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Full length Research Paper

Antibacterial and comparative hypoglycemic effect of *Anacardium occidentale* leaves

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Diabetes is one of the most prevalent metabolic disorders characterized by hyperglycemia, glycosuria, altered lipid and protein metabolism. It affects nearly 25% of the World population. An ethnomedical survey revealed that *Anacardium occidentale* parts are used as a remedy for the treatment of diabetes mellitus and as antibacterial decoctions in the treatment of infectious diseases. In this study, the ethylacetate effluent of ethanolic fraction of *A.occidentale* leaf at 300mg/kg bw had the best hypoglycemic activity in alloxan induced diabetic rats compared to other effluents at same dose and the standard drug-metformin. However, the ethylacetate effluent at 200mg/kg bw had equivalent hypoglycemic activity with metformin. The spectral analyses of the ethylacetate effluent revealed the presence of esters. The antibacterial effect of the stepwise fractions of *A.occidentale* leaf revealed that hexane inhibits *Salmonella typhi* (10mm), ethylacetate inhibits *Staphylococcus aureus* (12mm) while ethanol inhibits *Kliebsella pneumonae* (11mm). The results indicate that *A.occidentale* leaves had significant hypoglycemic and antibacterial potentials which can be exploited for drug development.

Keywords: Hypoglycemia, glycosuria, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is the fourth leading deadly disease worldwide affecting millions of people. In 1995, about 84 million people were estimated by World Health Organisation to have the disease and that by 2025, the number will increase to 300million. It is clinically treated by the use of insulin and oral hypoglycemic drugs (Onoagbe *etal* 1999).The treatments serve as palliatives and are ineffective in stemming down the ailment to a terminal state hence necessitating the search for alternative medications. NIDDM (type 2) diabetes account for 90 percent of diabetic cases (WHO, 2002). Insulin resistance and beta cell dysfunction are the metabolic abnormalities in the type 2 diabetes (Saad., *etal*). Glycemic control is one of the targets for managing diabetes mellitus. Studies have confirmed that for the type 2 diabetes, effective control of blood glucose substantially decrease the risk of developing diabetic complications. Management of diabetes with insulin and oral hypoglycemic agents has certain drawbacks which include ineffectiveness, short shelf life and requirement of constant refrigeration. The use of oral hypoglycemia drugs like sulfonylureas and biguanides is also associated with side effects (Rang and Dale, 1991). *Anacardium occidentale* parts are used as medicinal plants by Nigerians for the treatment of diabetes mellitus and bacterial infections. The research reports the hypoglycemic and antibacterial effects of the *A.ocidentale* leaves.

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MATERIALS AND METHODS

Materials

A.occidentale leaves were obtained from Minna and surburbs and identified in the Department of Biological Sciences, Federal University of Technology, Minna. White albino rats weight ranging from 80g to 200g were purchased and used for the study. Clinical strains of the bacterial species were obtained from the General Hospital, Minna and cultured.

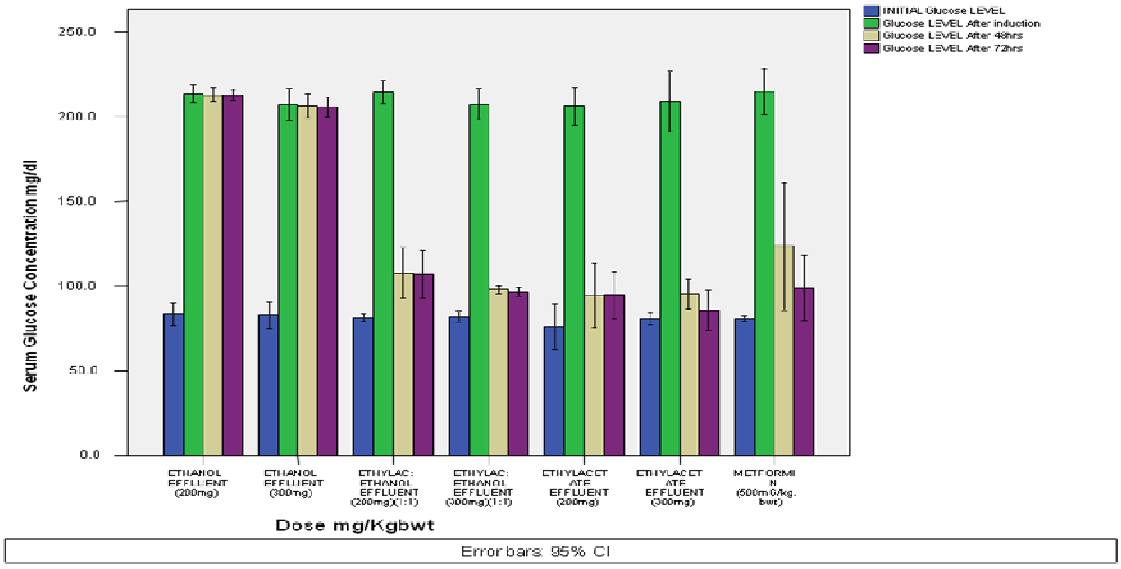


Figure 1. Comparative Effect of Metformin and different column effluents of ethanolic fraction of leaf extract of *A.occidentale* on serum glucose concentration in rats.

Chemicals and Reagents

Chemicals used were of analytical grade and obtained from reputable companies such as: May and Baker Ltd,Sigma, BDH chemicals Ltd. The chemicals included: Alloxan, n-hexane, ethylacetate, ethanol and dimethylsulphoxide. Metformin was obtained from pharmacy store in Minna.

The Reagents included: Glucose kits.

Equipment

Spectrophotometer (Spectronic 20D^{+}), Autoclave, GC-MS (GC 6890N and 5973 series, MS selector), I.R. spectrometer (Schimadzu FTIR Type).

Methods

Induction of Diabetes

Induction of diabetes was carried out by intraperitoneal injections of 200mg/kg bw alloxan to rats fasted for 18hrs period. One week (7 days) after injection, the rats were fasted again and initial fasting blood glucose levels determined. Animals that had glucose level above 150mg/dl were selected and used for the study.

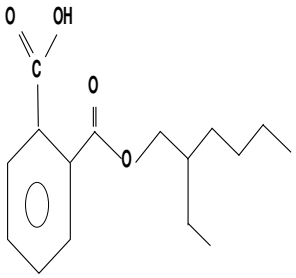
Administration of fractions

A set of rats divided into 7 groups of 3 rats each were used. Group IC and IIC consisted of rats administered (200,300mg/kg bw) ethylacetate effluent. Group IIIC and IVC consisted of rats administered (200,300mg/kg bw) ethylacetate: ethanol (1:1) effluents. Groups Vc and VIc consisted of rats given (200,300mg/kg bw) ethanol effluent while Group VIIc were given 500mg/kg bw metformin as standard drug. All the effluents were suspended in 1% DMSO. The groups were observed for 72hrs. Hypoglycemic activities were recorded using blood glucose lowering effect as parameter.

Antibacterial Activity (Ayepola and Adeniyi, 2008).
28.0g of nutrient Agar was dissolved in a litre of distilled water and sterilized at 121^{o}C for 5 mins using autoclave. After sterilization, it was allowed to cool to 45^{o}C before dispensing on sterile petri dishes and finally allowed to solidify before use. The test organism was inoculated into each of sterile nutrient broth in test tube and incubated at 37 ^{o}C for 3hrs. A cork borer of size 7mm was sterilized at 160 ^{o}C for 1hr which was used to bore holes in agar plates. The fractions (1.0mg/ml) were then dispensed after inoculation of test bacteria. The plates were then incubated at 37 ^{o}C for 2hrs. After incubation, the zones of inhibition were measured as activities of fractions.

RESULTS

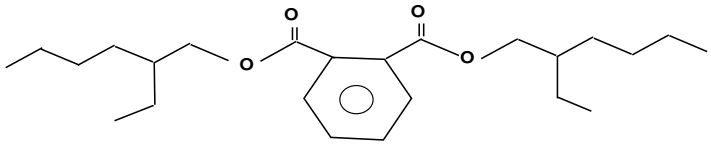
Figure 1 depicts the comparative effect of *A.occidentale* column fractions and metformin. Figure 2 and Figure 3 shows the structures of the esters as determined by GC-MS, while plates I-III represents antibacterial effect of the fractions.



Formula	$C_{16} H_{22} O_4$
Molecular wt.	278
CAS No.	4376-20-9

1,2 – Benzenedicarboxylic acid, mono(2-ethyl)ester

Figure 2. Structure of 1, 2-Benzenedicarboxylic acid, mono(2-ethyl)ester



Bis (2-ethylhexyl) phthalate

Formula	$C_{24} H_{38} O_4$
Molecular wt.	390
CAS No.	117-81-7

Figure 3. Structure of Bis (2-ethylhexyl) phthalate

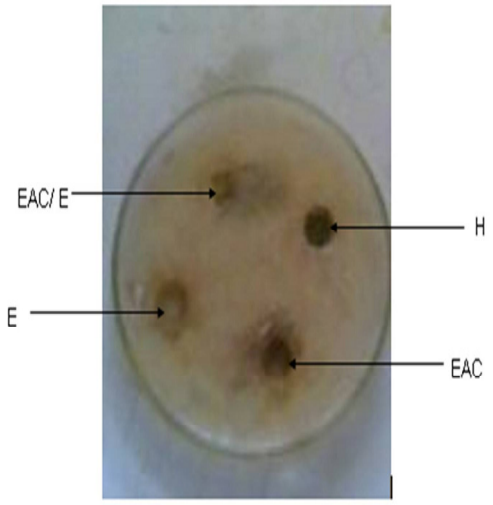


Plate 1.Effect of stepwise fraction of *A.occidentale* leaf extract on *klebsiella pneumonae*.only ethylacetate/ethanol and ethanol inhibits *klebsiella*.
(Key:H-hexane, EAC-ethylacetate,EAC/E-ethylacetate/ethanol,E-ethanol).

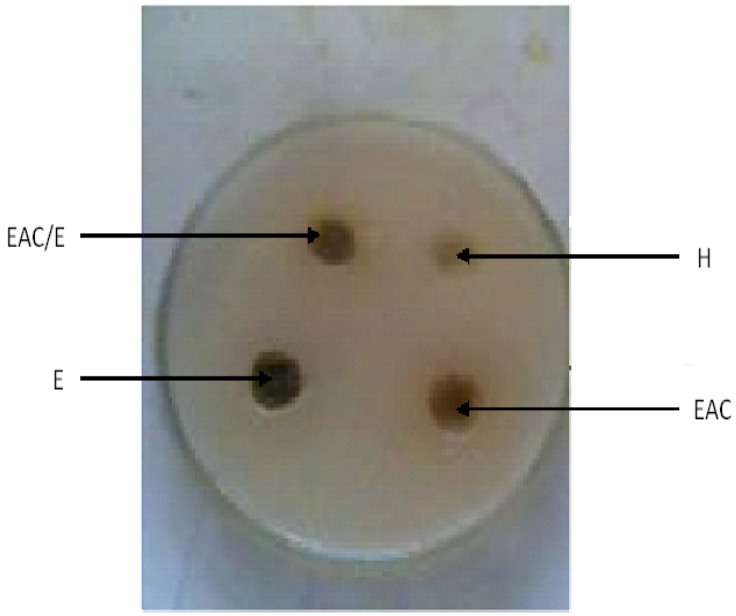


Plate 2. Effect of stepwise fraction of *A.occidentale* leaf extract on *salmonella typhi.*
(Key:H-hexane, EAC-ethylacetate,EAC/E-ethylacetate/ethanol,E-ethanol).

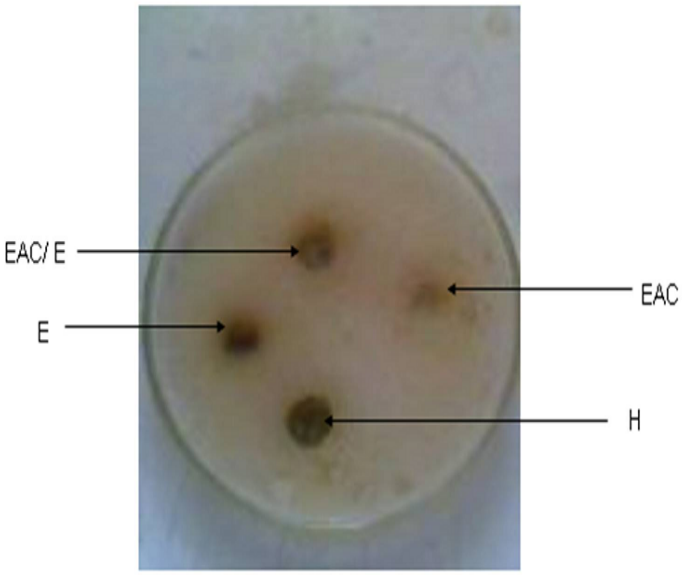


Plate3. Effect of stepwise fraction of A.occdentale leaf extract on *staphylococcus aureus*..
only ethylacetate fraction inhibits staphylococcus aureus.
(Key:H-hexane, EAC-ethylacetate,EAC/E-ethylacetate/ethanol,E-ethanol).

DISCUSSION

The results of comparative hypoglycemic effect of column (purified) effluents of ethanolic fraction of A.occidentale leaf are depicted in figure 1. The fractions were compared with the standard hypoglycemic drug (Metformin). The latter is an oral antidiabetic drug of biguanide origin known to exert its effects in the liver thereby inhibiting gluconeogenesis. It is usually well tolerated with minimal side effects, though with some

patients developing gastrointestinal side effects but exceedingly rare (Nathan, *etal*., 2009; Haries *etal*., 2009; Noor, *etal*., 2009). The ethylacetate fraction (200mg/kg.bw) gave the same hypoglycemic potential with the metformin drug which is an indication that the active principles in the fraction were soluble in ethylacetate in its purified state and may posses same antidiabetic effects but may differ in mode of action. Thus, the bioactive principles are soluble in intermediate polar solvents. However, the ethylacetate fraction (300mg/kg. bw) had a lower hypoglycemic activity which may be due to the physiological variations in the animals investigated. Thus, the bioactive principles are soluble in intermediate polar solvents.

The results of the antibacterial activity of the stepwise fraction of A. occidentale leaf were as presented in plates I, II and III. The antibacterial activity revealed that the antibacterial principles that inhibits Salmonella typhi (gram negative) are soluble in less polar solvents while that of Staphylococcus aureus (gram positive) are soluble in intermediate polar solvents. However, the active principles that inhibits Kliebsella pneumonae (grasm negative) resides in solvents of varying polarities. The activity of the fractions may be attributed to the presence and action of the phytochemicals. This is in agreement with the reports of Ayepola and Adeniji (2008) on the antibacterial activity of leaf extracts of Eucalyptus camaldulensis in which it was reported that the activity of the extracts was due to the presence of the phytochemicals especially polyphenolic compounds and volatile oils. The mechanism of action of tannins is based on their ability to bind proteins thereby inhibiting protein synthesis (Ayepola and Adeniji, 2008). It was also reported by Kudi (1999) that A.occidentale had good invitro antibacterial activity against E.coli and Pseudomonas species. Akinpelu (2001) reported that bark extract of A.occidentale exhibited invitro antimicrobial activity against 13 of 15 microbes examined. In the same study, the extracts of A.occidentale leaf was reported to exhibit activity against gram negative bacterium *Helicobacter pylori* which is a causative agent of stomach ulcer.

The spectral studies (GC-MS) of the most active fraction (ethylacetate) as presented in Figure 2 and 3 revealed that the likely active hypoglycemic principles contained in the fraction are: 1,2- benzenedicarboxylic acid, mono(2-ethylhexyl) ester and Bis (2-ethylhexyl) phthalate. This finding is in agreement with that of Nageshwara and Mahesh (2000) who reported that the antidiabetic activity of Casia auriculata leaves was linked to di-(2-ethylhexylpthalate). This also implies that the bioactive principles were isolated without loss of activity and hence crude extracts of A. occidentale leaf can be standardized and used as phytomedicine. The two bioactives are known to exert their effect by activating the gamma peroxisome proliferators activated receptors of the rat liver which facilitates the binding of insulin to receptors hence sensitizes the glucose uptake by the cells. The thiolidinediones are oral hypoglycemic drugs found to act like the two bioactives. Although, the administration of insulin sensitizers like thiolidinediones to diabetics are known to cause or induce toxicity and liver cancer through the activation of gamma PPARs, researches are ongoing to eliminate the toxicity (Moses *etal*., 2004)

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