African Journal of
Biochemistry and Molecular Biology Research

ISSN : 1595-7926

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJBMBR.v3i2.10474

Phytochemical and Anti-Sickling Properties of Aqueous Extract of *Pennisetum purpureum* Schumach (Elephant Grass) Shoots

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Apr 22, 2026	May 20, 2026	Jun 1, 2026	Jun 6, 2026

Abstract

Sickle cell disease remains a major haematological disorder associated with haemoglobin S polymerization and erythrocyte sickling, highlighting the need to explore plant-derived compounds with potential anti-sickling activity. This study aimed to determine the anti-sickling potential of the aqueous extract of young shoots of *Pennisetum purpureum* using microscopy after 2% sodium metabisulphite-induced sickling of sickle red blood cells. The findings showed that *Pennisetum purpureum* extract significantly reversed many sickled cells to normal-shaped cells, with the percentage reversal depending on both concentration and exposure time. At 250 µg/mL, sickle cell reversal was 88.82% at 0 min, 86.09% at 30 min, and 85.19% at 60 min, whereas the control without extract showed no reversal. At 500 µg/mL, the reversal percentages were 80.14% at 0 min, 92.27% at 30 min, and 90.51% at 60 min. At 1000 µg/mL, reversal increased from 79.13% at 0 min to 94.82% at 30 min and 96.50% at 60 min. Phytochemical analysis indicated the presence of aromatic and alkylic compounds, including quercetin, epicatechin, resveratrol,

vanillic acid, ellagic acid, and kaempferol. These compounds may contribute to anti-sickling activity through hydrophobic interactions that interfere with haemoglobin S polymerization and stabilize the hydrophobic erythrocyte lipid bilayer membrane. This study contributes to phytomedicine and sickle cell research by demonstrating the potential of *Pennisetum purpureum* young shoot extract as a source of bioactive compounds with anti-sickling properties, although further biochemical, toxicological, and clinical validation is required.

Keywords: *Pennisetum purpureum*; Anti-Sickling Activity; Sickle Cell Disease; Phytochemical Compounds; Haemoglobin S Polymerization.

INTRODUCTION

A terrible hereditary condition that primarily affects people of African origin is sickle cell disease (Elendu *et al.,* 2023). It results from a mutation in the beta subunit of haemoglobin where valine replaces the normal amino acid glutamic acid at the sixth residue, producing an aberrant haemoglobin, haemoglobin S (Hoban *et al.,* 2016). Deoxyhaemoglobin polymerizes and distorts red blood cells in the absence of oxygen, causing them to become rigid and encased in tiny blood vessels, obstructing capillaries and microvasculatures. These abnormal cells which block the vessels, are less flexible, leading to vaso-occlusive crises, tissue damage, and many complications (Patel *et al.,* 2023). It becomes difficult for organs to receive oxygen, causing stroke, pain at bone joints, myocardiac infarctions, which can lead to sickle cell crises and death (Nelson *et al.,* 2022).

In the developed countries such as the United States, sickle cell disease is managed with medications that are primarily aromatic compounds. Some of these drugs are hydroxyurea, Voxelotor and L-Glutamine (Vichinsky *et al.,* 2019). An advanced stage of treatment is a bone marrow transplant, which entails introducing the normal beta globin gene into sickle cell patients' bone marrow cells. These cells will develop into normal bone marrow cells that can generate normal red blood cells and result in a cure (Luzzatto, 2023). The bone marrow is the site for erythrocyte production. However, conventional medicine is expensive and has side effects. The cost of carrying out bone marrow transplant is thousands of dollars and even most Americans cannot afford it. In Nigeria and Africa in general, such treatments are not feasible for most sicklers at this time. As a result, there is need for alternative means to manage sickle cell anaemia.

Many plants have been useful and effective in treating many ailments in Africa (Wink, 2015). In recent years, the use of plants has garnered attention as potential adjunctive therapies for sickle cell disease (Mukherjee *et al.,* 2022). Studies have demonstrated that crops such as *Cajanus cajan* (Osuagwu, 2010), *Carica papaya* (Mojisola *et al.,* 2009) etc., can be used to manage sickle cell disease. Many plants can be tested for anti-sickling properties, which will help identify those that have anti-sickling potentials.

Pennisetum purpureum is a perennial tropical grass that is rich in protein and contains a variety of minerals, vitamins, anti-oxidants and phytochemicals that support the immune system by scavenging poisons (Okoye, 2023). *Pennisetum purpureum* is typically used as a forage, an ornamental plant, and to prevent erosion. However, in some part of Igbo speaking people of south eastern Nigeria, it has been a traditional prehistoric delicacy where it is commonly called 'achara'. The young shoots and leaves are consumed in soups among these Igbo communities and can also be boiled to make tea.

This study investigated the anti-sickling potential and phytochemical constituents of aqueous extract of *Pennisetum purpureum* shoots.

MATERIALS AND METHODS

Collection and Preparation of Plant Extracts

Fresh *Pennisetum purpureum* shoot was purchased from a reputable vendor at Ogige market at Nsukka town in Enugu state. The plant sample was identified at the University of Nigeria, Nsukka Herbarium in the Department of Plant Science and Biotechnology and was given a voucher number (UNN/13151). Extraction of the plant was done according to Olutayo *et al.* (2018). The shoots of *Pennisetum purpureum* were chopped into tiny pieces and weighed. Exactly 300 grams of the chopped sample was homogenized with 600 mL of distilled water with an electric blender. The resulting mesh was sieved and filtered to obtain the aqueous extract which was transferred into vials and stored in a refrigerator.

Blood Collection

Haemoglobin A and S were obtained from blood drawn from two volunteers from St. Anthony's Medical Diagnostic Laboratory, Nsukka, whose genotypes were ascertained by cellulose acetate electrophoresis at pH 8.6. Using a sterile 5-cc syringe, the blood was extracted from a visible vein and put into tubes containing EDTA to avoid clotting while

maintaining the integrity of the blood samples. The blood samples were kept in an ice-filled cooling flask.

Washing of Red Blood Cells

The red blood cells were washed three times with 1 % NaCl and centrifuged at 4000 rpm using a lab centrifuge, with the supernate (serum) being disposed after each wash with the aid of a Pasteur pipette. The red blood cells at the bottom of the test tube were used for the microscopy test.

Microscopy of the Aqueous Extract of *Pennisetum purpureum* Shoots on SS Red Blood Cells

To investigate the anti-sickling properties of the aqueous extract of the shoot of *Pennisetum purpureum*, a drop of 2 % sodium metabisulphite was added to a drop of SS red blood cells and placed on the slides of a bright field microscope with x40 lens magnification. The role of the 2 % sodium metabisulphite was to deoxygenate the red blood cells and convert them to all sickled shape. A picture was taken as the control. A drop of 2 % sodium metabisulphite and SS red blood cells were added to different doses of the plant extracts (250 µg/mL, 500 µg/mL and 1000 µg/mL) on the slide and covered with a cover slip. The pictures of the slides containing the different concentrations of the extracts mixed with the deoxygenated SS red blood cells were taken at different time intervals (0, 30 and 60 minutes). The red blood cell shapes were observed and compared to the control.

Quantification of Phytochemicals by Gas Chromatography-Flame Ionization Detector

The analysis of phytochemicals was performed on a BUCK M910 gas chromatography equipped with a flame ionization detector.

RESULTS

Phytochemicals Present in the Aqueous Extract of *Pennisetum purpureum* Shoot.

The result presented in table 1 below indicates the phytochemicals present in *Pennisetum purpureum* aqueous shoot extract as detected using the gas chromatography-flame ionization detector. Twenty-four compounds were detected including quercetin, catechin, gallocatechin, resveratrol, vanillic, and ellagic acid. Most of these compounds are aromatic, alkylic and therefore are hydrophobic. These aromatic and alkylic compounds

may show intracellular anti-sickling activity by interfering with the hydrophobic process of haemoglobin S polymerization. The aromatic and alkylic compounds by their hydrophobicity may also play an anti-sickling role on the erythrocyte lipid bilayer membrane.

Table 1: Phytochemicals present in the Aqueous extract of *Pennisetum purpureum* shoot

Ret Time [min]	Area [pA *s]	Amt/Area	Amount [ppm]	Name
2.238	2.05171	1.36613e-1	2.80290e-1	Kaempferol
4.081	4.09096	1.38567e-1	5.66872e-1	Catechin
5.295	4.87321	1.38829e-1	6.76541e-1	Quercetin
6.089	23.27275	1.40214e-1	3.26317	Luteolin
7.092	26.47255	1.40302e-1	3.71416	Artemetin
8.015	26.02094	1.40283e-1	3.65030	Retusin
8.870	26.63064	1.40254e-1	3.73506	Ellagic acid
9.672	39.15755	1.40370e-1	5.49654	Vanillic acid
10.427	52.35582	1.40312e-1	7.34616	Apigenin
11.142	26.04455	1.40231e-1	3.65226	Naringenin
11.815	16.54009	1.39995e-1	2.31553	Hesperidin
12.456	11.60985	1.39821e-1	1.62330	Isorhamnetin
13.063	12.00846	1.39789e-1	1.67862	Myricetin
13.640	9.46470	1.39508e-1	1.32040	Epicatechin
14.191	8.99107	1.39458e-1	1.25388	Daidzein
14.716	7.93991	1.37551e-1	1.09214	Genistein
15.218	7.09294	1.39093e-1	9.86580e-1	Apigenin
15.697	6.49283	1.38974e-1	9.02332e-1	Linamarin
16.156	6.12330	1.38854e-1	8.50246e-1	Gallocatechin
16.596	5.26150	1.38453e-1	7.28470e-1	Resveratrol
17.426	3.81342	1.37935e-1	5.26005e-1	Tangeretin
18.192	2.25179	1.32176e-1	2.97633e-1	Naringin
18.556	2.33815	1.37002e-1	3.20333e-1	Hesperidin
19.309	2.17443	1.33989e-1	2.91350e-1	Silymarin
Total:			**47.03401**	

Anti-Sickling Potential of Aqueous Extract of Shoot of *Pennisetum purpureum*

Results from figure 1 to 3 indicate the anti-sickling potential of *Pennisetum purpureum* aqueous shoot extract on SS red blood cells for 250, 500, and 1000 µg/mL concentrations. The percentage of sickling was calculated for the control (2 % sodium metabisulphite + SS red blood cells). In comparison, the percentage of reversal of sickled red blood cells to normal red blood cells was calculated upon adding the different concentrations of *Pennisetum purpureum* aqueous shoot extract (2 % sodium metabisulphite + SS red blood cells + plant extract). The *Pennisetum purpureum* aqueous shoot extract reversed many of the sickled red blood cells to normal red blood cells with the highest concentration (1000 µg/mL) showing the highest percentage reversal of sickling of 96.50 % in 60 minutes when

compared to the control (with 100 % sickled cells). Other concentrations of *Pennisetum purpureum* aqueous shoot extract (250 µg/mL and 500 µg/mL) also showed a significant anti-sickling effect. This revealed that the anti-sickling potential of the extract was concentration and time dependent.

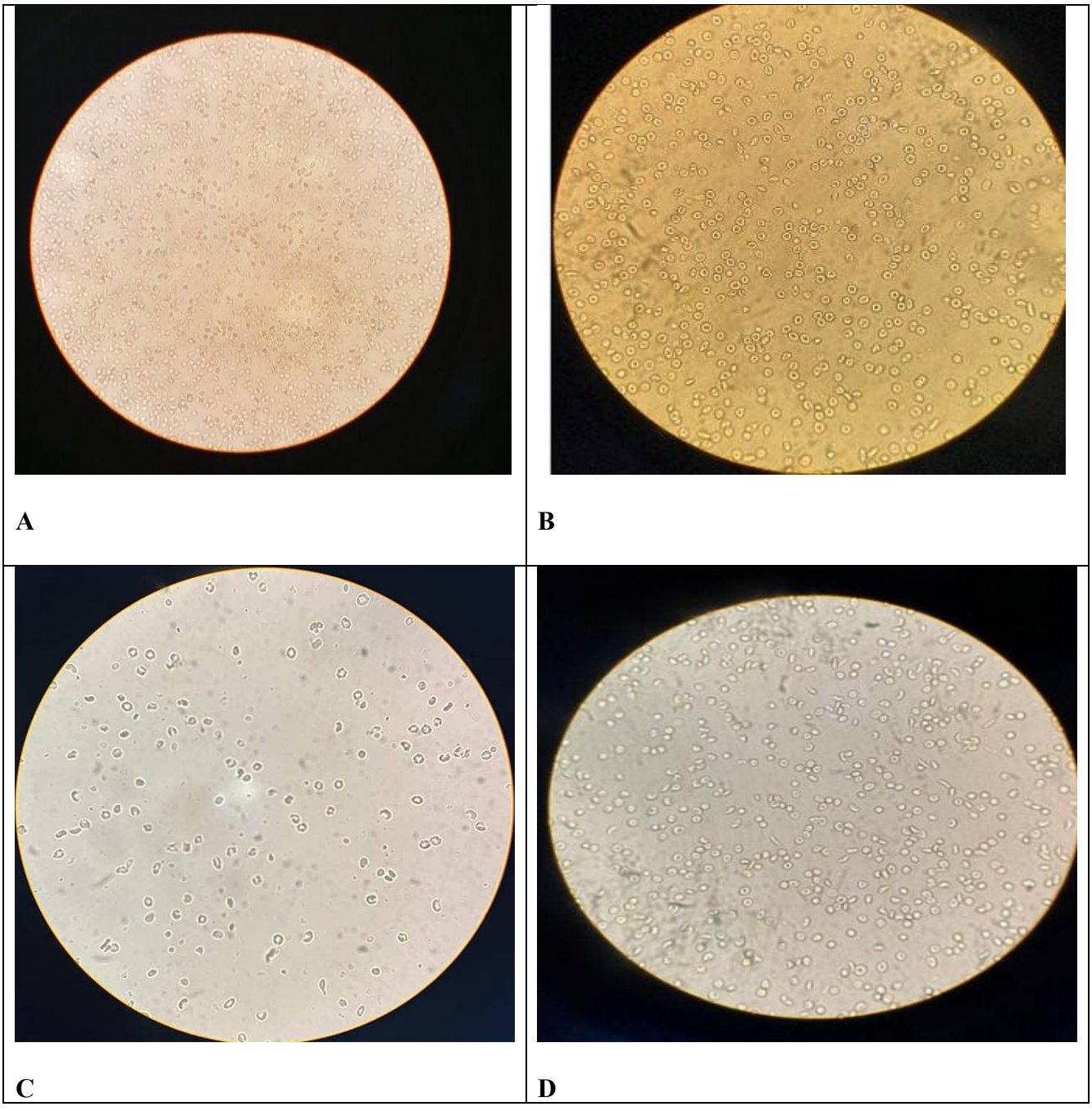

Figure 1: Microscopy of 250 µg/mL of aqueous extract of shoot of *Pennisetum purpureum* on SS red blood cells at 0,30 and 60 minutes.

A is the control (2% sodium metabisulphite + SS red blood cells, % of sickled red blood cells = 100. B showed 250 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 2% sodium metabisulphite + SS red blood cells at 0 minute, % of reversed sickled red blood cells = 88.82. C showed 250 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 2 % sodium metabisulphite + SS red blood cells at 30 minutes, % of reversed sickled red blood cells = 86.09. D showed 250 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 2 % sodium metabisulphite + SS red blood cells at 60 minutes, % of reversed sickled red blood cells = 85.19.

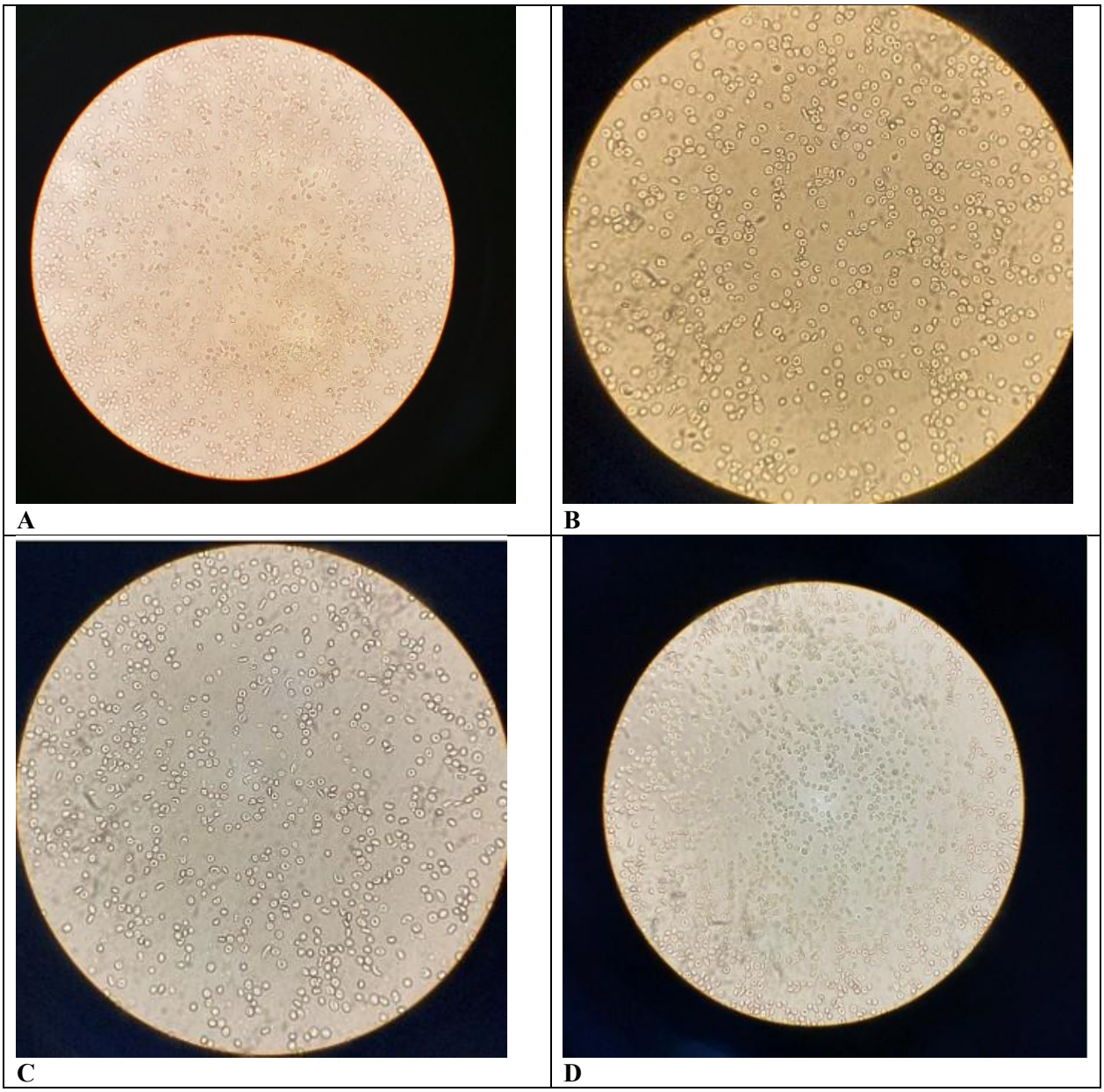

Figure 2: Microscopy of 500 µg/mL of the aqueous extract of shoot of *Pennisetum purpureum* on SS red blood cells at 0, 30 and 60 minutes.

A is the control (2% sodium metabisulphite + SS red blood cells), % sickled red blood cells = 100. B showed 500 µg/mL of *Pennisetum purpureum* aqueous shoot extract + 2 % Sodium metabisulphite + SS red blood cells at 0 minute, % reversed sickled red blood cells = 80.14. C showed 500 µg/mL of *Pennisetum purpureum* aqueous shoot extract + 2 % Sodium metabisulphite + SS red blood cells at 30 minute, % reversed sickled red blood cells = 92.27. D showed 500 µg/mL of *Pennisetum purpureum* aqueous shoot extract + 2 % Sodium metabisulphite + SS red blood cells at 60 minute, % reversed sickled red blood cells = 90.51.

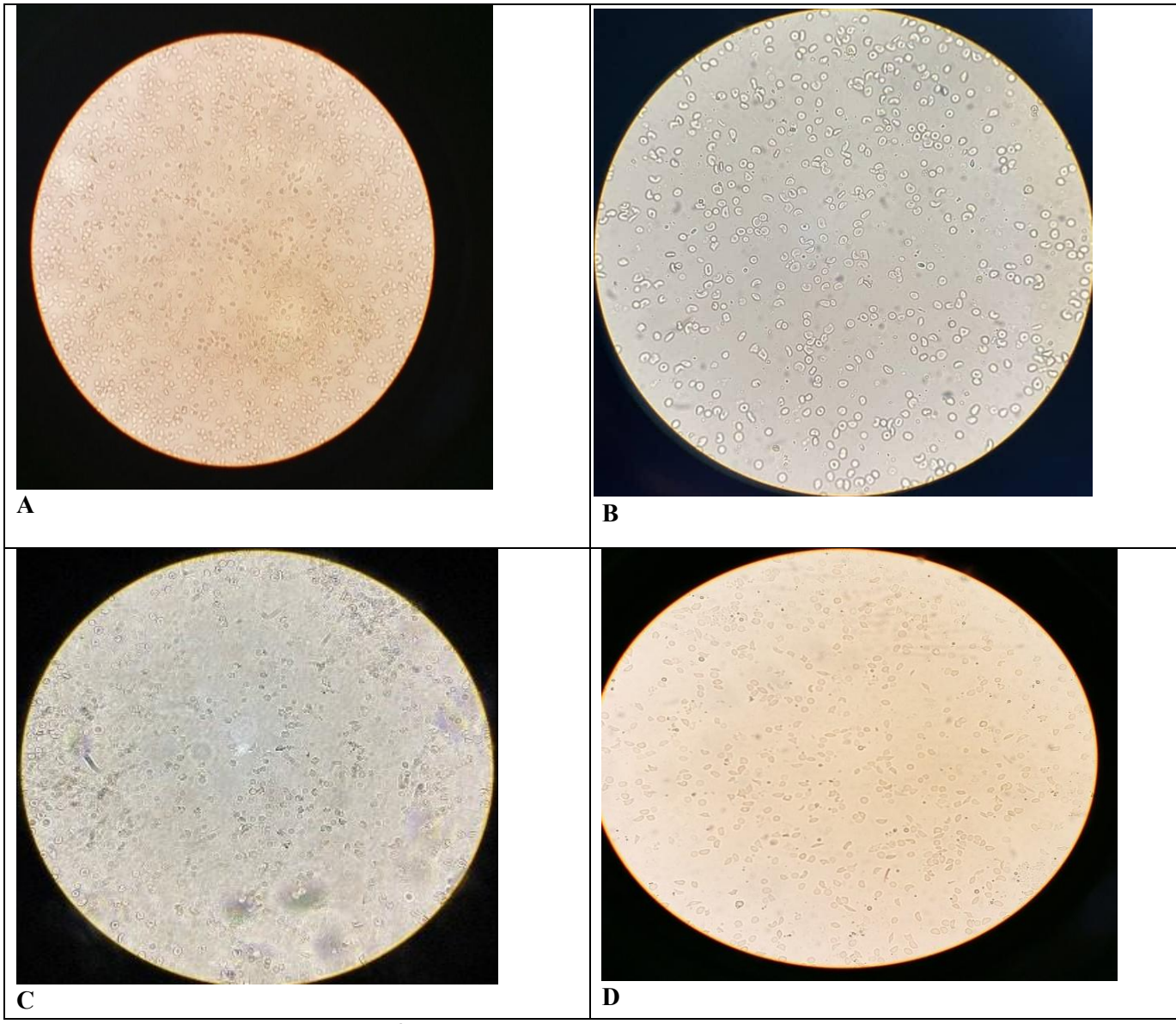

Figure 3: Microscopy of 1000 µg/mL of the aqueous extract of shoot of *Pennisetum purpureum* on SS red blood cells at 0, 30 and 60 minutes.

A is the control (2% Sodium metabisulphite + SS red blood cells), % sickled red blood cells = 100. B showed 1000 µg/mL of *Pennisetum purpureum* aqueous shoot extract + Sodium metabisulphite + SS red blood cells at 0 minute, % reversed sickled red blood cells = 79.13. C showed 1000 µg/mL of *Pennisetum purpureum* aqueous shoot extract + 2 % Sodium metabisulphite + SS red blood cells at 30 minutes, % reversed sickled red blood cells = 94.82. D showed 1000 µg/mL of *Pennisetum purpureum* aqueous shoot extract + Sodium metabisulphite + SS red blood cells at 60 minutes, % reversed sickled red blood cells = 96.50.

DISCUSSION

The experimental results presented in this work provide evidence that the shoot of the aqueous extract of *Pennisetum purpureum* has anti-sickling potentials. The data from microscopic studies was obtained by the application of 2 % sodium metabisulfite which induces the sickling of haemoglobin S red blood cells through deoxygenation (Chikezie, 2011).

The presence of the aqueous shoot extract of *Pennisetum purpureum* showed significant reversal of sickled cells to normal cells that was concentration dependent with the highest concentration of the extract (1000 µg/mL) exhibiting the greatest percentage of reversal of sickled red blood cells of 96.50 % at 60 minutes when compared to the control with 100 % sickled cells.

The anti-sickling potentials determined by microscopy may reflect the quantity and quality of diverse aromatic and alkylic bio-active molecules contained by the aqueous shoot extract of *Pennisetum purpureum.*

The phytochemical data obtained from screening samples of the aqueous shoot extract of *Pennisetum purpureum* revealed the presence of bio-active compounds such as polyphenols, flavonoids, alkaloids, tannins, saponins. Some of them are resveratrol, quercetin, kaempferol, naringenin, genistein and vannilic acid. Aromatic and alkylic functional groups have been demonstrated to contribute to the anti-sickling potentials of bio-active compounds. The work of Ekeke and Shode through the phytochemical screening of *Cajanus cajan* seeds revealed the presence of phenylalanine as its anti-sickling agent (Ekeke and Shode, 1985).The search for aromatic and alkylic functional groups of bio-active molecules from plants as potential anti-sickling agents requires knowledge of the

molecular structure of sickle cell haemoglobin, oxy-haemoglobin and the erythrocytes multi-functional plasma lipid bilayer membrane (Pagare *et al.,* 2024).

Haemoglobin has four subunits of two alpha and two beta globins. Each containing a haem prosthetic group with four Fe^{2+} atoms in which each cooperatively bind and release molecular oxygen to the tissues for metabolism (Chiabrando *et al.,* 2014).

In 1949, Linus Pauling, through x-ray diffraction data, illustrated that the sickle cell disease mutation which by natural selection of the gene, conferred a health advantage against malaria infections to its carriers. The mutation was on the beta globin gene resulting in the substitution of an abnormal amino acid valine, an alkylic hydrophobic force enhancer that replaced the normal amino acid glutamic acid at the sixth residue of the beta subunit (Pauling *et al.,* 1949). Upon deoxygenation, the abnormal sickle haemoglobin with each abnormal valine hydrophobic enhancer form polymers with other contingent deoxy-haemoglobin S polymers and forms hydrophobic association with aromatic macromolecules which interact with the red blood cell plasma membrane and distorts the cell from its spherical form to a sickle shape. The hydrophobic contact points between the abnormal valine residue at the sixth position of each of the four beta subunits and the lipid aromatic and alkylic molecules that make up the lipid bilayer of the erythrocyte plasma membrane are consequently thought to be competitive sites for the bio-active molecules from these plants to initiate their anti-sickling action (Chikezie *et al.,* 2013). Thus, the mechanism of reversal of sickled erythrocytes may result from the competitive hydrophobic interactions of the bio-active molecules from the plant extracts to the inherent hydrophobic macromolecules that form the cytoskeleton of the erythrocyte membrane. Also, their competitive action with the abnormal valine molecules at the sixth residue of the beta-chains may shorten and inhibit further polymerization of deoxy-haemoglobin S as an anti-sickling measure.

CONCLUSION

The study showed that *Pennisetum purpureum* aqueous shoot extract had visible anti-sickling effect. Phytochemical analysis revealed the presence of aromatic and alkylic compounds which might be contributing to intracellular anti-sickling activity by interfering with haemoglobin S polymerization and may also play an anti-sickling role on the

erythrocyte lipid bilayer membrane. Hence, *Pennisetum purpureum* aqueous shoot extract can be used as a natural therapeutic alternative for managing sickle cell disease.

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