Phytochemical, Mineral Composition and Anti-hyperlipidemic Effects of Processed Pentaclethra macrophylla Seeds on High fat Diet and Streptozocin-Induced Diabetic Wistar rats.

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Abstract
Background:
Diabetes mellitus is a heterogeneous chronic metabolic disorder that is associated with various complications such as hyperlipidemia which can subsequently lead to vascular damage and coronary heart diseases. Thus, lowering of plasma lipid levels is essential for progression to vascular diseases and the risk of developing coronary heart diseases. Thus, the aim of this study was to evaluate the antihyperlipidemic effects of the Pentaclethramacrophyllaseeds in high fat diet and streptozotocin-induced diabetic wistar rats.

Materials and Methods:
The seeds of Pentaclethramacrophyllawere processed into different stages (raw, 1st cooking, 2nd cooking, fermented and fermented and salted) and compounded with rat feeds. Wistar rats weighing between 175-240g were used for this study. The rats were divided in eight (8) groups. Groups 2 to 8 were fed with high fat diet (20% sucrose +30% lard + 50% standard diet) for four (4) weeks and then injected with 40mg/kg streptozotocin (STZ) in distilled water to induce diabetes. Blood glucose and Lipid profile of animals were analyzed 6 days after STZ injection and feeding to confirm hyperlipidemia and hyperglycemia. 50% rat feed was substituted with 50% of the various processed Pentaclethra macrophylla seeds and used to feed the animals. Metformin was administered daily by intra-gastric gavages for 2, 4, 6 and 8 weeks. Glucose was estimated by glucometer and lipids spectrophotometrically measured in all groups after 2, 4, 6 and 8 weeks of treatment.

Results:
Preliminary photochemical investigation of Pentaclethra macrophyllaseeds revealed decrease in phytochemicals as processing progressed. The result also showed different important minerals that may have disease preventive role. Lipid profile results in rats showed that there was a significant (p<0.05) increase, triglycerides, total cholesterol, and low density lipoprotein (LDL)-cholesterol and a decrease in high density lipoprotein (HDL)-cholesterol in high fat diet and streptozotocin-induced diabetic rats compared to the normal control. Treatment of high fat diet and streptozotocin-induced diabetic rats with various processed Pentaclethramacrophylla seeds over a period of eight (8) weeks significantly (p<0.05) reduced the levels of plasma, total cholesterol, triglycerides and LDL-cholesterol and increased HDL-cholesterol compared to rats not fed with various processed Pentaclethramacrophylla seeds.

Conclusions:
The various processed Pentaclethra macrophylla seeds revealed phytochemical contents although in decreased order as processing progressed and important minerals that can
prevent diseases. The various processed Pentaclethra macrophylla seeds also exhibited hypolipidemic activities in high fat diet and streptozotocin-induced diabetic wistar rats for the 8-weeks of treatment. This provides a valid scientific basis for using it in the treatment of diabetes in Nigerian folk medicine and as source of functional foods providing essential micronutrient preventing progression to cardiovascular diseases.

**Key words**: Streptozotocin, processed, Pentaclethramacrophylla, hyperlipidemia

### 1.0 Introduction

Diabetes is a very serious disease that occurs due to chronic hyperglycemic and subsequently leads to complications in carbohydrate, fat and protein metabolism. The disease arises due to insulin resistance, inadequate secretion or both. Pathological changes associated with these affect key organs like the liver, muscle and fat tissues leading to micro and macro vascular diseases such as nephropathy, neuropathy, atherosclerosis, hypertension etc imposing tremendous pain and financial burden on sufferers (Momo et al., 2006). According to WHO and others, it is likely to be the leading cause of morbidity and mortality. Although there are chemotherapies that are used to effectively manage diabetes, however, these substances have associated adverse effect and most times not readily available thus, the search for multi-therapeutic agents to end this agony. WHO have strongly encouraged the use of plant based therapy as an alternative therapy especially in developing countries where there are inadequate medical facilities. Several medicinal plants that are readily available are constantly being tested with the hope of developing therapies with no associated adverse effects. *Pentaclethra macrophylla*, belongs to the Mimosoideae subfamily. It is commonly known as African oil bean seeds and Ugba in southern-western Nigeria (Ladipo et al., 1993). The seeds are eaten as oil bean “salad” or used as soup condiment also, serve as animal feed. The seeds of the plant are used in Nigerian folk medicine to treat several ailments. The seed have been reported to exhibit hypoglycemic potentials, hyperlipidemia, and anti-diabetes effects (Monago et al., 2004, Ighodaro et al., 2012). Hyperlipemia is an associated complication of diabetes mellitus. The metabolic dysregulation causes high levels of total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein in the blood accelerating progression to vascular damage, atherosclerosis and coronary heart diseases. Decreasing the content of lipid level is essential for preventing the formation of dangerous lipoproteins and decreases the risk of development of micro and macro vascular diseases associated with diabetes. The study was thus undertaken to evaluate the phytochemical and mineral composition of processed *Pentaclethra macrophylla* seeds and anti-hyperlipidemic effects in high fat fed and streptozotocin-induced diabetic wistar rats.

### 2.0 Materials and Methods

#### 2.1 Collection of Plant Samples

The seeds of *Pentaclethra macrophylla* were obtained from Nkwo-Orodo in Mbaiteoli Local Government Area of Imo state, identified by Prof. B.E Okoli in the Plant Science and Biotechnology Department, University of Port Harcourt, Rivers State, Nigeria. The seeds were sorted, cleaned stored in air tight bags for subsequent use.

#### 2.2 Method of Processing the *Pentaclethra macrophylla* Seeds

The seeds were processed into different stages of treatment, first is the raw samples, which was later subjected to different cooking times by boiling in water for 16-18hours (1st cooking) and the rough testae removed (unfermented). The second stage of processing is called
The cotyledons of the dehulled seeds were removed, boiled again for 30 minutes (2nd cooking). Then the seeds were slice, dried and ground into powder and left overnight in water at room temperature to ferment and subsequently salted.

2.3 Determination of Phytochemical Compositions
The processed Pentaclethra macrophylla seeds were subjected to different phytochemical tests using standard methods AOAC (1990).

2.4 Determination of Saponin Content
The Saponin content was obtained using two extractions techniques. First solvent was acetone for extraction of crude lipid and the second was methanol for the extraction of the saponins proper. 2 grams of the sample was taken into soxhlet extraction using a 250ml round bottom flask for 4 hours using both solvents. After the extraction period elapse, the content in the flask were dried in an oven and weighed to know the saponin content. The saponin content of the sample was calculated:
% Saponin = (weight of saponin)/(weight of the ground sample) x 100

2.5 Determination of Alkaloid Content
In the processed seeds, alkaloid content was determined by the gravimetric method described by Harborne (1973). Five gram quantity of the ground samples was mixed with 50 ml of acetic acid solution in ethanol (10%). The content was vigorously mixed and allowed to settle for a while. Few drops of concentrated NH₃/NaOH solution was added to allow for the precipitation of the alkaloids and subsequently filtered. The precipitate obtained was dried in an oven at 60°C for 30 minutes and weighed.
% Alkaloid = (W₂ – W₁) / W x 100
W = weight of sample
W₁= weight of filter paper only
W₂ = weight of filter paper + precipitate formed.

2.6 Determination of Flavonoid Content
Flavonoids determination was done by taken 10 g of the processed sample and mixed with 100ml of methanol. After extraction, the whole content was filtered using 125 mm Whatman filter paper no. 42 and put into a crucible for evaporation to dryness and later weighed again.
% Flavonoid = weight of saponin x100 /weight of sample

2.7 Determination of Tannin Content
Tannin content was determined by taking 7g of the processed seeds and dissolved in distilled water. Varying concentrations of tannic acid were used as standards. 250 µl of the Folin-Ciocalteu reagent was added to tubes for dilution. After mixing and vigorously shaken, the mixture was allowed to rest for a period of five minutes thereafter, 2.5 ml of 7% sodium carbonate aqueous solution was added and allowed to rested again for 90 minutes. The resulting blue colour solution absorbance was taken at a wavelength of 760 nm using a spectrophotometer.
Tannin percentage = (x %) – (y %)
x % = percentage of the total phenolic compounds (g/100g dried seeds)
y % = content of the non-tannin phenols (g per 100 grams dried seeds).

2.8 Determination of Cyanogenic Glycoside
The alkaline picrate method was used for the determination of cyanogenic glycoside concentration. A small amount of the sample (3.0 g) was transferred to 50 ml distilled water
inside a flask which was incubated overnight, filtered and used for the determination. KCN solution ranging from 0.1 mg/ml to 1.0 mg/ml cyanide were set up and used to plot the standard curve. 1ml of the filtrate, 4 ml of alkaline picrate solution were added and incubated in water bath for 20 minutes. The colour obtained was recorded at 490 nm wave length against a sample blank.

Cyanogenic glycoside content (mg/100 g) = \( C \) (mg) \times 10 / weight of processed sample

2.9 Determination of Mineral Composition
The mineral composition of the processed seeds was determined using Atomic Absorption Spectrophotometer (AAS). The processed seeds were air dried and crushed to a very fine powder in a Creston high speed grinder, and 2 grams of sample transferred to a clean crucible. It was ashed at 550°C for about 3 hours. 15 ml of concentrated HCl and 6ml hot H\(_2\)NO\(_3\) were later added and transferred to a beaker. The content was placed on a hotplate and then heated to dryness at 100°C. After dilution with distilled water, samples were analyzed with the Atomic Absorption Spectrophotometer (AAS).

2.10 Procurement of Experimental Animals
Wistar rats weighing between 175-240g were used for this research study. The animals were procured at the animal house of the Department of Biochemistry, University of Port Harcourt, Choba, and Rivers State, Nigeria. The animals were housed in plastic metal top cages in the animal House of the Department of Biochemistry, University of Port Harcourt, weighed and divided into Eight (8) groups of sixteen rats each, such that the average weights were approximately equal. They were fed with standard diet and allowed access to clean water for a period of one week for acclimatization. The experiment was conducted in accordance with the ethical guidelines of the use of laboratory animals.

2.11 Experimental induction of diabetes and Compounding of Feeds
The rats in groups 2 to 6 were fed with high fat diet (20% sucrose +30% lard + 50% standard diet) for four weeks and then injected with 40mg/kg streptozotocin (STZ) in distilled water to induce diabetes. Group 1 served as normal control. Blood glucose and Lipid profile of animals were analyzed six (6) days after STZ injection and feeding. 50% rat feed was substituted with 50% of the various *Pentaclethra macrophylla* treatments used to feed the animals as presented in Table1. Metformin was administered daily by intra-gastric gavages. At the end of weeks 2, 4, 6 and 8 weeks, blood samples were collected from four rats in each group that were fasted overnight for estimation of lipid profile.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Group Identity</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>Normal feed + water</td>
</tr>
<tr>
<td>2.</td>
<td>Negative.control</td>
<td>High fat diet + streptozotocin (40mg/kg)</td>
</tr>
<tr>
<td>3</td>
<td>Metformin (Positive control)</td>
<td>High fat diet + streptozotocin (40mg/kg) + metformin(50mg/kg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>4</td>
<td>Raw</td>
<td>High fat diet + streptozotocin (40mg/kg) + feed compounded with the raw sample</td>
</tr>
<tr>
<td>5</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; cooking</td>
<td>High fat diet + streptozotocin (40mg/kg) + feed compounded with second treatment</td>
</tr>
<tr>
<td>6</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; cooking</td>
<td>High fat diet + streptozotocin (40mg/kg) + feed compounded with the third treatment</td>
</tr>
<tr>
<td>7</td>
<td>Fermented</td>
<td>High fat diet + streptozotocin (40mg/kg) + feed compounded with fourth treatment</td>
</tr>
<tr>
<td>8</td>
<td>Salted and fermented</td>
<td>High fat diet + streptozotocin (40mg/kg) + feed compounded with the fifth treatment</td>
</tr>
</tbody>
</table>

### 2.12 Determination of Biochemical Parameters
The glucose in plasma was assayed using the (multiCarein<sup>TM</sup>), containing a glucose strips and glucometer while Lipid profile was determined using Randox Diagnostic Kits (Crumlin, England). Plasma total cholesterol concentration was assayed by the method of Richmond, (1973). Plasma triglyceride (TG) concentration was assayed using the method of (Abel <em>et al</em>., 1952). Plasma HDL-cholesterol concentration was assayed using the method of Lopes-Virella, (1973). Plasma LDL- cholesterol (LDL-C) concentration was by (Friedewald <em>et al</em>., 1972).

### 2.13 Statistical Analysis
The data were analyzed for statistical differences between treatment groups, by means of one-way analysis of variance (ANOVA). In all, P< 0.05 was considered significant. Data are presented as Mean± S.E.M. (standard error in the mean).

### 3.0 Results

#### 3.1 Phytochemical Composition Processed of <em>Pentaclethramacrophylla</em> Seeds
Different processing stages of <em>Pentaclethra macrophylla</em> seeds and its phytochemicals results are given in Table 2. In the table, phytochemical contents reduced as processing progresses except in Tanin which showed significant (p<0.05) increase in second cooking, while the cyanogenic glycosides did not show significant (p>0.05) difference in all stages of processing.
Table 2: Phytochemical Composition (%) of Processed *Pentaclethra macrophylla* Seeds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw</th>
<th>1st Cooking</th>
<th>2nd Cooking</th>
<th>Fermented</th>
<th>Salted &amp; Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>23.28 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.48 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.39 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.81 ± 0.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.44 ± 0.57&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>33.93 ± 0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.37 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.58 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.41 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.87 ± 0.25&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saponin</td>
<td>14.26 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.01 ± 0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.55 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.09 ± 0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.62 ± 0.16&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tannin</td>
<td>11.78 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.06 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.20 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.39 ± 0.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.34 ± 0.14&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>0.004 ± 0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.003 ± 0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.003 ± 0.00&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>0.003±0.00&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>0.003±0.00&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values presented are mean ± standard deviation of four determinations. Mean values in each row with different small letter superscripts are statistically significant at p ≤ 0.05.

3.2 Mineral Composition of Different Processing Stages of *Pentaclethra macrophylla* Seeds

The mineral concentrations of the different processing stages of *Pentaclethra macrophylla* seeds were given in Table 3. Calcium concentration was highest in salted and fermented (773.15mg/kg) and least in 2nd cooking (467.50mg/kg). Manganese was highest in raw sample (16.30mg/kg) and lowest in 2nd cooking (8.30mg/kg). Salted and fermented has the highest zinc concentration of 195.90mg/kg while 1st cooking has the least concentration of 96.45mg/kg. Iron concentration was highest in fermented stage (161.30mg/kg) and least in 2nd cooking (107.15mg/kg). The raw seed had the highest magnesium concentration of 3,348.05mg/kg while 2nd cooking had the least value of 911.45mg/kg. Potassium concentration was highest in raw seeds (7,623.85mg/kg) and lowest in fermented stage (282.20mg/kg). Salted and fermented stage had the highest sodium concentration of 11,097.95mg/kg while 1st cooking stage had the lowest value of 182.90mg/kg.

Table 3: Mineral Composition (mg/kg) of Processed *Pentaclethra macrophylla* Seeds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw</th>
<th>1st Cooking</th>
<th>2nd Cooking</th>
<th>Fermented</th>
<th>Salted &amp; Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>471.70</td>
<td>568.05</td>
<td>467.50</td>
<td>701.70</td>
<td>773.15</td>
</tr>
<tr>
<td>Manganese</td>
<td>16.30</td>
<td>12.15</td>
<td>8.30</td>
<td>9.80</td>
<td>10.35</td>
</tr>
<tr>
<td>Zinc</td>
<td>162.65</td>
<td>96.45</td>
<td>125.45</td>
<td>138.50</td>
<td>195.90</td>
</tr>
<tr>
<td>Iron</td>
<td>141.50</td>
<td>148.00</td>
<td>107.15</td>
<td>161.30</td>
<td>140.85</td>
</tr>
<tr>
<td>Magnesium</td>
<td>3,348.05</td>
<td>2,451.15</td>
<td>911.45</td>
<td>1,159.15</td>
<td>1,253.15</td>
</tr>
<tr>
<td>Potassium</td>
<td>7,623.85</td>
<td>2,098.05</td>
<td>297.75</td>
<td>282.20</td>
<td>2,642.65</td>
</tr>
<tr>
<td>Sodium</td>
<td>351.95</td>
<td>182.90</td>
<td>1,881.05</td>
<td>353.20</td>
<td>11,097.95</td>
</tr>
</tbody>
</table>

3.3 Total Cholesterol Concentration (mmol/L) of Diabetic Rats Fed with Processed *Pentaclethramacrophylla* Seeds
The result from different processed of *Pentaclethra macrophylla* seed on Total Cholesterol concentration is presented in Table 4. The values obtained showed significant (p<0.05) increase in Total Cholesterol concentration all weeks of treatment in the negative control when compared to the normal control, however, there was decrease in treatment with the processed seeds of *Pentaclethra macrophylla*. In week 2, the groups treated with processed *Pentaclethra macrophylla* seeds showed no significant (p > 0.05) change in total cholesterol concentration. In week 4, the groups treated with fermented *Pentaclethra macrophylla* (PM) seeds had significant (p<0.05) reduction while in the 8th week, 2nd cooking and fermented treatment groups have significant reduction (p<0.05) compared to normal and negative control group.

**Table 4:** Total Cholesterol Concentration (mmol/L) of Diabetic Rats Fed with Processed *Pentaclethramacrophylla* Seeds

<table>
<thead>
<tr>
<th>Duration</th>
<th>Normal Control</th>
<th>Negative Control</th>
<th>Positive control</th>
<th>Raw</th>
<th>1st Cooking</th>
<th>2nd Cooking</th>
<th>Fermented</th>
<th>Salted&amp; Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd Week</td>
<td>1.35 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.30 ± 0.02&lt;sup&gt;bcdefgh&lt;/sup&gt;</td>
<td>2.02 ± 0.18&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>2.22 ± 0.36&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>2.05 ± 0.02&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>2.18 ± 0.17&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>2.20 ± 0.21&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>± 2.20 ± 0.25&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>4th Week</td>
<td>1.35 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.20 ± 0.16&lt;sup&gt;bcdegh&lt;/sup&gt;</td>
<td>1.82 ± 0.10&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.99 ± 0.19&lt;sup&gt;bcdfgh&lt;/sup&gt;</td>
<td>1.73 ± 0.06&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.92 ± 0.11&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.60 ± 0.21&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>± 1.82 ± 0.12&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>6th Week</td>
<td>1.32 ± 0.21&lt;sup&gt;aceg&lt;/sup&gt;</td>
<td>1.99 ± 0.19&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.59 ± 0.14&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.99 ± 0.21&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.51 ± 0.11&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.68 ± 0.18&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>± 1.38 ± 0.02&lt;sup&gt;bcdefgh&lt;/sup&gt;</td>
<td>± 1.72 ± 0.21&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>8th Week</td>
<td>1.35 ± 0.21&lt;sup&gt;acefgh&lt;/sup&gt;</td>
<td>1.74 ± 0.34&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.43 ± 0.12&lt;sup&gt;abcdedefgh&lt;/sup&gt;</td>
<td>1.59 ± 0.14&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.47 ± 0.06&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>1.26 ± 0.04&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>± 1.26 ± 0.15&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>± 1.57 ± 0.07&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values presented are mean ± standard deviation of four determinations. Mean values in each row with different small letter superscripts are statistically significant at p ≤ 0.05.

**3.4 Processing Stages of Pentaclethramacrophylla on Triglycerides Concentration**

The result of different processed stages of *Pentaclethra macrophylla* on triglyceride concentration in plasma is presented in Table 5. The result showed significant (p < 0.05) increase of plasma triglyceride in the negative control compared to the normal control in all the weeks. In week 4, the raw, 2nd and fermented treated groups had significant (p<0.05) decreased in the plasma triglycerides compared to positive control while in week 6, the salted and fermented group had significant (p<0.05) decrease compared to all other groups. In week 8, 2nd and fermented groups had significant decrease compared to other treated groups.

**Table 5:** Triglycerides Concentration (mmol/L) of Diabetic Rats Fed with Processed *Pentaclethramacrophylla* Seeds

<table>
<thead>
<tr>
<th>Duration</th>
<th>Normal control</th>
<th>Negative control</th>
<th>Positive control</th>
<th>Raw</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; cooking</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; cooking</th>
<th>Fermented</th>
<th>Salted&amp; fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Week</td>
<td>0.5125± 0.0096&lt;sup&gt;bdefgh&lt;/sup&gt;</td>
<td>1.9075± 0.3424&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6025± 0.0479&lt;sup&gt;bcdefgh&lt;/sup&gt;</td>
<td>0.7125± 0.1650&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>± 0.6675± 0.1806&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>0.760± 0.1078&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>± 0.7425± 0.0714&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; Week</td>
<td>0.5125± 0.0096&lt;sup&gt;bdefgh&lt;/sup&gt;</td>
<td>1.3925± 0.1282&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8000± 0.1899&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4925± 0.1756&lt;sup&gt;bdefgh&lt;/sup&gt;</td>
<td>± 0.6075± 0.0206&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>0.4725± 0.1028&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>± 0.3675± 0.0206&lt;sup&gt;bcdedefgh&lt;/sup&gt;</td>
<td>± 0.7500± 0.1781&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Values presented are mean ± standard deviation of four determinations. Mean values in each row with different small letter superscripts are statistically significant at p ≤ 0.05.

### 3.5 Different Processing Stages of *Pentaclethra macrophylla* Seeds on HDL Cholesterol Concentration.

The different processed stages of *Pentaclethra macrophylla* seeds on HDL cholesterol concentration is given in Table 6. In the table, the valued showed that in all the weeks of treatment, plasma HDL cholesterol of negative control decreased significant (p < 0.05) when compared to other groups. In the 2nd and 4th week, the raw showed significant increase in HDL compared to other processes groups. While in the 6th week, the 2nd cooking had significant (p<0.05) increase and in the 8th week, 2nd cooking and fermented groups had significant(p<0.05) increase in HDL compared to other treated groups.

<table>
<thead>
<tr>
<th>Duration</th>
<th>Normal Control</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Raw 1st Cooking</th>
<th>2nd Cooking</th>
<th>Fermented</th>
<th>Salted &amp;Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd Week</td>
<td>1.5500 ± 0.2082</td>
<td>0.6675± 0.0479b</td>
<td>1.2350 ± 0.0943c</td>
<td>1.7075 ± 0.1808</td>
<td>1.0025± 0.1367</td>
<td>1.3225± 0.1115</td>
<td>1.2925± 0.1302</td>
</tr>
<tr>
<td>4th Week</td>
<td>1.5500 ± 0.2082</td>
<td>0.6275± 0.0957b</td>
<td>1.0200 ± 0.2118</td>
<td>1.5500 ± 0.0424</td>
<td>0.9850± 0.2439</td>
<td>1.0600± 0.1349</td>
<td>0.9425± 0.1196</td>
</tr>
<tr>
<td>6th Week</td>
<td>1.5500± 0.2082</td>
<td>0.2500± 0.1913b</td>
<td>0.9300± 0.1349</td>
<td>1.4000 ± 0.4618</td>
<td>1.3675± 0.1452</td>
<td>1.4525± 0.1592</td>
<td>1.3250± 0.1258</td>
</tr>
<tr>
<td>8th Week</td>
<td>1.5500± 0.2082</td>
<td>1.3000± 0.1414b</td>
<td>1.4025 ± 0.1646</td>
<td>1.5250± 0.0957</td>
<td>1.6000± 0.1826</td>
<td>1.8150± 0.0238</td>
<td>1.8200± 0.0356</td>
</tr>
</tbody>
</table>

Values presented are mean ± standard deviation of four determinations. Mean values in each row with different small letter superscripts are statistically significant at P ≤ 0.05.

### 3.6 Different processing stages of *Pentaclethra macrophylla* seed on LDL cholesterol concentration

The different processed stages of *Pentaclethra macrophylla* seed on LDL cholesterol concentration in all the weeks of treatment is presented in Table 7. The plasma LDL cholesterol of the negative control increase significantly (p<0.05) compared to the normal control in weeks of treatment with different processed seeds *Pentaclethra macrophylla*. However, all treated groups showed significant (p<0.05) decrease in plasma LDL cholesterol comparable to positive and normal controls.
The different processed seeds of Pentaclethra macrophylla: et al., 2001). Manganese exhibit antioxidant function, boost the immune system and ferments. Free fatty acids mobilization from periphery tissues. Hyperglycemia and dyslipidemia affects the progression of coronary heart disease and linked to a decreased factor of cardiovascular diseases in diabetes (McCarty, 2005). Magnesium is a pigment found in of chlorophyll. Magnesium rich diets have been reported to stimulate cardiac functions (Edeoga and Enata 2001).

Minerals are the inorganic elements in plants. In recent times, researchers have started developing interest in them. Recent studies suggest that calcium as modifiers of diabetes risk (Liu et al., 2001). Manganese exhibit antioxidant function, boost the immune system and modulate enzyme activity (Talwar, 1989). Zinc functions in regulation of uptake glucose profile mediated by the insulin action. Iron is an essential metal for hemoglobin synthesis of erythrocytes, oxidation – reduction reactions, and cellular proliferation (Fernandez-Real et al., 2005). Magnesium is a pigment found in of chlorophyll. Magnesium rich diets have been linked to a decreased factor of cardiovascular diseases in diabetes (McCarty, 2005).

Hyperglycemia and dyslipidemia affects the progression of coronary heart disease and increases the mortality rate in diabetic patients. Elevated serum lipids are due to increased free fatty acids mobilization from peripheral fat depots. The different processed seeds of Pentaclethra macrophylla showed anti-hyperlipidemic effects. The lowering of the cholesterol concentrations while increasing the concentration of HDL cholesterol is an

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Table 7: LDL - Cholesterol Concentration (mmol/L) of Diabetic Rats Fed with Processed Pentaclethra macrophylla Seeds

<table>
<thead>
<tr>
<th>Duration</th>
<th>Normal Control</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Raw</th>
<th>1st Cooking</th>
<th>2nd Cooking</th>
<th>Fermented</th>
<th>Salted &amp; Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd Week</td>
<td>0.1925 ± 0.0619&lt;sup&gt;def&lt;/sup&gt;</td>
<td>0.5175± 0.01276&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3500± 0.1074&lt;sup&gt;efh&lt;/sup&gt;</td>
<td>0.1925± 0.1115&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2100 ± 0.0163&lt;sup&gt;acde&lt;/sup&gt;</td>
<td>0.2100 ± 0.0140&lt;sup&gt;acde&lt;/sup&gt;</td>
<td>0.1850± 0.0058&lt;sup&gt;adefgh&lt;/sup&gt;</td>
<td>0.2375± 0.0579&lt;sup&gt;acdefgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>4th Week</td>
<td>0.1925 ± 0.0619&lt;sup&gt;acde&lt;/sup&gt;</td>
<td>0.5046± 0.1275&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3200± 0.0141&lt;sup&gt;af&lt;/sup&gt;</td>
<td>0.2225± 0.1182&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1250 ± 0.0192&lt;sup&gt;adefgh&lt;/sup&gt;</td>
<td>0.2050 ± 0.3697&lt;sup&gt;acde&lt;/sup&gt;</td>
<td>0.1475± 0.5188&lt;sup&gt;adefgh&lt;/sup&gt;</td>
<td>0.1300± 0.0082&lt;sup&gt;adefgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>6th Week</td>
<td>0.1925 ± 0.00619&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.3300± 0.1457&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2625± 0.0450&lt;sup&gt;cdefg&lt;/sup&gt;</td>
<td>0.1875± 0.1144&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0625 ± 0.0330&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>0.1500 ± 0.0216&lt;sup&gt;acde&lt;/sup&gt;</td>
<td>0.1875± 0.3403&lt;sup&gt;acdefg&lt;/sup&gt;</td>
<td>0.1475± 0.0050&lt;sup&gt;acdefgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>8th Week</td>
<td>0.1925 ± 0.0619&lt;sup&gt;adef&lt;/sup&gt;</td>
<td>0.2125± 0.0287&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>0.1225± 0.0403&lt;sup&gt;cdefgh&lt;/sup&gt;</td>
<td>0.1800± 0.0516&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1475 ± 0.0222&lt;sup&gt;adefgh&lt;/sup&gt;</td>
<td>0.1300 ± 0.0183&lt;sup&gt;adefgh&lt;/sup&gt;</td>
<td>0.1175± 0.0096&lt;sup&gt;adefgh&lt;/sup&gt;</td>
<td>0.1575± 0.0150&lt;sup&gt;adefgh&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values presented are mean ± standard deviation of four determinations. Mean values in each row with different small letter superscripts are statistically significant at p ≤ 0.05.

3.7 Discussion
Plants are rich in different classes of bioactive components that help to prevent the occurrences of diseases in the body. These substances have been isolated and used for pharmacological purposes because they influence biological processes. They have been powerful anti-hypertensive, anti-inflammatory, antifungal, anti-hyperglycemic and anti-hyperlipidemic agents. Others have also been shown to exhibit anti-microbial activity by stimulation and inhibition of microorganism cell wall (Awoyinka et al., 2007). From this study, phytochemicals determined decreased as processing progressed from raw to salted and fermented Pentaclethra macrophylla seeds. The flavonoids act as antioxidants and scavenge free radicals. The Saponins exhibit hyperglycemia, hypercholesterol and antidiabetic properties (Lewis and Elvin-Lewis, 1995). The processed Pentaclethra macrophylla seeds have negligible amounts of cyanogenic glycosides and cardiac glycoside that have been reported to stimulate cardiac functions (Edeoga and Enata 2001). Hyperglycemia and dyslipidemia affects the progression of coronary heart disease and increases the mortality rate in diabetic patients. Elevated serum lipids are due to increased free fatty acids mobilization from peripheral fat depots. The different processed seeds of Pentaclethra macrophylla showed anti-hyperlipidemic effects. The lowering of the cholesterol concentrations while increasing the concentration of HDL cholesterol is an
implication of this effect. Similar results were also reported by (Monago et al., 2004, Akinmoladun et al., 2007, Ayoola et al., 2008).

4.0 Conclusion
In conclusion the processed Pentaclethramacrophylla seeds have hypolipidemic potentials in high fat diet and streptozotocin-induced diabetic rats during the 8-weeksof treatment andthis confirms its use in traditional medical practice in Nigeria.

References


Ighodaro, I and Chioma, Osigwe. (2012). Hypoglycaemic activity of aqueous extract of


