Antifalcemic activity, osmotic fragility test of red blood cells and mineral element contents of: *Cyttaranthus congolensis and Hypoxis angustifolia p*lants used in the management of sickle cell disease in Kwilu province Democratic Republic of Congo (DRC).

Abstract.

The objective of the present study was to determine the composition in mineral elements, the antifalcemic activity and the osmotic brittleness test, of the twenty three extracts of two plants *Cyttaranthus congolensis*, *Hypoxis angustifolia* used in the management of sickle cell disease by the traditional healers in Kwilu province. Mineral composition analyses of these two plants were performed using X-ray fluorescence spectrometric method. Twenty three(23) mineral elements were identified in each of these two plants among others: Potassium (K), Phosphorus (P), Calcium (Ca), Sodium (Na), Magnesium (Mg), Sulfur (S), Chlorine (Cl) and trace elements such as: Aluminum (Al), Silicon (Si), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Nickel (Ni), Copper (Cu), Zinc (Zn), Selenium (Se), Bromine (Br), Molybdenum (Mo), Tin (Sn), Iodine (I), Barium (Ba) and Lead (Pb). Mineral elements related to sickle cell disease are: Iron (Fe), Zinc (Zn), Selenium (Se), Copper (Cu), Calcium (Ca), Magnesium (Mg) and Manganese (Mn). Of all these elements, Potassium and Calcium are in higher content while Lead, Tin, Bromine, Copper and Nickel are in trace amounts. The antifalcemic activity of *Cyttaranthus congolensis* and *Hypoxis angustifolia* extracts, was tested using the Emmel test. The results obtained showed a significant *in vitro a*ntifalcemic activity for both plants. The osmotic fragility test used to confirm the Emmel test showed that the hemolysis rate decreased with increasing NaCl concentration. In the presence of anthocyanins, the antihemolytic activity at different NaCl concentrations was higher than the control. This indicates the action of the extracts of these plants on the osmotic fragility of red blood cell membrane.

Keywords: *Cyttaranthus congolensis*, *Hypoxis angustifolia*, osmotic fragility, sickle cell disease, hemolysis, mineral elements, fluorescence.

Introduction

Medicinal plants are an important source of health care worldwide (Srivastava, 2000; Hamilton, 2004). Traditional medicines are used extensively due to population growth and inaccessibility to modern medicines (Augustino and Gillah, 2005). Nearly 80% of the population relies on traditional medicine for their primary health care, according to the World Health Organization (WHO, 2002 In Africa, this demand is not only a result of the inaccessibility to modern medicines and their the high costs, but traditional medicine, which is very often considered a more appropriate treatment method (Marshall, 1998).

In the Democratic Republic of Congo, urban and rural populations are increasingly turning to the use of medicinal plants for their health problems (Betti, 2002). Sickle cell disease a hereditary disease affects nearly 5% of the world's population. It is caused by a mutation in the hemoglobin beta globin gene and is characterized by severe anemia, vaso-occlusive crises and high susceptibility to both viral and bacterial infections (Mpiana et al., 2014). This disease is characterized by a loss of mineral elements important in the functioning of the body (Kitadi et al., 2021 d).

Minerals are natural chemical elements that the body uses to activate certain biochemical processes. They include functionally important inorganic compounds such as iron (Fe) in hemoglobin and cytochrome or zinc (Zn) in insulin [Bruneton, 2002].

It is also established that plant leaves are potential sources of minerals and vitamins and are apparently inexpensive (Kitadi et al., 2021e). In order to scientifically validate the phyto-therapeutic richness of the DRC, two plants were chosen: *Cyttaranthus congolensis* and *Hypoxis angustifolia*, used in the Congolese pharmacopoeia for the treatment of sickle cell disease. The general objective of this study was to determine the mineral composition of the two plants used for the management of sickle cell disease.

2. Material and methods

The leaves of *Cyttaranthus congolensis*, and the bulbs of *Hypoxis angustifolia* used in this study were collected in May and July 2016 in Masi-Manimba Territory, Kwilu Province, whose geographical coordinates are: 5° 02′ 01″ South, 18° 50′ 01″ East. These samples were dried in the dark at the University of Kikwit in the Basic Science Laboratory. They were then grounded to a fine powder. . Total extracts of anthocyanins from both plants showed significant antifalcitrant activity. The analysis of the results was done with Excel. The osmotic fragility test was done in order to have an idea on the action of the extracts of these two plants on the osmotic fragility of red blood cell membrane.

2.1. Antifalcemic activity

The blood sample was mixed with the plant extracts at different concentrations (with physiological water as dissolution solvent). The Emmel test was used to evaluate the antifalcemic activity (Courtejoie and Hartaing, 1992). In this study, the Emmel test was modified as previously reported by Mpiana et al., (2007a). Microscopic images were obtained using a MOTIC 30207598 light microscope. A KODAK EASY SHARE C613 camera was used to digitize the micrographs. These were then processed using the MOTIC images 2000 software, version 1.3.

2.2. Detection of mineral elements

Detection and quantification of mineral elements was done by the X-ray fluorescence spectrometric method. This method of analysis allows determination of several elements in the same sample. A quantity of the powder was compressed into pellets through the hydraulic press for each plant and the resulting pellets were introduced into the X-ray fluorescence spectrophotometer for reading. ~~The~~ Analysis of the results was done with Excel.

2.3 Osmotic fragility test

Red blood cell (RBC) fragility was determined as previously described by Mpiana et al.,(2010c). Cells were placed in a graded series of hypotonic saline solutions buffered at pH 7.4 with 150 mM phosphate. Concentrations ranging from 0.2% to 0.9% NaCl were obtained in a final volume of 10 mL. A 10 µL sample of washed erythrocytes was added to 1990 µL of each hypotonic saline solution and immediately mixed by inverting several times. The tubes were allowed to stand for 150 min at room temperature (25°C). To determine the effect of anthocyanin extracts, Ten microliters (10 µL) of extract (30 mg/mL) was added to 1980 µL of each hypotonic saline solution, followed by 10 µL of RBCs and the mixture treated as described previously. The number of unlysed erythrocytes at

each saline concentration was determined by light microscopy (OLYMPUS×21) and hemacytometer (Neubauer cell). Hemolysis was calculated using the following equation:

Number of erythrocytes after 150 min×100/number of erythrocytes inoculated (0 min). [2]

The mean corpuscular fragility (determined from the saline concentration causing 50% hemolysis of red blood cells) was obtained from a lysis (%) versus NaCl concentration curve.

The effect of anthocyanins or organic acids on the ability of sickle cells to hydrate while resisting hemolysis following hyposmotic (hypotonic) shock is exploited as an index of their biological activity. This index reflects the effect of these metabolites on the membrane stability of erythrocytes.

The effect of anthocyanins on red blood cell membrane fragility is assessed by comparing the % hemolysis rate of SS blood cells alone and those treated with anthocyanins (Ngbolua, 2012, Kitadi et al., 2020 b).

3. RESULTS AND DISCUSSION

Figure 1 shows a micrograph of SS blood alone

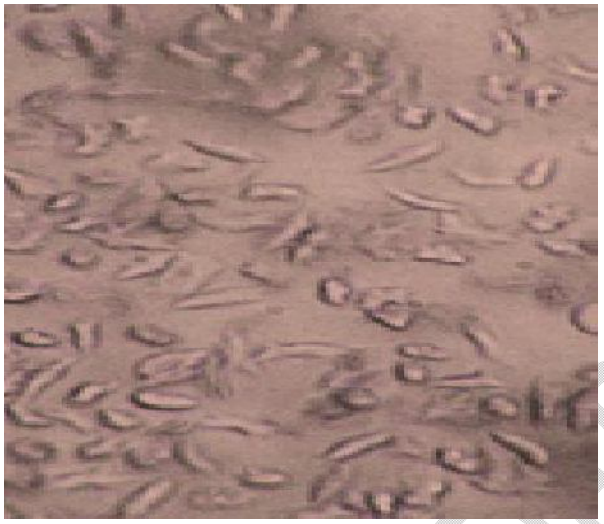

Figure 1: Optical micrograph of untreated (control) SS blood erythrocytes [0.9% NaCl, 2% Na2S2O5, 500 X magnification].

Micrographs of SS blood in the presence of total extracts of *Cyttaranthus congolensis* leaves is shown in Figure 2.

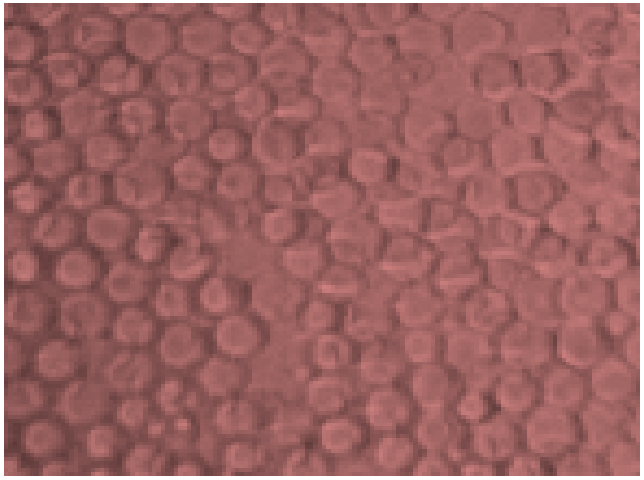

Figure 2: Optical microphotographs of SS blood erythrocytes treated with the total extracts of Cyttaranthus congolensis leaves, [NaCl 0.9%, Na2S2O5 2%, Magnification: 500 X].

Comparing figure 2 to figure 1 representing SS blood alone used as a control, we notice that figure 2 shows that the majority of erythrocytes in SS blood, although under hypoxic conditions, return to their normal shape. This modification of the morphology of sickle cells indicates an antifalcemic activity of the total extracts of these plants taken as an illustration.

This behavior was already observed for anthocyanins and organic acids extracted from other plants (Mpiana et al, 2009; Tshibangu, 2009; Kitadi, 2016). Our results therefore confirm those obtained in previous studies [Mpiana et al, 2009]. Table 1 gives mineral content of these two plants.

Table 1: Mineral content of *Hypoxis angustifolia* and *Cyttaranthus congolensis* plants used in the management of sickle cell disease

ELTS	***Hy.angustifoli***	***Cyt.congolens***	***Vigna unguic*** ***Famata et al(***	***Vigna unguiculata*** ***Kure et al (2016)***	***Alchornea co*** ***Ebenyi et al (***	***Alchornea cordifo*** ***Philip et al (2016)***	***A.senegalensis*** **(Yisa *et al*)**	***V. unguiculata*** ***(YOKA et al***
Cu	4,94	4,49	-	-	3,70	58,35	130,00	197,00
Fe	56,91	790,1	6,0	9,88	1,40	192,5	1800,00	197,00
Mn	10,19	46,87	-	1,00	1,25	32,5	290,00	11,6
Se	ND	ND	5,72	6,10	4,12	-	480,00	19,9
Mg	550,10	3477,53	-	-	3,60	-		
Zn	25,93	47,88	10,00	190,22	5,60	22,00	1350	24,5

Hy : *Hypoxis*, *Cyt* : *Cyttaranthus*, *ungui* : *unguiculata*, V : *Vigna*

The Kure et *al* (2016) identified four mineral elements including Fe, Mn, Cu and Zn in *Vigna unguiculata.*

The content of Fe in *Cyttaranthus congolensis* plant in this study is higher than that obtained in the bulbs of *Hypoxis angustifolia*. It is higher than that obtained by the other researchers except in the leaves of Annona senegalensis. The lowest content of this element is that obtained by Ebenyi et *al* (2017). Cu has a high content in *Vigna unguiculata* in study reported by Yoka et al (2014) as was the case for Fe while the lowest content of this element was obtained by Ebenyi et al (2016) or 3.70 ppm. The content of this element (Fe) for this study is between that of Philip et *al* (2016) found in the leaves of *Alchornea cordifolia* and in the leaves of *Cyttaranthus congolensis.* The element Zn is found in high content in *Annona senegalensis* studied by Yisa's et al while it is in low content in *Alchornea cordifolia* studied by Ebenyi et al. That of the two plants in this study is between *Cyttaranthus congolensis* and *Alchornea cordifolia* studied by Philip et al for *Hypoxis angustifolia* and between that of the seeds of *Vigna unguiculata* studied by Kure et al and

that of the bulb of *Hypoxis angustifolia* in this study. Se was not quantified in the two plants of this study its high content was obtained in *Annona senegalensis* studied by Yoka et al. The highest content of Mn obtained in this research is that in *Cyttaranthus congolensis* i.e. 46.87 ppm but low compared to that obtained by Yisa et *al* in leaves of *Annona senegalensis*. It was also noticed that the Fe content obtained by Yisa et *al* (1800.00 ppm) is higher compared to that of these two plants under study. It follows that the Mg content is higher in the leaves of *Cyttaranthus congolensis*, it is low in the leaves of *Alchornea cordifolia* studied by Ebenyi et *al*.

A similar study conducted by Elenga et *al*. (2015) on *Phytolacca dodecandra*, a kind of wild *spinach*, and *Spinacia oleracea*, or *spinach*, and by Houmkali (1982) on *Salvinia nymphellula*, a species of aquatic fern, showed similar results to those obtained in this study for the content of iron, an element that is essential for the survival of living beings because, it is iron hemoglobin that conveyed oxygen via the blood to the tissues.

In order to assess the osmotic fragility of sickle cell, the rate of hemolysis of erythrocytes at different NaCl concentrations, in the presence and absence of *anthocyanin* extracts was measured as shown in Figure 3.

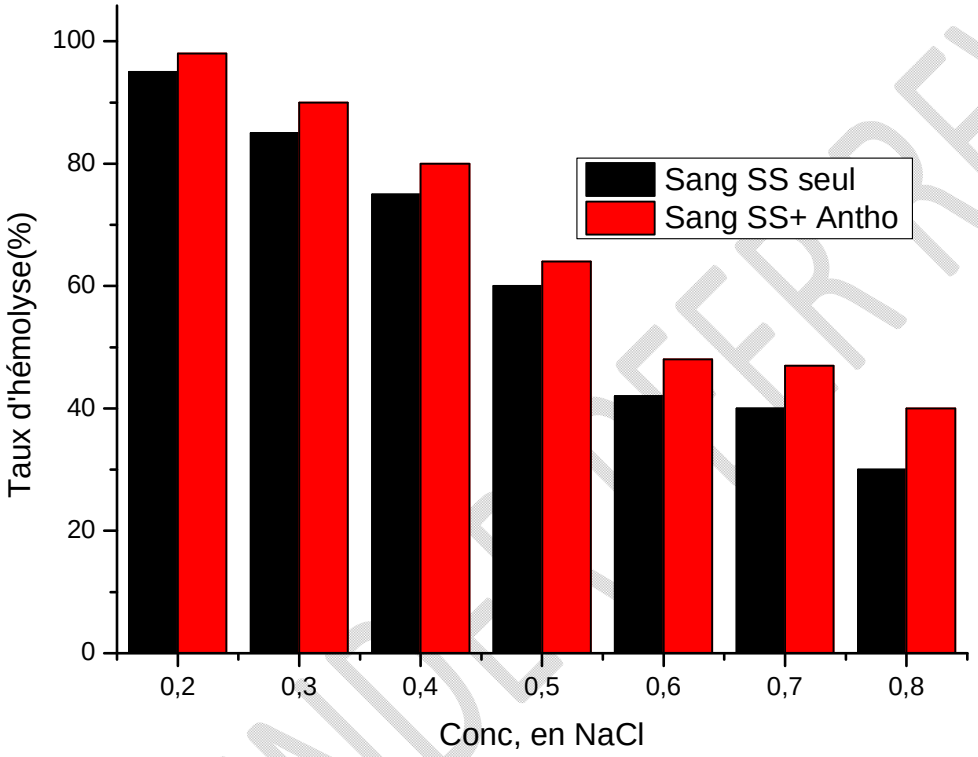

Figure 3 NaCl osmotic fragility test in %.

The rate of hemolysis decreases with increasing NaCl concentration (Fig. 3). In the presence of anthocyanins, the antihemolytic activity at different NaCl concentrations is higher than the control. This means that anthocyanins improve the hydration capacity of blood cells. The rehydration of cells prevents polymerization of HbS and falciformation of red blood cells (Mpiana et al., 2010 d).

Anthocyanins could therefore, in addition to their probable action on HbS solubility, have a direct action on the erythrocyte membrane probably on the phospholipids of erythrocyte membranes.

Indeed, the membrane of sickle cells is generally more rigid than that of normal erythrocytes. This results in a decrease in the efflux of Ca^{++} ion from the cell. The intracellular accumulation of calcium ions leads to cellular dehydration and consequently to an intracellular increase in HbS concentration. This increase in HbS concentration inside red blood cells accelerates polymerization of HbS, which precipitates on the membrane and thus enhances its rigidity (Kambale et al., 2013). Water leaving the cells can accumulate in the cellular interstices and cause generalized edema leading to hand-foot syndrome in young children with sickle cell disease.

Rehydration of cells by anthocyanins could therefore lead to the reduction of edema. The main pathways of dehydration of sickle cell red blood cells are the co-transport (K-Cl) and the K^+ channel activated by a calcium influx, called Gardos channel. The latter is active upon deoxygenation inducing polymer formation and increased membrane permeability to mono- and divalent cations, including the calcium ion Ca^{++}. The transient increase in intraglobular ionized calcium is sufficient to activate the Gardos K^+ channel and induce potassium and water leakage from red blood cells and reticulocytes (Kitadi et al., 2021e).

Conclusion

The objective of this study was to determine the mineral composition, the antifalcemic activity and the osmotic fragility test of the extracts of the two medicinal plants used in traditional Congolese medicine in the Kwilu province in the management of sickle cell disease. Results obtained showed a clear correction of the falciformity of the red blood cells because these falciform red blood cells recovered their normal form in contact with the total aqueous extracts of these plants. In addition to this correction, the extracts of these plants showed a high antihemolytic activity because it was found that the rate of hemolysis decreased with increasing NaCl concentration. This means that anthocyanins improve the hydration capacity of blood cells. The rehydration of cells prevents HbS polymerization and falciformation of red blood cells (Mpiana et al., 2010 d).

Results obtained from the fluorescence spectrophotometric method show that both plants contain 23 distinct mineral elements each. These results also show that calcium and potassium are the most abundant in the leaves of *Cyttaranthus congolensis* than in the bulbs of *Hypoxis angustifolia*. On the other hand, the iron content is by far higher in the leaves of *Cyttaranthus congolensis* than in the bulbs of *Hypoxis angustifolia*. Other elements such as magnesium, zinc, selenium, manganese, copper and cobalt present in these two plants play important roles in sickle cell disease. Magnesium, in addition to being a cofactor of enzymes, reduces the number of abnormal erythrocytes in sickle cell patients and improves the hydration of red blood cells. Comparing the mineral contents of this research with those of the other research teams, a clear difference emerges which is at least due to the method used by each team and also to the mineral composition of the soil where these different plants are harvested.

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