Antisickling activity and photodegradation effect of anthocyanins extracts from *Alchornea cordifolia* (Schumach & Thonn) and *Crotalaria retusa*

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Résumé:

L'activité antifalcémiante des extraits d'anthocyanes tirés d'*Alchornea cordifolia (A. cordifolia)* et de *Crotalaria retusa (C. retusa)* a été évaluée en utilisant le test d'Emmel. La chromatographie préparative sur couche mince utilisant le méthanol comme éluant a donné deux fractions (R_f : 0.76 ; 0,68) pour *A. cordifolia* et utilisant l'éthanol comme éluant a fourni une fraction (R_f : 0,74) pour le *C. retusa*. La fraction isolée d'*A. cordifolia*, dont le Rf = 0,68, a montré la plus grande activité. La photodégradation des anthocyanes, des extraits totaux et de la fraction isolée d'*A. cordifolia* et de *C. retusa* à été prouvée à l'aide de la lampe UV à 366 nm. L'élucidation des structures des composés isolés est en cours.

Mots clés : *Alchornea cordifolia, Crotalaria retusa,* anthocyanes, activité antifalcémiante et photodégradation.

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Summary

The antisickling activity of extracts of anthocyanins from *Alchornea cordifolia (A. cordifolia)* and *Crotalaria retusa (C. retusa)* was evaluated using Emmel's test. Preparative TLC with methanol provided two fractions (R_f: 0.76; 0.68) for *A. cordifolia*, and using ethanol, it provided one fraction (R_f: 0.74) for *C. retusa*. The second fraction (Rf: 0.68) isolated from *A. cordifolia* has exhibited the highest activity. The photodegradation of anthocyanins, of the total extracts and of the isolated fraction from *A. cordifolia* and *C. retusa* using 366 nm UV lamp has been proved. Structural elucidation of isolated compounds is in progress.

Key words: *Alchornea cordifolia, Crotalaria retusa,* anthocyanins, antisickling activity and Photodegradation.

Introduction

The drepanocytosis or sickle cell disease (SCD) is a genetic and hereditary disease. It is among serious health problems around the world, especially in the sub-Saharan Africa. Around 20% of some African populations are heterozygous AS. In Central Africa, 25 to 30% of populations are affected (1-5). Two percents of the Democratic Republic of Congo (DRC) populations are homozygous SS, approximately one million of individuals. Children are the most concerned. About 80% of anaemic children no medically treated die before their fifth birthday (6). SCD is spilled today on the 5 continents because of populations migrations (7, 8).

Several therapeutic options were used to fight against this disease which results from the replacement of the glutamic acid by the valin in β^6 position of haemoglobin. Unfortunately, no effective curative therapy is available nowadays. The current therapeutic approaches that exist are very expensive for most of African populations. Some are either toxic or present risks of HIV/AIDS infection (9, 10).

African populations affected by this disease resort to the traditional medicine (phytotherapy) to treat themselves. So, phytotherapy is explored more and more as a therapeutic alternative (11, 12). DRC with its 47% of African tropical forests possesses a large biodiversity. It may act as a source of interesting plants containing molecules with high antisickling activity.

Recently, we published a number of studies on the antisickling activity of some Congolese plants used in traditional medicine against SCD (6, 13). We also showed that anthocyanins are the main active principles of these plants. Anthocyanins are found in large quantities in plants kingdom, and they have antioxidant properties, protecting cells against free radicals generated during metabolic processes. In the best of our knowledge, these natural pigments have not yet been reported to exhibit antisickling effects before our previous similar findings (13-20).

However, it is known that anthocyanins are unstable towards some physico - chemical parameters such as heat, pH, light, etc.; while medicinal plant are commonly exposed to sun light by traditional practitioners. In this paper we verify the antisickling activity of anthocyanins extracted from *A. cordifolia* and *C. retusa* and the effect of the light on this activity.

Material and methods

Plant material

Plant material (leaves) used in this study was collected from *A. cordifolia* and *C. retusa* growing at the Université de Kinshasa site, Kinshasa (D.R. Congo) and was authenticated by the INERA (Institut National d'Etudes et Recherches Agronomiques/Faculté des Sciences, Université de Kinshasa). Voucher specimen numbers are respectively 4438 and 1319.

Extraction

The dried and powdered leaves (10 g) were repeatedly extracted by cold percolation with water (200 ml × 1) for 48 hrs. Fractions were filtered, mixed and the solvent was evaporated under reduced pressure using a rotary evaporator. Extraction of anthocyanins was then done using 100 g of dried powdered plant material with distillated water and diethyl ether according to the universal procedures (21).

Biological material

Blood samples used to evaluate the antisickling activity of the plant extracts in this study were taken from known sickle cell disease adolescent patients attending the "Centre de Médecine Mixte et d'Anémie *SS*" and "Centre Hospitalier Monkole", both located in Kinshasa area, D. R. Congo. In order to confirm their *SS* nature, the above-mentioned blood samples were first characterized by Hb electrophoresis on cellulose acetate gel, as previously reported (6). They were found to be *SS* blood and were then stored at ± 4° C in a refrigerator.

Biological activity

Blood sample is put in contact with plant extracts at different concentrations (with the

physiologic solution as the dilution solvent) according to Emmel's test procedure (22). In this study, Emmel's test was performed as previously reported (6).

Fractionation

The thin layer chromatography (TLC) was run on Merck plate with methanol for *A. cordifolia* and ethanol for *C. retusa* as eluting solvents. The developed plates were revealed by UV light at 254 nm and 366 nm. Separation was realised by preparative chromatography using silica gel with the same solvents.

Photodegradation

The photodegradation was studied using a CAMAG UV lamp with a maximum wavelength around 366 nm during different time periods. Solution's absorbances were measured using a ZUZI UV-2200 spectrophotometer.

Data analysis

A 6× zoom CANNON-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.

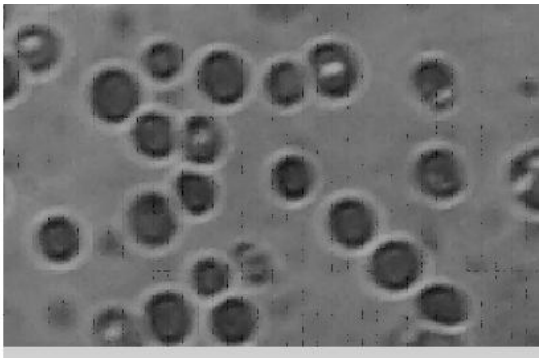

Fig. 2: Morphology of drepanocytes treated with anthocyanins extracts from A. cordifolia (standard 500X)

Figures 2 and 3 show that mixed together with crude extracts of anthocyanins, the majority of sickle-shaped erythrocytes are reversed into normal and biconcave shape.

Results

Antisickling activity of anthocyanins total extracts

Figures 1, 2 and 3 illustrate respectively the morphology of *SS* blood erythrocytes (standard) and that of *SS* blood erythrocytes in the presence of crude extracts of anthocyanins from *A. cordifolia* and *C. retusa.*

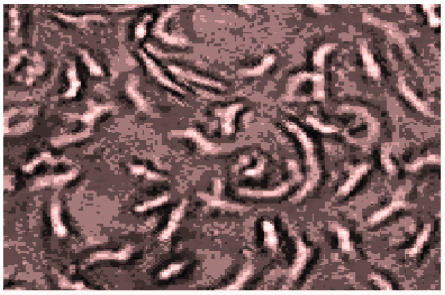

Fig.1: Morphology of drepanocytes none treated SS blood (standard 500X)

Figure 1 show that, the standard contains the majority of sickle-shaped erythrocytes; this confirms the SS nature of the blood.

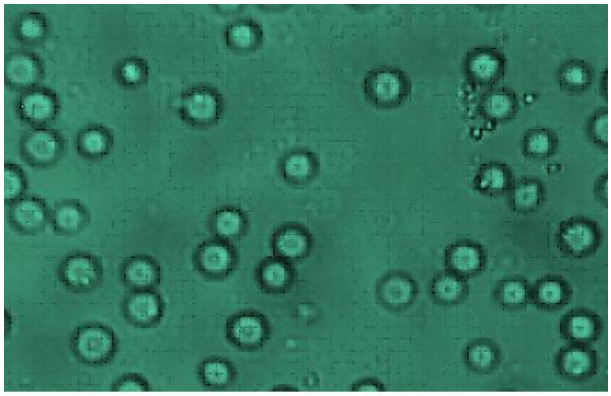

Fig. 3: Morphology of drepanocytes treated with anthocyanins extracts from C. retusa (500X)

Antisickling activity of isolated fractions of anthocyanins

Methanol was used to separate crude extract of anthocyanins into two different fractions

which R_f are 0,76 and 0,68 from *A. cordifolia* and ethanol was used to separate crude extract of anthocyanins into one fraction with a R_f 0.74 from *C. retusa*.

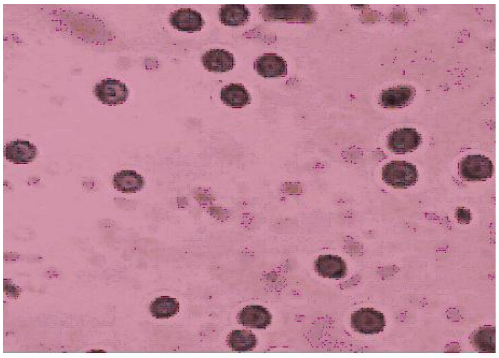

Fig. 4: Morphology of SS erythrocytes treated by the second anthocyanins fraction from A. cordifolia (500X)

The figures 4 and 5 show that the erythrocytes have been normalized compared to the standard (fig. 1). Therefore, these fractions of anthocyanins are responsible for the antisickling activity of *A. cordifolia* and *C. retusa.*

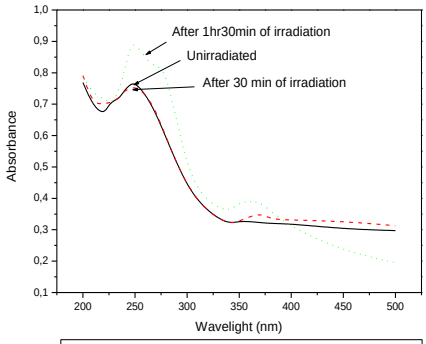

Fig. 6: UV-visible spectra of crude extract of anthocyanins from *A. cordifolia* unexposed and exposed to UV lamp during 30 min and 1hr30min

The figures 6 and 7 show the spectra of crude extracts of anthocyanins (unexposed) with an absorption band at 274 nm assigned to π-π* transition of flavylium ion which is

Antisickling activity of one of the fraction (R_f 0.68) obtained from *A. cordifolia* and the fraction from *C. retusa* was tested.

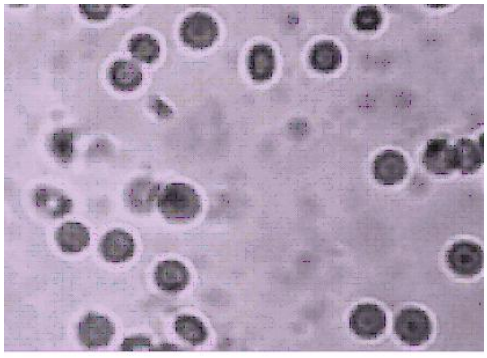

Fig. 5 : Morphology of SS erythrocytes treated by the anthocyanins fraction from C. retusa (500X)

Photodegradation of isolated fraction of anthocyanins

Figures 6, 7, 8 and 9 show UV-visible spectra of crude extracts and isolated fractions unexposed and exposed to UV lamp.

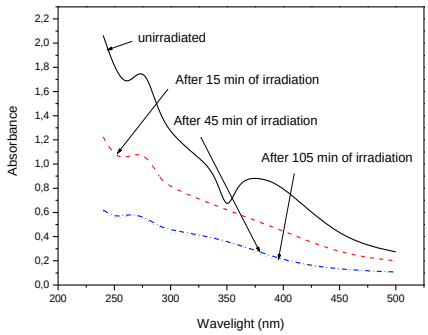

Fig. 7: UV-visible spectra of crude extract of anthocyanins from *C. retusa* unexposed and exposed to UV lamp during 15 min, 45 min and 105 min.

the basic structure of anthocyanins (14, 15).When exposed to UV lamp, new bands appear.

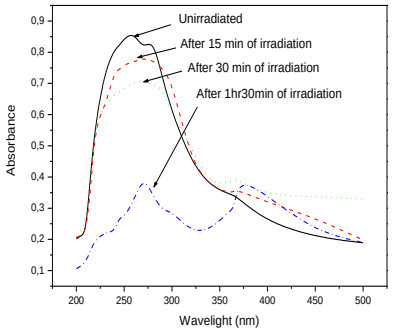

Fig. 8: UV-visible spectra of isolated fraction of anthocyanin from *A. cordifolia* unexposed and exposed to UV lamp during 15 min, 30 min and 1hrs30min.

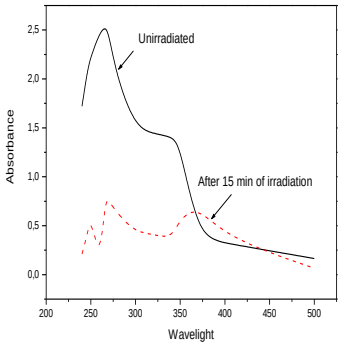

Fig. 9: UV-visible spectra of isolated fraction of anthocyanin from *C. retusa* unexposed and exposed to UV lamp during 15 min

The same band (274 nm) appears for unexposed isolated fraction as shown in figure 8 and 9. New bands appear also after exposition to UV lamp.

Discussion

The modification of the drepanocytes shape upon treatment with crude extracts of anthocyanins (Fig. 2 and 3) illustrates the antisickling activity of these extracts and hence justifies the use of *A. cordifolia* and *C. retusa* in traditional medicine (6-8, 12). This activity may be due to the fraction with the R_f= 0.68 for *A. Cordifolia* and with R_f= 0.74 for *C. retusa*. The structural determination of these fractions is still debatable. These results confirm our previous reports (13-20). So, anthocyanins might represent a rational and potential antisickling plant derived drug candidate for sickle cell disease patients. Anthocyanins have antioxidant properties, protecting drepanocytes against free radicals produced within erythrocytes cytoplasm. Indeed, a number of known altered processes associated with sickle cell disease can lead to the hyper-oxidation, including auto-generation of oxygen free-radicals. It is known that the glucose metabolism is reduced in sicklers and compromises the main source of reducing power (NADPH) for cellular metabolism. In fact, the activity of the glutathione reductase system is very low, leading to the hyperhaemolysis of drepanocytes (23).

This study suggests that anthocyanins extract may play a role in both inhibiting polymerization of S haemoglobin and scavenging free radicals within the erythrocytes. This provides possible putative mechanisms for earlier reports on the antisickling properties of anthocyanins of some Congolese plants and their use in the management of sickle cell disease by Congolese traditional healers.

However, anthocyanins are unstable towards some physical and chemical parameters such as temperature, UV-visible radiation, pH, etc (13-15). Since these plants are generally dried under the sun light in traditional medicine, we needed to check the behaviour of purified anthocyanins under UV-visible radiation. The UV radiation induces anthocyanins' spectra modifications. This phenomenon is probably due to the deterioration of anthocyanins by light leading to the formation of new chemical entities in the medium. These new chemical entities may cause either an increase (fig. 6) or a

decrease of the absorption of the solution (fig. 6, 7, 8, 9). This illustrates the instability of anthocyanins under light in general and under UV light in peculiar. It is therefore imperious to preserve theses plants from light when they are used against the sickle cell disease.

Acknowledgement

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