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Research Article

Pharmacognostic Standardization and Antidiabetic Evaluation of the Methanolic Extract of the Seeds of *Pterocarpus Santalinoides* L'herit Ex DC- Family Fabaceae

*Ani A.C[1], Ezugwu, C.O[2]

[1,2]*Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Enugu State Nigeria.*

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ABSTRACT

Diabetes remains a major public health concern affecting about 2.8% of the global population. Chronic hyperglycaemia of diabetes is frequently associated with long-term damage, dysfunction, and failure of different organs. With multiple risk factors, delayed diagnosis, life-threatening complications, failure of the current therapies, and financial costs, there is a need to look for alternative treatment.

Methods: Acute toxicity and anti-diabetic property of the methanolic extract of *P.santaliniod*es were evaluated on mice and alloxan induced diabetic rats respectively. Twenty alloxan induced diabetic rats were used for both chronic and sub chronic test. For both test methods, two dose levels (250mg/kg, 500mg/kg) were chosen. Water (5ml) and glybenclamide (5mg/kg) were used for negative and positive control respectively. Data obtained were analysed using analysis of varaiance (ANOVA) and T-test. The plant material was subjected to pharmacognostic studies, and it includes physicochemical analysis, phytochemical evaluation, determination of extractive value, histo-chemical analysis and microscopic analysis of the powdered crude drug.

Results: The 250mg/kg dose and 500mg/kg dose were statistically significant at $p<0.05$ at day 1,6,9,12,24 and 6, 9, 12, 24 relative to the placebo for chronic respectively. For sub chronic study, statistical significance was seen only for 500mg/kg on day 10 relative to placebo at $p<0.05$. Phytochemical analysis of the plant revealed alkaloids, resins, steroids,terpenoids,flavonoids, proteins, carbohydrates, reducing sugars, oils, acidic compounds,cardiac glycosides, tannins and saponins. Physicochemical analysis (total ash {4.7%}, water soluble ash {1%}, acid insoluble ash {12%}, sulphated ash 1.25%}); extractive value (ethanol extractive value {2.2%}, chloroform extractive value {10%}); histochemical analysis (lignified tissue, calcium oxalate, protein, starch, fat and oil and cellulose cell wall) and microscopic analysis of the powdered (branched multicellular non glandular trichomes, elongated unicellular non-glandular trichomes, epidermal cell of the testa,starch globules, annular xylem vessel, peristerm of raphae, large irregularly shaped calcium oxalate and layer of peristerm containing pigment).

Conclusions: Finally, *P. santaliniodes* possess anti-diabetic property which may be linked to the phytoconstituent and thus could serve as lead drug.

Keywords*:* *Pterocarpus, santalinoides*, Phytochemicals,Histo-chemical, Powder analysis, Physicochemical, Toxicity test,Antidiabetic, Acute model, Subchronic model.

INTRODUCTION

Diabetes found its place in antiquity through Egyptian manuscripts dating back to 1500 B.C.[1]Diabetes mellitus is a metabolic disorder characterized by the presence of chronic hyperglycaemia resulting from impairment in the metabolism of carbohydrates, lipids and proteins. It presents with three cardinal signs such as polyuria, polydipsia and polyphagia.[2] The pathogenic process ranges from autoimmune destruction of the pancreatic b-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action.[3] The two most common types of diabetes mellitus are type 1diabetes (T1D) and type 2 diabetes (T2D).T1DM is a disorder of glucose homeostasis characterized by autoimmune destruction of the insulin-producing pancreatic beta-cell that progressively leads to insulin deficiency, resultant hyperglycaemia[4] and life-long dependence on exogenous insulin, thus it is regarded as an autoimmune disease.[5] T2M occurs when the tissues are resistance to the insulin hormone produced[6] and a lack of adequate compensation by the beta cells which lead to a relative insulin deficiency. There have been a spike in the prevalence of diabetes in country such as Florida.[7] Globally, WHO projects that deaths resulting from diabetes will double between 2005 and 2030.[8] In deed diabetes mellitus has impacted negatively on social, economic and public health, owing to the high morbidity of diabetic complications.[9] Burden resulting from diabetes has been estimated[10] and American Diabetes Association after adjusting for inflation, stipulated a 26% increase in economic cost from 2012 to 2017 owing to the increased prevalence and increased cost per person with diabetes.[11]

Diabetic complications are classified into macro vascular and micro vascular symptoms with neuropathy,

*Author for Correspondence: aniangela598@gmail.com

nephropathy and retinopathy being the major micro vascular complications.[6] Diabe53tic kidney disease is the leading cause of end-stage renal disease[12] with fourteen times increase in mortality risk.[13] Other well-known complications of diabetes includes hypertension, erectile dysfunction, infections, delayed wound healing etc.[2,14] gastrointestinal complications,[15,16] heart failure with worse prognosis,[17] blindness,[18] dyslipidaemia[19] and diabetic foot ulcers.[19] Associated with the emergence of diabetes are central obesity[20] , intrauterine exposure to maternal diabetes[21], entero-viral infection in early childhood[22], complex inheritance-environment interaction[23] and medical conditions.[24] Effective diagnosis can be done via three basic parameters; A1C, oral glucose tolerance test (OGTT) and fasting blood sugar.[25] The accuracy of A1C has been questioned due to associated short comings in the face of anaemia, haemolysis and iron deficiency carbamylation and acidosis.[25] As an alternative artificial intelligence in the form of K-nearest neighbour,[26] fructosamine and glycated albumin reflecting the glycation of serum protein and glycated albumin respectively offers better accuracy.[27]

Management guidelines are geared towards maintaining a tight glycaemia control which essentially delays the onset of complications from diabetes. In the core of diabetes management lies lifestyle modification and weight reduction.[28] Exercise and diet has been shown to facilitate a reversal of insulin resistance.[29] Diabetes mellitus being a disease with several complications will involve the management of varying health problems. In effect, overall care of diabetes necessitates multidisciplinary care is needed.[30] In the heart of diabetes management are drugs such as metformin, sulphonyureas, glinide, thiazilidinediones, gliptins, alpha glycosidase inhibitors and sodium glucose co transporter 2 inhibitors etc. Despite the use of these drugs in diabetes management, glycaemia control is usually not achieved partly due to clinical inertia[9] in addition to their side effect.[31] Data suggesting treatment failures have also emerged. For instance, metformin failure has been shown to occur more rapidly in clinical practice than in clinical trials.[32] Failures of non-pharmacologic therapy and oral anti-hyperglycaemic agents burdens patients with a heavy history of uncontrolled hyperglycaemia.[33] Thus, the search an ideal therapy becomes one of the top priorities in combating this disease.

Over the last few decades the role of medicinal plants as a primary tool in the preservation of health and management of diseases is realized with great concern. From prehistoric time, man has used natural product to alleviate diseases.[34] Several bioactive compounds have been isolated from natural origin[35,36] and some of these natural products has served as lead compounds in drug discovery as well as a precursor in modern medicine.[35,37] Oftentimes after isolation, purification and characterization, the final use of the active component sometimes tally with their ethno medical use making ethno medicine key to uncovering the earth's medicinal reserve.[34,38] Natural products used in drug development have the incomparable advantages of abundant clinical experiences and unique diversity of chemical structures and biological activities.[39]

The medical significance of leguminous plant has prompted their use as natural biopharmaceutics for improving modern and postmodern human health.[40] The genus Pterocarpus, a family of Fabaceae (Leguminosae) consists of about 35 species. Pterocarpus soyauxii also contain chemical compounds.[41] Phytochemical analysis of P. santaliniodes revealed the presence of numerous secondary metabolites with massive structural diversity.[42] Some of the species have been studied and was found to contain 14 compounds[43] which are likely responsible for its pharmacological activities.[44,45] Ethanol exhibited more extractive capacities relative to pet ether, ethyl acetate.[46] On comparison, the leaves contains the bulk of the active principles, followed by the bark while the reverse was the case qualitatively.[47] P. santalinoides has been confirmed to possess anagelsic and antibacterial properties,[48] increase haematological parameters (hemoglobin, platelet index and PCV),[49] exhibit antidiabetic property,[50] improve serum lipid level,[51] have hepatoprotective property,[52] antioxidant,[53] have anti-diarrheal effect[54] and antipyretic activity.[55] The anti-diarrheal effect was synergistic on combination with 5:5 methanol extracts of Alchornea cordifolia and P. santalinoides against multi-drug resistant diarrhoegenic bacterial infection.[56] The antibacterial properties agrees with other findings.[46,57] The analgesic property is attributed to a morphine type alkaloid based on the IR spectral data gotten and thus could serve as a potential source of morphine[58] and the antioxidant property as a result of flavonoid.[59] The aqueous extract was also effective against Klebsiella pneumonia.[60]

Additionally,P. santalinoides has nutritional properties, anti-nutritional property in the likes of oxalate, phytic and hydrogen cyanide[61] and minerals.[48] Analysis of the seed showed that processing increased the concentration of the nutritive component.[62] Above all, acute toxicity testing confirmed the safety of the drug. Thus P. santalinoides is important in the field of pharmacology and therapeutics to explore alternative medicine in the treatment of different diseases in human and animals.

Standardization of medicinal plant is an ambiguous task due to its heterogeneous composition in the form of whole plant, plant part and extract thereof. The marked growth in the worldwide phyto-therapeutic market has made standardization even more pressing.[63] To ensure reproducibility of herbal products, proper control of the starting product remains pertinent. The first step in ensuring reproducible qualities is via authentication. Despite the evolution of modern techniques, pharmacognostic evaluation remains more reliable and invaluable. According to WHO[64] macroscopic and microscopic description of a medicinal plant remains the first step in establishing the identity and degree of purity of a plant material. Indeed macrospical and microscopical studies of phyto drug are pertinent to establishing botanical quality control.

Animals have historically played a critical role in the exploration and characterization of disease pathophysiology, target identification and in the evaluation of novel therapeutic agents and treatments in vivo.[65] In vivo studies prior to a drug becoming commercially

available is crucial because in vitro studies do not possess the ability to provide quantitative results of absorption, distribution, metabolism, and excretion in animal and human models.[66] Animal models have a long history in the field of diabetes research. Due to the increase in the prevalence of diabetes mellitus worldwide, the diabetic rat models are believed to play an important role in elucidating the pathogenesis of human diabetes and its complications, which are essential for investigating and developing novel drugs for diabetes and its complications.[65] The inbred and outbred mice showed comparable phenotypic variation, suggesting that the potential contribution from genetic heterogeneity to the total phenotypic variation is relatively small, and that the phenotypic variation is primarily influenced by experimental, analytical and environmental variation.[67] Animal models for diabetes include chemical, surgical (pancreatectomy) and genetic manipulations. Animal models for type 1 diabetes range from animals with spontaneously developing autoimmune diabetes to chemical ablation of the pancreatic beta cells while type2 diabetes is modelled in both obese and non-obese animal models with varying degrees of insulin resistance and beta cell failure.[68] Chemical method of diabetes induction using alloxan induces histological changes in the intestine but at dose lower than 200, degeneration of the various layers of the duodenum remains minimal.[69] Thus, animals offer a feasible model to study the pathogenesis of diabetes, develop and test new therapeutics.

Having established the limitation of oral hypoglycaemic agent and high mortality resulting from poor management, diabetes management has earned a global interest. In effect searching an ideal therapy has become one of the top priorities in combating this disease, thus underscoring the timeliness of this work. It will also justify or invalidate the use of P.santalinoides in the management of diabetes by the locals.

MATERIALS AND METHODS

Plant material collection and authentication:

Seeds of *P. santalinoides*werecollected from a farm in Oduma, Aninri LGA, Enugu state, South Eastern Nigeria. Identification was done by a taxonomist, (Mr. Felix Nwafor) in Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka.

Experimental Animals

Adult mice were used for the experiment. They were obtained from the Department of Pharmacology and Toxicology animal house, University Of Nigeria Nsukka. They were allowed to acclimatize for some time with constant feeding and free access to water.

Preparation of the plant extracts of *P. santalinoides*

Extraction was carried out using the method described standard method[56] with slight modifications. The seeds of *P.santalinoides* were air-dried after washing with distilled water at room temperature, pulverized into fine powder using an electric blender. Two hundred and fifty grams (250 gm) of the powdered sample was macerated in 1.25 litres of methanol (80%) with intermittent stirring to aid extraction for 24 hours. The marc was further macerated for 24 hours, filtered through a (cotton wool plugged funnel) to obtain a clear filtrate and added to the other filtrate. The filtrate was concentrated under reduced pressure in a rotary evaporator and was stored in refrigerator at a temperature of 4-8°C until needed for analysis.

Determination of Percentage Yields of Crude Extracts

The percentage yield for the plant sample was calculated as the amount of crude extract recovered in mass compared with the initial mount of powdered plant materials used.[57] It is presented in percentage (%) and the yield percentages were calculated as followed:

Weight of extract / weight of sample × 100

Extractive values:

The protocol was carried out according to established standards.[64] 5grams of the powdered drug were macerated with 100ml of absolute ethanol and chloroform in a closed flask for twenty-four hours with intermittent shaking. The mixtures were filtered rapidly while avoiding losses. Finally, 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and subsequently dried to a constant weight and weighed. The percentages of solvent soluble extractives were calculated with respect to air dried drug.

Administration of Plant Extract and sample collection

Twenty adult rates were used for the acute and sub chronic test study respectively. They were randomly distributed into four groups (A, B, C and D). Two dose levels (250mg/kg and 500mg/kg) of the methanolic extract of *P.santalinoides* were selected. Varying dose volumes were administered to each group from 100mg/ml and 1mg/ml stock solutions of the extract and standard respectively depending on the weight and intended dosage (either 250mg/kg or 500mg/kg).

Acute Toxicity Test (LD_{50})

The acute toxicity study of the methanolic crude extract of *Pterocarpus santalinoides* was assessed by giving oral administration of the drug to albino mice using the standard method described and[70] adopted by some researchers.[55,71] Briefly, the tests involved two phases. The first phase involved the determination of the toxic range. The mice were placed in three groups (n = 3) and the extract (10, 100 and 1000 mg/kg) suspended in distilled water was administered orally. The treated mice were constantly observed for the next 4hrs, then intermittently for the next 6hrs, then over a period of 24hrs for the number of deaths and behavioural changes. Since no death was encountered in first stage, the second phase was initiated. In this phase, three groups (n = 1) of mice were used for each dose. Each group received different doses of the extract (p. o.) 1600mg/kg, 2900 mg/kg and 5000mg/kg of the methanolic extract of *P.santalinoides* respectively. The animals were observed for lethality or signs of acute intoxication for the next 24hrs. The LD_{50} is the geometric mean of the highest non-lethal dose and the least toxic dose. Where '**a'** is the lowest dose that brought death and '**b'** is the highest dose that did not bring death.

Preliminary Qualitative Study (Phytochemical analysis)

Phyto-chemical screening of *Pterocarpussantaliniodes* extract for the active constituents was done according to standard protocol.[72,73]

Effect of Test Drug on Blood Sugar Level in Alloxan induced hyperglycemic Rats

Twenty-eight rats of either sex weighing 132-211gm were selected for this study. They were kept fasting for 36 hours prior to the experiment however with constant access to water. Initial blood was collected (tail blood) from every rat in order to ensure their non-diabetic state. Freshly prepared 10% aqueous solution of alloxan monohydrate was injected intra-peritonially to all the rats in a dose of 150 mg/ kg body weight. A 5% glucose solution was made available for the animals so as to prevent alloxan induced phase of hypoglycaemia. Next day, at the end of 48hrs of alloxan administration, blood samples were collected from all the rats and then rats. Subsequently, the rats were divided into four groups A, B, C and D having 5 each for acute and sub chronic study. A 100mg/ml concentration of aqueous solution of test drug was administered to the rats of group A and B orally in a dose of 250mg/kg and 500mg/kg of body weight daily for twenty-four hours and fourteen days respectively while group C and D received 5ml of water and 5mg/kg of glybenclamide respectively.

Sterilization of Materials

The sterilization of glass wares was done with water, detergent and disinfectant. The working tables were also swabbed with 75% ethanol before and after the experiment. The cages and eating utensils were cleaned on a daily basis**.**

Macroscopic Examination

Morphological characteristics of the seed of *Pterocarpus santalinodes* evaluated comprised of shape, size, taste, fracture, texture, colour and odour.

Physiochemical analysis

The dried powdered seed of *P.santaliniod*es was subjected to standard procedures according to WHO guidelines for the determination of various physicochemical parameters[64] and adopted by.[74]

Total ash value: About 3 g of air-dried powder of *P.santaliniodes* was weighed into a tarred silica crucible. The sample was spread in an even layer and ignited by gradually increasing the increasing the heat to 500-600°C until it is white, indicating the absence of carbon. It was cooled, weighed and percentage of total ash with reference to the air-dried powdered drug was calculated.

Acid insoluble ash: The ash obtained in the above method was boiled with 25 ml of dilute HCl for 5 minutes. The residue was collected on ash less filter paper and washed with hot water, ignited, cooled and weighed. The percentage of acid insoluble ash with reference to the air-dried drug was calculated.

Water soluble ash: The total ash obtained was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at allow temperature. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated.

Sulphated ash: Accurately weighed 1 g of the powder was put into a silica crucible previously heated to redness for 10 minutes and moistened with sulfuric acid (~1760 g/l) TS. The crucible containing the powder was heated gently to remove the excess of acid and ignited at about 800 °C until all the black particles have disappeared. Again, it was moistened with sulfuric acid (~1760 g/l) TS and reignited. A small amount of ammonium carbonate R was added and reignited to constant weight (WHO, 2012).

Moisture content: The powdered drug sample (3 g) was placed on crucible without preliminary drying after accurately weighing it in a tarred evaporating dish and dried at 105 °C for 5 hours, followed by weighing. The drying was continued until two successive readings matched each other or the difference between two successive weighing was not more than 0.25% of constant weight. Constant weight was reached when two consecutive weightings after 30 minutes in a desiccator showed not more than 0.01g difference.[75]

Powder analysis

A judicious quantity of the sample powder was mounted on clean glass slide and clarified with chloral hydrate. One drop of chloral hydrate was dropped and passed over a Bunsen burner repeatedly until bubbles formed. This signified the successful clearing of the tissue. Prepared samples were then observed for identification of diagnostic characters. The test was carried out in line with established protocol and adopted by killer.[76]

Histo-chemical analysis

The powdered samples were treated separately on microscope slides and observed under the light microscope for the presence of chemical substances which include cellulose, starch, proteins, fats and oils, lignified tissue, calcium carbonate and calcium oxalate crystals. It was done according to WHO guideline methods[64] and as implemented.[40,77] Below are the methods;

Test for cellulose: To the powdered plant materials, N/50 iodine and 60% sulphuric acid was added. A blue colour indicates the presence of cellulose.

Test for lignin: The samples were mounted in phloroglucinol and 2drops of hydrochloric acid added. A red colour would indicate the presence of lignin. The intensity of red colour would indicate the extent of lignifications.

Test for starch: The various samples were mounted separately in N/50 iodine. A blue colour indicates the presence of starch.

Test for calcium oxalate crystals: The powdered samples were cleared in chloral hydrate solution. The presence of bright structures of definite shapes and size indicate the presence of calcium oxalate crystals. After the addition of a few drops of conc. Sulphuric acid (80%) and re-view under the microscope, calcium oxalate crystals disappeared. This confirms the presence of calcium oxalate crystals in the sample.

Test for oil: The different samples were mounted in Sudan IV reagent. A pink color in any of the structures would indicate the presence of oils.

Calcium carbonate: Crystals or deposits of calcium carbonate dissolve slowly with effervescence when acetic acid or hydrochloric acid is added.

Statistical Analysis

The experimental results are presented as mean ± SEM (Standard error of the mean). All statistical analyses were performed using IBM SPSS. The differences among groups were evaluated by oneway analysis of variance (ANOVA), Duncan's multiple range test and T-test. A level of $p < 0.05$ was used as the threshold for statistical significance.

RESULTS

Organoleptic characters of powdered seed of Pterocarpus santaliniodes

The powdered seed material was bland, pale green in colour, coarse texture and odourless.

Phytochemical Analysis

Phytochemical anlaysis of methanolic extract of Pterocarpussantalinoides showed the presence of alkaloids, resins, steroids,terpenoids,flavonoids,proteins,carbohydrates, reducing sugars, oil, acidic compounds,cardiacglycosides,tannins,saponins.The result of phytochemical screening are summarised in Table-1

Macroscopic features of the seed

The seed of P. santaliniodes has distinctive characteristics. It is scarlet colored with an oblong shape.

Determination of the percentage yield

150gram/250gram × 100 = 60%

Determination of Extractive values: These are useful for the evaluation of a crude drug. It gives an idea about the nature of the chemical constituents present in the crude drug. Useful for the estimation of constituents extracted with the solvent used for extraction. Employed for material for which as yet no suitable chemical or biological assay exists.

Table 1: Results of Qualitative phytochemical screening of the Methanolic seed extract of *Pterocarpussantaliniode*s

Secondary Metabolite	Relative Abundance
Alkaloids	+
Resins	+++
Steroids	+++
Terpenoids	+++
Flavonoids	++
Proteins	+
Carbohydrates	++
Reducing Sugars	+++
Oils	++
Cardiac Glycosides	+++
Tannins	+++
Saponins	+++

Key (-) = absent (+) = present in small quantity
(++) = moderately present (+++) = Absolutely present

Table 2: The Result of the Histochemical Evaluation of content of *P. santaliniodes*

S.no.	Diagnostic character	Reagent used	Observation
1	Lignified tissue	1:1Phloroglucinol and conc.HCl	Cherry red
2	Calcium oxalate	Chloralhydrate/aceticacid	Needle shaped
3	Calcium Carbonate	Acetic acid	Effervescence absent
4	Protein	Ninhydrin	Violet and golden yellow
5	Starch grains	Ethanol and N/50 Iodine solution	Blue
6	Fats and oils	Sudan red IV	Brick red
7	Cellulose cell wall	N/50 Iodine and 80% Sulphuric acid	Blue

Table2: Powder sample of leaf showed presence of cellulose cell walls, lignin, protein, fat and oil and starch. The starch granule appeared in clutters (multiple aggregations) similar to wheat starch. On Ninhydrin application a violet and golden yellow colour showed the possible presence of different amino acids. On addition of acetic acid there was no evolution of effervescence confirming the absence of calcium bicarbonate.

Table 3: Result of The physico-chemical analysis of *P. santaliniodes*

Test	Observations
Total ash	4.7%
Water soluble ash	1%
Acid insoluble ash	4%
Moisture content	12%
Sulphated ash	1.25%
Ethanol extractive values	2.2%
Chloroform extractive values	10%

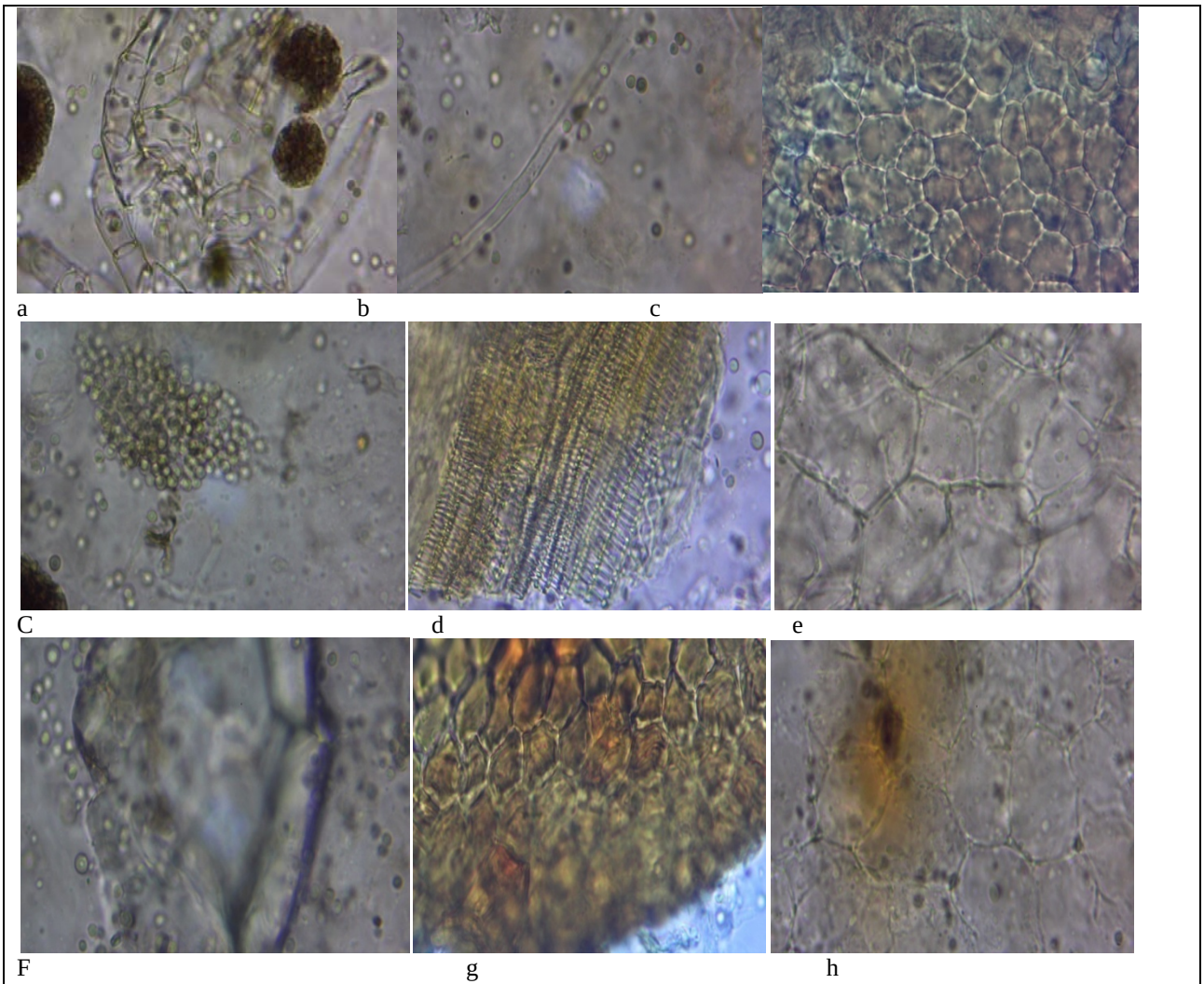

Fig1: The various diagnostic character of powdered crude drug. **a)** Branched unicellular non glandular trichomes **b)**Elongated unicellular non glandular trichomes **c)**Epidermal cells of testa **d)**Group of starch granules **e)**Group of annular xylem vessels **f)**Peristerm of raphae **g)**Large irregularly shaped calcium oxalate **h))** Peristermcontaing pigment **i)**Layer of peristerm

Table 4: The Result of Acute Toxicity Test of *P. santaliniodes*

Phases	**Dose(mg/kg)**	**Weight(g)**	**Dose(mg)**	**Volume(ml)**	**Conc(mg/ml)**	**Death**
I	10	20.02	0.2	0.2	1	0
		20.08	0.2	0.2	1	0
		18.49	0.18	0.18	1	0
	100	21.36	2.14	0.21	10	0
		42.79	4.28	0.43	10	0
		32.96	3.3	0.33	10	0
	1000	29.04	29.04	0.29	100	0
		32.79	32.79	0.33	100	0
		27.09	27.09	0.27	100	0
II	1600	42.08	67.33	0.34	200	0
	2900	43.46	126.03	0.63	200	0
	5000	34.29	171.45	0.86	200	0

Figure4: In the acute toxicity test, no death was recorded in all doses of the extract within the 48h observation; hence the LD50 could not be determined. Hyperactivity was noticed and this effect may be due to the presence of morphine like alkaloid.

Table 5: Effect of Methanolic extracts *P.santaliniodes*on body weight of diabetic rats

Doses (mg/kg)	**0hr**	**1hr**	**3hr**	**6hr**	**9hr**	**12hr**	**24hr**
500mg/kg Extract	523±1.5	484±1.3	433±2.0	****** 217±1.4	****** 130±4.8	****** 81±1.9	****** 76±1.3
250mg/kg Extract	496±1.4	* 246±2.3#	235±2.4#	* 222±2.2	** 144±1.1	** 82±7.5	** 82±1.7
5ml/kg Water	600±0.0	595±1.0	451±2.0	582±2.1	568±6.4	513±1.2	557±8.5
5mg/kg GB	597±6.5	590±2.2	567±3.9	** 367±4.9	****** 147±4.6	****** 90±1.5	** 73±1.1

Result expressed as mean±SEM (n=5). **=extremely significant ($p<0.05$) relative to placebo. *=slightly significant ($p<0.05$) relative to placebo. b=significant compared to GB

Table 6: Effect of methanolic extract of *P. santaliniodes* on blood glucose level of diabetic rat for acute model

Doses (mg/kg)	**0hr**	**1hr**	**3hr**	**6hr**	**9hr**	**12hr**	**24hr**
500mg/kg Extract	523±1.5	484±1.3	433±2.0	** 217±1.4	** 130±4.8	** 81±1.9	** 76±1.3
250mg/kg Extract	496±1.4	* 246±2.3#	235±2.4#	* 222±2.2	** 144±1.1	** 82±7.5	** 82±1.7
5ml/kg Water	600±0.0	595±1.0	451±2.0	582±2.1	568±6.4	513±1.2	557±8.5
5mg/kg GB	597±6.5	590±2.2	567±3.9	** 367±4.9	** 147±4.6	** 90±1.5	** 73±1.1

Result expressed as mean±SEM (n=5). **=extremely significant ($p<0.05$) relative to placebo. *=slightly significant ($p<0.05$) relative to placebo. b=significant compared to GB.

Table 7: Effect of the Methanolic extract of *P.santaliniodes*on blood glucose level of diabetic rat for subchronic model

Dose (mg/kg)	0hr	3hr	7hr	10hr	14hr
500mg/kg Extract	420±1.1	293±2.6	386±2.0	* 253±2.2	316±2.3
250mg/kg Extract	600±0.0	415±185	487±1.1	333±2.3	390±1.0
5ml/kg water	403±1.4	427±40	527±4.6	587±2.1	582±3.1
5mg/kg GB	600±0.0	425±303	434±2.6	** 86±5.9	** 177±9.6

Result expressed as mean±SEM (n=3).$p<0.05$ as compared with control group (one way ANOVA followed by Dunnet t-test 2 sided). **=extremely significant ($p<0.05$) relative to placebo. *=slightly significant ($p<0.05$) relative to placebo.

Table 8: Overall percentage reduction in Blood glucose level (BGL)

Treatements	Chronic	Subchronic
500mg/kg Extract	85%	-24.7%
250mg/kg Extract	83.5%	-35%
5ml/kg water	7.17%	+44%
5mg/kg GB	87%	-70.5%

(+)= Indicate increase in BGL (-) = Indicate reduction in BGL

DISCUSSION

Methanol extract tested positive for most of the phyto-constituents signifying the high elution capacity of methanol as opposed to other less polar solvent.[78] According to WHO guidelines, elucidation of physicochemical properties is important in the standardization of medicinal plant.[79] Ash values are helpful in determining the quantity of earth impurities, in effect the quality and purity of a crude drug, especially in the powdered form. It is also be used to remove all traces of organic matter, which may otherwise interfere in an analytical determination. Total ash less than 31.06% global maximum shows hygienic processing methods. Acid insoluble ash consists of silica and silicate which has an abrasive effect on the punches during production process. About 12% moisture content of seed powder is similar to the 9% moisture content on the leaf powder.[80] Diagnostic character serves as a marker for medicinal plant. The main principles of powder identification is to identify the diagnostic character and purity of the sample after being processed, especially into powder, based on the attributes of the original materials, such as its cell shape, crystal structure, form of tissue and histochemical reaction.[81] In this way, the purity of the identified materials and the microstructure of the adulterating substances can be observed fundamentally. The method of microscopic

identification is also much more intuitive, speedy, accurate and available than organoleptic inspection and the physical and chemical identification. The powdered crude drug of *P. santaliniodes* constitutes branched multicellular non-glandular trichomes, elongated unicellular non glandular trichomes, annular xylem vessel, peristerm of raphae, calcium oxalate, fat globules and epidermal cells of the testa.

Acute toxicity testing measures the adverse effects that occur within a short time after the administration of single dose of a chemical agent.[82] It enables the establishment of the therapeutic index. Findings from 24 hours acute toxicity testing of *P.santaliniodes* extract showed no toxicity at dosage range up to 5000mg/kg as no mortality was recorded. This re-validates the assertions of no toxicity (55) and zero lethality.[83] Although no mortality was recorded, behavioural changes were manifested. The animal displayed heightened physical activity and aggressiveness which support the hypothesis of potential presence of morphine alkaloid (55). Diabetes result in chronic hyperglycaemia.[84] Therefore, any drug or plant extract that reverses the hyperglycaemic state can be said to ameliorate the condition. Alloxan monohydrate (150mg/kg) was injected intraperitoneally into the albino rat. Pre-induction fast period of 36 hours resulted in rapid diabetes induction [85] after a single dose administration of alloxan.[86] A freshly prepared aqueous alloxan was used owing to the fact that alloxan is unstable with a half-life of about 1.5minutes and thus could easily disintegrate when left to stand in aqueous solutions into non-diabetogenic alloxanic acid due to spontaneous decomposition.[87-89] During the course of the experiment, 5% dextrose solution was provided in a drinking bottle so as to counter the initial hypoglycemic effect which accounts for high mortality rate.[90] The result from the studies indicated that intraperitoneal administration of alloxan produced consistent hyperglycemia in experimental animal. Alloxan induce diabetes via the selective necrosis of the beta cells and exhaustion of beta cells ultimately results in hyperglycemic state.[89]

In table 6, for the acute diabetic model, treatment of the animals with 250mg/kg resulted in overall 83.46% reduction in blood glucose level (from 496±1.4 to 82±1.7). Higher dose of the extract, 500mg/kg body weight caused 85% reduction in sugar level (from 523±1.5 to 76±1.3) (Table 6). The differences between groups were statistically significant at $p<0.05$ at day 6, 9, 12, 24 and day 1, 6,9,12 and 24 for 500mg/kg and 250mg/kg respectively relative to placebo. However, when compared to the standard drug, glybenclamide (5mg/kg), statistical difference at $p<0.05$ occurred at 1st and 3rd day for 250mg/kg. Glybenclamide, in comparison to the placebo was different at day 6,9,12 and 24 with overall 87% reduction in blood glucose level (from 597±6.5 to 73±1.1). These observations validate the ethno medical use of concoctions containing *P.santaliniodes* extract as effective anti-diabetics. The ANOVA result re-enforces the above conclusion. For chronic data, the $F_{cal>}F^{0.05}{}_{3,12}(3.49)$ at day 1,6,9,12 and 24 while in sub chronic, $Fcal>F^{0.05}{}_{3,8}$ (4.07) at day 10, thus we reject Ho and conclude that the effect of the treatment means is not the same. At $p<0.01$, $Fcal> F^{0.01}{}_{3.8}$ (5.95) at day 9, 12 and 24 for chronic while no significance was seen at any day for sub chronic.

Results presented in table 7 for sub chronic diabetes model, extract at all doses reduced the blood glucose level in alloxan induced diabetic rats. A 24.7% (420±1.1 to 316±2.3) and 35% (600±0.00 to 390±1.0) blood glucose reduction for 500mg/kg and 250mg/kg respectively were recorded. Relative to the placebo, a statistical difference ($p<0.05$) was seen on day 10 but when compared to the standard drug, glybenclamide (5mg/kg) the effect was not statistically different ($p<0.05$). Glybenclamide relative to placebo was significantly different at day 12 and 24 with 70.5% overall reduction in BGL (from 600±0.00 to 177±9.6).

In summary, *P. santaliniodes* posseses anti diabetic property which could result from abundant phyto constituents therein or better still to a definite combination of the chemical constituents present in the plant.

Limitations of the study include inability to assay the concentration of the extract on the pancreas, possible healing effect on the pancreas and unstable storage conditions owing to electricity outage.

CONCLUSION

In summary, *P. santaliniodes* has been established to possess anti-diabetic properties which can be attributed to the plants numerous phytoconstituents contained in the plant.These observations also validate the ethno medical use of concoctions containing *P.santaliniodes* extracts for diabetes.

Further investigation should focus on re-evaluation of the anti-diabetic property of *P. santaliniodes* with focus on the extracts healing capacity on the pancreas and on the isolation, purification and characterization of the bioactive peptides.

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DECLARATION

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Consent for publication: Not applicable

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