

***In Vitro* antisickling and Free Radical Scavenging Activities of *Kigelia africana* (LAM) Stem Bark**

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Abstract: *Kigelia africana* dried bark has been recommended in the management of sickle cell anemia by traditional practitioners. The aim of this study was to evaluate *in vitro* antioxidant and sickling inhibitory activities of *Kigelia africana*. Quantitative estimation of phenolic compounds was performed using colorimetric method in hydro-ethanolic extract (70%) and decocted. The sickling inhibitory activity was determinate according to Emmel method and the evaluation of antioxidant properties was carried out using the method of scavenging the free radical DPPH. The obtained results indicated that *Kigelia africana* species contains flavonoids (8.61 ± 1.08 mg QE and 9.78 ± 1.14 mg QE/g of dry weight) and total phenols (9.48 ± 0.19 mg GAE/g and 11.11 ± 0.22 mg GAE /g of dry weight) in decocted and hydroethanolic extract respectively. The IC₅₀ values of the ethanolic extract and the decocted were 0.320 ± 0.01 and 0.468 ± 0.04 mg/mL respectively. The *in vitro* sickle-formation inhibition test indicated the value of 89% and 82.36% for the ethanolic extract and the decocted at 10mg/mL respectively. Those values were higher than 80% which was the phenylalanine value. Both extracts showed antioxidant and sickling inhibitory activities. Overall, there could have a correlation between these activities and phenolic compound content in this studied plant extracts. These results would justify the use of this plant in rural environment.

Keywords: *Kigelia africana*, Phenolic Compounds, Antisickling Activity, Antioxidant Activity, Cote d'Ivoire

1. Introduction

In Côte d'Ivoire, according to the National Program for the Promotion of Traditional Medicine, nearly 1,421 species of medicinal plants are involved in traditional Ivorian medicine. These inventoried species appear to be a source of new active molecules that help to develop new effective and easily accessible drugs [1]. Several ethnobotanical surveys of plants used in the treatment of chronic and metabolic diseases have been carried out in Côte d'Ivoire [2, 3]. Mates and Sanchez-Jimenez [4] reported in their work that several chronic and metabolic conditions were related to oxidative

stress. Indeed, oxidative compounds are implicated in many diseases as a trigger or associated with complications. Some chronic conditions such as sickle cell disease are linked to oxidative stress although it is a genetic disease due to a hemoglobin mutation [5]. The Clinical signs of sickle cell disease are vaso occlusive seizures, hemolytic anemia and susceptibility to infection [6]. These clinical signs commonly observed in sickle cell patients could be treated with medicinal plants. The verification of this hypothesis began with an ethnobotanical investigation of plants used in the management

of sickle cell disease in eastern Côte d'Ivoire [3]. During this investigation, *Kigelia africana* was mentioned as a plant species that has an antisickling activity. *Kigelia africana* (Lam.) Benth belongs to the Bignoniaceae family. *Kigelia africana* occurs throughout tropical Africa, particularly in western, central and southern regions. It is widely used in West Africa by the traditional healers as an herbal remedy for the treatment of various ailments [7]. Some studies have highlighted its analgesic, anti-inflammatory and antimicrobial activities [8].

The purpose of this study was to assess the antisickling activity of *K. africana*. To achieve this goal, an evaluation of the sickling inhibitory and antioxidant activities were carried out.

2. Materials and Methods

2.1. Plant Material

The stem barks of *Kigelia africana* (Lam.) Benth collected in the Indenie-Djouablin region have been identified at the National Floristic Centre (CNF). The barks have been cleaned, cut and air-dried at room temperature (25°C). After three week drying, the plant was powdered with a Severin® brand grinder.

2.2. *Kigelia Africana* Extracts Preparation

2.2.1. Hydroethanolic Extract

Hydroethanolic extract has been made according to the protocol described below [9]. One hundred grams (100g) of the grinder plant were soaked in a liter of 70% hydroethanolic solution. The mixture was homogenized 10 times, for 2 minutes each time, using a Severin® brand blender. The resulting homogenate was wrung out with a square of white cloth, and then filtered three times on hydrophilic cotton and once on Whatman paper (3 mm). The filter was evaporated at 45°C using a Venticell®. The extract obtained was named EKA.

2.2.2. Decoction

According to Konkon [10], one hundred grams (100 g) of powdered plant were brought to a boil for 15 minutes in 2L of distilled water and the mixture has been cooled down to room temperature (25°C) and filtered three times on cotton and once on Whatman 3 filter paper. The resulting filtrate was dried at 50°C in the oven. The extract obtained was named DKA.

2.3. Antioxidant Properties

2.3.1. Total Phenol Content

The determination of phenolic compounds contents was made using [11] method. One (1) mL of Folin-Ciocalteu reagent was added to the plant extract at the concentration of 1 mg/mL in a test tube. After 3 minutes, 1 mL of 20% sodium carbonate solution (p/v) was added to the test tube and supplemented at 10 mL with distilled water. After 30 minutes, the absorbance was read at 745 nm, compared to methanol as blank, on a Jenway 7315 spectrophotometer. A standard range,

from 0.1 mg/mL Gallic acid stock solution, was used to determine the concentration of phenols in the sample. The result was expressed in mg GAE/g.

2.3.2. Flavonoid Content

The total flavonoid content was determined using direct quantification by the aluminum chloride method [12]. The calibration curve was made up using 0.1 mg/ml Quercetin stock solution. At a volume of 0.5 mL of plant extract, 0.5 mL of distilled water, 0.5 mL of aluminum chloride, 0.5 mL of potassium acetate and 2 mL of distilled water were added. After 30 minutes, the absorption was read at 415 nm compared to methanol as control. A standard range from 0.1 mg/mL Quercetin stock solution was used to determine the amount of flavonoids in EKA or DKA. The result was expressed in mg QE/g.

2.3.3. Antioxidant Activity Assessment

DPPH (2, 2 diphenyl-1-picrylhydrazyl) is the commonly used substrate for assessing antioxidant activity because of its stability in free radicals form. It is absorbed into visible at the wavelength of 517 nm. The experimental protocol used to study the trapping activity of DPPH was described by Parejo [13] with some slight modifications.

2.4. Evaluation of Sickling Inhibitory Activity

2.4.1. Blood Sampling

An agreement was obtained from the Ethics Committee and an informed consent has been approved by each voluntary sickle cell patient selected at Yopougon University Hospital in Hematology Clinical Department. To be included in the study, blood should come from homozygous sickle cell patient. The genotype of hemoglobin was confirmed by the electrophoresis method. The voluntary patient must not have had a blood transfusion for at least two months prior to the blood sampling. The venous blood of each volunteer was taken in a tube (EDTA). These samples were placed in a cooler and transported to the Immunity Biology Pole of the Pasteur Institute of Cote d'Ivoire (IPCI).

2.4.2. Sickling Inhibitory Test

The test was conducted by using Emmel method [14] which was slightly modified by Mpiana [15]. The blood sample was centrifuged for 3 minutes at 3000 rpm and the supernatant was removed using a Pasteur pipette. One (1) mL of washed red blood cells was suspended in 1 mL of physiological buffer (NaCl 0.9%). EKA and DKA solutions at 5 and 10 mg/mL were prepared. A volume of 50 µL of extract was homogenized with 50 µL of washed blood in a test tube. A volume of 50 µL of sodium meta-bisulfite (2%, w/v) was added to the mixture. The tube was sealed with paraffin. Negative and positive controls have been prepared. Negative control was prepared by mixing 50 l of washed blood with 50 l of physiological buffer and 50 µL of sodium meta-bisulfite (2%, w/v). The positive test was prepared by mixing 50 µL of washed blood with 50 µL of a phenylalanine solution at 10mg/mL and 50 µL of sodium meta-bisulfite (2%, w/v). The tubes were placed in a darkroom for 120 minutes.

Morphological analysis of the erythrocytes and counting of sickle cells were carried out by X40 observation under a microscope. Sickling inhibitory activity was expressed as a percentage of sickle cells formed in the presence of plant extracts in relation to the number of sickle cells present in the negative control. This activity was determined by the formula listed below:

$$AA = (P_0 - P_1) / P_0 \times 100$$

AA: sickling inhibitory activity; P₀: sickle cell levels in the control; P₁: sickle cell levels in the presence of plant extract. The experiment were triplicate and the data were subjected to one-way variance analysis (ANOVA) and the differences between the samples were determined by the T-student and Tukey multiple comparison test using the Graph Pad Prism 7.0 program.

Table 1. Phenolic content and scavenging activity of the aqueous and ethanolic extracts of *Kigelia Africana*.

Parameters Plants Extract	Polyphenols total (mg GAE /g)	Flavonoids (mg QE/g)
EKA	11.11±0.22 ^a	9.48±1.14 ^a
DKA	9.48±0.19 ^b	8.61±1.08 ^a

3.2. DPPH Scavenging Activity

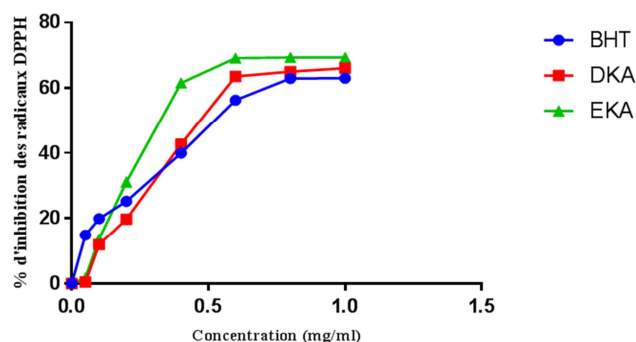


Figure 1. % inhibition of DPPH for DKA, EKA and BHT.

The CI_{50} values for EKA and DKA were 0.320 ± 0.01 and 0.468 ± 0.04 mg/mL respectively. BHT (0.5 ± 0.67 mg/mL) was used as a reference molecule (Figure 1). These results are comparable to those of Iram [18] and Evenamede [16]. These authors showed that *Kigelia africana* CI_{50} was about 0.300 mg/mL. Overall, there was no significant difference between the anti-free radical activity (DPPH) of DKA, EKA and BHT. This anti-free radical activity could be due to the presence of antioxidant molecules in the extracts analyzed. Therefore, it could have a correlation between the phenolic compound of the plant extract and its anti-free radical activity [19]. According to Sahgal [20], low inhibition percentage would indicate a proton transfer capacity. However, these extracts could serve as free radical inhibitors or trappers, possibly acting as primary antioxidants [19]. Polyphenols and their derivatives prevent the oxidation of hemoglobin into methemoglobin and inhibit the generation of free radicals [21].

3. Results and Discussion

3.1. Total Phenolic and Flavonoids Compounds Contents

Quantitative analysis of the EKA and DKA phenolic compounds was determined from the Gallic acid calibration curve, equation $Y = 8.1544X$ and $R^2 = 0.9732$. Total flavonoids were determined from the Quercetin calibration curves, equation $Y = 1.36x - 0.0181$ and $R^2 = 0.9976$. Polyphenols concentration of EKA (11.11 ± 0.22 mg GAE /g of dry weight) was higher than that of DKA (9.48 ± 0.19 mg GAE /g of dry weight). The total flavonoid content of EKA and DKA follows the same trend as the polyphenols concentration. The flavonoid content of EKA (9.78 ± 1.14 mg QE/g of dry weight) was higher than that of DKA (8.61 ± 1.08 mg QE/g of dry weight). The results were presented in Table 1. These results are consistent with those of Essam [16] and Abdulkadir [17].

3.3. Sickling Inhibitory Activity

Morphological analysis of red blood cells treated with NaCl at 0.9% and $Na_2S_2O_4$ 2% without plant extract showed in figure 2 that all red blood cells lost the rounded, biconcave shape to adopt the characteristic sickle shape. While, the red blood cells treated with plant extracts maintained their rounded and biconcave shape. The blood cells treated with DKA were showed on figures 3 at 5 mg/mL and figure 4 at 10mg/mL. The inhibitory activities of DKA at 5 and 10 mg/mL were 51% and 82.36% respectively. The sickle-formation inhibitory activity of EKA at concentrations of 5 and 10 mg/mL, were 63.33% and 89% respectively (figures 5 and 6). The sickle-formation inhibition activity was dose-dependent. Activities were recorded in Table 2. These results confirm Kplé study [22]. These authors highlighted this dose response relationship between the sickling inhibitory activity and plant extracts concentration.

Table 2. EKA and DKA antisickling activity.

Extracts	Concentration: 5 mg/mL	Concentration: 10 mg/mL
EKA	63.33 %±1.11	89 %±0.6
DKA	51 %±4.66	82.36 %±3.77

Sickle-formation inhibition could be explained by the presence of polyphenols and its derivatives, known for their protein interaction properties [23, 24]. This interaction would compete with hemoglobin S aggregation processes and then could inhibit the erythrocyte sickle-formation process [25]. Polyphenols would inhibit the oxidation of Fe^{2+} in Fe^{3+} by competing with 2,3-DGP [26]. At the concentration of 10mg/mL, there was no significant difference between the activities of EKA ($89 \pm 0.6\%$) and DKA ($82.36 \pm 3.77\%$). At this concentration the solvent had no influence on *Kigelia Africana* sickling inhibitory activity.

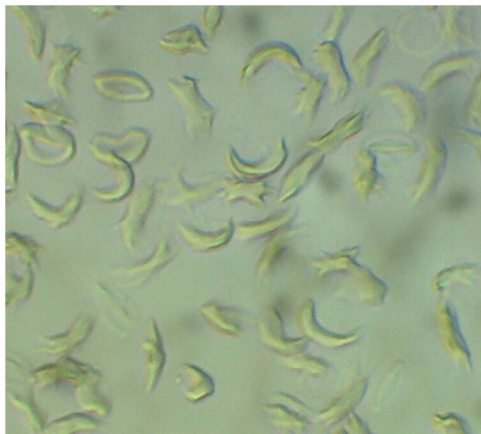


Figure 2. Morphology of SS Blood Sickle Cell in the presence of $\text{Na}_2\text{S}_2\text{O}_4$ 2%.

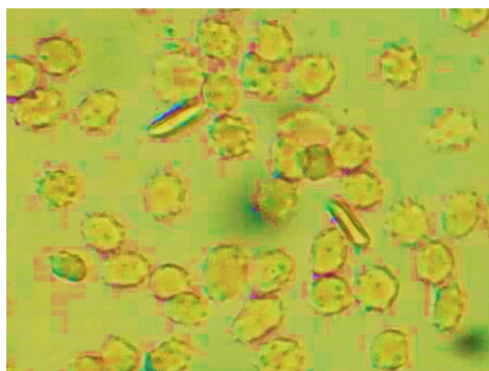


Figure 3. Morphology of Sickle Cell Blood treated with 5mg / mL of DKA.

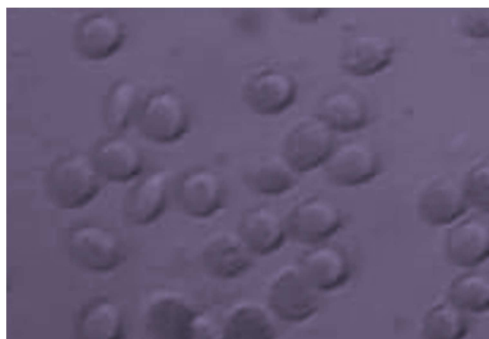


Figure 4. Morphology of Sickle Cell Blood treated with 10mg / mL de DKA.

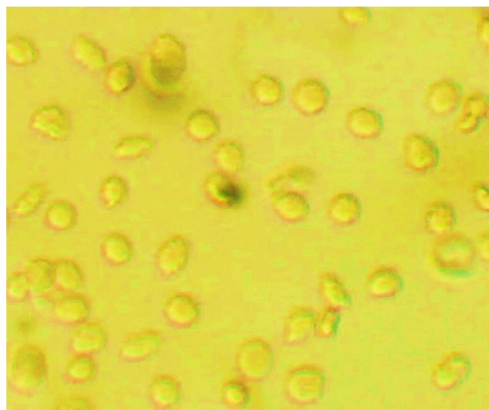


Figure 5. Morphology of Sickle Cell Blood treated with 5mg / mL of EKA.

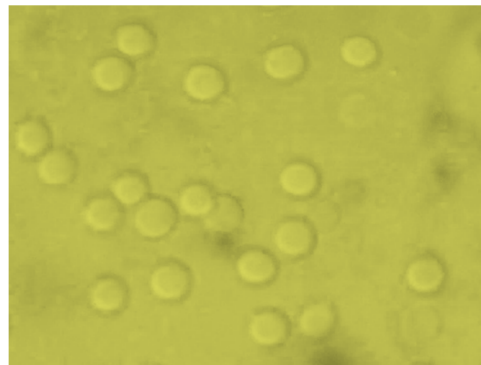


Figure 6. Morphology of Sickle Cell Blood treated with 10 mg / mL de EKA.

4. Conclusion

The barks of *Kigelia africana* contain phenolic compounds. The presence of these chemicals would give to *Kigelia africana* its anti-radical and sickle-formation inhibitory activities. There would be a correlation between phenolic compound content, antiradical and sickling inhibitory activities. These both properties could justify the use of this plant in traditional Ivorian medicine.

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