

ANTIHYPERGLYCAEMIC AND ANTIOXIDANT EFFECTS OF *ADENIA LOBATA* ENGL.
(PASSIFLORACEAE) IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

The antihyperglycaemic and antioxidant activities of a Ghanaian medicinal plant namely *Adenia lobata* Engl (Passifloraceae), used to treat diabetes mellitus in traditional medicine, was investigated. The dried stem powder of *A. lobata* was successively extracted by Soxhlet with petroleum ether and 70% ethanol to obtain the crude petroleum ether (PEAL: yield = 1.1w/w %) and ethanol (EEAL: yield = 5.4 w/w %) extracts. The extracts were assessed for their antihyperglycaemic and antioxidant activities. The antihyperglycaemic activity of PEAL and EEAL were determined in streptozotocin-induced diabetic rats (70 mg/kg body weight). Five groups of diabetic rats were given 150, 300 and 600 mg/kg body weight of PEAL and EEAL orally once daily for 20 days. Glibenclamide (5 mg/kg body weight) was used as positive control while distilled water (5 ml) acted as the normal diabetic control. The blood glucose levels were monitored initially for 6 hours and subsequently over 24 days. Both extracts exhibited statistically significant ($p < 0.001$) antihyperglycaemic activity throughout the study period, with EEAL showing the greatest activity. The antioxidant properties of the petroleum ether and ethanol extracts of *A. lobata* (PEAL and EEAL) were evaluated using five assays; total phenolic content, total antioxidant capacity, reducing power, DPPH scavenging effect and lipid peroxidation activity. In all these assays, the antioxidant properties increased with increasing concentration of the extracts.

Key words: Antihyperglycaemic, antioxidant, diabetes, *Adenia lobata*

Introduction

Diabetes mellitus is a chronic metabolic disorder in which the pancreas produces little or no insulin to meet the body's needs or the body cannot effectively use the insulin that is produced (Herfindal and Gourley, 1996). This results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. The development of this disease is associated with an increased presence of free radicals in the body. The production of these radicals leads to oxidative stress on the body and results in cellular damage (Kuhn, 2003; Johansen *et al.*, 2005). This damage may lead to many diseases such as diabetes, hypertension and stroke (Chen *et al.*, 2002). Antioxidants are protective agents that inactivate the reactive oxygen species (ROS) which caused cell damage (Tiwari and Rao, 2002). Hypoglycaemic medicinal plants with antioxidant agents may be beneficial in diabetes because of their ability to mop up ROS (Mendel, 1997). In Ghana, the use of medicinal plants in various ethno-botanical literatures has been reported (Irvine, 1961; Mshana *et al.*, 2000; Abbiw, 1990), while several reputable traditional health practitioners continue to make claims for medicinal plants in the management of diabetes. It is in this direction that the stem of *A. lobata*, a woody climber, which grows in the West African rainforests and used in treatment of diabetes (Irvine, 1961) was investigated.

Materials and Methods

Plant material

The stem of *A. lobata* was collected from Bobiri forest in Ghana on December 2009 by Mr Osafo Asare, a herbalist. Authentication was done at the Department of Botany, University of Ghana, Legon by a botanist, Mr. Amponsah. A voucher specimen with number (KNUST/HM1/2010/S-005 for *A. lobata*) is deposited at the Faculty of Pharmacy and Pharmaceutical Science herbarium.

Method of extraction

The stem of *A. lobata* was air-dried for ten days at room temperature and milled into a coarse powder. The powder (650.0 g) was Soxhlet extracted sequentially using petroleum ether (PEAL) and 70% ethanol (EEAL). The extracts were concentrated to viscous liquids under reduced pressure using a rotary evaporator. The viscous liquids were further evaporated to solvent-free, semi-solid masses over water bath (yield; PEAL: 1.1 % w/w, EEAL: 5.4 % w/w) and kept in a desiccator until needed for use. The preliminary phytochemical evaluation of part of powdered stem of *A. lobata* using standard methods as described by Harbone, 1998. Revealed the presence of reduced sugars, steroids and phenolic compounds. Large scale Soxhlet extraction by 70% v/v ethanol was done for 3 kg of the powder.

Animals

Adult male sprague-dowley rats (280-295 g) were housed at a standard condition of room temperature and supplied with standard pellet food with tap water *ad libitum* in animal house at the Department of Pharmacology, University of Ghana, Legon. The rats were obtained from the Centre for Scientific Research into Plant Medicine at Mampong-Akwapim, Ghana. All rats were treated in accordance with the National Institute of Health Guidelines for the care and use of laboratory animals (NIH, Department of Health and Human Services publication Number 85, revised 1985).

The research protocol was approved by the College of Health Sciences Ethics Committee.

Preliminary phytochemical screening of ethanol extract of *A. lobata*

The preliminary phytochemical screening of ethanol extract of the stem of *A. lobata* was carried out using standard procedures described by Harbone (1998).

Assessment of Antioxidant effects

Total Phenolic Content

The Folin-Ciocalteu's reagent with tannic acid as standard as described by Slinkard and Singleton (1977) was used to determine the total phenolic content of the extracts. Aliquots (1 ml) of tannic acid and each extract (0.50, 0.75, 1.00 and 1.50 mg/ml) were added to 1 ml distilled water and 1ml Folin-Ciocalteu's reagent in a test tube. The samples were allowed to stand after mixing for 5 minutes at 25°C in an incubator. A 2% sodium bicarbonate solution (1 ml) was added and the mixture incubated for 2 hours at 25°C. The test tubes were centrifuged at 3000 rpm for 10 minutes and the absorbances of supernatants determined at 760 nm. Distilled water (1 ml) was added to 1 ml Folin-Ciocalteu's reagent and used as blank after going through the same process. Three replicates were prepared for each concentration of the extracts and tannic acid. The total phenolic acid was expressed as Tannic acid equivalent (TAE) per weight of the extract (mg/g).

Total antioxidant capacity

The total antioxidant capacity was determined using the method described by Prieto *et al.* (1999). Different concentrations of extracts (0.50, 0.75, 1.00, 1.50 mg/ml) and ascorbic acid as standard were added 3 ml of solution (0.6 M H₂SO₄, 28 mM Na₂HPO₄ and 4 mM ammonium molybdate). The samples were incubated for 90 minutes at 25°C after shaking, and were centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was then determined at 695 nm. Distilled water (1 ml) was used as blank processed in the same way. The total antioxidant capacity was expressed as ascorbic acid equivalent (AAE) per dry weight of extract (mg/g).

Reducing Power

This assay was based on the method described by Oyaizu (1986). using *N*-propyl gallate as standard. One millilitre of extracts (0.50, 0.75, 1.00, 1.50 mg/ml) or *N*-propyl gallate was added to 1ml of water, mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated for 20 minutes at 50°C, trichloroacetic acid (2.5 ml) was added and samples centrifuged at 3000 rpm for 10 minutes. The absorbance of the mixture was measured at 700 nm after 2.5 ml of the supernatant has been mixed with 2.5 ml distilled water and 0.5 ml of 0.1% FeCl₃. The blank (1 ml distilled water, 2.5 ml phosphate buffer and 2.5 potassium ferricyanide) in a test tube was processed in the same pattern. The reducing power was expressed as *N*-propyl gallate equivalent (NPGE) per dry weight of extract (mg/g).

Free radical scavenging activity

The method described by Blois (1958). Was used to determine the free radical scavenging activity of the extracts. One millilitre of methanolic DPPH solution (20 mg/l) was added firstly to 3 ml of extract solution, then to 3ml of pure methanol (blank). The mixtures were incubated for 30 minutes and the various absorbances were measured at 517 nm. The reference drug was *N*-propyl gallate (1-30 mg/ml). The percentage inhibition of DPPH by the samples was calculated according to the formula:

$$\% \text{ inhibition} = \frac{(A - A_s)}{A} \times 100$$

where A is absorbance of control;
 A_s the absorbance of sample.

The IC_{50} values (the concentration in mg/ml at 50% inhibition) were determined from the curves between percentage inhibition and logarithmic sample concentration.

Linoleic acid autoxidation

This assay is based on the method according to Mitsuda *et al.* (1996). The extracts (0.50-1.50 mg/ml) dissolved in absolute alcohol were compared directly with *N*-propyl gallate dissolved in absolute alcohol, as the reference antioxidant. Equal volumes (2 ml) of the extracts and 2.5% linoleic acid in absolute ethanol, 4 ml of 0.05 M phosphate buffer (pH-7) and 1.9 ml of distilled water were put into test tube with screw cap and incubated in the dark at 40°C for 7 days. Two millilitres of the sample solutions were added to 1 ml of 20% aqueous trichloroacetic acid solution and 1 ml of 0.6% aqueous thiobarbituric acid solution. The samples were placed in boiling water bath for 10 minutes, allowed to cool to room temperature, and then centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatants was measured at 490 nm. The % inhibition of linoleic acid autoxidation was calculated as follows:

$$\% \text{ Inhibition} = \frac{(C_o - C_i) - (D - D_i)}{(C_o - C_i)} \times 100$$

where

C_o (Full reaction mixture) is the degree of lipid peroxidation in the absence of antioxidant

C_i is the underlying lipid peroxidation before the initiation of lipid peroxidation

D is any absorbance produced by the extract

D_i is the absorbance produced by the extract alone.

Assessment of antihyperglycaemic effect of STZ-induced diabetic rats

The bioassay model employed was according to the description of Nagarajan *et al.* (2005) with slight modification.

Induction of diabetes in rats

Streptozotocin (0.588 g) was dissolved in freshly prepared 0.1M citrate buffer of pH-4.5. The normal blood glucose level of the rats was taken and was found to be in the range of (3.8-4.9 mmol/l). Rats were injected intraperitoneally with a single dose of 70 mg/kg body weight streptozotocin after 12 hours fast. After injection, rats were given free access to feed and water. After a rest period of 48 hours, diabetes was confirmed by determining the fasting blood glucose levels. Rats with blood glucose level above 10.0 mmol/l were selected for the experiment.

Effect of extract on diabetic rats

The diabetic rats were randomly divided into five groups of five animals each: Groups A, B, C, D and E. The petroleum ether, PEAL and 70% v/v ethanol, EEAL were suspended in 2% tragacanth solution. Groups A, B and C were given oral doses of 150, 300 and 600 mg/kg body weight of PEAL respectively. Group D was given an oral dose of 5 mg/kg body weight of glibenclamide, a standard anti hyperglycaemic agent, as positive control, and Group E was given 5 ml/kg body weight distilled water as normal control. The experiment was repeated using EEAL with new sets of animals.

Blood glucose level measurement

Blood glucose levels were initially monitored hourly over six hours starting at 0 hour after drug administration to see the immediate effects of both extracts on the diabetic rats. The glucose level was subsequently monitored on a three-day interval basis over the next 24 days for *A. lobata* extracts. Blood samples were collected from tail vein using Accu-Chek® glucometer (Roche Diagnostics GmbH, Mannheim Germany). Throughout the study period, rats received standard diet and water every morning.

Statistical analysis

All the data provided in this study represents means \pm S.E.M. The results were analysed by one-way ANOVA followed by Bonferroni's multiple comparison test to establish significance ($p < 0.001$) between the treated and the control groups.

Results

Antihyperglycaemic effect of extracts

The blood glucose levels of the untreated diabetic rats (diabetic control) after 6 hours increased by 28.4 % (Table 1).

The blood glucose levels of diabetic rats treated with PEAL at doses of 150, 300 and 600 mg/kg body weight decreased by 19.9, 32.2 and 47.1 % respectively after the 6th hour.

Table 1: Effect of PEAL in streptozotocin-induced diabetic rats after 6 hours

TREATMENT AND DOSE	BLOOD GLUCOSE LEVEL (mmol/l)						
	TIME (hrs)						
	0	1	2	3	4	5	6
PEAL (150 mg/kg)	25.6±2.0	24.3±2.2	23.7±2.2	23.1±2.2	21.9±2.0	21.0±1.9	20.5±1.8 (19.9)
PEAL (300 mg/kg)	24.5±1.1	21.0±0.7	19.9±0.9	18.9±1.0	18.3±0.9	17.4±0.9	16.6±0.7 (32.2)
PEAL (600 mg/kg)	27.4±2.4	19.9±1.0	19.0±0.9	17.2±1.7	16.0±1.5	15.4±1.4	14.5±1.2 (47.1)
GB (5 mg/kg)	23.2±3.0	18.7±2.4	15.9±2.1	13.3±2.2	12.7±2.1	12.1±2.0	10.5±1.5 (54.7)
Control (5 ml/kg)	24.4±0.9	25.6±1.4	25.3±1.7	26.7±0.8	27.3±1.1	28.0±0.9	28.4±1.0 (-28.4)

All values are expressed as mean ± S.E.M. (N = 5); Number in the parentheses denotes percentage reduction (blood glucose level) after 6 hours; PEAL = petroleum ether extract of the stem of *A. lobata*; GB = Glibenclamide; PEAL treated groups were compared with control group ($p < 0.001$)

Table 2: Effect of PEAL in streptozotocin-induced diabetic rats after 24 days

TIME (days)	TREATMENT AND DOSE				
	PEAL (150 mg/kg)	PEAL (300 mg/kg)	PEAL (600 mg/kg)	GB (5 mg/kg)	Control (5 ml/kg)
	Blood	Glucose	Level	(mmol/l)	
0.0	25.6±2.0	24.5±1.3	27.4±2.4	23.2±3.0	24.4±0.9
3.0	19.5±1.9	16.1±0.5	16.2±1.1	14.7±0.2	31.8±0.4
6.0	17.7±1.7	12.9±0.6	12.0±0.2	12.5±0.4	32.0±0.4
9.0	15.7±1.9	11.8±0.4	11.4±0.2	10.4±0.3	32.3±0.3
12.0	14.6±1.9	10.4±0.4	10.1±0.3	9.7±0.3	32.2±0.3
150.0	14.0±1.7	9.2±0.4	8.8±0.3	7.6±0.2	32.4±0.4
18.0	13.4±1.8	8.4±0.4	8.0±0.3	7.5±0.2	32.1±0.3
21.0	12.6±1.8	7.6±0.3	7.0±0.2	6.1±0.2	32.3±0.3
24.0	12.0±1.8	6.9±0.3	6.2±0.2	4.7±0.4	32.7±0.2
	(53.1)	(71.8)	(77.4)	(79.7)	(-34.0)

All values are expressed as mean ± S.E.M. (N=5); Number in the parentheses denotes percentage reduction (blood glucose level) after 24 days; PEAL = petroleum ether extract of the stem bark of *A. lobata*; GB = Glibenclamide; PEAL treated groups were compared with control group ($p < 0.001$).

Those treated with EEAL at doses of 150, 300 and 600 mg/kg had their blood glucose levels lowered by 36.7, 41.1 and 51.7 % respectively within the same period (Table 2). Glibenclamide, the standard antidiabetic drug (5 mg/kg) lowered the blood glucose levels by 54.7 %. The extracts at the selected doses and glibenclamide showed significant antihyperglycaemic activities ($p < 0.001$) as compared to the diabetic control group 6 hours after treatment. On the 24th day, untreated diabetic (diabetic control) rats had 34.0 % increase in blood sugar level. PEAL administered at 150, 300, and 600 mg/kg body weight decreased the blood glucose levels by 53.1, 71.8 and 77.4 respectively (Table 2).

Table 3: Effect of EEAL in streptozotocin-induced diabetic rats after 6 hours

TREATMENT AND DOSE	BLOOD GLUCOSE LEVEL (mmol/l)						
	TIME (hours)						
	0	1	2	3	4	5	6
EEAL (150 mg/kg)	25.5±1.5	21.4±1.0	19.6±1.4	18.2±1.0	16.9±1.0	16.4±1.0	15.9±1.1 (36.7)
EEAL (300 mg/kg)	27.5±1.2	22.9±1.1	20.6±0.8	19.4±1.2	18.9±1.3	17.6±0.7	16.2±0.5 (41.1)
EEAL (600 mg/kg)	30.2±0.7	21.5±1.9	19.5±1.4	17.8±0.9	16.8±0.7	16.2±0.7	14.6±0.6 (51.7)
GB (5 mg/kg)	23.2±3.0	18.7±2.4	15.9±2.1	13.3±2.2	12.7±2.1	12.1±2.0	10.5±1.5 (54.7)
Control (5 ml/kg)	24.4±0.9	25.6±1.4	25.3±1.7	26.7±0.8	27.3±1.1	28.0±0.9	28.4±1.0 (-16.4)

All values are expressed as mean ± S.E.M. (N = 5); Number in the parentheses denotes percentage reduction (blood glucose level) after 6 hours; EEAL = 70% Ethanol extract of the stem bark of *A. lobata*; GB = Glibenclamide; EEAL treated groups were compared with control group (p<0.001).

On the other hand, EEAL at 150, 300, and 600 mg/kg body weight decreased the blood glucose levels by 71.4, 78.2 and 82.8 % respectively (Table 4). Glibenclamide (5 mg/kg body weight) lowered the blood glucose by 79.7 %. Throughout the study, EEAL at all the selected doses exhibited the greatest antihyperglycaemic activity compared to those of PEAL. The antihyperglycaemic activity of EEAL at 600 mg/kg body weight was comparable to that of the standard drug, glibenclamide (Table 4). Both extracts (PEAL and EEAL) and the standard drug demonstrated statistically significant antihyperglycaemic activity (p<0.001) when compared to the diabetic control group.

Table 4: Effect of EEAL in streptozotocin-induced diabetic rats after 24 days

TIME (days)	TREATMENT AND DOSE				
	EEAL (150 mg/kg)	EEAL (300 mg/kg)	EEAL (600 mg/kg)	GB (5 mg/kg)	Control (5 ml/kg)
	Blood	Glucose	Level	(mmol/l)	
0.0	25.5±1.5	27.5±1.2	30.2±0.7	23.2±3.0	24.4±0.9
3.0	15.1±1.2	13.0±1.5	12.2±1.1	14.7±0.2	31.8±0.4
6.0	13.5±1.1	11.6±1.5	10.7±0.9	12.5±0.4	31.9±0.4
9.0	12.0±1.0	10.5±1.2	9.6±0.7	10.4±0.3	32.3±0.3
12.0	11.0±0.9	9.1±1.1	8.5±0.3	9.7±0.3	32.2±0.3
15.0	9.8±0.6	7.8±0.8	7.2±0.1	7.6±0.2	32.4±0.4
18.0	9.3±0.5	7.6±0.7	6.3±0.1	7.5±0.2	32.1±0.3
21.0	7.9±0.4	7.1±0.7	5.9±0.1	6.1±0.2	32.3±0.3
24.0	7.3±0.2	6.0±0.4	5.2±0.1	4.7±0.4	32.7±0.2
	(71.4)	(78.2)	(82.8)	(79.7)	(-34.0)

All values are expressed as mean ± S.E.M. (N=5); Number in the parentheses denotes percentage reduction (blood glucose level) after 24 days; EEAL = 70% ethanol extract of the stem bark of *A. lobata*; GB = Glibenclamide; EEAL treated groups were compared with control group (p<0.001).

Antioxidants activity of extracts

Total phenolic content (TPC) of *A. lobata* extracts

The absorbance of *A. lobata* extracts increased with increasing concentration of the extracts. The total phenolic content expressed as tannic acid equivalent (TAE) in mg/g weight of extract for EEAL was 0.623 ± 0.040 and 0.513 ± 0.041 for PEAL (Table 5).

Table 5: Antioxidants activities of extracts

Test	Parameter	Sample		
		EEAL	PEAL	NPG
Total phenolic content	TAE(mg/g)	0.623 ± 0.0400	0.513 ± 0.0410	
Antioxidant capacity	AAE(mg/g)	0.687 ± 0.0640	0.556 ± 0.0520	
Reducing power	NPG(mg/g)	0.645 ± 0.0990	0.348 ± 0.0390	
FRSA	IC ₅₀	0.5210 ± 0.0057	2.106 ± 0.0082	0.2917 ± 0.0054
Lipid autoxidation	IC ₅₀	0.5985 ± 0.0081	8.593 ± 0.0900	0.4217 ± 0.0061

EEAL = 70% Ethanol extract of *Adenia lobata*; PEAL = Petroleum ether extract of *Adenia lobata*; NPG = *N*-propyl gallate; TAE = Tannic acid equivalent; AAE = Ascorbic acid equivalent; FRSA = Free radical scavenging activity; IC₅₀ = Effective concentration at 50% inhibition

Total antioxidant capacity (TAC) of *A. lobata* extract

PEAL and EEAL showed relatively good antioxidant capacities. EEAL exhibited a higher total antioxidant capacity than PEAL. The total antioxidant capacity expressed as ascorbic acid equivalent (AAE) mg/g dry weight of extract were 0.687 ± 0.064 for EEAL and 0.556 ± 0.052 for PEAL (Table 5). EEAL is therefore a better antioxidant than PEAL.

Reducing power of *A. lobata* extracts

The absorbance of the extracts increased with increasing concentration of the extracts. EEAL exhibited a higher reducing power than PEAL. The reducing power expressed as *N*-propyl gallate equivalent (NPGE) mg/g dry weight of extract were 0.645 ± 0.099 for EEAL and 0.348 ± 0.039 for PEAL. EEAL is therefore a better antioxidant than PEAL (Table 5).

DPPH scavenging effect of *A. lobata* extract

The free radical scavenging activity of *A. lobata* extracts expressed as percentage inhibition of DPPH shows concentration-dependent activity for all the extracts. The IC₅₀ values of the *N*-propyl gallate, EEAL and PEAL were 0.2917 ± 0.0054 , 0.5210 ± 0.0057 , 2.106 ± 0.0082 mg/ml respectively (Table 5). EEAL was found to be the most active free radical scavenger of the two extracts.

Lipid peroxidation of *A. lobata* extract

The IC₅₀ values of lipid autoxidation inhibition were 0.5985 ± 0.0081 for EEAL and 8.593 ± 0.0090 mg/ml for PEAL. The reference drug, *N*-propyl gallate, had an IC₅₀ of 0.4217 ± 0.0061 mg/ml (Table 5). All the extracts and *N*-propyl gallate showed concentrations-dependent inhibition of lipid peroxidation activities.

Discussion

Antihyperglycaemic of extracts

Diabetes is a global health problem and its complications are associated with an increased presence of free radicals that lead to elevated stress in the body (Mendel, 1997; Johansen *et al.*, 2005). Elimination of the generated free radicals and lipid peroxidation products can prevent further damage to the body tissue in diabetes. Free radicals and oxidative stress have been implicated in the aetiology of diabetes and its complications. The present study demonstrated the antihyperglycaemic activity of the petroleum ether extract (PEAL), 70% ethanol extract (EEAL) of *A. lobata* in streptozotocin-induced diabetic rats in acute and sub-chronic treatment study periods. All the extracts progressively lowered the blood glucose levels in the diabetic rats throughout the study period. Blood glucose levels measured in the first 6 hours were to see the short term effect of the extracts following administration of the extracts. After one to six hours following drug treatment using 600 mg/kg body weight of EEAL, it was observed that the blood glucose lowering effect was more than the effects observed when an equivalent dose of PEAL was used. Evaluation of blood glucose level after 20 days was to see the sub-chronic or long term effect of the test drugs. EEAL at 600 mg/kg body weight was more active than PEAL in this long term treatment. The differences in activities of the extracts may be

due to the type of constituents that are present in the extracts. EEAL having been obtained using a relatively polar solvent principally contained polar constituents, whereas PEAL principally contained non-polar constituents. Poly-phenols, sterols and flavonoids found in ethanol extract of *Butea monosperma* were found to be responsible for antihyperglycaemic effect in streptozotocin-induced diabetic rats (Bavarva and Narasimhacharya, 2008). From the preliminary phytochemical screening, reducing sugars and phenolic compounds were found in EEAL and steroids were found in PEAL, and these may be partly responsible for the antihyperglycaemic activity of the extract.

Antioxidants of extracts

Five different assays namely, total phenolic contents, total antioxidant capacity, reducing power, DPPH scavenging effect, and lipid peroxidation, were used to evaluate the antioxidant capacities of both *A. lobata* and extracts. Though *A. lobata* was among four medicinal plants assessed qualitatively based on the DPPH preliminary studies for their antioxidant potential (Mamyrbekova-Bekro *et al.*, 2008), extensive quantitative work has not yet been reported. The tests on both petroleum ether and ethanolic extracts of *A. lobata* (PEAL, EEAL) showed relatively good antioxidant capacities. The total phenol was assayed based on the reduction of phosphomolibdate salts to form a blue complex that was detected quantitatively at 760 nm. The phenolic content of PEAL and EEAL was expressed as tannic acid equivalent and was found to increase with increasing concentration of the extracts. The total antioxidant capacity of the extracts determined as ascorbic acid equivalent also increased with increasing extract concentration. The reducing capacity of a compound also served as a significant indicator of its potential antioxidant activity (Hsu *et al.*, 2006). The reductive ability of the extracts was measured in relation to their capacity to reduce Fe^{3+} to Fe^{2+} . PEAL, EEAL and the reference antioxidant, *N*-propyl gallate showed concentration-dependent reduction of Fe^{3+} to Fe^{2+} . From the results (Table 5), *N*-propyl gallate and EEAL were better antioxidant agents than PEAL. PEAL and EEAL exhibited their capabilities to scavenge DPPH. The percentage DPPH scavenging effect increased with increasing concentration of the extracts. The order of potency as defined by IC_{50} in mg/ml showed that the ethanol extract (EEAL of $\text{IC}_{50} = 0.5210 \pm 0.0057$) had better scavenging ability than the petroleum ether extract (PEAL of $\text{IC}_{50} = 2.106 \pm 0.0082$). In all cases, the *N*-propyl gallate of $\text{IC}_{50} = 0.2917 \pm 0.0054$ was found to have the best scavenging ability (Table 5). Phenolic compounds are known to possess DPPH radical scavenging property (Velioglu *et al.*, 1998) and may be responsible for the effects observed in the extracts of *A. lobata*. The results show that EEAL and PEAL are capable of inhibiting the formation of peroxides and hydroperoxides by removing free radicals. This inhibition process could prevent chronic vascular complications such as neuropathy, retinopathy, nephropathy and heart disease (Alexandra *et al.*, 2003). Generally, the polar extracts (EEAL) had relatively good antioxidant potentials than their respective petroleum ether extracts (PEAL) as shown, and this justified the traditional formulation as an aqueous decoction (Irvine, 1961; Abbiw, 1990).

5.0 Conclusion

It is reported in this study for the first time that the extracts of *A. lobata* exhibited promising antihyperglycaemic activity in streptozotocin-induced diabetic rats. This study has also established the antioxidant potentials of the stem of *A. lobata*. The extracts of these medicinal plants were found to be capable of removing free radicals that caused damage to body tissues. The antioxidant activity of the extracts may represent a protective mechanism against reactive oxygen species (ROS) associated with chronic hyperglycaemia and diabetic complications.

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