Original Research Article

Influence of particle size on the phytochemical, antioxidant and anti-inflammatory properties of powder of trunk bark *Parkiabiglobosa* (JACQ) (FABACEAE-MIMOSADEAE)

ABSTRACT

ABSTRACT:

Aims: The present study aim was to evaluate influence of particle size on the phytochemical, antioxidant and anti-inflammatory properties of powder of trunk bark *Parkiabiglobosa.*

Study design: This is an experimental study

Place and Duration of Study: The study was realized in the Phytomedicine and Medicine Research-Development (LR-D/PM) laboratories of the Health Sciences Research Institute (IRSS) of Ouagadougou in Burkina Faso**.**

Methodology: The powder was sieved and classified into four particle sizes: coarse, moderately fine, fine, and very fine. An aqueous decoction was then made from these powders. The extraction yields, qualitative phytochemical composition, and the estimation of polyphenol compound content were determined. The antioxidant effect of the extracts was obtained using four antioxidant models: ABTS, DPPH, FRAP, and LPO. The anti-inflammatory activity was evaluated against the soybean 15-lipoxygenase.

Results: Decreasing the particle size from coarse to very fine resulted in an approximately two-fold increase in extraction yield (from 12.19 to 29.05); an approximately two-fold increase in antioxidant and anti-inflammatory activities: ABTS (from 4.1 ± 0.1 to 1.4 ± 0.3 µg/mL); DPPH (from 7.3 ± 0.1 to 3.1 ± 0.3 µg/mL); FRAP (from 885.6 ± 84 to 6973 ± 21 mmol EAA/g), and LOX (from 34.09 ± 1.43 to 2.88 ± 0.65 µg/mL). Moreover, the modification of the particle size has also improved the LPO inhibitory activity and total phenolic and flavonoid contents. The increased surface area in contact with the extraction solvent may explain the interesting effect of the very fine powder

Conclusion: The very fine powder offers more significant antioxidant and anti-inflammatory activities. Therefore, this powder can be suggested to develop a phytomedicine against inflammatory diseases

Keywords: Particle size, antioxidant, anti-inflammatory, ParkiabiglobosaBurkina faso

1. INTRODUCTION

Humans have always used plants in the management of their health problems. Nowadays, scientific and pharmacological studies allow the synthesis of active ingredients. Unfortunately, the side effects of these synthetic products, combined with the fact that most of them are not always available for most people in some parts of the world (Sub-Saharan Africa and South Asia), limit the effective management of health problems [1,2]. Therefore, these reasons prompted the search for other alternatives, including discovering new active ingredients mainly from natural sources such as medicinal plants.
Indeed, medicinal plants constitute a vast reservoir of natural substances, which are pharmacologically active phytochemicals [2]. The World Health Organization (WHO) estimates that about 80% of the African population uses medicinal plants for their health needs for cultural, geographical, and financial reasons [3] In Burkina Faso, of 2,067 known species, 1,033 (50%) are traditionally used for common pathologies [4]. The genus *Parkia* regroups numerous species distributed mainly in all tropical countries [5]. These plants treat various ailments, including diabetes, wounds, skin diseases, diarrhea, measles, cough, and conjunctivitis. Several secondary phytochemicals (triterpenes, phenolic acids, flavonoids, …) have been identified and isolated (5,6). Moreover, many studies have reported the pharmacological activities of the different extracts from the *Parkia* genus, including anticancer, antioxidant, antidiabetic, antimalarial, antimicrobial, and anti-inflammatory [5,7]. In Burkina Faso rural areas, *Parkiabiglobosa* (*P. biglobosa*) (Jacq). R. Br. (Fabaceae-Mimosoideae) is widely used against infection, hypertension, diabetes, and hemorrhoids [7]. WHO encourages, recommends, and promotes quality traditional herbal medicines in national health care programs because of the tremendous use of these treatments [8]. Therefore, this enthusiasm for plants has allowed the formulation of new phytomedicines such as FACA® and SAYE® tea in Burkina Faso, respectively, used to manage sickle cell disease and malaria [5,6]. A previous survey performed by our team has revealed that *P. biglobosa* can be used against hemorrhoids. Based on these data, our teams have initiated scientific studies on this plant, including the quality assessment of the powder and the optimization of the extraction process [9]. The present study was undertaken to evaluate the influence of particle size on the phytochemical, anti-inflammatory, and antioxidant parameters of the powder of *Parkiabiglobosa*.

2. MATERIAL AND METHODS

2.1 Végétal material

The trunk bark of *Parkiabiglobosa* is harvested in Yako during the dry and rainy seasons (May and August 2020). The plant part was authenticated, and a specimen was deposited at the herbarium of the National Center for Scientific and Technological Research (CNRST) under number 8757.

2.2 Chemical reagents and solvents

Ascorbic acid, lipoxygenase, linoleic acid, trolox, Zileuton, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Potassium hexacyanoferrate [$K_3Fe(CN)_6$], trichloroacetic acid, trichloro-ferrate [$FeCl_3$], ethyl acetate, ethanol, and methanol analytical grade purchased from Sigma Chemical Co. (St. Louis, MO, USA). The water was distilled in our laboratory.

2.3 Preparation of different particle size classes of *P. biglobosa* dry powder

The dry powder of *P. biglobosa* was sieved following a method from the European Pharmacopoeia 9th edition [10]. Four particle sizes of powder were thus set up according to the sieve meshes: - 1.25 and 0.400 µm mesh sieves give coarse powder; - 0,315 and 0,200 µm afforded a moderately fine powder; - mesh sieves of 0.160 and 0.125 µm give fine powder; - 0.125 and 0.09 µm mesh sieves produced very fine powder. Thus, to obtain the coarse powder, a test sample of 50 g of raw powder was placed on the sieve 1.25 and 0.400 µm and sieved until nothing passed through the sieve 0.400 µm. The residue on this sieve (0.400 µm), corresponding to the coarse powder, was then collected. The operation was repeated until at least 10 g of powder was obtained. The residue passed through the 0.400 µm sieve was used to obtain the moderately fine powder. The other particle size classes were obtained according to the same principle with the corresponding sieves.

2.4 Determination of the residual moisture content (RMC)

The sieved powders' residual moisture content (RMC) was determined according to the thermogravimetry method [11]. The principle of the method is based on the water loss of the plant material by desiccation. The powders were placed in the halogen dryer programmed at a time and temperature equal to 10 min and 105°C, respectively. The machine displays the residual humidity at the end of the drying operation. The tests were carried out in triplicate.

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2.5 Determination of the extraction yield

The aqueous decoction was used to perform the extraction. In brief, a test portion of each particle-size powder was dispersed in distilled water at 1/25 (m: v). After homogenization, each mixture was allowed to boil for 20 min in a flask connected to a reflux condenser to minimize water loss. After cooling, the mixtures were filtrated on a fine mesh nylon cloth, and the collected filtrates were centrifuged at 2000 rpm for 10 min. The centrifuged filtrates were collected and freeze-dried for further analysis.

The yield of the extraction procedure was calculated using the following equation[12]:

$$\text{Yield } (\%) = (\frac{\text{Mf}}{\text{Mi}}) \times 100$$

Mf: mass of dried extract (g) or final mass;
Mi: initial mass or test portion of plant material (g)

2.6 Phytochemical screening

The qualitative phytochemical analysis was assessed by the thin layer chromatography [13]. The extracts were dissolved in methanol; 5 µL were deposited on the silica gel plate 1 cm from the edge of the plate and with a gap of 1 cm between the deposits. After drying, the plate was deposited in a covered migration tank (saturation migration) containing the following mobile phase: ethyl acetate- formic acid- water (90-1-1,v/v/v) for flavonoids detection. The solvent system, mixture ofethyl acetate, methanol and distilled water (7-1-1, v/v/v) used to reveal tannins, triterpenes/sterols and saponosids. After migration and drying, the plates were revealed by spraying with the specific reagents. These included sulphuric vanillin and Libermann Burchard reagent for terpenic and steroidal compounds.Sulphuricanisaldehydeit used for saponosides. The iron chloride for tannins and Neu reagent used for flavonoids. However, for the detection of tannins, saponosides, sterols and triterpenes, the plates were heated for 5 minutes at 110°C on a hot plate to reveal stains of various colors. Observations were made with visible light and a dual wavelength (254 and 366 nm) UV lamp.

2.7 Determination of total phenolics

Total phenolic compounds were determinedaccording Lezoulet al. method [14]. It is based on reducingFolin&Ciocalteu reagent (FCR)in an alkaline medium by phenolics. It resulting the formation of ablue-colored complex whichhas a maximum absorption at 760 nm. The intensity of the blue color depends of the total phenolic content. Briefly, in a test tube containing 1 mL of 1 mg/ml concentration, 1 mL of FCR 2N, and 3 mL of a 20% sodium carbonate solution were added. The experiment was repeat in triplicate. The blank control, it distilled water instead of the extract. After 40 min at the ambient temperature, the absorbance of the mixture was measured at 760 nm with a spectrophotometer (Agilent 8453). From the standard curve drawn with gallic acid (1-5 µg/mL), the total phenolic concentration of each extract was provided by the formula:

$$\text{TPC} = (\text{CTube}/\text{Ci}) \times \text{D}$$

TPC: Total phenolic content of the extract expressed as mg Gallic acid equivalent (GAE) /g extract;
CTube: Concentration (mg GAE/mL) in the dosing tube;
Ci: Concentration (in mg/ml) of the stock solution;
D: Dilution factor.

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2.8 Determination of total flavonoids

The determination of flavonoids realized according to JacoboV et al, method [15]. Approximately 2 mL of 1 mg/mL methanol extract was mixed with 2 mL of 2% $AlCl_3$ aluminum trichloride (in methanol). After 40 min incubation, absorbance was measured at 415 nm using the 8453 Agilent spectrophotometer. The control tube consists of 2 mL methanol with 2 mL $AlCl_3$. Quercetin was used as a reference compound, and its absorbance was measured under the same conditions. The tests were carried out in triplicate. The amount of flavonoids in the extracts expressed as mg quercetin equivalent per gram of extract (mg QE/g) was determined according to the following formula:

$$\mathbf{TFlav} = (\mathrm{A} \times \mathrm{m0})/(\mathrm{A0} \times \mathrm{m})$$

TFlav: Total flavonoid content of the extract expressed in mg QE/g;
A: Absorbance of the extract;
A0: Absorbance of quercetin;
m0: Mass (mg) of quercetin
m: Mass (mg) of the extract

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2.9 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reduction test

The anti-radical activity was realized according to Sanna et al, method. [16]. Nine dilutions were made from the 1 mg/mL extract and Trolox. Twenty (20) µL of these solutions (extract/ Trolox) were placed in the wells of a 96-well microplate. 200 µL (0.04 mg/mL) of DPPH solution was added. After 30 minutes of incubation, absorbance was read using a Bio-Rad 680 model Belgium spectrophotometer at 490 nm. The blank was made with 200 µL of DPPH and 20 µL of ethanol. The IC_{50} concentration required to scavenge 50% of DPPH radicals was determined using a graph plot percentage inhibition against sample concentration.Each concentration was assayed in triplicates, and the experiment was repeated thrice.

$$\mathbf{\%\ Inhibition\ = [(A0 - A1)/A0] \times 100}$$

A0 = Absorbance of the control
A1 = Absorbance of the sample or standard

2.10 ABTS (2, 2' - azinobis - [3-ethylbenzothiazoline-6-sulfonic acid])

The method used was describedby Arciszewskaet al. [17]. In 5 mL of distilled water, a mass of 19.2 mg of ABTS plus 3.312 mg of potassium persulfate added. The mixture was kept at room temperature and in the dark for 12 hours. In 220 mL of analytical ethanol, 4.5 mL of the mixture was diluted. Nine solutions were obtained from each sample extract at 1 mg/ml concentration. Trolox was used as a reference substance. The wells of a 96-well microplate were filled with 200 µL of ABTS solution mixed with 20 µL of the extractor Trolox at different concentrations. The plate was then incubated for 30 minutes at room temperature, and the absorbances were read using the spectrophotometer (Bio-Rad 680, UV-wise) at 415 nm. The control was prepared with 20 µL ethanol and 200 µL ABTS. All measurements were performed in triplicate.
The percentage of inhibition (% inhibition) was calculated according to the formula:

$$\mathbf{\%\ Inhibition\ = [(A0 - A1)/A0] \times 100}$$

A0 = Absorbance of the control;
A1 = Absorbance of the sample or standard.

The absorbance inhibition curve as a function of the extract or Trolox concentration was plotted to determine the 50% inhibitory concentration (IC_{50}).

2.11Ferric Ion Reduction Test (FRAP)

The spectrophotometric method described by Marchi et al. [18] allows us to evaluate the reducing power of the sample. In a test tube containing 0.5 mL of sample solution (at a concentration of 1 mg/mL), 1.25 mL of phosphate buffer (0.2 M, pH 6.6), and 1.25 mL of potassium hexacyanoferrate [K_3Fe (CN) $_6$,1%] were added. The mixture was heated to 50°C in a water bath for 30 minutes. 1.25 mL of trichloroacetic acid (10%) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. Three aliquots of 0.625 mL were placed in 3 tubes; then, 0.625 mL of distilled water and 0.125 mL of a 1% fresh aqueous $FeCl_3$ solution were added. A blank without extract was prepared under the same conditions. The reading was made at 700 nm against a standard curve of ascorbic acid. The reducing capacity of the extracts was expressed in mmol Ascorbic Acid Equivalent (AAE) /g dry extract according to the following formula:

$$\mathbf{C} = [(\mathbf{c} - \mathbf{D})/(\mathbf{M} \times \mathbf{Ci})] \times \mathbf{100}$$

C = Concentration of reduced compounds in mmol AAE/g dry extract;
c = Concentration of the sample;
D = Dilution factor of the stock extract solution;
M = Molar mass of ascorbic acid (176 g/mol);
Ci = concentration of the stock extract solution

2.12 Lipid peroxidation inhibition test (LPO)

The inhibitory activity of the extracts on lipid peroxidation was determined using 2-thiobarbituric acid on a rat liver homogenate. According to a previous report, the liver homogenate was obtained from WISTAR rats. The $FeCl_2$–H_2O_2 was used to induce peroxidation of liver homogenate according to a modified method of Sombié et al. [19]. An amount of 0.2 mL of the extract at 1.5 mg/mL was mixed with 1 mL of 1% rat liver homogenate.Then 50 mL of $FeCl_2$ (0.5 mM) and 50 mL of H_2O_2 (0.5 mM) added. The mixture was incubated at 37°C for 60 minutes.So 1 mL trichloroacetic acid (15%) and 1 mL 2-thiobarbituric acid (0.67%) were added, and mixture was heated in boiling water for 15 minutes. Absorbance was read at 532 nm with a spectrophotometer (Bio-Rad 680, UV-wise). Ascorbic acid was used as a reference product. The ability of the extracts to inhibit lipid peroxidation was expressed as a percentage inhibition according to the following formula:

$$\%\ \mathbf{Inhibition} = \mathbf{1} - [(\mathbf{A1} - \mathbf{A2})/\mathbf{A0}] \times \mathbf{100}$$

A0 = Control absorbance (without sample);
A1 = Absorbance with the sample;
A2 = Absorbance without liver homogenate

2.13.Lipoxygenase inhibition test

Inhibitiontest of lipoxygenase by the extracts realized according Lončarić et al [20]. Dilution solutions were made from stock aqueous extract at a concentration of 8 mg/mL.And 3.75 µL of each dilute solution and 146.25 µL of a lipoxygenase solution (820.51 U/ml) were mixed in the wells of the enzyme microplate. The reaction was initiated by adding 150 µL of a linoleic acid substrate solution (1.25 mM), and absorbance variations were read at 234 nm using the spectrophotometer (Victor Nivo, France). The tests were performed in triplicate. Zileuton was used as a reference substance.
The inhibitory activity of lipoxygenase expressed as a percentage of inhibition was determined using the following equation:

$$\%\ \mathbf{Inhibition} = [(\mathbf{ODcontrol} - \mathbf{ODsample})/\mathbf{ODcontrol}] \times \mathbf{100}$$

OD control: optical density of the control;
OD sample: optical density of the sample

2.14. Statistical analysis of the data

The results of the antioxidant and anti-inflammatory activities were analyzed using Graph Pad Prism software version 8.00. The statistical analysis was performed by a two-way analysis of variance (ANOVA) followed by Dunett's multiple comparison tests. The differences were considered significant for a *P*-value less than 0.05 (*P<.05*) compared to the control or reference. The results were presented as a mean ± standard deviation

3. RESULTS AND DISCUSSION

3.1. The amount of the different fractions obtained after sieving analysis different particle sizes of powder

After fractionation of the raw *P. biglobosa* powder, four particle size classes were obtained and recorded in Table 1 and Figure 1.

Table (1): Quantity of each fraction obtained after sieving

Fraction Particle size (µm)	**quantity (g)**	
	Rainy season	**Dry season**
Coarse (0.4 - 1.25)	50	50
Moderately fine (0.2 - 0.315)	46.05	51.39
Fine (0.1 - 0.125)	22.45	32.64
Very fine (0.09 - 0.1)	16.46	14.58

The pictures of the powder obtained before and after fractionation can be seen in Figure 1.

Figure (1): Different powders obtained after sieving, A= Coarse powder; B= Moderately fine powder; C= Fine powder; D= Very fine powder

2.2 Residual moisture content of powders

The residual moisture content values of each powder particle size were recorded in Table.

Table (2): Residual moisture content of the different powders

Particle size fraction (µm)		Residual moisture content (%)	
		Rainy season	Dry Season
Coarse	**(0.4 - 1.25)**	6.76 ± 0.18	7.25 ± 0.28
Moderately fine	**(0.2 - 0.315)**	6.80 ± 0.13	7.57 ± 0.20
Fine	**(0.1 - 0.125)**	7.26 ± 0.52	8.06 ± 0.46
Very fine	**(0.09 - 0.1)**	8.08 ± 0.42	7.89 ± 0.53

The residual moisture content of the powders ranged from 6.76 0.8% to 8.08 0.42%, with an average of 7.45 0.24%. The highest RMC was obtained with the very fine powder in the rainy season. The lowest RMC was 6.76 ± 0.8%, obtained with coarse powder in the rainy season. Independently of the season, it can be observed that the more the particle size decreases, the more the residual moisture content increases. Although the fine fraction obtained in the dry season was higher than the very fine powder, it can be noticed that the differences were not statistically significant ($P > .05$).

The residual moisture content values were less than 10%. According to several published papers [21], a low moisture content is required to guarantee good product preservation. Consequently, our results indicate that the fractions are well-conserved, have a good appearance, and are without microbial changes.

2.3 Extraction yield of the different powder fractions

the different yield values obtained from the powders presented in the table below.

Table (3): Yield (%) obtained after decoction extraction

Fraction Particle size (µm)		Yield (%)	
		Rainy Season	Dry Season
Coarse	**(0.4 - 1.25)**	14.30**	12.20**
Moderately fine	**(0.2 - 0.315)**	13.54**	13.81**
Fine	**(0.1 - 0.125)**	15.24*	16.41*
Very fine	**(0.09 - 0.1)**	24.34	29.05

**P* < *.05* vs. Very fine; ***P* < *.001* vs. Very fine

Extraction yields were ranged from 12.20 to 29.05%. The highest yield was obtained with the very fine powder obtained from *P. biglobosa* trunk bark collected in the dry season. The coarse powder of the same period gave the lowest yield. Overall, extraction yields increased with the decrease in particle size.
The very fine powder fraction obtained the highest extraction yield, suggesting efficient extraction needs low particle size. One of the reasons that can be advanced to explain this result is that small particle sizes increased the surface area exposed to the solvent. In addition, it promotes dissolution of a more significant number of solvent-soluble substances, thereby increasing yield [22, 23, 24]. The extraction yield depends upon other factors, such as edaphic factors, the extraction method, and the collection period.[9]. The yield of very fine powder collected during the dry season was higher (29%) than the collected during the rainy season[24].

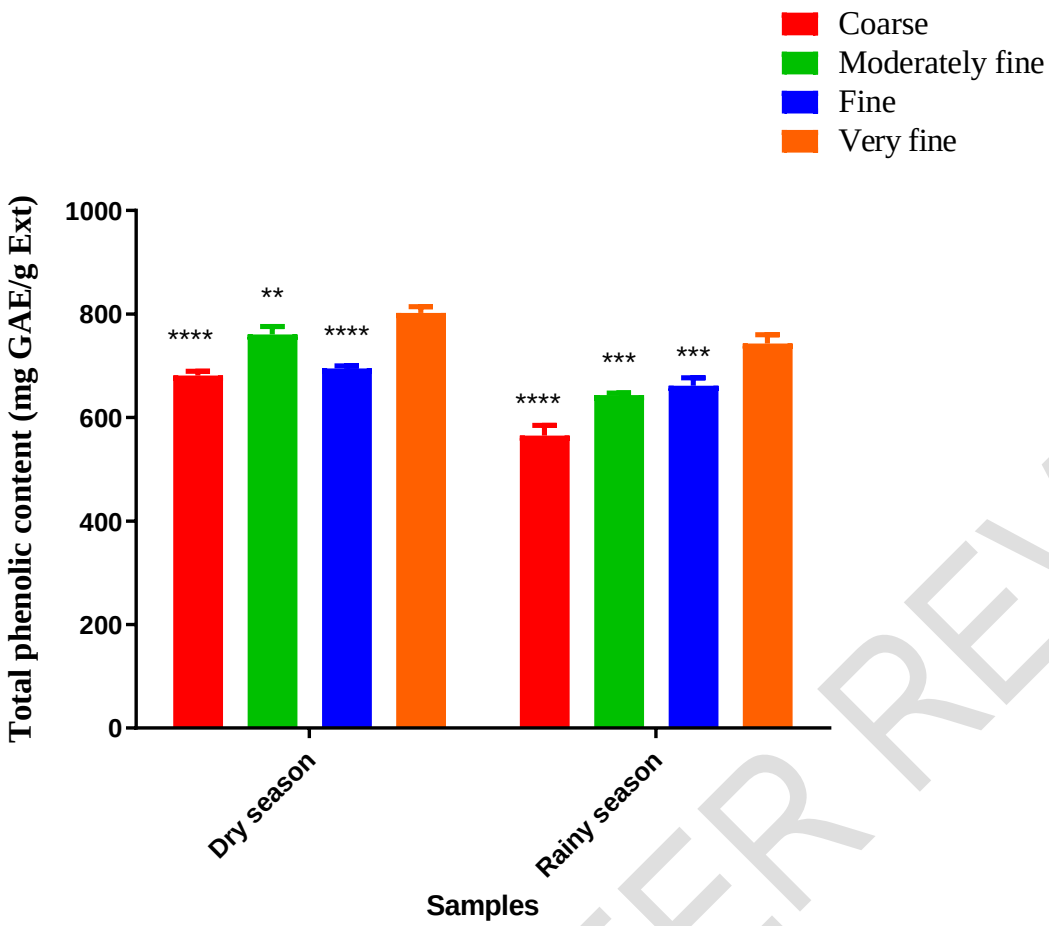
2.4 Phytochemical groups identified in the different fractions
The secondary metabolites such as flavonoids, tannins, saponins, sterols, and triterpenes were all observed in each fraction independently of the season and particle size.

Table (4): Phytochemical constituents of powders of different sizes

Metabolites	Rainy season	Dry season
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Stérols/Triterpènes	+	+

The secondary metabolites sought were all highlighted in powders of different sizes.
Phytochemical analyses performed by thin-layer chromatography revealed the presence of many chemical groups, including tannins, flavonoids, sterols, triterpenes, and saponosides. These results are in line with previous reports on the phytochemical content of the genus Parkia species, of which *P. biglobosa* [5, 6]. It can be noticed that the particle size did not influence the qualitative phytochemical content of the different powders.

2.5 Total phenolic content
Figure 1 shows the total phenolic contents of the extracts obtained in the fractions of different particle sizes.

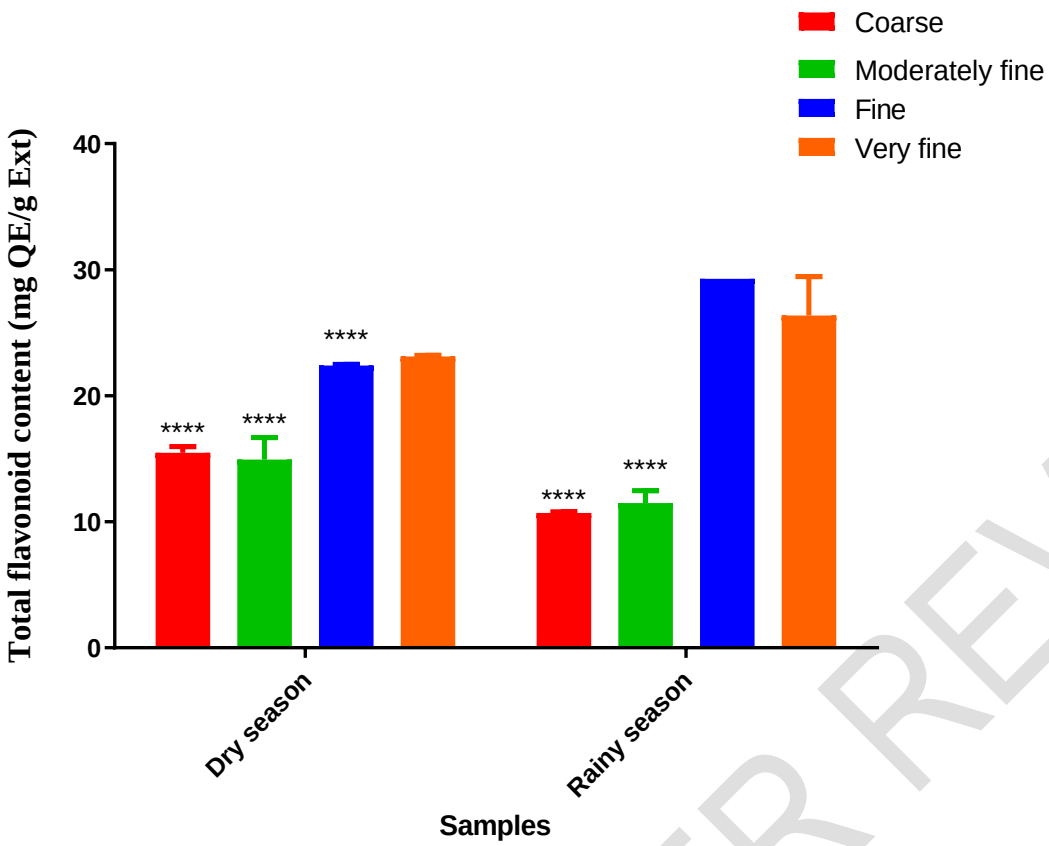


Figure(2): Total phenolic content in the fraction extracts.
The results were compared to the very fine powder, independently of the season. ** = *P < .05; *** P < .001, and **** = P < .0001.*

Total phenolics were ranged from 565.27 ± 19 to 802 ±12 mg GAE /g. The very fine powder of the dry season gave the highest polyphenols content. The lowest content was obtained from the wet season coarse powder. In the rainy season, total phenolic content increased with the decrease of the particle size, with a statistically significant difference.

2.6 Total flavonoid content

The determination of total flavonoids in the various extracts allowed theresults presented in Figure 2



Figure(3): Total flavonoid content of different extracts.

The results were compared to the very fine powder for the powder obtained during the dry season and to the fine fraction for those collected in the rainy season; **** = $p < .0001$*** = $p < .001$ vs. very fine

Concentrations ranged from 10.70 ± 0.1 to 29.27 ± 0.0 mg QE /g. The fine powder of the rainy season gave the highest concentration of flavonoids; this result was statistically not significant ($p > 0.05$) compared to the very fine powder. The coarse powder of the rainy season gave the lowest concentration of flavonoids.

2.7 Antioxidant activities

The results of antioxidant activities are shown in Table 5.

Table (5). Antioxidant effect of the different fractions

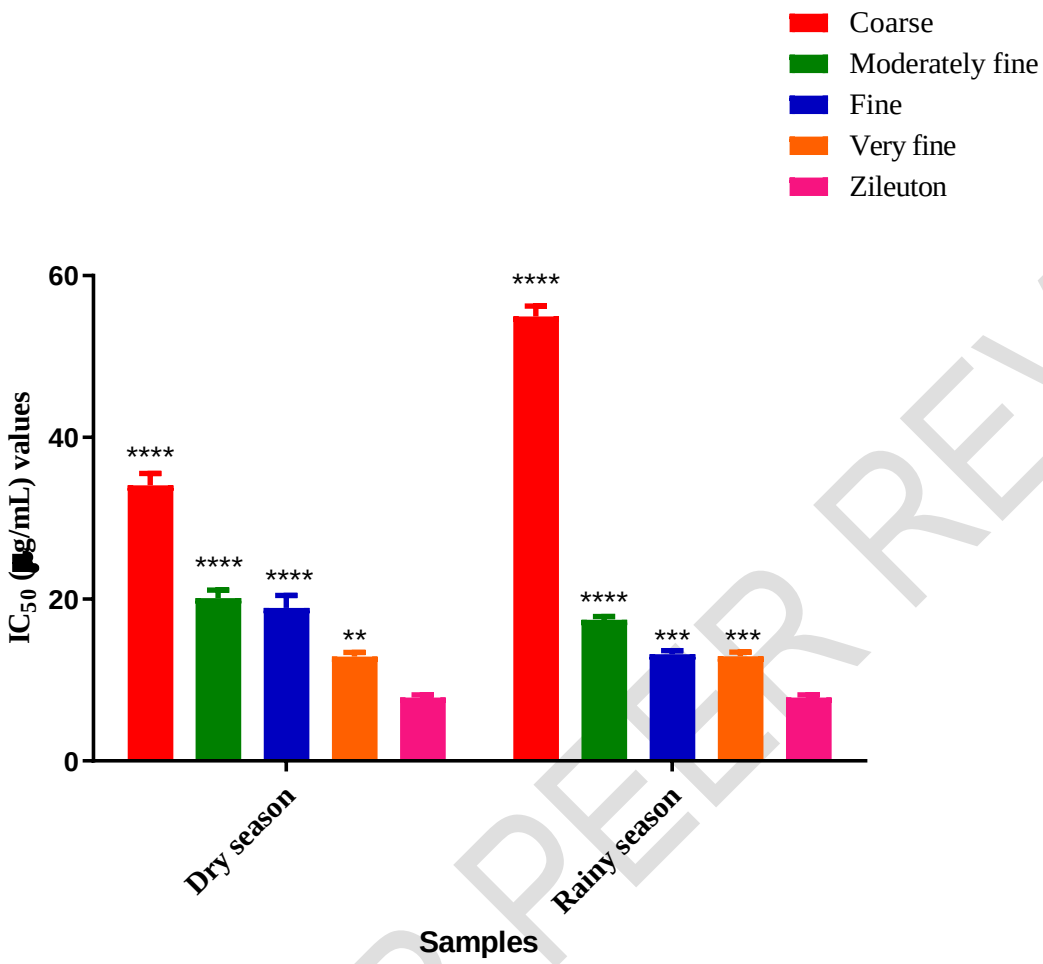
	Dry season				Rainyseason			
	DPPH$^{\alpha}$	**ABTS$^{\beta}$**	**FRAP$^{\gamma}$**	**LPO$^{\delta}$**	**DPPH$^{\alpha}$**	**ABTS$^{\beta}$**	**FRAP$^{\gamma}$**	**LPO$^{\delta}$**
Samples								
Coarse	5.5 ± 0.8*	4.1 ± 0.1***	885.7 ± 84.8 ***	40.7 ± 0.4***	7.4 ± 0.2	4.9 ± 0.2***	3005.5 ± 184.1**	49.3 ± 2.3****
Moderately fine	6.2 ± 0.2	3.1 ± 0.1***	2110.8 ± 54.8****	43.9 ± 0.0****	7.0 ± 0.3	3.6 ± 0.1****	1549.0 ± 19.6 ***	39.8 ± 1.8****
Fine	5.7 ± 0.2	2.5 ± 0.2	3372.6 ± 104.5****	41.7 ± 0.7****	5.5 ± 0.4**	2.5 ± 0.3	2700.8 ± 77.8***	23.6 ± 0.4
Very fine	3.6 ± 0.6***	1.4 ± 0.3**	**6973.0 ± 21.3**	48.4 ± 0.4****	3.2 ± 0.5****	1.9 ± 0.1	**3671.4 ± 194.8**	43.4 ± 1.6****
Trolox	**6.9 ± 0.2**	**2.2 ± 0.1**	-------	-------				
Ascorbicacid		-----		**26.6 ±1.1**				

DPPH and ABTS results are expressed by their IC_{50} (µg/mL) values ($^{\alpha}$ and $^{\beta}$, respectively, for the DPPH and ABTS tests); the results were compared to the Trolox. The estimation of reducing compounds measured by the FRAP test was expressed as mmol Ascorbic Acid equivalent/g ($^{\gamma}$), and the results were compared to the very fine fraction. The lipid peroxidation results ($^{\delta}$) are expressed as a percentage of inhibition; the results were compared to Ascorbic acid. **P < .05; **P < .01 ; ***P < .001 ; ****P < .0001*

Our results regarding the antioxidant effect confirm the potential of the different extracts to scavenge free radicals. The antioxidant effect was measured using four antioxidant tests, including ferric ion reducing (FRAP test), lipid peroxidation (LPO test), and radical scavenging (DPPH and ABTS tests). In most assays, the very fine powder, independent of the season, was the more potent. For instance, in the LPO test, this powder was more efficient compared to ascorbic acid. Therefore, these results indicate that reducing particle size may influence the antioxidant activity of the extract. Several other reports have reported that particle size can modify a product's antioxidant properties [22, 23].

The antioxidant effect of medicinal plants can be explained by numerous compounds, including polyphenols, coumarins, and stilbenes [25]. All the extracts were rich in polyphenol compounds, although the fine and very fine powders were the richer. So, the interesting antioxidant effect could be explained by the high polyphenol content of the extracts.

2.11 Lipoxygenase inhibition Activity (LOX)
The 50% inhibition concentrations of lipoxygenase by the different extracts are presented in Figure 3.



Figure(4): IC_{50} values obtained after inhibition of the 15-lipoxygenase.
For statistical analysis, the results were compared to Zileuton.*****P* < .0001; ****P* < .001*

The IC_{50} values were ranged from 54.94 1.2 to 12.88 0.6 µg/mL. The lowest IC_{50} was obtained from very fine dry season powder. The highest IC_{50} was obtained from the coarse powder collected from *P. biglobosa* trunk bark during the rainy season. All the fractions were significantly statistically different from Zileuton, which displayed an IC_{50} value of 7.84 0.3 µg/mL. The IC_{50} values decreased with the decrease in particle size.
The lipoxygenase pathway (LOX) is one of the pathways of the inflammatory process that leads to the production of pro-inflammatory compounds known as leukotrienes. The results showed an excellent inhibitory effect of the different extracts on the 15-lipoxygenase. As previously advanced, it can be seen that the reduction of particle size increases the anti-lipoxygenase effect of the different powders. Globally, the extracts prepared from trunk bark collected during the rainy season were more potent in inhibiting lipoxygenase, suggesting that the season influences the anti-inflammatory effect as measured through the *in vitro* inhibition of lipoxygenase. The lipoxygenase inhibition may be explained by the presence of phytochemicals known to act as potent lipoxygenase inhibitors. These include tannins, flavonoids, terpenoids, alkaloids, and saponins [26, 27].

4. CONCLUSION

Parkiabiglobosa is a plant widely used in our country to treat various disorders. The powder of the trunk bark of the plant collected during the dry and rainy seasons was sieved into four particle sizes, giving the (i) coarse, (ii) moderately fine, (iii)

fine, and (iv) very fine fractions. The qualitative phytochemical search indicates that all the fractions contain tannins, flavonoids, sterols, triterpenes, and saponins. Furthermore, the antioxidant and anti-inflammatory results demonstrated the potential of the different fractions, mainly the very fine fraction, at scavenging free radicals and inhibiting lipoxygenase. These significant results may be correlated with the high content of phenolic and flavonoid compounds. To the best of our knowledge, this is the first report on the phytochemical, antioxidant, and anti-lipoxygenase properties of powders of *P. biglobosa* trunk bark with different particle sizes. These results pave the way for developing a phytomedicine, mainly with the very fine powder, to treat inflammatory diseases, including hemorrhoids.

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