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X-ray fluorescence characterization of some medicinal plants traditionally used as aphrodisiac in the treatment of male sterility in Kenge City, Democratic Republic of the Congo

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ABSTRACT

Introduction

Male infertility refers to the inability of a man to impregnate a woman. It is a public health issue among men of reproductive age. Certain minerals, such as zinc, selenium, iron, and magnesium, are essential to produce healthy sperm and for maintaining male reproductive function. Medicinal plants containing these minerals can improve sperm quality and increase the chances of conception in men suffering from infertility.

Purpose

The aim of this research was to analyze the elemental composition of four medicinal plants traditionally used for their aphrodisiac properties, with a particular emphasis on their application in the treatment of male sterility.

Methods

The mineral content of selected plants was assessed by X-ray fluorescence using Energy Dispersive XRF Spectrometer (EDXRF).

Results

The findings reveal significant concentrations of various minerals in the studied medicinal plants, providing crucial information about their elemental composition. Thirty mineral elements were identified, including six macro elements (Na, Mg, P, S, K, Ca), 15 micro elements (Al, Si, Mn, Ni, Cu, Mo, Pb, Ti, V, Cr, Hg, Br, Rb, Sr, and Cd), three important elements in the diet of spermatozoa (Fe, Zn, Mg), and seven non-relevant elements. These results can have direct implications for the medicinal properties of the plants, especially in the context of treating male sterility.

Conclusions

Among the selected medicinal plants, *Mondia whitei* is rich in minerals as evidenced by principal component analysis (PCA) and could potentially contribute to improving overall health and indirectly promoting male fertility.

INTRODUCTION

Over the years, traditional medicinal plants have been extensively used in developing countries to address various diseases (Ndaba et al., 2020; Ngbolua et al., 2019; Ngbolua et al., 2011a, b). This practice is influenced by cultural factors, declining purchasing power, the ease of access, and their widespread availability. In many African regions, including Madagascar, a significant portion of the population tends to rely on traditional plant-based treatments without scientific evaluation (Ngbolua et al., 2011a, b). Medicinal plants represent a valuable heritage for humanity, particularly for marginalized communities in developing countries like the Democratic Republic of Congo (DRC), where these plants play a crucial role in primary healthcare and subsistence (Mulwele et al., 2016; Mulwele et al., 2023).

In the DRC, the utilization of medicinal plants contributes to the valorization of traditional pharmacopoeia, sustainable management of genetic resources under the Convention on Biological Diversity (CBD), specifically through Access and Benefit-Sharing (ABS) agreements (Djolu et al., 2023; Masengo et al., 2023a, b; Masengo et al., 2021a, b). This approach supports the development of effective and protected phytomedicine formulations, addressing conditions such as male sterility (Ndongo et al., 2019; Ndongo et al., 2023).

Medicinal plants contain biogenic salts essential for their growth and development, representing a relatively small subset of essential elements. While organic nutrition focuses on carbonaceous compound production through processes like photosynthesis, mineral nutrition in plants involves acquiring biogenic salts from the soil. Essential elements play specific roles in plant metabolism, and their absence can lead to deficiency symptoms related to these roles. As plants form the basis of the food chain, the mineral nutrients assimilated by plants become part of the constituent matter of animals, including humans. Plants not only contribute significantly to medical care in the absence of a modern medicinal system but also serve as a source of food providing essential biogenic salts crucial for metabolism (Ngbolua et al., 2021).

In the Kwango province, especially in the City of Kenge and its surroundings, various plant species—both woody and herbaceous such as *Hymenocardia acida* Tul. (Hymenocardiaceae), *Landolphia lanceolata* K Schun Pichon (Apocynaceae), *Mondia whitei* Hook F Skeels (Apocynaceae), and *Pentadiplandra brazzeana* Baill (Pentadiplandraceae)—are known for their aphrodisiac properties and are used in the treatment of male sterility. However, the mineral content of these species, crucial for maintaining metabolism and growth, has not been analyzed.

This study aims to investigate the mineral composition of these four plant species commonly used by traditional practitioners for treating male sterility. The focus is on determining the concentration of biogenic salts in these species using X-ray fluorescence analysis, a reliable and cost-effective analytical method for determining the chemical composition of various materials, including plant species (Terzano et al., 2019; Borges et al., 2020).

METHODS

X-ray fluorescence spectrometry (SFX or FX, XRF, X-ray fluorescence) is a non-destructive fundamental chemical analysis technique that identifies and determines most of the chemical elements in a sample. The technology can be used in a variety of materials such as minerals, ceramics, cement, metals, oils, water, glasses and so on in solid or liquid forms. It enables the analysis of all elements of sodium (11) uranium (92) at concentrations from ppm to 100 per cent, particularly reproducible, and also enables the identification and proportional analysis of all elements S starting with carbon in a variety of samples: minerals, metals, oils, alloys, powders, ceramics, polymers, cement, glasses, etc. (Eberhart, 1997).

Principle of fluorescence

It is an elementary spectrometer method that allows for the qualitative and quantitative analysis of elements but does not disclose their specificities. The principle is based on the analysis of radiation that emits an X-ray-excited sample. The sample to be studied is placed under the X-ray beam, and the atoms that make up the sample pass from its basic state to the excited state (Patnaik, 2004). Excited states are unstable, and when releasing energy, atoms tend to return to their basic states, especially photons X. Each atom emits a photon of energy at a clean wavelength with a clean electronic configuration (Mendham et al., 2005). This phenomenon is X-ray fluorescence, the secondary emission of X-rays that constitute the atoms of the sample. At the same time, analysis of secondary X radiation enables understanding the nature of chemical elements present in samples, such as mass concentrations. The spectrum of X-rays emitted by matter is characteristic of the composition of the sample (Loth, 2014; Tiana et al., 2003).

Preparation of the samples

In the case of biomaterials, such as medicines, measuring water content before mineralization is interesting, and bioactive salt content is always returned to the sample's dry mass. Our samples are placed in a 48-hour oven at 110 °C.

Determination of the mineral composition by spectrometry with x-ray fluorescence

The examination of x-ray fluorescence or the spectrometry of atoms begins with samples extracted from plants (Hy, Mo, and EP). 300 grams of collected plant parts, specifically the bark of Hymenocardia acida, the roots of Landolphia lanceolata, Mondia whitei, and Pentadiplandra brazzeana, were dried using a BLUE M drying oven at 37 °C. Once dried, these samples were crushed using an A-10 ANALYTICAL MILL OVERLOAD (TEKMAR COMPANY) and a SCIENTIFIC crusher from THOMAS USA. The resulting powders (500 µm) were then placed in labeled glass bottles. These powders were later transferred to sealed plastic bottles and labeled for mineral analysis.

For each extract, 5 grams of powder were weighed using a SARTORIUS CPA 2250 precision balance and mixed with 20 % binder (1 gram of Fluxane in 250 grams of BM 00021 CEREOX). After homogenizing the products mechanically, mussel preparation and disinfection were carried out for the production of pellets using a mechanical pelletizer. The formed disc was then placed in a clean polyethylene packaging, labeled according to the vegetable extract code, and stored in a desiccator containing silica gel. This preparation aimed to subject the samples to x-ray fluorescence spectroscopy to detect the various minerals present in each extract.

The machine used for this purpose incorporates multiple systems, including electrodes, an excitation tube, and an amplifier. Its operating principle involves transforming atomic rays caused by x-rays into spectra for mineral detection in the extracts.

Coding of the samples according to the following order

- **EsB22.130**: Hy (Echant Biological Sciences 2022 num 130 *for Hymenocardia acida),*
- **EsB22.131**: (Echant Biological Sciences 2022 num 131 for *Landolphia lanceolata),*
- **EsB22.132**: Mo (Echant Biological Sciences 2022 num 132 for *Mondia whitei*),
- **EsB22.133**: EP (Echant Biological Sciences 2022 num 133 for *Pentadiplandra brazzeana*).

Determination of the water content

This stage involves preparing beakers ranging from 5 to 10 ml. The process includes washing them with distilled water, drying them using the BLUE M drying oven at temperatures between 40 to 105 °C. Afterward, the vacuum weight of the beaker (Weight) is measured. Subsequently, the beaker, along with 1 gram of extract, is weighed, placed in a desiccator, and then subjected to a 3-hour drying period in the oven. Following this, it is left to rest for 20 to 30 minutes before measuring the combined weight of the beaker and the 1 gram of extract post-drying. This entire procedure is repeated three times, and calculations are performed using the following formula:

$$Humidity \; = \frac{\text{Moisture mass} - \text{dry mass}}{\text{Moisture mass}} x\; 100$$

Determination of the content of proteines

The protein content was performed from the Kjeldahl method. The objective is to measure the total nitrogen rate of vegetables. This experiment is very important because nitrogen plays an important role in the growth of plants (NBN, 2000). The nitrogen rate was evaluated by the following formula:

$$N = \frac{[(V_1 - V_0) * \mathrm{C}(H^+) * M_N] * [100 + W_{H_2O}]}{\mathrm{m} * 100}$$

Where: V_1 is volume, in millilitres, of sulphuric acid used for the proportioning of the sample; V_O is volume, in millilitres, of sulphuric acid used in the dummy trial; $C(H^+)$ is the concentration out of H_3O^+ of the sulphuric acid, in moles per liter (if, for example, 0,01 mol/L of sulphuric acid is used $C(H^+) = 0{,}02$ mol/L) because the sulphuric acid is a diacid; M_N is the molar mass of nitrogen, in grams per mole (= 14); m is the mass, in grams, of the sample of ground dried with the air; is the water content, expressed as a percentage in mass, when the sample of ground is dried out of drying oven (Rouessac et al., 2004).

In addition to the dpd tests for moisture content and protein, the data obtained on biogenic salts were statistically analysed by software R studio and SPSS, whose main analysis components and correlations were used. Analysis of major components allows the determination of the relationship between variables (biogenic salts) and individuals (plants). This analysis is always used for quantitative variables.

RESULTS

Table 1 provides the average values of minerals found in the four selected medicinal plants.

Table 1:
Mineral content of four selected plant species

N°	Elements	Unit	*H. acida*	*L. lanceolata*	*M. whitei*	*P. brazzeana*
1	Na	mg/kg	67,58 ±5,75	77,67 ±5,97	50,48±4,99	94,13±6,75
2	Mg	mg/kg	2751,91 ±200,90	3073,55 ±259,44	5829,08±460,94	1541,63±180,99
3	Al	mg/kg	79,86 ±6,00	8924,30 ±688,21	1113,13±96,03	64,04±5,99
4	Si	mg/kg	737,40 ±61,87	2375,93 ±180,38	8584,87±645,70	363,84±28,42
5	P	mg/kg	509,11 ±22,73	534,92 ±21,77	1182,09±78,12	762,11±51,02
6	S	mg/kg	517,35±36,98	6070,48 ±68,90	1341,33±105,93	6585,11±370,69
7	Cl	mg/kg	830,27 ±63,49	94,95 ±8,70	182,29±15,89	52,38±4,31
8	K	g/kg	5,69± 0,49	8,79 ± 0,65	21,74±1,19	9,77±0,74
9	Ca	g/kg	11,58 ±0,67	12,20 ±0,80	11,48±0,66	1,96±0,60
10	Ti	mg/kg	5,36 ±0,33	11,97 ±0,53	100,14±7,15	3,85±0,25
11	V	mg/kg	0,63 ±0,03	0,69 ±0,03	0,13±0,05	0,79±0,04
12	Cr	mg/kg	3,17 ±0,28	1,13 ±0,11	3,91±0,30	1,87±0,17
13	Mn	mg/kg	28,91 ±2,49	47,68±3,06	69,11±7,14	32,34±2,15
14	Fe	mg/kg	88,52 ±8,70	192,18±10,60	1134,39±99,64	63,57±4,43
15	Ni	mg/kg	6,53 ±0,47	0,56 ±0,04	1,00±0,10	0,71±0,05
16	Cu	mg/kg	8,14 ±0,75	8,03 ±0,68	7,14±0,68	2,18±0,20
17	Zn	mg/kg	17,43 ±1,02	23,71 ±1,85	28,40±2,67	2,20±0,15
18	As	mg/kg	ND	< 0.50	ND	ND
19	Se	mg/kg	0,74 ±0,07	< 0.44	< 0.44	< 0.44
20	Br	mg/kg	6,37 ±0,54	0,85 ±0,07	0,44±0,05	19,59±1,46
21	Rb	mg/kg	12,89 ±0,99	8,35 ±0,75	10,55±0,97	29,88±2,54
22	Sr	mg/kg	45,55 ±6,05	124,02 ±17,48	87,17±7,56	1,86±0,13
23	Y	mg/kg	4,03 ±0,27	ND	ND	ND
24	Zr	mg/kg	0,46	ND	ND	ND
25	Nb	mg/kg	1,91 ±0,25	0,95 ±0,05	ND	ND
26	Mo	mg/kg	0,31 ± 0,03	0,21 ±0,01	0,12±0,01	ND
27	Cd	mg/kg	< 0.15	< 0.15 ±	< 0.15±	< 0.15±
28	I	mg/kg	0,19 ± 0,09	0,22 ±0,04	0,15±0,07	0,19±0,10
29	Ba	mg/kg	10,19 ± 1,84	15,70 ±1,52	20,98±2,54	ND
30	Pb	mg/kg	ND	4,04 ±0,28	0,39±0,03	79,06±6,05

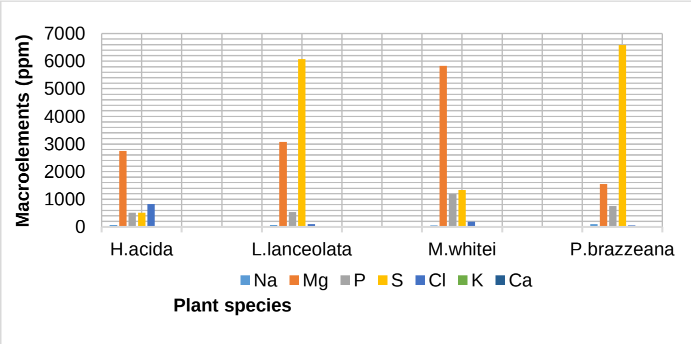
NB % = (g/kg)/10 = (mg/kg)/10000; < X below the limit of detection; ND not measured, non given.

This **Table** indicates that 30 biogenic salts are identified in each of these four selected plants. In addition to all plants, potassium and calcium are the main biogenic salts. The highest values of these elements were 21740 mg/kg of potassium in *Mondia whitei* and 12200 mg/kg of calcium in *Landolphia lanceolata*. The lowest concentrations of these two dominant elements were found in the *Pentadiplandra brazzeana* species, the 1960 mg/kg of calcium or 5690 mg/kg of potassium in the Hymenocardia acida species. These results are like those of others (Sykorova et al., 2006; Kitadi et al., 2019; Kitadi et al., 2021).

To well analyze the composition of various plant minerals, we have again identified these different biogenic salts in major elements: Na, Mg, P, S, Cl, K et Ca (**Figure 1**), Al, Si, Mn, Ni, Cu, Mo, Pb, Ti, V, Cr, As, Br, Rb, Sr and Cd **(Figures 2a and 2b)**, and known important biogenic salts for their effects on the food of the sperm: Fe and Zn **(Figure 3)**. The **table** also shows that the seven elements are not given, and in the case of Pb in *H. acida*, all plants except *L. lanceolata* are Pb, all plants except Y and Zr are excluded in *H. acida*, *M whitei* is Nb, *P. brazoana* is Nb, and *P. brazzeana* is Mo and Ba. Furthermore, by comparing the non-detectable elements (NDs) of the four plants studied, the table is concluded as follows: - *H. acida* and *L. lanceolata* have 2 NDs each, - first -

Pb and Have - second - Y and Zr – *M. whitei* has 4 NDs (Ease, Y, Zr and Nb) - finally, *P. brazzeana* contains 6 NDs.

Figure 1:
Content of macroelements of these four species of our samples



The results of this graph concerning macroelements show that Na and Mg are traces in 4 species, but the highest values are 94130 mg/kg of *P. brazzeana* and 58920 mg/kg of *M. whitei*. In addition, there are very remarkable mineral values, such as Mg, P, K, and Na in the species of *M. whitei*, Na in the species of *P. brazzeana*, Cl in the species of *H. acida*, and Ca in the species of *L. lanceolata*.

Figure 2a:
Content of microéléments of these four plants

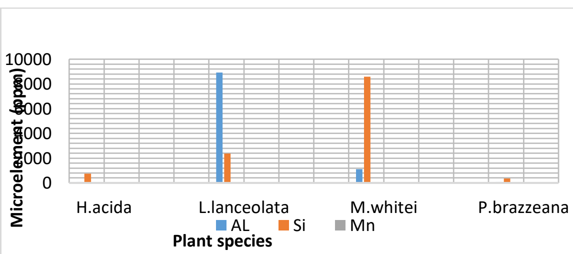
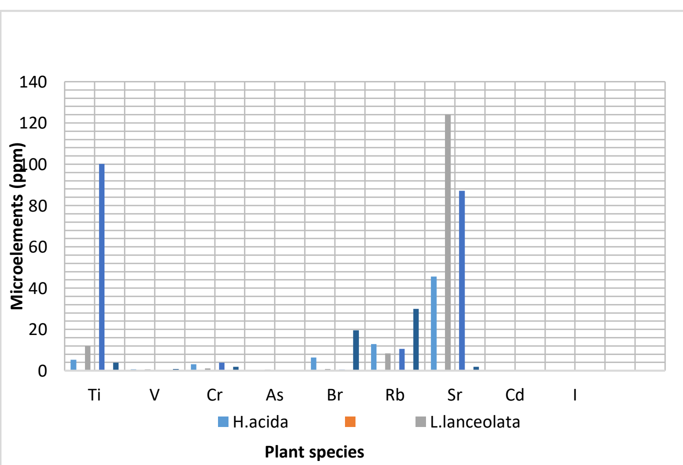
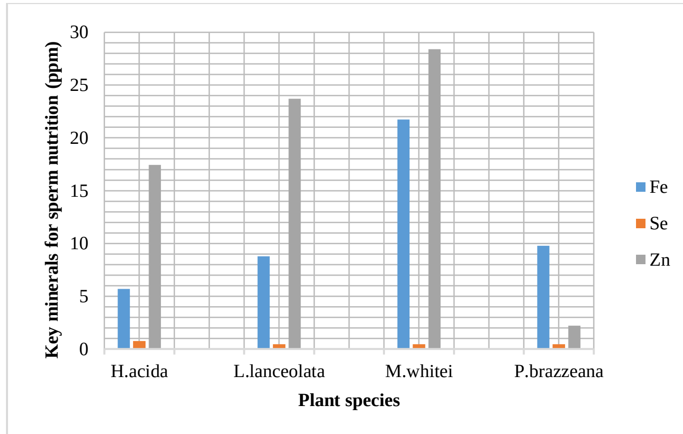


Figure 2b:
Content of microéléments of these four plants (continuation)



The results of Figures 2a and 2b show that the amount of microelement in these four species is even more significant in both species. *Mondia whitei* contains a considerable amount of Ti, Cr, Mn, and Sr, and *Pentadiplandra brazzeana* contains elements such as V, Br, Rb, and Pb. Other miner elements are in the form of traces of four other vegetable species (*Hymenocardia. acida* and *Landolphia lanceolata*).

Figure 3:
Plants content of biogenic salts



The findings from Figure 3 indicate that *Mondia whitei* exhibits the highest concentrations of Fe and Zn, reaching 1134 and 9 mg/kg, respectively. In contrast, *H. acida* shows the highest concentration overall, with 740 mg/kg, while *L. lanceolata*, *M. whitei*, and *P. brazzeana* are below the detection limit, with concentrations less than or equal to 440 mg/kg.

Principal Component Analysis

To identify the relationship between the variables (biogenic salts) and the entities (plants), we employed Principal Component Analysis (PCA). The dataset comprises 4 entities (plants) and 30 variables (biogenic salts).

Overall, based on the PCA results, as depicted in Figures 4a and 4b, it is evident that the highlighted entities make the most significant contributions to the plan's construction, while the highlighted variables are the ones best represented on the plan.

Figure 4a:
Graph of the individuals

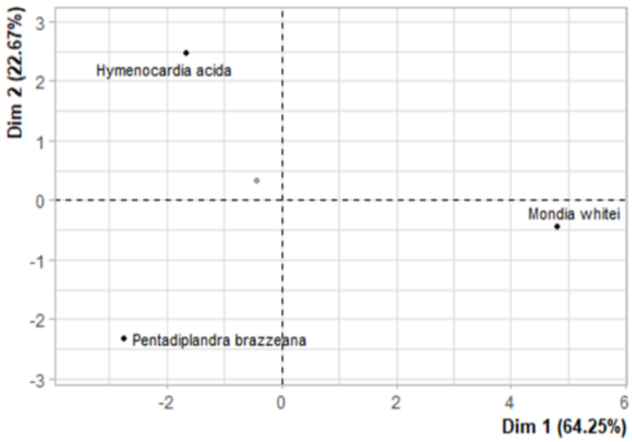


Figure 4b:
Graph of the variables (ACP)

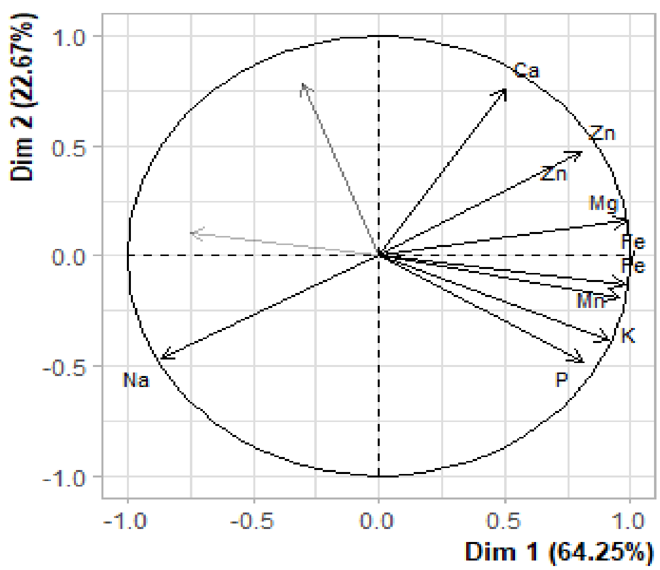
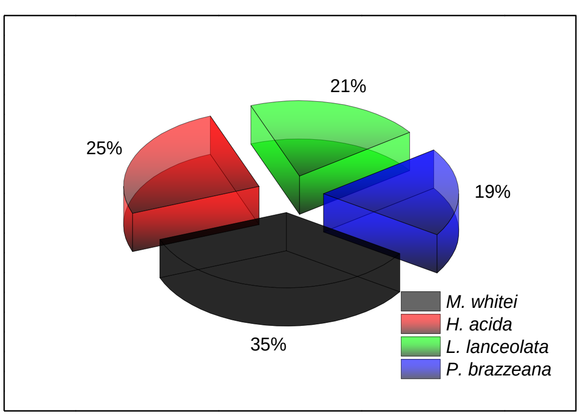


Figure 5:
Water content of the extracts



The findings from Figure 5 indicate a substantial difference with a 5% threshold between the mean values of the 'moisture bus (F-Calc') calculated frequency rates, where (17.61) is higher than (F-Tab) tabular frequency (4.39). It is noteworthy that the order of these values is *Mondia whitei* ≥ *Hymenocardia acida* ≥ *Landolphia lanceolata* ≥ *Pentadiplandra brazzeana*.

Figure 6:
Content of matter nitrogenized by the Kjeldahl method

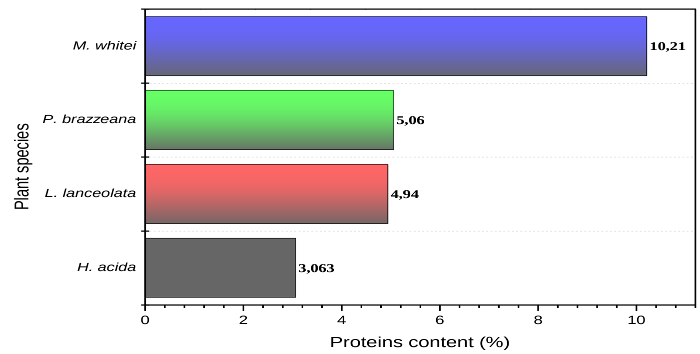


Figure 6 exhibits similar results to Figure 5, indicating a significant difference between treatments at a 5% confidence threshold. Because (F-Cal') calculated frequency (21.17) is higher than (F-Tab) tabular frequency (4.39). Notably, *Mondia whitei* demonstrates nitrogen content higher than that of the other species.

DISCUSSION

Given that the study focuses on male sterility and considering that three essential elements have been identified for spermatozoa nutrition, our findings align with other authors who have demonstrated the significance of iron for oxygen transport and storage in the body, as well as its role in the immune system, growth, and energy metabolism. The World Health Organization (WHO) highlights iron deficiencies as the most common and widespread nutritional disorders globally. The medical implications include problematic pregnancies, abnormal physical and cognitive development, an increased risk of infant morbidity, and reduced productivity in adults (Dos Anjos et al., 2000).

The concentrations of potassium found in our study are mostly comparable to or of the same order of magnitude as those observed in lower plants such as *Lentinula edodes*, *Lentinus cladopus*, *Pleurotus orida*, and *Pleurotus djamor*. For instance, the potassium content in Pleurotus djamor is approximately 36,340 mg/kg, exceeding values found in *Justicia secunda* Vah L. However, our values are significantly higher than those reported by other authors for the leaves of *Annona senegalensis* (Kitadi et al., 2022). This indicates that the elemental content varies significantly depending on the plant type and soil quality (Mallikarjuna et al., 2012; Yisa et al., 2010).

According to our study, the iron contribution is higher in all four species, contrary to the recommended daily nutritional intake of iron, which is 9 mg/day for men and 16 mg/day for women. Our iron content results contradict those reported for beef and veal but are like the values observed in locusts (*Locusta migratoria*) based on their diet (Martin et al., 2001). Zinc is a trace element that plays multiple roles in men's reproductive health. It is crucial for the conversion of testosterone into its active form. Additionally, a correlation seems to exist between seminal zinc levels and male fertility, with hypofertile men having significantly lower levels compared to fertile men. The recommended daily zinc intake is 14 mg/day for men and 12 mg/day for women, and our results are comparable to studies on beef and veal, as well as values observed in *Rhynchophorus phoenicis* larvae. However, zinc levels in our four vegetable species are considerably higher. Zinc deficiencies pose a significant public health concern, particularly for infant and maternal health. They can lead to delayed growth, skeletal and sexual maturation delays, skin lesions, diarrhea, alopecia, loss of appetite, and increased susceptibility to infections due to compromised immune system function. Zinc plays a crucial role in hormonal balance, supports fertility (Rahmani et al., 2004; Latham, 2001), and acts as a cofactor in the delta-5 reductase metabolism of testosterone, producing dihydrotestosterone, or the delta-9 desaturases metabolism of prostaglandins. This zinc action, essential for hormonal receptors, differentiation, or growth promoters, explains its positive effects on cell multiplication or differentiation, as well as the potential teratogenic effects of zinc deficiencies.

Copper is an essential trace element that plays a vital role in various metabolic processes. It is known to intervene in cartilage quality, bone mineralization, the synthesis and regulation of neurotransmitter peptides, immunity, and iron metabolism. Copper also plays a significant role in the oxidative metabolism of glucose, making it essential for the proper functioning of the myocardium. Zinc is a vital element in human, animal, and plant nutrition, playing a major role in growth, development, testicular maturation, neurological functions, and immunocompetence. It is involved in the activity of numerous enzymes. Studies have demonstrated that zinc supplementation reduces the incidence of infections, vaso-occlusive pain crises, the production of tumor necrosis factor-alpha, and the reduction in oxidative stress markers.

Chronic zinc toxicity, coupled with copper deficiency, can lead to adverse effects such as anemia, neutropenia, and a diminished immune response. Selenium is touted as an antioxidant par excellence, helping combat oxidative stress (Kabuyi et al., 2017; Mukeba et al., 2020). It has been observed that the selenium content in the seminal fluid of hypofertile men is lower compared to fertile men.

Although the selenium content in these four plants is almost negligible, most of selenium's biological functions rely on selenoproteins, such as glutathione peroxidases, which antioxidant enzymes constitute a primary defense line for the body (Noël et al., 2003; Parat et al., 1997). The determination of mineral content or proteins provides insights into the nutritional quality of the analyzed sample. Micronutrients, encompassing minerals and vitamins, play a crucial role in the nutritional value of food. Micronutrient deficiencies can have significant health consequences, contributing to growth defects, disruptions in immune functions, mental and physical development, and reproductive issues that may not always be corrected through food supplementation (Latham, 2001). It is worth noting that for women of reproductive age, the importance of folic acid is well-known, and supplementation is recommended to prevent neural tube malformations. Folate, a B-complex vitamin, is also a nutrient of interest for men, essential for spermatogenesis. In a study, a combination of zinc and folic acid over 25 weeks showed a significant increase in total sperm count (Wai et al., 2002). The analysis of these four plants (*Hymenocardia acida, Landolphia lanceolata* root barks, *Mondia whitei*, and *Pentadiplandra brazzeana*) revealed 30 biogenic salts, in contrast to the 13 mineral elements studied in *Euphorbia heterophylla*, including potassium, calcium, iron, titanium, zinc, manganese, rubidium, strontium, copper, nickel, bromine, and lead (Bruno et al., 2016).

CONCLUSIONS

The X-ray fluorescence characterization of medicinal plants traditionally used as aphrodisiacs in the treatment of male sterility in Kenge City, Democratic Republic of the Congo, has provided valuable insights into the elemental composition of these plants. The analysis revealed significant concentrations of various minerals, which may contribute to the plants' medicinal properties. This study has paved the way for exploring the therapeutic potential of selected plants and integrating them into broader healthcare practices, while also promoting cultural preservation and sustainable utilization.

Results Applications

- **Therapeutic development:** The findings from this study can guide further research in developing therapeutic interventions for male sterility using selected medicinal plants. Understanding the elemental composition can aid in formulating targeted and effective treatments.
- **Quality control in herbal medicine:** X-ray fluorescence characterization can be employed as a tool for quality control in the production of herbal medicines. Establishing standards based on mineral content ensures consistency and efficacy in traditional medicine formulations.
- **Cultural and traditional knowledge preservation:** The study contributes to the documentation and preservation of traditional knowledge regarding medicinal plants. Integrating scientific analysis with traditional practices helps bridge the gap between conventional and traditional medicine.
- **Health education:** The results can be utilized for health education initiatives in the community. Educating the public about the medicinal properties of these plants, based on scientific evidence, can promote informed and responsible use.
- **Ecological conservation:** Understanding the elemental composition of medicinal plants can also have implications for ecological conservation. Efforts to sustainably harvest and cultivate these plants can be informed by knowledge of their mineral content, contributing to biodiversity conservation.

Thus, the obtained results will serve as a scientific foundation to explore the therapeutic potential of these plants in the treatment of male sterility. Additionally, they contribute to the preservation of traditional knowledge and promote responsible utilization of these natural resources.

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Ethical Approval: Nil required

Conflicts of Interest: None declared.

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