Research Article

Antisickling and Antihemolytic Mechanism of *Spirulina platensis* (*Oscillatoriaceae*): A Nutraceutical Commonly Used in Cameroon

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Sickle cell anaemia (SCA) is a widespread genetic disease in Africa, associated with chronic hemolytic anaemia and vaso-occlusive and infectious complications. The most commonly used means of management and treatment such as blood transfusions and allografting are expensive and predispose patients to the risk of infections. This research study aimed at evaluating the antisickling and antihemolytic activities of aqueous extracts of *Spirulina platensis* from Cameroon for optimising the management of this disease using natural substances. The *Spirulina platensis* harvested in Nomayos-Yaounde was dried, crushed, and macerated for 24 h in distilled water and the filtrate was freeze dried. The determination of the inhibition rates of falciformation induced by 2% sodium metabisulfite (MBS) and the sickling reversibility rate was carried out at different concentrations (100, 200, 400, 800, and 1600 ± μg·mL⁻¹) of the spirulina extract at different time intervals (2 h, 4 h, and 24 h). Aspirin, hypotonic solution, Triton X-100, and hydrogen peroxide were used as hemolytic inducers and the antihemolytic activity of the extract was studied at 800 ± μg·mL⁻¹ and 1600 ± μg·mL⁻¹ using the colorimetric method. The extraction yield was 14.015%. The maximum duration of induced falciformation was 2 h 30 min and the percentage of falciformation increased from 27.99 ± 3.15% (at the initial time) to 91.44 ± 3.70%, giving a falciformation induction rate of 69.3%. The falciformation inhibition rate after 2 h 30 min ranges from 15.10 ± 0.60% to 66.09 ± 4.69% for the concentration of 100 ± μg·mL⁻¹ to 1600 ± μg·mL⁻¹ of the spirulina extract. The rate of inhibition of falciformation was found to be dose-dependent. The best concentration of the extract was 1600 ± μg·mL⁻¹. The reversibility rate of falciformation at 800 ± μg·mL⁻¹ and 1600 ± μg·mL⁻¹ varied from 37.54 ± 6.35% to 82.34 ± 5.63% as a function of time. 1600 ± μg·mL⁻¹ was the most active concentration after 24 h. In addition, the extract improved the Fe²⁺/Fe³⁺ ratio with an increase in the rates of 69.78 ± 8.81 and 69.78 ± 13.82 at 800 ± μg·mL⁻¹ and 1600 ± μg·mL⁻¹, respectively. According to each inducer at 800 ± μg·mL⁻¹ and 1600 ± μg·mL⁻¹, respectively, of the spirulina extract, the following rates of inhibition of hemolysis were found: 53.03 ± 9.46% and 96.67 ± 5.77% (aspirin); 80 ± 8.66% and 71.25% (hypotonic solution); 36.56 ± 9.53% and 45.67 ± 22.55% (Triton X-100); 24.26 ± 9.55% and 36.76 ± 1.27% (hydrogen peroxide). At the end of this study, the best antickling activities were obtained at the concentrations 800 ± μg·mL⁻¹ and 1600 ± μg·mL⁻¹. It also has antihemolytic properties on various hemolysis inducers at concentrations 800 ± μg·mL⁻¹ and 1600 ± μg·mL⁻¹ with inhibition rates varying from 36% to 96%.
1. Introduction

Sickle cell disease (SCD) is a serious, inherited, autosomal recessive genetic disorder, in which red blood cells take on a sickle shape instead of their normal disc shape. This haemoglobinopathy is caused by the replacement of hydrophilic glutamic acid by hydrophobic valine at position six (6) of the β chain of hemoglobin. This substitution alters its affinity for oxygen and its solubility under conditions of low oxygen pressure. The result is a polymerization of hemoglobin S inside the red blood cells leading to its sickling [1]. These sickled cells acquire a rigid structure which makes their circulation difficult in the microvessels, causing vaso-occlusion and various complications of the disease [2], such as painful crises, increased susceptibility to infections, hyperhaemolysis causing anaemia, splenomegaly, and increased production of endogenous free radicals, mainly the 'OH radical which is very abundant in sickle cell patients [3].

Worldwide, this haemoglobinopathy affects more than 50 million people in the homozygous SS form and 250 million heterozygous AS carriers [4]. Sickle cell disease is at its highest prevalence in Sub-Saharan Africa and is now a real public health problem for most Black African countries. According to the World Sickle Cell Organization, out of 500,000 sickle cell children born each year, 400,000 are in Africa. In Central and West Africa, 20–40% of people are carriers of the sickle cell trait. In Cameroon, the heterozygous AS prevalence is estimated at 25–30% and 2% in its homozygous SS form [5]. Sickle cell disease remains very deadly; 50%–90% of children with sickle cell disease die before their 5th birthday if they are not supported [6].

In Cameroon, hospitals and healthcare centers are insufficient and an access to medical care is not easy for the rural population. The management of the disease takes into account the preventive aspects such as the folate supplementation, the prevention of infections, oral hydration with alkaline water, rest, putting on oxygen, keeping warm, prescription of analgesics, and blood transfusion when the hemoglobin level is low [7]. The curative component consists of bone marrow transplantation and genetic therapy [7]. However, the various drug managements for this disease which have been recommended to patients demonstrated many limitations both in terms of cost and risks related to incompatibility and drug toxicity problems.

As a result, patients from low- and middle-income countries prefer phytotherapy for the management of the disease. Niprisan® and Ciklavit® are a formulation which appeared to be safe and effective in reducing severe painful crises. They are reported to inhibit the polymerization of the hemoglobin S and possess antioxidant activities [8]. Several plant extracts have demonstrated antischickling properties through the inhibitory effect of hemoglobin S polymerization, the reversibility of sickling, or the increase of the Fe²⁺/Fe³⁺ ratio. These include the hydroethanolic extract of Ficus sycomorus leaves [9], aqueous extract of Hemodya [10], aqueous extracts of Zanthoxylum heitzii [11], hydroethanolic extract of Theobroma cacao [12], aqueous extracts of Phaseolus vulgarus L. [13] and Rubia cordifolia L. [14], leaves of Rhaphiostylis beninensis [15], and Woodfordia fruticosa (L.) Kurz leaves [16], among others. Other studies demonstrated that nutraceutical and nutrition supplement have beneficial effects on the management of sickle cell diseases [17, 18]. The beneficial effects of these nutraceutical and nutrition in the management of sickle cell disease are attributed both to their higher levels of micro-nutrients (vitamins and minerals) and bioactive molecules (phenolic compounds and amino acids) composition, as well as the antiradical and antioxidant properties of these bioactive compounds [13–18].

Based on a systematic review on Spirulina platensis, it is a blue-green alga of the cyanobacteria family, endowed with nutritional and therapeutic properties [19]. The aqueous extract of Spirulina platensis from Cameroon is rich in macronutrients, micronutrients, and bioactive phytochemicals including polyphenols, flavonoids, beta carotene, phycoerythrin, phenolic acids, and calcium spirulan endowed with antioxidant activities [20]. In addition, a recent in vitro study on sickle cell patients reveals that S. platensis supplementation reduces a number of seizures, blood transfusions, and hospitalizations in children with sickle cell disease [21]. However, no study has highlighted in vivo on the antischickling and antihemolytic mechanism of the S. platensis aqueous extract on the red blood cells of sickle cell patients. In order to contribute to the improvement of the management of the disease and demonstrate the in vitro antischickling and antihemolytic activities of S. platensis, we decided to explore the antischickling and antihemolytic properties of the aqueous extract of S. platensis.

2. Materials and Methods

2.1. Types of Study, Blood Sample Collection, and Ethical Consideration. The experimental study was performed after an ethical clearance issued by the Regional Committee for Ethics Research for Human Health Center (clearance N° 676./CRERSH/2019) and the patient’s written consent was obtained; 27 patients’ confirmed sickle cell blood samples were collected including 10 women and 17 men between the ages of 10 and 40 who had been attending routine consultations at the Hemato-Oncology department of the Yaoundé Central Hospital. The patients who were in a state of crisis and those who have been transfused during the last three (03) months preceding the study were excluded. The samples were taken from the patients’ elbow crease by healthcare personnel and about 5 mL of blood was collected in EDTA tubes.

2.2. Preparation of the Extract of S. platensis. The green-blue algae (S. platensis) were collected in Nomayos in Yaoundé, the capital city of Cameroon. It was oven dried at temperatures between 37 and 45°C for three days. Fifty grams (50 g) of S. platensis powder were soaked in 1000 mL of distilled water, homogenized for 1 h and kept at room temperature (25°C) for 24 h before filtration with Whatman paper no. 4. The residues were reextracted in the same condition. The total filtrate was lyophilized and the extraction yield was calculated according to the following formula:
cells was calculated from the following formula:

\[ R = \left( \frac{\text{mass of the crude extract obtained}}{\text{mass of initial powder}} \right) \times 100. \]  

The powder of *S. platensis* obtained was then stored in a refrigerator protected from light for further assays.

### 2.3. Preparation of the Erythrocyte or Hematocrit Suspension.

The blood sample collected in EDTA tubes was centrifuged at 3000 rpm for 5 min, and the pellet recovered was washed 3 times with iso-saline PBS (10 mM potassium phosphate buffer, pH 7.4; 154 mM of NaCl) solution. Each step of washing consisted of suspension of the red blood cells (RBCs) into iso-saline phosphate buffer (PBS) followed by centrifugation (3000 rpm; 5 min). After the last centrifugation, the pellet was again suspended in a solution of iso-saline PBS to make a 10% hematocrit.

### 2.4. Determination of the Antifalcemic Activity of the Aqueous Extract of *S. platensis*: In Vitro Induction of Sickling.

One hundred (100 μL) microlitres of blood hematocrits were mixed with 100 μL of 2% sodium metabisulphite solution (final concentration) and then incubated at room temperature (25°C). 10 μL of this mixture was diluted in 1990 μL of Marciano liquid and the cells were counted under light microscope (40X) using the Malassez cells. Phenylalanine was used as a positive control at the same concentrations as the extract or phenylalanine [23].

#### 2.4.1. Determination of the Inhibitory Activity of the Aqueous Extract of *S. platensis* on Sickling.

In the tubes, containing 100 μL of the extract at different concentrations, 50 μL of SS blood and 50 μL of a 2% sodium metabisulphite (2%, W/V) final solution’s concentration were added. Then, they were incubated at room temperature (25°C) for 2 h 30 min. Total red blood cells and sickle cells were counted under a light microscope (40X) using the Malassez cells. Phenylalanine was used as a positive control at the same concentrations as the extracts. The inhibition rate (INH) was calculated by the following formula:

\[ \text{INH} = \left( \frac{f_0 - f_n}{f_0} \right) \times 100, \]  

where \( f_0 \) is the percentage of maximum sickling in the presence of HbS + 2% MBS blood and \( f_n \) is the minimum sickling percentage in the presence of HbS + 2% MBS + extract blood [23].

#### 2.4.2. Determination of the Effect of Extracts of *S. platensis* on the Reversibility of Sickling Cells.

The effect of the extract on the reversibility of sickling was achieved by incubating 50 μL of blood at room temperature (25°C) with 50 μL of the extract at different concentrations for 2 h, 4 h, and 24 h. Percent sickling was calculated before and after incubations. The percentage of sickling was then determined as mentioned above. The sickling reversibility rates (% R) are calculated according to the following formula:

\[ (%R) = \frac{f_0 - f_n}{f_0} \times 100, \]  

where \( f_0 \) is the percentage of initial sickling and \( f_n \) is the percentage of minimum sickling obtained in the presence of the extract or phenylalanine [23].

The concentrations of the extract which showed the best antisickling activities (800 and 1600 μg·mL\(^{-1}\)) were used to investigate the *in vitro* Fe\(^{2+}/\)Fe\(^{3+}\) ratio and the antihemolytic activity of the aqueous extract of spirulina.

#### 2.4.3. Evaluation of the Effect of Extracts of *S. platensis* on the Fe\(^{2+}/\)Fe\(^{3+}\) Ratio of Hemoglobin S.

In the presence of distilled water, the red blood cells undergo considerable osmotic pressure. The water diffuses into the interior of the cell through the membrane and causes hemolysis. Oxyhemoglobin and methemoglobin, constituting the hemolysate, absorb at 540 nm and 630 nm, respectively. The method was described by the authors in [20] and reported by the authors in [10].

The percentages of oxyhemoglobin (Fe\(^{2+}\)) and methemoglobin (Fe\(^{3+}\)) in each tube were calculated using the following formulas:

\[ \text{Fe}^{2+} = \frac{[\text{DOFe}^{2+} - \text{DOFe}^{3+}]}{\text{DOFe}^{2+}} \times 100; \quad \text{Fe}^{3+} \]  

\[ = 100 - \% \text{Fe}^{2+}. \]

The ratio \( R \) of oxyhemoglobin (Fe\(^{2+}\)) to methemoglobin (Fe\(^{3+}\)) was calculated as follows: \( R = \% \text{Fe}^{2+}/\% \text{Fe}^{3+}. \) The rate of increase (TA) of the Fe\(^{2+}/\)Fe\(^{3+}\) ratio was calculated by the following relationship:

\[ \text{TA} = \frac{R_{\text{essay}} - R_{\text{control}}}{R_{\text{essay}}} \times 100. \]  

### 2.5. In Vitro Antihemolytic Property of *S. platensis*. The antihemolytic effect of the aqueous extract of *S. platensis* was achieved at 800 and 1600 μg·mL\(^{-1}\), which showed better antisickling activities. Four models of hemolytic inducers were used including the hypotonic medium, salicylic acid, Triton X-100, and H\(_2\)O\(_2\) in the absence of the extract to determine the concentration of the inducer which provided the maximum hemolysis.

#### 2.5.1. Hemolytic Activity Induction of Aspirin and the Antihemolytic Effect of *S. platensis*. To 4.5 mL of the solution of NaCl (4.5 mg·mL\(^{-1}\)), 50 μL of aspirin at concentrations 0 and 0.7 mg·mL\(^{-1}\) was added. The control tube received the
same volume of PBS and then 500 μL of the erythrocyte suspension was added to each tube, homogenized and incubated at 37°C for 30 min. After centrifugation (3000 rpm; 5 min), the absorbance of the supernatant was measured at 540 nm [24].

To determine the effect of the *Spirulina platensis* extract on hemolysis induced by aspirin, five hundred microlitres of the aqueous extract of *S. platensis* at different concentrations (800 and 1600 μg·mL⁻¹) were added to 250 μL of hematocrit (10%) and incubated for 5 min at room temperature and then 25 μL of aspirin (0.7 mg·mL⁻¹) and 1750 μL of NaCl (0.45%) were added. The mixture was homogenized, incubated for 1 h at 37°C, and centrifuged (3000 rpm, 5 min). The optical density of the supernatants was read at 540 nm against the blank and the standard (quercetin) [25].

2.5.2. Hemolytic Activity Induction of Hypotonic NaCl and the Antihemolytic Effect of *S. platensis*. In order to determine the concentration of NaCl which leads to a maximum lysis of red blood cells, 100 μL of hematocrit (10%) was added to 5 mL of NaCl at different concentrations (3.5–8.5 mg·mL⁻¹), to a negative control (isotonic NaCl 9 mg·mL⁻¹), and to a positive control (distilled water). After 30 min of incubation at room temperature (25°C), the mixture was centrifuged (3000 rpm, 10 min) and the supernatant was read at an absorbance of 540 nm [26].

Fifty (50) microlitres of the extract at different concentrations (800 and 1600 μg·mL⁻¹) were mixed with 50 μL of hematocrit (10%) after 10 min incubation at room temperature and 2.5 mL of NaCl (0.35%) was added. The mixture was incubated at 37°C for 30 min and then centrifuged (3000 rpm, 10 min). The absorbance of the supernatant was read at 540 nm. For the control, the extract was replaced by the same volume of NaCl (0.35%) and a standard (quercetin) [26].

2.5.3. Hemolytic Activity Induction of Triton X-100 and the Antihemolytic Effect of *S. platensis*. Eight hundred microlitres of Triton X-100 at different concentrations (0.001%, 0.03%, 0.04%, 0.05%, 0.1%, and 1%) were mixed with 2.2 mL of PBS (0.2 M, pH = 7.4 containing 0.9% NaCl) and 500 μL hematocrit (10%). The mixture was incubated for 1 h at 37°C and centrifuged (3000 rpm, 5 min). The absorbance of the supernatant was measured at 540 nm. The control was prepared in the same manner with the absence of Triton X-100 [23].

A volume of 500 μL of extracts (800 and 1600 μg·mL⁻¹) was added to 250 μL of hematocrit (10%), the mixture was incubated for 5 min at room temperature, and then 400 μL of the Triton X-100 (1%) was added. After incubating for one hour at 37°C followed by centrifugation (3000 rpm; 10 min), the absorbance of the supernatant was read at 540 nm. For the control prepared, the extract was replaced by the same volume in PBS and a standard (quercetin) [27].

2.5.4. Hemolytic Activity Induction of Hydrogen Peroxide H₂O₂ and the Antihemolytic Effect of *S. platensis*. 500 μL of H₂O₂ at concentrations of 9%, 6%, 3%, 1%, 0.5%, 0.3%, and 0.2% were mixed with 250 μL of hematocrit (10%). After 3 h of incubation at 37°C, this solution was adjusted with 4.500 mL of PBS and centrifuged (3000 rpm, 10 min). The absorbance of the supernatants was read at 540 nm. Controls were prepared by replacing H₂O₂ with distilled water for the positive control and PBS for the negative control [28]. The percent hemolysis for all tests was calculated by applying the following formula [29]:

\[
\% \text{ hemolysis} = \frac{\text{Abs du test} - \text{Abs of control}}{\text{Abs du test}} \times 100
\]

where Abs = absorbance.

To determine the effect of *S. spirulina* on the inhibition activity of H₂O₂, 250 μL of hematocrit (10%) and 500 μL of the extract at different concentrations (800 and 1600 μg·mL⁻¹) were incubated for 30 min at room temperature followed by 500 μL of H₂O₂ (9%). After incubating for 3 h at 37°C, the final volume was adjusted to 4500 μL by PBS and centrifuged (3000 rpm; 10 min), and the optical density was read at 540 nm. The standard was carried out in the same manner using the quercetin as a reference molecule [28].

2.6. Statistical Analysis. Results are presented as the mean ± standard deviation. All the tests were carried out by group of quadruplets. Distribution test was checked before carrying out analysis. All the data were analyzed using one-way ANOVA followed by Dunnett’s post hoc test for multiple comparisons with the software SPSS version 21.0.

3. Results

3.1. In Vitro Induction of Sickling with Sodium Metabisulphite (MBS) 2% and Antisickling Properties of the Extract of *S. platensis*. Figure 1(a) shows the kinetic induction of falcification using 2% metabisulphite. It appears that SS blood added to 2% MBS (W/V) induced an increase in the percentage sickling of red blood cells at a time-dependent manner up to a maximum of 91, 38% after 2 h 30 min (Figure 1(a)). The treatment of homozygote SS red blood cells with both aqueous extract of *S. platensis* and phenylalanine after induction with metabisulphite (2%) showed a significant and linear reduction of sickling cells (Figure 1(b)). There was no significant difference in the reduction of falcification between phenylalanine and the extract of *S. platensis* (Figure 1(b)). However, there were no similarities on the morphology of red blood cells between the negative control (blood cell induced with MBS 2%) and those induced with MBS 2% and treated with *S. platensis* or phenylamine (Figure 2).

3.2. Reversibility of Sickling and the Fe²⁺/Fe³⁺ Ratio of the Aqueous Extract at Different Concentrations. We determined the effect of incubation time and extract concentration of *S. platensis* on the reversibility of sickling. The results showed that the reversibility of sickling increases with a time- and concentration-dependent manner of the aqueous extract of *S. platensis* (Figure 3). These results showed that the maximum reversibility (39.75%) of sickling...
is reached at a concentration of 1600 μg·mL$^{-1}$ after 2 hours (Figure 3(a)). It increased to 60% for the same concentration after 4 h (Figure 3(b)) and then to 84% after 24 h for the same concentration of S. platensis (Figure 3(c)). The results showed that there is no significant difference between the effect of the S. platensis extract and phenylalanine on the reversibility of sickling during all these times (Figures 3(a)–3(c)). The kinetics of the rate of reversibility as a function of time at 800 μg·mL$^{-1}$ and 1600 μg·mL$^{-1}$ of the extract of S platensis (Figures 4 and 5) showed a higher reversibility of sickling with a rate of 82% after 24 h (Figures 4(a) and 4(b)). Similarly, it emerges from our study that at the concentrations 800 μg·mL$^{-1}$ and 1600 μg·mL$^{-1}$, extracts of Spirulina platensis and quercetin significantly increase the Fe$^{2+}$/Fe$^{3+}$ ratio, with significant growth rates ($p < 0.05$) found at 800 μg·mL$^{-1}$ (Figure 4(c)).
We noted a change of morphology of red blood cells and the reduction of number of sickle red blood cells (Figures 5(a)–5(b)).

3.3. In Vitro Antihemolytic Property of S. platensis. Many studies have shown that the plasma membrane of human red blood cells of sickle cell patients was confronted with different molecules inducing a variable stability of the latter depending on the environment in which they are found [26]. Thus, we have determined the antihemolytic properties of the aqueous extract of *S. platensis* subjected to different mechanisms of hemolysis. The results obtained (Figure 6(a)) show maximum and significant *(p < 0.05)* hemolysis induction with salicylic acid of 83.94 ± 4.05% at the concentration of 0.7 mg·mL⁻¹ of salicylic acid compared to the negative control. After the treatment of hemolytic induction with the aqueous extract of *S. platensis* at the concentrations 800 µg·mL⁻¹ and 1600 µg·mL⁻¹, we noted that the inhibition of the hemolytic activity significantly increased *(p = 0.0001)*, respectively, from 53 to 96% with the concentration of *S. platensis*, while the percentage of inhibition of hemolysis decreased from 76 to 53% with quercetin (Figure 6(b)).

Significant variation of sodium chloride homeostasis can induce hemolysis of the red blood cell membrane. The maximum hemolysis noted (78.05%) with the hypotonic NaCl solution was found at 0.35% (Figure 7(a)). After treatment with *S. platensis*, we noted that at 800 µg·mL⁻¹, we found a higher inhibitory activity (80%) compared to 71% of inhibition at 1600 µg·mL⁻¹. The results demonstrated that *S. platensis* induced higher inhibition activity at lower concentration. Similar tendency was noted with quercetin (Figure 7(b)).

Hydrogen peroxide is a product of oxidative stress produced in the mitochondria by NAPDH oxidase. The level of hydrogen peroxide increased in the condition of anoxia. The effect of the hydrogen peroxide concentration on hemolysis of membrane mitochondria was explored. Our results demonstrated that a maximum membrane hemolysis of red blood cells (82.21%) could be induced by hydrogen peroxide at the concentration of 0.875% (Figure 8(a)). After treatment with the extract of *S. platensis*, we found an increase in the inhibition percentage from both *S. platensis* and quercetin as the concentration is increasing (Figure 8(b)). None of these inhibitions of hemolysis activities went beyond 50%.

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**Figure 3**: Reversibility of sickling cells at different concentrations of *S. platensis* and different times of incubation: (a) 2 h, (b) 4 h, and (c) 24 h.
Triton X-100 is a commonly used detergent and it is widely used to lyse cells to extract protein or organelles or to permeabilize the membranes of living cells. We studied the effect of *S. platensis* on the inhibition of hemolysis with Triton X-100. The results showed that at the concentration of 1%, the Triton X-100 induces a total hemolysis of red blood cells of sickle cell patients (Figure 9(a)). The treatment of Triton X-100 hemolysis with *S. platensis* or quercetin at the concentration of 800 $\mu$g·mL$^{-1}$ and 1600 $\mu$g·mL$^{-1}$ significantly and gradually reduced hemolysis up to 49–65% for quercetin and 38–46% for *S. platensis* (Figure 9(b)).

![Figure 4: Reversibility rates of sickling cells incubated at different times and the Fe$^{2+}$/Fe$^{3+}$ ratio of *S. platensis*: (a) 800 $\mu$g·mL$^{-1}$. (b) 1600 $\mu$g·mL$^{-1}$. (c) Fe$^{2+}$/Fe$^{3+}$.
](image)

**4. Discussion**

Research investigation of antisickling properties of medicinal plants and nutraceuticals has increased in recent years. The alternative management of the disease using phyto-medicines has proven to not only reduce crisis but also reverse sickling cells, stabilize the membrane of the red blood cells, and avoid its hemolysis. Antisickling properties of several medicinal plants from Cameroon and Africa have been documented [11, 13, 23, 30–34].

Extraction is an essential step in the process of the evaluation of the biological activity of natural substance. Many parameters can significantly affect the yield of extraction of molecules from natural products. The yield obtained in our study was 14.015%. This value is lower than that obtained by the authors in [20], which, upon aqueous extraction of *S. platensis*, obtained a yield of 16.84%. This variation could be explained by the difference in the level of solubility of solvents used, lyophilization process, degrees of purity, time of agitation, or also the type of filtration which affects the nature of the molecules present in the solution [35, 36].

Moreover, the aqueous extract of *S. platensis* used in this study was previously used for the determination of nutrients, trace elements, carotenoids, phycocyanin, and phytochemical composition using standard methods. The results showed that *S. platensis* contains protein (375.5 + 0.7 g/kg-dw), lipids (301.2 + 11.9 g/kg-dw), carbohydrates (243.9 + 9.9 g/kg-dw), and fibers (313.2 g/kg-dw). The HPLC profile revealed the presence of polyphenols (21.2 + 1.18 mg eq. QE/g Ext.), flavonoids (56.4 + 6.47 mg eq. QE/g Ext.), and phenolic acid such as caffeic and coumaric acids. Iron was the micro-nutrient found in majority but copper, manganese, zinc, and...
selenium were also found. The percentage of phycocyanin was 16.15% while carotenoids were 3.8% [20].

Aqueous extracts of several phytomedicines have demonstrated significant \textit{in vitro} antisickling activity. The antisickling action mechanism of the medicinal plants \textit{in vitro} included inhibition of sickling, reversibility of falcification, increase of the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio, and osmotic fragility of membrane of red blood cells, as well as the

\textbf{Figure 5:} Images of reversibility of red blood cells after 24 hours of incubation treated with phenylalanine or the extract of \textit{S. platensis} at concentrations 800 $\mu$g·mL$^{-1}$ and 1600 $\mu$g·mL$^{-1}$. (a) 800 $\mu$g·mL$^{-1}$ of the extract of \textit{S. platensis}. (b) 1600 $\mu$g·mL$^{-1}$ of the extract of \textit{S. platensis}. (c) 800 $\mu$g·mL$^{-1}$ of phenylalanine. (d) 1600 $\mu$g·mL$^{-1}$ of phenylalanine.

\textbf{Figure 6:} Hypotonic hemolysis with salicylic acid: (a) induction of hemolysis and (b) inhibition of hemolysis with \textit{S. platensis} or quercetin.
antioxidant activity. Among these, medicinal plants included herbal drugs such as Hemodya and niprisan (renamed Nicosan) with *Piper guineense*, *Pterocapus osun*, *Eugenia caryophyllum*, and *Sorghum bicolor* as components; Ciklavit (*Cajanus cajan* seed extract as base), aqueous extracts of *Zanthoxylum zanthoxyloides* roots, Ajawaron HF complex with *Cissus populnea* as the main component; aqueous and alcoholic extracts of *Terminalia catappa* leaves; and *Carica papaya* unripe fruit and dried leaf extracts [8, 10].

Our study demonstrated that the aqueous extract of *S. platensis* inhibited the sickling cell significantly, in a dose-dependent concentration of up to 66.09% at 800 μg·mL⁻¹. It acts positively on the reversibility of sickling cells in a dose-dependent manner and increases the Fe²⁺/Fe³⁺ ratio significantly. Several studies showed the correlation between the antisickling activity of natural products and its chemical composition. The aqueous extract of *S. platensis* possesses a higher level of amino acids like phenylalanine which has been reported to demonstrate significant antisickling property [37, 38]. Active constituents of medicinal plants and naturally occurring compounds, known as antisickling agents, are rich in aromatic amino acids, phenolic compounds, and antioxidant nutrients which are thought to be responsible for their observed antisickling action [39]. Studies indicated that *in vivo* vitamin and mineral supplements such as vitamins C and E, zinc, and magnesium or treatment with a combination of high dose antioxidants can reduce the percentage of irreversibly sickled cells [40, 41]. Phenylalanine, tyrosine, and arginine exhibited the antisickling properties which are attributed to their activity of inhibiting the polymerization of hemoglobin S and improving the Fe²⁺/Fe³⁺ ratio [42–44]. The Fe²⁺/Fe³⁺ ratio was

![Figure 7](image7.png)

**Figure 7:** Hypotonic hemolysis of NaCl: (a) induction of hemolysis and (b) inhibition of hemolysis with *S. platensis* or quercetin.

![Figure 8](image8.png)

**Figure 8:** Hypotonic hemolysis of H₂O₂: (a) induction of hemolysis and (b) inhibition of hemolysis with *S. platensis* or quercetin.
used to assess an increase or decrease in the affinity of hemoglobin for oxygen. This increase of the Fe$^{2+}$/Fe$^{3+}$ ratio found in our study due to the presence of *S. platensis* implies the conversion of deoxyhemoglobin S into oxyhemoglobin S by the extract and therefore promotes antisickling activity. The higher level of the Fe$^{2+}$/Fe$^{3+}$ ratio can be explained by the presence of a higher concentration of micronutrients such as selenium, zinc, copper, vitamins E and A, and other bioactive molecules (polyphenols, flavonoids, and carotenoids) present in the *Spirulina platensis* aqueous extract [20, 45]. Moreover, the involvement of these bioactive amino acids and their aromatic groups in the inhibition and reversibility of sickling has been demonstrated in some studies [13, 46]. Thus, the antisickling activity of the extract of *S. platensis* on the mechanism of the antisickling activity of red blood cells can be attributed to the presence of phenolic compounds and aromatic amino acids such as tyrosine, phenylalanine, and arginine found in a large concentration in *S. platensis* [20, 47].

In our study, we found a close result between the activity of the crude extract of *S. platensis* and that of phenylalanine (standard) but with a significantly higher activity of the spirulina extract. This could be explained by the fact that the molecules found in the extract act in a synergistic manner either in the inhibition of the polymerization hemoglobin S, the improvement of the Fe$^{2+}$/Fe$^{3+}$ ratio, the conversion of methemoglobin into oxyhemoglobin, or the stabilization of the existing oxyhemoglobin form, thus increasing their affinity to oxygen and the reversibility of the sickling cells. This corroborates the overlap of curves observed in the reversibility activity we obtained.

Several substances can be used to induce *in vitro* hemolysis of red blood cells using different mechanisms including salicylic acid, Triton X, H$_2$O$_2$, and hypotonic NaCl. The movement of fluids in the red blood cell which leads to hemolysis implies osmosis and tonicity of the cell membrane. Hypotonic solutions lead to cell swelling and eventual rupture or lysis if the resultant osmotic movement of water is great enough. In the case of red blood cells, this is referred to as hemolysis.

The most abundant cells in the human body are the erythrocytes, which can have several biological and morphological characteristics depending on the physiological conditions. These cells have been widely exploited in drug transport, gas, and other useful molecules in biological fluids. The polyunsaturated fatty acids (PUFA) and hemoglobin molecules which are redox active oxygen transport molecules and potent promoters of activated oxygen species mainly target the erythrocytes [48] Oxidative mutation of the erythrocyte membrane lipids and proteins may be responsible for hemolysis accompanied with several factors of hemoglobinopathies, oxidative drugs, excess transition metals, various radiations, and deficiencies in erythrocyte antioxidant coordination [48–50]. The magnitude of hemolysis appeared to be much more overwhelming, when red blood cells were exposed to any toxicant like hydrogen peroxide [48]. These high rates of hemolysis, even at low concentrations of hydrogen peroxide, are thought to be due to the fact that hydrogen peroxide causes oxidative damage to the cytoplasmic membrane following the lipid peroxidation of the polyunsaturated fatty acids present [51]. Indeed, when H$_2$O$_2$ crosses the plasma membrane, it can cause the degradation of the heme of hemoglobin by the oxidation of Fe$^{2+}$ ions to Fe$^{3+}$, with the production of hydroxyl radicals (HO$^\cdot$) which are very unstable and highly reactive by the Fenton reaction. These radicals induce a chain of lipid peroxidation leading to lysis of red blood cells [52]. The *Spirulina platensis* extract has been shown to protect against hemolysis induced by hydrogen peroxide. In addition,
several studies have shown that some of the phenolic compounds, in particular the flavonoids, have antifree radical properties by neutralizing or scavenging free radicals [53]. Moreover, polyphenols are known to chelate transition metals such as Fe$^{2+}$, thus reducing the rate of the Fenton reaction by electron transfer. They can also prevent oxidation caused by the hydroxyl radical [54] and prevent the passage of H$_2$O$_2$ through the erythrocyte membrane and the generation of free radicals [55].

The results of hemolysis induced by Triton X-100 at different concentrations showed significant hemolysis of Triton X-100 at the concentrations used. At 1% of Triton X-100 concentration, there is a maximum and significant hemolysis (100%). This result corroborates with that of [56], who obtained 100% hemolysis at the 1 Mm concentration of Triton X-100. The total hemolysis may be due to the chemical nature of Triton X-100. Indeed, Triton X-100 has the ability to disrupt the membrane of red blood cells or cells in general. Triton X-100 is a nonionic synthetic detergent which consists of a hydrophilic polar part and a hydrophobic tail. This molecule interacts with the hydrophobic parts of the lipids on the erythrocyte membrane until saturation and then causes the disruption of the membrane. At very high concentrations of Triton X-100, the red blood cells will be completely solubilized in the form of micelles or liposomes [27]. However, in our study, the antihemolytic properties of the aqueous extract of S. platensis show a significant hemolysis inhibitory activity. The mechanism of its inhibitory effects of the S. platensis extract includes the ability to interact with the external polar poles of the lipid bilayer on the membrane red blood cells and prevent its solubilization and therefore prevent hemolysis. The aqueous extract of S. platensis has shown the protective effects of the erythrocyte membrane in the presence of several agents inducing hemolysis. Our results corroborate with those of [13], who obtained 80% hypotonic hemolysis in the absence of the extract. This is justified by the fact that in the isotonic medium, the extracellular concentrations of electrolytes are almost equal to its intracellular. The antihemolytic effect of the S platensis aqueous extract on hemolysis induced by hypotonic solution could be attributed to its richness in mineral salts [20], which establishes a balance of concentration and osmotic pressure through its ability to bind to aquaporin and prevent water from entering the red blood cell [26]. This higher antihemolytic activity is also due to the presence of antioxidants and antifree radical molecules in S. platensis, mainly its richness in polyphenols [57], flavonoids, phycocyanin, carotenoid, and antioxidant vitamins and minerals (vitamin E, selenium, and zinc) [20] which would protect the membrane of red blood cells from lipid peroxidation [58, 59].

5. Conclusion

The aqueous extract of S. platensis from Nomayos in Cameroon showed antisickling properties and allows the reversibility of sickling cells in a dose-dependent manner. It presented an antihemolytic property at the concentrations 800 μg·mL$^{-1}$ and 1600 μg·mL$^{-1}$ on various hemolysis inducers. In the future, the investigation of the antisickling mechanism of the different fractions of the aqueous extract of S. platensis will be made in order to identify the fraction responsible for the antisickling effect observed in this study.

Data Availability

The data used to support the findings of this study are included within the article.

Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Committee for Ethics Research for Human Health Center (N°676./CRERSH/12/04/2019).

Consent

Informed consent was obtained from all subjects who participated in the study and their parents or guardian.

Conflicts of Interest

The authors declare there are no conflicts of interest.

Authors’ Contributions

Pieme Constant Anatole conceptualized and supervised the study and was responsible for project administration; Nkwikeu Nya Josela Prudence was responsible for the methodology; Teguem Tchoulegheu Apollinaire and Nkenmeni Djamnou Célestin were responsible for software; Pieme Constant Anatole, Teguem Tchoulegheu Apollinaire, Chetcha Chemegni Bernard, and Nkwikeu Nya Josela Prudence validated the study; Yembeau Natacha Lena and Feudjio Alfoditte Flore were responsible for statistical analysis; Teguem Tchoulegheu Apollinaire was responsible for investigation and writing the original draft; Biapa Nya Prosper Cabral and Pieme Constant Anatole reviewed and edited the manuscript; and Choupo Arnaud Cyrille visualized the study. All the authors have read and agreed to the published version of the manuscript.

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References

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