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RESEARCH ARTICLE

NUTRITIONAL COMPOSITION, CONSTITUENTS, AND ANTIOXIDANT ACTIVITY OF POWDER FRACTIONS OF *FICUS DICRANOSTYLA* MILDBREAD LEAVES

Tabi Omgba Yves[1], Tsague Marthe Valentine[2]*, Deli Markusse[3], Tembe Achick Estella[1], Ngono Mballa Rose[4], Fokunang N. Charles[1], Ngadjui Tchaleu Bonaventure[5], Dimo Théophile[6], Ndongo Embola Judith[7], ZeMinkande Jacqueline[8]

[1]*Department of Pharmacotoxicology and Pharmacokinetics, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaounde, Cameroon.*
[2]*Department of Biomedical Sciences, Faculty of Sciences, University of Ngaoundere, Ngaoundere, Cameroon.*
[3]*Department of Biological Sciences, Faculty of Sciences, University of Maroua, Maroua, Cameroon.*
[4]*Department of Pharmacology and Traditional Medicine, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I.*
[5]*Department of Chemistry, Faculty of Sciences, University of Yaounde I, Yaounde, Cameroon.*
[6]*Department of Physiology, Faculty of Sciences, University of Yaounde I, Yaounde, Cameroon.*
[7]*Department of Physiological Sciences/Biochemistry, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I.*
[8]*Department of surgery and specialties,Faculty of Medicine and Biomedical Sciences, University of Yaoundé I.*

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***Address for Correspondence:**
Tsague Marthe Valentine, Department of Biomedical Sciences, Faculty of Sciences, University of Ngaoundere, Ngaoundere, Cameroon. Tel- (+237)670518061.
E-mail: *tsague.marthe@yahoo.fr*

Abstract

Background and objective: The research on nutritional and health-benefiting compounds has increased in leafy vegetables such as *Ficus dicranostyla*.The current study was conducted to evaluate the effect of sieved-based fractionation on nutrient content, polyphenolic components, and antioxidant activities of *Ficus dicranostyla* leaves powder**.**
Methods: Dried leaves powder was sieved at three size classes (<125 µm, between 250 and 125 µm, ≥ 250 µm) and unsieved powder was produced and characterized for proximate composition, minerals, total phenolic, and flavonoids contents, as well as antioxidant activity. Nutrient quantities were expressed in g/100g of crude leaves.
Results: Statistics resultsrevealed a significant difference among fractions composition, as well as antioxidant activity with DPPH and FRAP. Proteins (19.60-26.08), lipids (5.33-10.40), and ash (11.44 16.60) contents were increased in small size fractions, while carbohydrates (40.19-50.88) and fibers (9.08–15.04) contents were lowered. Similarly, minerals namely iron, copper, magnesium, and zinc increased (3.26-9.68, 9.09-24.41, 1.38-2.33, and 3.79-8.55 g/100 g, respectively). In addition, phenolic compounds and flavonoids, as well as antioxidant activity were also higher in smaller powder particles. The powder fraction of < 125 µm (smaller particle) showed the highest phenolic content and antioxidant activity, demonstrating the importance of sizing in the profitability of functional food ingredients.
Conclusion: Data analysis clearly demonstrates that *Ficus dicranostyla* leaf powder could be recognized as a source of nutritive compounds and antioxidants. *Ficus dicranostyla* derived functional ingredients could be prepared for the nutritive purpose and oxidative stress-related disease prevention.
Keywords: Antioxidant, *Ficus dicranostyla*, mineral compositions.

INTRODUCTION

Wild edible plants are consumed across the world and often traded in urban markets, particularly in African countries. In Cameroon, they serve as sources of food, herbal tea and have the therapeutic virtues[1-4].The leaves of *Ficus dicranostyla* contain phenolics as well as nutrients such as carbohydrates, lipids, proteins, and minerals[3,5,6]. Some research work has shown that there are species that have therapeutic virtues, due to the presence of biologically active compounds, and can thus be presented as functional foods or food medicines[4,7]. Of capital functional importance, antioxidant activity has always been the basis of many

bioactivities in humans. To this end, the majorities of phytochemicals have very good antioxidant activity against free radicals and present themselves as potential active ingredients for the healing of patients, as well as the treatment of diseases that induce oxidative stress, thus improving the state. nutrition of the population[8,9].

F. dicranostyla (*Moraceae* family) is a wild edible plant found in savannas and Guinean forests, on rocky hills, and laterite slabs[10]. This plant grows from Cameroon to Senegal and is strongly present from Uganda to Zambia[11,12]. An edible plant, its flowers, leaves, bark, and stems are part of the various traditional meals of the Far North region of Cameroon[13]. Similar to other *Ficus* species, its leaves could be considered a source of nutrients and phytochemicals[14]. Some previous studies have shown that solvent extracts of *F. dicranostyla* possessed antioxidant activities[1]. These leaves could therefore fit the description of an ideal complementary food matrix and a significant challenge that can increase consumption is keeping its nutritional value through its supply chains and/or processing into functional food.

Successive grinding followed by sieving of the fractionating powder as a new tool or new approach to improve plant nutrients, polyphenols, and antioxidants of plant matrices hasreceived much attention[15]. Indeed, particle size affects extraction yield and thus health-promoting compounds. Fine particle fractionation has been associated with the enrichment of nutrients in several plant parts, including fruits, leaves, roots, and barks[16-20], where it has been found that molecules are distributed according to the size of the particle. Therefore, this study aimed to assess the influence of particle size fractionation by sieving on proximate, minerals, and phenolic phytochemical compounds as well as the antioxidant activity of *F. dicranostyla* powders.

MATERIALS AND METHODS

Plant material

The Plant material used in the present study consisted of the leaves *F. dicranostyla* Milabread (Collector: R. Letouzey number 6951; Herbarium number 8618 SRF/ Cam). The leaves were harvested in July 2021 in the locality of Mokolo, located in Maroua, the capital of the Far North Region of Cameroon. The fresh mature leaves were harvested in the morning with care. Once in the laboratory, these leaves were thrilled and washed with tap water to remove dust and sticky dirt. Clean leaves drained and air-dried in a ventilated oven at 40°C for 24 hours. The dried leaves were then grounded to obtain a fine powder adapted to the different meshes of the sieve.

Plant grinding

The electric BIOBASE Disintegrator grinder (Model MPD-102, No: 61 South Gongye Road Jinan City, China; Serial Nº: 20020020) supplied with a sieve drilled with 1 mm trapezoidal holes were used for grinding dried *F. dicranostyla* leaves. Grinding was operated at 1400 r/min for 1 minute in ambient air.

Powder sieving

The grounded leaves of *F. dicranostyla* were fractionated with the meshes of an electric laboratory sieve (MINOR.) according to their particle size. The sieving process is based on the separation of particles of granular material through several sieves of decreasing mesh size. In practice, the *F. dicranostyla* leaf powder was divided into two batches: the first batch consisted of unscreened powder which had served as a control. While the second batch was sieved and obtained different particle sizes (Figure 1). Two selected sieves of different meshes (125 and 250 µm) were installed on the shaken MINOR sieve operating by vertical vibration at 0.5 mm vibration amplitude. For each batch, 50 g of mother powder was poured onto the upper sieve, and the sieving was unrolled for 15 minutes. After that, the amount of powder retained on each sieve was collected and then weighed. This allowed us to produce three powder fractions of different particle size classes: less than 125 µm (called<125 µm), between 125 and 250 µm (denoted 125-250 µm), and greater than 250 µm (called ≥ 250 µm) and here they have been called large (L), medium (M) and small (S). The powder fractions obtained were then packaged in polyethylene bottles and stored at 4°C for future research work. Powder recovery or fraction mass (%) was calculated and defined as the ratio of the mass of powder dry matter collected on each sieve to the weight of the total sieved powder. Fraction mass was calculated according to the following formula:

$$\text{Fraction mass (\%)} = \frac{\text{Weight of each powder fraction}}{\text{weight of total sieved powder}} \times 100$$

Proximate analysis of plant powders

The content of moisture, carbohydrates, proteins, fiber, fat, and ash of vegetable fractions was determined. The determination of moisture content was carried out following the official AOAC[21] method. Indeed, leaf powder (5 g) was oven-dried at 103±2 °C for 24 h, then transferred to a desiccator and finally allowed to cool to room temperature. The ash content was assessed by incinerating 5 g of leaf powder in a muffle furnace at 550°C for about half a day or until a constant mass of greyish-white ash was obtained[21]. Total protein content was determined after mineralization by the Kjeldahl method[22], and colorimetric determination was described by Devani *et al.*,[23,], with a nitrogen conversion factor of 6.25. Crude fat content was determined in 5 g of powder sample by soxhlet extraction method using hexane as extraction (8 h) solvent[24]. Carbohydrate content was determined according to Dubois *et al.*,[25] methods using sulfuric acid. The experiment was done in three replicates.

Determination of some minerals

The mineral composition had been determined on ashes, obtained by incineration of the powders in a muffle furnace at 550°C[26]. All these minerals were extracted after dissolving ash (1 g) in 10 mL of hydrochloric acid (1.5 N) and then the mixture was heated on a hotplate until completely dry. Then a few drops of H_2O_2 and 5ml of deionized water were added and made up to 25ml in a calibrated flask. The solutions obtained had been used for the determination in triplicate of copper (Cu), iron (Fe), zinc (Zn), and

magnesium (Mg) by Atomic Absorption Spectrophotometry (AAS) (Hitachi, Tokyo, Japan).

Determination of phenolic bioactive compounds

Extraction of phenolic compounds from powder samples

The particle size extraction of phenolic compounds had already been carried out by the Deli *et al.*,[16] protocol, in which the maceration of dried plant powder with a solvent extract had been chosen as the extraction methodology in order to minimize the destruction of the active ingredients of the matrix. A mass of 2 g of the parent or sieved powder had been dissolved in 20 mL of the methanol/water (70/30, v/v) solvent mixture. The mixture had been stirred at 300 rpm using a magnetic stirrer (Pierron MT 15140) for 24 hours at room temperature, and then this solution had been filtered through Whatman N°1 filter paper to eliminate insoluble residues. The filtrate was then made up to 15 mL by adding extraction solvent and stored at 4°C for further work.

Determination of total phenolic content (TPC)

To determine total phenolic content (TPC) was carried out as previously described[27] using a Folin-Ciocalteu reagent (diluted up to 10 times). Initially, 20 µL of hydromethanolic extract of the samples had been mixed with 2,980 µL of distilled water. Then, 500 µL of freshly prepared 10% (v/v) Folin-Ciocalteu reagent and 400 µL of a saturated solution of sodium carbonate Na_2CO_3 (20%, w/v) were added. The resulting mixture had been well homogenized and allowed to equilibrate for 30 min at room temperature in the dark. All readings were recorded in triplicate at 760 nm in a UV-Vis spectrophotometer. A calibration curve (R2=0.98) had been produced from standard solutions of gallic acid (40 to 280 g/L, R2=0.99). The total content of phenolic compounds was expressed in milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Determination of flavonoid content (FLC)

The flavonoid content had been determined by spectrophotometry according to the method of Dewanto *et al.*,[28]. To 0.1 ml of each extract added 2.4 ml of distilled water followed by 0.15 ml of 5% (w/v) sodium nitrite (Na_2NO_2) was added. After 6 minutes, a volume of 0.3 ml of 10% aluminum chloride ($AlCl_3 \cdot 6H_2O$) (w/v) was added. The whole mixture was kept at room temperature for 5 minutes and 1 ml volume of 4% (w/v) sodium hydroxide (NaOH, 1 M) was added at the end. The absorbance of the solution was measured at 510 nm using UV/visible spectrophotometry against the extraction solvent used as a blank. The results were expressed in milligrams of rutin equivalents per gram of dry weight (mg RE/g DW), based on a calibration curve derived by linear regression, established from different concentrations (20 to 140 g/L; R2=0.99) of rutin as standards.

Determination of *in vitro* antioxidant activities

DPPH radical scavenging assay

Antioxidant activity was first evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. The role of the DPPH was to measure the hydrogen atom or the electron donation capacity of the extracts[29]. A volume of 0.5 mL of hydromethanolic extract of plant powder or ascorbic acid (used as reference) at different concentrations (0.025, 0.05, 0.1, 0.5, 1, and 5, 10, 100 mg/mL) was reacted with a volume of 2 mL of a 0.1 mM methanolic DPPH solution in the dark for 1 hour at room temperature and the absorbance (Abs) was measured at 517 nm using UV/visible spectrophotometry. The radical scavenging activity was estimated as a percentage of DPPH discoloration using below equation:

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Control Abs} - \text{Sample Abs}}{\text{Control Abs}} \; x\,100$$

IC_{50} value, which is the inhibitory concentration of the crude extract that could scavenge 50% DPPH radical or inhibit oxidation by 50% was determined from a plot of inhibition percentage against extract concentration. The IC_{50} value is inversely related to the activity and a lower IC_{50} value means higher antioxidant activity. Ascorbic acid standard showed the IC_{50} value of 15.69 ±1.96 µg/mL.

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) was also chosen for specification Fe^{3+} reducing to Fe^{2+} capability of analyzed powder extracts[30]. Indeed 1 mL of the extract from each leaf powder sample had been mixed in 2.5 mL of phosphate buffer (200 mM, pH 6.6) and in a volume of 2.5 mL of potassium ferricyanide solution [$K_3Fe(CN)_6$] (1%, w/v). The mixture had been incubated in a water bath at 50°C for 30 minutes, then left to cool, after adding a volume of 2.5 ml of trichloroacetic acid solution (10%, w/v) and finally centrifuged at 3000 rpm for 10 minutes. The supernatant (2.5 ml) had been removed and mixed with a volume of 2.5 ml of distilled water and a volume of 0.5 ml of $FeCl_3$ (1%, w/v), left to react for another 10 minutes at room temperature, and absorbance values were measured at 700 nm. A calibration curve was drawn from the straight line obtained with the ascorbic acid solution used as a reference at different concentrations. Ferric reducing antioxidant power is determined by referring to this calibration curve of ascorbic acid and expressed in terms of mg ascorbic acid equivalent per g of dry weight (mg AAE/g DW) of a sample. The formation of an intense blue color indicates a stronger antioxidant capacity.

Statistical analysis

Data were presented as mean±standard deviation with three replications per sample. A one-way ANOVA test with one factor (particle size) was used to determine the occurrence of statistically significant differences. The level of statistical significance was set at $p<0.05$. Duncan's multiple range tests was used to determine the degree of significance of the difference between the two means. Stat graphics centurion version 16.1 was used for this purpose. Sigma plot 11.0 was used to plot graphs. Pearson correlation coefficients were used to examine correlations between total phenolic and mineral content, and antioxidant activity. Principal Component Analysis (PCA) was conducted to analyze the degree of correlation between chemical composition and antioxidant properties of powders.

RESULTS AND DISCUSSION

Powder recovery

From the statistical analysis, we observed that the small fraction <125 µm was more abundant in the raw powder of *F. dicranostyla* (43.89%), followed by the Medium (32.46%) and the Large portion (23.65%). The different-mesh sieving process provided sufficient quantities of all considered particle size classes since the lower mass fraction retained on the different-mesh sieves was always above 23%. Thus, the sieving allowed us to separate the leaf particles of *F. dicranostyla* into particle size classes composed of particles of very different sizes. Noumi *et al.*,[20] reported similar observations on *Eucalyptus* leaves powder which passes freely through the sieved openings. Also, the sieving procedure was reported efficient for *H. sabdariffa* and *D. glomerata* powders, as obtained granulometric had well different in size[17].

Table 1: Changes in contents of proximate composition and some minerals of *F. dicranostyla* leave powders of different sizes.

Constituents (g/100 g DW)	Powder fractions			Unsieved powder
	< 125 µm	125 – 250 µm	≥ 250 µm	
Moisture	5.73±0.30^{b}	5.33±0.29^{b}	4.07 ±0.30^{a}	4.07±0.40^{a}
Total proteins	26.08±1.59^{c}	24.49±1.72^{c}	19.60±0.66^{a}	21.93±1.22^{b}
Total lipids	10.40±0.40^{d}	8.20±0.20^{c}	5.33±0.23^{a}	6.33±0.50^{b}
Carbohydrates	38.04±1.31^{a}	40.19±1.76^{b}	50.88±1.91^{d}	44.97±1.12^{c}
Total fibers	9.08±0.51^{a}	10.71±0.63^{b}	15.04±0.51^{d}	12.80±0.48^{c}
Total ash	16.60±1.20^{d}	12.02±1.00^{ab}	11. 3.26±0.24^{a}	13.60±0.9^{bc}
Iron	9.68±0.66^{c}	9.79±0.57^{c}	44±0.70^{a}	5.58±0.10^{b}
Copper	24.41±2.01^{d}	15.98±1.10^{c}	9.09±0.08^{a}	15.10±0.14^{b}
Magnesium	2.33±0.01^{c}	0.16±0.01^{a}	1.38±0.01^{b}	1.50±0.14^{b}
Zinc	8.55±0.08^{d}	6.23±0.08^{c}	3.79±0.06^{a}	4.69±0.10^{b}

Values are expressed as mean±standard deviation of three independent determinations. Values in the same row followed by the same letter are not significantly different (p< 0.05) according to Duncan's multiple range test (n=3).

Table 2: Percentage DPPH scavenging activity (%) and IC_{50} (mg/mL) values of *F. dicranostyla* powder extracts.

Concentrations (mg/mL)	Powder fractions			Unsieved powder
	< 125 µm	125 – 250 µm	≥ 250 µm	
0.25	16.91±2.18^{d}	6.46±0.54^{b}	2.42±0.80^{a}	10.65±2.78^{c}
0.50	25.53±1.40^{d}	18.75±0.55^{b}	13.28±4.83^{a}	22.39±1.89^{c}
1.00	39.17±1.63^{c}	35.31±2.56^{b}	27.05±1.36^{a}	38.90±2.91^{bc}
1.50	63.98±3.25^{b}	59.01±2.35^{b}	51.01±3.56^{a}	56.02±3.58^{ab}
2.00	74.74±1.56^{c}	72.63±4.14^{b}	63.53±1.17^{a}	71.78±0.74^{b}
IC_{50} (mg/mL)	1.15±0.05^{a}	1.40±0.04^{b}	1.65±0.05^{d}	1.45±0.05^{c}

Values are given in mean±standard deviation of three independent determinations (n = 3). Values in the same row followed by the same letter are not significantly different at p<0.05, according to Duncan's multiple range tests.

Proximate value

The moisture, lipid, proteins, ash, carbohydrates, and fiber contents of *F. dicranostyla* leaves powder were: 4.07, 6.33, 21.93, 13.60, 44.97, and 12.80 g/100 g of dried weight (DW), respectively (Table 1).The low level of moisture of *F. dicranostyla* leaves powder (generally lower than 10%) was highly predictable, as a good storage ability of analyzed powders[31]. This nutritional value of *F. dicranostyla* leaves powder was higher than those reported for three consumed leafy vegetables of the North-West Region of Cameroon, namely *Xymalos monospora*, *Mentha longifolia*, and *Amaranthus sp* (12.23; 10.34 and 7.8 g/100 g DW, respectively), lipids (1.032; 14.67 and 1.44 g/100 g DW, respectively) and carbohydrates (17.73; 27.05 and 20.13 g/100 g DW, respectively)[3]. Interestingly, higher ash content suggests that *F. dicranostyla* leaf powder could be a source of minerals. If 100 g of *F. dicranostyla* leaves powder provides 21.93 g/100 g DW of proteins, this indicates that vegetable leaves could be rich sources of protein. The Recommended Dietary Allowance (RDA) of protein for children, adult males, adult females, pregnant women, and lactating mothers are 28; 63; 50; 60, and 65 g, respectively. On the other hand, its fiber contents are important in improving the digestive system, controlling blood glucose levels in diabetes and cholesterol levels in cardiovascular diseases, preventing constipation as well as reducing the risk of colon cancer[32-34]. It can be also noted that fractionation of *F. dicranostyla* leaves powder according to the particle size resulted in significant (p<0.05) differences in the powder composition (Table 1). The moisture (4.07 to 5.73 g/100 g), lipid (5.33 to 10.40 g/100 g), protein (19.60 to 26.08 g/100 g), and ash (11.44 to 16.60 g/100 g) contents expressed in dried basis, indicated an increase with reducing powder particle size, contrary to carbohydrate and fiber contents which were more concentrated the larger fractions. Therefore, the larger powder fraction was enriched in fiber and carbohydrate contents, whereas the smallest powder fraction was enriched in lipid, proteins, ash, and moisture contents. Results of the present study were consistent with the fact that the smaller particle is richer in ash, lipid, and proteins, because fibrous plant parts are harder to grind, resulting in larger particles[35-37]. On other hand, the results of moisture may be explained by the high surface of small particles, facilitating the absorption of

surrounding air humidity. Similar observations were previously made for *Dichrostachys glomerata*, *Boscia senegalensis*, and *Hibiscus sabdariffa* powders[16] and *on Eucalyptus grandis* powders[38]. According to these reports, powder fraction with smaller particle size was found to possess higher ash content and smaller fiber content (hemicellulose and cellulose).

Mineral analysis

A total of four mineral elements (iron, copper, magnesium, and zinc) were determined in powder fractions and unsieved powder from *F. dicranostyla* leaves, as shown in Table 1.

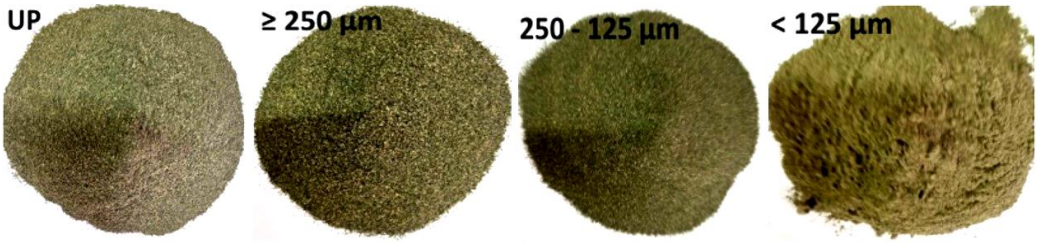

Figure 1: Unsieved powder (UP) from dried leaves of *F. dicranostyla* and its corresponding powder fractions (< 125, 125 – 250, and ≥ 250 µm) with different particle sizes.

From the results, there was a demarcation in the amount of copper (15.10±0.14 mg/100 g DW) followed by iron and zinc with 5.58±0.10 mg/100 g DW and 4.69±0.10mg/100gDW, respectively; while magnesium content (1.50±0.14 mg/100 g DW) was the lowest. The mineral contents of *F. dicranostyla* leave powder were higher than those of some leafy vegetables of the South-West Region of Cameroon; *Amaranthus dubius* (0.06; 0.069 and 0.26mg/100g), *Gnetum africanum* (0.75; 0.02 and 0.71 mg/100 g) and *Vernonia amygdalina* (0.17; 0.05 and 0.80 mg/100 g) for copper, iron, and zinc respectively[39]. In addition, results showed that the four mineral contents varied significantly (p<0.05) depending on the powder particle sizes. The mineral values for different particle sizes increased from 3.26 to 9.68 g/100 g; 9.09 to 24.41 g/100 g; 1.38 to 2.33g/100 g and 3.79 to 8.55 g/100 g DW, respectively, for iron, copper, magnesium, and zinc. Thus, the mineral contents increased inversely to the particle sizes of *F. dicranostyla* fractions.

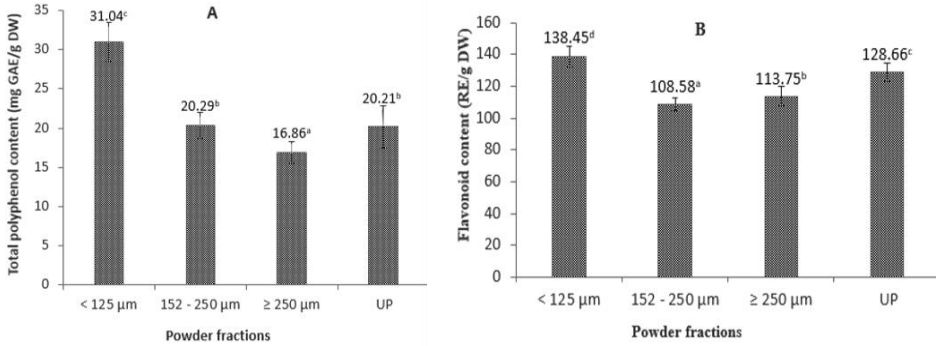

Figure 2: Total phenolic contents (A) and flavonoid contents (B) in *F. dicranostyla* leave powders with different particle sizes.

GAE: gallic acid equivalents, **RE:** rutin equivalents, **UP:** unsieved powder. Bars with different superscripted letters differ significantly (p< 0.05) according to Duncan's multiple range test (n=3).

Overall, the Small fraction showed the highest mineral contents, while the large fraction showed the lowest. Similar results in terms of the effect of particle size on mineral contents were observed by Deli *et al.*,[18,19] for *Hibiscus sabdariffa* calyx and *Dichrostachys glomerata*fruit powders. Obtained results in this study also support the earlier reported hypothesis in several research studies[35,38], which suggested that the finest powders from grounded and sieved vegetable matrices would be more concentrated in minerals than large particle powders. These authors reported the highest ash contents in the smallest particle sizes, as the higher ash contents suppose the higher mineral contents of the food matrix. Minerals contained in this leafy vegetable could be of great interest for health such as antioxidant capacity.

Total phenolic and flavonoid contents

Total phenolic content (TPC) and flavonoid content (FLC) in *F. dicranostyla* Figures with different particle sizes are shown in Figures 2 A and B. It was found that TPC and FLC were significantly affected by the particle size (p<0.05). With the decrease in particle size processed by plant grinding and sieve fractionation, the highest TPC and FLC (31.04 mg GAE/g DW and 138.45 RE/g DW, respectively) were observed in a small fraction (< 125 µm particle size), while particle size of ≥ 250 µm exhibited the lowest TPC and FLC (16.86 mg GAE/g DW and 108.58 RE/g DW, respectively). Meanwhile, the TPC and FLC of powder fraction of Medium fraction (125 – 250 µm) and that of unsieved powder (UP) were: 20.29 mg AGE/g and 113.75 RE/g DW; 20.21 mg AGE/g and 128.66 mg RE/g DW, respectively. Obtained results permitted to

sort of the *F. dicranostyla* powder samples by descending order according to their TPC, as well as in FLC: Smaller particle sizes > medium > unsieved powder > larger particle. Yao *et al.*,[1] reported the total phenolic content of 1.78 mg GAE/g from an ethanolic extract from *F. dicranostyla* leaves. Interestingly, the total phenolic content of *F. dicranostyla* leaves in this study was higher than those of previous studies on aqueous and methanolic extracts of *Ficus carica* leaves with TPC ranging from 4.72 to 6.90 mg GAE/g WD[40]. Also, the *F. dicranostyla* leaves powders to have the highest TPC compared to other wild edible leafy vegetables, including *Xymalos monospora*, *Mentha longifolia*, and *Amaranthus sp* leaves with 18.1; 24.2, and 16.7 mg/g GAE/g, respectively[3]. Results are consistent with other studies in terms of the effect on particle size[16,35,41]. The high amount of total polyphenol and flavonoid compounds are indicative of stronger antioxidant capacity. Indeed, phenolic compounds such as flavonoids have been reported to possess antioxidant properties. Therefore, a matrix rich in phenolic and flavonoids has many essential roles in decreasing the risk of various human diseases[42].

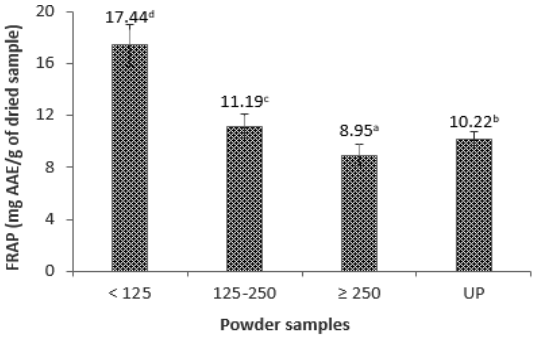

Figure 3: Ferric reducing antioxidant power (FRAP) of the powder fractions and unsieved powder from *F. dicranostyla* leaves.

AAE: ascorbic acid equivalents, **UP:** unsieved powder. Bars with different superscripted letters differ significantly ($p< 0.05$) according to Duncan's multiple range test (n=3).

Antioxidant activity

DPPH radical scavenging activity

Results of the scavenging activity of *F. dicranostyla* leave powders with different particle sizes on DPPH radicals are shown in Table 2. Powder fractions extracts and the unsieved powder showed noticeable free radical scavenging activities in a concentration-dependent manner. The scavenging activity increased as the concentration increased for each individual powder extract. Similar to this observation, previous research studies also reported that plant extracts are capable of trapping the DPPH free radical in a dose-dependent manner[43,44]. Particularly, the IC_{50} value, which is the inhibitory concentration of the crude extract that could scavenge 50% DPPH radical or inhibit oxidation by 50% was determined from a plot of inhibition percentage against extract concentration. The IC_{50} value is inversely related to the activity and a lower IC_{50} value means higher antioxidant activity. Obtained IC_{50} values varied significantly ($p<0.05$) depending on the powder particle sizes. The smaller powder with a particle size of < 125 µm showed the highest DPPH scavenging activity with an IC_{50} of 1.15 mg/mL followed by 125-250 µm and unsieved powder with IC_{50} of 1.15 and 1.45 mg/mL, whereas powder fraction of ≥ 250 µm had the lowest DPPH free radical scavenging activities (IC_{50} of 1.65 mg/mL). Compared to ascorbic acid (IC_{50} value of 0.015 mg/mL) used in this study as a reference antioxidant, powder fractions of *F. dicranostyla* were 7-11 times less active against DPPH free radicals.

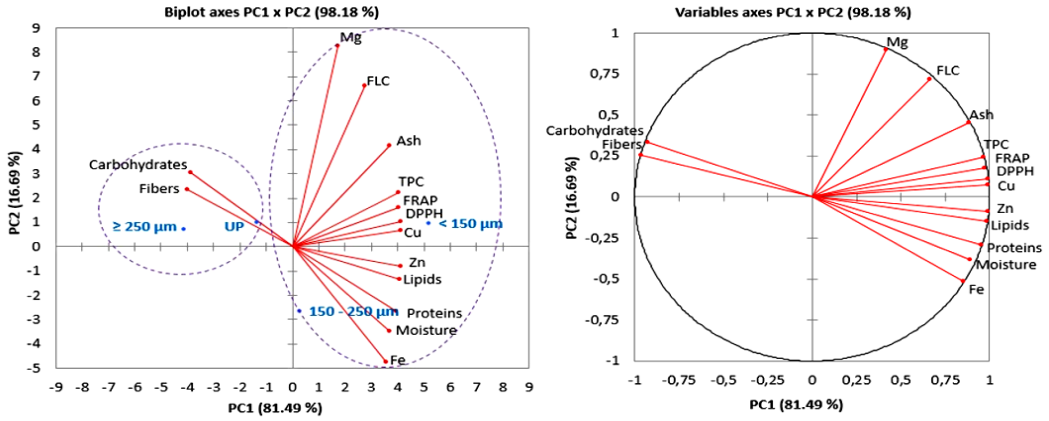

Figure 4: Representation of the variables of *F. dicranostyla* powder samples on the principal components PC1 and PC2.

PC: principal components, **UP:** unsieved powder, **TPC:** total phenolic content, **FLC:** flavonoid content

These results correlate well with the obtained above results on total polyphenol content and flavonoids. Antiradical activity depends on the content of phenolic compounds that behave like antioxidants, due to the reactivity of phenols[45]. This suggests a contribution of these phenolic compounds to the antioxidant activity of *F. dicranostyla* powders.

Ferric reducing antioxidant power

FRAP is another assay used to determine the antioxidant property of *F. dicranostyla* powder samples (Figure 3). Generally, FRAP values indicate all electron–donating reductants in the sample extracts. FRAP values of analyzed powder extracts varied from 8.95 (powder fraction of ≥250 µm) to 17.44 mg AAE/g of the dried sample (powder fraction of <125 µm), showing the highest in <125 µm powder, while powder fraction of ≥250 µm revealed the lowest activity. Similar to DPPH, the high activity of the powder extracts may be ascribed to their phenolic antioxidant compounds such as TPC and FLC. These phytochemicals contribute significantly to the antioxidant properties of plant extract, which could react with free radicals to stabilize and terminate radical chain reactions by donating an electron. The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators[46,47].

Principal components analysis

Principal components analysis (PAC) was performed in order to compare all powder fractions and unsieved powder on the basis of their proximate and phytochemical constituents, and antioxidant activity. Thus, analyzed parameters were organized into principal components using axes PC1 and PC2, which expressed 98.49% variation among *F. dicranostyla* powder samples. The first ax (PC1) explains 81.50% of total variability, while PC2 explained only 16.69 variations. The representation of powder samples on the PC1×PC2 plot denoted a separation of powder samples according to the particle size effect (Figure 4). Globally, it was observed that variables were organized into two main groups: powder fractions of <125 µm (smaller) and that of 125-250 µm (medium) were mostly positioned on the right side of PC1, while powder fractions of 250 µm ≥ (larger) and unsieved powder (control sample), on the left side of the PC1. In general, total phenolic, flavonoid, minerals elements (zinc, copper, magnesium, and iron), lipids, and proteins contents were positively correlated with each other and higher in the smallest powders of <125 µm and 125-250 µm when compared to powder with large particle size (≥250 µm), richer in carbohydrates and fibers contents. The powder fraction of ≥250 µm (larger particle powders) and unsieved powder were lesser rich in minerals and phenols and also lower FRAP and DPPH radical scavenging. Indeed, IC_{50} radical scavenging was negatively correlated with DPPH radical scavenging. It was observed in Figure 5 that the total phenolic and flavonoids among other constituents contributed to increasing in FRAP and DPPH radical scavenging. Highly significant correlations ($r > 0.99$; $p<0.001$) were revealed between total phenolic contents and FRAP, total phenolic contents and DPPH radical scavenging ($r > -0.95$; $p< 0.001$). A stronger correlation significantly between FRAP and DPPH radical scavenging assays ($r > -0.94$; $p<0.001$) can be attributed to the fact that both methods are based on similar reactions mechanism. Moreover, significant correlations were found between flavonoids and FRAP ($r > 0.73$; $p<0.01$) and between flavonoids and DPPH radical scavenging ($r > -0.69$; $p<0.01$). Additionally, a positive correlation was seen between total phenolic and flavonoid contents ($r > 0.79$; $p<0.01$) with was in agreement with the studies reported by Nguimbou *et al.*,[48]. Several studies showed that the antioxidant capacity of plant material is very well-correlated with total phenolic compounds and the contribution of phenolic compounds to the overall antioxidant activity is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators[46,47]. Similar correlations were reported on other plant extracts[16,48].

CONCLUSIONS

This study demonstrated the importance of edible *F. dicranostyla* leaves powder, as a valuable natural resource rich in macronutrients, some minerals, and phenolic bioactive compounds associated with antioxidant activities. The study revealed that *F. dicranostyla*is rich in protein, lipids, ash, minerals, flavonoids, and phenolic compounds. The extracts also reveal marked antioxidant activities. Compounds were distributed according to the powder particle size. In general, total phenolic, flavonoids, minerals, lipids, and protein contents, and the highest antioxidant activity were more concentrated in small particle fractions (< 125 µm), contrary to carbohydrates and fibers contents that were more found in large size plant fractions (≥ 250 µm). The relationship between the particle size distribution and antioxidants analyses demonstrated that sizing in functional food ingredients is important. *F. dicranostyla* could be a natural source of nutrients and a candidate for the development of alternative solutions to treat oxidative stress-related illnesses.

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AUTHOR'S CONTRIBUTION

Y Tabi Omgba: writing original draft, conceptualization. **MV Tsague:** methodology, formal

analysis, conceptualization. **M Deli:** data curation, investigation. **AE Tembe:** editing, data interpretation. **R Ngono Mballa:** investigation, conceptualization. **C Fokunang N:** data curation, investigation. **B Ngadjui Tchaleu:** critical review. **T Dimo:** methodology, formal analysis, conceptualization. **J Ndongo Embola:** investigation, conceptualization. **J Ze Minkande:** study design, supervision. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

No conflict of interest is associated with this work.

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