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Pharmacognostic Studies and Antidiabetic Evaluation of the Ethanolic Root Extract of *Anacardium occidentale* Linn (Anacardiaceae) in Mice

PN Olotu[1*], IA Olotu[2], MB Kambasha[1], A Ahmed[3], U Ajima[4], TL Ohemu[1], LD Ior[5], VA Okwori[1], J David[6], UFB Abdulfattah[1], EU Onche[7]

[1]*Department of Pharmacognosy & Traditional Medicine,University of Jos, Jos, Nigeria*

[2]*Department of Biochemistry, University of Jos, Jos, Nigeria*

[3]*Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria, Nigeria*

[4]*Department of Pharmaceutical Chemistry, University of Jos, Jos, Nigeria*

[5]*Department of Pharmacology, University of Jos, Jos, Nigeria*

[6]*Department of Medical Biotechnology, National Biotechnology Development Agency, Abuja, Nigeria*

[7]*School of Chemistry, University of Manchester, UK*

ABSTRACT

Anacardium occidentale is well established in folklore medicine and is reputed to be effective against renal and skin infections and is also used to treat tooth decay and acne. This study was carried out to explore the pharmacognostic and anti-diabetic activity of the ethanol extract of Anacardium occidentale root in both normal and Streptozotocin induced diabetic mice. The diverse features of the powdered root were observed macroscopically using the standard description of terms. The powder is light brown in colour, rough to touch with characteristic odour and taste. Some of the characteristic features of the plant observed microscopically were multicellular multiseriate medullary rays crossing parenchymatous cells, prism of calcium oxalate crystals spread across the cell and fibre groups forming sheaths with prisms on them. The crude drug was found to have a moisture content of 15% w/w while the total ash, water soluble ash and acid insoluble ash values were 2.67% w/w, 34% w/w and 10% w/w respectively. Phytochemical evaluation of the extract revealed the presence of alkaloid, tannins, anthraquinones, flavonoid, and terpenes/steroids. Streptozotocin-induced diabetic and non-diabetic mice were administered intraperitoneally with ethanol extract of the root at 500 mg/kg, 250 mg/kg and 125 mg/kg respectively, for six hours. There was a significant ($p<0.05$) reduction in the level of blood glucose of the mice after administration .The results from this study suggest that the ethanolic extract of the root possesses some hypoglycemic properties and could therefore have potential for diabetes management.

Keywords: *Anacardium occidentale*, Streptozotocin, Phytochemicals, Alkaloid, Flavonoid and terpenes/steroids

INTRODUCTION

Diabetic mellitus is a metabolic disorder that is rapidly becoming a global epidemic and the World Health Organisation (WHO) has projected that by 2025, three hundred million people could be affected worldwide [1,2]. Some of the complications that may arise due to Diabetic mellitus include coronary artery disease, and cerebrovascular system, renal dysfunction, neurological disorders and may eventually lead to premature death [1,3]. Some of the treatment regimens presently being employed include insulin, oral anti hyperglycemic agents and inhibitors of α-glycosidase enzyme. Alternative and complementary medications including plant products are also available [4]. About 80% of the world population still relies on traditional medicines for primary healthcare and this is mainly in the developing countries [5]. Plants of various origins have been exploited effectively over many

generations for therapeutic purposes [6]. Herbalists in Nigeria use a variety of herbal preparations to treat variety of ailments including many microbial ailments and diabetes mellitus [7].

Anacardium occidentale is a woody vine which may be small trees or shrubs. The stems often get woody with age and may produce milky or resinous juice. The leaves are simple and often pinnately compound or trifoliate, mostly alternate; stipules none or very small, flowers are regular (actionomorphic), perfect or most often imperfect (dioecious). The fruit is a berry or berry-like drupe which is usually oily. Fruit diversity is very high and a myriad of morphological types are commonly found in the family [8].

The Hausa tribe in Northern Nigeria use *Anacardium occidentale* in the management of pain of various categories, such as migraine, sickle cell crisis and menstrual pain by taking decoction of the stem bark. The decoction of the root and stem is also used in same country as an anti-inflammatory and anti-diarrhoeal agent. Sometimes, the Surinam uses the seed of cashew as a wormicide to kill the larva of butterfly. A number of local communities in the Amazon region use the fruit juice and tea of the bark as diarrhea remedies [9].

The antimicrobial activities of *A. occidentale* extracts are well established [10]. Its fruits were shown to exhibit antibacterial activity against *Helicobacter pylori*; the causative organism of stomach ulcers [5]. It has been found to also possess anti-viral [11] and anti-fungal activities [12]. The stem-bark and leaves of the plant have also been reported to possess blood glucose lowering activities [13] while radical scavenging activities has been reported for the shoot [14] and the leaves [15]. The present study is therefore aimed at evaluating the Pharmacognostic profile of the plant and also to investigate the anti-diabetic properties of the ethanolic root extract in mice.

MATERIALS AND METHODS

Plant collection, identification and preparation

The roots of *Anacardium occidentale* Linn (Anacardiaceae) were collected from the Federal College of Forestry Jos, Plateau State, Nigeria in March 2015. The roots were identified and also authenticated at the Department of Horticulture and Landscape Technology, Federal College of Forestry, Jos, Nigeria. The roots were cut into pieces and dried under the shade and subsequently reduced to powder form with the aid of a mortar and pestle and the powder was then stored in an air-tight container until ready for use.

Plant extraction

The powdered roots of *Anacardium occidentale* (80 g) was extracted by maceration using 80% ethanol as the solvent for 72 h with intermittent shaking at 2 h intervals. The extract was then filtered with Whatman No. 1 filter paper. A rotary evaporator (Rotavapour R 210, Buchi, Switzerland) was used to concentrate the extract at 40oC and the yield determined. The dried extract stored in a refrigerator until it was required for use.

Animals

Healthy Swiss albino mice weighing between 20-30 g of both sexes were used. The animals were fed with grower mesh (poultry feed) and water ad-libitum and kept in standard cages under laboratory condition. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals and the study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences of the University of Jos.

Chemicals and reagents

All the solvents used in the study were of Analytical grade.

Microscopy

This involves the anatomical study of the plant part with each distinguishing character noted and pictures taken. In the procedure, the powdered sample of the root was cleared and mounted in glycerol and observed under the microscope as previously described [16].

Phytochemical screening

This is a useful step in detecting the presences of specific classes of phytoconstituents in the extract. This was carried out using standard methods [16,17].

Quantitative evaluation

Moisture can aid the microbial spoilage of crude drugs and its determination is important in assessing the stability and resistance to deterioration of crude drugs. Ash values are helpful in evaluating the quality and purity of crude drugs especially in powder form while extractive values are used in determining the amount of active constituents extracted with solvents from a given amount of medicinal plant material. Standard methods and procedures were used to determine the moisture content, ash values (total ash and acid insoluble ash values) and extractive values (alcohol soluble and water soluble values) [16].

Acute toxicity studies

A total of ten healthy animals of equal numbers of male and female mice were used and each received a single oral-dose of 2000 mg/kg body weight of *Anacardium occidentale* extract dissolved in distilled water. The animals were observed individually after administration, once during the first 30 min, periodically during the first 24 h and daily thereafter for a period of 7 days. Changes in the skin and fur, eye and mucus membrane (nasal), breathing during this period were noted. Also, responses like salivation, lacrimation, perspiration, piloerection, urinary incontinence, ptosis, drowsiness, gait, tremors and convulsion were similarly noted [18].

Experimental animals

Fifty adult mice (50) were obtained from the animal housing unit of the University of Jos. Forty (40) of the mice were scheduled to be used for the experiment. The animals were fed for three (3) weeks until they attained a weight of 20 to 30 g. They were maintained in normal and standard laboratory conditions of temperature with a 12 h light-dark cycle and adequate ventilation. The mice were fed with Commercial diet (vital feed) and water ad-libitum. Food however was withheld for about 12 h before the experiment but access to water was allowed.

Induction of diabetes

Diabetes was induced in all of the animals by intraperitoneal injection of 50 mg/kg body weight of streptozotocin freshly prepared with water as a vehicle. Diabetes was confirmed 2-3 days later in the animals and the fasting blood sugar levels were determined using a one touch glucometer by taking blood sample from the tail vein of the mice. All mice were fasted overnight before the Streptozotocin (STZ) was administered.

The animals were divided into five (6) groups of 3 rats each and treated as follows:

Group I: Normal control (saline).

Group II: Streptozotocin treated control (50 mg/kg. i.p).

Group III: Streptozotocin (50 mg/kg. i.p)+*A. occidentale* root extract (125 mg/kg. p.o)

Group IV: Streptozotocin (50 mg/kg. i.p)+*A. occidentale* root extract (250 mg/kg. p.o)

Group IV: Streptozotocin (50 mg/kg. i.p)+*A. occidentale* root extract (500 mg/kg. p.o)

Group V: Streptozotocin (50 mg/kg. i.p)+standard drug, Metformin (500 mg/kg, p.o)

Plant extracts and standard drug metformin (500 mg/kg) and saline were administered with the help of feeding cannula. Blood samples were withdrawn at each point (0, 2, 6, 16, 24 h) from the tail vein of the animal after drug and plant extract administration and tested using the glucometer.

Statistical analysis

The results are expressed as mean ± Standard Deviation (SD) using one-way Analysis of variance (ANOVA) followed by student's t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups. A significant change was considered acceptable at $p<0.05$.

RESULTS

Microscopy results are as shown in Figure 1 and the results of chemo microscopy are as shown in Table 1. The quantitative values of the powdered stem are presented in Table 2 and Phytochemical Constituents and hydroethanolic extract of Anacardium occidentale is presented in Tables 3 and 4.

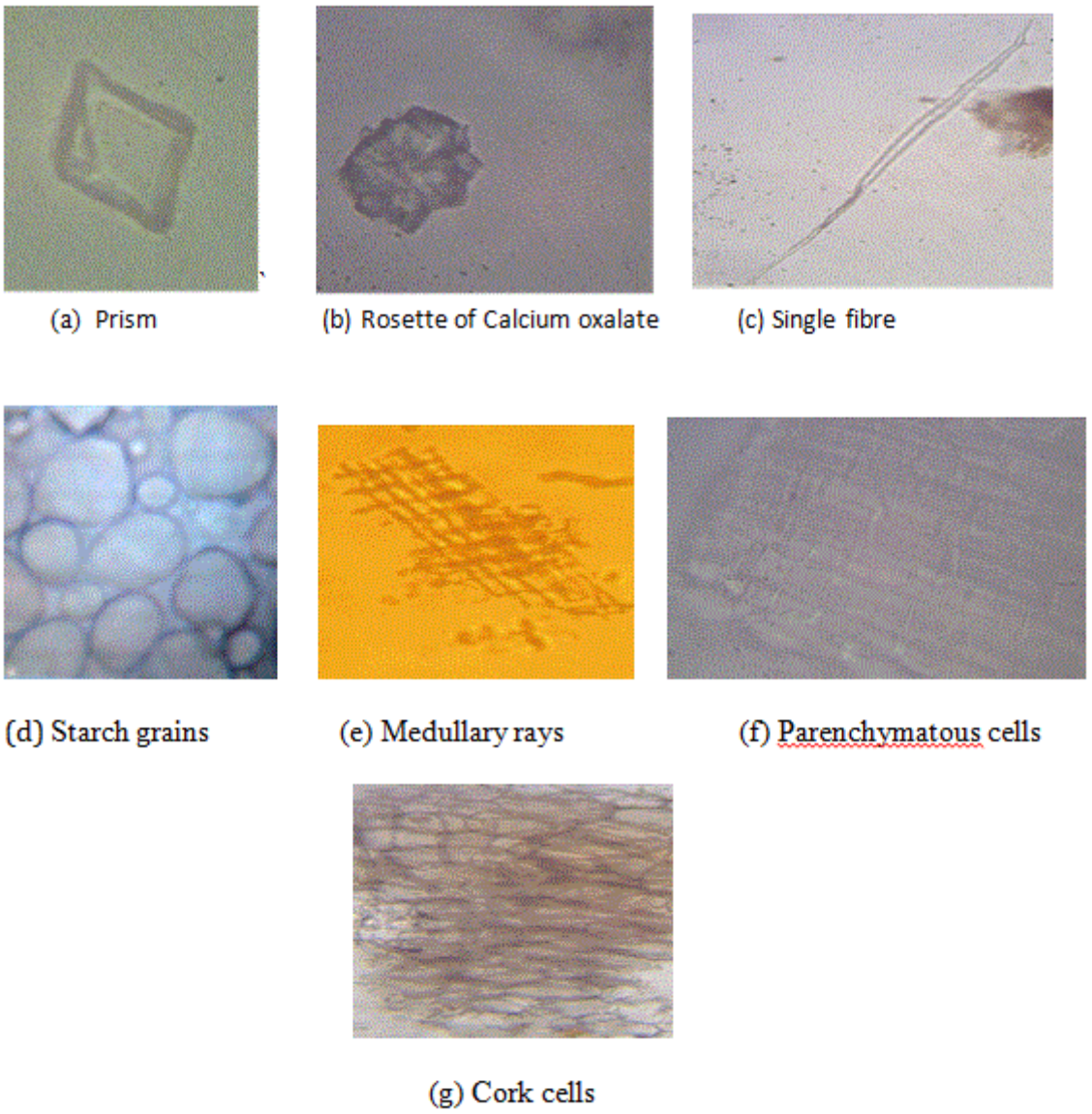

Figure 1: Pharmacognostic characters of *Anacardium occidentale* powdered root

Table 1: Summary of the results of chemo microscopy

Test	Observation	Inference
Cellulose	Blue-black colour observed	Cellulose present
Lignin	Red colour observed	Lignin present
Calcium oxalate crystals	Dissolution of the crystals	Calcium oxalate present
Starch grains	Blue-black	Starch grains present
Proteins	Dark pink colour observed	Proteins present

Table 2: Results for the quantitative values of the powdered stem of *Anacardium occidentale*; n=3

Test	Stem powder (%w/w)
Moisture content	6.79
Total ash-value	1.9
Acid insoluble ash-value	1.28
Water soluble ash-value	0.8
Alcohol extractive value	0.97
Water soluble extractive value	0.58

Table 3: Summary of the phytochemical constituents of *Anacardium occidentale*; Key: += present and -= absent

Test for	Observations	Inferences
Tannins		
lead sub-acetate	A white colour changed was observed	Hydrolysable tannins
Anthraquinons		
fully oxidized bound	A pink colour in the ammonia lower phase	+
Saponins		
frothing	Frothing which persisted on warming	+
Heamolytic	Complete heamolysis of red blood cells	+
Cardiac glycosides		
Legal	A deep red colour which faded to brownish yellow	+
Kedde	An immediate violet colour which faded gradually through reddish brown to brown yellow with a whitish crystalline solid precipitate	+
Lieberman's	A colour change from violet to blue and then green	+
Salkowski's	A reddish brown colour at the interface	+
Kella-Kiliani'	A brown ring at the interface	+
Flavonoids	A pink colour formation	+
Alkaloids		
Mayer's reagent	No cream precipitate	+
Dragendorff's reagent	No orange precipitate	+
Wagner's reagent	No reddish precipitate	+
Tannic acid	No black precipitate	+
Picric acid	No yellow precipitate	+

Table 4: Effect of hydroethanolic extract of *Anacardium occidentale* root on blood glucose; Values are expressed as Mean ± SD (n=6). Values in each column with different superscript (a-b) are significantly different ($p<0.05$)

Dose administered (mg)	1 h	2 h	3 h	6 h
500	175.69 ± 11.15ab	169.00 ± 5,57ab	158.33 ± 7.64ab	141.67 ± 16.19a
250	190.67 ± 9.02b	181.67 ± 9.07b	171.67 ± 7.77b	158.00 ± 7.810a

125	167 ± 6.51a	152.33 ± 8.62a	142.00 ± 8.54a	130.67 ± 18.01a
Standard drug (metformin)	168.00 ± 9.85a	156.67 ± 12.01a	153.67 ± 16.44ab	142.69 ± 15.37a

DISCUSSION

The macroscopic features of the powdered root were observed using the standard description of terms. The powder is light brown in colour, rough to touch with characteristic odour and taste. Some features of the plant seen were multicellular multiseriate medullary rays crossing parenchymatous cells, prism and rosette of calcium oxalate crystals spread across the cell, numerous spindle fiber with tapering ends, starch grains with centric helium and group of fibres forming sheaths with prisms on them. These features could be used pharmacognostically for identification and differentiation. The moisture content of the crude drug was found to be 14% w/w and this falls within the acceptable limit for crude drugs [19]. As far as practicable, moisture must be eliminated from crude drugs, as high moisture content can lead to deterioration due to fungal growth [20]. The total ash value, water soluble ash value and acid insoluble ash value were 2.67% w/w, 34% w/w and 10% w/w, respectively. The above data is also indicative of a high purity of the powdered plant material as all traces of extraneous or organic matter were removed [20]. The results of the extractive values showed that the percentage of alcohol extractive value was 6% w/w and that of water extractive value was 9% w/w. This implies that there was neither adulteration nor substitution with the drug.

Streptozotocin is a very useful compound for inducing type one diabetes in experimental animals and it was employed in the present study due to its selective toxicity to the β-cells of the pancreatic islets. Streptozotocin-induced diabetic and non-diabetic mice were administered intraperitoneally with the hydroethanolic extract of *Anacardium occidentale* root at 500 mg/kg, 250 mg/kg and 125 mg/kg, respectively, for six hours. There was a significant ($p<0.05$) decrease in the level of blood glucose of the mice after administration. Phytochemical screening of the aqueous extract of *Anacardium occidentale* root revealed the presence of alkaloids, Tannins, Anthraquinones, flavonoids, and terpenes/steroids. It can therefore be assumed that the hypoglycaemic effect of the extract may be due to some of these phytoconstituents. A number of mechanisms have been proposed for the blood glucose lowering effects of medicinal plants such as decreased intestinal absorption of glucose or even restoration of the function of pancreatic tissues thereby leading to an upsurge in insulin production [21]. The results obtained indicate that the extract may be acting via one or a combination of the above mechanisms. Additional work is on-going in order to possibly isolate and identify the specific active ingredient(s) responsible for the observed actions.

CONCLUSION

The results from this study suggest that the hydroethanolic extract of the root of *Anacardium occidentale* possesses anti-diabetic activities and could be used for the management of diabetes and associated metabolic alterations.

REFERENCES

[1] Chinaka, O.N., et al., *J Pharmacol Toxicol*, **2012**. 7(4): p. 181-191.
[2] Amos, A.F., McCarty, D.J. and Zimmet, P., *Diabetes Med*, **1997**. 14: p. S1-S85.
[3] Jayatilake, G.S., *J Nat Prod*, **1993**. 56: p. 1805-1810.
[4] Sabo, C.E., Michael, S.R. and Temple, L.L., *Diabetes Educ*, **1999**. 25: p.945-956.
[5] Kubo, J., Faye, O. and Gaye, M., *J Agric Food Chem*, **1999**. 47(2): p. 533-537.
[6] World Health Organization, Geneva, **2002**. p. 20-25.
[7] Olukoya, D.K., Idika, N. and Odugbemi, T. J., *Ethnopharmacol*, **1993**. p.112-157.
[8] Susan, P.K., Plant classification: United States Department of Agriculture (USDA) Natural resources conservation service, **2009**. p. 11-25.
[9] Onasanwo, S.A., et al., *Nig J Physiol Sci*, **2012**. p. 65-71.
[10] Akinpelu, D.A., *Fitoterapia*, **2001**. 72: p. 286-287.
[11] Gonçalves J.L.S., et al., *J Ethnopharmacol*, **2005**. (3): p. 403-407.
[12] Schmourlo, G., *J Ethnopharmacol*, **2005**. p. 563-568.
[13] Ojewole, J.A., *J Clin Pharmacol*, **2003**. 25(3): p.199.
[14] Roach, P.D., *Clin Exp Pharmacol Physiol*, **2003**. 30: p. 5-6.
[15] Abas, F., *Food Chem*, **2006**. 95: p. 566-573.
[16] Evans, W.C., W.B. Saunders Company Ltd, London, **2009**. p. 334-335, 340-344, 542-578.

[17] Sofowora, A., Spectrum book Ltd, Nigeria, **2008**. p. 200-202.
[18] Organization for Economic Cooperation and Development, Acute oral toxicity-acute toxic class method, **2002**.
[19] African Pharmacopoeia, OAU/STRG Scientific Publication No. 3 Lagos, Nigeria, **1986**. 78: p. 142.
[20] Kadam, P.V., *Int J Pharm Phytopharmacol Res*, **2011**. 1 (6): p. 350-353.
[21] Malviya, N., Jain, S. and Malviya, S., *Acta Pol Pharm*, **2010**. 67 (2): p. 113-118.