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# Ameliorative Effects of Ethanolic Leaf Extract of *Physalis angulata* (Ewe Koropo) on Diabetic-Induced Wistar Rats in South West Nigeria

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## Abstract

Excessive consumption of foods high in calories, lack of exercise and oxidative stress play crucial roles in diabetic physiopathology, and if not diagnosed and treated early, damages to kidney, eyes, heart and nerves are inevitable. Medicinal plants have long been utilized in traditional medicine for the treatment of diseases. In this study, qualitative and quantitative phytochemicals analyses of ethanolic extract of *Physalis angulata* were carried out using standard biochemical methods. In addition, evaluation of the antioxidant activity and inhibitory potential of the plant extract against key enzymes associated with hyperglycemia-a symptom that characterizes diabetes were done. Antioxidant activities were determined using DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate), FRAP (Ferric Reducing Antioxidant Power), ABTS (2, 2l-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), lipid peroxidation assays while the alpha–glucosidase and α-amylase inhibitory activities were also evaluated. The ethanolic extract of *Physalis angulata* exhibited significant strong scavenging activity on DPPH, lipid peroxidation, reducing power, ABTS activity as compared with the standard drug Ascorbic Acid against the diabetic group ($p<0.05$). Furthermore, the ethanolic extract did exhibit significant α-amylase and α–glucosidase activities with IC50 (23.88, 15.10), when compared with standard drug acarbose with IC50 (24.75, 41.74). The findings of this study indicate that the ethanolic extract of *Physalis angulata* leaves possess anti hyperglycemic properties; and thus provide pharmacological benefits to the ethnomedical use of this plant in the treatment, management and control of type 2 diabetes mellitus.

## Keywords

Oxidative stress • Hyperglycemia • Diabetes mellitus

## Introduction

Diabetes mellitus is a life-threatening endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water when blood glucose levels remain high. Diabetes is commonly accompanied with polydipsia, polyuria, microvascular problems involving eyes, kidney and peripheral nerves as well as cardiovascular problems such as hypertension [1-4]. Currently, diabetes is the fifth major cause of death in the world affecting 366 million individuals globally and this figure is projected to increase to a staggering 552 million by the year 2030 as reported by the International Diabetes Federation [5-8]. More specifically, Nigeria population is greatly affected by diabetes because it is the fourth major cause of death in the country affecting all race groups. Also the black community is at risk because of rapid lifestyle and cultural changes [9-13].

A hormone produced by the βcells at the pancreatic islets of Langerhans helps to regulate blood glucose levels that rise after food intake. In diabetic patients, this hormone known as insulin is either under produced or cells fail to utilize the produced insulin, thus resulting into hyperglycemia-high blood sugar level. With regards to this, a therapeutic approach to treat diabetes is important [14-17]. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase. Alpha glucosidase and alpha amylase are the important enzymes involved in the digestion of carbohydrates. Alpha Amylase is involved in the breakdown of long chain carbohydrates and alpha glucosidase breaks down starch and disaccharides to glucose [18,19]. They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes [20].

For a long time natural products from plants have been used for the treatment of diabetes, mainly in developing countries where the resources are limited and affordability and access to modern treatment is a problem [12]. Extensive research has been carried out to screen the bioactivity of these inhibitors because of their significant importance in health care and medicine [21]. Plant food rich in polyphenols have been reported to cause effects similar to insulin in the utilization of glucose and act as good inhibitors of key enzymes like alpha amylase and alpha glucosidase associated with type 2 diabetes and lipid peroxidation in tissues [18]. Studies have also shown that the bioactivity of polyphenols in plants is linked to their antioxidant activity and many of these plants also possess hypoglycemic properties. Consequently, imbalance between cellular generation and scavenging capacity of free radicals elicits tissue damage associated with DM pathology [4]. Also, incidents of oxidative stress induced neurological disorders mediated by inhibition of enzymatic activities connected with neurotransmission have been reported in experimental diabetic rats [2]. As a follow up to these findings, it is obvious that understanding the relationship between oxidative stress and DM pathology has the potentials to expand the therapeutic intervention options against the pathogenesis and progression of the disease.

Natural products are a major source of new natural drugs and their use as an alternative medicine for treatment of various diseases has been increased in the last few decades. In comparison to the formulated drugs, the herbs and spices have fewer side effects. In recent years, in view of their beneficial effects, use of spices or herbs is gradually increasing not only in developing countries but also in developed countries. Plant

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leaves have been used as herbal medicine for their healing properties since ancient times. Some bioactive compounds within these plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloids, tannin, flavonoid and phenolic compounds. Their concentrations may vary in different plants which result in unique medicinal properties for a specific plant.

*Physalis angulata* is an annual, herbaceous plant which belongs to Solanaceae family. It is known by different names, including camapu; cutleaf ground cherry; wild tomato, mullaca, winter cherry etc. In Southwest Nigeria, it's known as Koropo. Its biological properties include anti-mycobacterial, anti-cancerous, anti-tumorous, anticoagulant, hypotensive, immune-stimulant etc. [10,14]. The plant prefers moist drained sandy loamy soil with full sun or partial shade; it is renowned as an effective stimulant for the immune system. The juice is used in the treatment of earache, jaundice, fever, bladder diseases etc. The fruit and other aerial parts are used in the treatment of boils, sores, cuts, constipation, intestinal and digestive problems [6], and used as an anti-mutagenic, anticoagulant, antispasmodic, antileucemis agents. Several research works have been done to justify the medicinal properties of this plant. However, to our knowledge, there is no report on the comparative inhibitory activities of *Physalis angulata* and in vivo studies on this plant. The present study was designed to evaluate the antioxidant and anti-diabetic activities of ethanolic extract of Physalis angulata in streptozotocin induced diabetic rats.

# Materials and Methods

## Plant materials and chemicals

Fresh leaves of *Physalis angulata* were collected from a garden around Pahayi Ilaro, Ogun state, south west, Nigeria. Authentication was done by Dr. Nodza George of the Department of Botany and Microbiology, University of Lagos. Assign with plant identification no LUT: 8242. Streptozotocin was purchased from Sigma Chemical Co. (St Louis, MO, USA). A one-touch glucometer was purchased from Roche Diagnostics GmbH (Mannheim, Germany) for the analysis of blood glucose (BG). All other chemicals were of analytical grade.

## Preparation of plant extract

Fresh *Physalis angulata* leaves were thoroughly rinsed and air-dried at room temperature for 15 days. The dried samples was grinded into fine powder using a Master chef electric blender Model MC-BL 1644 and stored in an air-tight container at room temperature until required for use.

## Ethanolic extract preparation

Ethanol extract of the plant was prepared by macerating 200 g of the dry powdered plant material in 1 litre of etanol in a clean 5 L round bottom flask and the closure was sealed with aluminum foil, then kept at room temperature for 72 hours (for thorough extraction). At the end of the 72 hours, the extract were filtered first through a Whatmann's filter paper number 1(125 mm) and funnel and then through into a clean beaker. The ethanol extract obtained after filtration was kept in a sterile bottle and freeze-dried.

## Experimental animal

Wistar Strain albino rats weighing 200 g to 250 g used in this study were purchased from the animal house of the University of Lagos, Nigeria. The animals were housed and maintained under standard laboratory conditions. They were fed with standard rat pellet diet.

## Experimental design

The animals were group as follows:

Group A: Normal control

Group B: Diabetic

Group C: Diabetic + leaf extract

Group D: Diabetic + Ascorbic Acid

Group E: Diabetic + Acorbose

## Diabetes induction

The animals were left to fast for 14 hours but had free access to water. Diabetes mellitus was induced by intra-peritoneal injection of a freshly prepared solution of streptozotocin (STZ) (at 60 mg/kg body weight) in 0.1 m cold citrate buffer (pH 4.5). The animals were allowed to drink 15% glucose solution overnight. The animals were then left for 72 hours after which the blood glucose levels were measured. Diabetes was confirmed from the fasting blood glucose using one touch select simple Glucometer.

## Phytochemical screening

Phytochemical analyses were carried out on the ethanolic extract using standard procedures to identify the constituents as described by the modified methods of Harbone (1973), Rajeshwar and Lalitha (2013).

## Qualitative analysis

**Test for tannins:** About 0.5 g of the sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

**Test for phlobatannins:** Crude extract (0.5 g of each plant sample was boiled with 2 ml of 1% aqueous hydrochloric acid for 10 minutes. Deposition of a red precipitate indicates the presence of phlobatannin.

**Test for saponin:** About 2 g of the sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observe the formation of emulsion.

**Test for flavonoids:** About 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated $H_2SO_4$.

**Testforsteriods:** About 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml $H_2SO_4$. The color changed from violet to blue or green in some samples indicating the presence of steroids.

**Test for terpenoids (salkowski test):** About 5 ml of each extract was mixed in 2 ml of chloroform, and concentrated $H_2SO_4$ (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

**Test for cardiac glycosides (keller-killani test):** About 5 ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Test for alkaloid: About 5 mg sample of the extract dissolved in 3 ml of acidified ethanol was warmed slightly and then filtered. Few drops of Mayer's reagent and 1 ml of Dragendroff's reagent were added to 1 ml of the filtrate and turbidity was observed.

Test for phenol: About 2 drops of 5% $FeCl_3$ was added to 1 cmβof the extracts in a test tube. A greenish precipitate indicates the presence of phenolics.

## Quantitative determination

**Estimation of tannins:** Plant extract (500 mg) of the sample was weighed and dissolved in 50 ml of distilled water, and shake for one hour. A 5 ml aliquot of the filtrate was mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 m potassium ferrocyanide. The absorbance was measured at 720 nm within 10 minutes.

**Estimation of total phenolic compound:** About 0.5 g sample of extract

was weighed and dissolved in 50 ml of water, 0.5 ml was taken and 0.1 ml of Folin-Ciocalteu reagent (0.5 n) was added, it was mixed and incubated at room temperature for 15 minutes. After this, 2.5 ml of sodium carbonate solution (7.5% w/v) was added and further incubated for 30 minutes at room temperature. The absorbance of the solution was measured at 760 nm. The concentration of total phenol was expressed as Gallic Acid Equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

**Total flavonoid content estimation:** About 1 ml of sample solution (100 µg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1 m potassium acetate and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol.

**Estimation of saponins:** The spectrophotometric method of Brunner (1984) was used for the analysis of saponins. Briefly, 1 g of the finely ground dried sample was weighed into a 250 ml beaker and 100 ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman number 1 filter paper into a 100 ml beaker containing 20 ml of 40% saturated solution of $MgCO_3$. The resulting mixture was again filtered to obtain a clear colorless solution. One milliliter of the colorless filtrate was pipette into a 50 ml volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to the marked level with distilled water. This was then allowed to stand for 30 minutes for a blood red color to develop. 0 ppm-10 ppm saponins standard was prepared from saponins stock solution. The standard solutions were treated similarly with 2 ml of 5% $FeCl_3$ solution as earlier described. The absorbance of the samples as well as standard saponin solutions was read after color development using a Jenway V6300 spectrophotometer at wavelength of 380 nm. Percentage saponin was calculated using the formula

% saponin =Abs of sample × Average gradient × Dilution factor Weight of sample × 10,000.

**Alkaloids:** The quantitative determination of alkaloids was done by distillation and titrimetric methods as described by Henry (1973). Briefly, 2 g of finely ground sample was weighed into 100 ml beaker and 20 mls of 80% absolute alcohol added to give a smooth paste. The mixture was transferred to a 250 ml flask and more alcohol added to make up to 1 g of magnesium oxide was then added. The mixture was digested in a boiling water bath for an hour and half under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a Buchner funnel. The residue was poured back into the flask and re-digested for another thirty minutes with 50 ml alcohol after which the alcohol was evaporated. Distilled water was added to replace the lost alcohol. When all alcohol has evaporated, 3 drops of 10% HCl was added. The whole solution was later transferred into 250 ml volumetric flask; 5 ml of Zinc acetate solution and 5 ml of potassium ferricyanide solution were thoroughly mixed together to give a homogenous mixture. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10 ml of the filtrate was transferred into a separating funnel and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10 ml of hot distilled water and transferred into a Kjeldahl tube with the addition of 0.2 g of selenium for digestion to a clear colorless solution. The clear colorless solution was used to determine Nitrogen using Kjeldahl distillation apparatus the distillate was back titrated with 0.01 N HCl and the titre value obtained was used to calculate the % Nitrogen using the formulae:

%N = Titre value × Atomic mass of Nitrogen × Normality of HCl × 100 Weight of sample (mg) % Alkaloid = % Nitrogen × 3.26

Where 3.26 is a constant.

**Glycosides:** About 10 ml of extract was pipette into a 250 ml conical flask. 50 ml Chloroform was added and shaken on a Vortex Mixer for 1 hour. The mixture was filtered into a conical flask. 10 ml pyridine and 2 ml of 2% sodium nitroprusside were added and shaken thoroughly for 10 minutes. 3 ml of 20% NaOH was later added to develop a brownish yellow color. Glycoside standard of concentration ranging from 0 mg/ml-5 mg/ml were prepared from 100 mg/ml stock glycoside standard. The series of standards 0 mg/ml-5 mg/ml were treated similarly like the sample above. The absorbance of sample as well as standards was read on a spectronic 21D Digital spectrophotometer at a wavelength of 510 nm. % Glycoside was calculated using the formula:

% Glycoside = Abs of sample × Average gradient × Dilution factor Weight of sample × 10,000.

**Steroids:** About 0.05 g of sample extract was weighed into a 100 ml beaker. 20 ml of chloroform-methanol (2:1) mixture was added to dissolve the extract upon shaking for 30 minutes on a shaker. The whole mixture until free of steroids. 1 ml of the filtrate was pipette into a 30 ml test tube and 5 ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37℃-40℃ for 90 minutes. It was cooled to room temperature and 10 ml of petroleum ether added followed by the addition of 5 ml distilled water. This was evaporated to dryness on the water bath. 6 ml of Liebermann Buchard reagent was added to the residue in dry bottle and absorbance taken at a wavelength of 620 nm on a spectronic 21D digital spectrophotometer. Standard steroids of concentration of 0 mg/ml-4 mg/ml were prepared from 100 mg/ml stock steroid solution and treated similarly like the sample as above.% steroid was calculated using the formula:% steroid=Absorbance of sample × Average gradient × Dilution factor Weight of sample × 10,000.

**Terpenoids:** About 0.50 g of sample was weighed into a 50 ml conical flask and 20 ml of 2:1 chloroform-methanol mixture was added, shaken thoroughly and allowed to stand for 15 minutes. The supernatant obtained was discarded, and the precipitate was re-washed with another 20 ml chloroform-methanol mixture for re-centrifugation. The resultant precipitate was dissolved in 40 ml of 10% Sodium Dodecyl Sulphate (SDS) solution.1 ml of 0.01 m ferric chloride solution was added to the above at 30 seconds intervals; shaken well, and allowed to stand for 30 minutes. Standard triterpenes of concentration range 0-5 mg/ml were prepared from 100 mg/l stock triterpenes solution from sigma-Aldrich chemicals, U.S.A. The absorbance's of sample as well as that of standard concentrations of triterpenes were read on a digital spectrophotometer at a wavelength of 510 nm.The percentage of triterpenes was calculated using the formula: Absorbance of sample × Average gradient × Dilution factor Weight of sample × 10,000.

Total antioxidant capacity determination: Solution of the sample extract (1 ml) was mixed with 3 ml of reagent solution (0.6 m sulphuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 950 C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 69 nm. The total antioxidant capacity was expressed as equivalent of ascorbic.

**DPPH radical scavenging activity assay:** An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75, 100 µg/ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the expression: % inhibition = [A0-A1] × 100/A0

Where A0 is the absorption of the blank sample and A1 is the absorption of the extract.

**Lipid peroxidation assay:** The reaction mixture containing liver homogenate (0.2 ml), Tris–HCl buffer (20 mm pH 7.0, 0.1 ml), $FeCl_2$ (2 mm, 0.1 ml), ascorbic acid (10 mm, 0.1 ml), and 0.5 ml plant extract (25 µg/ml-100 µg/ml) in a final volume of 1 ml. The reaction mixture was incubated at 37°C for 1 hour. Lipid peroxidation was measured as malondialdehyde

(MDA) using trichloroacetic acid (TCA), thiobarbituric acid (TBA) and HCl (TBA-TCA reagent: 0.375 % w/v TBA; 15 % w/v TCA and 0.25 N HCl). The incubated reaction mixture was mixed with 2 ml of TBA-TCA reagent and heated in a boiling water bath for 15 minutes. After cooling, the flocculent precipitate was removed by centrifugation at 10,000 rpm for 5 minutes. Finally, malondialdehyde concentration in the supernatant fraction was determined spectrophotometrically at 535 nm. Ascorbic acid was used as standard.

**Reducing power assay:** Various concentrations of the extracts (20 µg/ml to 100 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 500°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 µg/ml to 16 µg/ml) was used as standard.

**ABTS radical scavenging assay:** Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. ABTS cation radical was produced by the reaction between 7 mm ABTS in water and 2.45 mm potassium persulfate, stored in the dark at room temperature for 12 h-16 h before use. ABTS solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5µl of plant extract to 3.995 ml of diluted ABTS solution, the absorbance was measured at 30 mm after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula

ABTS+ scavenging effect% = (AB-AA) × 100/AB

Where AB is absorbance of ABTS radical + ethanol

Where AA is absorbance of ABTS radical + sample extract

**Standard**

Inhibitory activity of alpha-amylase on Physalis angulate: The activity of α-amylase was performed according to the chromogenic method described. Briefly 120 µl of ethanolic extract of *Physalis angulata* (20 mg/ml) in DMSO was mixed with 480 µl of distilled water and 1.2 ml of 0.5% w/v soluble potato starch in 20 mm phosphate buffer pH 6.9 containing 6.7 mm sodium chloride in a test tube. The reaction was initiated (0 min) by addition of 600 µl of enzyme solution (4 units/ml in distilled water), 600 µl of the mixture was withdrawn after 3 min into separate test tubes containing 300 µl DNSA color reagent (1 g of 3, 5-dinitrosalicylic acid (96 mm), 30 g of sodium potassium tartarate and 20 ml of 2 N sodium hydroxide to a final volume of 100 ml in distilled water) and transferred to a hot water bath maintained at 85°C -90°C for 15 minutes. Afterwards the reaction mixture in each tube was diluted with 2.7 ml distilled water and the absorbance measured at 540 nm (Shimadzu UV-160 spectrophotometer, Kyoto, Japan). Test incubations were also prepared for 5, 7.5, 10, 15 and 20 mg/ml of *Physalis angulata* to study the concentration dependent inhibition. For each concentration, blank incubations were prepared by replacing the enzyme solution with 600 µl in distilled water at the start of the reaction. Control incubations, representing 100% enzyme activity were conducted in a similar manner, replacing *Physalis angulata* with 120 µl DMSO. All the tests were run in triplicate. Net absorbance (A) due to the maltose generated was calculated as:

A540 nm *Physalis angulata* = A540 nm Test – 540 nm Blank from the value obtained the percentage (w/v) of maltose generated was calculated from the equation obtained from the maltose standard calibration curve (0-0.1% w/v maltose). The level of inhibition (%) was calculated as: % inhibition = 100% reaction (at t=3 minutes) Where, % reaction = Mean maltose in sample × 100/ Mean maltose in control.

**Alpha-glucosidase inhibition assay:** The effect of ethanolic extract of *Physalis angulata* on rat intestinal α-glucosidase activity was assayed according to the method of Matsui et al., with slight modifications. Briefly 0.5 mg protein equivalent of crude α-glucosidase enzyme was incubated with different concentrations of ethanolic extract of *Physalis angulata* for 5 min before initiating the reaction with substrates maltose (6 mm) and sucrose (45 mm), in a final reaction mixture of 1 ml of 0.1 m phosphate buffer pH 7.2. The reaction mixture was incubated for 20 and 30 min at 37 °C for substrates maltose and sucrose, respectively. The reaction was stopped by adding 1.0 ml of Tris base and α-glucosidase activity was determined by monitoring the glucose released from maltose and sucrose by glucose oxidase method. Enzyme inhibition data were expressed as IC50 value (The concentration of *Physalis angulata* required to inhibit 50% of α-glucosidase activity).

The enzyme kinetics on inhibition of α-glucosidase activity by ethanolic extract of *Physalis angulata* was studied using different concentrations of substrate maltose (5, 10, 15, 20 and 25 mm) were incubated with α-glucosidase in the absence of inhibitor and with 7.5 mg/ml and 15 mg/ml, for *Physalis angulata* in phosphate buffer pH 7.2 (0.1 m) at 37 °C, and amount of glucose formed was determined by glucose oxidase method. Double reciprocal plots of enzyme kinetics were constructed according to Line weaver and Burk method to study the nature of inhibition. Km and Vmax values were calculated from Line weaver-Burk plots (1/s vs. 1/v) (Line weaver & Burk, 1934).

# Results

## Statistical analysis

The experimental data obtained were expressed as Mean ± S.E.M. The difference between the extract and standard were compared using One way Analysis of Variance (ANOVA) followed by Duncan (Control vs. Test) using the SPSS Software Version 20 $P<0.05$ was considered statistically significant.

# Discussion

There are quite number of plants and plant derived compounds that have been used for the management of diabetes in order to control blood sugar level, as synthetic anti-diabetic drugs have adverse side effects in humans. With the increasing incidence of diabetes mellitus in the world, this disorder still remain life threatening and if not diagnosed and treated early, could damage vital organs in the body.

The ethanolic leaves extract of *Physalis angulata* can be a source of herbal medicine to efficiently treat human diseases. The results of our qualitative and quantitative phytochemical screening as shown in Tables 1 and 2 revealed the presence of important compounds like alkaloid, steroids, flavonoid, tannin, glycosides, terpenoids, phenolics, saponins. Many kind of natural products, such as terpenoids, alkaloids, flavonoids, phenolic, and some others, have shown antidiabetic potential [9]. The presence of tannins may also be responsible for antidiabetic properties [7].

**Table 1**. Qualitative phytochemical analysis of ethanolic *Physalis angulata* leaves extract.

| S/N | Test | *Physalis angulata* Leaves Extract |
|---|---|---|
| 1 | Tannins | + |
| 2 | Phlobatanins | - |
| 3 | Saponin | + |
| 4 | Flavonoids | + |
| 5 | Steroids | + |
| 6 | Terpenoids | + |
| 7 | Glycosides | + |
| 8 | Alkaloids | + |
| 9 | Phenols | + |

Keys: + =Test substance present, - =Test substance absent

**Table 2**. Quantitative phytochemical analysis of ethanolic *Physalis angulata* leaves extract.

| S/N | Test | *Physalis angulata* Leaves Extract |
|---|---|---|
| 1 | Tannin | 18.94 ± 0.085 |
| 2 | Phenol | 28.93 ± 0.115 |
| 3 | | Flavonoid |
| 4 | Alkaloid | 31.11 ± 0.05 |
| 5 | Steroid | 19.56 ± 0.04 |
| 6 | Terpenoid | 17.08 ± 0.21 |
| 7 | Saponin | 40.12 ± 0.09 |
| 8 | Glycoside | 26.96 ± 0.16 |

DPPH scavenging activity is a free radical compound that has been used to determine the free radical scavenging capacity of biochemical samples due to its stability (in radical form) and its reproducibility. The DPPH assay is often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damage caused by oxidative stress. The inhibitory effect of *Physalis angulata* leaf extract on DPPH activity is shown in Table 3. From the result, it was observed that *Physalis angulata* showed a significant different when compared with the Diabetic control group ($p<0.05$). This inhibitory effect might be due to the present of phytochemicals. In addition, the standard drug (Ascorbic acid) had higher inhibitory effect than our plant extract.

**Table 3**. Antioxidant Activities of Ethanolic *Physalis angulata* Leaves Extract.

| Treatment | Lipid peroxidation | FRAP | DPPH | ABTS |
|---|---|---|---|---|
| Control | 21.06 ± 0.1310 | 23.40 ± 0.2164 | 27.13 ± 0.1438 | 25.41 ± 0.0201 |
| Diabetic control | 96.92 ± 0.2040 $^{a}$ | 92.10 ± 02510 $^{a}$ | 84.16 ± 0.2764 $^{a}$ | 79.83 ± 0.0251 $^{a}$ |
| Diabetic + Extract(200 mg) | 53.21 ± 0.0970 $^{ab}$ | 66.11 ± 0.0010 $^{ab}$ | 50.29 ± 0.4879 $^{ab}$ | 39.34 ± 0.2079 $^{ab}$ |
| Diabetic + Ascorbic Acid(100 mg) | 58.84 ± 0.1481 $^{ab}$ | 68.24 ± 0.0033 $^{ab}$ | 58.18 ± 0.2875 $^{ab}$ | 42.94 ± 0.2109 $^{ab}$ |

Values are expressed as mean ± SEM, n = 4, a = values are significantly different when compared to normal control ($P<0.05$), b = values are significantly different when compared to diabetic control ($P<0.05$).

FRAP = Ferric Reducing Antioxidant Power

DPPH = 2, 2-diphenyl-1-picryl-hydrazyl-hydrate

ABTS = 2, 2l-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid

Lipid peroxidation refers to the oxidative degradation of lipids. In this process free radicals take electrons from the lipids (generally in cell membranes), resulting in cell damage. In this present finding, the Physalis angulata extract showed a significant different when compared with the diabetic group ($p<0.05$). Our standard drug (Ascorbic acid) has slightly higher inhibitory effect than the plant extract ($p<0.05$).

Ferric reducing antioxidant power (FRAP) is one of the most rapid test and very useful for routine analysis. It is used to estimate the antioxidant capacity by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2, 4, 6-tri-(2-pyridyl)-s-triazine) and $FeCl_3 .6H_2 o$. This depend on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III) TPTZ) complex to ferrous tripyridyltriazine Fe (II) TPTZ) at low pH. In this present study, the plant extract tested showed good antioxidant potential relatively to our standard drug. Physalis angulata extract showed a significant different when compared to the diabetic group ($p<0.05$). This antioxidant property could be due to the various phytochemicals present.

ABTS scavenging assay is a widely accepted model to determine the total antioxidant activity. It measures the relative ability of antioxidants to scavenge the ABTS generated in aqueous phase, as compared with a Trolox (water soluble vitamin E analogue) standard. It was observed from our result that *Physalis angulata* showed a significant different ($p<0.05$) as compared to the diabetic group.

Alpha amylase inhibitory activity is responsible for hydrolyzing starch by breaking it into low molecular weight products such as glucose before absorption. In this study, ethanol extract of *Physalis angulata* leaves was investigated for its capability against alpha-amylase activity. The results showed (Table 4) that the plant extract significantly inhibited alpha-amylase activity in a dose-dependent manner. The inhibitory activity of the standard drug is slightly higher than the plant extract at IC50 values of 24.75 µg/ml and 23.88 µg/ml respectively. Inhibition of alpha amylase can lead to reduction in post prandial hyperglycemia [16]. Thus, the inhibition of alpha-amylase activity can reduce postprandial hyperglycemia and prevent the risk of diabetes development [11,19]. Alpha glucosidase is an enzyme present in the small intestine, used for the cleavage of disaccharides into glucose. From the result (Table 5), the standard drug has higher inhibitory activity than the plant extract with IC50 of 41.74 µg/ml and 15.10 µg/ml respectively. There are many natural constituents available in plants with alpha glucosidase inhibition activity. Although, inhibition of alpha glucosidase may be challenging in the control of diabetes [1], it can be speculated that the phytochemicals present in *Physalis angulata* might be responsible for the antioxidant and antidiabetic properties of the plant.

**Table 4**. Comparison between the *in vitro* inhibitory effect of *Physalis angulata* extract and acarbose on alpha-amylase.

| Concentration (µg/ml) | % Inhibition of Plant PAE±(SEM) | % Inhibition of Acarbose |
|---|---|---|
| 25 | 47.09$^{a}$ ± 0.2367 | 64.45$^{c}$ ± 0.2367 |
| 50 | 67.14$^{a}$ ± 0.2619 | 73.98$^{c}$ ± 0.2619 |
| 75 | 68.77$^{a}$ ± 0.2367 | 78.91$^{c}$ ± 0.2367 |
| 100 | 81.15$^{a}$ ± 0.1556 | 88.19$^{c}$ ± 0.1556 |
| IC50 (µg/ml) | 23.88 | 24.75 |
| 26.96 ± 0.16 | 26.96 ± 0.16 | 26.96 ± 0.16 |

**Table 5**. Comparison between the *in vitro* Inhibitory effect of *Physalis angulata* extract and acarbose on alpha-glucosidase.

| Concentration µg/ml) | Percentage Inhibition | |
|---|---|---|
| | Ethanol extract | Acarbose |
| 25 | 41.54$^{a}$ ± 0.0493 | 26.96 ± 0.16 |
| 50 | 51.56$^{a}$ ± 0.1443 | 26.96 ± 0.16 |
| 75 | 72.01$^{a}$ ± 0.0953 | 26.96 ± 0.16 |
| 100 | 77.73$^{a}$ ± 0.0721 | 26.96 ± 0.16 |
| IC50 (µg/ml) | 15.10 | 26.96 ± 0.16 |

Values are expressed as mean ± SEM, n=4, plant extract was compared with acarbose at $P\leq0.05$.

## Conclusion

It was concluded that the leaf extract contains phytochemicals such as alkaloid, saponin, Steroids, flavonoid, tannin, glycosides and phenolics. The results of our antioxidant and anti-diabetic tests also concluded that the ethanolic leaf extract of *Physalis angulata* possesses potent antioxidant and anti-diabetic activities. These properties provide some biochemical basis for the use of *Physalis angulata* (leaf) for management of diabetes mellitus.

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