Phytochemical Profile of Annona Muricata (Soursop) and Dose-Dependent Biochemical, Haematological, Antioxidant, Inflammatory and Reproductive-Hormonal Effects of Its Leaf Phenolic Extract in Female Wistar Rats

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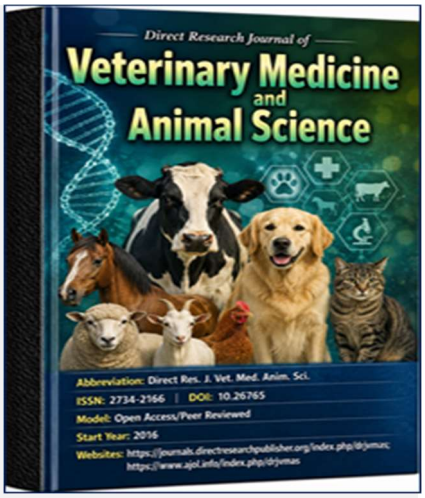
ABSTRACT

Background: Annona muricata L. (soursop) leaves are widely used in ethnomedicine, yet integrated safety data on repeated oral exposure to the leaf phenolic extract across several organ systems remain scarce. Aim and objectives: This study aimed to characterise the phytochemical, sugar, amino acid, vitamin and fatty-acid composition of A. muricata leaves and to determine the dose-dependent effects of its leaf phenolic extract on hepatic, renal, lipid, haematological, antioxidant, inflammatory and reproductive-hormonal parameters in female Wistar rats. Methods: Composition was profiled by high-performance liquid chromatography (HPLC) and gas chromatography (GC). Twenty female Wistar rats were randomly assigned to four groups (n = 5): distilled-water control and the extract at 100, 300 and 500 mg/kg body weight by oral gavage daily for 14 days. Serum biochemistry, leucocyte indices, antioxidant indices, cytokines and hormones were measured and compared by one-way ANOVA with post-hoc testing ($p < 0.05$). Results: Chromatography tentatively identified acetogenins, alkaloids, flavonoids, phenolic acids, phytosterols, sugars, amino acids, vitamins and saturated and unsaturated fatty acids; only gallic acid, ascorbic acid, glucose, mannose and palmitic acid were quantified. At 100 mg/kg, only CUPRAC differed significantly from control. At 300 mg/kg, AST, ALT, cholesterol, triglycerides, monocyte percentage, FRAP, TEAC, GPx, SOD, FSH and estrogen were significantly altered. At 500 mg/kg, AST (103.12 → 138.35 IU/L), ALT, BUN, creatinine and uric acid rose, whereas ALP, total protein, albumin, globulin, cholesterol and triglycerides fell; all seven antioxidant indices, IL-1β, IL-6, TNF-α, neutrophil percentage, FSH, LH and estrogen increased, while lymphocyte and monocyte percentages and IL-10 decreased. HDL and LDL were unchanged. Implications: The extract shows no significant hepatic or renal change at 100 mg/kg but dose-related hepatic, lipid, redox and endocrine alterations from 300 mg/kg and hepatorenal, immunological and hormonal stress at 500 mg/kg. These findings caution against high-dose use; histopathology and longer studies are needed before any safe human dose is proposed.

***Keywords**: Annona muricata, phenolic extract, HPLC, GC, subacute toxicity, female Wistar rats, oxidative stress, cytokines*



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INTRODUCTION

Natural products derived from medicinal plants remain a major source of therapeutic agents and continue to attract considerable scientific interest because of their broad pharmacological potential and comparatively low cost relative to synthetic drugs (Newman and Cragg, 2020). Annona muricata L. (Annonaceae), widely known as soursop or graviola, is a small evergreen tree cultivated throughout the tropics for its edible fruit and extensively used in ethnomedicine for the management of diabetes,

hypertension, inflammation, microbial infections, malaria and cancer (Badrie and Schauss, 2010; Moghadamtousi et al., 2015; Coria-Téllez et al., 2018). Phytochemical investigations have shown that virtually every part of the plant, particularly the leaves, is rich in acetogenins, alkaloids, flavonoids, phenolic acids and other secondary metabolites believed to underlie its broad spectrum of bioactivity, including antioxidant, anti-inflammatory, antimalarial, antidiabetic and anticancer properties (Moghadamtousi et al., 2015; Wahab et al., 2018; Abdussalam-Mohammed and Darwish, 2020; Ugbogu et al., 2026). A growing body of chromatographic and metabolomic work has continued to expand the known chemical inventory of the plant and to refine understanding of its structure–activity relationships (Wolfender et al., 2019).

Despite this widespread ethnomedicinal use, and the growing tendency of the general population, including pregnant women, to self-medicate with herbal preparations, comprehensive data on the safety profile of A. muricata leaf phenolic extract across multiple organ systems remain scarce. In most jurisdictions, including Nigeria, herbal products are not subjected to the same regulatory scrutiny as conventional pharmaceuticals before they reach end users, and the paucity of pre-registration toxicological data is therefore of particular public-health concern. Existing reports have largely evaluated isolated endpoints, such as acute lethality, hepatoprotection against a specific chemical insult, or antioxidant activity in vitro (Adewole and Ojewole, 2009; Arthur et al., 2011; Usunobun, 2014), so that the composite hepatic, renal, haematological, antioxidant, inflammatory and reproductive-hormonal picture of repeated oral exposure has, to our knowledge, rarely been reported within a single integrated study.

Recent toxicological work has produced mixed signals. A single 2000 mg/kg dose of A. muricata leaf ethanol extract produced no abnormal biochemical toxicity in Sprague Dawley rats, although mild histological changes were seen in the kidney and liver (Zubaidi et al., 2023), whereas a 42-day sub-chronic study of an aqueous fruit extract in Wistar rats reported lower serum triglycerides and liver enzymes (Agbabiaka et al., 2024). A recent review of the botany, phytochemistry, clinical trials and toxicity profile of the species stresses the need for dose-defined repeated-dose data (Vieira et al., 2025; Ugbogu et al., 2026), and the neurotoxic potential of annonaceous acetogenins such as annonacin remains a concern for prolonged consumption (Champy et al., 2004; Chan et al., 2020). Because the chemistry and toxicity of the extract depend on plant part, solvent and dose, a dose–response study in a single integrated model is needed.

The aim of this study was to characterise the phytochemical composition of A. muricata and to evaluate the dose-dependent effects of its leaf phenolic extract in female Wistar rats. The specific objectives were to: (i) profile the phytochemical, sugar, amino acid, vitamin and fatty-acid composition of A. muricata [state plant part(s)] by HPLC and GC; (ii) determine the effects of 100, 300 and 500 mg/kg body weight of the extract, given orally for 14 days, on liver enzymes, renal indices, serum proteins and lipid profile; (iii) assess its effects on leucocyte indices and serum antioxidant biomarkers; (iv) measure its effects on pro- and anti-inflammatory cytokines and reproductive hormones; and (v) identify the lowest dose at which significant changes occur. It was hypothesised that the extract would alter these endpoints in a dose-dependent manner.

MATERIALS AND METHODS

This was a quantitative, laboratory-based, controlled experimental (post-test, four parallel groups) study combined with analytical phytochemistry. A repeated-dose design was chosen to detect dose-related effects on several organ-system markers; the 14-day duration and the doses of 100, 300 and 500 mg/kg were selected. Independent variable: extract dose; dependent variables: serum biochemical, haematological, antioxidant, cytokine and hormone measures.

Chemicals and reagents

Absolute methanol, ethanol, chloroform, n-hexane, sulphuric acid, ferric chloride solution, Mayer's reagent, Dragendorff's reagent, Folin–Ciocalteu reagent, gallic acid standard, Ellman's reagent, reduced glutathione (GSH) (BOC Sciences, Shirley, NY 11967, USA; AH2026/03/12-23) , and commercial diagnostic/ELISA kits for liver enzymes, renal indices, lipid profile, antioxidant biomarkers, cytokines and reproductive hormones were of analytical grade and were obtained from RayBiotech Life, Inc.,GA, USA; RB26/5/12125A.

Experimental site

The study was carried out at the Laboratory Animals Unit and the Animal Health Technology Laboratory of the Teaching and Research Farm, Oyo State College of Agriculture and Technology, Igboora, Oyo State, Nigeria (7°15′ N, 3°30′ E). Animals were housed at room temperature, low humidity, 12 h light/dark cycle, cage density of 5 rats, feed ad libitum with compound feed. HPLC/GC and ELISA analyses were performed at Nigeria Institute of Medical Research, Lagos, Nigeria.

Ethical consideration

The study protocol was approved by Oyo State College of Agriculture and Technology Institutional Animal Care and Use Committee/Research Ethics Committee with approval number; OYSCATECH/IBR/2025/009. All procedures complied with the ARRIVE 2.0 guidelines and national/institutional rules on the care and use of

laboratory animals. Blood was collected by cardiac puncture under ketamine injection at 2mg/kg, followed by cervical dislocation to euthanizing the rat.

Plant collection and identification

Fresh, apparently healthy leaves, mature fruit pulp and seeds of A. muricata were collected from farms within Ibarapa Local Government Area of Oyo State, Nigeria. The plant material was identified at the Department of Animal Health Technology, Oyo State College of Agriculture and Technology, Igboora (7°15′ N, 3°30′ E). The plant was authenticated by a taxonomist at Department of Forestry Technology, Oyo State College of Agriculture and Technology, Igboora and a voucher specimen (O/IBR/2025/009) was deposited.

Preparation of the phenolic leaf extract

The leaves were washed free of dust and debris, air-dried under shade to a constant weight, and pulverised into fine powder using a mechanical grinder. 500 g of the powder was macerated in 1000 mL] absolute methanol for 48h] at 37 °C with constant rotary agitation, filtered, and the residue was re-extracted with 80% aqueous methanol in two further rounds of cold maceration. The pooled filtrates were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at temperature], then lyophilised to obtain the phenolic extract of A. muricata leaf yielded 20% w/w. The extract was stored at 4 °C until use and was dissolved in explon for gavage at 100 mL/kg.

Determination of total phenolic content

Total phenolic content of the extract was estimated by the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965) and expressed as mg gallic acid equivalent (GAE) per 100 g of extract. Total flavonoid content was determined by HPLC. Results are reported in the Results section.

Qualitative phytochemical screening

Standard qualitative tests were used to screen the extract for alkaloids (Mayer's and Dragendorff's reagents), flavonoids (sodium hydroxide test), phenolic compounds (ferric chloride test), tannins (gelatin test), and saponins, steroids/triterpenes and carbohydrates using established protocols (Harborne, 1998).

Chromatographic profiling

The phytochemical, sugar, amino acid, vitamin and fatty-acid composition of A. muricata leaves and macerated was characterised using gas chromatography (GC-MS) for volatile and semi-volatile constituents, including fatty acids (Alali et al., 1999), and high-performance liquid chromatography (HPLC) for non-volatile phenolics, sugars, amino acids and vitamins (George et al., 2015), with thin-layer chromatography (TLC) as a preliminary screen (Marston, 2011; Azman et al., 2022). Compounds were tentatively identified by comparison of retention times, peak areas and, where applicable, mass spectra with reference libraries and authentic standards. Because acetogenins are large and thermolabile.

Experimental animals and design

Twenty female Wistar rats weighing between 120-130g obtained from College Laboratory Animals Farm were acclimatised for one week under standard laboratory conditions with free access to feed and water. Females were used to determine the reproductive hormone. Animals were randomly allocated into four groups of five rats: Group 1 (control) received distilled water only; Groups 2, 3 and 4 received the A. muricata leaf phenolic extract orally by gavage at 100, 300 and 500 mg/kg body weight, respectively, once daily for 14 consecutive days.

Sample collection

Throughout the 14-day dosing period, animals were observed daily for clinical signs of toxicity and mortality, and body weight was recorded every two days. At the end of the treatment period, animals were humanely sacrificed and blood samples collected by cardiac puncture into plain and EDTA sample bottles for serum and whole-blood analyses, respectively. Serum was separated by centrifugation and stored at −20 °C pending analysis.

Determination of liver function biomarkers, renal indices and lipid profile

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by the Reitman–Frankel colorimetric method, and alkaline phosphatase (ALP) by the King–Armstrong method (Reitman and Frankel, 1957 for AST and ALT; King and Armstrong, 1934 for ALP). Total protein was determined by the Biuret method and albumin by the bromocresol green method, with globulin obtained by difference. Blood urea nitrogen (BUN), serum creatinine and uric acid were determined using standard enzymatic-colorimetric diagnostic kits, while total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides were determined enzymatically using commercial diagnostic kits.

Haematological analysis

Total white blood cell (WBC) count, differential leucocyte counts (neutrophils, lymphocytes, monocytes and eosinophils) and platelet counts were determined from

EDTA whole blood using an automated haematologyanalyser, with confirmation by manual microscopy of Leishman-stained blood films.

Determination of serum antioxidant biomarkers

Serum cupric ion-reducing antioxidant capacity (CUPRAC), ferric-reducing antioxidant power (FRAP), Trolox-equivalent antioxidant capacity (TEAC), paraoxonase-1 (PON-1) activity, glutathione peroxidase (GPx) activity, superoxide dismutase (SOD) activity and catalase (CAT) activity were determined using established spectrophotometric assay methods.

Determination of serum inflammatory cytokines

Serum concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumour necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions.

Determination of serum reproductive hormones

Serum follicle-stimulating hormone (FSH), prolactin (PROL), progesterone (PROG), estrogen (ESTRO) and luteinizing hormone (LH) were quantified by ELISA following the manufacturers' protocols.

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM), n = 5 animals per group. Normality (Shapiro–Wilk) and homogeneity of variance (Levene) were assessed. Group differences were analysed by one-way ANOVA followed by [Dunnett's test (vs control) or Tukey's test, recommended in place of unadjusted LSD]; Welch ANOVA or the Kruskal–Wallis test was used when assumptions were violated, and a linear-trend test was used to assess dose-dependence. Exact p values and effect sizes should be reported; because many endpoints were examined, p-values were adjusted for interpreted as exploratory. Analyses were performed with GraphPad Prism 10 version. Values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Phytochemical, nutrient and fatty-acid composition of A. muricata

Chromatographic profiling revealed that A. muricata possesses a chemically diverse composition (Tables 1–5). HPLC analysis of the phytochemical profile (Table 1) detected carotenoids, acetogenins, alkaloids, phenolic acids, flavonoids, phytosterols, isoflavones and benzylisoquinoline alkaloids, including beta-carotene, annonacin, anonaine, gallic acid, ascorbic acid, catechin, epicatechin, beta-sitosterol, daidzein, genistein, quercetin, kaempferol, rutin, apigenin, reticuline, atherospermine, coreximine, annomuricin, ferulic acid, vitexin, emodin, swainsonine, casuarine and liriodenine. Gallic acid (19.72 ppm) and ascorbic acid (7.28 ppm) were the only constituents for which quantitative external-unit values were obtained, while quercetin recorded the highest chromatographic peak area (5811.64), followed by kaempferol (713.83), annonacin (473.63), anonaine (434.07) and rutin (284.32). This diverse acetogenin–alkaloid–flavonoid profile is consistent with previous reports on A. muricata leaf (Leboeuf et al., 1982; Moghadamtousi et al., 2015; Gavamukulya et al., 2014) and may partly explain the pharmacological effects observed in the treated animals, although the administered extract itself was not profiled and peak areas are not concentrations. Comparable HPLC-based quantification of flavonoids and phenolic acids has been reported for ethanolic and aqueous soursop leaf extracts (George et al., 2015), while flavonoid aglycones such as quercetin and kaempferol are widely recognised for their antioxidant and enzyme-modulatory activity across plant species (Panche et al., 2016).The sugar profile (Table 2) revealed eight constituents' glyceraldehyde, glucose, mannose, fructose, xylose, arabinose, rhamnose and galactose with glucose (85.03 ppm) and mannose (17.26 ppm) quantitatively determined. The amino acid profile (Table 3) revealed seventeen amino acids, including both essential (valine, threonine, isoleucine, leucine, phenylalanine, methionine, histidine, tryptophan) and non-essential (glycine, alanine, serine, proline, glutamic acid, aspartic acid, arginine, tyrosine, cysteine) residues (lysine was not detected), with glycine and histidine recording the highest peak areas. The vitamin profile (Table 4) tentatively identified nine vitamins (detection of vitamin B12 in a plant requires confirmation) spanning water- and fat-soluble classes (vitamins B1, B2, B3, B6, B12, C, A, E and K), while the fatty-acid profile obtained by GC (Table 5) identified twelve saturated and unsaturated fatty acids, with linoleic and oleic acids as the predominant unsaturated constituents and palmitic acid (330.59 ppm) as the only quantitatively determined fatty acid, a distribution broadly comparable with that reported for soursop seed oil (Elagbar et al., 2016, Jawonisi et al., 2026). Collectively, this nutrient-rich, polyphenol-dense composition in which tannins, saponins and other minor secondary metabolites have also been documented in related phytochemical surveys of the species (Mueller-Harvey, 2006; Baskar et al., 2007; Gessner et al., 2017) may contribute to both the reported therapeutic potential and the dose-dependent physiological effects of A. muricata leaf extract described.

Effect on liver function biomarkers

Repeated administration of the phenolic extract produced

Table 1: Phytochemical profile of Annona muricata (soursop) determined by HPLC.

Component	Retention time	Area	Height	External units
Beta-carotene	0.316	86.8640	14.655	0.0000
Annonacin	1.266	473.6330	22.797	0.0000
Anonaine	2.750	434.0705	9.398	0.0000
Gallic acid	4.450	197.1980	4.310	19.7198 ppm
Ascorbic acid	5.466	72.8245	3.648	7.2824 ppm
Catechin	6.483	162.2395	8.421	0.0000
Epicatechin	7.333	75.4030	7.671	0.0000
Beta-sitosterol	7.950	92.5435	5.225	0.0000
Daidzein	8.783	95.6260	7.584	0.0000
Genistein	9.533	77.9850	9.269	0.0000
Quercetin	11.050	5811.6360	126.744	0.0000
Kaempferol	12.166	713.8265	24.852	0.0000
Rutin	13.700	284.3240	9.644	0.0000
Apigenin	15.233	245.5730	11.194	0.0000
Reticuline	16.250	85.2605	3.815	0.0000
Atherospermine	17.616	256.0670	6.669	0.0000
Coreximine	19.416	115.0640	6.312	0.0000
Annomuricin	20.766	83.3550	6.217	0.0000
Ferulic acid	21.566	60.0270	11.597	0.0000
Vitexin	22.766	179.6870	12.877	0.0000
Emodin	24.016	76.4155	11.096	0.0000
Swainsonine	24.850	60.0230	9.346	0.0000
Casuarine	25.683	102.0710	15.111	0.0000
Liriodenine	26.783	62.4090	10.289	0.0000

Values represent chromatographic detector response; external units are reported only where quantified against a reference standard.

Table 2: Sugar profile of Annona muricata (soursop) determined by HPLC.

Component	Retention time	Area	Height	External units
Glyceraldehyde	2.016	72.1750	4.953	0.0000
Glucose	3.700	780.2615	36.224	85.0327 ppm
Mannose	5.883	172.6115	7.368	17.2612 ppm
Fructose	7.966	218.0285	9.401	0.0000
Xylose	15.500	239.7985	7.384	0.0000
Arabinose	17.233	537.5750	15.641	0.0000
Rhamnose	20.500	116.6740	6.812	0.0000
Galactose	23.866	103.7470	5.971	0.0000

Table 3: Amino acid profile of Annona muricata (soursop) determined by HPLC.

Component	Retention time	Area	Height	External units
Glycine	3.700	2483.6360	51.692	0.0000
Alanine	5.883	638.5950	11.905	0.0000
Serine	7.966	766.6820	15.791	0.0000
Proline	9.116	79.0650	4.317	0.0000
Valine	9.950	61.3690	3.726	0.0000
Threonine	10.500	113.2400	3.356	0.0000
Cysteine	11.300	66.3180	3.036	0.0000
Isoleucine	11.850	97.0710	3.200	0.0000
Leucine	12.333	52.8440	2.775	0.0000
Aspartic acid	12.816	50.9550	2.923	0.0000
Glutamic acid	15.500	589.0080	12.079	0.0000
Histidine	17.233	2185.9035	34.139	0.0000
Phenylalanine	19.400	67.9570	1.324	0.0000
Methionine	20.500	56.2040	1.274	0.0000
Arginine	21.066	56.1150	2.884	0.0000
Tyrosine	21.983	69.4570	4.682	0.0000
Tryptophan	23.083	95.0080	4.888	0.0000

Table 4: Vitamin profile of Annona muricata (soursop) determined by HPLC.

Component	Retention time	Area	Height	External units
Vitamin B3 (niacin)	1.116	195.5690	20.431	0.0000
Vitamin B6 (pyridoxine)	2.716	40.3240	3.099	0.0000
Vitamin C (ascorbic acid)	4.250	100.1665	6.910	0.0000
Vitamin B1 (thiamine)	6.250	52.4320	3.042	0.0000
Vitamin A (beta-carotene)	7.366	63.6220	2.364	0.0000
Vitamin B2 (riboflavin)	8.800	113.5430	2.524	0.0000
Vitamin E (tocopherol)	10.366	59.6850	3.836	0.0000
Vitamin K (phylloquinone)	13.600	35.7890	2.937	0.0000
Vitamin B12 (cobalamin)	16.366	49.8440	2.273	0.0000

Table 5: Fatty acid profile of Annona muricata (soursop) determined by GC.

Component	Retention time	Area	Height	External units
Caproic acid (C6:0)	3.300	161.3740	10.716	0.0000
Caprylic acid (C8:0)	4.033	113.5575	10.189	0.0000
Capric acid (C10:0)	5.016	3730.4660	428.479	0.0000
Lauric acid (C12:0)	6.450	204.5580	16.666	0.0000
Myristic acid (C14:0)	7.150	919.4540	112.889	0.0000
Palmitic acid (C16:0)	7.533	2744.7565	168.099	330.5946 ppm
Stearic acid (C18:0)	7.816	1989.2175	232.966	0.0000
Oleic acid (C18:1)	8.600	2907.6740	362.983	0.0000
Linoleic acid (C18:2)	9.200	15299.7240	1702.296	0.0000
Arachidic acid (C20:0)	10.200	203.8050	8.880	0.0000
Lignoceric acid (C24:0)	10.550	130.7630	5.897	0.0000
Behenic acid (C22:0)	11.483	480.4960	51.482	0.0000

Table 6: Effect of Annona muricata leaf phenolic extract on serum liver enzymes of Wistar rats (Mean ± SEM).

Parameter	Control	Treatment A (100 mg/kg)	Treatment B (300 mg/kg)	Treatment C (500 mg/kg)
AST (IU/L)	103.12 ± 3.85	104.45 ± 4.21	119.30 ± 6.54[a]	138.35 ± 6.93[ab]
ALT (IU/L)	54.34 ± 1.52	56.42 ± 2.32	68.34 ± 4.22[a]	82.62 ± 2.38[ab]
ALP (IU/L)	164.41 ± 4.23	168.60 ± 2.74	170.94 ± 2.62	143.85 ± 2.28[a]

Values with superscript [a] differ significantly from control and [b] differ significantly from Treatment A ($p < 0.05$).

a clear, dose-dependent rise in the transaminases (Table 6). Serum AST increased from 103.12 ± 3.85 IU/L in the control group to 138.35 ± 6.93 IU/L ($p < 0.05$) at 500 mg/kg, while ALT rose from 54.34 ± 1.52 IU/L to 82.62 ± 2.38 IU/L over the same dose range. At 300 mg/kg, AST (119.30 ± 6.54 IU/L) and ALT (68.34 ± 4.22 IU/L) were already significantly higher than control ($p < 0.05$). In contrast, ALP declined progressively from 164.41 ± 4.23 IU/L in the control to 143.85 ± 2.28 IU/L ($p < 0.05$) at the highest dose.AST and ALT are cytoplasmic enzymes released into circulation following hepatocellular injury, so their significant elevation at 500 mg/kg suggests that repeated exposure to the extract at the highest dose compromised hepatocyte membrane integrity, whereas 100 mg/kg was comparable to control and 300 mg/kg produced an intermediate, significant rise, indicating a graded dose–response rather than a threshold effect (hepatotoxicity was not confirmed histologically). This pattern is consistent with reports that A. muricata leaf preparations, although hepatoprotective at low to moderate doses in models of chemically induced liver injury (Adewole and Ojewole, 2009; Arthur et al., 2012; Usunobun, 2014), can themselves precipitate hepatic stress when administered at high concentrations, most likely because of the cumulative pro-oxidant action of alkaloids and acetogenins at doses higher than those used traditionally (Chan et al., 2020). Evaluations of acute and sub-chronic toxicity of soursop leaf extracts have similarly reported a dose-dependent transition from a protective to a mildly hepatotoxic profile once threshold doses are exceeded (Arthur et al., 2011). The concurrent decline in ALP, an enzyme more closely linked to biliary and bone turnover, may reflect reduced hepatobiliary synthetic activity rather than cholestasis, although this warrants confirmation by histopathology. Notably, all animals survived to the end of the study.

Effect on renal function and lipid profile

Renal indices likewise showed a dose-dependent rise (Table 7). BUN increased from 6.60 ± 0.78 mmol/L in the control to 9.74 ± 1.12 mmol/L ($p < 0.05$) at 500 mg/kg, while uric acid rose from 0.48 ± 0.020 to 0.72 ± 0.056 mmol/L ($p < 0.05$) over the same range (Table 7). Serum creatinine followed a similar trajectory (Table 7), climbing from 0.46 ± 0.03 mg/dL in control to 0.706 ± 0.03 mg/dL ($p < 0.05$) at the highest dose. Total protein, albumin and globulin all fell significantly at 500 mg/kg (Table 7), from 58.37 ± 1.43, 16.41 ± 2.05 and 49.66 ± 3.24 g/L in control to 42.75 ± 2.15, 12.35 ± 1.46 and 32.05 ± 2.54 g/L, respectively ($p < 0.05$). Total cholesterol and triglycerides decreased markedly from 300 mg/kg onward (no change

Table 7: Effect of Annona muricata leaf phenolic extract on renal indices and lipid profile of Wistar rats (Mean ± SEM).

Parameter	Control	Treatment A (100 mg/kg)	Treatment B (300 mg/kg)	Treatment C (500 mg/kg)
BUN (mmol/L)	6.60 ± 0.78	6.52 ± 0.94	6.80 ± 1.04	9.74 ± 1.12[a]
Uric acid (mmol/L)	0.48 ± 0.020	0.47 ± 0.034	0.46 ± 0.031	0.72 ± 0.056[a]
Creatinine (mg/dL)	0.46 ± 0.03	0.42 ± 0.02	0.426 ± 0.02	0.706 ± 0.03[a]
Total protein (g/L)	58.37 ± 1.43	59.42 ± 1.84	59.02 ± 1.16	42.75 ± 2.15[a]
Albumin (g/L)	16.41 ± 2.05	16.44 ± 1.33	16.24 ± 2.34	12.35 ± 1.46[a]
Globulin (g/L)	49.66 ± 3.24	50.16 ± 2.42	49.86 ± 3.12	32.05 ± 2.54[a]
Total cholesterol (g/dL)	1.96 ± 0.02	1.92 ± 0.04	0.76 ± 0.01[a]	0.79 ± 0.02[ab]
HDL (mg/dL)	49.4 ± 0.43	50.6 ± 0.36	50.3 ± 0.34	50.7 ± 0.56
LDL (mg/dL)	51.2 ± 0.5	53.0 ± 0.4	54.6 ± 0.76	54.0 ± 0.52
Triglyceride (mg/dL)	61.37 ± 0.38	62.0 ± 0.40	48.21 ± 0.56[a]	43.42 ± 0.47[ab]

Values with superscript [a] differ significantly from control and [b] differ significantly from Treatment A ($p < 0.05$).

Table 8: Effect of Annona muricata leaf phenolic extract on leucocytic indices of Wistar rats (Mean ± SEM).

Parameter	Control	Treatment A (100 mg/kg)	Treatment B (300 mg/kg)	Treatment C (500 mg/kg)
WBC (×10³/mm³)	6.72 ± 0.43	6.32 ± 0.12	6.04 ± 0.65	7.10 ± 1.01
Neutrophils (%)	28.61 ± 2.67	29.63 ± 1.27	30.14 ± 1.09	50.16 ± 3.74[a]
Lymphocytes (%)	54.34 ± 2.67	54.72 ± 3.18	59.59 ± 0.65	39.09 ± 9.08[a]
Monocytes (%)	9.36 ± 3.20	8.14 ± 1.94	6.04 ± 1.42[a]	4.62 ± 3.13[a]
Eosinophils (%)	1.29 ± 0.12	3.10 ± 0.14	2.89 ± 0.11	1.61 ± 0.33
Platelets (×10⁶/mm³)	536.24 ± 8.33	568.12 ± 7.23	562.86 ± 4.04	546.28 ± 1.67

Values with superscript [a] differ significantly from control ($p < 0.05$).

at 100 mg/kg), whereas HDL and LDL were unchanged across groups. Elevated urea and creatinine are recognised markers of impaired glomerular filtration, and the concurrent hypoproteinaemia and hypoalbuminaemia observed at 500 mg/kg suggest either reduced hepatic protein synthesis or increased renal protein loss. The marked reduction in cholesterol and triglycerides at higher doses is consistent with the reported hypolipidaemic activity of A. muricata leaf polyphenols, which have been shown to modulate lipid metabolism via inhibition of HMG-CoA reductase and enhanced lipoprotein clearance, although at 500 mg/kg such a steep fall may equally reflect general metabolic stress rather than a purely beneficial hypolipidaemic action. Overall, 100 mg/kg was the highest dose without significant hepatic or renal change; hepatic enzymes, cholesterol and triglycerides were already altered at 300 mg/kg and clear renal stress emerged at 500 mg/kg. A formal no-observed-adverse-effect level cannot be assigned without histopathology, and 300 mg/kg is best regarded as a provisional lowest-observed-adverse-effect level for the affected endpoints (cf. Arthur et al., 2011).

Effect on leucocytic indices

The extract significantly altered leucocytic indices at the highest dose (Table 8). Total WBC count was highest at 500 mg/kg (7.10 ± 1.01 × 10³/mm³) relative to control (6.72 ± 0.43 × 10³/mm³). Neutrophil percentage rose sharply from 28.61 ± 2.67% in control to 50.16 ± 3.74% ($p < 0.05$) at the highest dose, while lymphocyte percentage fell reciprocally from 54.34 ± 2.67% to 39.09 ± 9.08% ($p < 0.05$). Monocyte percentage declined progressively with dose, from 9.36 ± 3.20% in control to 4.62 ± 3.13% ($p < 0.05$) at 500 mg/kg, whereas eosinophil percentage and platelet count remained comparatively stable across all groups.This leucocyte response neutrophilia with concomitant lymphopenia and monocytopenia at the highest dose indicates a shift in immune cell distribution favouring innate over adaptive immunity, and mirrors previous findings that phenolic-rich plant extracts can activate neutrophils and modulate cytokine-driven leucocyte trafficking (Chabot-Richards and George, 2014; Mayadas et al., 2014), and is broadly consistent with dose-related shifts in blood hematology previously reported for soursop-treated rats (Syahida et al., 2012). The stability of platelet and eosinophil counts across doses suggests that the extract, at least within 14 days of exposure, does not compromise thrombopoiesis or provoke an allergic-type response (McBrien and Menzies-Gow, 2017).

Effect on serum antioxidant biomarkers

All seven antioxidant biomarkers increased at 500 mg/kg (Table 9); CUPRAC rose from 100 mg/kg, FRAP, TEAC, GPx and SOD from 300 mg/kg, whereas PON-1 and CAT changed only at 500 mg/kg. CUPRAC rose from 3.06 ± 0.02 µmol/L in control to 9.14 ± 0.32 µmol/L ($p < 0.05$) at 500 mg/kg; FRAP and TEAC increased from 29.46 ± 1.23 and 38.13 ± 2.67 µmol/L to 40.45 ± 1.34 and 52.48 ± 1.64 µmol/L, respectively ($p < 0.05$); and PON-1 rose from29.76 ± 2.42 to 40.23 ± 3.42 µmol/L ($p <0.05$). The endogenous enzymatic antioxidants GPx, SOD and CAT similarly increased with dose, from 130.83 ± 1.96, 206.36 ± 9.64 and 8.32 ± 1.03 in control to 180.38 ± 1.52 ($p < 0.05$), 356.36 ± 6.58 ($p < 0.05$) and 14.6 ± 1.13 ($p < 0.05$), respectively, at the highest dose. This coordinated up-regulation of both non-enzymatic (CUPRAC, FRAP, TEAC, PON-1) and enzymatic (GPx, SOD, CAT) antioxidant capacity is consistent with the high polyphenolic and flavonoid content demonstrated in the

Table 9: Effect of Annona muricata leaf phenolic extract on serum antioxidant biomarkers of Wistar rats (Mean ± SEM, µmol/L).

Parameter	Control	Treatment A (100 mg/kg)	Treatment B (300 mg/kg)	Treatment C (500 mg/kg)
CUPRAC	3.06 ± 0.02	5.16 ± 0.08[a]	7.12 ± 0.16[ab]	9.14 ± 0.32[abc]
FRAP	29.46 ± 1.23	29.32 ± 1.22	32.84 ± 1.23[a]	40.45 ± 1.34[ab]
TEAC	38.13 ± 2.67	39.23 ± 1.38	47.26 ± 1.54[a]	52.48 ± 1.64[ab]
PON-1	29.76 ± 2.42	29.86 ± 0.78	30.42 ± 0.66	40.23 ± 3.42[a]
GPx	130.83 ± 1.96	131.62 ± 1.56	172.42 ± 1.66[a]	180.38 ± 1.52[ab]
SOD	206.36 ± 9.64	209.36 ± 8.23	288.44 ± 7.48[a]	356.36 ± 6.58[ab]
CAT	8.32 ± 1.03	8.62 ± 1.05	8.72 ± 1.07	14.6 ± 1.13[a]

CUPRAC = cupric reducing antioxidant capacity; FRAP = ferric reducing ability of plasma; TEAC = Trolox equivalent antioxidant capacity; PON-1 = paraoxonase type-1; GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase. Superscript [a] differs from control, [b] from Treatment A and [c] from Treatment B ($p < 0.05$).

Table 10: Effect of Annona muricata leaf phenolic extract on serum inflammatory cytokines of Wistar rats (Mean ± SEM, pg/mL).

Parameter	Control	Treatment A (100 mg/kg)	Treatment B (300 mg/kg)	Treatment C (500 mg/kg)
IL-1β	35.33 ± 1.83	35.55 ± 1.52	35.03 ± 1.44	55.43 ± 1.51[a]
IL-6	59.34 ± 1.32	60.05 ± 2.12	59.94 ± 1.54	72.34 ± 2.26[a]
IL-8	42.44 ± 1.52	42.35 ± 1.67	42.54 ± 1.02	42.74 ± 0.94
TNF-α	101.36 ± 1.23	102.06 ± 1.18	101.28 ± 1.24	133.25 ± 1.74[a]
IL-10	42.34 ± 1.32	42.55 ± 0.87	42.34 ± 1.02	30.48 ± 1.82[a]

IL = interleukin; TNF-α = tumour necrosis factor-alpha. Superscript [a] differs from control ($p < 0.05$).

phytochemical profile (Table 1), and supports the well-documented free-radical-scavenging and redox-modulating activity of A. muricata leaf constituents such as quercetin, kaempferol, rutin and catechins (Baskar et al., 2007; Ayoola et al., 2008; George et al., 2015). Comparable dose-related increases in serum and tissue antioxidant capacity have been reported for other polyphenol-rich plant extracts fed to farm and laboratory animals (Gessner et al., 2017). While an increase in antioxidant capacity is generally interpreted as beneficial, the parallel rise seen at 500 mg/kg alongside evidence of hepatorenal stress (Tables 6 and 7) suggests that part of this response may represent a compensatory adaptive up-regulation of the endogenous antioxidant defence system to counter extract-induced oxidative challenge at the highest dose, rather than a purely protective effect (Pandey and Rizvi, 2009). Because no oxidative-damage marker (e.g. malondialdehyde) was measured and the enzymes were assayed in serum, the compensatory interpretation remains hypothetical.

Effect on inflammatory cytokines

Only the highest dose produced significant changes in the cytokine panel (Table 10). IL-1β rose from 35.33 ± 1.83 pg/mL in control to 55.43 ± 1.51 pg/mL ($p < 0.05$) at 500 mg/kg, and IL-6 increased from 59.34 ± 1.32 to 72.34 ± 2.26 pg/mL ($p < 0.05$) over the same range. TNF-α similarly rose from 101.36 ± 1.23 to 133.25 ± 1.74 pg/mL ($p < 0.05$), while the anti-inflammatory cytokine IL-10 fell from 42.34 ± 1.32 to 30.48 ± 1.82 pg/mL ($p < 0.05$). IL-8 remained essentially unchanged across all groups. The concurrent rise in the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α alongside a fall in the regulatory cytokine IL-10 at 500 mg/kg indicates a shift toward a pro-inflammatory state at this dose, which is consistent with, and mechanistically compatible with, the neutrophilia and hepatorenal stress markers observed in the same treatment group (Tables 6, 7 and 8). Such a cytokine profile has been associated with tissue-level oxidative and inflammatory injury in other plant-extract toxicity models (Gessner et al., 2017), although the same phenolic constituents have, at lower doses, been reported to exert anti-inflammatory and analgesic effects in comparable animal models (De Sousa et al., 2010), underscoring the dose-dependent, biphasic nature of the extract's immunomodulatory activity and reinforcing the interpretation that 500 mg/kg approaches, or exceeds, the tolerable upper limit for this extract under the present 14-day exposure regimen.

Effect on reproductive hormones

The extract produced a dose-dependent increase in the gonadotropic and steroid hormone panel (Table 11). FSH rose from 6.16 ± 0.51 IU/L in control to 13.41 ± 2.09 IU/L ($p < 0.05$) at 500 mg/kg, LH increased from 3.14 ± 0.24 to 6.22 ± 0.42 IU/L ($p < 0.05$), and estrogen rose from 29.6 ± 1.66 to 35.6 ± 1.53 IU/L ($p < 0.05$). FSH (10.22 ± 1.00) and estrogen (33.6 ± 1.24) were already significantly higher at 300 mg/kg. Prolactin and progesterone were highest at the intermediate dose (300 mg/kg), at 12.0 ± 1.64 and 7.0 ± 2.35 IU/L, respectively, but these increases relative to control were not statistically significant.The significant elevation of FSH, LH and estrogen indicates that the extract alters circulating gonadotropin and estrogen concentrations, consistent with an effect on hypothalamic–pituitary–gonadal signalling (Marques et al., 2015). Functional consequences for follicular development or fertility cannot be inferred because oestrous-cycle stage, ovarian histology and fertility outcomes were not assessed, and rats of ≈ 90 g may have been pre-pubertal,

Table 11: Effect of Annona muricata leaf phenolic extract on serum reproductive hormones of Wistar rats (Mean ± SEM, IU/L)

Parameter	Control	Treatment A (100 mg/kg)	Treatment B (300 mg/kg)	Treatment C (500 mg/kg)
FSH	6.16 ± 0.51	7.24 ± 2.35	10.22 ± 1.00[a]	13.41 ± 2.09[a]
Prolactin	10.0 ± 1.00	11.0 ± 1.87	12.0 ± 1.64	10.0 ± 1.64
Progesterone	5.0 ± 0.45	6.6 ± 0.51	7.0 ± 2.35	6.2 ± 1.00
Estrogen	29.6 ± 1.66	30.0 ± 1.38	33.6 ± 1.24[a]	35.6 ± 1.53[a]
LH	3.14 ± 0.24	3.38 ± 0.08	4.88 ± 0.24	6.22 ± 0.42[a]

FSH = follicle stimulating hormone; LH = luteinizing hormone. Superscript [a] differs from control ($p < 0.05$).

in which case hormone values would not represent adult cyclicity. The reproductive safety of the extract during pregnancy and active fertility therefore remains unestablished pending longer-term studies with cycle staging.Taken together, the present findings indicate that A. muricata leaf phenolic extract is a dose-sensitive bioactive preparation: at 100 mg/kg there was no significant hepatic, renal, haematological, cytokine or hormonal change, whereas from 300 mg/kg dose-related hepatic, lipid, redox and endocrine alterations emerged, and at 500 mg/kg they were accompanied by measurable renal, immunological and inflammatory changes. Converted to a human-equivalent dose (rat dose × 0.162), 100, 300 and 500 mg/kg correspond to about 16, 49 and 81 mg/kg.

Limitations and future work

This study has limitations: small groups (n = 5), a short 14-day exposure, a single sex, no histopathology or organ weights, no oxidative-damage markers, no oestrous-cycle staging, an incompletely characterised extract and largely non-quantified phytochemical profiling. Many endpoints were compared, which raises the risk of false-positive findings. Future work should include 28- and 90-day studies following OECD 407/408, histopathology, extract standardisation, and verification of compound identity by LC-MS/MS.

CONCLUSION

This study shows that Annona muricata possesses a chemically diverse composition (tentatively identified by chromatography) and that its leaf phenolic extract produces dose-dependent changes in female Wistar rats after 14 days of oral administration. At 100 mg/kg no significant hepatic, renal, haematological, cytokine or hormonal change was observed, although CUPRAC was raised. At 300 mg/kg AST, ALT, antioxidant indices (FRAP, TEAC, GPx, SOD), FSH and estrogen were significantly higher and cholesterol, triglycerides and monocyte percentage were significantly lower. At 500 mg/kg the extract significantly altered liver enzymes, renal indices, serum proteins, lipids, leucocyte distribution, antioxidant biomarkers, cytokines and reproductive hormones, indicating hepatorenal and immunomodulatory stress. Adverse-effect signals therefore begin at 300 mg/kg, and no safe human dose can be inferred without histopathology, longer exposure and quantified phytochemistry. Sub-chronic (28–90-day) studies with histopathology, oxidative-damage markers and oestrous-cycle-controlled endocrine assessment are recommended.

DATA AVAILABILITY STATEMENT

The raw data from the research will be available on request.

AUTHOR CONTRIBUTIONS

Oladejo A.O; Conceptualization, methodology, investigation, formal analysis, writing, and supervision;
Mustapha T.B.; Methodology, investigation, and formal analysis
Maruf N, B.; Methodology, investigation, and formal analysis

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this study.

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