UMYU Scientifica, Vol. 3 NO. 3, September 2024, Pp 130 – 140
https://doi.org/10.56919/usci.2433.015
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Phytochemical Analysis and Antisickling Activity of some Medicinal Plants from Sokoto, Nigeria

Sumayya Ahmed Ayuba[1]* and Andrew Onu[2]
[1]Department of Biochemistry, Faculty of Science, Sokoto State University, Sokoto, Nigeria
[2]Department of Biochemistry and Molecular Biology, Faculty of Chemical and Life Science, Usmanu Danfodiyo University Sokoto, Nigeria

ABSTRACT
Sickle cell anemia occurs due to the polymerization of abnormal hemoglobin S resulting from decreased oxygen tension. Ultimately, this causes alterations in red blood cell structure, resulting in anemia. This study, therefore, examined the phytochemical properties and the antisickling activity of some selected plants (*Khaya senegalensis, Vernonia amagdalina, Ficus carica, Cassia nigricans, and Ficus sycomorus*) that have been reported to be used by traditional medical professionals in the management of sickle cell anemia. The plants were exhaustively extracted using cold methanol maceration. Each plant's methanolic extract was subjected to qualitative and quantitative phytochemical testing to ascertain its phytocompound composition. To determine the sickling reversal ability of the plant extracts, sickling was induced in red blood cells (RBCs) by adding sodium metabisulfite (2 %) and then treated with 10 µg/ml of the extracts. Osmotic fragility test was used to investigate the membrane stabilizing effect of selected extracts (250 µg/mL) on the solubility of hemoglobin S and the integrity of the sickle cell membrane. From the results, it was observed that the plants investigated showed the presence of alkaloids, saponins, terpenes, and flavonoids. Only anthraquinones was found absent in all plant extracts tested. The study also revealed a high antisickling activity by the plants. The extracts significantly ($p<0.05$) reversed sickling cells with *K. senegalensis* (95.29 ± 5.62 %), *V. amagdalina* (92.19 ± 6.91 %), *F. carica* (88.32 ± 3.98 %), *C. nigricans* (92.26 ± 5.01 %) and *F. symcomorus* (92.11 ± 6.31 %). Regarding membrane stabilizing potential, *F. carica stem bark* demonstrated a considerably greater membrane stabilizing potential (IC_{50} = 3.98 ± 0.51 mg/mL) followed by *C. nigricans* leaves (5.01 ± mg/mL). The methanolic extract of the plants studied demonstrated high potency in maintaining erythrocyte membrane integrity and altering the polymerization of sickle cell hemoglobin at increasing concentrations.

ARTICLE HISTORY
Received May 02, 2024
Accepted July 20, 2024
Published July 27, 2024

KEYWORDS
Sickle cell anemia, Anti-sickling properties, Medicinal plants, Phytocompounds

INTRODUCTION
Sickle cell anemia (SCA) is a prevalent heritable illness with significant global significance and carries notable public health implications, particularly within Africa (Paintsil *et al.*, 2022). "Based on available data, 43 million people have the sickle cell trait, and 4.4 million people worldwide are estimated to be affected with sickle cell anemia" (Global Burden of Disease Study 2013 Collaborators, GBDSC, 2015). Sub-Saharan Africa bears the highest burden, with approximately 80 % of Sickle cell anemia cases (Adigwe *et al.,* 2023). Alarmingly, the rate of death for children under five years old within this population ranges from 50 % to 80 % (Oguntibeju, 2023). The severe impact of the disease in this region is further compounded by limited access to comprehensive healthcare services (Acharya *et al.*, 2023). This condition is characterized by red blood cell (RBCs) sickling, hemolytic anemia, and vaso-occlusion, resulting in excruciating pain, inflammation, pulmonary hypertension, organ damage, and other chronic morbidities (Khan *et al.*, 2022). One amino acid alteration in adult hemoglobin, principally expressed in red blood cells (RBCs), is responsible for the pathophysiology of sickle cell anemia. (Fernandes, 2017). This molecular disorder is starting to become a public health concern. Regretfully, no effective treatment is currently available.

Several treatments have been investigated. Despite various modalities that endeavor to mitigate its trajectory, there remains a dearth of curative interventions for Sickle cell anemia (SCA) (Innocent Iba *et al.*, 2022). The therapeutic options accessible for individuals predominantly impacted by Sickle cell anemia (SCA), such as bone marrow transplantation or gene therapy, remain elusive due to various barriers and limitations (Fontana *et al.*, 2023). The only disease-modifying treatments for acute and chronic SCA problems are hydroxyurea and

Correspondence: Sumayya Ahmed Ayuba. Department of Biochemistry, Faculty of Science, Sokoto State University, Sokoto, Nigeria. ✉ ayubasumayya@gmail.com. Phone Number: +234 703 935 4675
How to cite: Ayuba, S. A., & Onu, A. (2024). Phytochemical Analysis and Antisickling Activity of some Medicinal Plants from Sokoto, Nigeria. *UMYU Scientifica*, 3(3), 130 – 140. https://doi.org/10.56919/usci.2433.015

continuous blood transfusions (Inusa *et al.*, 2019). Hydroxyurea administration exhibits a two-fold impact in Sickle cell anemia (SCA), comprising the augmentation of fetal hemoglobin (HbF), known for its safeguarding effect against the deleterious consequences of sickle hemoglobin (HbS) and the prevention of polymerization within the red blood cells, thereby ameliorating the pathophysiology associated with SCA (Rankine-Mullings, 2017). Furthermore, hydroxyurea as a preventive measure in recurring painful crises and chest symptoms, demonstrates a notable reduction in hospital admissions and transfusion requirements (Green *et al.*, 2016). Nonetheless, the potential long-term toxicity associated with this therapeutic agent has raised valid concerns within the scientific community (Nevitt, 2017).

In low-and middle-income countries, traditional medicines, including phytomedicines, are extensively employed by many families afflicted by Sickle cell anemia (SCA), with utilization rates reaching up to 80 % (Oniyangi and Cohall, 2020). These remedies, readily accessible and culturally embraced, are predominantly employed for managing painful episodes and other complications associated with SCA (Busari, 2017). Research has shown numerous potential therapeutic agents that prevent cellular adhesion, reduce RBC destruction, oxidative and membrane damage, or boost nitric oxide bioavailability for people with SCA (Amujoyegbe, 2015; Vaishnava and Rangari, 2016; Imaga, 2017). Nonetheless, orthodox medical healthcare practitioners in resource-limited regions generally discourage the utilization of traditional medicines in Sickle cell anemia (SCA) due to a dearth of scientific understanding regarding their efficacy and safety, as highlighted by the World Health Organization (WHO, 2013).

In summary, Sickle cell anemia (SCA) is a prevalent hereditary disease with significant global implications, particularly in Africa, where limited access to comprehensive healthcare services exacerbates the burden of the condition, resulting in high mortality rates among children. Despite advancements, effective treatments remain limited. Traditional medicine, particularly medicinal plants, offers potential therapeutic alternatives. In this context, this study aims to investigate the potential antisickling properties of medicinal plants, including *Khaya senegalensis, Vernonia amagdalina, Ficus carica, Cassia nigricans,* and *Ficus sycomorus,* commonly used by traditional healers in Sokoto, Nigeria, to manage SCA. The study seeks to explore the efficacy of these plant extracts in reversing sickling cells and stabilizing the sickle cell membrane, with the ultimate goal of identifying natural remedies that could contribute to the management of sickle cell anemia. Hence, the specific objectives of the study are to (1) Assess the qualitative and quantitative phytochemical composition of the selected medicinal plant extracts; (2) Determine the antisickling activity of the plant extracts on red blood cells (RBCs) induced by sickle under varying concentrations; (3) Evaluate the effect of the plant extracts on the solubility of hemoglobin S and the integrity of the sickle cell membrane using the osmotic fragility test; (4) Compare the reversal activity of sickling cells and the membrane stabilizing potential among the different plant extracts; and (5) Validate the traditional use of these medicinal plants by exploring there *in vitro* antisickling efficacy as potential alternative treatments for sickle cell anemia.

MATERIALS AND METHODS

Study Area

The research was carried out in Sokoto, Nigeria, from October 2021 to March 2022. Sokoto is in the extreme northwest of the country on the national border with the Republic of Niger. It lies between latitude 13^{0}05'N and longitude 05°15'E (Figure 1) and has almost 4.2 million people per square mile (NPC, 2006). Hausa and Fulani ethnic groups comprise most of the city's population, and agriculture is the primary business (Okeowo and Fatoba, 2022).

Collection of Data

A semi-structured questionnaire (Ali *et al.*, 2022) was employed to gather ethnobotanical information from a purposively selected group of participants, including herb vendors, conventional healers, farmers, and people with practices and beliefs typical of the community. The questionnaire was meticulously developed in English to ensure cultural and linguistic appropriateness. Nevertheless, conversations with herb vendors and conventional healers were conducted in the indigenous Hausa language within their locale to enhance effective communication. Forty-three (43) individuals were chatted with, and the individuals gave their opinions voluntarily and one at a time to ensure anonymity. The information gathered comprised the following: age, sex, marital status, employment, academic background, native names for plants used in SCA treatment, portion of plant utilized, preparation/extraction technique, and treatment approach. This research followed the Helsinki Declaration (WMA, 2008).

Collection and Identification of Medicinal Plants

The plants (Table 1) specimens used for this research were collected with the assistance of local herb vendors and traditional healers. They provided valuable assistance as well as Hausa names for the plants. The plants were identified at the Usmanu Danfodiyo University Herbarium, Sokoto, Nigeria, and voucher specimens (*khaya senegalensis*: UDUSH/ANS/0835; *Cassia nigricans:* UDUSH/ANS/0836; *Vernonia amagdalina*: UDUSH/ANS/0837; *Ficus carica*: UDUSH/ANS/0838; *Ficus sycomorus*: UDUSH/ANS/0839) were generated and stored.

Preparation and Extraction of Plant Extract

The plants collected were air-dried for 3 weeks. The dried plants were then milled into powder, after which the powdered plant materials (100 g each) were individually

de-waxed with n-Hexane using a Soxhlet extractor to remove lipophilic compounds (Virot *et al.*, 2007). Afterward, the residue (300 g) was individually macerated with 2 L methanol/water (70:30, v/v) at room temperature for 72 h. It was then filtered and further concentrated using a rotary evaporator set at 50^{0}C under decreased pressure. The concentrated extract was diluted with water to a final volume of 200 mL and sonicated. Freezing and freeze-drying the supernatant produced dried plant extract. Before usage, the dried plant extract was diluted in phosphate-buffered saline (PBS) at pH 7.4 to the required concentrations (Lins *et al.*, 2018).

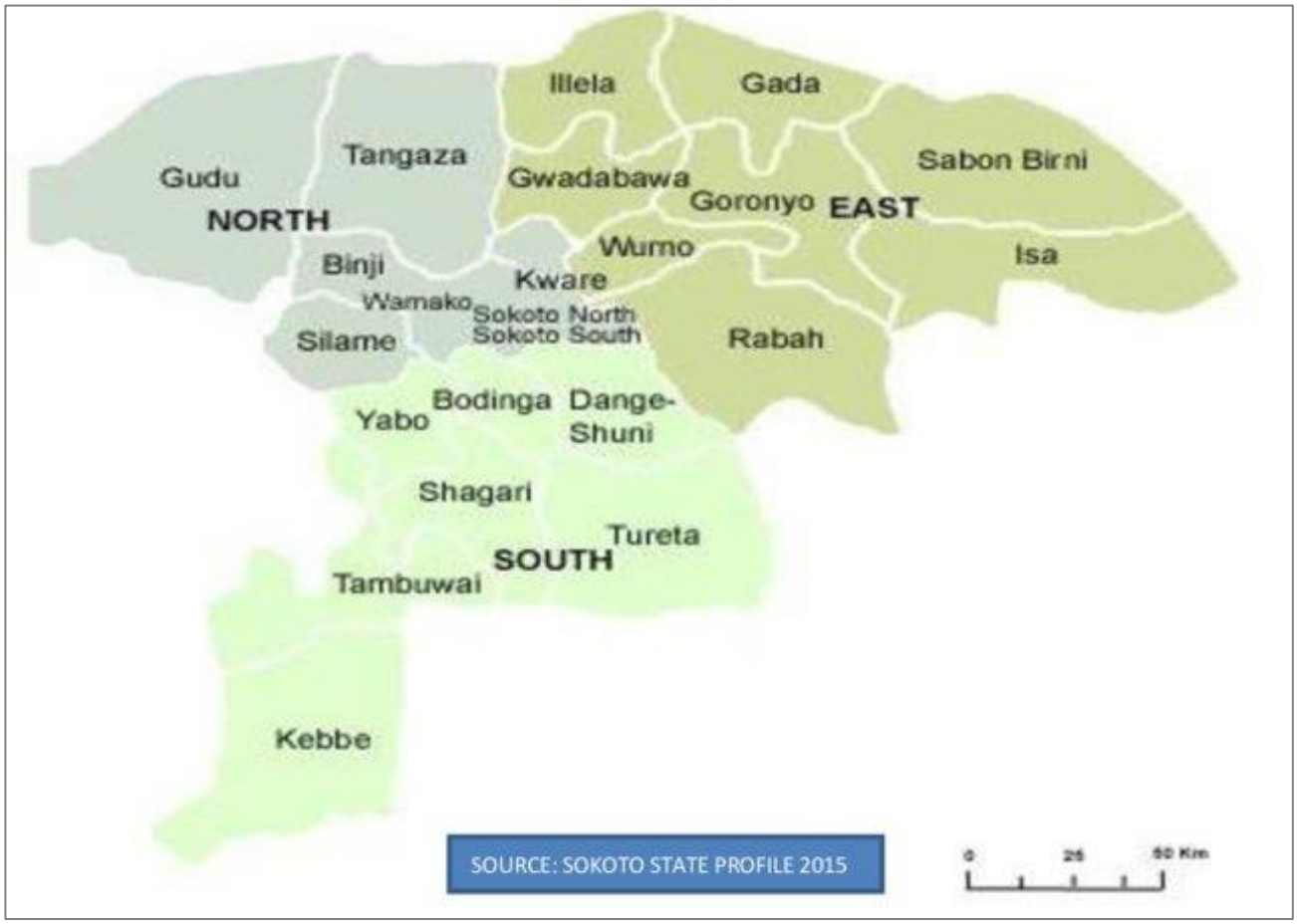

Figure 1: Sokoto State, Nigeria

Table 1: Plants Used in Sokoto to Manage Sickle Cell Anemia

Scientific Name	Family	Local Name	Portion of Plant Utilized	Preparation/Extraction	Administration Method
Khaya senegalensis	*Meliaceae*	Mahogany (Madachi)	Stem bark	Decoction	Applied locally
Vernonia amagdalina	*Asteraceae*	Bitter leaf (Chusar-doki)	Leaves	Decoction	Taken orally
Ficus carica	*Moraceae*	Common fig	Stem bark	Decoction	Taken orally
Cassia nigricans	*Leguminosae - Caesalpinioideae*	Black grain (Tafasa)	Leaves, Whole plant	Decoction	Taken orally
Ficus sycomorus	*Moraceae*	Fig-mulberry (Baure)	Stem bark, Leaves	Decoction	Taken orally

Blood Sample Collection

The blood sample used in this study for assessing the antisickling properties of the plant extracts was obtained from adolescent patients diagnosed with Sickle cell anemia and attending Specialist Hospital, Sokoto. None of the patients recently received blood transfusion from individuals with normal hemoglobin genotype. An electrophoresis test was used to verify the homozygote HbS/HbS (SS) status of participants in this study. Blood

samples were taken and kept in sodium EDTA tubes for the experiment. Every patient who took part in the trial gave written informed permission. Sokoto State Ministry Ethics Committee has also approved all study protocols (UDUTH/HREC/2018/No.668).

Qualitative Phytochemicals Screening

Phytochemical tests were conducted to detect alkaloids, tannins, saponins, flavonoids, and glycosides following Harborne (1999) and Muthukumaran *et al.* (2016).

Alkaloids Assay

Harborne (1999) outlined the procedure used in detecting alkaloids in extracts. After mixing 3 mL of 10 mg/mL plant extract with one milliliter of 1 % (v/v) HCl, the product was heated for 20 minutes. After cooling, the resultant solution was filtered. Two drops of Mayer's reagent were applied to one milliliter of filtrate. A creamy precipitate was detected to suggest the presence of alkaloids.

Tannins Assay

For tannins (Muthukumaran *et al.*, 2016), 0.5 g of sample was dissolved in 20 mL of distilled water and filtered. Ferric chloride drops of 0.1 percent were added. The formation of a blue-black or brownish-green color suggested the presence of tannins.

Saponins Assay

In the test for saponins (Harborne, 1999), two (2 g) of the plant extract were dissolved and boiled in 20 mL of distilled water in a water bath for 10 min, cooled, and filtered. Five milliliters (5 mL) of distilled water and 10 mL of the filtrate were intensely mixed. A continuous, persistent froth showed the presence of saponins.

Flavonoids Assay

To test the presence of flavonoids in the extracts, 3 mL of the aqueous extract was mixed with 2 mL of 1 % (v/v) of aluminum solution. Yellow coloration appears when flavonoids are present (Muthukumaran *et al.*, 2016).

Glycosides Assay

Using the Keller-Killani test, glycosides were detected. Five milliliters of aqueous plant extract were mixed with two milliliters of acetic acid and one drop of ferric chloride solution. Afterward, concentrated sulfuric acid (1 mL) was added. The Deoxysugar property of cardenolides was demonstrated by a brown ring at the contact (Chaitanya Latha *et al.*, 2017).

The quantitative phytochemical analysis of extracts was performed according to the method described by Harborne (2005). Terpenes, alkaloids, saponins, flavonoids, tannins, and volatile oils were examined in the extracts.

Quantitative Phytochemicals Evaluation

Estimation of Total Alkaloids

The gravimetric method of Harborne (2005) was used to estimate alkaloids. To a 250 mL beaker, 1 g of extract sample was added, and 200 mL of 10 % acetic acid in ethanol was added, covered, and left to settle for 4 h. The extract was then filtered and concentrated to one-third of the original volume in a water bath. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The solution was allowed to settle, and the precipitate was collected and washed with 15 % ammonium hydroxide, followed by filtration. The alkaloids residue was dried in an oven, weighed, calculated, and expressed as a percentage of the weight of the sample analyzed.

Estimation of Total Flavonoids

Flavonoids content of the extract was estimated using the method of Bao *et al.*, 2005. A 1.0 mL of the test extract and 5 % $NaNO_2$ solution were added to 1 ml of distilled water, and then 75 mL of a 10 % $AlCl_3.H_2O$ solution was added after 5 minutes. This was followed by adding 0.5 ml of 1M sodium hydroxide after 5 minutes. The solution was mixed thoroughly and left at room temperature for 10 minutes. The rise in absorbance was determined using a UV-visible spectrophotometer set at 510 nm. A standard quercetin calibration curve was used to calculate the total flavonoids content. Results are presented as milligram of quercetin equivalents (QE) per gram of extract.

Estimation of Total Tannins

Total tannins were estimated using the Folins-Ciocalteu method (1927). 0.1 mL of the extract was combined with 6.5 mL of distilled water, then 0.5 mL of Folins-Ciocalteu reagent was added, followed by 1.5 mL of 20 % sodium carbonate, then incubated for 60 minutes. The absorbance of the sample was measured at 725 nm using a spectrophotometer. The results were expressed as micrograms of tannic acid equivalents per gram of extract. A standard tannic acid calibration curve was used to calculate the total tannins content and then expressed as mg tannic acid equivalent (TAE)/g.

Estimation of Saponins

Obadoni and Ochuko's (2001) method was adopted to determine saponins. A conical flask containing 100 g of the pulverized samples was added to 100 ml of 20 % ethanol. The flask was heated at 55°C for four hours with constant stirring in a hot water bath. The mixture was then filtered, and the process was repeated with another 200 mL of 20 % ethanol to exhaustively extract the residue. The extracts were reduced to 40 ml in a water bath set at 90°C. The concentrated extract was then moved into a 250 ml separating funnel, and 20 ml of diethyl ether was added and shaken vigorously. The mixture was allowed to settle and show distinct layers. The aqueous layers was collected, while the ether layer was discarded. The process was repeated twice using 60 ml of

N-butanol. Then, 10 ml of 5 % sodium chloride solution was used to wash the combined n-butanol extracts. The remaining solution was then heated to a dried constant weight in a water bath. The saponins content was calculated as a percentage.

Estimation of Terpenoids

Estimating terpenes was done using the Indumathi *et al.* method Indumathi *et al.* (2014). Dried plant extract (100 mg) was combined with 9 mL of ethanol for 24 hours with constant shaking. Then, the extract, after filtration, was partitioned with 10 mL of petroleum ether using a separating funnel. The ether extract was separated into a clean, pre-weighted beaker and then dried in an oven until a constant weight was obtained. The ether was evaporated, and total terpenoids contents was measured as a percentage of the weight of the sample analyzed.

Antisickling Assay

Antisickling assay was conducted employing Ngbolua *et al.*'s (2015) methodology. Concisely, a blood sample was combined with an equal amount of 2 % sodium metabisulfite after being diluted with 150 mM phosphate buffer saline (30 mM of NaH_2PO_4, 120 mM of Na_2HPO_4, and 150 mM of NaCl). A drop of the combination was put on a microscope slide with a cover slip while the aqueous plant extract was there. Para-hydroxybenzoic acid was the positive control, and biological solution was the negative control. The cover slip's edges were securely covered with paraffin to generate an oxygen-depleted atmosphere.

Erythrocyte Membrane Stability Activity

The erythrocytes' osmotic fragility analyzes extracts' membrane stabilizing impact under hypotonic disruption incubation or osmotic stress. One milliliter of each extract (250 µg/mL) and 0.05 mL of blood obtained from a Sickle cell anemia patient were introduced to a ten-milliliter reaction tube that held four milliliters of buffered saline at various concentrations (ranging from 0.00 - 0.80 %) with a pH of 7.4. After a 24-hour incubation period at a temperature of 25°C, the mixture was centrifuged for 15 minutes at 3000 rpm. At 540 nm, the supernatant's absorbance was measured compared to a blank consisting of 0.85 % buffered saline solution. Plotting the percentage of lysis (%) against the concentration of NaCl yielded the mean corpuscular fragility, which was derived from the concentration of saline that led to 50 % destruction of RBC.

Statistical analysis

Data is shown as mean ± SEM (standard error of mean). GraphPad Prism 6 (registered trademark of GraphPad Software, Inc.) was used to run all statistical tests. One-way ANOVAs and Bonferroni correction conducted comparisons for multiple comparison tests. A p-value of < 0.05 was statistically significant. Results were then expressed as the mean ± S.E.M. of 5 replicate experiments.

RESULTS

Phytochemical Screening of Plant Extracts

The result of the phytochemical analysis (Table 2) showed that saponins, tannins, terpenes, steroids, and alkaloids were found in all the plants studied, while anthraquinones were found to be absent in all plants tested. Flavonoids were present in *V. amagdalina* leaves, *C. nigricans* leaves, and *F. sycomorus* leaves extracts but absent in *K. senegalensis* stem bark, *F. carica* stem bark, *and F. sycomorus* stem bark extracts. All samples, except for *C. nigricans*, contained cardiac glycosides.

Table 2. Qualitative Phytochemical Constituents of *K. senegalensis* stem bark, *Vernonia Amagdalina* leaves extract, *Ficus Carica* Stem bark extract, *Cassia nigricans* leaves, *Ficus sycomorus* Stem bark extract and *Ficus sycomorus* leaves

	K. senegalensis stem bark	*Vernonia Amagdalina* leaves extract	*Ficus Carica* Stem bark extract	*Cassia nigricans* leaves	*Ficus sycomorus* stem bark extract	*Ficus sycomorus* leaves
Saponin glycosides	ND	ND	ND	*	ND	*
Alkaloids	*	*	*	*	*	*
Flavonoids	ND	*	ND	*	ND	*
Saponins	*	*	*	*	*	*
Tannins	*	*	*	*	*	*
Terpenes	*	*	*	*	*	*
Steroids	*	*	*	*	*	*
Anthraquinones	ND	ND	ND	ND	ND	ND
Volatile Oils	*	*	ND	ND	*	*
Cardiac glycosides	*	*	*	ND	*	*

* Indicates presence of phytocompound, 'ND' indicates that the phytocompound was not detected.

Table 3. Quantitative Phytochemical Components of *F. sycomorus* leaves extract, *C. nigricans* leaves extract, *V. amagdalina* leaves extract, *F. sycomorus* stem bark extract, *K. senegalensis* stem bark extract and *F. carica* Stem bark extract

	F. sycomorus leaves extract	*C. nigricans* leaves extract	*V. amagdalina* leaves extract	*F. sycomorus* stem bark extract	*K. senegalensis* stem bark extract	*F. carica* stem bark extract
Alkaloids (%)	0.58 ± 0.009^{a}	0.69 ± 0.003^{a}	0.58 ± 0.008^{a}	2.87 ± 0.012^{b}	2.52 ± 0.031^{c}	2.12 ± 0.182^{d}
Tannins (mg TE/g)	3.50 ± 0.001^{a}	*	3.50 ± 0.001^{a}	2.14 ± 0.012^{b}	4.64 ± 0.063^{c}	*
Flavonoids (mg QE/g)	0.68 ± 0.001^{a}	0.90 ± 0.003^{b}	0.68 ± 0.002^{a}	*	*	*
Saponins (%)	*	*	*	4.51 ± 0.004^{a}	*	2.51 ± 0.238^{b}
Volatile Oil (%)	*	*	*	*	4.48 ± 0.073	*
Terpenes (%)	*	*	*	*	*	3.15 ± 0.015

Values are presented as mean ± SEM, n=5, values with different superscript are significantly (P<0.05) different.

The alkaloids content of *F. sycomorus* stem bark (0.58 ± 0.009 %) was considerably (P<0.05) greater than that of other plant extracts investigated, according to the quantitative content of the phytocompounds as displayed in Table 3. However, the alkaloids content of *F. sycomorus* (0.58 ± 0.009 %), *C. nigricans* (0.69 ± 0.003 %), and *V. amygdalina* leaves extracts (0.58 ± 0.00 %) did not vary significantly (P>0.05). There was no significant (P>0.05) change in the tannins content of *F. sycomorus* leaves extract (3.50 ± 0.001 mg TE/g)) and *V. amagdalina* leaves extract (3.50 ± 0.001 mg TE/g), but they were both significantly (P<0.05) higher than that of *F. sycomorus* stem bark (2.14 ± 0.012 mg TE/g) and significantly (P<0.05) lower than that of *K. senegalensis* stem bark extract (4.64 ± 0.063 mg TE/g). For flavonoid content, *C. nigricans* leaves extract (0.90 ± 0.003 mg QE/g) was significantly (P<0.05) higher than the flavonoids content of both *F. sycomorus* leaves extract (0.68 ± 0.001 mg QE/g) and *V. amagdalina* leaves extract (0.68 ± 0.002 mg QE/g). Meanwhile, it was also observed that the saponins' content of *F. sycomorus* stem bark extract (4.51 ± 0.004 %) and *F. carica* stem bark extract (2.51 ± 0.238 %) was significantly (P<0.05) distinct.

Antisickling Assay

It was confirmed that 2 % sodium metabisulfite induced sickling in the blood of SCA patients. Microscopic observation for physiological alterations in sickling was recorded and presented on Plate 1. Subsequently, the sickling reversal assay of the studied plant extracts was observed and presented in Figure 2. The result of induction of sickling with sodium metabisulphite (2 %) showed that *K. senegalensis* stem bark extract had the highest sickling reversal potential (95.29 ± 0.67 %), and its sickling reversal potential was significantly (P < 0.05) higher compared to all the other extracts studied. The *F. sycomorus* leaves extract (91.76 ± 0.71 %), *C. nigricans* leaves extract (92.26 ± 0.71 %), *V. amagdalina* leaves extract (92.19 ± 0.67 %), and *F. sycomorus* stem bark extract (92.11 ± 1.07 %) was not significantly (P > 0.05) different when their sickling reversal potential was compared. However, *F. carica* stem bark extract (88.32 ± 1.55 %) had the least reversal effect.

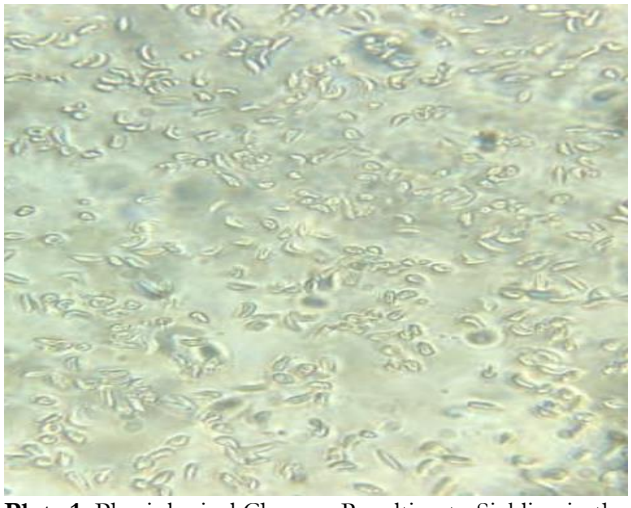

Plate 1: Physiological Changes Resulting to Sickling in the Presence of 2 % Sodium Metabisulfite

Erythrocyte Membrane Stability Activity

Regarding the erythrocyte membrane stability, the result revealed a significant (P<0.05) proportion of the percentage of hemolysis of the red blood cells decreased

while increasing the concentration of the buffered saline solution at 250 µg/mL of each of the extracts (Figure 3). Ranking of the extracts by their ability to stabilize the erythrocyte membrane was calculated by determining the lowest concertation (IC50 %) (Table 4) of the buffered saline that stabilizes 50 % of the RBC membrane in the presence of 250 µg/mL of the extracts. Among the plant extracts examined, *F. carica* stem bark extract showed the best capacity to preserve membrane integrity at (3.98 ± 0.51 %) buffered solution, while *F. sycomorus* stem bark extract showed the lowest at (6.31 ± 0.33 %) buffered saline. The next best extract to stabilize the erythrocyte membrane is *C. nigricans* leaves at (5.01 ± 0.72 %) buffered saline. *K. senegalensis* stem bark (6.02 ± 0.92 %), *V. amagdalina* leaves extract (6.90 ± 0.48 %), *F. sycomorus* stem bark extract (6.31 ± 0.33 %), and *F. sycomorus* leaves (6.17 ± 084 %) were found to be higher than the control (5.62 ± 0.49 %) hemolysis of the RBCs.

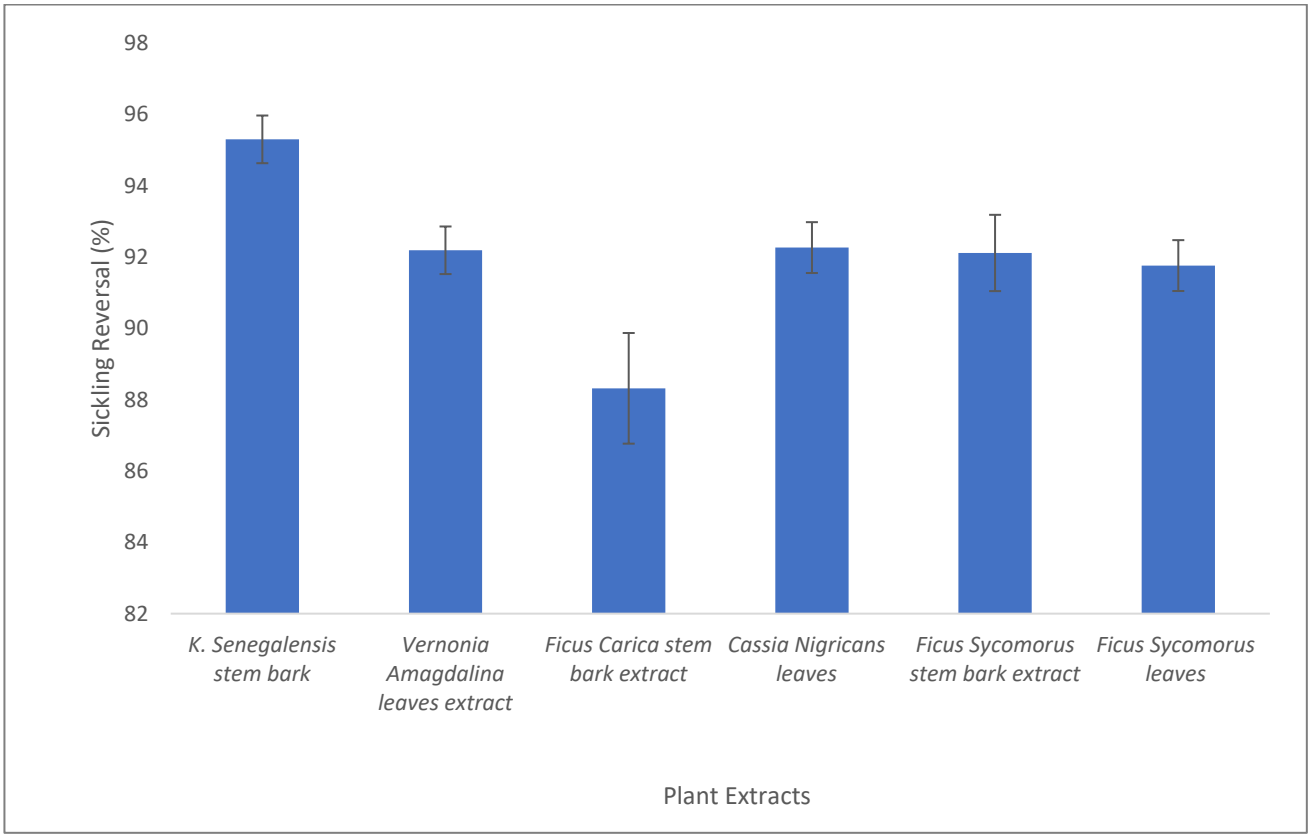

Figure 2: Effect of Sodium Metabisulfite (2 %) and Extracts on Sickling Reversal Ability of Sickle Cell Red Blood Cell

Table 4: IC_{50} of Saline Solution Containing 250 µg/ml of Extract Protecting Erythrocyte Membrane Fragility

Plant extract	**IC_{50}**
K. senegalensis stem bark	6.02 ± 0.92
V. amagdalina leaves extract	6.9 ± 0.48
F. carica stem bark extract	3.98 ± 0.51
C. nigricans leaves	5.01 ± 0.72
F. sycomorus stem bark extract	6.31 ± 0.33
F. sycomorus leaves	6.17 ± 084
Control	5.62 ± 0.49

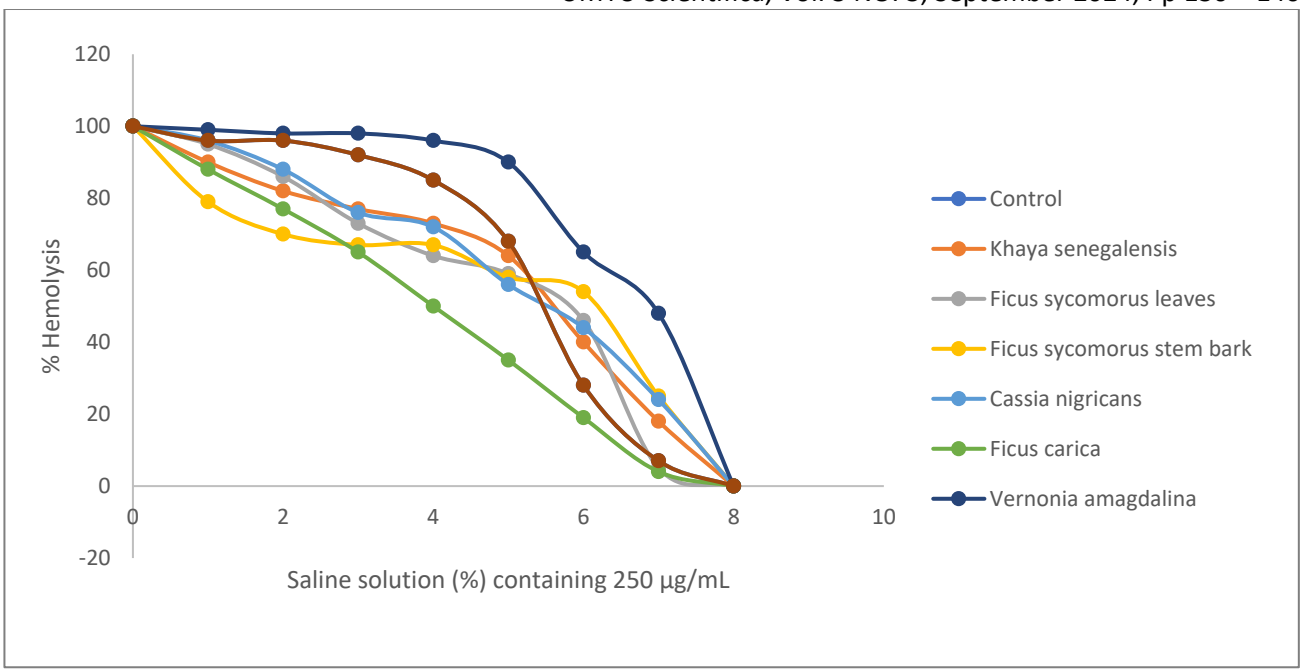

Figure 3. Percentage of Hemolysis of Sickling Cells after Treatment with 250 µg/ml of Extract.

DISCUSSION

Sickle Cell Anemia (SCA) remains a global health concern, affecting populations across diverse geographical regions (Musa *et al.*, 2021). According to numerous studies, SCA is one of the most severe hereditary disorders, posing a significant threat to individuals worldwide (Odame and Jain, 2020; Sachdev *et al.*, 2021; Tebbi, 2022). In the quest for effective therapeutics, the discovery of novel drugs has garnered attention, with a primary focus on exploring the chemical constituents of medicinal plants. Qualitative phytochemical screening of plant extracts serves as a fundamental step in elucidating the pharmacological potential of these natural sources. This research presents a comprehensive analysis of qualitative tests conducted on extracts from *K. senegalensis*, *V. amagdalina, F. carica*, *C. nigricans,* and *F. sycomorus*. The qualitative analysis of various extracts revealed the presence of multiple bioactive compound classes, including alkaloids, flavonoids, tannins, and terpenes. Plant tissues' structural and chemical properties influence the permeability, solubility, and extraction efficiency of these bioactive compounds by different solvents (Pauline *et al.*, 2013).

Consequently, the variations in the qualitative and quantitative composition of phytochemicals in the extracts significantly impact their biological activities (Pauline *et al.*, 2013). Over the past few years, there has been a surge in interest in plant-based medicine attributable to the development of a large number of drugs that are isolated or developed based on natural products (Singh *et al.*, 2020; Süntar 2020; Najmi *et al.*, 2022). Evaluation of medicinal plants based on traditional knowledge presents a strategy for developing novel drugs. Several plant-based drugs/formulations have proven effective in reducing the SCA crisis, successfully demonstrating the reverse sickling properties of medicinal plants (Krasias, 2021; Ohiagu, 2021; Yembeau *et al.*, 2022). Studied plants in this research are very important plants used in the traditional medicinal system in Sokoto State, NorthWestern Nigeria. Therefore, the study assessed the plants' ability to prevent sickling *in vitro*.

All the tested plant extracts displayed the presence of most of the secondary metabolites. Secondary metabolites in plant extracts have long been recognized for their medicinal properties, including alkaloids, flavonoids, tannins, and saponins (Twaij & Hasan, 2022). The presence of these bioactive compounds in tested plant extracts underscores their potential therapeutic significance. Previous research has recognized several medicinal plants, including *Cissus populnea, Vigna unguiculata, Cajanus cajan,* and aloe vera, for their abundance in tannins, saponins, alkaloids, flavonoids, steroids, and phenolic compounds. As supported by authors, these compounds have been correlated with antisickling properties (Elufioye *et al.*, 2020; Joel *et al.*, 2023).

Researchers have identified three principal targets for developing antisickling compounds in the quest for efficacious therapeutic interventions targeting sickle cell anemia. These targets encompass modification of the hemoglobin (Hb) gene, inhibiting HbS polymerization, and enhancing structural resilience within red blood cell membranes (Oder *et al.*, 2016; Mishra *et al.*, 2021). Among the plants examined, *F. carica* stem bark extract emerged as a promising candidate, demonstrating notable membrane stability activity. Compared to control, all plant extracts under investigation showed much greater reversal sickling action, *Khaya senegalensis* is the most potent.

According to reports, hydroxyurea exerts its effects by modulating erythrocyte membrane deformability and influencing hematological parameters, thereby serving as a mechanism to alleviate sickling (Renó *et al.*, 2021). The probable reason for the observed inhibition might be due

to the phytochemicals' interaction with the RBC membrane or interference with the polymerization of Hb molecules. Alkaloids are important secondary metabolites present in all the plant extracts studied in this research, and the presence of flavonoids has been associated with antisickling activities in the bark of *Fagara tesmannii* used extensively in South-West Cameroun (Yembeau *et al.*, 2022). Compared to the control group, the percentage of hemolysis was comparatively lower in *F. carica* stem bark and *C. nigricans* leaves extracts. Red blood cells treated with *F. carica* stem bark extract exhibited a marginally improved resistance to a hypotonic solution. The compounds in the plant extracts might be interacting with the RBC membrane, providing it with more structural integrity.

Reports have shown the antisickling effects of extracts high in phenolic compounds, aromatic amino acids, and greater antioxidant capabilities (Mishra *et al.*, 2021). Most of the extracts of the studied plants were rich in tannins, flavonoids, and alkaloids, which may account for the antisickling properties that were observed.

The findings of the membrane stabilization assay revealed that the plant extracts effectively suppressed the hypotonicity-induced hemolysis of sickle cell trait red blood cells. These results corroborate those of the osmotic fragility assay, wherein the extracts demonstrated a decrease in the percentage of hemolysis of sickle RBC. Previous research suggests that the presence of flavonoids and other phytoconstituents may confer antiinflammatory qualities, even though the precise mode of action of the extract remains elusive (Akinpelu *et al.*, 2017).

Summarily, sickle cell anemia (SCA) continues to pose a significant global health challenge, impacting populations across diverse geographical regions. Extensive research efforts have underscored the severity of SCA and emphasized the urgent need for effective therapeutic interventions. Exploring novel drugs, particularly those derived from medicinal plants, has emerged as a promising avenue for pursuing viable treatments. Our research has contributed to this endeavor by conducting a comprehensive qualitative analysis of plant extracts from *K. senegalensis, V. amagdalina* leaves, *F. carica*, *C. nigricans*, and *F. sycomorus*, revealing the presence of diverse bioactive molecules, including phenolics and alkaloids. These findings are significant, highlighting the potential pharmacological importance of these natural sources in managing sickle cell anemia (SCA).

Furthermore, evaluating medicinal plants based on traditional knowledge offers a valuable strategy for developing novel drugs. Past successes in utilizing plant-based drugs/formulations to mitigate SCA crises highlight the promising therapeutic properties of medicinal plants. Detecting secondary metabolites in the analyzed plant extracts, including alkaloids, flavonoids, tannins, and saponins, underscores their potential therapeutic significance. Previous research has also identified several medicinal plants rich in these bioactive compounds, correlating with their antisickling properties. Additionally, our study has identified promising antisickling activities in extracts such as *F. carica* stem bark, with notable inhibition of sickling observed. The mechanism of action of these extracts may involve interactions with erythrocyte membranes or interference with Hb polymerization, possibly mediated by the presence of alkaloids and flavonoids. The membrane stabilization assay results further support the antisickling potential of the plant extracts, highlighting their ability to suppress hypotonicity-induced hemolysis of sickle cell trait red blood cells. While the precise mechanism of action remains elusive, the presence of flavonoids and other phytoconstituents may confer antiinflammatory properties, contributing to their therapeutic efficacy. This research underscores the importance of exploring natural sources for novel antisickling compounds, offering promising avenues for developing effective treatments for SCA.

CONCLUSION

This study showed that the plants contained varied concentrations of phytocompounds, including flavonoids and alkaloids. They also demonstrated antisickling activity *in vitro* with *F. sycomorus* stem bark extract, the most effective in maintaining the integrity of RBC, while *Khaya Senegalensis* stem bark was the best extract in reversing sickling. The membrane stabilization assay results further support the antisickling potential of the plant extracts, highlighting their ability to suppress hypotonicity-induced hemolysis of sickle cell trait red blood cells. Even though the precise mechanism of action remains elusive, the presence of flavonoids and other phytonutrients may confer antiinflammatory properties, contributing to their therapeutic efficacy.

This study's *in vitro* antisickling action validates traditional healers' usage of these plants, and this action might be attributed to phytocompounds. This research underscores the importance of exploring natural sources for novel antisickling compounds, offering promising avenues for developing effective treatments for SCA.

REFERENCES

Acharya, B., Mishra, D. P., Barik, B., Mohapatra, R. K., & 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*(1). **[Crossref]**

Adigwe, O. P., Onoja, S. O., & Onavbavba, G. (2023). A Critical Review of Sickle Cell Disease Burden and Challenges in Sub-Saharan Africa. *Journal of Blood Medicine*, *14*(April), 367–376. **[Crossref]**

Akinpelu, B. A., Makinde, A. M., Amujoyegbe, O. O., Isa, M. O., Nwobiko, V. C., Akinwotu, A. O., ... & Oyedapo, O. O. (2017). Evaluation of anti-inflammatory and antisickling potentials of Archidium ohioense Schimp. ex Mull extracts. IOSR Journal of Pharmacy and Biolgical Sciences, 12(1), 18. **[Crossref]**

Ali, H.H., Souidi, Z., Benarba, B., & Belhouala, K. (2022). An ethnobotanical study of the traditional uses of medicinal and aromatic plants of the regions of Relizane (Northwest Algeria). International Journal of Environmental Studies, 80, 612 - 634. **[Crossref]**

Amujoyegbe, O. O., Idu, M., Agbedahunsi, J. M., & Erhabor, J. O. (2016). Ethnomedicinal survey of medicinal plants used in the management of sickle cell disorder in Southern Nigeria. *Journal of ethnopharmacology*, *18 5*, 347–360. **[Crossref]**

Busari, A. A., & Mufutau, M. A. (2017). High prevalence of complementary and alternative medicine use among patients with Sickle cell anaemia in a tertiary hospital in Lagos, South West, Nigeria. *BMC complementary and alternative medicine*, *17*(1), 299. **[Crossref]**

Chaitanya Latha, B., Ahalya, S., Divya Naidu, P., Mounica, K., & Ravi Kumar, A. (2017). Phytochemical evaluation of Andrographis paniculata, Cassia angustifolia and Eclipta alba. *Indian Journal of Research in Pharmacy and Biotechnology*, *5*(2), 160-163.

Elufioye, T. O., Williams, B. M., & Cyril-Olutayo, M. C. (2020). Identification of the anti- sickling activity of Anogeissus leiocarpus and in silico investigation of some of its phytochemicals. Avicenna Journal of Medical Biochemistry, 8(1), 1-14. **[Crossref]**

Fernandes, Q. (2017). Therapeutic strategies in Sickle Cell Anemia: The past present and future. *Life Sciences*, *178*, 100–108. **[Crossref]**

Fontana, L., Alahouzou, Z., Miccio, A., & Antoniou, P. (2023). Epigenetic Regulation of β-Globin Genes and the Potential to Treat Hemoglobinopathies through Epigenome Editing. *Genes*, *14*(3). **[Crossref]**

Global Burden of Disease Study 2013 Collaborators (2015). Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet (London, Engla nd)*, *386* (9995), 743–800. **[Crossref]**

Green, N. S., Manwani, D., Qureshi, M., Ireland, K., Sinha, A., & Smaldone, A. M. (2016). Decreased fetal hemoglobin over time among youth with sickle cell disease on hydroxyurea is associated with higher urgent hospital use. *Pediatric Blood & Cancer*, *63*(12), 2146. **[Crossref]**

Harborne J. B. (1999). An overview of antinutritional factors in higher plants. In: Secondary plants products. Antinutritional and beneficial actions in animal feeding Caygill JC and Mueller- Harvey I, eds. Nottingham Univ Press, UK, 7-16.

Harborne, J. B. (2005). Phytochemical Method: A Guide to Modern Techniques of Plant Analysis, 3rd ed., New Delhi; Springer Pvt. Ltd, 5-16, 22.

Indumathi, C., Durgadevi, G., Nithyavani S., & Kothandaraman, G. (2014). Estimation of terpenoid content and its antimicrobial property in Enicostemma litorrale. *International Journal of ChemTech Research*, 6(9), 4264-4267.

Imaga, N.A. (2017). Phytomedicines and nutraceuticals: alternative therapeutics for sickle cell anaemia. **[Crossref]**.

Innocent Iba, Itoro Udo, & Nsima Andy. (2022). Potentials of ethnobotanicals and nutraceuticals in the management of sickle cell disease. *World Journal of Biology Pharmacy and Health Sciences*, *10*(1), 086–102. **[Crossref]**

Inusa, B. P. D., Hsu, L. L., Kohli, N., Patel, A., Ominu-Evbota, K., Anie, K. A., & Atoyebi, W. (2019). Sickle cell disease—genetics, pathophysiology, clinical presentation and treatment. *International Journal of Neonatal Screening*, *5*(2). **[Crossref]**

Joel, A. A., Mireille, K. K. T., Mattieu, K. A. K., Ange, K. G. K. N. D., Marcelin, K. K. A., Sitapha, O. U. A. T. T. A. R. A., ... & Joseph, D. A. (2023). Phytochemical investigation and antisickling properties of a Poly-herbal formula on the HBSS Red Blood Cells. Journal of Pharmacognosy and Phytochemistry, 12(1), 260-265. **[Crossref]**

Khan, S. A., Damanhouri, G. A., Ahmed, T. J., Halawani, S. H., Ali, A., Makki, A., & Khan, S. A. (2022). Omega 3 fatty acids - Potential modulators for oxidative stress and inflammation in the management of sickle cell disease. *Jornal de Pediatria*, *98*(5), 513–518. **[Crossref]**

Krasias, I. (2021). The antisickling properties of medicinal plants, insights in botanical medicine. American Journal of Molecular Biology, 11(4), 165-189. **[Crossref]**

Lins, P. G., Pugine, S.M.P., Scatolini, A.M., & Pires de Melo, M. (2018). In vitro antioxidant activity of olive leaf extract (*Olea europaea L.*) and its protective effect on oxidative damage in human erythrocytes. *Heliyon 4* (2018) e00805. **[Crossref]**

Mishra, S., Sonter, S., Dwivedi, M. K., & Singh, P. K. (2021). Antisickling, anti-inflammatory and anti-oxidant potential of Rubia cordifolia L.: A traditionally used medicinal plant of Amarkantak, Madhya Pradesh (India). Medicinal Plants-International Journal of Phytomedicines and Related Industries, 13(3), 499-514. **[Crossref]**

Munung, N. S., Nembaware, V., de Vries, J., Bukini, D., Tluway, F., Treadwell, M., Sangeda, R. Z., Mazandu, G., Jonas, M., Paintsil, V., Nnodu, O. E., Balandya, E., Makani, J., & Wonkam, A. (2019). Establishing a Multi-Country Sickle cell anaemia Registry in Africa: Ethical Considerations. *Frontiers in genetics* , *10*, 943. **[Crossref]**

Musa, H. H., El-Sharief, M., Musa, I. H., Musa, T. H., & Akintunde, T. Y. (2021). *Scientific African*, *12*, e00774. **[Crossref]**

Muthukumaran, P., Saraswathy, N., & Aswitha, V. (2016). Assessment of Total Phenolic, Flavonoid, Tannin Content and Phytochemical Screening of Leaf and Flower Extracts from *Peltophorum pterocarpum* (DC.) Backer ex K.Heyne: a comparative study. *Pharmacogn. J.* **[Crossref]**

Najmi, A., Javed, S. A., Al Bratty, M., & Alhazmi, H. A. (2022). Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. Molecules, 27(2), 349. **[Crossref]**

National Population Commission (2006) PHC Priority Tables.

Neto, V. M. D. S. (2023). Probing the Antisickling Power of Distinct Drugs in Sickle Cell Disease (Doctoral dissertation).

Nevitt, S. J., Jones, A. P., & Howard, J. (2017). Hydroxyurea (hydroxycarbamide) for Sickle cell anaemia. *The Cochrane database of systematic reviews*, *4*(4), CD002202. **[Crossref]**

Ngbolua, K., Herintsoa, R., Hajatiana, R. Mudogo, D., Tshilanda, D., Tshibangu, D. S. T. & Mpiana, P. T. (2015), "In vitro antierythrocyte sickling effect of lunularic acid of natural origin," *International Blood Research & Reviews*, 4(3), 1–6. **[Crossref]**

Odame, I., & Jain, D. (2020). Sickle cell disease: Progress made & challenges ahead. Indian Journal of Medical Research, 151(6), 505-508. **[Crossref]**

Oder, E., Safo, M. K., Abdulmalik, O., & Kato, G. J. (2016). New developments in anti-sickling agents: can drugs directly prevent the polymerization of sickle haemoglobin in vivo?. British journal of haematology, 175(1), 24-30. **[Crossref]**

Oguntibeju, O. O. (2023). *A Review of the Relationship between the Immune Response , Inflammation , Oxidative Stress , and the Pathogenesis of Sickle Cell Anaemia.*

Ohiagu, F., Chikezie, P., & Chikezie, C. (2021). Sickle hemoglobin polymerization inhibition and antisickling medicinal plants. J. Phytopharmacol, 10, 126-133. **[Crossref]**

Okeowo, G., & Fatoba, I. (2022). "State of States 22 Edition" (PDF). *Budgit.org*. BudgIT.

Oniyangi, O., & Cohall, D. H. (2020). Phytomedicines (medicines derived from plants) for sickle cell disease. *Cochrane Database of Systematic Reviews*, (9). **[Crossref]**

Paintsil, V., Amuzu, E. X., Nyanor, I., Asafo-Adjei, E., Mohammed, A. R., Yawnumah, S. A., Oppong-Mensah, Y. G., Nguah, S. B., Obeng, P., Dogbe, E. E., Jonas, M., Nembaware, V., Mazandu, G., Ohene-Frempong, K., Wonkam, A., Makani, J., Ansong, D., Osei-Akoto, A., & Sickle cell anaemia in Sub-Saharan Africa Consortium (2022). Establishing a Sickle cell anaemia Registry in Africa: Experience from the Sickle Pan-African Research Consortium, Kumasi-Ghana. *Frontiers in genetics*, *13*, 802355. **[Crossref]**

Rankine-Mullings, A. E., & Owusu-Ofori, S. (2017). Prophylactic antibiotics for preventing * pneumococcal infection in children with Sickle cell anaemia. *The Cochrane database of systematic reviews*, *10*(10), CD003427. **[Crossref]**

Renó, C. O., Maia, G. A. S., Nogueira, L. S., de Barros Pinheiro, M., Rios, D. R. A., Cortes, V. F., & de Lima Santos, H. (2021). Biochemical evaluation of the effects of hydroxyurea in vitro on red blood cells. Antioxidants, 10(10), 1599. **[Crossref]**

Sachdev, V., Rosing, D. R., & Thein, S. L. (2021). Cardiovascular complications of sickle cell disease. Trends in cardiovascular medicine, 31(3), 187-193. **[Crossref]**

Singh, B., Singh, B., Kishor, A., Singh, S., Bhat, M. N., Surmal, O., & Musarella, C. M. (2020). Exploring plant-based ethnomedicine and quantitative ethnopharmacology: Medicinal plants utilized by the population of Jasrota Hill in Western Himalaya. Sustainability, 12(18), 7526. **[Crossref]**

Süntar, I. (2020). Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. *Phytochemistry Reviews*, *19*(5), 1199-1209. **[Crossref]**

Tebbi, C. K. (2022). Sickle cell disease, a review. Hemato, 3(2), 341-366. **[Crossref]**

The World Medical Association (2008) "Declaration of Helsinki: ethical principles for medical research involving human subjects," pp. 1–5. **[Crossref]**

Twaij, B. M., & Hasan, M. N. (2022). Bioactive secondary metabolites from plant sources: types, synthesis, and their therapeutic uses. *International Journal of Plant Biology*, *13*(1), 4-14. **[Crossref]**

Vaishnava, S., & Rangari, V.D. (2016). A review on phytochemical and pharmacological research - remedy for Sickle cell anaemia. *International Journal Pharmaceutical Science Research*, 7(2):472-81. **[Crossref]**

Virot, M., Tomao, V., Colnagui, G., Visinoni, F. & Chemat, F. (2007). New microwave-integrated Soxhlet extraction. An advantageous tool for the extraction of lipids from food products, *J. Chromatogr.* A 1174-138e144. **[Crossref]**

WHO, (2013). Centers for Disease Control and Prevention. *Lancet,* **381**: 142-51.

Yembeau, N. L., Biapa Nya, P. C., Pieme, C. A., Tchouane, K. D., Kengne Fotsing, C. B., Nya Nkwikeu, P. J., ... & Telefo, P. B. (2022). Ethnopharmacological Study of the Medicinal Plants Used in the Treatment of Sickle Cell Anemia in the West Region of Cameroon. Evidence-Based Complementary and Alternative Medicine, 2022. **[Crossref]**