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Comparative Influences of Extracts of Various Parts of *Annona muricata* (Soursop) on Basal Lipid Profile and Plasma Fatty Acid Synthase in Wistar Rats

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Abstract
Annona muricata (Soursop) contains high levels of annonaceous acetogenins and essential oils to which most of its ethnomedicinal benefits are attributed.
This study was carried out to evaluate the effect of extracts of various parts of Annona muricata on the lipid profile and plasma fatty acid synthase (FAS) of adult Wistar rats.
Five groups of Wistar rats (6 rats per group) received Annona muricata extracts at doses of 100, 200, 400, 600 and 800 mg/kg for 28 days via oral gavage. The rats were sacrificed on the 29th day, and body weights and organ weights were obtained. Plasma lipids and plasma FAS were determined using appropriate assay kits as per manufacturer's protocol.
The fruit pulp and leaf extracts demonstrated brought about marked and significant decreases in plasma FAS activity, when compared to the stem and root bark extracts ($p < 0.05$). However, only the fruit pulp extract produced significant and dose-dependent anti-atherogenic and hypolipidemic potential, with reductions in total cholesterol : high density cholesterol (TCHL: HDL) and LDL: HDL ratios (5.41 to 4.31, and 1.71 to 0.79, respectively) as doses of the fruit pulp extract were increased, relative to the root bark extract and control groups ($p < 0.05$).
The fruit pulp and stem-bark extracts demonstrated better anti-lipidemic and anti-cholesterolemic agents in rats than the leaf and root-bark extracts.
Keywords: *Annona muricata,* Fruit, Stem bark, Leaf, Lipid profile, Fatty acid synthase

Introduction
Annona muricata is a shrubby plant commonly sourced in Nigeria, especially the rain forest regions of the South-Eastern plains and Niger-Delta basins. The major phytochemicals reported to be present in *Annona muricata* are alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, phytosterols, terpenoids and proteins [1]. Among the chemical constituents found in *A. muricata*, the alkaloids (reticulin, coreximine, coclarine and anomurine) [2] and essential oils (beta-caryophyllene, delta-cadinene, epi-α-cadinol and alpha-cadinol) [3] stand out. Numerous ethnomedicinal properties have been ascribed to the plant, including, wound healing [4], anti-arthritic, anticonvulsant [5, 6], anti-diabetic and hypolipidemic [7 - 9]; anti-inflammatory and anti-nociceptive [10 - 12], antioxidant [4, 13 - 15], anti-hypertensive [16], antiparasitic [17], anti-plasmodial [18], hepatoprotective [19], gastroprotective, and antineoplastic effects [20, 21]. A study by Adewole *et al*. [22] showed that daily intraperitoneal injection of streptozotocin-induced diabetic Wistar rats with methanolic leaf extract of *A. muricata* (100 mg/kg) for two weeks significantly reduced their blood glucose concentration. In addition, the extract at the same dose significantly decreased plasma TCHOL, LDL, TGs and VLDL cholesterol. These beneficial effects have been linked to the antioxidant and hypolipidemic potential of extract, as well as its protective effects on pancreatic β-cells [9]. Ethanol extract of the stem bark of *A. muricata* has also been reported to possess antidiabetic and hypolipidemic properties against alloxan- induced diabetic rats [8].
Fatty acid synthase is a multi-enzyme protein that catalyzes fatty acid biosynthesis. Its main function is to catalyze the synthesis of palmitate (C16:0, a long-chain saturated fatty acid) from acetyl-CoA and malonyl-CoA, in the presence of NADPH. This enzyme plays a central role in the *de novo* lipogenesis in humans. By the action of its seven active sites, FAS catalyzes all the reaction steps in the conversion of acetyl-CoA and malonyl-CoA to palmitate and other long chain fatty acids [24].
The present study was carried out to determine the effects of extracts of various parts of *A. muricata* on basal lipid profile and plasma FAS activity in Wistar albino rats.

Materials and Methods
Experimental rats
The rats used were adult albino male Wistar rats with weights between 100 g – 150 g. The rats were supplied by Mr. Silvanus Innih of the Anatomy Department, University of Benin, Benin City, housed in the Department of

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Biochemistry animal house, and were acclimatized for one week before the study. They were fed standard rat chow and water *ad libitum*. A written approval for the study was obtained from the Research Ethics Committee Guideline Principles on Handling of Animals of the College of Medicine, University of Benin (CMR/REC/2014/57), and was strictly adhered to.

Preparation of *Annona muricata* crude extracts

A large quantity of fresh parts of the plant was collected from trees in house-hold gardens in Benin City and around University of Benin vicinity, Edo state, Nigeria. The plant was identified by Dr Bamidele of the Department of Plant Biology and Biotechnology, University of Benin and authenticated by Professor Idu of the same Department. A voucher specimen number UBHa 0205 was deposited at the Herbarium of Department of Plant Biology and Biotechnology, University of Benin. The samples (leaves, roots, stem bark and pulp) were washed and pulverized separately after drying at room temperature (about 25 ^{o}C) for 4 weeks. Each pulverized plant sample was macerated in methanol for 48 hours after which it was filtered through cheese cloth. The obtained extracts were then concentrated *in vacuo* using rotary evaporator to obtain viscous gels which were air-dried to gel-like solids. The gel-like crude methanolic extracts gotten from the various parts of the plant were reconstituted to obtain a stock solution using distilled-deionized water as solvent. Each reconstituted crude extract was stored in small-capped plastic containers in a refrigerator at -4 ^{o}C until used.

Administration of extracts

The extracts were administered with the aid of a gavage, acting as an oro-gastric tube. Utmost care was taken not to inflict oral or oesophageal injuries on rats.

Sub-chronic toxicity assessment

During period of usage, extracts were administered to the rats based on calculated doses per weight of rat (*i.e*. equivalent volume). This dose calculations were done weekly per weight of rats for the sub-chronic studies, as the weekly weights of the rats per group were recorded *i.e*. day 0, day 7, day 14, day 21 and day 28. Untreated rats (group one) served as the control and was administered 2 ml of distilled water [4, 23, and 25].

Observations in sub-chronic assessment (clinical signs and mortality)

The rats were observed for signs of weakness, increased or decreased appetite, weight loss and other physiological changes including mortality. Clinical signs to be assessed before dosing, immediately and 4hrs after dosing, include level of sedation, restlessness, change in nature of stool, urine and eye colour, excretion of worms, diarrhoea, haematuria, uncoordinated muscle movements etc. the animals will be observed for toxic symptoms such as weakness or aggressiveness, food refusal, loss of weight, diarrhoea, discharge from the eyes and ears, noisy breathing and mortality [26, 27, 28].

Experimental protocol for sub-chronic toxicity studies

Various methanolic extracts of the plant parts (fruit pulp, leaf, stem-bark and root-bark) were administered at increasing doses from 100 mg/kg (group 2), 200 mg/kg (group 3), 400 mg/kg (group 4), 600 mg/kg (group 5) and 800 mg/kg (group 6). The group 1 rats were given 2 ml of distilled water (0 mg/kg) and served as the control. Each group had six (6) rats each.

Blood sample collection for sub-chronic assessment

At the end of the experimental period of 28 days, the rats were sacrificed after euthanizing them, then whole blood (5 ml) collected into heparin bottles and centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. The plasma samples were transferred into plain sample bottles and immediately taken to the University of Benin Teaching Hospital, Hematology Department, for plasma lipid profile analysis using ready-to-use Randox® kits; Plasma total cholesterol (TCHL, 2176CH), triacylglycerol (TAG, 1039TR), high density lipoprotein (HDL, CH203), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Estimation of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were done using the equations:

$$\text{VLDL} = \text{Concentration of triacylglycerol}/5 \quad \text{---------- 1}$$

$$\text{LDL – Cholesterol Concentration} = \text{Total Cholesterol} - \text{VLDL} - \text{HDL cholesterol} \text{------ 2}$$

Plasma fatty acid synthase (FAS) was assayed using the MyBioSource® ELISA kit following the prescribed procedures based on sandwich enzyme-linked immuno-sorbent assay technology. A blue color product that changes into yellow after adding acidic stop solution is positive and the intensity of the color yellow is proportional to the FAS and measured spectrophotometrically at 450 nm in a microplate reader, followed by the calculation of FAS concentration from a standard curve.

Statistical analysis

Data were entered into the Microsoft Excel spread sheet (v.10) prior to descriptive analysis. The data are presented as mean ± SEM, and were analyzed using the Duncan's multiple range analyses of variance, ANOVA. Correlation analyses were done using Pearson's correlation (p=0.05) of the Statistical Package for Social Sciences, SPSS®, Version 21.0, IBM Corp., Armonk, NY, USA. Histograms and line plots were done using Graph Pad software®, Prism 5, Version 5.01, La Jolla, CA, USA. Values of $p < 0.05$ were considered significant.

Results

Table 1. Lipid profile of rats administered fruit pulp extract of *Annona muricata*

Group	TCHL (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	82.00 ± 2.70	204.33 ± 6.02	15.17 ± 2.04	25.97 ± 4.14	40.87 ± 1.20
100 mg/kg	76.67 ± 3.96	186.60 ± 7.34	16.83 ± 1.8	22.53 ± 5.07	37.20 ± 1.47
200 mg/kg	80.33 ± 3.04	226.67 ± 15.10	17.17 ± 8.83	17.83 ± 5.72	45.33 ± 3.02
400 mg/kg	78.83 ± 2.88	210.33 ± 18.47	18.67 ± 0.67	18.10 ± 5.69	42.07 ± 3.69
600 mg/kg	82.17 ± 3.95	221.33 ± 9.90	18.33 ± 1.67	17.73 ± 4.23	44.27 ± 1.84
800 mg/kg	81.83 ± 3.52	239.00 ± 13.56	19.00 ± 1.81	15.03 ± 5.52	48.96 ± 3.00
F-value	9.842	18.903	6.830	11.729	17.027
p-value	0.006	0.000	0.002	0.000	0.000

Values are presented as mean ± SEM (n = 6). *P* value less than 0.05 ($p<0.05$) indicates significant change, by one-way Duncan's multiple range ANOVA.

Table 2. Lipid profile of rats administered leaf extract of *Annona muricata*

Group	TCHL (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	71.00 ± 0.00	199.00 ± 0.00	17.00 ± 0.00	14.20 ± 0.00	39.80 ± 0.00
100 mg/kg	90.00 ± 0.00	209.00 ± 0.00	17.00 ± 0.00	31.20 ± 0.00	41.80 ± 0.00
200 mg/kg	92.00 ± 0.00	197.00 ± 0.00	23.00 ± 0.00	29.60 ± 0.00	39.40 ± 0.00
400 mg/kg	87.33 ± 2.35	201.00 ± 12.12	19.40 ± 1.60	26.84 ± 2.86	40.36 ± 2.42
600 mg/kg	83.00 ± 1.90	203.33 ± 8.57	20.00 ± 2.56	22.33 ± 4.92	40.61 ± 1.71
800 mg/kg	73.33 ± 2.80	200.67 ± 5.71	18.67 ± 1.05	14.87 ± 2.46	39.70 ± 1.00
F-value	27.904	11.930	18.992	12.302	3.918
p-value	0.000	0.017	0.000	0.000	0.058

Values are presented as mean ± SEM (n = 6). *P* value less than 0.05 ($p<0.05$) indicates significant change, by one-way Duncan's multiple range ANOVA.

Table 3. Lipid profile of rats administered stem-bark extract of *Annona muricata*

Group	TCHL (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	87.00 ± 1.00	212.50 ± 7.00	11.00 ± 0.00	33.50 ± 1.00	42.57 ± 1.00
100 mg/kg	83.83 ± 6.00	269.50 ± 12.00	12.50 ± 0.80	19.10 ± 0.00	53.90 ± 2.00
200 mg/kg	75.50 ± 1.00	220.83 ± 16.00	15.33 ± 1.00	17.33 ± 0.00	44.16 ± 3.00
400 mg/kg	74.83 ± 2.00	215.33 ± 21.00	10.50 ± 1.00	21.26 ± 4.00	43.06 ± 4.00
600 mg/kg	84.60 ± 4.00	219.20 ± 13.00	14.40 ± 2.00	26.36 ± 7.00	43.84 ± 3.00
800 mg/kg	81.20 ± 4.00	225.20 ± 7.00	14.60 ± 2.00	21.56 ± 6.00	45.04 ± 1.00
F-value	17.833	27.003	11.289	29.112	27.190
p-value	0.000	0.000	0.001	0.000	0.000

Values are represented as mean ± SEM (n = 6). *P* value less than 0.05 ($p<0.05$) indicates significant change, by one-way Duncan's multiple range ANOVA.

Table 4. Lipid profile of rats administered root-bark extract of *Annona muricata*

Group	TCHL (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	78.33 ± 2.00	240.00 ± 31.00	10.66 ± 0.90	19.66 ± 8.00	20.50 ± 4.00
100 mg/kg	87.00 ± 4.00	226.75 ± 11.00	14.75 ± 2.00	26.90 ± 7.00	45.35 ± 2.00
200 mg/kg	84.80 ± 1.00	228.20 ± 21.00	12.00 ± 1.00	27.16 ± 2.00	45.64 ± 4.00
400 mg/kg	83.00 ± 4.00	253.33 ± 26.00	14.66 ± 2.00	17.66 ± 0.70	50.66 ± 5.00
600 mg/kg	85.60 ± 4.00	215.83 ± 23.00	11.33 ± 1.00	31.16 ± 8.00	43.16 ± 5.00
800 mg/kg	81.00 ± 3.00	262.50 ± 47.00	13.00 ± 2.00	31.16 ± 8.00	52.50 ± 9.00
F-value	29.923	12.365	11.276	31.182	27.002
p-value	0.000	0.000	0.001	0.000	0.000

Values are presented as mean ± SEM (n = 6). *P* value less than 0.05 ($p<0.05$) indicates significant change, by one-way Duncan's multiple range ANOVA.

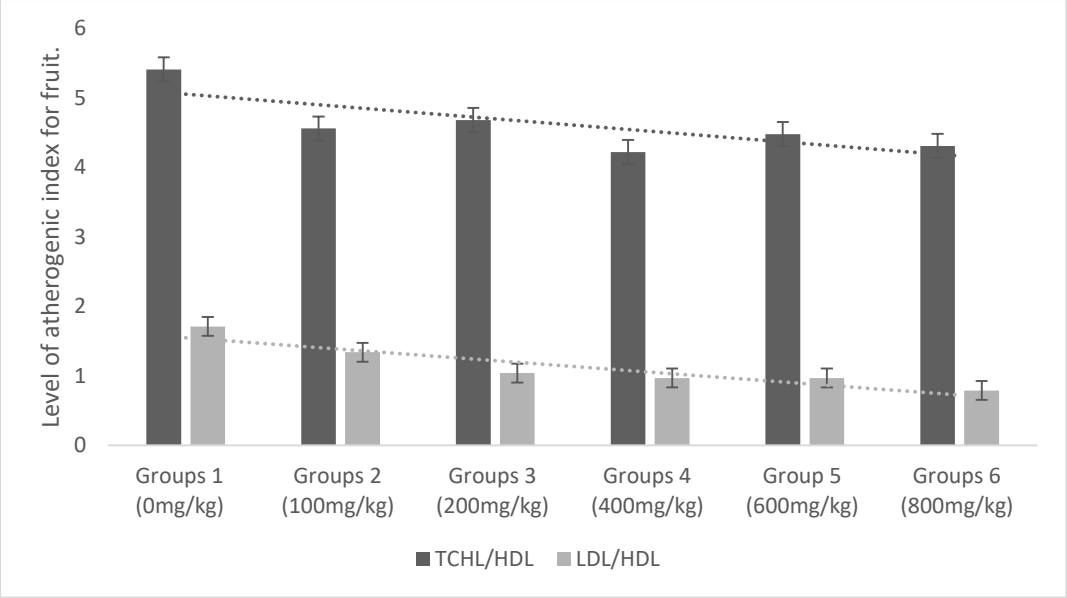

Figure 1. Atherogenic indices of plasma cholesterol: HDL and LDL: HDL for rats administered fruit pulp extract of *Annona muricata.* The Pearson's correlation between both indices, $r^2 = 0.892$ indicated a significant correlation, $p=0.017$ ($p<0.05$).

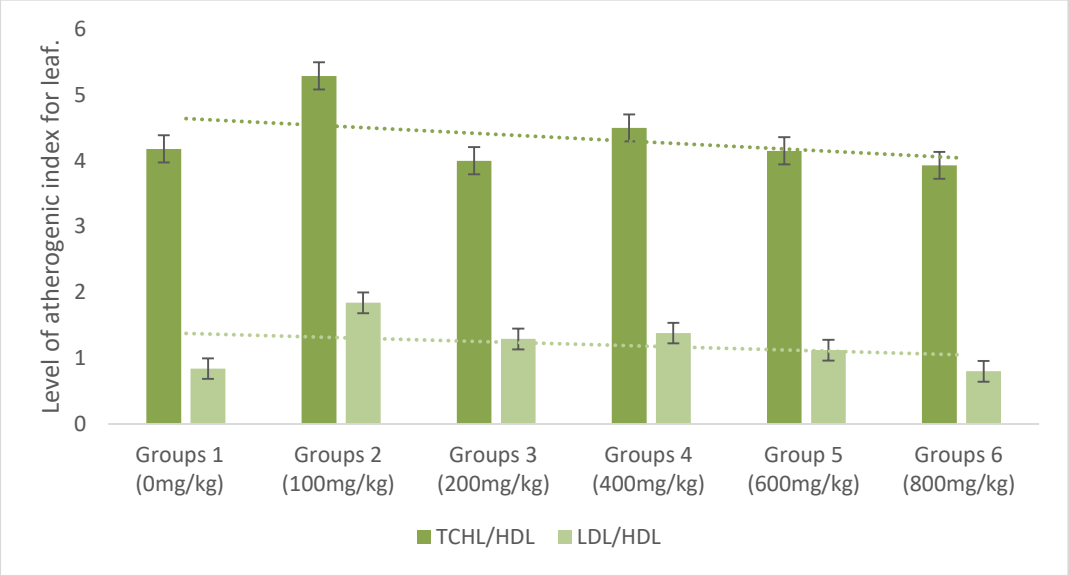

Figure 2. Atherogenic indices of plasma cholesterol: HDL and LDL: HDL for rats administered leaf extract of *Annona muricata.* The Pearson's correlation between both indices, $r^2 = 0.865$ indicated a significant correlation, $p=0.026$ ($p<0.05$).

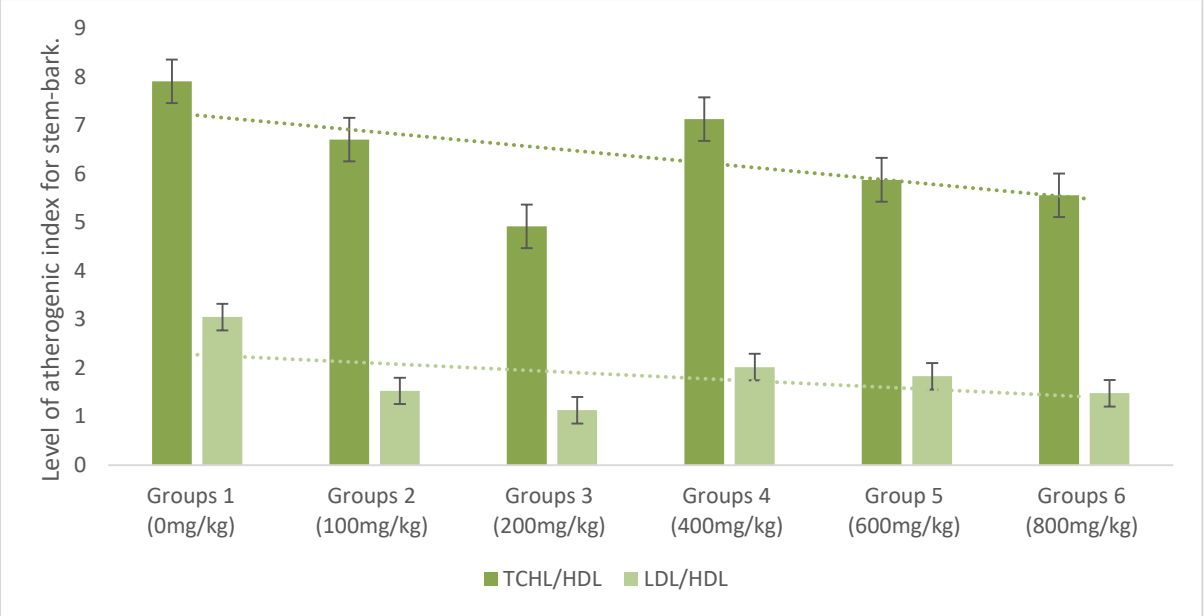

Figure 3. Atherogenic indices plasma cholesterol: HDL and LDL: HDL for rats administered stem-bark extract of *Annona muricata*. The Pearson's correlation between both indices, $r^2 = 0.897$ indicated a significant correlation, $p=0.015$ ($p<0.05$).

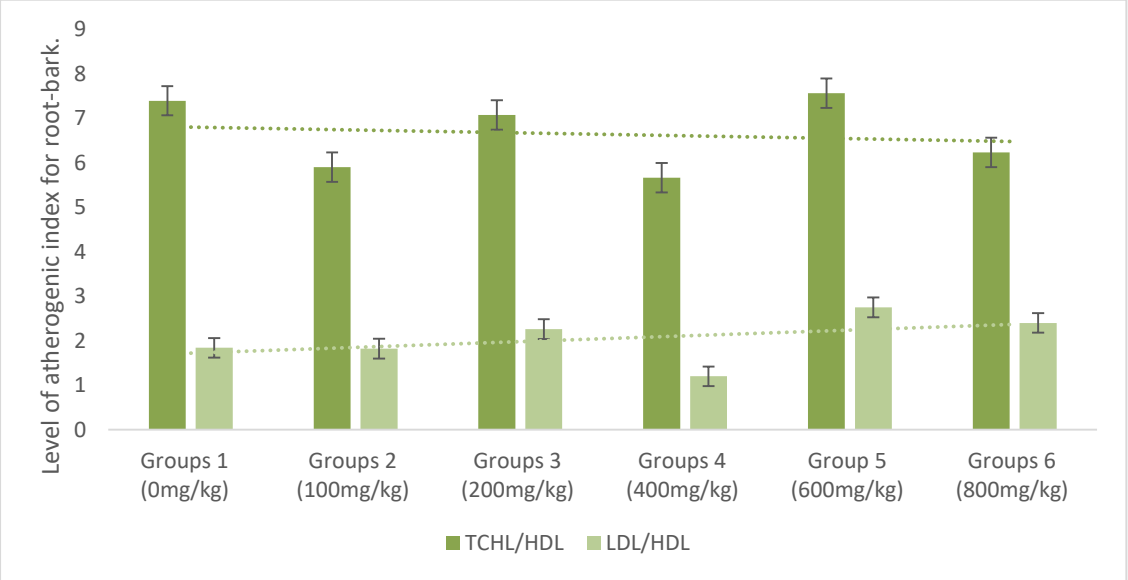

Figure 4. Atherogenic indices of plasma cholesterol: HDL and LDL: HDL for rats administered root-bark extract of *Annona muricata*. The Pearson's correlation between both indices, $r^2 = 0.681$ did not give a significant correlation, $p=0.137$ ($p>0.05$).

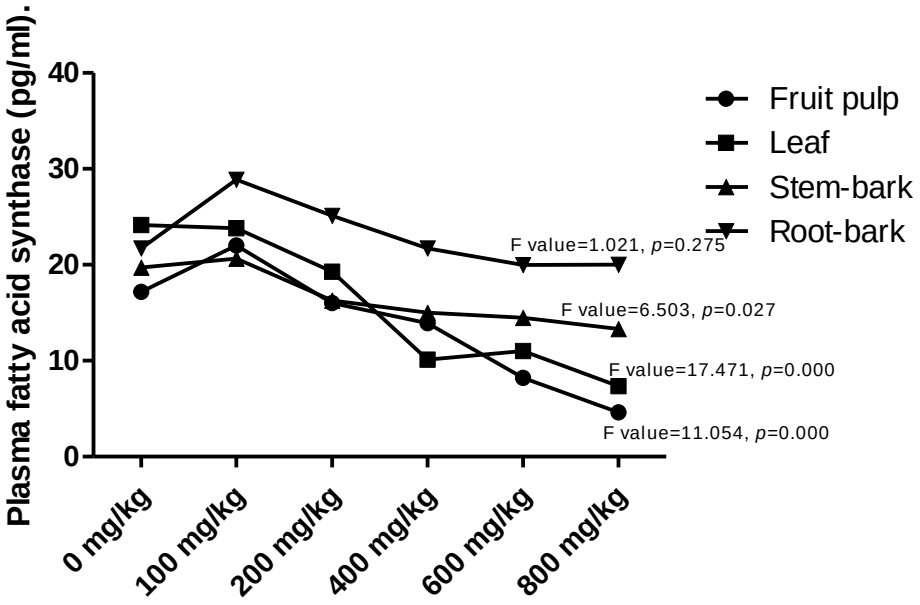

Figure 5. Plasma fatty acid synthase profile of rats administered extracts of *Annona muricata*

Table 5. Organ weights of rats administered *Annona muricata* fruit pulp extract

Groups	Brain	Lungs	Liver	Pancreas	Right kidney	Left kidney	Spleen	Heart	Colon*
0 mg/kg	0.88±0.10	0.87±0.10	4.16±0.10	0.55±0.02	0.35±0.03	0.41±0.03	0.49±0.04	0.46±0.40	0.70±0.20
100 mg/kg	0.83±0.10	0.93±0.10	3.47±0.20	0.49±0.02	0.27±0.02	0.33±0.02	0.38±0.02	0.43±0.10	0.72±0.20
200 mg/kg	0.84±0.10	0.92±0.10	3.81±0.20	0.55±0.04	0.37±0.02	0.40±0.10	0.60±0.10	0.48±0.02	0.66±0.10
400 mg/kg	0.77±0.10	0.84±0.10	3.59±1.00	0.61±0.10	0.33±0.03	0.39±0.03	0.40±0.03	0.45±0.10	0.81±0.20
600 mg/kg	0.78±0.10	0.61±0.10	3.51±0.10	0.58±0.10	0.41±0.10	0.48±0.10	0.54±0.10	0.45±0.10	0.79±0.20
800 mg/kg	1.00±0.20	0.97±0.20	3.67±0.10	0.40±0.10	0.43±0.40	0.48±0.10	0.44±0.10	0.41±0.10	0.84±0.30
F-value	18.299	11.303	16.003	28.411	14.208	26.195	10.670	48.519	14.042
p-value	0.000	0.000	0.000	0.002	0.000	0.025	0.000	0.050	0.001

Values are represented as mean ± SEM (n = 6). *P* value less than 0.05 ($p<0.05$) indicates significant change, by one-way Duncan's multiple range ANOVA. * Weight of 2 cm from the distal part of the anus.

Table 6. Organ weights of rats administered *Annona muricata* leaf extract

Groups	Brain	Lungs	Liver	Pancreas	Right kidney	Left kidney	Spleen	Heart	Colon*
0 mg/kg	0.88±0.10	0.99±0.10	3.44±0.20	0.51±0.60	0.73±0.10	0.37±0.04	0.41±0.04	0.58±0.10	0.63±0.10
100 mg/kg	0.85±0.10	0.81±0.10	3.59±0.30	0.62±0.10	0.55±0.10	0.35±0.04	0.39±0.05	0.41±0.04	0.83±0.10
200 mg/kg	0.96±0.10	0.81±0.10	3.66±0.30	0.52±0.03	0.51±0.05	0.38±0.02	0.43±0.02	0.50±0.08	0.72±0.10
400 mg/kg	0.73±0.10	0.76±0.10	3.55±0.20	0.47±0.04	0.39±0.02	0.31±0.02	0.38±0.01	0.38±0.03	0.70±0.10
600 mg/kg	0.84±0.10	0.73±0.10	3.17±0.30	0.37±0.10	0.29±0.04	0.26±0.10	0.28±0.05	0.30±0.05	0.42±0.10
800 mg/kg	0.93±0.10	0.96±0.10	3.45±0.10	0.43±0.10	0.52±0.04	0.36±0.02	0.42±0.01	0.39±0.02	0.64±0.10
F-value	16.005	51.924	22.805	33.824	62.935	15.204	34.726	21.207	44.063
p-value	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000

Values are represented as mean ± SEM (n = 6). *P* value less than 0.05 ($p<0.05$) indicates significant change, by one-way Duncan's multiple range ANOVA. * Weight of 2 cm from the distal part of the anus.

Table 7. Organ weights of rats administered *Annona muricata* stem-bark extract

Groups	Brain	Lungs	Liver	Pancreas	Right kidney	Left kidney	Spleen	Heart	Colon*
0 mg/kg	0.86±0.10	0.76±0.10	3.82±0.10	0.53±0.10	0.65±0.10	0.40±0.10	0.43±0.10	0.45±0.10	1.06±0.10
100 mg/kg	0.80±0.10b	0.78±0.10	3.40±0.10	0.47±0.10	0.42±0.10	0.42±0.10	0.45±0.10	0.48±0.10	0.66±0.10
200 mg/kg	0.97±0.10	0.80±0.10	3.35±1.00	0.69±0.10	0.67±0.10	0.56±0.10	0.60±0.10	0.61±0.10	0.96±0.10
400 mg/kg	0.83±0.10	0.98±0.1	3.82±0.10	0.49±0.10	0.49±1.00	0.43±0.10	0.45±0.10	0.46±0.10	0.94±0.10
600 mg/kg	0.86±0.10	0.98±0.2	4.03±1.00	0.49±0.10	0.50±0.10	0.38±1.00	0.40±0.10	0.37±0.10	0.63±0.10
800 mg/kg	0.59±0.10	0.71±0.1	3.95±0.10	0.39±0.10	0.42±0.10	0.36±0.10	0.39±0.10	0.47±0.10	0.64±0.10
F-value	21.883	46.207	15.382	26.710	33.182	21.033	42.039	52.337	19.410
p-value	0.000	0.000	0.001	0.000	0.000	0.000	0.002	0.000	0.000

Values are represented as mean ± SEM (n = 6). *P* value less than 0.05 ($p<0.05$) indicates significant change, by one-way Duncan's multiple range ANOVA. * Weight of 2 cm from the distal part of the anus.

Table 8. Organ weights of rats administered *Annona muricata* root-bark extract

Groups	Brain	Lungs	Liver	Pancreas	Right kidney	Left kidney	Spleen	Heart	Colon*
0 mg/kg	1.11±0.00	0.90±0.07	3.57±0.40	0.23±0.10	0.65±0.10	0.45±0.06	0.48±0.04	0.41±0.10	0.64±0.20
100 mg/kg	1.00±0.00	0.70±0.04	3.47±0.10	0.30±0.10	0.43±0.04	0.34±0.30	0.35±0.03	0.41±0.10	0.47±0.10
200 mg/kg	0.80±0.00	1.00±0.00	3.51±0.20	0.48±0.10	0.48±0.20	0.37±0.02	0.39±0.20	0.43±0.10	0.54±0.10
400 mg/kg	0.70±0.10	0.87±0.02	4.02±0.20	0.30±0.05	0.35±0.05	0.43±0.10	0.38±0.20	0.42±0.02	0.45±0.10
600 mg/kg	0.90±0.10	0.83±0.20	3.46±0.20	0.39±0.10	0.42±0.10	0.40±0.02	0.40±0.20	0.41±0.04	0.57±0.10
800 mg/kg	0.80±0.00	0.80±0.05	3.91±0.20	0.35±0.10	0.32±0.30	0.34±0.01	0.39±0.20	0.35±0.60	0.52±0.10
F-value	10.863	13.274	16.526	19.208	24.921	19.663	14.529	10.113	22.108
p-value	0.024	0.011	0.001	0.000	0.000	0.000	0.000	0.029	0.000

Values are represented as mean ± SEM (n = 6). *P* value less than 0.05 ($p<0.05$) indicates significant change, by one-way Duncan's multiple range ANOVA. * Weight of 2 cm from the distal part of the anus.

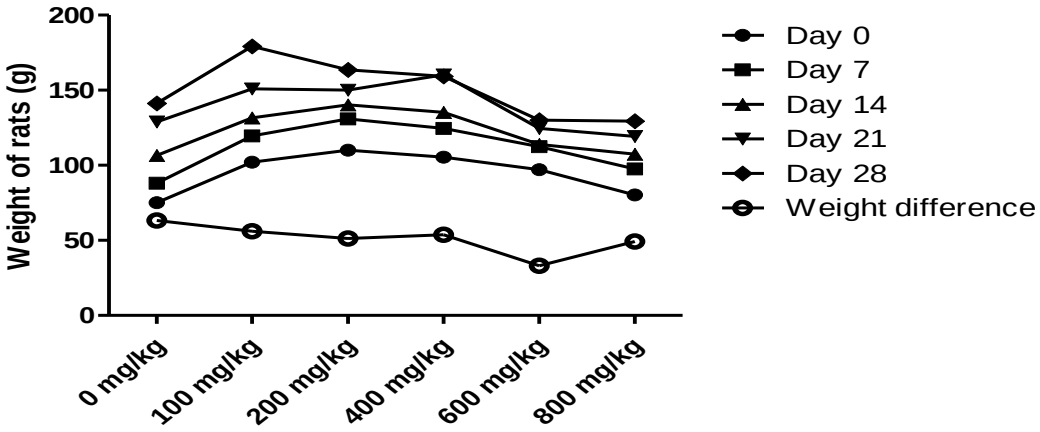

Figure 6. Weights of rats administered *Annona muricata* fruit pulp extract

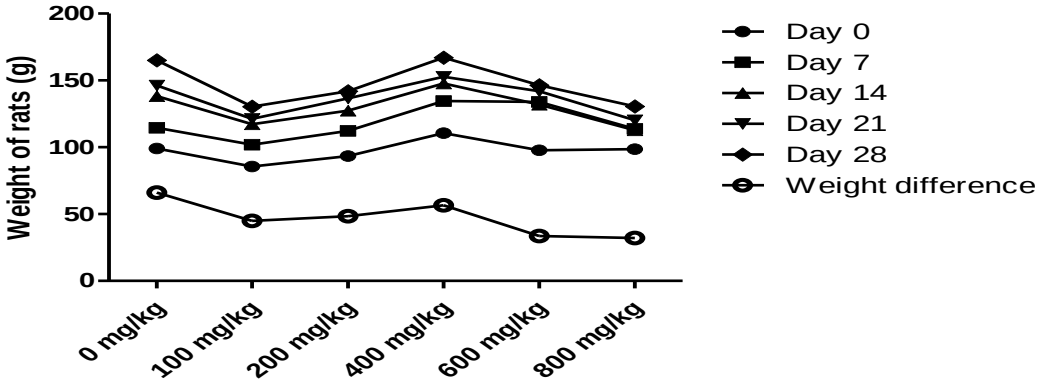

Figure 7. Weights of rats administered *Annona muricata* leaf pulp extract

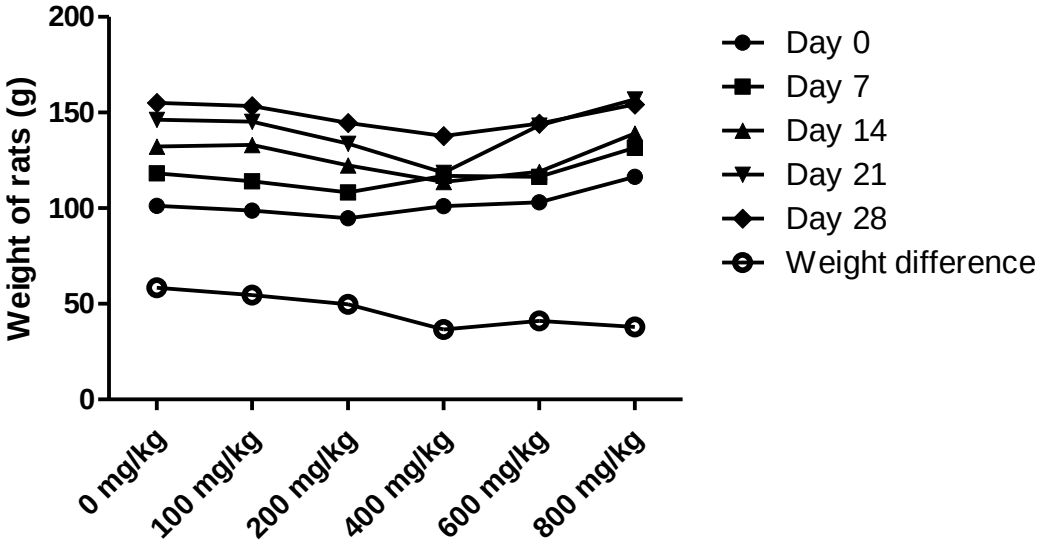

Figure 8. **Weights of rats administered *Annona muricata* stem-bark pulp extract**

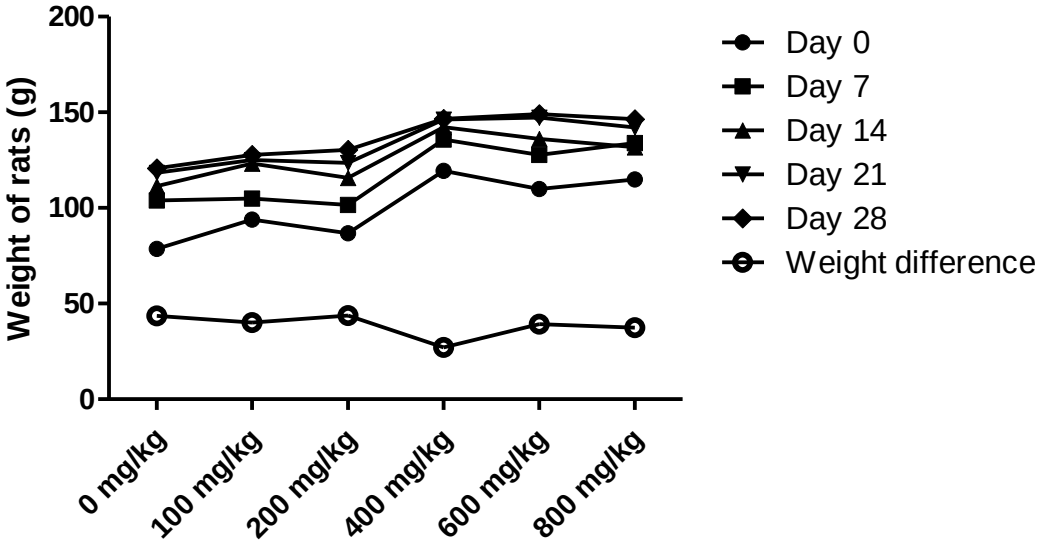

Figure 9. Weights of rats administered *Annona muricata* root-bark pulp extract

Discussion

It has been established that atherosclerosis is a serious complication produced by dyslipidemia which eventually causes coronary heart disease. Studies have shown that the number of hyperlipidemic patients is continuously increasing. Life style, especially high fat diet, is a predominant risk factor for in hyperlipidemia. Numerous studies have indicated that diet regulation and drug therapy to control blood cholesterol reduce coronary heart disease-associated morbidity and mortality [29]

Disorders of lipid metabolism are the primary risk factor for cardiovascular disease. The known lipid-lowering drugs (statins, fibrates and bile acid sequestrates) regulate the lipid metabolism through different mechanisms but also have many side effects in patients [30]

Essential oils has been demonstrated to prevent dyslipidemia; triterpenes prevents LDL oxidation [30], camphene which is a monoterpene and a product of the isoprenoid biosynthetic pathway possesses a lipid-lowering effect through the reactivation of lipolysis enzymes for early clearance of lipids (plasma total

cholesterol, LDL-cholesterol and triglyceride) levels from circulation [30], phenylpropanoids suppresses liver lipid synthesis [28].

According to Jun et al. [30], essential oils isolated from Melissa officinalis cause reductions in rate of fatty acid synthesis, decrease in cellular TAG and cholesterol concentrations which were attributed to decreased expression of SREBP-1c and its responsive genes in fatty acid synthesis, including FAS, SCD1, and ACC1. These essential oils induced bile acid synthesis in HepG2 cells and reduced the nuclear form of SREBP-2, a key transcription factor in hepatic cholesterol synthesis, suggesting that intake of fruit pulps with phytochemicals possessing pleasant scent, like Annona muricata could have beneficial metabolic effects, especially on lipid status. Kossouoh et al. [3], Fekam et al. [31] and Soheil et al. [32] have reported that Annona muricata is very rich in essential oils (volatile fatty acids) attributing for its fruit pulpy scents and soured taste as well.

Studies of the influence of the various extracts on the basal lipid profile of the rats; plasma total cholesterol (TCHL), plasma triacylglycerol (TAG), plasma high density lipoprotein (HDL), plasma low density lipoprotein (LDL) and plasma very low density lipoprotein (VLDL), were carried out and the data obtained were used to determine the atherogenic indices of the groups. Based on the observed patterns of the atherogenic indices, the group treated with fruit pulp extract demonstrated a better anti-atherogenic and hypolipidemic potentials compared to the other extracts as represented in figure 1 and table 1; the TCHL: HDL and LDL: HDL ratios showed a progressive down-ward trend (5.41 to 4.31 and 1.71 to 0.79, respectively) with increasing administered doses of the fruit pulp extract, compared to the control. There was however, a significant correlation between TCHL: HDL and LDL: HDL ratios, i.e., r2 = 0.892, p=0.017. In our previous research [4, 23], the concentrations of alkaloids, phenols and flavonoids were determined in the various fractions of Annona muricata methanol extracts using petroleum–ether, trichloromethane, ethyl acetate, methanol, and methanol–water (90: 10), in the order of increasing polarity. We observed high content flavonoids in the fruit pulp chloroform and fruit pulp methanol-water fractions; significant correlations were observed, i.e. TCHL: HDL versus fruit pulp chloroform fraction (r2 = 0.582, p=0.021) and fruit pulp methanol-water (r2 = 0.670, p=0.008) fraction; while LDL: HDL versus fruit pulp chloroform fraction (r2 = 0.853, p=0.037) and fruit pulp methanol-water (r2 = 0.890, p=0.015) fraction.

In the present study, the leaf extract did not show a good potential to significantly reduce the basal plasma lipid status of the rats used for the studies, when compared to the group administered the fruit pulp extract, although there was significant correlation between TCHL: HDL and LDL: HDL (r2 = 0.865, p =0.026).

Agu et al. [4] and Agu [23] reported the highest alkaloid content in the leaf methanolic fraction (correlation with TCHL: HDL was r2 = 0.594, p =0.024 and LDL: HDL r2 = 0.610, p =0.001) and leaf petroleum ether fraction (correlation with TCHL: HDL was r2 = 0.890, p =0.011 and LDL: HDL r2 = 0.859, p =0.005). Usunomena and Okolie [33], Agbai et al. [34], Adewole et al. [22] and Adeyemi et al. [35] had previously reported the hypolipidemic and anti-atherogenic properties of Annona muricata leaf, though on pathologically challenged animals.

Similar observation was observed for the group treated with root-bark extract (table 4 and figure 4), as LDL: HDL was increased markedly and TCHL: HDL gave unstable pattern (1.84 to 2.40, and 7.39 to 6.23, respectively for group 6 compared to the control group); with increasing concentration of extract, LDL: HDL demonstrated an increasing trend in contrast to TCHL: HDL. This observation was confirmed by the Pearson's correlation, r2 = 0.681 (p=0.137) indicating a possible absence of hypocholesterolemic mechanisms observed for the fruit pulp and leaf, though Agu (2016) and Agu et al. (2017a) reported the highest content of total phenol in the root-bark methanol fraction (correlation with TCHL: HDL was r2 = 0.673, p=0.048, and LDL: HDL was r2 = 0.513, p=0.247). The group administered the stem-bark extract was next to the fruit pulp group in terms of hypolipidemic and anti-atherogenic potential (TCHL: HDL reduced from 7.91 in control to 5.56 in group 6 after an initial increase to 7.13 in group 4, while LDL: HDL reduced from 3.05 in control to 1.48 in group 6, after an initial increase to 2.02 in group 4).

TCHL: HDL demonstrated a significant correlation with LDL: HDL, i.e. r2 = 0.897 (p=0.015). Agu et al. [4] and Agu [23] had reported a high content of total phenol in the methanol Stem-bark fraction of Annona muricata; correlations of r2 = 0.739 (p=0.024) and r2 = 0.801 (p=0.006).

Serum fatty acid synthase was progressively reduced with administered Annona muricata extracts; the fruit pulp and leaf showed superior significant influences on the lipogenic enzyme. Fatty acid synthase (FAS) is the sole enzyme of de novo fatty acid synthesis, and is highly expressed in most human cancers; its inhibition is selectively cytotoxic to human cancer cells [36, 37], suggesting the selective influence and specificity of Annona muricata on this enzyme [20, 21].

Conclusion

The fruit pulp and stem-bark extracts treated groups demonstrated better potential as anti-lipidemic, anti-cholesterolemic and FAS inhibiting agents, compared to the leaf and root-bark extracts treated groups. This could be attributed to the high content of essential oils as reported in the literature [3, 4, 13, 23, and 32].

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