

Effect of Storage on the Bioactivity of Drepanoalpha[®] (An Anti-Sickle Cell Disease Polyherbal Formula) and Comparative Biochemical Profile of Different Batches

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Abstract

Drepanoalpha[®] is a poly-herbal formula (PHF) used in Democratic Republic of the Congo for the management of Sickle Cell Disease (SCD), a genetic disease due to the presence of hemoglobin S in the blood which could in hypoxia conduct to the formation of tactoids, the leading cause of erythrocyte sickling that plays a key role in the pathophysiology of SCD like vaso-occlusion (due to the loss of membrane elasticity) and hemolytic anemia. The aim of the present study was to evaluate the storage time effect on the bioactivity and the secondary metabolites (anthocyanis and flavonoids) and minerals (Fe, Mn, Zn and Cu) profiles of this PHF. Results revealed that flavonoids content of different batches is of the same order of magnitude: the percentage of ratio flavonoids/total polyphenols was 5.3% (batch A); 5.0% (batch B); 4.7% (batch C). However, the anthocyanins content of PHF varies according to the storage time. The percentage of ratio anthocyanis/total polyphenols was 3, 2% for batch A; 4, 7% for batch B; 8, 3% for batch C. Results revealed no significant difference between the average values of total ash (11.34 ± 0.35 84 g/100 g DM for A; 11.5 ± 0.12 84 g/100 g DM for B and 10 ± 0.84 84 g/100 g DM for C) of three batches (threshold: 5%). The different batches of Drepanoalpha[®] contain Fe (0.535 ± 0.107 mg/100 g for A; 0.517 ± 0.103 mg/100 g for B; 0.54 ± 0.108 mg/100 g for C), Mn (4.366 ± 0.786 mg/100 g for A; 4.7 ± 0.799 mg/100 g for B; 4.318 ± 0.734 mg/100 g for C), and Zn (2.293 ± 0.321 mg/100 g for A; 2.389 ± 0.334 mg/100 g for B; 2.338 ± 0.327 mg/100 g for C) and are free of Cu. The micronutrient values of batches A, B and C are not significantly different (threshold: 5%) from each other. At 250 µg /mL, there is no significant difference (threshold: 5%) in anti-oxidative activity between the different batches. All samples displayed strong anti-sickling activity indicating thus at room temperature, the storage time (one or two years) has no effect on the bioactivity, flavonoids content and mineral profile of the PHF. It is desirable that Drepanoalpha[®] could be formulated as syrup or capsule for a double blind, placebo-controlled and randomized cross-over clinical trials in SCD patients.

Keywords

Sickle Cell Disease, Traditional Medicine, Medicinal Foods, Anti-sickling Activity, Polyphenols

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1. Introduction

Sickle Cell Disease (SCD) is a genetic disorder due to the presence of hemoglobin S in the blood which could in hypoxia conduct to the formation of tactoids, the leading cause of erythrocyte sickling that plays a key role in the pathophysiology of SCD like vaso-occlusion (due to the loss of membrane elasticity) and hemolytic anemia [1, 2].

In the Democratic Republic of Congo (DRC), one million people suffer from this disease.

Moreover, 80% of children with SCD who do not have special medical coverage die before the age of five. This disease is therefore one of the causes of morbidity and mortality in the country [3-6]. Current treatment of SCD includes drugs like hydroxyurea (HU) which was approved by the U.S. Food and Drug Administration and the European Medicines Agency but expensive for population of rural zones in Africa. However, it was reported that HU induces toxic effects when administered for a long time [7]. Studies have thus suggested that one possible way to provide inexpensive, effective and readily available drugs is to investigate the therapeutic potential of indigenous plants, because they could contain putative molecules with health benefits in SCD [8-13].

Drepanoalpha[®] is a poly-herbal formula (PHF) from medicinal-based food plants used in Democratic Republic of the Congo for the management of Sickle Cell Disease (SCD). The efficacy and the safety of this PHF have been scientifically validated *in vitro* as well as *in vivo* [14-19]. Drepanoalpha[®] is administered after pre-infusion of one to two teaspoons of the powder into boiling water. However, the daily implementation of this process makes it somewhat difficult to use this remedy, especially in rural areas with low electricity coverage. Also, it does not offer all the guarantees of conservation of the infused which must be administered in the hours following its preparation. In addition, the administration volume (± 20 mL) does not promote good treatment compliance in the patient. Finally, the infused obtained does not guarantee a reproducibility of the doses and consequently that of the pharmacological effects in SCD patients.

In order to obtaining in the short term a standardized phytomedicine which could be registered by the competent authorities and presented in the form of syrup and capsule with a precise and homogeneous dosage, a lyophilizate obtained from the infused powder of Drepanoalpha[®] was chosen as raw material. However, to reassure us that the lyophilizate thus obtained will preserve the therapeutic properties of the powder of Drepanoalpha[®], the present study was carried out with the aim of evaluating the storage time

effect on the bioactivity and the secondary metabolites (anthocyanins and flavonoids) and minerals (Fe, Mn, Zn and Cu) profiles of three different batches (March 2014: A, May 2015: B and July 2015: C) of this PHF.

2. Material and Methods

2.1. Description of Drepanoalpha[®]

Drepanoalpha[®] is a dietary supplement. It supplements the sickle cell patients' nutrition with essential proteins and minerals. In fact, Drepanoalpha[®] powder contains 17% crude protein, 5.70% fat, 6% crude fiber and 55.33% carbohydrates and has an energy value of 1482.07 kJ. In addition, Drepanoalpha[®] contains iron (9.0 mg/100 g), magnesium (1.4 mg/100 g), calcium (4.8 mg/100 g), zinc, manganese, potassium, phosphorus and vitamin C. Phytochemical studies have shown that anthocyanins and organic acids are the main active ingredients of Drepanoalpha[®]. In addition to its nutritional properties, Drepanoalpha[®] has shown an ability to increase the combined hemoglobin level of antifalcemic activity (normalization rate >80%), anti-haemolytic and anti-oxidative ($ED_{50} = 0.604 \pm 0.028$ $\mu\text{g/mL}$).

Drepanoalpha[®] reduces the frequency of sickle cell crises and improves the general condition of treated patients. With a lethal dose (LD_{50}) greater than 4000 mg/kg in Wistar rats and 16000 mg/kg in guinea pigs, the product may be considered non-toxic under normal conditions of use. In addition, the product did not exhibit toxicity to immune cells and blood clotting factors [14, 16]. For its use, approximately 80 ml of boiling water should be placed in a cup and the amount of powder of Drepanoalpha[®] corresponding to the patient's age, or 1 to 2 teaspoons, added. After infusion for 30 minutes, the supernatant is collected after filtration using a tea sieve or a very clean cloth. The infused thus obtained will be administered three times a day at a rate of 20 ml per dose. This infused cannot be used beyond 24 hours after its preparation. To be effective, Drepanoalpha[®] must be consumed continuously for at least 6 months; only then will the maintenance doses come [19]. From the above Drepanoalpha[®] represents an opportunity for an emerging country like the DRC to obtain rapid and adequate therapeutic responses. In this study, we used three different batches of Drepanoalpha[®] produced during the following periods: March 2014 (batch A); May 2015 (batch B) and July 2015 (batch C). These powders have been used as plant material for proximate, phytochemical and pharmacological investigations.

2.2. Infusion and Lyophilization

30 g of Drepanoalpha[®] powder from batches A, B and C were infused in 400 mL of boiling water for 30 minutes. The

filtrate was then lyophilized using a LabCongo brand lyophilizer for 72 hrs. The lyophilized extracts were stored in sealed brown bottles.

2.3. Preparation of Test Samples

The test samples are solutions prepared after reconstitution of lyophilizates in an appropriate solvent. The samples used for the quantitative analyzes of the total polyphenols and flavonoids were prepared by dissolving 20 mg of lyophilisate in 100 ml of distilled water to obtain a solution of 200 µg/ml.

However, for the anthocyanins, 60 mg of lyophilizate were dissolved in 10 ml of distilled water to obtain a solution of 6000 µg/ml.

For the evaluation of the anti-oxidative and anti-radical activity, 50 mg of lyophilizate of each batch were dissolved in 20 ml of distilled water to obtain a solution of 2500 µg/ml.

Serial dilutions were then made with distilled water to obtain concentrations of 500µg/ml, 250µg/ml and 100µg/ml.

To prepare the various test samples intended for the evaluation of anti-sickle cell activity (Emmel test), 128 mg of each lyophilizate were dissolved in 20 ml of physiological solution (0.9% NaCl) in order to obtain solutions of 6400 µg/mL.

From these solutions, different sequential dilutions (dilution factor 2) have been carried out using the physiological solution until concentrations of 100, 50 and 25 µg/mL were obtained.

2.4. Quantitative Determination of Phytochemical Groups

2.4.1. Determination of Total Polyphenols

The determination of the total polyphenols has been carried out with the Folin-Ciocalteu colorimetric reagent as previously reported [20].

(i). Preparation of the Folin-Ciocalteu Reagent

To prepare the Folin-Ciocalteu reagent, 10 g of sodium tungstate and 2.5 g of sodium molybdate has been dissolved in 70 mL of distilled water. Then 50 ml of (85%, d = 1.71) phosphoric acid and 10 mL of concentrated hydrochloric acid (37%, d = 1.19) were added

The mixture obtained was boiled under reflux for ten hours. Then 15 g of lithium sulphate and a few drops of bromine were added. The resulting solution was again boiled for 15 minutes. After cooling, the solution was brought to (increased to) 100 mL with distilled water. Before each use, the reagent was diluted 10 times using distilled water.

(ii). Dosage

200 µL of each test sample (200 µg/mL) was added to 1 mL of

diluted Folin-Ciocalteu reagent. After homogenization, the mixture is incubated for four minutes. After this incubation period, 800 µL of a 7.5% sodium carbonate solution is added to the mixture which is again incubated for 30 minutes in the dark at room temperature. The absorbance of all the samples is measured at 765 nm using a UV-Visible spectrophotometer. For the preparation of the blank, we operated in the same way except that instead of the extract, we put 200µl of distilled water alone. The concentration of the total polyphenols was calculated as gallic acid equivalents.

2.4.2. Determination of Flavonoids

The quantification of flavonoids was carried out as previously reported [21]. Briefly, 100 µL of each test sample were mixed with 4 mL of distilled water and 0.3 mL of a 5% sodium nitrite solution. After 5 minutes of contact, 20 µL of 10% of aluminum chloride solution was added. 2 mL of 1 M sodium bicarbonate solution was added to the mixture and the whole was diluted in 10 mL of distilled water after 5 minutes of rest. The whole was vortexed and the absorbance measured at 510 nm using a UV-Visible spectrophotometer. For the preparation of the blank, we operated in the same way except that instead of the extract, we put 100 µL of distilled water. The flavonoids were quantified as quercetin equivalents.

2.4.3. Determination of Anthocyanins

Total anthocyanin content was determined by the differential pH method as described by Giusti and Wrolstad [22].

(i). Preparation of Potassium Chloride Buffer (0.025 M)

In a 100 mL volumetric flask, 180 mg of potassium chloride are dissolved, with stirring, in approximately 80 mL of distilled water. After complete dissolution, the solution is acidified with a few drops of concentrated hydrochloric acid until a pH of 1 is checked using a pH meter. The volume of the preparation is then brought to the gauge mark by adding distilled water.

(ii). Preparation of Sodium Acetate Buffer (0.025 M)

In a 100 mL volumetric flask, 340 mg of sodium acetate are dissolved in approximately 80 mL of distilled water. After complete dissolution with stirring, about 1 mL of glacial acetic acid is added to the resulting solution to give it a pH of 4.5. After checking this pH value with a pH meter, the volume of the preparation is brought to the gauge mark by adding distilled water.

(iii). Dosage

0.5 mL of lyophilizates reconstituted in distilled water (6000 µg/mL) were mixed with 3.5 mL of the potassium chloride

buffer. The mixture was then homogenized using a vortex. After 15 minutes, the absorbance was measured at 515 nm and 700 nm using a UV-Visible spectrophotometer. The same mixture was then combined with 3.5 ml of sodium acetate buffer. After 15 minutes, the absorbance was again measured at 515 nm and 700 nm using a UV-Visible spectrophotometer. For the preparation of the white (bank), we operated in the same way except that instead of the extract, we added 0.5 mL of distilled water. The total anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalents in 100 g of the dried sample and was calculated according to the following formula:

$T_{AC} = \frac{(AxPMxDFx100)}{\epsilon x C}$; Where A is the absorbance $A = (A_{515} - A_{700})p^H 1 - (A_{515} - A_{700})p^H 4.5$; PM is the molecular weight of cyanidin-3-glucoside = 449.2; DF is the sample dilution factor, ϵ is the molar absorbance of cyanidin-3-glucoside = 26900; and C is the buffer concentration in mg/mL.

2.5. Evaluation of the Anti-radical Activity of the Extracts

The evaluation of the anti-radical activity on hydrogen peroxide (H₂O₂) was performed according to the method of Ruch *et al.* [23].

Briefly, 0.2 mL of reconstituted lyophilisate (500 µg/mL) was incubated with 0.12 mL of a solution of hydrogen peroxide (30 volumes) for 10 minutes at room temperature. Absorbance was measured at 230 nm. Ascorbic acid (500 µg/mL) was used as a positive control. The inhibition percent was calculated as follows: % Inhibition = $\frac{A_0 - A_i}{A_0} \times 100$; where A₀ is the absorbance of the standard and A_i is the absorbance in the presence of the extracts.

2.6. Evaluation of the Antioxidative Activity

The evaluation of the antioxidant activity of the extracts was carried out using the method of Prieto *et al.* [24].

2.6.1. Preparation of Ammonium Molybdate Reagent

To prepare the ammonium molybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), 100 mL of distilled water was acidified using 3.27 mL of concentrated sulfuric acid. Then 436.6 mg of ammonium molybdate and 436.8 mg of sodium phosphate were dissolved. The solution thus obtained was brought (increased) to 300 ml by addition of distilled water.

2.6.2. Dosage

0.2 mL of test samples (100, 250 and 500 µg/mL) and 2 mL of molybdate reagent were mixed in different test tubes. The tubes were corked with cotton wool and incubated at 95°C

for 90 minutes in a water bath. After cooling to room temperature, the absorbance was measured at 695 nm with a UV-Visible spectrophotometer. The activity of the extracts was expressed in mg equivalent of ascorbic acid per gram of extract. Distilled water and vitamin C were used as negative and positive controls, respectively.

2.7. Mineral Analysis

The analysis of the minerals present in the lyophilizates of different batches of Drepanoalpha[®] powder (A, B and C) was carried out by determining the ashes, identifying the mineral elements (Iron, Zinc, Manganese and Copper) and their dosage.

2.7.1. Determination of Ashes

The total ash of the Drepanoalpha[®] powder of different batches (A, B and C) was determined by the calcination/mineralization method [25]. In calibrated porcelain crucibles (C₁), 2 g of the different Drepanoalpha[®] powders (C₂) were weighed on an analytical balance (ES 220A). These powders were then calcined on a hot plate at 550°C until the white smoke disappeared, then placed in a muffle furnace (HEARAEUS) at a temperature of 600-630°C for 4 hours until the white ashes was obtained). The weight (C₃) of these different ashes was then determined. The determination of the organic matter content is made by calculating the weight difference according to the following formula:

Organic matter content (MO%) = $\frac{C_2 - C_3}{C_2 - C_1} \times 100$; Where

MO%: Organic matter content; C₁: weight of the empty crucible; C₂: initial weight (before calcination); C₃: final weight (after calcination). The ash rate is calculated according to the following relation: Ash rate (%) = 100 - MO%.

2.7.2. Identification and Quantification of Minerals

From the total ashes of our three different samples (lots A, B and C), we identified iron, zinc, manganese, copper and then measured the last three elements as previously described [18, 25, 26].

2.8. In vitro Anti-sickle Erythrocyte Activity

The study was submitted for approval to the Ethics Committee of the Department of Biology (Faculty of Science) of the University of Kinshasa (Ref.: CBD/FSC/MMJ/039/MM/2016). Blood samples were provided by free and informed consent of sickle cell patients. Therefore, all the measures have been taken to ensure so that the different blood samples do not present any risk of infection for the patients.

2.8.1. Collection and Storage of Blood Samples

The collection of a 5 ml blood sample on EDTA in a ratio 1:5 (one volume of EDTA for four volumes of blood) is stored at 4°C for a period not exceeding 3 days before use. Sickle cell blood samples were obtained at the "Centre de Médecine Mixte et d'Anémie SS (Centre Mabanga)" located in Yolo-Sud (Kalamu, Kinshasa city). To be collected, the blood should be (come) derived from homozygous sickle cell patients whose hemoglobin status has been proven by electrophoresis of hemoglobin (on alkaline pH cellulose acetate gel) and who have not be transfused within four months before blood test, regardless of age and gender.

2.8.2. Emmel Test

The blood sample was mixed with dissolved lyophilizate

solutions at different concentrations in physiological solution (0.9% NaCl) as the dissolving solvent. The control consists of diluted sickle cell blood without extract. The effect of the different extracted samples is observed by optical microscopy after 24 hours and 48 hours of exposure under hypoxia and isotonic conditions to evaluate the duration of persistence of the antifalcemic effect. A digital camera was used to record microscopic images of the erythrocytes obtained. These micrographs were then processed by the computer software MOTIC images 2000, version 1.3 [3-6, 11-14, 18-20].

3. Results and Discussion

3.1. Efficiency of Freeze-drying

After infusion and lyophilization of the Drepanoalpha[®] powder, the amount of lyophilizate obtained (%) is presented in figure 1.

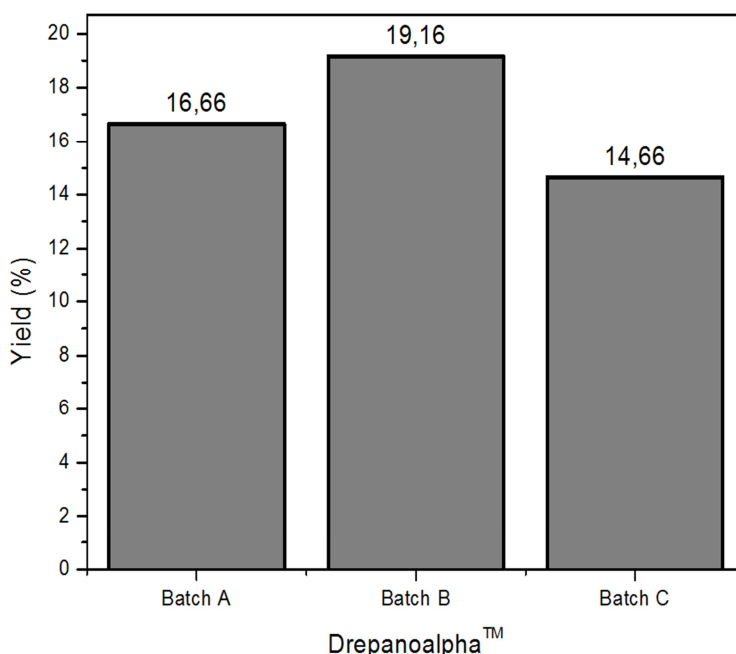


Figure 1. The yield of freeze-drying.

It appears from this figure that after freeze-drying of our different batches, starting from 30 g of Drepanoalpha[®] powder, the efficiency obtained for batch A is 16.66%, or 5 g. For the batch B the yield was 19.16%, or 5.75 g and for batch C 14.66%, or 4.40 g. For this purpose, we found that batch B had a high efficiency compared to batches A and C.

3.2. Determination of Phytochemical Markers

The content of different batches of the lyophilizate of Drepanoalpha[®] in phytochemicals are shown in Table 1. The values given are averages. These results represent in each case the mean \pm standard deviation of three measurements.

Table 1. Average values of phytochemicals contained in Drepanoalpha[®].

Batch	Total polyphenols (mg GAE/g)	Flavonoids (mg EQE/g)	Anthocyanins (mg CE/g)
A	108.745 \pm 0.120	5.715 \pm 0.070 (0.053)	3.500 \pm 0.470 (0.032)
B	84.580 \pm 0.350	4.220 \pm 0.000 (0.050)	4.005 \pm 0.233 (0.047)
C	92.745 \pm 0.100	4.330 \pm 0.000 (0.047)	7.680 \pm 0.000 (0.083)

It is also apparent from this table that the flavonoids content of different batches is of the same order of magnitude.

However, their anthocyanin content varies according to the storage time. The best rate of anthocyanins is obtained with

batch C of July 2015. These results show that the flavonoids are relatively chemically stable whereas the anthocyanins are sensitive to the shelf life of the Drepanoalpa[®] samples (ratio flavonoids or anthocyanins/total polyphenols).

Thus, flavonoids can therefore be selected as phyto-markers for the traceability and standardization of this drug. According to [3-9, 21] and [22], the bioactivity of plant

species traditionally used in the treatment of sickle cell disease in the DRC is attributed among other things to the presence of anthocyanins.

3.3. Micronutrient Analysis

Table 2 gives the organic matter and the total ash content of Drepanoalpa[®]

Table 2. Drepanoalpa[®] organic matter and total ash content.

Drepanoalpa [®]	Batch		
	A	B	C
Organic matter (g/100 g DM)	89.01	88.38	90.84
Total ashes (g/100 g DM)	11.34 ± 0.35	11.5 ± 0.12	10 ± 0.84

It can be seen from this table that there is no significant difference between the average values of organic matter and total ash of three batches (threshold: 5%).

Table 3 gives the results of qualitative analysis of micronutrients contained in Drepanoalpa[®]:

Table 3. Qualitative analysis of Drepanoalpa[®] micronutrient

Micronutrients	Batch		
	A	B	C
Copper	-	-	-
Iron	+	+	+
Manganese	+	+	+
Zinc	+	+	+

(Legend: + denote the presence of the searched element; - denote the absence of the searched element)

It can be seen from this table that the different batches of Drepanoalpa[®] contain Iron, Mn, and Zn and are free of Cu.

The quantitative analysis of three minerals present in the Drepanoalpa[®] powder (batches A, B and C) is given in Table 4.

Table 4. Quantitative micronutrients analysis

Micronutrients (%)	Batch		
	A	B	C
Iron	0.535 ± 0.107	0.517 ± 0.103	0.54 ± 0.108
Manganese	4.366 ± 0.786	4.7 ± 0.799	4.318 ± 0.734
Zinc	2.293 ± 0.321	2.389 ± 0.334	2.338 ± 0.327

This table shows that the micronutrient values (Fe, Mn and Zn) of batches A, B and C are not significantly different (threshold: 5%) from each other. The presence of zinc in this product, which is a cofactor of anti-oxidative enzymes, would strengthen the immune system and the antioxidant defense system of diseased subjects and thus prevent them against bacterial infections. In fact, it has been shown that zinc supplementation increases the activity of natural killer cells (NK cells) and the CD4⁺/CD8⁺ ratio in sickle cell subjects. In this case, Drepanoalpa[®], as a dietary supplement, would boost the immune system of sickle cell disease patients [14, 18, 27].

The presence of iron as a hematopoietic factor would prevent hemolytic anemia in sickle cell patients [14, 18]. The presence in this PHF of manganese, which has antioxidant properties, would regulate the actions of free radicals, limiting the oxidative stress disorders. It plays an important role in the construction of bones and joints that would be beneficial for sickle cell patients [26]. Compared to previous work, the quantitative analysis of iron revealed a rate of 9% but we found that under our operating conditions a low iron content which varies from 0.517 to 0.54%.

This could be due to the nature of the soil and the type of microclimate from which the samples came, hence the importance of standardization.

3.4. Antioxidative Activity

The anti-oxidative activity of batches A, B and C is presented in Table 5. The values given are mean ± standard deviation of three measurements.

Table 5. Evaluation of the anti-oxidative activity

Batch	Phosphomolybdate (mgAAS/mL)	
	500 µg/ml	250 µg/ml
A	0.131 ± 0.004	0.106 ± 0.001
B	0.118 ± 0.006	0.098 ± 0.001
C	0.130 ± 0.004	0.103 ± 0.001
Vit C	0.588 ± 0.041	0.384 ± 0.010

It can be seen from this table that at 250 µg/mL, there is no significant difference (threshold: 5%) in anti-oxidative activity between the different lots. However, at 500 µg/mL the batches A and C are more active than lot B. In any case, Drepanoalpa[®] has anti-oxidative properties. For this purpose, it is well indicated for a better management of sickle cell disease because it could prevent hemoglobin S against the cascades of oxidative reactions of which the red blood cell is the seat.

3.5. Anti-radical Activity

The anti-radical activity of the different batches (A, B and C) obtained are shown in Table 6.

Table 6. Anti-radical activity of different batches of Drepanoalpha[®]

Batch	% Inhibition of H ₂ O ₂
A (500 µg/ml)	2.980 ± 0.596
B (500 µg/ml)	2.690 ± 0.457
C (500 µg/ml)	2.850 ± 0.399

Hydrogen peroxide is not known to be directly responsible for oxidative stress; however, its oxidation capacity is due to its ability to generate the hydroxyl radical which is highly oxidizing. The maintenance of hydrogen peroxide at a very low level in the body is therefore of considerable interest for sickle cell patients. However, at the concentration of 500

µg/mL, the anti-free radical activities of vitamin C, batches A, B and C are only 8.7%, 2.980%, 2.690% and 2.850% respectively. Thus, these results show that the mechanism of action Drepanoalpha[®] in the fight against oxidative stress is not that of reducing hydrogen peroxide in water.

3.6. Anti-sickling Activity

The results of the anti-sickling activity of extracts from different batched of Drepanoalpha[®] are given in Figure 2 below.

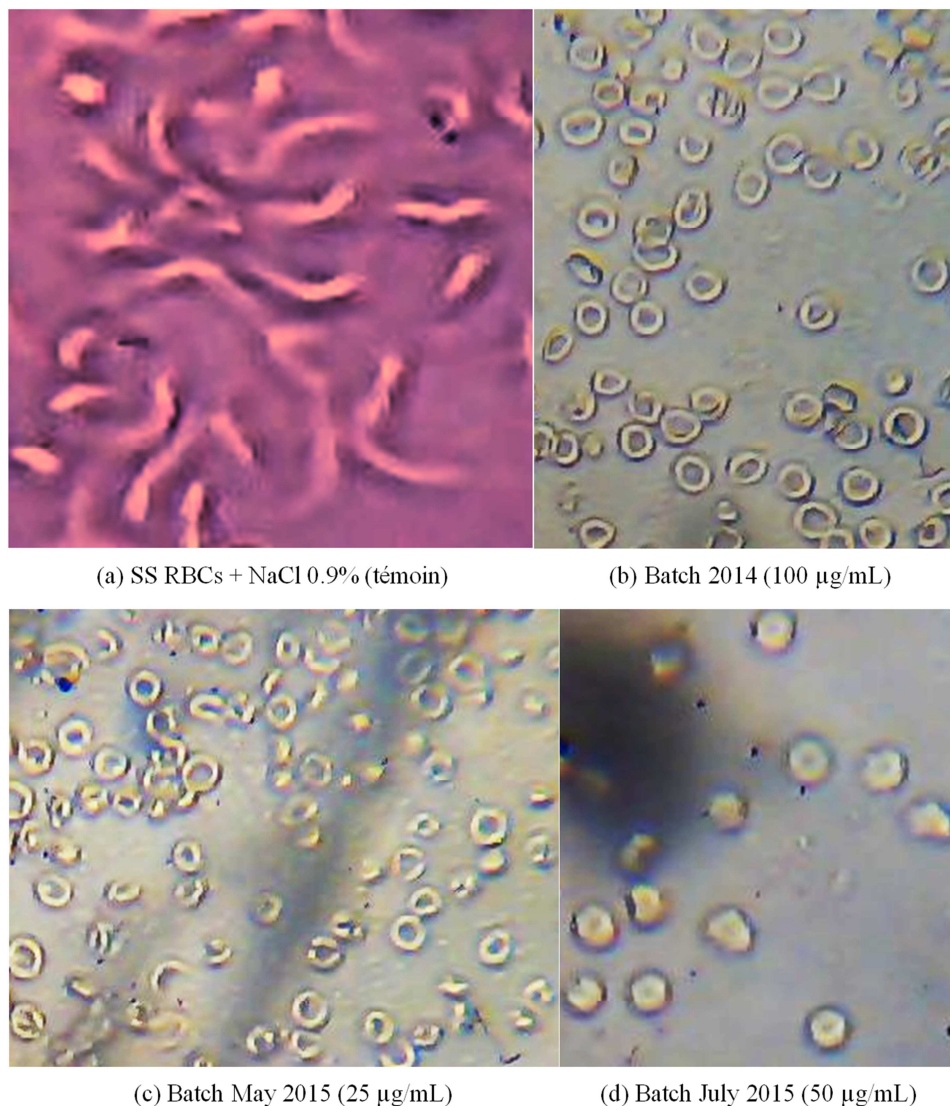


Figure 2. Phenotype of untreated sickle cells (a) or treated with lyophilisate of Drepanoalpha[®] (b-d) (NaCl 0.9%, Na₂S₂O₅ 2%, X500).

As can be seen in figure 2 (a), all erythrocytes are sickled. In our experimental conditions, this falciformation was induced by the combined actions of 2% sodium metabisulfite and paraffin, all contributing to create hypoxia. On the other hand, in figures 2 (b) to (d), we see that for the same SS blood treated with the extracts of different batches of Drepanoalpha[®], red blood cells return to the normal

biconcave form, indicating that the shelf life of Drepanoalpha[®] samples has no effect on the antisickling activity of this drug.

4. Conclusion and Suggestions

The aim of the present study was to evaluate the storage time

effect on the bioactivity and the secondary metabolites (anthocyanins and flavonoids) and minerals (Fe, Mn, Zn and Cu) profiles of this PHF. Results revealed that flavonoids content of different batches is of the same order of magnitude. However, the anthocyanins content of PHF varies according to the storage time. Results revealed no significant difference between the average values of total ash of three batches (at threshold 5%). The micronutrient values of batches A, B and C are not significantly different from each other. At 250 µg /mL, there is no significant difference in anti-oxidative activity between the different batches. All samples displayed strong anti-sickling activity indicating thus at room temperature, the storage time (one or two years) has no effect on the bioactivity, flavonoids content and mineral profile of Drepanoalpha®. It is desirable that Drepanoalpha® could be formulated as syrup or capsule for a double blind, placebo-controlled and randomized cross-over clinical trials in SCD patients.

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