Phytochemical and antidiabetic studies of ethanolic extracts and fractions of the fruits of *Solanum anomalum* Thonn. Ex. Schumach.

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ABSTRACT

The antidiabetic potential of crude ethanolic extract (452 and 678mg/kg), aqueous fraction(452mg/kg) and N-hexane fraction(452mg/kg) of the fruits of *Solanum anomalum* was evaluated on Alloxan-induced diabetic Rats and the results compared to the standard drug, Glibenclamide(2.5mg/kg). Animals were divided into six (6) groups of six Rats each.

Pretreatment with Alloxan was carried out with 150mg/kg and animals with blood glucose levels (BGL) above 200mg/dl in 72 hours were considered diabetic. Study was carried out in acute case (upto 6 hours) and in prolonged treatment (14 days). Results show a significant (p<0.001) reduction in blood glucose level of the diabetic rats in both acute and prolonged treatment comparable to Glibenclamide. The LD50 of the extract was determined to be 2260 ± 131.78mg/kg using the method of Tainter and Miller. Phytochemical analyses of the fruit show the presence of Alkaloids, Saponins, Cardiac glycosides, Terpenes, Flavonoids and Tanins.

Ethanolic extract and fractions of the fruits of *Solanum anomalum* were therefore found to possess significant antidiabetic effect.

Keywords: Antidiabetic, *Solanum anomalum*, Blood glucose level, LD50, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of high global public health significance. It is characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both (1). It affects the metabolism of carbohydrates, fats, proteins and electrolytes in the body, leading to severe complications such as hypoglycaemia, diabetic ketoacidosis, thirst, polyuria, lack of energy, visual blurriness and weight loss (2-4).

Diabetes is classified as Type 1, Type 2 and Gestational diabetes. Type 1 diabetes (previously known as insulin-dependent, juvenile or childhood –onset, is characterized by deficient insulin production. Type 2
diabetes (formerly called non-insulin dependent or adult-onset) results from the body’s ineffective use of insulin. Type 2 diabetes comprises 90% of people with diabetes around the world and is largely the result of excess body weight and physical inactivity.

Gestational diabetes is hyperglycaemia with blood glucose values above normal but below those diagnostic of diabetes, occurring during pregnancy (5).

World Health Organization (WHO) projects that diabetes will be the 7th leading cause of death in 2030. In 2014, the global prevalence of diabetes was estimated to be 9% among adults aged 18 years and above. In 2012, diabetes was the direct cause of 1.5 million deaths. More than 80% of diabetes deaths occur in low- and middle-income countries (5).

The plant, Solanum anomalum (Children’s tomato) is a shrub up to 2 meters tall, usually armed with prickles up to 5mm long on stem, branches and midrib of the leaves, family, Solanaceae.

The fruit is a globose (ball-shaped) berry 5-9 mm in diameter, green when young, shiny red when mature (6). They taste bitter and are normally eaten by older people, often times with palmwine. In Nigeria the fruits are used as a laxative and as a digestive. In a particular village called Ikot Nta Itumbonuso in Ini Local Government Area of Akwa Ibom State, a young child with obvious symptoms of Splenomegalgy, a diseased condition known locally as Ikpakup, was reported to have recovered fully by the mother after repeatedly eating the raw fruit every morning. In Ghana, fruit juice was applied to sores on the ears to alleviate pain (7). It contains Saponins, Cardiac glycosides, anthraquinones, terpenes, flavonoids and alkaloids (8). This paper reports the antidiabetic potential of Solanum anomalum fruit extracts/fractions.

**MATERIALS AND METHODS**

**Plant Material**

Fresh fruits of Solanum anomalum were collected in June, 2012 from a farmland in Obot Ndom Itumbonuso in Ini Local Government Area of Akwa Ibom State, Nigeria. The Plant was identified by Dr. (Mrs.) M.E.Bassey, a plant taxonomist in the Department of Botany and Ecological Studies, University of Uyo. The plant specimen and voucher number UUH: No 75(a) was deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

**Plant preparation and Extraction**

The ethanolic extract was prepared by maceration (cold extraction) of 250.01g of the air-dried, powdered fruits of S. anomalum using 50% ethanol in distilled water (v/v) in an extracting jar. This set up was allowed to stand for 72 hours with occasional shaking. The extracts were filtered, concentrated until constant weights were achieved and stored in a refrigerator at 2-8°C for use in subsequent experiments. This procedure was repeated 3 times for maximal extraction (yield 69.6%).
Partitioning and Fractionation
150g of the crude extract was partitioned with 100ml of n-hexane and 100ml of distilled water. The ethanolic extract was dissolved in distilled water and subsequent partitioning with n- hexane to obtain the n-hexane fraction and the aqueous fraction. The aqueous fraction was subsequently partitioned with ethanolic extract and n-hexane to obtain the n-hexane fraction and then the aqueous fraction. The fractions were separately concentrated to dryness in vacuo at 40°C to give the dried fraction.

Phytochemical Screening
Preliminary phytochemical screening of the ethanolic extract was carried out using the standard procedures (9, 10) to identify the different phpto-constituents present in the extract.

Animals
The animals (mice and rats) of both sexes were obtained from University of Uyo animal house. Rats weighing (100-140g), mice weighing (30-35g). They were maintained on standard animal pellets (Guinea feed) and water ad libitum. Housed in cages to acclimatize to the animal house in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo and maintained under standard conditions (12 light and 12h dark cycle, 25±2°C).

Acute Toxicity
The method of Tainter and Miller (11) was used for the determination of Median Lethal Dose (LD50). The Ethanolic extract of Solanum anomalum was injected intraperitoneally into the mice. Seven groups of six (6) mice per group were used for the tests. Groups 1-7 were injected with 1000, 1500, 2000, 2200, 2300, 2700 and 2800 respectively. Physical signs of toxicity such as drowsiness, stretching, reduced mobility, decreased breathing rate were observed after 30 minutes of sample administration. Mortality in each group within 24 hours was recorded, percentage mortality calculated and the percentage was transformed into probit values by referring to the tables for “transformation of percentage to probits. A graph of percentage death in (probits) was plotted against log-dose and the dose corresponding to probit 5 i.e 50% was read to be the LD50.

Induction of Diabetes
The animals were fasted overnight and experimental diabetes was induced by single intra peritoneal injections of freshly prepared solution of alloxan (150mg/kg body weight) in distilled water. Control rats were injected with distilled water alone.
After 72hr, rats with hyperglycaemia (blood glucose level range above 200mg/dl were considered as diabetic and used for the treatment.

Experimental Design
The diabetic animals were divided into six groups of 6 rats each and treated as follows:
Group 1: Negative control group: diabetic rats were given 10 ml/kg/day of normal saline for 14 days.
Group 2: Positive control: diabetic rats given 2.5mg/kg/day of glibenclamide orally in aqueous solution for 14 days as the standard oral hypoglycaemic agent for comparison.
Group 3: diabetic rats were given middle dose of crude extract 452 mg/kg in aqueous solution for 14 days.
Group 4: diabetic rats were given high dose of crude extract 678 mg/kg in aqueous solution for 14 days.
Group 5: diabetic rats were given 425 mg/kg of the aqueous fraction per orally for 14 days. Group 6: diabetic rats given 452 mg/kg/day of the N-hexane fraction per orally for 14 days.

All the diabetic rats were weighed using electronic balance before commencement of the treatment. The respective doses for the different groups were administered per orally to the diabetic rats daily for 14 days. On the first day, acute monitoring of the blood glucose levels of the diabetic rats was carried out at 0 hr, 1 hr, 2hr, 4hr and 6 hr, using a glucometer with blood obtained from the tail tip of each rats.

**Statistical Analysis**
Data are reported as mean to standard error of the mean (SEM) and were analyzed statistically using one way ANOVA followed by Tukey–Kramer multiple comparison test and values of P<0.001 and 0.05 were considered significant.

**RESULTS**
Preliminary Phytochemical screening revealed the presence of alkaloids, saponins, cardiac glycosides, terpenes, flavonoids and tannins.
The LD50 was determined to be 2260+ 131.78mg/kg. The crude extract (452 and 678 mg/kg) produced dose-dependent reductions in blood glucose level (BGL) in alloxan-induced diabetic rats relative to control during acute studies. These effects were statistically significant (p<0.01-0.001) and progressed for 6 hours. The effect of the doses of the crude extracts used (452-678mg/kg) at the end of 6 hours as well as that of N-hexane fraction were more significant than that of the standard drug, glibenclamide (Table 1 and Fig.1).
On prolonged treatment (14 days), the extract and fractions produced sustained reductions of BGL in diabetic rats. These reductions were significant (p< 0.001) when compared to control. The effects of the doses of the crude extract were comparable to that of N-hexane fraction but less than that of the standard drug, glibenclamide, 2.5 mg/kg, on day 14(Table 2 and Fig.2)

**DISCUSSIONS**
The crude ethanolic extracts/n-hexane fraction demonstrated significant antidiabetic activity in alloxan induced diabetic rats (Tables 1&2; Figures 1&2). Some phytochemical compounds such as polysaccharides (12), terpenes and tannins (13), and alkaloids (14) have been implicated in the antidiabetic activities of plants. Phytochemical studies of the extract revealed the presence of terpenes, saponins, tannins and alkaloids. These constituents may in part be responsible for the observed significant activity of this extract either singly or synergistically. Some plants have been observed to exert hypoglycaemic activity by
stimulating insulin release from the cells of islets of Langerhans or its release from bound insulin \( (15,16) \). Other plants act through extra pancreatic mechanisms by inhibition of hepatic glucose production \( (17) \). Over-production and inadequate utilization of glucose by the tissues is the fundamental base of hyperglycaemia in diabetes mellitus \( (18) \). Current drugs used in the management of diabetes such as metformin, pioglitazone, acarbose and miglitol have side effects such as liver toxicity, cardiovascular risk, flatulence, abdominal bloating \( (19, 20) \). Extracts of *Solanum anomalum* if found to be devoid of the above side effects would serve as a lead for the production of newer and probably less expensive antidiabetic drug.

**Table 1 Antidiabetic Effect Of Ethanolic Extract And Fractions Of Solanum anomalum ON ALLOXAN-Induced Diabetic Rats In Acute Study**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DOSE (mg/kg)</th>
<th>BLOOD GLUCOSE LEVEL (mg/dl) in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>10ml</td>
<td>241.8±3.96 252.8±6.96 263.0±5.63 268.0±4.85 277.2±6.52</td>
</tr>
<tr>
<td>CRUDE EXTRACT</td>
<td>452</td>
<td>248.7±7.07 203.4±5.88C 165.0±5.40c 149.0±6.32c 116.8±5.63c</td>
</tr>
<tr>
<td>N-HEXANE FRACTION</td>
<td>452</td>
<td>241.0±8.01 197.8±5.19C 162.8±4.44C 147.2±5.41c 123.6±3.34c</td>
</tr>
<tr>
<td>AQUEOUS FRACTION</td>
<td>452</td>
<td>244.4±8.09 240.8±4.83a 237.2±5.89b 232.4±5.08b 226.4±6.46c</td>
</tr>
<tr>
<td>GLIBENCLAMIDE</td>
<td>2.5</td>
<td>248.6±3.72 155.8±2.71c 153.8±3.31c 148.2±5.70c 137.0±9.52c</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. Significant at \(^a p< 0.05, ^b p< 0.01, ^c p< 0.001\). When compared to control. \( n=6 \)

**Table 2 Anti Diabetic Effect Of Ethanolic Extract And Fractions Of Solanum Anomalum On Alloxan-Induced Diabetic Rats in Prolonged Study**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DOSE (mg/kg)</th>
<th>BLOOD GLUCOSE LEVEL (mg/dl) in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>10ml</td>
<td>241.8±3.96 293.4±8.11 290.0±3.52 283.8±3.91</td>
</tr>
<tr>
<td>CRUDE EXTRACT</td>
<td>452</td>
<td>248.7±7.07 151.2±4.11a 146.4±4.96a 122.6±6.80a</td>
</tr>
<tr>
<td>N-HEXANE FRACTION</td>
<td>452</td>
<td>249.6±3.82 131±7.63a 126.0±3.84a 122.0±5.83a</td>
</tr>
<tr>
<td>AQUEOUS FRACTION</td>
<td>452</td>
<td>244.4±8.09 171.2±8.84a 150.0±3.74a 128.6±4.12a</td>
</tr>
<tr>
<td>GLIBENCLAMIDE</td>
<td>2.5</td>
<td>248.6±3.72 212.4±6.62a 147.4±2.41a 133.4±2.08a</td>
</tr>
</tbody>
</table>

Data are expressed as mean \( ± \) SEM. Significant at \( p< 0.001 \), when compared to control \( n=6 \)
CONCLUSION

Crude ethanolic extracts and fractions of the fruits of *Solanum anomalum* have potential antidiabetic activity on diabetic rats. The crude ethanolic extracts contain bioactive phyto-constituents which possibly may be responsible for the antidiabetic effect of the fruits.

However, further studies should be carried out to elucidate the exact molecular and cellular mechanism(s) of action.
REFERENCES

10. 137-143.

