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Appraisal of anti-diabetic and phytoconstituents of the methanol stem bark extract of *Anisopusmannii* in alloxan induced diabetic rats

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Abstract

This study was carried out to investigate the phytochemicals and also analyze the anti-diabetic effects of the stem bark extracts of Anisopusmanniiwith a view of determining the efficacy of the plant as an anti-diabetic. Fresh stem bark of Anisopusmanniiwas collected from konduga Local Government Area, Borno State and was air-dried under shade, pulverized to powder and extracted with 85% methanol using soxhlet extraction technique to afford a gummy mass weighing 14.57 g. The crude methanol extract (60 g) was fractionated with n-haxaneand then subjected to phytochemical evaluation and anti-diabetic activity. The results revealed the presence of saponins, flavonoids, cardiac glycosides, carbohydrates, tannins, terpenes and steroids. The anti-diabetic effects were tested on alloxan-induced diabetic rats, the extracts showed significant (P<0.05) decrease in fasting blood glucose level in diabetogenic rats at 1st hours (8.10±3.20) to 4th hours (7.08±2.02) at the dosage of 250 mg/kg of methanol crude extract. Similarly, significant (P<0.05) dose dependent decrease in FBG level were observed at 500 mg/kg at all the study time range from (7.66±2.26) to (6.18±2.140). Therefore, this study support the folkloric claim for the use of this plant Anisopusmanniias a remedy to diabetes.

Key words: *Anisopusmannii*, diabetic, antidiabetic
Abbreviation: FBG- Fasting blood glucose

Introduction

The use of plants as medicine is an ancient practice common to all societies especially the African society. This practice continues to exist in the developing nations. It is on this basis that researchers keep on working on medicinal plants in order to produce/develop the best drugs that have physiological uses (Usman and Osuji, 2007).Man uses plants forvarious reasons; the most useful of it all is the management of different diseases (Mustapha, 2013). The affordability, availability and accessibility rendered Africans to plants as the primary source of healthcare management. Such traditional medicines often are herbs and other plant materialswhose efficacy and safety has a very wide claimed (Adzu *et al*., 2004).

*Anisopusmanii*is a small genus of plants in the dogbane family first described as a genus in 1895 (Brown, 1895). The group is endemic to tropical Africa (Brown, 1895). It is a familiar herb in the traditional medicinal

preparations in Northern Nigeria, where a decoction of the leaves is used as remedy for diabetes mellitus, diarrhoea and pile(Aliyu *et al*., 2011).

Diabetes mellitus is a common and very prevalentdisease affecting the citizens of both developed anddeveloping countries. It is estimated that 25% of theworld population is affected by this disease. Diabetesmellitus is caused by the abnormality of carbohydratemetabolism which is linked to low blood insulin levelor insensitivity of target organs to insulin (Maiti *et al*., 2004). A lot of concerted effort has been made in the treatment of diabetesthrough different route for the discovery of newer drugs simplydue to the existing limitations of synthetic drugs.

There is no existing herbal formulation with scientifically proven efficacy in north-eastern Nigeria. Hence, this study will appraisethe traditional claim of the use of *Anisopusmannii* as anti-diabetic drug.

Sample Collection and Identification
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The herbarium sample of the plant material was collected from Dalwa village in Konduga Local Government Area of Borno State, Nigeria. The sample was identified and authenticated by a plant taxonomist from the Department of Biological Sciences University of Maiduguri, Borno State, Nigeria. Professor S.S. Sanusi, Specimen voucher number 7019A was allocated and deposited at the herbarium of Postgraduate Research Laboratory Department of Chemistry, University of Maiduguri.

Sample Preparation and Extraction

The fresh plant sample was cleaned and air-dried under shade at room temperature for seven days. The air-dried plant material was pulverized into powder using wooden mortar and pestle. One kilogram (1kg) of the powdered stem bark was extracted exhaustively using soxhlet apparatus for seven days using 85% methanol as the solvent. The extract was concentrated to dryness on water bath at 45^{o}C.The extract was weighed, labeled and kept for further work

Fractionation of Crude Methanol Extract

Sixty gram (60 g) of the *Anisopus mannii* methanol extract (AMME) was successively partitioned with *Anisopus mannii* n-hexane (AMNH), *Anisopus mannii* ethyl acetate (AMEA), *Anisopus mannii* n-butanol (AMNB) and *Anisopus mannii* residual aqueous portions (AMRA) respectively. The partitioned portions obtained were tested to evaluate the antidiabetic and phytochemical properties. The portion that showed better activity was subjected to further purification through column chromatography and the different portion obtained was subjected to TLC; However, similar fraction were pooled on the basis of their Rf values for further anti-diabetic effects. The pooled portion was also subjected to preparative thin layer chromatography (PTLC) and phytochemical test; to determine likely compounds that is responsible for anti-diabetic effects. Spectroscopic analyses of the pure compound were determined for identification

Results

Table 1. Phytochemical Evaluation of crude, n-hexane, n-butanol and ethylacete of *Anisopusmanii*

Test	CRE	NHE	NBE
1. Carbohydrates			
i. Molisch's test	+	-	+
ii. Barfoed's test	-	-	-
iii. Fehling's test	+	-	+
iv. Combine reducing sugar	+	-	+
v. Ketoses (Selivanoff's test)			
2. Tannins			
i. Ferric chloride test	-	-	-
ii. Lead acetate test	+	-	+
3. Phlobatannins	-	-	-
4. Glycosides			
i. Free Anthraquinones	-	-	-
ii. Combine Anthraquinones	-	-	-
5. Cardiacglycosides			
i. Keller-killiani's test	+	+	+
6. Steroids			
i. Liebermann-Burchard's test	+	+	+
7. Terpenes	+	+	+
8. Saponins			
i. Frothing test	+	-	+
ii. Fehling solution test	+	-	+
9. Flavonoids			
i. Shinoida's test	+	+	+
ii. Ferric chloride test	-	-	-
iii. Sodium hydroxide test	-	-	-
10. Alkaloids			
i. Dragendroff's test	-	-	-
ii. Confirmatory test for alkaloids	-	-	-

Key: (+) = Present, (-) Absent, CRE =Crude extract, NHE = n-hexane extract, NBE = n-butanol extract.

Table 2: Effects of the Oral Administration of Methanol Stem Bark Extract of *AnisopusMannii*on Fasting Blood Glucose Level in Diabetic Wister Strain Albino Rats.

Treatment	0hrs ml/mol	30min ml/mol	1hrs ml/mol	2hrs ml/mol	3hrs ml/mol	4hrs ml/mol
Normal control+1ml water	3.80± 0.23[a]	4.22±0.35[b]	4.22±0.32[a]	4.26±0.30[b]	4.04±0.18[b]	3.80±0.13[b]
Diabetic control 1ml water	8.62±2.63[a]	7.98±2.66[b]	8.60±2.96[b]	9.98±2.60[b]	7.86±2.76[b]	11.02±4.89[b]
Non Diabetic+250mg Extract	3.68±0.38[a]	3.80±0.42[b]	2.90±o.86[a]	2.72±0.81[a]	2.48±0.76[b]	2.62±0.83[b]
Non Diabetic+500mg Extract	3.24±0.15[a]	4.06±0.31[b]	3.76±0.09[a]	3.70±o.o8[b]	3.68±0.15[b]	3.46±0.12[b]
Diabetic+250mg Extract	9.32±2.65[b]	8.24±3.45[b]	8.10±3.20[b]	7,28±2.65[b]	7.48±2.97[b]	7.08±2.92[b]
Diabetic+500mg Extract	8.68±2.21[b]	7.66±2.26[b]	6.98±2.23[b]	6.76±2.45[b]	6.60±2.09[b]	6.18±2.14[b]
Diabetic+1 ml/kg glibenclamide	11.28±2.90[b]	7.66±2.56[b]	7.86±3.11[b]	7.62±2.66[b]	6.84±2.55[b]	5.36±1.87[b]
Diabetic+1ml/kg insulin	18.24±4.80[a]	18.40±40[b]	16.52±4.79[a]	13.66±3.60[a]	12.50±3.27[b]	10.74±3.68[b]

Values with superscript ‘a’ are statistically significant at P<0.05
Values with superscript ‘b’ are not statistically significant at P>0.05

Discussion

Phytochemical analysis of *Anisopus Mannii* stem bark extract indicates the presence of saponins, flavonoids, cardiac glycosides, carbohydrates, tannins, terpenes and steroids. There is no anthraquinones, the chemical constituents present in the extract have been reported to possess many therapeutic values. For instance, Cardiac glycosides are the drug of choice in the treatment of congestive heart failure (Boyce and Christy, 2004) likewise Saponins are very useful in the management of inflammatory conditions of the respiratory tract (Finar, 1989; Trease and Evans, 1989). Sui *et al*., 1994; Anila and Vijayalakshmi, 2002 reported in their separate findings that saponins have hypoglycemic and anti-diabetic effects. Flavonoids have also been reported to possess hypoglycemic and anti-diabetic effect (Ahmad *et al*., 2000).

Treatment of diabetic rats with the extract in table 2 showed a significant reduction in

Fasting blood glucose (FBG) at the dose tested. The hypoglycaemic activity was comparable with that of the standard drug glibenclamide and insulin in which the extract showed significantly ($P<0.05$) lowered blood glucose from the 2 hrs to the 4th hrs with percentage of 32.62% to 52.48% of glibenclamide. The diabetic control rats showed significantly higher reduction level of fasting blood glucose at 4th hrs, lower body weight, slight increase in both feed and water intake compared to normal control and diabetic rats treated with the different doses of extract and standard anti-diabetic drugs. This is consistent with earlier reports (Urmila *et al*, 2003; Maiti *et al.*, 2004; Maiti *et al.*, 2005; Ugwu *et al.*, 2011), and are established features of diabetes. Therefore the results of this study have revealed the hypoglycaemic effects of *Anisopus mannii* in normal and alloxan induced diabetic rats. *Anisopus mannii treatment* resulted in a significant reduction in the serum glucose levels in normal and diabetic rats at 500 mg/kg dose an effect similar to that of sulphonylureas, which produce hypoglyceamia in normal and diabetic rats by stimulating the pancreatic beta-cells to release more insulin (Goth, 1985).

Therefore this study revealed the presence of an active principle(s) in *Anisopus mannii* which supported it application as agents of lowering blood glucose levels by traditional healers.

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