

ANTIDIABETIC ACTIVITY OF *ANACARDIUM OCCIDENTALE* IN ALLOXAN – DIABETIC RATS

S. Abdullahi and G.A. Olatunji

Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria

Corresponding author: olatunji.ga@unilorin.edu.ng

ABSTRACT

This study investigated the hypoglycemic effect of inner bark extract of Anacardium occidentale Linn. (Anacardiaceae) in normal (normoglycemic) and in alloxan-induced diabetic rats. The inner reddish bark of the plant was extracted with ethanol and screened for hypoglycemic activity in a model of alloxan-induced diabetes in rat species. Bioactivity-guided fractionation of the ethanolic extract led to fractions that displayed diverse hypoglycemic effects at doses of 34.0, 200.0 and 300.0 mg/kg body weight. The alloxan-diabetic rats showed significant reduction in plasma glucose level after treatment with the fractions. These results lend support to the validity of the folkloric use of A. occidentale in the treatment of diabetes mellitus type II.

Keywords: *Anacardium occidentale*, ethanol extract, hypoglycemic activity, diabetes mellitus, rats.

INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from the vascular disease (Cleide *et al.*, 2004). More than 400 species of plants have been reported to display hypoglycemic effect, but only a few of them have been scientifically investigated (Cleide *et al.*, 2004; Patel *et al.*, 1986). *Anacardium occidentale* is the *Anacardium* species (cashew) widely distributed throughout the African countries; it is popularly used as an antidiabetic herbal remedy in many African countries folklore medicine.

Most of the studies carried out on this plant

were directed towards the leaf, stem-bark, nut-shell, fruits and flowers. (Kamtchouing *et al.*, 1998; Sokeng *et al.*, 2001; Alexander-Lindo *et al.*, 2004; Ojewole, 2003; Olatunji *et al.*, 2005; Leonard *et al.*, 2006). However, investigations carried out specifically on the inner-soft bark extract of *A. occidentale* as a separate tissue are near to none. The possibility of the interactive effects of the constituents of the outer cracked bark on the overall therapeutic efficacy of the extract had not been considered. These effects may be synergistic, antagonistic or neutral. The present study is therefore aimed at the extraction of just the inner-bark of *A. occidentale*, partial characterization of its constituents, screening of the extract as natural hypoglycemia.

MATERIAL AND METHODS**Apparatus and Reagents**

IR spectra of the fractions were recorded on a computerized BUCK SCIENTIFIC 500 spectrophotometer. ¹H-NMR spectra were obtained on a BRUCKER spectrometer Model ARX-200 in C₂H₅OH-d₆, chemical shift (δ) in ppm are reported relative to internal TMS. Open column chromatography was performed with Kieselgel S (Riedel-de Haen) 70 – 230 mesh. Thin Layer Chromatography (TLC) was carried out with precoated silica gel (60F₂₅₄MERCK, Germany) plates. TLC bands were visualized under UV lamps at 254 and 366 nm or with vanilin spray reagent.

Plant Material

The inner-bark of *A. occidentale* was collected from the premises of the University of Ilorin, Ilorin, Nigeria. The material was authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Extraction and fractionation

The inner-bark of *A. occidentale* was dried in the laboratory at room temperature and grinded into powder form. 1.0 kg of the air-dried blended inner-bark of the plant was macerated with ethanol at room temperature. After filtration, the solution was concentrated to dryness under reduced pressure to give 77.3 g (7.73%). 10.0 g of the crude ethanolic extract was chro-

matographed on silica gel and eluted in succession with n-hexane, n-hexane/diethylether (2:1), n-hexane/diethylether (1:1) and ethanol / diethylether (1:1). 50 ml fractions were collected and a total of 48 fractions were collected. The fractions collected were simultaneously monitored by TLC whereby fractions with identical R_f values were combined accordingly. These were concentrated, weighed and labeled appropriately as shown in Table 1.

Phytochemical Tests of Fractions of *A. occidentale*

The chromatographic fractions of *A. occidentale* were tested for the presence of alkaloids, terpenoids, lipids, polyphenols and flavonoids following standard methods (Trease and Evan, 1978; Tracey *et al.*, 1980; Ikan *et al.*, 1969; Stahl *et al.*, 1969). The results are shown in Table 2.

Animals

A total of thirty (30) male wistar rats weighing between 150 and 200g obtained from the Animal Breeding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria were used for the study. All animals were maintained (6 per cage) in a standard environmental condition and were allowed free access to a standard commercial diet and water *ad libitum*.

Table 1: Chromatographic fractions from *A. occidentale* inner bark crude extract

Fraction	Fraction	Solvent	Colour	Weight (mg)
8 – 12	A	Hex/Et ₂ O (2:1)	Yellow	70
13 – 16	B ₁	EtOH/Et ₂ O (1:1)	Yellow	28
	B ₂	EtOH/Et ₂ O (1:1)	Off-white	22
17 – 19	C	EtOH/Et ₂ O (1:1)	Yellow	290
22 – 24	D	EtOH/Et ₂ O (1:1)	Reddish-brown	1058.6
26 – 28	E	EtOH/Et ₂ O (1:1)	Light-brown	133.6
29 – 36	F	EtOH/Et ₂ O (1:1)	Light-brown	388.1
37 – 48	G	EtOH/Et ₂ O (1:1)	Light-brown	369.2

Table 2: Chromatographic components of the inner-bark of *A. occidentale* positive to phytochemical screening

Test	Fractions Positive to Test
Polyphenols	D, E, F and G
Flavonoids	D, E, F and G
Alkaloids	B ₂
Terpenoids	A
Lipids	C

Hypoglycemic Activity Assays

The crude extract, fractions D, E, F and G were evaluated for hypoglycemic activity following standard methods (Ata-ur-Rahman *et al.*, 2007; Olatunji *et al.*, 2005). Diabetes was induced in adult rats by intraperitoneal injection of alloxan 65 mg/kg. After the induction of diabetes, the rats were divided into two groups, one group serves as the control and the other as the test group. The animals were then kept for overnight fasting. Fasted animals with plasma glucose levels higher than 200 mg/dl were selected for the study. The test group of overnight fasting rat was fed with test samples in 2 ml of distilled water. The control rats received 2 ml of distilled water only. Blood samples were drawn at different time intervals. Before drawing the blood, the tails were dipped in water for 30 seconds and the blood was drawn by cutting the tail tip. The blood sugar in the serum was estimated using a glucometer. The percentage decrease in glycemia in the blood sample was calculated using the formula:

$$\% \text{ decrease in glycemia} = \frac{G_o - G_x}{G_o} \times 100$$

where G_o = Initial glycemia; and G_x = Glycemia at time x.

RESULTS AND DISCUSSION

Fractionation of the ethanolic extract of the inner-bark of *A. occidentale* between solvents gave four bioactive fractions: fractions D, E, F and G which are active at diverse doses of

300.0, 34.0 and 200.0 mg/kg respectively. Other fractions were in relatively small amounts that could not be used to test for bioactivity (Tables 3 – 7). Phytochemical screening of the various fractions revealed the presence of alkaloids, terpenoids, lipids, polyphenols and flavonoids (Leonard *et al.*, 2006; Edy *et al.*, 2007; Trevisan *et al.*, 2006) (Table 2). During a routine screening of the plant extract for antidiabetic effects, it was observed that the more polar fractions of *A. occidentale* inner-bark that proved positive for both polyphenols and flavonoids (Edy *et al.*, 2007; Trevisan *et al.*, 2006) in the general phytochemical tests are active in the hypoglycemic assays. Of the four fractions, fraction D displayed the highest hypoglycemic activity with approximately 20% decrease in glycemia.

The IR spectra of the four fractions showed broad absorption bands in the range of 3400 – 3500 cm^{-1} which are characteristics of –OH stretching vibrations. There are also absorptions at 1237-1238 cm^{-1} , 1100-1200 cm^{-1} which indicate the C–O stretching vibrations. The weak absorptions in the range 1610-1640 cm^{-1} are typical of alkenes. The ^1H NMR spectrum of fraction E revealed many overlapping signals. Among other resonances, there are signals at δ_{H} 0.8 – 1.0ppm (m), δ_{H} 1.20 – 1.40ppm (s), δ_{H} 3.6 – 3.8ppm (m) and δ_{H} 7.0 – 7.2ppm (broad singlet). The broad singlet at 7.0 – 7.2 ppm showed the presence of aromatic groups which would be in agreement with results of the phytochemical screening that indicates presence of phenols and flavonoids.

Previous studies have demonstrated that the crude extracts of *A. occidentale* had numerous biological applications (Barcelos *et al.*, 2007; Konan *et al.*, 2007; Olajide *et al.*, 2005; Ojewole, 2004; Franca *et al.*, 1993; Kubo *et al.*, 1994). Reports showed that the aqueous, alcoholic and hexane extracts from the barks and leaves of *A. occidentale* had hypoglycemic effects (Ojewole, 2003; Alexander-Lindo *et al.*, 2004; Sokeng *et al.*, 2001; Kamtchoung *et al.*, 1998). In the present work we showed that the

ethanolic extract of the inner-bark of *A. occidentale* reduced the serum glucose level in diabetic rats but to our astonishment, the values did not return to those of normal controls (Table 3).

Table 3: Hypoglycemic activities of crude extract on diabetic and normal rats after 30 minutes.

Animal	Body Weight (g)	Initial Glucose Level (mg/dl)	Glucose Level After 30 Minutes
Diabetic Rats			
Rat 1	168	208	130
Rat 2	142	140	90
Control (normal) Rats			
Rat 3	228	85	89
Rat 4	192	94	95
Rat 5	226	78	44

The crude extract produced significant hypoglycemic activity after intraperitoneal injection of alloxan at a dose of 700.0 mg/kg body weight. The percentage decrease in glycemia in the test sample as a function of time is calculated by applying the formula:

$$\% \text{ decrease in glycemia} = \frac{G_o - G_x}{G_o} \times 100$$

Where G_o = initial glycemia, and G_x = glycemia at 30 minutes. Thus

$$\% \text{ decrease in glycemia} = \frac{174 - 110}{174} \times 100 = 36.8\%$$

Table 4: Hypoglycemic activities of fraction D on diabetic rats after 30 minutes

Animal	Body Weight (g)	Initial Glucose Level (mg/dl)	Glucose level After 30 Minutes (mg/dl)
Rat 1	122	488	374
Rat 2	109	422	370
Rat 3	152	436	354

Fraction D produced significant reductions in the blood glucose level at a dose of 300.0mg/kg body weight. The percentage decrease in glycemia in the test sample is calculated as follows:

$$\% \text{ decrease in glycemia} = \frac{448.7 - 366}{448.7} \times 100 = 18.4\%$$

Table 5: Hypoglycemic activity of fraction E on diabetic rats after 30 minutes

Animal	Body weight (g)	Initial Glucose Level (mg/dl)	Glucose Level After 30 Minutes (mg/dl)
Rat 1	225	218	181
Rat 2	217	225	204
Rat 3	145	482	396

Fraction E produced significant hypoglycemic activity at a dose of 30mg/kg body weight. The percentage decrease in glycemia in test sample is calculated as follows.

$$\% \text{ decrease in glycemia} = \frac{308.3 - 260.3}{308.3} \times 100 = 15.6\%$$

Table 6: Hypoglycemic activity of fraction F on diabetic rats after 30 minutes

Animal	Body weight (g)	Initial Glucose Level (mg/dl)	Glucose Level After 30 Minutes (mg/dl)
Rat 1	129	313	252
Rat 2	181	137	120

Fraction F produced significant hypoglycemic activity at a dose of 200.0mg/kg body weight. The percentage decrease in glycemia in the test sample is calculated as follows:

$$\% \text{ decrease in glycemia} = \frac{225 - 186}{225} \times 100 = 17.3\%$$

Table 7: Hypoglycemic activity of fraction G on diabetic rats after 30 minutes

Animal	Body weight (g)	Initial Glucose Level (mg/dl)	Glucose level After 30 minutes (mg/dl)
Rat 1	126	156	136
Rat 2	130	181	227

Fraction G produced hypoglycemic activity at a dose of 200.0mg/kg body weight. The percentage decrease in glycemia is calculated as follows:

$$\% \text{ decrease in glycemia} = \frac{156 - 136}{156} \times 100 = 12.8\%$$

Edy *et al.*, 2007; Trevisan *et al.*, 2006 are among several researchers who have demonstrated that polyphenols and flavonoids act as anti-oxidants. Salam *et al.*, 2008 showed that some flavonoidal compounds such as psibaptigenin and hesperidin isolated from various plants act as anti-diabetic agents by selectively targeting PPAR- γ (Peroxisome proliferators-activated receptor-gamma), a nuclear receptor that plays an essential role in insulin resistance and metabolic syndrome.

CONCLUSION

Based on our current findings, the four fractions of *A. occidentale* that are active in the hypoglycemic assays proved positive to both polyphenols and flavonoid compounds. Similarly, our results showed that the fractions displayed significant reductions in the blood glucose level and this effect may be on account of the presence of polyphenols and flavonoids in the plant extract. The results showed that the oral administration of inner-bark extract of *A. occidentale* had a beneficial effect on the diabetic state reducing the hyperglycemia. Studies with purified and isolated polyphenols and flavonoids from the plant extract are underway to further elucidate their actual structures and pro-

pose mechanism of action.

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