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Study of the antiradical and anti-sickle cell activities of the methanolic extract of the leaves of *Gliricidia sepium*, an African herbal medicine used for the treatment of *SS* sickle cell disease

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Abstract

The objective of this study is to evaluate the antiradical and antisickling activities of the methanolic extract of the leaves of Gliricidia sepiumon sickled hemoglobins and to identify the nature of metabolites molecules responsible for these activities. The radical DPPH• is used to measure the antioxidant power of the methanolic extract. For the anti-sickling activity, reversibility of the sickle cells is studied, according to the incubation time of the extract compared to the controls (physiological water and phenylalanine) on blood samples from SS sickle cell patients. For this purpose, concentrations of 0.05; 0.5; 5 and 10 mg/mL of the methanolic extract were brought into contact with type SS sickle cells after causing their sickling with a 2% solution of sodium metabisulphite solution. The evaluation was carried out every 30 minutes for 120 minutes. The results of the Emmel test revealed that the application of the methanolic extract of the plant at 0.5 mg/mL and0.05 mg/mL on sickled red blood cells shows remarkable activity on the reversibility of red blood cell sickling in 120 min, even greater than that of the reference, which is phenylalanine. Moreover, for the two concentrations 10 and 5 mg/mL of the methanolic extract of leaves ofGliricidia sepium , the percentage of sickle cells decreases over time. This demonstrates the anti-falsemic power of the plant because. In fact, for the negative control, the number of sickle cells increases over time. However, the evaluation of the antioxidant activity shows a fairly high IC50 (13.5733 ± 0.0538 mg/mL), showing that the molecules responsible for the antioxidant activity do not necessarily act on the sickled red blood cells

Keywords: *Gliricidia sepium , anti-sickling activity, anti-free radical activity*

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I. Introduction

For years, plants have been an important reservoir of bioactive molecules in the case of the management of many diseases [1–4]**.** Plant organs participate in different functions with varying physico-chemical and metabolic reactions. These metabolic variabilities are the physiological adaptation responses to environmental conditions and stresses [5,6].

Sickle cell anemia or *SS* anemia is an endemic genetic disease in certain populations of tropical regions [7–9]. It is due to the replacement of glutamic acid in normal hemoglobin by valine in position six of the beta chain of globin. The resulting pathological hemoglobin (HbS) is poorly soluble in the absence of oxygen and causes polymerization which leads to the sickling of red blood cells [10–12]. This change in the shape of the red blood cells makes them fragile and less flexible, which leads to their early hemolysis and various infarctions. According to the WHO, nearly 5% of the world's population carry a gene responsible for a hemoglobin abnormality [13]. The majority of people with this disease live in sub-Saharan Africa with prevalence varying between 10 and 40% [14]**.** The homozygous form *SS* is manifested by anemia, susceptibility to infections and by bone and/or abdominal pain attacks. In addition, sickle cell disease via vaso-occlusive crises can lead to the generation of free radicals and therefore lead to oxidative stress. In the absence of appropriate management of this form *SS*, 50% of children die before the age of 5 years [15]. Today, access to drugs to relieve the disease is becoming more and more difficult. As a result, people are increasingly turning to medicinal plants for the management of this particular disease [16,17]. *Gliricidia sepium*, which is a plant used by a medical office specializing in natural medicine for the management of sickle cell disease, has been targeted in this present

study. Studies done on this plant have shown its high nutritional value for ruminants and that it could be used to maintain livestock and even promote modest gains during the dry season in tropical areas [18,19]. However, this plant has been the subject of a few chemical studies [20,21]and it then becomes interesting to determine its anti-free radical and anti-sickness activities to justify its use in natural medicine by the aforementioned medical office.

II. Materials and methods

Blood samples

The sample is blood of non-transfused patients whose *SS* sickle cell anemia is confirmed by electrophoresis. The blood is obtained by venipuncture at the level of the fold of the elbow in a tube containing EDTA (anticoagulant). The blood sample is kept at ± 4°C in the refrigerator.

Plant material

The choice for *Gliricidia sepium*is justified by spectacular results obtained by a medical practice specializing in natural medicine. Indeed, after using the leaves of the plant, the state of health of patients with *SS* sickle cell disease improved considerably. The plant material was collected in Mbour [14° 24′ 20″ north, 16° 51′ 20″ west], a town located in western Senegal, about 80 km from Dakar. The plant material used consists of the powder of the leaves of *Gliricidia sepium*. After harvest, the plant material is dried in the dark and then pulverized using an electric grinder. The powder obtained is packaged and subsequently used for the various chemical tests.

Extraction procedure

The secondary metabolites were extracted by maceration to avoid possible degradation of the thermo-degradable molecules present in the plant. For this purpose, methanol is used as extraction solvent for the evaluation of antioxidant and anti-sickling activities. *Gliricidia Sepium* leaf powder (100 g) were macerated for 48 h in 200 mL of methanol. The macerate is filtered, and the solid residue is taken up and then returned to macerate twice in 200 mL of methanol. The three fractions are combined, dried with magnesium sulphate, then concentrated using a rotary evaporator. The residue obtained will be used to prepare the solutions to be tested.

Antioxidant activity

The antioxidant test was carried out using the DPPH• method [22]**.** The DPPH• solution is obtained by dissolving 10 mg of solid DPPH• in 250 mL of methanol away from light after stirring for 30 min. A sample stock solution of 40 mg/mL is prepared by dissolving 80 mg of dry extract in 2 mL of methanol. Then a concentration range was prepared with a 1/2 dilution factor. 0.2 mL of these different concentrations is taken and introduced into test tubes and mixed with 7.8 mL of the purple methanolic solution of DPPH• previously prepared. The tubes are stirred manually for a few seconds then incubated for 30 min away from light, the reading is carried out by a spectrophotometer at a wavelength of 517 nm using methanol as a blank. The prepared ascorbic acid is used as the reference antioxidant.

Anti-sickling activity

The anti-sickle cell activity of the extract was measured using the Emmel test according to the protocol described by Imaga *et al.* [23]with slight modifications. A drop of blood is placed on a slide, in contact with a substantially equivalent drop of sodium metabisulphite (2%) and a drop of extract at different concentrations. Spreading and mixing are done with the edges of the coverslip; then nail polish is coated around the lamella thus preventing any entry of air. For each sample, a first test is carried out directly on fresh blood which has not yet undergone any manipulation (control).Observations under an optical microscope at magnification 40, after 15 min of incubation, makes it possible to search for sickle-shaped red blood cells. Every 30 min, a test is carried out, which gives for each of them a total of five tests respectively at T_0, T_{30}, T_{60}, T_{90} and T_{120}. The time T_0 is actually the first reading taken 15 min after having deprived the red blood cells of oxygen.

III. Results and Discussion

Antioxidant activity of the total methanolic extract of *Gliricidia Sepium*

The antioxidant activity of methanolic extract of leaves of *Gliricidia Sepium* for different concentration are listed in Table 1. Figure 1 which represent the inhibitory power versus the extract concentration allows to determine the IC50 (13.5733±0.0538 mg/L) of methanolic extract of leaves of *Gliricidia Sepium*.

Table 1: Inhibitory power (IP) of the methanolic extract of *Gliricidia Sepium*

Concentration (mg/mL)	IP (mg/mL)	IC50 (mg/mL)
0.3125	0.93262937	13.5733±0.0538
0.625	2.26455141	
1.25	5.90578507	

2.5	12.2558596	
5	22.6909841	
10	40.097384	
20	70.5589388	

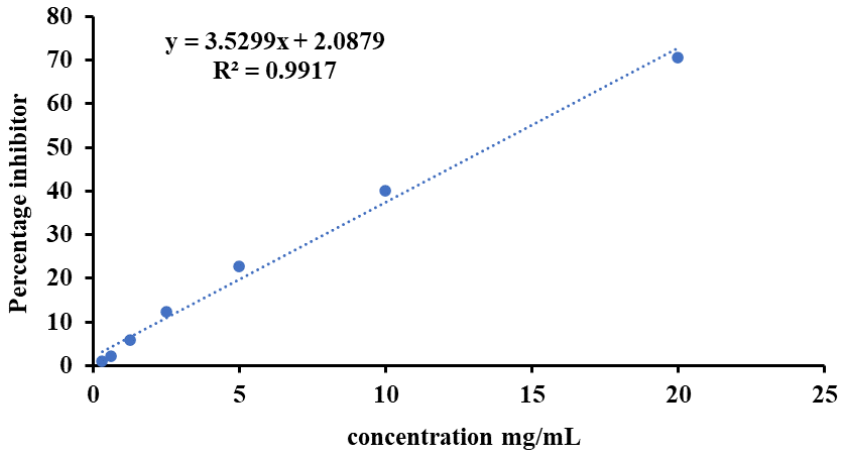


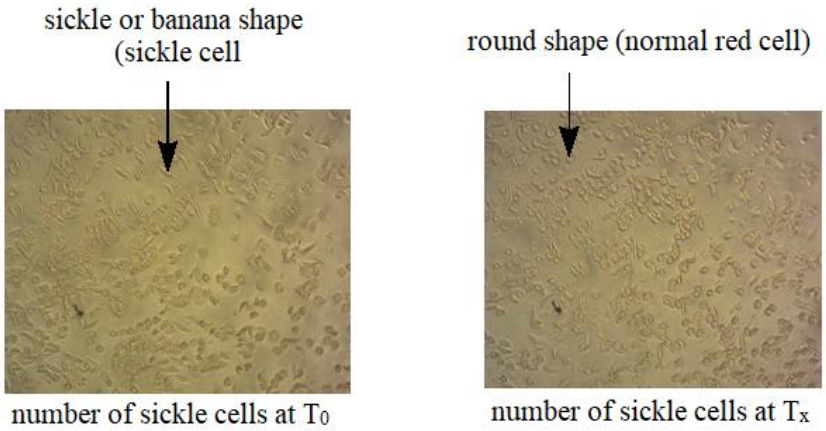
Figure 1. Percentage inhibitor of *Gliricidia sepium* as a function of concentration

III.1.1. Anti-sickling activity of *Gliricidia Sepium*

The residual sickle cells over the time in the blood sample treated by methanolic extract of *Gliricidia sepium* or by phenylalanine as reference are presence in the following figures 2-5 and picture 1.

A count is made on each slide to determine the level of residual sickle cells. The percentage of residual sickle cells represents the ratio between the number of sickle cells at Tx and the number of sickle cells at To.

$$\%\ residual\ sickle\ cells = \frac{average\ sickle\ \ cells\ at\ Tx}{average\ sickle\ cells\ at\ T0} \times 100$$



Picture 1. Number of sickle cells from T_0 to T_x.

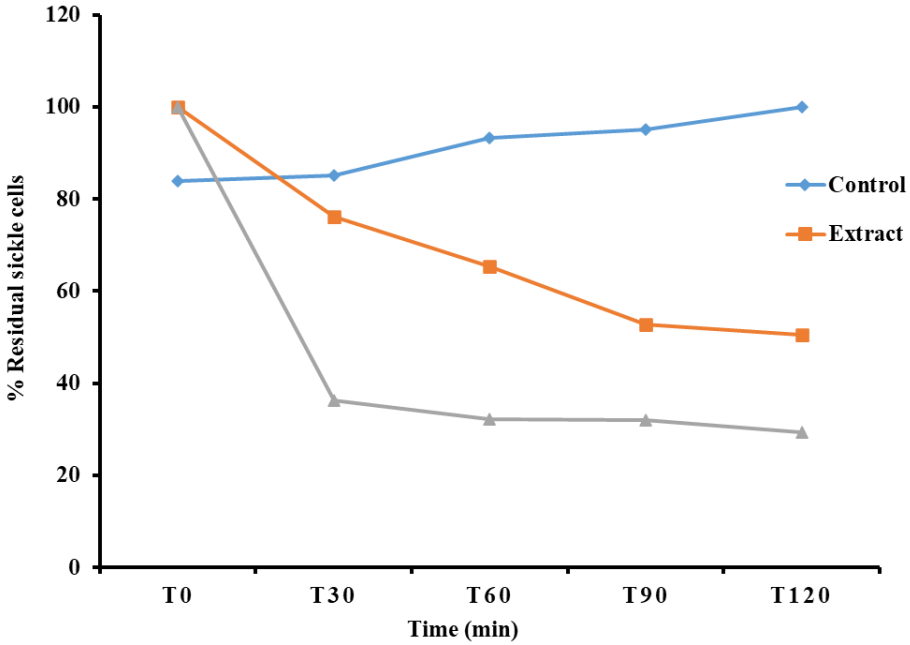


Figure 2. Evolution of residual sickle cells as a function of time at 10 mg/Ml

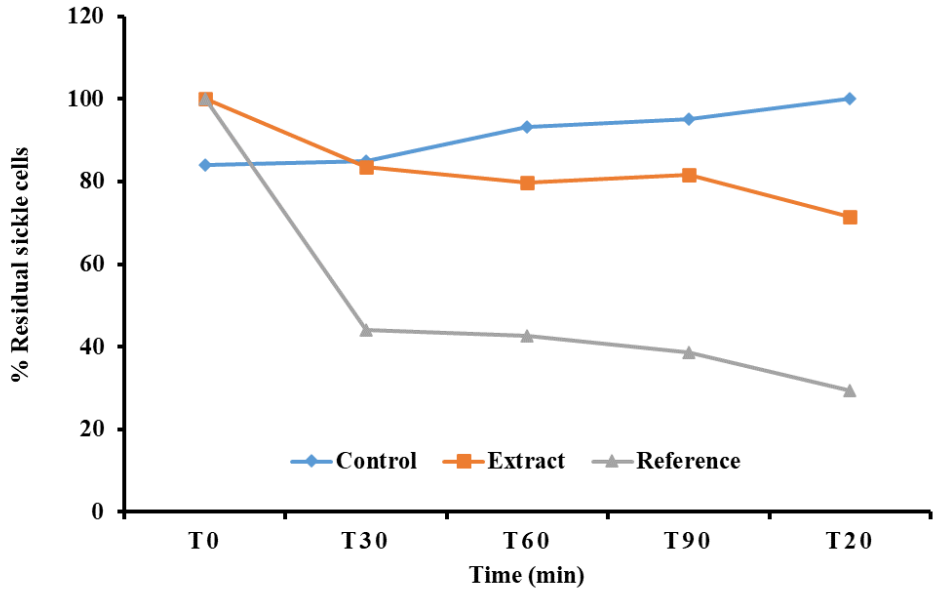


Figure 3. Evolution of residual sickle cells as a function of time at 5 mg/mL

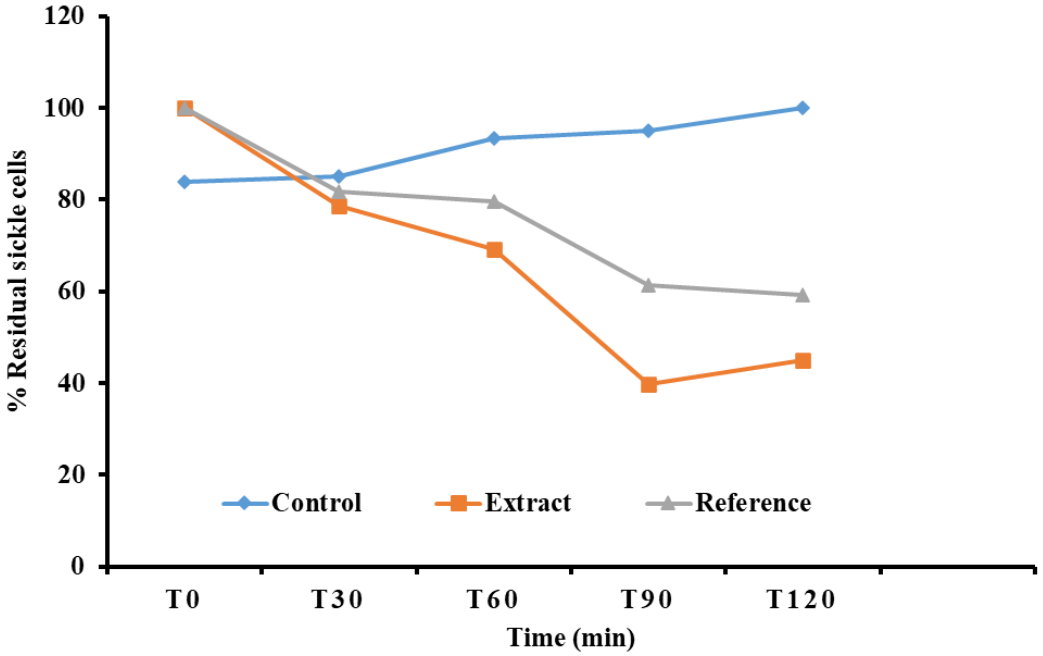


Figure 4. Evolution of residual sickle cells as a function of time at 0.5 mg/mL

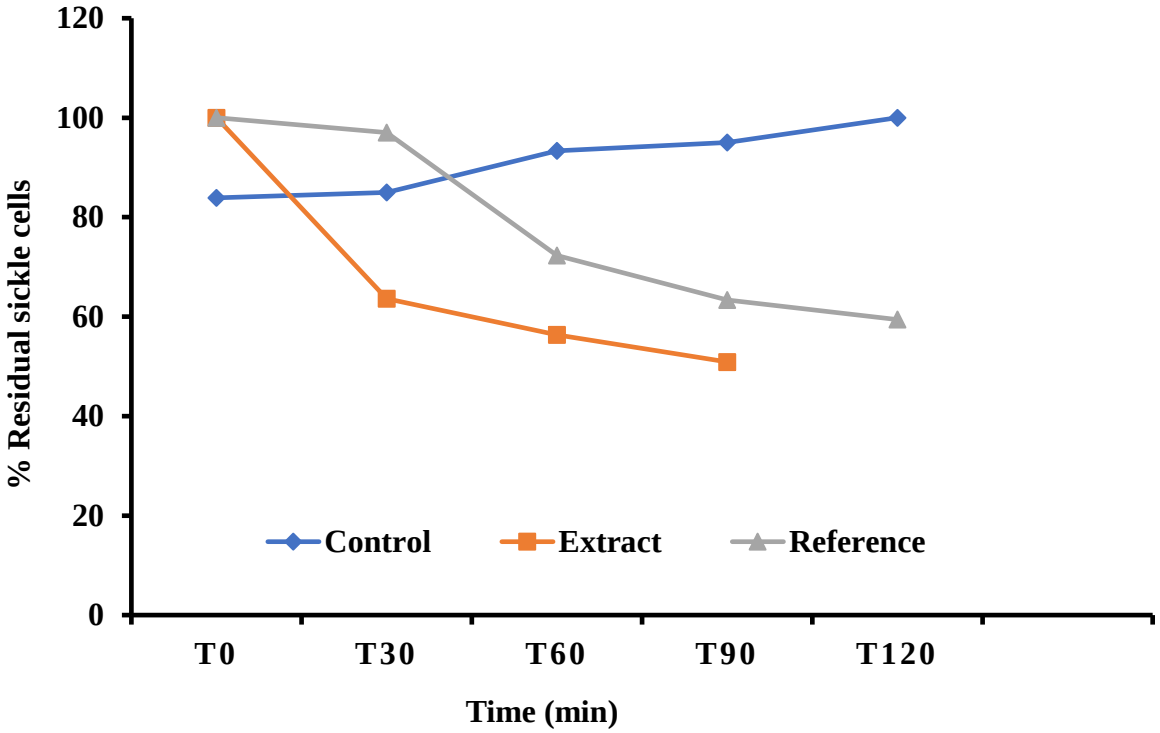


Figure 5. Evolution of residual sickle cells as a function of time at 0.05 mg/mL

The antioxidant potential was evaluated by the colorimetric technique using the radical DPPH• (2,2-diphenyl-1-picryl-hydrazyl). The DPPH• radical is one of the most commonly used substrates for the rapid and direct assessment of antioxidant activity due to its stability and simplicity of analysis. The violet-colored DPPH• radical is reduced to a yellow-colored compound in the presence of antiradical compounds. With regard to the results (Table 1), the methanolic extract of the leaves of *Gliricidia sepium*has a relatively low antioxidant activity (IC50 = 13.5733±0.0538 mg/mL) comparatively to ascorbic acid (0.143 mg/mL) used as reference. This result agrees with the low content of polyphenolic compounds, revealed by the results of the phytochemical

screening. Indeed, an overview of the literature reports that phenolic compounds, because of their hydroxyl groups, express their antioxidant activity by trapping free radicals or by chelating certain ions [24,25].

In this study, an evaluation of the anti-sickling activity of *Gliricidia sepium*was conducted by incubating the *SS* red blood cells for the necessary time in the methanolic extract of the leaves of *Gliricidia sepium* and counting the number of residual sickle cells according to the Emmel test. Figures 2-5 and Picture 1 show the evolution of the percentages of sickled red blood cells as a function of time. The results of the anti-sickling activity reveal that for the two concentrations 10 and 5 mg/mL of the methanolic extract of leaves of *Gliricidia sepium*, the percentage of sickle cells decreases over time. This demonstrates the anti-sickle cell power of the plant because for the negative control, the number of sickle cells increases over time. Moreover, after 120 minutes of incubation, we find that for a concentration of 5 mg/mL, the methanolic extract causes a return to be sickling up to about 30% against 70% for the phenylalanine positive control. The anti-sickling activity is more accentuated with the extract concentration of 10 mg/mL, after 120 minutes of incubation. Indeed, it causes a reversal of sickling up to 50 %. Our results corroborate those of Oduola *et al*.[26]**.**The positive control, phenylalanine remains more active than the extract for the two concentrations 10 and 5 mg/mL over time. On the other hand, the results obtained and compared with those of the blank controls give an anti-sickling activity greater than the reference, at the respective concentrations of 0.5 mg/mL and 0.05 mg/mL. These observations suggest that the methanolic extract of this plant contains compounds with anti-sickling activities, all of which justifies their use in the traditional treatment of sickle cell *SS*. Several authors have demonstrated the importance of tropical plants in the management of sickle cell disease[27–29]. The activity of these plants is linked to the presence of a wide variety of biologically active substances, including amino acids capable of reversing sickling[30–32]**.**At high concentrations (5 and 10 mg/mL), the extract is less active than the reference (Figures 2 and 3). Decreasing the concentrations (0.5 and 0.05 mg/mL), the methanolic extract becomes more active than the reference (Figures 4 and 5). The negative control shows no activity on the reversibility of the sickle cells because a maximum of sickle cells is observed at 120 minutes. It is also noted that the evolution curves of the residual sickle cells for the methanolic extract, and the reference are superimposed. This result may suggest the presence of phenylalanine or analogous compounds in the leaves of *Gliricidia sepium*. It could also be associated with the significant anti-sickling activity of the methanolic extract on erythrocytes. Indeed, phenylalanine is involved in inhibiting the polymerization of deoxygenated hemoglobin and reversing sickling. These results obtained in the study of the activity of the methanolic extract of the leaves of *Gliricidia sepium* confirm the results of the *BIOKENEYA* medical office regarding the use of this plant in the management of *SS* sickle cell disease.

IV. Conclusion

The results obtained on *Gliricidia Sepium*show a relatively weak antioxidant activity whereas its anti-sickle cell activity is greater than those of phenylalanine which is the reference. However, this activity is concentration dependent, and the maximum is obtained for extract concentration of 0.5 and 0.05 mg/mL. These observations suggest that the methanolic extract of this plant contains compounds with anti-sickling activities, all of which justifies their use in the traditional treatment of sickle cell disease *SS*. The results of the anti-sickle cell activity also reveal that for higher concentrations (5 and 10 mg/mL) of the methanolic extract of *Gliricidia sepium* leaves, the percentage of sickle cells decreases with time. This fact demonstrates the anti-falsemic power of the extract from the leaves of the plant because for the negative control, the number of sickle cells increases with time. These observations suggest that the methanolic extract of this plant contains compounds with anti-sickling activities, all of which justifies their use in the traditional treatment of sickle cell *SS*.

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Our great and profound gratitude goes to Gora SEYE, Director of *BIOKENEYA*, a traditional office medicine, who provided us with the sample of the plant, *Gliricidia sepium*, he uses to treat sickle cell disease. The encouraging results obtained by Gora SEYE led us to carry out this study which confirmed its results.

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