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In vitro Antisickling Activity of Anthocyanins Extract of a Congolese Plant: *Alchornea cordifolia* M. Arg.

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The Antisickling activity of ethanolic and aqueous extract of the leaves of *Alchornea cordifolia* L. was evaluated *in vitro* using Emmel test. The aqueous extract showed a higher activity. Anthocyanins crude extract of the leaves of this Congolese plant exhibited attractive antisickling activity, thus, supporting the claims of the traditional healers and suggesting a possible correlation between the chemical composition of this plant and its uses in traditional medicine.

Key words: *Alchornea cordifolia*, aqueous extract, anthocyanins, antisickling activity

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INTRODUCTION

Higher plants produce a wide array of secondary metabolites that have therapeutic and pharmaceutical applications. These plants, without knowledge of the specific physiologically active substances they contain, have been of service to humans over many centuries and across all cultures (Balick and Cox, 1996; Shetonde, 2005). With the progressive loss of biodiversity all over the world, especially in the tropics, society is not only losing present benefits from current use but is being deprived of the option of future availability. Medicinal plants provide meaningful inputs for drugs; their loss through extinction could lead to irreplaceable loss. Despite the intensive investigation of terrestrial flora, only a small percentage of the approximately 250,000-500,000 species of higher plants have been systematically investigated chemically and pharmacologically. In this regard, large areas of tropical rainforests remain virtually untapped. Consequently, the potential to find new and interesting bioactive natural products from among myriads of plants in different settings and ecosystems is very great (Cragg *et al*., 1999; Kumar, 2004; Petlevski *et al*., 2001).

The Democratic Republic of Congo is one of the biodiversity-rich countries in the world and home to a large number of endemic species (Brenan, 1978). Most Congolese plants have not yet been studied scientifically whereas others have proven to be sources of chemically and pharmacologically exciting natural products (Mpiana *et al*., 2007). In addition, no much information has been documented in scientific literature; and as in other parts of the world, information on herbal medicine in DRC has been dominated by oral tradition (Shetonde, 2005; Kambizi and Afolayan, 2001; McGaw *et al*., 2000). It is therefore urgent to intensify the chemical investigation of Congolese plants in order to establish their therapeutic values, as well as for accurate documentation of the knowledge and experience of our traditional healers.

In this study, we report the antisickling activities of anthocyanins and alkaloids crude extracts of *A. cordifolia* M. Arg. leaves which, is a plant highly used in Congolese traditional medicine for the treatment of drepanocytosis (Mpiana *et al*., 2007).

Drepanocytosis, also known as sickle cell anaemia, is a genetic disease resulting from the inheritance of two abnormal allelomorphic genes that control the formation of the β chains of Haemoglobins (Hb). The symptoms of this disease result from an ultimate aggregation of haemoglobins in the HbSS erythrocytes due to lowered oxygen tension, resulting in the characteristic distorted sickled shapes. Sickling of blood cells is the cause of precocious haemolysis of erythrocytes and various complications of SS subjects (Kambizi and Afolayan, 2001; McGaw *et al*., 2000; Lehninger, 1994; Gentilini, 1986; Voet and Voet, 1998; Mehanna, 2001; Moody *et al*., 2003). Sickle cell anaemia has been affecting people of African origin for centuries (Mehanna, 2001; Bernardin, 1999; Neuwinger, 2000).

There have been several approaches to the management and prevention of crises in sickle cell patients. Since sickle cell anaemia is an inherited trait, the hope of finding a cure is still elusive and the available form of management still consists of dealing with symptoms as they occur in the various crises. For the management of severe anaemia, patients are often transfused with blood while in less severe anaemia the haematocrit is built up as circumstances permit. The various painful crises are managed with the use of analgesics and the precipitating causes of the crises are usually dealt with at the same time. Antibiotics and antimalarials are hence used as appropriate. Sickling reversal agents such as urea, hydroxyurea, carbamylating agent cyanate, testosterone and progesterone in low doses have been tried at various times but some have been found to be very toxic (Moody *et al*., 2003).

Plants used in traditional medicine have been shown to contain antisickling principles *in vitro* (Sofowora and Isaac, 1971; Sofowora, 1975). Recently, we reported a good number of plants used in Congolese traditional medicine which possess attractive antisickling activities and which could be therapeutically exploited in the treatment of sickle cell anaemia (Mpiana *et al*., 2007). *Alchornea cordifolia* M. Arg., *syn. A. cordata* Benth, *Shousboea cordifolia* Schoumach (Euphorbiaceae), is one of the above-mentioned plants which exhibited strong antisickling activity. It is a small tree of approximately 4-5 m that is widespread in many regions of the world (Kerharo and Adam, 1985). Known as Mbuzi mbunzi (Kikongo, DRC) (Mpiana *et al*., 2007; Kambu, 1990), *A. cordifolia* has a wide variety of applications in African traditional medicine. These include the relief of various diseases such as drepanocytosis, fever, cough, scabies, respiratory and urinary trucks infections, hemorroides etc. (Kerharo and Adam, 1985; Courtejoie and Hartaing, 1992).

In our continuing search for antisickling active natural products from plants, we discovered that a crude *A. cordifolia* leaf aqueous extract displayed promissing antisickling activity. This activity was evaluated *in vitro* on SS blood using Emmel's test (Courtejoie and Hartaing, 1992) and is expressed as the normalization of sickled cells. We have determined the Minimal Concentration of Normalization (MCN) of sickled cell erythrocytes for both the aqueous crude extract and that of the chemical class of natural products responsible for this activity, the anthocyanins.

MATERIALS AND METHODS

Plant material: Leaf materials used in this study were collected between February and June 2006 from *A. cordifolia* Schrub growing at the Université de Kinshasa site, Kinshasa (DRC) and were authenticated by Mr. B.L. Nlandu of the INERA (Institut National d'Etudes et Recherches Agronomiques) where a voucher specimen, F. Billiet and B. Jadin N°4020 was deposited.

Extraction: The dried and powdered plant material (leaves, 10 g) was repeatedly extracted by cold percolation with 95% EtOH (100 mL×1) and water (200 mL×1) for 48 h. Fractions were filtered and the solvent was evaporated under reduced pressure using a rotary evaporator. A chemical screening was then done on the aqueous extract of the leaves of *A. cordifolia*. Several classes of compounds were identified, including alkaloids, polyphenols (tanins, quinones, flavonoids, anthocyanins and leucoanthocyanins), terpenoids and saponins. Extraction of anthocyanins and alkaloids was then done using 100 g of dried powdered plant material with distilated water and diethyl ether according to well known standard methods (Bruneton, 1999).

Biological material: Blood samples used to evaluate the antisickling activity of the plant extracts in this study were obtained from known drepanocitary adolescent patients attending the Centre de Médecine Mixte et d'Anémie SS and Centre Hospitalier Monkole, both located in Kinshasa area, DRC. In order to confirm their SS nature, the above-mentioned blood samples were first characterized by electrophoresis on cellulose acetate gel, as previously reported (Mpiana *et al*., 2007). These samples were authenticated as SS blood and were then stored at ±4°C in a refrigerator.

Biological activity: Blood sample was added in contact with plant extracts at different concentrations using physiologic solution (NaCl 0.9%) as the dilution solvent, according to Emmel's test procedure (Courtejoie and Hartaing, 1992). In this study, Emmel's test was performed as previously reported by Mpiana *et al*. (2007).

Data analysis: A zoom 6xCANON-type digital camera was used to convert the photonic micrograph image into a digital image, which was then digitalized using a MOTIC image 2000 1.3 software on Windows XP. This software allows the determination of the average diameter, surface as well as the perimeter of the observed erythrocytes.

RESULTS AND DISCUSSION

Plant parts used in this study are those used by traditional healers. Figure 1 and 2 shows the morphology of SS blood erythrocytes (standard) and that of SS blood erythrocytes in the presence of *A. cordifolia* aqueous extract.

A normalization of erythrocytes of SS blood sample treated with *A. cordifolia* extract indicates the influence of the extract on the sickleness of cells as previously reported for other Congolese plants (Mpiana *et al*., 2007). Figure 3 shows the normalization of sickled cells with the plant aqueous extract concentration. This normalization increases with the extract concentration and reach a maximum and constant value at 48.8 μg mL^{-1} (MCN). This corresponds to a normalization rate of 73.3%.

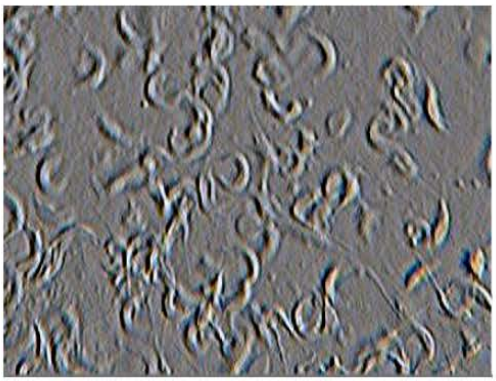

Fig. 1: Morphology of drepanocyte of non treated SS blood standard, (ordinary view X 500)

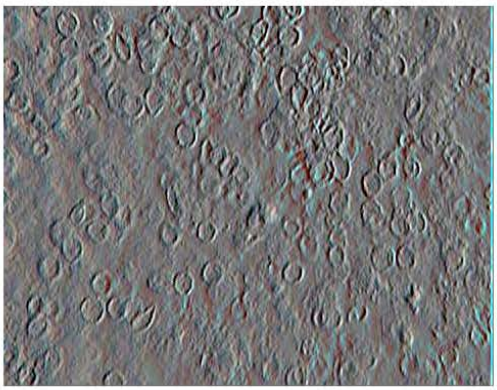

Fig. 2: Morphology of drepanocytes treated with aqueous extract of *A. cordifolia* (48.8 μg mL^{-1}), (ordinary view X 500)

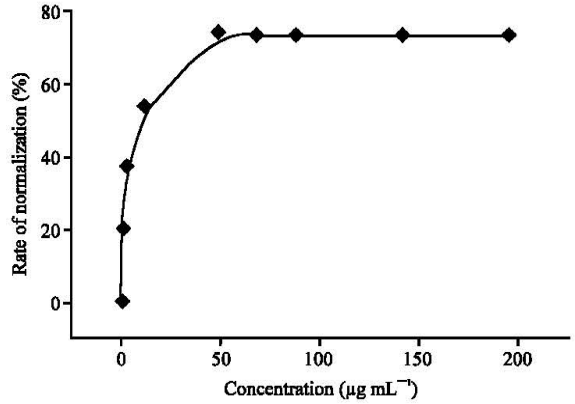

Fig. 3: Concentration-dependent antisickling effect of *A. cordifolia* aqueous extract

Taking into consideration both the chemical screening results and the solubility of different chemical groups (Bruneton, 1999), we supposed that the antidrepanocytary activity of *A. cordifolia* aqueous extract would be due to the presence of anthocyanins or alkaloïds, which are present in this plant. We then decided to focus the continuation of our investigation on these two chemical groups.

Figure 4 and 5 shows the morphology of SS blood erythrocytes in the presence of *A. cordifolia* anthocyanins and alkaloids extracts, respectively.

These morphological SS blood cells were observed in anaerobic conditions, i.e., after deoxygenation of haemoglobin. As it can be seen, the antisickling activity due to anthocyanins extract is higher than that of the alkaloids extract. This normalization increases with the extract concentration and reach a maximum and constant value at 0.097 µg mL^{-1} (MCN), corresponding to a normalization rate of 93.3% for the anthocyanins (Fig. 6). For the alkaloids extracts, the normalization remains less than 20%.

The average radius, perimeter and surface determined are shown in Table 1. However, the average radius for the erythrocytes of non traited SS blood could not be determined; because sickled cells of none treated SS blood is not circular. The average radius appeared after treatment of SS blood cells with *A. cordifolia* extract, indicating the re-appearance of the normal form of blood cells.

Statistical treatments (student's test applied with a probability threshold of 0.05) (Azoulay and Cohen, 1969) enabled the determination of a significant difference between the average values of both the perimeter and the surface of erythrocytes on the images, thus confirming the modification of the erytrhocytes form in presence of *A. cordifolia* aqueous extract.

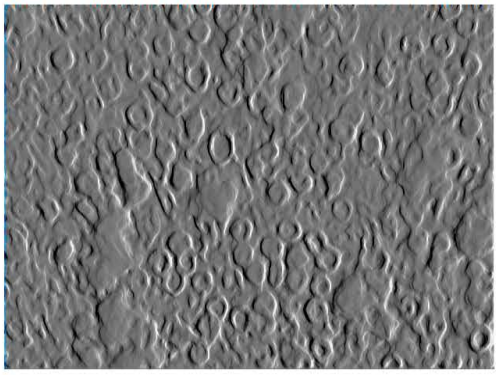

Fig. 4: Morphology of SS blood erythrocytes treated with anthocyanins extract of *A. cordifolia* (3.1 µg mL^{-1}) (ordinary view X 40)

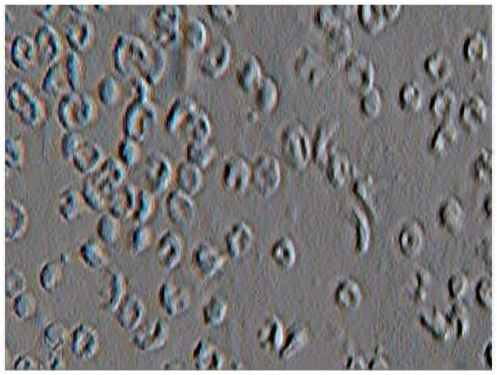

Fig. 5: Morphology of SS blood erythrocytes treated with alkaloïds extract of *A. cordifolia* (3.1 µg mL^{-1}), (ordinary view X 40)

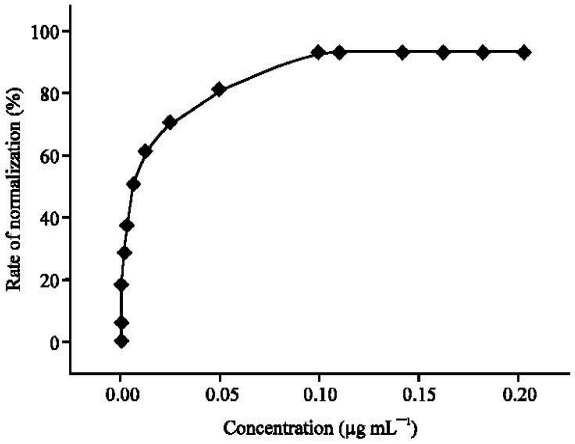

Fig. 6: Concentration-dependent antisickling effect of anthocyanins of *A. cordifolia*

Table 1: Average values of radius, perimeter and surface of erythrocytes before and after treatment with a solution of 48.8 μg mL^{-1} of *A. cordifolia* aqueous extract

Parameters	Non treated SS blood	Treated SS blood
Radius (μm)	-	3.09±0.48
Perimeter (μm)	29.24±2.83	19.39±1.25
Surface (μm^2)	21.32±1.91	30.44±4.11

We concluded that the ability of the extracts to normalize the SS blood erythrocytes in this study, may represent a rational explanation for the use of this plant in treating drepanocytosis by Congolese traditional healers. To the best of our knowledge, *A. cordifolia* has not yet been reported to exhibit antisickling effects. Further studies, involving the isolation and characterization of secondary metabolites from this plant are recommended.

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REFERENCES

Azoulay, E. and O. Cohen, 1969. Cours et Exercices de Statistique. Société d'Edition d'Enseignement Supérieur, Paris.

Balick, M.J. and P.A. Cox, 1996. Plants, people and culture: The science of ethnobotany. The Scientific American Library, New York.

Bernardin, F., 1999. Résultats et indications actuelles de l'allogreffe de moelle dans la drépanocytose. Path. Biol., 97: 59-64.

Brenan, J.P.M., 1978. Some aspects of the phytogeography of tropical Africa. Ann. Missouri Bot. Garden, 65: 437-478.

Bruneton, 1999. Pharmacognosie: Phytochimie-plantes médicinales. Tec and Doc, Paris.

Courtejoie, J. and I. Hartaing, 1992. Laboratoire et Santé. Saint Paul, Kinshasa.

Cragg, G.M., M.R. Boyd, R. Khanna, R. Kneller, T.D. Mays, K.D. Mazan, D.J. Newman and E.A. Sausville, 1999. International collaboration in drug discovery and development: The NCl experience. Pure Applied Chem., 71 (9): 1619-1633.

Gentilini, M., 1986. Médecine Tropicale. Flammarion, Paris.

Kambizi, L. and A.J. Afolayan, 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. J. Ethnopharmacol., 77 (3): 5-9.

Kambu, K., 1990. Eléments de Phytothérapie Comparée, Plantes Médicinales Africaines. CRP, Kinshasa.

Kerharo, J. and J.G. Adam, 1985. La pharmacopée sénégalaise traditionnelle, plantes médicinales et toxiques. Vigot Frères, Paris.

Kumar, P., 2004. Valuation of medicinal plants for pharmaceutical uses. Curr. Sci., 86 (7): 930-937.

Lehninger, A., 1994. Principe de Biochimie. Flammarion, Paris.

McGaw, L.J., A.K. Jäger and Van Staden, 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. J. Ethnopharmacol., 72 (2): 247- 263.

Mehanna, A.S., 2001. Sickle cell anemia and antisickling agent then and now. Curr. Med. Chem., 8 (2): 79-88.

Moody, J.O., O.O. Ojo, A.A. Omotade, P.E. Olumese and O.O. Ogundipe, 2003. Anti-sickling potential of Nigerian herbal formula (Ajawaron HF) and the major plant component (*Cissus populnea* L. CPK). Phytother. Res., 17 (10): 1173-1176.

Mpiana, P.T., D.S.T. Tshibangu, O.M. Shetonde and K.N. Ngbolua, 2007. *In vitro* antidrepanocytary activity (anti-sickle cell anaemia) of some Congolese plants. Phytomedicine, 14: 192-195.

Neuwinger, H.D., 2000. African Traditional Medicine. Mepharm Publisher, Stuttgart.

Petlevski, R., M. Hadzija, M. Slijepcevic and D. Juretic, 2001. Effect of antidiabetes herbal preparation on serum glucose and fructosamine in NOD mice. J. Ethnopharmacol., 75: 181-184.

Shetonde, O.M., 2005. Phytochemical investigation of a Congolese plant *Biophytum umbraculum* (Oxalidaceae). M.Sc. Thesis, University of Botswana.

Sofowora, E.A. and W.A. Isaac, 1971. Reversals of sckling and crenation in erythrocytes by the root extract of *Fagara xanthoxyloides*. Lloydia, 34: 389.

Sofowora, E.A., 1975. Proceedings of Current Concepts in the Management of Sickle Cell Disease, Williams, G. (Ed.). New Orleans, USA, New-Orleans Sickle Cell Foundation.

Voet, D. and J.G. Voet, 1998. Biochimie. 2nd Edn. De Boeck Université, pp: 123-125.