Original Research Article

Secondary metabolites and mineral elements of *Manotes expansa* and *Aframomum Alboviolaceum* leaves collected in the Democratic Republic of Congo

ABSTRACT

Background and Aims: *Manotes expansa* Sol. ex Planch. and *Aframomum alboviolaceum* (Ridl.) Schum. are two plants belonging respectively to the family *Connaraceae* R.Br. and *Zingiberaceae* Martino widely used in traditional medicine for the treatment of eye diseases, fever, headaches, gastritis as well as asthma. The aim of the present study is the valorization of these two plants collected in the Democratic Republic of Congo by a quantitative and qualitative analysis of secondary metabolites and mineral elements in their leaves.
Materials and Methods: The determination of secondary metabolites in the leaves of *Manotes expansa* and *Aframomum alboviolaceum* was carried out by UV-Visible spectrophotometry and X-ray fluorescence spectrophotometry for the identification and quantitative analysis of mineral elements.
Results: The results showed that the leaves of these two plants are rich in phenolic compounds, i.e. 442.2 mgEqAG/g for the leaves of *A. alboviolaceum*, 370.64 mgEqAG/g for the red leaves and 282.64 mgEqAG/g green leaves of *M. expansa*. Although being part of the same plant, the red and green leaves of *M. expansa* presented a totally different phytochemical profile. The contents of condensed tannins, anthocyanins and flavonoids are respectively 0.3%, 0.68% and 3.29% for the leaves of *A. alboviolaceum*; 0.58%, 0.36% and 6.89% for the red leaves, and 0.65%, 0.26% and 7.55% for the green leaves of *M. expansa.* The mineral content in the leaves of both plants remains dominated by the high concentration of potassium (K), calcium (Ca), Magnesium (Mg), Manganese (Mn) and Iron (Fe).
Conclusion: The high content of phenolic compounds and essential trace elements makes the leaves of *M. expansa* and *A. alboviolaceum* potential candidates to alleviate several health problems in Africa in general and particularly in the Democratic Republic of Congo.

Keywords: Manotes expansa, Aframomum alboviolaceum, mineral content, secondary metabolites, Democratic Republic of Congo

1. INTRODUCTION

Long time ago, plants have been used by humans for various needs including food and medicine. Almost 40% of currently available drugs have been derived directly or indirectly from natural precursors from plants. The therapeutic power of plants is always associated to the different chemical compounds often found in the plant kingdom (secondary metabolites and mineral elements) [1][2]. The study of plant chemistry has always been a topical issue despite its antiquity, this is mainly due to the fact that the plant kingdom represents an important source of huge variety of bioactive molecules that are used in food industry, cosmetology and pharmacy etc. Among these compounds, Anthocyanins, alkaloids, saponins, tannins, terpenes and flavonoids etc. [3]. In addition, the leaves of plants are also sources of mineral elements and vitamins that are available to everyone. The human body for example consists of about 1.9% of mineral elements in the form of macro and micro nutrients. These elements must be supplied regularly through diet [4][5]. Deficiency of mineral elements in the human body can lead to serious diseases such as hemoglobin synthesis disorders, inflammatory anemia, fatigue, growth retardation, neural tube defects, immune depression, osteoporosis, muscle spasms, nervous disorders etc. [6].

The main objective of the present study is to enhance the use of medicinal plants in the diet and in the management of certain diseases. To do this, a particular choice was made on *Manotes expansa* Sol. ex Planch. and *Aframomum alboviolaceum* (Ridl.) K.Schum..

These two plants belonging respectively to the family *Connaraceae* R.Br. and *Zingiberaceae* Martinov are widely used for their therapeutic properties in management of several diseases such as: eye diseases, fever, headaches, gastritis, asthma, amoebiasis, painful menstruation, tuberculosis, diarrhea, dysentery, anemia, etc. [7][8][9]. Thus, a qualitative and quantitative study of different secondary metabolites and mineral elements present in the leaves of these two plants collected in the Democratic Republic of Congo was carried out.

2. MATERIALS AND METHODS

2.1 Materials

The leaves of *M. expansa* (green and color) and *A. albovialaceum* were collected in Kinshasa, precisely in the commune of Mont-Ngafula in March 2019 (DRC). These samples were identified and authenticated at the Herbarium of the Institut National d'Etudes et de Recherches Agronomiques (INERA) at the Faculty of Sciences of the University of Kinshasa. The leaves of these plants were dried in the open air and protected from sunlight for two weeks, and then ground to powder using an electric grinder (Sinbo).

2.2 Methods

2.2.1 Chemical screening in solution

The phytochemical screening in solution was carried out using the method described by Békro [10].

2.2.2. Thin layer chromatography (TLC)

The standard protocol of Wagner was used in order to highlight certain secondary metabolites by spots of different colors on chromatograms [11].

2.2.3. Determination of secondary metabolites

2.2.3.1. Determination of total polyphenols

The content of total polyphenols was determined by the method of Folin-Ciocalteu [12]. Briefly, 200µL of the extract was mixed with 1mL of freshly prepared Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 0.8 mL of 7.5% sodium carbonate. The whole set was incubated at room temperature for 30 minutes and read against a blank using a UV-visible spectrometer type JENWAY 7315 at 760 nm. A calibration curve of gallic acid at different concentrations (50µg/mL to 350µg/mL in 80% methanol), prepared under the same conditions with the extract was plotted for the calculation of the total polyphenol concentration expressed in milligrams of gallic acid equivalent per gram of dry plant matter (mgEAG/g).

2.2.3.2 Determination of flavonoids and tannins

The percentages of flavonoids and tannins in the samples of plant leaves were determined following the experimental protocol described by Dohou et al (2003) [13].

2.2.3.3 Determination of anthocyanins

The determination of anthocyanins was done according to the methodology of Lebreton et al (1967) [14].

2.2.4 Determination of minerals by x-ray fluorescence spectrometry

Minerals content was determined done using an X-ray fluorescence spectrophotometer. The preparation of samples and measurements for the quantification of the mineral elements by X-ray fluorescence were carried out following the procedure described by Kabengele et al (2020) [15].

3. RESULTS AND DISCUSSION

3.1 Chemical screening

3.1.1 Chemical screening in solution

The results of phytochemical screening in solution of *A. alboviolaceum* and *M. expansa* leaves show the presence of polyphenolic compounds (flavonoids, Anthocyanins, leuco-anthocyanins, Tannins, saponins, steroids and free quinones) while the triterpenoids, alkaloids and bound quinones are absent in *A. alboviolaceum* leaves. For *M. expansa green* and red leaves, a similar phytochemical profile was found regarding the presence of polyphenolic compounds. The triterpenoids are also present, while the free alkaloids and quinones are absent in red leaves.

3.1.2 Chemical screening by TLC

The result of the chemical screening by TLC of leaves of *A. alboviolaceum* and *M. expansa* confirmed the presence of triterpenes, anthracene derivatives, alkaloids and flavonoids as presented in figures 1,2,3 and 4.

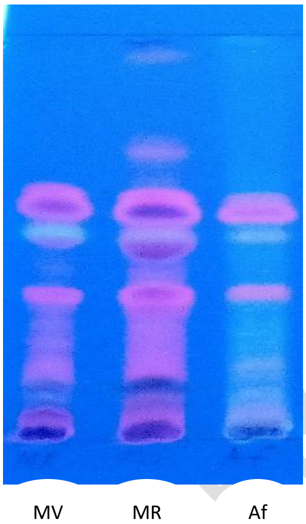

Fig.1. Chromatogram of the methanolic extract of *A. alboviolaceum* and *M. expansa* for anthraquinons
Mobile phase: Ethyl acetate/Methanol/Water (50 :8,5 :6,5).
Detection: UV at 366nm

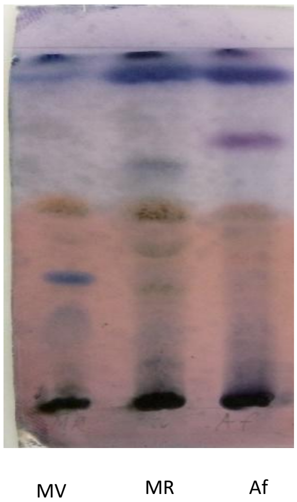

Fig.2. chromatogram for terpenes
Mobile phase : Toluene/ Ethyl acetate (31 :2,5).
Detection : Sulfuric anysaldehyde

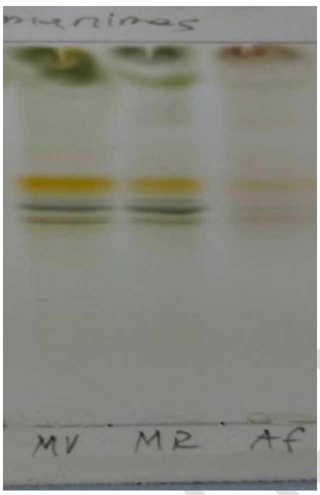

Fig.3. Chromatogram for alkaloids
Mobile phase : Toluene/ ethyl acetate/ dietthylamine (35 : 10 : 5).
Détection : $NaNO_2$ 5%.

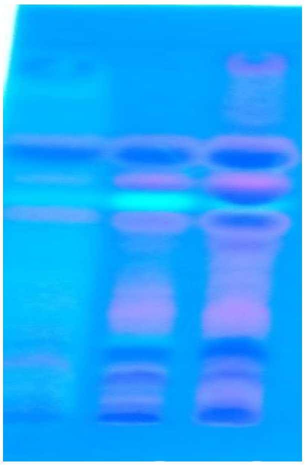

Fig. 4. chromatogram for flavonoids. Elution system: ethyl acetate/ formic acid/ glacial acetic acid/ water (50: 6.5: 6.5: 13.5)
Detection: Neu reagent and UV at 366 nm

The TLC results presented in figures (1, 2, 3, 4) above show the presence of phenolic compounds (flavonoids, anthraquinones, terpenes) and alkaloids. The anthracene compounds revealed by the presence of red spots on figure 1 after revelation at 366 nm and 10% ethanolic KOH. The terpenoids are identified by the blue spots on the chromatogram at figure 2 after revelation with sulfuric anysaldehyde and heating at 100°C for 10minutes. Figure 3 shows the spots corresponding to the presence of alkaloids which are yellow in color after revelation with a 5% $NaNO_2$ solution. The blue fluorescence on the chromatogram of figure 4 confirms the presence of flavonoids in the 3 samples after spraying with Neu reagent and UV lamp at 366 nm.

The results of the chemical screening in solution and TLC prove that the leaves of these two plants are rich in phenolic compounds. It is worth mentioning that, although being part of the same plant (*M. expansa*), the green and red leaves revealed some differences in the presence or not of some metabolites and their TLC profile is slightly different. These observed differences in the composition of *M. expansa* leaves are the results of the evolution and distribution of metabolites as a function of the maturity of different parts of the plant [16].The comparison of result of this study with the one conducted by Inkoto et al (2018) on *A. Alboviolaceum* shows a similar phytochemical profile except for alkaloids. The results obtained from this study for *Manotes longiflora* are similar to the one conducted by Kablan in 2008 [17][18].

3.2 Secondary metabolite determination

3.2.1 Total polyphenol content

The total polyphenol content expressed as milligram gallic acid equivalent per gram of dry matter (mgEqAG/g) in leaves of two species was determined from the linear regression equation of the gallic acid calibration curve. The results obtained show that *A. alboviolaceum* leaves have the highest total polyphenol content of 442.2mgEqAG/g. 370.64mgEqAG/g for red leaves and 282.64mgEqAG/g green leaves of *M. expansa*.

3.2.2 Flavonoid, tannin and anthocyanin contents

The flavonoid, tannin and anthocyanin contents expressed in % are shown in the following table 1:

Table 1: concentrations of flavonoids, tannins and anthocyanins in the three leaf samples.

Métabolites	Concentration (%)		
	M. expansa (green)	*M. expansa (red)*	*A. alboviolaceum*
Flavonoïds	7.55	6.89	3.29
Anthocyanins	0.26	0.36	0.68
Hydrolysable Tanins	2.14	1.87	1.67
condensed Tanins	0.65	0.58	0.30

3.3 Determination of minerals by X-ray fluorescence spectrometry

X ray fluorescence spectrometry analysis on powders of *A. alboviolaceum and M. expansa* (red and green part) allowed the quantification of several mineral elements. The results are presented in Table 2 below.

Table 2: Concentration of mineral elements in the samples

	Concentration (mg/Kg)		
Eléments	***M. expansa (Red)***	***M. expansa (green)***	***A. aboviolaceum***
Potassium (K)	**18360.2 ±103.8**	**5898.1 ±289,1**	**28830.0 ±203.7**
Phosphore (P)	**3769.0 ±73.2**	**1238.0 ±56.1**	**3862.4 ±43.8**
Calcium (Ca)	**3594.3 ±92,1**	**11480.0 ±102,3**	**794.0 ±12.7**
Sodium (Na)	**4570.0 ±101.4**	**5840.8 ±122.9**	**590.0 ±11.8**
Magnésium (Mg)	**556.0 ±29.1**	**1465.0 ±32.2**	**576.7 ±19.1**
Soufre (S)	**3655.3 ±142,8**	**3226.0 ±207,1**	**3683.0 ±192.8**
Chlore (Cl)	**231.7 ±8.7**	**178.8 ±11.6**	**1410.2 ±63.4**
Manganèse (Mn)	**179.9 ±17.1**	**886.2 ±34.8**	**64.8 ±7.7**
Aluminium (Al)	**380.2 ±12.8**	**414.4 ±13.0**	**248.9 ±17.5**
Neodymium (Nd)	**18.7 ±2.8**	**8.6 ±2.1**	**28.6 ±5.2**
Fer (Fe)	**236,0 ±21,2**	**271,0 ±27,8**	**90.0 ±8.2**
Zinc (Zinc)	**38,8 ±3.8**	**8,3 ±3.2**	**52.8 ±3.4**
Cuivre (Cu)	**15.7 ±2.1**	**7.5 ±0.6**	**20.4 ±1.3**

Strontium (Sr)	**6.3 ±0.2**	**41.2 ±6.2**	**-**
Rubidium (Rb)	**99.8 ±23.9**	**22.8 ±9.1**	**111.8 ±12.3**
Brome (Br)	**225.9 ±42.2**	**66.0 ±2.1**	**7.6±0.9**
Argent (Ag)	**5.7 ±0.6**	**-**	**8.9 ±2.1**
Silicium (Si)	**1129.0 ±179.2**	**1532.0±186.3**	**1386.0±207.9**
Titane (Ti)	**19.7± 2.4**	**32.6 ± 5.3**	**11.6 ±1.2**
Nickel (Ni)	**12.9 ±2.1**	**13.4 ±3.2**	**3.5 ±0.8**

The mineralogical study carried out by the X-ray fluorescence method allowed the characterization of 20 elements including calcium (Ca), sodium (Na), potassium (K), phosphorus (P), magnesium (Mg), sulfur (S), Chlorine (Cl), Aluminum (Al), Manganese (Mn), Iron (Fe), Zinc (Zn), Copper (Cu), Strontium (Sr), Rubidium (Rb), Bromine (Br), Silicon (Si), Neodymium (Nd), Nickel (Ni), Titanium (Ti) and Silver (Ag).

This table 2 shows that all three samples contain the 7 macroelements (K, P, Ca, Na, Mg, S and Cl). Indeed, potassium is the most abundant element in *A. alboviolaceum*, which was also confirmed by the study result by Latham & Kumbuta (2010). Ca is the most abundant element in the green part of *M. expansa* and K in the red part.

The comparison of the results of this study with the one carried out by Enzonga et al (2019) in Congo Brazzaville and the one carried out by Herzog et al (2015) in Ivory Coast on *A. alboviolaceum* seeds reveals huge discrepancies in the concentration values of elements such as Mg, Fe, Ca, P [19][20].

The concentrations of mineral elements such as K, Ca, Zn, Cu, Br, Mn, Ni found in the study of Agbo et al (2020), on the leaves of *M. expansa* in Ivory Coast are far lower than those found in the same plant harvested in DRC. This may be attributed to the different chemical composition of the harvest soil [21]. It should be noted that the element Sr was not detected in *A. alboviolaceum* and the element silver was not identified in the green part of *M. expansa*.

Manotes expansa leaves are very rich in mineral elements, especially Iron which is found in high content in both red and green parts with concentrations of 236.0 and 271.0 ppm respectively. Iron is one of the constituents of hemoglobin and it is also involved in oxygen transport in the cell; thus *M. expansa* can be used in anemia cases because of its high iron content [22]. Consumption of *M. expansa* leaves can be considered as an alternative solution to this problem of deficiency of iron element. High concentration of Zinc in red leaves of *M. expansa* and *A. alboviolaceum* confirms their use in strengthening the immune system and preventing some diseases of microbial origin [21].

4. CONCLUSION

This work focused on the phytochemical profile of the leaves of *A. alboviolaceum* and *M. expansa*. The aim was to identify and quantify the mineral elements and secondary metabolites present in the leaves of these two plants. The results of chemical screening by TLC and in solution revealed that the leaves of *A. alboviolaceum* and *M. expansa* are rich in secondary metabolites including polyphenols (flavonoids, saponins, tannins, anthocyanins, leuco-anthocyanins), alkaloids, terpenoids, steroids and free quinones. Quantitative analysis of the different secondary metabolites present in the leaves of these two plants allowed the determination of different concentrations of polyphenolic compounds.

The results showed that the leaves of *A. alboviolaceum* are very rich in total polyphenols compared to the leaves of *M. expansa*. A high level of flavonoids was obtained in the green colored leaves of *M. expansa*; anthocyanins and saponins were found in high amounts in the leaves of *A. alboviolaceum*. Mineralogical determination on the powder of each sample revealed the presence of 20 mineral elements, namely Ca, Na, K, P, Mg, S, Cl, Al, Mn, Fe, Zn, Cu, Sr, Rb, Br, Si, Nd, Ni, Ti and Ag. It appears from these results that the leaves of these two plants are very rich in mineral elements and secondary metabolites, this can justify their multiple uses in the treatment of several diseases.

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