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Phytochemical and mineral profiling of anti-fertilizer plants used by pygmy women in the town of Mbandaka in the Democratic Republic of the Congo: Case of *Ipomoea involucrata* P.Beauv. and *Piptadeniastrum africanum* (Hook.f.) Brenan

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ABSTRACT

This study was carried out to determine the phytochemical and mineral composition of *Piptadeniastrum africanum* (Hook.f.) Brenan and *Ipomoea involucrata* P. Beauv., two plants presumed anti-fertilizers, were cited during an ethnobotanical survey carried out in 2018 among indigenous women in Mbandaka and the surrounding area. Micrography was carried out, followed by determination of mineral composition by ICP-AES and SAA, and the TLC for a highlight of the various phytochemical compounds making up these two plants. The study determined the relative structure of the multiple cells and tissues making up these plant drugs, including numerous raphids characteristic of *Ipomoea involucrata* and calcium oxalate crystals characteristic of *Piptadeniastrum africanum*. Among the chemical compounds, those directly linked to female anti-fertility have been highlighted. These include flavonoids, tannins, phenolic compounds, and terpenoids. Elemental analysis revealed mineral salts and trace elements directly influencing reproduction, such as iron, zinc, calcium, sodium, and arsenic. Even if these data make it possible to justify the activity of these two plants in reproductive health, it is, however, necessary for an in-depth study on the in vitro evaluation to determine the clear anti-fertilizing activity of these plants used, raw, by indigenous women.

Keywords: Contraception, Native women, Heavy metals, Raphides

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Introduction

Birth spacing still does not sit well with the conjugal duty of women in the Democratic Republic of Congo, particularly in traditional pygmy societies, where women constantly seek to ingratiate themselves with their husbands or to be the favorite in the case of polygamy (Bahuchet and Maret, 2000). To this end, pygmy women, reputed to be sexually active, often resort to all their ancestral pharmacopeia to satisfy the sexual appetite and shrink the vagina or space births (Kabena *et al.*, 2018). Ethnobotanical studies have revealed that these practices are often observed among low-income people due to the often inaccessible cost of allopathic medicines and the cultural acceptability of traditional system medicines (Diame, 2010).

However, scientific work continues to prove that the biological effects induced by these medicinal plants are due to secondary metabolites and chemical compounds, which mainly act on the reproductive system (Enitome, 2017).

At the same time, with the economic situation, we are witnessing a change in the practices and aspirations of women, in terms of sexuality, who increasingly resort to the ancestral pharmacopeia, no longer to satisfy only their sexual appetite but rather especially to space births.

Although the contraceptive methods of traditional medicine appeal more to Pygmy women in the town of Mbandaka, the unmet need for contraception remains among a good number of women, most of whom are afraid of the harmful side effects of these traditional practices (Zieran *et al*., 2004; Lazare, 2017).

As the recipes are prepared in a raw way, they contain all the chemical compounds present in the plant. As a result, even compounds with a toxic effect (heavy metals) on the reproductive system would be directly administered. This represents a danger to the health of indigenous women, who frequently use these plants as the contraception method for excellence. No in-depth study has yet been carried out to identify the phytochemical compounds linked to anti-fertilizing activity or to determine their net activity.

Based on these observations, we propose to undertake a study to determine the composition of secondary metabolites and minerals (mineral salts and trace elements) of two medicinal plants, *Piptadeniastrum africanum*, and *Ipomoea involucrata*, frequently used successfully as contraceptive agents in the traditional pharmacopeia of the pygmies of the City of Mbandaka in the Democratic Republic of Congo. The results of this study would serve as a springboard for the design of better-quality contraceptives and would contribute to the enhancement of the traditional pharmacopeia of this region.

Materials and Methods

Biological material

The biological material consists of leaves of *Ipomoea involucrata* (Convolvulaceae) and bark of *Piptadeniastrum africanum* (Fabaceae) collected at the Botanical Garden of Eala in the town of Mbandaka, in the former Province of Equateur, Democratic Republic of Congo in July 2021.

The identification of the plants collected in the field was carried out at the Herbarium of the Botanical Garden of Eala in Mbandaka and/or at the Laboratory of Systematic Botany and Plant Ecology of the Department of Biology of the Faculty of Sciences of the University of Kinshasa (Herbarium IUK) under herbal numbers: Devred 744 and Pauwels 247 for *Piptadeniastrum africanum* and Carrington 80 and Robyns 4264 for *Ipomoea involucrata*.

Conditioning

Harvested leaves and bark were dried respectively for one and two weeks at room temperature (± 27°C) in the shade (in the absence of sunlight).

Sample spraying

After the drying time has elapsed, the biological materials are reduced to a fine powder using a Moulinex grinder and then sifted using a USA Standard testing Steve brand sieve with a diameter of 1 mm. This made it possible to obtain a fine powder with a grain size of ± 1 mm.

Microscopic examination of powders (Micrography)

This review is based on the fact that the cellular elements undergo a simple slippage during spraying, which affects their shape, content, or associations very little. I did not understand. Each plant is characterized by one or more particular histological elements; these cellular forms are also found in the powder and make it possible to characterize it. It, therefore, makes it possible to detect falsification quickly from a botanical point of view.

Thus, the examination is carried out after heating the preparation based on fine powders and Steimetz's reagent, and the results are obtained after focusing at 40X magnification of an optical microscope.

Phytochemical analysis

The fine powders obtained after grinding the leaves and the bark served as base materials for obtaining the extracts (methanolic or ethanolic) to be used.

The different chemical analyses are carried out by phytochemical screening according to the method described by Kapepula *et al.* (2016). This is a qualitative analysis based on coloring and/or precipitation reactions carried out on dry plant drugs of *Piptadeniastrum africanum* and *Ipomoea involucrata*.

Determination of elemental composition by ICP-AES and SAA

The contents of the following minerals: P, Ca, Mg, Na, Co, K, Zn, Mn, Ni, Se, Pb, Cd, Al, Ba, Cr, and As were evaluated by Coupled Plasma Optical Emission Spectroscopy by Induction (ICP-AES). Atomic Absorption Spectroscopy (AAS) was used for Iron and Copper according to the method detailed by Mukeba (2021). The concentrations of the elements in the solution were determined by comparing the sample's and standard solutions' respective light intensities. The results were then transferred to the sample in mg kg^{-1}. These analyses were carried out in triplicate for each of the species studied under the following working conditions:

ICP-AES working conditions

- Instrument : ICAE S (Optima 8300 Perkin Elmer, USA);
- Power : 1500W;
- Plasma gas flow : 8 L/n;
- Nebulizer : 0.70 L/min;
- Auxiliary gas flow : 0.20 L/n;
- Plasma burn height : 5–22 mm
- Reading time : 1-5s (max 45s);
- Flow time : 1s (max 10s);
- View: Radial.

SAA working conditions

- Instrument : SAA (Anat 400 Perkin Elmer, USA),
- Flame temperature : 2800°C,
- Acetylene pressure : 0.9-1.0 bar,
- Atmospheric pressure : 4.5–5 bar,
- Reading time : 1 to 10 seconds (maximum 60 seconds),
- Flow time : 3-4 seconds (maximum 10 seconds).

Results and Discussion

Micrographic profile of herbal drugs

The microscopic profile of the leaf powder of *Ipomoea involucrata* is shown in Figure 1 below:

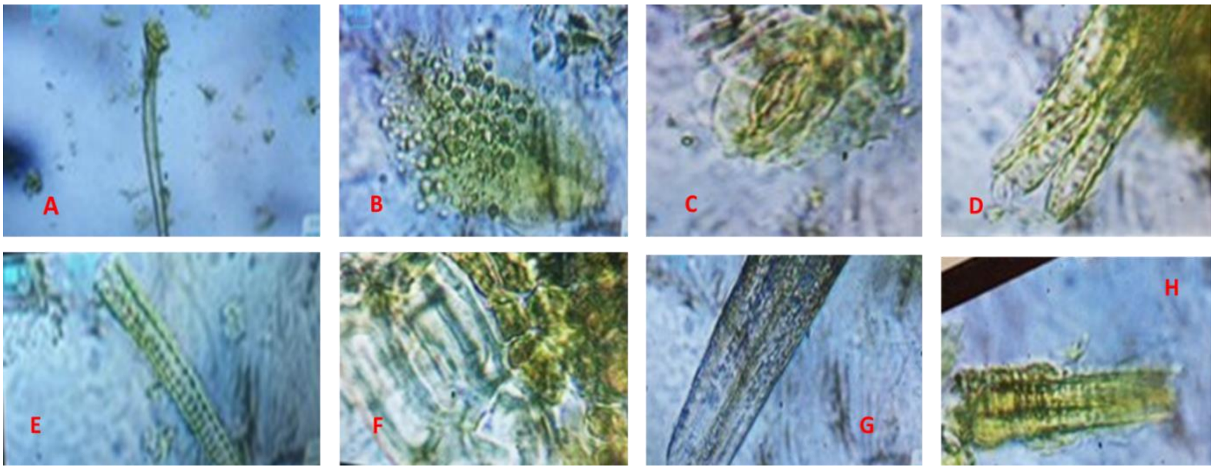

Figure 1. Microscopic profile of *Ipomoea involucrata*: Tectorial hairs (A), oil droplets (B), stomata (C), Sclerotic fibers (D), Vessels Fragment (E), Sclerides (F), Raphides (G) and conducting vessels (H).

The microscopic profile of *P. africanum* bark powdered is shown in Figure 2 below:

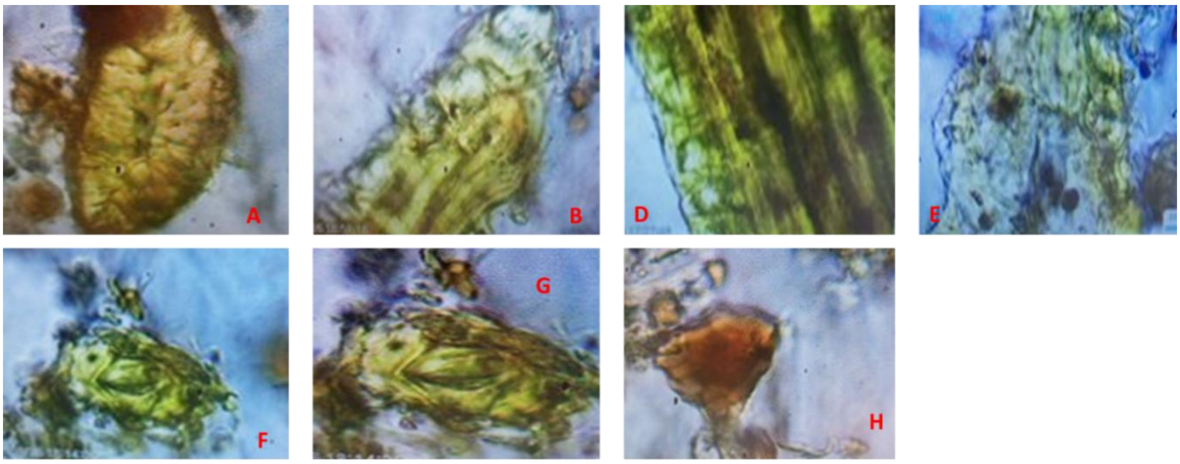

Figure 2. Microscopic profile of *P. africanum*: Sclerides (A), sclerotic fibers (B), parenchyma (C), tracheids+starch grains (D), tanniferous cells (E), sclerids (F) and crystals (G).

Therefore, the histological elements obtained for each species are characteristic of the powders of the plants studied.

It is imperative to point out the presence of numerous raphides in *Ipomoea involucrata*. The study by Hao and Zhong (2008) proved that raphides produce severe toxic reactions by facilitating the passage of toxins through the skin when the tissue containing the raphides also contains these toxins. Furthermore, plants containing calcium oxalate raphide can injure the skin and mucous membranes and cause irritation, numbness, and a burning sensation with swelling (John *et al.*, 2005).

In the current state of our knowledge, there is no data on the microscopy of the powders of these plants. These characteristic elements, therefore, constitute a database for the identification and authentication of these medicinal plants for further research.

Elemental composition of plants by ICP-AES and SAA

Analyzes revealed the presence of mineral salts and trace elements in different proportions in the two plants, as shown in Tables 1 and 2 below:

Table 1. Concentration of mineral salts expressed in mg kg^{-1} (ppm) of dry matter (Average ± SD, n=3).

Element	Mineral salt concentration expressed in mg kg^{-1} (ppm)	
	Ipomoea involucrata	*Piptadeniastrum africanum*
Potassium	15073 ± 348.19	4643.7 ± 35.34
Phosphorus	1808.9±121.43	3709.5 ± 201.53
Calcium	18863 ± 64.29	673 ± 8.30
Sodium	1138.7 ± 7.77	3064.7 ± 41.681
Magnesium	6011 ± 49.57	530.27 ± 7.45

Significant concentrations of mineral salts were found in both species. It should be noted high concentrations of potassium (15073 mg kg^{-1}) and calcium (18863 mg kg^{-1}) in the leaves of *Ipomoea involucrata* and those extremely low in calcium (673 mg kg^{-1}) and magnesium (530.27 mg kg^{-1}) in the bark of *Piptadeniastrum africanum*.

Table 2. Concentration of trace elements expressed in mg kg^{-1} (ppm) of dry matter (Average ± SD, n=3).

Elements	Mineral salt concentration expressed in mg kg^{-1} (ppm)	
	Ipomoea involucrata	*Piptadeniastrum africanum*
Iron	279.87 ± 6.16	435.030 ± 23.95
Zinc	14.88 ± 7.05	73.307 ± 12.00
Copper	27.19 ± 0.58	78.040 ± 0.99
Manganese	186.70 ± 1.94	3035.000 ± 9.07
Selenium	684.03 ± 228.41	488.100 ± 55.15
Cobalt	20.50 ± 19.76	16.050 ± 8.27
Cadmium	21.24 ± 6.13	20.060 ± 9.27
Arsenic	201.35 ± 32.17	82.490 ±78.07
Nickel	67.00 ± 51.67	61.500 ± 33.97
Aluminum	1775.00 ± 2.65	1032.700 ± 9.81
Barium	66.14 ± 5.25	1048.000 ± 5.97
Lead	ESD	419.07.000 ± 348.25

Aluminum (1775 mg kg^{-1} and 1032.7 mg kg^{-1}) and selenium (684.03 mg kg^{-1} and 488.1 mg kg^{-1}) are elements present in large quantities in the two plants. In addition, high concentrations of lead (419.07 mg kg^{-1}), barium (1048 mg kg^{-1}), iron (435.03 mg kg^{-1}), and manganese (3035 mg kg^{-1}) are found in the bark of *Piptadeniastrum africanum* and that of arsenic (201.35 mg kg^{-1}) in the leaves of *Ipomoea involucrata*.

These results show the presence of heavy metals in the two samples analyzed, including those with physicochemical properties, allowing them to cross biological barriers and accumulate in tissues, thus causing damage to the reproductive health of women. These include, among others, cadmium, aluminium, nickel, arsenic, selenium and lead. In relation to these elements, previous studies show, in particular, that:

- Cadmium has the potential to induce the appearance of endometrial cancer (Wolf and Akesson, 2008; Cho *et al.*, 2013);
- Cadmium has the ability to accumulate in the placenta, so it is toxic for the development of the fetus (Skakkebaek *et al.*, 2001);
- Women's exposure to cadmium and arsenic leads to the depletion of follicular capital. In women exposed to cadmium and arsenic, the average time required for conception is 4.70 ± 2.27 months, while it is 1.72 ± 1.31 months in unexposed women ($p < 0.0001$) (Mukendi *et al.*, 2018);
- Several experimental and epidemiological studies have shown that nickel (Ni 2+) is genotoxic (Costa *et al.*, 2002; Chen *et al.*, 2003).

Furthermore, nickel is recognized as having a carcinogenic potential (Denkhaus and Salnikow, 2002). In 1990, nickel and nickel compounds were classified by the International Agency for Research on Cancer (IARC) in group 1 "potential carcinogen for humans".

Nickel is also an allergen responsible for contact dermatitis (UKWIR, 1996);

- Many studies have explored the potential existence of a relationship between lead exposure and reproductive effects. The results are contradictory, but overall, the available data suggest a link between blood lead and the occurrence of abortions, preterm deliveries, impaired sperm, and therefore, male fertility, as well as female fertility (Amara *et al.*, 2016);
- Aluminum is one of the toxic substances that can affect male and female fertility.

Phytochemical composition by TLC

TLC analysis showed the presence of flavonoids and phenolic acids in both plants. These results agree with those found by Ateufack *et al.* (2015); Esien *et al.* (2017); Mbiantcha *et al.* (2017) and Sinan *et al.* (2020).

The leaves of *Ipomoea involucrata* contain chlorogenic acid, anthraquinones, coumarins, terpenoids, as well as gallic tannins and the bark of *Piptadeniastrum africanum* contains anthocyanins, terpenoids, gallic and catechin tannins.

The significant presence of these phytochemicals may help justify their use as a means of birth spacing by Aboriginal women based on reports of previous studies that demonstrate that flavonoids and phenolic compounds block ovulation and destroy the estrous cycle. Sharma *et al.* (2013) also show that tannins, alkaloids, and saponins contribute to interrupting the estrous cycle. Furthermore, these compounds lead to a reduction in the weight and diameter of the uterus, of its myometrium and endometrium, indicating an uterotrophic effect.

The same study also shows that saponins, tannins, flavonoids and cardiac glycosides contribute to reducing egg count.

The search for alkaloids gave a negative result for both species. This is different from the results of Esien *et al.* (2017) and Sinan *et al.* (2020).

Conclusion and Recommendations

The general aim of this study was to evaluate the phytochemical, qualitative and quantitative chemical composition, as well as the characteristic elements of *Piptadeniastrum africanum* and *Ipomoea involucrata,* plants used by pygmy women in Mbandaka.

The phytochemical composition was determined to detect the presence of active ingredients to understand the use of these plants as a means of contraception in indigenous pharmacopeia. In addition, the chemical analysis was done to have an overall view of the different chemical elements that the raw recipes used would contain and the micrography made it possible to ensure that there was no involuntary falsification, which could mow the results of the analyses, in the powders used as biological study material.

The characteristic histological elements allowing the identification of these two species have been highlighted. This study shows that these two species have various secondary metabolites that directly impact female fertility, namely flavonoids, tannins, terpenoids and phenolic compounds, as demonstrated by previous studies. This justifies the use of these plants as a means of birth spacing. Moreover, certain minerals found in the drugs of these two species are considered to have direct positive or negative effects on female fertility. This is the case for calcium, sodium, potassium, magnesium, arsenic, nickel, cadmium, lead and aluminum.

Thus, we suggest that extensive studies on the clear activity of these plants in reproductive health be carried out and that the active principles responsible are isolated with a view to the development of "organic contraceptive pills" to improve the use of these plants in traditional reproductive health care.

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