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Controlled Differential Sieving (CDS), phytochemical profiling, and antioxidant activity of *Senna italica* (Fabaceae) Leaves

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Abstract

Aims and scope: *Senna italica* is recognized for its nutritional value and therapeutic potential. Various parts of the plant exhibit pharmacological properties, including antioxidant, hypolipidemic, and antibacterial activities. The main objective of this work was to evaluate the effects of the Controlled Differential Sieving (CDS) process on the phytochemical composition and antioxidant properties of *S. italica* leaves powder fractions.

Methods: The harvested leaves are washed, dried, crushed, pulverized and sieved on a column of sieves with decreasing mesh sizes of 355, 250, 200 and 125 µm. The choice of sieve was based on the results of the particle size and their availability. The fractions retained on each sieve were cleaned, weighed and subjected to evaluation of the antioxidant property. *In vitro* tests were used to determine their metabolite content and their antioxidant activities.

Results: The results showed a significant increase ($p<0.05$) in total polyphenol content as particle size decreased, rising from 55.47 ± 2.30 mg GAE/g DM in the ≥355 µm fraction to 75.81 ± 1.51 mg GAE/g DM in the ≤125 µm fraction. The coarse fraction has the highest IC_{50} (1.15±0.16 µg/mL), while the finest has the lowest IC_{50} (0.95±0.05 µg/mL) synonymous with greater free radical trapping capacity. Regarding FRAP, The coarse powder has the lowest values (42.90±0.92 mg/g DM) synonymous with low reducing power while the finest fraction has the highest value of iron reducing power which is 59.91±1.90 mg/g DM.

Conclusion: *S. italica* leaf powders have been demonstrated to possess antioxidant properties, with these characteristics being amplified in fine powder fraction.

Keywords: *Senna italica* leaves powder; Controlled Differential Sieving; Particle size; phytochemical composition; Antioxidant properties

1. Introduction

In recent years, pharmacological and nutritional research has focused increasingly on plants due to their abundance of bioactive compounds with beneficial effects on health and nutrition, and their comparatively low toxicity levels [1]. A notable example is *Senna italica*, a shrub whose all parts (leaves, flowers, pods, seeds, stems and roots) are extensively utilized in traditional medicine. The specific posology of this plant is determined exclusively by traditional healers [2]. It is found in semi-arid and arid regions, as well as dry thickets and meadows. It is also present in the grassy savannahs of dry regions of tropical Africa, from sea level to 1850 m altitude [3]. It is present in Cameroon, in the savannah of the far north region [4]. The decoction of *S. italica* leaves is used there to treat stomach aches (laxative properties). Pharmacological analyses of its leaves have confirmed the presence of antioxidant [5], antihyperglycemic,

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hypolipidemic and anti-obesity properties [6], as well as anti-inflammatory, antipyretic and analgesic properties [7]. In addition, it has been shown to have purgative [8], stimulant effects on the uterine contraction [9], and to act as an acaricide [10], antibacterial [11], anticancer [12], antifungal [13], insecticide [14] and anthelmintic [4]. In the process of using *S. italica* in treatments, various extraction methods of bioactive compounds use organic solvents like alcohol, ether, acetone, methane, hexane. However, these techniques have various limitations and the yield of the extracts is influenced by critical parameters such as temperature and the nature of the solvent [15]. These include the thermal destruction of thermolabiles compounds [16]. The negative interference of extraction solvent residues with bioactive molecules, with the environment and the health of consumers [17]. Faced with these problems, certain techniques such as microwave-assisted extraction, ultrasonic extraction and extraction with supercritical fluids have been developed as innovative extraction techniques. However, it should be noted that these techniques also involve the use of solvents, which can result in the loss of sensitive biomolecules [18]. The work of Prasad and colleagues revealed that extraction by pressurized fluids can cause a conformational change and denaturation of proteins [19]. To address these limitations, a new process Controlled Differential Sieving (CDS) was developed. This solvent-free method allows for the extraction of a wide range of bioactive compounds and shows considerable promise for producing functional powders for food and therapeutic applications. The process encompasses a series of steps, including drying, grinding, and sieving, which result in particles with variable physicochemical and functional characteristics [20]. This process leads to the production of solid particles ranging in size from 0.1 to 700 µm. The substance under consideration was derived from a dry plant matrix. The pharmacological and nutritional properties of powders have been demonstrated to be closely related to their particle size [21]. This technology, when applied to a range of plant substrates, has highlighted the concentration effect of bioactives and functionalities in the fine fractions of *Salix alba* powders [22], *Adansonia digitata* [23], and numerous others [24].

To the best of our knowledge, no study on *S. italica* has employed CDS as a means of extraction. The present study has been conducted for the purpose of evaluating the effect of Controlled Differential Sieving (CDS) on the phytochemical moleculs and antioxidant properties of the powder fractions of *S. italica* leaves.

2. Materials and method

2.1. Plant material

The plant material used in this study was the leaves of *Senna italica* (Fabaceae). The leaves were collected in November 2020 in the Dogba Maroua locality (Far North Region; Cameroon). The plant was identified at the Department of Biological Sciences of the University of Ngaoundéré, Laboratory of Biodiversity and Sustainable Development, and this identification was confirmed at the National Herbarium under No. 7845/SRF/Cam by comparison with the specimen collected by Bounougou.

Figure 1 *Senna italica* plant

2.2. Production of *Senna italica* leaves powder in laboratory

Initially, the leaves of *S. italica* were meticulously washed and dried in a shaded area for a duration of seven days. Subsequently, the leaves were pounded in a wooden mortar in order to reduce their size and thus facilitate their passage through the ultra-centrifugal electric crusher brand Biobasedisintegrator MPD-1102. This crusher is equipped with a 24-tooth rotor with a diameter of 99 mm and a trapezoidal mesh screen. The grinding was carried out in batches of 100 g of powder at 12,000 rpm for 1 mm in ambient air following the protocol described by Becker and his collaborators [26]. The powder was separated into three distinct parts. The first part was used for a particle size distribution of the *S. italica* leaves powder. The second part underwent sieving using an electric sieve, while the third part, designated as the unsieved powder (UP), served as the reference powder for subsequent analysis and the particle size distribution of the powder.

2.3. Particle size distribution of the powder of *Senna italica* leaves

The particle size distribution of the powder (2 g) unsieved of the leaves of *S. italica* was determined by laser diffraction using a branded particle size analyser (Mastersizer 3000, Malvern Instruments France, Orsay, France). The underlying principle of this method is based on the diffraction of a laser beam as it passes through the powder; the light diffracted by the particles is measured using a detector. The device utilises high-pressure air to disperse particles at ambient temperature. For each measurement, the obscuration level was set at 2 % in order to avoid multiple diffusion. This was achieved by adjusting the dispersion conditions to an air pressure of 1.5 bar. The experiment was conducted using a 30 % air pressure and 30 % flow rate of feed, in addition to a 2.5 mm hopper length for powder samples of *S. italica* leaves. The measurement of particle size was conducted in accordance with the principles of volume equivalent spherical diameters. The positional parameters that are characteristic of the particle size distribution D_{10}, D_{50} and D_{90} were measured, where D_X signifies that X % of the particles have a diameter smaller than D_X. The span is defined as the interim interval on the median diameter and is used to measure the spread of the particle size distribution of powder. The following equation is used to calculate the span:

$$Span=(D_{90} - D_{10})/D_{50}$$

2.4. Controlled Differential Sieving of *Senna italica* leaves powder

It was conducted in accordance with the protocol delineated by Baudelaire [20]. The fundamental principle of this method entails the passage of a mass of powder through a column of sieves, with the sieves exhibiting a decreasing mesh size, thereby resulting in the classification of powders according to their decreasing size [27]. Consequently, 100 g (the initial mass) of finely ground powder were deposited on the first sieve of a column comprising four sieves of decreasing sizes (355 µm, 250 µm, 200 µm and 125 µm). Subsequently, all components were positioned on the platform of an ENDECOTTS brand electric sieve (Minnor 1332-06). This apparatus induced a vertical vibratory movement in the column. Following a period of 10 minutes during which vibration was sustained, the apparatus was deactivated. Thereafter, the column was meticulously extracted, and the sieves were detached in a sequential manner. The powders retained (fractions) on each sieve and on the collecting basin (located at the bottom of the column) were collected and weighed in order to determine their mass yield. The initial mass of the powder introduced for sieving is denoted m1, and the mass of the powder remaining on the sieve or in the basin is denoted m2.

$$Masse\ yield\ (\%) = m2/m1\ X\ 100$$

2.5. Determination of the content of secondary metabolites in the powder fractions of *Senna italica* leaves

2.5.1. Total polyphenol

The total polyphenol content was determined by the Folin-Ciocalteu method [28], which is based on the reduction of the Folin-Ciocalteu reagent to blue-coloured tungsten and molybdenum oxide by polyphenols in the environmental kaline. The intensity of this blue colouration provides information on the total polyphenol content of the mixture. The chromatic response exhibited an absorption maximum at 760 nm, and its intensity was directly proportional to the quantity of polyphenols present in each sample. The extraction process involved the amalgamation of 0.25 g of each fraction in 25 mL of a water-methanol mixture (30:70 (v/v)), which was then subjected to shaking (300 rpm) at laboratory room temperature. The temperature should be maintained at 24 ± 2 °C for a period of 24 hours. The mixtures were then filtered using Wattman No. 4 filter paper. In order to achieve this objective, a series of six gallic acid solutions of varying and increasing concentrations (20, 40, 60, 80, 100 and 120 mg/L) were introduced into six tubes for calibration purposes. The preparation of a seventh tube was then undertaken, containing 50 µL of the aqueous extract. Subsequently, 400 µL of 35 % Na2CO3 and 200 µL of Folin-Ciocalteu reagent were added to the seven tubes, which were then agitated. The resulting mixture was then subjected to incubation in the dark at 40 °C for a period of 20 minutes.

Subsequent to this, the absorbance were measured at a wavelength of 760 nm using a UV/visible spectrophotometer (Biochrom Limited, Cambridge, CB, United Kingdom) with a white made from distilled water. A regression équation calibration curve y=2,18x+0,033 with R^2 = 0,97 was constructed using a range of aqueous solutions of gallic acid. It is imperative to note that all tests were repeated at least three times. The results were expressed as milligrams of gallic acid equivalent per gram of dry matter, referring to the calibration curve.

2.5.2. Flavonoids

The method of analysis employed was the colorimetric method [29], which is based on the formation of the flavonoid-aluminum complex in the presence of sodium nitrate and aluminum chloride. The complex exhibits a yellow colouration, with maximum absorption occurring at 510 nm. In order to achieve this, a mass of 0.5 g of plant powder was stirred for 1 hour and 30 minutes with 20 mL of the methanol-distilled water-acetic acid extraction solvent mixture (140:50:10 V/V). Subsequently, the mixture was subjected to a centrifugation process at 3500 rpm for a duration of 20 minutes. The resulting pellet was collected in a 50 mL beaker, and the volume was then adjusted with distilled water. Subsequently, 0.1 mL of the suitably diluted extract was added to 2.4 mL of distilled water and 0.15 mL of Na_2NO_2 (5 %). Following a period of six minutes at ambient temperature (24 ± 2 °C), 0.3 mL of freshly prepared $AlCl_3$, $6H_2O$ (10 %) was added to the solution. Following a five-minute period of rest, maintained at a temperature of 24 ± 2 °C, 1 mL of 1 M NaOH was added, and the optical density was measured at 510 nm on a blank containing the extraction solvent (methanol-water-distilled acetic acid). The results were expressed as milligrams of rutin equivalent per gram of dry matter (mg RE/g DM) with reference to the regression equation calibration curve y = 1,48x+0,21 with R^2 = 0,94.

2.5.3. Condensed tannins

The measurement of condensed tannins, otherwise known as proanthocyanidins, was conducted in the presence of concentrated sulfuric acid [30]. The procedure involved the depolymerisation of the tannins in an acidic methanolic medium, followed by a reaction with vanillin. This process led to the transformation of the tannins into anthocyanidins of a specific red colour, which could be measured by spectrophotometry at 500 nm. The procedure involved the introduction of 1 mL of polyphenolic extract and 0.1 g of PVPP (polyvinylpolyrolidone) into a tube containing 1 mL of distilled water. The mixture was stirred for a period of one hour, after which it was subjected to a centrifuge. The upper layer, constituting a tannic solution, was collected for further analysis. Subsequently, 0.05 µL of the suitably diluted extract was mixed with 3 mL of vanillin (4 %), followed by the addition of 1.5 mL of concentrated sulfuric acid. Subsequent to the process of homogenisation, the mixture was then subjected to incubation at ambient temperature for a period of 30 minutes. Absorbance was measured at a wavelength of 500 nm against a blank containing pure methanol. The condensed tannin content was determined with reference to a standard range of catechin (0 to 0.600 mg/mL). The tannin content was calculated and expressed as catechin equivalent per dry matter range with reference to the regression equation calibration curve y = 2,73x+0,02 with R^2 = 0,99.

2.5.4. Carotenoids

The carotenoid content was evaluated by means of the colourimetric method [31]. To this end, 2.5 g of powder were mixed with 25 mL of acetone. In order to limit the probable release of tissue acids contained in the sample or isomerization, 0.75 g of magnesium carbonate was added to the mixture. Following a 10-minute period of stirring, the mixture was subjected to centrifugation (603 g for 10 minutes at 1°C), after which the upper layer of the mixture was collected. This operation was repeated on three consecutive occasions until a colorless residue was obtained. The filter was transferred to a separatory funnel containing petroleum ether, and approximately 150 ml of distilled water was added slowly to avoid the formation of an emulsion. The two aqueous and ethereal phases were then separated by partitioning. This process was repeated on three consecutive occasions to ensure the complete removal of acetone. Subsequent to the removal of the aqueous phase, the ethereal phase (containing the carotenoids) was collected in a 25 mL Erlenmeyer flask, and the final volume was adjusted to the mark with petroleum ether. Subsequently, 7.5 g of anhydrous sodium sulfate was added to the solution with the objective of removing any residual water. The spectrophotometer was then utilised to measure the optical density of the solution at a wavelength of 450 nm, with the resultant data being employed to calculate the carotenoid content.

2.6. Evaluation of the anti-radical properties and the total reducing power of the powder fractions of *Senna italica* leaves

2.6.1. Determination of the anti-radical power at DPPH

The anti-radical activity at DPPH (2,2-diphenyl-1-picrylhydrazyl) was evaluated according to the protocol described by Zhang and Yasumori, [32]. In this test, antioxidants reduce DPPH which changes from purple to yellow, the color intensity of which is inversely proportional to the capacity of the antioxidants present in the medium. Briefly, 0.5 mL of

each methanolic solution of the extracts at different concentrations (0.2; 0.4; 0.6; 0.8; 1; 1.2; 1,4 mg/mL) was added to 2 mL of the DPPH solution (0.025 g/L). In parallel, a negative control is prepared by mixing 0.5 mL of methanol with 2 mL of the methanolic DPPH solution. The absorbance reading is carried out against a blank prepared for each concentration at 517 nm after 60 min of incubation in the dark and at room temperature. The positive control is represented by a solution of a standard antioxidant; ascorbic acid, the absorbance of which was measured under the same conditions as the samples and for each concentration, the test was repeated 3 times. The inhibitory concentration 50 % (IC_{50}) values were determined graphically by identifying the concentration of extract that reduces 50 % of DPPH.

2.6.2. Determination of the Ferrie Reducing-Antioxidant Power (FRAP) of the powders

The total reducing power of *Senna italica* powders was evaluated according to the method originally developed by Dongmo *et al*. [33]. This method is based on the ability of each powder to reduce iron (III) to iron (II). Six test tubes were prepared, with each containing 1 g of powder, 2.5 mL of phosphate buffer solution (0.2 M, pH 6.6), and 2.5 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] to 1 %. The tubes were then subjected to an incubation period of 30 minutes at a temperature of 50 °C within a water bath. Thereafter, 2.5 mL of 10 % trichloroacetic acid was added, and the mixture was subjected to centrifugation at 1000 rpm for 10 minutes at a temperature of 25 °C. Thereafter, 2.5 mL of the obtained mixture was transferred and combined with 2.5 mL of distilled water and 0.5 mL of a 0.1 % aqueous solution of FeCl3. The resulting mixture was then analysed spectrophotometrically at a wavelength of 700 nm. A calibration curve was constructed utilising the ascorbic acid concentrations employed as a reference standard. The total reducing power was expressed in milligram equivalent of ascorbic acid per 100 mL of extract (mg of ascorbic acid/100 mL of extract).

2.7. Statistical analysis

During the course of the experimental process, the trials were replicated on three occasions. The mean values and standard deviations were then calculated using Excel version 2010. A one-way analysis of variance (ANOVA) was conducted in order to ascertain the significance of the results. The Duncan test was performed to classify the means using the STATGRAPHICS Centurion 16.1.18 software. The Sigmaplot 11.1 software was utilized to conduct the plot of the particle size distribution curve, while the Excel Microsoft Office 2013 software was employed for the regression lines required for the calculation of the IC_{50} of the DPPH test.

3. Results

3.1. Granulometrics distribution of *Senna italica* leaves powder particles

In the field of powder technology, the study of the particle size distribution is of paramount importance. This is due to the fact that the size of the particles is one of the key physical parameters that must be measured in order to gain a comprehensive understanding of the composition of the powder. As illustrated in Figure 2, the results of the particle size distribution of powder particles from *S. italica* leaves demonstrate a distribution width of 1.61. The size of these particles varies between 0.179 µm and 635.12 µm. The particle uniformity was found to be 0.66, and the characteristics D_{10}, D_{50} and D_{90} were determined to be 62.86 µm, 272.10 µm and 503.62 µm, respectively. These values reflect that 10 %, 50 %, and 90 % of the particles in the powder have a diameter smaller than these sizes, respectively.

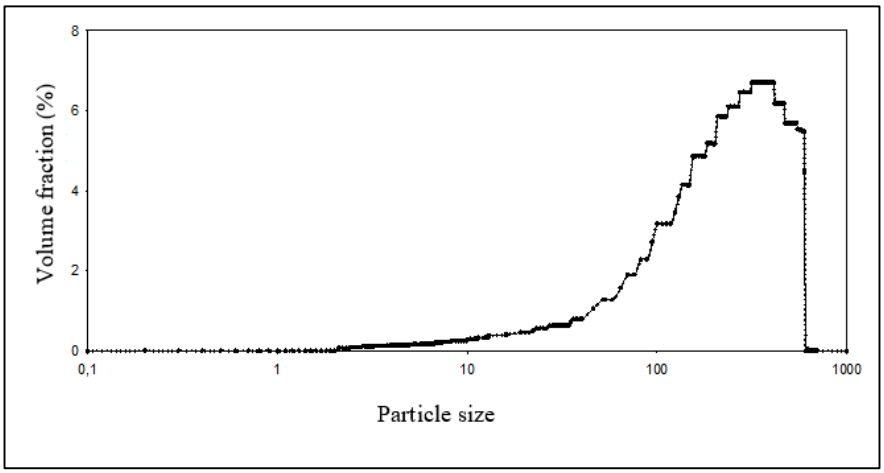

Figure 2 Logarithmic curve of distribution of particles contained in *Senna italica*

3.2. Mass yields of the particle size fractions of the unsieved powder of *Senna italica* leaves

Table 1 presents the values of the mass yields of each powder fraction retained after CDS. The variability in yields is found to be statistically significant (P≤0.05) when considering the fractions. The proportion of powders exhibiting the highest mass yield was found to be the fraction ≥355 µm, accounting for 25.26 % of the total. Conversely, the fraction ≤125 µm was found to be the proportion exhibiting the lowest mass yield, accounting for 16.12 % of the total.

Table 1 Mass yields of different particle size fractions of *Senna italica* leaf powder after CDS Values represent means and standard deviations (n = 3)

Granulometrics Fractions	Mass yields (%)
≥355 µm	25.26 ± 1.13^{e}
355 – 250 µm	22.38 ± 0.83^{d}
250 – 200 µm	17.72 ± 0.97^{b}
200 – 125 µm	18.34 ± 1.21^{c}
≤125 µm	16.12 ± 1.43^{a}

Values represent Means ± standard deviations (n = 3). The different superscript letters on the values in the mass fraction column indicate a significant difference (p≤0.05) .

3.3. Contents of some secondary metabolites in the powder fractions of *Senna italica* leaves

Table 2 presents the contents of some secondary metabolites in the particle size fractions of the powders of *S. italica* leaves. The contents of total polyphenols and condensed tannins in the raw powders of *S. italica* are 65.83±2.29 mg GAE/g DM and 38.40±1.38 mg CE/g DM, respectively. The content of these compounds increased significantly (p<0.05) in the fractions, with those of small size having the highest values. Thus the contents of total polyphenols vary from 55.47±2.30 mg GAE/g DM in the coarse fractions to 75.81±1.51 mg GAE/g DM in the fine fractions. Condensed tannins vary between 30.51±1.12 mg CE/g DM and 54.35±1.24 mg CE/g DM, flavonoids vary between 35.12±1.35 and 59.55±0.61 mg RE/g DM, carotenoids vary between 12.72±1.10 and 15.77±0.97 (mg/100 g DM).

Table 2 Contents of total polyphenols, flavonoids, condensed tannins and carotenoids from particle size fractions and unsieved powder from *Senna italica* leaves

Powders	Total Polyphenols (mg GAE/g DM)	Flavonoids (mg RE/g DM)	Condensed Tannins (mg CE/g DM)	Total Carotenoids (mg/100 g DM)
UP	65.83±2.29	42.05±1.63	38.40±1.38	14.09±1.39
≥355	55.47±2.30**	35.12±1.35*	30.51±1.12**	12.72±1.10*
355 - 250	57.06±2.01*	40.03±0.61	31.18±2.1**	12.82±1.04*
250 - 200	60.79±1.50*	43.58±0.72	39.61±1.29	14.75±1.15
200 125	72.53±2.26*	58.58±1.01**	43.75±1.28*	15.45±1.1
≤125	75.81±1.51**	59.55±0.61**	49.35±1.24**	15.77±0.97

Values represent Means ± standard deviations (n = 3). *p<0.05, **p<0.01: significant difference compared to UP. Unsieved Powder, GAE: Gallic Acid Equivalents, RE: Rutin Equivalents, CE: Catechin Equivalents, CGE: Cyanidin 3-Glucosidic Equivalents, DM: Dry Matter, UP: Unsieved Powder.

3.4. DPPH antiradical and reducing activity of powder fractions of *Senna italica* leaves.

Figure 4 illustrates the effect of extract concentration or ascorbic acid on DPPH antiradical activities. It note that the more the concentration of the powder increases, the more the percentage of trapping of DPPH radicals increases linearly in the chosen concentration range. This linear increase made it possible to determine the IC50 (Inhibitory Concentration) for which 50 % of radicals are trapped. Table 3 presents the anti-radical activity and the total reducing power of the powder fractions of Senna italica leaves. The test results show that the raw powder values are 0.96±0.06 mg/mL (IC50) for the DPPH test and 49.89±1.21 mg/g DM for the FRAP test. IC50 values decreased significantly (p<0.05) with particle size. The coarse fraction (≥355 µm) has the highest IC50 (1.15±0.16 µg/mL), while the finest has the lowest IC50 (0.95±0.05 µg/mL) synonymous with greater free radical trapping capacity. Regarding FRAP, the coarse

powders have the lowest values (42.90±0.92 mg /g DM) synonymous with low reducing power while the finest fraction has the highest value of iron reducing power which is 59.91±1.90 mg /g DM.

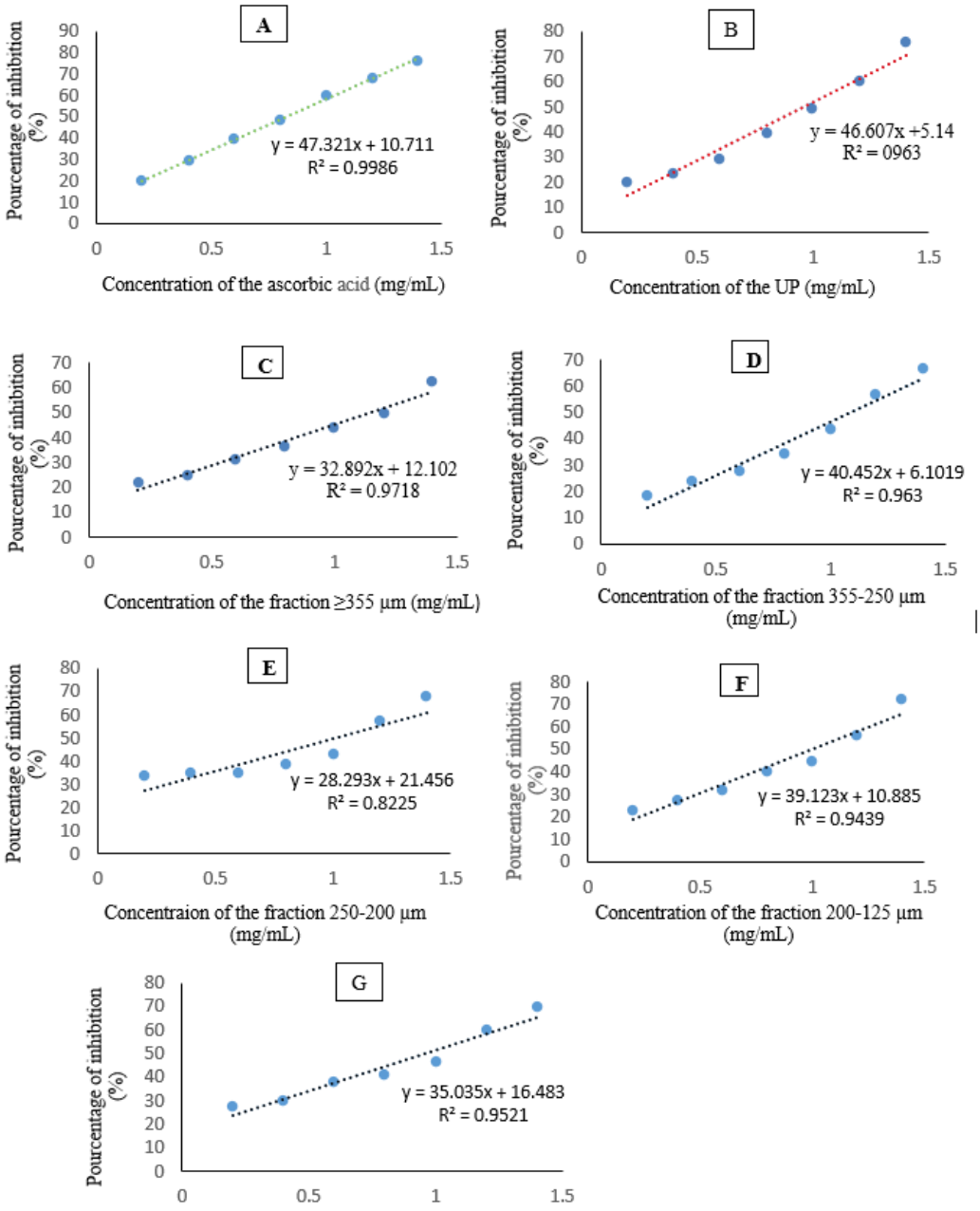

Figure 3 Regression lines for calculating the IC_{50} (Inhibitory Concentration at 50 %): (A): ascorbic acid; (B): unsieved powder; (B): fraction ≥355 µm; (D): 355-250 µm; (E): fraction 250-200 µm; (F): fraction 200-125 µm and (G): ≤125 µm of *S. italica*

Table 3 Antioxidant activities of the fractions powdery and unsieved powder of *Senna italica* leaves

Powders	DPPH (IC_{50} mg/mL)	FRAP (mg/g DM)
Ascorbic acid	0.83±0.07	62.11±1.78
UP	0.96±0.06	49.89±1.21**
≥355	1.15±0.16a *	42.90±0.92b***
355 - 250	1.08±0.05a*	46.94±1.17a***
250 - 200	1±0.09a*	50.38±0.87**
200 125	0.99±0.05	55.94±1.14a*
≤125	0.95±0.05	59.91±1.90b

Values represent Means ± standard deviations (n = 3). ap<0.05; b p<0.01 significant difference compared to UP. *p<0.05; **p<0.01; ***p<0.001 significant difference compared to ascorbic acid. DPPH: 2.2-diphenyl-1-picrylhydrazyl, FRAP: Ferrie Reducing-Antioxidant Power, IC_{50}: 50 % Inhibitory Concentration.

4. Discussion

It is evident that *S. italica* powder exhibits a relatively uniform structure with regard to the extent data (extent <2). The normal distribution of powders around its median value (272 µm) clearly justifies the choice of sieves 125, 200, 250, and 355 µm. The yield values obtained are consistent with the characteristics of a normal distribution, as observed, and demonstrate an equitable distribution of powders across the designated classes. The distribution of particles is contingent on the inherent characteristics of the base material, as well as the treatments undergone by the powder, namely the drying method and the ease of grinding. It is evident that each plant tissue possesses distinct mechanical properties, which are attributable to the unique characteristics of their respective tissues [34]. Furthermore, the morphology of the powder particles, the velocity and duration of the movement exerted during sieving, and the chemical composition are additional factors that are likely to influence the particle size distribution [35]. During the sieving process, the powder obtained from the leaves of *S. italica* exhibited minimal cohesion, as it adhered to the walls of the sieves, where its particles aggregated. Déli and his collaborators made similar observations in relation to *Boscia senegalensis* powder Deli *et al.* [25]. Raihane and his collaborators [36] found that the greater the vibration of a cohesive powder, the more its particles organise and compact. This phenomenon is a limiting factor that hinders the sifting of powders and consequently the distribution of particles according to their size.

The secondary metabolites evaluated in the fractions of *S. italica* leaves exhibited an increase in their content when transitioning from coarse to finer powders. These results are consistent with those reported by Ngatchic and his collaborators, and Déli and his collaborators [25], who also observed an increase in the content of secondary metabolites in the finest fractions. The present study found that there was an increase in the content of secondary metabolites in the finest fractions of *S. italica* leaves. This finding is consistent with those of Ngatchic *et al.*, [23], and Déli *et al.,* [25] who also observed an increase in the content of secondary metabolites in the finest fractions. The increase in the content of phenolic compounds with the reduction in particle size of powders was also noted by Karam et al. [34] for the fruits of *Grewia coriacea* Mast. As posited by Zaiter *et al.,[*22] the elevated levels of secondary metabolites observed in this study serve to reinforce the fundamental premise of CDS, which posits the accumulation of bioactive molecules within discrete particulate fractions. The same elevated levels of secondary metabolites were also observed in green tea leaves, *Salix alba* Bark, leaves of *Scrophularia nodosa* and *Hedera helix* by Beker *et al.,* [26]. However, the aerial parts of *Hypericum perforatum* and *Achillea millefolium* were also analysed. The present study, in accordance with previous studies, indicates a greater concentration of phenolic compounds in the finest powder fractions. The intracellular localization of these compounds may also provide a theoretical basis for this observation. It has been demonstrated that simple phenolic compounds, such as benzoic acid and benzaldehyde, or their derivatives, are generally linked covalently to plant cell wall polysaccharides, forming ester linkages with arabinose hemicellulose groups or with a lignin core [25]. Other polyphenols, such as tannins, have been observed to accumulate in vacuoles, while flavonoids remain in the cytosol where they are mainly synthesised in free form [37].

It has been established that the carotenoids responsible for the red, orange and yellow colouration of plants are located in the chromoplasts [38]. In order to make them bioaccessible, it is necessary to break the cell walls through grinding. This process enables the cell to be deconstructed, the contents of its wall to be dispersed (e.g. cellulose, hemicellulose, pectins, proteins), and the active ingredients to be released [39]. Conversely, sieving allows the particles to be separated according to their cut. Consequently, it can be hypothesised that the most substantial particles in plant powders are, a

priori, derived from less fragile plant components, frequently corresponding to the elements of the plant cell wall, which are hypothesised to contain fewer active or phenolic compounds [27].

In vitro analysis of the antioxidant activity of the fractions leaves of *S. italica* by the DPPH test revealed that the inhibitory concentration (IC_{50}) decreases with particle size. This finding indicates that the lower the IC_{50} of the extract, the greater its capacity for scavenging free radicals. Conversely, the FRAP test demonstrates that higher values indicate greater anti-radical activity, thereby validating the reliability of the experimental findings. The low IC_{50} value and the highest iron reducing power value of the finest fraction (≤125 µm) clearly demonstrate that the fine powders have the highest activity. The presence of bioactive compounds, including total polyphenols, flavonoids, condensed tannins, carotenoids, and vitamin C, in the fine fractions can be attributed to the strong anti-radical, reducing, and antioxidant activities observed.

An in vitro analysis was conducted on the antioxidant activity of the fractions of *S. italica* leaves, employing the DPPH (2,2-diphenyl-1-picrylhydrazyl) test. The results of this analysis indicated that as the particle size decreased, the inhibitory concentration (IC_{50}) also decreased. This finding indicates that the lower the IC_{50} of the extract, the greater its capacity for scavenging free radicals. Conversely, the FRAP test demonstrates that higher values indicate greater anti-radical activity, thereby validating the reliability of the experimental findings. The low $\mathbf{IC_{50}}$ value and the highest iron-reducing power value of the finest fraction (≤125 µm) clearly demonstrate that the fine powders have the highest activities. The presence of bioactive compounds, including total polyphenols, flavonoids, condensed tannins, carotenoids, and vitamin C, in the fine fractions can be attributed to the strong anti-radical, reducing, and antioxidant activities observed.

These bioactive compounds are recognized for their diverse biological activities, notably their antioxidant properties, which involve the capture of free radicals, thereby impeding their destructive effects (Becker *et al.,* 2016). Consequently, the Ferrie reducing-antioxidant power (FRAP) is associated with the phenolic composition. Li and his collaborators observed that the antioxidant and chelating capabilities of plant extracts increase in proportion to their phenolic compound content, thereby elucidating the prevailing relationship between the phenolic compound content of *S. italica* leaf fractions and their antioxidant power (Li *et al.*, 2015).

5. Conclusion

The bioactive molecules, phenolic compounds and carotenoids present in *S. italica* powders are concentrated in fine particles (≤125 µm) during the CDS process. It is evident that fine powders demonstrate the highest antioxidant properties. Consequently, CDS emerges as a process for concentrating secondary metabolites. Its employment in the enhancement of bioactive compound and pharmacological properties is both feasible and recommended. The potential applications of CDS extend to various domains, including food supplements, functional foods, and nutraceuticals. Consequently, the ≤125 µm fraction of leaf powder from *S. italica* could be utilized as a nutraceutical to combat stress and obesity.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have no conflict of interest related to this publication.

Statement of ethical approval

This study was approved by the ethics committee of the University of Ngaoundere.

Authors contributions

Study concept and design (DA, FD, SNA, NYN, SDS), acquisition analysis and interpretation data (DA, FN, NYN), drafting the manuscript (DA, FD, NYN, SDS), critical of the manuscript for important intellectual content (DA, FD, SNA, NYN, SDS) and study supervision (FD, NYN, SDS). All authors had full access to all of the data in study. All authors are responsible for the integrity of data and the accuracy of data analysis and gave the publishing approval of this manuscript.

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