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Oxidative and Haemolytic Properties of Aqueous Extract of *Pennisetum purpureum* Shoots as Remedy to Sickle Cell Disease

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Abstract

Reported anti-sickling activity of *Pennisetum purpureum* shoots has prompted interest in their effects on haemoglobin oxidation and erythrocyte stability. This study assessed the oxidative and haemolytic effects of an aqueous extract of *P. purpureum* shoots. UV–visible spectroscopy was performed over 400–700 nm to monitor oxyhaemoglobin absorbance at 540 and 576 nm and methaemoglobin formation at 630 nm. Samples were examined in the absence of extract and at extract concentrations of 250, 500, and 1,000 µg/mL, with measurements obtained at 0, 30, 60, and 90 minutes and 24 hours. Erythrocyte lysis was assessed spectrophotometrically across a saline concentration gradient. In the presence of the extract, the oxyhaemoglobin absorbance ratio (A576/A540) decreased from 1.04 to 0.91, while absorbance at 630 nm increased from 0.05 to 0.42, indicating methaemoglobin formation. Haemoglobin oxidation increased with extract concentration and incubation time. The haemolysis assay also indicated an erythrocyte-stabilising effect. These findings demonstrate concentration- and time-dependent haemoglobin oxidation alongside erythrocyte-stabilising activity under the tested conditions. The study provides preliminary laboratory evidence to guide further investigation of the extract's potential relevance to sickle cell disease management.

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Keywords: *Pennisetum purpureum*; Haemoglobin Oxidation; Methaemoglobin; Haemolysis; Sickle Cell Disease

INTRODUCTION

Sickle cell disease is a debilitating genetic disorder that affects over 12 % of Africans and people of African descent (Hassel, 2010). The disease is due to the production of abnormal haemoglobin S, which is caused by a mutation in the beta globin gene where adenine is replaced by thymine at codon 6 of the beta globin gene (Arishi *et al.,* 2021) which causes a substitution of glutamic acid by valine at the sixth residue (Ma *et al*., 2023). This causes an aberrant form of haemoglobin called haemoglobin S. This change in the sicklers red blood cells is due to the hydrophobic bonds formed by valine (Rees *et al.,* 2010).

Under conditions of low oxygen, the red blood cells become rigid and get trapped in small blood vessels, triggering acute painful episodes, depriving tissues of oxygen-rich blood, blocking the capillaries and micro-vasculatures, making it difficult for organs to receive oxygen and damaging organs such as spleen, heart, liver, lungs and kidneys which can lead to sickle cell crises and death (Hassel, 2010).

In developed nations, drugs which are mostly aromatic compounds are used to manage sickle cell disease such as crizanlizumab, voxelotor and hydroxyurea (Migotsky *et al.,* 2022). An advanced stage of treatment is by using bone marrow transplant, which involves transplanting the beta globin gene into premature sickle cell bone marrow which will mature into normal bone marrow and lead to cure. The bone marrow is the site for the synthesis of red blood cells (Pelley, 2007). Conventional medicine is expensive, and the cost of carrying out bone marrow transplant is very high. In Nigeria and Africa in general, such treatments are not feasible for most sicklers especially in rural communities. As a result, there is need for a cheap and alternative means to manage sickle cell anaemia.

Many plants have been useful and effective in treating various ailments in Africa (Gurib-Fakim, 2006). Research has shown that sickle cell disease can be managed with crops such as *Cajanus cajan* (Osuagwu, 2010). Some of these crops might oxidize haemoglobin and cause haemolysis of red blood cells, leading to deleterious and aggravated condition among sickle cell patients, who resort to self-help and treatment to manage their sickle cell ailment. Therefore, it is necessary to assess their oxidative capacities and haemolytic potentials.

Pennisetum purpureum is a perennial tropical grass native to the African grasslands. It belongs to the family poaceae (Paudel *et al.,* 2018). In Nigeria, *Pennisetum purpureum* is commonly called Ciyawa-giwa in Hausa, Achara in Igbo, and fafa in Yoruba, and especially used in the Southeastern Nigeria as vegetable in soup (Okoye, 2023). *Pennisetum purpureum* is rich in protein and contains a variety of minerals and vitamins including vitamin C, calcium, potassium and magnesium, as well as antioxidants and phytochemicals that support the immune system by scavenging dangerous poisons and helps to protect the body from infections (Okoye, 2023). Therefore, as a remedy to sickle cell disease management it has the advantage of a desirable and common-food crop in the sub-Saharan region of Africa, where toxicity concerns are considered of minor importance.

In this work, the oxidative and haemolytic effect of the aqueous shoot extract of *Pennisetum purpureum* is accessed by UV-Visible spectroscopy and haemoglobin under saline gradient stabilization.

MATERIALS AND METHODS

Collection and Preparation of Plant Extracts

The fresh shoot of *Pennisetum purpureum* was purchased from a reputable vendor in Ogige market at Nsukka in Enugu State, Nigeria. The plant sample was identified at the University of Nigeria, Nsukka Herbarium in the Department of Plant Science and biotechnology with a voucher number (UNN/13151). The plant extracts were prepared according to the methods of Olutayo *et al.* (2018). The fresh shoot of *Pennisetum purpureum* was chopped into small chunks. Exactly 300 grams of the chopped sample was weighed and homogenized with 600 mL of distilled water using an electric blender. The aqueous extract of *Pennisetum purpureum* was obtained by sieving and filtering the resultant mesh with a stainless steel 80 mesh lab sieve with a pore size of 0.2 mm and a Whatman no. 1 filter paper with a pore size of 11 µm. Then it was put into vials and kept in a refrigerator.

Blood Collection

Two volunteers from St. Anthony's Medical Diagnostic laboratory in Nsukka provided two venous blood samples (AA and SS), which were then verified by electrophoresis using cellulose acetate paper and cellulose acetate buffer at pH 8.6. Using a sterile 5-cc syringe, the blood was extracted from a visible vein and placed in tubes

containing EDTA to avoid clotting and maintain the integrity of the blood samples. The blood samples were kept in an ice-filled cooling flask.

Washing Blood Cells

The red blood cells were washed 3 times using 1 % normal saline. The solution with the blood and saline was thoroughly mixed by shaking and centrifuged at 4000 rpm for 10 minutes. After centrifugation, the serum was removed using a Pasteur pipette. The washing procedure was repeated 3 times to ensure that the plasma components were eliminated. The bottom of the tube which contained the red blood cells was used for further analysis.

Extraction of Haemoglobin from Haemolysate Using Sephadex Chromatography

The washed red blood cells were lysed with distilled water to obtain the haemolysate. Sephadex G-25 chromatography was used to extract the haemoglobin from the haemolysate eliminating the membrane and other materials present. 10 mM potassium phosphate buffer with pH 7.8 was used to equilibrate the column. The haemolysate was carefully added on top of the column and the haemoglobin eluted out.

Oxidative Studies of *Pennisetum purpureum* Aqueous Shoot Extract on Haemoglobin Using UV-Visible Spectroscopy

UV-Visible spectroscopy was applied to investigate the oxidative effect of *Pennisetum purpureum* aqueous shoot extract on haemoglobin A and S. UV-Visible spectroscopy was carried out using the method of Lui *et al.* (2018). The instrument was set at the range of 400 nm to 700 nm. Normal oxy-haemoglobin spectra shows the presence of 3 peaks at 540 nm, 576 nm with methaemoglobin in the oxidized Fe^{3+} species at 630 nm. The peaks at 540 nm and 576 nm represent the binding of oxygen to ferrous iron (Fe^{2+}). The 630 nm band reflects oxidation of oxyhaemoglobin to ferric (Fe^{3+}) or methaemoglobin which has lost its capacity to bind oxygen (Ezeawgula and Onwubiko, 2016). Exactly 900 µL of potassium phosphate buffer was used as the baseline. The oxyhaemoglobin spectra was obtained by diluting 100 µL of haemoglobin A and S in 900 µL of the same buffer. Exactly 100 µL of the haemoglobin was added to the different concentrations of the *Pennisetum purpureum* aqueous shoot extract and observed for 0, 30, 60, 90 minutes and 24

hours. The absorbance ratio of the β peak (576 nm) and α peak (540 nm) was recorded. The absorbances at the 630 nm band of oxidation were evaluated.

Haemolytic Study of Aqueous Shoot Extract of *Pennisetum Purpureum* on SS Red Blood Cells

In the haemolytic study of aqueous shoot of *Pennisetum purpureum,* eleven test tubes with different volumes of saline and distilled water were used. The test tubes were labelled 0-10. Exactly 100 µL of SS red blood cells were added to each test tube and used as the control. The *Pennisetum purpureum* aqueous shoot extract was tested at its highest concentration (1000 µg/mL). 100 µL microliters of *Pennisetum purpureum* aqueous shoot extract was added to each test tube. UV-visible spectroscopy was used to detect how fast the red blood cells were breaking and the concentration of the haemoglobin being released in the solution. The absorbance of the selected peak at 577 nm of haemoglobin was plotted against the test tube number with the saline/distilled water gradient. A slope indicated how the red blood cells were breaking. The slope will be straight if the red blood cells were not breaking. If the red blood cells were breaking, the slope will be downwards.

RESULTS

Effect of the Aqueous Extract of Shoot of *Pennisetum purpureum* on the Oxidation of haemoglobin AA

The results of the oxidative studies of aqueous extract of shoot of *Pennisetum purpureum* on haemoglobin indicated a slight decrease in the absorbance maxima of the ratio of the beta peak (576 nm) and alpha peak (540 nm) on addition of the plant extracts to the haemoglobin from 1.04 to 0.91. This decrease in absorbance increased over time. As the absorbance decreased, the concentration of haemoglobin also decreased slightly from 0.07 mM to 0.06 mM, while there was a gradual increase in the absorbance of the 630 nm band with time from 0.05 to 0.42 which was due to oxidation. At the early stages, there was insignificant oxidation but when the haemoglobin was incubated with the plant extracts over a period of time, there was a gradual increase in absorbance of the 630 nm band indicating oxidation. Oxidation was time dependent.

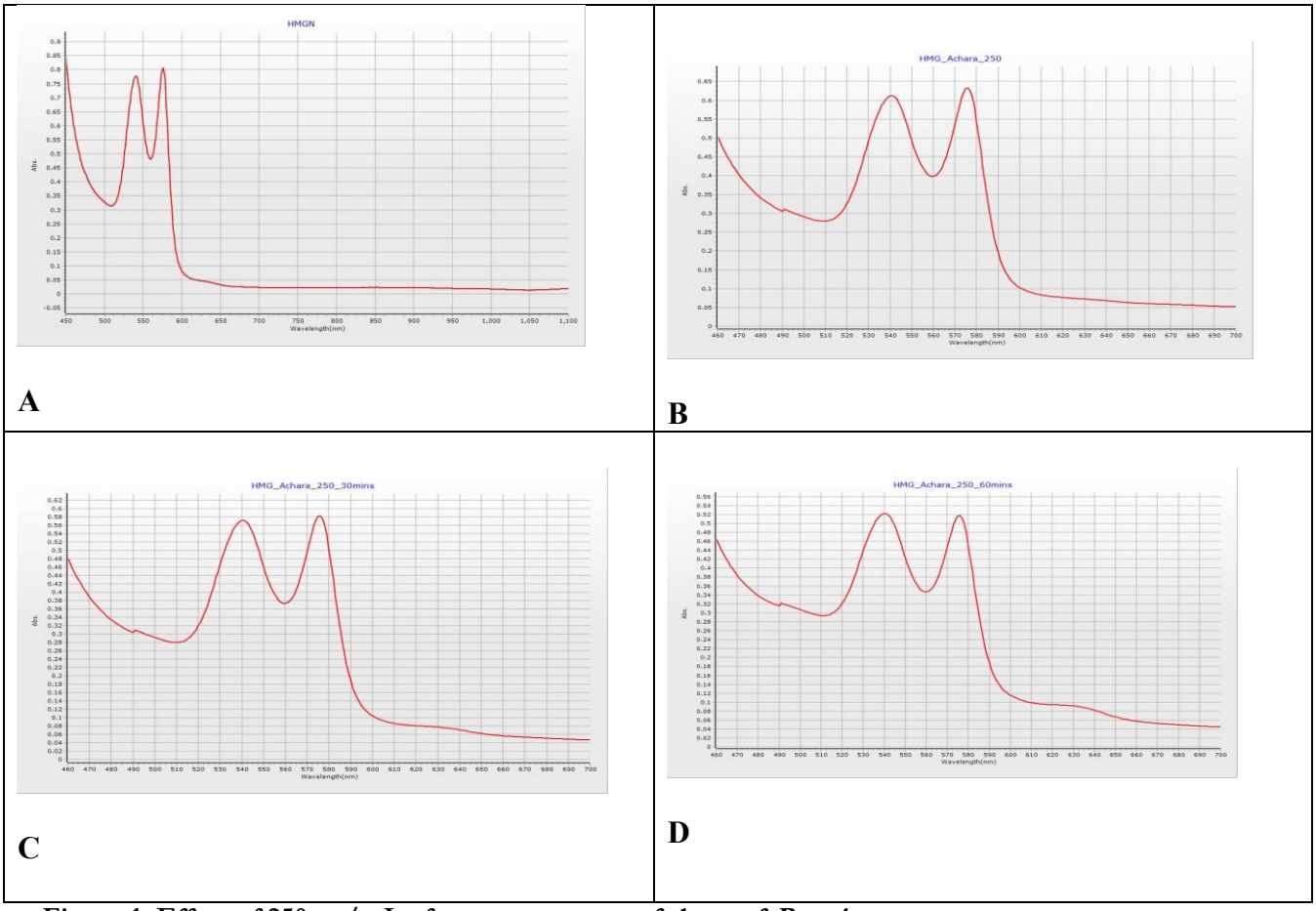

Figure 1: Effect of 250 µg/mL of aqueous extract of shoot of *Pennisetum purpureum* on oxidation of haemoglobin AA at different time intervals.

A is the CONTROL (Spectrum of oxy-haemoglobin A + 900 µl of potassium phosphate buffer only), Concentration of haemoglobin is 0.07 mM. Haemoglobin purity = 1.04, absorbance at 630 nm= 0.05 mM. B Showed a UV-Visible scan of 250 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 0 minutes. Absorbance ratio of 576nm and 540nm peak = 1.03 , absorbance at 630nm band = 0.07 , Concentration of haemoglobin = 0.07 mM. C showed a UV-Visible scan of 250 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 30 minutes. Absorbance ratio of 576 nm and 540 nm peak = 1.02, absorbance at 630 nm band = 0.08, Concentration of haemoglobin = 0.07 mM. D showed a UV-Visible scan of 250 µg/mL of aqueous shoot extract of *Pennisetum purpureum* mixed with 100µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 60 minutes. Absorbance ratio of 576 nm and 540 nm peak = 1, absorbance at 630 nm band = 0.09, Concentration of haemoglobin = 0.06 mM.

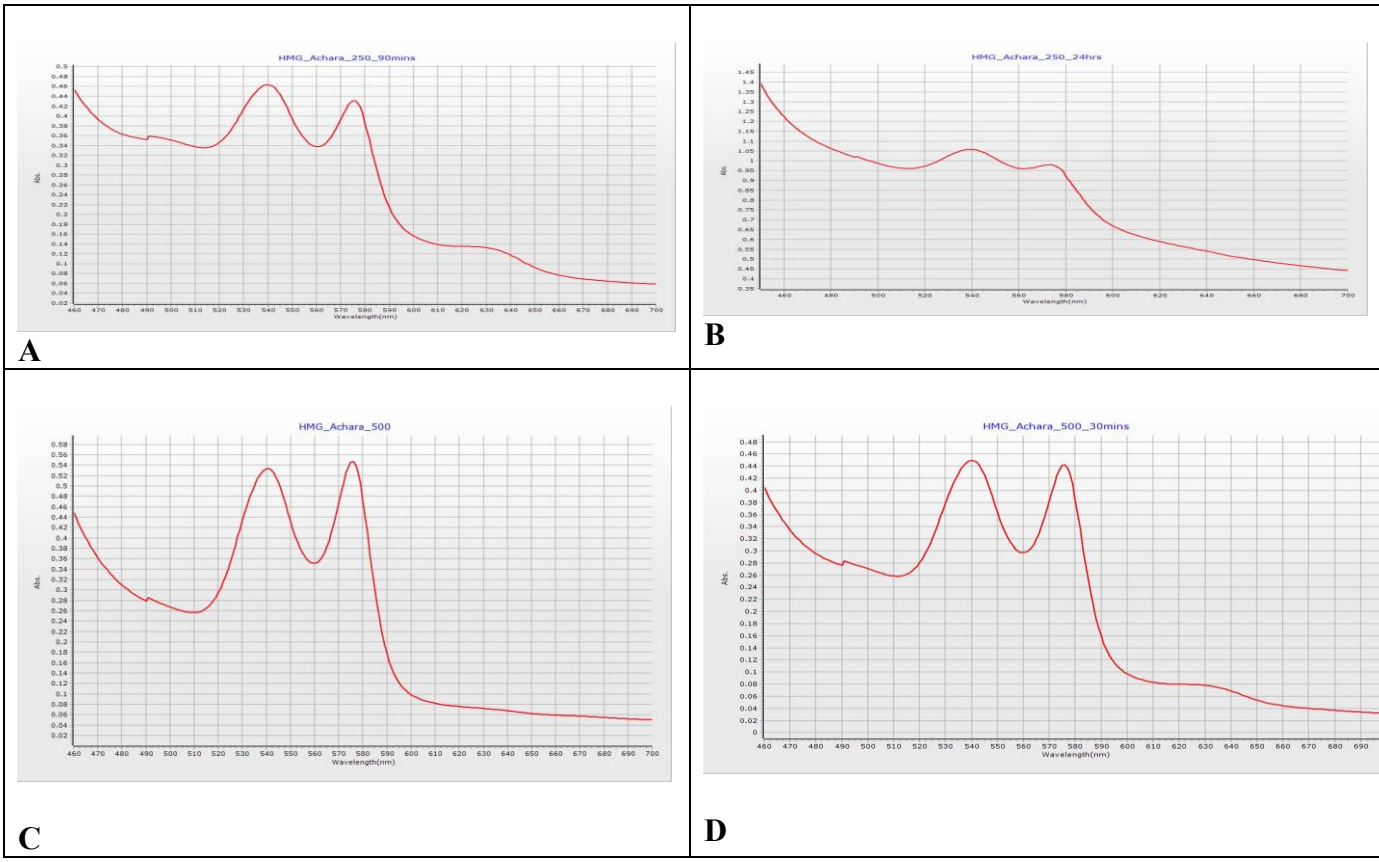

Figure 2: Effect of 250 µg/mL and 500 µg/mL of aqueous extract of shoot of *Pennisetum purpureum* on oxidation of haemoglobin AA.

A is a UV-Visible scan of 250 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 90 minutes. Absorbance ratio of 576 nm and 540 nm peak = 0.93, absorbance at 630 nm band = 0.13, Concentration of haemoglobin = 0.06 mM. B is a UV-Visible scan of 250 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 24 hours. Absorbance ratio of 576 nm and 540 nm peak = 0.92, absorbance at 630 nm band = 0.56, Concentration of haemoglobin = 0.06 mM. C is a UV-Visible scan of 500 µg/mL of aqueous shoot extract of *Pennisetum purpureum* mixed with 100µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 0 minutes. Absorbance ratio of 576 nm and 540 nm peak = 1.04, absorbance at 630nm band = 0.07, Concentration of haemoglobin = 0.07 mM. D is a UV-Visible scan of 500 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 30 minutes. Absorbance ratio of 576 nm and 540 nm peak = 0.98, absorbance at 630 nm band = 0.08, Concentration of haemoglobin = 0.06 mM.

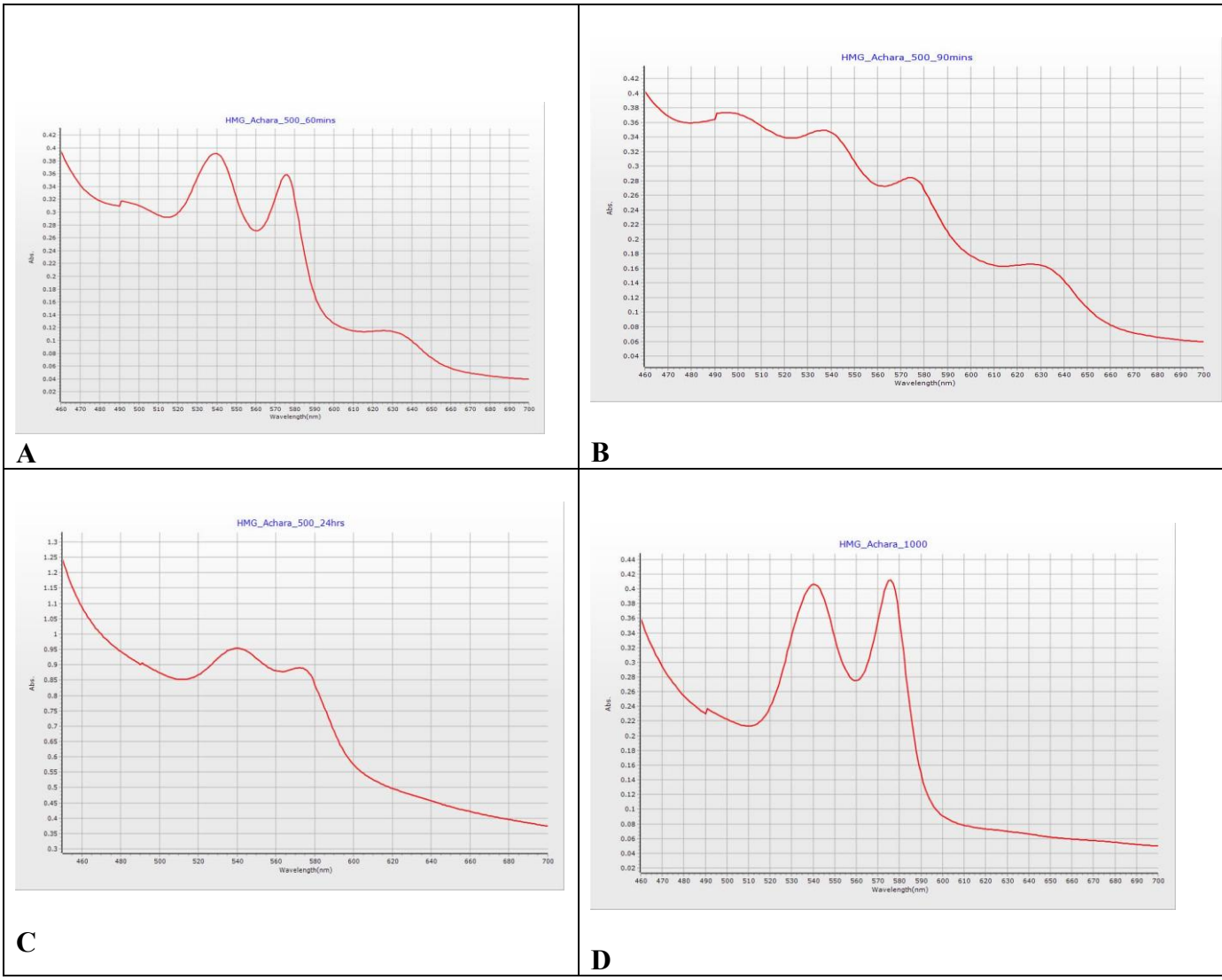

Figure 3: Effect of 500 µg/mL of aqueous extract of shoot of *Pennisetum purpureum* on oxidation of haemoglobin AA.

A showed a UV-Visible scan of 500 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 60 minutes. Absorbance ratio of 576 nm and 540 nm peak = 0.92, absorbance at 630 nm band = 0.11, Concentration of haemoglobin = 0.06 mM. B showed a UV-Visible scan of 500 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 90 minutes. Absorbance ratio of 576 nm and 540 nm peak = 0.8, absorbance at 630 nm band = 0.16, Concentration of haemoglobin = 0.05 mM. C showed a UV-Visible scan of 500 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 24 hours. Absorbance ratio of 576 nm and 540 nm peak = 0.93, absorbance at 630 nm band = 0.48, Concentration of haemoglobin =

0.06 mM. D showed a UV-Visible scan of 1000 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 0 minute. Absorbance ratio of 576 nm and 540 nm peak = 1, absorbance at 630 nm band = 0.07, Concentration of haemoglobin = 0.06 mM.

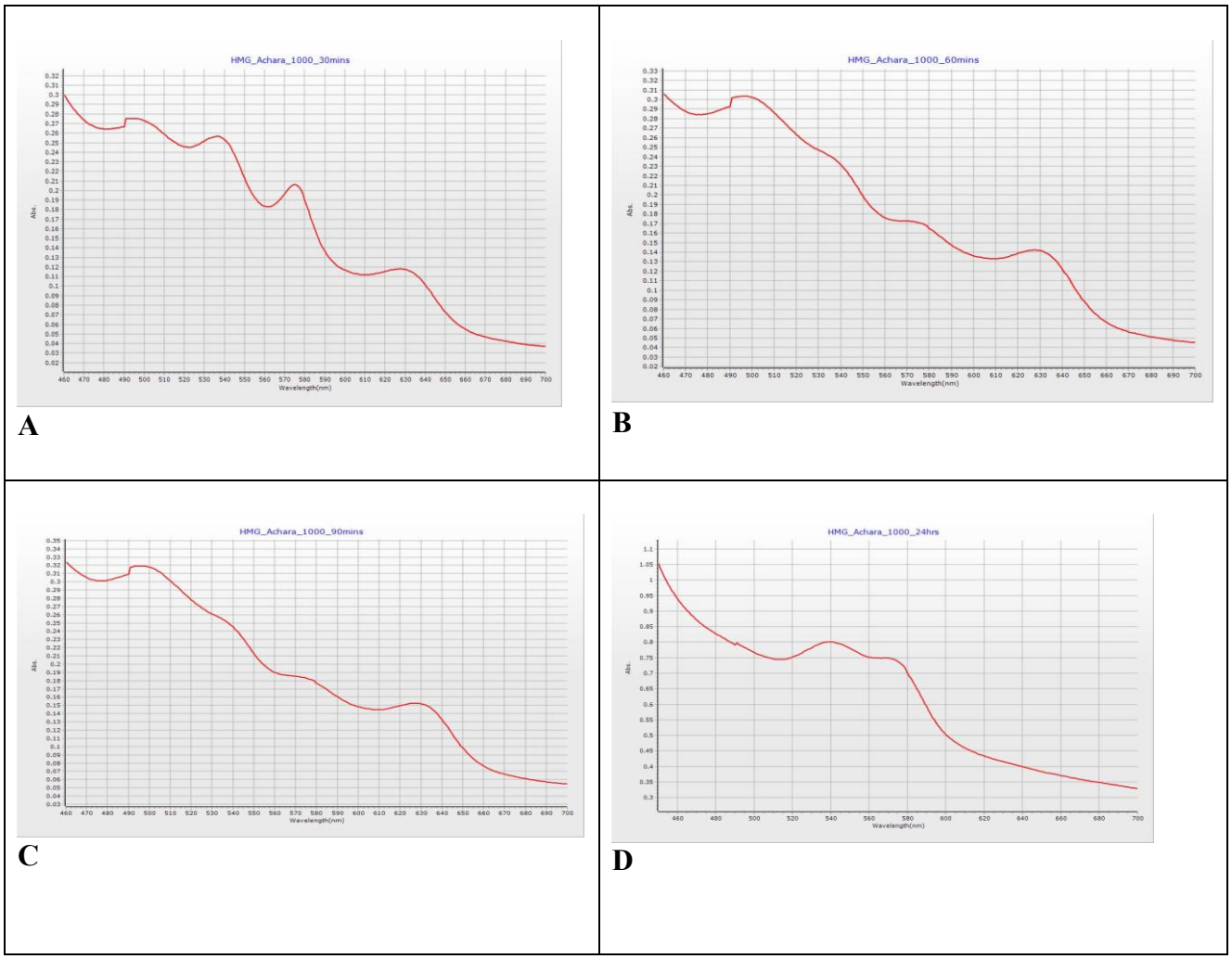

Figure 4: Effect of 1000 µg/mL of aqueous extract of shoot of *Pennisetum purpureum* on oxidation of haemoglobin AA.

A showed a UV-Visible scan of 1000 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 30 minute. Absorbance ratio of 576 nm and 540 nm peak = 0.84, absorbance at 630 nm band = 0.12, Concentration of haemoglobin = 0.05 mM. B showed a UV-Visible scan of 1000 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 60 minute. Absorbance ratio of 576 nm and 540 nm peak = 0.74, absorbance at 630 nm band = 0.14, Concentration of haemoglobin = 0.05 mM. C showed a UV-Visible scan of 1000 µg/mL

of *Pennisetum purpureum* aqueous shoot extract mixed with 100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) at 90 minute. Absorbance ratio of 576 nm and 540 nm peak = 0.72, absorbance at 630 nm band = 0.15, Concentration of haemoglobin = 0.05 mM. D showed a UV-Visible scan of 1000 µg/mL of *Pennisetum purpureum* aqueous shoot extract mixed with100 µl of AA haemoglobin and 900 µl of Potassium phosphate buffer (baseline) after 24 hours. Absorbance ratio of 576 nm and 540 nm peak = 0.91, absorbance at 630 nm band = 0.42, Concentration of haemoglobin = 0.06 mM.

Haemolytic Effect of the Aqueous Extract of Shoot of *Pennisetum Purpureum* on SS Red Blood Cells

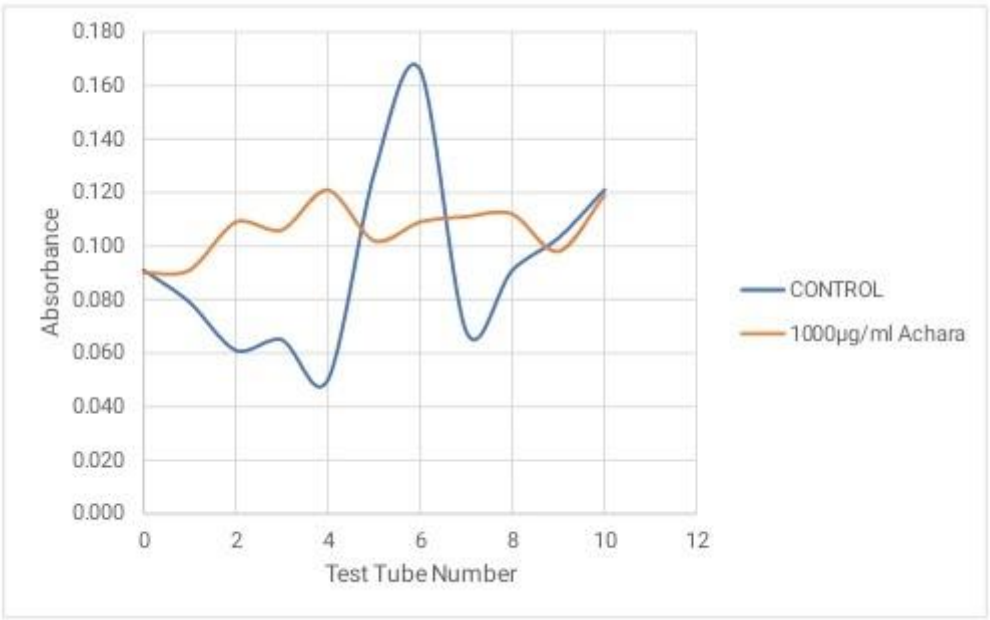

Figure 5: Haemolytic effect of the aqueous extract of *Pennisetum purpureum* shoot for the 1000 µg/mL concentration.

The haemolysis curve was plotted for the control and the addition of the plant extract. Eleven test tubes were used for the haemolysis study. Test tube 0 to 10 comprised 0 µL to 1000 µL saline and 1000 µL to 0 µL distilled water, respectively to form a saline gradient with distilled water. The absorbance at 577 nm was plotted against each test tube number. The plant extract showed a stabilizing haemolytic effect compared to the control.

DISCUSSION

The oxidative properties of *Pennisetum purpureum* aqueous shoot extract was determined from the absorbance changes of oxyhaemoglobin monitored between 400 nm and 700 nm by UV-Visible spectroscopy. Normal oxyhaemoglobin spectra shows the presence of two peaks at 540 nm and 576 nm. The first two peaks at 540 nm and 576 nm represent the alpha and beta peak, respectively. They show the binding affinity of oxygen to ferrous (Fe^{2+}) haemoglobin while the third peak at 630 nm indicates the oxidation of ferrous (Fe^{2+}) haemoglobin to ferric (Fe^{3+}) or methaemoglobin which has lost its capacity to bind oxygen (Ezeawgula and Onwubiko, 2016).

Results from UV-Visible spectroscopy indicated that *Pennisetum purpureum* aqueous shoot extract has a mild oxidative effect on oxy-haemoglobin A which was time dependent. There was a slight decrease in the absorbance maxima of the ratio of the β-peak (576 nm) to the α-peak (540 nm) from 1.04 to 0.91 with increase in time. There was also a gradual increase in absorbance at the 630 nm band from 0.05 to 0.42 which indicated a mild oxidation. This shows that while the *Pennisetum purpureum* plant is widely consumed in West Africa, its capacity to oxidize haemoglobin even at high concentrations of the plant extracts is minimal as demonstrated from this work. This study is consistent with previous studies which indicate that several local plants in Africa such as *Allum sativum,* commonly known as garlic which has been reported to exert an anti-oxidant effect in vitro on sickle red blood cells (Balasenthil *et al.,* 2000). Anti-oxidant capacitiy has also been reported from *W.fruticosa* leaves, flowers and bark (Shringika *et al.,* 2021). Recent studies have shown that the oxidative phenomenon plays a significant role in the pathophysiology of sickle cell disease. The oxidant stress may contribute to the sickling process with the formation of dense cells, development of vaso-occlusion and shortened red blood cell survival. The oxidant damage in sickle red blood cells is most likely the consequence of the inherent instability of haemoglobin S, which results in increase in free radical generation in association with impaired anti-oxidant defense (Wetterstroem *et al.,* 1984).

Pennisetum purpureum aqueous shoot extract also showed a stabilizing haemolytic effect on SS red blood cells treated with aqueous shoot extract of *Pennisetum purpureum* in a gradient made up of mL ratios of distilled water and saline solution (1 % NaCl) and their absorbances taken at 577 nm wavelength. Haemolytic agents tend to cause rapid breakage of red blood cells while anti-haemolytic agents prevent the breakage of red blood cells.

Previous research on plants such as *Carica papaya* were tested for haemolysis and *Carica papaya* leaf reduced haemolysis and protected erythrocyte membrane integrity under osmotic stress conditions (Imaga and Adepoju, 2010). *Cajanus cajan* leaves and seeds, *Zanthoxylum zanthoxyloides* leaf also increase the red blood cell resistance to haemolysis (Ameh *et al.,* 2012; Mamatchi *et al.,* 2025).

CONCLUSION

Pennisetum purpureum aqueous shoot extract has a mild oxidative effect on haemoglobin which was dependent on time. *Pennisetum purpureum* aqueous shoot extract also showed a stabilizing haemolytic effect on sickled red blood cells when compared to the control. This indicates that *Pennisetum purpureum* aqueous shoot extract can be used as a natural therapeutic agent for management of sickle cell disease.

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