Original Research Article

Nutritional PotentialAnd The Effect of Ethanol And Aqueous Extracts of PlukenetiaConophora Leaf On The Body Weight and Blood Glucose Concentrations Of Diabetic Rats

ABSTRACT

Proximate composition and energy value, macro-mineral concentration, percentage daily value (%DV) , nutrient rich index (NRI) and the effect of ethanol and aqueous extracts of *Plukenetiaconophora*leaves on the body weight and blood glucose concentration of diabetic rats were determined using standard methods.The pharmacological model was 120mg/kg b.w. (via intraperitoneal) alloxan monohydrate induced diabetic rat model, with the ethanol and aqueous extracts respectively administered orally at 200mg/kg and 500mg/kg respectively and glibenclamide administered as reference control at 5mg/kg b.w. Chemical analysis showed that all the samples were rich in crude fiber (17.7% ; %DV: 63.21), ash (11.57%) , carbohydrate (57.14%; %DV :47.71), energy (254.05Kcal/100g ; %D.V. : 19.96) . Spectrophotometric analysis data revealed Potassium concentration (68.07mg/kg; %DV: 1.45) as highest while Sodium concentration (0.51mg/kg; %D.V.: 0.02) was the lowest. The potassium to sodium ratio was high (136.1) while the sodium to potassium ratio was very low (0.008) and these ratios may reveal good cardiovascular health effect of the leaf. Nutrient Rich Index (NRI) based on macronutrients and macro minerals was estimated to be 16.47. Data on the effect of the ethanol

extract and aqueous extract of the leaves of*P.conophora* on the body weight and blood glucose level of the rats revealed that the extracts significantly ($p< 0.05$) , time and dose dependently restored the body weights towards normal compared to the diabetic control. The extracts also significantly ($p< 0.05$), time and dose dependently lowered blood glucose concentration towards normal in treated rats. The nutritional and nutraceutical potential of the leaves of *P.conophora* shown in this study suggest its possible use in food as cheap, effective and safe anti -diabetic agent for the management of diabetes mellitus and its associated complications.

Key words:Nutritional potential, Diabetes mellitus, Plukenetiaconophora, nutrientrich index, percentage daily value.

Introduction

Nutrition, chemotherapy and exercise make up the trio in the management of diabetes mellitus.The amount of calorie compared to the amount of nutrients consumed by diabetics is therefore vital.Ranking by nutrient density is one of the nutrient profiling strategy employed to promote health and prevent disease (WHO/IASO,2014) Nutrient density means a measure of the nutrients provided per calorie of food.(Hunter & Cason, 2014)).Ordering foods by nutrient density is a method used in comparing foods by the proportion of nutrients in them. Nutrient dense foods are seen to be the opposite of energy dense foods . The Nutrient Rich Index (NRI) uses validated nutrient profiles against accepted measures of a healthy diet (JAND,2013)). One

of the current goals of researchers in the world today is to discover new drugs for the development of new products with high therapeutic efficacy and low toxicity profile. To accomplish this, more attention has been drawn to medicinal plants in recent years. Medicinal plants are reported to be good sources of compounds with potential therapeutic properties (Okigbo*et al.,* 2019). They are used for the management of diabetes, erectile dysfunction, and cardiovascular, neurodegenerative, and inflammatory diseases (Gupta et al.,2017).

Diabetes mellitus has been reported as a burden of disorder in the structure and function of biological systems (Lozano *et al.*, 2010). According to Okoroh *et al.*, (2021), diabetes mellitus is a non-communicable disease which has been singled out as a major factor in the endocrine region of the bio system responsible for the crisis in the metabolism of biomolecules such as fats, carbohydrates and proteins. Diabetes mellitus is a contributory factor to impaired vision, stroke, kidney failure, cardiovascular diseases (WHO, 2016), Its prevalence has been rapidly on the increase particularly among middle- and low- income nations like Nigeria . Infact, the World Health Organization, WHO (2016) highlighted diabetes mellitus to be the 7^{th} leading cause of deaths by 2030.The implication is that diabetes mellitus presents a major challenge to researchers and health care systems around the globe .Diabetes mellitus is defined as a group of metabolic diseases of endocrine origin indicated when there is high glucose level in the blood over a prolonged period and large amount of sugar in urine detected because of complete or relative lack of insulin resulting from the impairment of insulin secretion ,insulin action or both (WHO,2014). Its symptoms include osmotic diuresis which eventually causes excessive loss of water from tissues, increased thirst, hunger, and high concentration of lipids in the blood (WHO, 2013). Today Insulin is mostly used in the treatment of diabetics and this is supported using a lot of antidiabetic compounds including sulfonylurea, biguanides and thiazolidinedioneand these

chemotherapeutic agents are expensive and have side effects on long time usage but there are natural medicines reported for therapy in the management of diabetes mellitus (Li et al., 2004).

Plukenetiaconophora(African walnut) formerly known as *Tetracarpidiumconophorum* otherwise known as Black walnut belongs to the vine family, Euphorbiaceae in the order Malpighiales of angiosperms in the plant kingdom(United States Department ofAgriculture, 2015) has been widely used in traditional medicine throughout the world.It is one of the top priority non-timber yielding plantspecies within the tropical lowland rainforest of South-west Nigeria and the Yorubas in Nigeria call it "Awasa" (Amusa et al., 2014). It is a Liane (climbing and twining woody plant) of the family Euphorbiaceae and could be more than 30m long. The plant is variously known in Nigeria as "Ukpa" (Igbo), "Okwe" (Edo) (Burkhill,2018). The plant bears capsular fruit that is 6-10cm long by 3-11cm wide and contains sub globular seeds of 2-2.5cm long wrapped in a thin brown shell. The kernel is eaten roasted or cooked as traditional snacks (masticatory) in Nigeria. It is contained in a pod which may house; one shelled nut (single), two shelled nut (double) and three shelled nut. The walnut shells could be black or brown from the plant. The nut is whitish upon cracking from the shell. The nut has a thin layer in between two halves (when a nut is divided into two equal parts) of nut. A bitter taste is usually observed upon drinking water immediately after eating the nuts. This could be attributed to the presence of chemical substances such as alkaloids (Ayodele,2017). Walnut plants are considered to be herbs in traditional Chinese medicine.They are said to detoxify kidneys, strengthen the back and knees, and moisten the intestines. It is believed to stop asthma and is prescribed to be taken between bouts of asthma, but not for acute asthma. It is used for elderly as a constipation cure and the bark is used as tea for laxative and chewed for toothache, it also helps to prevent and control high blood pressure (Wikipedia,2018).

Increase in the population of the world, ignorance and abject poverty has drastically affected the quality of health of mankind. Actually, world scientists have really made great impact on the prevention and management of diabetes mellitus. However, this endocrine driver of metabolic crisis is still a serious source of concern to scientists in the global community being a major cause of death, biochemical abnormalities and health complications, disability and economic problem (Okoroh,2018).

Synthetic drugs are very expensive, scarce and have side effects. This implies that diabetes mellitus is still a challenge to the world researchers. There is therefore need to engage further in a nutritional and nutraceutical study towards discovering new drugs in nature to manage diabetes mellitus and its associated complications in humansThe aim of this study was to investigate thenutritional Potential and the effect of ethanol and aqueous extracts of plukenetiaconophora leaf on the body weight and blood glucose concentrations of diabetic rats **..**The nutritional and nutraceutical information shown in this study would aid to educate people in the locality on the nutritional and medicinal benefits of the leaves of plukenetiaconophorain their in their locality and also enhance its biodiversity.

Materials and Methods

Sample Collection and Preparation

Plukenetiaconophora freshleaves were acquired from umuogbuaguvillage in Igboeze North Local Government of Enugu State in the southeast Geopolitical Zone of Nigeria during the rainy season and were authenticated in the department of Pharmacognosy , Faculty of Pharmaceutical Sciences, Gregory University , Uturu, Nigeria . The plant specimen (GUU/23/04) was preserved

in the Departmental herbarium for future reference. They were cleaned with water, allowed to air dry for a week, milled, and stored in a container with clear labels for proximate, mineral analysis.

Proximate composition determination

Proximate composition was determined using the Association of Official Analytica Chemists (AOAC,2016). Moisture content(MC) and dry matter (DM) were determined using the oven drying method (925.10), Protein content by Kjedhal method (960.52), crude lipid content by the Soxhlet method (2003.05), Crude fiber by the Hennenberg and Stohmann method, ash content by dry ashing method (muffle furnace) 923.03 and carbohydrates by differential method 100-(fat+protein +ash+ fiber + MC) as reported by (Onyeike& Acheru,2002). Energy value was estimated using Atwater factor method by multiplying the values of protein, fat and carbohydrates respectively using 4Kcal/g, 4Kcal/g and 9Kcal/g respectivel(Onyeike&Acheru, 2002)

Determination of macro-mineral concentrations

The AOAC official method (AOAC International, 2016) was adopted with slight modification. Ashing releases the minerals, whose quantity in the analyte can be determined by Atomic Absorption Spectrophotometry.Two grammes of dry powdered leaf sample was placed in a porcelain crucible and ashed at 450^{o}C for 5-6hr. The ash was thendissolved in 2mL concentrated HNO_3, and heated on a low heat for 1min. Then, it was cooled and filtered through Whatman No.42 filter paper into 50mL volumetric flask. The clear solution volume was made up to 20mL

with deionized water. A blank was also prepared using similar experimental procedure. The macro-mineral and trace metal levels of the samples were determined by Atomic Absorption spectrophotometry by using an AVANTA GBC flame spectrometer and hollow cathode lamps. Determination of Calcium, potassium, magnesium, sodium and phosphorus, elemental concentrations in the test sample were performed using the method of calibration curve according to the absorption concentration. Several solution of different known concentration were prepared and the elemental concentration in unknown sample was determined by extrapolation from the calibration curve based on Beer's law. All sample concentration were obtained in parts per million (PPM) and were reported as mg/kg dry weight of material. This procedure was repeated for trplicate samples.

Calculation

Concentration (mg/kg) = concentration (PPM) x 10

Where PPM= concentration obtained from the machine in parts per million.

10 = conversion factor from PPM to mg/kg.

Estimation of the proximate and macro-mineral nutrient potential (%DV) and Nutrient Rich Index.

Percentage daily values (%DV) were estimated by comparing the current samples with a 2,000 calorie reference diet, for adults and children aged 4 and above (Nutritional Data, 2011). It was calculated as follows; %DV = (Amount of nutrient / RDA) X 100.Nutrient rich index(NRI) was calculated as follows ; NRI = Sum of nutrients to encourage – Sum of nutrients to limit.

Preparation of P.conophora leaves ethanol and aqueous extracts

Ethanol extraction and aqueous extraction of the leaves were conducted as reported by AOAC International (2016).The dried leaves were pulverized with a manual grinder and weighed with an electronic balance to obtain a mass of 400g (ground dry weight sample) which was well packaged and labelled. Ethanol extraction and water extraction were carried out at Biochemistry laboratory, Gregory University, Uturu .To every mass of 200g of the pulverized material, 1000ml of 70%V/V of ethanol and 1000ml of distilled water were used for soaking and the bottles were shaken intermittently. After 48hrs,first filtration process was done using clean white cotton material already immersed into the ethanol .Second filtration was done using Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator at a temperature of 55^{o}C and the concentrate was subjected to evaporation using a water bath regulated at a temperature of 55^{o}C until a residue which weighed 40g was obtained as extract. The residuewas stored in a refrigerator until further experimental use. The percentage yield was 20% (w/v). This was calculated as follows:

$$\text{Extract percentage yield (\%)} = \frac{\text{weight of extract}}{\text{weight of dry ground power}} \times \frac{100}{1}$$

Procurement of animal

Eighty-Four (84) female Wisterlbinorats weighing between 180-200g will be procured ,Their weights taken and they will be divided into Nine groups of Nine (9) rats per group, acclamatized for a week separately in a cage, fed and given access to water *ad libitum.*

List 1-Experimental Design for anti-diabetic screening

S/N	Group	Treatment
1.	Normal Control	Water + Basal diet
2.	Diabetic Control	Water + Basal Diet + Alloxan monohydrate (120mg/kg b.w.)
3.	DC+GLE_5 treatment	Water + Basal Diet + Alloxan monohydrate (120mg/kg b.w.) + Glibenclamide (5mg/kg b.w.)
4.	DC+PCE_{200} treatment	Water + Basal Diet +Alloxan monohydrate (120mg/kg b.w) + PCE (200 mg/kg b.w)
5.	DC+PCE500 treatment	Water + Basal Diet +Alloxan monohydrate (120mg/kg b.w) + PCE (500mg/kg b.w)
6.	DC+PCA_{200} treatment	Water + Basal Diet + (Alloxan monohydrate (120mg/kg b.w.) + PCA (200mg/kg b.w.)

7.	DC+PCA_{500} treatment	Water + Basal Diet + Alloxan monohydrate (120mg/kg b.w.) + PCA (500mg/kg b.w.)

Note:NC= Normal control group; DC = Diabetic control group; DC+GLB_5 = Diabetic control treated with 5mg/kg b.w.glibenclemide group; DC+PCE_{200} = Diabetic control treated with 200mg/kg b.w.Plukenetiaconophora leaves ethanol extract; DC+PCE_{500} = Diabetic control treated with 500mg/kg b.w.*Plukenetiaconophora* leaves ethanol extract; DC + PCA_{200}= Diabetic control treated with 200mg/kg b.w.*Plukenetiaconophora* leaves aqueous extract ; DC+PCA_{500} = Diabetic control treated with 500mg/kg b.w.*Plukenetiaconophora* leaves aqueous extract.

Animal Handling

Ethical consideration

Animal handling was conducted in accordance with the International Guidelines for the Care and Handling of Experimental Animals (National Institute of Health, 2011), and the study protocol was duly obtained from the Faculty of Basic Medical Sciences Research and Ethical Committee, Gregory University, Uturu, Abia State, Nigeria.

Pilot Study

The pilot study will be carried out to determine the dose of alloxan monohydrate to be used for the induction of diabetes in the experimental rats. Threewistar albino rats after acclimatization

will be administered three different doses of alloxan monohydrate (100mg/kg,120mg/kg and 150mg/kg b.w. respectively) via intraperitonial(I.P.) route.After 72hr of administration, the rats will be fasted and blood collected via tail cutting and their fasting blood glucose level is tested to confirmhyperglycemiastatus.

Acute Toxicity Study on crude extracts

Oral acute test willbe performed using the Organization of Economic Cooperation and Development (OECD) guideline for testing of chemicals 423 (OECD, 2002).

Induction of Experimental Diabetes Mellitus

The method of Burcelin et al., (2013) was modified and used .After acclimatization of the animals for a period of 7days, the five(4) rats in Normal control group were placed on normal diet of guinea growers mash . The 24rats (n=4 rats/group) in the other 6 groups were fasted overnight and induced diabetes using a single intraperitoneal injection of alloxan (120mg/kg bw). Alloxan (Sigima, USA) at a dose of 120mg/kgb.w was prepared in fresh and cold normal saline solution and administered immediately to the animals. The animals were first weighed using an electronic scale (TH 500) and their base line fasting blood glucose level taken using Fine Test Auto-coding^{TM} Premium Blood Glucose Monitoring System and Blood Glucose Strips via tail vein cut before they were injected with alloxan monohydrate. The animals in the normal control group were injected normal saline alone. After 72hr of administration, the rats were again fasted and blood collected via tail cutting and their fasting blood glucose level were tested which confirmed hyperglycemia. Treatment of the animals with the ethanol extract and aqueous extract of *P.conophora* and glibenclamide reference drug was done immediately after the last

alloxan injection. Blood glucose and body weight of the animals were determined after5th, 10^{th}, 15^{th} and 20^{th} day of commencement of treatment during the study. Glibenclamide and the extracts were given (1ml per animal) once daily by intragastric gavage to the experimental groups undergoing treatment while the normal control group was given water only (1ml per animal) once daily . The extracts and glibenclamide (reference drug) were kept in plastic bottles with cap tightly sealed before and after each use, stored in the refrigerator, protected from direct sunlight to prevent spoilage throughout the time of animal treatment.

Data analysis

Data on proximate composition and mineral concentration were statistically analyzed by a one-way analysis of variance (ANOVA) using SPSS/PC + package. Multiple comparisms of differences between means were conducted by using Fisher's Least Significance Difference (LSD). Significance was accepted at a p-value of less than 0.05 ($p < 0.05$)

Results and Discussion

The proximate composition and energy value of the leaves of P.conophora are shown in Table 1. The findingsofthis research showed that moisture content was 9.02 ± 0.03% d.w. This value was lower than the value reported by Okoroh et al., (2021) for Citrus lanatus, Okoroh et al.,

(2017) for P.ostreatus samples (AVOS, WWS, and SAWCS) , for F.carpensis leaves (Okoroh et al., 2019) and Uzoekwe et al., (2015). This implies that too much water in the leaves of P.conophora makes them to have low shelf life thereby decomposing easily. According to Olutiola*et al.,* (1991), the amount of water in foods show their water activity.Water activity could be employed (Uraih&Izuagbe, 1990) tomeasure howstable and how susceptiblethese food substances could be when microorganisms want to contaminate them. Moisture helps food to digest easily.The drying of the leaf sample reduced its moisture content and drying could serve as a measure of preserving the leaf samples .Drying makes themstaylonger.The amount of food nutrients in the leaves will also relatively increase because of drying

The ash content of the dry leaves of P.conophora in this study wasfound to be 11.57 ± 0.01%. This value was higher than the one reported by Okoroh et al (2021) for C.lanatus, Okoroh et al., (2017)for P.ostreatus samples(AVOS, SAWCS AND WWS), Sumaira et al., (2016), Uzoekweet al., (2015), Olaniyi et al., (2018), for F.capensisleavesandC.cujete leaves respectively. Ash content reflects the mineral values of the dry leaves of P.conophora.

The value of the crude protein in this study was lower than the values reported by Okoroh et al.,(2021) for C.lanatus seeds., Olaniyi etal.,(2018); and Okoroh et al.,(2017) also reported low protein contents for F.carpenses leaves, S. virosa leaves, C.cujtee leaves, F.capenses leaves and fruiting bodiesof organically cultivated.ostreatus (SAWCS, WWS and AVOS) respectively. Proteins are building blocks in the cells and tissues for growth and repair. Consumption of appreciable amount of the leaves of P.conophora in diets may serve as goodsources of plant protein.

The crude lipid value of P.conophora leaves reported in this research was much higher than the values reported by Okoroh et al., (2017) for P.ostreatus samples (SAWCS) but lower than the

value reported for C.lanatus seeds(Okoroh et al., 2021), for peeled and unpeeled fruit seeds of Anarcadiun occidentale andA. Hypogaea (Okoroh and Onuoha , 2019). Natural plant foods (seeds, nuts and leaves) are good sources of oils rich in monounsaturatedandpolyunsaturated fatty acids and are classified as essential part of optimal diets for the prevention of CVD by leading health experts.

This study revealed a high crudefiber , high carbohydrate content and appreciable energy values. The appreciable energy valuecould be dueto the carbohydrate content of the sample.Okoroh et al., (2021) reported higher crude fiber and energy values but lower total carbohydrate content for C.lanatus seeds. The crude fiber content was higher in P.ostreatus samples (SAWCS, AVOS andWWS) but the total carbohydrate and energy (Okoroh et al., (2017), were comparable. Fibre consists of non-nutrient substances such as lignin and cellulose.It also has cell wall polymers.These materials cannot be digested by humans.Nutritionally,fibre is importantbecause it helps to clean the intestinal tract.It also maintains the movement of the intestine in a peristaltic way(Mukhopadhyay and Guha,2015). Fibre helps to lower the rate of absorption of glucose in the digestive tract.Fibre also removes cholesterol. Since, the findings in this study indicated that the mushroom samples are rich in fibre; they are therefore good in the diet of diabetic patents. Mathenge (1997) also reported that fibre is essential in the diet because it helps to maintain bulk and also the movement of the intestine by the process of peristalsis via surface extension of food in the digestive tract. This implies that the consumption of these leaf sample will produce healthy condition, make food digestion easy and cure certain nutritional disorders. However, consumption of too much with high concentration of fibre may cause irritation of the intestine. It may also decrease the availability of nutrients.

Table 1: Proximate composition and energy value of the leaves of Plukenetiaconophora

Proximate Component	Concentration (%)
Ash	11.57 ± 0.01
Dry Matter	90.98± 0.02
Moisture	9.02 ± 0.03
Crude Lipid	1.73 ± 0.01
Crude Protein	2.84 ± 0.01
Crude Fiber	17.70 ± 0.01
Total Carbohydrate	57.14 ± 0.01
Calorificvalue (Kcal/100gsample)	254.05 ± 0.09

Values represent Mean ± SD; (Number of Tests, n = 3)

Macro-mineral, Sodium/Potassium ratio and Potassium/Sodium ratio

The potassium value indicated in this study was high while the sodium value was low (Table 2). These values of potassium and sodium were much lower than those reported by Okoroh et al.,(2021) for C.lanatus;Uzoekwe et al., (2015) for F.carpensis and Okoroh et al., (2021) for P.ostreatus samples. Potassium is one of the most essential minerals that the body needs to fulfil balanced ion chemistry.Potassium is the major intracellular ion that all cells in the body use.It helps to keep mineral balance in these cells (Pohl *et al.,* 2013). Potassium and Na create electrical potential difference across cell membrane.This electrical pressure difference helps the muscles to contract, transmission of ions across the neurons as well as normal function of the heart associated vascular system (Mikko *et al.,* 2006). Since mushrooms are very rich in potassium, they may be used to prevent hypertension.This is because diets containing very low K concentration (Aburto *et al.,*2013) may result tohigh blood pressure.Extreme lack of K in the body causes a deficiency diseases calledhypokalemia.

The values of calcium, magnesium and phosphorus shown in this study were lower than those reported by Okoroh et al., (2021) for C.lanatus, Uzoekwe et al.,(2015) for bark and leaves of F. carpensis and Okoroh et al., (2019) for F.capensis. Calcium is essentialin cell functionand

chemistry. Calcium is needed in signal transduction, fertilization, muscle contraction as well astransmission along the nerve.Itdoes the work of a second messenger (Brini*et al.,* 2013).A lot ofenzymes utilize Ca as a cofactor. It is required in bone and teeth formation. Calcium from the extracellular fluid helps to establish electrical pressure difference acrossa cell membrane that is in excited state(Brini*et al.,* 2013). Magnesium is important inthe body because it helps in metabolism.It plays great role inspecificneuromuscular activitiesas well as actions in organs and systems linked with the heart and its associated vessels (Ryan, 1991).. The potassium sodium ratio indicated in this study was extremely high while the sodium potassium ratio was very low indicating that the leaves may be good in diets for cardiovascular health.Low level of K may cause hypertention(Aburto et al., 2013).

Table 2a: Macro-mineral composition of the leaves of Plukenetiaconophora

Mineral	Concentration (mg/kg d.w)
Sodium (Na)	0.51 ± 0.02
Potassium (K)	68.07 ± 0.07
Phosphorus (P)	4.50 ± 0.03
Calcium (Ca)	15.06 ± 0.06
Magnesium(Mg)	8.21 ± 0.02

Values represent Mean ± SD; (Number of Tests, n = 3).

Table 2b: Sodium to potassium Na/K) and Potassiun to sodium(K/Na) ratio of the leaves of Plukenetiaconophora leaves

Ratios	values
Na/K	0.008
K/Na	136.1

Nutrient potential (%DV) and Nutrient Rich Index (NRI) ofP.conophora leaf

The nutrient potential (%DV) and the Nutrient Rich Index(NRI) of P.conophora leaf are shown in Table 3.The total %DV of the nutrients that encourage (protein, fiber, calcium,potassium, magnesium and phosphorus) was greater than the total %DV of the nutrients that limit (lipid, sodium and carbohydrate) resulting to a NRI of 16.47.Okoroh et al., (2018) reported higher proximate and fiber nutrient density value for samples of Pleurotus ostreatus (SAWCS, AVOS and WWS) cultivated by substrate organic supplementation technique compared to the result obtained in this study. The results showed that the leaves of P.conophora presented good nutritional potential when consumed in appreciable amount in terms of daily serving. Ranking by nutrient density is one of the nutrient profiling strategy employed to promote health and prevent disease (WHO/IASO,2014)Nutrient density means a measure of the nutrients provided per calorie of food.(Hunter and Cason, 2014)).Ordering foods by nutrient density is a method used in comparing foods by the proportion of nutrients in them. Nutrient dense foods are seen to be the opposite of energy dense foods . The Nutrient Rich Index (NRI) uses validated nutrient profiles against accepted measures of a healthy diet (JAND,2013)

Table 3 :Nutrient potential (%Daily values) of P.conophora leaf

Nutrient (g)	Amount in 100Kcal of	Daily reference	%Daily value

	P.conophorasample	value (g)	
Crude protein	2.84 ±0.03	50	5.68
Crude lipid	1.73±0.01	20	8.65
Crude fiber	17.70 ±0.01	28	63.21
Total carbohydrate	57.14±0.01	125	47.71
Calcium	15.05±0.06	1300	1.16
Potassium	68.07±0.07	3500	1.94
Magnesium	8.21±0.02	40	0.20
Sodium	0.51±0.02	2300	0.002
Phosphorus	4.50±0.03	700	0.64

Nutrient rich index =Sum of nutrients to encourage – Sum of nutrients to limit

= 72.830-56.362 = 16.47

Effect of ethanol extract and aqueous extracts of P.conophora leaf on Body weights of the diabetic rats.

Table 4: Changes in the body weight (s) of rats in seven groups during the experimental period of 20days.

Groups/ Treatment	BEFORE INDUCTION	AFTER 5DAYS	AFTER 10DAYS	AFTER 15 DAYS	AFTER 20 DAYS
NC	145.53±0.28^{c}	146.02±0.11^{e}	147.43±0.53^{f}	148.34±0.31^{d}	151.63±0.34^{f}
DC	148.38±0.30^{d}	147.03±0.14^{f}	146.00±0.12^{e}	144.17±0.24^{b}	143.30±0.15^{a}
DC+GLB$_5$	145.09±0.08^{c}	142.44±0.37^{b}	141.52±0.08^{b}	143.35±0.09^{a}	147.65±0.17^{c}
DC+PCE$_{200}$	144.32±0.41^{b}	143.23±0.50^{c}	142.00±0.03^{c}	145.02±0.12^{c}	148.12±0.31^{d}
DC+PCE$_{500}$	143.06±0.11^{a}	142.14±0.18^{b}	148.45±0.49^{g}	149.00±0.51^{e}	150.11±0.26^{e}
DC+PCA$_{200}$	145.17±0.25^{c}	144.30±0.50^{d}	144.38±0.22^{d}	145.89±0.26^{c}	147.83±0.64^{c}
DC+PCA$_{500}$	143.19±0.28^{a}	139.95±0.08^{a}	138.68±0.54^{a}	145.27±0.42^{c}	146.22±0.13^{b}

Values are mean±SD for 4rats in each group. Values in the same column with different superscripts are significantly different at $p < 0.05$. NC= Normal control group; DC = Diabetic control group; $DC+GLB_5$ = Diabetic control treated with 5mg/kg b.w.glibenclemide group; $DC+PCE_{200}$ = Diabetic control treated with 200mg/kg b.w.Plukenetiaconophora leaves ethanol extract; $DC+PCE_{500}$ = Diabetic control treated with 500mg/kg b.w.*Plukenetiaconophora* leaves ethanol extract; $DC + PCA_{200}$= Diabetic control treated with 200mg/kg b.w.*Plukenetiaconophora* leaves aqueous extract ; $DC+PCA_{500}$ = Diabetic control treated with 500mg/kg b.w.*Plukenetiaconophora* leaves aqueous extract.

The major intention of this research work was to investigate the anti-hyperglycemic effects of ethanol extract and aqueous extract of Plukenetiaconophoraleaves in diabetic animal model. Alloxan induced diabetic rats was used as a major experimental model to establish antidiabetic properties of the plant extracts in the rats. Glibenclemide was used as a reference treatment because it is well established as one of the standard drugs for the management of diabetes mellitus. Body weights and blood glucose concentrations were bioindicators used to justify the therapeutic effects of the extracts monitored at 5days, 10days, 15days and 20days respectively.

The body weights of the rats in the seven groups were monitored during the experimental period of 20days as shown in Table 5 .After 5days of induction, there wasincrease in the mean body weights of rats; NC(146.02±0.28g) compared to thediabeticcontrol group but there was significant difference ($p < 0.05$) between the mean body weights of rats in all the groups except between DC+GLB5 group and DC+PCE500 group where there was no significant difference ($p > 0.05$).After 10days of induction, there was significant increase($p < 0.05$) in the mean body weights of rats in NCgroup (147.43±0.53g), DC+PCE500 group (148.45±0.49g) compared to the in DC group (147.03± 0.1g) with a significant decrease in mean body weight. There was significant difference ($p < 0.05$)between the mean body weights of rats in all the groups.After 15 days of induction, there was significant increase in the mean body weights of rats in the normal control group, all treated groups but there wasa decrease in the mean body weight of rats in the

diabetic control group. There was no significant difference ($p > 0.05$) between the mean body weights of rats in DC+PCE200 group, DC+PCA200 and DC+PCA500 respectively but there was significant difference($p < 0.05$) between these groups and the NC group, DC+GLB5 group. After 20 days of induction, there was increase in mean body weights of rats in NC group and all the treated groups respectively but there was a decrease in mean body weight of rats in the diabetic control group. There was no significant difference ($p > 0.05$) in the mean body weights of rats in DC+GLB5 groups andDC+ PCA200 groups but there was a significant difference ($p < 0.05$) between the mean body weights of all other groups .

Diabetes mellitus caused by the administration of alloxan is associated with loss in body weight. The loss in body weight may be due to increase in muscle wastage coupled with loss of tissue proteins(Lenzen, 2008) . The induced diabetic rats exhibited significant decrease in body weight but treatment with PCE extract, PCAextract and glibenclamide brought a reverse in the trend by causing a dose and time dependent appreciation in the body weights of the diabeticratswhile alloxan administration negatively affected the body weight of the non-treated diabeticrats. The improvement in the body of the treated animals showed that the extracts prevented muscle / tissue damage caused by the condition of hyperglycemia. It was observed that the body weight of the rats treated with the ethanol extract and aqueous extract of the leaves of P.conophora respectively were higher than that treated with glibenclemide on the 20^{th} day after induction. In diabetes, body weight may reduce because the body cells could not use glucose properly as source of energy. Proteins are used instead, leading to a decrease in body protein content and reduction in body weight .

Table 5: Effects of ethanol extract and aqueous extracts of the leaves of P.conophora on blood glucose levels (mg/dl) of rats in the seven groups during the experimental period of 20days.

Groups/ Treatment	BEFORE INDUCTION	AFTER 5DAYS	AFTER 10DAYS	AFTER 15DAYS	AFTER 20DAYS
NC	91.73±0.20^{f}	93.38±0.50^{a}	92.35±0.44^{a}	90.46±0.28^{a}	90.70±0.33^{a}
DC	87.49±0.42^{c}	276.47±0.47^{e}	278.55±0.33^{g}	279.51±0.35^{g}	283.75±0.25^{g}
DC+GLB$_5$	89.97±0.06^{d}	274.61±0.47^{d}	246.05±0.18^{e}	160.52±0.02^{d}	109.08±0.11^{f}
DC+PCE$_{200}$	94.25±0.25^{g}	265.30±0.20^{b}	222.68±0.10^{b}	135.13±0.14^{c}	102.91±0.14^{b}
DC+PCE$_{500}$	90.53±0.45^{e}	270.15±0.08^{c}	226.24±0.25^{c}	174.19±0.27^{e}	106.13±0.20^{d}
DC+PCA$_{200}$	84.61±0.27^{b}	277.35±0.35^{f}	230.44±0.68^{d}	122.23±0.10^{b}	105.18±0.30^{c}
DC+PCA$_{500}$	82.98±0.76^{a}	286.77±0.49^{g}	255.30±0.05^{f}	186.53±0.55^{f}	107.18±0.17^{e}

Values are mean±SD for 4rats in each group. Values in the same column with different superscripts are significantly different at $p < 0.05$**. NC= Normal control group; DC = Diabetic control group; DC+GLB$_5$ = Diabetic control treated with 5mg/kg b.w.glibenclemide group; DC+PCE$_{200}$ = Diabetic control treated with 200mg/kg b.w.*Plukenetiaconophora* leaves ethanol extract; DC+PCE$_{500}$ = Diabetic control treated with 500mg/kg b.w.*Plukenetiaconophora* leaves ethanol extract; DC + PCA$_{200}$= Diabetic control treated with 200mg/kg b.w.*Plukenetiaconophora* leaves aqueous extract ; DC+PCA$_{500}$ = Diabetic control treated with 500mg/kg b.w.*Plukenetiaconophora* leaves aqueous extracts.**

After 5days of induction, there was significant increase in the blood glucose level of all the groups compared to the normal control and there was significant difference ($p < 0.05$) between the blood glucose levels of all the groups. After 10days of induction, the blood glucose levels of the NC group and all the treated groups was lower than compared the diabetic control group. There was a significant difference ($p < 0.05$)between the blood glucose levels of all the groups.After 15 days and 20days of induction, there was significant decrease in the blood glucose levels of rats in the NC group and all the treated groups compared to the diabetic group where there was a significant rise in blood glucose level. Also after 10days and 20 days of induction, there was significant difference ($p < 0.05$) between the blood glucose levels of all the groups.Both extracts used in the treatment of the rats caused a significant($p < 0.05$) dose and time dependent reduction in blood glucose levels of the diabetic rats. This study shows that ethanol extract and aqueous extracts of P.conophora leaves have anti-hyperglycemic properties and may be used in the management of diabetes mellitus. The reason why plasma blood glucose reduced may be either due to a rise in plasma insulin level in the diabetic rats which may

stimulate the beta cells of the islets of Langerhans to secrete pancreatic insulin. Another reason may also be because the transport of blood glucose to peripherial tissues may have been improved due to the enhancement of insulin insensitivity. This report is in line with the work of Agrawal et al., (2010) who examined oyster mushroom extracts and found out that it has beneficial effects on sugar control.The result of the effect of the ethanol extracts and aqueous extracts of the leaves of P.conophora on blood glucose level and body weight showed that the extracts lowered blood glucose level and increased the body weight in the treated diabetic rats. This effect may be because the plasma insulin level was now increased in diabetic rats which may have influenced the stimulation of the beta cells in islets of Langerhans (Alarcon-Aguilara et al ., 1998).It isalso possible that extract could have enhanced insulin secretion by beta cells or there is an increased sensitivity of target tissues for insulin or it may be because glucose metabolism has been improved . The presence of dietary fiber may also be responsible for the hypoglycemic effect of the plant extracts. Dietary fiber has been revealed by science asan agent that reduces glucose absorption, decreases the rate of gastric emptying and binds to cholesterol to eliminate it (Chen and Raymond, 2008). Consumption of the leaves of P.conophora is therefore encouraged in diabetic patients.

Today around the world, diabetes is increasing in alarming rate because of people eat more of high density calorie foods and live sedentary lifestyle . Currently, WHO recommended the importance to investigate and explore hypoglycemic agents of plant origin due to the fact that plants used in trado-medicine have less side effect when compared to their orthodox counterparts (Alarcon-Aguilara et al ., 1998)). Plants are considered a more potent healer from ancient times because they promote repair mechanism in a natural way(Mahomoodally et al., 2005).

Conclusion

Proximate data revealed that the leaves of P.conophora are rich in ash, fiber, total carbohydrate and appreciable level of lipid and protein . They are therefore good sources of these nutrients. Macromineral concentration data showed high levels of potassium, calcium, magnesium, phosphorus and low level of sodium in the plant sample.The potassium to sodium ratio was high. They can possibly serve as good sources of these minerals to aid metabolism and enhance body physiology. The Nutrient Rich Index (NRI) of P.conophora was found to be very appreciable. This suggests that the plant leaf has good nutritional potential and also affordable for the locals .

Data on the effect of the ethanol extract and aqueous extract of the leaves of P.conophora on the body weight and blood glucose level of the diabetic rats revealed that the extracts significantly,time and dose dependently restored the body weights towards normal. The extractsalso significantly, time and dose dependently lowered blood glucose in treated animal. The results therefore showed the nuraceutical potential of the plant leaf sample, suggesting its possible use as an anti hyperglycemic agent for the management of diabetic mellitus and its associated complications.

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