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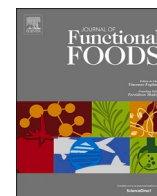
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Efficacy of nutritional plant-based extracts from *Aframomum citratum* and *Xylopia parviflora* in mitigating epididymal and excrement fat accumulation in DIO-C57BL/6 mice

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ABSTRACT

Obesity is a major global health issue, frequently exacerbated by high-fat diets (HFDs), which contribute to adverse metabolic outcomes. This study investigates the efficacy of extracts from nutritional plants *Aframomum citratum* (C.Pereira) K.Schum and *Xylopia parviflora* Spruce in modulating selected anthropometric parameters and fat deposition in DIO-C57BL/6 mice. Over 30 days, we observed that the extracts significantly attenuated body mass index (BMI) and apparent fat mass index (aFMI) in treated mice compared to HFD controls. Notably, *X. parviflora* at 200 mg.kg⁻¹ BW (body weight) effectively reduced both visceral and subcutaneous adipose tissues, while orlistat served as a comparative benchmark. Lipid profile assessments revealed significant decreases in triglycerides, total cholesterol, and fatty acid contents in the epididymal fat pads of treated animals. Gas chromatography-mass spectrometry (GC-MS) analysis confirmed these findings and also indicated that treatment with the extracts enhanced the excretion of fatty acids, suggesting a potential mechanism of lipid malabsorption. Furthermore, histological analyses revealed a marked decrease in adipocyte size in treated groups, indicating a considerable decline in fat deposition. These findings suggest that bioactive compounds, particularly phenolic acids and flavonoids, found in *A. citratum* and *X. parviflora* extracts exhibit significant anti-obesity properties, positioning them as promising natural therapeutic agents for managing diet-induced obesity. Future research should focus on elucidating the mechanisms responsible for these effects and assessing their clinical applicability. This study contributes valuable insights into the role of dietary interventions in obesity management, with implications for the development of novel therapeutic strategies.

Abbreviations: ABC, ATP binding cassette; AC, *Aframomum citratum*; aFMI, apparent fat mass index; AMPK, AMP-activated protein kinase; AT, Adipose tissue; ATGL, Adipose triglyceride lipase; aSWAT, Apparent subcutaneous adipose tissues; aVWAT, Apparent visceral adipose tissues; BAT, Brown adipose tissue; BMI, Body mass index; BS, Body surface; BW, Body weight; C/EBP, CCAAT/enhancer-binding protein; CHO, Cholesterol; CFTRI, Central food technological research institute; CPCSEA, Control and supervision of experiments on animals; DAG, Diacylglycerol; DBT, Department of Biotechnology; FAS, Fatty acid synthase; FAMES, fatty acid methyl esters; FFA, Free fatty acid; GC-MS, Gas chromatography-mass spectrometry; GR, Growth rate; HFD, High-fat diet; MAECI, Ministry of Foreign Affairs and International Cooperation; MAG, Monoacylglycerol; MUFA, Monounsaturated fatty acid; ND, Normal diet; NPC1L1, Niemann-Pick C1-like 1; ORL, Orlistat; PPAR γ , Peroxisome proliferator-activated receptor gamma; PUFA, Polyunsaturated fatty acid; SFA, Saturated fatty acid; STD, Standard, STE: Sterols; TAG, Triacylglycerol; TLC, Thin layer chromatography; TWAS, The World Academy of Science; WAT, White adipose tissues; XP, *Xylopia parviflora*.

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1. Introduction

Obesity is a major global health challenge, representing the most prevalent nutritional disorder in both developed and developing nations (Horta et al., 2023). It is defined by an abnormal or excessive accumulation of body fat, often stemming from an imbalance between caloric intake and energy expenditure (Ogden et al., 2013). Obesity involves a complex interplay of genetic, behavioral, and environmental factors, resulting in a dysfunction of the satiety center in the brain and the body's ability to regulate energy homeostasis (Farooqi & O'Rahilly, 2006). This leads to the storage of excess energy in adipose tissue (AT), which is associated with a host of metabolic disorders, including type 2 diabetes, cardiovascular disease, and certain cancers (Kahn et al., 2006). Notably, 95 % of obesity cases are attributed to exogenous or primary causes, largely related to lifestyle and diet, while only 5 % result from endogenous, genetic, or hormonal factors (Cummings & Overduin, 2007).

A widely accepted models for studying obesity are the high-fat diet (HFD)-induced obese rodent (mice and rats) models. Prolonged consumption of an HFD is known to significantly increase body weight and fat deposition, particularly in abdominal and visceral regions, which are strongly linked to insulin resistance, hyperleptinemia, and other metabolic dysfunctions associated with obesity (Burnett et al., 2017; Kershaw & Flier, 2004). Research shows that central obesity, characterized by fat accumulation in the intra-abdominal region, poses a greater risk for metabolic syndrome compared to peripheral obesity, where fat is predominantly stored in the femorogluteal region (Després & Lemieux, 2006). These patterns of fat distribution vary by gender, ethnicity, and age, influencing an individual's risk for obesity-related complications and their overall quality of life (Strack et al., 2022).

Current strategies to combat obesity include bariatric surgery, lifestyle modifications such as diet and physical activity, and pharmacotherapy (Chopra et al., 2020; Nwakibani et al., 2022). Medications like orlistat, lorcaserin, and liraglutide, which target appetite regulation and satiety, have been approved for clinical use. However, their side effects, which include liver and kidney damage, nausea, and vomiting, limit their long-term application and patient compliance (Apovian et al., 2015; Astrup, 2010). For example, orlistat, despite being associated with gastrointestinal side effects like oily stools and flatulence, continues to be utilized in obesity management because it effectively inhibits fat absorption (pancreatic lipase inhibition), thereby facilitating weight loss and enhancing metabolic health when used alongside a low-calorie diet (O'Meara et al., 2004). Therefore, there is an urgent need for novel anti-obesity agents that are both effective and have minimal adverse effects (Thornton et al., 2020).

In recent years, attention has shifted toward natural products and bioactive compounds that can modulate energy metabolism without the harmful side effects seen with conventional drugs (Atanasov et al., 2021; Nwakibani et al., 2019). Compounds such as curcumin, resveratrol, and berberine have gained considerable attention for their ability to influence adipogenesis, lipid metabolism, and oxidative stress pathways, making them promising candidates for obesity management (Aware et al., 2022; Konstantinidi & Koutelidakis, 2019). Building on our previous research, where we characterized the chemical profiles (Nwakibani et al., 2023; 2022) and highlighted the antioxidant, liver-protective, and enzyme-inhibitory properties of hydro-ethanolic extracts derived from *Aframomum citratum* and *Xylopia parviflora* in both cell-based and animal models (Nwakibani et al., 2019, 2021; Nwakibani et al., 2020a; 2022), our current research shifts focus to explore how these extracts influence adipose tissue metabolism and fecal lipid excretion in DIO-C57BL/6 mice. This study aims to explore their anti-obesity potential by assessing their impact on weight gain, fat accumulation, fecal excretion and various metabolic parameters.

2. Material and methods

2.1. Chemicals and reagents

Casein was obtained from Indian Casein Company (Mumbai, India), while AIN 93Vx vitamin and AIN 93 M mineral mixtures were sourced from MP Biomedicals Pvt. Ltd. (Mumbai, India). Biochemical reagents were acquired from Agape Pharmaceutical (Kerala, India), and Himedia (Mumbai, India) supplied additional dietary materials. Sucrose and lard were purchased locally (Mysore markets, India). Solvents, including methanol, isopropanol, and hexane, were provided by Spectrochem Pvt. Ltd. (Mumbai, India). Throughout the study, ultrapure, triple-distilled, and MilliQ water was prepared using a Millipore water purification system (Merck, Mumbai, India) to ensure minimal metal contamination in all reagents and buffers. Additional chemicals and solvents were procured from Sigma Chemicals Co. (St. Louis, MO, USA) or Merck Pvt. Ltd. (Mumbai, India).

2.2. Plant materials and sample preparation (extraction)

Aframomum citratum (C.Pereira) K.Schum and *Xylopia parviflora* Spruce fruits, collected from different locations within West Cameroon, were obtained following the methodology outlined by Nwakibani et al. (2022). One hundred grams of these plants underwent cleaning and subsequent storage at room temperature ($30 \pm 6^\circ\text{C}$). These materials were then pulverized and subjected to extraction through stirring with a hydroalcoholic solution composed of 70 % ethanol and 30 % water, following the previously outlined method (Nwakibani et al., 2020b). The resultant lyophilized extracts were preserved at 4°C , and for animal studies, an equivalent proportion (1:1) of the hydroethanolic solvent was utilized to dissolve the extracts.

2.3. Animal models and experimental design

2.3.1. Induction of obesity

Four to six-week-old C57BL/6 male mice ($n = 56$) with an average weight of 23 ± 2 g were procured from the CSIR-Central Food Technological Research Institute (CFTRI) animal facility subsequent to mandatory institutional approval (approval number CFT/IAEC/197/2020). The study procedures strictly adhered to the guidelines set forth by India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for the ethical use of laboratory animals. Throughout the experiment, every effort was made to minimize any discomfort or distress experienced by the animals, ensuring a humane approach in all aspects of their care and handling. Animals were housed under standard environmental conditions, including a temperature range of $22\text{--}25^\circ\text{C}$, a 12-h light/dark cycle, and a relative humidity maintained at 50 ± 10 %. They had access to standard chow (normal diet) and water ad libitum. Following a 6-day acclimatization period, mice were randomly assigned to seven groups, each consisting of 8 animals. One group received a standard diet (ND) with a fat content of 10 % of total calories, while the remaining groups were fed a high-fat diet (HFD) comprising 60 % of total calories from fat for 18 weeks (Fig. 1). From the HFD-fed groups, only those animals exhibiting a weight gain exceeding 30 % were selected and distributed into six groups, each containing seven mice.

2.3.2. Animal grouping and drug injections

Prior to treatment, mice underwent a 2-day acclimatization period. Group randomization was based on body weight (BW), with the experimental layout as follows: mice fed a regular diet with saline (ND + saline); mice fed a HFD with (HFD + saline); mice fed a HFD with orlistat (50 mg.kg^{-1} BW, HFD + ORL50); mice fed a HFD with *X. parviflora* extract (100 mg.kg^{-1} BW or 200 mg.kg^{-1} BW; HFD + XP100 or HFD + XP200); and mice fed a HFD with *A. citratum* extract (100 mg.kg^{-1} BW or 200 mg.kg^{-1} BW; HFD + AC100 or HFD + AC200). Orlistat, as well

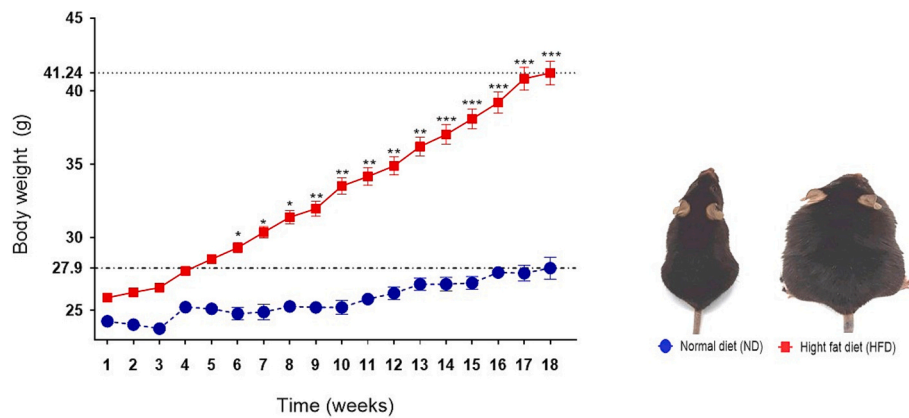


Fig. 1. Representation of body weight over time in C57BL/6 mice on normal diet (ND) and high-fat diet (HFD) regimens. The values represent the mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs HFD mice at week 1.

extracts, were prepared in saline. The doses selected for this study were based on our prior investigations that evaluated the bioactivity and safety of *X. parviflora* and *A. citratum* extracts in animal models (Nwakibian et al., 2022). The chosen concentrations align with those used in previous metabolic studies and were scaled to correspond to feasible human-equivalent doses using body surface area normalization (Reagan-Shaw et al., 2008). Given that the extrapolated human dose falls within the range of dietary intake levels (an intake of 500–1100 mg/day), these concentrations was considered safe and achievable through oral consumption (Busia, 2024). Oral treatment was administered every 24 h for a duration of 30 days.

2.4. Measurement of anthropometric parameters

Body weights (BW) were monitored daily, and animal feces were collected before euthanasia. Following a 12-h fast, waist circumferences were estimated and body height was measured by recording the nasal-to-anal distance to the nearest 0.1 mm with a caliper while the mice were under anesthesia, ensuring precision by minimizing any potential movement. White adipose tissues (WAT), including epididymal, perirenal, retroperitoneal, inguinal, and subcutaneous fat pads, along with brown adipose tissue (BAT), were extracted, cleansed, weighed, and then preserved at -80°C for subsequent analyses. Body weight increase (BWI), growth rate (GR), body surface (BS), body mass index (BMI), and apparent fat mass index (aFMI) were subsequently computed as previously outlined by (Gargiulo et al., 2014).

2.5. Lipidomic profile of feces samples and epididymal adipose tissues

2.5.1. Total lipids extraction and quantification

Total lipids were isolated from the epididymal fat pads and excrement samples of representative mice ($n = 5$) using the Folch method (Folch et al., 1957). Total triglycerides and total cholesterol content were estimated using colorimetric methods described by Fletcher (Fletcher, 1968), Searcy and Bergquist (Searcy & Bergquist, 1960).

2.5.2. Fatty acid profile of feces samples and epididymal adipose tissues by GC-MS

The free fatty acid (FFA) profile (FAMES: fatty acid methyl esters) of total lipids extracted from epididymal tissues and excrement samples was analyzed using gas chromatography–mass spectrometry (GC-MS), following the method outlined by Prasad et al., 2019 with modifications as described by Nwakibian et al., 2022. Fatty acid methyl esters (FAMES) were generated by esterifying epididymal tissues and excrement samples-extracted lipids in a 1 mL BF_3 -methanol complex solution at 65°C for 30 min. To facilitate quantification, 50 μL of an internal standard, heptadecanoic acid ($1\text{ mg}\cdot\text{mL}^{-1}$), was added to each sample

prior to methylation. After cooling for 10 min, a water: hexane solution (1:1, v/v) was introduced, and the upper phase containing the FAMES was carefully transferred to GC vials. The FAMES were separated using an Agilent DB-23 column ((50 %-cyanopropyl)-methylpolysiloxane; 60 m length, 0.25 mm ID, 0.25 μm film thickness) on an Agilent 7890B GC-5977 A mass spectrometer system (M/S Agilent Technologies, Singapore). A sample volume of 1 μL was injected with a 20:1 split ratio, while helium was used as the carrier gas at a flow rate of 1 mL/min. The temperature programming began at 40°C , held for 1 min, then increased to 130°C at $70^{\circ}\text{C}/\text{min}$ with a 1-min hold. Subsequently, it was raised to 170°C at a rate of $6.5^{\circ}\text{C}/\text{min}$, followed by a further increase to 200°C at $2.75^{\circ}\text{C}/\text{min}$, with a hold of 6 min. A final temperature increase brought the system to 210°C at $40^{\circ}\text{C}/\text{min}$ for 2 min, and then to 230°C at $20^{\circ}\text{C}/\text{min}$, held for 7 min. The mass spectra were recorded under electron impact (EI) ionization at 70 eV. Fatty acids were identified by comparing spectra against the NIST version 2.0 g library across an m/z range of 40–500 Da

2.5.3. Separation and analysis of excrement lipid classes by thin layer chromatography (TLC)

Non-polar lipids were isolated utilizing a solvent system consisting of Petroleum ether: Diethyl ether: Acetic acid (70:30:01 V/V) following the method outlined by Prakash, Selvi, Vijayaraj and Kudachikar (2021). The lipid extract obtained from fecal samples of mice fed with either a standard diet or a high-fat diet was diluted with chloroform, and 5 μL of the solution was applied onto TLC plate. Subsequently, the plate was developed using the aforementioned solvent system to separate non-polar lipids such as triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG), free fatty acids (FFA), and sterol esters (STE). Visualization of the separated lipids was achieved by exposing the TLC plate to iodine vapors, followed by documentation of the resulting images.

2.6. Histological analyses of adipose tissues

Sections of epididymal tissues from three representative mice per group were preserved in 10 % formalin, and thin slices measuring 4–5 μm were affixed to glass slides. Hematoxylin and eosin (H&E) staining was applied to the adipose tissue sections, followed by capturing digital images at $\times 10$ magnification with a high-resolution camera-equipped optical microscope (BX51, Olympus, Tokyo) linked to a computer. Cell sizes were quantified using FIJI software, with cells at the image periphery excluded from analysis.

2.7. Statistical analysis

All the data were subjected to statistical analysis, and to assess

significant differences between groups, we employed one-way analysis of variance (ANOVA), followed by multiple comparisons using the Bonferroni post hoc test. Data visualization was accomplished with GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, USA). All results are presented as mean $\pm$ standard deviation (SD).

3. Results

3.1. *A. citratum* and *X. parviflora* modulate some body anthropometric parameters in DIO-C57BL/6 mice

To examine the effects of *A. citratum* and *X. parviflora* on selected anthropometric parameters, we measured the body weights (BW), waist circumference (WC) and height (H) of mice from all the groups across the experimental period. After 4 weeks of treatment, the experimental results showed a significant increase in BS ($p < 0.001$) and BMI ($p < 0.01$) in HFD-fed control animals as compared with normal diet animals. Even more, aFMI significantly ($p < 0.001$) increased in the HFD-fed control animals as compared with normal diet animals (Table 1). Whereas, no significant ($p > 0.05$) difference in growth rate (GR) was observed between the HFD-fed control animals and normal diet animals. However, *X. parviflora* at 200 mg.kg⁻¹ BW significantly ($p < 0.05$) attenuated BMI, BS and aFMI in the HFD treated animals. A similar result was seen in HFD-fed animals with orlistat at 50 mg.kg⁻¹ BW as compared with the HFD-fed control (Table 1), but no effect was observed in HFD-fed animals and treated with *A. citratum* extracts. In contrast, treatment with *X. parviflora* at 200 mg.kg⁻¹ BW and orlistat at 50 mg/kg BW led to a significant reversal of GR ($p < 0.001$) when compared to the HFD-fed control group, with GR values of -13.85 for *X. parviflora* and -10.65 for orlistat, respectively.

3.2. Extracts from *A. citratum* and *X. parviflora* reduce adipose tissue weight in DIO-C57BL/6 mice

Administering treatment to mice for 30 days resulted in changes of fat depot weights. Fat pad weights were normalized to each mouse's body weight and expressed in grams (g), as shown in Table 2. A high-fat diet caused obesity in mice compared to those fed a normal diet after 18 weeks, as shown by a significant increase in the ratio of all the five types of fat pad mass to body weight, including the epididymal fat pad ($p < 0.01$), perirenal ($p < 0.01$), retroperitoneal ($p < 0.05$), inguinal ($p < 0.05$) and axillary ($p < 0.05$) fat pads. However, treatment of HFD-fed

animals with orlistat at 50 mg.kg⁻¹ BW significantly attenuated the weight of epididymal fat pad ($p < 0.001$), retroperitoneal ($p < 0.001$) and axillary ($p < 0.05$) fat pads. Similar to orlistat, extracts at 200 mg.kg⁻¹ BW decreased the weight of the epididymal fat pad ($p < 0.05$), perirenal ($p < 0.01$), retroperitoneal ($p < 0.05$) and inguinal ($p < 0.05$) fat pads. There was no significant effect of the high-fat diet feeding and treatments at different doses on brown adipose tissues (BAT). However, as shown in Table 3, both extracts at 200 mg.kg⁻¹ BW significantly reduced ($p < 0.01$) the total apparent visceral adipose tissues (aVWAT) with no effect ($p > 0.05$) on apparent subcutaneous adipose tissues (aSWAT). Compared to the extracts, orlistat showed the highest activities.

3.3. *A. citratum* and *X. parviflora* extracts lower lipid accumulation in epididymal fat pads and excrement in DIO-C57BL/6 mice

Mice's epididymal fat pads and excrements underwent a change in lipid content after 30 days of treatment (Fig. 2A, B). The results showed significantly higher triglycerides (an increase of 36.22 % ($p < 0.01$)), total cholesterol (an increase of 67.57 % ($p < 0.001$)) and total free fatty acid (an increase of 35.32 % ($p < 0.01$)) levels of epididymal fat pads in HFD-fed control mice as compared with normal diet fed mice (Fig. 2A). The same trends were reversely observed in feces sample with a significant ($p < 0.001$) decrease of triglycerides (70.93 %), total cholesterol (87.95 %) and total free fatty acid (75.38 %) levels in the HFD-fed control mice as compared with normal diet fed mice (Fig. 2B). Treatment with orlistat at 50 mg.kg⁻¹ BW and extracts at both concentrations (100 and 200 mg.kg⁻¹ BW) reduced triglycerides, total cholesterol and total free fatty acid content (31.16–45 % triglycerides, 26.92–58.44 % cholesterol and 24.83–42.50 % total free fatty acid content) in epididymal fat pads compared with the HFD-fed control mice (Fig. 1A). In the feces sample (Fig. 2B), triglycerides (62.12 %), total cholesterol (77.22 %) and total free fatty acid (90.38 %) levels were significantly ($p < 0.001$) increased with orlistat at 50 mg.kg⁻¹ BW compared to the HFD-fed control mice. *X. parviflora* and *A. citratum* were able to significantly ($p < 0.01$) increase the total cholesterol and total free fatty acid content levels at 200 mg.kg⁻¹ BW. However, no significant ($p > 0.05$) difference in total triglyceride content was observed between the HFD-fed control mice and HFD-fed treated mice.

3.4. Effect of *A. citratum* and *X. parviflora* extracts on the epididymal fat pads and excrement lipid profile

Gas chromatography–mass spectrometry (GC – MS) analysis was conducted to examine the epididymal and feces free fatty profiles of all experimental groups (Table 4 and Table 5). We confirmed the presence of six (06) FFAs in the epididymal fat pads and eleven (11) FFAs in the excrement fat samples of the HFD-fed mice group, both ranging from C12:0 to C20:1 and, where palmitic (C16:0), stearic acid (C18:0), oleic (C18:1) and linoleic (C18:2) acids were predominant. Consistent findings were observed in HFD-fed mice treated with extract at 100 mg.kg⁻¹ BW, indicating comparable responses in this model. Table 4 illustrates that in the epididymal fat pads of HFD-fed control mice, the level of palmitoleic acid (C16:1) decreased, while both stearic acid (C18:0) and linoleic acid (C18:2) levels significantly increased in comparison to mice on a normal diet. However, treatment with extracts resulted in a slight, non-significant ($p > 0.05$) increase in palmitoleic acid (C16:1) levels. In contrast, both stearic acid (C18:0) and linoleic acid (C18:2) levels were reduced following treatment with either orlistat (50 mg.kg⁻¹ BW) or *X. parviflora* (200 mg.kg⁻¹ BW). In the fecal samples (Table 5), HFD-fed control mice showed a significant rise in stearic acid (C18:0) and a decrease in linoleic acid (C18:2) levels compared to normal diet-fed mice. Notably, orlistat (50 mg.kg⁻¹ BW) significantly ($p < 0.01$) reversed these effects, restoring the levels of both stearic and linoleic acids. Meanwhile, treatment with extracts produced only modest, non-significant changes ($p > 0.05$) compared to the HFD-fed control mice.

Table 1

Effect of *A. citratum* and *X. parviflora* extracts on anthropometric in DIO-C57BL/6 mice.

Groups of experimental animals	BMI	BS	GR	aFMI
ND	1.84 $\pm$ 0.07	76.26 $\pm$ 3.78	3.86 $\pm$ 3.86	0.01 $\pm$ 0.00
HFD	2.64 $\pm$ 0.19**	102.20 $\pm$ 5.09***	5.28 $\pm$ 2.78	0.03 $\pm$ 0.00***
HFD + ORL50	2.15 $\pm$ 0.25#	89.11 $\pm$ 8.61*##	-10.95 $\pm$ 6.24***##	0.01 $\pm$ 0.00##
HFD + XP100	2.37 $\pm$ 0.30**	95.73 $\pm$ 11.33**	-1.95 $\pm$ 5.76	0.04 $\pm$ 0.00***
HFD + XP200	2.04 $\pm$ 0.22#	85.78 $\pm$ 5.37##	-13.06 $\pm$ 5.00***##	0.02 $\pm$ 0.00**
HFD + AC100	2.36 $\pm$ 0.21**	95.05 $\pm$ 8.30**	4.16 $\pm$ 4.18	0.04 $\pm$ 0.00***
HFD + AC200	2.36 $\pm$ 0.22**	94.72 $\pm$ 5.24**	5.60 $\pm$ 5.52	0.02 $\pm$ 0.00##

The values represent the mean $\pm$ SD. # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ vs the HFD group; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs ND group. BMI: Body mass index, BS: Body surface, GR: Growth rate, aFMI: Apparent fat mass index, WS: Waist circumference, ND: Normal diet, HFD: High fat diet, ORL: Orlistat, XP: *Xylopiia parviflora*, AC: *Aframomum citratum*.

Table 2

Effect of hydroalcoholic nutritional plant extracts on adipose tissue weights expressed as a ratio of the body weight.

Groups of experimental animals	Epididymal WAT (g)	Perirenal WAT (g)	Retroperitoneal WAT (g)	Inguinal WAT (g)	Axillary WAT (g)	BAT (g)
ND	0.43 ± 0.06	0.11 ± 0.02	0.23 ± 0.01	1.40 ± 0.18	0.49 ± 0.35	0.37 ± 0.01
HFD	3.89 ± 0.34***	0.49 ± 0.06***	1.39 ± 0.18***	2.71 ± 0.53*	1.30 ± 0.28*	0.28 ± 0.06
HFD + ORL50	2.16 ± 0.37*** #	0.32 ± 0.03	0.67 ± 0.1 ^{##}	1.53 ± 0.45 [#]	0.49 ± 0.21 [#]	0.33 ± 0.05
HFD + XP100	4.04 ± 0.11***	0.38 ± 0.05*	1.16 ± 0.22***	2.34 ± 0.62	1.30 ± 0.26*	0.47 ± 0.04
HFD + XP200	2.01 ± 0.29***	0.24 ± 0.07 [#]	0.75 ± 0.07* ^{##}	1.28 ± 0.47 [#]	1.03 ± 0.31	0.32 ± 0.05
HFD + AC100	4.44 ± 0.24***	0.53 ± 0.05***	1.27 ± 0.16***	2.50 ± 0.57	1.34 ± 0.35*	0.43 ± 0.09
HFD + AC200	2.23 ± 0.03*** [#]	0.14 ± 0.03 ^{###}	0.91 ± 0.07**	1.61 ± 0.47 [#]	1.01 ± 0.15	0.36 ± 0.04

The values represent the mean ± SD. #p < 0.05, ##p < 0.01, and ###p < 0.001 vs the HFD group; *p < 0.05, **p < 0.01, and ***p < 0.001 vs ND group. ND: Normal diet, HFD: high-fat diet, ORL: Orlistat, XP: *Xylopia parviflora*, AC: *Aframomum citratum*.

Table 3Effect of *A. citratum* and *X.parviflora* extracts on total apparent subcutaneous (aSWAT) and visceral (aVWAT) adipose tissues weights expressed as a ratio of the body weight.

Groups of experimental animals	aVWAT (g)	aSWAT (g)	WAT + BAT (g)
ND	0.26 ± 0.09	0.94 ± 0.45	0.50 ± 0.18
HFD	1.92 ± 1.01***	1.73 ± 0.42**	1.58 ± 0.53***
HFD + ORL50	1.05 ± 0.56** [#]	1.10 ± 0.12	0.95 ± 0.32 [#]
HFD + XP100	1.86 ± 1.11***	1.83 ± 0.53	1.62 ± 0.56***
HFD + XP200	1.33 ± 0.84** [#]	1.15 ± 0.61	1.10 ± 0.41**
HFD + AC100	2.08 ± 1.19***	2.15 ± 0.80**	1.83 ± 0.63***
HFD + AC200	1.43 ± 0.92** [#]	1.67 ± 0.65*	1.33 ± 0.49**

The values represent the mean ± SD. #p < 0.05, ##p < 0.01, and ###p < 0.001 vs the HFD group; *p < 0.05, **p < 0.01, and ***p < 0.001 vs ND group. ND: Normal diet, HFD: high-fat diet, ORL: Orlistat, XP: *Xylopia parviflora*, AC: *Aframomum citratum*.

The total FFAs were distributed in saturated and unsaturated fatty acids as shown in Fig. 3A and B for epididymal fat pads and excrement samples respectively. In comparison to the other groups, the mice that were fed high-fat diets (HFDs) exhibited significantly elevated levels of both saturated and unsaturated fatty acids in their epididymal fat pads (Fig. 3A). Similarly, the analysis of excrement samples revealed a markedly lower concentration of both types of fatty acids (Fig. 3B). Treatment with orlistat at 50 mg.kg⁻¹ BW and extracts at 200 mg.kg⁻¹ BW significantly (p < 0.001) reduced (38.41–40.50 %) the level of unsaturated fatty acids in epididymal fat pads. However, the amount of epididymal unsaturated fatty acids was significantly (p < 0.05) decreased (44.84 %) by *X. parviflora* at 200 mg.kg⁻¹ BW. Thin layer chromatography of feces samples from normal diet and high-fat diet fed C57BL/6 mice displayed the presence of triacylglycerol (TA), free fatty acid (FFA), sterols (STE) and cholesterol (CHO), but they were more prominent in normal diet C57BL/6 mice (Fig. 4B). Treatment with orlistat at 50 mg.kg⁻¹ BW and *X. parviflora* at 200 mg.kg⁻¹ BW extracts alone resulted in a significant (p < 0.001) increase in the level of both saturated and unsaturated fatty acids (Fig. 3B).

3.5. Hydroalcoholic extracts from *A. citratum* and *X. parviflora* ameliorated HFD-induced fat deposition in epididymal fat pads and excrement samples

The adipocyte size analysis in adipose tissue by H&E staining (Fig. 5)

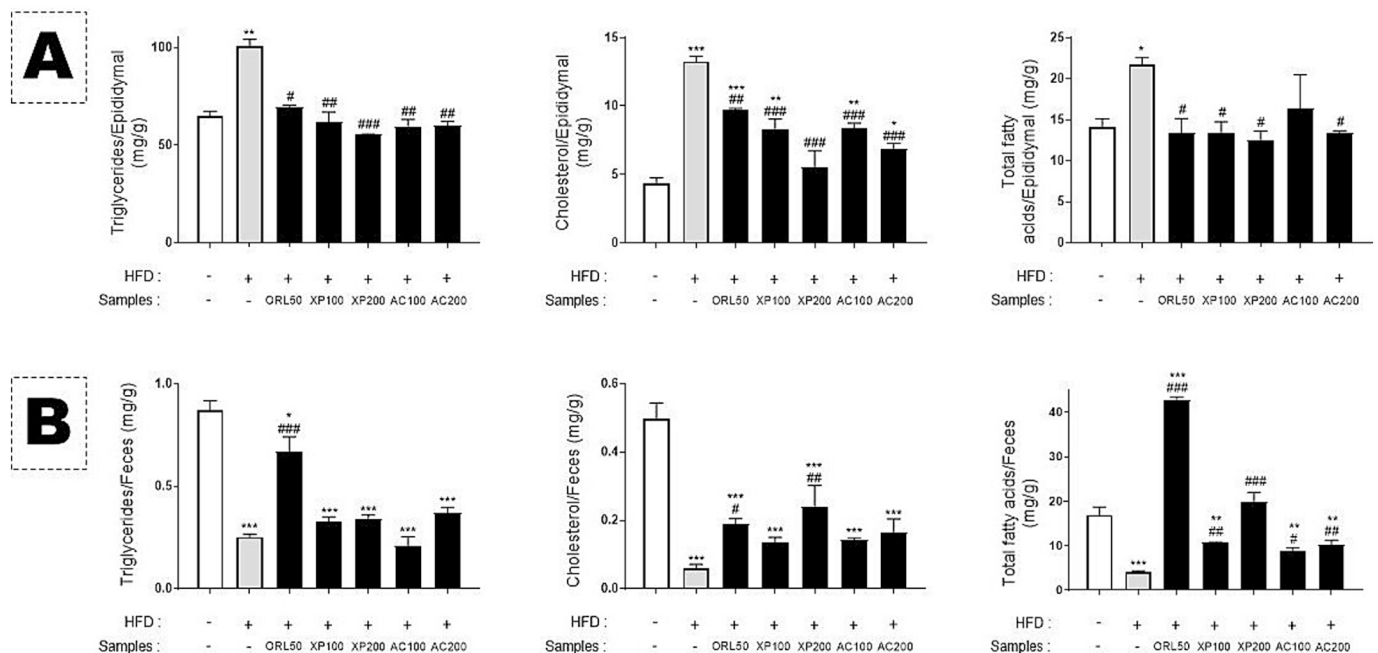


Fig. 2. Effect of *A. citratum* and *X.parviflora* nutritional plant extracts on epididymal and excrement triglyceride, cholesterol and the total FFA contents. (A) Effect of extracts on epididymal lipid contents. (B) Effect of extracts on excrement lipid contents. The values represent the mean ± SD. #p < 0.05, ##p < 0.01, and ###p < 0.001 vs the HFD group; *p < 0.05, **p < 0.01, and ***p < 0.001 vs ND group.

Table 4

Fatty acid profile of epididymal fat from different experimental groups after 30 days of treatment.

Groups of experimental animals	ND	HFD	HFD + ORL50	HFD + XP100	HFD + XP200	HFD + AC100	HFD + AC200
Group of fatty acids	Amount of fatty acids in 1 mg of total fatty acid extracted from epididymal fat (%)						
C12:0	0.06 ± 0.00	0.00	0.25 ± 0.01	0.22 ± 0.04	0.23 ± 0.03	0.20 ± 0.00	0.21 ± 0.04
C14:0	1.38 ± 0.01	1.25 ± 0.08	1.45 ± 0.01	1.91 ± 0.28	1.47 ± 0.17	1.44 ± 0.05	1.47 ± 0.00
C15:0	0.18 ± 0.00	0.00	0.11 ± 0.00	0.00	0.14 ± 0.85	0.00	0.10 ± 0.00
C16:0	22.90 ± 0.57	22.95 ± 0.12	24.60 ± 0.13	18.98 ± 0.91	11.53 ± 0.63***	10.43 ± 0.32***	22.56 ± 0.03
C16:1	14.29 ± 0.01	4.85 ± 0.15**	3.93 ± 0.01**	6.66 ± 0.28**	6.77 ± 0.18**	14.15 ± 0.90**	5.45 ± 0.02**
C18:0	1.59 ± 0.04	3.32 ± 0.18*	3.73 ± 0.07*	3.66 ± 0.71*	10.86 ± 0.39***	3.59 ± 0.04*	3.00 ± 0.01*
C18:1	47.45 ± 0.46	46.71 ± 0.85	50.13 ± 0.35	49.70 ± 0.50	48.93 ± 0.03	25.29 ± 0.95***	48.22 ± 0.03
C18:2	11.26 ± 0.14	20.89 ± 0.80**	15.49 ± 0.27*	18.12 ± 0.22*	9.49 ± 0.17**	34.32 ± 0.95***	18.29 ± 0.02*
C18:3	0.20 ± 0.01	0.00	0.00	0.32 ± 0.00	0.34 ± 0.09	0.46 ± 0.21	0.28 ± 0.00
C20:1	0.68 ± 0.02	0.00	0.27 ± 0.22	0.38 ± 0.04	0.42 ± 0.04	0.37 ± 0.08	0.37 ± 0.02

The values represent the mean ± SD. #p < 0.05, ##p < 0.01, and ###p < 0.001 vs the HFD group; *p < 0.05, **p < 0.01, and ***p < 0.001 vs ND group. ND: Normal diet, HFD: high-fat diet, ORL: Orlistat, XP: *Xylopiia parviflora*, AC: *Aframomum citratum*.

Table 5

Fatty acid profile of feces sample from different experimental groups after 30 days of treatment.

Groups of experimental animals	ND	HFD	HFD + ORL50	HFD + XP100	HFD + XP200	HFD + AC100	HFD + AC200
Group of fatty acids	Amount of fatty acids in 1 mg of total fatty acid extracted from feces sample (%)						
C11:2	0.1 ± 0.00	0.00	0.00	0.00	0.00	0.00	0.00
C12:0	0.11 ± 0.01	0.4 ± 0.05	2.10 ± 0.00	0.24 ± 0.01	0.12 ± 0.03	0.11 ± 0.00	0.17 ± 0.03
C14:0	0.6 ± 0.01	1.4 ± 0.03	1.62 ± 0.00	1.37 ± 0.02	1.16 ± 0.01	1.35 ± 0.09	1.36 ± 0.05
C14:1	3.58 ± 0.01	0.00	0.00	0.00	0.00	0.00	0.00
C15:0	3.24 ± 0.81	0.22 ± 0.05	8.30 ± 4.30**	0.29 ± 0.09*	0.32 ± 0.16*	0.28 ± 0.01*	0.33 ± 0.01*
C16:0	21.74 ± 0.31	22.24 ± 0.02	8.77 ± 4.09***	21 ± 0.11	11.91 ± 11.66***	22.43 ± 0.03	22.82 ± 0.03
C16:1	0.57 ± 0.00	0.48 ± 0.00	1.84 ± 0.14	0.83 ± 0.01	12.07 ± 11.62***	0.61 ± 0.01	0.60 ± 0.01
C17:0	0.06 ± 0.00	0.46 ± 0.03	6.53 ± 3.21***	0.13 ± 0.01	0.20 ± 0.06	0.15 ± 0.01	0.14 ± 0.00
C18:0	8.53 ± 0.13	28.48 ± 0.11***	7.86 ± 2.31***	25.28 ± 0.13***	37.85 ± 0.37***	30.25 ± 0.11***	30.71 ± 0.10***
C18:1	26.07 ± 23	37.56 ± 0.07*	48.16 ± 3.61***	38.24 ± 0.08**	27.46 ± 0.58**	35.58 ± 0.16*	34.09 ± 0.13*
C18:2	27.96 ± 0.3	5.76 ± 0.03***	11.51 ± 4.44***	6.31 ± 0.00***	4.30 ± 0.06***	5.31 ± 0.02***	5.12 ± 0.04***
C18:3	1.26 ± 0.02	0.00	1.29 ± 0.26	0.16 ± 0.01	0.08 ± 0.00	0.00	0.00
Group of fatty acids	Amount of fatty acids in 1 mg of total fatty acid extracted from feces sample (%)						
C19:0	0.00	0.84 ± 0.03	0.00	0.73 ± 0.01	0.00	0.00	0.00
C19:1	0.63 ± 0.01	0.00	0.00	0.00	0.00	0.00	0.00
C20:0	1.53 ± 0.03	0.00	1.85 ± 0.37	0.00	1.04 ± 0.01	0.80 ± 0.02	0.86 ± 0.02
C20:1	2.12 ± 0.03	2.1 ± 0.00	0.59 ± 0.00	3.23 ± 0.15	2.38 ± 0.03	2.77 ± 0.03	2.78 ± 0.00
C20:2	0.00	0.00	0.17 ± 0.00	0.37 ± 0.03	0.33 ± 0.01	0.36 ± 0.01	0.34 ± 0.01
C20:3	0.28 ± 0.00	0.00	0.66 ± 0.00	0.52 ± 0.00	0.24 ± 0.01	0.00	0.37 ± 0.00
C20:4	0.46 ± 0.00	0.00	0.14 ± 0.0	0.29 ± 0.01	0.23 ± 0.02	0.00	0.31 ± 0.01
C22:0	1.06 ± 0.00	0.00	0.00	0.00	0.32 ± 0.04	0.00	0.00
C22:3	0.00	0.00	0.00	0.92 ± 0.03	0.00	0.00	0.00

The values represent the mean ± SD. #p < 0.05, ##p < 0.01, and ###p < 0.001 vs the HFD group; *p < 0.05, **p < 0.01, and ***p < 0.001 vs ND group. ND: Normal diet, HFD: high-fat diet, ORL: Orlistat, XP: *Xylopiia parviflora*, AC: *Aframomum citratum*.

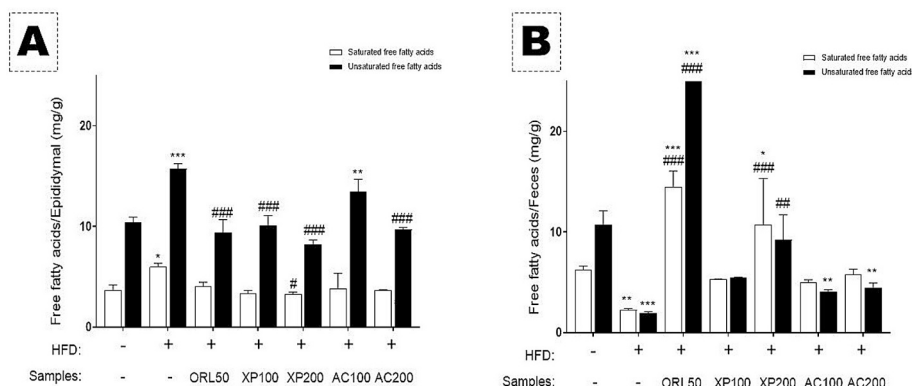


Fig. 3. Effect of the nutritional plant extracts on the epididymal and excrement-saturated/–unsaturated free fatty formation in DIO-C57BL/6 mice. Effect of extracts on saturated and unsaturated fatty acid content in (A) epididymal fat pads and (B) feces samples of different mouse groups after 30 days of treatment. The values represent the mean ± SD. #p < 0.05, ##p < 0.01, and ###p < 0.001 vs the HFD group; *p < 0.05, **p < 0.01, and ***p < 0.001 vs ND group.

revealed that orlistat treatments significantly ($p < 0.001$) decreased epididymal adipocyte area distribution by bringing the cells about to the half of what is observed in HFD-fed mice. Extracts treatments at both concentrations (100 and 200 mg.kg⁻¹ BW) resulted in a statistically

significant ($p < 0.001$) decrease in adipocyte cell size distribution to 390–549 μm² (Fig. 4 A). In excrement samples, photographic images show different appearances between groups. In fact, the color of feces samples of high-fat diet fed mice treated with orlistat and extracts at 200

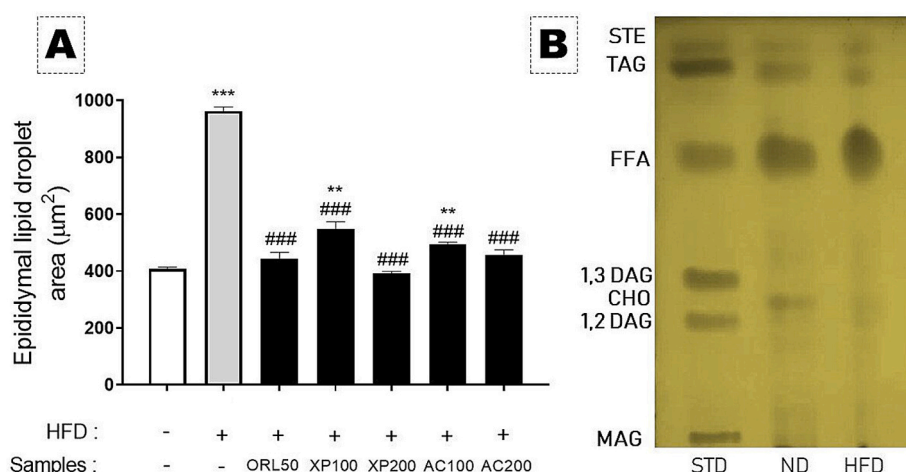


Fig. 4. Effect of *A. citratum* and *X. parviflora* on epididymal lipid droplet formation in DIO-C57BL/6 mice. (A) Effect of the nutritional plant extracts on epididymal lipid droplet formation. (B) TLC profile of feces samples from normal and high-fat fed C57BL/6 mice. ND: Normal diet, HFD: High fat diet, STD: Lipid standard, TAG: Triacylglycerol, DAG: Diacylglycerol, MAG: Monoacylglycerol, FFA: Free fatty acid, STE: Sterols, CHO: Cholesterol. The values represent the mean $\pm$ SD. # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ vs the HFD group; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs ND group.

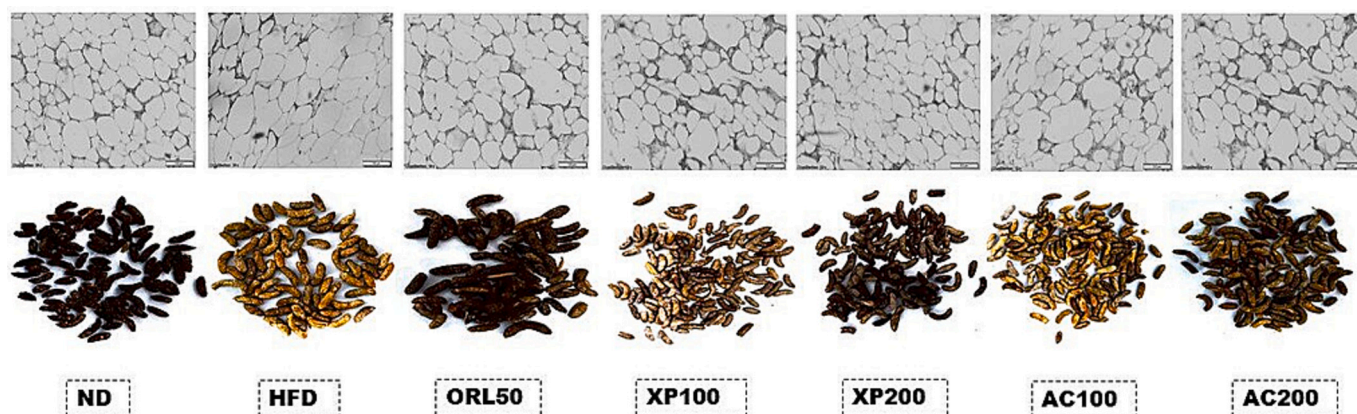


Fig. 5. Representative histological and photography images of epididymal tissues and excrement samples from different mouse groups. The eWAT sections were stained with hematoxylin n and eosin (H&E), and the magnification is 100.

mg/kg BW was more pronounced (dark-brown) and closer to the normal diet fed mouse groups compared to the negative control. However, the appearance of excrement samples from mouse groups treated with the lower concentration of extracts was both similar to that of normal diet high-fat diet fed mice without treatment (Fig. 5).

4. Discussion

Obesity is a multifaceted disorder characterized by excessive adipose tissue accumulation, leading to various health issues, including metabolic syndrome, cardiovascular diseases, and insulin resistance (Kahn et al., 2006). Adiposity, or the presence of excess fat, contributes to systemic inflammation and metabolic dysregulation, further exacerbating obesity-related complications (Kanneganti & Dixit, 2012). Recent studies have demonstrated that dietary interventions, such as the inclusion of specific plant extracts, can influence adipose tissue dynamics by reducing fat deposition and modulating lipid profiles (Ahmad, 2022; Ríos-Hoyo & Gutiérrez-Salmeán, 2016). Additionally, fecal fat accumulation serves as an indicator of lipid absorption and metabolism; alterations in fecal fat content can reflect the efficacy of dietary strategies in managing obesity (Ríos-Hoyo & Gutiérrez-Salmeán, 2016). Understanding the interplay between obesity, adiposity, and fecal fat accumulation is crucial for developing effective interventions aimed at

reducing fat accumulation and improving metabolic health (Cani & Jordan, 2018).

After 4 weeks of treatment with *A. citratum*, *X. parviflora* extracts and orlistat subsequent to obesity induction, the HFD-fed control mice exhibited a significant increase in body weight and BMI compared to normal diet fed mice. This aligns with the understanding that high-fat diets contribute to obesity, as demonstrated in other studies. For instance, (De Moura Dias et al., 2021) reported that a high-fat diet resulted in increased body weight and BMI in mice, indicating the effectiveness of the diet in inducing obesity. The increase in BW and BMI in the HFD-fed rodents is a common finding in obesity research, reinforcing the model's validity. It was also reported a significant increase in fat mass index (aFMI) in the HFD-fed control mice compared to normal diet mice. This finding is consistent with other research that has documented similar results in high-fat diet models. For example, a study by Yang et al., 2024 demonstrated that high-fat feeding leads to lipid accumulation in adipose tissue and increased fat mass, supporting the idea that FMI is a reliable indicator of body fat content and its elevation in obese models is a hallmark of metabolic syndrome. Interestingly, while BW, BMI, and aFMI were significantly altered, no significant difference in growth rate (GR) was observed between the HFD-fed control mice and those on a normal diet. This suggests that while the HFD impacts weight and fat accumulation, it does not necessarily influence

overall growth (An et al., 2022). This finding could imply that the energy balance in these mice is maintained despite the increase in fat mass, possibly due to compensatory mechanisms. Similar observations have been made in studies where HFD did not affect linear growth, indicating that the body might prioritize energy storage over growth under these conditions (Buettner et al., 2007; Sampey et al., 2011). The most notable results emerged with the administration of *X. parviflora* at 200 mg.kg⁻¹ BW, which significantly attenuated BMI, BS, and aFMI in HFD-treated mice. This is particularly interesting as it suggests a potential therapeutic effect of *X. parviflora* in managing obesity-related parameters. For instance, *Xylopiia parviflora* has been shown to improve lipid metabolism, likely due to its rich content of polyphenols and flavonoids, which exert antioxidant and anti-inflammatory effects (Nwakiban et al., 2023). These compounds are known to enhance energy expenditure and reduce lipogenesis, thereby lowering fat accumulation (Nwakiban et al., 2022). The efficacy of *X. parviflora* is comparable to that of orlistat, a well-known anti-obesity medication, which also significantly reversed GR in HFD-fed mice. This indicates that both orlistat and *X. parviflora* not only reduced obesity-related parameters but also positively influenced growth regulation, possibly by improving metabolic function and energy homeostasis (Henness & Perry, 2006). Similar findings were reported by Liu et al., 2017, where herbal extracts demonstrated significant weight loss effects and improved metabolic profiles in HFD-induced obesity models. Conversely, *A. citratum* did not exhibit significant effects on these parameters. This could suggest varying mechanisms of action or bioavailability of the compounds within these extracts. In contrast to our previous in vitro findings (Nwakiban et al., 2021), where *A. citratum* effectively reduced lipid accumulation in human adipocyte cell models, the present study found slight and no significant effects in vivo. These effects can vary widely based on concentration, duration of treatment, and the specific pathways they target (Nwakiban et al. 2023). However, this discrepancy highlights the potential for differential responses in cellular models versus whole-organism contexts, underscoring the need for further investigation into its bioactive mechanisms.

The findings of our study indicate that treatment with *A. citratum* and *X. parviflora* extracts reduced fat tissue weight in DIO-C57BL/6 mice after a 30-day administration. Specifically, the study observed a normalization of fat depot weights when adjusted for body weight. As expected, the HFD led to a significant increase in various fat pad weights compared to mice on a normal diet. This is consistent with existing literature, which demonstrates that a HFD promotes obesity through the accumulation of visceral fat, particularly in the epididymal, perirenal, retroperitoneal, inguinal, and axillary regions (Hariri & Thibault, 2010). A study by Misawa et al., 2015 showed that HFD in C57BL/6 mice resulted in a significant increase in body weight and fat mass, corroborating our results. The increase in fat pad weights is primarily due to lipid accumulation and hypertrophy of adipocytes, as observed in other studies that evaluated the impact of dietary fats on adipose tissue development (Choe et al., 2016; Jéquier, 2002). In our research, the epididymal fat pad demonstrated a notable increase along with the other fat depots, reinforcing the notion that HFD is a significant contributor to adiposity. Treatment with extracts at 200 mg.kg⁻¹ BW and orlistat showed significant decreases in specific fat depots. The treatment with extracts resulted in decreased weights of the epididymal, retroperitoneal, and inguinal fat pads. These findings align with our earlier research (Nwakiban et al., 2019), where we highlighted the pancreatic lipase inhibitory effects of the studied nutritional plant extracts. This action closely resembles that of orlistat, a widely recognized medication for weight management (Heck et al., 2000; Henness & Perry, 2006). Similar studies, such as those by Ji et al., 2019, support the notion that specific phytochemicals in plant extracts may modulate lipid metabolism and fat storage. Moreover, our study shows that both extracts at 200 mg.kg⁻¹ BW significantly reduced total apparent visceral adipose tissue (aVWAT), whereas no significant effect was observed on apparent subcutaneous adipose tissues (aSWAT). This is crucial as visceral fat is more closely associated with metabolic disorders and cardiovascular diseases

compared to subcutaneous fat (Chiba et al., 2007; Jung et al., 2016). The selective mitigation of aVWAT without affecting aSWAT suggests a potential therapeutic role for extracts in managing obesity-related complications without adversely affecting energy reserves stored in subcutaneous fat. Interestingly, despite the pronounced effects of extracts on fat depot weights, our findings indicated no significant impact of HFD feeding mice and treatments on brown adipose tissue (BAT). This observation is important as BAT is known for its role in thermogenesis and energy expenditure (Suchacki & Stimson, 2021). Previous studies, such as those by Fj et al., 2021, have shown variable effects of dietary interventions on BAT, suggesting that while certain dietary compounds can modulate white adipose tissue (WAT), they may not significantly affect BAT.

Our study demonstrates the significant impact of extracts from *X. parviflora* and *A. citratum* on lipid metabolism in high-fat diet (HFD)-induced obese mice, particularly affecting epididymal fat pads and lipid accumulation in feces. Epididymal fat pads in HFD-fed control mice exhibited significant increases in triglycerides, total cholesterol, and total fatty acid contents compared to normal diet mice. This aligns with other studies on HFD-induced obesity, where excessive calorie intake leads to lipid accumulation in adipose tissues (Friedman, 2000; Kopelman, 2000). For instance, a study by He et al., 2020 observed elevated triglycerides and cholesterol levels in epididymal fat pads of HFD-fed mice, supporting our findings. The observed decrease in lipid content within epididymal fat pads following treatment, particularly at a dosage of 200 mg.kg⁻¹ body weight, indicates a potential lipolytic activity of these extracts. This effect may stem from their influence on lipogenic and lipolytic pathways, possibly involving the downregulation of fatty acid synthase (FAS) or the upregulation of adipose triglyceride lipase (ATGL). These pathways are known to play critical roles in the balance of lipid synthesis and breakdown, suggesting that the extracts may act by shifting this balance toward lipid mobilization. Studies by Singh et al., 2020 reported similar mechanisms in *Hibiscus sabdariffa* extract-treated mice, which showed reduced lipogenesis and increased fatty acid oxidation. Moreover, Chaudhary et al., 2015 demonstrated a 30–50 % reduction in fat pad weight and lipid content in HFD-induced obese mice treated with *Camellia sinensis* extract. The data also show the inverse relationship in fecal lipid content, where HFD-fed mice had significantly lower excretion of triglycerides, cholesterol, and total free fatty acids. This is likely due to increased lipid absorption and retention in adipose tissue under HFD conditions, a common observation in obesity models (Arias et al., 2024; Dahik et al., 2020). Treatment with extracts and orlistat, significantly increased lipid excretion, consistent with findings in studies where compounds like UP601 (a botanical composition) showed enhanced fecal lipid excretion after HFD treatment (Yimam et al., 2017). The reduction of lipid content in epididymal fat pads and the increase in lipid content in feces upon treatment with *X. parviflora* and *A. citratum* suggest these extracts act through a mechanism similar to that of orlistat, promoting fat excretion while reducing fat storage. Moreover, the selective increase in cholesterol and total free fatty acid content without a significant change in triglycerides at certain doses may indicate different modes of action on various lipid species, which is a topic worth further exploration (Kimura et al., 2016).

The gas chromatography-mass spectrometry (GC-MS) analysis reveal nuanced responses in lipid profiles, particularly in relation to key fatty acids like palmitoleic (C16:1), stearic (C18:0), and linoleic acid (C18:2), indicating possible metabolic changes induced by the extracts. In the untreated HFD-fed mice, a decrease in palmitoleic acid (C16:1) and an increase in both stearic acid (C18:0) and linoleic acid (C18:2) levels of epididymal fat pads are indicative of lipid metabolism disturbances commonly associated with obesity. Palmitoleic acid, a known lipokine, plays a critical role in regulating insulin sensitivity and fatty acid oxidation (Cao et al., 2008). The decrease in C16:1 level in the HFD-fed mice aligns with findings of impaired lipokine signaling in obesity models (Stefan et al., 2010). Treatment with *A. citratum* and *X. parviflora* extracts resulted in a slight, though not statistically significant, increase

in palmitoleic acid levels, suggesting a limited restoration of lipokine function. While the changes were not significant, even modest increases in C16:1 could still be of interest given its potential role in improving insulin sensitivity and reducing lipid accumulation (Liang et al., 2024; Z.-H. Yang et al., 2011). In contrast, treatment with orlistat and *X. parviflora* extracts led to decreases in both stearic acid (C18:0) and linoleic acid (C18:2), suggesting beneficial shifts in saturated and polyunsaturated fatty acid profiles. Elevated levels of stearic acid, a saturated fatty acid, have been associated with lipotoxicity and inflammation in adipose tissue (Swinburn et al., 2004). The observed post-treatment reduction in C18:0 may indicate decreased lipotoxicity, potentially reflecting improved fatty acid oxidation or lower lipid accumulation in adipocytes. Linoleic acid, an essential omega-6 fatty acid, is important for maintaining membrane fluidity and modulating inflammatory responses. The slight decrease in C18:2 levels following treatment with *X. parviflora* extracts could signify a rebalancing of omega-6 and omega-3 fatty acids, which is often disrupted in obesity (Simopoulos, 2016). The fecal lipid profiles provide additional insight into how dietary treatments impact lipid absorption and excretion. In the HFD-fed mice, a significant increase in C18:0 levels and a decrease in C18:2 suggest metabolic disruption and possible malabsorption, as commonly seen in obese models. These changes may also indicate altered microbial lipid processing, given the influence of gut microbiota on fatty acid composition and absorption in obesity (Clarke et al., 2014). Treatment with orlistat, significantly reversed these alterations, normalizing the levels of both stearic and linoleic acids. Orlistat's known mechanism of action (limiting fat absorption) likely explains these effects, as stearic acid, being less readily absorbed compared to other saturated fats, would accumulate in feces (Hauptman et al., 1992). The restoration of linoleic acid levels further suggests that orlistat might help rebalance polyunsaturated fatty acid (PUFA) profiles, which are frequently disrupted in obesity. On the other hand, *A. citratum* and *X. parviflora* extracts produced only modest, non-significant changes in fecal fatty acid profiles, suggesting that their influence on fat absorption and excretion is less pronounced compared to orlistat. However, the trend toward reduced stearic acid levels might hint at a mild lipase-inhibitory effect, warranting further exploration. Investigating these extracts at higher doses or over extended treatment periods could provide more conclusive data. Additionally, exploring the role of gut microbiota in mediating these effects could provide valuable insights into the mechanisms of action.

In alignment with previous findings, these data were presented to delineate the effects of the extracts on both saturated (SFA) and unsaturated fatty acids (UFA). This approach allows for a clearer understanding of the distinct impacts on fatty acid profiles. Indeed, HFD-fed mice, both saturated (SFA) and unsaturated fatty acids (UFA) were significantly elevated in the epididymal fat pads, suggesting increased fat storage in adipose tissues. The same trend was inversely observed in excrement samples, with HFD-fed mice showing a lower amount of both SFAs and UFAs (MUFAs, PUFAs), indicative of reduced fat excretion (Basson et al., 2021). This finding aligns with earlier research where high-fat diets are shown to promote fat retention in tissues while inhibiting fecal fat excretion (Jamar & Pisani, 2023). Treatment with orlistat at 50 mg.kg⁻¹ BW and hydroethanolic extracts at 200 mg.kg⁻¹ BW led to a significant decrease in the level of unsaturated fatty acids in epididymal fat pads. This suggests that the extracts may also inhibit fat accumulation in adipose tissues, possibly through modulating lipid metabolism or reducing lipogenic enzyme activity. Similar effects were observed in studies of plant-based treatments on lipid profiles, such as those using *Camellia sinensis* or *Garcinia cambogia*, which also reduced adipose tissue FFAs in HFD-induced obesity models (Hursel et al., 2009; KIM et al., 2008). Interestingly, both orlistat and *X. parviflora* extract at 200 mg.kg⁻¹ BW significantly increased the levels of both SFA and UFA in excrement samples. This result highlights the compounds' ability to enhance fecal fat excretion. Orlistat's effect on fecal fat excretion has been well-documented, as it blocks dietary fat absorption by inhibiting

pancreatic lipase (Katimbwa et al., 2022; Sumithran & Proietto, 2014). The increase in fecal fat excretion with *X. parviflora* suggests that the extract may also promote lipid malabsorption or impair fat digestion, possibly through a similar mechanism or by modulating intestinal lipid transporters (such as NPC1L1 or ABC transporters), as shown in studies by (Ji et al., 2019).

Histological analysis of adipose tissue using Hematoxylin and Eosin (H&E) staining revealed a significant reduction in adipocyte cell size in mice treated with orlistat and plant extracts. This result is consistent with previous studies indicating that orlistat, reduces fat absorption, leading to decreased lipid accumulation in adipose tissues and consequently smaller adipocyte size (Katimbwa et al., 2022). Also, the significant reduction in adipocyte size with plant extract treatments aligns with findings from other plant-based studies. For example, *Camellia sinensis* and *Garcinia cambogia* extracts were shown to reduce adipocyte hypertrophy in obesity models by modulating lipid metabolism, enhancing lipolysis, and reducing lipogenesis (Hursel et al., 2009; KIM et al., 2008). The results suggest that the hydroethanolic extracts in this study may exhibit similar mechanisms, inhibiting lipid accumulation and promoting adipocyte size reduction. This could be linked with the bioactive compounds in the extracts, such as polyphenols and flavonoids, which have been shown to inhibit adipogenesis and stimulate fat breakdown in preadipocytes and adipocytes (Cory et al., 2018; Nwakibani et al., 2022). Polyphenolic compounds may activate AMP-activated protein kinase (AMPK) and increase the expression of peroxisome proliferator-activated receptor gamma (PPAR γ) and CCAAT/enhancer-binding protein (C/EBP), leading to enhanced fatty acid oxidation and reduced lipid storage (Duarte et al., 2021; J et al., 2013; Wang et al., 2021). Photographic evidence of fecal samples from the various treatment groups revealed notable differences in the appearance of excrement, particularly in mice treated with orlistat and the extracts at 200 mg.kg⁻¹ BW. The feces from these groups were darker in color (dark-brown), resembling those of normal diet-fed mice more closely than the feces from HFD-fed mice without treatment. In contrast, excrement samples from mice treated with the lower concentration of plant extracts (100 mg.kg⁻¹ BW) were visually similar to those from the untreated HFD-fed mice. The dark-brown coloration of the feces in treated mice is indicative of increased fat excretion, which is consistent with orlistat's known mechanism of action. This phenomenon has been reported in other studies, where fecal lipid content increased significantly in HFD-fed mice treated with orlistat (Katimbwa et al., 2022; J. Yang et al., 2023). The darker feces could be a visual manifestation of this increased fat content, as unabsorbed lipids are expelled through the digestive system. The similarity in fecal appearance between the higher concentration of plant extract-treated groups and the orlistat group suggests that the hydroethanolic extracts may also enhance fecal fat excretion, possibly by inhibiting intestinal fat absorption or modulating lipid transporters (Ji et al., 2019). Moreover, plant extracts rich in bioactive compounds like saponins, flavonoids, and polyphenols have been reported to promote fat excretion by altering bile acid metabolism and improving gut health, thereby enhancing fat elimination via feces (Af & Vm, 2021; Dzah et al., 2023; Marrelli et al., 2016; Oakenfull, 1981). This could explain the increased fat excretion observed in mice treated with the 200 mg.kg⁻¹ BW concentration of plant extracts in this study.

The safety profile of *X. parviflora* and *A. citratum* extracts has been previously explored in various studies, with no reported acute or chronic toxic effects at comparable doses (Meva et al., 2024; Oladoja et al., 2024). Consistent with these findings and due to our previous study (Nwakibani et al., 2022), our experimental model did not exhibit any observable signs of toxicity, including abnormal behavior and mortality, further supporting the non-toxic nature of the administered doses. These results suggest that the tested extracts are well tolerated and safe for potential therapeutic applications.

5. Conclusion

In conclusion, this study demonstrates the significant anti-obesity effects of hydroethanolic extracts of *X. parviflora* and *A. citratum* in DIO-C57BL/6 mice, primarily through their modulation of lipid metabolism and fat accumulation. Treatment with *X. parviflora* at 200 mg. kg⁻¹ BW led to decreases in body weight, body mass index (BMI), and fat mass index (FMI), suggesting its therapeutic potential for managing obesity. The notable decrease in epididymal, retroperitoneal, and inguinal fat pads, along with the normalization of apparent visceral adipose tissue (aVWAT), underscores the extract's ability to selectively reduce visceral fat, a key contributor to metabolic disorders, without depleting subcutaneous fat reserves. The significant increase in fecal fat excretion further suggests that these extracts enhance lipid elimination by modulating intestinal fat absorption, similar to orlistat's action. Gas chromatography-mass spectrometry (GC-MS) analysis revealed decreases in fatty acids such as palmitic, stearic, and oleic acids in epididymal fat, indicating a dual mechanism of fat reduction and enhanced excretion. The histological analysis supported these findings, showing reduced adipocyte size, highlighting the extracts' potential in inhibiting fat storage and promoting lipolysis. While *X. parviflora* demonstrated significant effects, *A. citratum* showed limited efficacy, emphasizing the need for further research into its bioactive compounds and mechanisms of action. Future studies should focus on identifying these compounds, their pharmacokinetics, and their role in obesity management to optimize their therapeutic potential. Both *A. citratum* and *X. parviflora* are widely used as spices in traditional African cuisine, which supports their safety for human consumption. Their common inclusion in daily meals suggests a long-standing history of dietary exposure without reported adverse effects, reinforcing their non-toxic nature.

CRedit authorship contribution statement

Achille Parfait Nwakibian Atchan: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Shilpa Talkad Shivashankara Murthy:** Writing – review & editing, Methodology, Formal analysis. **Paolo Magni:** Writing – review & editing. **François Ferriere:** Writing – review & editing. **Gabriel Agbor Agbor:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Jules-Roger Kuiate:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Uma Venkateswaran Manjappara:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Ethical statement

The study procedures strictly adhered to the guidelines set forth by India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for the ethical use of laboratory animals (approval number CFT/IAEC/197/2020).

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findings to the broader scientific community.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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