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SCAN ME

Research Paper

PHARMACOGNOSTIC PROFILING AND ANTIDIABETIC EFFECTS OF LEAVES OF *Vitellaria paradoxa* C.F GAERTN (SAPOTACEAE)

Odoh, Uchenna Estella and Obiano, Ogochukwu Victoria

Department of Pharmacognosy and Environmental Medicine,
Faculty of Pharmaceutical Sciences, University of Nigeria,
Nigeria.

Abstract

Diabetes mellitus is a complex disorder of carbohydrates, fats, and proteins metabolism that is primarily as a result of a deficiency or complete lack of insulin secretion by the beta cells of the pancreas or resistance to insulin. This research was aimed at evaluating the antidiabetic effects of the methanol extract of leaves of *Vitellaria paradoxa* in alloxan induced diabetic rats and also to establish the pharmacognostic standards of the leaves of *Vitellaria paradoxa* for proper and easy identification. The powdered leaves of *Vitellaria paradoxa* was subjected to extraction with methanol by cold maceration and the yield after extraction was 21.25 %. Phytochemical analysis, quality control standards were carried out using standard methods and acute toxicity test was also carried out using method described by Lorke. Phytochemical screening revealed the presence of proteins, alkaloids, tannins, carbohydrates, reducing sugars, glycosides, saponins, flavonoids, resins, steroids, oils; and acidic compounds were absent. Diabetes mellitus was induced in twenty (20) rats by single intraperitoneal injection of alloxan monohydrate (140 mg/kg). Two days after induction, antidiabetic study was carried out using the hyperglycemic (> 200 mg/dl) rats which were divided into groups (n=4) based on the closeness of their blood glucose level to receive oral administration of the extract (200, 400 and 800 mg/kg) , glibenclamide (5mg/kg) and water respectively. Their blood glucose level was measured before 0 hour and at 0.5, 1, 2 and 4 hours after treatment. Similar study as above was carried out on normal rats. The extracts at doses of 200, 400 and 800 mg/kg showed reduction (38.5, 40.9 and 59.6 % respectively) in hyperglycemic rats on the seventh day; 28.1, 34.1 and 58.2 % respectively in normoglycemic rats on the first day. The results of the pharmacognostic studies revealed the presence of unicellular non glandular trichome, upper epidermal cells with no stomata, epidermal cells with numerous paracytic stomata, phloem parenchyma cells, annular xylems with phloem cells, calcium oxalate e.t.c. and presence of pith, pericycle, midrib, collenchymas, xylem and phloem

cells from the transverse section of the leaf. Pharmacognostic standardization results were as follows: total ash (3.55 %), acid insoluble ash (2.61 %), water soluble ash (5.92 %), sulphated ash (7.77 %), water soluble extractive (1.86 %), alcohol soluble extractive (5.46 %) and moisture content (6.42 %). It can be concluded that the methanol extract of leaves of *Vitellaria paradoxa* possesses antidiabetic properties on alloxan induced diabetic rats.
Key words: *Vitellaria paradoxa,* Diabetes mellitus, Phytochemica analysis, Standardization.

INTRODUCTION

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period [1]. Diabetes mellitus is a hereditary, metabolic disease characterized by hyperglycemia and eventual glycosuria. It is caused by the inability of tissues to carry out normal metabolism of carbohydrates, fats and proteins, due to an absolute or relative lack of insulin [2]. Diabetes mellitus is a disease caused by deficiency or diminished effectiveness of endogenous insulin [3]. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. Diabetes mellitus is a complex disorder of carbohydrate, fat, and protein metabolism that is primarily as a result of a deficiency or complete lack of insulin secretion by the beta cells of the pancreas or resistance to insulin. The disease is often familial but may be acquired, as in Cushing's syndrome, as a result of the administration of excessive glucocorticoids [4]. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications [5]. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma [6]. Serious long-term complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes [5]. Diabetes was one of the first diseases described [7] with an Egyptian manuscript from c. 1500 BCE mentioning "too great emptying of the urine" [8]. The first

described cases are believed to be of Type 1 diabetes [8]. As of 2013, 382 million people have diabetes worldwide [9]. Type 2 makes up about 90 % of the cases [10]. This is equal to 8.3 % of the adult population [9] with equal rates in both women and men [10]. More than 80 % of diabetic deaths occur in low and middle-income countries [11]. In 2014, the International Diabetes Federation (IDF) estimated that diabetes resulted in 4.9 million deaths. The World Health Organization (WHO) estimated that diabetes resulted in 1.5 million deaths in 2012, making it the 8th leading cause of death [5]. The discrepancy between the two estimates is due to the fact that cardiovascular diseases are often the cause of death for individuals with diabetes; the IDF uses modeling to estimate the amount of deaths that could be attributed to diabetes [12]. More than 80 % of diabetic deaths occur in low and middle-income countries [11]. The greatest increase in rates was expected to occur in Asia and Africa, where most people with diabetes will probably live in 2030 [13]. The increase in rates in developing countries follows the trend of urbanization and lifestyle changes, including a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present [13]. Chronic hyperglycemia in diabetes mellitus is the cause of many late complications characterized by kidney disease, blindness and the risk of cardiovascular disease and also available drugs often encounter difficulties in their treatment because of side effects. However, herbal treatment is an alternative in the treatment of this pathophysiology in as much as plants are used to cure diabetes [14]. Currently available synthetic anti-diabetic agents produce serious side effects like hypoglycemia, coma. Glucosidase and lipase inhibitors, which have been in use, give rise to certain side effects. Lipase inhibitors produce a weight loss in patients and some others cause hepatotoxicity, abdominal pain, flatulence, diarrhoea and hypoglycemia [15].

Plants have long time history of its utilization of in prevention or treatment of diseases. More than 200 species of plants possess antidiabetic properties which were evaluated mostly by screening tests. These plants are categorized according to their proved mode of action and are classified into those that act by inhibiting glucose absorption from intestine, increasing insulin secretion from the pancreas, inhibiting glucose production from hepatocytes, or enhancing glucose uptake by adipose and muscle tissues [16]. Healthcare professionals caring for diabetic patients need to be aware of phytotherapy to incorporate phytomedicine into their practices and should undertake more responsibility in relation to these kinds of therapies that are commonly-used throughout the world [17].

Vitellaria paradoxa Gaertner (Sapotaceae) which is also known as Shea tree and *Butyrospermum parkii* grows naturally in the wild in the dry savannah belt of West Africa from Senegal in the West to Sudan in the East, and onto the foothills of the Ethiopian highlands. It occurs in 19 countries across the African continent, namely Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Ethiopia, Ghana, Guinea Bissau, Côte d'Ivoire, Mali, Niger, Nigeria, Senegal, Sierra Leone, South Sudan, Sudan, Togo, Uganda, Democratic Republic of the Congo, and Guinea. A testa found at the site of the medieval village of Saouga is evidence of shea butter production by the 14th century [18]. Shea butter is the fat extracted from the kernels of *Vitellaria paradoxa* and contains high levels of UV-B absorbing triterpene esters, including cinnamic acid, tocopherols (vitamin A), and phytosterols. Shea butter does contain a high percentage of unsaponifiables, such as phytosterols (campesterol, stigmasterol, beta-sitosterol, and alpha-spinosterol) and triterpenes (cinnamic acid esters, alpha- and beta-amyrin, parkeol, buytospermol, and lupeol), and hydrocarbons such as karitene [19]. Shea butter is composed of five principal fatty acids: palmitic, stearic, oleic, linoleic, and

arachidic [20]. Scientific investigations have shown that *Vitellaria paradoxa* it has antioxidant properties [21; 22], antidiabetic activity [14], antimicrobial [23].

The aim of this research is to evaluate the antidiabetic effects of the methanol extract of leaves of *Vitellaria paradoxa* in alloxan-induced diabetic rats and establish the pharmacognostic standards of the leaves of *Vitellaria paradoxa* for proper and easy identification.

Fig 1: Picture of *Vitellaria paradoxa* leaf plant

MATERIALS AND METHODS

Collection and Identification of Plant Material

The leaves of *Vitellaria paradoxa* were collected from Botanical garden of University of Nigeria, Nsukka in Enugu State in June and authenticated by a botanist; Mr. Amos of Department of Botany, University of Nigeria, Nsukka

Preparation of Plant Material

The *Vitellaria paradoxa* leaves were properly dried under shade drying for 3 weeks and were ground into very fine powder. The powdered leaves were weighed using a

weighing balance and the weight was recorded. The powder was kept in a bottle and was used for extraction, phytochemical analysis, microscopic examination and quality control parameters evaluations. Fresh leaves were also kept for the transverse section of the leaf.

Animals Used

Thirteen (13) mice weighing between 15-31 g and forty (40) albino rats weighing between 59 - 82 g of either sex procured from the Department of Veterinary Medicine, University of Nigeria were used for the study. The animals were grouped based on the closeness of their body weight and were housed in a clean metal cage with four (4) animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 ^{0}C with 12 hour day/ night cycle). They were fed with standard feed and water ad libitum. The animals were acclimatized to laboratory conditions for 7 days prior to the experiment.

Extraction

A 500 g of the powdered leaves was weighed out and macerated at room temperature with 2.5 litres of methanol for 3 days with shaking at interval. At the end of the 3 days, the macerates were collected by filtration and the residue discarded. The macerates were evaporated to dryness using rotary evaporator. The extract were collected and weighed. The percentage yield of the extract was calculated.

Phytochemical Analysis

The phytochemical test was performed in order to detect the presence or absence of major secondary plant metabolites of pharmacognostic importance which includes: alkaloids, steroids, tannins, saponins, flavonoids, oils, and glycosides etc following standard procedures [24].

Pharmacological Evaluation

Determination of Acute Toxicity (Ld_{50})

Acute toxicity tests are generally the first test conducted and provide data on the relative toxicity likely to arise from a single or brief exposure. Standardized tests are available for oral, dermal and inhalation exposures. In the test, a single dose of the drug is used in each animal for the determination of gross behavior and LD_{50} (the dose which has proved to be lethal (causing death to 50 % of the tested group of animals)) is usually an initial step in the assessment and evaluation of the toxic manifestations and provides information on health hazards likely to arise from short-term exposure to drugs.The method as described by Lorke [25] was used for this study. The test involved two phases. The first phase involved the determination of the toxic dose range. Mice are placed in 3 groups of 3 animals each and treated with test substance at 10, 100, and 1000 mg/kg respectively. The treated animals are observed for 24 hour for deaths. The number of dead animal and death pattern in the first phase determines the doses used for the second phase. In the second phase, 4 different doses of the tests agent are administered as predetermined in the earlier phase of the study. Animals are observed for lethality and signs of acute intoxication for 24 hour. The LD_{50} is calculated as the geometric mean of the highest non lethal dose and the least toxic dose.

Induction of Diabetes Mellitus in Rats

The animals were fasted for 12 hours with free access to water and feed prior to induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate dissolved in water for injection at a dose of 140 mg/kg body weight. The diabetic state was confirmed 48 hours after alloxan injection by glucosuria and hyperglycemia. Rats with fasting blood

glucose level of 200 mg/dl and above were considered diabetic and selected for the experiment and grouped based on the closeness of their blood glucose levels [26].

Determination of Antidiabetic Effects of *Vitellaria paradoxa* on the Fasting Blood Sugar level of Normoglycemic Rats

Twenty (20) rats were used in this study and the animals were divided into five groups of five rats each and treated as follows:

GROUP A: Normal rats were administered orally with aqueous solution of methanol extract of *Vitellaria paradoxa* leaves (200 mg/kg).

GROUP B: Normal rats were administered orally with aqueous solution of methanol extract of *Vitellaria paradoxa* leaves (400 mg/kg).

GROUP C: Normal rats were administered orally with aqueous solution of methanol extract of *Vitellaria paradoxa* leaves (800 mg/kg).

GROUP D: Normal rats were administered orally with aqueous solution of glibenclamide (5 mg/kg).

GROUP E: Normal rats were administered with water (0.5 ml/kg).

They were fasted overnight for 12 hr. At the end of the fasting period, Groups, B and C ere given 200, 4000 and 400 mg/kg doses of the extract orally. Group D received 5 mg/kg of Glibenclamide as the positive control and Group E received water only as negative control.

The drugs were administered to the animals and observed for 4h. Blood samples were withdrawn from the tail vein of each animal rat at 0, ½, 1, 2 and 4 h interval and their blood sugar level determined and recorded using Accu-check Active Glucometer and strips (Roche Diagnostics GmbH, Germany) [26].

Determination of Antidiabetic effEcts of *Vitellaria paradoxa* on the Fasting Blood Sugar level of Hyperglycemic Rats

Twenty (20) rats were used in this study and the animals were divided into five groups of four rats each and treated as follows:

GROUP A: Diabetic rats were administered orally with aqueous solution of methanol extract of *Vitellaria paradoxa* leaves (200 mg/kg).

GROUP B: Diabetic rats were administered orally with aqueous solution of methanol extract of *Vitellaria paradoxa* leaves (400 mg/kg).

GROUP C: Diabetic rats were administered orally with aqueous solution of methanol extract of *Vitellaria paradoxa* leaves (800 mg/kg).

GROUP D: Diabetic rats were administered orally with aqueous solution of glibenclamide (5 mg/kg).

GROUP E: Diabetic rats were administered with water (0.5 ml/kg).

They were fasted overnight for 12 hr. At the end of the fasting period, Groups, B and C ere given 200, 4000 and 400 mg/kg doses of the extract orally. Group D received 5 mg/kg of Glibenclamide as the positive control and Group E received water only as negative control.

The drugs were administered to the animals daily for a period of 7 days. Blood samples were withdrawn from the tail vein of each animal rat for the acute study at 0, ½, 1, 2 and 4 h and then at day 0, 1, 3, 5 and 7 days for the prolonged study and their blood sugar level determined and recorded using Accu-check Active Glucometer and strips (Roche Diagnostics GmbH, Germany) [26].

Determination of Pharmacognostic and Quality Control Parameters

Preparation of Sample for Microscopical Examination

Powdered Microscopy

The powdered leaves were sieved to produce fine powders suitable for examination. A 2 drops of chloral hydrate were added to moisten the powdered sample. It was covered with a cover slip and this was passed across the flame of a burner repeatedly until bubbles occurred and then it was allowed to cool. A 2 drops of glycerin were added for clarity of the structures and the slide was viewed under the microscope to reveal microscopically characters which were observed and noted [27].

Transverse Section of Plant Material

The plant material (leaf) was cut with the aid of microtone. The section was then transferred into staining jar and stained in a safranin for 5 minutes. The safranin was drained off and the material was washed off three times with distilled water. It was washed again with 97 % alcohol for two times. The section were counterstained in 1 % fast green for 5 minutes and washed with absolute alcohol for about 3 - 4 times. Then, the section was transferred in staining jar containing 50/ 50 alcohol/ xylene and washed until it became clear. Pure xylene was used to finally clear the section. The section was then mounted and viewed under the microscope and the picture was taken with the use of photomicroscope [27].

Determination of Ash Values

The ash content of a crude drug is generally taken to be the residue that remained after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter that is added for the purpose of adulteration. Determination of ash value helps in the identification of crude drugs and their cleanliness. It also provides information relative to its adulteration with inorganic

matter. The methods adopted for the determination of ash values follow the specification given by Odoh *et al*., [28] and United States Pharmacopoeia [29].

Total Ash Value

The total ash value represents the amount of the residual substance not volatized on ignition at 450 ^{0}C. It is used to exclude drugs which have been coated with chalk, lime or calcium sulphate to improve their appearance. It usually consists of carbonates, phosphates, silicates and silica.

A tarred nickel crucible was ignited to a constant weight at dull red heat, cooled and stored in a desiccator. A 2 g of the powdered leaves was weighed into the crucible and was heated gently until at 100-105 ^{0}C until all the moisture had been driven off and the material completely charred. The heat was increased until most of the carbon had been vaporized, after which the material was heated at about 450 ^{0}C to make the residue carbon free. The heating and cooling were continued until a constant weight was achieved.

Acid Insoluble Ash

This is the ash in dilute hydrochloric acid; it represents the method for measuring the amount of silica matter such as sand and siliceous earth (non-pharmacological ash) in a crude herb. The total ash gotten in (a) above was transferred to a beaker containing 25 ml of dilute hydrochloric acid, heated to boiling on a water bath for 5 minutes and filtered through an ashless filter paper. The beaker and crucible were washed repeatedly with water and the washing passed through the filter paper until free from acid. The filter paper was dried in an oven, folded into narrow cone, then inserted into a tarred nickel crucible and heated at 150 ^{0}C until it was completely ashed. The residue was heated more strongly and cooled in a dessicator after which the crucible was re-weighed.

Water Soluble Ash

Water soluble ash is that part of total ash content which is soluble in water and it is good indicator of substitution or adulteration or the presence of exhausted materials substituted in the genuine drug. A nickel crucible was ignited to a constant weight at 450 ^{0}C and 2 g of the powdered leaves were weighed into a crucible. The crucible with the drug was ignited at low heat, initially to burn off the carbon content and the heat was gradually increased until all the carbon was burnt off. The crucible was cooled in a dessicator and reweighed, and heating was continued until a constant weighed was obtained. The content of the crucible was transferred into a small beaker, 25 ml of distilled water was added and the beaker content were boiled for 5 minutes, after which it was filtered through an ashless filter paper. The filter paper together with the residue was dried in the oven and compressed into a small or narrow cone. This was then transferred into the crucible and heating continued until the ashless filter paper was eliminated, the weighed was noted.

Sulphated Ash

Sulphated ash produces a more consistent ash. In this method, all oxides and carbonates are converted to sulphates at a higher temperature. A nickel crucible was ignited to a constant weight at a dull red heat in the oven. A 2 g of the powdered plant material was spread over the bottom of the crucible which was weighed. The plant material was moistened with dilute sulphuric acid and ignited at low heat initially to burn off the carbon content. The crucible was cooled in a dessicator. More dilute acid was added and heating continued to about 800 ^{0}C with occasional cooling and re-weighing until a constant weight was obtained.

Determination of Extractive Yields

The determinations of water and alcohol soluble extractives are used as a means of evaluating drugs, the constituents of which are not readily estimated by other means. In some cases, the amount of a drug soluble in a given solvent is an index of its purity. The method used here are in conformity with the recommendation of Odoh *et al*., [28] and United States Pharmacopoeia [29].

Water Soluble Extractives

A 5 g of the powdered plant material was weighed and placed in a 250 ml stoppered conical flask. 100 ml of distilled water was added and the stopper was firmly replaced. The contents of the flask were shaken mechanically for 6 hours and were then allowed to macerate for a further 18 hours for a total of 24 hours and were filtered. A 20 ml of the filtrate was evaporated to dryness in a 25 ml beaker over a water bath. The residue was dried to constant weight at 105 ^{0}C and was weighed.

Alcohol Soluble Extractives

A 5 g of the powdered plant material was weighed and placed in 250 ml stoppered conical flask. 100ml of 90 % alcohol was added and the stopper was firmly replaced. The content of the flask of was shaken mechanically for 6 hours and was then allowed to macerate for a further 18 hours for a total of 24 hours and was filtered. A 20 ml of the filtrate was evaporated to dryness in a 25 ml beaker over a water bath. The residue was dried to a constant weight of 105 ^{o}C and was weighed.

Determination of Moisture Content

A tarred evaporating dish was heated to a constant weight and stored in a dessicator. A 2 g of the powdered material was added to the dish and kept in an oven maintained at a temperature of 105 ^{0}C. It was allowed to dry until a constant weight was achieved. The difference in weight of the evaporating dish was noted.

Statistical Analysis

All the experimental results were expressed as mean ± standard error of mean (S.E.M). The significance or difference between means was determined using one – way analysis of variance (ANOVA). Comparison was done by Dunnett's test. Dunnett t - tests treat one group as a control and compare all other groups against it. $P < 0.05$ were considered as significant.

RESULTS

Percentage Yield of Extract

The yield of methanol extract was 51.25 g (21.25 % w/w)

Results of Phytochemical Analysis of Powdered Leaves of *Vitellaria paradoxa*

The result shows that leaves contains carbohydrates, reducing sugars, proteins, oils, alkaloids, glycosides, saponins, tannins, flavonoids, resins, steroids, and terpenoids (Table 1).

Table I: Results of Phytochemical Analysis of Powdered leaves of *Vitellaria paradoxa*

Phytochemical Constituent	Inference
Carbohydrates	+
Reducing sugars	+
Proteins	+
Oils	+
Alkaloids	+
Glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Resins	+
Steroids	+
Terpenoids	+
Acidic compounds	-

Key: - = Absent, + = Present

Result of Acute Toxicity Studies

There was no death in the first and second stage in all groups after 24 hours. Thus the LD_{50} of the methanol extract of *Vitellaria paradoxa* was found to be greater than 5000 mg/kg (Table 2) which shows that the extract is safe.

Table 2: Result of Acute Toxicity Studies

Phase	Dose (mg/kg)	No of Animals	No of Deaths
I	10	3	0/3
	100	3	0/3
	1000	3	0/3
II	1600	1	0/1
	2400	1	0/1
	3600	1	0/1
	5000	1	0/1

Results of the Antidiabetic Studies

The results of the antidiabetic studies of the extract of *Vitellaria paradoxa* reveal dose dependent reduction of diabetes in both normoglycemic and hyperglycemic rats (Tables 3 and 4).

Table 3: Results of the Effects of the extract of *Vitellaria paradoxa* on Fasting Blood Sugar Level of Normoglycemic Rats

Treatment	Dose (mg/kg)	Blood Glucose Level (mg/dl)				
		0 h	½ h	1 h	2 h	4 h
Extracts	200	192 ± 0.91	191 ± 0.91 (0.5)	184 ± 1.41 (4.2)	180 ± 0.91 (6.3)	138 ± 1.78 (28.1)
	400	185 ± 1.78	184 ± 1.41 (0.5)	157 ± 1.08 (15.1)	151 ± 0.71 (18.4)	122 ± 1.35 (34.1)
	800	196 ± 1.08	192 ± 1.08 (2.0)	174 ± 1.08 (11.2)	113 ± 1.58 (42.3)	82 ± 1.58 (58.2)
Glibenclamide	5	158 ± 0.91	154 ± 1.68 (2.5)	150 ± 0.91 (5.1)	129 ± 0.91 (18.4)	23 ± 1.47 (85.4)
Water (0.5 ml/kg)	Nil	148 ± 0.91	152 ± 1.08	156 ± 1.08	156 ± 1.08	181 ± 1.78

Each value of the blood glucose level represents the mean ± SEM; n = 4 in each group. Experimental groups were compared with control group. Values are statistically significant at P < 0.05 vs 0 h. The values in parenthesis represent the % decrease in fasting blood glucose level.

Table 4: Results of the Effects of the Extract of *Vitellaria paradoxa* on Fasting Blood Sugar Level of Hyperglycemic Rats

Treatment	Dose (mg/kg)	Blood Glucose Level (mg/dl)							
		Day 1					Day 3	Day 5	Day 7
		0 h	½ h	1 h	2 h	4 h			
Extracts	200	540 ± 0.91	533 ± 0.71 (1.3)	532 ± 0.71 (1.5)	523 ± 1.29 (3.1)	520 ± 0.91 (3.7)	482 ± 0.82 (10.7)	385 ± 1.47 (28.7)	332 ± 1.58 (38.5)
	400	389 ± 1.08	385 ± 1.58 (1.0)	343 ± 1.47 (11.8)	322 ± 1.08 (17.2)	320 ± 0.82 (17.7)	318 ± 0.91 (18.3)	310 ± 0.82 (20.3)	230 ± 0.82 (40.9)
	800	453 ± 1.47	444 ± 0.91 (2.0)	443 ± 1.47 (2.2)	320 ± 1.08 (29.4)	313 ± 1.08 (30.9)	216 ± 1.35 (52.3)	192 ± 0.91 (57.6)	183 ± 0.91 (59.6)
Glibenclamide	5	492 ± 1.08	486 ± 1.35 (1.2)	332 ± 1.11 (32.5)	323 ± 0.91 (34.3)	180 ± 0.91 (63.4)	158 ± 0.91 (67.9)	152 ± 1.08 (69.1)	112 ± 0.91 (77.2)
Water (0.5 ml/kg)	Nil	450 ± 1.08	453 ± 0.91	460 ± 1.08	464 ± 1.41	470 ± 0.91	_	_	_

Each value of the blood glucose level represents the mean ± SEM; n = 4 in each group. Rats used as negative control died after the first day. Experimental groups were compared with control group. Values are statistically significant at P < 0.05 vs 0 h. The values in parenthesis represent the % decrease in fasting blood glucose level.

Results of Microscopic Studies

The results of microscopic studies reveal the presence of unicellular non glandular trichome, large upper epidermal cells with no stomata, large phloem parenchyma cells, large epidermal cells with numerous paracytic stomata, annular xylems with phloem cells, isolated fibre from midrib, small prism calcium oxalate and midrib, pith, pericycle, epidermal hair (trichome), collenchyma, phloem, xylem from the transverse section of *Vitellaria paradoxa* leaf (Plates 1-9).

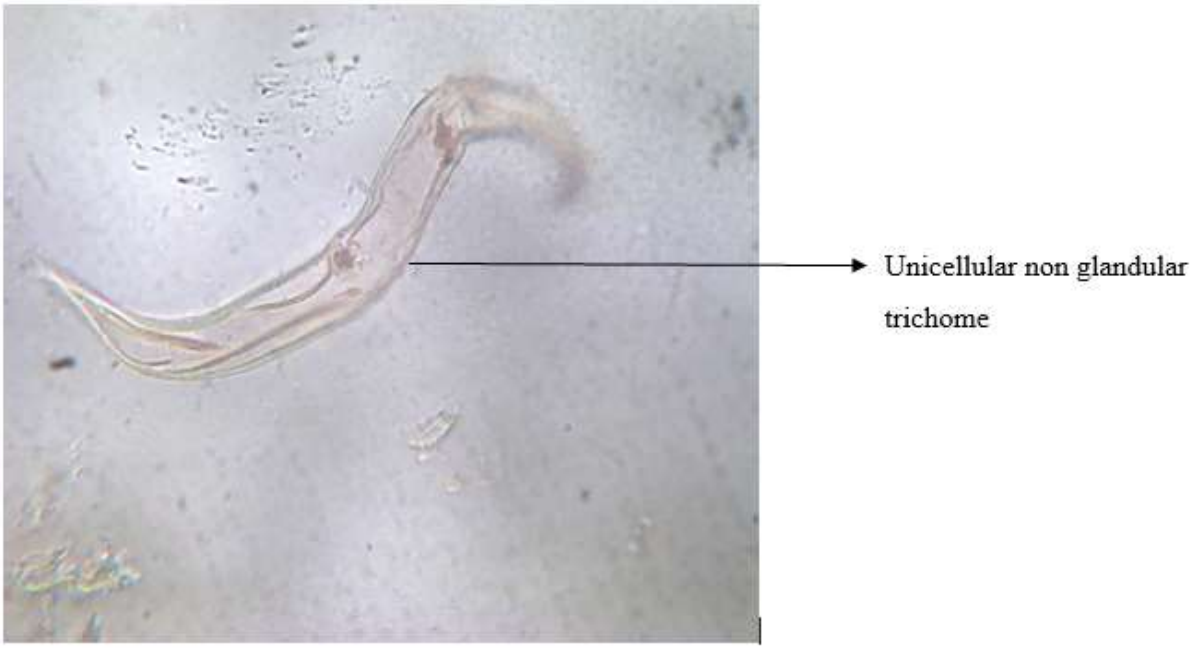

Plate 1: Unicellular non Glandular Trichome

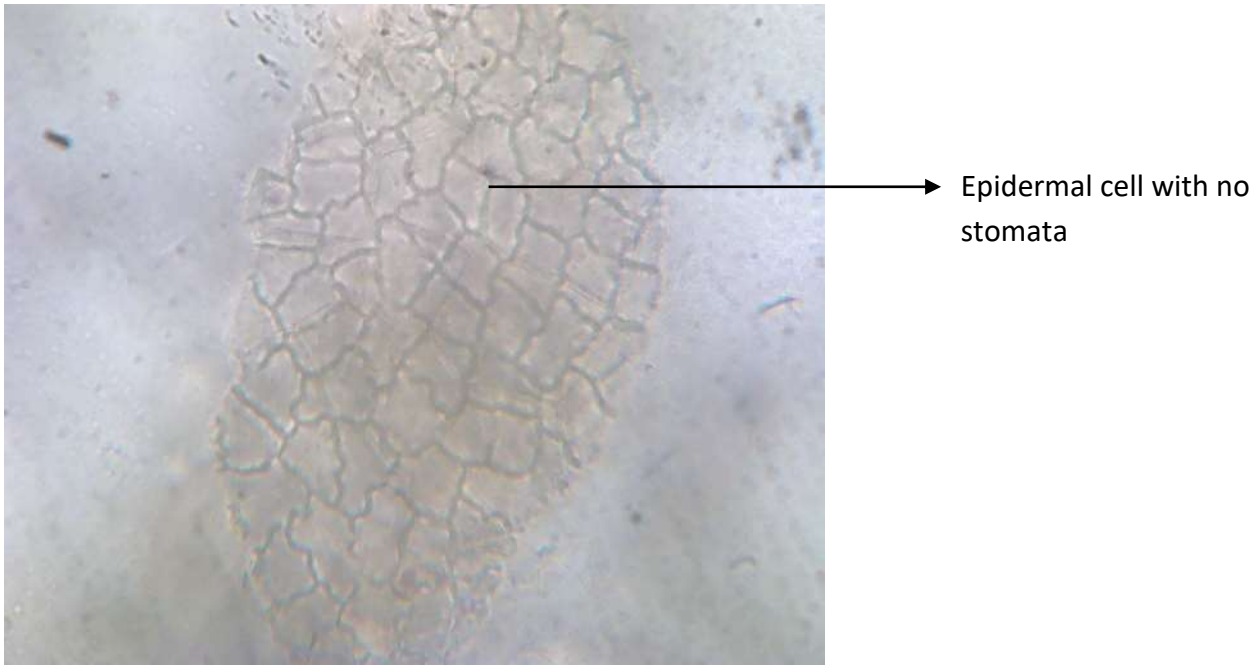

Plate 2: Large Upper Epidermal cells with no Stomata

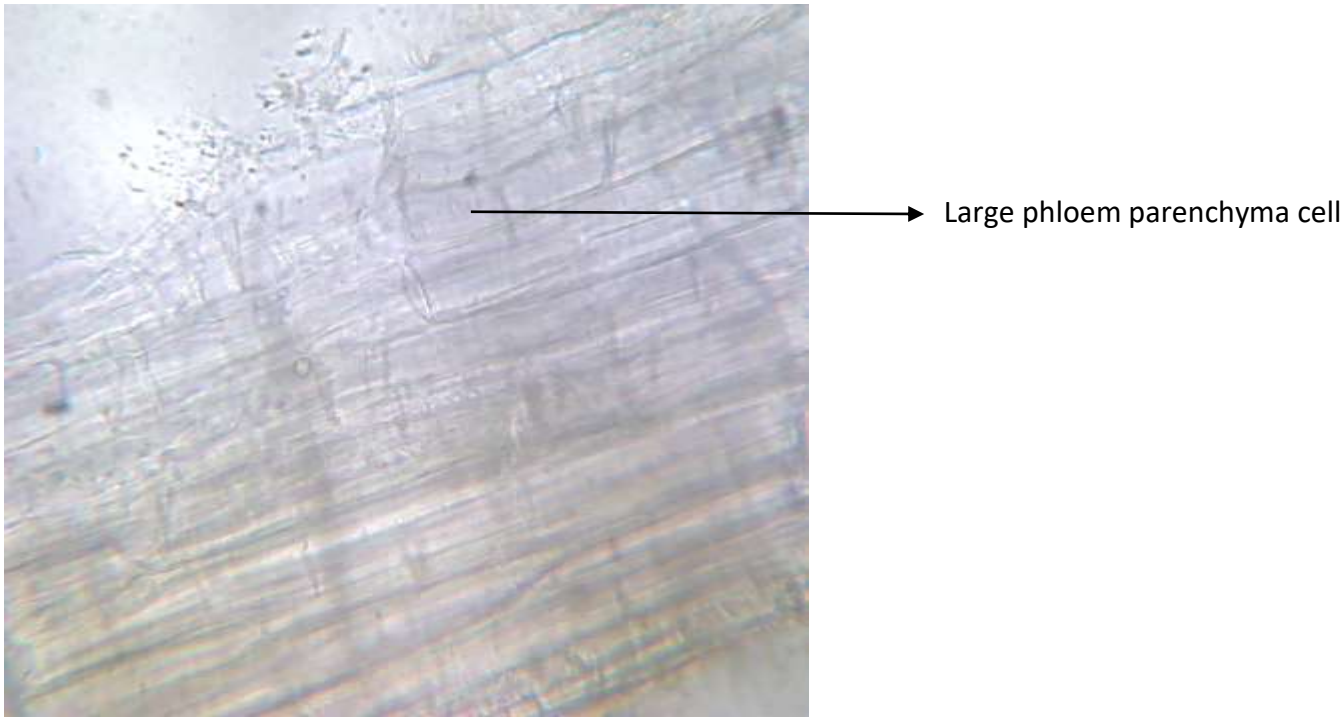

Plate 3: Large Phloem Parenchyma Cells

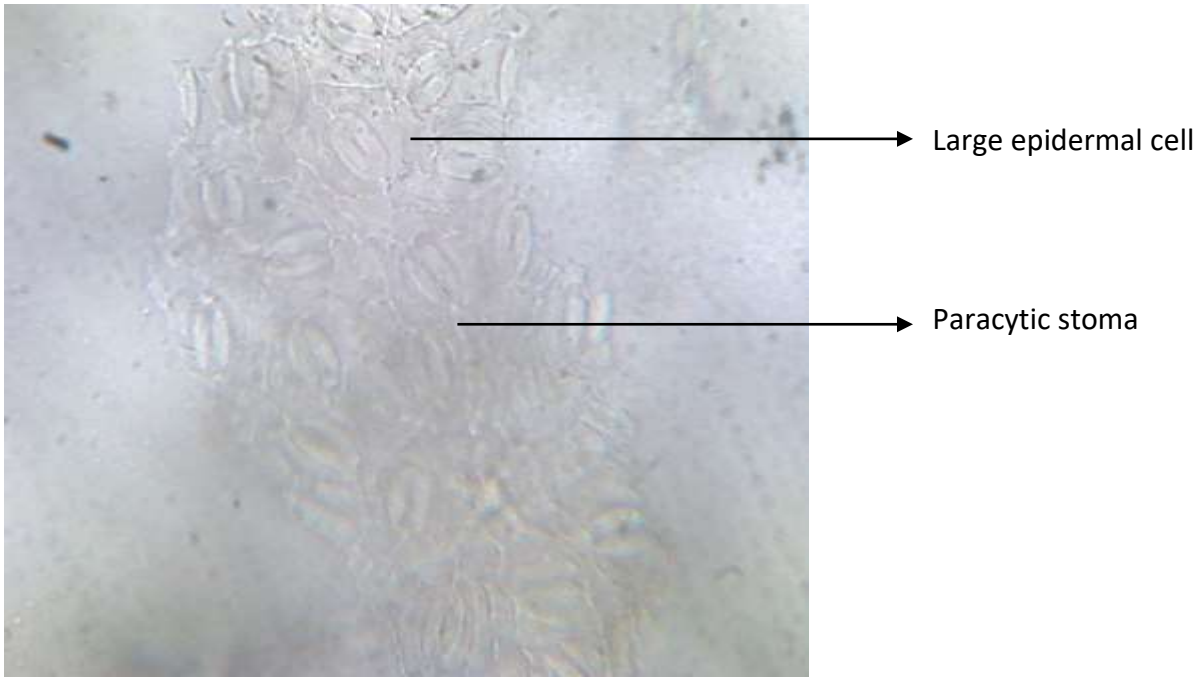

Plate 4: Large Epidermal Cells with numerous Paracytic Stomata

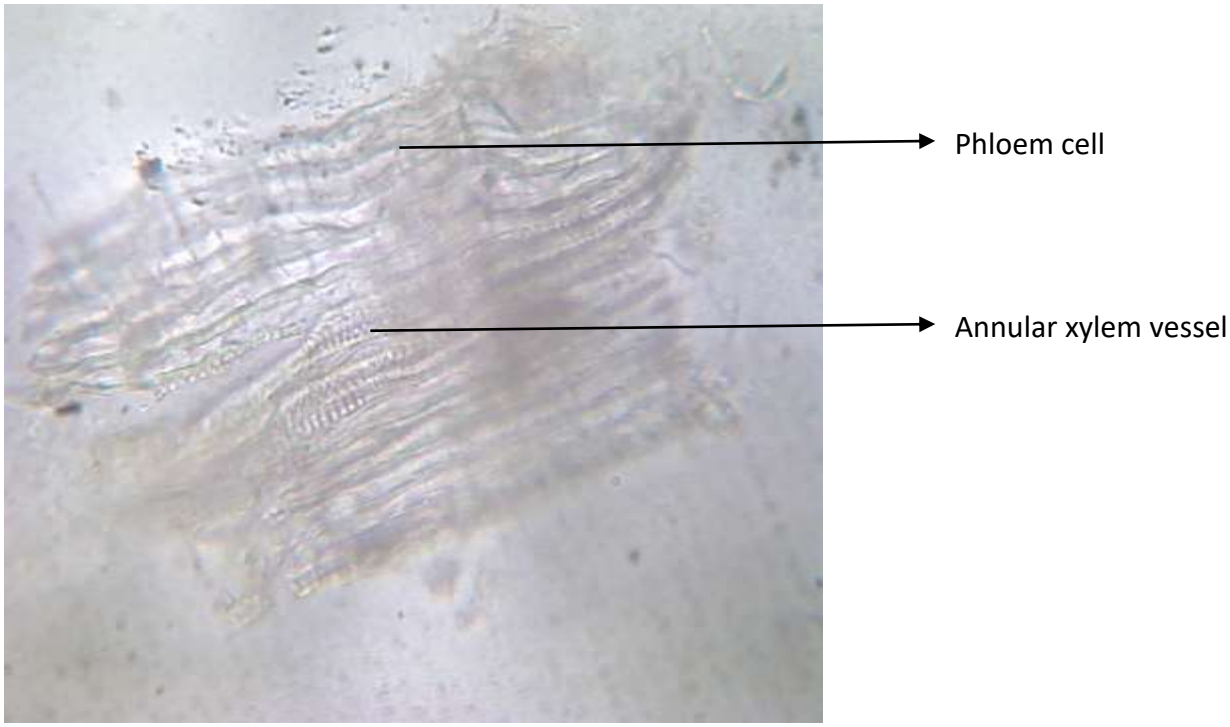

Plate 5: Annular Xylems with Phloem Cells

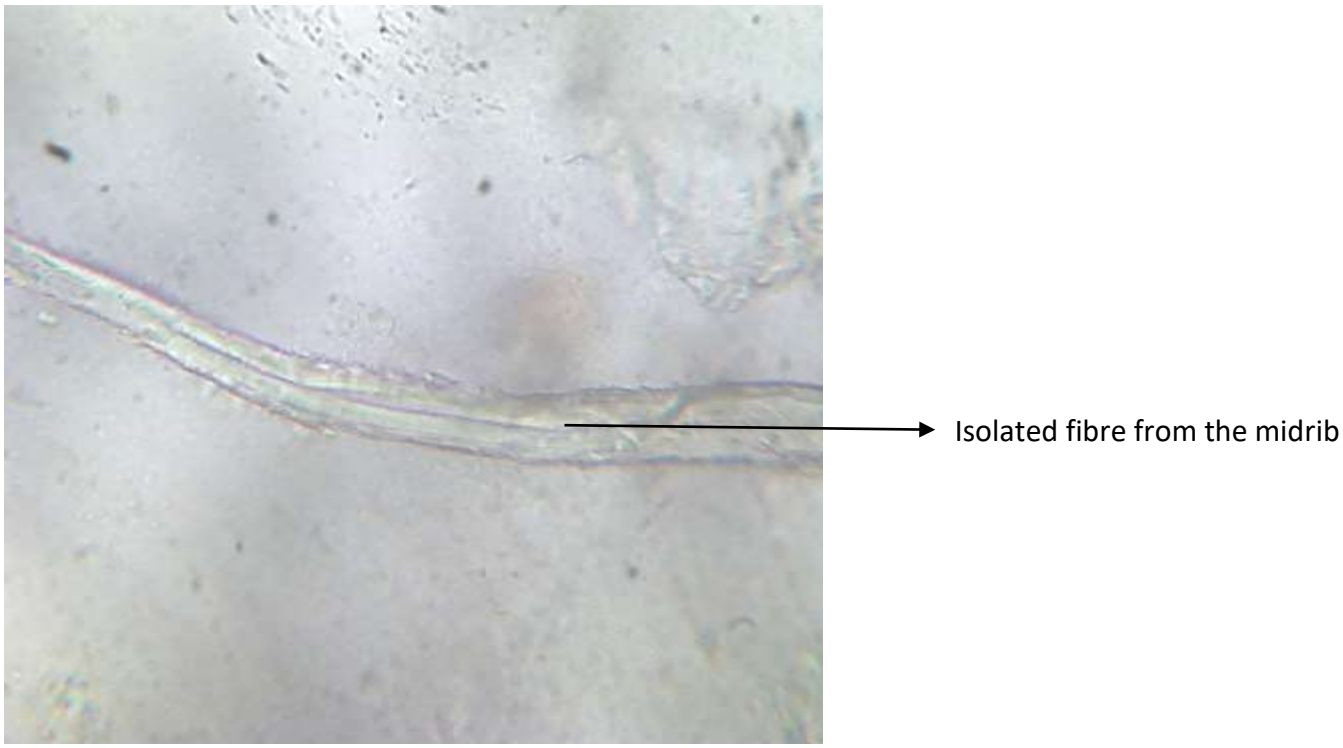

Plate 6: Isolated Fibre from the Midrib

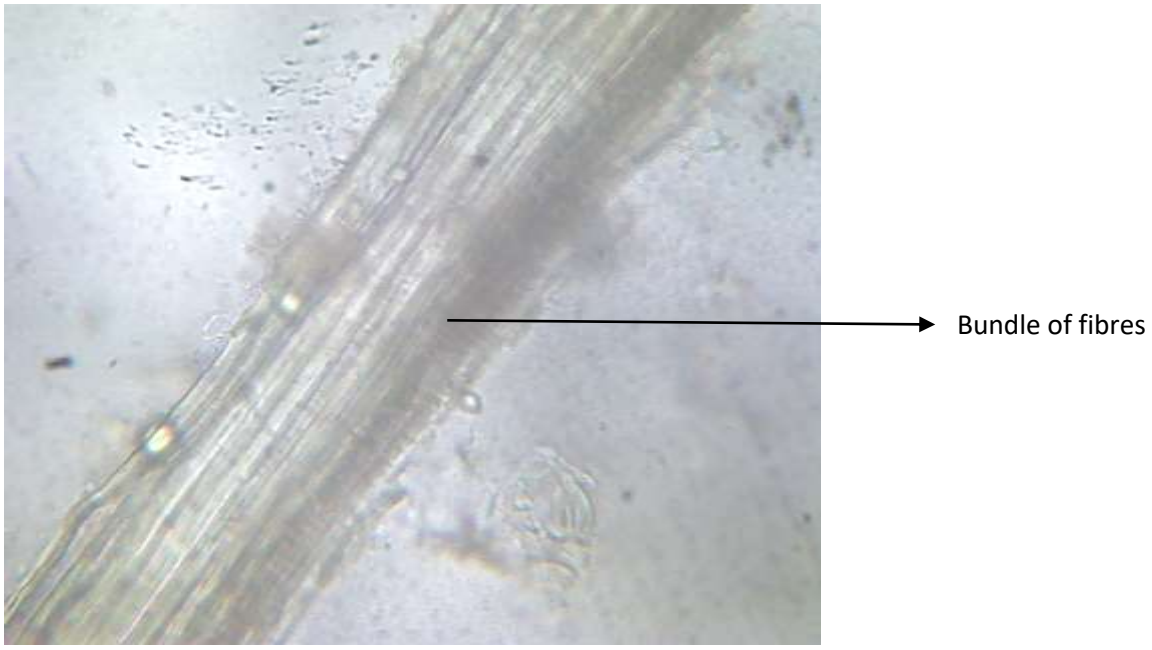

Plate 7: Bundle of Fibres from the Midrib

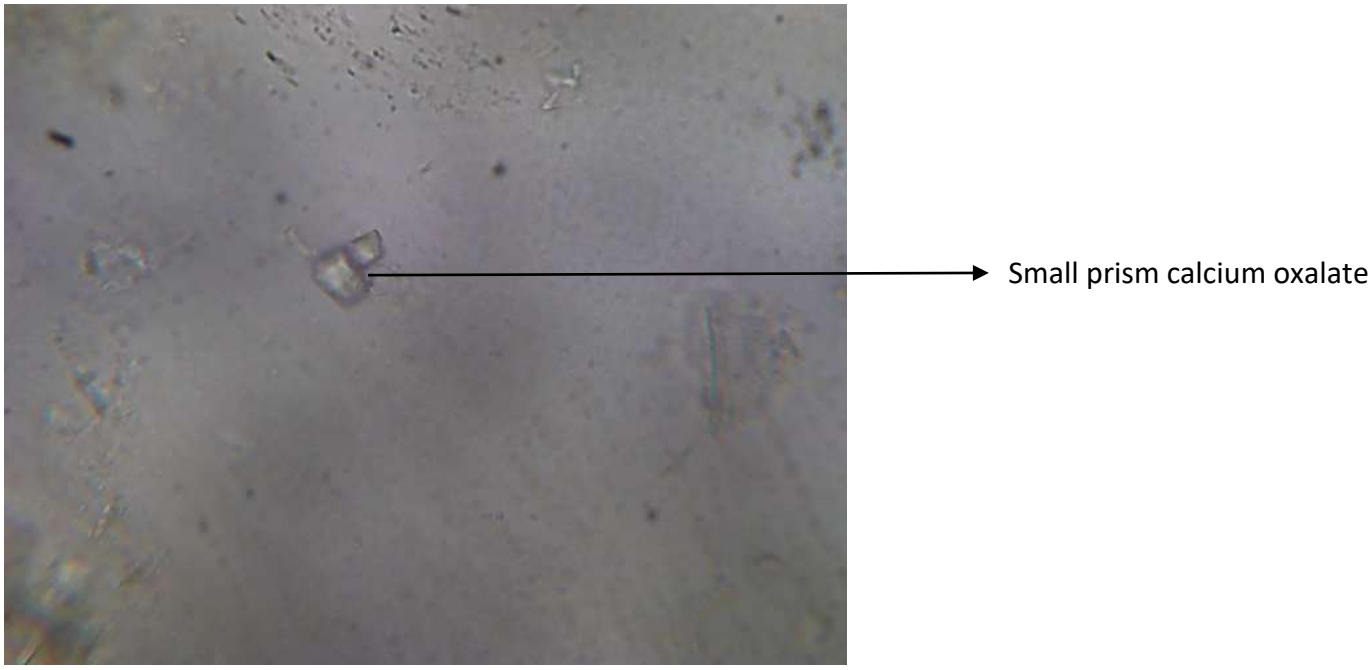

Plate 8: Prism of Calcium Oxalate

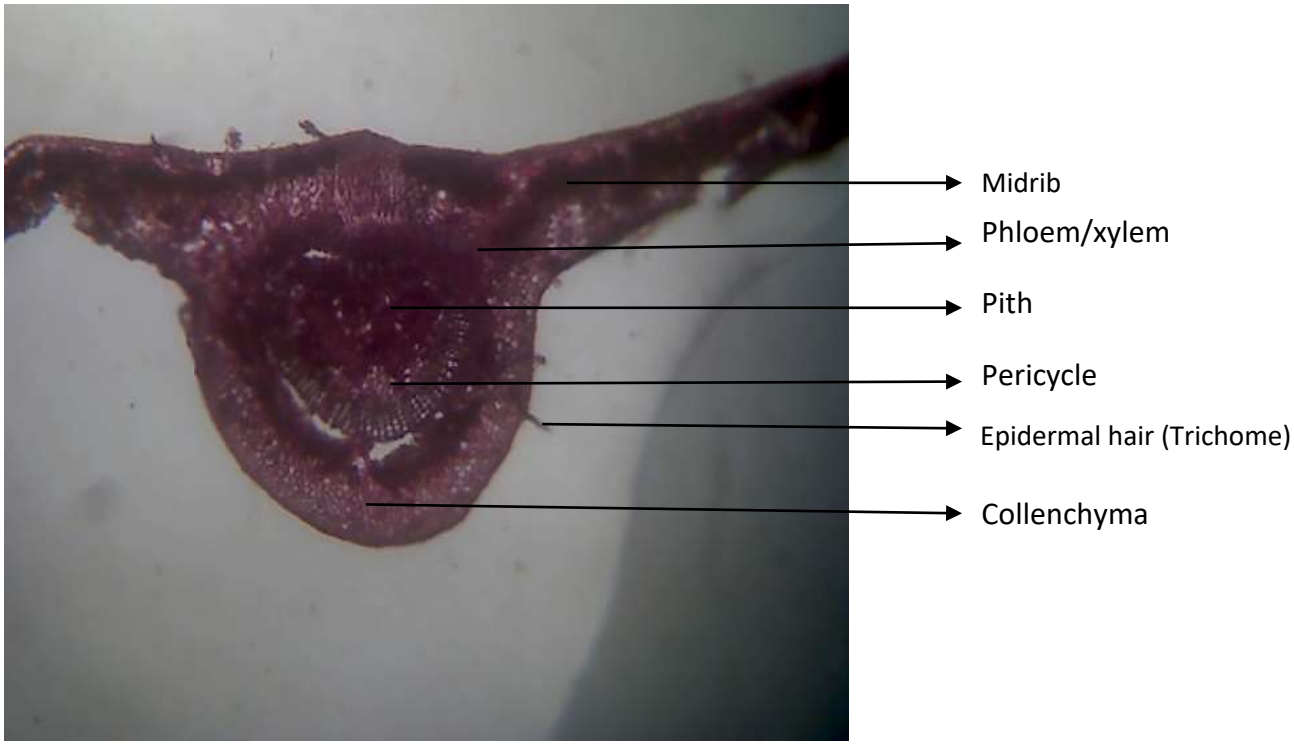

Plate 9: Transverse Section of *Vitellaria paradoxa* Leaf

RESULTS OF QUALITY CONTROL PARAMETERS

The results of the quality control parameters and their percentage composition are presented as follows in Table 5.

Table 5: Results of Quality Control Parameters

Parameter	Composition (%)
Total ash	7.75
Acid insoluble ash	2.16
Water soluble ash	2.32
Sulphated ash	2.55
Water soluble extractive	4.86
Alcohol soluble extractive	5.46
Moisture content	6.42

DISCUSSION

Diabetes mellitus is the world's largest growing metabolic disorder, and as the knowledge on the heterogenicity of this disorder is advanced, the need for more appropriate therapy increases [30]. Currently available synthetic antidiabetic agents

produce serious side effects like hypoglycemic coma and hepatorenal disturbances [31]. Following WHO's recommendation for research on the beneficial use of medicinal plants in the treatment of diabetes mellitus [32], investigations on hypoglycemic agents derived from medicinal plant also gained momentum.

The importance of phytotherapy in treatment of Type 2 diabetes mellitus seems to gradually increase in coming years. Phytotherapy can be effective in prevention of diabetes and its complications as well as optimization of the treatment and life standards. As in case of many chronic metabolic diseases, the mechanism is closely related, particularly in diabetes, to oxidative stress and inflammation in the body. Therefore, due to the antioxidant properties herbs should be considered for both prevention and treatment of diabetes. It is a well known fact that herbs constitute a part of culture in the whole world for quite a long time and that nutrients have beneficial effects on health. It is quite important to utilize and use the richness of the nature in the most efficient way. Special attention should be paid to these treatments, which are traditionally used by people for hundreds of years and use of which is gradually on the increase [17].

Because the safety and efficacy are the ultimate goals, to ensure the reproducible quality of the herbal drugs, the exact identification and quality assurance of the raw materials are essential [33]. Mostly, the herbal materials are supplied to the market is shrunken, twisted, rolled and deformed and without trade name and proper identification. So, such drugs can easily be adulterated or substituted.

Identification of the different classes of phytochemical constituents of the plant is an important parameter, which gives an indication of the pharmacological active metabolites present in the plant [34]. The presence of saponins, tannins, alkaloids in the plant parts studied (leaves) calls for an in-depth study on the plant. The metabolites are

of various pharmacological importance. The presence of saponins in this plant could be responsible for the traditional use of shea butter in the relaxation of muscles and in the treatment of sprains, wounds and colds as practiced generally in Nigeria.

The result of the antidiabetic study indicates that methanol extract of *Vitellaria paradoxa* was found to reduce the glucose level in alloxan monohydrate induced diabetic animals. The dose (800mg/ kg) showed the most significant reduction in blood glucose level compared to 200 mg/kg and 400 mg/kg in both normoglycemic and hyperglycemic rats. Thus, 800 mg/kg can serve as a lead for the formation of antidiabetic agent.

Microscopy also plays an important role in drug identification. The importance of epidermal characters, in general, are widely recognized in taxonomic considerations and in many cases these are successfully used in the identification of taxa at genus as well as species levels[35]. Similarly, studies in stomata have a great taxonomic as well as pharmacognostic value in proper identification of medicinal plants [36].

The application of pharmacognostic protocols such as macromorphology, micromorphology, organoleptic tests, ash value, histochemical studies and UV fluorescence study will help in identifying genuine drugs because these tests result in specific results for a particular drug. The physical parameters are almost constant for a plant therefore these are helpful in setting standards for a crude drug. Various physicochemical parameters were evaluated for the leaf as mentioned in WHO guidelines. These parameters are important for detection of drug adulteration or improper handling of raw materials [37]. One such parameter is ash value, which gives an idea of inorganic composition and other impurities in a plant drug. The total ash value is also important for detection of metal, salts, and silica [38]. The results obtained for ash values are of tremendous important in quality control especially for the

determination of percentage yield and detection of adulteration. In a study done by [39], ash values were used to detect the presence of any siliceous contamination and presence of any water soluble salts. Alcohol and water soluble extractive values indicate the presence of adulterants, faulty processing and poor quality of the drug. Total ash and acid insoluble ash contents are important indices to determine quality and purity of herbal medicines. There are always chances of microbial growth when the crude drug is stored for a longer period of time and the moisture content of crude drug is directly related to its stability and consequently with the shelf life of crude drug. The lower the moisture content, the higher will be the stability of that drug and chance of microbial growth will be less and vice versa [40].

CONCLUSION

It can be seen from this research that *Vitellaria paradoxa* contains proteins, alkaloids, tannins, terpenoids, carbohydrates, reducing sugars, glycosides, saponins, flavonoids, resins, steroids, oils; and acidic compounds were absent. The result of the antidiabetic study showed that methanol extract of *Vitellaria paradoxa* possesses antidiabetic activity in alloxan induced diabetic rats and hypoglycemic effects in normal rats. In addition, this research established the pharmacognostic studies and quality control parameters of the leaves of *Vitellaria paradoxa.* The results obtained can serve in the identification and preparation of a monograph of *Vitellaria paradoxa* from the family of Sapotaceae and its possible inclusion in the Pharmacopoeia. Further studies are required towards isolating, purifying and characterizing these bioactive phytoconstituents which could serve as lead molecules to the development of better antidiabetic agent.

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