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Influence of particle size fraction and bioactive compound contents on the biological activities of *Solanum torvum* L. leaf powder extracts

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ABSTRACT

This study investigates the bioactive compound content and biological activities of raw powder extracts and particle size fractions from *Solanum torvum* leaves. The leaves, harvested from Douala, were processed into powders and subsequently divided into different fractions. Methanolic extracts of these powders were analyzed for their content of bioactive compounds (total polyphenols, total flavonoids, and condensed tannins), antioxidant (DPPH, ABTS, FRAP assays), and antimicrobial (bacterial and fungal) activities. The particle size fraction of 250-400 µm constitutes the largest proportion (33.41%) of the raw powder. The highest contents of bioactive compounds are found in the 200-250, 400-500, and < 125 µm fractions for total polyphenols (44.62 ± 0.19 mg GEA/100 ml extract), total flavonoids (14.47 ± 0.37 mg QE/100 ml extract), and condensed tannins (22.79 ± 0.12 mg CE/100 ml extract), respectively. The antioxidant activity of extracts from 500-800µm fraction improved their DPPH and ABTS assays by

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31.50% and 40.44% compared to the raw powder. The same fraction (500-800 µm) and the raw powder extracts demonstrated bactericidal activities, while the 125-200, 200-250, and ≥ 800 µm fraction extracts exhibited moderate and significant antifungal activities against several bacterial and fungal strains, respectively. These biological activities are primarily attributed to the average flavonoid contents, which become more accessible in extracts after fractionation. This method of processing powder suggests that no plant powder should be disregarded or rejected due to its low content of bioactive compounds.

1 Introduction

The practice of incorporating powder from various plants into dishes to enhance their nutritional value, particularly micronutrients such as iron, carotenoids, and zinc, has become commonplace for many consumers, including manufacturers, patients, and researchers (Siekmans et al. 2017; Boateng et al. 2019). Many are aware that these food matrices contain not only micronutrients but also a wide variety of bioactive compounds, such as polyphenols, flavonoids, and tannins, which are essential in managing numerous diseases (Jucá et al. 2020; Borokini et al. 2022; Osei-Owusu et al. 2023). Various biological activities, including antioxidant, antimicrobial, anticancer, antidiabetic, antiobesity, and antitumoral activities, have been associated with these compounds and have been reported by various researchers (Loganayaki et al. 2010; Fombang and Saa 2016; Djoueudam et al. 2019; Senizza et al. 2021). These results have led to an ever-increasing search for plant matrices rich in bioactive compounds, to the detriment of those low in them. This situation could potentially lead to a loss of biodiversity.

Several authors have identified a range of bioactive compound contents that significantly affect the variations in desired biological activities. For instance, Loganayaki et al. (2010) tested thirteen extracts. They found that the highest radical scavenging activity (DPPH) and ferric-reducing antioxidant power were reported in the fruit and leaves of *S. nigrum*, while *S. torvum* matrices corresponded to low total polyphenol contents. Similarly, Djoueudam et al. (2019) also found that the total polyphenols and flavonoids responsible for bacteriostatic and bactericidal activities vary in content. Therefore, high polyphenol contents in a plant matrix do not necessarily correlate with the most significant biological activities. Based on these observations and the health benefits consumers seek, plant matrices with low polyphenol contents should not be neglected. This is especially relevant since the literature already shows a clear distinction between plant matrices with low bioactive compound contents (fruit, stem, and leaves of *S. torvum*, *Manihot esculenta*, *Hibiscus sabdariffa* calyces, etc.) and those that are particularly rich in them (*Moringa oleifera* leaves, spirulina, etc.) (Nouman et al. 2016; Ravani et al. 2018; Tao et al. 2019).

In response to the escalating demand for plant matrices rich in bioactive compounds, many researchers have opted to increase the specific surface area of plant matrices by processing them into powders (Deli et al. 2019, 2020). *S. torvum* leaf powders have been the focus of various studies (Loganayaki et al. 2010; Ramamurthy et al. 2012; Acharyya and Khatun 2018; Djoueudam et al. 2019; Senizza et al. 2021; Osei-Owusu et al. 2023; Murugesan et al. 2024). This small shrub leafy vegetable belongs to the Solanaceae family and is widely distributed in India, Thailand, China, tropical America, and sub-Saharan Africa (Ramamurthy et al. 2012). In Cameroon, it is commonly grown near homes as wild eggplant. Recent phytochemical analyses of *S. torvum* leaf powder and their particle size fractions have revealed low contents of total polyphenols, flavonoids, and condensed tannins (Unpublished work). However, the previous researchers have not addressed the impact of these low contents on biological activities such as antioxidant, antibacterial, and antifungal activities. Each particle size fraction is characterized by the specific surface area of its particles, a major physical characteristic that influences compound extraction. Nabil et al. (2020) and Wu et al. (2022) reported that the bioactive content of plant powders increases proportionally with their specific surface area. However, they also demonstrated that an increase in the specific surface area of powders does not necessarily guarantee an increase in the content of bioactive compounds. Given that the recognized biological activities of bioactive compounds are strongly correlated not only with their presence but also with their content in a food matrix (Prithvira 2019; Gong et al. 2020), it is evident that the biological activities of a powder could be enhanced if its particle size fractions made the bioactive compounds more accessible, even if they were present in low content in the plant powder. Therefore, this study aimed to evaluate the contents of bioactive compounds in extracts of *S. torvum* leaf powder, its particle size fractions, and their effects on antioxidant, antibacterial, and antifungal activities.

2 Material and methods

2.1 Sampling and preparation of *S. torvum* leaves powders

S. torvum leaves were harvested from the field of Douala, Cameroon. These leaves were washed with clean water and dried at 25°C using an electric dryer for 5 hours. The dried leaves were ground using a Moulinex grinder (4500W, 220-240 V, France) and sieved through a 1000 µm mesh sieve to produce a powder (Assiéné et al. 2021). This powder was stored in airtight containers at 4°C until further analysis.

2.2 Determination of particle size fractions of powder

The particle size of the resulting powder was determined through sieving. A column of AFNOR sieves, decreasing in size (1000, 800, 710, 500, 400, 250, 125, 100, 50, 40 µm) down to the collector, was utilized for this purpose (Melcion 2000). A mass (M_1) of powder was placed on top of a stack of sieves and subjected to a 50 Hz vibration for 15 minutes using a Merck-type vibrator. Particles smaller than the sieve mesh passed through (Mi), while those larger were retained (Mj). Each mass was weighed to determine the particle size distribution (Rp). Depending on the sieve size, this distribution was expressed for each sample. The particle size fractions were inferred based on the mass of the sieved particles according to the formula 1.

$$R_p = \left(\frac{M_i}{M_1}\right) x\ 100 \quad (1)$$

2.3 Preparation of extracts

Extracts were prepared following the Senizza et al. (2021) method. The maceration technique was employed to obtain these extracts. Powders derived from *S. torvum* leaves were extracted with methanol at a ratio of 1:2 (mass: volume) for 48 hours at room temperature. Methanol was chosen as the extraction solvent due to its superior extraction efficiency for phytochemicals and antioxidants from *S. torvum* (Ramamurthy et al., 2012). After extraction, the samples were filtered using Whatman No 1 filter paper and dried at 40 °C using a rotary evaporator for 30 minutes. All extracts were maintained in an oven at 40 °C until a constant weight was achieved. The dried extracts were stored in amber glass bottles (to mitigate light effects) at room temperature for a maximum of 6 months. The extraction process was conducted on each fraction of particle size.

2.4 Determination of the bioactive compound contents of the extracts

2.4.1 Total polyphenol

The total polyphenol content was determined using a modified version of the method described by Makkar et al. (2007). The 100 µl of the extract was added to 200 µl of ten-fold diluted Folin Ciocalteu reagent. This mixture added 200 µl of sodium carbonate (7.5% w/v), then vortexed and incubated in the dark at room temperature for ten minutes. Subsequently, 1000 µl of distilled water was added, and the solution was vortexed again. The absorbance was measured at 760 nm using a spectrophotometer (BK-UV1600 PC visible spectrometer, China). The total polyphenol content was calculated based on a calibration curve established using gallic acid as a standard and expressed in milligrams of Gallic Acid Equivalent per 100 millilitres of extract (mg GAE/100ml Extract).

2.4.2 Total flavonoid

The total flavonoid content was determined by following a slightly modified version of the method described by Chang et al. (2002). An extract (500 µl) was combined with 500 µl of a freshly prepared aluminium chloride solution (2% w/v). This mixture was incubated in the dark at room temperature for fifteen minutes. The absorbance was then measured at 430 nm using a spectrophotometer (BK-UV1600 PC visible spectrometer, China). The total flavonoid content was calculated using a calibration curve and expressed in milligrams of quercetin equivalent per 100 milliliters of extract (mg QE/100 mL Extract).

2.4.3 Condensed tannin

The content of condensed tannins was assessed using a modified version of the method described by Ogboru et al. (2015). An extract volume of 50 µl was mixed with 3 mL of 4% (w/v) vanillin in 80% methanol and 750 µl of concentrated hydrochloric acid. Then, the mixture was vortexed and incubated at 30˚C for 20 minutes. The absorbance was measured at 550 nm using UV/visible spectrophotometry (BK-UV1600 PC visible spectrometer, China), with a hydromethanolic solvent (70/30) serving as the blank. The tannin content was calculated by comparing it with the standard curve prepared using a standard catechin solution (0.2 g/l). The results were expressed as milligrams of catechin equivalent per 100 milliliters of extract (mg CE/100 mL Extract).

2.5 Determination of antioxidant activities

2.5.1 DPPH radical-scavenging activity assay

The radical-scavenging activity was determined following the method outlined by Ramamurthy et al. (2012). For this, extracts from *S. torvum* leaves (0.3mL) were combined with 2.7 mL of 0.5 mM methanolic 1,1-diphenyl-2-picrylhydrazyl (DPPH). The mixture was incubated at 37 °C for 30 minutes, after which the absorbance was measured spectrophotometrically at 517 nm. The radical-scavenging activity (%RSA) was assessed as a percentage of DPPH discoloration using formula 2.

$$\%\ RSA = ([A_{DPPH} - A_S]/A_{DP\,PH})\ x\ 100 \quad (2)$$

RSA_{50} (or IC_{50}) represents the sample concentration required to reduce the total free DPPH radical by 50%. A_S denotes the absorbance of the DPPH solution with the sample extract, while A_{DPPH} refers to the absorbance of the DPPH solution without the extract. Ascorbic acid was utilized as the standard.

2.5.2 ABTS radical scavenging activity assay

The procedure was conducted following the method given by Galla et al. (2017), with some modifications. The $ABTS^+$ radical was

generated by reacting 7 mM $ABTS^+$ with 2.45 mM potassium per sulphate ($K_2S_2O_8$). After incubation at room temperature in the dark for 16 hours, the solution was diluted to achieve an absorbance of 0.70 ± 0.02 at 734 nm. The $ABTS^+$ solution (1 mL) was then added to the test sample (10 µl), thoroughly mixed, and incubated for 30 minutes. The absorbance of the mixture was measured at 734 nm. Ascorbic acid (Vitamin C) was used as the standard. The radical scavenging activity (%RSA) was calculated using the formula 3.

$$\% \text{ RSA} = ([A_{ABTS} - A_S]/A_{ABTS}) \text{ x } 100 \quad (3)$$

RSA_{50} (or IC_{50}) represents the sample concentration required to reduce the total free ABTS radical by 50%. A_S denotes the absorbance of the ABTS solution with the sample extract and A_{ABTS} refers to the absorbance of the ABTS solution without the extract.

2.5.3 Ferric reducing antioxidant power (FRAP) assay

The reducing power of iron (Fe^{3+}) extracts was assessed following the method described by Padmaja et al. (2011), with minor modifications. Approximately 25 µl of each diluted extract (2 mg/mL in methanol) was introduced into a new microplate, followed by 25 µl of 1.2 mg/mL Fe^{3+} solution. The plates were pre-incubated for 15 minutes at room temperature. Subsequently, 50 µl of 0.2% ortho-phenanthroline was added to achieve final concentrations of 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, and 1.95325 µg/mL. The mixtures were further incubated for 15 minutes at room temperature, after which the absorbance was measured at 505 nm using a UV/Visible light spectrophotometer (Infinite M200 TECAN, Swiss) against a blank (comprising 25 µl methanol + 25 µl Fe^{3+} + 50 µl ortho-phenanthroline). Ascorbic acid (Vitamin C) was used as the positive control. From the obtained Optical Density, reducing percentages were calculated for each concentration and used to determine the RC_{50} from dose-response curves.

2.6 Determination of antimicrobial activities

2.6.1 Antibacterial activity

As Newton et al. (2002) described, the broth microdilution method was employed for susceptibility testing of bacterial species using 96-well microtiter sterile plates. Crude extracts were dissolved in a 5% dimethylsulfoxide (DMSO) solution and diluted with Mueller Hinton broth to achieve stock concentrations of 2000 µg/mL for the extracts, 1000 µg/mL for fractions, and 500 µg/mL for the isolated compounds. Serial two-fold dilutions of the test substances were performed with Mueller Hinton broth to yield a volume of 100 µl/well, resulting in concentration ranges of 1000-0.96 µg/mL, 500-0.96 µg/mL, and 250 to 0.96 µg/mL, respectively. One hundred microliters of each bacterial suspension obtained from the Centre Pasteur du Cameroun (CPC) (containing approximately 1.5×10^6 CFU/mL) was added to the respective wells containing the test samples and thoroughly mixed to yield final concentrations ranging from 500-0.48 µg/mL for the extract, 250-0.48µg/mL for the fraction, and 125 to 0.48µg/mL for the isolated compounds. The solvent control, 5% DMSO, did not exhibit inhibitory effects on bacterial growth. Ciprofloxacin at a concentration of 125 to 0.48µg/mL was used as the standard reference. The assay microtiter plates were incubated at 37 °C for 24 hours. Inhibitory concentrations of the extracts were detected following the addition of 50 µl of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, South Africa) and further incubation at 37 °C for 30 minutes. Viable bacteria convert the yellow dye of p-iodonitrotetrazolium chloride to a pink color. This assay is based on the ability of living cells to transform the INT dye tetrazolium ring into a pink-colored formazan structure due to the action of mitochondrial and other dehydrogenases within the cell. The lowest concentration at which no visible color changes were observed was considered the Minimum Inhibitory Concentration (MIC). Bactericidal concentrations were determined by adding 50 µl aliquots of the preparations (without INT), which showed no visible color change after subculture from MIC assays, into 150 µl of extract-free Mueller Hinton broth. These preparations were further incubated at 37 °C for 48 hours, and bacterial growth was revealed by adding INT as described above. The smallest concentration at which no color changes were observed was considered the Minimum Bactericidal Concentration (MBC). The tests were performed in duplicates. The ratio MBC/MIC was calculated to determine the bactericidal (MBC/MIC $\leq$ 4) and bacteriostatic (MBC/MIC > 4) effects.

2.6.2 Antifungal activity

Yeast inocula were prepared from 48-hour-old cultures by selecting 2 to 3 colonies and suspending them in a sterile saline (NaCl) solution (0.9%). The absorbance was read at 530nm and adjusted with the saline solution to match a 0.5 McFarland standard solution, corresponding to approximately 106 yeast cells/mL. Conidia suspensions of dermatophyte species were prepared from 10-day-old cultures. The number of conidia was determined using a spectrophotometer and adjusted with a sterile saline (NaCl) solution (0.9%) to an absorbance of 0.600 at 450 nm, corresponding to a final concentration of about 1 x 10^4 spores/mL (Venugopal and Venugopal 1992).

The Minimum Inhibitory Concentration (MIC) of each extract was determined using broth microdilution techniques according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards, NCCLS) for yeasts (M27-A2). Stock solutions of the test extracts were prepared in a 5% aqueous dimethylsulfoxide (DMSO) solution and diluted with Sabouraud Dextrose Broth

(SDB) to achieve a 1 mg/mL concentration. This was serially diluted two-fold to obtain a concentration range of 500 to 0.24 µg/mL for extracts and 125 to 0.24 µg/mL for compounds. The final concentration of DMSO in the well was less than 1% (preliminary analysis with 1% DMSO did not inhibit the growth of the test organisms).

The negative control well consisted of 195 µl of SDB and 5 µl of the inoculum (containing about 10^6 cells/mL for yeast and 1 x 10^4 spores/mL for filamentous fungi). The plates were covered with a sterile lid and incubated on a shaker at 37 °C for 48 hours (for yeasts) and at 28 °C for 7 days (for dermatophytes). MICs were assessed visually after the corresponding incubation period and were defined as the lowest product concentration at which no growth or virtually no growth was observed. The assay was repeated three times.

For the Minimal Fungicidal Concentration (MFC) determination, 5 µl aliquots from each well that showed no growth of microorganisms were replicated into 195 µl of SDB and incubated at 37 °C for 48 hours (yeasts) and at 28 °C for 7 days (dermatophytes). The lowest concentration that yielded no growth after subculturing was taken as the MFC. Nystatin (for yeasts) and griseofulvin (for dermatophytes) were positive controls.

2.7 Statistical analysis

Analyses were conducted in triplicates. Microsoft Excel 2016 software was utilized for the calculation of means and standard deviations, as well as for plotting the curves. The software Statgraphic Centurion 15.2 (StatPoint Technologies, Inc, Warrenton, Virginia, USA) was employed to analyze variance and for separating means using the Duncan multiple range test at $P < 0.05$. XLStat 2016 was used for principal components analysis, which established relationships between particle size fractions of *S. torvum* leaf powders, the mass of particles, bioactive compounds, and antioxidant activities.

3 Results and discussion

3.1 Particle size fractions and their mass distribution in *Solanumtorvum* leaf powder

The particle size distribution of *S. torvum* leaf powders (Figure 1) allowed for identifying seven distinct particle size fractions: < 125, 125-200, 200-250, 250-400, 400-500, 500-800, and ≥ 800 µm. Significant differences were observed between the masses of particles across these seven fractions, and among the tested fractions, the 250-400 µm fraction had the highest mass (33.41%). These differences can be attributed to the particle size that characterizes each fraction comprising the powder. The mass of each fraction can either negatively or positively influence the content of bioactive compounds. The accessibility of bioactive compounds significantly depends on the specific surface area of each particle during extraction (Rodriguez et al. 2019). Similar results regarding the particle size distribution of various plant powders have been reported by many authors (Bahar and Khalili 2015; Wu et al. 2022).

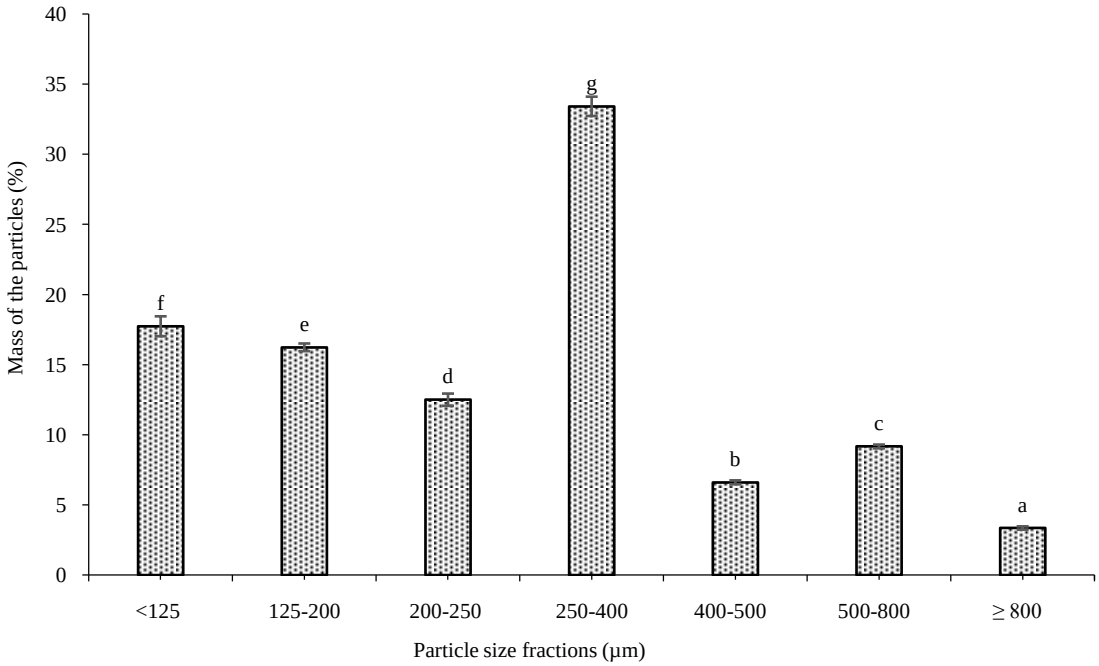

Figure 1 Mass distribution of particle size fractions in *S. torvum* leaf powder; Data are mean of five replicates; Columns without common letters differ significantly at LSD P<0.05.

Table 1 Contents of bioactive compounds in extracts of *S. torvum* leaf powder and its particle size fractions

S. torvum leaves powders	Total polyphenols mg GAE/100mL Extract	Total flavonoids mg QE/100 mL Extract	Condensed tannins mg CE/100 mL Extract
Powder (Ø < 1000 µm)	43.63 ± 0.15^{cd}	10.97 ± 0.75^{bc}	17.73 ± 0.27^{e}
Particle size fraction (µm)			
≥ 800	40.43 ± 0.32^{a}	9.62 ± 0.18^{a}	13.01 ± 0.59^{a}
500 – 800	43.60 ± 0.27^{cd}	11.79 ± 0.36^{cd}	13.51 ± 0.51^{ab}
400 – 500	43.88 ± 0.24^{de}	14.47 ± 0.37^{f}	12.93 ± 0.85^{a}
250 – 400	44.14 ± 0.20^{e}	12.62 ± 0.87^{de}	14.21 ± 0.61^{bc}
200 – 250	44.62 ± 0.19^{f}	10.37 ± 0.33^{ab}	16.56 ± 0.60^{d}
125 – 200	41.12 ± 0.19^{b}	11.62 ± 0.20^{c}	14.77 ± 0.22^{c}
< 125	43.37 ± 0.15^{c}	12.77 ± 0.60^{e}	22.79 ± 0.12^{f}

Mn ± SD - Means ± Standard Deviation; Means in the same column for each compound, with different superscripts, are significantly different from each other respectively ($p < 0.05$). GAE : Gallic Acid Equivalent; QE: Quercetin Equivalent; CE : Catechin Equivalent

3.2 Bioactive compound contents in *S.torvum* leaf powder extracts and its particle size fractions

3.2.1 Total polyphenol content

The total polyphenol content of extracts from the powder and its particle size fractions is 43.63 ± 0.15 mg GAE/100mL for the powder (Table 1). This is significantly lower (296.4 mg GAE/100mL extract) than the total polyphenol content reported by Djoueudam et al. (2019) in *S. torvum* leaf powder extracts. The polyphenol content varies significantly across various fractions, ranging from 40.43 ± 0.32 to 44.62 ± 0.19 mg GAE/100mL extract. As the particle size decreases, the accessibility of total polyphenol content increases. Among the tested fractions, 200-250 µm has shown the highest polyphenol content (44.62 ± 0.19 mg GAE/100 mL extract).

These results can be attributed to the grinding process, during which the dry leaves of *S. torvum* are reduced to small particles of varying sizes, facilitating the dissemination of polyphenols. This mechanical effect increases the specific surface area of the leaves, enhancing the exposure of polyphenols to the extraction solvent (methanol) and thereby facilitating their extraction in greater quantities. Similar observations were made by Nabil et al. (2020) on particle size fractions of a plant powder (*Opuntia ficus-indica*), further corroborating the results obtained in this study.

However, these findings contrast with those reported by Wu et al. (2022), who demonstrated that a smaller powder particle size (increases specific surface area) does not always improve the accessibility of polyphenols (catechin). This can be explained by the fact that certain compounds, such as fibers, can limit the grinding of leaves by forming agglomerates, thereby preventing the dissemination of compounds in the smaller particle-size fractions. This results in a concentration of these compounds in the larger particle size fractions, as evidenced by the work of Deli et al. (2019) on grinding and sieving for the fractionation of plant powders.

3.2.2 Total flavonoid content

The total flavonoid content of powder extracts of *S. torvum* leaves is 10.97 ± 0.75 mg QE/100 mL extract, which is lower than the 16.70 mg QE/100mL reported by Djoueudam et al. (2019). Flavonoid content significantly varies across particle size fractions, ranging from 9.62 ± 0.18 to 14.47 ± 0.37 mg QE/100mL extract (Table 1). As the particle size decreases, a significant increase in flavonoid content is exhibited (from 9.62 ± 0.18 to 12.77 ± 0.60 mL QE/100 mL extract). However, an optimum is observed in the 400-500 µm fraction (14.47 ± 0.37 to QE/100 mL extract).

As previously demonstrated with total polyphenols, the presence of polysaccharides (fibers) or heterosides (glycosides) linked to flavonoids in the leaves, and these are similar to the results reported by Prithvira (2019) those who suggested that the dissemination of flavonoids across different particle size fractions may associate their accessibility to the solvent during extraction. These results align with those reported by Wu et al. (2022) on the flavonoid (catechin) content of particle size fractions of a plant powder (*Rhizoma dioscoreas oppositae*).

Flavonoids, an essential group of natural polyphenolic compounds characterized by their flavan core, are primarily found in vegetables, fruits, and plant-based beverages. Due to their multiple biological activities, including antimicrobial, antioxidant, antidiabetic, and anticancer properties (Maizuwo 2017; Dongmo et al. 2023; Murugesan et al. 2024), these are an indispensable component in the field of health.

3.2.3 Condensed tannin content

As demonstrated in Table 1, the accessibility of condensed tannins to the solvent during extraction significantly increases as the particle size decreases (corresponding to an increase in specific leaf surface area). The values for the powders are 17.73 ± 0.27 mg CE/100 mL extract, and they range from 12.93 ± 0.85 to 22.79 ± 0.12 mg CE/100 mL extract for the particle size fractions. The fraction < 125 µm has the highest content, at 22.79 ± 0.12 mg CE/100 mL extract.

The previously mentioned factors in polyphenols can explain these results and align with findings Deli et al. (2019) reported on *Dichrostachys glomerata* and *Hibiscus sabdariffa* powders. Moreover, complexation-free polyphenols in the leaves may also promote their dissemination across various particle size fractions, particularly the finest ones (Guerriero et al. 2018), further explaining this result. Several authors have reported similar results with superfine particle sizes (Meng et al. 2019). Tannins, a group of polyphenolic compounds, are known for their biological activities (such as antioxidant and antimicrobial properties) and their role in managing several chronic diseases (Nouman et al. 2016; Rodriguez et al. 2019; Nabil et al. 2020).

3.3 Antioxidant activities of S. torvum leaf powder extract

3.3.1 DPPH radical scavenging activity

Antioxidant activity encompasses the prevention of free radicals, the neutralization of existing free radicals, and the repair of damaged biomolecules (Makkar et al. 2007). These activities are specific to pure molecules or plant extracts with this health-promoting capacity. The antioxidant capacity of *S. torvum* leaf powder extracts and their particle size fractions were evaluated and presented in Table 2. It is expressed as the concentration in µg/mL of extract required to scavenge 50% free radicals (RSa_{50}). Therefore, the lowest RSa_{50} value is considered to have the greatest antioxidant capacity for *S. torvum* powder extracts.

Results of the study revealed that the powder extracts and their particle size fractions exhibit antiradical activity, with concentrations varying significantly from one fraction to another. The most potent concentration among particle size fractions was found in the 500-800 µm fraction, at 11.40 ± 2.40 µg/mL extract, while the concentration of the powder is 36.20 ± 2.16 µg/mL extract. This represents a 31.50% improvement in the extract's antiradical activity due to the powder's fractionation. However, compared with ascorbic acid (vitamin C) (7.80 ± 1.40 µg/mL), extracts from the 500-800 µm fraction and the powder are 1.46 and 4.64 times less active against DPPH radicals, respectively. This result suggests that the extracts' bioactive compounds contribute to the studied powder's antiradical activity. Total polyphenols, flavonoids, and condensed tannins, known for their antioxidant activity (Ramamurthy et al. 2012; Ravani et al. 2018; Nabil et al. 2020), show no significant correlation (Table 3) with the DPPH antiradical activity of the powder extracts studied. This indicates that the accessibility of bioactive compounds in the extracts is insufficient to ensure the highest scavenging activity. Therefore, the contents of these bioactive compounds in the extracts are a key factor that needs to be taken into account to ensure the greatest antiradical activity. This observation is supported by the fact that the closest antiradical activity to vitamin C is not ensured by the particle size fraction with the highest contents of bioactive compounds. Deli et al. (2019) report that extracts' DPPH antiradical/antioxidant capacity becomes increasingly important as bioactive compound contents become more accessible, corresponding to smaller particle sizes of plant powder. This result

Table 2 Antioxidant activities of extracts from *S. torvum* leaf powder and its particle size fractions (µg/mL)

S. torvum leaves powders	DPPH (RSa_{50})	ABTS(RSa_{50})	FRAP (RC_{50})
Powder(Ø < 1000 µm)	36.20 ± 2.16^{d}	45.00 ± 2.39^{ef}	24.00 ± 1.52^{b}
Particle size fraction (µm)			
≥ 800	28.60 ± 2.66^{c}	48.80 ± 3.55^{f}	30.00 ± 3.42^{de}
500-800	11.40 ± 2.40^{a}	18.20 ± 2.74^{b}	33.00 ± 2.96^{ef}
400-500	78.60 ± 3.09^{g}	32.20 ± 2.85^{c}	37.00 ± 2.44^{g}
250-400	47.40 ± 2.65^{e}	55.00 ± 2.18^{g}	28.20 ± 1.65^{cd}
200-250	57.80 ± 3.28^{f}	55.40 ± 3.38^{g}	26.20 ± 1.14^{bc}
125-200	17.40 ± 1.50^{b}	42.00 ± 2.67^{de}	23.00 ± 1.04^{b}
< 125	35.00 ± 3.01^{d}	37.60 ± 2.15^{d}	34.60 ± 1.43^{fg}
*Vitamin C	7.80 ± 1.40^{a}	11.20 ± 1.68^{a}	18.00 ± 1.00^{a}

Mn ± SD - Means ± Standard Deviation; Means in the same colunm with different superscripts are significantly different from each other ($P < 0.05$), RSa_{50} - 50% Scavenging activity required to scavenge 50% of free radicals; RC_{50} - Reduction Concentration required to reduce 50% of Fe^{3+}* Reference

Table 3 Pearson correlation coefficients between particle mass, bioactive compound contents, and antioxidant activities

	DPPH	FRAP	ABTS	Mass of the particles	Total Flavonoids	Total polyphenol	Condensed tannins
DPPH	1						
FRAP	0.331 (0.422)	1					
ABTS	0.298 (0.473)	-0.573 (0.137)	1				
Mass of the particles	-0.045 (0.914)	-0.503 (0.203)	0.220 (0.600)	1			
Total Flavonoids	0.475 (0.233)	0.614 (0.105)	-0.422 (0.297)	-0.132 (0.755)	1		
Total polyphenol	0.534 (0.172)	0.211 (0.615)	-0.026 (0.950)	0.242 (0.563)	0.423 (0.295)	1	
Condensed tannins	-0.061 (0.884)	0.003 (0.994)	0.096 (0.820)	0.309 (0.455)	0.039 (0.926)	0.243 (0.560)	1

r (p): Correlation coefficient (probability threshold)

contrasts with those obtained in this study. Certain phenolic compounds, free or not, in varying contents in the extracts could explain these differences.

3.3.2 ABTS radical scavenging activity

The ABTS radical scavenging activity (RSa_{50}) of *S. torvum* leaf powder extracts varies significantly across particle size fractions (Table 2). The most potent concentration in µg/mL of extract is closest to that of ascorbic acid, the reference antioxidant used in this study. Among the extracts of the particle size fractions, the most potent concentration is found in the 500-800 µm fraction at 18.20 ± 2.74 µg/mL extract. The concentration for the powder is 45.00 ± 2.39 µg/mL extract, representing a 40.44% improvement in the antioxidant activity of the extracts. However, these concentrations (18.20 ± 2.74 µg/mL and 45.00 ± 2.39 µg/mL) are respectively 1.62 and 4.01 times less active against the ABTS radical than vitamin C (11.20 ± 1.68 µg/mL).

The antioxidant activity of the extracts studied is attributed to the bioactive compounds accessible in the powder (total polyphenols, flavonoids, and condensed tannins (Ramamurthy et al., 2012; Ozcan et al., 2014). These compounds show no significant correlation with the concentrations (RSa_{50}) obtained (Table 3). This may be explained by the reasons previously mentioned for DPPH radical scavenging activity. The antioxidant activity of *S. torvum* leaf powder extracts, evaluated via the DPPH and ABTS assays, depends not only on the accessibility of the bioactive compounds but also on their content in the extracts. However, in this study, the highest antioxidant activity is not ensured by the extracts with the highest bioactive compound contents.

3.3.3 Ferric-reducing antioxidant power (FRAP)

The FRAP assay assesses the antioxidant potential of foods by measuring their ion-reducing capacity (Djoueudam et al. 2019). This capacity is attributed to the bioactive compounds present in the food (Ramamurthy et al. 2012). In this context, the concentrations of *S. torvum* leaf powder extract needed to reduce iron by 50% (Table 2). The data revealed that all extracts possess a reducing capacity for Fe^{3+} ions, which significantly varies across different particle size fractions. This observation corroborates the antioxidant potency of the bioactive compounds in the powder (Kumar and Pandey 2013). However, the fraction of 125-200 µm and the powder do not exhibit a significant difference in their $Fe^{3\pm}$ reducing concentration, and this fraction possesses 1.27 times less antioxidant power (23.00 ± 1.04 µg/mL extract) compared to vitamin C (18.00 ± 1.00 µg/mL extract). Several studies have identified flavonoids as the primary bioactive compounds responsible for Fe^{3+} reduction (Makkar et al. 2007; Kumar and Pandey 2013; Prithvira 2019). The average flavonoid content in the extracts of the 125-200 µm fraction (11.62 ± 0.20 mg EQ/100 mL extract) and powders (10.97 ± 0.75 mg EQ/100 mL extract) does not show a significant difference (Table 1). This further confirms that the antioxidant capacity of the studied extracts depends not only on the accessibility of the bioactive compounds but also on their concentration in the extract, even if it is low.

3.4 Relationship between bioactive compound content, their antioxidant activity, and grouping of *S.torvum* leaf powder extracts

Data analyzed from *S. torvum* leaf powder extracts revealed a weak correlation between the content of bioactive compounds and antioxidant activity. However, a multivariate analysis will facilitate a comprehensive understanding of the interactions among these variables, thereby enabling a more accurate characterization of the powder extracts. A principal component analysis was conducted (Figure 2). The first principal component (F1) accounted for 60.90% of the observed variations among variables, while the second principal component (F2) accounted for 15.28%.

The correlation circle (Figure 2a) identifies two major groups of variables characterized by positive correlations. The first group comprises total polyphenols, total flavonoids, DPPH, and FRAP, while the second group includes condensed tannins, ABTS, and

particle mass. These two primary groups indicate that the antiradical/antioxidant activity (DPPH), more than the Fe^{3+} reducing power (FRAP), is primarily attributed to flavonoids among the analyzed polyphenols. Condensed tannins contribute minimally to the antioxidant activity (ABTS). However, the mass of the particles containing them should be considered.

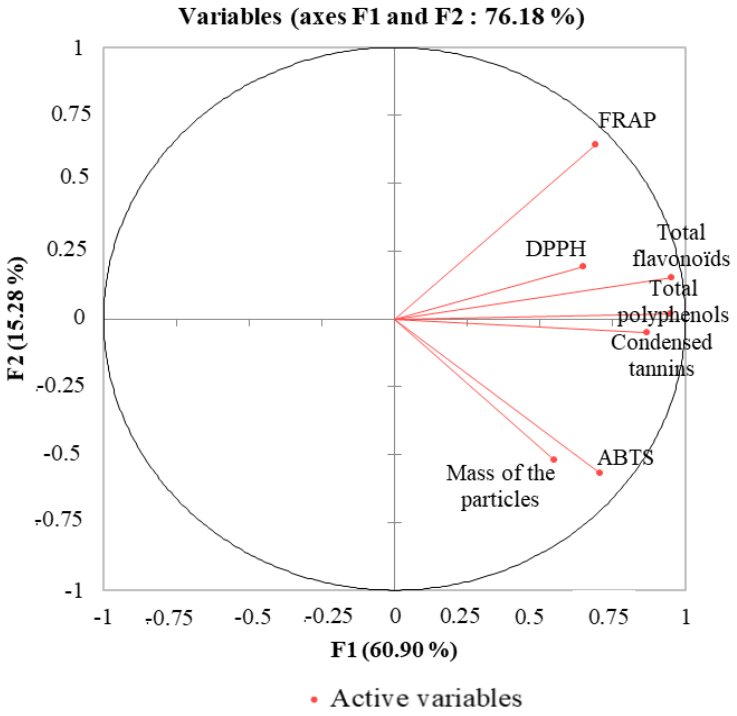

Figure 2a Correlations between the mass of the particles, bioactive compounds contents and antioxidant activities

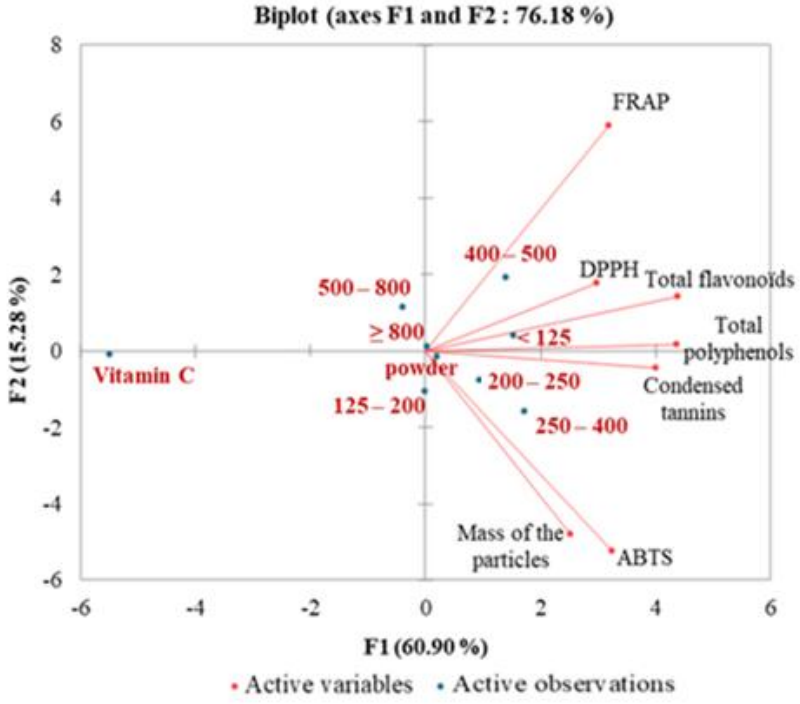

Figure 2b Regrouping *S. torvum* leaves powder extracts according to the mass of particle size fractions, bioactive compounds contents, and their antioxidant activities

Table 4 Antibacterial activities of extracts from *S. torvum* leaf powder and its particle size fractions

S. torvum leaves powders	Parameters	Bacteria								
		PA	SA	EC	KP	SF	SD	Stm	ST	SE
Powder (Ø < 1000 µm)	MIC (µg/mL)	>500	>500	>500	>500	>500	>500	250	250	>500
	MBC (µg/mL)	ND	ND	ND	ND	ND	ND	500	500	ND
	MBC/MIC	ND	ND	ND	ND	ND	ND	2	2	ND
Particle size fraction (µm)										
≥ 800	MIC (µg/mL)	>500	250	>500	>500	>500	>500	>500	>500	>500
	MBC (µg/mL)	ND	500	ND	ND	ND	ND	ND	ND	ND
	MBC/MIC	ND	2	ND	ND	ND	ND	ND	ND	ND
500-800	MIC (µg/mL)	250	>500	>500	250	>500	250	>500	>500	>500
	MBC (µg/mL)	500	ND	ND	500	ND	500	ND	ND	ND
	MBC/MIC	2	ND	ND	2	ND	2	ND	ND	ND
400-500	MIC (µg/mL)	500	500	500	500	500	250	500	500	>500
	MBC (µg/mL)	>500	>500	>500	>500	>500	500	>500	>500	ND
	MBC/MIC	ND	ND	ND	ND	ND	2	ND	ND	ND
250-400	MIC (µg/mL)	500	500	250	500	500	>500	500	500	>500
	MBC (µg/mL)	>500	>500	500	>500	>500	ND	>500	>500	ND
	MBC/MIC	ND	ND	2	ND	ND	ND	ND	ND	ND
200-250	MIC (µg/mL)	>500	>500	>500	>500	>500	>500	>500	>500	>500
	MBC (µg/mL)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND	ND
125-200	MIC (µg/mL)	>500	>500	>500	>500	>500	>500	>500	>500	>500
	MBC (µg/mL)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND	ND
< 125	MIC (µg/mL)	>500	>500	>500	>500	>500	>500	>500	>500	>500
	MBC (µg/mL)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND	ND
*Amoxicilline	MIC (µg/mL)	/	0.5	/	/	/	/	/	/	/
	MBC (µg/mL)		1							
	MBC/MIC		2							
*Ciprofloxacine	MIC (µg/mL)	1	/	0.25	0.5	0.25	0.5	0.5	0.5	0.5
	MBC (µg/mL)	4		1	2	2	1	2	1	2
	MBC/MIC	4		4	4	0.125	2	4	2	4

PA: *Pseudomonas aeruginosa* (ATCC10145), SA: *Staphylococcus aureus* (ATCC25922), EC: *Escherishia coli* (ATCC10536), KP: *Klebsiella pneumonae* (ATCC13883), SF: *Shigella flexineri* (NR518), SD : *Shigella dysenteria* (CPC), Stm : *Salmonella typhimurium* (CPC), ST: *Salomonella typhi* (ATCC6539), SE: *Salmonella enteritidis* (ATCC13076), ND: Not Determined, MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, MBC/MIC: Determines the bactericidal (MBC/MIC ≤ 4) or Bacteriostatic (MBC/MIC > 4) effects of the extracts, 0 ≤ MIC< 10µg/mL: Very good activity, 10 ≤ MIC< 125µg/mL: Good activity, 125 ≤ MIC< 250µg/mL: Significant activity, 250≤ MIC<500µg/mL: Moderate activity, MIC> 500µg/mL: Low activity (Kuete et al. 2010), *Standard

Table 5 Antifungal activities of extracts from *S. torvum* leaf powder and its particle size fractions

Solanumtorvum leaves powders extract	Parameters	Yeast				Dermatophyte		
		CA	CK	CP	CN	TM	MA	EF
Powder(Ø < 1000 µm)	MIC (µg/mL)	>500	>500	>500	>500	>500	>500	>500
	MFC (µg/mL)	ND	ND	ND	ND	ND	ND	ND
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
Particle size fractions (µm)								
≥ 800	MIC (µg/mL)	500	500	250	250	250	>500	>500
	MFC (µg/mL)	>500	>500	500	500	500	ND	ND
	MFC/MIC	ND	ND	2	2	2	ND	ND
500-800	MIC (µg/mL)	500	>500	>500	500	250	>500	500
	MFC (µg/mL)	>500	ND	ND	>500	500	ND	>500
	MFC/MIC	ND	ND	ND	ND	2	ND	ND
400-500	MIC (µg/mL)	>500	>500	>500	>500	>500	>500	>500
	MFC (µg/mL)	ND	ND	ND	ND	ND	ND	ND
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
250-400	MIC (µg/mL)	>500	>500	>500	>500	>500	>500	>500
	MFC (µg/mL)	ND	ND	ND	ND	ND	ND	ND
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
200-250	MIC (µg/mL)	>500	>500	>500	>500	125	>500	>500
	MFC (µg/mL)	ND	ND	ND	ND	250	ND	ND
	MFC/MIC	ND	ND	ND	ND	2	ND	ND
125-200	MIC (µg/mL)	>500	>500	250	250	>500	>500	>500
	MFC (µg/mL)	ND	ND	500	500	ND	ND	ND
	MFC/MIC	ND	ND	2	2	ND	ND	ND
< 125	MIC (µg/mL)	>500	>500	>500	>500	>500	>500	>500
	MFC (µg/mL)	ND	ND	ND	ND	ND	ND	ND
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
*Nystatine	MIC (µg/mL)	0.5	0.25	1	0.25	/	/	/
	MFC (µg/mL)	2	1	2	1			
	MFC/MIC	4	0.25	2	0.25			
*Griseofulvine	MIC (µg/mL)	/	/	/	/	0.25	1	0.5
	MFC (µg/mL)					1	2	2
	MFC/MIC					0.25	2	4

CA: *Candida albicans* (CPC), CK: *Candida krusei* (CPC), CP: *Candida parasilosis* (CPC), CN: *Cryptococcus neoformans* (CPC), TM: *Trichophyton mentagrophytes* (CPC), MA: *Microsporium audouinii* (CPC), EF: *Epidermophyton flocosum* (CPC), ND: Not Determined, MIC = Minimum Inhibitory Concentration, MFC : Minimum Fungicidal Concentration, MFC/MIC: Determines the bactericidal (MBC/MIC ≤ 4) or Bacteriostatic (MFC/MIC > 4) effects of the extracts, 0 ≤ MIC< 10µg/mL: Very good activity, 10 ≤ MIC< 125µg/mL: Good activity, 125 ≤ MIC< 250µg/mL: Significant activity, 250≤ MIC<500µg/mL: Moderate activity, MIC> 500µg/mL: Low activity (Kuete et al. 2010), *Standard

When these two major groups are plotted on the F1xF2 factorial plane, these accounts for 76.18% (Figure 2b) of the interactions between these variables (bioactive compounds and antioxidant activities) and the factors (powder extracts), four groups of powder extracts can be distinguished: 400-500 and < 125, 200-250 and 250-400, 125-200, and 500-800 µm. All these groups are characterized by antioxidant activities due to bioactive compounds in all extracts, which become accessible after extraction. Among these four, only the 125-200 µm and 500-800 µm fractions exhibit the highest antioxidant activities, comparable to that of vitamin C, the reference antioxidant. The antiradical/antioxidant (DPPH) and ion-reducing (FRAP) activities of the 500-800 µm fraction are attributed to their average flavonoid content. The antioxidant activity (ABTS) of the 125-200 µm fraction is weak due to condensed tannins. The fraction ≥ 800 µm and powder are underrepresented on the F1xF2 factorial plane. This suggests that when the content of bioactive compounds is low in a plant powder, it would be beneficial to fractionate it into finer particles. This would enhance the accessibility and content of bioactive compounds, thereby improving biological activities.

3.5 Antimicrobial activities and influence of bioactive compound contents of powder and its particle size fractions of *S. torvum* leaf extracts

3.5.1 Antibacterial activities

The antibacterial properties of *S. torvum* leaf powder extracts were assessed against gram-positive bacterial strains and clinical bacterial isolates obtained from the Centre Pasteur du Cameroun (CPC). These include *Pseudomonas aeruginosa* (PA: ATCC10145), *Staphylococcus aureus* (SA: ATCC25922), *Escherichia coli* (EC: ATCC10536), *Klebsiella pneumoniae* (KP: ATCC13883), *Shigella flexneri* (SF: NR518), *Shigella dysenteriae* (SD: CPC), *Salmonella typhimurium* (Stm: CPC), *Salmonella typhi* (ST: ATCC6539), and *Salmonella enteritidis* (SE: ATCC13076). The microdilution broth susceptibility assay was employed for this purpose, as noted by Newton et al.(2002).

The results indicate that all powder extracts exhibit antibacterial activity, ranging from moderate (250 ≤ MIC < 500µg/mL) to low (MIC > 500µg/mL) (Table 4) (Kuete et al. 2010). The powder and the 500-800 µm fraction demonstrated bactericidal activity against two (Stm, ST) and three (PA, KP, SD) bacteria strains, respectively. This antibacterial activity is attributed to phenolic compounds in the *S. torvum* leaf powder extracts (Acharyya and Khatun 2018; Senizza et al. 2021), which are made more accessible by particle size distribution. It is observed that even a low content of phenolic compounds is sufficient to exhibit antibacterial activity, as these activities are observed at micro concentrations of the extracts. Consequently, the obtained polyphenol contents are adequate. However, the observed weak antibacterial activities can be attributed to the lipophilic nature of all the isolates, which hinders their entry into the hydrophilic periplasmic space of bacterial strains of isolates (Dongmo et al. 2023). These results affirm that *S. torvum* leaves are a valuable source of bioactive compounds used in traditional medicine for treating infectious diseases (Loganayaki et al. 2010; Senizza et al. 2021; Rajapaksha and Premathilake 2020; Jan et al. 2024).

3.5.2 Antifungal activities

The antifungal properties of *S. torvum* leaf powder extracts are presented in Table 5. These extracts were evaluated against clinical fungal isolates obtained from the Centre Pasteur du Cameroun (CPC), including *Candida albicans* (CA: CPC), *C. krusei* (CK: CPC), *C. parasilosis* (CP: CPC), *Cryptococcus neoformans* (CN: CPC), *Trichophyton mentagrophytes* (TM: CPC), *Microsporium audouinii* (MA: CPC), and *Epidermophyton flocosum* (EF: CPC).

All powder extracts showed antifungal activities ranging from significant minimum inhibitory concentrations (125 ≤ MIC < 250µg/mL) to low minimum inhibitory concentrations (MIC > 500µg/mL). The powder extracts demonstrated low activity (MIC > 500 µg/mL) against all studied fungal strains. The fraction 125-200 µm showed moderate activities (MIC = 250 µg/mL) against two yeast strains (CP and CN), while the fraction 200-250 µm fraction exhibited significant activity (MIC = 125 µg/mL) against the dermatophyte, and the ≥ 800 µm fraction displayed moderate activities against the two yeasts (CP and CN) and the dermatophyte. The activities of these fractions are fungicidal. Bioactive compounds (total polyphenols, flavonoids, and condensed tannins), made more accessible in the extracts studied after particle size distribution, may explain these results (Kumar and Pandey 2013; Senizza et al. 2021). Interestingly, it was not the extracts from the particle size fractions with the highest contents of bioactive compounds that produced the most significant results. However, the weak solubility of isolates in polar solvents can also account for the observed low activities. These results justify using these leaves as a medicinal plant for treating certain skin conditions and gastric diseases.

Conclusion

Evaluating the bioactive compound contents in leaf powder extracts, their particle size fractions, and their impact on biological activities leads to the following conclusions. When the content of bioactive compounds is low in a plant powder, such as the *S. torvum* leaf powder, which is considered of little value in terms of its biological activity, one effective method to enhance these activities is to proceed with the fractionation of the powders. This process improves the accessibility of bioactive compounds (polyphenols, flavonoids, condensed tannins) and increases their content in the powder extracts, particularly in the extracts of the

finest particle-size fractions. This enhancement has specific positive effects on their antioxidant (DPPH, ABTS, FRAP), antibacterial (*S. typhimurium, S. typhi, P. aeruginosa, K. pneumoniae, S. dysenteriae*), and antifungal (*C. parapsilosis, C. neoformans, T. mentagrophytes*) activities. Flavonoids appear to be the primary bioactive compounds responsible for these activities. The mass of the particle size fraction must be considered. This method of processing powder suggests that no plant powder should be disregarded or rejected due to its low content of bioactive compounds.

Author's contribution

ASSIENE AGAMOU Julien Armel: Conceptualization, Investigation, Methodology, Data Analysis, Writing-Original draft, Writing-review and editing; DJEUKEU ASONGNI William: Methodology; Data analysis, Review original draft; ASSIENE OYONG Damase Serge: Methodology, Data Analysis, Review original draft TIZE Zra: Methodology, Data analysis; OBONO NDONG Tatiana Léa, MBANGO EKE Pauline, MBOUBE NGONGANG Oriane, KEUBING FEUDJIO Suzanne Rose: Methodology; FONGNZOSSIE FEDOUNG Evariste: Supervision, Methodology, Review original draft

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Declaration of interest

The authors declare that they have no conflict of interest.

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