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Phytochemical screening and Antidiabetic activity of extracts of *Citrullus lanatus rind* in Alloxan-Induced Diabetic Albino Mice

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Abstract

A number of plants products among which the protein-rich Cucurbitaceae seeds are commonly used in traditional medicine with increasing acclaimed efficacy against diabetes mellitus. In this study the anti-diabetic effect of ethanol and petroleum ether extracts of Citrullus lanatus (watermelon) rind in alloxan-induced diabetic mice have been investigated. Phytochemical screening of the petroleum ether and ethanol extracts of the rind was carried out and diabetes was induced in mice by the injection of 150 mg/kg (i.p.) of alloxan monohydrate freshly dissolved in physiological saline. Doses (150, 200 and 250 mg/kg) of the extracts were administered each to a group of five diabetic mice in the study. The activity was compared with reference standard glibenclamide (2 mg/kg) and negative control of physiological saline. Treatment of the alloxan-induced diabetic mice with the extracts of Citrullus lanatus rind decreased the raised blood glucose levels significantly ($P<0.05$) in a dose-dependent manner (with ethanol extract more effective than the petroleum ether extract). Results of phytochemical screening of the rind extracts have indicated the presence of steroids, terpenoids, saponins, tannins, alkaloids, reducing sugar, flavonoids, and anthraquinones. The results revealed that both extracts of the rind of this plant have anti-diabetic potential. Results have also shown that the ethanol extract was more potent than the petroleum ether extract which is in agreement with the reported claim that ethanol extract in combination with diet supplement has significant antidiabetic activity in albino mice. Moreover, the classes of phytochemicals observed in this plant extracts have previously been observed to contribute to hyperglycemic effects.

Keywords: Alloxan monohydrate, *Citrullus lanatus*, diabetes mellitus, rind, glibenclamide, albino mice.

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1. Introduction

Diabetes mellitus is a major illness of the human race implicated with numerous clinical manifestations. It is a clinical syndrome characterized by chronic hyperglycemia with impaired carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, or decreased sensitivity of the tissues to insulin [1]. As has been reported [2] diabetes mellitus is ranked seventh among the leading causes of death and is considered third when its fatal

complications are taken into account. It has been reported that the chronic hyperglycemia of diabetes is associated with complications like renal failure, coronary artery disorder, neurological complications, cerebro-vascular disease, blindness, and limb amputation, long term dysfunctions and failure of various organs and eventually premature death [3]. According to World Health Organization (WHO) projections [4], the diabetes population is likely to increase to 300 million or more by the year 2025. Diabetes mellitus has also been projected to exceed a prevalence of 380 million by the year 2030, with the type 2 accounting for about 90% of cases worldwide [5]. The disease has an increasing prevalence worldwide. It is rapidly spreading in Africa today as a result of rapid uncontrolled urbanization and westernization of dietary habits. A widely varying prevalence across the African continent has been reported [6]: Benin 3%; Mauritania 6%; Cameroon 6.1%; Congo 7.1%; Zimbabwe 10.2%; Democratic Republic of Congo 14.5%. The WHO suggests that Nigeria has the greatest number of people living with diabetes in Africa, with an estimated burden of about 1.7 million which will increase to 4.8 million by 2030 [7]. The incidence and prevalence of diabetes mellitus has continued to increase in Nigeria, despite a great deal of research and resources [8,9,10].

High cost of conventional drugs with their relatively high incidence of side effects is one important problem with the modern medicine. The management of diabetes without any side effect is still therefore a major challenge. According to the WHO [11], approximately 80% of the world's population currently uses herbal medicines in healing different ailments. Among the estimated 400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemically [12,13]. There is therefore a need for a planned activity guided phyto-pharmacological evaluation of herbal drugs. The anti-diabetic potential of *Citrullus lanatus* (watermelon) was evaluated [14] in vivo where albino mice were fed experimental diet containing none, 10% watermelon flesh powder or 1% watermelon rind ethanol extract. Results have shown that supplementation with rind ethanol extract significantly decreased blood glucose level and increased serum insulin levels. Feeding with flesh powder induced moderate changes but which were not statistically significant. In the present research determination of the anti-diabetic effect of ethanol and petroleum ether extracts of watermelon rind alone - and not when mixed in diet supplement - in diabetic mice is the aim of the study.

2. Experimental

Plant material

Citrullus lanatus was purchased from Unguwar Rimi market Kaduna north, Kaduna state, Nigeria. The rind was removed, washed with water and bench dried under room temperature for eleven days in the absence of direct sunlight. It was then ground into powder using moter and pestle. The powdered material was weighed and kept in an airtight polyethene bag.

Sample extraction

300g of the dried powdered rind was socked in 1 litre of petroleum ether (60-80 ^{o}C) with frequent shaken for three days after which it was filtered using filter paper. The remains was again socked with another 1 litre of the solvent for two days and filtered. The solvent was recovered from the filtrate by using a rotary vacuum evaporator to afford the petroleum ether extract. The same procedure was repeated on the residue, after drying for two days, using ethanol and the solvent removed by using a rotary vacuum evaporator to get the ethanol extract.

Phytochemical screening

Phytochemical screening was carried out on the two extracts (petroleum ether and ethanol) of *Citrullus lanatus* rind using standard procedures and tests [15,16] to determine the presence of alkaloids, tannins, terpenoids, flavonoids, reducing sugar, anthraquinones, and saponins.

Pharmacological study

Animals

White albino mice weighing 22-25g of both sexes were obtained from the department of biochemistry Kaduna State University, Kaduna-Nigeria. The mice were kept in clean and dry aluminium cages under standard environmental conditions of temperature, relative humidity, and with feeding and water. The mice were fasted, however, for 12 hours before experimentation. The experimental methods involving animals have been approved by Animal Research Ethics Committee of Kaduna State University, Kaduna-Nigeria.

Experimental design

Diabetes was induced by a single intraperitoneal injection of 150mg/kg body weight alloxan monohydrate freshly dissolved in physiological saline 0.9% v/w immediately before used to overnight fasted albino mice. After 7 days, animals with fasting plasma glucose level ≥7.0 mmol/dL (or ≥126 mg/dL) or more were considered diabetic, as reported [17] elsewhere, and employed in the study. The mice were then grouped into 5 groups of five mice each as follows:

Group I: Served as positive control and received glibenclamide (2 ml/kg body weight)
Group II: Received petroleum ether extract at 150 mg/kg body weight
Group III: Received petroleum ether extract at 200 mg/kg
Group IV: Received petroleum ether extract at 250 mg/kg

Group V: Received Ethanol extract at 150 mg/kg
Group VI: Received Ethanol extract at 200 mg/kg
Group VII: Received Ethanol extract at 250 mg/kg
Group VIII: Served as negative control receiving physiological saline (10 ml/kg)

The animals were treated once and fasting blood glucose concentrations were measured at 0, 1, 3, 6 and 12 hours. Blood samples were taken by a snip-cut at the tip of the tail and blood glucose levels were measured with a glucometer (a ONE TOUCH Ultra easy blood glucose monitoring system, LifeScan Europe Division of Cilag GmbH international 6300 Zug Switzerland).

Statistical analysis: All the values of fasting blood glucose were expressed as mean ± standard error of mean (SEM.) and analyzed for ANOVA followed by Student's t-test. Differences between groups were considered significant at $P < 0.05$ levels.

3. Results and Discussion

Results

Phytochemical screening

The results of phytochemical studies on both the petroleum ether and ethanol extracts of the rind of *Citrullus lanatus* have been provided in table 1.0.

Table 1.0: Phytochemical analysis of the extracts of C*itrullus lanatus* rind

Chemical Constituents	Petroleum ether extract	Ethanol extract
Steroids	+	+
Alkaloids	-	+
Terpenoids	+	+
Anthraquinones	+	+
Flavonoids	+	+
Reducing sugar	-	+
Saponins	+	-
Tannins	-	+

Key: + = Present; - = Absent

Pharmacological study

The effects of the petroleum ether and ethanol extracts of the rind of *Citrullus lanatus* on fasting blood glucose levels in alloxan monohydrate-induced diabetic albino mice have been given in table 2.0.

Table 2.0: Blood glucose levels in alloxan induced diabetic mice

		Blood glucose levels in (mmol/L) Sampling time in hours				
Test material	**Groups**	**0**	**1**	**3**	**6**	**12**
Glibenclamide (positive control)	Group I	9.8±2.5	10.3±2.8	6.9±3.3	5.6±2.6	3.4±1.3
Petroleum extract						
150 mg/kg	Group II	8.7±2.3	7.9±1.5	6.4±2.8	5.6±3.3	4.2±2.4
200 mg/kg	Group III	8.9±3.6	6.9±2.8	5.2±1.6	4.1±2.9	3.4±1.3
250 mg/kg	Group IV	9.3±3.3	7.4±1.6	5.1±1.1	3.8±2.5	2.3±0.7
Ethanol extract						
150 mg/kg	Group V	9.6±2.8	9.0±2.4	7.4±0.5	4.2±1.9	3.3±1.4
200 mg/kg	Group VI	8.1±3.8	7.2±3.3	6.1±2.7	4.6±2.9	3.1±1.6
250 mg/kg	Group VII	9.2±2.4	8.1±1.7	6.2±2.7	3.9±3.6	2.1±2.8
Physiological saline (negative control)	Group VIII	9.0±3.2	8.5±3.2	8.9±2.8	9.4±1.6	8.6±3.7

P<0.05 with respect to both Glibenclamide and Physiological saline; The data was analyzed using Student's t-test and all results were expressed as mean ± SEM.

Discussion

The results of phytochemical screening have indicated the presence of steroids, terpenoids, flavonoids, and anthraquinones in both the petroleum ether and ethanol extracts. Saponins have been found to be present in the petroleum ether extract only and reducing sugars, tannins and alkaloids were detected in the ethanol extract only. These results are in agreement with that obtained by some researchers [18] who reported that saponins, alkaloids,

tannins, phenols, and flavonoids were present in the seed and rind of Citrullus lanatus. In another study [19], after successive extractions with petroleum ether, chloroform and ethyl acetate, the ethanol extract of the residue of the seeds indicated the presence of tannins and phenols, proteins, alkaloids, flavonoids, carbohydrates, cardioglycosides, terpenoids and the absence of steroids and saponins; with the saponins been found only in the petroleum ether extract and steroids found in both the petroleum ether and chloroform extracts.

The antidiabetic activities of the extracts tested in this study could be attributed to the presence of flavonoids, terpenoids, alkaloids, saponins and anthraquinones, which have been shown to be hyperglycaemic by many researchers. A flavonoid-rich fraction isolated from guava (*Psidium guajava*) leaves has been reported [20] to have lowered blood glucose in humans. Another flavonoid-rich fraction extracted from *Tamarindus indica* and tested in both the alloxan and fructose induced hyperglycaemia in rats showed a significant ($p < 0.05$) reduction in elevated blood glucose level after 8 hours for 400 mg/kg dose and after 16 hours for the 200 mg/kg dose [21]. Saponins could also be responsible for the hypoglycaemic activity. For instance, ginseng and its saponins have been shown to lower blood glucose in alloxan-treated diabetic and normal mice[20]. Saponins in another study [22] have also been shown to reduce serum glucose in elderly patients with hyperglycemia. Terpenoids have also been shown to lower blood glucose in rats [23] and anthraquinones have been said to prolong hypoglycaemic effect in diabetic mice [24,25]. Two anthraquinones isolated from the ethanol extract of rhubarb rhizome have been reported [26] to have anti-diabetic properties. Alkaloids have also been implicated to have hypoglycaemic activity and it was found that the neem seed kernel powder contained hypoglycaemic alkaloids that lowered blood sugar levels in alloxan-induced diabetic rabbits [27].

Alloxan monohydrate destroys β-cells of Islets of Langerhans of the pancreas resulting in a decrease in endogenous insulin secretion and paves ways for the decreased utilization of glucose by body tissues [28]. It results in elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides [29]. In the present study the percentage increase of blood glucose in the untreated group appeared to be higher than that in the treated group. Comparison of the average values of blood glucose levels in the treated and untreated (control) groups of alloxan-induced diabetic mice suggested some favourable antidiabetic effect of *C. lanatus*. However, statistical analysis using student's t-test revealed that there is a statistically valid difference between the treated and the control groups. Both the petroleum ether and ethanol extracts exhibited significant ($P<0.05$) anti-diabetic effect at 6 h and 12 h at the doses of 200 and 250 mg/kg. From the results it can be suggested that both extracts exhibited dose dependent action in a similar mechanism as glibenclamide i.e., by stimulation of surviving β-cells to release more insulin [30]. The antidiabetic activity of raw watermelon rind has been studied in mice using streptozotocin to induce diabetes and results have shown that blood glucose level was significantly decreased [14] The results of the present study, using extracts of the rind, have been in agreement with the previous findings. The results have shown that the ethanol extract was more effective in decreasing blood glucose level in the experimented mice than the petroleum ether extract.

4. Conclusion

The results of this study have revealed that both extracts of the rind of the plant have anti-diabetic potential as they have significantly reduced the fasting blood glucose levels in alloxan induced diabetic mice. Results have also shown that the ethanol extract was more potent than the petroleum ether extract. The results, therefore, support the traditional usage of the plant material for the management of diabetes mellitus. However, the mechanism of action of the extracts needs to be studied and the specific phytochemicals responsible for the activity isolated and characterized.

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