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Evaluation of the Antioxidant and Anti-diabetic Effect of *Mucuna puriens* Extract

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Authors' contributions

The work was undertaken with collaboration among all the authors. Author NNN designed the study, carried out the extraction and phytochemical analysis with author NUD and wrote the first draft. Authors AEE, RMU and CNO carried out the in-vivo experiments and analysis of the results. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the scientific basis for the anti-diabetic and antioxidant activity of *Mucuna pruriens* (Fabaceae) leaf ethanolic extract using alloxan-induced diabetic rats, DPPH and ABTS assay.
Place and Duration of Study: Department of Chemistry/Biochemistry Federal University Ndufu Alike, Ebonyi State, Nigeria between October 2013 and May 2014.
Methodology: The polyphenol content was determined using Folin-Ciocaltu method and their linear relationship with antioxidant activity was evaluated using linear regression analysis. The antioxidant activity was determined using 1, 1-Diphenyl, 2-picrylhydrazyl (DPPH) and (2, 2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical assay. The active ingredients in

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the ethanolic extract were isolated using HPLC method. Also the ant-diabetic activity was determined in vivo using alloxan-induced diabetic wister rats.
Results: Ethanol extract showed the highest phenolic content as well as highest antioxidant activity. A strong relationship was found between phenolic contents and antioxidant activity. The HPLC analysis indicates the presence of gallic acid, caffeic acid, p-coumaric acid, quercetin and (+)-catechin. The ethanolic extract at the concentration of 400 mg/kg significantly ($P<0.001$) increased the intracellular antioxidant enzymes and reduced the elavated serum lipids and showed more active than the reference drug (metformin).
Conclusion: Based on the obtained result, the antioxidant and anti-diabetic activity demonstrated by *Mucuna pruriens* leaf extracts provide good evidence to support the traditional use of this plant in treatment of diabetics.

Keywords: Mucuna pruriens; antioxidant; phenolic; anti-diabetic, alloxan-induced.

1. INTRODUCTION

Reactive oxygen species (ROS), such as superoxide radicals, hydroxyl (OH) radicals and peroxyl radicals, are natural by-products of the normal metabolism of oxygen in living organisms with vital roles in cell signalling [1]. However, environmental factors such as pollutants, tobacco smoke and UV radiation contribute in generation of free radicals [2]. The development of many pathophysiological conditions including diabetes, hypertension, atherosclerosis, cancer and the process of aging have been attributed to oxidative stress, which is an excessive production of reactive oxygen species (ROS) above the body's antioxidant capacity [3,4].

Literature reports have shown that diabetes mellitus is associated with oxidative stress, resulting to an increased production of reactive oxygen species (ROS), including superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) or reduction of antioxidant defence system [5,6]. Antioxidants such as ascorbic acid, glutathione, tocopherols and reactive oxygen scavenging enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) have all been reported to prevent the formation of ROS [7,8]. It is well established that the risk of cardiovascular disease due to atherosclerosis enhance with increasing concentration of total cholesterol and augmented levels of triglycerides in the plasma [9,10].

The evaluation of traditional plants treatments for diabetes has been recommended by World Health Organization (WHO), due to undesirable side effect or contraindications of synthetic drugs [11]. Medicinal plants have been identified and widely used worldwide to address a variety of health problems [12,13].

Herbal medicine has been the main source of medication in various part of Africa including Nigeria. *Mucuna pruriens* is a plant of the Fabaceae family, typically found in tropical regions and used for various purposes in traditional medicine in several countries.

In India and West Africa for example, it is used against snake bites [14,15]. It is also used as a uterine stimulant and aphrodisiac [16]. In Honduras, Central America, Africa and Guinea, this plant is also used as food [17]. In Nigeria, the leaf of this plant has been used by traditional healers in the treatment of diabetics. Although extensive studies have reported the antioxidant properties of *Mucuna pruriens*, the phenolic identification data are still insufficient and incomplete; in particular, quantitative data on phenolics in the species are still missing.

The objectives of this study were to: (1) compare and evaluate total antioxidant capacity of ethanolic, methanolic and acetonic extracts of *Mucuna prurines* L. leaves (2) identify and quantify major phenolic compounds in the active extract as antioxidant by HPLC; (3) determine the relationship between antioxidant activity and phenolic compounds of the extracts to confirm that phenolic constituents are responsible for antioxidant activity of the plants and (4) evaluate the anti-diabetic activity of the extracts in *Alloxan-induced diabetic Wistar rats*.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

1, 1-Diphenyl, 2-picrylhydrazyl (DPPH), Gallic acid, P-coumaric acid, vanillic acid, Caffeic acid, P-hydroxybenzoic acid, ferulic acid, (+)-catechin, quercetin were obtained from Sigma Aldrich

(Steinheim Germany), The ChemEnzyme kit (Japan) was used for the determination triglyceride (TG), total cholesterol (TC) and high-density lipoproteins (HDL). SOD and GPx were evaluated using appropriate kits from Randox (Antrim, UK)., All other chemicals and solvents were of the highest commercial grade from Merck (KGaA, Germany) or from Sigma (Steinheim Germany). Plant part used in this research was the leaf extract of *Mucuna pruriens*. The plant materials were obtained from Benin City and authenticated by Professor Ozekwe of the department of Botany, university of Benin and the voucher specimen No 37232 deposited at the herbarium of the University of Benin. All other chemicals and solvents were of the highest commercial grade purchased from Merck (KGaA, Germany) or from Sigma (Steinheim Germany).

2.2 Preparation of Plant Extracts

Leaves of *M. pruriens* were collected at Benin City on the month of September 2013 and authenticated by Professor Ozekwe of the department of Botany, university of Benin.

About 300 grams of dried and coarsely grounded leaves of the specimen were extracted with 300 ml each of Methanol, Ethanol, and Acetone in a Soxhlet apparatus for 36 hrs. After extraction, the solvent was filtered and then evaporated by Rotavapor (N-1200 Japan). The percentage yields based on the dried starting materials were 38%, 41% and 55%, for ehanolic, methanolic and acetoinc extracts, respectively. The powders were stored in the dark at 4°C until being used.

2.3 Determination of Total Phenolic Content

The total phenolic content of the crude extracts of each plant was determined by Folin-Ciocalteu method as described by Wolfe et al. [18] with slight modification. Briefly, 5 ml of Folin-Ciocalteu reagent (1:10 v/v distilled water) was mixed with 1.0 ml of each ethanol extract (1 mg/ml) and 4 ml (75 g/l) of Sodium carbonate. The resulting mixture was vortexed for 15 seconds and then allowed for colour development within interval of 30 min at 35°C. Shimadzu UV–VIS spectrophotometer (Unico Japan) was used to measure the absorbance at 765 nm. The total phenolic was calculated using equation obtained from a standard gallic acid calibration curve y = 6.2548 x 0.0925, R2 = 0.9962 and then expressed as mg of gallic acid equivalent per gram.

2.4 DPPH Radical Scavenging Activity Assay

The determination of DPPH assay was carried out according to Silva et al. [19] with little modification. A solution of DPPH (5 mL, 23.6 µg/mL in ethanol) was mixed with aliquot portion of the sample, and then incubated for 30 min. The absorbance of each sample was taken at 517 nm. Ascorbic acid (0.9, 1.9, 3.9, 4.9, 6.9 µg/mL) was used as positive reference. The estimation of percentage DPPH scavenged was determined from the equation: DDPH scavenging activity = 100 × (Ac – As)/Ac where Ac is the absorbance of the control and As is the absorbance of the sample. IC_{50} values calculated and indicate the concentration of the sample required to reduce the absorbance by 50% at 517 nm.

2.5 Trolox Equivalent Antioxidant Capacity Assay (TEAC)

The determination of ABTS free radical-scavenging activity of each sample was obtained according to the method described by RE et al. [20]. The persulfate oxidation of ABTS was used for the generation of free radical cation $ABTS^+$. Breifly, a mixture of potassium persulfate (2.45 mM) and ABTS (7.0 mM) was allowed to stand overnight at room temperature in the dark to develop radical cation $ABTS^+$. The resulting solution was diluted with ethanol and the absorbance obtained at 734 nm 12 hours prior to use. A solution of the radical cation $ABTS^+$ (5 mL), was mixed with aliquot portion of each sample and the decrease in absorbance at 734 nm was measured after 10 min. Trolox (1.1, 1.7, 2.3, 2.9, 3.5 µg/mL) was used as positive reference. IC_{50} values calculated and indicate the concentration of the sample required to decrease the absorbance at 517 nm by 50%. All experiments were performed in triplicate. The DPPH and TEAC data were expressed as IC_{50} (mg/mL).

2.6 High Performance Liquid Chromatography (HPLC) Analysis of the Extract

Chromatographic analyses were carried out using Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC (RSLC) systems (Thermo Fisher Scientific Inc., MA, USA). The separation

was carried out on a C18 Prevail column (150 · 2.1 mm, 2 lm), coupled to a rapid separation pump (LPG-3400RS), rapid separation diode array detector (DAD-3000RS) and Ultimate 3000RS auto sampler (WPS-3000). The acquisition data, peak integration, and calibrations were performed with Dionix Chromeleon software (Version 6.80 RS 10).

2.6.1 Chromatographic conditions

The method of Chuanphongpanich [21] was used to determine the phenolic composition of the ethanolic extract of *Mucuna pruriens* using HPLC. The mobile phase consisted of solvent A (acetonitrile), solvent B (acetic acid solution at pH 3.0), and solvent C (methanol). The system was run with the following gradient elution program: 0 min, 5%A/95%B; 10 min, 10%A /80%B /10%C; 20 min, 20%A /60%B /20%C and 30min, 100%A. The initial condition for equilibration of the column was carried out on a 5 min post run. The analysis was carried out at constant flow rate at 1 ml/min and the injection volume was 20 µl. For UV detection, the optimization of the wavelength program was carried out to monitor phenolic compounds at their respective maximum absorbance wavelengths as follows: λ 280 nm held for 18.0 min, changed to λ 320 nm and held for 6 min, and finally changed to λ 380 nm and held for the rest of the analysis and the diode array detector was set at an acquisition range from 200 nm to 700 nm. The detection and quantification of Gallic acid, (+)-Catechin, Vanillic acid, and Caffeic acid was done at 280 nm, of P-coumaric acid, and rutin hydrate at 320 nm, and of Quercetin, at 380 nm, respectively.

2.6.2 Standard and sample preparation

An approximate weight of 0.005 g of the analyte in methanol was weighed into the 50 ml volumetric flask to prepare the standard solution (100µg/ml) of each phenolic compound. The mixed standard solution was prepared by dilution the mixed stock standard solutions in methanol to give a concentration of 10 µg/ml for each polyphenols. All standard solutions were stored in the dark at 5°C and were stable for at least one month.

Serial dilution of the stock standards with methanol was used to prepare the calibration curves to yield 1.25 - 20 µg/ml for Gallic acid, (+)-Catechin, Vanillic acid, P-coumaric acid, rutin hydrate, EA; 0.5 - 8.0 µg/ml for Caffeic acid, and 0.375 - 6.0 µg/ml for Quercetin. The calibration curves were constructed from chromatograms as peak area vs. concentration of standard.

A solution of ethanolic extract of *Mucuna pruriens* at a concentration of 5 mg/ml was prepared in ethanol by vortex mixing (Branson, USA) for 30 min. The samples were stored in the dark at low temperature (5°C). Spiking the sample solution with phenolic standards was done for additional identification of individual polyphenols.

2.6.3 Induction of Alloxan-induced diabetic Wistar rats

Male Wistar rats (*Rattus norvegicus allivias*), weighing 190-230 g were obtained from Sonad Animal laboratory, Benin. Animals were housed six per standard rat cage, in a room with a 12:12 h light/dark cycle with controlled temperature of (27 ± 1°C). There were six rats per group in each experiment. The procedures were carried out according to institutional guidelines for animal care and use. The induction of Hyperglycemia was through intravenous injection of alloxan. The animals were kept under observation. The animals were tested for glucosuria using Diastex strips After 48 hrs [22]. 14 days after the alloxan injection, rats with fasting blood glucose levels greater than 200mg/dL were considered diabetic. The experiment was carried out according to a previously described method [22,23].

2.6.4 Experimental design

The rats were divided into the six groups, each with six animals. Group I (NC): Normal rats treated with vehicle (saline) alone; Group II (AC): Alloxan-induced diabetic rats treated with saline as vehicle alone; Group III (MPEE+100): Diabetic rats treated with *Mucuna pruriens ethanolic extract* at the dose of 100 mg kg−1 body weight (p.o); Group IV (MPEE+200) : Diabetic rats treated with *Mucuna pruriens ethanol extract* at the dose of 200 mg kg−1 body weight (p.o); Group V (MPEE+400): Diabetic rats treated with *Mucuna pruriens ethanol extract* at dose of 400 mg Kg-1 body weight (p.o); Group VI (Met+150): Diabetic rats treated with metformin, 150 mg Kg-1 body weight (p.o).

2.6.5 Estimation of antioxidant enzymes activities

The collection of blood of the treated rats was carried out using EDTA coated tubes. The evaluation of the activities of SOD (EC: 1.15.1.1)

and GP_X (EC: 1.11.1.9) were obtained using commercial kits. CAT (EC: 1.11.1.6). The determination of the activity was carried out according to Aebi [24] and expressed as K or U/gHb. The total hemoglobin of samples was measured by a hemoglobin Reagent kit.

2.6.6 Estimation of blood lipids

The blood of anesthetized pre-treated rats were collected and allowed to clot. Then, the clotted blood was centrifuged at 2500 rpm for 5 min. Triglycerides, total cholesterol and high-density lipoproteins were estimated using commercial kits. Low-density lipoproteins and very low-density lipoproteins were calculated by formula [25] using LDL = TC – HDL – (TG/5), where TC= total cholesterol, HDL= high density lipoprotein and TG= triglyceride.

2.7 Statistical Analysis

All data are presented as means ± S.D. Data analysis were carried out using one-way analysis of variance (ANOVA) followed by Duncan's test to analyze the difference. Comparisons between groups and between times points were made by Differences were considered significant when p-values were less than 0.05. All statistical analyses were performed using SPSS (SPSS Inc, Chicago, USA).

3. RESULTS AND DISCUSSION

The evaluation of the antioxidant properties of medicinal plants, especially those traditionally used in folk medicine is of particular interest. In assessing the antioxidant properties of medicinal plants, it is recommended that more than one extraction system is used. With this in mind, we chose to extract the raw materials with solvent of varying polarity. The result of the total phenolic contents and antioxidant activity of the plant extracts are shown in Table 1. Ethanolic showed the highest extractive value of phenolic content (155.89mg.GAE/g) and antioxidant activity in *Mucuna pruriens.* Generally, total phenolics correlate with redox and antioxidant capacities as measured by TEAC or DPPH methods [26].

Various literature reports indicate a linear relationship between total phenolic contents and antioxidant activity [27-28]. A linear regression analysis was used to evaluate a direct correlation between the three methods in the plant species. This study showed that there is strong correlations between total phenolic content and antioxidant activity (r^2 = 0.937) of *Mucuna pruriens*.

Phenolic acid and flavonoids are well known to possess antioxidant activity. One major phenolic compound widely occurring in the plant kingdom especially in fruits and vegetable is phenolic acid. Selected phenolics in the extracts was separated and identified by HPLC method. Extraction was performed with aqueous methanol due to its protective role, which prevents phenolic compounds from being oxidized by enzymes, such as phenoloxidase [29]. Column employed in separating phenolic compounds is exclusively reversed-phase. Since polyphenols absorb in the ultraviolet region, the identification of each compound was based on a combination of retention time and spectral matching. The amount of phenolic compounds detected in the extracts is presented in Table 2 and the results are expressed in mg/100 g dry sample.

Plants with phenolic constituent are very important because of their free radical scavenging ability resulting from the presence of hydroxyl group. The HPLC analysis indicates the presence of gallic acid, caffeic acid, p-coumaric acid, quercetin and (+)-catechin in *Mucuna puriens* extract. Generally, antioxidant property of flavonoids depends on the structure and pattern of substitution of the hydroxyl group [29]. The presence of 3' 4'-orthodihydroxy configuration in

Table 1. Total polyphenol contents, DPPH radical scavenging activity and Trolox equivalent antioxidant capacity (TEAC) of *Mucuna puriens* extracts

Specie	Sample extract	Total polyphenol (mg·GAE/g)	DPPH (IC50—mg/mL)	TEAC (IC50—mg/mL)
M. pruriens	EE	155.89 ± 0.37	15.721 ± 0.81	6.009 ± 1.08
	ME	72.11 ± 0.33	69.943 ± 1.01	27.032 ± 1.12
	AE	153.13 ± 0.14	13.062 ± 0.13	6.028 ± 1.01
	Positive reference	-	8.730 ± 0.07	6.110 ± 0.13

Data represented as Mean ± SD (n = 3). Ethanolic extract (EE), Methanolic extract (ME), Acetonic extract (AE), Positive reference DPPH: ascorbic acid. Positive reference TEAC: Trolox

Table 2. Content of isolated phenolic compounds in *Mucuna pruriens* ethanolic extract

Standard	Content, mg/100g dry sample[a]
Gallic acid	2.9± 0.07
Caffeic acid	1.6 ± 0.04
P-coumaric acid	1.9 ± 0.03
Vanillic acid	ND
Quercetin	1.5 ± 0.21
(+)-Catechin hydrated	1.1 ± 0.05
Rutin hydrate	ND

[a] *Each value is the mean (mg/100 g dry sample) of three replications; ±, Standard deviation; ND, Not detected.*

B ring and 4-carbonyl group in C ring is an essential requirement for effective radical scavenging activity. Also, a catechol-like in ring C with the presence of 3-OH group or 3- and 5-OH group is beneficial for antioxidant activity of flavonoids. Electron delocalization from ring B increases radical scavenging activity, as a result of presence of C2-C3 double configuration with a 4-keto arrangement [30].

Quercetin possesses a catechol-like structure in ring B with a 2, 3-double bond in conjunction with a 4-carbonyl group in ring C, which allows for delocalization of phenoxy radical electron to the flavonoids nucleus.

It has been reported that 3, 4-position of dihydroxylation on the phenolic ring in caffeic acid showed increased antioxidant activity [31].

Caffeic acid is expected to possess good antioxidant activity due to additional conjugation in the propenoic side chain, which might increase the electron delocalization by resonance, between aromatic ring and propenoic acid [30].

The antioxidant and cardioprotective role of catechin hydrate and rutin have been attributed to their ability to reduce oxidative stress, lipid peroxidation, free radical generation and low density lipoprotein (LDL) cholesterol oxidation [32]. It is therefore anticipated that the phenolic acids, flavones and flavonols isolated from this plant extract (Fig. 1) might contribute immensely for their antioxidant activity.

Alloxan is a hydrophilic and chemically unstable pyrimidine derivative; with its toxic nature it can cause a massive reduction in insulin release by the destruction of the pancreatic β cells, inducing hyperglycaemia. In diabetes mellitus, hyperglycemia can inactivate antioxidant enzymes like SOD, CAT and GPX through glycatination thereby resulting in induced oxidative stress which in turn causes lipid peroxidation. In Alloxan-induced diabetes, reduction in antioxidant enzymes levels and enhanced lipids peroxidation has been well documented [33,34]. One major role of SOD in enzymatic antioxidant defense system is to scavenge the superoxide radicals by converting them to H_2O_2 and molecular oxygen.

The observed decrease in SOD activity in alloxan-induced rats could result from inactivation by H_2O_2 or by glycosylation of the enzyme, which have been reported to occur in diabetes. CAT and GPX are involved in the elimination of H_2O_2 [35].

From our findings, the extract of *Mucuna pruriens* increased the activity of intracellular antioxidant enzyme in dose dependent manner (Table 3). When compared to the alloxan-induced diabetic rats, the activity of SOD, CAT and GPx significantly increased (51%, $P<.0001$), (40%, $P<.0.001$) and (58%, $P<0.001$) respectively at the concentration of 400 mg/kg of the extract. The extract showed higher activity when compared with the standard drug (Metformin).

In type 2 diabetes, dyslipidemia is characterized through increased levels of serum triglycerides (TG) and reduced levels of serum high density lipoproteins (HDL), while total cholesterol (TC) and low density lipoproteins (LDL) may be either normal or marginally elevated [35]. This elevation of serum lipids has been attributed to the abnormalities in pancreatic beta cell and thus affects the insulin secretion. In this study, the diabetic rats (induced by alloxan) showed a persistent rise in blood serum lipids levels after 14 days with the characteristic features of diabetes mellitus. The administration of ethanolic leaf extract of *Mucuna pruriens* at all the three doses significantly reduced the elevated TG, TC, LDL, VLDL and raised the level of HDL in a dose independent manner (Table 4). This could be attributed to the presence of antilipidemic compounds that may inhibit or activate some

enzymes involved in triacylglycerol metabolism. We anticipate that the mechanism by which ethanolic extract of *Mucuna pruriens* exhibited its antihyperglycemic action may be by increasing the secretion of insulin from pancreatic β cells or its release from the bound form. However, further work to ascertain the exert mechanism of action is required.

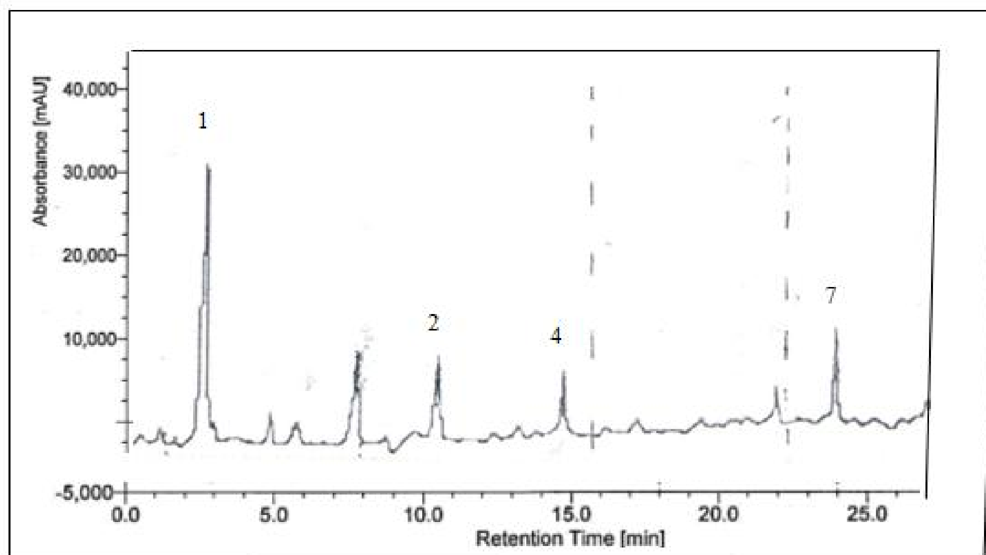

Fig. 1. HPLC chromatogram of *Mucuna pruriens ethanolic* leaves extract. Peaks: 1, gallic acid; 2, (+)-catechin; 4, caffeic acid; 7, quercetin

Table 3. Effect of ethanolic extracts of *Mucuna pruriens* on intracellular antioxidant enzymes in alloxan-induced Rats

Group	Blood intracellular	Antioxidant	Enzymes
	SOD (u /g Hb)	CAT (u/mg Hb)	GPx (K/g Hb)
Normal control	2529.0 ± 0.06	48.2 ± 0.21	2.3 ± 0.13
Alloxan-induced	1533.4 ± 0.15	30.8 ± 0.11	1.2 ± 0.22
Std Metformin	1930.1 ± 0.26	39.9 ± 0.18	1.8 ± 0.31
MPEE (100 mg/kg)	1622.0 ± 0.29	32.2 ± 0.48	1.1 ± 0.17
MPEE (200 mg/kg)	1907.3 ± 0.15	38.5 ± 0.61	1.5 ± 0.33
MPEE (400 mg/kg)	2318.5 ± 0.54	43.1 ± 0.83	1.9 ± 0.22

MPEE: Mucuna pruriens ethanolic extract, rats used in each group = 6, SOD: Superoxide dismutase, CAT: catalase, GPx: glutathione peroxidase.

Table 4. Effect of ethanolic extract of *Mucuna pruriens* on serum lipid profile in alloxan-induced diabetic Rats

Group	Serum lipids (mg/dl)				
	TG	TC	HDL	LDL	VLDL
Normal control	79.1 ± 2.06	73.2 ± 2.21	31.7 ± 1.13	13.3 ± 3.13	7.8 ± 4.13
Alloxan-induced	139.8 ± 1.03	109.5 ± 3.11	19.3 ± 1.22	28.4 ± 1.19	15.4 ± 3.23
Std Metformin	97.2 ± 4.41	89.6 ± 2.18	24.9 ± 3.17	19.2 ± 0.43	10.6 ± 2.29
MPEE (100 mg/kg)	126.7 ± 3.29	101.2 ± 4.48	20.3 ± 2.25	23.6 ± 0.22	12.1 ± 1.17
MPEE (200 mg/kg)	113.4 ± 2.15	93.5 ± 2.61	23.5 ± 1.33	18.0 ± 0.31	9.8 ± 2.23
MPEE (400 mg/kg)	81.2 ± 1.54	85.2 ± 1.53	28.1 ± 4.22	15.3 ± 0.15	8.1 ± 3.25

MPEE: Mucuna pruriens ethanic extract, rats used in each group = 6, TG: serum triglycerides, TC: total cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein.

4. CONCLUSION

The ethanolic leaf extract of *Mucuna pruriens*, used widely in Nigeria traditional medicine in the treatment of diabetics, exhibited good antioxidant activity when compared to standard ascorbic acid and trolox and also showed good anti-diabetic activity with reference to metformin drug. The experimental data from this study may support the use of *Mucuna puriens* leaf extract in the treatment of diabetes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Animal House and Use Committee, Faculty of Medicine and Health Sciences, Ebonyi State University (ethical approval number: EB/FMHS/BC-UUH/00771). The rats were handled in accordance with guidelines for care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Aruoma OI, Cuppette SL. Antioxidant methodology; In *in vivo* and in vitro Concept; AOCS Press: Champaign, IL, USA. 1997;142–169.
2. Cavas L, Yurdakoc K. An investigation on the antioxidant status of the invasive alga *Caulerpa racemosa* var. cylindracea (Sonder) Verlaque; Huisman, et Boudoresque (Caulerpales, Chlorophyta). J. Exp. Mar. Biol. Ecol. 2005;325:189–200.
3. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44-84.
4. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002; 82:47-95.
5. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed. Pharmacother. 2005;59:365-73.
6. Vincent AM, Russell JW, Low P and Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. Endocr. Rev. 2004;25:612-28.
7. Zhang XF, Tan BK. Antihyperglycaemic and anti-oxidant properties of *Andrographis paniculata* in normal and diabetic rats. Clin. Exp. Pharmacol. Physiol. 2000;27:358-63.
8. Lin YF, Tsai HL, Lee YC, Chang SJ. Maternal vitamin E supplementation affects the antioxidant capability and oxidative status of hatching chicks. J. Nutr. 2005;135:2457-61.
9. Davy G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, Patrono C. In-vivo formation of 8-iso-prostaglandin F2a and platelet activation in diabetes mellitus: Effects of improved metabolic control and vitamin E supplementation. Circulation. 1999;99:224-9.
10. Kwiterovich Jr PO. Detection and treatment of elevated blood lipids and other risk factors for coronary artery disease in youth. Ann. NY Acad. Sci. 1995;748:343-332.
11. Day C. Traditional plant treatments for diabetes mellitus: Pharmaceutical foods. Br. J. Nutr. 1998;80:5-6.
12. Cowan MM. Plants products as anti-microbial agents. Clin. Microbiol. Rev. 1999;12:564-582.
13. Goh SH, Chuah CH, Mok JSL, Soepadmo E. Malaysian medicinal plants for the treatment of cardiovascular diseases. Pelanduk Publication, Malaysia. 1995;162.
14. Siddhuraju P, Vijayakumari K, Janardhanan K. Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC). J. Agric. Food Chem. 1996;44(9):2636-2641.
15. Chikagwa-Malunga SK, Adesogan AT, Sollenberger LE, Badinga LK, Szabo NJ, Littell RC. Nutritional characterization of *Mucuna pruriens.* Effect of maturity on the nutritional quality of botanical fractions and the whole plant. Anim. Feed Sci. Technol. 2009;148(1):34-50.
16. Amin KMY, Khan MN, Zillur-rehman S, Khan NA. Sexual function improving effect of *Mucuna pruriens* in sexually normal male rats. Fitoterapia. 1996;67(1):53-56.

17. Diallo OK, Kante S, Myhrman R, Soumah M, Cissé NY, Berhe T. Increasing farmer adoption of *Mucuna pruriens* as human food and animal feed in the republic of Guinea. Proceedings. Tegucigalpa, 2000. Tegucigalpa, International Center for Information on Cover Crops (CIDICCO). 2002;60-72.
18. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J. Agric Food Chem. 2003;51(3):609-614.
19. Silva, TMS, Camara CA, Lins, ACS, Barbosa-Filho, JM, Silva EMS, Freitas BM, Santos FAR. Chemical composition and free radical scavenging activity of pollen loads from stingless bee *Melipona subnitida* Ducke. J. Food Compos. Analys. 2006;19:507–511.
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical. Bio.Med. 1999;26:1231–1237.
21. Chuanphongpanich S, Phanichphant S. Method development and determination of phenolic compounds in broccoli seeds samples. Chiang Mai J Sci 2006;33:103-7.
22. M. Eidi, A. Eidi, and H. Zamanizadeh, Effect of *Salvia officinalis* L. leaves on serum glucose and insulin in healthy andstreptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 2005; 100(3):310–313.
23. Kameswara Rao B, Kesavulu MM, Giri R, Appa Rao C. Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook. fruit powder in alloxan-diabetic rats. J. Ethnopharmacol. 1999;67: 103-109.
24. Aebi H. Catalase in method. In: Vergmeyer HU. (ed.) Enzymatic Analysis. Academic Press, New York. 1974;673.
25. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of LDL C in plasma without use of the preparative ultracentrifuge. Clin. Chem. 1972;18:449-502.
26. Tabart, J, Kevers C, Pincemail J, Defraigne JO, Dommes J. Antioxidant capacity of black currant varies with organ, season, and cultivar. J. Agr. Food Chem. 2006;54:6271–6276.
27. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 2006;97:654–660.
28. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 2003;81:321–326.
29. Harborne JB. Phytochemical methods. A Guide to modern techniques of plant analysis. Chapman and Hall: London, UK. 1998;40–106.
30. Bors W, Heller W, Michael C, Saran M. Radical chemistry of flavonoids antioxidants. Advances in Experimental Medicine and Biology. 1990;264:165–170.
31. Kim, DO, Lee CY. Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. Critical Review Food Science and Nutrition. 2004;44(4): 253–273.
32. Thielecke F, Boschmann M. The potential role of green tea catechins in the prevention of the metabolic syndrome - a review. Phytochemistry. 2009;70(1):11-24.
33. Oyedemi S, Bradley G, Afolayan A. Antidiabetic activities of aqueous stem bark extract of Strychnoshenningsii Gilg in Streptozotocin-nicotinamide Type 2 diabetic rats. Iranian J. Pharm. Res. 2011;10:221-228.
34. Mohammadi S, Montasser Kouhsari S, Monavar Feshani A. Antidiabetic properties of the ethanolic extract of *Rhus coriaria* fruits in rats. DARU. 2010;18:270-275.
35. Lin YF, Tsai HL, Lee YC, Chang SJ. Maternal vitamin E supplementation affects the antioxidant capability and oxidative status of hatching chicks. J. Nutr. 2005;135:24.

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