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Antioxidant properties of granulometric classes and solvent extraction of *Diospyros mespiliformis* Hochst. ex A. fruits powder

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ABSTRACT

The influence of solvent extractions (ethanolic and hydroethanolic extract) and extraction by controlled differential sieving processing (CDSp) on chemical content, *in vitro* and *in vivo* antioxidant capacity of *Diospyros mespiliformis* fruits powders was investigated. *D. mespiliformis* fruits powder were finely ground and fractionated by CDSp into four particle size fractions (< 50 µm; 50 – 180 µm; 180 – 315 µm; and > 315 µm). The chemical components determined were carotenoids, vitamin C, vitamin A and saponin. The *in vitro* antioxidant activity was carried out using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. To evaluate the *in vivo* antioxidant activity against high fat diet (HFD) induced hyperlipidemia in rats; the different particle size powder fractions, ethanolic and hydroethanolic extract of *D. mespiliformis* fruits were administered orally (600 mg/kg, po) for 30 days, with HFD and the effect of extract on enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT) and peroxidase were estimated in the blood, heart, liver and kidney. Toxicity parameters like alanine transaminase and aspartate aminotransferase were evaluated in these organs. The < 50 µm fraction contained higher carotenoids (19.27 ± 0.21 mg/100 g DW) and vitamin A (3.21 ± 0.03 mg/100 g DW) than the other fractions, unsieved powder (6.87 ± 0.11 mg/100 g DW for carotenoids and 1.15 ± 0.02 mg/100 g DW for vitamin A) ethanolic extract (7.02 ± 0.30 mg/100 g DW for carotenoids and 1.17 ± 0.04 mg/100 g DW for vitamin A) and hydroethanolic extract (3.56 ± 0.19 mg/100 g DW for carotenoids and 0.59 ± 0.03 mg/100 g DW for vitamin A) of *D. mespiliformis* fruits. The vitamins were highly concentrated in ethanolic extract (12.22 ± 0.02 mg/100 g DW) followed by powder fraction of < 50 µm (11.11 ± 0.41 mg/100 g DW). The different samples of *D. mespiliformis* fruits powder improved the SOD, catalase, peroxidase, alanine transaminase and aspartate aminotransferase levels significantly as compared to the control group. The present study has revealed that the fraction, < 50 µm powder of *D. mespiliformis* fruits have significant *in vivo* antioxidant activity than the ethanolic extract and can be used to protect tissues from oxidative stress.

INTRODUCTION

Oxidative stress has been implicated in the development and progression of many diseases such as inflammation, cancer, coronary heart disease, atherosclerosis and Alzheimer's disease (Yamaguchi et al., 2000; Soobrattee et al., 2006; Jones, 2008). Normally, animal cells are equipped with many defense mechanisms against

oxidative stress including glutathione (GSH), vitamins C and E, catalase (CAT), superoxide dismutase (SOD) and various peroxidases (Kryston et al., 2011; Nimse and Pal, 2015). Synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used in processed foods. It has been reported that these compounds have some side effects and are carcinogenic (Botterweck et al., 2000; Sasaki et al., 2002). The side effects have necessitated further research for alternative sources of antioxidants such as natural products. Although there is limited scientific validation of the safety of natural products, it is generally accepted that natural products are safer compared to synthetic ones (Vongtau et al., 2005; Oluyemi et al., 2007).

Plants have been identified as a huge source of structurally diverse and important antioxidant compounds with potentials for development into novel therapeutic molecules (Rout et al., 2009). Special attention has been paid to fruits, as they are a rich source of phenolic compounds (Manganaris et al., 2014; D'Angelo, 2020). The indigenous fruit trees for example *Diospyros mespiliformis* Hoschst ex A. DC which are sources of edible fruits rich in vitamins and minerals (Magaji, 2019). The plant is reportedly one of the most important genera of Ebenaceae whose species have been used over the millennia in traditional medicinal systems including Ayurveda, Chinese and African folklores (Jigam et al., 2012; Sara et al., 2018). It is widely used in parts of Africa and a number of chemical constituents of therapeutic importance have been isolated (Abba et al., 2016). The leaves are used in sleeping sickness, malaria, headache and anthelmintic (Adzu et al., 2002 ; Belemtougri et al., 2006) and extraordinary remedy for fever and for wounds, barks and roots are used to treat malaria, pneumonia, syphilis, leprosy, dermatomycoses and diarrhea, facilitation of delivery and as psycho-pharmacological drug (Mohamed et al., 2009). A traditional food plant in Africa, the fruit has potential to improve nutrition.

There are several techniques which are used in extracting bioactive compounds from medicinal plants. The extraction of antioxidant components of most plant materials strongly depends on the nature of extraction techniques and solvent (Hayouni et al., 2007; Jakopic et al., 2009). The extraction from a plant material is mainly affected by the vigor of the extraction procedure which may differ from one sample to another (Jakopic et al., 2009). However, the efficiency of solid/liquid extraction processes is affected by critical processing parameters, such as temperature, nature of solvent, structure of solid matrix (mainly particle size) and extraction time (Franco et al., 2007; Maria et al., 2009). Conventional extraction methods usually involve water or organic solvents and may result in the noticeable degradation of components (Penchev, 2010; Karam et al., 2016). On the other hand, both the particle size of the plant matrix and the temperature of the extraction process are easily manipulated physical conditions. The limitations of some techniques which include; lengthy extraction time, requirement of costly and high purity solvents, evaporation of the large amount of solvent, poor extraction selectivity and thermal decomposition of thermolabile compounds (Luque de Castro and Garcia-Ayuso, 1998; Wang and Weller, 2006), more promising extraction techniques referred to as the nonconventional techniques were developed which include ultrasound assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, pulsed electric field assisted extraction, supercritical fluid extraction and pressurized liquid extraction (Azmir et al., 2013). But, Ma et al. (2008) concluded that phenolic compounds from the barks of *Citrus reticulata* became unstable at elevated temperatures (above 50°C) with ultrasound-assisted extraction. Prasad et al. (2010) found that pressurized fluid extraction was responsible for a conformational change and denaturation of proteins. But there is also a use of solvent and the loss of sensitive biomolecules (Piriou, 2012). Recently, alternation method of drying and grinding process and CDSp extraction (grinding and controlled differential screening) refers in combinations of drying, grinding, and controlled sieving processes, has received increasing attention due to the raising desire to develop ecological extraction technologies of natural and active ingredients (Baudelaire, 2013; Becker et al., 2017; Karam et al., 2016; Zaiter et al., 2016; Zaiter et al., 2018). The sieving process separates plant powders by granulometric differentiation through sieves of decreasing mesh, leading to selective distribution of bioactive molecules in the different granulometric (particles sizes) fractions (Becker et al., 2016; Zaiter et al., 2018).

Previous studies indicated that the different particle size fractions, unsieved powder and solvents extracts of *D. mespiliformis* fruits have antihyperlipidemia activity and a source of polyphenol compounds which depend on particle size fraction. However, none of the published report described the chemical components and the antioxidant capacities of different particle size fractions, unsieved powder of *D. mespiliformis* fruits. Therefore, the objective of the present study was to determine the influence of particle size of *D. mespiliformis* fruits on the carotenoids, vitamin C, vitamin A and saponin content and *in vitro* and *in vivo* antioxidant capacity compared to unsieved powders and solvent extracts.

MATERIALS AND METHODS

Plant material

The dried fruits of *D. mespiliformis* were collected during

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the month of February, 2017, in Kaélé, Far-North region of Cameroon, and were transported to the laboratory of Biophysics, Food Biochemistry and Nutrition at the University of Ngaoundere.

Preparation of ethanolic and hydroethanolic extracts of *D. mespiliformis* fruits

To prepare the solvents extracts, 100 g of *D. mespiliformis* fruits powder was mixed with ethanol in the ratio of 1/10 (w/v) and hydroethanol (50%) in the ratio 1/10 (m/v) and stand under stirring (Variomag Poly) for 24 h at 18°C to improve the extraction. Thereafter, the homogenate obtained was then filtrated with Whatman No 1 filter paper and ethanol was removed using a rotary evaporator (BUCHI - R210/215) at 40°C and under reduced pressure of 17,500 Pa. Finally, the frozen extract (-18 C) was put into the freeze dryer chamber for 48 h and under pressure of 10 Pa. The temperature of freeze dryer was at -60°C. The collected powder extract was conditioned in polyethylene bags and stored at 10°C until analysis.

Production of powder fractions

For the production of granulometric fraction, an electric Ultra-Centrifugal Mill ZM 200 (Haan, Germany) supplied with 24-tooth rotor of 99 mm and trapezoid holes a mesh sieve of 1 mm and operated at 6,000 rpm was used to make whole powder of *D. mespiliformis* fruits. About 1.5 kg of the *D. mespiliformis* fruits were slowly ground by 50 g batches, operating by impact and shearing effects. Grinding was performed at room temperature (approximately 20°C). This rotor speed was chosen as a compromise between grinding efficiency and local temperature increase in plant parts during grinding, as the latter is known to be enhanced at high rotor speed and lead to bioactive compounds alteration (Karam et al., 2016; Zaiter et al., 2016).

The sieving process is based on the separation of particles from a granular material by making them pass through several sieves of decreasing mesh size (315, 212 and 180 µm in the current study). Ground plants were sieved with the Analysette 3 Spartan apparatus (Fritsch, Idar-Oberstein, Germany), operating by vertical vibration. Basically, 100 g ground plant sample was sieved in permanent vibratory mode at 0.5 mm amplitude for 10 min. The fraction of the powder retained on each sieve was recovered and weighed for the calculation of the mass fraction of each granulometric class. A sample of unsieved plant powder was kept for comparison purposes. Resulting plant powders were then put in sealed polyethylene plastic bags and stored at 10°C until analyses.

After grinding, ground *D. mespiliformis* fruits were sieved. The sieving process is based on the separation of particles from a granular material by making them pass through several sieves of decreasing mesh size (315, 180 and 50 µm in the current study). 100 g ground powder were sieved with the vibratory sieve shaker Analysette 3 Spartan (Fritsch, Idar-Oberstein, Germany) at 0.5 mm vibration amplitude for 10 min. A sample of unsieved plant powder was kept for comparison purposes. Resulting plant powders were then put in sealed polyethylene plastic bags and stored at 10°C until analyses.

Determination of vitamin C content

The indophenol method (Tomohiro, 1990) was use. This method is based on the oxidation-reduction reaction between indophenol and vitamin C.

Standardization of indophenol solution

To each of the three the volumetric flasks 5 mL of 10% glacial acetic acid solution (99 – 100% purity) was pipetted, followed by 2 mL of standard ascorbic acid solution (1 mg/mL distilled water). The mixture was titrated with indophenol solution until a faint pink color appeared at about 30 s, and then volume V (1) was recorded. To the three flasks 7 mL of 10% glacial acetic acid solution were added, followed by equal volume of water to that of indophenol used and the mixture was titrated against indophenol solution until a pink color was formed. Then a titration of the blank with the indophenol solution to the turnaround was performed and volume V (2) was noted.

Exactly, 1 g of sample was weighed and then ground in a mortar with a little Fontaine bau sand and 10% acetic acid; then the volume was adjusted to 50 mL in a volumetric flask with the acetic acid solution, the mixture was filtered immediately. Ten milliliters of the sample were transferred into conical flasks and titrated with indophenol solution until a pink color appeared, then the volume A was noted. In a second step, 10 mL of acetic acid was introduced into a beaker, a volume of water equivalent to the volume of the indophenol A solution was added. The contents of each beaker were titrated with the indophenol solution and the corresponding volume B was recorded.

The amount of vitamin C equivalent to 1 mL of indophenol solution is expressed as follows:

$$C = \frac{Cs}{V(1) - V(2)}$$

$$Qc(mg/100g) = \frac{(A-B) \times C \times VT \times 100}{Vi \times S}$$

Determination of vitamin A contents and carotenoids contents

The carotenoids are extracted with the hexane-acetone mixture: 30/70 (v/v) and are subjected to reading with a spectrophotometer between 430 and 450 nm (AOAC, 1990).

To do this, 1 g of sample was weighed, 30 mL of the 30/70 (v/v) hexane-acetone mixture was added, the whole was heated under reflux for 1 h and then cooled and filtered. The filtrate was washed with distilled water in a separatory funnel, the lipid phase was transferred to a 25 mL flask and adjusted to volume with hexane. This solution was diluted 1/10th with hexane and the optical density was read between 430 and 450 nm to determine the maximum absorbance.

The concentration of carotenoid was expressed in milligram per 100 g of dry weight (DW) and calculated according to the formula:

$$C = \frac{OD * f}{196 * m}$$

OD: the optical density obtained for maximum absorption; f: dilution factor and m: mass of test powder sample (g). The quantity of carotenoids in the diluted solution for spectrophotometric reading is QC with QC = CV.

According to the conversions of Coultate (1988), 6 µg carotenoids = 1 µg vitamin A.

Determination of saponin content

The determination of saponin content was determined by the method according to Hiai et al. (1976). In an acidic medium vanillin reacts with an OH group in position C-3 to give a chromogenic complex with a maximum absorbance at 544 nm.

For the extraction of the saponins, 0.2 g of sample has been introduced in a tube. Then 10 mL methanol (70 %) was added. The mixture was stirred for 1 h and centrifuged at 3500 rpm for 20 min; the supernatant was recovered in a tube and the volume noted constitutes the saponin extract.

For the assay, 0.125 mL of saponin extract was placed in test tubes and 1 mL of distilled water was added. A volume of 0.5 mL of 1/10 (v/v) prepared FolinCiocalteu reagent, 0.125 mL of vanillin reagent (8% vanillin in ethanol) and 1.25 mL of 72% sulfuric acid were added successively to the test tubes. The tubes were then vortexed and incubated at 60°C for 10 min and cooled in an ice bath and the absorbance was measured at 544 nm. The standard range was performed in the same way using a 1 g/mL saponin stock solution. The amount of saponin was expressed in milligram of saponin per 100 g of dry product (mg/100 g) from the regression equation DO = aQ + b established with the standard range.

The quantity of saponins in the test sample (Q) was determined by referring to the calibration curve of the regression equation Optical Density DO = Xa. The result was expressed in equivalent mg of saponins/100 g MS.

Determination of DPPH free radical scavenging activity assay

The DPPH free radical scavenging activity of the solvents extracts, unsieved powders and different particle size fractions of *D. mespiliformis* fruits was determined by DPPH assay, and ascorbic acid (vitamin C) was used as a standard as described by Zhang and Yasumori (2004) with modification.

The electron donating capacity of the extracts was measured by whitening of the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) cation radical. This assay is based on the ability of antioxidants to scavenge the DPPH cation radical. Here, 2 mL of 0.1 mM DPPH methanolic solution was added to 0.5 mL hydromethanolic extract of plant sample at different concentrations (0.025, 0.05, 0.1, 0.5, 1, 5, 10, 100 mg/mL). The mixture was thoroughly stirred and incubated in the dark for 1 h at room temperature. After that, absorbance of the mixture was measured at 517 nm by UV/visible spectrophotometry. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage of DPPH radical scavenging potential of the compounds and standard control (ascorbic acid) were calculated from the equation below.

$$\text{Inhibition of free radical (\%)} = \frac{\text{(Control absorbance-Sample absorbance)} \times 100}{\text{(Control absorbance)}}$$

The antioxidant activity was expressed as the concentration required to cause 50% DPPH scavenging, referred to as IC_{50} (µg/ml).

Animals

Healthy male Wistar albino rats weighing 200 – 300 g were procured from the animal house of National School of Agro-Industrial Sciences of the University of Ngaoundere in Cameroon. The animals were kept under standard environmental conditions of room temperature (25 ± 1°C), relative humidity (35 to 60%) and 12 h light and dark cycle in the animal house of biochemistry and nutrition laboratory. The rats were fed with a standard laboratory diet and water *ad libitum*. The animals were left to acclimatize to laboratory conditions for at least two weeks before experiment.

High fat diet induced hyperlipidemic model – antioxidant study

The rats were fed with a high-fat diet (HFD) composed of 300 g of yolk, 2 g of cholesterol, 250 g of coconut oil and 50 g of soya oil for 30 days, as described by Hamlat et al. (2008) and Ngatchic et al. (2013), with some modifications. The rats were divided into 11 groups, with 5 rats in each group. Treatment was done at the same time as induction for 30 days between 07:00 and 10:00 h. Group I served as normal control, the rats were fed with normal pellet diet and treated with vehicle (distilled water); group II was negative control rats fed with HFD and treated with vehicle (distilled water); group III which served as positive control received HFD and vitamin C (20 mg/kg of body weight), groups IV, V, VI, VII, VIII and IX hyperlipidemic rats were fed with HFD treated orally with *D. mespiliformis* powder fractions < 50 µm, 50 - 180 µm, 180 - 315 µm, > 315 µm respectively and at a dose of 600 mg/kg of body weight. Groups X and XI hyperlipidemic rats were fed with HFD treated orally with ethanolic and hydroethanolic extracts *D. mespiliformis,* respectively.

Preparation of organs homogenate and blood serum

At the end of 30 days of treatment, animals were fasted overnight and weighted. Afterwards, they were anesthetized with ether petroleum. The blood samples were collected from the neck by cardiac puncture. The blood samples collected were centrifuged at 3000 rpm for 10 min at 4°C. The supernatant (serum) obtained was stored at -4°C.

After blood collecting, liver, kidney and heart were removed from three rats in each group. The liver, kidney and heart harvested were cleaned of adhering tissue and homogenized in phosphate buffer (0.1 M pH 7.4 having 0.15 M KCl). The homogenate was centrifuged and the supernatant were stored at -20°C until used for the determination of oxidative stress markers.

Determination of lipid peroxidation

Malondialdehyde (MDA), an index of lipid peroxidation, was determined using the method described by Yagi (1976) with little modification, a major indicator of oxidative stress. This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid (TBA) producing TBA reactive substance, a pink chromogen, which can be measured spectrophotometrically at 532 nm. Thus, 100 µL of the sample, 400 µL of TBA reagent and 80 µL of HCl was thoroughly stirred and incubated in a boiling water bath for 15 min. After that, the mixture was cooled in a cold-water bath for 30 min, and mixture was centrifuged at 3000 rpm for 5 min at 25°C. The absorbance was measured on a UV/visible spectrophotometer. The results were expressed in terms of the MDA content in µmol per milligram of protein using molar extinction coefficient of MDA ($\varepsilon = 1.53*10^5$ L/mmol/cm), according to the formula below: $C = DO / \mathcal{E}. L$

Determination of superoxide dismutase activity

Superoxide dismutase activity was estimated by the method of Beauchamp and Fridovich (1971). The reaction mixture consisted of; 0.2 mL of sample introduced in 2.5 of 0.1 M carbonate buffer solution at pH 10.2. Then, 0.3 mL of aqueous solution of adrenalin was added to the mixture to trigger the reaction. After homogenization, the absorbance of the mixture was measured every 30 s until 150 s in order to follow an increase of absorbance at 480 nm. 300 µL of distilled water were used in a reference tube. The SOD activity was thereafter expressed as units per milligram of protein.

Determination of catalase activity

Catalase activity was determined according to the method of Sinha (1972). This method is based on the direct measurement of the decrease in absorbance at 240 nm due to $2O_2$ consumption by CAT. Thus, 1 mL of phosphate buffer (0.1 M) at pH 7.0 and 0.4 Ml of H_2O_2 (0.2 M) were added to 0.1 mL of the sample contained in the tube. The reaction was stopped at 30, 60 and 90 s by adding 2 mL of dichromate/acetic acid mixture (5/95, v/v). Absorbance was measured at 620 nm, and CAT activity expressed in units per milligram of protein using molar extinction coefficient of CAT (e = 0.036 L/mmol/cm).

The results were expressed by the protein content determined according to the method of Lowry et al. (1951), MDA content (lmol/mg protein), CAT activity (units/mg protein) and SOD activity (units/mg protein) were determined in the organ homogenates and blood serum.

Determination transaminase

These hepatotoxicity markers of alanine transaminase (ALT) and aspartate transaminase (AST) were estimated using INMESCO kits following the methods described by the manufacturers.

Statistical analysis

Results were expressed as mean + standard deviation and all statistical comparisons were made by means of one-way ANOVA test followed analysis and P – values less

Table 1. Carotenoid, vitamin A, vitamin C and saponins contents of powders (CDSp fractions, unsieved powder, hydroethanolic and ethanolic extract).

Samples	Carotenoids (mg/100g DW)	Vitamin A (mg/100g DW)	Vitamin C (mg/100g DW)	Saponins (mg mg/100g DW)
F : < 50 µm	19.27 ± 0.21^{f}	3.21 ± 0.03^{f}	11.11 ± 0.41^{f}	9.41 ± 0.08^{d}
F : 50 - 180 µm	9.59 ± 0.08^{e}	1.60 ± 0.01^{e}	8.46 ± 0.03^{c}	8.34 ± 0.54^{c}
F : 180 - 315 µm	8.24 ± 1.52^{d}	1.37 ± 0.02^{d}	8.21 ± 0.05^{b}	7.33 ± 0.19^{b}
F : > 315 µm	2.12 ± 2.01^{a}	0.35 ± 0.00^{a}	6.44 ± 0.00^{a}	3.55 ± 0.03^{a}
Unsieved powder	6.87 ± 0.11^{c}	1.15 ± 0.02^{c}	9.86 ± 0.02^{d}	3.26 ± 0.04^{a}
Hydroethanolic extract	3.56 ± 0.19^{b}	0.59 ± 0.03^{b}	10.22 ± 0.03^{e}	11.37 ± 0.06^{e}
Ethanolic extract	7.02 ± 0.30^{c}	1.17 ± 0.04^{c}	12.22 ± 0.02^{g}	11.62 ± 0.08^{e}

F, Fraction; DW, dry weight. Means ± standard deviations with different superscripted letters within the same line differed significantly ($p < 0.05$).

than or equal to 0.05 were considered significant. Duncan's multiple ranking test was used to rank the averages. All of these analyses were done using the Stat Graphics Plus 5.0 software and curves plotted using the Excel 2010 software.

RESULTS AND DISCUSSION

Vitamin, carotenoids and saponin contents

The result of carotenoids, vitamin A, vitamin C and saponin contents in different powder fractions, unsieved powder, ethanolic extract and hydroethanolic extract of *D. mespilifomis* fruits is shown in Table 1.

Table 1 shows that, carotenoid, vitamins A and vitamin C contents were more concentrated in the lower particle size. The carotenoids (19.27 ± 0.21 mg/100g DW) and vitamin A (3.21 ± 0.03 mg/100g DW) contents of particle size of < 50 were much higher than those of other CDSp powder fractions, unsieved powder and solvent extracts (ethanolic and hydroethanolic). On the other hand, the vitamin C content of the ethanolic extract was 12.22 ± 0.02 mg/100g DW, which was more than the < 50 µm (11.11 ± 0.41 mg/100g DW) particle size. Comparing different powder fractions, the highest vitamin C concentration was obtained from < 50 µm (11.11 ± 0.41 mg/100g DW) particle size. The particle size of > 315 µm had a lower vitamin C (6.44 ± 0.00 mg/100g DW)
concentration than the other fractions.

The results of vitamin C content showed that small particle size fractions were more concentrated with vitamin C than large particle size fractions. These results are not similar to those found by Deli et al. (2020) on the calyxes of *Hibiscus sabdariffa* powder fractions; they found no significant difference between the different particle size fractions. In addition, Djantou (2016) showed that, they were no significant difference ($p < 0.05$) between mango powders of small particles size and those of large particles size. Our study showed that the ethanolic extract has shown high vitamin C content compared to all CDSp powder fractions of *D. mespilifomis* fruits. These results are similar to those found by Deli et al. (2020) on the CDSp powder fractions and ethanolic extract of the calyxes of *H. sabdariffa*. The abundance of vitamins C in the fractions of < 50 µm may be explained by the fact that the finest particles would be individualized during grinding and would pass preferentially to the smaller particle size during sieving.

The results of this study show that, the particle size fractions were highly concentrated in carotenoids and vitamin A than the solvent extracts (ethanolic and hydroethanolic). A very significant difference ($p < 0.001$) between carotenoids content in the < 50 µm fraction (19.27 ± 0.21 mg/100g DW), than ethanolic extract (7.02 ± 0.30 mg/100g DW).

The vitamin A and carotenoid contents were highly concentrated in the ethanolic extract compared to the hydroethanolic extract. There was a statistically significant difference ($p < 0.01$) between the vitamin A content of the particle size of < 50 µm (3.21 ± 0.03 mg/100g DW) and the ethanolic extract (1.17 ± 0.04 mg/100g DW). The < 50 µm fraction was more concentrated with vitamin A than the other fractions and solvent extracts (ethanolic and hydroethanolic). The highest carotenoid contents (19.27 ± 0.21 mg/100g DW) was obtained using the particle size of < 50; but the smallest carotenoid contents were obtained using large particle size of > 315. Carotenoid content was significantly affected by the particle sizes. It impairs in particle size processed by superfine grinding performed by CDSp; carotenoid content was raised. We noted there was a high significant difference ($p < 0.01$) between the carotenoid content of the particle size < 50 µm (19.27 ± 0.21 mg/100g DW) and the ethanolic extract (7.02 ± 0.30 mg/100g DW).

Thus the carotenoid contents of the other fractions are 9.59 ± 0.08 mg/100g DW for the fraction of 50 – 180 µm, 8.24 ± 1.52 mg/100g DW for the fraction of 180 – 315 µm,

2.12 ± 2.01 mg/100g DW for the fraction of > 315 µm, 6.87 ± 0.11 mg/100g DW for the unsieved powder, 3.56 ± 0.19 mg/100g DW for the hydroethanol extract. Comparing these two extraction methods, the particle size of < 50 µm has a higher vitamin A and carotenoid contents compared to the other particle size fractions and solvent extracts (ethanolic and hydroethanolic).

Carotenoids are major determinants of the organoleptic and nutritional quality of fruits and pigments responsible for the colors (orange and yellow) of fruits and vegetables, and their appearance indicates low levels or degradation of chlorophyll (Yahia and Ornelas-Paz, 2010; Gross, 2012). These metabolites are not only responsible for the red or bright orange color, but in humans, the antioxidant properties of carotenoids are believed to be responsible for their involvement in the prevention of chronic diseases (Linnewiel et al., 2009).

A diet rich in carotenoids may reduce the risk of cancer, muscle degeneration and sunburn-induced skin damage (Wang et al., 2008) and may prevent the development of certain chronic diseases such as cancer and cardiovascular disease (Wright et al., 2003; Li et al., 2015). There is a reduced risk of coronary heart disease or infarction when carotenoid intake is high (Kritchevsky, 1999).

The increase in vitamin A and carotenoids contents would be due not only to the sensitivity of vitamin A to different chemical factors such as oxygen, temperature, pH, light (Loveday and Singh, 2008). The major causes of carotenoid destruction during processing and storage are enzymatic and non-enzymatic oxidation. As a result, the transformation leads to a loss of coloring and biological activities of the carotenoids. The carotenoids are located in the chloroplasts, themselves surrounded by the cell walls (Vishnevetsky et al., 1999). Our result showed that grinding could liberate bioactive compounds which have antioxidant activity. In addition, the grinding of vegetables results in a breakdown of the plant cell structure, facilitating micronutrients release and improvement of their bioavailability (Lemmens et al., 2010; Svelander et al., 2011; Knockaert et al., 2012; Moelants et al., 2012; Palmero et al., 2013; Deli et al., 2020). Their release and incorporation into the micelles depend on the level of disruption of plant tissues and cells during the grinding and digestion process (Low et al., 2015). Fragmentation of food (fruits and vegetables) into small pieces by mechanical action (cutting, chopping, homogenizing or grinding) improves the bioavailability of carotenoids.

The saponin content of CDSp fractions, unsieved powder, hydroethanolic and ethanolic extracts of *D. mespilifomis* fruits are presented in Table 1. These levels also show that as the particle size increases the saponins decrease. There is a very significant difference ($p < 0.001$) between the large particle size fraction and the solvents extracts. According to Table 1, the saponin content of *D. mespiliformis* fruits, of the different particle size fractions differs significantly ($p < 0.05$) from that of the extracts. It is 11.62 ± 0.08 mg/100g DW for ethanolic extract, 11.37 ± 0.06 mg/100g DW for hydroethanolic extract, particle size < 50 µm (9.41 ± 0.08 mg/100g DW), particle size 180 – 315 µm (7.33 ± 0.19 mg/100g DW) and particle size 50 – 180 µm (8.34 ± 0.54 mg/100g DW).

D. mespiliformis fruits are not only a source of minerals but also a source of carotenoids and vitamin A. When compared to usual fruits, these contents are high in *D. mespiliformis* fruits and more important in the < 50 µm fraction.

DPPH radical scavenging assay

The efficacy of the antioxidant was based on the availability to scavenge free radicals. In order to evaluate this efficacy, the diphenyl-picrylhydrazyl method was used. The antioxidant activity of *D. mespiliformis* was measured in terms of radical scavenging ability, using DPPH method. The degree of discoloration indicates the scavenging potential of the antioxidants present in the extracts (Molyneux, 2004).

For DPPH free radical scavenging potential of the CDSp powder fractions, unsieved powder, solvent extracts of *D. mespiliformis* fruits and vitamin C, the IC_{50} values are represented in Figure 1. The IC_{50} values of scavenging DPPH radicals for particle size < 50 µm and vitamin C were 0.034 ± 0.015 and 0.004 ± 0.002 mg/mL respectively. In this current study, the IC_{50} of particle size < 50 µm of *D. mespiliformis* demonstrated significantly higher free radical scavenging effect compared to the other powder fractions, unsieved powder and solvent extracts. The results from DPPH radical scavenging activity were found to be high in vitamin C (IC_{50} = 0.004 ± 0.002 mg/mL) followed by particle size < 50 (IC_{50} = 0.034 ± 0.015 mg/mL), ethanolic extract (IC_{50} = 1.037 ± 0.204 mg/mL), hydroethanolic extract (IC_{50} = 1.111 ± 0.133 mg/mL), powder fraction of 180 – 315 µm (IC_{50} = 1.201 ± 0.218 mg/mL), unsieved powder (IC_{50} = 1.475 ± 0.136 mg/mL), powder fraction of 50 – 180 µm (1.489 ± 0.160 mg/mL) and powder fraction of > 315 µm (IC_{50} = 2.308 ± 0.241 mg/mL). These results showed that DPPH radical scavenging potential is not dependent on the particle sizes.

Carotenoids and vitamin A are excellent scavengers of radical species due to their conjugated double bond system (Packer et al., 1981; Mortensen et al., 2001; Skibsted, 2012). Its antioxidant activity is linked to its long polyene chain, which allows them to act with radicals by simple electrophilic addition and electron transfer and to neutralize singlet oxygen (Valko et al., 2006). Thus, this activity is important in that fraction of *D. mespiliformis* fruit which is probably due to its high carotenoid content which could have acted with radicals by simple electrophilic addition and electron transfer, which neutralized it. The antioxidant efficiency of the smaller particle size fraction

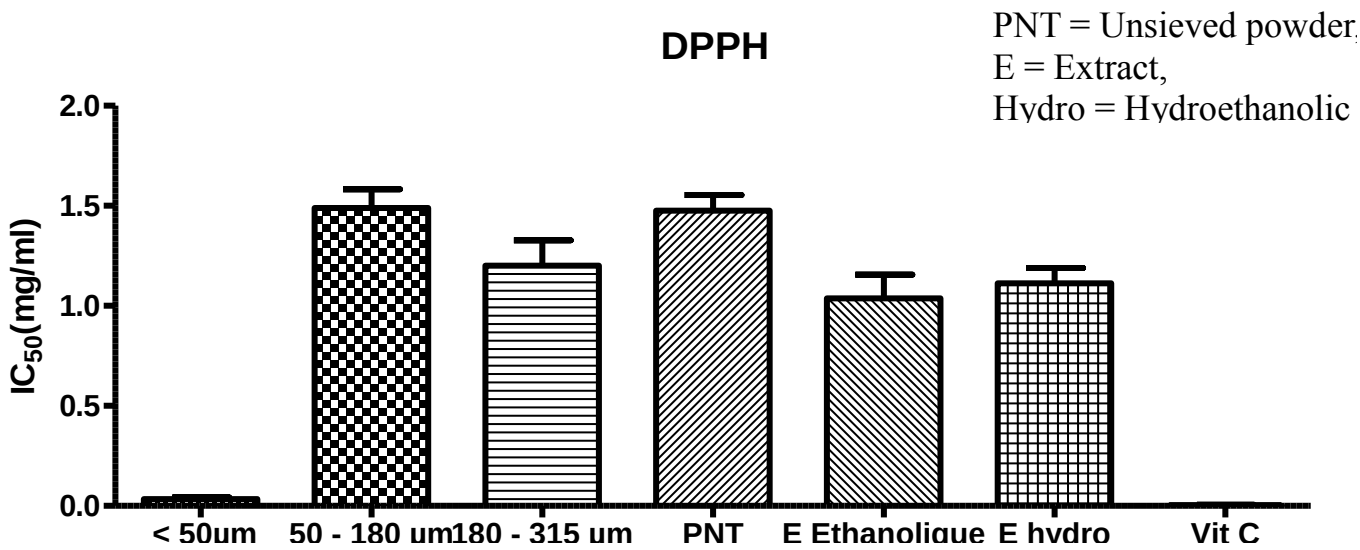

Figure 1. Values of IC_{50} of *D. mespiliformis* fruits.

could also be due to the saponins and vitamins present in these fine particles of the *D. mespiliformis* fruits, which are known as antioxidant substances with the ability to trap radical species and reactive forms of oxygen. The main mechanism of action of phenolic compounds is the scavenging of free radicals by the transfer of the H atom to the DPPH, which is then transformed into a stable DPPH molecule (Popovici et al., 2010).

This antioxidant efficacy could be also explained by carotenoids and vitamin A contents, which are higher in the particle size fraction < 50 µm compared to that of the ethanolic extract. Vitamin A has an important antioxidant capacity due to its long carbon chain carrying multiple conjugated unsaturations (Landete, 2013) and the antioxidant activity of carotenoids is essentially based on the scavenging of the superoxide anion or peroxide radicals. This result may also be explained by the fact that this particle size fraction concentrates molecules in small quantities that are endowed with antioxidant activity.

The objective of this part was to see the effect of CDS extraction on vitamins, carotenoids, saponins and antioxidant activity of *D. mespiliformis* fruit powders and to compare it to the conventional method (ethanolic and hydroethanolic extracts).

The results obtained show that *D. mespiliformis* fruits are rich in bioactives components. Fractionation by the PTC method gives a better result than the hydroethanolic and ethanolic extracts in terms of antioxidant activity, as the powder fraction < 50 µm has a higher antioxidant activity than the solvent extracts. The powder fraction < 50 µm has a yield comparable to that of the ethanolic extract. This fraction is positioned as the most effective fraction against CVD, so it would be important to see how it would act *in vivo.*

In vivo antioxidant effect of the different particle size fractions, unsieved powder and solvent extracts of the fruits of *D. mespiliformis*

MDA is a widely used parameter for assessing oxidative damage to lipids. Lipid peroxidation reactions define a set of chain reactions that often result in the production of MDA, a compound that is toxic to the cell; a high level in the body is indicative of the presence of reactive oxygen species (ROS) and toxicity.

As for CAT and SOD, they are enzymes involved in the process of eliminating reactive species from oxygen. The increase in their activity is synonymous with good antioxidant power in the plasma or organ in question. Table 2 shows the influence of the HFD on the MDA content, CAT and SOD activities of animals treated with atorvastatin and untreated rats. The HFD diet significantly ($p < 0.05$) increases the MDA content and decreases the activity of CAT and SOD in most of the tissues analyzed, including blood, liver, heart and kidneys.

Effect of the hyperlipidemic diet on the concentration of MDA

The results obtained in this study indicate that lipid peroxidation increased in the negative control group corresponding to the animals which only received the HFD, this is revealed by the high levels of MDA (2.09 ± 0.01 µM), compared to the positive control group (1.08 ± 0.02 µM) which corresponds to the animals which received HFD and atorvastatin (Table 3). Pretreatment with the different granulometrics fractions and solvent extracts of *D. mespiliformis* fruits at a dose of 600 mg/kg with the HFD

Table 2. Effect of hyperlipidic diet on oxidative stress parameters in rats.

	Parameters	Normal diet	Hyperlipidic diet
Blood	MDA (µM)	1.15 ± 0.05^{bc}	2.09 ± 0.01^{b}
	CAT (Units/mg)	139.15 ± 10.48^{e}	33.19 ± 4.00^{a}
	SOD (µUnits/mg)	97.53 ± 3.93^{f}	47.12 ± 3.87^{a}
Liver	MDA (µM)	1.23 ± 0.19^{abc}	2.95 ± 0.36^{c}
	CAT (Units/mg)	139.05 ± 6.07^{d}	45.96 ± 5.63^{a}
	SOD (µUnits/mg)	106.20 ± 3.50^{f}	19.65 ± 0.74^{a}
Kidney	MDA (µM)	1.22 ± 0.26^{a}	2.05 ± 0.01^{e}
	CAT (Units/mg)	182.31 ± 6.68^{d}	49.54± 5.10^{a}
	SOD (µUnits/mg)	81.98 ± 1.65^{d}	18.70 ± 1.65^{a}
Heart	MDA (µM)	1.15 ± 0.05^{bc}	2.66 ± 0.26^{e}
	CAT (Units/mg)	114.28 ± 14.45^{d}	19.42 ± 2.95^{a}
	SOD (µUnits/mg)	87.23 ± 2.52^{g}	37.06 ± 0.30^{a}

F, Fraction. Means ± standard deviations of five rats from each group; show significance when compared with positive control (vitamin C treatment) with different superscripted letters within the same line differed significantly ($p < 0.05$).

Table 3. Malondiodehyde levels in rats fed HFD and treated with particle size fractions, unsieved powder and solvent extracts of the *D. mespiliformis* fruits.

Samples	MDA (µM) blood	MDA (µM) heart	MDA (µM) liver	MDA (µM) kidney
Negative Control	2.09 ± 0.01^{e}	2.66 ± 0.26^{e}	2.95 ± 0.36^{c}	2.05 ± 0.01^{e}
Normal Control	1.15 ± 0.05^{bc}	1.04 ± 0.01^{a}	1.08 ± 0.03^{a}	1.23 ± 0.19^{abc}
Positive Control	1.08 ± 0.02^{a}	1.03 ± 0.05^{a}	1.07 ± 0.03^{a}	1.27 ± 0.05^{abc}
F : < 50 µm	1.16 ± 0.02^{c}	1.78 ± 0.14^{c}	1.05 ± 0.03^{a}	1.53 ± 0.04^{cd}
F : 50 - 180 µm	1.14 ± 0.03^{bc}	1.21 ± 0.02^{ab}	1.15 ± 0.03^{a}	1.09 ± 0.23^{ab}
F : 180 - 315 µm	1.23 ± 0.04^{c}	1.74 ± 0.08^{c}	1.25 ± 0.09^{ab}	1.72 ± 0.072^{c}
F : > 315 µm	1.62 ± 0.03^{d}	1.74 ± 0.09^{c}	1.44 ± 0.03^{b}	1.72 ± 0.09^{c}
Unsieved powder	1.19 ± 0.05^{cd}	2.11 ± 0.08^{d}	1.23 ± 0.02^{a}	1.01 ± 0.08^{a}
Hydroethanolic extract	1.10 ± 0.02^{ab}	1.23 ± 0.06^{b}	1.10 ± 0.02^{a}	1.42 ± 0.14^{bcd}
Ethanolic extract	1.19 ± 0.02^{cd}	1.09 ± 0.00^{ab}	1.19 ± 0.04^{a}	1.47 ± 0.53^{cd}

F, Fraction. Means ± standard deviations of five rats from each group; show significant when compare with positive control (vitamin C treatment) with different superscripted letters within the same line differed significantly ($p < 0.05$).

significantly reduced the MDA levels. The results show that MDA levels in blood samples were significantly reduced in the animals treated with the powder fractions of < 50 µm (1.16 ± 0.02 µM), 50 – 180 µm (1.14 ± 0.03 µM) and 180 – 315 µm (1.23 ± 0.04 µM), compared to the animals which received the solution of > 315 µm fraction (1.62 ± 0.03 µM) and the unsieved powder (1.19 ± 0.05 µM). However, no significant difference was observed between the < 50 µm, 50 – 180 µm and 180 - 315 µm fractions and solvent extracts ($P > 0.05$).

In the heart, only the animals treated with powder fraction of 50 – 180 µm fraction showed low MDA compared to those in the normal control group and those in the positive control group. In animals which received fractions of < 50 µm, 180 – 315 µm and > 315 µm on the other hand; have MDA levels which were significantly reduced compared to the negative control. There was no significant difference in MDA levels between animals treated with the particle size fractions and extracts (ethanolic and hydroethanolic).

In the kidney, MDA was significantly reduced in the animals treated with powder fractions of < 50 µm, 50 – 180 µm and 180 – 315 µm with values of 1.16 ± 0.02 µM, 1.14 ± 0.03 µM and 1.23 ± 0.04 µM respectively, compared to the > 315 µm fraction and unsieved powder with values of 1.62 ± 0.03 µM and 1.19 ± 0.05 µM respectively.

Table 4. Superoxide dismutase content of extracts and granulometric fraction of *D. mespiliformis* fruits.

Samples	SOD (µM) blood	SOD (µM) heart	SOD (µM) liver	SOD (µM) kidney
Negative Control	47.12 ± 3.87^{a}	37.06 ± 0.30^{a}	19.65 ± 0.74^{a}	18.70 ± 1.65^{a}
Normal Control	97.53 ± 3.93^{f}	87.23 ± 2.52^{g}	106.20 ± 3.50^{f}	81.98 ± 1.65^{d}
Positive Control	73.34 ± 0.25^{d}	88.33 ± 1.16^{g}	83.24 ± 0.64^{d}	67.91 ± 1.65^{c}
F : < 50 µm	87.69 ± 2.30^{e}	71.28 ± 1.44^{e}	98.59 ± 0.10^{e}	78.48 ± 1.65^{d}
F : 50 - 180 µm	105.03 ± 2.11^{g}	66.00 ± 1.41^{d}	81.67 ± 1.27^{d}	78.60 ± 1.65^{d}
F : 180 - 315 µm	84.25 ± 0.40^{e}	60.17 ± 1.98^{c}	70.56 ± 1.91^{c}	70.30± 1.65^{c}
F : > 315 µm	73.35 ± 0.45^{d}	51.74 ± 1.16^{b}	36.47 ± 0.78^{b}	55.54 ± 1.65^{b}
Unsieved powder	51.30 ± 0.48^{c}	56.53 ± 0.04^{c}	71.43 ± 1.41^{c}	69.98 ± 1.65^{c}
Hydroethanolic extract	50.27 ± 0.37^{b}	78.27 ± 0.89^{f}	84.33 ± 1.68^{d}	65.91 ± 1.65^{c}
Ethanolic extract	46.59 ± 0.65^{bc}	73.76 ± 3.54^{e}	82.77 ± 1.67^{d}	66.63 ± 1.65^{cd}

F, Fraction. Means ± standard deviations of five rats from each group; show significance when compared with positive control (vitamin C treatment) with different superscripted letters within the same line differed significantly ($p < 0.05$).

Table 5. Catalase levels in rats fed with different extracts and particle size fractions of the fruits of *D. mespiliformis.*

Samples	CAT (µM) blood	CAT (µM) heart	CAT (µM) liver	CAT (µM) kidney
Negative Control	33.19 ± 4.00^{a}	49.54± 5.10^{a}	45.96 ± 5.63	18.70 ± 1.65^{a}
Normal control	139.15 ± 10.48^{f}	182.31 ± 6.68^{d}	139.05 ± 6.07	81.98 ± 1.65^{d}
Positive Control	111.33 ± 12.04^{c}	203.70 ± 4.54^{e}	174.82 ± 1.25	67.91 ± 1.65^{c}
F : < 50 µm	133.53 ± 17.07^{e}	201.63 ± 3.44^{e}	158.43 ± 3.48	78.48 ± 1.65^{d}
F : 50 - 180 µm	112.97 ± 12.11	197.50 ± 8.17^{e}	129.00 ± 8.2	78.60 ± 1.65^{d}
F : 180 - 315 µm	90.30 ± 5.37^{b}	174.19 ± 8.16^{d}	89.72 ± 4.66	70.30± 1.65^{c}
F : > 315 µm	55.36 ± 4.07^{b}	110.59 ± 5.39^{b}	84.69 ± 4.56	55.54 ± 1.65^{b}
Unsieved powder	57.69 ± 3.44^{b}	181.91 ± 4.99^{d}	82.49 ± 14.15	69.98 ± 1.65^{c}
Hydroethanolic extract	70.59 ± 0.53^{b}	139.03 ± 4.71^{c}	96.01 ± 0.39	65.91 ± 1.65^{c}
Ethanolic extract	89.28 ± 6.93^{c}	177.44 ± 3.23^{d}	112.82 ± 3.11	66.63 ± 1.65^{cd}

F, Fraction. Means ± standard deviations of five rats from each group; show significance when compared with positive control (vitamin C treatment) with different superscripted letters within the same line differed significantly ($p < 0.05$).

Compared to solvent extracts, there is no significant difference ($P > 0.05$) between small particle size and medium particle size powder fractions.

In the liver, the MDA level is significantly reduced in the fractions < 50 µm, 50 – 180 µm and 180 – 315 µm, > 315 µm, unsieved powder, solvent extracts compared to the negative control. Compared to solvent extracts, there is no significant difference between small and medium fractions.

Activity of catalase and superoxide dismutase

The activities of catalase and superoxide dismutase obtained in the blood, kidney, liver and heart of animals treated with the different granulometric fractions, unsieved powder and solvent extracts (ethanolic and hydroethanolic) of the *D. mespiliformis* fruits are showed in Tables 4 and 5. The catalase activity was significantly lowered in the normal control group than negative control (untreated animals) but was significantly increased when compared to groups treated with different powder fractions of *D. mespiliformis* fruits. The < 50 µm fraction expressed the highest catalase activity compared to the other powder fractions, unsieved powder and solvent extracts. In general, catalase activity was particle size dependent; indeed, the potential catalase enhances when particle size impairs. The ethanolic extract showed higher catalase activity than hydroethanolic extract in all organs (heart, liver, and kidney) and serum. The > 315 µm fraction and unsieved powder showed the least activity compared to other samples.

The result of SOD activity showed that the particle sizes of 50 – 180 µm (105.03 ± 2.11 µM in blood) have the highest superoxide dismutase activity compared to the other CDSp powder fractions, unsieved powder and solvent extracts (ethanolic and hydroethanolic extracts) of

D. mespiliformis fruits. In general, the 50 – 180 µm fraction was more efficient than positive control (atorvastatin) and the SOD increased when particle size of powder fractions decreased. In the blood, the SOD activity decreased significantly in the animals treated with ethanolic (46.59 ± 0.65 µM) and hydroethanolic (50.27 ± 0.37µM) extracts compared to the < 50 µm fractions (87.69 ± 2.30 µM), 50 - 180 µm fractions (105.03 ± 2.11 µM) and 180 - 315 µm fractions (84.25 ± 0.40 µM), the > 315 µm fraction (73.35 ± 0.45 µM) and unsieved powder (51.30 ± 0.48 µM). In the heart, liver and kidney, SOD activity was decreased in the animals treated with the > 315 µm fraction.

The particles sizes fractions and solvent extracts (ethanolic and hydroethanolic) of the *D. mespliformis* fruits showed activity against stress by induction of catalase synthesis and superoxide dismutase. This activity would be due to the presence of phenolic compounds in these particle size fractions and the solvent extracts (ethanolic and hydroethanolic). Compounds as vitamin C, Vitamin A and carotenoids could be responsible potential antioxidant. As a large number of works carried out on antiradical activity have shown, phenolic compounds, and more particularly flavonoids, are recognized as potential antioxidant substances with the capacity to trap radical species and reactive forms of oxygen (Javanovic et al., 1994; Yanagimoto et al., 2004). Phenolic compounds therefore exhibit significant antioxidant activity that is mainly based on the redox properties of their hydroxyl group and the structural interactions between different parts of their chemical structure (Visioli et al., 1998; Djeridane et al., 2006).

Administration of the various fractions and extracts of *D. mespiliformis* fruits at a concentration of 600 mg/kg with the HFD significantly reduced MDA levels. The results obtained show that the administration of the different fine particle size classes of *D. mespilifomis* fruits and atorvastatin concomitantly with the HFD diet for 30 days significantly reduced ($p < 0.05$) the concentration of MDA levels in the different organs. This reduction could depend on the particle size on one hand, the type of extraction solvent on the other, or the 600 mg/kg dose, which induces the reduction of stress markers. The increase in MDA levels in untreated animals could be explained by the generation of ROS following consumption of the HFD diet. This mechanism of reduced peroxidation in HFD rats treated with these particle size classes would be due to an increase in the antioxidant enzymes: catalase and superoxide dismutase. These enzymes act as lines of defense in the cell against the pro-oxidative effect of free radicals (Piechota-Polanczyk and Fichna, 2014; Żukowski et al., 2018).

SOD, catalase and peroxidase are able to eliminate free radicals and other reactive species. SOD is one of the most important antioxidant defense enzymes that scavenge superoxide anion ($O_2^{\circ-}$) by converting to hydrogen peroxide (H_2O_2) thus impair the toxic effects due to this free radical and the HFD diet.

Catalase is an antioxidant enzyme largely distributed in all animal tissues. The enzyme is known to protect the system from highly reactive hydroxyl radicals through hydrogen peroxide decomposition (Favier, 2003). It acts in synergy with SOD; their role is to accelerate the dismutation of hydrogen peroxide into water and molecular oxygen (Marfak, 2003). This hemoprotein protects tissues from hydroxyl radicals, which are highly reactive (Sathishsekar and Subramanian, 2005). A number of substances of food origin such as vitamins E and C, carotenes and polyphenols oppose the propagation of free radicals, very often forming from one highly reactive radical to another much less reactive radical. They are capable of neutralizing a single free radical per molecule (Servais, 2004).

The small particle size fraction, < 50 µm followed by the fraction 50 - 180 µm show higher or greater SOD and CAT activity than the other large particle size fractions and the solvent extracts in all organs. This enhancement in animals treated with the finer particle size powders indicates that this particle size class releases more of the active ingredients present in these powders compared to the solvent extracts. The release of these active ingredients in higher amounts may have caused an increase in detoxification capacity through improved free radical scavenging compared to these extracts.

Effect of particle size fractions, unsieved powder and solvent extracts of the fruits of *D. mespiliformis* on transaminases

The concentrations of transaminases in organs such as heart, liver and kidney of the animals in the groups are shown in Tables 6 and 7. It can be seen from these tables that these concentrations (ALT and AST) decreased significantly in the animals treated with the different particle size fractions, unsieved powder and solvent extracts of *D. mespiliformis* fruits compared to the animals in the negative control group.

These tables also show significant reductions in AST and ALT levels in all organs of animals in the groups treated with < 50 µm and 50 - 180 µm compared to animals treated with large fractions. On the other hand, no significant difference between animals treated with the extracts (ethanolic and hydroethanolic) and the smaller fractions was noted. In view of the results, the administration of *D. mespiliformis* fruits does not affect liver function. The analysis of variance shows that there are no significant differences ($p > 0.05$) between the transaminase levels of the animals treated with the solvent extracts, unscreened powder and particle size fractions of the fruits of *D. mespiliformis* and those treated with vitamin C as well as the animals in the normal control group.

The aminotransferases (ALT and AST) are markers of

Table 6. Effect of different particle size fractions, unsieved powder and solvent extracts of *D. mespiliformis* fruit on organs level of AST enzyme.

Sample	AST kidneys	AST liver	ASAT heart
Negative control	69.0 ± 1.94^{a}	63.33 ± 2.80^{a}	23.33 ± 0.58^{a}
Normal control	126.0 ± 6.92^{e}	161.0 ± 2.48^{e}	103.97 ± 2.67^{e}
Positive control	134.11 ± 4.2^{f}	147.06 ± 3.15^{d}	102.66 ± 2.30^{e}
F: < 50 µm	109.25 ± 6.5^{d}	117.81 ± 2.7^{c}	93.71 ± 2.30^{d}
F: 50 - 180 µm	100.8 ± 1.77^{c}	111.06 ± 2.31^{c}	91.50 ± 0.86^{d}
F: 180 – 315 µm	86.41 ± 5.53^{b}	76.26 ± 1.80^{b}	62.01 ± 2.63^{b}
F: > 315 µm	75.30 ± 1.04^{a}	80.70 ± 1.17^{b}	64.70 ± 3.50^{b}
Unsieved powder	96.85 ± 2.09^{c}	83.07 ± 2.32^{b}	70.25 ± 1.11^{c}
Hydroethanolic extract	96.65 ± 0.84^{c}	81.77 ± 1.75^{b}	69.57 ± 0.54^{c}
Ethanolic extract	93.14 ± 0.84^{bc}	76.11 ± 1.57^{b}	62.69 ± 0.93^{b}

F, Fraction. Means ± standard deviations of five rats from each group; show significance when compared with positive control (vitamin C treatment) with different superscripted letters within the same line differed significantly ($p < 0.05$).

Table 7. Effect of different particle size fractions, unsieved powder and solvent extracts of *D. mespiliformis* fruit on organs level of ALT enzyme.

Sample	ALT kidneys	ALT liver	ALT heart
Negative control	172.78 ± 1.94^{f}	127.85 ± 0.30^{e}	118.1 ± 2.99^{f}
Normal control	112.38 ± 1.94^{b}	114.22 ± 1.48^{c}	79.53 ± 0.81^{ab}
Positive control	96.62 ± 1.94^{a}	101.06 ± 1.15^{a}	74.71 ± 4.95^{a}
F: < 50 µm	101.1 ± 1.94^{a}	99.73 ± 1.05^{a}	83.1 ± 1.37^{bc}
F: 50 - 180 µm	117.57 ± 1.94^{bc}	98.93 ± 1.31^{a}	83.65 ± 2.31^{bcd}
F: 180 – 315 µm	123.68 ± 1.94^{cd}	119.26 ± 1.80^{d}	87.55 ± 3.45^{bde}
F: > 315 µm	128.11 ± 1.94^{de}	117.6 ± 1.87^{cd}	88.43 ± 0.67^{de}
Unsieved powder	131.62 ± 1.94^{e}	107.63 ± 2.32^{b}	89.1 ± 1.17^{e}
Hydroethanolic extract	131.27 ± 1.94^{e}	109.21 ± 2.01^{b}	86.18 ± 1.21^{bde}
Ethanolic extract	126.94 ± 1.94^{de}	101.34 ± 1.57^{a}	80.23 ± 0.93^{b}

F, Fraction. Means ± standard deviations of five rats from each group; show significance when compared with positive control (vitamin C treatment) with different superscripted letters within the same line differed significantly ($p < 0.05$).

liver damage and can thus be used to access liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (Pramyothin et al., 2006). ALT and AST are located in the cytoplasm and mitochondria of liver cells in high concentrations but low in the blood. ALT is more hepato-specific than AST because it is more sensitive to hepatic damage (Pratt and Kaplan, 2000; Al-Habori et al., 2002). In this study, there were no changes in the ALT and AST levels, which reveal that the extracts did not affect the liver function or metabolism.

The chemical composition of *D. mespiliformis* could provide important clues to the identification of chemical compounds responsible for its effect on liver enzymes. Indeed, this plant is rich in flavonoids, molecules known to be hepatoprotective (Narayana et al., 2001; Thabet et al., 2018).

Multivariate analysis can summarize the variability of a complex data set and present it in a most interpretable form, such as principal components. The principal components analysis (PCA) of chemical characteristics and antioxidant capacity of powders issued from *D. mespiliformis* were carried out. Figure 2 show that the eigenvalues and approximately 100% of the variance of the original data were explained by both analyses. The PCA explains 80.82% of the total variation which exists in this system with a contribution of 61.38% for the axis F1 and 19.43% for the axis F2. It is observed that the carotenoids, vitamin A, vitamin C and saponins are highly

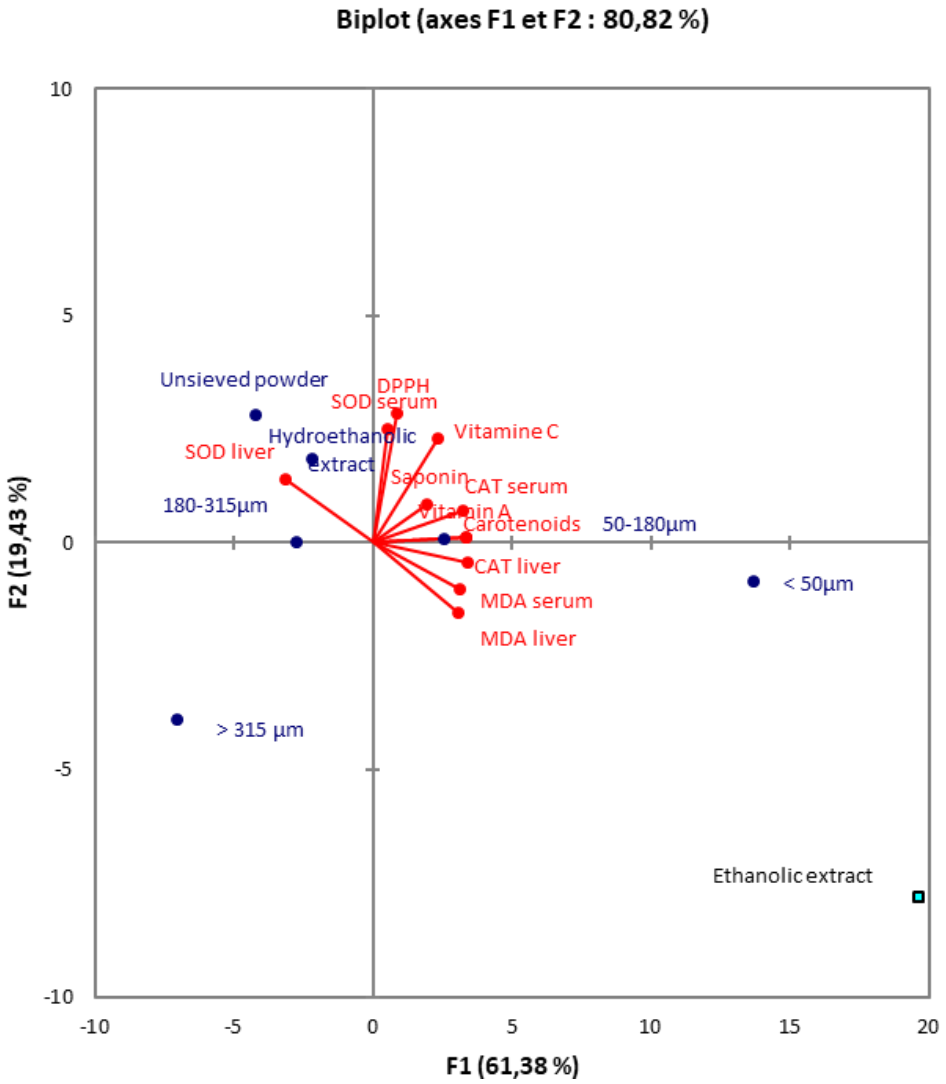

Figure 2. Principal components analysis of saponins, vitamins contents and antioxidant properties of *D. mespiliformis* powders (CDSp fractions, unsieved powders, and solvents extract).

positively correlated with the enzymatic activities of CAT and MDA, while negatively correlation was observed with SOD levels. These results suggest that carotenoids, vitamin A, vitamin C and saponins, may be important contributors to the antioxidant capacity. The results showed significant positive correlation between carotenoids, vitamin A and saponins with the values of serum CAT activity and correlation between vitamin C with serum SOD and DPPH activities. As shown in Figure 2, saponins and vitamins jointly contributed to the increase in DPPH, CAT and SOD activities and decrease MDA in finer powder fractions (< 50 µm and 50 – 180 µm). Thus, the antioxidant power of < 50 µm and 50 – 180 µm derived particularly from its high level in carotenoids and vitamins. The powder fractions 180 – 315 µm, unsieved powder and hydroethanolic extract are lower in vitamins and saponins but, a positive correlation was found with serum SOD activity. The grinding followed by controlled differential sieving process was developed in this study and compared to solvent extractions. PCA showed the contribution of carotenoids and vitamins in the smaller particle size of the powder, the higher the proportions, and also to exhibit higher preventive antioxidant activity as compared to coarser particles, ethanolic and hydroethanolic extract. However, ethanolic extract was essentially rich in total carotenoid contents. There is considerable evidence to suggest that the grinding followed by CDSp is helpful for improving the carotenoid, vitamin and saponins contents

the antioxidant activities of *D. mespiliformis* powder fruits.

Conclusion

The replacement of solvent extracts by CDSp may be advantageous because of its implication on human health. Different powder fractions, unsieved powder and solvent extracts of *D. mespiliformis* fruits can be a source of natural antioxidants. The results showed that the < 50 µm powder fraction was the potential antioxidant and had the highest content in compounds such as carotenoids and vitamins. The *in vivo* antioxidant activity suggest that the smaller particle size of powder fractions may be useful against HFD induced hyperlipidemia possibly due to its anti-hyperlipidemic and antioxidant effect.

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