in the blood stream and cannot be converted to glycogen for storage by the liver due to low insulin secretion and concentration as well as insulin resistance. Clinically, insulin resistance is usually characterized by decreases in glucose uptake; utilization and storage at normal or increased concentrations of circulating insulin (Hunter and Garvey, 1998; Sesti, 2006). In this study, hyperglycemia was induced by alloxan. All rats treated with alloxan had significant increase in glucose concentration. This is an indication of hyperglycemia in the experimental animals. Alloxan is a well appreciated diabetogenic agent used in inducing hyperglycemia in many studies using animal model (Zanatta *et al*., 2007; Atawodi *et al*., 2014; Thilagam *et al.*, 2020). Alloxan is a 5,5-dihydroxyl pyrimidine-2,4,6-trione, a toxic analog of glucose (Ighodaro *et al*., 2018; Wu *et al*., 2020; Gunawan *et al.*, 2021). It 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 (Rohila and Ali, 2012). Deficiency in glucose homeostasis can confer glucose toxicity on key organs like the liver and other peripheral abnormalities (Rossetiti *et al*., 1990; Pietrangelo, 2007).

In view of this study, low glucose concentrations were measured in animals treated with aqueous and ethanol seed extracts of *Xylopia aethiopica and Trichilia emetica*. This is an indication of antihyperglycemic potentials of these extracts. The Results obtained for glucose reduction potentials were comparable to near normal and positive control groups treated with glibenclimide which is a standard drug. The antihyperglycemic properties may be attributed to enhanced utilization of glucose by tissues via glycolysis, tricarboxylic acid cycle, the shunt pathway for endogenous storage. In addition, pathways that enhance reduction in insulin secretion capacity of pancreatic β- cells and resistance may have also been inhibited. Part of the molecular mechanism underlying pancreatic beta cell destruction in the alloxan induced hyperglycemic rats is the generation of reactive oxygen species (ROS), significant contributors to the etiology of glucose toxicity in insulin dependent organ like the liver for the hemostasis of glucose (Robertson *et al*., 2007; Gerber and Rutter, 2017; Macdonald *et al*., 2017). Groups administered with alloxan only exhibited marked reduction in non-enzymic (GSH)and enzymic (CAT and SOD) antioxidant makers while MDA, index for lipid peroxidation showed significant increase in the hepatic tissues. The decreased concentration of these enzymes may be attributed to the quick response in combating free radicals generated by alloxan. Various studies have shown that diabetes mellitus is associated with oxidative stress, leading to an increased production of reactive oxygen species (ROS), including superoxide radical ($O_2•^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•) (Aruoma, 1998; Ighodaro, 2018; Asmat *et al*., 2016; Rehman and Akash, 2017). The mechanism of alloxan induced chronic toxicity is integrated in a free radical

generating capacity via the Fenton reaction (Ebelt *et al*., 2000; Rohilia and Ali, 2012). Alloxan is very unstable and have the capacity to inhibit the activities of many functionally important sulfhydryl (-SH) groups in proteins and enhance lipid peroxidation products. It exhibits continuous redox cycle reactions to produce highly reactive oxygen species which include the superoxide radical anion (O2.-) and hydroxyl (OH) radical. The superoxide radical anion can undergo dismutation reaction to H_2O_2 by SOD an enzymic antioxidant that is found in all tissues and organs. CAT prevents the accumulation of H_2O_2 and subsequent generation of highly reactive OH radical by decomposing the hydrogen peroxide to water and molecular oxygen (Ray *et al*., 2007; Ślesak *et al*., 2007Sánchez-Valle *et al*., 2012;). Low activity of oxidative stress marker enzymes can lead to increased production of peroxidation products can in turn cause disruption to glucose homeostasis (Yang *et al*., 2011; Pitocco *et al*., 2013). In the test groups, there was marked elevation of these antioxidant enzymes and decrease in the formation of lipid peroxidation product (MDA). This is an indication of improved antioxidant capacity exhibited by the extracts which tends to ameliorate the toxicity and diabetogenic properties associated with alloxan. Several studies have shown the capacity of antioxidant enzymes to prevent or reverse the toxic effect of chronic hyperglycemia in the cells. Ability of alloxan to oxidize or attack sulfhydryl groups has been established by many studies to be directly involved in the production of its toxic products (Comporti *et al*., 1993; El-Alfy *et al*., 2005 Macdonald Ighodaro *et al*., 2017). Inhibition of reaction pathway leading to the formation of these toxic products and their autoxidation may have offered a possible mechanism in protecting sulfhydryl groups from participating in continuous redox cycle reactions.

In the study, it can be concluded that combination of various bioactive components found in the aqueous and ethanol 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.

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