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Research Article
Antioxidant and Antihyperlipidemic Properties of Different Granulometric Classes of *Adansonia digitata* Pulp Powder

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Abstract

Background and Objectives: Baobab pulp is widely used nowadays for its richness in bioactive compounds. Recent studies have shown that the bioactive composition and biological activities of plants can be improved by splitting the powder. However, the granulometric class with the most concentration of bioactive compounds varies from one plant to another. This work aimed at evaluating the effect of particle size of *Adansonia digitata* pulp powder on its antioxidant and antihyperlipidemic activities. **Materials and Methods:** Three particle size classes, <50 µm, 100-50 µm and >100 µm, were obtained by the Comminution and controlled Differential Screening method (CDS-extraction) and were studied. The phenolic content, *In vitro* antioxidant activity of different granulometric classes were evaluated by DPPH, ABTS and power reducing tests were carried out. The *in vivo* antioxidant and antihyperlipidemic properties were evaluated by hyperlipidemia induction in rats. **Results:** We found that the highest phenolic contents and *in vitro* antioxidant activity were obtained for the <50 µm fraction. This fraction mostly reduced the level of Malondialdehyde (MDA) and increased the activity of Superoxide dismutase (SOD) and Catalase significantly ($p<0.05$) in plasma and various organs of rats as compared to the unsieved powder. Maximum reduction in Triglycerides (TG), total cholesterol (TC) and LDL-C as well as the greatest increase in HDL-C was also observed with this fraction. **Conclusion:** This study revealed that phenolic content and these activities increased with the decrease in particle size. A significant correlation was then observed between the content of phenolic compounds and the biological activities. Fraction <50 µm could therefore be used in the nutraceutical industry for the formulation of new products with a higher value in bioactive compounds.

Key words: *Adansonia digitata*, particle size, phenolic compounds, antioxidants, antihyperlipidemic activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Native to tropical Africa, The Baobab (*Adansonia digitata*) plant is widely used in traditional African medicine[1]. It is recognized by the population as "the pharmacist's tree" because all its parts are exploited for their therapeutic and nutritional virtues[2]. In Cameroon, we are mostly interested in the pulp. It is traditionally used as antipyretic, analgesic, anti-diarrheal, anti-dysenteric and in the treatment of smallpox and measles[3]. Numerous studies have shown that the baobab pulp has several biological effects, such as hepatoprotective activities[4,5], anti-tumor[6], anti-diabetic[7], anti-hyperlipidemic[8] and antioxidant properties[9,10]. Baobab pulp helps fight oxidative stress and hyperlipidemia, which are considered to be the main risk factors for cardiovascular diseases[11,12], which are currently the main causes of death worldwide. The antioxidant and anti-hyperlipidemic activities of *A. digitata* pulp are associated with the high content of bioactive compounds including polyphenols, flavonoids, tannins[13,14] as well as vitamin C, whose content are 6 times greater than that oflime[15]. Due to its richness in these compounds, the baobab pulp is an ingredient well appreciated and widely used in the nutraceutical industry[16]. However, to exploit the antioxidant and antihyperlipidemic properties of the pulp efficiently, these molecules must first be extracted or made available in a suitable food form. Extraction techniques such as hydrodistillation, ultrasonic and microwave extraction have been widely used for this purpose[17,18]. However, it is known that the efficiency of extraction is influenced by some critical parameters such as temperature and the nature of the solvent[19,20]. Indeed, these techniques induce thermal destruction of thermolabile compounds and the solvents used are most often known as harmful to health[21]. Another way of enhancing compound extraction could be to grind and sieve the plant. Indeed, fine grinding reduces particle size, thickens particle size distribution and increases specific surface area, which leads to the improvement of it activities[22-24]. To achieve this, the Comminution and controlled Differential Screening method (CDS-extraction) which involves grinding and sieving processes was used to increase the content of some bioactive compounds in the powder. This method does not use solvents and produces a wider range of active ingredients[25]. Some studies have evaluated the effect of this process on the antioxidant activity of certain plants, among which Zaiter *et al.*[26] showed that *Salix alba* (L) powder, obtained after grinding and sieving, exhibited maximum antioxidant activity in the 50-100 µm fraction. Also, Becker *et al.*[27] have shown that fractionation has improved the antioxidant activity of the resulting powders of *Hypericum perforatum* and *Achilea millefolium* with a high activity in the 100-80 µm fraction. Deli *et al.*[28] showed that the 0-180 µm fraction of *Boscia senegalensis*, 180-212 µm of *Dichrostachys glomerata* and 212-315 µm of *Hibiscus sabdarifa* were those that presented the most antioxidant activities. According to above data, the antioxidant activity and bioactive compounds varies according to the nature of plant and granulometric classes. The major goal of this research was to evaluate the influence of particle size on the antioxidant and antihyperlipidemic properties of *Adansonia digitata* pulp powder.

MATERIALS AND METHODS

***Adansonia digitata* powder production:** *Adansonia digitata* fruits were purchased from the Ngaoundere local market, in the Adamaoua Region of Cameroon. The woody pericarp of the Baobab fruit was broken and the dried pulp was crushed without seeds using a pestle and mortar. An ultra-centrifugal Mill ZM 200 (Retsch, France) was used to obtain the fine ground powder. After grinding, the powder was sieved with a vibratory sieve shaker (analysette 3 Spartan Fritsh, idar-oberstein, Germany) which helped obtain the following granulometric class: <50 µm, 100-50 µm and >100 µm. Granulometric classes were weighted and then stored in sealed polyethylene plastic bags at 4°C until analysis.

Determination of Phytochemical composition

Extraction of total phenolic compounds: Extraction of polyphenols from granulometric classes of *Adansonia digitata* were carried out using the method of Kim *et al.*[29] with some modifications. Only 2 g of each powder was macerated during 24 h through stirring in 20 mL of methanol/Water 70/30 (v/v). The methanol/water extract was then centrifuged at 3500 rpm for 20 min. The supernatant layer was filtered, brought to 15 mL and stored at 4°C before analysis.

Total phenolic content: Total phenolic content was determined according to the method described by Wafa *et al.*[30]. Only 0.02 mL of samples was added to test tubes containing 2.98 mL of distilled water followed by an addition of 0.5 mL of folin-ciocalteu reagent solution (1/10) and 0.4 mL of sodium carbonate (Na_2CO_3, 20%). The sample was thoroughly mixed and vortexed. After 20 min of incubation at room temperature, absorbance was measured at 760 nm against a blank on a UV-visible spectrophotometer. Gallic acid (0.2 g mL^{-1}) were used for calibration and total phenolic content was expressed as gram of gallic acid equivalents per100 g of dry weight (g GAE/100 g DW).

Total flavonoid content: The determination of total flavonoid content was conducted as previously described by Dewanto *et al.*[31] and 0.1 mL of each extract were mixed with 2.4 mL of distilled water and 0.15 mL of 5% sodium nitrite (Na_2NO_2) solution. Subsequently, the tubes were held at 25°C for 6 min and 0.3 mL of 10% aluminium chloride ($AlCl_3$, $6H_2O$) was added in each test tube and maintained at 25°C for 5 min. Lastly, 1 mL 1 M of sodium hydroxide (NaOH) was added and vigourously mixed. Samples were immediately measured (510 nm) against a blank on a UV-visible spectrophotometer. Total flavonoid content was calculated as gram of rutin equivalent per 100 ram of dry weight (g RE/100 g DW) against a standard curve of rutin (0.1 g L^{-1}).

Condensed tannin content: The condensed tannin content of each granulometric class was evaluated according to the method described by Sun *et al.*[32] with slight modifications; 0.05 mL of each extract was mixed with 3 mL of 4% vanillin and 1.5 mL concentrated sulfuric acid was added. The mixture was stirred and then kept at room temperature for 30 min. The absorbance was measured at 500 nm against a blank on a UV-visible spectrophotometer. A standard cube was prepared using catechin solutions (0-0.6 mg mL^{-1}). The condensed tannin content was expressed as gram of catechin equivalent per 100 g of dry Weight (g CE/100 g DW).

In vitro antioxidant activity

DPPH radical scavenging activity assay: The DPPH radical scavenging activity was evaluated according to the method described by Zhang and Hamauzu[33]. 2 mL of 0.025 g L^{-1} DPPH solution in methanol/water (70/30) was mixed with 0.5 mL plant extract. The reaction mixture was incubated in the dark for 1 h and its optical density was recorded at 517 nm against the blank. For the control, 2 mL DPPH methanol/water solution was mixed with 0.5 mL of methanol/water (70/30). The DPPH radical scavenging activity was expressed in terms of IC_{50} values that refers to the smallest concentration of antioxidants required to scavenge 50% of the DPPH radical.

ABTS cation radical scavenging activity assay: The ABTS radical scavenging activity was measured according to the method described by Re *et al.*[34] with slight modifications. ABTS solution was generated by mixing 6.62 mg of potassium persulfate and 38.4 mg of ABTS reagent in a glass beaker, then 10 mL distilled water was added and then the mixture was perfectly mixed. This solution was kept away from light and left to stand for 16 h at room temperature. The resulting $ABTS^{+\cdot}$ solution was diluted with the absolute ethanol to get a blue-green coloration with an absorbance of 0.700±0.02 at 734 nm. Then 0.15 mL of each sample was added to 2 mL of ABTS radical solution. The decrease of absorbance was measured at 734 nm. The half maximal inhibitory concentration (IC_{50}), which refers to the smallest concentration of antioxidants required to scavenge 50% of the ABTS radical cation, was calculated.

Reducing power: Reducing power was determined referring to the method described by Oyaizu[35] with slight modifications. 1 mL sample solution was mixed with 2.5 mL of 0.2 M phosphate buffer (PH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. 2.5 mL of 10% trichloracetic acid was added to the mixture which was then centrifuged at 3000 rpm for 15 min. 2.5 mL of upper layer solution was mixed with 2.5 mL of water and 1 mL of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. Increased absorbance indicated an increase in reducing power. A standard cube was prepared using ascorbic acid (0.1 mg mL^{-1}). The reducing power was expressed as gram of ascorbic acid equivalent per 100gram of dry Weight (AAE/100 g DW).

Diet formulation and animal experiments: Healthy male wistar rats, weighing about 200-300 g were reared at the animal house of the National School of Agro-industrial Sciences of the University of Ngaoundere, Cameroon. The animals were maintained under controlled conditions with a temperature of 25±2°C, humidity of 50±10% and a 12/12 light/dark cycle. The animals were fed *ad libitum* with normal laboratory chow standard pellet diet. The animals were allowed to acclimatize for 7 days before beginning the experiments. All the procedures were in strict accordance with the guidelines established by the Department of Biological Sciences of the University of Ngaoundere.

Hyperlipidemia and oxidative stress induction: Hyperlipidemia and oxidative stress were induced by a lipid-rich diet containing 300 g of egg yolk, 2 g of cholesterol, 250 g of coconut oil and 50 g of soya oil, as described by Aissatou *et al.*[36]. Various works including those of Garait[37], Hsu and Yen[38] showed that a diet high in fats was able to induce oxidative stress in rats and increase the level of lipid in the blood.

Evaluation of *in vivo* antioxidant and antihyperlipidemic properties: The male Albino rats (wistar strain) were divided into eight groups (08) with each group containing 5 rats.

Group 1: normal group in which the rats were daily administered standard diet. Group 2: negative group in which the rats were daily administered a hyperlipidemic diet. Group 3: the antihyperlipidemic positive control group in which the rats were daily administered atorvastatin (10 mg kg^{-1} day^{-1}) along with a hyperlipidemic diet. Group 4:the antioxidant positive control group in which the rats were daily administered ascorbic acid (20 mg kg^{-1} day^{-1}) along with a hyperlipidemic diet. Group 5, group 6 and group 7 in which the rats were daily administered respectively <50 µm, 100-50 µm and >100 µm powder of *Adansonia digitata* (250 mg kg^{-1} day^{-1}) along with a hyperlipidemic diet and group 8: in which, the rats were daily administered the unsieved powder (250 mg kg^{-1} day^{-1}) along with a hyperlipidemic diet.

After 28 days of experiment, the animals were put under fasting for 24 h and blood sample was collected by jugular puncture under diethyl ether anesthesia. The blood samples collected were centrifuged at 3000 rpm for 15 min. Serum samples obtained were collected in dry tubes and kept at -20°C for biochemical analysis. Immediately after collecting the blood, organs such as the liver, kidney and heart were removed and washed in 0.09% NaCl solution. 0.5 g of the liver, heart and kidneys were ground separately in a mortar, by mixing with 4.5 mL of 0.1 M phosphate buffer, pH 7.4 and then centrifuged for 10 min at 3000 rpm. The homogenates obtained were stored at -20°C for the evaluation of oxidative stress parameters.

Measurement of malondialdehyde (MDA) level: MDA content was determined by the thiobarbituric acid reaction (TBARS) method described by Yagi[39]. 100 µL of sample and 400 µL of TBA reagent were introduced into test tubes and then sealed. The mixture was heated in a water bath at 100°C for 15 min; then cooled in a cold water bath for 30 min. The tubes were opened to allow the gases formed during the reaction to evaporate. The mixture was then centrifuged at 3000 rpm for 5 min at 25°C. The absorbance of the supernatant was read at 532 nm.

Superoxide dismutase (SOD) and catalase (CAT) activity: The activity of superoxide dismutase was evaluated by the method described by Beauchamp and Fridovich[40]. An aliquot of 0.2 mL of sample was introduced into 2.5 mL of carbonate buffer (pH 10.2). The reaction began with the addition of 0.3 mL of freshly prepared adrenaline to the mixture. After homogenization, the final mixture was read at 480 nm every 30 sec up to 150 sec.

The method described by Sinha[41] was used to assess the activity of catalase. In a tube, 0.1 mL of sample and 1 mL of pH 7.0 phosphate buffer were mixed. To this mixture was added 0.4 mL of H_2O_2 (0.2 M) and the reaction was stopped after 30, 60 and 90 seconds by the addition of 2 mL of a dichromate/acetic acid solution ($K_2Cr_2O_7$ 5% prepared in glacial acetic acid). The change in absorbance was noted after reading at 620 nm.

Lipid profile and transaminases analysis: Serum determinations were performed by an automatic biochemical analyzer. Levels of total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) were determined with a specialized reagent kit using a colorimetric method according the procedure described by Richmond[42]. Low density lipoprotein (LDL-C, VLDL-C) were calculated according to the Friedewald *et al.*[43] method.

Statistical analysis: Each experiment and measurement were performed in triplicate. The results were presented as Mean±Standard deviation. One way analysis of variance (ANOVA) was carried out (level of significance $\alpha = 0.05$) followed by multiple comparisons of DUNCAN using the Statgraphic package (Statgraphic Plus for windows, version 15.1.0.2 Manuggistic inc, USA). XLSAT 2020 was used for principal components analysis (PCA) to show the correlations between variables and the various fractions of plant powder.

RESULTS AND DISCUSSION

Phenolic compounds: Total phenolic content, flavonoid content and condensed tannin content of granulometric classes and unsieved powder of *Adansonia digitata* fruit pulp is presented in Table 1. It appears from Table 1 that the content of total polyphenols of the different particle size classes varies from 7.95±0.35 to 10.68±0.13 mg GAE g^{-1} DW. The flavonoid content varies from 5.77±0.04 to 7.40±0.11 mg RE g^{-1} DW and the content of condensed tannins varies from 3.82±0.02 to 5.44±0.10 mg EC g^{-1} DW. It is generally observed that the content of phenolic compounds increases when the particle size decreases from >100 µm to <50 µm. Thus, the fraction <50 µm had the highest content of total phenolic compounds (10.68±0.13 mg GAE g^{-1} DW), flavonoids (7.40±0.11 mg RE g^{-1} DW) and condensed tannins (5.44±0.10 mg EC g^{-1} DW) compared to the unsieved powder which had a total polyphenol content of 7.71±0.02 mg GAE g^{-1} DW, flavonoids of 5.98±0.06 mg RE g^{-1} DW and condensed tannins of 4.51 ± 0.30 mg EC g^{-1}

Table 1: Phenolic compounds of different granulometric classes and unsieved powder

	Samples			
Phenolic compounds	Unsieved powder	>100 µm	100-50 µm	<50 µm
Total phenolic content (mg GAE g^{-1} DW)	7.71±0.02[a]	7.95±0.35[a]	9.07±0.05[b]	10.68±0.13[c]
Flavonoid content (mg RE g^{-1} DW)	5.98±0.06[a]	5.77±0.04[a]	5.61±0.39[a]	7.40±0.11[b]
Condensed tannin content (mg CE g^{-1} DW)	4.51±0.30[b]	3.82±0.02[a]	4.35±0.07[b]	5.44±0.10[c]

The data were expressed as Mean±Standard deviation of triplicate (n = 3). Means in the same row with different letters were statistically different (p<0.05)

Table 2: Antioxidant activities of different granulometric classes and unsieved powder

Samples	DPPH IC_{50} (mg mL^{-1})	ABTS IC_{50} (mg mL^{-1})	Reducing power (mgEAA g^{-1} DW)
>100 µm	0.62±0.01[c]	1.75±0.02[d]	3.76±0.02[a]
100-50 µm	0.73±0.01[d]	1.67±0.03[c]	3.85±0.03[b]
<50 µm	0.55±0.01[b]	1.46±0.03[b]	4.09±0.05[c]
Unsieved powder	0.74±0.02[d]	1.64±0.01[c]	3.81±0.07[a]
Vitamin C	0.01±0.00[a]	0.01±0.00[a]	/

The data were expressed as Mean±Standard deviation of triplicate (n = 3). Means in the same column with different letters were statistically different (p<0.05). *In vivo* antioxidant activity of granulometric class powder of *A. digitata*

DW. This fraction therefore had an improved content of total polyphenols, flavonoids and condensed tannins in the unsieved powder by 38.52, 23.74 and 20.62% respectively. This could be explained by the fact that the reduction in the size of the particles led to an increase in the specific surface area of the particles and thus exposed the hydrophilic groups of the phenolic compounds to easily react with water or solvents, which increased the bioavailability of molecules[44]. On the other hand, this could also be explained by the fact that the ultra-fine grinding ruptured the wall or the structures which, when linked to polyphenols prevent their bioavailability. During sieving, these structures (fibers and more rigid carbohydrates) go into the large size classes while polyphenols of smaller sizes go into the small particle size classes[45].

***In vitro* antioxidant activity:** The antioxidant activities of each granulometric classes and unsieved powder evaluated by DPPH test, ABTS test and reducing power are reported in Table 2. The anti-radical activity with DPPH and ABTS were expressed in IC_{50}. It should be noted that the smaller the IC_{50} the greater is its antioxidant activity.

The DPPH method was used to measure the ability of the antioxidants contained in the different particle size classes to stabilize the DPPH radical. Thus it appears from Table 2 that the ability to inhibit the DPPH radical varies significantly ($p<0.05$) from one size class to another with IC_{50} falling between 0.55 and 0.73 mg mL^{-1}. Thus, the particle size class <50 µm is the one which exhibited the best inhibition of the DPPH radical with an IC_{50} of 0.55±0.01 mg mL^{-1} followed by the fraction, >100 µm (IC_{50}: 0.62±0.01 mg mL^{-1}) and lastly 100-50 µm (IC_{50}: 0.73±0.01 mg mL^{-1}). These inhibitions were however lower than that of vitamin C taken as a reference (IC_{50}: 0.01±0.00 mg mL^{-1}). This higher activity in fraction <50 µm could be due to the action of total polyphenols, flavonoids and higher tannins in this fraction as presented in Table 1. Indeed, they are able through their structure rich in hydroxyl groups to trap and neutralize free radicals by yielding their protons or electrons. Several previous studies have also shown that the ultrafine fractions were those which exhibited the highest DPPH anti-radical activity due to their content of phenolic compounds[26,46]. Indeed, the increase in the antioxidant activity is due to the fact that when particle size is small the antioxidant compounds are better extracted. With regards to the inhibition of the ABTS radical, we observe in Table 2 that, similarly to the anti-radical activity with DPPH, the inhibition of the ABTS radical increases with the reduction in particle size. IC_{50} values are between 1.46 and 1.75 mg mL^{-1}. The strongest inhibition was recorded for the fraction <50 µm (IC_{50}: 1.46±0.03 mg mL^{-1}) followed by 100-50 µm (IC_{50}: 1.67±0.03 mg mL^{-1}) and finally (IC_{50}: 1.75±0.02 mg mL^{-1}). The fraction <50 µm exhibited greater ABTS inhibition than that of the unsieved powder (IC_{50}: 1.64±0.01 mg mL^{-1}). However, this inhibition was much lower than that of vitamin C taken as a reference (IC_{50}: 0.01±0.00 mg mL^{-1}). The higher activity in fraction <50 µm could be due to the action of high total polyphenols, flavonoids and tannins in this fraction as presented in Table 1. Indeed, it has been shown that these molecules are capable, by their structure rich in hydroxyl groups, of trapping and neutralizing free radicals by yielding their protons or their electrons.

The reducing power of a compound may serve as an indicator of its potential antioxidant activity. The test assesses the electro transfer potential of the different granulometric classes with higher values of reducing power, indicating a better antioxidant activity. From Table 1, it can be seen

similarly to the DPPH and ABTS tests that reducing power increases with the reduction in particle size from >100 µm to <50 µm. Thus the highest reducing activity was recorded for the fraction <50 µm with a value of 4.09 mg EAA g^{-1} DW followed by the fraction 100-50 µm (3.85 mg EAA g^{-1} DW) and the fraction >100 µm (3.76 mg EAA g^{-1} DW). The reducing activities observed indicate the presence of proton-donating compounds in the different particle size classes leading to the reduction of Fe^{3+} to Fe^{2+}. The reducing power of the fraction <50 µm was higher compared to that of the unsieved powder (3.81±0.07 mg EAA g^{-1} DW). The higher reducing power of the fraction <50 µm could be linked to components such as phenolic substances which are higher than those found in the other fractions. Indeed, phenolic compounds, particularly flavonoids and condensed tannins reduce and inactivate oxidants thanks to the presence of hydroxyl groups in their structures which can serve as electron donors. Overall, we find that the fractions which have the best trapping powers of the free radicals DPPH and ABTS, also show the best reducing capacities of Fe^{3+}. This indicates the presence of antioxidant molecules in the fractions which intervene by two types of mechanisms of action.

Effect of different granulometric class powder of *A. digitata* on lipid peroxidation: Malondialdehyde (MDA) is considered to be one of the end products of the oxidation of polyunsaturated fatty acids mediated by free radicals. The high levels of MDA therefore mark the presence of oxidative stress. The level of Malondialdehyde in the serum, the kidneys, the liver and the heart of the different groups of rats subjected to the different diets is presented in Table 3. From this Table, it appears that the consumption of a hyperlipidemic diet for 28 days favored a significant increase in lipid peroxidation (negative control) in all the tissues studied compared to normal control. Furthermore, there is an overall significant reduction ($p < 0.05$) in the MDA levels after the same treatment period in the various batches having received the fractions of the pulp of *A. digitata*. However, this reduction was greater when the particle size of the powder decreased. Thus, the rats which received the fraction <50 µm were those which had the greatest reduction in the rate of malondialdehyde, particularly in the plasma, the kidneys and the heart compared to those who received the unsieved powder and in the negative control group. Indeed, this fraction made it possible to bring MDA levels close to that of the normal control, thus showing the preventive effect of the fraction <50 µm against lipid peroxidation. Furthermore, it can be seen that at the dose of 250 mg kg^{-1} of body weight, this same fraction had achieved a reduction in MDA comparable to that of vitamin C taken as standard in the kidneys. However, this reduction was significantly very low in plasma and liver compared to that of vitamin C. Furthermore, we found that in the liver, the fraction >100 µm exhibited the best reduction in the level of MDA followed by the fraction <50 µm. These reductions were, however, better than those of rats given the unsieved powder. These data is in concordance with the results of antioxidant activities carried out *in vitro*. Thus, this reduction observed in the rate of malondialdehyde by the *A. digitata* powder could be due to the phenolic compounds contained in these powders. Indeed, several studies have shown that polyphenols, particularly flavonoids, have the ability to trap free radicals, which gives them an inhibitory effect on the peroxidation of membrane lipids. In addition, through their ability to chelate pro-oxidant metals, they prevent the production of free radicals through the fenton reaction.

Effect of different granulometric classes of *A. digitata* powder on SOD and CAT levels: SOD and catalase are the most important antioxidant enzymes in the body's defense system. SOD catalyzes the disproportionation of the superoxide $O_2^{\bullet-}$ anion into H_2O_2 and oxygen while catalase catalyzes the reduction of hydrogen peroxide H_2O_2 into water molecules. These enzymes therefore reduce the toxic effects of free radicals in the body[47]. Table 4 shows the activity of SOD

Table 3: Effect of different granulometric classes of *Adansonia digitata* powder on plasma, kidney, Liver and Heart MDA levels

	MDA levels (µmole mL^{-1})			
Samples	Plasma	Kidney	Liver	Heart
>100 µm	1.48±0.13^{c}	1.42±0.12^{de}	1.02±0.09^{b}	1.03±0.04^{e}
100-50 µm	1.18±0.10^{b}	1.43±0.09^{e}	1.37±0.11^{c}	0.86±0.03^{cd}
<50 µm	1.07±0.06^{b}	1.03±0.15^{a}	1.31±0.12^{c}	0.81±0.06^{bc}
UP	1.10±0.11^{b}	1.06±0.03^{ab}	1.40±0.10^{c}	0.91±0.03^{d}
NC	1.05±0.05^{b}	1.22±0.06^{bc}	1.06±0.11^{b}	0.74±0.08^{b}
PC	0.63±0.08^{a}	1.26±0.04^{cd}	0.67±0.09^{a}	0.12±0.05^{a}
Neg C	1.97±0.13^{d}	1.84±0.11^{f}	3.39±0.15^{d}	2.49±0.06^{f}

UP: Unsieved powder, NC: Normal control, PC: Positive control, Neg C: Negative control. The data were expressed as mean±standard deviation of triplicate (n = 3). Means in the same columm with different letters were statistically different ($p<0.05$).

Table 4: Effect of different granulometric classes of *A. digitata* powder on SOD and CAT activities

	SOD levels (µmole mL^{-1})			
Samples	Plasma	Kidney	Liver	Heart
SOD activity (µmole mL^{-1})				
>100 µm	50.00±6.43^{b}	36.44±5.72^{b}	53.39±6.86^{b}	56.77±4.38^{b}
100-50 µm	66.40±4.24^{c}	41.50±11.73^{b}	60.45±6.48^{bc}	60.78±5.57^{b}
<50 µm	68.02±6.41^{c}	47.01±6.30^{bc}	71.82±7.81^{cd}	63.97±5.74^{b}
Unsieved powder	48.39±3.25^{b}	51.18±5.57^{bc}	80.27±8.10^{d}	50.93±6.54^{b}
NC	62.34±3.52^{c}	42.16±8.52^{b}	52.84±5.74^{b}	58.14±5.48^{b}
PC	69.07±4.24^{c}	62.24±5.86^{c}	81.25±8.83^{d}	84.71±6.61^{c}
Neg C	20.92±5.91^{a}	13.11±2.64^{a}	16.39±4.63^{a}	19.23±5.44^{a}
CAT activity (µmole mL^{-1})				
>100 µm	2.32±0.21^{a}	3.60±0.65^{b}	2.61±0.68^{ab}	2.58±0.14^{ab}
100-50 µm	2.64±1.12^{a}	3.68±1.30^{b}	4.29±0.15^{c}	2.43±0.19^{ab}
<50 µm	5.19±1.40^{c}	3.74±0.75^{b}	2.80±0.41^{abc}	2.62±0.05^{ab}
Unsieved powder	4.72±1.04^{bc}	2.70±0.06^{ab}	2.90±0.20^{abc}	4.16±0.09^{d}
NC	2.75±0.41^{a}	6.35±1.60^{c}	2.79±0.65^{abc}	2.60±0.63^{ab}
PC	2.77±0.30^{ab}	3.38±1.06^{b}	3.99±0.99^{bc}	6.03±1.74^{c}
Neg C	1.70±0.36^{a}	1.19±0.07^{a}	2.32±0.87^{a}	2.11±1.17^{a}

NC: Normal control; PC: Positive control, Neg C: Negative control, The data were expressed as Mean±Standard deviation of triplicate (n = 3). Means in the same columm with different letters were statistically different ($p<0.05$)

and catalase in the plasma, kidneys, liver and heart of rats having received the particle size classes of *Adansonia digitata* at a dose of 250 mg kg^{-1} bw.

We observe generally and similarly to the rate of MDA a significant variation. On all the organs studied, the enzymatic activities of these two enzymes increased with the reduction in particle size. Among the particle size classes, the fraction <50 µm is that which presented the maximum activity of SOD and catalase in the liver, kidney, plasma and the heart. However, compared to the unsieved powder, this fraction showed better activity in the plasma and the heart with regards to SOD and in plasma and kidneys for the activity of catalase. The enzymatic activities of SOD and catalase in the fraction <50 µm at a dose of 250 mg kg^{-1} bw were comparable if not superior to those of normal control. This result sufficiently proves that the fraction <50 µm reinforces the antioxidant potential of the unsieved *Adansonia digitata* powder. Thus, it is possible to think that the phenolic compounds contained in this fraction acted not only as a scavenger of free radicals but also played a role in improving the synthesis of antioxidant enzymes which in turn protect cells against reactive oxygen species.

Antihyperlipidemic proprieties

Effect of different granulometric classes of *A. digitata* powder on lipidemia: Figure 1shows the serum level of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides of rats which received the different particle size fractions of *Adansonia digitata* after four weeks of the experiment. Compared to normal control, the high-fat diet resulted in an increase in total cholesterol, LDL-cholesterol, triglycerides and a reduction in HDL-cholesterol in rats who received no treatment (negative control). In rats treated with different fractions and with atorvastatin (positive control), we observe a significant decrease ($p<0.05$) in total cholesterol, LDL-cholesterol and an increase in HDL-cholesterol compared to the unsieved powder and the negative control. The finest fraction <50 µm, is the one which mostly improved the lipid profile of the rats subjected to hyperlipidemic treatments compared to the unsieved powder as regards total cholesterol, LDL-cholesterol and HDL-cholesterol.

The great reduction in the lipid level in the fraction <50 µm could be explained by the effect of phenolic compounds, in particular flavonoids whose level is highest in this fraction. In fact, flavonoid have several antihyperlipidemic activities. They increase fecal excretion of cholesterol and triglycerides, complex bile salts, block the synthesis of cholesterol by inhibiting HMG-COA reductase and finally suppress the transcription gene responsible for fatty acid synthesis[48]. These results concur with Alhassan *et al.*[49] who revealed that the aqueous extracts of *Adansonia digitata* pulp induced a reduction in the total cholesterol level and in LDL-cholesterol in hyperlipidemic rats.

Effect of granulomeric classes of *Adansonia digitata* pulp powder on TC/HDL and LDL-C/HDL-C ratio of rats subjected to different treatments: Hyperlipidemia is a major risk factor for the development of arteriosclerosis, which is a major

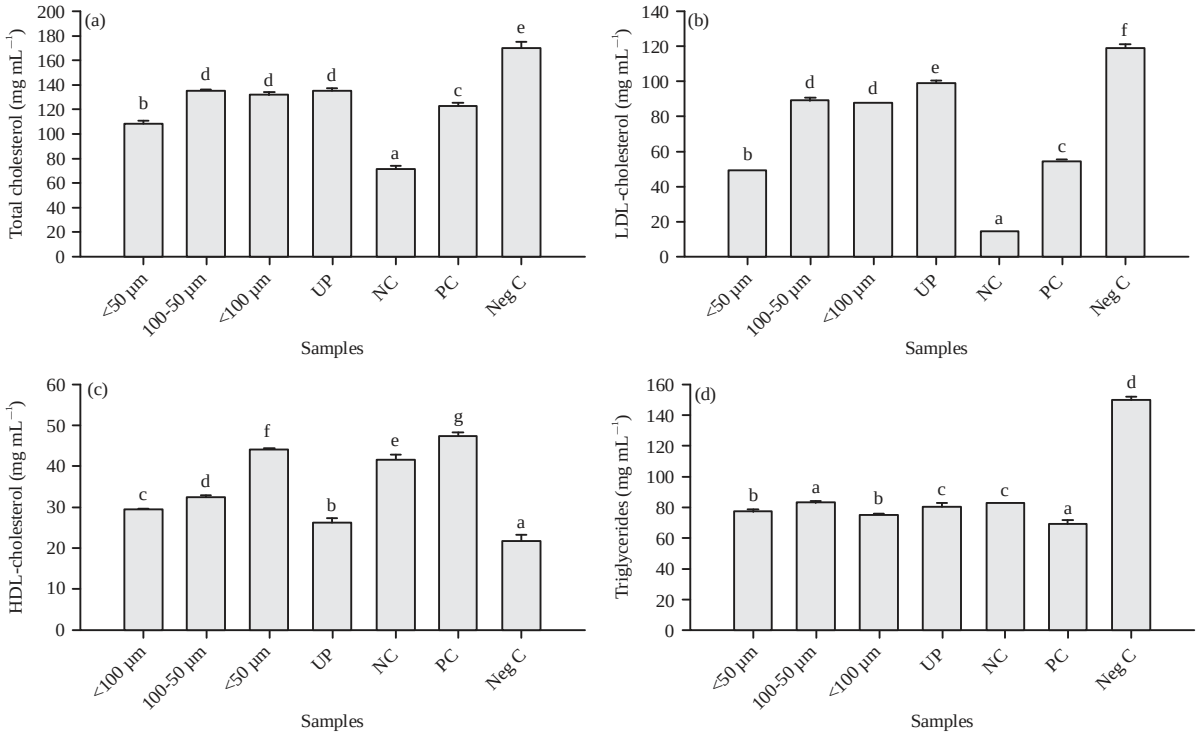

Fig. 1(a-d): (a) Serum level of total cholesterol, (b) LDL-cholesterol, (c) HDL-cholesterol and (d) Triglycerides of rats having received the different granulometric classes of *Adansonia digitata* after four weeks of experimentation

UP: Unsieved powder, NC: Normal control, PC: Positive control, Neg C: Negative control. The data were expressed as Mean±Standard deviation of triplicate (n = 3). Means with different letters were statistically different (p<0.05)

Table 5: TC/HDL and LDL-C/HDL-C ratio of rats subjected to different treatments

Samples	CT/HDL	LDL/HDL
≥100 µm	4.21±0.24^{d}	2.77±0.21^{bc}
100-50 µm	4.53±0.19^{e}	2.99±0.18^{bc}
<50 µm	2.75±0.03^{c}	1.14±0.03^{b}
UP	4.34±0.29^{d}	2.60±1.32^{c}
NC	1.69±0.06^{a}	0.30±0.05^{a}
PC	2.28±0.11^{b}	0.96±0.10^{a}
Neg C	7.41±0.71^{f}	5.09±0.65^{d}

UP: Unsieved powder, NC: Normal control, PC: Positive control, Neg C: Negative control. The data were expressed as Mean±Standard deviation of triplicate (n = 3). Means in the same columm with different letters were statistically different (p<0.05)

health problem worldwide. As such, reducing the risk of arteriosclerosis by decreasing the level of lipid in the blood is an important factor. As such, the ratio of TC/HDL and LDL-C/HDL-C of rats subjected to different lipid-lowering treatments is presented in Table 5. We observed a significant decrease in the atherogenic index and LDL-C/HDL-C risk ratio in the groups that received the different particle size fractions of *A. digitata* and atorvastatin compared to the hyperlipidemic control group. This therefore suggests that at a dose of 250 mg kg^{-1} bw, consumption of the fraction <50 µm makes it possible to reduce more the risk ratio compared to the unsieved powder and to the negative control.

Principal Component analysis (PCA) of phenolic compounds, antioxidant and antihyperlipidemic activity of granulometric classes of *Adansonia digitata* pulp powder: Phenolic content, antioxidant and anti-hyperlipidemic proprieties of the different granulometric classes and the unsieved powder of *Adansonia digitata* were analyzed by principal component analysis (PCA) to evaluate the different contributions of phenolic compounds on the biological proprieties studied and to better visualize the impact of particle size on the composition of bioactive compounds and the antioxidant and antihyperlipidemic activities (Fig. 2).

The first two main components (F1 and F2) explain 84.08% of the variability of the set of parameters studied. The F1 axis separates the fraction <50 µm from the other fractions and from the unsieved powder and represents 56.23% of the variability. It is defined positively for phenolic compounds,

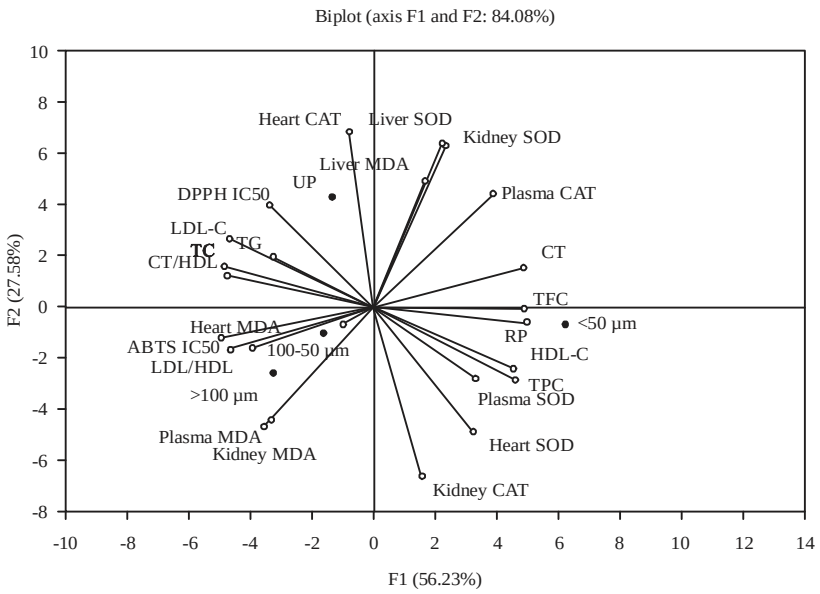

Fig. 2: Principal component analysis of different granulometric classes of *Adansonia digitata* in relation to the different variables studied

flavonoids, tannins, total reducing power the level of HDL-C, SOD and catalase and negatively for the IC_{50} of DPPH and ABTS tests, MDA, LDL-C, CT and TG and CT/HDL and LDL/HDL ratios. With regard to the relationship between the bioactive compounds evaluated and the various variables studied, a strong and positive correlation was observed between the total reducing power and phenolic compounds (r = 0.951), flavonoids (r = 0.934) and tannins (r = 0.956). Also Between the plasma SOD activity and the phenolic compounds (r = 0.903) and tannin (r = 0.638); Between the plasma CAT activity and flavonoids (r = 0.781) and tannins (0.855) content; Between the HDL-C level and the phenolic compounds (r = 0.984), flavonoids (r = 0.864) and tannins (r = 0.813). And a strong and negative correlation was observed between the IC_{50} of DPPH and ABTS and the phenolic compounds with respective values r = -0.650 and r = -0.847 ; Between the tannins and the level of plasma MDA (r = -0.841); Between the phenolic compounds, flavonoids, tannins and the LDL level (r = -0.927, r = -0.925 and r = -0.803 respectively) between the TG level and flavonoids (r = -0.802). This confirms that the phenolic compounds, the flavonoids and the tannins are directly associated with the antioxidant and antihyperlipidemic activities as described by several authors[48,49].

In addition, the comparison observed between the fraction <50 µm and the phenolic compounds, flavonoids and tannins confirms the fact that this fraction is the richest in these bioactive compounds. This observation explains the increasing of the enzymatic activities (SOD and CAT) and HDL-C level. As well as the IC_{50} in the DPPH and ABTS test, the lowest levels of MDA, TG, LDL-C, CT and the CT/HDL and LDL/HDL ratios were observed. Thus, this fraction improved various parameters compared to the other fractions and to the unsieved powder.

CONCLUSION

In this study the influence of the particle size on the content of phenolic compounds and the antioxidant and antihyperlipidemic activities of the pulp of *Adansonia digitata* was studied. The highest content of phenolic compounds and maximum antioxidant and antihyperlipidemic proprietor were observed with the fraction <50 µm. This fraction shows protection against hyperlipidemia by improving the blood lipid profile by reducing the level of malondialdehyde and by raising the level of antioxidant enzymes in the organs. Its effect was correlated with their content of bioactive compounds. This study therefore shows the link between particle size and the antioxidant and antihyperlipidemic activities of the baobab pulp.

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