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Influence of Cooking and Fractionation of Momordica Balsamina L. (Cucurbitaceae) Leaves on Hypolipidemic and Antioxidant Properties in Rats Fed a High-fat Diet

Sali Abraham Adoum
The University of Ngaoundere
Rosane Soh Matsinkou
rosanesoh@gmail.com
The University of Ngaoundere
Innocent Djakwardam
The University of Ngaoundere
Pièrre Jidibe
University of Ngaoundere
Josiane Therese Metsangang Ngatchic
The University of Ngaoundere

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Abstract

Background

Momordica balsamina leaves are widely consumed for their nutritional and therapeutic properties. This study evaluated the effect of cooking and granulometric fractionation of *M. balsamina* leaf powders on their bioactive compound content, antioxidant activities, and anti-hyperlipidemic properties.

Methods

Leaves were divided into raw and cooked batches, dried, ground, and fractionated into five granulometric classes (> 355 µm; 355–200 µm; 200–125 µm; 125–50 µm; < 50 µm). The contents of total phenolic compounds, flavonoids, and condensed tannins were determined, and antioxidant activity was evaluated *in vitro* while *in vivo* hypolipidemic and antioxidant properties were studied in hyperlipidemic *Wistar* rats, with analysis of lipid parameters, transaminases, and oxidative stress markers.

Results

Cooking influenced bioactive compound content, with the 50–125 µm fraction of cooked leaves (D2) showing the highest content, and best antioxidant and hypolipidemic activities. D2 reduced total cholesterol (53.28%), triglycerides (48.72%), and LDL-c (86.35%), while increasing HDL-c by 194%. Hepatic parameters and oxidative markers showed a trend towards normalization, suggesting a hepatoprotective and antioxidant effect. Hypolipidemic and antioxidant activities were correlated with polyphenol, flavonoid, and tannin content.

Conclusion

Cooking and fractionation of *M. balsamina* leaves enhance their antioxidant and hypolipidemic properties, thereby justifying their potential use in preventing cardiovascular diseases and oxidative stress, and underscoring their value as a functional food ingredient for promoting cardiovascular health.

1. INTRODUCTION

Rapid urbanization and nutritional transition have profoundly altered dietary habits, with a marked increase in the consumption of saturated fats, refined sugars, and ultra-processed foods. These dietary changes, combined with increasing sedentary lifestyles, have contributed to the rise of non-communicable chronic diseases, particularly cardiovascular diseases (CVDs). According to the World Health Organization, CVDs caused nearly 17.9 million deaths in 2019, representing the leading cause of global mortality. This burden is particularly concerning in low- and middle-income countries, where nutritional transition is occurring rapidly, often without adequate support [1]. Among the main risk

factors for CVDs are dyslipidemias, characterized by elevated total cholesterol, low-density lipoprotein (LDL), and triglycerides, associated with decreased high-density lipoprotein (HDL). These abnormalities promote lipid accumulation in the arterial wall and initiate the atherosclerotic process [2]. Oxidative stress plays a central role in this mechanism, amplifying lipid oxidation, inflammation, and endothelial dysfunction. Management of dyslipidemias relies on hygienic-dietary measures, including reducing saturated fat intake, increasing fiber consumption, and regular physical activity [3]. When these interventions are insufficient, pharmacological treatment is generally initiated, notably with statins, inhibitors of the key enzyme in cholesterol biosynthesis (HMG-CoA reductase) [3]. Although these treatments have demonstrated their effectiveness in reducing cardiovascular risk, their prolonged use can be associated with side effects (myopathies, liver disorders, or intolerances) that may affect therapeutic adherence. Moreover, these treatments do not always directly address associated oxidative mechanisms [3]. In this context, research is turning towards complementary approaches, particularly through the valorization of plant matrices rich in bioactive compounds.

Leafy vegetables play an important role in this dynamic due to their high content of polyphenols, flavonoids, carotenoids, antioxidant vitamins, and minerals. These compounds are recognized for their ability to modulate lipid metabolism, inhibit lipoprotein oxidation, and strengthen endogenous antioxidant systems [4]. However, the biological expression of these compounds depends not only on their presence in the raw plant but also on the technological processes applied before consumption. Indeed, technological treatments influence the stability, release, and bioavailability of bioactive compounds. Cooking, widely used to improve palatability and sanitary safety, can induce physicochemical transformations: thermal degradation of certain thermolabile vitamins, modification of phenolic structures, or conversely, increased bioavailability through cellular matrix disruption. Thus, its impact on biological activity can be either deleterious or beneficial depending on the conditions applied [4]. Meanwhile, mechanical processes such as grinding and granulometric fractionation are promising technological strategies. Reducing particle size increases specific surface area and can promote the release of intracellular compounds. Sieving fractionation allows concentrating certain secondary metabolites in fine fractions, potentially richer in bioactive compounds and more bioavailable. Using this technique, several authors have demonstrated that powder fractionation concentrates polyphenols in finer particles [5, 6, 7, 8]

Among these plant resources, *Momordica balsamina L*., belonging to the Cucurbitaceae family, is widely consumed in tropical regions of Africa and Asia. Its leaves have high nutritional density and contain various phenolic compounds and micronutrients [9]. Several studies have reported its antioxidant, hypolipidemic, antidiabetic, and antibacterial properties [10, 9] However, despite its potential, its technological and functional valorization remains limited. To date, few studies have explored the combined impact of technological processes, such as cooking and sieving fractionation, on the hypolipidemic and antioxidant properties of *M. balsamina* leaves. Thus, this work aims to evaluate the hypolipidemic and antioxidant properties of *M. balsamina* leaves, raw or cooked, fractionated into different granulometries, in *Wistar* rats fed a high-fat diet.

2. MATERIAL AND METHODS

2.1 Material

2.1.1 Plant material

The plant material used in this study was *Momordica balsamina* leaves, harvested in June 2025 at Mbé, in the Vina department of the Adamaoua Region, Cameroon. Professor MAPONGMETSEM, a botanist at the University of Ngaoundere, Faculty of Science, Department of Biological Sciences, identified the samples.

2.1.2 Experimental animals

The study was conducted on adult male *Wistar* rats (*Rattus norvegicus*), aged 3 months and weighing 200-230g. The animals were housed at the animal facility of the Laboratory of Biophysics, Food Biochemistry and Nutrition (LABBAN), National School of Agro-Industrial Sciences (ENSAI), University of Ngaoundere, Cameroon. They were kept under standard conditions: temperature (25°C), relative humidity (50–60%), adequate ventilation, and a 12-hour light/dark cycle. The rats had *ad libitum* access to standard feed and tap water. Handling and care were in accordance with the European Council Directive on animal protection (EEC Council Regulation 86/609) [11].

2.2 Methods

2.2.1 Harvesting, pre-treatment, and initial processing of *M. Balsamina* leaves

Once harvested, *M. balsamina* leaves were transported to the laboratory where they were washed with running water to remove impurities. Some of the leaves were subjected to cooking according to traditional cooking methods. Cooking time was set based on interviews with local residents and literature evidence. For this, 100 g of leaves were immersed in 1.5 L of boiling distilled water in a covered stainless steel pot. The average cooking temperature was 95 ± 2°C for 10 minutes, measured with a food thermometer probe. The leaves were then quickly cooled with water and drained before drying. In total, 10 replicated samples (weighing 100 g each) of cooked leaves and 10 replicated samples of raw leaves (control) were dried in a ventilated hot air dryer set at 45°C for 24 hours and then stored for further analysis.

2.2.2 Grinding and Fractionation of Powders

After drying, the cooked and raw leaves were separately ground using an ultra-centrifugal mill ZM 200 (Haan, Germany) operating at 12,000 rpm with a 1 mm mesh sieve. The powder fractions were produced on an Analysette 3 Spartan apparatus (Fritsch, Idar-Oberstein, Germany). To this end, 100 g of crude powder were poured onto a sieving column with decreasing mesh sizes of 355, 200, 125, and 50 µm, ending with the collection pan. The system was left to vibrate at an amplitude of 0.5 mm for 10 min,

generating powders of respective sizes > 355, 355 – 200, 200 – 125, 125 – 50 µm, and < 50 µm. The powders on the respective sieves and the collection pan (powder fractions) were then collected, packed in polyethylene bags, and stored at 10°C until analysis.

2.2.3 Determination of Phytochemical composition

Extraction and determination of phenolic compounds content

Phenolic compounds were extracted from formulated powders according to the method described by Biyanzi et al. (2018) with some modifications. 5 g of powder were extracted with 100 mL of 80% methanol by mechanical shaking (OSCILL 10, PROLABO, France) for 1 hour at room temperature. The extract was filtered through Whatman No. 1 filter paper; the residue was recovered and extracted again under shaking for 1 hour with the same volume of 80% methanol. The operation was repeated twice. The combined filtrates were transferred to a 25 mL volumetric flask and made up to the mark with distilled water, then transferred to a dark or amber bottle for analysis [12].

Total phenolic compounds (TPC) in powder extracts were determined according to the protocol described by Wafa et al. (2014) [13]. 200 µL of extract was placed in a tube with 1.2 mL of distilled water. Subsequently, 200 µL of 1/10th Folin-Ciocalteu reagent and 400 µL of 20% Na2CO3 solution were added, respectively. The tubes containing the samples and the blank were vortexed. After agitation, the mixture was incubated in a water bath at 40°C for 20 min. Absorbance was measured at 725 nm using a UV spectrophotometer (Shimadzu UV–VIS 1605, Tokyo, Japan). Calibration was performed using an aqueous solution of gallic acid (0.2 g/L), and results were expressed as mg gallic acid equivalent/g dry matter.

Determination of flavonoid content

The total flavonoid content (TFC) was determined using the colorimetric method described by Dewanto et al. (2002) [14]. The extract solution (100 µL) was added to a volumetric flask containing 2.4 mL of distilled water and 150 µL of sodium nitrite (Na2NO2, 5%). After 5 min, 300 µL of aluminum chloride (AlCl3, 6H2O, 10%) was added. After 5 min, 100 µL of sodium hydroxide (NaOH, 1 N) was added to the mixture and vortexed. The samples were kept at room temperature for 5 min and the absorbance recorded at 510 nm. A rutin solution (0.1 g/L) was used as a standard, and the total flavonoid content was expressed as milligrams of rutin equivalent per 100 g of dry matter (mg RE/100 g DM).

Determination of condensed tannins content

The condensed tannin content was determined according to the protocol of Sun et al. (1998) [15]. 50 µL of extract solution was mixed with 3 mL of 4% vanillin and 1.5 mL of concentrated H_2SO_4, and the mixture was incubated at room temperature for 30 min. The absorbance was measured at 500 nm, and a tannic acid solution (0-0.600 mg/mL) was used as a standard. The condensed tannin content was expressed as milligrams of tannic acid equivalent per 100 g of dry matter (mg TAE/100 g DM).

2.2.4 Determination of antioxidant activity

DPPH radical scavenging activity assay

The DPPH radical scavenging activity of the methanolic/aqueous extracts was measured according to the method of Zhang & Hamauzu (2004), with slight modifications [16]. For this determination, the extract sample (25 mg/mL) was first diluted in a methanol/water mixture (70/30, v/v) to obtain solutions with concentrations of 0.025-5 mg/mL. Then, each solution (0.5 mL) was added to 2.0 mL of 0.2 g/L methanolic DPPH solution. The reaction mixture was incubated in the dark for 60 min at room temperature (25 ± 2°C), and the optical density was recorded at 517 nm using the extraction solvent as a blank. The percentage of DPPH radical scavenging effect was calculated as follows:

$$Scavenging\ activity\ (\%) = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

The antioxidant activity was expressed as the IC50 value (mg/mL), which represents the concentration required to scavenge 50% of DPPH radicals. IC50 values were determined by plotting the DPPH scavenging effect (%) against concentration and extrapolating. Ascorbic acid served as a reference standard.

Total Reducing Power

The total reducing power of the methanolic/aqueous extracts was investigated using the method developed by Oyaizu (1986) [17]. A volume of extract (2.5 mL) of each sample was mixed with 2.5 mL of phosphate buffer (200mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The mixture was incubated in a water bath at 50°C for 30 min, cooled, mixed with 2.5 mL of 10% trichloroacetic acid solution, and centrifuged (Thermo Scientific, Heraeus Megafuge 8R centrifuge, Osterode am Harz, Germany) at 3,000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 1.0% FeCl3, allowed to react for 10 min at room temperature, and the absorbance was measured at 700 nm. Ascorbic acid solution was used as a standard. The reducing power was expressed as milligrams of ascorbic acid equivalent per g of dry matter (mg AAE/g DM).

2.2.5 Induction and treatment of hyperlipidemia

Induction of Hyperlipidemia

Hyperlipidemia was induced according to the method of Ngatchic et al. (2020), based on the work of Garait (2006) who demonstrated the effectiveness of a high-fat diet in inducing stress and hyperlipidemia [18]. Sixty-five (65) normocholesterolemic rats were randomly divided into two main groups:

- A control group (n = 5): fed a normal diet (ND) for 28 days;
- A test group (n = 60): fed a high-fat diet for 28 days.

The basic constituents of the high-fat diet were egg yolk (100 g of egg yolk provides approximately 1.14 g of cholesterol) and pork fat (lard), chosen for their high content of saturated fat (Table 1) [19].

Table 1
Composition of différent diets

Constituents		Normal Diet (g/kg)	High Fat Diet (g/kg)
Proteins	Fish powder	200	100
Carbohydrates	Corn starch	590	290
	Sucrose	50	50
Lipids	Pork fat	-	150
	Egg yolk	-	300
	Soybean oil	50	50
Others	Cellulose	50	-
	Minerals	50	50
	Vitamin b complex	10	10

Treatment of hyperlipidemia

After induction, the rats were divided into twelve (12) experimental groups (5 per cage) and treated as follows:

- A normal control group: normocholesterolemic rats receiving distilled water (10 mL/kg body weight)
- A hyperlipidemic control group: hyperlipidemic rats without treatment (distilled water only)
- A reference control group: hyperlipidemic rats treated with atorvastatin (10 mg/kg body weight)
- Nine experimental groups receiving: hyperlipidemic rats treated with an aqueous suspension of powdered fractions < 50 µm, 50–125 µm, 125–200 µm, 200–355 µm, and > 355 µm, as well as crude powder of cooked and raw *M. balsamina* leaves at a dose of 250 mg/kg body weight.

The animals had free access to water throughout the experimental period, which lasted 28 days.

2.2.6 Animal sacrifice and biochemical analysis

Animal sacrifice

At the end of the treatment period, animals were fasted for 24 hours, and then anesthetized with ketamine (10 mg/mL) and diazepam (5 mg/mL) via intraperitoneal injection. Blood was collected from the jugular vein and centrifuged (4000 rpm, 15 minutes) to obtain serum, which was stored at -20°C for biochemical analysis. The liver was excised, rinsed with saline solution, and weighed. A 0.8 g sample of liver tissue was homogenized in 4% phosphate buffer and centrifuged at 4000 rpm for 15 minutes. The resulting supernatant was stored at -20°C for subsequent oxidative stress assessment.

Biochemical Analyses

The treatment's effects on lipid profiles, liver and kidney function, and oxidative stress were assessed through various biochemical parameters. Lipid profiles included total cholesterol [20], HDL cholesterol [21], triglycerides [22] and LDL cholesterol [23]. Liver function was evaluated via alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [24], while kidney function was assessed through creatinine levels [25]. Oxidative stress was measured by superoxide dismutase (SOD) activity [26], catalase (CAT) activity [27] and malondialdehyde (MDA) levels [28]. Enzymatic and colorimetric methods were used for analysis.

2.2.7 Statistical Analysis

All analyses were performed in triplicate, and results are presented as mean ± standard deviation. Statistical significance of differences between sample means was determined by analysis of variance (ANOVA) followed by Tukey's test with a 95% confidence level using Statgraphics Centurion 16.1. Principal component analysis (PCA) was used to classify and correlate factors defining antioxidant activity and physicochemical properties, performed using XL-STAT 2019.

3. RESULTS

3.1 Bioactive profile of cooked and raw *M. balsamina* leaf powders

Table 2 shows the bioactive composition of cooked and raw *M. balsamina* leaf powders, with varying particle sizes. Cooked leaf powders (D1-D4) generally exhibited higher levels of total polyphenols, flavonoids, and condensed tannins compared to raw leaf powders (F1-F4). The unsieved powder (PNT) had the lowest levels of these bioactive compounds.

Particle size significantly impacted bioactive compound availability, with finer fractions showing higher contents than coarser fractions. Notably, the intermediate fraction D2 (50–125 µm) from cooked leaves concentrated the highest levels of total polyphenols (62.33 ± 0.03 mg GAE/g DM), flavonoids (15.17 ± 0.06 mg RE/g DM), and condensed tannins (2.03 ± 0.01 mg TAE/g DM), representing increases of 1.92, 1.32, and 1.35 times, respectively, compared to the unsieved powder.

Table 2
Bioactive composition of cooked and raw *M. balsamina* leaf powders

Fractions		Total polyphénols (mg GAE/g DM)	Total flavonoids (mg RE/g DM)	Condensed tannins (mg TAE/g DM)
≤ 50µm	**D1**	55.35 ± 0.02^{de}	13.09 ± 0.01^{d}	1.95 ± 0.02^{bc}
	F1	47.83 ± 0.02^{efg}	12.14 ± 0.04^{e}	1.60 ± 0.01^{de}
50–125µm	**D2**	62.33 ± 0.03^{fg}	15.17 ± 0.06^{e}	2.03 ± 0.01^{bc}
	F2	57.50 ± 0.07^{g}	13.24 ± 0.01^{g}	1.65 ± 0.02^{e}
125–200µm	**D3**	52.74 ± 0.01^{cd}	13.94 ± 0.02^{d}	1.93 ± 0.01^{b}
	F3	43.65 ± 0.15^{ef}	12.28 ± 0.14^{f}	1.52 ± 0.01^{de}
300µm	**D4**	45.23 ± 0.03^{bc}	12.06 ± 0.0^{b}	1.75 ± 0.02^{ab}
	F4	39.49 ± 0.06^{cd}	10.36 ± 0.04^{d}	1.44 ± 0.01^{cd}
PNT	**D5**	32.45 ± 0.05^{a}	11.46 ± 0.01^{a}	1.50 ± 0.01^{a}
	F5	24.47 ± 0.03^{b}	9.41 ± 0.01^{c}	1.28 ± 0.01^{b}

D1-D5: powder fractions from cooked leaves, F1-F5: powder fractions from raw leaves, PNT: unsieved powder, GAE: gallic acid equivalent, RE: rutin equivalent, TAE: tannic acid equivalent. Values are mean ± standard deviation (n = 3). Different superscript letters within the same column indicate significant differences ($p < 0.05$) according to Tukey's multiple range test.

3.2 Antioxidant activity

The analysis of the antioxidant activity of cooked and raw *M. balsamina* leaf powders reveals promising results (Table 3). The IC50 DPPH values range from 0.203 mg/mL to 0,698 mg/mL, indicating significant antioxidant potential. Notably, fractions D2, D1, F2, and F1 exhibit the highest antioxidant activity, with IC50 values of 0.203 mg/mL, 0,218 mg/mL, 0,272 mg/mL, and 0.282 mg/mL, respectively. Furthermore, the reducing power of the powder samples varies significantly, with values ranging from 5.53 to 13.80 mg AAE/g MS. The best reducing powers are observed in fractions F2, D3, D2, and F3, with values of 13.80 mg AAE/g MS, 13.14 mg AAE/g MS, 12.45 mg AAE/g MS, and 10.15 mg AAE/g MS, respectively.

Table 3
Antioxidant Activity of *M. balsamina* Powders

Fractions		IC50 DPPH (mg/mL)	Reducing powder (mg AAE/g DM)
≤ 50µm	**D1**	0.218 ± 0.17^{b}	8.50 ± 0.97^{e}
	F1	0.282 ± 0.40^{d}	8.86 ± 0.40^{f}
50–125µm	**D2**	0.203 ± 0.10^{a}	12.45 ± 0.29^{h}
	F2	0.272 ± 0.26^{c}	13.80 ± 0.20^{j}
125–200µm	**D3**	0.377 ± 0.46^{e}	13.14 ± 0.59^{i}
	F3	0.407 ± 0.17^{f}	10.15 ± 0.40^{g}
300µm	**D4**	0.411 ± 0.17^{g}	5.52 ± 0.49^{a}
	F4	0.496 ± 0.10^{h}	6.64 ± 0.1^{c}
PNT	**D5**	0.566 ± 0.17^{i}	7.64 ± 0.66^{d}
	F5	0.698 ± 0.20^{j}	5.53 ± 0.91^{b}

D1-D5: powder fractions from cooked leaves, F1-F5: powder fractions from raw leaves, PNT: unsieved powder, GAE: gallic acid equivalent, RE: rutin equivalent, TAE: tannic acid equivalent. Values are mean ± standard deviation (n = 3). Different superscript letters within the same column indicate significant differences ($p < 0.05$) according to Tukey's multiple range test.

3.3 Effect on experimental *Wistar* rats

3.3.1 Effect on body weight

The results in Table 4 show that a hyperlipidemic diet led to significant weight gain in the hyperlipidemic control group (47 ± 3.34g before treatment, 34 ± 3.02g after treatment). However, consumption of cooked and raw *M. balsamina* leaf powder resulted in decreased body weight. The most notable reductions were seen with the 50–125 µm fractions (D2: 50.25 ± 3.03g to -24 ± 2.61g; F2: 38.25 ± 3.44g to -23 ± 2.79g), followed by the ≤ 50 µm fractions (D1: 42 ± 3.85g to -21 ± 3.24g; F1: 41.75 ± 0.62g to -22.5 ± 1.93g). These effects were comparable to the reference medication (Atorvastatine: 46.5 ± 1.84g to -25.5 ± 5.57g).

Table 4
Effect of *M. balsamina* leaf powder on body weight gain in hyperlipidemic rats

Groups		Weight gain before treatment	Weight gain after treatment
Normal Control		10.5 ± 0.95^{a}	9.75 ± 2.52^{k}
Hyperlipidemic Control		47 ± 3.34^{h}	34 ± 3.02^{l}
Reference control		46.5 ± 1.84^{g}	-25.5 ± 5.57^{a}
≤ 50 µm	**D1**	42 ± 3.85^{de}	-21 ± 3.24^{e}
	F1	41.75 ± 0.62^{d}	-22.5 ± 1.93^{d}
50–125 µm	**D2**	50.25 ± 3.03^{i}	-24 ± 2.61^{b}
	F2	38.25 ± 3.44^{c}	-23 ± 2.79^{c}
125–200 µm	**D3**	42.25 ± 1.79^{e}	-18 ± 4.06^{g}
	F3	37.75 ± 2.49^{b}	-20.75 ± 4.36^{f}
300 µm	**D4**	46.5 ± 1.84^{g}	-15.5 ± 2.32^{h}
	F4	43.5 ± 3.27^{f}	-22.25 ± 4.11^{d}
PNT	**D5**	46.5 ± 1.84^{g}	-12.5 ± 2.10^{j}
	F5	38.25 ± 0.94^{c}	-14 ± 2.73^{i}

D1-D5: powder fractions from cooked leaves, F1-F5: powder fractions from raw leaves, PNT: unsieved powder. Values are mean ± standard deviation (n = 5). Different superscript letters within the same column indicate significant differences ($p < 0.05$) according to Tukey's multiple range test.

3.3.2 Effect on lipid profile

The results in Table 5 show that the *M. balsamina* powder fractions have beneficial effects on the lipid profile in hyperlipidemic rats. The D2 fraction (50–125 µm, cooked leaves) reduces total cholesterol by 53.3% (211.83 to 98.96 mg/dL), triglycerides by 48.7% (156.66 to 80.33 mg/dL), and LDL-cholesterol by 86.4% (159.73 to 21.80 mg/dL), and increases HDL-cholesterol by 194.2% (20.76 to 61.10 mg/dL). The D1 fraction (≤ 50 µm, cooked leaves) shows similar effects, reducing total cholesterol by 52.9% (211.83 to 99.73 mg/dL), triglycerides by 47.3% (156.66 to 82.56 mg/dL), and LDL-cholesterol by 85.4% (159.73 to 23.28 mg/dL), and increasing HDL-cholesterol by 188.6% (20.76 to 59.93 mg/dL). The F2 (50–125 µm, raw leaves) and F1 (≤ 50 µm, raw leaves) fractions also show beneficial effects on lipid parameters, but to a lesser extent. Furthermore, the D1 and D2 fractions significantly reduce LDL-c/HDL-c ratios (95.1%

and 95.5%) and TC/HDL-c ratios (83.7% and 84.2%), indicating a reduction in atherogenic risk (Table 6). These effects are comparable to those of atorvastatin.

Table 5
Effects of *M. balsamina* powder fractions on lipid profile in hyperlipidemic rats

Groups		CT (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)
Normal Control		98.33 ± 0.75a	79.16 ± 0.82a	50.16 ± 0.17cd	32.33 ± 0.74a
Hyperlipidemic Control		211.83 ± 2.90g	156.66 ± 0.41h	20.76 ± 0.57a	159.73 ± 3.22g
Reference control		99.26 ± 0.32b	81.06 ± 0.24bc	60.66 ± 0.43g	22.38 ± 0.36b
≤ 50 µm	**D1**	99.73 ± 0.13b	82.56 ± 0.39c	59.93 ± 0.52fg	23.28 ± 0.57b
	F1	107.8 ± 0.61d	85.90 ± 0.49d	48.73 ± 0.31c	41.88 ± 0.88e
50–125 µm	**D2**	98.96 ± 0.63b	80.33 ± 0.3b	61.10 ± 0.15g	21.80 ± 0.68b
	F2	103.63 ± 0.37c	82.66 ± 0.62c	58.60 ± 0.28f	28.50 ± 0.26c
125–200 µm	**D3**	103.33 ± 1.82c	82.50 ± 0.34c	51.73 ± 1.09d	35.10 ± 2.52d
	F3	109.03 ± 0.58d	91.86 ± 0.56f	53.40 ± 1.10e	37.26 ± 1.10d
300 µm	**D4**	107.46 ± 0.83d	86.90 ± 1.06d	48.83 ± 0.35c	41.25 ± 1.38e
	F4	111.26 ± 0.52e	102.46 ± 2.29f	49.60 ± 0.51c	41.17 ± 0.50e
PNT	**D5**	116.5 ± 0.87f	95.03 ± 0.62e	45.10 ± 0.17b	52.39 ± 0.88f
	F5	121.46 ± 1.22f	123.20 ± 1.96g	46.46 ± 0.40b	50.36 ± 1.20f

D1-D5: powder fractions from cooked leaves, F1-F5: powder fractions from raw leaves, PNT: unsieved powder. Values are mean ± standard deviation (n = 5). Different superscript letters within the same column indicate significant differences ($p < 0.05$) according to Tukey's multiple range test.

Table 6
Effects of *M. balsamina* powder fractions on atherogenic parameters in hyperlipidemic rats

Groups		***LDL-c/HDL-c (mg/dL)***	***TC/HDL-c (mg/dL)***
Normal Control		0.64 ± 4.35^{c}	1.96 ± 4.41^{cd}
Hyperlipidemic Control		7.69 ± 5.64^{f}	10.20 ± 5.08^{h}
Reference control		0.36 ± 0.83^{a}	1.63 ± 0.74^{a}
≤ 50 µm	D1	0.38 ± 1.09^{a}	1.66 ± 0.25^{ab}
	F1	1.16 ± 2.83^{e}	2.21 ± 1.96^{e}
50–125 µm	D2	0.35 ± 4.53^{a}	1.61 ± 4.20^{a}
	F2	0.48 ± 0.92^{b}	1.76 ± 1.32^{bc}
125–200 µm	D3	0.67 ± 2.31^{c}	1.99 ± 1.66^{cd}
	F3	0.69 ± 1.00^{c}	2.04 ± 0.52^{b}
≥300 µm	D4	0.84 ± 3.9^{d}	2.20 ± 2.37^{e}
	F4	0.83 ± 0.98^{d}	2.24 ± 1.01^{ef}
PNT	D5	1.16 ± 5.17^{e}	2.58 ± 5.11^{f}
	F5	1.08 ± 3.00^{e}	2.61 ± 3.05^{fg}

D1-D5: powder fractions from cooked leaves, F1-F5: powder fractions from raw leaves, PNT: unsieved powder. Values are mean ± standard deviation (n = 5). Different superscript letters within the same column indicate significant differences ($p < 0.05$) according to Tukey's multiple range test.

3.3.3 Effect on hepatic and renal parameters

The data presented in Table 7 indicate that *M. balsamina* leaf powder fractions have varying effects on hepatic and renal parameters. The D2 fraction (50–125 µm, cooked leaves) exhibits the most beneficial effects, with significant reductions in ASAT/ALAT (1.49 ± 1.50), creatinine (0.79 ± 0.01 µmol/mL), and urea (36.56 ± 0.61 µmol/mL) compared to the hyperlipidemic control group (1.97 ± 0.72, 1.60 ± 0.17 µmol/mL, and 53.33 ± 1.97 µmol/mL, respectively). These values are similar to those of the normal control group and the reference control group, indicating a significant protection of liver and kidney function ($p < 0.05$). In contrast, the D4, F4, PNT D5, and F5 groups show significantly higher values for these parameters, suggesting a lesser protective effect. Overall, the D2 fraction appears to have the most promising effects on hepatic and renal parameters.

Table 7
Effect of *M. balsamina* leaf fractions on Ritis quotient, plasma creatinine, and urea in experimental rats

Groups		ASAT/ALAT (µmol/mL)	Creatinine (µmol/mL)	Urea (µmol/mL)
Normal Control		1.58 ± 1.33^{ab}	0.69 ± 0.10^{a}	36.53 ± 2.65^{a}
Hyperlipidemic Control		1.97 ± 0.72^{e}	1.60 ± 0.17^{f}	53.33 ± 1.97^{f}
Reference control		1.49 ± 0.66^{a}	0.76 ± 0.05^{ab}	37.40 ± 1.48^{bc}
≤ 50 µm	**D1**	1.59 ± 0.52^{ab}	0.90 ± 0.10^{bcde}	38.06 ± 0.89^{bcd}
	F1	1.59 ± 0.75^{ab}	0.92 ± 0.06 ^{bcde}	38.40 ± 0.75^{bcde}
50–125 µm	**D2**	1.49 ± 1.50^{a}	0.79 ± 0.01^{abc}	36.56 ± 0.61^{a}
	F2	1.55 ± 2.33^{ab}	0.81 ± 0.03^{abcd}	36.66 ± 0.66^{a}
125–200 µm	**D3**	1.55 ± 0.90^{ab}	0.95 ± 0.13^{cde}	38.40 ± 0.81^{bcde}
	F3	1.52 ± 0.77^{ab}	0.96 ± 0.11^{de}	38.73 ± 0.32^{bcde}
300 µm	**D4**	1.66 ± 1.47^{c}	0.99 ± 0.11^{e}	39.40 ± 0.75^{cde}
	F4	1.64 ± 1.13^{c}	1.01 ± 0.07^{e}	40.06 ± 0.55^{de}
PNT	**D5**	1.65 ± 1.41^{c}	0.99 ± 0.10^{e}	40.30 ± 0.70b
	F5	1.71 ± 0.63^{cd}	1.02 ± 0.06^{e}	40.70 ± 0.85^{de}

D1-D5: powder fractions from cooked leaves, F1-F5: powder fractions from raw leaves, PNT: unsieved powder. Values are mean ± standard deviation (n = 5). Different superscript letters within the same column indicate significant differences ($p < 0.05$) according to Tukey's multiple range test.

3.3.4 Effect on oxidative enzymes

Figure 1 and Fig. 2 illustrate the activities of superoxide dismutase (SOD) and catalase in the liver and kidneys of various experimental animal groups. The hyperlipidemic group shows a significant decrease ($p < 0.001$) in SOD and catalase activity compared to the normal group, indicating substantial oxidative stress induced by the high-fat diet. Among the fractions, D2 and D1 exhibit the best results, with SOD increase rates of 109.98% (86.03 ± 0.81 U/g of organ) in the liver and 128.06% (79.8 ± 0.28 U/L) in plasma, respectively, for fraction D2, and 106.49% (84.60 ± 1.10 U/g of organ) in the liver and 125% (78.73 ± 0.78 U/L) in plasma for fraction D1. Catalase activity in the liver and plasma of groups D1-D5 and F1-F5 is similar to that of the positive control group (ATOR), and significantly higher than that of the hyperlipidemic group (GH) ($p < 0.05$).

PNT: unsieved powder; GN: normal control group, GH: hyperlipidemic control group, ATOR: reference control group. Values are mean ± standard deviation (n = 5). Different superscript letters indicate significant differences ($p < 0.05$) according to Tukey's multiple range test.

PNT: unsieved powder, GN: normal control group, GH: hyperlipidemic control group, ATOR: reference control group. Values are mean ± standard deviation (n = 5). Different superscript letters indicate significant differences ($p < 0.05$) according to Tukey's multiple range test.

3.3.5 Effect on MDA

The malondialdehyde level in the liver and kidneys of the different groups of rats subjected to various treatments is shown in Fig. 3A and B. Overall, these figure shows that the MDA level observed in the liver and plasma of hyperlipidemic rats (GH) increased significantly ($p < 0.05$), approximately 152% (in the liver) and 293% (in plasma) compared to the normal group (GN). This elevation of MDA is consistent with an increased production of reactive oxygen species (ROS), resulting from excessive lipid oxidation. Administration of the powdered fractions reduced the MDA level to normal, as there was no significant difference with the normal control at the end of the treatment.

PNT: unsieved powder, GN: normal control group, GH: hyperlipidemic control group, ATOR: reference control group. Values are mean ± standard deviation (n = 5). Different superscript letters indicate significant differences ($p < 0.05$) according to Tukey's multiple range test.

3.3.6 Principal Component Analysis

The correlation matrix of analysis presented in Fig. 4 showed a strong and positive correlation between total phenolic compounds and flavonoids ($r = 0.841$), with condensed tannins ($r = 0.865$) and with the percentage of inhibition ($r = 0.977$). Between HDL-C level and phenolic compounds ($r = 0.859$), flavonoids ($r = 0.761$), and tannins ($r = 0.707$). Between hepatic superoxide dismutase (SODf) and total phenolic compounds, flavonoids, and condensed tannins, respectively $r = 0.924$, $r = 0.913$, and $r = 0.896$. Additionally, a strong and negative correlation was observed between total cholesterol and total phenolic compounds, flavonoids, and condensed tannins with respective values $r = -0.980$, $r = 0.896$, and $r = -0.909$; Between plasma MDA level with total phenolic compounds ($r = -0.884$), with flavonoids ($r = 0.805$), and with tannins ($r = 0.704$); Between phenolic compounds, flavonoids, tannins, and ALAT level ($r = -0.942$, $r = -0.866$, and $r = -0.751$ respectively); Between creatinine level and phenolic compounds, flavonoids ($r = -0.856$, $r = 0.813$).

WG: Weight gain after treatment, TPC: Total Polyphenols Content, TFC: Total Flavonoids Content, CTC: Condensed tannin content, UR: Urea, CRT: Creatinine, RP: Reducing powder, TG: triglyceride, CT: total cholesterol, LDL-c: LDL cholesterol, HDL-c: HDL cholesterol, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CATh: hepatic catalase, CATs: serum catalase, SODh: hepatic superoxide dismutase, SODs: serum superoxide dismutase

4. Discussion

The analysis of the results obtained in this study highlights the significant impact of particle size and thermal treatment on the bioactive compound content of *M. balsamina* leaves. Table 2 shows that fine fractions consistently exhibit higher levels of total polyphenols, flavonoids, and condensed tannins than coarse fractions, suggesting that reducing particle size improves the availability of these compounds. Thermal treatment also appears to play an important role in releasing bioactive compounds. Heat breaks down cell wall structures, releasing polyphenols bound to fibers and improving their extractibility and bioavailability. This is consistent with the results of Turkmen et al. (2005) and Dewanto et al. (2002), who showed that thermal treatment can increase polyphenol content and improve their bioavailability [29, 30]. The increase in total polyphenol, flavonoid, and condensed tannin values in fine fractions can be explained by several factors. Firstly, reducing particle size leads to an increase in the specific surface area of particles, exposing hydrophilic groups of phenolic compounds to react easily with water or solvents, thereby increasing their bioavailability [31]. Additionally, ultra-fine grinding can break down structures that bind polyphenols, preventing their bioavailability [8]. Finally, sieving may also play a role in the distribution of bioactive compounds. More rigid fibrous and carbohydrate structures are retained in larger particle size classes, while smaller polyphenols are concentrated in smaller size classes [7]. This explains why fine fractions are richer in bioactive compounds than coarse fractions.

Building on the findings from Table 2, the results in Table 3 demonstrate that *M. balsamina* powders possess significant antioxidant activity, with IC50 DPPH values ranging from 0.203 mg/mL to 0.698 mg/mL. This is likely due to the high content of phenolic compounds, including polyphenols, flavonoids, and tannins, which are known to neutralize free radicals and exhibit reducing power [32, 33] The strong antioxidant activity of fractions D2, D1, F2, and F1 can be attributed to their ability to donate electrons or protons, thus scavenging reactive oxygen species (ROS) and preventing oxidative damage. The Fe^{3+} ¯reducing assay is a well-known method for assessing the antioxidant capacity of a variety of biological samples, such as plant extracts, food, beverages, and pharmaceuticals. It is based on the reduction of ferric ions (Fe3+) to ferrous ions (Fe2+) in the presence of antioxidants acting as reducing agents [34]. The reducing power of these fractions may also contribute to their antioxidant effect, possibly by modifying the redox state of metal ions, such as iron, and preventing the formation of ROS.

The analysis of the effects of *M. balsamina* leaf powder on body weight gain in hyperlipidemic rats, presented in Table 4, reveals that *M. balsamina* powder fractions significantly reduced body weight gain in hyperlipidemic rats. The 50–125 µm fractions (D2 and F2) exhibited the most pronounced effects, followed by the ≤ 50 µm fractions (D1 and F1). These results are consistent with those of Kgosana et al (2025), who reported that *M. balsamina* leaf-rich diet reduced body weight in obese rats [35]. The presence of phenolic compounds in these powder fractions as results have previously showed could explain the observed effects on weight. Through the inhibition of digestive enzymes linked to obesity, the modulation of neurohormones and peptides involved in food intake, and the enhancement of the growth of helpful gut microorganisms while suppressing pathogenic ones, these phytonutrients function as powerful anti-obesity agents.[36, 37].

The combined results from Tables 5 and 6 indicate that *M. balsamina* powder fractions exhibit beneficial effects on lipid profile and atherogenic parameters in hyperlipidemic rats. Specifically, the D2 fraction (50–125 µm, cooked leaves) reduces total cholesterol, triglycerides, and LDL-cholesterol, while increasing HDL-cholesterol. Furthermore, the D1 and D2 fractions significantly decrease LDL-c/HDL-c and TC/HDL-c ratios, indicating a reduction in atherogenic risk (Table 6). It has been discovered that *M. balsamina* improves lipid profiles by raising high-density lipoprotein cholesterol and decreasing low-density lipoprotein (LDL) cholesterol. Through their modulation of enzymes involved in cholesterol manufacture and promotion of the removal of LDL from the bloodstream, the flavonoids and sterols found in *M. balsamina* help to achieve this lipid-regulating action. This hypocholesterolemic action is supported by its antioxidant qualities [38, 39, 40] The concomitant decrease in LDL-c/HDL-c and TC/HDL-c ratios suggests an anti-atherogenic potential of *M. balsamina* leaves, likely contributing to the prevention of cardiovascular disorders associated with dyslipidemia, in line with previous studies on the hypolipidemic effects of *M. balsamina* rich in phenolic compounds [9]. Moreover, *M. balsamina* aids in preventing the production of oxidized LDL, a crucial component in the development of atherosclerosis, by lowering oxidative stress, which can lead to the oxidation of LDL cholesterol. This defense against oxidative damage lowers the chance of blood vessel plaque development, which benefits cardiovascular health even more.

The liver secretes many enzymes, including transaminases (ALT and AST), and is involved in a range of metabolic processes. These parameters serve as biological indicators of liver disease [41]. These liver-related biological markers were found to be significantly increased in the blood of rats given a high-fat diet, indicating liver damage and dysfunction resulting from such a diet (Table 7). We observed an elevation of AST and ALT in the control group receiving a hyperlipidemic diet without treatment. Since these enzymes are cytoplasmically localized and released into the bloodstream following cellular damage, the inability to protect the liver's structural integrity may be the cause of the elevated blood levels of AST and ALT [42]. The amount of ALT in the blood indicates the extent of cell membrane damage, while the amount of AST indicates mitochondrial damage, as 80% of this enzyme is found in mitochondria [43]. Administration of *M. balsamina* leaf powders significantly reduced these values, with the fine fractions showing greater efficacy and values approaching those of the normal control.

Our results also showed a significant ($p < 0.05$) increase in plasma creatinine and urea levels in *Wistar* rats fed a high-fat diet without treatment. According to Steven *et al.* (2023), the rats' liver and kidneys accumulated fat because of their high-calorie diet [44]. The buildup of fat in the liver causes rats to develop fatty liver disease, or hepatic steatosis, which accounts for the rise in the levels of liver biochemical markers (AST and ALT). However, these animals' kidneys' increased fat content hinders them from fulfilling their functions, particularly blood purification. As metabolic waste products such urea and creatinine are removed less frequently, their blood content rises [44]. Administration of *M. balsamina* powder fractions significantly reduced plasma urea and creatinine levels. Specifically, fraction D2 (50–125 µm, cooked leaves) significantly reduced AST/ALT, creatinine, and urea levels, indicating protection against liver and kidney damage. This may be due to the presence of bioactive compounds such as phenols, flavonoids, proanthocyanidins, steroids, terpenoids and alkaloids in *M. balsamina* leaf

as previous reported [45, 46]. Quercetin present in *M. balsamina* is known to preserve the function of the liver in acute alcoholic injury by upregulating the expression of Interleukin 10 and Oxygenase-1 and thus inhibiting NLRP3 inflammasome activation and inflammatory factor secretion [47].

Superoxide dismutase and catalase are the most important antioxidant enzymes in the body's antiradical system. SOD catalyzes the dismutation of the superoxide anion ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), while catalase degrades hydrogen peroxide (H_2O_2) into water (H_2O) [48, 49]. Figure 1 and Fig. 2 show a decrease in the activity of these enzymes in untreated hyperlipidemic rats. Indeed, hyperlipidemia is known to promote triglyceride accumulation, lipid peroxidation, and increased mitochondrial H_2O_2, gradually depleting protective enzymatic systems such as superoxide dismutase and catalase. This phenomenon is widely described in the literature, notably by Jeong et al. (2025) who demonstrate that obesity and lipid excess increase ROS production and decrease antioxidant enzymes, and by Schmatz et al. (2017) who confirm a systematic reduction of catalase in obese patients [50, 51]. The effect of powdered fractions on the activities of these enzymes could be explained by an increased ability of powdered fractions to restore enzymatic defense, probably due to a better release of active compounds after gentle cooking and particle size reduction. These observations are in line with the work of Becker et al. (2017) and Deli et al. (2019), who show that the particle size of plant materials directly influences the bioavailability of antioxidants, as well as the work of Souda et al. (2018) on *Momordica* leaves, which demonstrate their exceptional antioxidant power linked to a high content of flavonoids, cucurbitacins, and phenols [7, 8, 52].

Malondialdehyde (MDA) is a final product of polyunsaturated fatty acid oxidation and is one of the most widely used biomarkers of tissue oxidative stress. An increase in MDA indicates an intensification of lipid peroxidation, thus oxidative damage to cell membranes, particularly in the liver and kidney, which are key organs of detoxification and excretion [53, 54]. Pathophysiologically, excess circulating lipids promote lipoprotein oxidation, notably LDL, generating free radicals capable of initiating lipid peroxidation chain reactions [53]. These reactions lead to the formation of toxic secondary products, including MDA, capable of altering membrane integrity, modifying proteins, and disrupting cellular enzyme functions. The high levels of MDA measured in the liver of untreated rats are explained by the central role of this organ in lipid metabolism and detoxification, making it particularly exposed to redox imbalances [54]. However, the granulometric fractions from cooked (D1-D5) and uncooked (F1-F5) leaves of *Momordica balsamina* induce a significant and progressive decrease in MDA levels in both organs studied, compared to the hyperlipidemic group at the statistical threshold $p < 0.05$. This decrease indicates effective inhibition of lipid peroxidation and protection of cell membranes against oxidative damage. The cooked fractions generally show a more marked effect than the uncooked fractions, suggesting that heat treatment (10-minute boiling) may promote the release of antioxidant compounds such as polyphenols, flavonoids, and vitamins by breaking down cell wall structures, thus facilitating their extractibility. These compounds are known for their ability to interrupt radical chain reactions [55]. Furthermore, fine particle size fractions are distinguished by a greater reduction in MDA levels, which can be attributed to better bioavailability of bioactive compounds. Fraction D2 shows the best result with a

reduction rate of approximately 53% (in the liver) and 71% (in plasma) compared to the hyperlipidemic group. The decrease in particle size increases the contact surface, promotes the release of antioxidant metabolites, and improves their intestinal absorption, thus enhancing their biological efficacy. These compounds can act either by direct scavenging of free radicals or by stimulating endogenous antioxidant systems, contributing to the reduction of MDA formation.

The Principal Component Analysis (PCA) carried out on lipid, biochemical, and antioxidant parameters, as well as bioactive compounds, revealed a clear structuring of the experimental groups and highlighted relationships between the different variables studied. The first two principal components explain 89.85% of the total variance, with a major contribution of the PC1 axis (82.02%), indicating a very good representation of the initial information. The results confirms that bioactive compounds such as phenolic compounds, flavonoids, and condensed tannins are directly associated with antioxidant and hypolipidemic activities as described by several authors [56, 57]

5. CONCLUSION

The study demonstrates that *M. balsamina* leaf powders, particularly the cooked and finely ground fractions (D1 and D2), exhibit significant antioxidant, hypolipidemic, and hepatoprotective effects in hyperlipidemic rats. The bioactive compounds, including polyphenols, flavonoids, and condensed tannins, are more readily available in cooked and finer particle size fractions, contributing to enhanced biological activities. The D2 fraction (50–125 µm, cooked leaves) shows the most promising results, with significant reductions in total cholesterol (53.3%), triglycerides (48.7%), and LDL-cholesterol (86.4%), as well as increased HDL-cholesterol (194.2%). Additionally, it improves antioxidant enzyme activities (SOD and catalase), reduces oxidative stress (MDA levels), and exhibits hepatoprotective and renal protective effects. The correlation analysis confirms that the bioactive compounds are directly associated with the observed biological activities, suggesting that Momordica balsamina leaf powder, particularly the D2 fraction, could be a valuable natural ingredient for the prevention and management of hyperlipidemia and related oxidative stress disorders.

Declarations

Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose and the corresponding author states that there is no conflict of interest.

Consent for publication

Not applicable

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Ethical approval

All experimental procedures were reviewed and approved by the Ethics Committee of the Postdoctoral Research Unit in Food Science and Nutrition, University of Ngaoundéré, under approval number 06-/2026.

References

1. **Dehghan A, Rezaei F, Aune D. A comparative assessment between Globorisk and WHO cardiovascular disease risk scores: a population – based study. Sci Rep. 2023;13:1–11. https://doi.org/10.1038/s41598-023-40820-3.**
2. **Feingold KR. Lipid and Lipoprotein Metabolism. Endocrinol Metab Clin North Am. 2022;51:437–58. org/10.1016/j.ecl.2022.02.008. https://doi.org/https://doi..**
3. **Goldstein JL, Brown MS. A Century of Cholesterol and Coronaries: From Plaques to Genes to Statins. Cell. 2015;161:161–72. https://doi.org/10.1016/j.cell.2015.01.036.A.**
4. **Hossain A, Khatun MA, Islam M, Huque R. Enhancement of Antioxidant Quality of Green Leafy Vegetables upon Different Cooking Method. Prev Nutr Food Sci. 2017;22:216–22. https://doi.org/https://doi.org/10.3746/pnf.2017.22.3.216.**
5. **Baudelaire E. Comminution and controlled differential screening method for the dry extraction of natural active principles. Brevet: PCT/FR2011/000561; 2013. p. 6.**
6. **Zaiter A, Becker L, Karam M, Dicko A. Effect of particle size on antioxidant activity and catechin content of green tea powders. J Food Sci Technol. 2016;53:2025–32. https://doi.org/10.1007/s13197-016-2201-4.**
7. **Becker L, Zaite A, Petit J, et al. How do grinding and sieving impact on physicochemical properties, polyphenol content, and antioxidant activity of Hieracium pilosella L. powders? J Funct Foods. 2017;35:666–72. https://doi.org/https://doi.org/10.1016/j.jff.2017.06.043.**
8. **Deli M, Baudelaire E, Josiane N, et al. Successive grinding and sieving as a new tool to fractionate polyphenols and antioxidants of plants powders: Application to Boscia senegalensis seeds, Dichrostachys glomerata fruits, and Hibiscus sabdariffa calyx powders. Food Sci Nutr. 2019;7:1795–806. https://doi.org/10.1002/fsn3.1022.**
9. **Thiaw M, Samb I, Genva M, et al. Momordica balsamina L.: A Plant with Multiple Therapeutic and Nutritional Potential—A. Rev Nutraceuticals. 2023;3:556–73. https://doi.org/10.3390/nutraceuticals3040040.**

10. Mashiane P, Shoko T, Manhivi V, et al. A Comparison of Bioactive Metabolites, Antinutrients, and Bioactivities of African Pumpkin Leaves (Momordica balsamina L.) Cooked by Different Culinary Techniques. Molecules. 2022;27. https://doi.org/https://doi.org/10.3390/molecules27061901.
11. Smith JA, Broek FAR, van den, Martorell JC, et al. Principles and practice in ethical review of animal experiments across Europe: summary of the report of a FELASA working group on ethical evaluation of animal experiments. FELASA Work Gr Ethical Eval Anim Exp. 2007;41:143–60.
12. Biyanzi P, Ndjouenkeu R, Mbofung CM, et al. Physicochemical Characteristics, Nutritional Value and Antioxidant Activity of Ziziphus mauritiana Fruit and Processing Product in Savannah Region of Cameroon. Int J Food Nutr Saf. 2018;9:75–99.
13. Wafa G, Amadou D, Larbi KM, Héla EFO. Larvicidal activity, phytochemical composition, and antioxidant properties of different parts of five populations of Ricinus communis L. Ind Crop Prod jo. 2014;56:43–51. https://doi.org/http://dx.doi.org/10.1016/j.indcrop.2014.02.036.
14. Dewanto V, Wu X, Adom K, Liu R. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. J Agric Food Chem. 2002;50:3010–4. https://doi.org/http://dx.doi.org/10.1021/jf0115589.
15. Sun B, Ricardo-da-Silvas JM, Pranger I. Critical Factors of Vanillin Assay for Catechins and Proanthocyanidins. J Agric Food Chem. 1998;46:4267–74. https://doi.org/10.1021/jf980366j.
16. Zhang D, Hamauzu Y. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chem. 2004;88:503–9. https://doi.org/10.1016/j.foodchem.2004.01.065.
17. Oyaizu M. Studies on Products of Browning Reaction: Antioxidative Activities of Products Browning Reaction Prepared from Glucosamine. Jpn J Nutr. 1986;44:307–17.
18. Garait B, LE STRESS OXYDANT INDUIT PAR VOIE METABOLIQUE (REGIMES ALIMENTAIRES) OU PAR VOIE GAZEUSE (HYPEROXIE). (2006) ET EFFET DE LA GLISODIN ®. In: Biologie cellulaire. Université Joseph-Fourier - Grenoble I. p 197.
19. Ngatchic JMT, Fomekong CG, Baudelaire NE, Njintang YN. Antioxidant and Antihyperlipidemic Properties of Different Granulometric Classes of Adansonia digitata Pulp Powder. Pakistan J Nutr. 2020;19:393–403. https://doi.org/10.3923/pjn.2020.393.403.
20. Naito HK. High-Density Lipoprotein (HDL) Cholesterol. Clin Chem. 1984;437:1207–13.
21. Gordon T, Castelli WP, Hjortland MC, et al. High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. Am J Med. 1977;62:707–14. https://doi.org/10.1016/0002-9343(77)90874-9.
22. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982;28:2077–80.
23. Friedewald W, Levy R, Fredrickson D. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin chemestry. 1972;18:499–502.

24. Reitman S, Frankel S. A Colorimetric Method for the Determination of Serum Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases. Am J Clin Pathol. 1957;28:53–6. https://doi.org/https://doi.org/10.1093/ajcp/28.1.56.
25. Bartels H, Cikes M. Chromogens in the Creatinine Determination of Jaffé. Clin Chim acta. 1969;26:1–10. https://doi.org/https://doi.org/10.1016/0009-8981(69)90278-2.
26. Misra HP, Fridovich I. The Role of Superoxide Anion in the Epinephrine and a Simple Assay for Superoxide Dismutase. J Biol Chem. 1972;247:3170–5. https://doi.org/10.1016/S0021-9258(19)45228-9.
27. Sinha AK. Calorimetric Assay of Catalase. Anal Biochem. 1972;47:389–94. https://doi.org/https://doi.org/10.1016/0003-2697(72)90132-7.
28. Placer A, Cushman L, Connor B. Estimation of Product of Lipid Peroxidation (Malonyldialdehyde) in Biochemical Systems. Anal Biochem. 1966;16:359–64. https://doi.org/https://doi.org/10.1016/0003-2697(66)90167-9.
29. Turkmen N, Sari F, Velioglu YS. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chem. 2005;93:713–8. https://doi.org/10.1016/j.foodchem.2004.12.038.
30. Dewanto V, Xianzhong W, ADom KK, Lui Rha. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. J Agric Food Chem. 2002;50:3010–4.
31. Prasedya ES, Frediansyah A, Martyasari NWR, et al. Effect of particle size on phytochemical composition and antioxidant properties of Sargassum cristaefolium ethanol extract. Sci Rep. 2021;11:17876. https://doi.org/10.1038/s41598-021-95769-y.
32. Bas TG. (2026) Dietary Polyphenols (Flavonoids) Derived from Plants for Use in Therapeutic Health: Antioxidant Performance, ROS, Molecular Mechanisms, and Bioavailability Limitations. Int J Mol Sci 2026, 27:1–55. https://doi.org/https://doi.org/10.3390/ijms27031404
33. Zahra M, Abrahamse H, George BP. (2024) Flavonoids: Antioxidant Powerhouses and Their Role in Nanomedicine. Antioxidants 13:. https://doi.org/https://doi.org/10.3390/antiox13080922
34. Gulcin Ïi, Alwasel SH. (2025) Fe 3 + Reducing Power as the Most Common Assay for Understanding the Biological Functions of Antioxidants. processes 13:1–20. https://doi.org/https://doi.org/10.3390/pr13051296
35. Kgosana MR, Mayimele NN. The use and benefits of Momordica balsamina L. (Nkaka) amongst Bantu people in southern Africa : From traditional food source to modern medicine. J Appl Pharm Sci. 2025;15:1–7. https://doi.org/10.7324/JAPS.2025.224194.
36. Aloo S, Ofosu FK, Kim N, Kilonzi SM. Insights on Dietary Polyphenols as Agents against Metabolic Disorders: Obesity as a Target Disease. Antioxidants. 2023;12:1–36. https://doi.org/https://doi.org/10.3390/antiox12020416.
37. Mamun A, Al, Rakib A, Mandal M et al. (2024) Polyphenols: Role in Modulating Immune Function and Obesity. Biomolecules 14:. https://doi.org/https://doi.org/10.3390/biom1402022

38. Wu Y, Tan F, Zhang T, et al. The anti – obesity effect of lotus leaves on high – fat – diet – induced obesity by modulating lipid metabolism in C57BL / 6J mice. Appl Biol Chem. 2020;63. https://doi.org/10.1186/s13765-020-00541-x.
39. Muronga M, Quispe C, Tshikhudo PP, et al. Three Selected Edible Crops of the Genus Momordica as Potential Sources of Phytochemicals: Biochemical, Nutritional, and Medicinal Values. Front Pharmacol. 2021;12:1–19. https://doi.org/10.3389/fphar.2021.625546.
40. Choudhary BR, Berwal MK, Ram H et al. (2022) Momordica balsamina L.: An unexploited vegetable crop rich in medicinal and nutritional properties. J Agric Ecol | 14:84–92. https://doi.org/https://doi.org/10.53911/JAE.2022.14212 Associate.
41. Daum G. Lipids of mitochondria. Biochim Biophys Acta. 1985;822:1–42. https://doi.org/https://doi.org/10.1016/0304-4157(85)90002-4.
42. Recknagel RO, Glende EA, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmac Ther. 1989;43:139–54. https://doi.org/https://doi.org/10.1016/0163-7258(89)90050-8.
43. Tang X, Gao J, Wang Y, et al. Effective protection of Terminalia catappa L. leaves from damage induced by carbon tetrachloride in liver mitochondria. J Nutr Biochem. 2006;17:177–82. https://doi.org/10.1016/j.jnutbio.2005.06.008.
44. Steven CJAPTV, Pascal TA, Justin BG, et al. Impact of a high-calorie diet on liver and kidneys in Wistar rats. Int J Biosci. 2023;23:27–37. https://doi.org/http://dx.doi.org/10.12692/ijb/23.2.27-37.
45. Zahra K, Malik MA, Mughal MS, et al. HEPATOPROTECTIVE ROLE OF EXTRACTS OF MOMORDICA CHARANTIA L. IN ACETAMINOPHEN-INDUCED TOXICITY IN RABBITS. J Anim Plant Sci. 2012;22:273–7.
46. Okoro SE, Ossai GE, Anacletus FC. Organo-protective Potentials of Momordica balsamina aqueous plant extract against cadmium-induced stress in wistar rats. World J Adv Res Rev. 2024;24:2010–9. https://doi.org/https://doi.org/10.30574/wjarr.2024.24.3.3727.
47. Liu S, Tian L, Chai G, et al. Function ameliorates alcohol-induced acute liver injury via. Food Funct. 2018. https://doi.org/10.1039/c8fo00650d.
48. Jomova K, Alomar SY, Alwasel SH, et al. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Arch Toxicol. 2024;98:1323–67.
49. Xie Y, Gu Y, Li Z, et al. Effects of exercise on different antioxidant enzymes and related indicators: a systematic review and meta-analysis of randomized controlled trials. Sci Rep. 2025;15:1–15. https://doi.org/10.1038/s41598-025-97101-4 1.
50. Jeong I, Lee J, Park S-J, Kim O-K. High fat diet enhances catalase loading into adipose tissue derived extracellular vesicles with limited effect on oxidative stress. Sci Afr. 2025;15. https://doi.org/10.1038/s41598-025-15594-5.
51. Schmatz R, Bitencourt MR, Patias LD, et al. Clinica Chimica Acta Evaluation of the biochemical, in fl ammatory and oxidative pro fi le of obese patients given clinical treatment and bariatric surgery. Clin Chim Acta. 2017;465:72–9. https://doi.org/10.1016/j.cca.2016.12.012.

52. Souda S, George S, Mannathoko N, et al. Antioxidant and Antibacterial Activity of Methanol Extract of Momordica Balsamina. IRA-International J Appl Sci (ISSN 2455–4499). 2018;10:7–17. https://doi.org/10.21013/jas.v10.n2.p1.
53. Ayala A, Muñoz MF, Argüelles S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med Cell Longev. 2014;2014:31. https://doi.org/10.1155/2014/360438.
54. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2016. https://doi.org/10.1016/j.ab.2016.10.021.
55. Toydemir G, Gultekin B, Hall RD, et al. Effect of food processing on antioxidants, their bioavailability and potential relevance to human health. Food Chem X. 2022;14. https://doi.org/10.1016/j.fochx.2022.100334.
56. Derouich M, Bouhlali EDT, Bammou M et al. (2020) Bioactive Compounds and Antioxidant, Antiperoxidative, and Antihemolytic Properties Investigation of Three Apiaceae Species Grown in the Southeast of Morocco. Scientifica (Cairo) 2020:. https://doi.org/10.1155/2020/3971041
57. Li Y, Zhu L, Guo C, et al. Dietary Intake of Hydrolyzable Tannins and Condensed Tannins to Regulate Lipid Metabolism. Mini Rev Med Chem. 2022;22:1789–802. https://doi.org/https://doi.org/10.2174/1389557522666211229112223.

Figures

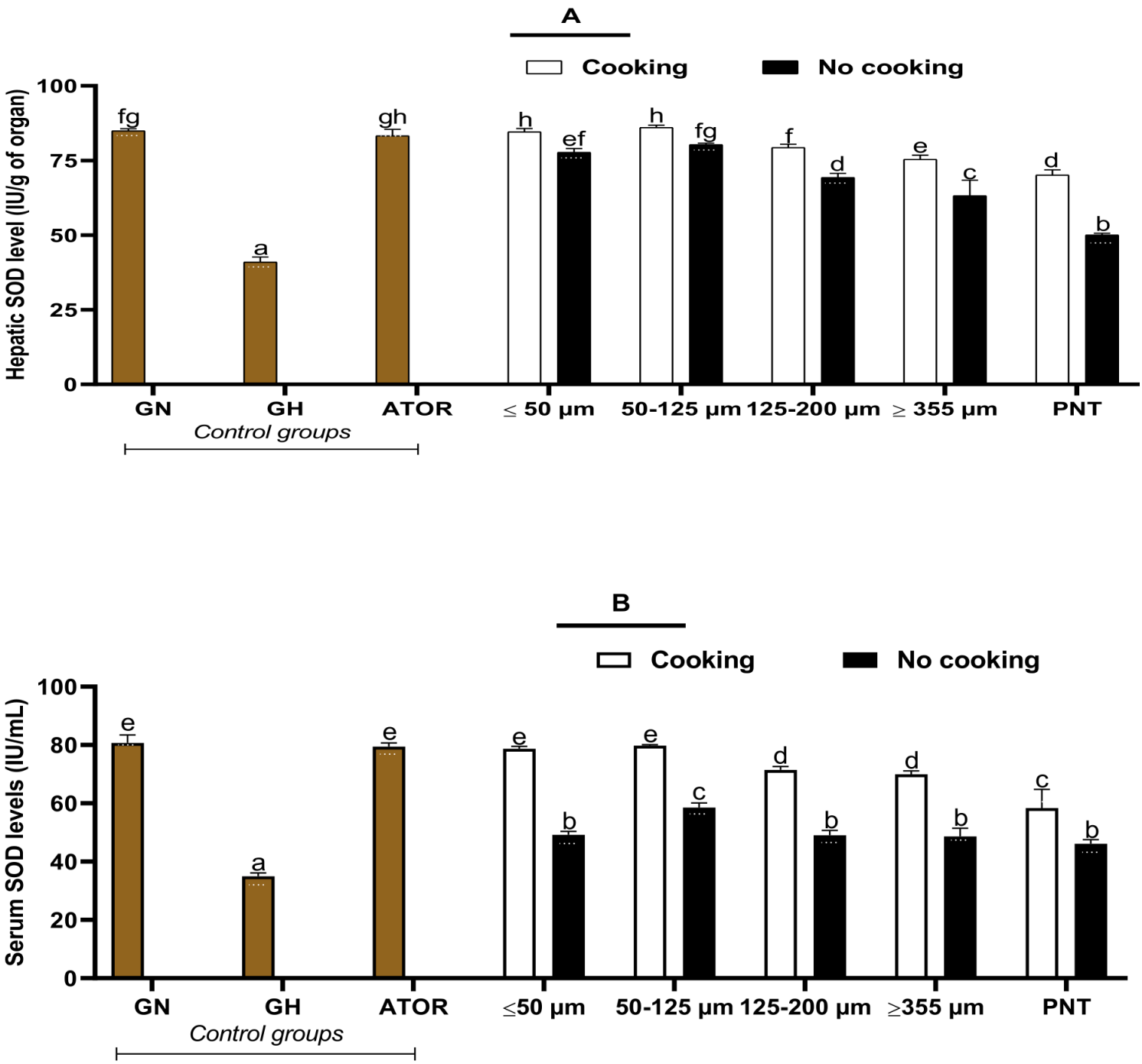


Figure 1

Variation in superoxide dismutase (SOD) levels in the liver (A) and serum (B) of rats undergoing hypolipidemic treatment

PNT: unsieved powder; GN: normal control group, GH: hyperlipidemic control group, ATOR: reference control group. Values are mean ± standard deviation (n=5). Different superscript letters indicate significant differences ($p<0.05$) according to Tukey's multiple range test.

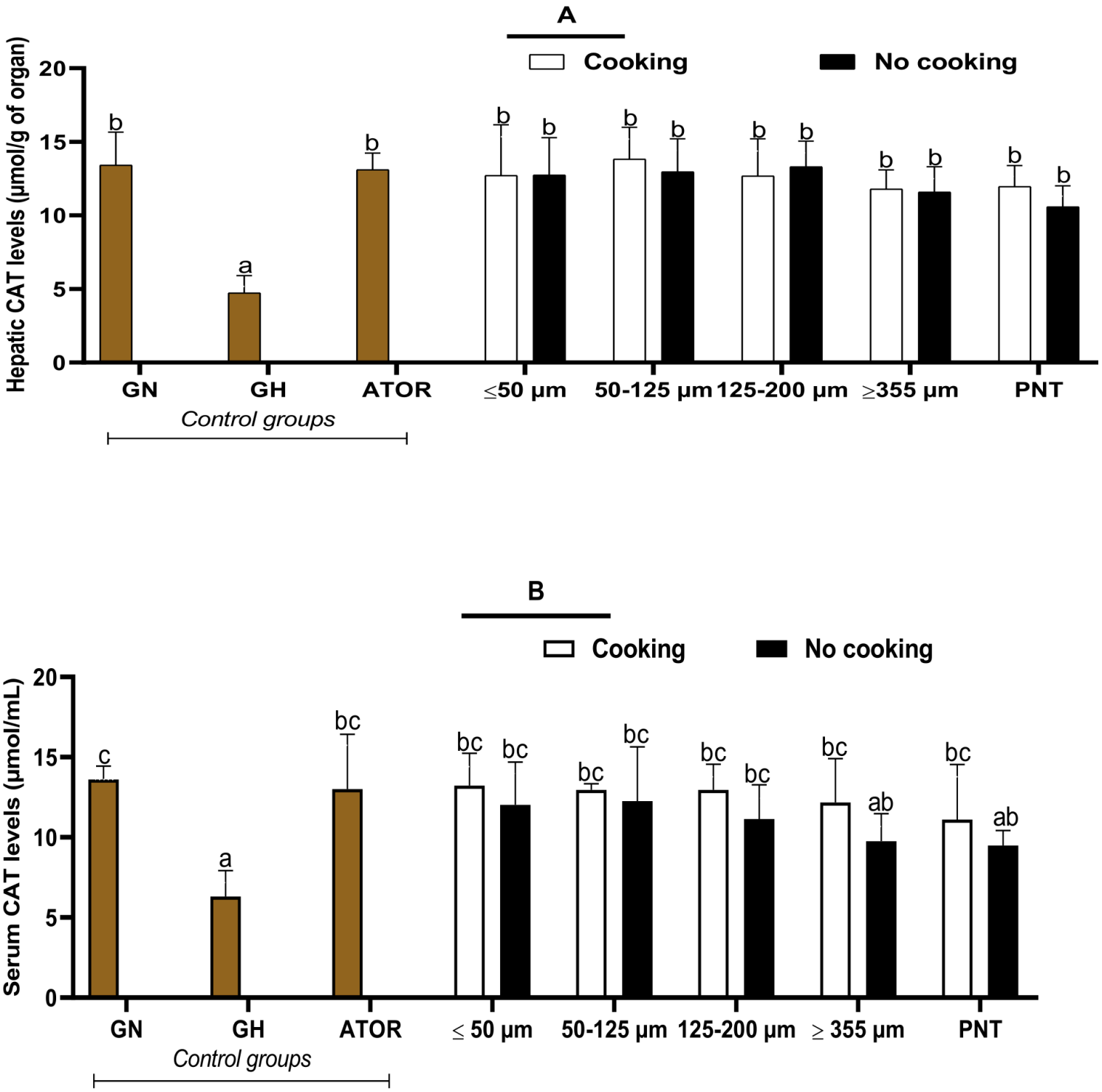


Figure 2

Variation in catalase (CAT) levels in the liver (A) and serum (B) of rats undergoing hypolipidemic treatment

PNT: unsieved powder, GN: normal control group, GH: hyperlipidemic control group, ATOR: reference control group. Values are mean ± standard deviation (n=5). Different superscript letters indicate significant differences ($p<0.05$) according to Tukey's multiple range test.

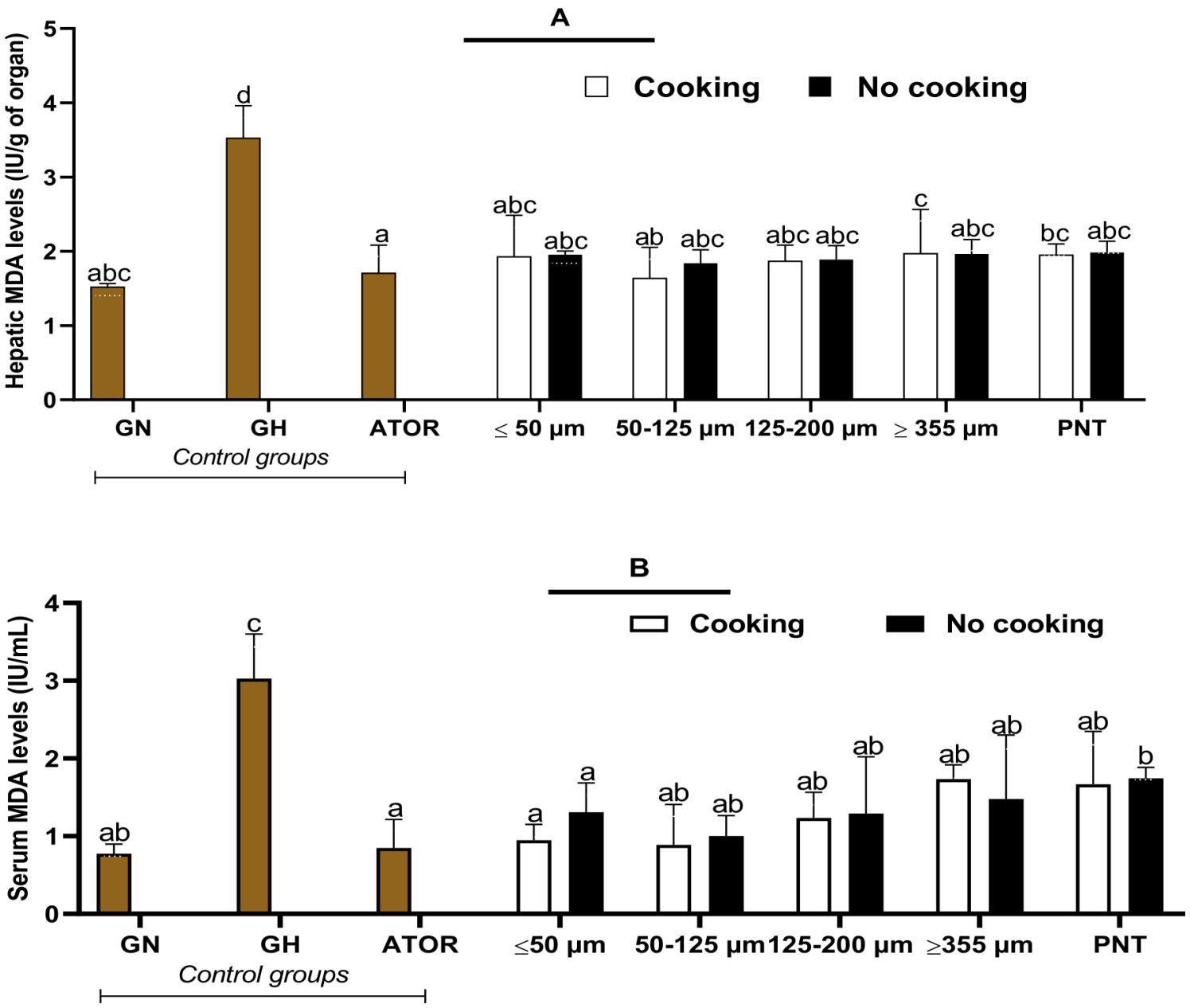


Figure 3

Variation in malondialdehyde (MDA) levels in the liver (A) and plasma (B) of rats undergoing hypolipidemic treatment

PNT: unsieved powder, GN: normal control group, GH: hyperlipidemic control group, ATOR: reference control group. Values are mean ± standard deviation (n=5). Different superscript letters indicate significant differences ($p<0.05$) according to Tukey's multiple range test.

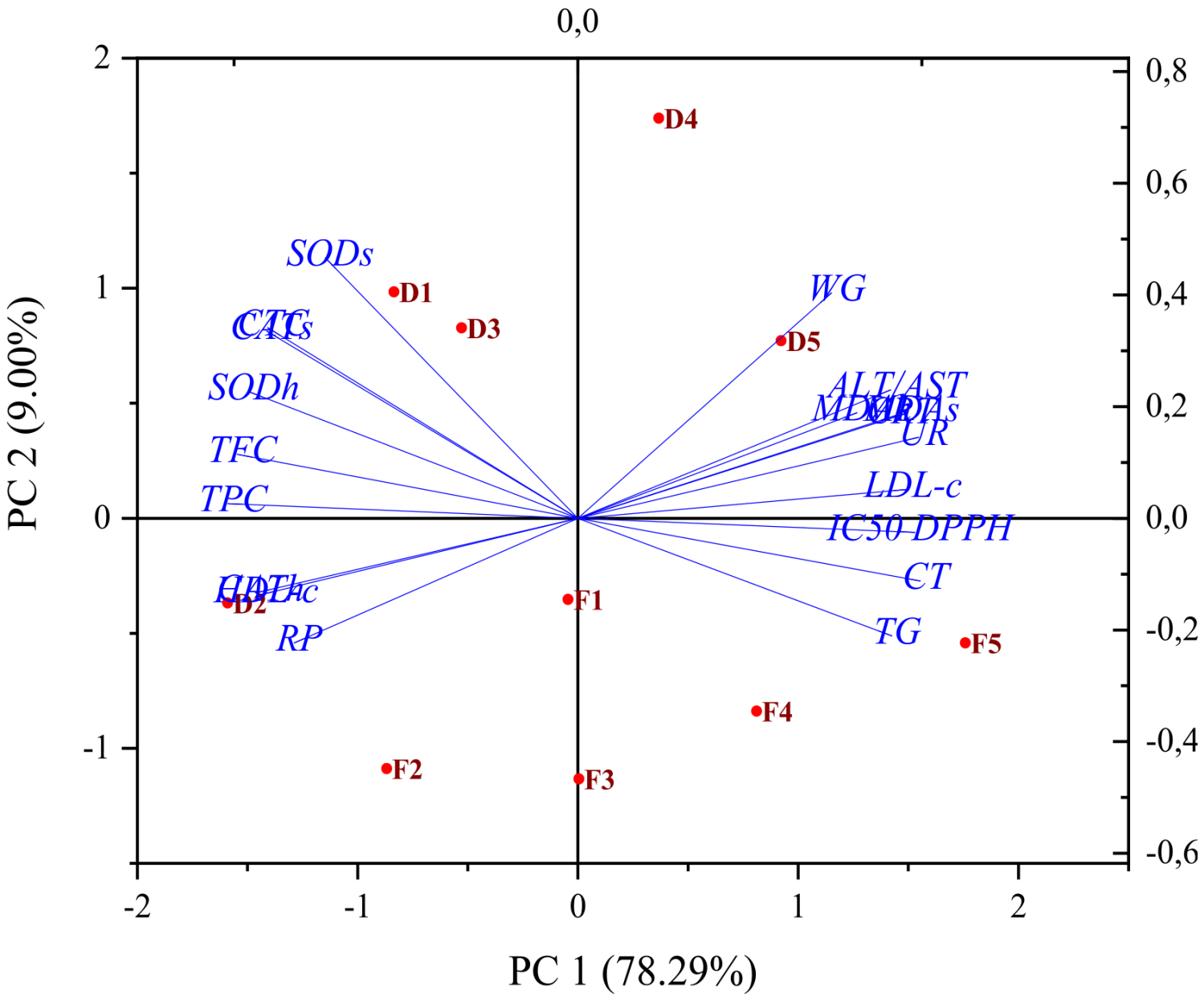


Figure 4

Principal Component Analysis (PCA) between bioactive compounds evaluated, antioxidant and hypolipidemic activities, and different granulometric fractions

WG: Weight gain after treatment, TPC: Total Polyphenols Content, TFC: Total Flavonoids Content, CTC: Condensed tannin content, UR: Urea, CRT: Creatinine, RP: Reducing powder, TG: triglyceride, CT: total cholesterol, LDL-c: LDL cholesterol, HDL-c: HDL cholesterol, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CATh: hepatic catalase, CATs: serum catalase, SODh: hepatic superoxide dismutase, SODs: serum superoxide dismutase