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ANTISICKLING ACTIVITY AND MINERAL CONTENT OF *HURA CREPITANS* L., *ALTERNANTHERA BETTZICKIANA* (REGLE) G. NICHOLSON AND *DISSOTIS BRAZZAE* COGN, PLANTS USED IN THE MANAGEMENT OF SICKLE CELL DISEASE IN KWILU PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO

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ABSTRACT

This study was initiated to determine the qualitative and quantitative mineral composition and the antisickling activity of three plants mostly used in Congolese traditional medicine for the management of sickle cell disease in Kwilu province, Democratic Republic of the Congo. Qualitative and quantitative analyses of the mineral composition of the leaves of *Alternanthera bettzickiana*, *Dissotis brazzae* and the barks of *Hura crepitans* harvested in Kwilu province were carried out using the fluorescence spectrometric and ICP-OES methods. The results obtained in this study showed that 23 mineral elements were present in the three plants studied, including macroelements such as Potassium (K), Phosphorus (P), Calcium (Ca), Sodium (Na), Magnesium (Mg), Sulfur (S), Chlorine (Cl) and micro elements such as: Aluminum (Al), Silicon (Si), Vanadium (V), Chrome (Cr), Manganese (Mn), Iron (Fe), Nickel (Ni), Copper (Cu), Zinc (Zn), Selenium (Se), Bromine (Br), Molybdenum (Molybdenum), Tin (Sn), Barium (Ba) and Lead (Pb). Some of these elements are related to sickle cell disease, such as Iron, Zinc, Selenium, Copper, Calcium, Magnesium and Manganese. Calcium and potassium were found in higher content while lead, brome, copper and nickel were found in trace content. The crude extracts of these plants have showed good correction of sickling of red blood cells which pass from the sickled form to the normal one. It has been also found that, the anthocyanins extracted from these three plants increase the hydration ability of red blood cells.

KEYWORDS: *Hura crepitans, Alternanthera bettzickiana, Dissotis brazzae*, sickle cell anemia, mineral elements, fluorescence.

INTRODUCTION

Medicinal plants represent an important source of health care in the world.[1,2] The rapid growth of population and the inaccessibility of modern medicines in some of developing countries have contributing to the increase of the demand for traditional medicines which is often considered as a treatment method.[3,4] According to the World Health Organization, nearly 80% of people depend on traditional medicine for their primary health care.[5] In the Democratic Republic of the Congo (DRC), urban and rural populations are increasingly turning to the use of medicinal plants to solve their health problems.[6] Sickle cell disease is one of the hereditary disease that affects nearly 5% of the world's population, it is caused by a mutation in the hemoglobin beta globin gene, and characterized by severe anemia, vaso-occlusive crisis and a high susceptibility to both viral and bacterial infections.[7] This disease is characterized by the loss of some mineral elements that are important in the normal functioning of the body.[8] Minerals are natural chemical elements that the body uses to activate certain biochemical reactions. They are among functionally important inorganic compounds such as iron (Fe) in hemoglobin and Cytochrome or zinc (Zn) in insulin.[9]

It is also established that leaves are potential sources of minerals and vitamins and are apparently inexpensive.[10] This study was done to determine the mineral composition of three plants (*Hura crepitans, Alternanthera bettzickiana, Dissotis brazzae*) used in Congolese traditional medicine for the management of sickle cell disease in Kwilu province, DRC, in order to explore the mineral composition of these Congolese phytotherapeutic plant.

MATERIAL AND METHODS

1. Mineral composition determination

The leaves of *Alternanthera bettzickiana* and *Dissotis brazzae* and the barks of *Hura crepitans* used in this study were harvested in May and July 2016 in Kwilu province (DRC). These samples were prepared (washed, dried and ground) at the basic science laboratory of the University of Kikwit. The detection and quantification of the mineral composition was done by the fluorescence spectrometric method (XEPOS 3) and ICP-OES (ARCOS).

2. Antisisckling essays

In order to evaluate the antisickling activity of plant extract samples, two *in vitro* antisickling assays were performed:

- **Emmel test**

Emmel test was performed as previously described.[11] Briefly, sickle cell blood was diluted with 150 mM phosphate buffered saline (NaH_2PO_4 30 mM, Na_2HPO_4 120 mM, NaCl 150 mM) and mixed with an equivalent volume of 2% sodium metabisulfite. A drop from the mixture was spotted on a microscope slide in the presence or absence of plant extracts and covered with a cover slip. Paraffin was applied to seal the edges of the cover completely to exclude air (Hypoxia condition). The red blood cells (RBC) images were treated with a computer assisted image analysis system (Motic Images 2000, version 1.3).

- **Osmotic fragility test**

The fragility of red blood cells (RBCs) was determined as previously reported by Mpiana *et al.*[11] The cells were placed in 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 made up in a final volume of 10 mL. A 10 µL sample of washed SS RBC as 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. To determine the effect of the anthocyanin extracts, 10 µL of extract (30 mg/mL) were added to 1980 µL of each hypotonic saline solution, then 10 µL of RBC added and the mixture treated as described earlier. The number of RBC not lysed at each saline concentration was determined using a photonic microscope (OLYMPUS×21) and a haemacytometer (Neubauer's cell). Haemolysis was calculated using the following equation.

Number of RBC after 150 min×100/number of RBC inoculated (0 min).

The mean corpuscular fragility (determined from the concentration of saline causing 50% haemolysis of the RBC) was obtained from a plot of lysis (%) versus NaCl concentration.[7]

4. Data analysis

Statistical data analysis and curves were processed using Microcal Origin 8.5 package software and Microsoft Excel.

RESULTS AND DISCUSSION

1. Elements contents

The table below shoes the macroelements content of the three plants studied.

Table 1: Macroelements content of the Three Plants.

Element	Concentration (g/Kg)		
	A. Bettzickiana	***D. brazzae***	***H. crepitans***
K	12.86 ±0.98	8.29±0.77	19.43±1.04
P	1.15 ± 0.10	1.39 ±0.15	2.47±0.17
Ca	25.71 ±1.23	1.91 ±0.19	37.60±4.45
Na	3.64 ± 1.45	2.17 ±0.26	1.58±0.38
Mg	3.12 ±0.24	9.43 ±0.84	4.89±0.22
S	1.51±0.20	1.36±0.14	3.73±0.20
Cl	0.38 ±0.06	0.13 ±0.01	5.94±0.21

As it can be seen in Table 1, the three plants studied contain seven macroelements especially K, P, Ca, Na, Mg, S and Cl. The contents of K, P, Ca, S and Cl are higher in the barks of *Hura crepitans* than in the leaves of the two others plants. It was also found that the contents Mg was higher in the leaves of *Dissotis brazzae* than in those of *A. Bettzickiana* and *H. crepitans*.

According to these results, *Alternanthéra Bettzickiana* is much richer in Ca followed by K, Mg, S and P, while in the leaves of *Dissotis brazzae* the Mg was revealed to be the most abundant, followed by K, Na, P, S and Cl.

Calcium remains the most abundant element in the barks of *Hura crepitans* followed by K, Cl, Mg, S, P and Na. The mineral composition of *Hura crepitans* have been determined previously by Muhamed *et al.*[12] Comparing our results to those obtained by these authors, it may be seen that Calcium, potassium and magnesium content obtained by Muhamed *et al* [12] where very lower than that obtained in this work. But values of Calcium and Potassium concentrations obtained in this work is at the same order of magnitude as that of *Ocimum basilicum* [13,14] Ecological factors can explain this difference.

Potassium and sodium are electrolytes needed for the body to function normally and help maintain fluid and blood volume in the body. Ca constitutes a large proportion of the bone, human blood and extracellular fluid; it is necessary for the normal functioning of cardiac muscles, blood coagulation and the regulation of cell permeability. In humans, Mg is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium. Magnesium in addition may play an important role as co-factor of enzymes, reduces the number of abnormal erythrocytes in sickle cell disease and improves the hydration of red blood cells.[15-17]

The trace element levels of the three plants studied are summarized in Table 2.

Table 2: Trace elements of the three plants.

Elements	Concentration (mg/Kg)		
	A. Bettzickiana	***D. brazzae***	***H.crepitans***
Al	902.79 ±67.10	58.30±8.48	315.04±20.74
Si	239.84 ±25.49	37.70±7.08	447.35±39.00
V	2.45±0.91	1.76±0.12	2.18±0.97
Cr	1.96±0.32	1.80±0.46	2.46±0.11
Mn	681.83±71.87	524.87±56.97	34.86±6.48
Fe	408.55±43.93	164.52±14.19	795.51±53.67
Ni	3.05±0.79	4.08±1.11	2.31±0.20
Cu	9.44±1.03	23.67±1.81	6.16±0.60
Zn	22.81±4.56	51.56±4.63	32.18±4.31
Se	ND	ND	< 0.80
Br	4.77±0.21	47.94±6.41	9,08±0.93
Mo	ND	1.71±0.34	ND
Sn	0.72±0.07	1.20±0.39	0.87±0.14
I	1.65±0.72	2.46±0.66	1.47±0.34
Ba	15.58±1.57	7.04±1.81	315.04±20.74
Pb	3.02±0.55	ND	1.19±0.01

From Table 2, it can be seen the trace mineral composition of the three plants, where we may found that, in *Alternanthera bettzickiana* Aluminum (Al) dominates in content while Iodine (I) is found in low concentration. The Iron (Fe) content was higher in *Dissotis brazzae* while the Tin (Sn) content was the lowest in this plant. In *Hura crepitans*, the Iron (Fe) content was higher than that of all the others elements identified in this plant, that of Selenium (Se) was the lower. The Iron content in this plant was the highest of all three plants, that may prove the use of the macerated extract of the bark of this plant by Kwilu Tradipractitioners to increase the hemoglobin level in most case of sickle cell patients. Indeed, iron is important in the production of hemoglobin. Iron deficiency makes the body produce fewer and smaller red blood cells, leading to anemia.[15,16]

By comparing the results of this research with that obtained by Muhamed et al[12] using *Hura crepitans*, it appears that the content of the same elements identified by this team were lower than that obtained in this study. But our values are in the same order of magnitude of that of other plants.[17,18] This difference may be due to the composition of the mineral elements in the soil where the different samples were collected.

It can be noticed the presence of other important trace elements like Zn, Cu, I… In fact, Zinc is a catalyst for many enzymes that are needed for red blood cell production; as a result, zinc deficiency may be associated with anemia. Impaired iron absorption can be caused by a decrease in trace elements as zinc, which is found in the structure of enzymes that coordinate or catalyst iron metabolism. Cu is also a component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin, an iron-oxidizing enzyme in blood. The observation of anaemia in Cu deficiency may probably be related to its role in facilitating iron absorption and in the incorporation of iron into haemoglobin.[15,17,18]

3.2. Antisickling activity

Figure 1 provides a micrograph of the SS blood alone while Figure 2 provides an illustrative micrograph of the SS blood when treated with a plants extract (*Dissotis brazzae* Cogn. leaf extract).

Figure 1: Optical microphotography of untreated SS blood erythrocytes (control) [NaCl 0.9%, $Na_2S_2O_5$ 2%, Magnification 500 X].

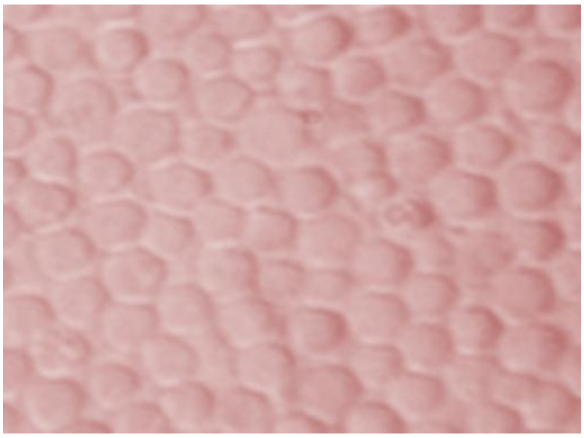

Figure 2: Optical microphotography of SS blood erythrocytes treated with crude extracts of *Dissotis brazzae* Cogn. leaves. [NaCl 0.9%, $Na_2S_2O_5$ 2%, Magnification: 500 X].

Figure 1 shows that this is indeed SS blood, as many sickle cell erythrocytes can be observed. This sickling is due to hypoxia created by the addition of 2% sodium metabisulfite.

On the other hand, figure 2 indicates that in the presence of crude aqueous extracts of these plants (*Dissotis brazzae* Cogn., *Alternanthera bettzickiana, and Hura crepitans*) the sickle cell are normalized. This normalization of SS blood erythrocytes in hypoxic conditions is partly experimental evidence which may justify the use of these plants in traditional medicine in Kwilu province for the management of sickle cell disease. The normalization of SS blood erythrocytes results in the reappearance of the circular shape of sickle cells. These results corroborate with those obtained previously by Mpiana *et al*.[7,11, 19-21]

3.3. Osmotic fragility

It was shown that antisickiling activity of some Congolese medicinal plants is due to anthocyanins.[7, 11,19-21] Then, the effect of anthocyanins extracts on the membrane stability of RBCs was evaluated by comparing the hemolysis rates of untreated and treated sickle RBCs in order to see the influence of anthocyanins one the osmotic fragility of drepanocytes. Figure 3 gives comparative hemolysis rates of untreated and sickle RBCs treated with anthocyanins from *Alternanthera bettzickiana* at different NaCl concentration.

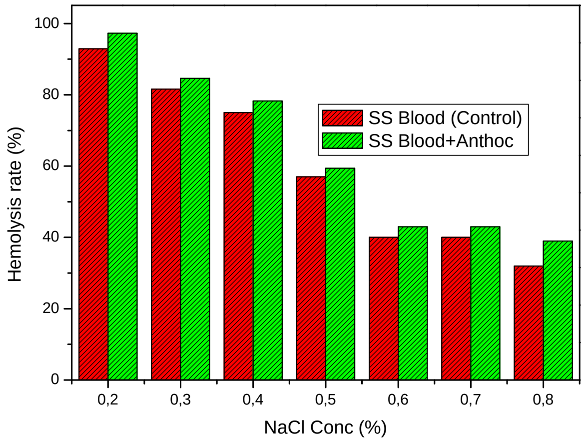

Figure 3: Hemolysis rates of untreated (control) and sickle red blood cells treated with anthocyanins from *Alternanthea bettzickianae* (Regle) G. Nicholson. (10µg/mL) at different NaCl concentration.

As it can be seen in figure 3, in presence of anthocyanins, the hemolysis is higher than in the control. This means that anthocyanins improve the blood cells hydratation ability. The cells rehydration prevents the polymerization of Hb S and the red blood sickling.[21]

CONCLUSION

The objective of this study was to determine the qualitative and quantitative mineral composition of *Hura crepitans, Alternanthera bettzickiana, Dissotis brazzae* used in traditional medicine in the province of Kwilu for the management of sickle cell disease. The Results obtained show that the three plants contain up to 23 distinct mineral elements, among which calcium and potassium were found to be the most abundant in the leaves of *A. Bettzickiana* and in the barks *of H. crepitans* while magnesium and potassium were found to be the most abundant in D. brazzeae. The high content of iron was found in *Hura crepitans*, which is an important element that increases the level of hemoglobin in sickle cell patients. This plant is widely used in the Kwilu province for the management of sickle cell disease usually in the case of lower level of hemoglobin. Other elements such as magnesium, zinc, selenium, manganese, copper, cobalt play an important role in sickle cell management.

The crude extracts of these three plants have showed an antifalcemic activity. The anthocyanins obtained from these three plants have increased the hydration ability of the red blood cells of sickle cell patients.

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