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Anti-diabetic efficacy of ethanol leaf extract of *Hibiscus surrattensis* (linn.) on alloxan -induced diabetic rats

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Abstract

Diabetes is a chronic disease caused by insufficient insulin production or the inability of the body to use the insulin produced. *Hibiscus surattensis L.* is a plant species from the genus *Hibiscus* (Malvaceae), with leaves that has been used in traditional medicine to treat diabetes. The ethanol leaf extract of *Hibiscus surattensis (Linn.)*, was evaluated for its anti-diabetic properties on alloxan-induced diabetic rats. The experimental design used was complete randomized type for all the studied parameters undertaken. The standard Qualitative and quantitative phytochemical screening of the plant extract gave positive test for saponins, alkaloids, flavonoids, phlabotanins, candiac glycoside and deoxysugar. Proximate analysis showed Moisture content (83.15 %), Crude liquid (1.4 %), Crude protein (1.75 %), Crude fibre (0.1%), Ash (13.46 %), Carbohydrate (0.14 %) and Calorie value (20.16 kcal). The medium lethal dose (LD50) was determined using intraperitoneal route which was calculated to be 4698.94 mg/kg. Animals in group 1; received 10mg/kg body weight/day of distilled water group 2; received 10 mg/kg of glibeniclamide, while group 3-5; received the low, medium and high doses of the plant extract. The anti-diabetic activities during acute and prolonged studies were investigated. Blood Glucose Level (BGL) was measured at intervals by using glucometer. The treatment was significant at (P = 0.05) causing reduction in BGL of the diabetic rats both in acute and prolong treatment. There were reductions in tissue degradation in treated diabetic rat compared to untreated rats

Keywords: *Hibiscus surattensis*; Diabetics; Alloxan; Glibenclamide; Phytoconstitueuts; Proximate; Blood Glucose Level (BGL)

1. Introduction

Hibiscus Surattensis, occur in tropical Africa and tropical Asia. It has been introduced into tropical America and is locally naturalized there [1, 2] *Hibiscus surattensis*, "Bush Sorrel" is a weak-stemmed, prostrate or climbing plant covered with soft hairs and scattered prickles. It flowers between the months of September-March [3, 4]. It is consumed as delicacy, used as candy and garnish [5]. Polyphenol compounds are present in the leaves, which also show anti-inflammatory activities. Study reported that consuming *Hibiscus* tea lowers blood pressure in a group of prehypertensive and midly hypertensive adults [6, 7, 8]. *Hibiscus surattensis*, commonly called "Ifot ebot" or "Afat Ibam" in Ibibio, while it called "Okpolimlin" in Yakurr language in Cross River State. In Tanzania leaf sap is taken to prevent miscarriage and treat vertigo, where as the root decoction is used as laxative [9, 10]. An ointment made from leaves of *Hibiscus surattensis*, and the leaves and root decorations is used to treat inflammations, gonorrhea, itching caused by chicken pox.

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Although a number of studies have been carried on this plant, there was no scientific report on the hypogylycamic activity, this therefore necessitated the investigation into the anti-diabetic property of the plant to confirm its use locally.

2. Material and methods

2.1. Materials and Methods Preparation of Plant Extract

Fresh leaves of *Hibiscus surattensis* (Linn) were collected on 26th of April, 2015 from a farm land at Ugep in Yakurr Local Government Area of Cross River State, Nigeria. Samples of the plant species were collected for authentication by a taxonomist in the department of Botany and Ecological Studies, University of Uyo, Uyo Nigeria. A Voucher specimen was deposited at the Departmental Herbarium with a voucher No: Oyomah, UUH3417 (Yakurr Cross River). The fresh leaves (3kg) were dried on the laboratory table for 2weeks. Two hundred and fifty grams (250g) of the dried powder of the leaf sample were extracted with 900ml of 95% ethanol for about 72hours with occasional agitation. The homogenate was filtered, and the filtrate was evapourated to dryness in vacuum at 40^0C using rotary evaporator. The yield was 9.4g. The extract was stored in a refrigerator at 4^0C until used.

2.2. Animals

Albino wistar rats of both sexes weighing between (80-110g) used for this experiment were obtained from the university of Uyo animal house. They were maintained on standard animal pallet and water ad libitum. Permission and approval for animal studies were obtained from College of Health Sciences Animal Ethic Committe. University of Uyo. The 10%, 20% and 30% of LD_{50} were used as working doses implying low, middle and high dose respectively.

2.3. Determination of LD50

The LD_{50} of the extract was estimated using swiss albino mice by intraperitoneal (i.p.route using the method of lorke [11]. The LD_{50} was calculated to be 4698.94mg/kg.

2.4. Induction of Diabetes

The method of [12] was used. The entire experimental animals were fasted for 24hours, they were re-weighed and 150mg/kg of alloxan monolydrate (BDH) was injected intraperitoneally to induce hyperglycemia. A rest perod of one hour was allowed during which rats were allowed access to food and water and the fructose developed fully during 72 hours of rest. Rats with moderate diabetes having persistent glycosuria (Indicated by Benedicts Qualitative Test), were considered diabetic and selected for the experiments.

2.5. Quantitative and Qualitative Phytochemical Screening

Standard qualitative and quantitative phytochemical tests were carried out with the leaf extract to elucidate the presence or absence of some bioactive compounds in the plant species. The standard procedure described by [13], were used for the quantitative phytochemical screening of the leaf extracts. The quantitative phytochemical composition of the leaf extracts were determined using the method described by [14, 10].

2.6. Proximate Analysis

The standard recommended method of the Association of Official Analytical Chemists were used for the determination of moisture content, Crude Protein, Crude Fat, Carbohydrate, Crude Fibre and Ash [15, 16].

2.7. Evaluation of Anti-diabetic Activity

The Blood Glucose Level (BGL) of the animals was monitored after alloxanisation in blood samples collected by amputation of the tail tip under mild anaesthesia. The blood was dropped on the dextrostix reagent pad. The strip was inserted into micropress or digital blood glucometer and the reading were noted [17]. After 72hours of rest, rats with BGL above 150mg/dl of blood glucose were selected for the experiment and divided into five groups of five animals each. The extracts 468.89, 939.78 and 1469.6mg/kg were orally administrated to the respective groups of animals. The reference drug glibenclamide (10mg/kg) and normal saline were also administered orally to two groups animals

representing reference and control groups respectively. The treatments were carried out in each group of animals for 14days. The blood glucose level was monitored after 0, 1, 3, 5, 7 and 24hours of administration of a single dose of the extract (for acute study) and at the end of 7 and 14days (for prolong treatment).

2.8. Statistical Analysis

Data obtained were analyses using the students t-test to determine statistical significance of the change in BGL $P<0.05$ was considered significant [18].

3. Results

3.1. Phytochemical Screening

Table 1 Phytochemical Screening of the Ethanol Leaf Extract of *Hibiscus surattensis*

Sr. No.	Secondary Metabolites	Test Done		Inference
1	Tanings	i	Ferric Chloride	ND
2	Saponnins	i	Frothing	+++
		ii	5%$NaCO_3$	+++
		iii	Fehling	+++
3	Alkaloids	i	Dragendoff's	+++
		ii	Hagger's	+++
4	Phlobatannins	i	Dilute HCl	ND
5	Cardiac-Glucoside	i	Keller-Killiani	+++
		ii	Sakowski	+++
6	Flawonoids	i	Shinoda	ND
7	Anthraquinones	i	Free Borntrager's	ND
8	Deoxy	i	Ferric Chloride	++
9	Terpenes	i	Acetic Anlydride and Conc.H_2SO_4	+

KEY: + = Present in low concentration; ++ = Present in moderate concentration; +++ = Present in high concentration; ND = Not detected

Table 2 Quantitative Estimate of Some Phytochemical Constituents in *Hibiscus surattensis*

Constituents	% composition
Saponnins	45.15 + 1.05
Alkaloids	48.72 + 0.54
Flavonoids	00.00 + 0.00
Cardiac-Glycoside	46.46 + 0.70

The value show are means (+ SEM) of triplicate

Table 3 Proximate Analysis of the Leaf of *Hibiscus surattensis*

Parameter	Percentage
Moisture Content	83.15%
Crude Protein	1.75%
Crude Lipid	1.4%
Ash	13.46%
Crude Fibre	0.1%
Carbohydrate	0.14%
Calorie Value (Kcal)	20.16 Kcal

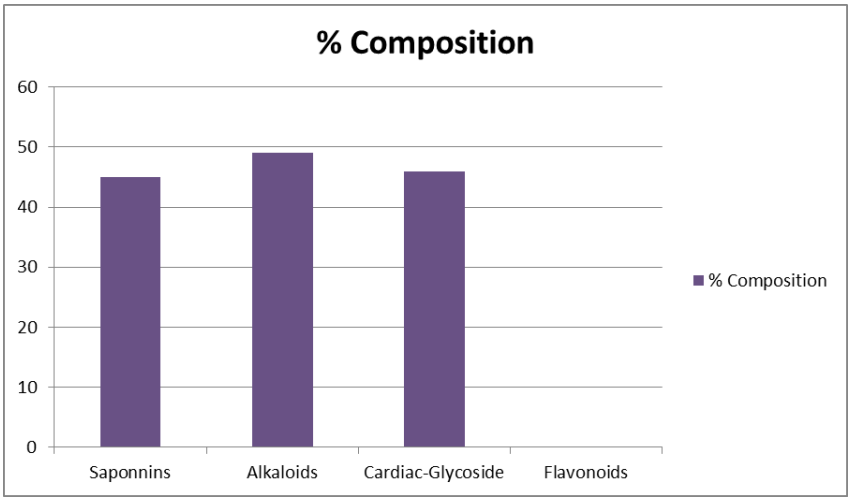

Figure 1 Quantitative Estimate of Some Phytochemical Constituents in *Hibiscus surattensis*

Table 4 Effects of Ethanol Leaf Extract of *Hibiscus surattensis* on the Blood Glucose Level of Alloxcan-Induced Diabetic Rats

Group	Treatment Doses of Drug	Acute Treatments Blood Glucose Level (mg/d/) (Mean ± SDM)					
		0 Hour	1 Hour	3 Hours	5 Hours	7Hours	24 Hours
1	10ml/kg distil water	566.1±7.914	262.2± 8.418	267.3±8.271	263.1±8.143	262.1±8113	276.4±9.482
2	10mg/kg glibenclamide	270.4 ±7.314	263.7±7.253	245.05±7.413	238.4±8.245	233.8±8.086	232.6±9.816
3	468.89/kg *H. Surattensis*	239.3 ± 5.281	236.6±9.823	223.3±8.178	209.6±8.791	200.7±8.917	194.5±9.738
4	939.78mg/kg Extract	255.5 ±7.412	253.2±6.810	245.2±8.123	246.0±8.123	232.3±9.516	194.5±9.738
5	1469.6mg/kg of Extract	243.8 ±8.418	239.1±6.642	238.6±6.423	224.3±8.251	217.1±8.035	199.7±6.968

Data are expressed as mean ± SEM for five animal per group (P = 0.05, 0.01) when compared to control

Table 5 Effects of Ethanol Leaf Extract of *Hibiscus Surattensis* on the Blood Glucose Level of Alloxcan-Induced Diabetic Rat (Prolonged treatment)

Group	Drug	Dose	Blood Glucose Level (mg/d/) (Mean ± SEM)			
			Initial	1st Day	7th Days	14th Days
1	Distilled water	10ml/kg	256.1±7.914	276.4±9.482	281.3±5.840	356.01±16.010
2	Gliben clamide	10mg/kg	270.4±7.314	232.6±9.816*	231.2±10.112*	226.4±9482**
3	Extracts	468.89mg/kg	239.3±5.281	194.5±9.738*	171.5±12.402*	156.3±6.313**
4	Extracts	939.78mg/kg	255.5±7.412	218.1±10.415*	195.3±9849*	168.6±1.304**
5	Extracts	1469.6mg/kg	243.8±8.418	199.7±6.968*	163.3±5.401*	126.3±9.236**

N = 5, *P<0.05, 0.01 compared to the control.

The results of phytochemical screening of the ethanol leaf extract of *Hibiscus surattensis* revealed a number of bioactive constituents, as summarized in Table 1 below which include Saponinins, Alkaloids, Cardiac-Glycoside, Terpenes and Deoxy-Sugar. Saponinins, Alkaloids, Cardiac-Glycoside were present in high concertration, while terpenes were present in low concentration.

The various doses of ethanol leaf extract of *Hibiscus surattensis* administered to alloxan- induced diabetic rats produced a significant (P<0.01) reduction in BGL of the diabetic rats in a dose dependent fashion in both acute and prolonged treatment compared to the control group. Hypoglycaemic effect during prolonged treatment (14 Days) was comparable to that produced by the standard reference drug (Table 5).

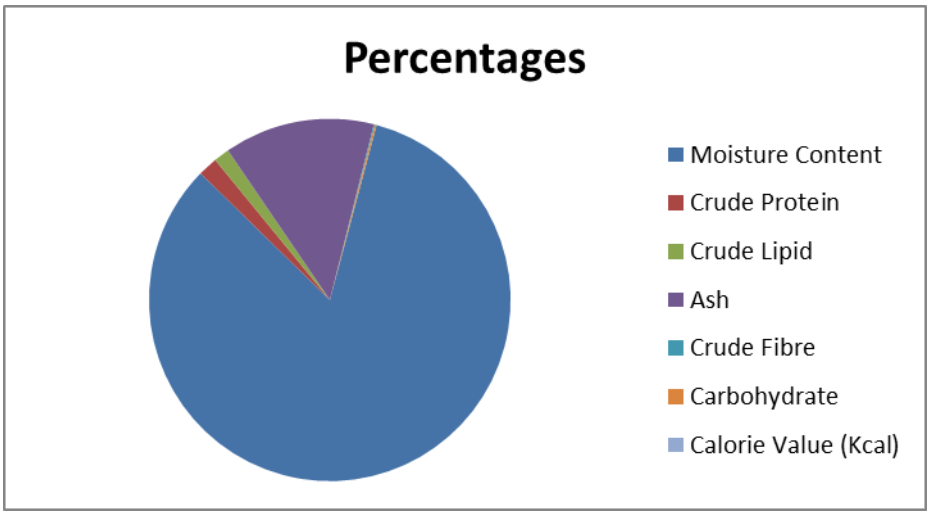

Figure 2 Pie-Chart Showing Proximate Analysis of *Hibiscus surattensis*

4. Discussion

Some plants have been used locally to treat diabetes and some have been proven scientifically and its hypolycaebimic effect reported. Evaluation of anti-diabetic activity of ethanol leaf extract of *Hibiscus surattensis* was carried out i alloxan-induced diabetic rats. Some secondary metabolites have been reported to be involved in anti-diabetic activity of many plants [19, 20].

The phytochemical screening constituents common in medicinal plant were Tannins, Terpenes, Flavonoids, Cardiac-Glycoside, Saponnins and Alkaloids [21, 22]. Flavonoids, Tannins and alkaloids have been implicated in antidiabetic activities of plants [23, 19, 4]. The qualitative phytochemical screening of the leaf of *Hibiscus surattensis* showed that

the plant is rich in Saponins, Alkaliod, Tannins and Cardiac-Gycosides. The proximate analysis showed some level of proteins made of amino acid which are essential in the body building, boosts immunity and thereby in proving health outcomes of the consumers [24], the presence of some level of carbohydrate is of great significance in meeting the energy needs of the person's physical and mental activities. Fibre is important for gasto-intestinal tract functions as well as preventing and managing a variety of disease [25, 26].

The anti-diabetic effects of the plant leaf extract were highly significant as when compared to the control. The observed reduction in the blood glucose level (BGL) of the diabetic rats by glibenclamide in this study portrays an in severe state of diabetes. Continuous treatment with the extract. For the period of 2weeks caused decrease in BGL of treated rats compared to untreated diabetic rats. This was followed by corresponding increase in body weight of the treated rats due to reduction in the degradation of the animal tissues. Diabetes is characterized by a severe loss in body weight due to loss or degradation of structural proteins [18]. This was alleviated by the treatment of the diabetic rats with ethanol leaf extract of *Hibiscus surattensis*.

The untreated alloxan-induced diabetic rats exhibited significant reduction in their body weight.

5. Conclusion

The results from this study revealed that the leaves of *Hibiscus surattensis* were rich in some medicinal metabolites. The proximate analysis of the leaves showed it is edible and safe for consumption. The parameters studied showed; it is good for diabetic patients. The ethanol leaf extract obviously reduced the fasting blood glucose levels in the treated diabetic rats and gradually reduced the metabolic breakdown of diabetic rats' tissue. This plant is recommended Therapeutically, Traditionally and pharmaceutically, for diabetic treatment.

Compliance with ethical standards

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Disclosure of conflict of interest

The Authors declare that there is no conflict of interests regarding the paper production.

Statement of ethical approval

The present research work was performed using animals for the experimental analysis.

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