Original Research Article

PHARMACOGNOSTIC PROFILE AND ANTI-DIABETIC ACTIVITY OF *Jatropha tanjorensis* LINN (EUPHORBIACEAE) LEAF ON ALLOXAN-INDUCED DIABETIC RATS.

ABSTRACT

Background: *Jatropha tanjorensis* has been used traditionally for the treatment of a variety of diseases, these include; renal problem, cardiovascular disease, hypertension, inflammation and in moderate depression. The pharmacognostic standardization of it's can be used in the development of it's monograph. Diabetes mellitus is disease that is responsible for millions of death yearly. Over the years, efforts have been made in the discovery of new bioactive compounds with Antidiabetic activity

Objective: The objective of the study was to evaluate the pharmacognostic profile and anti-diabetic activity of methanol leaf extract of *Jatropha tanjorensis* on alloxan-induced diabetic rats.

Method: The phytochemical analysis, pharmacognostic profile and acute toxicity study were done using standard methods. The anti-diabetic activity of the methanol leaf extract of *J. tanjorensis* was investigated by using normal and alloxan-induced diabetic rats for acute and sub-acute studies.

Results: Phytochemical screening revealed alkaloids, flavonoids, carbohydrates, reducing sugars, cardiac glycosides, saponins and tannins. The moisture content obtained was 5.67% w/w. The total ash value was 8.39%w/w, acid-insoluble ash value was 0.72% w/w and water-soluble ash value was 3.91% w/w. There was no mortality or any signs of behavioral changes or toxicity observed after oral administration of *Jatropha tanjorensis* up to the dose of 5000 mg/kg body weight in mice. In the sub-acute anti-diabetic study for 14days, there was a significant ($p<0.05$) dose-dependent anti-diabetic effect of the methanol leaf extract of *J. tanjorensis* of 11.76, 55.51 and 77.65% blood glucose level reduction for 100, 200 and 400 mg/kg respectively, when compared with the negative control (- 23.18 %)and 67.29 % for positive control; Glibenclamide (5 mg/kg)which has less activity.

Conclsion: From this study, it shows that *J. tanjorensis* leaf have anti-diabetic property, justifying its use in traditional medicine for the treatment of diabetes mellitus. The Pharmacognostic profile can be used for a monograph of the plant for its proper identification and quality control.

Key words: Diabetes, Hyperglycemia, *Jatropha tanjorensis*, Medicinal Plant, Pharmacognostic Profile, Alloxan.

1. INTRODUCTION

Diabetes mellitus, one of the major public health problems worldwide, is a metabolic disorder of multiple etiologies distinguished by a failure of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism as a result of defects in insulin secretion and/ insulin action [1]. According to International Diabetes Federation (IDF) report, elevated blood glucose is the third uppermost risk factor for premature mortality, following high blood pressure and tobacco use globally [2].

In 2019, according to IDF report, 463 million adults (20-79yrs) were living with diabetes, and by 2045 this will rise to 700 million. 79 % of adults with diabetes were living in low- and middle-income countries. 1 in 5 people who are above 65yrs have diabetes and have caused 4.2 million deaths [3]. In Nigeria, there is an overall pooled prevalence of 5.77 % with 11.7million Nigerians (1 out of every 17 adults) are living with diabetes.

Diabetes can be managed through pharmacological and non-pharmacological approaches [4]. Different extracts from medicinal plants have also been used traditionally to manage diabetes globally, and these are considered as relatively inexpensive, less toxic and with relatively little or no side effects [5].

Jatropha tanjorensis(J.L. Ellis and Saroja) belonging to the Euphorbiaceae family is a common plant of field crops, in rainforest zones of West Africa including Nigeria [6]. It is commonly called “Hospital too far”, “Catholic vegetable”, “Iyana ipaja” or “Ugu-Oyibo”. Its primary use is for fencing while its secondary use are a source of edible leafy vegetables and medicine, prepared locally in most Southern Nigeria by collecting the leaves and squeezing out the juice

[7]. *J. tanjorensis* leaf exhibit low antioxidant and very low haemagglutinationtitre value, the latter indicting low toxicity on red blood cells. The leaf extract has hypoglycemic properties and is taken as a remedy against diabetes [8]. It is popular as a natural remedy against malaria infection and hypertension in Southern Nigeria where they drink the squeezed-out juice. However, there is no scientific validation to these claims. Research has shown that fresh *Jatropha tanjorensis* leaves contain a high water and low protein content. The trace elements, zinc, iron and selenium are in concentrations comparable to those found in food regarded as good dietary sources of these elements. The leaf extract also possesses antimicrobial properties and inhibit the growth of *S. aureus* and *E. coli* [9]. *J. tanjorensis* plant leaves are popularly consumed in Nigeria as soup and as a tonic with the claim that it increases blood volume. The leaves have anti-anemic effects (blood replenishing potentials). The leaf was found to contain some important biogenic principles for rapid hemopoiesis in the bone marrow [10]. *J. tanjorensis* leaf is a potent anti-HIV agent (effective against HIV-1 vector) [11]. The leaves are also employed traditionally in the treatment of renal problem, cardiovascular disease, hypertension, inflammation and in moderate depression. The aim of this study was to find out the scientific basis of the use of *Jatropha tanjorensis* in the management of diabetes used by traditional practitioners using methanol leaf extracts on alloxan-induced diabetic rats.

Figure 1: Pictorial representation of Jatropha tanjorensis Linn (Euphorbiaceae)

2. MATERIALS AND METHODS

2.1 Collection and preparation of plant materials

The leaves of *Jatropha tanjorensis* were freshly collected from a private garden in the locality of Nsukka Local Government Area, Enugu State in July, 2020. The plant was identified by Mr. Felix, a taxonomist in University of Nigeria, Nsukka and voucher specimen (PCG/UNN/0378) deposited in the Herbarium of Department of Pharmcognosy and Environmental Medicine of same University. The leaves were air dried at room temperature for four days then further dried in an oven at 40˚C for 6 hr. The crispy leaves were ground into powder and filtered using a sieve aperture of 1.0mm. The fine powder was preserved in moisture-free airtight container and used for phytochemical analysis, microscopic and anti-diabetic evaluations.

2.2 Animals

Healthy mixed sexes of Wistar Albino mice and rats were purchased from the Animal Farm, Department of Pharmacology and toxicology, University of Nigeria, Nsukka. The animals were

examined and acclimatized to the environmental conditions and were housed in aluminum cages floored with saw-dust with provision of food and water a week prior the experiment.

2.3 Preparation of extract

A 650 g of the powdered leaf sample was macerated in 3litres of methanol (Analytical grade) for 72 hr. The suspension was filtered and the resulting filtrate was evaporated to dryness over a water bath to obtain a sticky extract. The percentage yield was then determined.

2.4 Phytochemical Analysis

Phytochemical analysis tests were carried out on the methanol leaf extract using standard methods [12, 13] to test for the presence of secondary metabolites such as alkaloid, carbohydrates, saponins, tannins, flavonoids, etc.

2.5 Pharmacognostic profile

2.5.1 *Fresh Leaf Microscopy*

Foliar epidermis of the adaxial (upper surface) and abaxial (lower surface) surfaces of the leaves were prepared by clearing method. The leaf samples were cleared by soaking in 3.5% sodium hypochlorite for 18 hr. Then, the epidermal strips of the leaf samples were scrapped gently with the aid of a pair of forceps and placed on a clean slide, and then stained with Safranin solution and covered with a cover slip [14]. The slides were viewed under a light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA) at x 40, x 100 and x 400 magnifications and photomicrographs were taken with a Moticam 2.0 image system with software (Motic Carlsbad, CA, USA) fitted to the microscope. The following parameters were observed and assessed; Epidermal cells, Stomata type, Stomata size (length and width), Stomatal density, Stomatal

index, Trichome parameters, Vein islet number, vein islet termination number and palisade ratio. All parameters were observed on both the adaxial and abaxial surfaces of the leaves [14].

Transverse section (TS) of the leaf was made using a Reichert sledge microtome following the procedures of [15] and [16)]. The sections were microtomed at 10 – 15 unimicrons and were picked with the aid of a camel hair brush from the tip of the microtome knife into separate Petri dishes containing 70% absolute alcohol and labeled appropriately. Safranine and Fast green served as biological stains in differentiating lignified tissues.

2.5.2 Chemomicroscopy

Chemomicroscopy conducted on the powders to determine the presence of starch, calcium oxalate crystals and lignified vessels. A judicious quantity of the sample was dropped on a glass slide. One drop of chloral hydrate was dropped and passed over a Bunsen burner repeatedly until bubbles formed. This signified the successful clearing of the tissues. The chemomicroscopy of the plant constituents such as Starch, Lignin, Cellulose, Tannins, Calcium oxalate crystals, Gums and Mucillages was done using appropriate reagents and following the standard chemomicroscopy techniques.

2.6 Acute toxicity study

The acute toxicity test was estimated in mice using Lorke's method of [17].

2.7 Induction of diabetes

The Albino rats were fasted overnight (12-14 hr) and their weight and fasting blood glucose level recorded with a weighing balance and glucometer respectively and induced diabetes by a single intraperitoneal injection (a volume of 1ml/kg) of freshly prepared alloxan monohydrate solution (Sigma-Aldrich, USA), (120mg/kg body weight). Alloxan was prepared by weighing according to the individual animal weight and solubilized with distilled water before injection. 10% glucose

solution bottles were kept in their cages for the next 24hrs to prevent hypoglycemia and also food was given to the animals 30 min after administration of alloxan. After 48hrs of alloxan injection, plasma blood glucose level of each animal was determined by taking blood from the tail and animals with a fasting blood glucose level above 200 mg/dl were included in the study while those that did not develop more than 200mg/dl glucose levels were excluded from the study [18, 19].

2.8 Experimental design

2.8.1 Acute diabetic study

The animals weighing (138.90 -190.19 kg) were divided into 5 groups (A-E)clearly differentiated using coloured permanent markers for the evaluation of fasting blood glucose level with 4 animals in each group. They were treated with the plant extracts two days after administration of alloxan excluding the diabetic control groups. Blood samples were drawn using the tail method for measuring blood glucose levels from each at the intervals of 0, 1, 3, 6, 9, 12 and 24hr during the study period. Groups A, B and C were given 100, 200 and 400mg respectively of the plant extracts, while Group D and E were given the diabetic controls Standard Glibenclamide, (5 mg/kg per day p.o.) and distilled water 5ml/kg (negative control) respectively and was recorded and analyzed.

2.8.2 Sub-acute anti-diabetic study

Fresh animals weighing (154.02-186.95kg) were used and also divided into 5 groups with 4 animals in each group except group A which had 5 animals. Same procedure as followed in acute-diabetic study was used except that fasting blood glucose level were examined using the tail method of blood extraction on 0, 3^{rd}, 7^{th}, 10^{th} and 14^{th} day. The results were carefully recorded and analyzed.

2.9 Statistical analysis

Numerical data obtained from the study were expressed as the mean values ± Standard Error of Mean (N=5). Differences among means of control tested groups were determined using one-way ANOVA, followed by Dunnett's multiple comparison test. A probability level of less than 5% ($p<0.005$) was considered significant.

3. RESULTS

3.1 Preliminary phytochemical Screening

The preliminary phytochemical screening tests carried out on the methanol leaf extract of *Jatropha tanjorensis* showed the presence of carbohydrates, reducing sugars, alkaloids, cardiac glycosides, saponins, tannins, flavonoids, and fixed oils [Table1].

Table 1: Result of the phytochemical analysis of methanol leaf extract of *J. tanjorensis.*

Test	**Class of compound**	**Result**
Molisch	Carbohydrate	+
Fehling's	Reducing sugar	+
Dragendorf	Alkaloids	+
Frothing	Saponins	+
Ferric chloride	Tannins	+
Ammonium solution	Flavonoids	+
Paper translucency	Oils	+
Salkowski	Aglycone and steroidal cardiac glycosides	+
Litmus paper	Acidity	+

KEY: + ---------- Present - ---------- Absent

3.2. Fresh Leaf Microscopy and Chemomicroscopy

Table 2: Summary of the fresh leaf microscopy of *J. tanjorensis*

Epidermal cell	The epidermal cells are polygonal to irregular in shape on the upper surface but irregularly shaped with wavy/undulated anticlinal cell walls on the lower surface.
Stomata type	The leaf is amphistomatic (stomata occur both on the upper and lower surfaces but more on the lower surface) with anomocytic type of stomata (lack of subsidiary cells)
Trichome	Covering unicellular trichomes are scarcely present.
Stomata density (mm^{-2})	Upper surface: 5.88 ± 0.00; Lower surface: 60.29 ± 1.47
Stomata length (µm)	Upper surface: 24.91 ± 0.00; Lower surface: 28.75 ± 0.74
Stomata width (µm)	Upper surface: 17.18 ± 0.00; Lower surface: 18.13 ± 0.26
Stomata index (%)	Upper surface: 1.18 ± 0.00; Lower surface: 14.10 ± 0.47
Stomata size (μm^2)	Upper surface: 427.95 ± 0.00; Lower surface: 521.55 ± 18.45
Palisade ratio	8.00 ± 0.41 (7 – 9)

Values are mean ± SEM, n=4

Table 3: Result of Chemomicroscopy of *J. tanjorensis* Leaf

Parameter	**Reagent(s)**	**Result**
Starch grains	Iodine solutuion	Present
Lignified tissues	Conc. Hydrochloricacid + Phloroglucinol	Present
Calcium oxalates	Iodine solution Conc. Sulphuric acid	Present; Prism and druse shape
Tannin	Ferric chloride	Present
Cellulose	Zinc chloride; Conc. Sulphuric acid	Present
Gum/Mucilage	Ruthenium red	Absent
Protein	Biuret reagent; Nihydrin	Present
Oil globules	Sudan IV reagent	Present

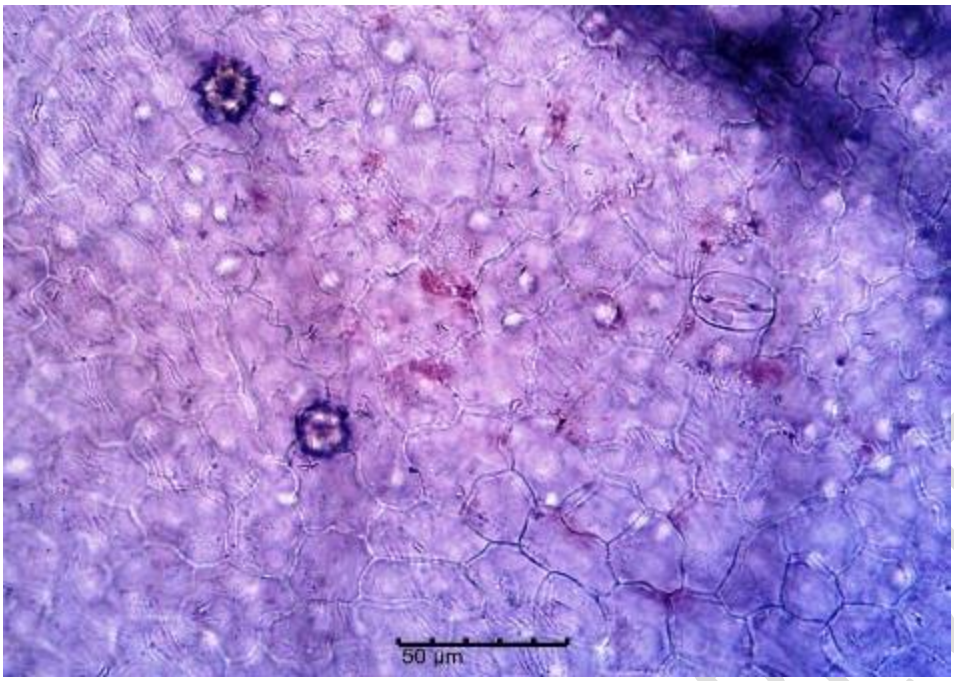


Figure 2: Upper surface of te leaf of *J. tanjorensis* showing polygonal irregularly shaped epidermal cells. Stomata are scarcely distributed and are of anomocytic type (X 400).

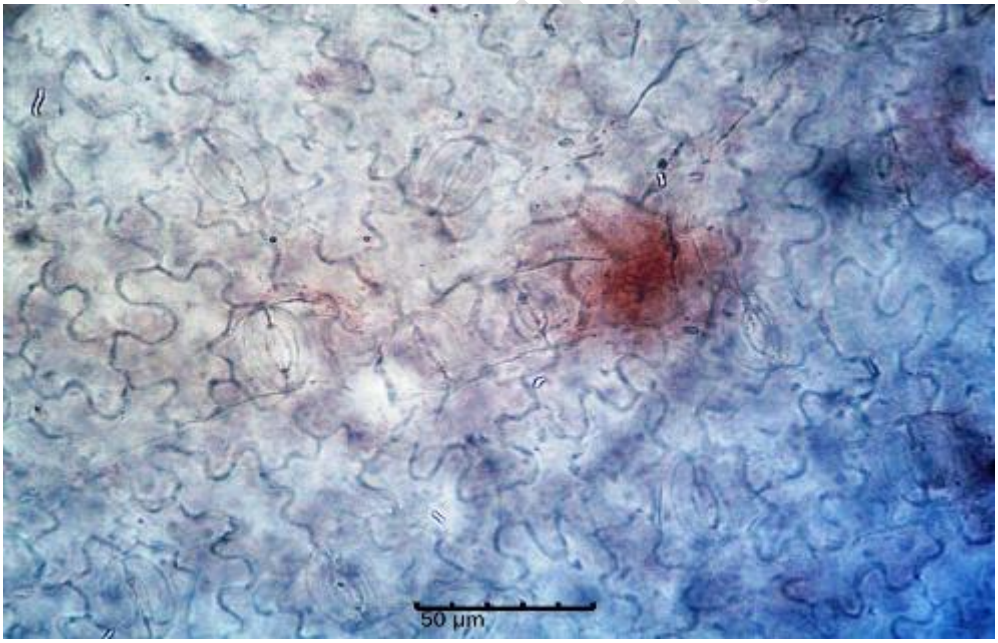
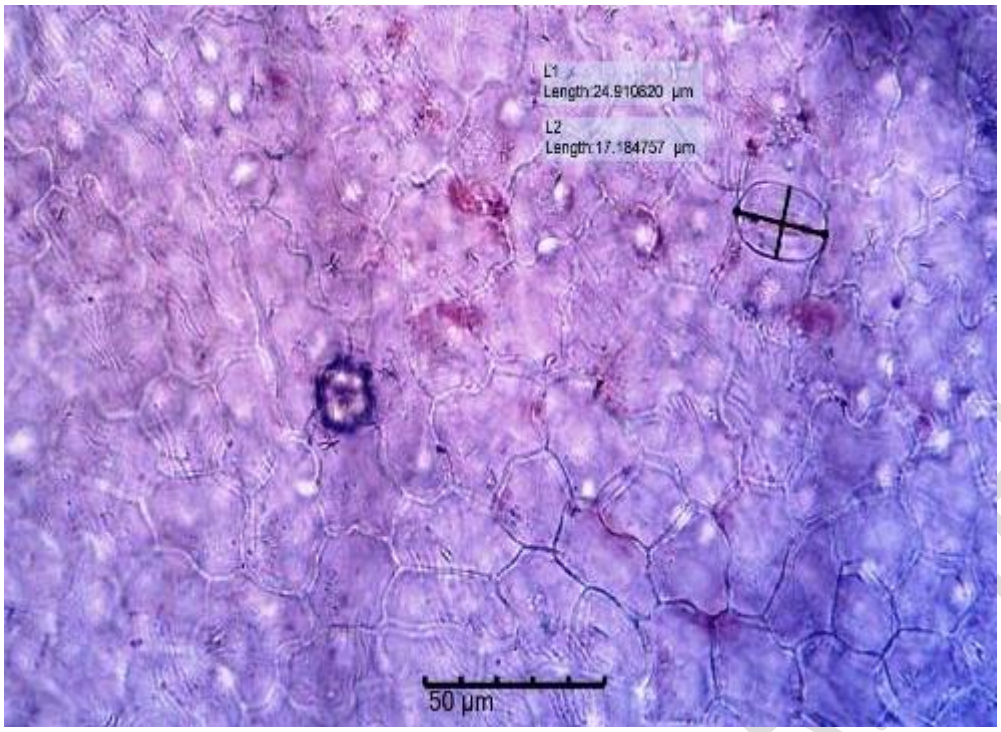


Figure 3: Lower epidermal surface of the leaf of *J. tanjorensis* showing irregularly shaped epidermal cells with wavy cell walls and anomocytic stomata (X 400)



X400

Figure 4: Quantitative measurement of the stomata on upper epidermal surface of the leaf of *J. tanjorensis* (X 400)

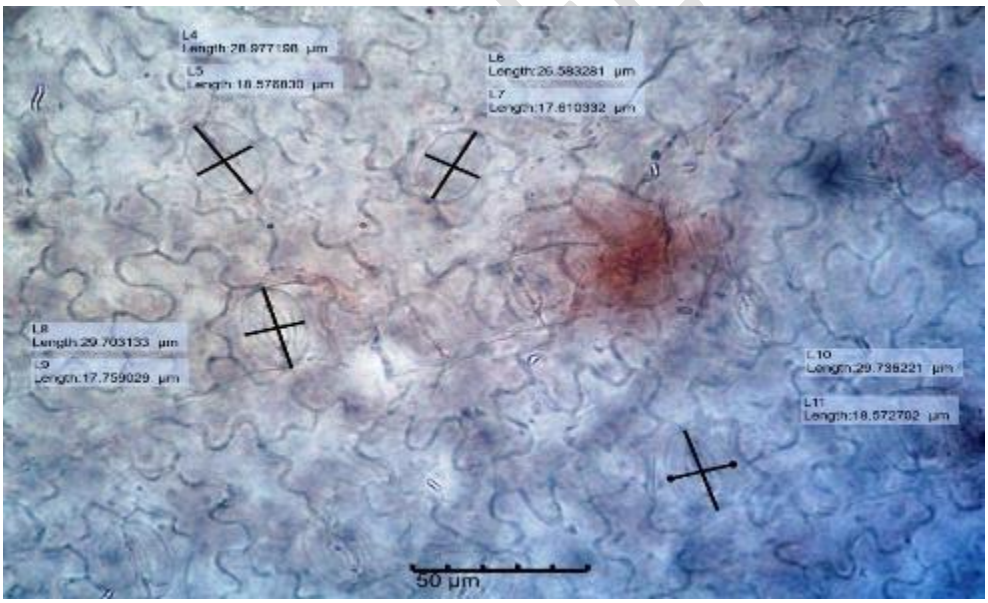


Figure 5: Photomicrograph of the Quantitative measurement of the stomata on lower epidermal surface of the leaf of *J. tanjorensis* (X 400)

Figure 6: Chemomicroscopy of the leaf powder showing a bundle of lignified vessel and fibre elements (X 400)

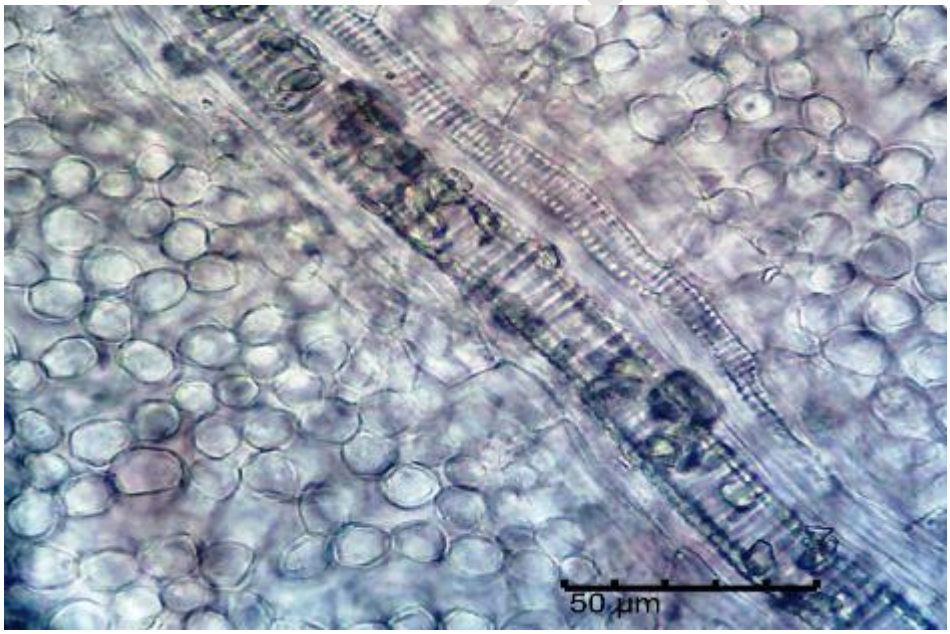


Figure 7: Photomicrograph of the leaf fragment showing arrangement of the palisade cells and vascular bundle within the veins of *J.tanjorensis* (X 400)

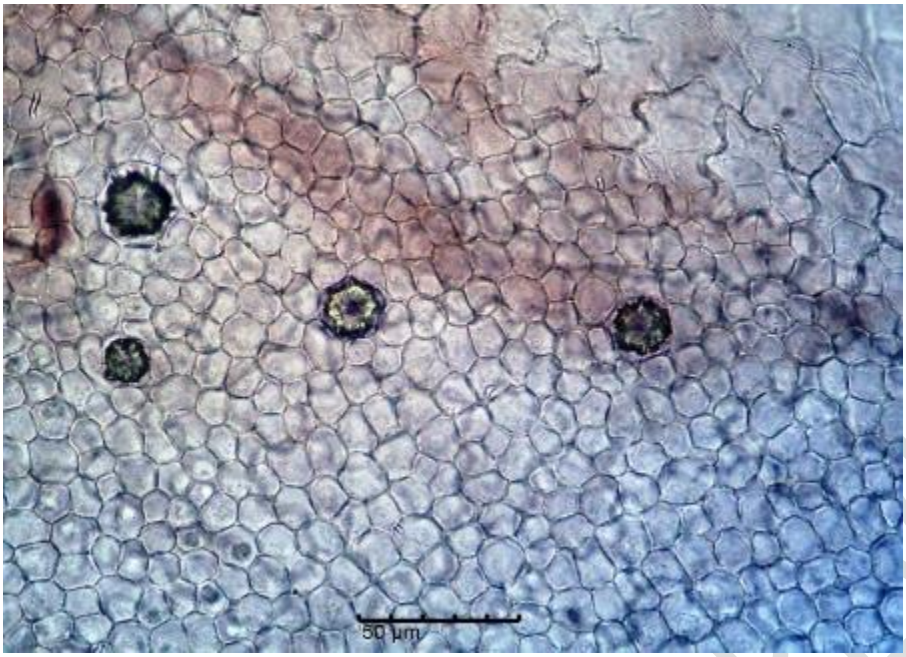


Figure 8: Photomicrograph of the leaf fragment showing the palisade cell, epidermal cells and some sphaeraphides (calcium oxalate) (X 400)

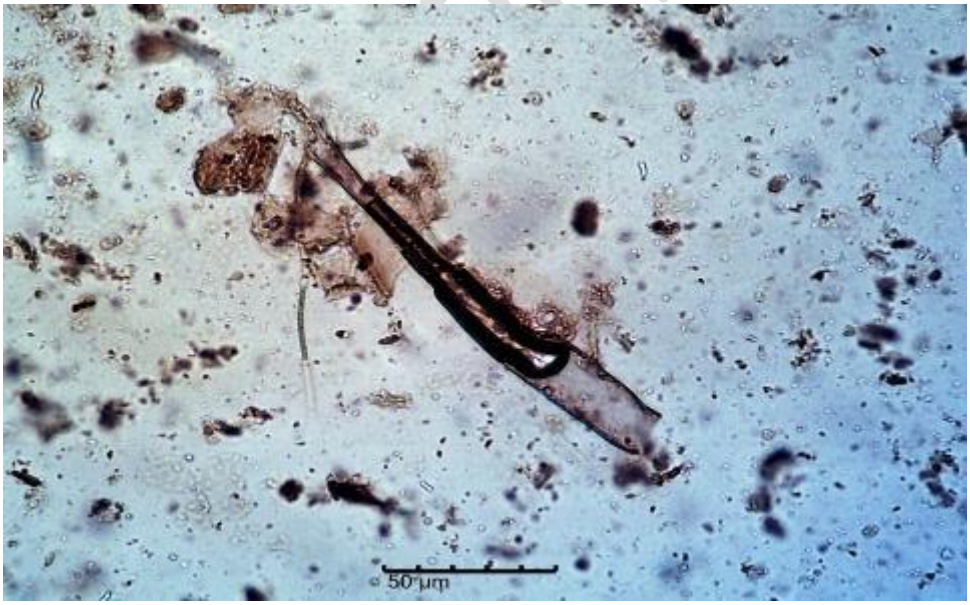


Figure 9: Photomicrograph of the leaf powder showing a fragment of unicellular Trichome (X 400)

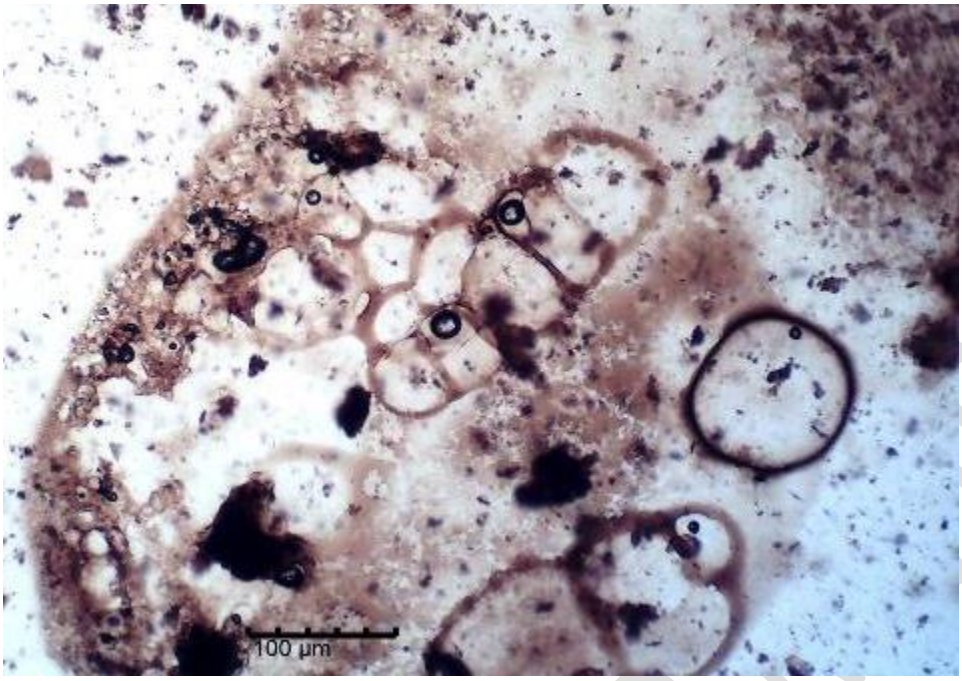


Figure 10: Photomicrograph of the leaf powder showing groups of secretory tissues (most likely laticiferous tissues) (X 400)

Figure 11: Transverse section of the leaf of *J. tanjorensis* (X 400)

3.3. Physicochemical Studies

Table 4 shows the values obtained for the analytical standards tested for. These include; Moisture content, ash values and Extractive values.

Table 4: Result of Analytical standards of the leaf powder of *J. tanjorensis*

S/N	Parameter	Value (% w/w)
1.	Moisture content (LOD)	5.67 ± 0.15
	Ash values	
2.	Total ash	8.39 ± 0.04
3.	Water soluble ash	3.91 ± 0.05
4.	Acid insoluble ash	0.72 ± 0.00
	Extractive values	
5.	Hexane soluble extractive value	8.50 ± 0.06
6.	Ethylacetate soluble extractive value	6.19 ± 0.04
7.	Alcohol soluble extractive value	13.51 ± 0.07
8.	Water soluble extractive value	15.75 ± 0.03

Values expressed in mean ± SEM, n= 3.

3.4 Results for Antidiabetic Studies of J.tanjorensis

The results of the acute antidiabetic study [Tables 5 and 6] and Sub-acute studies [Tables 7 and 8] are shown below.

Table 5: Results of acute anti-diabetic activity of methanol extract of *Jatropha tanjorensis*

Treatment group	**Dose (mg/kg)**	**Blood glucose level (mg/dl)**

		0h	1h	3h	6h	9h	12h	24h
Extract	100	310.25±66.70	181.00±41.80	162.50±51.50*	145.25±27.60*	119.25±18.03*	170.00±71.70*	78.75±10.50*
	200	432.75±92.80	277.25±1.14	205.50±59.10*	146.00±51.40*	98.00±19.20*	84.75±8.24*	65.75±5.51*
	400	486.00±50.70	353.25±86.80	336.25±92.80	99.25±7.32*	90.50±11.20*	67.25±2.75*	62.75±2.43*
Gb	5	552.00±33.25	394.25±79.50	282.50±33.40*	185.00±75.72*	103.75±23.14*	83.00±14.50*	69.00±4.18*
Distilled water (5 ml/kg)	-	518.00±64.69	488.00±68.60	532.25±41.20	580.25±19.75	574.50±25.50	593.00±7.00	600.00±.00

Values are mean ± Standard Error of Mean (N=4) with level of significance, *p<0.05 level compared with the negative control (one-way ANOVA followed by Dunnett's multiple comparison test)

Table 6: Results of the percentage blood glucose reduction of methanol extract of *J. tanjorensis*in the acute antidiabetic study

Treatment group	Dose mg/kg	Blood glucose level reduction (%)					
		1h	3h	6h	9h	12h	24h
Extract	100	41.66	47.62	53.18	61.56	45.21	74.62
	200	35.93	52.51	66.26	77.35	80.42	84.81
	400	27.31	30.81	79.58	81.38	86.16	87.09
Gb	5	28.52	48.82	66.49	81.20	84.96	87.50
Distilled water (5 ml/kg	-	5.79	-2.75	-12.02	-10.91	-14.48	-15.83

Gb – Glibenclamide

Table 7: Results of the sub-acute antidiabetic effect of the crude methanol extract of *J. tanjorensis*

Treatment group	Dose mg/kg	Blood glucose level (mg/dl)				
		Day 0	**Day 3**	**Day 7**	**Day 10**	**Day 14**
Extract	100	365.67±37.17	334.00±76.45	322.67±44.71*	107.00±05.50*	110.00±08.16*
	200	492.75±58.67	318.50±01.00	218.75±58.25*	121.00±29.54*	229.25±57.06*
	400	432.20±87.65	237.75±68.56*	96.60±05.71*	86.40±07.57*	84.20±09.25*
Gb	5	600.00±.00	380.50±86.62	196.25±65.53*	85.25±12.43*	87.00±16.52*
Distilled water (5 ml/kg)	-	459.50±48.08	494.50±41.36	566.00±28.32	578.00±16.55	600.00±.00

Values are mean ± SEM (N=4) *p<0.05 level compared with the negative control (one-way ANOVA followed by Dunnett's multiple comparison test), Gb (Glibenclamide)

Table 8: Results of the percentage blood glucose reduction of the methanol extract of *J. tanjorensis* in sub-acute antidiabetic study

Treatment groups	Dose mg/kg	Blood glucose level reduction (%)			
		Day 3	**Day 7**	**Day 10**	**Day 14**
Extract	100	8.66	11.76	70.74	69.92
	200	35.36	55.61	75.44	53.48
	400	44.99	77.65	80.01	80.52
Gb	5	36.58	67.29	85.79	85.50
Distilled water (5 ml/kg)	-	-7.62	-23.18	-25.79	-30.58

Gb - Glibenclamide

4. DISCUSSION

The bioactive constituents synergistically present in the plant (Table 1) may be responsible for the observed anti-diabetic and other therapeutic effects of the plant. However, the alkaloids, saponins and flavonoids contained in the plant extract have been verified to possess the antidiabetic effect of the plant by enhancing the activity of hexokinase and phosphofructokinase, resulting in glucose transport, carbohydrate digestion and absorption and also involved in Insulin secretion respectively [20].

In recent times, however, there has been an increase in consciousness of the need for standardization of medicinal plant extracts, especially for those with potential therapeutic uses [21-23]. Microscopic features [Table 2 and 3, Figures 2-11] could be used pharmacognostically for identification and differentiation. The water-soluble extractive value [Table 4] indicated the presence of water-soluble matters such as sugars, amino acids and vitamins derived from plants while the alcohol soluble extractive values indicate the presence of polar compounds, also indicating that this plant sample can best be extracted with an alcohol-based solvent. The moisture content obtained was 5.67 % w/w which is low when compared to the African Pharmacopoeia limit of moisture content for vegetable drug (8 – 14 % w/w) and may less likely degrade due to hydrolytic reactions and enzymatic activation during storage. The ash value indicates the presence of inorganic ions and used to determine the quality and purity of crude drug. The values show that there was neither adulteration nor substitution. High ash value indicates the presence of impurities.

There was no mortality or any signs of behavioral changes or toxicity observed after oral administration of *Jatropha tanjorensis* up to the dose of 5000 mg/kg body weight in mice used for the study.

The result of the acute anti-diabetic study shows that low dose (100 mg/kg) of the methanol extract of *Jatropha tanjorensis* has quick on set antidiabetic action (41.66 %) compared to the other doses and the standard drug (28.52 %) when compared with the negative control [Tables 5 and 6]. At the sixth hour, 100, 200 and 400 mg/kg of the extract and the standard drug have significant antidiabetic effect of 53.18, 66.26, 79.58 and 66.49 % respectively. The result of the sub-acute anti-diabetic study shows that there is a dose-dependent anti-diabetic effect of the methanol extract of *Jatropha tanjorensis* when compared with negative control group which received distilled water. The 400 mg/kg gave the highest anti-diabetic effect (44.99 %) with the statistical significance of 0.035 when compared to the standard drug (36.58) on day three [Tables 7 and 8]. On day fourteen, all the doses studied had outstanding significant effect. The hyperglycemic model was used to screen the anti-hyperglycemic activity of plant extracts. An excessive amount of glucose in the blood induces the insulin secretion. This secreted insulin will stimulate peripheral glucose consumption and control the production of glucose through different mechanisms [24].

The effect of Glibenclamide, a standard oral anti-diabetic used in this study on blood glucose reduction, has been attributed to enhanced beta cells of the pancreas resulting in secretion of larger amounts of insulin. So, the mechanism behind this anti-hyperglycemic activity of plant extracts involves an insulin-like effect, probably through peripheral glucose consumption or enhancing the sensitivity of beta cells to glucose, resulting in increased insulin release [25]. Alloxan produces hyperglycemia by a selective cytotoxic effect on pancreatic beta cells. One of the intracellular phenomena for its cytotoxicity is through generation of free radicals demonstrated both in vivo and in vitro [26]. Administration of glucose solution after inducing diabetes with alloxan was to prevent the hypoglycemic shock associated with alloxan.

5. CONCLUSION

The methanol leaf extract showed that *Jatropha tanjorensis* possesses anti-diabetic properties at various doses studied. This research support the inclusion of this plant in traditional anti-diabetic preparations and shows appreciable results to support the traditional claims of the plant extract. The pharmacognostic standards obtained will assist in the preparation of a monograph of *Jatropha tanjorensis* for proper identification of the plant.

Ethics Statement

All animal experiments Complied with the ARRIVE act; and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines.

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ABBREVIATIONS

J.tanjorensis: *Jatropha tanjorensis*

GB: Gilbenclamide

ANOVA: One Way Analysis of Variance.