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## Study of the antioxidant potential, polyphenol content, and mineral composition of *Cordyla pinnata*, a plant for food and medicinal use of the Senegalese pharmacopoeia

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### RESEARCH ARTICLE

### ABSTRACT



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Oxidative stress, caused by reactive oxygen species, is known to cause oxidation of biomolecules, leading to cell damage and oxidation of important enzymes, resulting in an unstable pathophysiological state. The antioxidant capacity of leaves, stems, and roots of *Cordyla pinnata* was determined by measuring the inhibition of the absorbance of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals by spectrophotometry. The polyphenol contents were determined with the same technique. The mineral contents were evaluated by atomic absorption spectrometry. The strongest inhibition of the DPPH radical after that of the control antioxidant ( $IC_{50} = 0.014$  mg/mL) was obtained with the ethyl acetate fraction of the leaf hydroethanolic extract ( $IC_{50} = 0.201$  mg/mL). For ABTS, the ethyl acetate fraction of the stem extract was more active ( $IC_{50} = 0.884 \times 10^{-3}$  mg/mL) than the other extracts and ascorbic acid ( $IC_{50} = 0.915 \times 10^{-3}$  mg/mL). The polyphenol content of the leaves, stems and roots extracts is between 66.33 and 142.67; 55.33 and 69.33; 67.67 and 116.00 EAT/g of dry extract, respectively. The contents of Fe, Na, Zn, K, Mg, and Ca are 0.0005, 0.0006, 0.0020, 0.0897, 0.0247, and 0.0273% for leaves, respectively. They are 0.0001, 0.0010, 0.0015, 0.0557, 0.0131, and 0.1357% for the stems, respectively. The mineral contents of the roots in the same order are 0.0002, 0.0013, 0.0013, 0.0140, 0.0096, and 0.0267%. Strong inhibition of free radicals and the chemical composition of various plant materials would justify the use of *C. pinnata* in the management of certain pathologies and nutritional deficiencies.

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### 1. Introduction

Oxidative stress, caused by reactive oxygen species (ROS), is known to cause oxidation of biomolecules leading to cell damage and oxidation of important enzymes resulting in an unstable pathophysiological state [1]. In fact, type-II diabetes, atherosclerosis, obesity, cancer, infectious diseases, and aging are physiological states responsible for an increase in the production of free radicals [2,3].

For several years, immunosuppressive treatments, broad-spectrum antibiotic therapy, or even soothing radiotherapy prescribed in pathologies have become considerable with many adverse effects [4]. In order to provide practitioners with new treatment options, researchers are trying to explore other more natural means, including herbal medicines. Therefore, many active ingredients with new biological activities are isolated from plants [5].

In recent years, medicinal plants have become increasingly present in global environment and development policy. Their use and preservation are cross-sectoral themes encompassing

health care, nature and biodiversity protection, economic promotion, trade, and various legal aspects [6-8].

Today, a large part of the world's population uses and depends on traditional medicine, and therefore on the use of plants and plant extracts. This is especially the case with local and indigenous populations in developing countries. This is because herbal medicines are not only cheaper than modern medicines; they are often the only ones available in remote areas [9].

In modern and traditional medicine, the use of antioxidant remedies is increasingly on the roadmap of practitioners to prevent the occurrence of reactive species in patients. Indeed, plants have secondary metabolites such as polyphenols, which constitute a very wide family of molecules reputed to have antioxidant capacity [10]. However, some minerals, which are not antioxidants, are redox catalysts of enzymes, which are the first line of defense against oxidative attacks. Therefore, their intake through food helps to maintain the oxidative balance [11].

This present study focused on the plant species found in xerophilic environments, *Cordyla pinnata* (Caesalpiniaceae), used in traditional medicine in the treatment of some pathologies and infections such as intestinal parasitosis, stomach pains, and diabetes. *Cordyla pinnata* is distributed mainly in Senegal, Niger, North of Nigeria, Togo, and northern Cameroon [12]. The study of its chemical composition revealed the presence of cordylasins A and B, mildbraedin in the extracts of its leaves. Cordylasins A and B are flavonol pentaglycosides. They are also present in other *Cordyla* species: *C. haraka* and *C. richardii* [13]. Mildbraedin has also been isolated from the leaves of *Mildbraediodendron excelsum* [14], of which it is the main constituent. Mildbraedin has also been detected in plants of *Cordyla* species. It is a minor component in *C. haraka* and *C. richardii* and the major component of *C. somalensis*. It is a kaempferol tetraglycoside [13]. Their biological activities have not been reported. Dieye et al. [15] also described for the first time the antimicrobial activity of *Cordyla pinnata* extracts in *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* with minimum inhibitory concentrations (MIC) of up to 1.302±0.451 mg/mL. Chromatographic profiling also showed the presence of several compounds. The purpose of this work is to contribute to the study of the antioxidant properties of *Cordyla pinnata* extracts and its composition in mineral elements.

## 2. Experimental

### 2.1. Material

#### 2.1.1. Plant material

The plant material consisted of *Cordyla pinnata* (Leaves, stems bark, and roots bark). The species was collected in Senegal at the end of June 2019 in Touba Diagl  in the rural community of Khelcom Birame (14° 29'21.3 "N 16° 04'53.2" W) of the Kaolack region. Drying was carried out at room temperature (25 °C), in the shade, in a ventilated room. A herbarium (Code: IFAN; number: 62942) has been deposited at the Fundamental Institute of Black Africa (IFAN). After drying, the leaves, stem bark, and root bark were ground separately with a mortar, then with an electric mill (Retsch KG, West-Germany). The powders obtained were stored in amber bottles, which were then kept protected from light and at room temperature.

#### 2.1.2. Reagents and solvents

DPPH, ABTS, Folin-Ciocalteu reagent, persulfate potassium, and ethyl acetate were supplied by Sigma-Aldrich (France). Ascorbic acid, tannic acid, and butanol were obtained, respectively, from UCB Pharma (Belgium), Merck (Germany), and Prolabo (Switzerland). The analytical grade 95° ethanol comes from the company Valdafrique (Senegal).

#### 2.1.3. Equipment

The absorbance measurements were carried out using a UV/Vis spectrophotometer (Thermo Fisher Scientific Evolution 300, Madison, Switzerland). The minerals were assayed by an atomic absorption spectrophotometer (ICE 3300 FL AA system, Thermo Scientific, China).

## 2.2. Methods

### 2.2.1. Extraction method

#### 2.2.1.1. Crude extraction

A quantity of 100 g of each powder was macerated in 1 L of ethanol 95°-water solvent (1:1, v:v) for three days at room temperature (25 °C). The homogenates obtained were successively filtered on cotton, then on Whatman filter paper. The filtrates are then concentrated to dryness using a rotary evaporator (Büchi R-124, Switzerland) at a temperature below 40 °C and at low pressure, to obtain the crude extracts [15].

#### 2.2.1.2. Liquid-liquid extraction

A 3 g sample of each crude extract, dissolved in pure water, was extracted three times with ethyl acetate. The combined acetate phases were evaporated to form the ethyl acetate fraction. The residual aqueous phase was extracted with butanol three times. The residual aqueous phase, as well as the butanol phase were evaporated separately to obtain a butanol fraction and an aqueous fraction [15].

### 2.2.2. Measurement of antioxidant capacity

Methods used to measure antioxidant capacity by inhibiting 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) are those described by Sarr et al. [16] with some modifications. The measurements were carried out for a range of concentration of each of the extracts. In fact, the partially soluble dry extracts in ethanol were dissolved in aqueous ethanol (1:1, v:v).

#### 2.2.2.1. Measurement of antioxidant capacity by the DPPH assays

A quantity of 4 mg of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) powder was dissolved in 100 mL of ethanol and the resulting solution was stored in the dark for 12 h. In each test tube containing 0.8 mL of a hydro-ethanolic solution (1:1, v:v) of the test extract, 3.2 mL of the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) solution were added. Ascorbic acid, used as a control, was also tested under the same conditions. The absorbance reading was taken after 30 minutes of incubation at room temperature (25 °C) on a spectrophotometer at 517 nm using the water-ethanol solvent mixture (1:9, v:v) as a blank. Three absorbance measurements were taken for each concentration tested (n = 3).

#### 2.2.2.2. Measurement of antioxidant capacity by ABTS assays

A quantity of 38.40 mg of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was previously dissolved in 10 mL of water before adding 6.75 mg of persulfate potassium. The resulting mixture was stored in the dark and at room temperature for 12 hours before use. This solution was diluted by mixing of ethanol to obtain an absorbance of 0.70 at 734 nm. The antioxidant capacity was measured by adding 2 mL of a hydro-ethanolic solution (1:1, v:v) of the extract to be tested to 2 mL of the ABTS solution. The extracts were tested at different concentrations. Ascorbic acid, used as a control, was tested under the same conditions. The absorbance reading was taken after 2 minutes of incubation at room temperature (25 °C) on a spectrophotometer at 734 nm using the ethanol-water mixture (3:1, v:v) as a blank. Three absorbance measurements were taken for each concentration tested (n = 3).

#### 2.2.2.3. Calculation of the percent inhibition

The method for calculating percentages of inhibition (PI) was recently described by Sarr et al. [16]. The percentage inhibition (PI) of the absorbance of the radical corresponds to:

$$PI = [(A_0 - A_1) \times 100] / A_0 \quad (1)$$

**Table 1.** Inhibition of radicals by leaf extract and its fractions.

Extracts	IC <sub>50</sub>	
	DPPH (mg/mL)	ABTS (×10 <sup>-3</sup> mg/mL)
Aqueous fraction	0.450±0.009	15.667±0.577
Ethyl acetate fraction	0.201±0.010	78.667±2.517
Butanol fraction	0.273±0.009	27.000±0.000
Crude extract	0.297±0.009	12.667±1.154
Ascorbic acid	0.014±0.000	0.915±0.000

**Table 2.** Inhibition of radicals by stem extract and its fractions.

Extracts	IC <sub>50</sub>	
	DPPH (mg/mL)	ABTS (×10 <sup>-3</sup> mg/mL)
Aqueous fraction	>3	91.063±6.386
Ethyl acetate fraction	0.805±0.001	0.884±0.251 <sup>a</sup>
Butanol fraction	0.839±0.004	18.000±1.751 <sup>b</sup>
Crude extract	0.945±0.000	18.510±2.462 <sup>b</sup>
Ascorbic acid	0.014±0.000	0.915±0.000 <sup>a</sup>

<sup>a</sup> There are no significant difference.<sup>b</sup> There are no significant difference.**Table 3.** Inhibition of radicals by root extract and its fractions.

Extracts	IC <sub>50</sub>	
	DPPH (mg/mL)	ABTS (×10 <sup>-3</sup> mg/mL)
Aqueous fraction	1.136±0.007	32.062±1.086
Ethyl acetate fraction	0.448±0.004	5.303±0.151
Butanol fraction	0.528±0.002	15.185±6.327 <sup>a</sup>
Crude extract	0.696±0.002	17.910±1.422 <sup>a</sup>
Ascorbic acid	0.014±0.000	0.915±0.000

<sup>a</sup> There are no significant difference.

A<sub>0</sub>: Absorbance of the solution of pure ABTS or DPPH; A<sub>1</sub>: Absorbance of the ABTS or DPPH solution after the addition of the extract was tested at an initial concentration and a given time.

#### 2.2.2.4. Determination of the IC<sub>50</sub>

The regression lines of inhibition (in percentage) of the absorbance of 2,2-diphenyl-1-picryl-hydrazyl and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) by extracts were plotted using Microsoft® Excel 2013. These made it possible to determine the concentrations inhibiting by half the absorbance of free radicals (IC<sub>50</sub>).

#### 2.2.3. Determination of total polyphenols

The concentration of extracts in total polyphenols is estimated by UV-visible absorption spectrophotometry using the Folin-Ciocalteu test [17]. The various extracts are dissolved before in aqueous ethanol (1:1, v:v). A quantity of 25 µL of each solution was taken and introduced into a tube. 125 µL of Folin-Ciocalteu reagent and 2 mL of distilled water were added to the tube. After standing for 5 minutes, 375 µL of sodium carbonate (10%) is added to the tube. The system is kept at 37 °C for 2 hours. The absorbance of the container of each tube was measured at the wavelength λ = 760 nm. The blank consists of 25 µL of ethanol, 125 µL of Folin-Ciocalteu reagent, 2 mL of distilled water and 375 µL of sodium carbonate (10 %). The concentrations of total polyphenols contained in the extracts are calculated by referring to the calibration curve obtained using tannic acid as a standard. Results are expressed in milligrams of equivalent tannic acid (mg EAT)/g of extract.

#### 2.2.4. Determination of minerals

##### 2.2.4.1. Mineral content measurement

Mineralization was performed using the method reported by Bennouna et al. [18]. A quantity of 0.5 g of each powder (Leaf powder, stem powder, and root powder) was separately mineralized with 2 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub> 95-97%), 6 mL of nitric acid (HNO<sub>3</sub> 37%) and 6 mL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>

50%). This mixture was heated at 100 °C for 30 minutes using a hot plate (Heat-stir UC 152, Stuart). After cooling, 25 mL of 0.1 M nitric acid was added, and the mixture was then filtered through Whatman paper. The filtrate obtained diluted to 1/10 was analyzed by an atomic absorption spectrophotometer (AAS). The mineral contents were evaluated as a percentage (%).

##### 2.2.4.2. Calculation of mineral content (%)

The method of calculating the percentages of minerals (MP) is as follows:

$$MP = [C_R \times (V_1 + V_2 + V_3 + V_4) \times 10/m_p] \times 100 \quad (2)$$

C<sub>R</sub>: Concentration read with atomic absorption spectrophotometer; V<sub>1</sub>: 2 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub> 95-97%); V<sub>2</sub>: 6 mL of nitric acid (37%); V<sub>3</sub>: 6 mL of hydrogen peroxide (50%); V<sub>4</sub>: 25 mL of 0.1 M nitric acid; m<sub>p</sub>: 0.5 g of plant powder.

##### 2.2.5. Statistical analysis

The statistical analysis of the results obtained was carried out with Microsoft® Excel 2013. For each test, three repetitions were performed. The results are presented in the form of mean value±standard deviation. For each set of samples, analysis of variance (ANOVA) was performed; and the p-value was obtained. The difference is considered significant, if the p value is less than 0.05. If two samples showed close results, a student test was performed for these two samples. A no significant difference is materialized by a letter (a and b) indicated in exponent on the mean value±standard deviation.

### 3. Results

#### 3.1. Measurement of antioxidant capacity

The results of the measurement of the antioxidant capacity of crude hydroalcoholic extracts and fractions of *C. pinnata* are presented in Tables 1-3.

**Table 4.** Total polyphenol content of crude hydroethanolic extracts and fractions of leaves, stems, and roots

Extracts	Total polyphenol content (mg EAT/g extract)		
	Leaf	Stem	Root
Aqueous fraction	66.33±10.11	64.00±17.78 <sup>b</sup>	67.67±6.11
Ethyl acetate fraction	142.67±10.97	69.33±1.53 <sup>b</sup>	116.00±12.49
Butanol fraction	87.00±9.00 <sup>a</sup>	59.00±20.42 <sup>b</sup>	80.33±10.21
Crude extract	89.67±7.50 <sup>a</sup>	55.33±12.50 <sup>b</sup>	91.33±8.96

<sup>a</sup> There are no significant difference.

<sup>b</sup> There are no significant difference.

**Table 5.** Mineral elements content (%).

Plant material	Mineral content (%)					
	Iron	Sodium	Zinc	Potassium	Magnesium	Calcium
Leaves	0.0005	0.0006	0.0020	0.0897	0.0247	0.0273 <sup>a</sup>
Stems	0.0001	0.0010	0.0015	0.0557	0.0131	0.1357
Roots	0.0001	0.0013	0.0013	0.0140	0.0096	0.0227 <sup>a</sup>

<sup>a</sup> No significant difference.

Statistical analysis of the IC<sub>50</sub> values obtained showed a significant difference, respectively, for the inhibition of ABTS (p = 3.785×10<sup>-14</sup>) and DPPH (p = 8.122×10<sup>-9</sup>) by leaf crude extract and its fractions (Table 1). However, the antioxidant reference showed higher capacity than that of the crude extract and its fractions (with an IC<sub>50</sub> of 0.014±0.000 mg/mL against DPPH and 0.915±0.000×10<sup>-3</sup> mg/mL against ABTS). Regarding DPPH, the ethyl acetate fraction was more active (IC<sub>50</sub> = 0.201±0.010 mg/mL), followed by the butanol fraction (IC<sub>50</sub> = 0.273±0.009 mg/mL), then the total extract (IC<sub>50</sub> = 0.297±0.009 mg/mL). The aqueous fraction remained the least active (IC<sub>50</sub> = 0.450±0.009 mg/mL).

The results in Table 1 also indicate a significant variation in the inhibition of ABTS depending on the leaf extract and its fractions. The total extract (12.667×10<sup>-3</sup>±1.154×10<sup>-3</sup> mg/mL) and the aqueous fraction (15.667×10<sup>-3</sup>±0.577×10<sup>-3</sup> mg/mL) were the most active. The IC<sub>50</sub> for the butanol fraction was (27.000×10<sup>-3</sup>±0.000×10<sup>-3</sup> mg/mL). The ethyl acetate fraction showed the lowest capacity (78.667×10<sup>-3</sup>±2.517×10<sup>-3</sup> mg/mL) against ABTS compared to other leaf extracts.

Table 2 shows the IC<sub>50</sub> values of DPPH and ABTS inhibition by stem extract and its fractions. A variation of the same order did note with the inhibition of DPPH depending on the nature of the extract as with the leaf extracts. The ethyl acetate fraction was more active with an IC<sub>50</sub> value of 0.805±0.001 mg/mL, followed by the butanol fraction (0.839±0.004 mg/mL), then the total extract (0.945±0.000 mg/mL). The aqueous fraction was less active with an IC<sub>50</sub> value greater than (> 3 mg/mL) towards DPPH. Analysis of variance of these results showed a significant difference (p = 9.617×10<sup>-10</sup>).

ABTS radical inhibition tests showed IC<sub>50</sub> values (Table 2) of the ethyl acetate fraction (0.884×10<sup>-3</sup>±0.251×10<sup>-3</sup> mg/mL) and the control antioxidant (0.915×10<sup>-3</sup>±0.000×10<sup>-3</sup> mg/mL) with a no significant difference (p = 0.845). No significant differences (p = 0.785) are also obtained between the butanol fraction (18.000×10<sup>-3</sup>±1.751×10<sup>-3</sup> mg/mL) and the total extract (18.510×10<sup>-3</sup>±2.462×10<sup>-3</sup> mg/mL) less active than those cited previously. The aqueous fraction (91.063×10<sup>-3</sup>±6.386×10<sup>-3</sup> mg/mL) remained the least active.

Table 3 shows the results of the antioxidant capacity of the root extracts. However, the evolution of antioxidant potential *in vitro* (for DPPH and ABTS) depending on the nature of the extract remains the same as with stem extracts. For inhibition of DPPH (p = 7.583×10<sup>-6</sup>), the IC<sub>50</sub> values were 1.136±0.007, 0.448±0.004, 0.528±0.002, and 0.696±0.002 mg/mL, respectively, for the aqueous fraction, the ethyl acetate fraction, the butanol fraction and the total extract. The ethyl acetate fraction inhibited ABTS more strongly with an IC<sub>50</sub> of 5.303×10<sup>-3</sup>±0.151×10<sup>-3</sup> mg/mL. The IC<sub>50</sub> values of the butanol fraction (15.185×10<sup>-3</sup>±6.327×10<sup>-3</sup> mg/mL) and the total extract (17.910×10<sup>-3</sup>±1.422×10<sup>-3</sup> mg/mL) were not significantly different (p = 0.507). The aqueous fraction had a lower antioxi-

dant potential with an IC<sub>50</sub> value of 32.062×10<sup>-3</sup>±1.086×10<sup>-3</sup> mg/mL.

### 3.2. Total polyphenol content

Table 4 presented the total polyphenol contents of crude hydro-ethanolic extracts and fractions of leaves, stems, and roots, respectively.

The results showed a higher total polyphenol content of the ethyl acetate fraction (142.67 mg EAT/g of extract) in the leaves (Table 4). Comparison of the butanol fraction (87.00 mg EAT/g of extract) and the total extract (89.67 mg EAT/g of extract) showed no significant differences (p = 0.713). The aqueous fraction showed the lowest polyphenol composition (66.33 mg EAT/g of extract).

Concerning stems (Table 4), the statistical analysis of the total polyphenol content of the aqueous fraction (64.00 mg EAT/g of extract), of the ethyl acetate fraction (69.33 mg EAT/g of extract), of the butanol fraction (59.00 mg EAT/g of extract) and of the total extract (55.33 mg EAT/g of extract) did not show significant differences (p = 0.693).

The analysis of the roots (Table 4) showed a significant difference (p = 0.002) with a higher content in the ethyl acetate fraction (116.00 mg EAT/g of extract), followed by the crude extract (91.33 mg EAT/g of extract). The butanol fraction (80.33 mg EAT/g of extract) did the lowest polyphenol content, after the aqueous fraction (67.67 mg EAT/g of extract).

### 3.3. Mineral content

The iron, sodium, zinc, potassium, magnesium, and calcium content of leaves, stems, and roots is presented in Table 5. Statistical analysis showed a significant difference (p = 1.981×10<sup>-29</sup>) with a higher iron content in leaves (0.0005 %) than in stems (0.0001 %) and roots (0.0002 %). However, atomic absorption spectrometry cannot distinguish Fe<sup>2+</sup> from Fe<sup>3+</sup>.

A significant difference (p = 1.163×10<sup>-6</sup>) was also found for the quantification of sodium in leaves, stems, and roots (Table 5). The results showed a higher sodium level in the roots (0.0013%), followed by the stems (0.0010%). The amount of this was lower in the leaves (0.0006%).

The statistical analysis of the results obtained from the quantification of zinc in the three parts of the plant gave a p value of 3.862×10<sup>-5</sup>. However, the percentages of zinc were 0.0020, 0.0015, and 0.0013%, respectively, in leaves, stems, and roots (Table 5).

Statistical analysis of the data showed significant differences in the case of potassium and magnesium (Table 5) with p values of 3.702×10<sup>-12</sup> and 8.719×10<sup>-94</sup>, respectively. The composition of potassium and magnesium (Table 5) according to the plant materials evolved in the same way. The potassium and magnesium contents were higher in the leaves (respectively, 0.0897 and 0.0247%), and lower in the roots

(respectively, 0.0140 and 0.0096%). The stems were made of 0.0557% potassium and 0.0131% magnesium.

The amount of calcium in the stem (0.1357%) of *Cordyla pinnata* was significantly higher than that in the leaves (0.0273%) and roots (0.0267%). Analysis of variance gave a *p* value of  $4.855 \times 10^{-12}$  (Table 5). However, the comparison of calcium levels in leaves and roots did not show significant differences (*p* = 0.422).

## 4. Discussion

### 4.1. Antioxidant capacity

The plants contain many bioactive substances that exhibit antioxidant properties. Various plants such as *Berberis vulgaris* L., *Ptilostigma thonningii* [19,20] have been reported exhibiting antioxidant capacity. The antioxidant potential of plant extracts can be measured through inhibition of DPPH and inhibition of ABTS. The ABTS assay measures the relative ability of antioxidants to scavenge ABTS generated in the aqueous phase. ABTS is generated by reacting with a strong oxidizing agent (e.g., potassium permanganate or potassium persulfate) with the ABTS salt. The reduction of the blue-green ABTS radical by hydrogen-donating antioxidants is measured by suppression of its characteristic long-wave absorption spectrum [21]. The DPPH is a stable free radical with an absorption band at 515 nm. It loses this absorption when reduced (electron transfer) by an antioxidant or a free radical species. The DPPH method is widely used to determine antiradical/antioxidant activity of purified phenolic compounds as well as natural plant extracts [22]. The inhibition of ABTS by extracts of *C. pinnata* has not been previously reported according to the available literature.

The antioxidant capacity of ethanolic extracts of leaves and stems by inhibiting DPPH is reported by Fall *et al.* [23]. However, the extraction methods are different. To do this, a quantity of 30 g of powdered leaves of *C. pinnata* was decocted twice for 30 minutes using 300 mL of ethanol. The resulting extract was filtered through Whatman paper and concentrated under reduced pressure using a rotary evaporator to obtain a dry extract. The same process was used to obtain the trunk bark extract. Fall *et al.* [23] obtained  $IC_{50}$  values of  $0.33 \times 10^{-3} \pm 0.11 \times 10^{-3}$ ,  $19.53 \times 10^{-3} \pm 0.42 \times 10^{-3}$  and  $21.07 \times 10^{-3} \pm 0.11 \times 10^{-3}$  mg/mL for ascorbic acid, trunk bark extract and *C. pinnata* leaf extract. The  $IC_{50}$  value of ascorbic acid  $0.33 \times 10^{-3} \pm 0.11 \times 10^{-3}$  mg/mL obtained by these latter is twenty times lower than that presented by this study  $0.014 \pm 0.000$  mg/mL. This difference may be due to the use of water as a solvent with ethanol as described in the experimental part. However, this difference is smaller with the crude leaf extracts  $21.07 \times 10^{-3} \pm 0.11 \times 10^{-3}$  and  $0.297 \pm 0.009$  mg/mL, and larger with the stem's crude extracts  $19.53 \times 10^{-3} \pm 0.42 \times 10^{-3}$  and  $0.945 \pm 0.000$  mg/mL.

Antioxidant activity tests on DPPH with the aqueous extract and fractions (Aqueous, methanolic, ethyl acetate) of *Aristolochia longa* show more or less similar results [24]. The aqueous extract and the aqueous, methanolic and ethyl acetate fractions obtained  $IC_{50}$  values of 0.355, 0.125, 0.199, and 0.221 mg/mL, respectively. However, the authors used different DPPH concentration and proportions in extract or fraction solution.

Regarding the ABTS assays, Mahamat *et al.* [25] report  $IC_{50}$  values of 0.472, 0.350, and 0.474 mg/mL of hydromethanolic, hydroacetic and aqueous extracts of *Bauhinia rufescens*. These values are significantly higher than those of *C. pinnata* extracts even there is a difference in the preparation methods of ABTS solution.

### 4.2. Total polyphenol content

Plants have secondary metabolites such as polyphenols. The latter constitute a very widespread family of molecules known to have antioxidant capacity [10]. In the present study,

the contents of total polyphenols of *C. pinnata* were evaluated. The latter are more concentrated in leaf extracts (66.33 and 142.67 mg EAT/g) than in stem extracts (55.33 and 69.33 mg EAT/g) and root extracts (67.67 and 116.00 mg EAT/g). These results correlate with the DPPH inhibition assays for which the inhibition with the leaf extracts is greater.

The strongest inhibition of the DPPH radical after that of the control antioxidant ( $IC_{50} = 0.014 \pm 0.000$  mg/mL) is obtained with the ethyl acetate fraction of the hydroethanolic leaf extract ( $IC_{50} = 0.201 \pm 0.010$  mg/mL) with the highest polyphenol content. However, this fraction does not inhibit ABTS more strongly. Sarr *et al.* [16] described a similar situation, where *Vitex doniana* extracts strongly inhibit DPPH and weakly ABTS absorbances. They concluded that the extracts could contain compounds that have a characteristic absorption band at 734 nm just like ABTS. This would have camouflaged the latter's inhibition. For ABTS, the ethyl acetate fraction of the stem extract was more active ( $IC_{50} = 0.884 \times 10^{-3} \pm 0.251 \times 10^{-3}$  mg/mL) than the other extracts and ascorbic acid ( $IC_{50} = 0.915 \times 10^{-3} \pm 0.000 \times 10^{-3}$  mg/mL).

However, the extracts and fractions of *C. pinnata* (Leaves, stems, and roots) have high contents of polyphenols compared to the hydro-ethanolic extracts of the leaves and stems bark of *Ptilostigma thonningii* as reported by Dieng *et al.* [26]. These authors reported polyphenol contents of  $6.16 \pm 0.12$  mg EAT/g extract and  $3.12 \pm 0.08$  mg EAT/g extract of stems bark and leaves, respectively.

### 4.3. Mineral content

Minerals are redox catalysts of enzymes which constitute the first line of defense against oxidative attacks [11]. Iron is present in the body in two forms: heme iron ( $Fe^{2+}$ ) and non-heme iron ( $Fe^{3+}$ ). Heme iron helps oxygen transport to red blood cells and muscles. It also plays an essential role in the destruction of peroxides. Non-heme iron is involved in the action of certain enzymes, but remains more toxic than heme iron [27]. Sodium plays an important role in maintaining water balance and cell permeability [28]. Zinc is a cofactor for the good activity of some enzymes, thus intervening in many functions such as metabolism. It also protects the functional groups of proteins [29]. The systematic review of the literature carried out by Boyle *et al.* [30] suggests a beneficial effect of magnesium supplementation on anxiety and stress. This supplementation would also induce an improvement in the condition of patients with depression [31]. Potassium is an important part of the cell. Its deficiency can lead to sugar intolerance [28]. Calcium is involved in the regulation of the structure and function of skeletal muscles, the polymerization of fibrin and the conduction of impulses in the nervous system [32]. It also plays a messenger role in the insulin hormonal response, thus improving glucose tolerance [28].

Mineral levels have not been reported for all plant material (Leaves, stems, roots) of *Cordyla pinnata*. Mineral content has been reported for other plant species. Alyemini *et al.* [33] reported contents of 0.016, 0.0780, 0.3740, 0.1850, 0.0038 and 1.2000% of iron, sodium, potassium, magnesium, and calcium, respectively, in Sesame black Saudi cultivars. The contents of iron, sodium, potassium, magnesium and zinc are much higher than those presented in this study. However, the zinc content of *C. pinnata* in this study (0.0020, 0.0015, and 0.0013% for leaves, stems, and roots, respectively) is a little close to that of sesame (0.0038%) described by Alyemini *et al.* [33].

The daily mineral intake recommended by the Food and Agriculture Organization of the United Nations is less than 1300 mg/day, 260 mg/day, 1.68 mg/day and 514 mg/kg/day for calcium, magnesium, iron, and zinc [34]. These contents are accumulated in 0.958 kg of stem powder, 1.052, 0.336, and 25.700 kg of leaf powder, respectively. The amount of powder that contains the recommended daily intake of zinc remains

high, especially since it is evaluated in kilograms of body mass. Descaillot and Laville report that a potassium intake greater than 1.5 g/day is accompanied by lower cardiovascular mortality [35]. This contribution could be obtained by a mass of 1.672 kg of leaf powders. Descaillot and Laville also reported that consuming 2.4 g/day of sodium increases the risk of cardiovascular events [35].

## 5. Conclusions

This work showed that crude polar extract and fractions of *C. pinnata* strongly inhibit the DPPH and ABTS radicals. The strongest inhibition of the DPPH radical was obtained with the ethyl acetate fraction of the leaf extract ( $IC_{50} = 0.201 \pm 0.010$  mg/mL) after the control antioxidant ( $IC_{50} = 0.014 \pm 0.000$  mg/mL). For ABTS, the ethyl acetate fraction of the stem extract was more active ( $IC_{50} = 0.884 \times 10^{-3} \pm 0.251 \times 10^{-3}$  mg/mL) than the other extracts and ascorbic acid ( $IC_{50} = 0.915 \times 10^{-3} \pm 0.000 \times 10^{-3}$  mg/mL). The contents of polyphenols and mineral elements found in different plant materials reveal that this plant is an important source of micronutrients. These levels would justify the use of *C. pinnata* in the management of certain pathologies and nutritional deficiencies.

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Conflict of interests: The authors declare that they have no conflict of interest. Ethical approval: All ethical guidelines have been adhered. Sample availability: Samples of the compounds are available from the author.

## CRedit authorship contribution statement

Conceptualization: Serigne Omar Sarr; Methodology: Pape Issakha Dieye; Software: Pape Issakha Dieye; Validation: Serigne Omar Sarr; Formal Analysis: Pape Issakha Dieye, Thierno Mouhamed Wane, Elhaj Ousmane Faye; Data Curation: Pape Issakha Dieye; Writing - Original Draft: Pape Issakha Dieye; Writing - Review and Editing: Rokha Gueye, Bara Ndiaye, Yerim Mbagnick Diop; Supervision: Serigne Omar Sarr.

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