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Mineral Element and Antioxidant Potential of Four Plants Used in Traditional Medecine in Brazzaville-Congo

Mikala Grâce Raycelia Merveille [a], Kadja Amani Brice [b], Nkounkou Loumpangou Célestine [a*], Bonazaba Milandou Longin Justin Clair [a], Mamyrbekova-Bekro Janat Akhanovna [b], Bekro Yves-Alain [b] and Ouamba Jean-Maurille [a]

[a] Unité de Chimie du Végétal et de la Vie (UC2V), Faculté des Sciences et Techniques, Université Marien Ngouabi, Brazzaville, Congo.
[b] Laboratoire de Chimie Bio Organique et de Substances Naturelles (LCBOSN), UFR/ SFA, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: celestinenkounkou1@gmail.com;*

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ABSTRACT

Background: Plants represent an important natural resource of secondary metabolites with therapeutic properties and nutrients, including minerals, which contribute to the proper functioning and well-being of the human body.
Aims: Four medicinal plants used in Congo-Brazzaville were selected in order to determine their mineral element content and assess their antioxidant activity.
Methodology: Mineral element content were determined by atomic absorption spectrometry with flame and flameless. The antioxidant activity assessed, using the DPPH radical scavenging test and iron reducing capacity (FRAP).
Results: Eight (08) macroelements and five (05) trace elements from these plants have been quantified. The antioxidant potential showed that organic extracts were more active than aqueous extracts. However, this antioxidant potential remains low compared to that of ascorbic acid used as the reference antioxidant. *Garcinia kola* (seeds) and *Ageratum conyzoides* (leafy stems) were rich in mineral elements. Similarly, *Cogniauxia podoleana* (leaves) and *Garcinia kola* (seeds) showed remarkable antioxidant potential.
Conclusion: The presence of thirteen mineral elements and the antioxidant potential are proved in these plants. Would therefore help to maintain the immunity of the human body by resisting various pathologies such as bacterial infections, malaria, inflammations and gastrointestinal disorders.

Keywords: Macroelements; trace elements; DPPH; FRAP; medicinal plants.

1. INTRODUCTION

Ageratum conyzoides Linn. (Asteraceae), *Aframomum alboviolaceum* Schum et Thonn (Zingiberaceae), *Garcinia kola* Heckel (Clusiaceae) and *Cogniauxia podoleana* Bail. (Cucurbitaceae) are four medicinal plants used to treat various illnesses in Africa, particularly in Brazzaville-Congo [1-3]. Studies by Mikala et al. indicate the presence of secondary metabolites such as alkaloids, phenolic compounds (coumarins, flavonoids and tannins), steroids and terpenes, which may be responsible for the antioxidant and vermicidal activities observed against *Lumbricus terrestris* [4]. The therapeutic properties of secondary metabolites can also be linked to the presence of nutrients that are essential for the body to function properly [5,6]. These nutrients include the mineral elements: macroelements and trace elements [7,8]. These nutrients are supplied to the body by the diet, and their deficiency or excessive supply could be detrimental to the body's health, leading to low immunity and, consequently, exposure to pathologies such as bacterial infections, diabetes, digestive problems, the proliferation of gastrointestinal parasites, etc [9,10]. Quantification of these mineral elements and assessment of antioxidant activity would therefore be necessary to better evaluate the therapeutic potential of the organs of these four plants.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

The plant material consisted of leafy stems of *Ageratum conyzoides* Linn, and *Aframomum alboviolaceum* Schum & Thonn, seeds of *Garcinia kola* Heckel, roots and leaves of *Cogniauxia podoleana* Bail. These various plant organs were collected in the south-east of Brazzaville, specifically in the Mayanga (4° 16‘5.394’’South; 15°11’‘12, 132’‘East) and Moukoundzi-Ngouaka (4° 16’ 21.747’’ South; 15° 14‘ 34.725" East) districts. They were first cleaned with water and dried in the shade at an ambient temperature of 25°C, then ground. The powders obtained were stored in jars and will be used for analysis.

2.2 Methods

2.2.1 Preparation of ash samples

A mass of 0.5 g of powder from each plant was placed in five separate crucibles. These powders were calcined in an oven (Nabertherm 30-3000°C) by gradual heating from 100 to 650°C for 5 h. After cooling, the crucibles were removed from the oven. A volume of 5 mL of nitric acid (1 mol/L) was then added to the ash

contained in the crucibles and dried on a hot plate. The remaining ashes were again calcined in the oven at 400°C for 30 min, and then recovered with 10 mL of nitric acid (1 mol/L). After filtration, each filtrate obtained was transferred to a 50 mL flask and made up to the mark with the nitric acid solution (1 mol/L) [11-13].

2.2.2 Preparation of aqueous extracts

A mass of 100 g of *Garcinia kola* seed powder was macerated in 1 L of distilled water for 24 h. For the species *Aframomum alboviolaceum*, *Cogniauxia podoleana* and *Ageratum conyzoides*, 100 g of plant powder was boiled in 1L of distilled water for 20 min. After filtration, the solutions were concentrated using a rotary evaporator (Heidolph). The dry extracts obtained were used to prepare selective extracts and assess antioxidant activity.

2.2.3 Preparation of selective extracts

2 g of aqueous dry extract from each plant was solubilized in 50 mL of distilled water. Then, using a separating funnel, the liquid-liquid extraction was carried out by successively adding 50 mL of each organic solvent in ascending order of polarity with exhaustion of the material (hexane, dichloromethane, ethyl acetate and n-butanol).

2.2.4 Preparation of the FRAP reagent

The FRAP reagent is composed of a mixture of three solutions with a volume ratio of 10:1:1. These were 25 mL of acetic acid-sodium acetate buffer solution (300 mM at pH = 3.6) plus 2.5 mL of 10 Mm TPTZ (2,4,6-Tri (2-pyridyl) -s-triazine) solution prepared in 40 mM hydrochloric acid and 2.5 mL of 20 mM iron III chloride prepared with distilled water. After stirring, the resulting homogeneous mixture was placed in a water bath at 37°C for approximately two hours [14-16]. This reagent was used freshly after preparation.

2.2.5 Quantification of mineral elements

The minerals were determined by flame and flameless atomic absorption spectrometry (Thermo Scientific iCE 3000 SERIES). The calibration range for each standard was prepared on the basis of a multi-element (Multielements of calibration standard) by diluting 10 mg/mL stock solutions with ultrapure water or 2% nitric acid [11-13]. The absorbances of the samples were measured at the specific wavelengths for each mineral element (Table 1) with reference to the goodbook.

The mineral element content was determined using the method described by the AOAC according to formula (1). The values obtained are expressed in milligrams per 100 g of plant powder [11, 13, and 17].

$$\text{Content in } \% = \frac{C\left(\frac{mg}{L}\right) * V(mL)}{m\ (g)} \times 100 \qquad (1)$$

C: Concentration of the element assayed in mg/L determined as a function of the calibration curve obtained with the SAA, M: mass of the plant material used.

2.2.6 Assessment of antioxidant activity

2.2.6.1 DPPH test

For each extract, a range of concentrations (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL) was prepared in absolute ethanol. 1 mL of these samples was added to 1.5 mL of ethanolic solution of DPPH radical (0.03 mg/mL). The mixture obtained was shaken well and left to incubate for 30 min at 25°C. The absorbance of the mixture was read at a wavelength of 517 nm using a UV-Visible spectrophotometer. Ascorbic acid (vitamin C) was used as the reference compound [4,18]. The percentage reduction of the DPPH radical was determined using the following formula (2):

Table 1. Wavelengths used to measure mineral compound

Macroelements	**Ca**	**Mg**	**P**	**K**	**Na**
Maximum wavelength (nm)	422,7	766,5	313,2	766,5	589,0

Trace elements	**Cr**	**Co**	**Cu**	**Fe**	**Li**	**Mn**	**Se**	**Zn**
Maximum wavelength (nm)	357,9	240,7	324,7	248,3	670,8	279,5	196,0	213,9

R (%) = (Ab - Ae) / Ab × 100 (2)

R: percentage reduction of the DPPH radical; Ab : absorbance of the blank; Ae: absorbance of the sample. The concentrations required to reduce 50% (CR50) of the radical were determined using Excel.

2.2.6.2 FRAP test

Antioxidant activity was assessed using the method developed by Benzie et al. [14], with a few modifications. Crude, selective aqueous extracts of the various plants were prepared in a concentration range from 0.78125 µg/mL to 200 µg/mL. The reaction mixture consisted of 3 mL of FRAP reagent plus 100 µL of crude aqueous or selective extract of the different plants. The absorbance of the mixture obtained was read at 593 nm on a UV-visible spectrophotometer, after incubation at room temperature in the dark for 4 min. The ascorbic acid (vitamin C) and iron sulphate monohydrate ($FeSO_4$) used as standard compounds were prepared under the same conditions as the extracts. The experiment was repeated three times.

The contents are expressed in microgram ascorbic acid equivalent per 100 grams of extract (µg EAA/100 g extract) and in microgram iron sulphate per 100 grams of extract (µg ESF/100 g extract) according to the formula (3):

$$c = \frac{Ci \times D}{C0} \times 100 \quad (3)\ [14]$$

With C = Quantity of reducing compounds in µg EAA/100 g extract or µg ESF/100 g dry extract ; ci = sample concentration read (µg/mL); D = the dilution of the extract stock solution and C0 = concentration of the extract stock solution (mg/mL).

Graph pad 5.01 software was used to process the data, using Tukey's test to determine significance at the 5% probability level. All results are significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Quantification of Mineral Elements

3.1.1 Macroelements content

The macroelements measured in the plant organs were Ca, Mg, P, K and Na (Table 2), with a predominance of Na (2570.466 to 21283.526 mg/100 g), the latter being involved in nerve conduction [19,20]. Magnesium content is highest in *G. kola* seeds (3989.430 mg/100g) and in *C. podoleana* leaves (1641.932 mg/100g). This mineral acts as an enzyme cofactor and cardiac regulator in the human body [19,20]. Calcium is more abundant in the leafy stems of *Ageratum conyzoides* (1355.064 mg/100g), a mineral that strengthens the bone system and skeletal rigidity [19,21].

These results show that the leafy stems of *A. conyzoides* are richer in macroelements, followed by *G. kola* seeds.

3.1.2 Trace element contents

The trace elements quantified in plant organs were Co, Cr, Fe, Li, Mn, Se and Zn (Table 3), with a predominance of Fe (3.238 to 76.003 mg/100g) and Zn (6.012 to 24.154 mg/100g). This mineral helps to combat iron-deficiency anaemia and is essential for blood regulation [19,20]. The highest iron content was found in G. kola seeds (76.003 mg/100g). As for zinc, its deficiency is particularly serious, especially in people taking diuretics, alcohol or having a vegetarian lifestyle. Zinc stimulates the immune system, neutralises copper overload and helps eliminate cadmium from cigarettes [22].

These results show the presence of macroelements and trace elements in the selected plants. Similar studies have shown relatively low levels of mineral elements in the seeds of *Garcinia kola* in Nigeria and the mesocarp of its fruits in Congo [23,24]. The same applies to the seeds of *Aframomum alboviolaceum* fruits [25].

The variations in mineral content in the study plants can be justified by the chemical composition of the soil at the place of harvest, but also by each plant's capacity to store these chemical elements [26]. The mineral composition of these plants could justify their use in traditional medicine to treat infections, spasms, cramps, pain, rheumatism, diabetes, cancer, parasites, etc. [27,28].

3.2 Assessment of Antioxidant Activity

3.2.1 Antioxidant profile using the DPPH radical scavenging method

Table 4 shows the concentrations of samples that reduce 50% of DPPH (CR50). These values are significant at $p<0.05$. Of all the plants studied, *A. alboviolaceum* showed the best activity, which is still lower than that of vitamin C.

Table 2. Macroelements measured in plants

Plants	Macroelements (mg/100 g)				
	Sodium	**Calcium**	**Magnesium**	**Phosphorus**	**Potassium**
A. conyzoides	21283,526	1355,064	3,105	312,846	3141,979
A. alboviolaceum	8055,427	48,0451	1,768	766,675	917,127
C. podoleana (leaves)	2570,466	48,719	1641,932	839,834	449,207
C. podoleana (roots)	4674,186	493,933	3,260	56,847	366,507
G. kola (seeds)	18720,439	451,861	3989,430	356,074	894,027

Table 3. Trace elements measured in plants

Plants	Trace elements (mg/100 g)							
	Chrome	**Cobalt**	**Copper**	**Iron**	**Lithium**	**Manganese**	**Selenium**	**Zinc**
A. conyzoides	0,918	0,013	6,177	6,177	7,425	1,924	Trace	24,154
A. alboviolaceum	0,723	0,012	3,975	3,975	2,024	2,531	9,242	17,798
C. podoleana (leaves)	1,001	0,012	3,238	3,238	1,702	0,387	0,033	6,0123
C. podoleana (roots)	0,579	0,014	4,577	4,575	4,652	3,017	3,346	11,410
G. kola (seeds)	0,684	0,006	5,891	76,003	5,657	1,037	8,082	15,502

Table 4. CR50 of the different extracts

Plants	Aqueous extracts	Ethylacetate extracts	n-butanol Extracts	Standard: Vitamin C
A. conyzoides	1,850±0,010	1,289±0,050	0,132±0,030	0,015±0,090
G. kola (seeds)	0,759±0,120	0,149±0,070	0,202±0,110	
A. alboviolaceum	0,098±0,010	0,026±0,014	0,061±0,070	
C. podoleana (roots)	8,125±0,150	0,971±0,120	1,658±0,170	
C. podoleana (leaves)	1,090±0,010	1,597±0,150	0,354±0,130	

CR_{50}±ESM; M = Mean values ± Standard error of means of six experiments; p<0,05

3.2.2 Antioxidant profile using the FRAP method

The results were expressed as ascorbic acid equivalent (EAA) (Fig. 1) and iron sulphate equivalent (ESF) (Fig. 2) whose calibration lines are respectively $y_{ascorbic\ acid} = 0.0074x - 0.0011$ and $R^2 = 0.9994$ and $y_{iron\ sulphate} = 0.0197x - 0.0187$ and $R^2 = 0.9991$.

The extracts showed antioxidant activity that varied according to the extraction solvent. Indeed, these quantities were higher for ethyl acetate extracts (156.02 to 1329.11 mg EAA/100 g extract). However, only the aqueous leaf extracts of *C. podoleana* (1163.829 mg EAA/100 g extract) and *A. alboviolaceum* (904.339 mg EAA/100 g extract) showed higher values (Fig. 1).

Fig. 2 shows the quantities of iron-reducing compounds in selective and aqueous extracts in iron sulphate equivalent. These contents are more remarkable for the ethyl acetate extracts (79.453 to 508.195 mg ESF/100g extract). Aqueous extracts of *C. podoleana* leaves (458.020 mg ESF/100 g extract) showed considerable reducing power, followed by leafy stems of *A. alboviolaceum* (348.635 mg EAA/100 g extract).

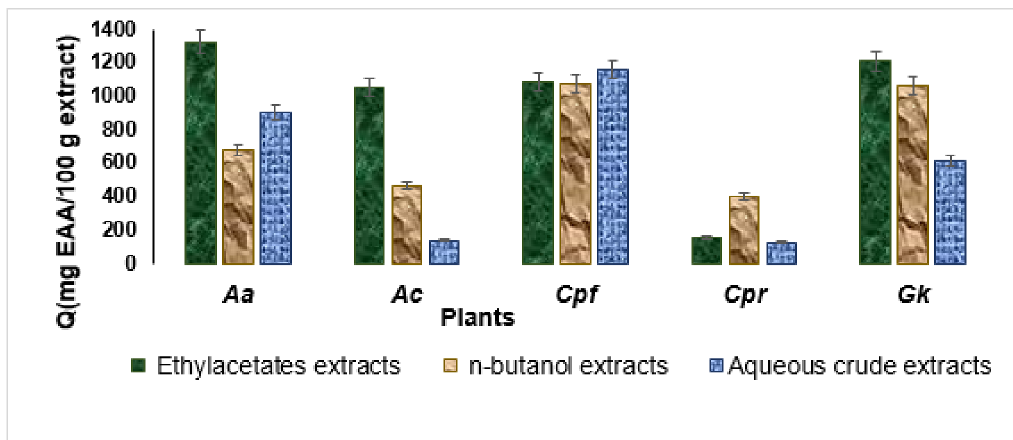

Fig. 1. Quantities of iron-reducing compounds in ascorbic acid per 100 g of dry extract
Aa: Aframomum alboviolaceum (leafy stems); Ac: Ageratum conyzoides (leafy stems); Cpf: Cogniauxia podoleana (leaves); Cpr: Cogniauxia podoleana (roots); Gk : Garcinia kola (seeds); (p<0,05)

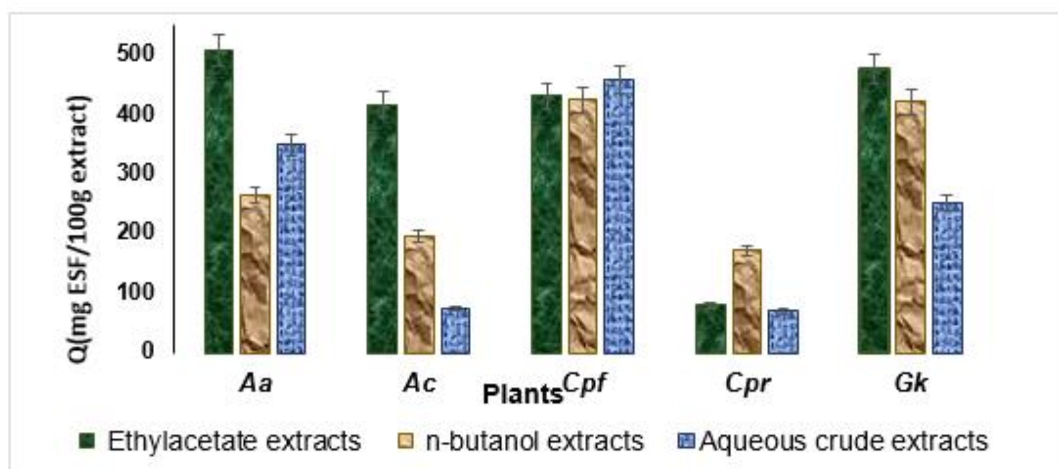

Fig. 2. Quantities of iron-reducing compounds in selective and aqueous extracts in iron sulphate equivalent
Aa: Aframomum alboviolaceum (leafy stems); Ac: Ageratum conyzoides (leafy stems); Cpf : Cogniauxia podoleana (leaves); Cpr : Cogniauxia podoleana (roots); Gk : Garcinia kola (seeds); (p<0,05)

These contents are more remarkable for the ethyl acetate extracts (79.453 to 508.195 mg ESF/100g extract). Aqueous extracts of *C. podoleana* leaves (458.020 mg ESF/100 g extract) showed considerable reducing power, followed by leafy stems of *A. alboviolaceum* (348.635 mg EAA/100 g extract).

The quantities of compounds reducing Fe^{3+} ions to Fe^{2+} in the extracts, expressed in ascorbic acid equivalent, are approximately double those expressed in iron sulphate equivalent. The ethyl acetate extract of the leafy stems of *A. alboviolaceum* exhibited the best antioxidant profile (1329.11 mg EAA/100g extract and 508.195 mg ESF/100g extract).

Furthermore, the antioxidant activity of the plants varied according to the nature of the extraction solvent. This may be justified by the type of compound present in these extracts, including polyphenols [4]. Optimum extraction of compounds with antioxidant properties is achieved using polar or moderately polar solvents [29,30]. The reduction methods used have different reaction mechanisms. The FRAP test is based on electron transfer, while the DPPH radical test is based on proton transfer [14-15]. Furthermore, according to the two methods used, the best antioxidant potential was observed with the leafy stems of *A. alboviolaceum*, the seeds of *G. kola* and the leaves of *C. podoleana*. The observed phytochemical composition of these plants, in particular phenolic compounds, could justify their antioxidant potential [4,31]. The hydroxyl groups in polyphenols are considered electron donors [32]. This reducing power may reveal their antioxidant capacity [14,15]. These results could justify their use in traditional medicine in the Congo.

4. CONCLUSION

Determination of mineral elements by flame and flameless atomic absorption spectrometry made it possible to quantify 13 mineral elements in the organs of selected plants. The results showed that, of all the elements measured, phosphorus, potassium, sodium, chromium, cobalt, copper and zinc were present in significant quantities in all the plants. These plants are an optimal source of minerals. Evaluation of antioxidant activity using the DPPH radical scavenging method and the FRAP test demonstrated the antioxidant potential of the selected plants. The selective extracts showed greater antioxidant power than the raw aqueous extracts. These high mineral contents, combined with the presence of secondary metabolites, could justify the antioxidant, anthelmintic, antimicrobial and antimalarial properties attributed to the organs of these plants in traditional medicine.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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