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Evaluation of the Anti-Sickling Cell and Cytotoxic Activities of Polar Extracts of *Terminalia avicennioides Root Bark Guill*. & *Perr*. (Combretaceae)

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Abstract

Background: Sickle-cell anemia is a hereditary, disabling disease that remains a public health concern in many countries, with the prevalence of sickle-cell trait in Africa ranging from 10 to 40% depending on the region. This study aims to evaluate the effects of *Terminalia avicennioides* root bark extracts on several parameters involved in sickle cell disease. **Method**: Anti-radical activity was assessed *in vitro* using the ABTS radical cation method with spectrophotometer analysis. Blood from individual with sickle cell disease (SS) was used to assess anti-sickling activity by the Emmel test, while normal blood was used to assess antihemolytic and cytotoxic activity respectively. **Results:** The results showed that **CE** had the highest anti-free radical activity with an IC_{50} = 0.068 mg/mL while **AqF** with an IC_{50} of 0.074 mg/mL had an activity close to that of vitamin C (IC_{50} = 0.070 mg/mL), used as a reference. However, **EAF** showed the best anti-sickling activity with 38.68% and 13% of residual sickle cells for 2.5 mg/mL and 20 mg/mL respectively, whereas for the negative control, 85% of sickle cells were counted. Two extracts, **CE** and **AqF**, exhibited mor interesting anti-hemolytic activity than quercetin, with IC_{50} values of 0.078 mg/mL and 0.082 mg/mL respectively, compared with 0.082 mg/mL for quercetin. Cytotoxicity results showed that all extracts induced leukocyte cell proliferation. The most active crude extract had a CP_{50} = 1.649, while arginine had a CP_{50} =0.005 at 48 h incubation. **Conclusion**: The bioactive polar compounds contained in *Terminalia avicennioides* root powder extracts and fractions make a significant contribution to enhancing the activities assessed. The results obtained show that the extracts present much more interesting activities on certain parameters than the positive controls, thus confirming the traditional use of this plant in the management of sickle cell disease.

Keywords

Sickle Cell Disease, Cytotoxicity, *Terminalia avicennioides*, Antiradical, Antihemolytic

1. Introduction

Sickle cell disease is a persistent, non-contagious congenital blood disorder. It encompasses a group of clinical syndromes that affect hemoglobin due to an abnormal hemoglobin genetic code. This abnormal hemoglobin, which causes deformation of the red blood cells, is inherited by children from their parents [1]. The main cause of sickle cell disease is a mutation in the gene responsible for hemoglobin production, known as the beta-globin gene (HBB). This point mutation occurs in the sixth codon of the β globin gene on chromosome 11. It results in the replacement of glutamic acid by valine, producing an abnormal hemoglobin called hemoglobin S (HbS), which differs in structure from normal adult hemoglobin (HbA) [2]. This causes HbS to become stiff and sticky, resulting in deformation and a reduced ability to circulate in small blood vessels [3]. This sickle-shaped deformation of the red blood cell, known as falciformation, is the cause of several complications.

The clinical manifestations of sickle cell disease are diverse, and can vary in severity from one individual to another. The characteristic symptom is recurrent episodes of intense pain, called vaso-occlusive crises, which result from the obstruction of blood flow in small vessels by sickle-shaped red blood cells. Fatigue, anemia and increased susceptibility to infection are also common symptoms of sickle cell disease [4]. Renal, ocular, splenic sequestration and osteonecrosis complications have also been described in this disease [5].

Sickle cell disease is the most widespread genetic disorder in the world, with 7.74 million people affected [6]. The African continent bears the heaviest burden, with over 90% of deaths in children under 5 [7]. According to recent data published by Galadani *et al.*, approximately 300,000 children are born with sickle cell disease worldwide every year [8]. Nearly 100,000 and 14,000 people suffer from the disease in the United States and the United Kingdom respectively [9] [10]. In Africa, sickle cell disease is a major public health problem, with the prevalence of sickle cell trait ranging from 10 to 40% depending on the region [11]. In the general population, for example, it is over 40% in DR Congo and 15% in Senegal [12].

Several strategies have been put in place to manage sickle cell disease patients, including advice on lifestyle, blood transfusion, allografts, engineering therapy and chemotherapy [13] [14].

Regarding chemotherapy, hydroxyurea has been the drug of choice approved since 1998 by the US FDA (Food and Drug Administration) for adults and recently in 2017 for children with sickle cell disease [15]. Hydroxyurea acts, among other things, to increase fetal hemoglobin (HbF) production, stop hemoglobin falciformation, reduce the frequency of painful events and the need for blood transfusion in children under 5 years of age [16]. Three other drug treatments for sickle cell disease have recently been approved by the US FDA. They are: **L-glutamine** (approved in 2017), required for the synthesis of glutathione, nicotinamide adenine dinucleotide and arginine. It helps to reduce the frequency of painful attacks and may act as an antioxidant compound [17]. **Crizanlizumab** (approved in 2019), this drug, administered by injection, can help reduce the frequency of painful attacks in adults and children over 16 by preventing blood cells from adhering to the inner walls of blood vessels [18]. **Voxelotor** (approved in 2019), is a first-class allosteric modifier of HbS, increasing its affinity for oxygen. This drug is used to treat sickle cell disease in adults and children over the age of 12. Taken orally, it can reduce the risk of anemia and improve blood circulation throughout the body [19]. Other plant-based treatments, known as phytomedicines, which have obtained marketing authorization in certain other countries, are widely used in the management of sickle cell disease patients. These include Niprisan, Ciclavit and Fagara [6] [20] [21]. Traditional medicine, which essentially uses plants to treat illnesses, is becoming increasingly attractive, not only because of its accessibility but also because of the lower cost of treatment. It is also a major source of new drugs. Today, according to some sources, 50% of the small molecules placed on the market for the treatment of cancers, and the most effective drugs for curing influenza or malaria, are still extracted or derived from plants [22].

It is in this context that we propose to explore the biological properties of the *Terminalia avicennioides* plant in the management of sickle cell disease.

Terminalia avicennioides Guill & Perr (Combretaceae) grows as a 7 - 8 m shrub, often branched at the base, in the savannahs of Africa. In Senegal, it is irregularly disseminated in the various wooded savannahs from Casamance to the Senegal River. It is traditionally used to treat a number of pathologies. Scientific studies have demonstrated *Terminalia avicennioides*' antibacterial, antifungal, antileishmanial, antidiarrheal and antidiabetic properties [23]-[26].

The aim of this article is to investigate the anti-oxidant, anti-sickling, antihemolytic and cytotoxic properties of different polar extracts of *Terminalia avicennioides* root in order to verify the relevance of its traditional use in the management of sickle cell disease in Senegal.

2. Materials and Methods

2.1. Plant Material

Terminalia avicennioides roots were harvested in the village of Keur Serigne Diabel in the department of Koungheul (Senegal). After identification by the botanical laboratory of Medicine, Pharmacy and Odontology at the Cheikh Anta Diop university in Dakar, the bark fibers detached from the root are dried in the dark at room temperature for 10 days. The bark is then cut into small pieces using pruning shears, and pulverized using a grinder. The resulting powder is stored at room temperature until use.

2.2. Biological Material

The biological material used for the anti-sickling activity test is SS sickle cell blood. This blood is collected from patients of all sexes and ages who come to the Diamniadio Children's Hospital biology laboratory for screening, after obtaining their consent. Samples are taken in EDTA tubes by venipuncture at the elbow. The identification of the type of sickle cell disease is first carried out by an Emmel test and then confirmed by electrophorese.

For the antihemolytic activity test, normal blood samples were taken from healthy student volunteers in the laboratory.

The cytotoxicity test was performed on red blood cell leukocytes from the same blood used for the anti-hemolytic test.

3. Methodology

3.1. Extraction and Fractionation

A 70 g mass of *Terminalia avicennioides* root powder was macerated in an Erlenmeyer flask containing 300 mL of hexane at room temperature for 72 hours with moderate agitation. After filtering with paper filter, the residue was taken up with 200 mL methanol following the same procedure. The combined filtrates were then reduced to dryness using a Büchi rotary evaporator fitted with a Büchi vacuum pump. The resulting dry methanolic crude extract was dissolved in distilled water, then fractionated with dichloromethane and ethyl acetate respectively to extract compounds according to their polarity. The various extracts and fractions thus obtained were then evaporated to dryness, identified as **CE** (crude extract), **EAF** (ethyl acetate fraction), **AqF** (aqueous fraction) and stored at 4˚C until use.

3.2. Anti-Free Radical Activity Test by ABTS Radical Scavenging

Antioxidant activity against the ABTS cation radical was carried out according to the method described by Pellegrini *et al.* [27], with slight modifications.

The ABTS solution (7 mM) was prepared in potassium persulfate (2.45 mM) and incubated for 12 to 16 hours in the dark at room temperature.

A volume of 1500 µL of this solution with absorbance between 0.600 and 0.850 nm was added to 50 µL of extract or standard (ascorbic acid) at different concentrations, and absorbance was measured at 734 nm after 10 min incubation in the dark. An ethanol control was used. Three readings were taken for each concentration tested, and the percentage inhibition was calculated according to the following formula:

$$Inhibion\ \% = \frac{Ac - Ae}{Ac} X100. \qquad \text{(Equation 1)}$$

With Ac = absorbance control; Ae = absorbance extract.

3.3. Anti-Sickling Activity Test

To study anti-sickling activity, Emmel tests was performed on blood from SS subjects [28]. Followed by observation under a light microscope at 40 X magnification and a sickle cell count.

A 100 µL volume of SS sickle cell blood is mixed in a tube with 100 µL of extract at different concentrations (20 mg/mL, 10 mg/mL, 5 mg/mL, and 2.5 mg/mL) then incubated for 1 hour at room temperature. After incubation, 100 µL of sodium metabisulfite solution ($Na_2S_2O_5$) is added. A smear of this mixture is taken, followed by morphological analysis under a light microscope and a sickle cell count at 40 X magnification.

Sickle cell counting is performed on five fields. For each field, both normal red blood cells and sickle cells are counted. The ratio between the number of sickle cells and total red blood cells is used to determine the sickle cell content of each solution.

$$Residual\ sickle\ cell\% = \frac{Sickle\ cell\ count}{Total\ red\ blood\ cell} X100. \qquad \text{(Equation 2)}$$

3.4. Antihemolytic Activity Test

The antihemolytic activity of *Terminalia avicennioides* root extracts was evaluated following the method described by Shabbir with minor modifications [29]. A volume of 0.5 mL of extract at different concentrations (0.125 mg/mL, 0.0625 mg/mL, 0.03125 mg/mL, 0.015625 mg/mL, 0.0078125 mg/mL and 0.00390625 mg/mL), was mixed with 0.5 mL of red blood cell suspension, then incubated at room temperature for 20 min. After incubation, 0.5 mL of hydrogen peroxide was added to the mixture for induction of oxidative degradation of membrane lipids. Similarly, a negative control was prepared with a similar volume of the mixture. A positive control consisting of quercetin was prepared under the same conditions. The mixtures thus obtained were centrifuged at 1000 G, for 10 min, and the anti-hemolytic activity was assessed spectrophotometrically at 540 nm. Percentage inhibition is calculated using the following formula.

$$Hemolysed\ red\ blood\ cell\% = \frac{A\,control - A\,test}{A\,control} X100. \qquad \text{(Equation 3)}$$

With A = Absorbance.

3.5. Cytotoxic Activity Test

The cytotoxic activity of extracts was assessed on leukocytes obtained from erythrocytes using the protocol written by Williams [30].

The CCK8 (cell counting Kit 8) calorimetric method proposed by Elabscience® was used to determine the activity of extracts on leukocytes. The principle consists in measuring the yellow-colored formazan obtained by reduction of CCK8 by lactate dehydrogenase (LDH) released by living leukocytes. The amount of formazan released is proportional to the increase in leukocyte proliferation. Concretely, 100 µL of cell suspension (5000 leukocytes) are deposited in the wells of a 96-well plate.

A volume of 10 μL of extract at different concentrations (0.05 to 3 mg/mL) was then added to each well. Arginine was used as the positive control. The negative control was prepared under the same conditions, replacing the extracts with physiological water. The plate was then pre-incubated for 24, 48 or 72 h in a 5% CO_2 incubator at 37˚C. A 10 μL solution of CCK8 is added to each well before re-incubating the plate for 3 hours. Absorbances were measured using an Elisa reader at 450 nm. Cell proliferation rates were calculated using the following formula.

$$Cell\ proliferation\ \% = \frac{A extract - A negatif\ control}{A extract} X100. \quad \text{(Equation 4)}$$

With A = Absorbance.

4. Results

4.1. Antioxidant Activity by Reduction of the ABTS Cation Radical

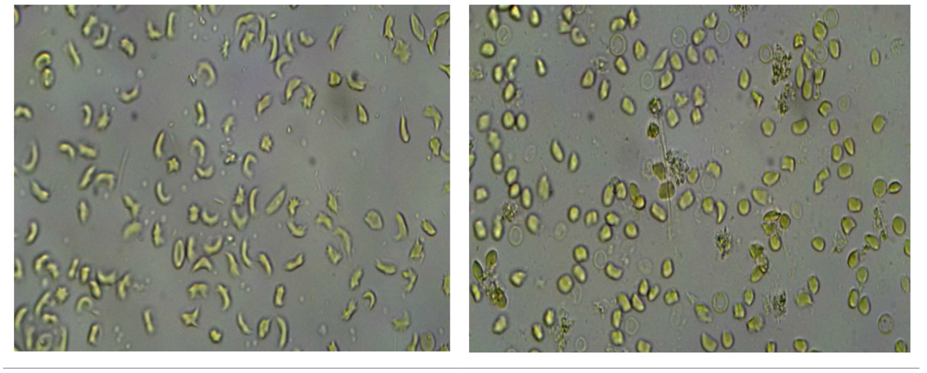
The results of the anti-radical activity of extracts and ascorbic acid at different concentrations, expressed as IC_{50}, are calculated from the linear regression equations obtained from the graph of percentage inhibition of the ABTS radical as a function of concentration. Table 1 below shows the IC_{50} values obtained with extracts and vitamin C.

Table 1. IC_{50} values for extracts and vitamin C.

Extracts	CE	AqF	EAF	Vit C
IC50 (mg/mL)	0.068 ± 0.002	0.074 ± 0.003	0.139 ± 0.006	0.070 ± 0.009

4.2. Anti-Sickling Activity

Figure 1 shows optical micrographs of sickle cells from the negative control (a) and **AEF** (b). The percentage of sickle cells is shown in Figure 2.



a) Blood SS without extract b) Blood SS with **EAF** 20mg/mL

Figure 1. Optical micrograph of sickle cells from control (a) and F. AE (b).

Figure 1 demonstrates that in hypoxic conditions (left fig), red blood cells adopt a sickle shape, confirming the SS nature of the blood samples (control). When sickle erythrocytes are mixed with ethyl **EAF** (right fig) in the indication condition of the investigation, the majority of the red blood cells have a normal morphology showing the inhibition activity of the **EAF**.

The results of the sickle cell count of the extracts after Emmel's test are shown in Figure 2 below. The sickle cell count of the negative control is higher than those obtained with the extracts. The anti-sickling effect of the **EAF** with 13% of residual sickle cells is slightly more significant than those obtained with the other extracts at 20 mg/mL. Indeed with the **CE**, **AQF** the residual sickle cells are respectively 17% and 20% at the same concentration. With the concentration of 2.5 mg/mL, the **EAF** and the **CE** have almost the same activity with respectively 38% and 37% of residual sickle cells found.

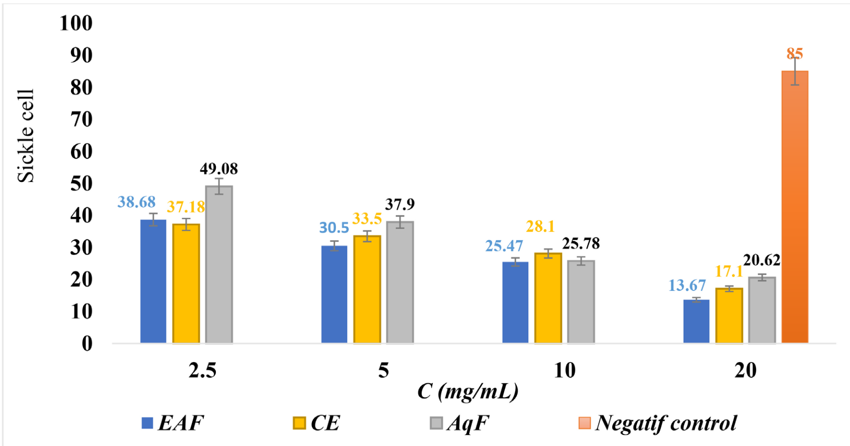


Figure 2. Percentage of residual sickle cell after treatment.

4.3. Anti-Hemolytic Activity

The results of the anti-hemolytic activity test obtained with extracts and quercetin as a function of concentration are shown in Figure 3 below:

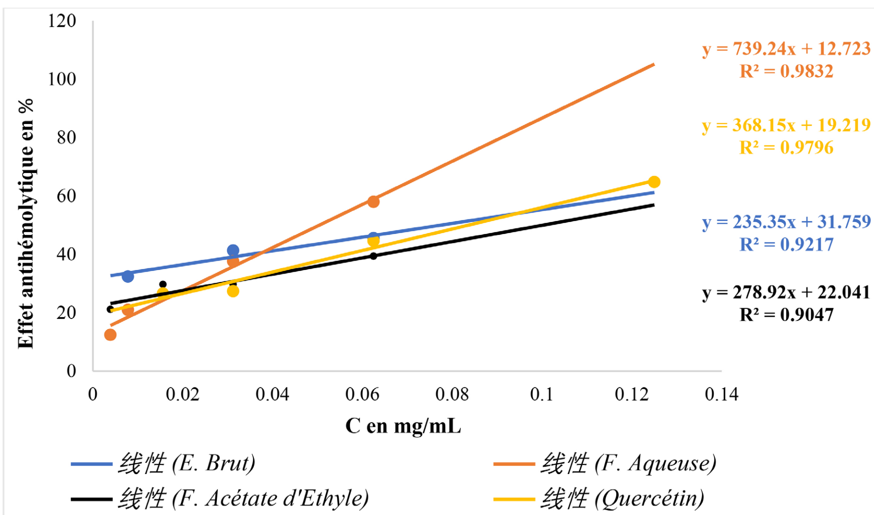


Figure 3. Anti-hemolytic activity of extracts and quercetin.

The IC_{50} values for extracts and quercetin obtained from the linear regression equations in **Figure 3** are given in **Table 2** below.

Table 2. IC50 values for extracts and quercetin.

Extracts	CE	AqF	EAF	Quercetin
IC50 (mg/mL)	0.078 ± 0.003	0.050 ± 0.001	0.100 ± 0.002	0.082 ± 0.001

4.4. Cytotoxic Activity of Extracts

The results of the cytotoxic activity of *Terminalia avicennioides* extracts on leukocytes showed that the extracts are not cytotoxic and induced leukocyte proliferation within 48 h. The same trend was observed for arginine used as a positive control. After 72 h of incubation, a decrease in proliferation was observed for all extracts and arginine, materialized by a slight increase in CP_{50} as shown in **Table 3** below.

Table 3. Proliferation concentration at 50%.

Incubation time	CE	AqF	EAF	Arginine
24 H	1.846 ± 0.015	1.846 ± 0.026	1.846 ± 0.046	0.017 ± 0.003
48 H	1.649 ± 0.010	1.759 ± 0.012	1.785 ± 0.062	0.005 ± 0.001
72 H	2.126 ± 0.061	3.995 ± 0.083	2.673 ± 0.014	0.008 ± 0.000

5. Discussion

The antioxidant activity of *Terminalia avicennioides* root bark powder extracts and fractions was measured spectrophotometrically at 734 nm *in vitro*, using the ABTS cation radical trapping technique. The extracts and polar fractions were all found to be active in ABTS inhibition, with IC_{50} values ranging from 0.118 to 0.225 mg/mL, compared with 0.145 mg/mL for vitamin C. The more active crude extract can be explained by a synergistic effect of the various polar compounds contained in the root barks of this plant. This significant inhibition is dose-dependent.

The work of Amou *et al.* has shown the presence of phenolic compounds, tannins, alkaloids and flavonoids, among others, in *Terminalia avicennioides* roots [31].

A significant relationship has been shown between the presence of polyphenolic and alkaloid compounds and anti-free radical and anti-inflammatory activity [32] [33]. The work of Maturin *et al.* showed an IC_{50} of 0.016 mg/mL for the methanolic extract of terminalia avicennioides roots against 0.024 mg/mL for ascorbic acid using the DPPH radical [34].

The ABTS cation radical is a good support for the study of antiradical activities, as it can be reduced by both proton and electron donors.

Falciformation inhibition activity was assessed using SS sickle cell blood incubated with the extracts for 1 hour before creating a hypoxic environment by adding sodium metabisulfite. The results show that all extracts inhibit falciformation in a dose-dependent manner. The ethyl acetate fraction proved the most active, with residual sickle cell counts ranging from 38.68% to 13.67% at concentrations of 2.5 and 20 mg/mL respectively, while the negative control showed a sickle cell count of 85%. The second-ranked crude methanolic extract was slightly less active, with residual sickle cell counts ranging from 37.18% to 17.1% for concentrations of 2.5 and 20 mg/mL respectively. The high polarity of the methanolic extract, with its abundance of polar secondary metabolites, does not explain the lower effect compared with the ethyl acetate fraction. These results are similar to those described by Mohamed *et al.*, where a dose-dependent effect was observed for the methanolic extract of *Adansonia digitata*, with a rate of non-sickle cells corresponding to 98% for a concentration of 1 g/mL [35]. A hydroalcoholic extract of *Ficus abutifolia* leaf at 1.5 mg/mL produced a 73.15% reduction in the number of sickle cells at 10% [36].

The antihemolytic activity of *Terminalia avicennioides* root bark was also evaluated in this study. Indeed, erythrocytes are particularly vulnerable to free radicals due to their polyunsaturated fatty acid-rich membrane and their association with hemoglobin [37].

In this study, hydrogen peroxide was used as a hemolysis inducer at 1/3 dilution. According to Shabbir *et al.*, it induces 98% hemolysis [29].

The antihemolytic activity results shown in **Figure 3** and **Table 2** demonstrated that the extracts have antihemolytic activities. **CE** and **AqF** with IC_{50} of 0.07 and 0.05 mg/mL respectively are more active than quercetin. Quercetin, with an IC_{50} of 0.08 mg/mL, is a reference antihemolytic. It is also known for its antioxidant and anti-inflammatory activities. Indeed, reactive oxygen species can cause oxidative damage to biological compounds such as proteins, lipids and DNA. According to the properties described above, quercetin can help prevent such damage [38]. Comparing our results with those of another study carried out on methanolic extracts of the leaves of two species of mangroves: *Bruguiera gymnorrhiza* and *Heritiera littoralis* [39], we found that *Terminalia avicennioides* extracts are far more active in inhibiting haemolysis than those of the leaves of these two mangroves, which have respectively IC_{50} of 311.29 μg/mL and 526.90 μg/mL. The same observation was made with the work of James and Alewo [40] on *Gymnema sylvestre* leaves with an IC_{50} = 29.83 mg/mL.

The extracts contain secondary metabolites such as flavonoids and polyphenols, which may be partly responsible for the anti-hemolytic effect observed. These phenolic compounds, particularly flavonoids, have been shown to neutralize or scavenge free radicals [41]. The study carried out by kumar *et al.* on *Sorghum bicolor* extracts shows that the ethyl acetate and aqueous extracts have respectively reversibility rate of 84% and 67%, corresponding to residual sickle cells of 16% and 33%. These results are very close to ours using the same solvents [42].

In addition, polyphenols act as chelators of transition metals such as Fe^{2+}, reducing the rate of the Fenton reaction. They are also effective in preventing hydroxyl radical-induced oxidation and blocking the penetration of H_2O_2 through the red cell membrane, as well as the subsequent formation of free radicals [43] [44].

The cytotoxic activity of the extracts measured on leukocytes using arginine as a positive control showed an identical evolution of their effects on leukocytes. Between 24 h and 48 h incubation, the proliferation concentration 50% (CP_{50}) decreased for all extracts and arginine, showing an increase in leukocyte numbers. From 72 h, this concentration began to increase for all extracts and arginine tested, indicating a decrease in leukocyte proliferation. This may be linked to a longer exposure time of leukocytes to the drugs. However, the crude extract with a CP_{50} equal to 2.126 mg/mL at 72 h of incubation proved to be the least toxic. The ethyl acetate fraction with a CP_{50} of 2.673 mg/mL has a cytotoxicity close to that of the aqueous fraction, while the arginine used as a reference has a CP_{50} of 0.08 mg/mL. The degree of bilateral correlation using SPSS software gives a p value < 0.05 thus showing good significance of the test.

Arginine is an amino acid considered an essential dietary supplement, particularly in certain circumstances in patients in a catabolic state or in the presence of an acute stress factor [45]. It contains a guanidine nitrogen group that binds to NO synthase and serves as a substrate for this enzyme in the production of nitric oxide (NO). The latter is a vasodilator that facilitates the circulation of red blood cells through the vessels [46].

6. Conclusion

The anti-sickle cell properties of *Terminalia avicennioides* root bark were investigated in this study. Because of its multiple therapeutic effects, the root fibers of this plant are commonly used in the preparation of tea and other beverages in Senegal. The results obtained for the various parameters studied show that the polar extracts of *Terminalia avicennioides* roots are endowed with anti-hemolytic, antioxidant and anti-sickling properties. The cytotoxicity test shows that these extracts are not cytotoxic to leukocytes, but rather induce their proliferation. These very encouraging results are in places more interesting than the positive controls. However, a major challenge lies in preserving and protecting this plant, which is currently under heavy attack from traditional practitioners and the general public. Further pharmacological and toxicity studies on the plant's secondary metabolites will be needed before a phytomedicine or nutritional supplement can be made available.

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Authors' Contributions

This work was carried out in collaboration among all authors. Authors CS designed the study and wrote the manuscript. Authors MS, OF, MN, NFC and AN supervised the experiments and managed the analyses of the study; authors NB, RB, MM, SMS conducted the experiments and collected the data. All authors read and approved the final manuscript.

Ethical Approval

Approval of the study (0228/2017/CER/UCAD) was obtained from the Research Ethics Committee of the Cheikh Anta Diop University of Dakar.

Conflicts of Interest

The authors of this article declare that they have no conflict of interest in the production of this work.

References

[1] Elendu, C., Amaechi, D.C., Alakwe-Ojimba, C.E., Elendu, T.C., Elendu, R.C., Ayabazu, C.P., *et al.* (2023) Understanding Sickle Cell Disease: Causes, Symptoms, and Treatment Options. *Medicine*, **102**, e35237. https://doi.org/10.1097/md.0000000000035237

[2] Akakpo-Akue, J., Ahon, G.M., Kplé, T.K.M., Djaha, L.M.L., Yvette, F., Ibrahime, S., *et al.* (2023) Effects of Methanolic and Aqueous Extracts of *Griffonia simplicifolia* (Fabaceae) on the Inhibition of Falciformation of Human Hb SS Erythrocytes. *Open Journal of Applied Sciences*, **13**, 24-35. https://doi.org/10.4236/ojapps.2023.131003

[3] Bunn, H.F. (1997) Pathogenesis and Treatment of Sickle Cell Disease. *New England Journal of Medicine*, **337**, 762-769. https://doi.org/10.1056/nejm199709113371107

[4] Lal, N. and Prajapati, P. (2024) Sickle Cell Disease: Causes, Pathophysiology, Diagnosis and Therapy. Chhatrapati Shahu Ji Maharaj University. https://doi.org/10.5281/zenodo.10807549

[5] Obeagu, E.I., Ubosi, N.I., Obeagu, G.U., Egba, S.I. and Bluth, M.H. (2024) Understanding Apoptosis in Sickle Cell Anemia Patients: Mechanisms and Implications. *Medicine*, **103**, e36898. https://doi.org/10.1097/md.0000000000036898

[6] Ahajumobi, N.E. and Asika, J.C. (2024) Afro Medicinal Plants a Promising Remedy for Sickle Cell Anemia. *International Blood Research & Reviews*, **15**, 26-37. https://doi.org/10.9734/ibrr/2024/v15i1332

[7] Delgadinho, M., Ginete, C., Santos, B., Mendes, J., Miranda, A., Vasconcelos, J., *et al.* (2022) Microbial Gut Evaluation in an Angolan Paediatric Population with Sickle Cell Disease. *Journal of Cellular and Molecular Medicine*, **26**, 5360-5368. https://doi.org/10.1111/jcmm.17402

[8] Galadanci, N., Phillips, S., Schlenz, A., Ivankova, N. and Kanter, J. (2024) Current Methods of Newborn Screening Follow-Up for Sickle Cell Disease Are Highly Variable and without Quality Assurance: Results from the ENHANCE Study. *International Journal of Neonatal Screening*, **10**, Article 22. https://doi.org/10.3390/ijns10010022

[9] https://www.google.com/search?q=%E2%80%9CSickle-Cell-Disease-Fact-Sheet-2024%E2%80%9D.&oq=%E2%80%9CSickle-Cell-Disease-Fact-Sheet-Accessed

[10] Spurway, A., George, S., Thompson, C. and Weeks, S. (2024) Sickle Cell Disease: Causes, Treatments and the Patient Experience.

https://www.pharmaceutical-journal.com/article/ld/sickle-cell-disease-causes-treatments-and-the-patient-experience

[11] World Health Assembly (2006) Sickle-Cell Anaemia: Report by the Secretariat. World Health Organization. https://iris.who.int/handle/10665/20890

[12] Ya, M.S., Mukuku, O., Lubala, T.K., Mutombo, A.M., Kanteng, G.W., Umumbu, W.S., *et al.* (2014) Drépanocytose chez l'enfant lushois de 6 à 59 mois en phase stationnaire: épidémiologie et clinique. *Pan African Medical Journal*, **19**, Article 71. https://doi.org/10.11604/pamj.2014.19.71.3684

[13] Kuriri, F.A. (2023) Hope on the Horizon: New and Future Therapies for Sickle Cell Disease. *Journal of Clinical Medicine*, **12**, Article 5692. https://doi.org/10.3390/jcm12175692

[14] Acharya, B., Mishra, D.P., Barik, B., Mohapatra, R.K. and Sarangi, A.K. (2023) Recent Progress in the Treatment of Sickle Cell Disease: An Up-to-Date Review. *Beni-Suef University Journal of Basic and Applied Sciences*, **12**, Article No. 38. https://doi.org/10.1186/s43088-023-00373-w

[15] Buhari, H.A., Ahmad, A.S. and Obeagu, E.I. (2023) Current Advances in the Diagnosis and Treatment of Sickle Cell Anaemia. *Newport International Journal of Biological and Applied Sciences*, **4**, 1-10. https://doi.org/10.59298/nijbas/2023/1.1.11111

[16] Yayo-Aye, M., Adjambri, A.E., Kouakou, B., N'guessan-Blao, R., Adjé, L.M., Kamagaté, T., *et al.* (2024) Impact of Hydroxyurea on Clinical and Biological Parameters of Sickle Cell Anemia in Children in Abidjan. *Mediterranean Journal of Hematology and Infectious Diseases*, **16**, e2024026. https://doi.org/10.4084/mjhid.2024.026

[17] Sadaf, A. and Quinn, C.T. (2020) L-Glutamine for Sickle Cell Disease: Knight or Pawn? *Experimental Biology and Medicine*, **245**, 146-154. https://doi.org/10.1177/1535370219900637

[18] Jacobs, J.W., Stephens, L.D., Chooljian, D.M., Sharma, D., Adkins, B.D. and Booth, G.S. (2024) Crizanlizumab and Sickle Cell Disease: When Should Medications Have Their Approval Status Revoked? *American Journal of Hematology*, **99**, 1016-1018. https://doi.org/10.1002/ajh.27275

[19] Yenamandra, A. and Marjoncu, D. (2020) Voxelotor: A Hemoglobin S Polymerization Inhibitor for the Treatment of Sickle Cell Disease. *Journal of the Advanced Practitioner in Oncology*, **11**, 873-877. https://doi.org/10.6004/jadpro.2020.11.8.7

[20] Imaga, N.A. (2013) Phytomedicines and Nutraceuticals: Alternative Therapeutics for Sickle Cell Anemia. *The Scientific World Journal*, **2013**, Article 269659. https://doi.org/10.1155/2013/269659

[21] Perampaladas, K., Masum, H., Kapoor, A., Shah, R., Daar, A.S. and Singer, P.A. (2010) The Road to Commercialization in Africa: Lessons from Developing the Sickle-Cell Drug Niprisan. *BMC International Health and Human Rights*, **10**, Article No. 511. https://doi.org/10.1186/1472-698x-10-s1-s11

[22] Faye, L. and Champey, Y. (2008) Plantes, médicaments et génétique. *Médecine/Sciences*, **24**, 939-946. https://doi.org/10.1051/medsci/20082411939

[23] Mann, A., Ibrahim, K., Oyewale, O., *et al.* (2012) Isolation and Elucidation of Three Triterpenoids and Its Antimycobacterial Activity of *Terminalia avicennioides*. *American Journal of Organic Chemistry*, **2**, 14-20. https://doi.org/10.5923/j.ajoc.20120202.03

[24] Mann, A., Banso, A. and Clifford, L.C. (2008) An Antifungal Property of Crude Plant Extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. *Tanzania Journal of Health Research*, **10**, 34-38. https://doi.org/10.4314/thrb.v10i1.14339

[25] Suleiman, M.M., Oyelowo, B.B., Abubakar, A., Mamman, M. and Bello, K.T. (2017) A Controlled Study to Investigate Anti-Diarrhoeal Effect of the Stem-Bark Fractions of *Terminalia avicennioides* in Laboratory Animal Models. *International Journal of Veterinary Science and Medicine*, **5**, 14-22. https://doi.org/10.1016/j.ijvsm.2017.04.002

[26] Yahaya, S.F., Suleiman, M.M., Mohammed, A. and Ibrahim, N.D.G. (2019) Anti-diabetic Potentials of Stem-Bark Extracts of *Terminalia avicennioides* on Alloxan-Induced Diabetic Rats. *Sokoto Journal of Veterinary Sciences*, **17**, 33. https://doi.org/10.4314/sokjvs.v17i2.5

[27] Re, R., Pellegrini, N., Proteggente, A., *et al.* (1999) Original Contribution Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radical Biology & Medicine*, **26**, 1231-1237.

[28] Sall, C., Seck, M., Faye, B., Dioum, M.D., Seck, I., Gueye, P.M., *et al.* (2016) Étude *in vitro* de l'effet antifalcémiant des globules rouges et de l'activité antioxydante d'extraits de la poudre de racines de *Maytenus senegalensis* Lam (Celestraceae). *International Journal of Biological and Chemical Sciences*, **10**, Article 1017. https://doi.org/10.4314/ijbcs.v10i3.9

[29] Shabbir, M., Khan, M.R. and Saeed, N. (2013) Assessment of Phytochemicals, Antioxidant, Anti-Lipid Peroxidation and Anti-Hemolytic Activity of Extract and Various Fractions of *Maytenus royleanus* Leaves. *BMC Complementary and Alternative Medicine*, **13**, Article No. 143. https://doi.org/10.1186/1472-6882-13-143

[30] Tripodi, D., Lyons, S. and Davies, D. (1971) Separation of Peripheral Leukocytes by Ficoll Density Gradient Centrifugation. *Transplantation*, **11**, 487-488. https://doi.org/10.1097/00007890-197105000-00010

[31] Amou, B., Ajayi, T.O. and Dada-Adegbola, H.O. (2025) Antimicrobial Activity of *Terminalia leiocarpa* Baill. and *Terminalia avicennioides* Guill. & Perr. Root Bark Extracts in Resistant Clinical Isolates. *Journal of Pharmacy & Pharmacognosy Research*, **13**, 633-646. https://doi.org/10.56499/jppres24.2010_13.2.633

[32] Musa, F.M., Muhammad-Idris, Z.K. and Wartu, J.R. (2024) Evaluation of Phytochemical, *in Vitro* Antibacterial and Rate of Kill Assay of *Terminalia avicennioides* Leaf against Some Bacteria Associated with Diarrhoea. *Science World Journal*, **19**, 229-239. https://doi.org/10.4314/swj.v19i1.30

[33] Maguirgue, K. (2022) Evaluation Phytochimique, Potentiels Antioxydants et Anti-Inflammatoires *in Vitro* des Extraits des Feuilles de *Commelina benghalensis* Linn (Commelinaceae). *International Journal of Biological and Chemical Sciences*, **16**, 2673-2684. https://dx.doi.org/10.4314/ijbcs.v16i6.17

[34] Mathurin, I., Paul., N., Xavier, W., Frederic Armel, N., Mboïssio Angelique, O., Marinette, S., *et al.* (2025) Chemical Characterization and Evaluation of the Antioxidant and Antibacterial Activities of Methanolic Extracts of the Root Bark of *Terminalia avicennioides* (Combretaceae). *World Journal of Organic Chemistry*, **12**, 1-7. https://doi.org/10.12691/wjoc-12-1-1

[35] Mohammed, S.A.M., Abdalla, E.I., Eisa, I.M., Mohamed, H.Y., Hassan, A.B. and Elbasheir, M.M. (2024) To Study the *in Vitro* Anti-Sickling Activity of Adansonia Digitata Fruits Extract on Sickle Cells. *European Journal of Medicinal Plants*, **35**, 384-392. https://doi.org/10.9734/ejmp/2024/v35i61234

[36] Diouf, I., Sene, M., Ba, A., Ndiaye, M., Sene, M., Ouedraogo, A., *et al.* (2025) Antisickling Properties of Hydro-Alcoholic Extract of Ficus Abutilifolia Leaves. *Journal of African Association of Physiological Sciences*, **12**, 31-37. https://doi.org/10.4314/jaaps.v12i2.4

[37] Diouf, S. (2019) Etude des activités antioxydante, antihémolytique et antifalcémiante d'une combinaison d'extraits méthanolique de: Carica Papaya, Hibuscis Sabdariffa et Terminalia avicennioides. Thèse d'exercice, Pharmacie, Cheikh Anta Diop de Dakar.

[38] Osredkar, J. (2024) Quercetin-Effects on Human Health. Intech Open. https://www.intechopen.com

[39] Karim, M.A., Islam, M.A., Islam, M.M., Rahman, M.S., Sultana, S., Biswas, S., *et al.* (2020) Evaluation of Antioxidant, Anti-Hemolytic, Cytotoxic Effects and Anti-Bacterial Activity of Selected Mangrove Plants (*Bruguiera gymnorrhiza* and *Heritiera littoralis*) in Bangladesh. *Clinical Phytoscience*, **6**, Article No. 8. https://doi.org/10.1186/s40816-020-0152-9

[40] James, O. and Alewo, I.M. (2014) *In Vitro* Antihemolytic Activity of Gymnema Sylvestre Extracts Against Hydrogen Peroxide (H_2O_2) Induced Haemolysis in Human Erythrocytes. *American Journal of Phytomedicine and Clinical Therapeutics*, **2**, 861-869.

[41] Kumar Kurre, M., Patra, S. and Patra, P.K. (2025) *In Vitro* Anti-Sickling Activities of *Sorghum bicolor* (L.) Moench. *International Journal of Advanced Research*, **13**, 326-332. https://doi.org/10.21474/ijar01/20570

[42] Hatia, S., Septembre-Malaterre, A., Le Sage, F., Badiou-Bénéteau, A., Baret, P., Payet, B., *et al.* (2014) Evaluation of Antioxidant Properties of Major Dietary Polyphenols and Their Protective Effect on 3T3-L1 Preadipocytes and Red Blood Cells Exposed to Oxidative Stress. *Free Radical Research*, **48**, 387-401. https://doi.org/10.3109/10715762.2013.879985

[43] Tsao, R. (2010) Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients*, **2**, 1231-1246. https://doi.org/10.3390/nu2121231

[44] Hapner, C.D., Deuster, P. and Chen, Y. (2010) Inhibition of Oxidative Hemolysis by Quercetin, but Not Other Antioxidants. *Chemico-Biological Interactions*, **186**, 275-279. https://doi.org/10.1016/j.cbi.2010.05.010

[45] Sadeghi, A., Taherifard, E., Dehdari Ebrahimi, N., Rafiei, E., Hadianfard, F. and Taherifard, E. (2023) Effects of l-Arginine Supplementation in Patients with Sickle Cell Disease: A Systematic Review and Meta-Analysis of Clinical Trials. *Health Science Reports*, **6**, e1167. https://doi.org/10.1002/hsr2.1167

[46] Korman, R., Hatabah, D., Brown, L.A., Harris, F., Bakhsi, N., Rees, C., *et al.* (2023) Mechanism(s)-of-Action for Arginine Therapy in Children with Sickle Cell Disease and Vaso-Occlusive Pain: Results of a Pharmacokinetics/Pharmacodynamics Study. *Blood*, **142**, 3861-3861. https://doi.org/10.1182/blood-2023-187793