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Physicochemical characterisation and phytochemical study of *Grewia coriacea* Mast powders: focus on polyphenols

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Abstract
This study is to contribute to the valorisation of *Grewia coriacea* Mast fruits consumed in Brazzaville, Republic of Congo. Physicochemical and phytochemical characterisations of different size classes (< 315 µm, between 315 and 500 µm, and > 500 µm) fruit powder. *Grewia coriacea* Mast presented the following chemical composition: proteins (7.36%), minerals (6.69%), lipids (0.08%), total sugars (3.89%) and fermentable sugars (1.57%). The zinc (8.60mg/100 DM) to potassium (2251.4 mg/100 g DM). calcium (343.0 mg / 100 g DM), magnesium (127.0 mg/100 g DM) and phosphorus (94.7 mg/100 g DM). vitamin C (6.25 mg/100 g DM). For the extraction of polyphenols, anthocyanins, flavonoids and condensed tannins, the best extraction yield was obtained for the < 315 µm size class, with an average of 20.4%, for all size classes, acetone and methanol were the best extraction solvents. Extraction of total polyphenols (3.85 mg eq GA/g DM)) and condensed tannins (8.22 (mg eq CT/g DM)) was improved in the < 315 µm size class; however, flavonoids (6.78 (mg eq QU/g DM). Anthocyanin contents of all size classes ranged between 44.57 and 60.41 mg / g DM.

Keywords: *Grewia coriacea* Mast, chemical composition, extraction yield, Study of phenolic phytochemical compounds.

Introduction

In recent decades, due to the increased interest in consumption of healthy food, a growing number of chemical investigations have been conducted on natural bioactive substances in wild plants. Food plants constitute an important group of non-woods forest products (NWFP). They are historically and today still an operating and intense marketing in Brazzaville, Republic of the Congo. *Grewia coriacea* Mast is a plant of Guineo-Congolese spontaneous flora that produces some edible fruits [1]. The genus *Grewia* belongs to the *Malvaceae* family that comprises 48 genera and more than 600 species worldwide, but 10 genera and 29 species in the Republic of Congo [2]. *Grewia coriacea* Mast is a widespread species that spontaneously grows in dense forests of Central Africa under the form of a tree of 4 - 25 m high and 12 - 40 cm diameter [3]. The flowers are arranged in terminal panicles from 3 to 10 cm long. The fruits are grouped into clusters of ovoid drupes of 2.5 to 4 cm long and 1.8 to 4 cm wide [1]. They are light green in the early stages of development and became black at maturity, where they are harvested for human consumption. The different parts of *Grewia coriacea* Mast find their applications in several areas: barks are used in medicine for treating intestinal diseases and syphilis [4]; fruits are used in the food industry for the manufacture of juices, syrups, and wines; wood is employed in the manufacture of plates, spoons, and forks, and finally the red decoction of fruits can also be used in dyeing [5]. In addition to these multiple interests, *Grewia coriacea* Mast is an important source of vitamine C and other

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nutrients [6]. *Grewia coriacea* Mast is a plant that contains natural bioactive substances: polyphenols, flavonoids, tannins and anthocyanins. [7]. Secondary metabolites are the subject of many researches, especially polyphenols, which constitute a family of over 8000 compounds, ubiquitous in the plant kingdom, and among them over 4000 flavonoids have been identified [8]. They are widely used in therapy as anti-inflammatory and antioxidant agents [9], for the prevention of many diseases related to oxidative stress and its associated pathologies such as cancer, diabetes, cardiovascular disorders and Alzheimer s disease [10]. According to [11], polyphenols is a family of biomolecules having diverse structures. The basic structural element that characterizes them is the presence of at least one benzene ring directly bonded to hydroxyl groups. The polyphenols are primarily recognised for their antioxidant activities: they are able to prevent diseases caused by free radicals [12]. They can trap free radicals, inhibit the enzymes responsible for the formation of free radicals, and are even chelators of some metal ions [13]. The use of flavonoids has experienced a tremendous interest these last decades (in particular anthocyanins that are used as potential food supplements), which has contributed to the discovery of the antioxidant power of these substances [14]. The total phenolic and flavonoid contents are two general indices widely used to represent the overall antioxidant capability in a sample [15]. This study proposes to enhance the physicochemical and phytochemical knowledge about fruits of *Grewia coriacea* Mast that remains still embryonic. It is in this perspective that the current study propose to conduct a physicochemical characterisation of *Grewia coriacea* Mast powders and to study the phytochemical interest of *Grewia coriacea* Mast extracts, with a view to identify the families of major active biomolecules confering antioxidant activity to this plant.

2. **Material and methods**

2.1. **Chemical products**

Folin-Ciocalteu reagent was obtained from VWR international (France). Gallic acid, quercetin, catechin, vanillin, and ascorbic acid were obtained from Sigma-Aldrich (France). Sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), and sodium hydroxide (NaOH) were supplied by Acros Organics (Belgium). Other reagents and solvents were purchased from Merck (Germ:,any).

2.2. **Plant material**

Fruits of *Grewia coriacea* Mast were purchased from the « Bouemba » market in Ouenzé district of Brazzaville, Republic of the Congo (4° 14’ 0.07” S 15° 16’ 8.88” E; elevation: 241 m) in June 2014. The fruits were washed and dried in the open air at 37 °C for 20 days and then finely ground using an electric ultra-centrifuge grinder ZM 200 (Retsch, France) supplied with 1 mm trapezoidal holes sieve. Grinding was operated at 6 000 rpm at room temperature (about 20 °C).

2.3 **Sample preparation**

After grinding, *Grewia coriacea* Mast powder was divided in two lots: a first lot of unsieved powder, which allowed determining the chemical composition. The second batch corresponds to sieved powders, divided into three fractions according to their particle size class: less than 315 µm (called < 315 µm), between 315 and 500 µm (noted 315 - 500 µm), and greater than 500 µm (referred as > 500 µm).

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2.4. **Extraction of phenolic compounds**

The selective extraction of the main families of phenolic compounds was performed according to the protocol described by [16], with some modifications: 1 g of unsieved powder was mixed with 10 mL of one of the following solvents: ethanol-water, acetone-water, and methanol-water (70/30 % (v/v)). The obtained extract was centrifuged in a 5702 R Eppendorf centrifugeat (France) at 1200 g for 15 min at room temperature (20 °C). The supernatant was collected and filtered on Whatman paper filter N°1 (Sigma Aldrich, France). Maceration was carried out twice successively at room temperature (20 °C) for 2 h. After that, the filtrates were mixed and stored at 4 °C until analysis.

2.5. **Chemical composition of powders**

2.5.1. **Water content**

Determination of water content was performed following the [17] method that is based on the measure of the mass loss of samples after stoving at 103 ± 2 °C until complete elimination of free water. The crucible was first cleaned, dried, and weighed (W_0 5 g of sample was put in the crucible and then the filled crucible was weighed (W_1) and placed in an oven at 105 °C for 5 h. The crucible was removed from the oven, then cooled down in a desiccator, before being weighted (W_2) again. Water content was finally expressed in weight percents with the formula below :

$$\boldsymbol{Water\ content}\ (\%) = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \qquad \text{(Equation 1)}$$

2.5.2. **Ash content**

Ash content in *Grewia coriacea* Mast powders was determined according to the [18] method. It consists in mineralising 3 to 5 g of powder (in a previously dried ceramic crucible) in a muffle furnace. The mineralisation crucible has first been cleaned, dried, and weighed (W_0). The crucible containing the wet product was placed in an oven at 105 °C for 24 h. After drying, the crucible was cooled down in a desiccator (P_2O_5) before being weighed again (W_2). After that, the crucible containing the dry sample was introduced into a muffle furnace at 550 °C for incineration for about 6 h, then cooled down in the dessicator and weighed once more (W_3). Ash content corresponds to the mass of product remaining in the crucible after incineration reported to total dry matter of the product. Equation 2 is used to express the ash content in weight percent on dry basis:

$$\boldsymbol{Ash\ content} = \frac{(W_3 - W_0)}{(W_2 - W_0)} \times 100 \qquad \text{(Equation 2)}$$

2.5.3. **Total fat content**

Lipids are determined according to the [18] by weight difference after Soxhlet extraction. To this aim, 5 g of *Grewia coriacea* Mast powder was placed in a cartridge previously dried for 1 h in an oven at 105 °C and cooled down in a desiccator. Then, the lipids contained in the powder were extracted for 6 h with diethyl ether and light petroleum. Last, solvent mixture was evaporated and extracted lipids were weighed.

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2.5.4. **Total protein content**

The total protein content was determined by the Kjeldahl method [17], based on the transformation of organic nitrogen into mineral nitrogen in ammonia form, $(NH_4)_2SO_4$, mediated by oxidative action of fuming sulphuric acid on organic matter in the presence of a catalyst (Na2SO4 + CaSO4 + Se). The total protein content was calculated using a 6.25 conversion factor, adapted to plant samples [18].2.5.5. **Total sugars content**

The total sugars content of *Grewia coriacea* Mast powders was determined by the method developed by [19]: 2 mL of extract was placed in a test tube with 1 ml phenol in 5% aqueous solution. 5 ml H_2SO_4 was quickly added in the solution and the mixture was immediately agitated. A yellow colour develops, which is stable for several hours. The tubes were placed in a water bath at 25 - 30 °C for 20 min and then cooled down in water at 20 °C. Absorbance was measured at 485 nm with Perkin Elmer Lambda 11 UV/visible spectrophotometer. Total sugars content was determined by reference to a standard glucose range.

2.5.6. **Reducing sugars content**

1 g powder sample was solubilised in 10 mL boiling distilled water for 15 min. Sugar extracts were assayed by the 3.5-dinitrosalicylic (DNS) acid method [20]: in the presence of a sugar, the DNS gives a reddish-brown complex (3-amino 5-nitrosalycilic acid), which has its absorption maximum at 540 nm. Total sugars content was determined by reference to a standard glucose range.

2.5.7. **Minerals content**

The levels of major minerals (K, P, Mg, Ca, Zn), were determined by atomic absorption spectroscopy after solubilisation of ashes in acidic medium. 1 g ash was solubilised in 10 mL hydrochloric acid and then the solution is completed to 100 mL with deionised water. The device used for mineral contents determination was an HGA 700 Atomic Absorption Spectrometer (Perkin-Elmer, USA) powered by an air-acetylene flame. For the extraction of calcium and magnesium, 10 mL lanthanum chloride (prepared by dissolving 18 g lanthanum oxide into 250 mL concentrated HCl) were added before filling the 100 mL flask up to the mark with deionised water.

2.5.8. **Phosphorus content**

Phosphorus content of powders of *Grewia coriacea* Mast was determined by the colorimetric method described by [21]. In an acid medium and in the presence of molybdate ammonium $(NH_4)_6MB_7O_{24}$, $4H_2O$), phosphates gives a phosphomolybdic complex, which, reduced by ascorbic acid, develops a blue colour with an absorption maximum at 690 nm. Absorbance measure was carried out against a blank prepared by adding 2 mL of the reaction solution to 10 mL deionised water. Phosphorus concentration was determined from a phosphate calibration range of 0 to 10 mg / L.

2.5.9. **Vitamin C content**

Vitamin C content was determined by a titrimetric method with dichloropheno-lindophenol reagent [22] with few modifications. 1.5 g powder was added to 150 mL of an aqueous solution of 4% (v/v) oxalic acid. The mixture was homogenised and filtered. 5 mL filtered

solution was two-fold diluted with a 4% (v/v) oxalic acid aqueous solution. This solution was titrated with a 0.01% (v/v) aqueous solution of 2.6-dichloro-phenolindophenol (colour indicator) until persistence of a pink colour for 15 s. The calibration curve was realised with an aqueous solution of 0.05% ascorbic acid. The results were expressed in milligrams of ascorbic acid equivalents per 100 g of dry matter.

2.6. **Particle size distribution**

Particle size distributions of *Grewia coriacea* Mast powders were determined by laser diffraction using a Mastersizer 3000 (Malvern Instruments Ltd., Worcestershire, UK) supplied with the dry dispersion unit Aero S at room temperature (20 °C). Powders were dispersed in the cell measurement at 2 mm hopper length, 1 bar feed pressure and 40% feed rate, in order to reach an obscuration in the range from 0.5 to 3 %. From particle size distributions, three characteristic size estimators were determined (Dv_{10}, Dv_{50}, and Dv_{90}). Dv_{50} is the diameter for which 50% (in volume) of powder particles were smaller; it represents the mean size of powders. D_{10} and D_{90} are similarly defined (diameters corresponding to 10% and 90% smaller particles, respectively) and characterise the size of the coarser and the finer fractions, respectively. In addition, span, which describes the width of the particle size distribution, was calculated according to equation 3:

$$\boldsymbol{Span} = \frac{\boldsymbol{Dv_{90} - Dv_{10}}}{\boldsymbol{Dv50}} \qquad \text{(Equation 3)}$$

2.7. Extraction yield

The extraction yield was calculated with the formula below [23]:

$$\boldsymbol{R}\ (\%) = \frac{\boldsymbol{M_{ext}}}{\boldsymbol{M_{ech}}} \times \mathbf{100} \text{ (Equation 4)}$$

Where R is the extraction yield (%); M_{ext}, extracted mass after solvent evaporation (mg), and M_{ech}, the dry weight of plant sample (mg).

2.8. **Determination of polyphenols**

Total polyphenols: the determination of total polyphenols was carried out according to the Folin-Ciocalteu method (FC) with few modifications [24]. 50 µL extract was mixed with 3 µL deionised water and 250 µL Folin–Ciocalteu reagent (1 N). After 8 min equilibration, 750 µL of 20% Na_2CO_3 and 950 µL deionised water were added to the extracts; after incubation for 30 min at room temperature (20 °C), absorbance was read at 765 nm with a UV/visible spectrophotometer PerkinElmer Lambda 11. Results were expressed in milligrams of gallic acid equivalents per gram of dry matter by referring to the calibration curve of gallic acid standard in the concentration range from 0 to 20 $mg.L^{-1}$.

Total flavonoids: total flavonoids were quantified according to the method described by [25]: 500 µL extract was added to 1500 µL of 95% methanol, 100 µL of 10% (w/v) $AlCl_3$, 100 µL of 1 M sodium acetate, and 2800 µL deionised water. The mixture was stirred and then incubated in the dark at room temperature (20 °C) for 30 min. Blank was prepared with the same protocol in which the extract was replaced by 95% methanol. Absorbance was measured at 415 nm with Perkin Elmer Lambda 11 UV/visible spectrophotometer. Results were expressed in milligrams of quercetin

equivalents per gram of dry plant material by referring to the calibration curve of quercetin standard in the concentration range from 0 to 50 $mg.L^{-1}$.

Condensed tannins: condensed tannins were determined by the method of vanillin in acidic medium described by [26]: vanillin reagent was prepared by mixing equal volumes of 8% (v/v) HCl aqueous solution, 37% (v/v) methanol, and methanol solution at 4% (m/v) of vanillin. The mixture was maintained at 30 °C before analysis. 200 µL extract was added to 1 mL vanillin reagent; the mixture was stirred and incubated in darkness at 30 °C for 20 min. Absorbance was measured at 500 nm with a Perkin Elmer Lambda 11 UV/visible spectrophotometer against a blank consisting of a mixture of equal volumes of 37% (v/v) methanol and 8% (v/v) HCl. (Results were expressed as milligrams of catechol equivalents per gram of dry plant material by reference to the calibration curve of catechol standard in the concentration range from 0 to 50 $mg.L^{-1}$.

Total anthocyanins: Anthocyanins were extracted following the method of [27] with some modifications. 1 g *Grewia coriacea* Mast powder was added to 20 mL of a 85/15 %(v/v) mixture of 95% (v/v) ethanol and 1.5 M HCl (). The extract was transferred in a 40 mL volumetric flask, whose volume was completed with the mixture of ethanol and hydrochloric acid and stored for 12 h at 4 °C. After filtration, absorbance was measured with a Perkin Elmer Lambda 11 UV/visible spectrophotometer at535 nm. Total anthocyanin content was determined by the application of the Beer-Lambert law and expressed as milligrams per gram of dry matter (equation 5):

$$C = \frac{AD}{\varepsilon L} \quad \text{(Equation 5)}$$

With: C, concentration of the solution in $mol.L^{-1}$; A, absorbance of the diluted sample at 535 nm; D, dilution factor; ε, molar absorption capacity of anthocyanins in acid-ethanol medium (98.2 $L.mol^{-1}.cm^{-1}$), L: optical path length (1 cm).

2.9. **Statistical analysis**

All analyses were carried out in triplicate. Results were expressed in terms of means ± standard deviations. Statistical analyses (ANOVA) were performed with Minitab 15 software (Minitab Inc., France). Single-way analysis of variance was conducted to determine the significance of differences between analytical results at $p < 0.05$ significance level.

3. **Results and discussion**

3.1 **Study of the chemical composition of unsieved *G. coriacea* Mast powder**

Tabl. 1

Table 1 shows the chemical composition of unsieved powder of *Grewia coriacea* Mast. It contained 9.50% water on wet basis, as well as 6.69% ash, 0.08% lipids, 7.36% proteins, 3.89% total sugars, 1.57% reducing sugars, and 6.25 mg vitamin C per 100 g on dry basis. These values are close to those reported by [28] for *Grewia tenax* moisture

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(13,02 %), Ash (5,20 %), and proteins (7,70%). Obtained results were also similar values reported by [6] for *Grewia coriacea* Mast. The water content was under 10%, which shows the good storage ability of *Grewia coriacea* Mast powder.

Tabl. 2

Table 2 shows that minerals varied in the range from 8.60 (zinc) to 2251.4 (potassium) mg / 100 g dry matter. These results indicate that potassium is the most abundant mineral in *Grewia coriacea* Mast powder, followed by calcium (343.0 mg / 100 g dry matter), magnesium (127.1 mg / 100 g dry matter), phosphorus (94.7) and Zinc. These values are similar to those reported by [28] for *Grewia flavescence*: 269 mg calcium and 8.77 mg potassium per 100 g dry matter. The needs in calcium are important for bone and teeth development [29]. A dietary allowance of 900 mg calcium per day is recommended for adults and children [30]. Potassium content of Grewia coriacea Mast in this study was higher than that of *Grewia flavescence* [28]. Potassium is an essential micronutrient as it acts in close cooperation with sodium to maintain the acid-base balance of the body [31].

3.2. **Particle size distribution**

Tabl. 3; Fig 1

Particle size distribution and granulometric parameters of sieved powders of *Grewia coriacea* Mast are presented in figure 1 and table 2. Particle size distributions of *Grewia coriacea* Mast powders were monomodal. Particle diameters of *Grewia coriacea* Mast powders ranged between 10 and 1000 µm. D_{50} were well comprised in the size ranges provided by the sieves, showing that the sieving procedure was successful in obtaining well different size classes. D_{10} and D_{90} were within or close to the ranges of sieve sizes, also confirming the suitability of the sieving procedure. The presence of particles larger than the bottom sieve size was also observed, especially for the 315 - 500 µm sample, which may be due to the fact that particles probably had non-spherical shapes, allowing them to pass through the sieves of mesh inferior to their mean size. Spans were small, indicating narrow particle size distributions and a high degree of uniformity of particle size in each size classes.

3.3. **Extraction yield**

Fig. 2

It appears on figure 2 that acetone gave the best extraction yields with an average of 20.4% for all size classes, while ethanol led to the lowest extraction performance (17.03% average extraction yield). Extraction yields significantly differed ($p < 0.05$) depending on the powder size class and extraction solvent. Better extraction was achieved for the < 315 µm sample (19.40%), followed by 315 - 500 µm (17.48%) and > 500 µm (16.43%) class sizes. The extraction yields obtained in this study are comparable to those obtained by [32, 33], with acetone being the best extraction solvent (19.29% on average).

3.4. **Total polyphenols content**

Tabl. 4

Total polyphenols content did not significantly vary according to the size class ($p > 0.05$). The smallest size class was expected to contain more active biomolecules, as larger particles correspond to fruit parts that are harder to grind such as fibres that contain less biomolecules and this was confirmed by results presented in table 4. Acetonic extracts record the highest levels of total polyphenols (3.85 mg eq AG / g dry matter) followed by methanolic and ethanolic extracts (3.63 and 3.20 mg eq GA/g DM, respectively). The work carried out by [34] confirms our findings as it evidenced that the fruit of *Grewia oligoneura* Sprague had a better total polyphenols content (16.27 mg eq GA / g DM) within 5 *Grewia* species.

3.5. **Total flavonoids content**

Tabl. 5

Statistically, total flavonoid contents in studied extracts (table 5) were similar ($p > 0.05$) and maximal for < 315 µm and 315 - 500 µm samples (6.65 and 6.78 mg eq QU / g DM on average, respectively). These values are very similar to those reported by [6]: 5.20 mg eq QU / g DM. As regards the extraction solvent, acetone and methanol remained the best extractors of flavonoids with averaged contents of 6.65 and 6.78 mg eq QU /g DM, respectively.

3.6. **Condensed tannins content**

Tabl. 6

The results of condensed tannins contents displayed in table 6 revealed a significant difference ($p < 0.05$) between all size classes. For all powder samples, methanol was more efficient for tannin extraction (7.19 and 5.44 mg eq CT / g DM on average for < 315 µm and 315 - 500 µm samples, respectively). The condensed tannins content decreased for the highest particle size. Whereas ethanol weakly extracted tannins (5.52 mg eq CT / g DM on average), extraction performance was increased with acetone, as this latter solvent has the ability to solubilise proanthocyanidins that are not soluble in ethanol [35-36]. The efficiency of condensed tannins extraction depends on their chemical nature, the type of employed solvent, and operating conditions [14]. This explains the significant differences observed for condensed tannin contents according to the type of extraction solvent ($p < 0.05$). 3.7.

Total anthocyanins content

Tabl. 7

Table 7 presents the anthocyanin contents of the three samples of different size class with significant difference ($p < 0.05$). Total anthocyanin contents ranged between 44.57 and 60.41 mg / g DM. For all powder samples, Acetone was more efficient for anthocyanins extraction (60.41 mg / g DM on average for < 315 µm and 315 - 500 µm samples, respectively). These values are similar to those reported by [37] for *Grewia assiatica* (72 mg / 100 g DM). The fruit of *Grewia coriacea* Mast is rich in anthocyanin

pigments, giving it a reddish colour. Anthocyanins enhance visual acuity, which allows considering therapeutic applications for extracts from *Grewia coriacea* Mast powders.

4. **Conclusion**

Physicochemical characterization and extraction of polyphenolic compounds of the fruits of *Grewia coriacea* Mast are crucial steps for the promotion of the bioactive principles of this plant. Antioxidant activity of the fruit of *Grewia coriacea* Mast can be explained by its significant proportions of phenolic compounds, flavonoids, condensed tannins, and anthocyanins . It was shown in the current study that extraction yields depend both on particle size class and employed solvent. Extraction solvent must be carefully chosen to preserve the plant biological activities. Besides, it appeared that the powder of size class inferior to 315 µm allowed maximising levels of total polyphenols and condensed tannins when acetone or methanol were used as extraction solvents. The 315 - 500 µm size class was preferable for the extraction of flavonoids. The total anthocyanin content, approximately equal to 60 mg / g DM for the different samples confirmed that *Grewia coriacea* Mast can be regarded as a Central African dye plant. *Grewia coriacea* Mast, owing to its significant polyphenols content that contribute to its antioxidant potential power, may also find its application in traditional medicine for the treatment of oxidative stress-related diseases.

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Table 1: Physicochemical analysis of unsieved powder of *Grewia coriacea* Mast fruits.

Components	Composition
Water (%)	9.50 ± 0.73
Crude Ash (% DM)	6.69 ± 0.09
Crude Fat (% DM)	0.08 ± 0.05
Proteins (% DM)	7.36 ± 0.71
Total sugars (% DM)	3.89 ± 1.03
Reducing sugars (% DM)	1.57 ± 0.08
Vitamin C (mg / 100 g DM)	6.25 ± 2.30

Table 2**:** Mineral contents of unsieved powder of *Grewia coriacea* Mast fruits.

Minerals	mg /100 g DM
Phosphorus	94.75 ± 1.82
Calcium	343.02 ± 0.51
Magnesium	127.16 ± 0.46
Potassium	2251.43 ± 1.66
Zinc	8.65± 2.30

Table 3: Granulometric parameters of the different size classes of *Grewia coriacea* Mast

Sieved powders	Dv_{10}, µm	Dv_{50}, µm	Dv_{90}, µm	Span,
< 315 µm	123.3 ± 3.1	154.1 ± 2.2	256.3 ± 4.3	1.85 ± 0.06
315 - 500 µm	322.2 ± 2.8	473.5 ± 3.5	697.0 ± 0.0	0.79 ± 0.00
> 500 µm	465.3 ± 54.3	697.7 ± 32.0	1053.3 ± 5.8	0.84 ± 0.13

Figure 1: Particle size distributions of the different size class of *Grewia coriacea* Mast powders.

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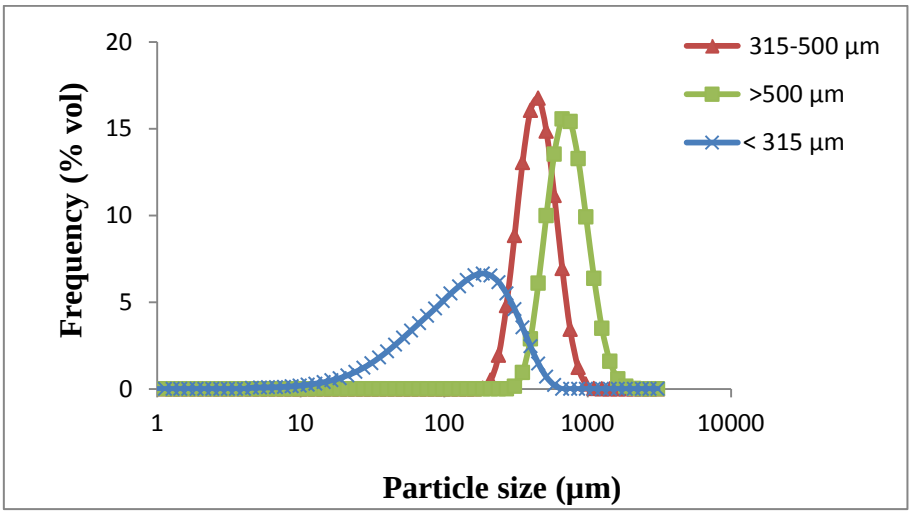

Figure 2: Extraction yields of the different size classes of *Grewia coriacea* Mast powder.

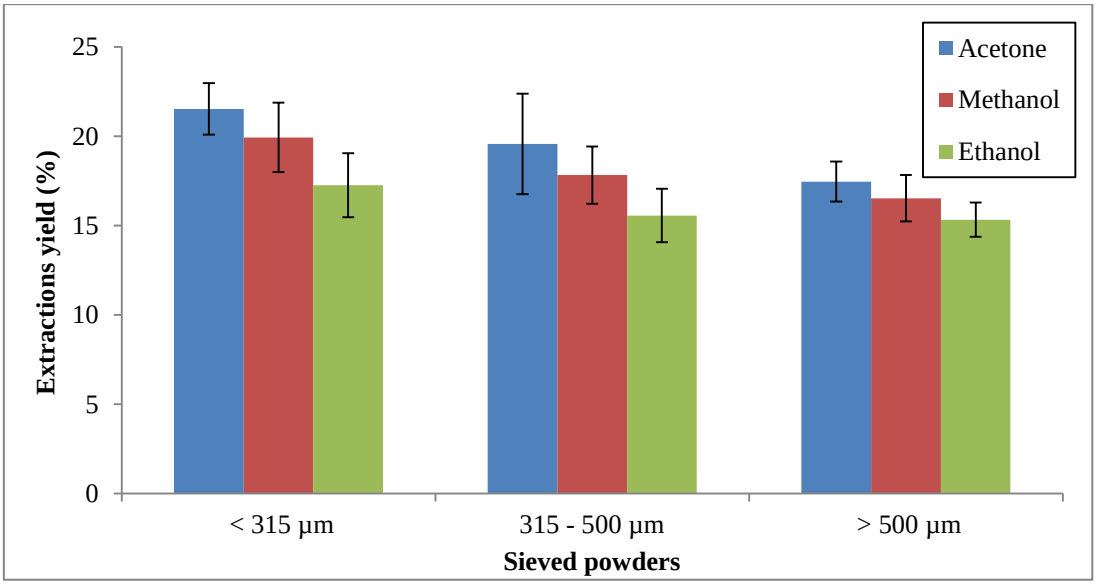

Table 4: Total polyphenols content of the different size classes of *Grewia coriacea* Mas

Sieved powders	Extraction solvents	TP content (mg eq GA/g DM)	Average
< 315 µm	Acetone	4.17 ± 2.71	3.63 ± 1.18
	Methanol	3.78 ± 1.79	
	Ethanol	3.59 ± 1.65	
315 - 500 µm	Acetone	3.82 ± 0.87	3.85 ± 2.05
	Methanol	3.63 ± 0.89	
	Ethanol	3.44± 1.79	
> 500 µm	Acetone	3.55 ± 1.66	3.28 ± 1.09
	Methanol	3.37 ± 0.32	
	Ethanol	2.92 ± 1.28	

TP:total polyphenols; GA:gallic acid; DM: dry matter

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Table 5: Total flavonoids content of different size classes of *Grewia coriacea* Mast powder.

Sieved powders	Extraction solvents	TP content (mg eq QU/g DM)	Average
< 315 µm	Acetone	8.54 ± 1.22	6.65 ± 1.70
	Methanol	6.81 ± 2.23	
	Ethanol	4.61 ± 1.67	
315 - 500 µm	Acetone	7.44 ± 2.44	6.78 ± 2.34
	Methanol	6.48 ± 2.33	
	Ethanol	5.42 ± 2.24	
> 500 µm	Acetone	4.98 ± 1.87	4.14 ± 1.07
	Methanol	3.87 ± 0.59	
	Ethanol	3.56 ± 0.77	

TF: total flavonoids; QU:quercetin; DM: dry matter.

Table 6: Contents in condensed tannins of the different size classes of *Grewia coriacea* Mast powder.

Sieved powders	Extraction solvents	TC content (mg eq CT/g DM)	Average
< 315 µm	Acetone	9.24 ± 2.13	8.22 ± 0.83
	Methanol	8.75 ± 0.21	
	Ethanol	6.69 ± 0.13	
315 - 500 µm	Acetone	8.19 ± 0.42	7.19 ± 1.45
	Methanol	8.63 ± 1.32	
	Ethanol	4.75 ± 2.63	
> 500 µm	Acetone	5.75 ± 0.83	5.44 ± 0.87
	Methanol	6.45 ± 0.46	
	Ethanol	4.12 ± 1.32	

TC: condensed tannins; CT: catechol; DM: dry matter.

Table 7: Contents in anthocyanins of the different size classes of *Grewia coriacea* Mast powder.

Sieved powders	Extraction solvents	TA contents (mg/g DM)	Average
< 315 µm	Acetone	62.67 ± 0.13	60.41 ± 0.62
	Methanol	59.66 ± 1.05	
	Ethanol	58.91 ± 0.64	
315 - 500 µm	Acetone	57.23 ± 0.77	54.89 ± 0.70
	Methanol	55.33 ± 0.16	
	Ethanol	52.12 ± 1.19	
> 500 µm	Acetone	48.05 ± 1.07	44.57 ± 1.04
	Methanol	47.25 ± 1.17	
	Ethanol	38.42 ± 0.88	

TA: total anthocyanins.