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Physico-Chemical Characterisation and Antioxidant Activity of the Powder of the leaves and extract *Vitex madiensis* Oliv. : Influence on the granulometric class.

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Abstract

Nowadays, a Numbers of studies have been carried out in the field of ethnopharmacology have shown sufficiently that many plants used in traditional medicine are effective on different pharmacological models. Under traditional conditions, they are less toxic compared to conventional drugs. The influence of the granulometric class the parameters of the chemical composition and on antioxidant activity were investigated. The main purpose of the study is to characterised the powder and evaluated antioxidant activity powder of the plant extracts of *Vitex madiensis* oliv. according granulometric classes. A crude extract was prepared using hydroalcoholic as the solvent and tested for its antioxidant potential. The best results reveal the interest of the grinding and sieving procedure to enhance the parameters of the chemical composition and antioxidant activity. The 0–180 μm granulometric class led also to the highest content in phenolic compounds (circa 54.7 mg gallic acid equivalent / g of dry matter). These results reveal the interest of the fine grinding and sieving procedure to enhance the parameters of the chemical composition and antioxidant, as significantly higher levels of bioactive compounds were found in ground and sieved fractions in comparison with unground ***Lippia multiflora*** **Moldenke leaves.**

Keywords : *Characterisation, powder, antioxidant, vitex madiensis, granulometric class.*

1. Introduction

The use of medicinal plants in the management and treatment of illnesses began for centuries. In more recent years, much research has been done ; is has been found that many plants have medicinal do indeed have medicinal values. [1]

For example of plants with anxiolytic, analgesic, diuretic and hepato-protective properties [2,3,4]. The manufacturing of traditional medicine draws a particular attention because of many bioactive compounds into plants serve as starting point for the development of enhanced versions of traditional medicines [5].

In an oxidative stress situation, the antioxidant system is no longer able to prevent cell damage caused by reactive oxygen species. These attack all biological materials (DNA, proteins, lipids). Oxygen Free Radicals (ROS) have been implicated in many diseases and the aging process. These free radicals, which cause damage to the tissues by oxidative stress, are generated by aerobic respiration, inflammation and lipid peroxidation. The antioxidant systems minimises or prevent the fatal effects of the ROS [6].

Antioxidant such as the phenolic compounds, belonging to the class of said compounds of a secondary plant metabolite sector exist in the healing and food plants and show a spectre of pharmalogical properties such as : antibacterial, anti-inflammatory, vasodilator, anticarcinogenic, anti-thrombotic, anti-atherogenic and analgesic amond so many other.

Ejaz Ahmed et al. [7] have evidenced an additional contribution of polyphenolic compounds to neuroprotective effects of catechin against Alzheimer's disease. Even more recently, it has been found that the presence of flavonoids in plants, mainly catechin, might be responsible for inhibitory and antioxidant activities [8].

Vitex madiensis Oliv. (Verbenaceae), is widely distributed in the eastern and western parts of Africa. Various parts of the plant are used by traditional medicine practitioners in the management and treatment of several disorders which include rheumatism, hypertension, cancer, and inflammatory diseases [1]. Few studies from *Vitex madiensis* Oliv., however the scientific information is very limited on the characterization and the antioxidant properties of the powder of this plant. The medicinal applications of Vitex madiensis have not been given a scientific base.

This study is based on the caracterisation and evaluation antioxidant activity to the particle size by fine grinding of powder of the leaves and extract *Vitex madiensis* Oliv.

The grinding of the *Vitex madiensis* Oliv. powder followed by sieving into four grading classes. The levels of polyphenols, flavonoids, tannins and antioxidant activity by the FRAP, DPPH tests were determined using UV / visible spectrometric methods.

The results of all grain size fractions were compared with that of the unground plant to evaluate the efficiency of the successive grinding/sieving process.

2. Materials and methods

2.1. Plants

The leaves of *Vitex madiensis* oliv. Were manually harvested in August 2015 in Republic of Congo. They were are air dried between 30 and 40 ° C for good moisture.

2.2. Chemicals Products

Chemicals Folin-Ciocalteu phenol reagent, 2,2′-azino-bis (3-ethylbenzothiazoline- 6-sulphonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity, Ferric-tripyridyltriazine (FRAP), gallic acid, sodium chloride, quercetin, hydrochloric acid, ethyl acetate, acetic acid, and standard ascorbic solution were acquired from Sigma-Aldrich Corporation. Stock solutions of standards were prepared in 70 % methanol at a concentration of 0.5 mg/mL and diluted when needed.

2.3. Plant grinding

Approximately 2 kg of dried *Vitex madiensis* oliv. leaves were crushed with the Ultra Centrifugal Mill ZM 200 (Retsch France) operating by impact and shearing effects. Grinding rotational speed was fixed at 12.000 rpm. Grinding rotational speed was performed at ambient temperature using 1 mm.

2.4. Plant powder sieving

Lots of about 200 g of grounds plants were sieved with a vibration amplitude for 15 min with the analysette 3 spartan apparatus (Fritsch, idar - Oberstein, Germany)

Different granulometric class, N 500 µm, 315-500 µm, 180-315 µm, b 180 µm, were obtained with appropriate 20 mm diameter sieves (Fritsch).

The powders retained on each sieve were stored under vacuum sealed in bags sealed at 10 °C before analysis.

2.5. Particle size

Particle of vitex madiensis Oliv. mean diameter was mesures using a laser diffraction particle size analyzer Dry way, by laser granulometry (Mastersizer 3000, Malvern Instruments, Malvern, UK) equipped with a wet sample unit.

A small sample amount was Disperseur sees dry under agitation and the particle size was measured successive times.

Analyses were carried out using a 2% obscuration level, obtained by adapting the dispersion conditions to sample flowability :50 % air pressure, 50 % feed rate, 2,5 mm hopper length for the whole unground plant, as well as N500 µm, 315–500 µm, and b 180 µm granulometric class ;

Particle size distribution was characterised by classical diameters (D10, D50, and D90, where Dx means that x % of the volume of particles has a diameter lower than Dx). and the span, a common parameter related to the width of particle size distribution, defined in eq. (1)

$$Span = \frac{D90-D10}{D50} \qquad \text{Eq. (1)}$$

2.6. Scanning electron microscopy (SEM)

Samples were deposited on a double-sided adhesive at room temperature. The coverslips were coated with gold palladium for 2X100s (Polaron SC7640, Thermo VG Scientific, England). Preparations were observed under SEM (Cambridge Stereoscan S240) at 15 kV.

2.7. Shape of particles

The shape of flour particles was analysed with a QICPIC (Sympatec GmbH, Germany) supplied with the LIXELL (Sympatec GmbH, Germany) dispersion module that was designed for suspensions and emulsions. The flour (0.25 g) was dispersed in 75 mL water (VWR International SAS, Fontenay-sous-Bois, France) to obtain a sufficient obscuration level (3 %) and to avoid particle superimposition. For the determination of shape parameters of flour particles, the diameter of a circle of equal projection area (EQPC) was preliminary determined with the apparatus. The EQPC is defined as the diameter of a circle that has the same area as the projection area of the particle. From the EQPC, the sphericity and convexity were determined. The sphericity value (S), ranging between 0 and 1, is the ratio between the perimeter of the equivalent circle to the real perimeter. The smaller the sphericity value, the more irregular the particle shape. In fact, irregular shape results in an increase in real perimeter for a given

projection area. The convexity, ranging also between 0 and 1, describes the compactness of a particle. Figure 1c shows a particle with a projection area A (light gray) leaving open a concave region of area B (dark red) on its right side. The convexity is defined as the ratio of the projection area it self (A) and the area of the convex hull (A+B). Without concave regions, the maximum theoretical convexity is 1. Windox software (Windox 5.4, 2007, Sympatec GmbH, Clausthal-Zellerfeld, Germany) was used in this study to determine the shape parameter distributions [9].

2.8. Colorimetric Analysis

The color descriptors of the flour samples were determined with a Chromameter CR210 (Minolta France S.A.S., Carrières-sur-Seine, France), calibrated against a light yellow standard. The flour was poured in a petri dish placed above the light source and covered with a white plate. Flour color was evaluated according to the Lab color space by three color coordinates (L*, a*, b*). L* represents the sample luminosity, varying from black (0) to white (100) ; a* represents the color varying from green (−) to red (+) ; the value for b* represents the color varying fromblue (−) to yellow (+). White ness index (WI) was calculated according to as follows [10].

$$WI = 100 - \sqrt{(100 - L)^2 + a^2} + b^2 \quad \text{Eq. (2)}$$

2.10. Rheological measurements and analysis

Rheological studies were carried out using a Kinexus-pro Rheometer (Malvern Instruments, Orsay, France). Controlled shear rate measurements were performed using cup and bob (C25 DINC 0026 SS ; PC 25 DINC 0041 SS). A sample of 10 mL was loaded into the cylinder and was allowed to equilibrate to maintain set temperature and applying shear rate from 0.1 to 100 s^{-1}. The flow behavior of the powder plant dilute dispersions juice were analysed at different temperature of 20, 40, 50 and 60° C and different concentrates of 0.2, 0.4, 0.8, 1.6, 3.2 % (w/v). Experimental data were evaluated and fitted according to the rheological model Power Law (Eq.3) [11].

$$\sigma = \eta\gamma^{n} \quad \text{Eq. (3)}$$

2.11. Wettability

The wettability is the time required for all the powder particles arranged at the water surface is wet. It is measured by FIL standard (1985) or the method of Niro Atomizer (1978).

2.12. Solubility

It is some essential criteria in the quality control of the powders destined to be incorporated in aqueous phase. The indication of solubility is measured according to the ADPI norm (2002b12) or the method of Niro Atomizer (1978 ; 13) and to determine the ability of a powder to be dissolved. After centrifugation series defined the pellet is weighed.

2.13. Powder composition

Analyses of powder composition (moisture, lipids, proteins, ashes, carbohydrates, water activity, reducing sugar) were performed for all granulometric class. Water content was mesured by weight loss after drying 2 g of powder at 105 °C for 5 h [14]. Total protein content determined by Kjeldhal method for quantification of nitrogen using a conversion factor 6.25 that is suitable for plants [15]. Fat content was quantified by so Soxhlet method [16] used 2 g of sample.

Mineral content was determined by putting 2 g of powder at 500 °C for 5 h until formation of white ashes [17]. Carbohydrate content was obtained by difference with other components. Its standard error was deduced from the standard errors of other components by propagation of uncertainties.

2.14. Extracts Preparation

The extraction of obtained powders (30 g) was carried out by cold maceration in a 70 % ethanolic hydroalcoholic solution (1 :5, v/v), stirred on a magnetic plate (700 rpm for 30 min). The solution are then clarified by filtration on whatman paper, concentrated by évaporation of alcohol under vacuum, and stored until analysis at low temperature (-10 °C).

2.15. Preparation of aqueous *Vitex madiensis* Oliv. dispersions for rheological analysis

Preparation of aqueous the plant powders for rheological analysis aqueous dispersions were prepared from dried powders at desired concentrations of 0.2, 0.4, 0.8, 1.6 and 3.2 (w/v). The dispersions were subjected to mild heating and stirring for partial dissolution of heterogeneous compounds in the powder. The prepared samples were equilibrated at room temperature for 48 h prior to dynamic viscosity analysis where the clear juice and water were taken as control. Concentrated aqueous dispersions were prepared by dissolving 1000 mg powder in 1.5-2.5 mL distilled water depending upon different critical volumes. The critical volume was estimated by crude test tube tilting (TTM) method [18, 19], where the volume of the dispersion was

considered, when meniscus did not flow or deform due its own weight. Tilting test was performed in duplicates and the measured values were expressed as their respective mean. The sample were equilibrated at room temperature for 20 h prior to viscosity analysis. Further visual inspections were carried out for both concentrated and dilute dispersions.

2.16. UV-visible analyses

UV-visible spectrophotometric analyses were carried out with an UV-visible spectrophotometer cary 50 Scan (Agilent, Santa Clara, California, USA).

2.16.1. Dosage of polyphenols

2.16.1.1. Determination of total phenolic content

Total polyphenols were quantified according to the Folin-Ciocalteu (FC) method [20]: 100 µL *Vitex madiensis* Oliv. powder extract was mixed with 500 µL Folin-Ciocalteu reagent and 400 µL of 7.5 % (w/v) Na_2CO_3. The mixture is stirred and incubated in the dark at room temperature for 10 min and the absorbance is measured at 760 nm by UV/vis. spectrophotometry (Agilent, Santa Clara, California). The results were expressed in milligrams of gallic acid equivalents per gram of dry matter by referring to the calibration curve established with gallic acid standards. This assay was triplicated.

2.16.1.2. Determination of flavonoid content

The determination of total flavonoids was carried out according to the method described by Dehpeur et al. [21]: 500 µL of powder extract was added to 1 500 µL of 95% methanol, 100 µL of 10% (w/v) $AlCl_3$, 100 µL of molar sodium acetate and 2.8 mL distilled water. The mixture was stirred, then incubated in the dark and ambient temperature for 30 min. Blank was realised with the same protocol by substituting 95 % methanol for the extract and absorbance was measured at 415 nm by UV/vis. spectrophotometry (Agilent, Santa Clara, California). The results were expressed in terms of milligrams of quercetin equivalents per gram of dry matter by referring to the calibration curve of the quercetin.

2.16.1.3. Determination of condensed tannin content

The condensed tannins are determined by the vanillin method in acid medium as described by Ba et al. [22]. Vanillin reagent was prepared by mixing equal volumes of 8 % (v/v) HCl and 4 % vanillin in some methanol (m/v). The mixture was maintained at 30 °C until performing the quantitative analysis. 200 µL of powder extract were added to 1 000 µL of vanillin reagent: the

mixture was shaken, then incubated in the dark at 30 °C during 20 min. The absorbance was measured at 500 nm by UV/vis. spectrophotometry (Agilent, Santa Clara, California) against a blank constituted of an equivalent mixture of methanol and 8 % HCl. Results were expressed as milligrams of catechol equivalents per gram of dry matter by referring to the calibration curve obtained from catechol standards.

2.16.2. Antioxidant activity

2.16.2.1. FRAP test

1 mL of the extract at different concentrations (from 0.007 to 2.5 mg/mL) is mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a 1% K3Fe potassium ferricyanide (CN)6 solution. The whole is incubated in a water bath at 50°C for 20 min, then 2.5 mL of 10% trichloroacetic acid is added to stop the reaction. The tubes are centrifuged at 3,000 rpm for 10 minutes. An aliquot (2.5 mL) of supernatant is combined with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl3 (ferric chloride) aqueous solution. The absorbance of the reaction medium is read at 700 nm against a similarly prepared blank, replacing the extract with distilled water to calibrate the apparatus (UV-vis spectrophotometer). The positive control is represented by a standard of an antioxidant, ascorbic acid, whose absorbance has been measured under the same conditions as the samples. An increase in absorbance corresponds to an increase in the reducing power of the extracts tested [23,24].

The percentage of iron reducing power is calculated by the following reaction:

$$Iron\ reducing\ power(\%) = \frac{A_0 - A_1}{A_0}$$ Eq. (4)

Ao absorbance of FeCl3 (-).

A1 absorbance of FeCl3, solution in the presence of the extract.

2.16.2.2. ABTS test

The method described by Muanda et al. [25] was used in this study, with slight modification. The ABTS radical anion solution was prepared by dissolving 1 mM AAPH and 2.5 mMin phosphate buffer saline solution (100 mM, pH 7.4) containing 150 mM NaCl. After incubation in a water bath at 68 °C for 20 min, the resulting ABTS solution was diluted with methanol to obtain a blue-green coloration with an absorbance of 0.70 ± 0.02 at 734 nm, then 2.94 mL of buffered ABTS radical anion solution was added. The decrease in absorbance was measured at 734 nm by UV/visible spectrophotometry. The ABTS radical scavenging activity, considered as closely related to antioxidant activity, was expressed in terms of half maximal inhibitory concentration

(IC50), which refers to the smallest concentration required to scavenge 50 % of the ABTS radical. The ABTS was triplicated.

The scavenging activity was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} . 100 \quad \text{Eq. (5)}$$

2.13.2.3. DPPH test

The method used is that of Bret et al (1995) [26] with some modifications. First, a methanolic solution of DPPH (200 µM) was prepared for each analysis and kept in the dark. 2.90 mL of the methanolic solution of DPPH (200 µM) was added to 100 µL of the extract to be studied. The solution was mixed and incubated at 37 °C for 30 min in a dark place.

Absorbance is measured at 517 nm against a blank of 2.90 mL of DPPH methanolic solution at 200 µM and 100 µL of methanol. The samples to be studied and the control are prepared under the same conditions. The decrease in absorbance is measured with a UV-visible spectrophotometer at 517 nm. Thus, the percentage inhibition of DPPH radicals is calculated according to the following equation:

$$\%\, inibition = \frac{(A_{controlled} - A_{extract})}{A_{controlled}} \times 100 \quad \text{Eq. (6)}$$

2.14. Statistical analyses

For all experiments, the average value and standard deviation from three replicates were calculated. Statistical analysis (XLSTAT add-on (Addinsoft, Paris, France) to Excel 2016 (Microsoft, Redmond, USA) was conducted using with $p < 0.05$ as significance level.

3. Results and discussion

3.1. Particle size distribution

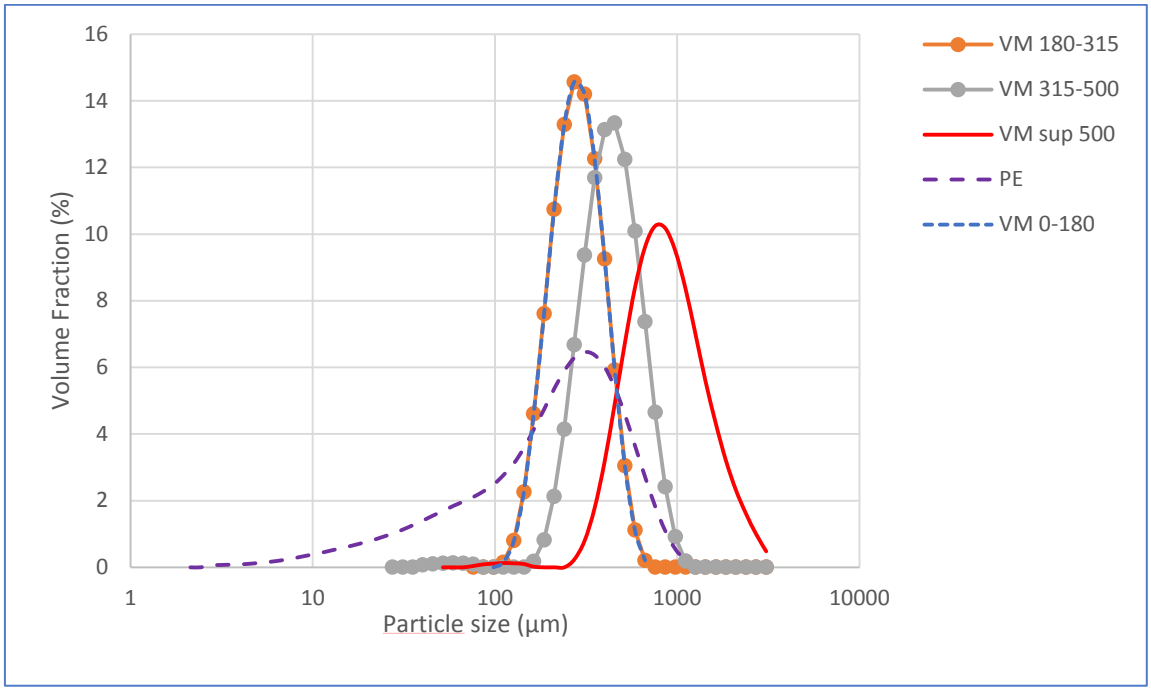

Figure 1: Particle size distribution of *Vitex madiensis* Oliv. leaf powders: granulometric classes and unsieved powder.

Table 1: Mean particle size and span of particle size distribution of granulometric classes and unsieved powder of *Vitex madiensis* Oliv. leaves.

Powder sample	Mean particle size D10 (µm)	Mean particle size D50 (µm)	Mean particle size D90 (µm)	Span (−)
< 180 µm	112,33 ± 2,08	181,67 ± 2,08	286,00 ± 3,46	0,96 ± 0.00
180 - 315 µm	193,66 ± 0,57	301,67 ± 1.52	464,00 ± 2,64	0.89 ± 0,02
315 - 500 µm	283,66 ± 1,52	459,33 ± 4,50	732,66 ± 7,63	0.99 ± 0,00
> 500 µm	500,00 ± 16,46	907,3 ± 0.03	1800,00 ± 0,10	1.42 ± 0,03
Unsieved powder	41,70 ± 0,30	217.67 ± 7.77	397,00 ± 28,83	1.61 ± 0.1

The particle size distribution of the particle size of *Vitex madiensis* oliv. presented in Figure 1 and the particle size parameters indicated in Table 1 show the presence of bimodal distributions with peaks around 454-13.33 µm and 310-14.02 µm respectively for particle size classes 315-500 µm and 180-315 µm.

The sieving process made it possible to separate the powder into separate granulometric classes.

The values of d50 are between 181.33 and 907.33 µm Class 0-180 µm has the lowest d50 and the highest class > 500 µm.

The span values show that the particle size distribution of *Vitex madiensis* oliv. particles > 500 µm is wider than those of other particle size classes.

3.2. Scanning electron microscopy

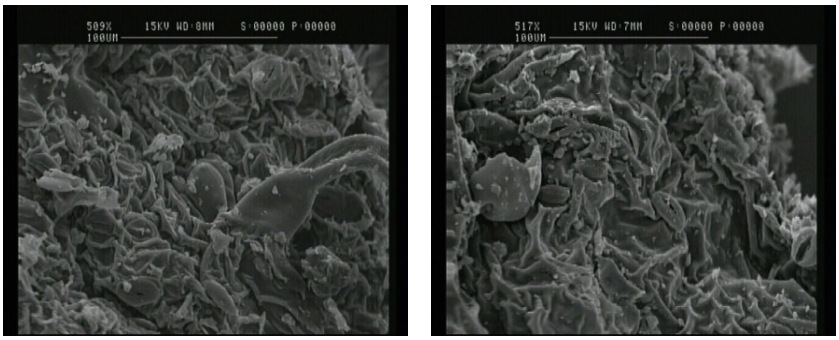

Figure 2: Microstructure of unsieved powder of *Vitex madiensis* Oliv. leaves imaged by scanning electron microscopy.

Figure 2 shows the scanning electron micrographs of unsieved powder of *Vitex madiensis* Oliv. leaves. Both large, medium, and small particles can be found in this plant powder, which was consistent with the grinding process that is expected to result in large particle size distributions. In addition, the significant presence of fibres justifies the difficulties of sieving.

3.3. Colourimetric Analysis

Table 2: Colour settings of *Vitex madiensis* Oliv. leaf granulometric classes and unsieved powder.

Granulometric class	L*	a*	b*	WI	BI
< 180 µm	50.95 ± 0.28	-1.70 ± 0.03	10.77 ± 0.07	49.75 ± 0.20	20.38 ± 0.22
180 - 315 µm	49.95 ± 0.28	-1.70 ± 0.01	10.63 ± 0.08	48.81 ± 0.22	20.50 ± 0.20
315 - 500 µm	48.11 ± 0.55	-1.26 ± 0.02	7.40 ± 0.03	47.57 ± 0.22	13.14 ± 0.20

> 500 µm	48.07 ± 0.56	-1.25 ± 0.03	7.40 ± 0.01	47.53 ± 0.01	13.13 ± 0.06

L* = lightness; a* = magenta-green colour component; b* = yellow-blue colour component; WI = whiteness index ; BI : browning index.

Colour of plant powders is of paramount importance because it can inform about the antioxidant activity of plant powders.

The luminance value (L *) is between 50.95 and 48.07. While the values a * (red-green color component) vary from -1.25 to -1.70 and those of b * (yellow-blue color component) vary in the range from 10.77 to 7.40 and the whiteness index is between 49.75 and 47.53 finally the browning index varies between 20.38 and 13.13.

According to the different particle size classes, the value of the luminance (L *) of the class 0-180 µm (50.95) is greater than that of the other classes of powders (180-315 µm, 315-500 µm and> 500 µm). The value of a * (red-green chromatic component) is higher in the class> 500 µm than in other size classes. The value b * (yellow-blue color component) is higher for the powder of class 0-180 compared to other classes. Moreover, the highest whiteness and browning indices corresponded to the smallest granulometric class (< 180 µm).

In fact, powders of different size classes were green in color because carotenoids are a vast set of natural pigments widely used in plants, where they are photosynthetic accessories and provide protection against oxidation [27]. According to the different particle size classes, the value of the luminance (L *) of class 0-180 is higher than that of the other classes of powders (180-315 ;315-500 and> 500). L * and BI decrease with respect to the effect of grinding.

3.5. Rehydration ability

3.5.1. Wettability of *Vitex madiensis* Oliv. leaf powders

Table 3: Wettability indices of *Vitex madiensis* Oliv. leaf granulometric classes and unsieved powder.

Granulometric classes	Wettability (s)
Unsieved powder	> 1 800

< 180 µm	> 1 800
180 - 315 µm	> 1 800
315 - 500 µm	> 1 800
> 500 µm	1 275 ± 51

The wettability index for all grain size classes is greater than 1800 seconds except the class greater than 500 µm, so its powders are not wettable. The particle size has a great influence on the wettability. The morphology (convexity / sphericity) and compositional factors must have a greater impact on this property.

3.5.2. Solubility of *Vitex madiensis* Oliv. leaf powders

Table 4**:** Solubility of *Vitex madiensis* Oliv. leaf granulometric classes and unsieved powder.

Granulometric classes	Solubility (%)
< 180 µm	31.60 ± 0.01
180 - 315 µm	25.69 ± 0.01
315 - 500 µm	21.51 ± 0.15
> 500 µm	11.20 ± 0.01

The solubility values according to the different granulometric classes show that they vary between 31.60% and 11%. Solubility deceased when the particle size was increased, which was expected as the surface-to-volume ratio is higher for small particles, allowing more water access to particle material. This insoluble character probably corresponds to the existence within the powder of a totally insoluble fraction or to the type of plant.

3.5. Chemical composition

Table 5: Chemical composition (moisture, proteins, ashes, lipids, reducing sugars, and non-sugar carbohydrates) on wet basis of all granulometric classes and unsieved powder of *Vitex madiensis* Oliv.

Powder sample	< 180 µm	180 - 315 µm	315- 500 µm	> 500 µm
Moisture (% w/w)	8.62 ± 0.01	8.52 ± 0.02	8.49 ± 0.04	8.46 ± 0.01
Protein (% w/w)	21.57 ± 0.00	21.47 ± 0.01	23.52 ± 0.08	18.82 ± 0.01
Ash (% w/w)	5.31 ± 0.08	5.36 ± 0.00	7.45 ± 0.08	7.93 ± 0.02
Lipid (% w/w)	1.9 ± 0.03	1.2 ± 0.01	0.9 ± 0.02	0.5 ± 0.08
Reducing sugar (% w/w)	2.06 ± 0.60	1.56 ± 0.02	1.03 ± 0.25	1.01 ± 0.42

resented data are mean ± standard deviation of triplicated measures; mean values in the same line followed by different superscripted letters were statistically different ($p < 0.05$).

Table 5 presents the results of the chemical composition of the four granulometric classes of the leaf powders of Vitex madiensis Oliv. It can be seen that the successive grinding and sieving process had a great influence on the chemical composition of studied plant powders. The water content of the granulometric classes was close to 8.5 % (w/w) and a small increase of water content was denoted when particle size was decreased. These values are close to those typically found for other types of powders: tea (6.6%) and flour (12.6%) for example [29]. The values of water activity in all classes are pockets (0.5). The soluble protein content of powders was high, ranging between 18.82 and 23.52 % (w/w); no clear influence of particle size was denoted on protein content. Fat content varied between 0.5 and 1.9 % (w/w) in granulometric classes and an increase in fat content was recorded when particles size was decreased it is obvious that *Vitex madiensis* Oliv. powders are not sources of fat.

Ash content of granulometric classes varied between 5.31 and 7.93 % (w/w); a moderate increase in ash content was found for higher particle size. Reducing sugars of granulometric classes ranged between 1.01 and 2.06 % (w/w) and a decreasing trend was evidenced when particle size was increased. Non-sugar carbohydrate content of granulometric classes was comprised between XX.X and 83.75 µm and increased with particle size: this was expected, as fibres, main components of the non-sugar carbohydrates, are hard to grind, thus resulting in large particles. This finding was consistent with previous studies involving other medicinal plants.

3.6. Total phenolic content

Table 6: Polyphenol content of hydromethanolic extracts of granulometric classes and unsieved powder of *Vitex madiensis* Oliv.

Granulometric class	Polyphenols (mg / g)	Flavonoids (µg.Eq Catechin / mg of extract)	Condensed tannins
< 180 µm	84.71± 0.02	56.00 ± 0.01	26.63 ±0.009
180 - 315 µm	82.00 ± 0.06	23.86 ± 0.001	17.25 ± 0.12
315 - 500 µm	42.43 ± 0.12	15.25 ± 0.004	16.25 ± 0.01
> 500 µm	20.19 ± 0.03	9.25 ±0.02	8.88 ± 0.09

Table 6 shows that total phenolics, flavonoids, and condensed tannins were more concentrated in smaller particles. This demonstrates the efficiency of the successive grinding and sieving process to discriminate plant powders into fractions having well-different contents in bioactive molecules. Also, an enrichment in investigated bioactime molecules was highlighted by these analyses: this is consistent with previous studies on other plants and can be explained by the probable high content in fibres of *Vitex madiensis* Oliv, as fibres are concentrated in higher-size particles, thus decreasing the content in other biomolecules.

3.7. In vitro antioxidant activity

Table 7: Radical scavenging assays (DPPH, FRAP, ABTS) for the different granulometric classes of *Vitex madiensis* oliv.

	DPPH	FRAP	ABTS
Granulometric class		IC50 (mg/L)	
< 180 µm	43.83 ± 0.01	49.70 ±0.09	67.28 ±0.08
180 - 315 µm	43.23 ± 0.02	44.70 ± 0.77	55.71 ± 0.66
315 - 500 µm	49.87 ± 0.1	47.47 ± 0.05	58.14 ± 0.02
> 500 µm	27.03 ± 0.04	56.27 ± 0.01	55.42 ± 0.06

As shown in Table 7, all extracts were able to trap free radicals. These radicals vary according to the different granulometric classes. The highest IC 50 is that of the class 0-180 µm µm (67.28).

Conclusion

The results of this study clearly demonstrate the importance of plant powders in the discovery of new therapeutic agents such as antioxidants. There are already many reports that have demonstrated antioxidant properties of extracts prepared from many plants. However, there are only a few Antioxidant studies found to have many side effects thanks to which new antioxidants from natural sources. However, only a few studies have attempted to study the antioxidants of the plant powder according to the different particle size classes as well as the characterization of its powders. Which makes this survey important?

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