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`Effect of Ethanol Extract of *Gnetum africanum* leaves on the Lipid Profile of Albino Rats

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Abstract: *The implication of hypercholesterolemia in metabolic maladies has informed research on a wide spectrum of diet-based interventions in lowering serum cholesterol levels. This work investigated the effect of ethanol extract Gnetum africanum leaves on the lipid profile of Wister rats. A total of 12 rats divided into two groups of 6 rats each were employed in the investigation. The first group (control) were fed rat chow while the second group (test) were fed a composite feed containing rat chow and 10% extract of G. africanum leaves. The lipid profile (total cholesterol, triglycerides, LDL, HDL) of the rats was assayed after 18 days of feeding.The result showed that the total cholesterol level in the test group (0.476 ± 0.077 mg/dL) was significantly lower ($p<0.05$) than the control group (0.872 ± 0.161); similarly, there was also a significant reduction in the level of triglycerides ($p<0.05$) in the test relative to the control group. However, there was no significant difference in the levels of both HDL and LDL when between both groups. We conclude that the leaves of G. africanum, aside its nutritional value in culinary, may also play a medicinal role in reducing serum cholesterol in humans, and has the potential to be developed into a potent non statin-based hypolipidemic agent.*

Keyword: *Gnetum africanum, leaves, lipid profile, hyperlipidemia, wistar rats & ethanol extract.*

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I. Introduction

Lipids remain vital component of the biochemistry of living organism, where they play roles as structural membrane components,in energy storage and supply,and in hormonal synthesis amongst other functions[1,2]. The integral function of lipids in cell metabolism has instigated immense interest in their study, leading to a further elaboration of their implication in health and disease conditions. It is now widely accepted that dysfunction in lipid metabolism can lead to several diseases including atherosclerosis and cardiovascular disorders [3].

In humans, diet affects serum lipid profile and by extension health status[4]; with the general consensus favouring the consumption of foods low in cholesterol, while rich in polyunsaturated fatty acids as against saturated ones [5]. Again, dietary intervention has been variously reported to be an important non-pharmacological way of reducing cholesterol in person with elevated cholesterol, such diet typically include plant sterols, fibre, soy products and Mediterranean foods [6].

G. africanum is a forest liana that grows in most parts of Sub-Saharan Africa, South America and subtropical Asia[7]. The leaves are widely used as an ingredient in soups and stews in Southern Nigeria and are much in demand for their nutritional and therapeutic properties-where it is used in ethno-medicine to treat a range of diseases including splenomegaly and sore throat[8]. This work, a continuation of previous effort to ascertain the therapeutic importance of the leaves *G. africanum*, investigated the hypolipidemic potential of the leaves.The work is unique in that it reports for the first time, to the best of our knowledge, the effect of the leaves on lipid profile of rats placed on a diet supplemented with *G. africanum* leaves. Whereas a previous attempt[9] investigated this potential, it did so by assessing the implication of the extract of the leaves on lipid indices when administered to rats via oral gavage for 7 days. We suppose our procedure will yield a better outcome as we extended the feeding time to 18 days, while also saving the rats the discomfiture of oral gavage that may lead to perturbations in the biochemical parameters assayed.

II. Materials And Method

2.1 Sample Collection and Preparation.

The fresh leaves of *G. africanum* used in this work were bought from *Eke –Awka*market in Awka, Anambra State, Nigeria and was authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University Awka, Nigeria.

The leaves were washed with distilled water and dried under room temperature for four days. Thereafter, the leaves were ground into fine powder using a manual blender. The obtained powder was macerated in 70% ethanol for 24 h, after which the solution was filtered and the ethanol evaporated using a Soxhlet apparatus. The obtained filtrate (extract) was used to supplement standard rat chow to obtain the experimental feed.

2.2 Experimental feed formulation

The leaf extract of *G. africanum* and standard rat chow were mixed in a ratio of 1: 10 to obtain the experimental diet. The composition of the pelletized commercial rat chow (Vital Grower's Feed) had a proximate composition of; crude protein (13%), fat (8%), crude fibre (15%), calcium (0.9%), phosphorous (0.35%), energy (2600 Kcal/kg).

2.3 Animal handling and grouping

A total of 18 male Wistar albino rats, purchased from the Animal farm of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, were used in the experiment. The animals were handled following standard ethical guidelines on animal handling and research. The rats were divided into three groups (Group1-3) of 6 rats each. The first group (Group 1) served as baseline; they were sacrificed shortly after purchase, and had their blood collected via cardiac puncture. The blood samples were centrifuged and the obtained sera used for biochemical investigations.The baseline gives the biochemical status of the rats prior use in the experiment, as experimental conditions and ambience where animals are kept combine to affect biochemical indices. Group 2 (test group) received the experimental feed made of 10% of the leaf extract of *G. africanum* and 90% of grower's mash for 18 days. The final cohort (Group 3) received only the grower's mash for 18 day and served as the experimental control.

2.4 Sample collection for biochemical investigation

After 18 days of feeding the animals (G1 & 3), they were fasted for 12 h, were weighed and thereafter anaesthetized using chloroform. Blood samples were collected from the rats via cardiac puncture. The obtained samples were centrifuged at 1000rpm for 30 min to obtain the sera, which was employed for the lipid profile analysis.

2.5 Lipid Profile investigation.

2.5.1 Estimation of serum total cholesterol

The cholesterol content of the serum was measured at 546nm using Auto-chemistry analyser (Mindray BA-88, China). The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

2.5.2 Estimation of serum triglyceride

The Triglyceride content of the serum was measured at 546nm using Auto-chemistry Analyser (Mindray BA-88). The triglycerides (GPO) method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase. The principle of this method was described by Fossati[10] who coupled the reaction with the classical Trinder reaction sequence. The reaction quantitates the total glycerides in serum including the mono and diglycerides, and the free glycerol fractions.

2.5.3 Estimation of serum low-density lipoprotein (LDL)

Again**,** this was analyzed using the Auto-chemistry Analyzer (Mindray BA-88) using the auto LDL™ Cholesterol Reagent.This was determined by measuring the amount of cholesterol remaining in the serum after precipitation with polyvinyl sulphate. The LDL content was estimated as the difference between the total cholesterol and the cholesterol remaining in the solution after precipitation.

2.5.4 Estimation of serum high-density lipoprotein (HDL)

This was determined using the Auto-chemistry Analyzer (Mindray BA-88) using the auto HDL™ Cholesterol Reagent. This was determined by measuring the amount of cholesterol remaining in the serum after precipitation of LDL, VLDL and Chylomicron by the addition of phosphotungstic acid and magnesium chloride. The HDL content was measured as the remaining cholesterol in the sample solution after precipitation.

2.6 Qualitative Phytochemical analysis of *G. africanum*.

Alkaloids: One mL of the extract was mixed with 5 mL of 2% HCl in a test tube, heated on water bath, and filtered. Of the filtrate, 2 mL was divided into two aliquots of 1 ml each. To the first portion, few drops of Wagner's reagent were added; occurrence of reddish-brown precipitate is taken as a positive test. To the second aliquot, 1 ml of Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids [11].

Saponins: The extract (1 ml) was boiled with 5 ml of distilled water, the soluble fraction of the mixture was decanted into two aliquots while still hot. The obtained filtrate was used for the following tests:

Emulsion test: Two drops of olive oil was added to 1 ml of the extract in test tube. The set up was mechanically agitated and observed for formation of emulsion; which indicates the presence of saponin.
Frothing test: Distilled water (3 mL) was added to 1 ml of the extract. 0.5 mL filtrate was diluted to 5 mL with distilled water and shaken vigorously for 2 minutes. Formation of stable froth head indicates the presence of saponin [12].

Flavonoids
Lead acetate test: To 1 ml of the filtrate, 1 ml of 10 % lead acetate solution was added. Appearance of a buff-coloured precipitate indicates the presence of flavonoids [13]
Ferric Chloride test: To 1 mL of extract in a test tube, a few drops of 10% $FeCl_2$ was added. A green-blue or violet coloration indicates the presence of a phenolic hydroxyl group [13]
Glycosides. To 2 mL of the extract, a few drops of Fehling's solution A and B wereadded; an orange-red precipitate suggests the presence of reducing sugar.
Tannins: A quantity, 2g of the ground leaves was boiled with 5ml of 45% of ethanol for 5 minutes. The mixture was cooled and filtered. The filtrate was used for the following tests:
i. Lead sub-acetate: To 1ml of the filtrate, 3 drops of lead sub-acetate solution was added. A gelatinous precipitate indicates the presence of tannins.
ii. Bromine Water: To 0.1ml of the filtrate, 0.5ml of bromine water was added and then observed for a pale brown precipitate.
iii. Ferric Chloride: 1ml of the filtrate was diluted with distilled water and 2 drops of ferric chloride was added. A transient greenish to black colour indicates the presence of tannins.

2.7 Statistical analysis.
All statistical analyses were performed using SPSS 23.0 (SPSS, Inc., Chicago, USA). In order to determine the variance between means of the different groups, one-way ANOVA tests were performed, followed by post-hoc test (Tukey HSD) to compare means. A *P* value <.05 was considered significant. Data are presented as mean ± standard deviation (SD).

III. Results

3.1 Table 1: showing mean concentrations of lipids in rats fed experimental feed containing extract of *G. africanum* leaves vs baseline and control.

	TOT. CHOL	TRIGLYCERIDES	LDL	HDL
Group				
Control	0.872±0.161	0.888 ± 0.269	0.035±0.019	0.268±0.043
Test	0.476±0.077*	0.263 ± 0.183*	0.049±0.013	0.233±0.085

Table showing the mean values (mmol/L) for total cholesterol (Tot. chol), triglycerides and low density lipoprotein (LDL), high density lipoprotein (HDL) in the three groups of rat. Values with same superscript (*) differ significantly (p< 0.05) when compared to the control.

4.6 TABLE 2: Qualitative phytochemical screening of leaves of *G. africanum*

Phytochemicals	Remark/Inference
Alkaloids	+
Saponin	+
Flavonoids	+
Glycosides	+
Tannins	+

Key: + present

IV. Discussion And Conclusion

As earlier noted, dietremains an important non-pharmacological way of reducing cholesterol in person with elevated cholesterol, and our research findings indicate that the leaves of *G. africanum* was able to significantly lower the total cholesterol level in the test rats compared to the control. The observed hypocholestrolemic effect is corroborated by the works of Udeh *et al.,*[14]whonoted a significant decrease ($p < 0.01$) in total cholesterol levels of diabetic rats administered methanol leaf extract (200 mg/kg bw) versus control administered distilled water. This suggests that the hypocholesterolemic principle in the leaves may be generally soluble in polar solvents.

Furthermore, the extract significantly decreased the concentration of triglycerides in the test group compared to the control. Again our work is corroborated the result of Udeh*et al.,* [14] who reported a similar trend with high concentration (800 mg/kg bw) causing a reduction in the triglycerides content of rats administered ethanol leaf extract of *G. africanum* compared to control. The observed reduction in triglyceride level may have

positive health roles. Triglycerides have been recently shown to be negatively correlated with HDL-C; which is in turn positively associated with better cardiovascular health outcomes[15].

In the present work, the levels of HDL and LDL were not significantly altered by the extract relative to the control, which is again similar to the findings of Udeh*et al.*, [14], who observed no significant change in the levels of both HDL and LDL in test rats administered 100-800 mg/kg bw of ethanol leaf extract of *G. africanum* compared to control rats administered distilled water.

The above observed pharmacological effects invariably stems from the phytochemistry of the investigated leaves, with preliminary phytochemical analysis revealing to contain tannins, alkaloids, saponin, flavonoids and glycosides.

V. Conclusion

In conclusion, the ethanol leaf extract of *G. africanum* used in the current work showed promise as a potential hypolipidemic agent by significantly decreasing the levels of total cholesterol and triglycerides. The result is more so pertinent considering that hyperlipidemia is a major trigger for a host of metabolic maladies, and hence the search for therapeutic agents that can lower lipid levels is the focal point for many scientific investigations. The statins remains the first choice drugfor managing hyperlipidemia, however, the high cost of potent statins [16] and low responsiveness in certain patients [17] makes it important that other non-statin based agents are explored.

Finally, our findings is still too rudimentary to draw a conclusion on the effect prolonged consumption of *G. africanum* will have on the lipid profile of humans. More studies will have to be done to further understand the long-term implication of consuming *G. africanum*leaf by employing a randomized, clinical trials.

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