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Original Article

In-Vitro Antisickling, Antioxidant, and Anti-Inflammatory Activities of Aqueous Stem Bark Extract of Zanthoxylu-m Zanthoxyloides

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ABSTRACT

Sickle cell disease is a group of haemoglobinopathies affecting large number of people globally especially Nigeria. The disease presents severe occlusive pain, and damages organ which consequently affect the quality of life of the patients. To provide alternative therapy with lesser side effects than pharmaceutical drugs used in the management of the disease, this study determined *in-vitro*, antisickling, antioxidants, and anti-inflammatory effects of aqueous stem bark extract of *Zanthoxylum zanthoxyloides.* After the aqueous extraction of the stem bark, the qualitative, quantitative, antioxidant, and anti-inflammatory properties were assessed. In addition, ethical approval from Kwara State University Teaching Hospital, Taiwo oke, Ilorin, Kwara State was obtained and blood samples was collected from sickle cell anaemia patients which was used to determine the anti-sickling property of the extract using the sickling reversal test. The result showed that the extract constitutes important phytochemicals including alkaloids, flavonoids, phenols, terpenes, and saponins. It reversed sickled erythrocyte by 58.11 %, inhibited polymerization and haemolysis, and exhibited both antioxidative, and anti-inflammatory properties as it inhibited the free radicals tested although at a significant lower rate compared to the standard used. Hence, aqueous stem bark extract of *Zanthoxylum zanthoxyloides* can be regarded as a potential source of therapeutic agents for the monitoring sickle cell disease. However, more studies are necessary to characterize, and identify the specific bioactive compounds responsible for the antisickling, antioxidative, and anti-inflammatory properties.

Keywords: Antisickling, osmotic fragility, polymerization inhibition, Zanthoxylum zanthoxyloides

INTRODUCTION

Sickle Cell Disease (SCD) is a group of genetic blood disorder characterized by mutation of haemoglobin β-gene (HbB) giving rise to abnormal haemoglobin (HbS with sickle-like shape) in red blood cells (Obeagu and Obeagu, 2023). The mutation of the beta gene is as a result of the replacement of glutamic acid with valine at position 6 of the hemoglobin beta-globin chain (Aboderin *et al.,* 2025). The sickled shape haemoglobin causes reduced transportation of oxygen, blockages in blood vessels, and increased breakdown of red blood cell which consequently results in anaemia, severe pains, and several other complications (Obeagu, 2020).

Sickle Cell Anaemia (SCA) is the most predominant type of sickle cell disease (Obeagu and Obeagu, 2023). It is a chronic haemoglobin disorder, characterized by vaso-occlusive episodes leading to fibroids and calcification factors like high level of reactive oxygen species and pro-inflammatory cytokines, endothelial destruction, and haemolysis (Owusu-Ansah *et al*., 2016). The main symptoms of SCA are anaemia, mild jaundice, bone joint pain, hepatosplenomegaly, retarded growth, vaso-occlusive crisis, and acute chest syndrome (Dafaalla and Humeida, 2021). SCA symptoms can be managed by the use of medications including hydroxyurea, crizanlizumab, L-glutamine and prescribed pain killers (Aboderin *et al.,* 2025). Blood Transfusion is also used to increase the normal erythrocytes, increase blood oxygen carrying capacity and reduce anaemia (Aboderin *et al.,* 2025). However, certain risks are associated with frequent blood transfusion by SCA patients, these include; blood-borne diseases, allergic and haemolytic reactions (Issa *et al.,* 2026), hemosiderosis, alloimmunization, transfusion-associated lung injury, and circulatory overload (Alharbi *et al.,* 2017). Due to chronic haemolysis, SCA affects

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

bone marrow by triggering marrow expansion, causing osteoporosis, necrosis, and infarction (Alsafwani *et al.,* 2017). The affected bone marrow can be replaced with healthy donor cells through stem cell transplant also known as haematopoietic stem cell transplantation (HSCT) (Kuriri, 2023). Gene therapy is also an emerging treatment which involves the modification of stem cells of the patient producing a healthy haemoglobin (Vaishnava and Rangari, 2019). Among the therapeutic approaches for the management of SCA is the use of pharmaceutical drug called hydroxyurea which reduces the occurence of painful crises, thereby reducing the requirement for blood transfusion in SCA patient (Quarmyne *et al.,* 2016). Hydroxyurea presents side effects such as headache, mild gastro intestinal symptoms, skin hyperpigmentation or darkening of nails (Agrawal *et al*., 2014). These side effects necessitate the need for safer, less expensive and more accessible alternative therapy which can complement or reduce the drugs' adverse effects.

Over the years, there is increased interest in the exploration of medicinal plants for the treatment and management of various ailment owing to their anti-sickling, anti-inflammatory, antioxidative, antiseptic, and antimicrobial properties (Aremu *et al.,* 2024). Some of the plants that have been used for the management of SCA are *Carica papaya, Cajanu cajan, Terminalia catappa,* and *Piper guineense* (Amponsah *et al.,* 2024).

Zanthoxylum zanthoxyloides is a medicinal plant that belongs to Rutaceae family. It is called African prickly ash in English, Orin ata in Yoruba, Fasar kwari in Hausa, and Ata ohu in Igbo (Mustapha *et al.,* 2026). It has been reported that the plant contains numerous bioactive compounds including alkaloids, tannins, flavonoids, and saponins which are responsible for its medicinal activities (Oladipo *et al.,* 2023). It has been traditionally used to fight infections, anti-malaria remedies, anaemia, and blood related conditions. Hence, this study aims to investigate the anti-sickling, antioxidative, and anti-inflammatory properties of the aqueous stem bark extract of the plant.

MATERIALS AND METHODS

Plant collection and preparation

The stem barks of *Zanthoxylum zanthoxyloides* was bought from Oja oba, Ilorin. The aqueous extract was obtained following the method stated by Alsuliam *et al.* (2022). Briefly, the barks was thoroughly washed, dried (at room temperature), and grinded. About 500 g of the powdered stem bark was soaked in 5 liters of clean distilled water for 72 hours, after which it was filtered with Whatman No. 1 filter paper, dried under vacuum, and then lyophilized.

Chemicals and Reagents

The chemical used for this study includes sodium metabisulphite. All others are of analytical grade.

Phytochemical Analysis of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides*

Qualitative Analysis

Qualitative detection of saponins, phlobatannins, phenolics, tannins, alkaloids, steroids, triterpenes, glycosides and flavonoids analysis of the extract was carried out following the method described by Odebiyi and Sofowora (1978).

Alkaloids

To 1cm^3 of 1% Hydrochloric acid in a test tube, 3cm^3 of the extract was added. The mixture was heated for 20 minutes, and filtered after it cooled down. Two drops of Wagner's reagent was added to 1 cm^3 of the filtrate. The presence of alkaloids was confirmed by a reddish-brown precipitate.

Tannins

Approximately 1cm^3 of freshly prepared 10 % potassium hydroxide was mixed with 1cm^3 of the extracts. The presence of tannins was confirmed by a dirty white precipitate.

Phenolics

About 2 drops of 5 % $FeCl_3$ was added to 1cm^3 of the extract in a test tube. A greenish precipitate indicated the presence of phenolics.

Glycosides

To 1cm^3 of the extract, 10 cm^3 of 50 % sulphuric acid was added, the mixture was heated in boiling water for 15 minutes, then, 10 cm^3 of Fehling's solution was added. The presence of glycosides was confirmed by a brick red precipitate.

Saponins

Frothing test: in a test tube containing 10ml of distilled water, 2ml of the extract was added and vigorously shaken for 2 minutes. Frothing indicated the presence of saponins.

Flavonoids

About 1ml of 10 % NaOH was added to 3ml of the extract. A yellow coloration indicated the presence of flavonoids.

Steroids

Salakowsti test: 5 drops of concentrated H_2SO_4 was added to 1ml of the extract. Red colouration indicated the presence of steroids

Phlobatannins

Approximately 1ml of the extract was added to 1 % HCl. A red precipitate indicated the presence of phlobatannins.

Triterpenes

About 5 drops of acetic anhydride was added to 1ml of the extracts. A drop of concentrated H_2SO_4 was added and the mixture was steamed for 1 hour and neutralized with NaOH followed by the addition of chloroform. A blue green color indicated the presence of triterpenes.

Terpenoids

About 5 ml of aqueous extract of the sample was mixed with 2 ml of $CHCl_3$ in a test tube 3ml of con. H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish-brown coloration confirmed the presence of terpenoids.

Quantitative Analysis

Determination of Total Phenolic Contents (TPC)

About 200 µL of distilled water was added to the extract at a concentration of 1 mg/mL, the mixture was treated with Folin-Ciocalteu reagent for 3 minutes, after which 2 mL of 20 % ($^{w}/_{v}$) sodium carbonate solution was added. Following incubation in the dark for 60 minutes, the absorbance was at 650 nm using T70 UV/VIS spectrometer. The blank made up of ethanol. All tests was replicated five times and results was expressed as mg of gallic acid equivalent per g (mg GAE/g) dry weight (Tamilselvi *et al*., 2012).

Calculation;

Concentration of Total Phenolic (mg gallic acid/g) =

Absorbance × Gradient Factor from Standard Curve × Dilution Factor

Determination of Total Flavonoid Contents (TFC)

The total flavonoid content was evaluated using the method of aluminium chloride colorimetric method following the procedure described by Kanchan *et al*. (2012). Briefly, 0.5 ml of the extract solution (1 mg/mL) was mixed with 1.5 ml of ethanol, 0.1 ml aluminium chloride solution, 0.1 ml potassium acetate solution and 2.8 ml distilled water. Sample blank was prepared in a similar manner by replacing aluminium chloride with distilled water. Distilled water was used in place of aluminium chloride solution as blank and absorbances were measured at 415 nm. Quercetin was used as a standard. The TFC values of the extracts was expressed as milligrams of quercetin equivalent per gram (mg QE/g) of extracts. TFC was calculated by using the following formula;

$$\text{Calculation; TFC (mg quercetin equivalents per g)} = \frac{R \times D.F \times V \times 100}{W}$$

Where, R = result obtained from standard curve

D.F = Dilution factor

V = Volume of stock solution

100 = 100 g dried plant

W = Weight of plant used in the experiment

Determination of Total Tannin Contents (TTC)

TTC was determined following the procedure described by Tamilsevi *et al.* (2012). Approximately 750 µl of distilled water, 500 µl Folin-Ciocalteu reagent and 1000 µL of 35 % sodium carbonate was added to 100 µl of the extract. The mixture was vigorously shaken, and diluted with 10 ml of distilled water, then incubated for 30 min at 37^0C. Finally, the absorbance was read at 725 nm. The TTC was expressed as mg of gallic acid equivalent per g (mg GAE/g) dry weight using the formula below:

Concentration of Total Tannin gallic acid equivalent =

Absorbance × Gradient Factor from Standard Curve × Dilution Factor

Determination of Total Alkaloid Contents (TAC)

Into a micro-centrifuge tube, 1.5 ml of the extract was placed and centrifuged for 20 minutes at 13000 rpm, after which 0.5 ml of the supernatant was poured into another tube, then Dragendorff's reagent of 0.2 ml was added, thoroughly mixed and subjected to centrifugation at 13000 rpm for 40 minutes. A 0.2 ml of concentrated nitric acid, and 0.8 ml of water was used to dissolve the residue. After which 0.2 ml was pipetted out, and mixed with thiourea solution of 1 ml. finally, the absorbance was read at 464 nm against a

blank constituting thiourea and nitric acid (Sreevidya and Mehrotra 2019).

Calculation;

Concentration of Total Alkaloid (Bismuth Nitrate pentahydrate equivalent)

Absorbance × Gradient Factor from Standard Curve × Dilution Factor

Determination of Total Saponin Contents (TSC)

The TSC determination was carried out using the procedure described by Baccou *et al.* (1977). An extract of 500 µl was added to 500 µl anisaldehyde reagent (0.5 %), this was mixed, and allowed to stand for 10 minutes, followed by the addition of 2 ml sulphuric acid reagent (50 %). The tube was cooled inside water bath for 10 minutes (temperature of 60 °C), removed after 10 minutes. Then, absorbance was read at 435 nm. The quantity of saponin was evaluated as saponin (diosgenin) equivalent from the calibration curve of standard saponin (100-1000 µg/ml)

Calculation;

Concentration of Total Saponin (Diosgenin equivalent) =

Absorbance × Gradient Factor from Standard Curve × Dilution Factor

Determination of Glycoside Contents

The concentration of glycoside was assessed using the procedure described by Tofighi *et al.* (2016). A 10 ml of Baljet's reagent was mixed with 10 g of N. oleander leaf and flower extract. This was incubated for 1 hour, and distilled water of 20 ml was added. At 495 nm, the absorbance was finally measured. The standard used was Securidaside. The concentration of cardiac glycoside was reported as mg securidaside equivalent (SE)/g

Determination of Terpenoids Contents

The total terpenoids was determined according to the procedure used by Malik *et al.* (2017). A 100 mg of dried extract was soaked in ethanol (9 ml) for 24 hours. After filtering the mixture, the filtrate was mixed with petroleum ether of 10 ml for further extraction utilizing separating funnel. The ether extract was segregated, and allowed to dry off (wf). The ether was vapourized and the terpenoids percentage yield was calculated by the formula: Initial weight (initial weight of the extract (wi) – final weight (wf) / initial weight (wi) X 100.

Collection of Blood Samples

Having obtained ethical approval from Kwara State Teaching Hospital, Taiwo Oke, Ilorin, and seek patients' consents, blood samples were collected from confirmed SCA patients of between ages 18 and 25 in sodium EDTA bottles.

In-Vitro Antisickling Screening

The procedure analyzed by Pauline *et al.* (2013) was used to determine the effect of *Zanthoxylum zanthoxyloides aqueous* extract on the reversal of erythrocyte sickling. The blood sample collected from SCA patient was washed twice by centrifugation at 1200 g using phosphate buffered saline (PBS; pH 7.4). A mixture of 100 µl of washed erythrocyte, and 100 µl of freshly prepared 2% sodium metabisulphite were placed in a cleaned Eppendorf tube and incubated for 2 hours at 37°C. The mixture was incubated for another 2 hours at 37°C after adding 100 µl of the extract. Finally, 10 µL of the incubated cells was diluted 100 times, a drop was placed on a slide and covered with a cover slip. The cells were then counted under Microscope at ×40 magnification. The percentage sickling was calculated using the formula;

$$\text{Percentage Sickling} = \frac{\text{Number of sickled cells}}{\text{Total number of counted cells}} \times 100$$

A control test was performed by replacing the extract with 100 µl of PBS. The experiment was repeated five times.

Polymerization Inhibition Test

The polymerization inhibition test was carried out using the method of Pauline *et al.* (2013). Briefly, a solution containing 0.88 ml of 2% sodium metabisulfite (freshly prepared), 0.1ml of extract (in the control, phosphate buffered saline was used instead) and 0.02 mL HbSS blood was poured into a cuvette and absorbance of the mixture was read at 700nm instantly,

for 30 minutes, at 2 minutes interval. The percentage rate of polymerization was calculated using the formula;

$$\text{Rate of polymerization (Rp)} = \frac{(\text{Final Absorbance} - \text{Initial Absorbance})}{30} \times 100$$

Osmotic Fragility Test

The method described by Nwaoguikpe *et al.* (2013) with minor modifications. A 5 ml of sodium chloride solution of concentrations ranging from 0 to 0.9 % was prepared and arranged serially in a test tube rack. In an eppendorf bottle, a 100 µl of extract and 900 µl of suspended RBC was mixed and incubated at 37ºC for 30 minutes. After incubation, 1 ml of each sodium chloride solution prepared and 10 µl of treated RBC were mixed and incubated for another 30 minutes at room temperature (25ºC). The contents was centrifuged at 4000 rpm for 5 mins. The supernatant of each test tube was poured into a cuvette and the absorbance of the released haemoglobin was read at 540 nm using a spectrophotometer. Erythrocyte osmotic fragility curve was prepared by plotting percent haemolysis against the sodium chloride concentrations.

Calculation:

$$\text{Osmotic Fragility} = \frac{\text{Optical density of test}}{\text{Optical density of distilled water}} \times 100$$

In-Vitro* Antioxidant Assays of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides

2, 2-Diphenyl-1-picryhydrazyl (DPPH) Radical Scavenging Assay

Free radical scavenging activity was determined according to the method of Mensor *et al*. (2001). Briefly, 2.5 ml of extract at varying concentrations (7.8125–1000 µg/ml), was added to 500 µl of 0.3 mM alcoholic solution of DPPH (Himedia, India). These mixtures were incubated for 30 minutes in dark, and absorbance were measured at 518 nm using UV-visible spectrophotometer (Systronics AU-2700, India). The positive control used was butylated hydroxytoluene (BHT). The experiments were performed in triplicates, and scavenging activity was expressed as percentage inhibition, using the following formula.

$$\% \text{ Scavenging} = ([\text{Abs}_{\text{control}} - \text{Abs}_{\text{samples}}]/ \text{Abs}_{\text{control}}) \times 100$$

Ferric Ion Reducing Power Assay (FRAP)

The method described by Benzie and Strain (1996) was used for this assay. Approximately 3.6 ml of FRAP solution was added to 0.4 ml of distilled water and incubated for 5 minutes at 37˚C. This solution was mixed with certain concentration of the plant extract (80 ml) and incubated at 37˚C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO4, 7H2O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions.

Hydroxyl Radical (OH.) Scavenging Assay

The procedure described by Smirnoff and Cumbes (1989) was followed with slight modifications to determine the scavenging activity of the extract using OH. Briefly, the mixture of 2 ml of the extract at varying concentrations; 7.8125 to 1000 mg/mL, 8 mM ferrous sulfate of 0.6 ml, 0.5 ml of 20 mM hydrogen peroxide, and 2 ml of 3 mM salicylic acid were incubated foe 30 minutes at 37^0C. After which 0.9 mL of distilled water was added to each vial, centrifuged at 4472 g for 10 minutes and absorbance read at 510 nm. The percentage OH. Scavenging activities of the extract was calculated using the formula:

$$\% \text{ Scavenging} = ([\text{Abs}_{\text{control}} - \text{Abs}_{\text{samples}}]/ \text{Abs}_{\text{control}}) \times 100$$

***In-Vitro* Anti-Inflammatory Assays**

Heat induced haemolysis

Fresh human blood was collected from a healthy person in a heparinized bottle, it was centrifuged for 5 minutes at 1100 g and then washed with normal saline thrice and later reconstituted as 10 % v/v suspension with phosphate buffer (pH 7.4)

The method used by Qamar *et al.* (2022) was adopted for this assay. Briefly, 1ml of extracts at various concentrations (7.8125 to 1000 µg) and 1 ml of suspended RBCs were mixed in tubes, incubated for 25 minutes at 50^0C, allowed to cool, then centrifuged at 1100 x g for 3 minutes, and with the use of spectrophotometer, the absorbance of the supernatant was observed at 560 nm. The negative control used was phosphate buffer, while the positive control was diclofenac sodium.

The process was carried out in triplicates and the percentage of inhibition was calculated as follows:

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance of control} - \text{absorbance of treated}}{\text{Absorbance of Control}}\right) \times 100$$

Proteinase Inhibitory Assay

A proteinase inhibitory assay was performed as described by Marrassini *et al.* (2018). The reaction mixture (1 ml; 0.03 mg trypsin and 0.5 ml Tris-HCl buffer) was incubated with 0.5 ml of the extract at different concentrations (7.1825 – 1000 µg) at 37^{0}C for 5 minutes. Approximately 0.5 ml of 0.8% (w/v) casein was later added and incubated for 20 minutes. The reaction was stopped by the addition of 2 ml of 70% perchloric acid, centrifuged and then measured the absorbance of the supernatant obtained at 280 nm.

Inhibition of Protein Denaturation

The anti-inflammatory effect of the extract using its ability to inhibit protein denaturation was evaluated following the protocol described by Deepthi *et al.* (2020) with slight modifications. A volume of 1 ml of extracts or of diclofenac sodium at different concentrations (7.8125 - 1000 μg/ml) was homogenised with 1 ml of aqueous solution of bovine serum albumin (5 %) and incubated at 27°C for 15 minutes. The mixture of distilled water and BSA constituted the control tube. Denaturation of the proteins was caused by placing the mixture in a water bath for 10 minutes at 70°C. The mixture was cooling inside the ambient room temperature, and the activity each mixture was measured at 660 nm. Each test was done three times. The following formula was used to calculate inhibition percentage:

$$\% \text{ Inhibition} = \{ [\text{Abs control} - \text{Abs extract}] \div \text{Abs control} \} \times 100.$$

STATISTICAL ANALYSIS

The data generated from the study will be presented as the mean standard error of the five replicates and subjected to a one-way analysis of variance (ANOVA). The data will be considered statistically different at ($p < 0.05$) using Graph Pad Prism version 9.0

RESULTS

Qualitative Phytochemical Analysis of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides*

As shown in table 1, *Zanthoxylum zanthoxyloides* contains more of alkaloids, flavonoids, tannins, and phenols than saponins, terpenoids and glycosides.

Table 1: Qualitative Analysis of Aqueous Stem Bark Extract of *Zanthoxylum Zanthoxyloides*

Phytochemicals	Result
Alkaloids	+
Flavonoids	++
Phenols	+++
Saponins	+
Terpenoids	+
Glycosides	+
Tannins	+
Anthraquinones	-

+ = present, ++ = moderately present, +++ = highly present, - = absent

Quantitative Phytochemical Analysis of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides*

The result as shown in table 2 indicates that *Zanthoxylum zanthoxyloides* contains a broad spectrum of secondary metabolites including alkaloids, flavonoids, steroids, terpenoids, tannins, phenols, glycosides, and saponins, this demonstrates that the extract contains bioactive compounds which may contribute to its bioactive effect. The highest constituent is the phenolic compounds (800.60 ± 2.08 mg GAE/g), followed by the flavonoids (54.21 ± 2.17 mg QE/g).

Table 2: Quantitative Analysis of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides*

Phytochemicals	Concentration
Alkaloids	12.88 ± 0.06 (µg AE/g)
Flavonoids	54.21 ± 2.17 (mg QE/g)
Steroids	23.07 ± 0.17 (µg Cholest/g)
Terpenoids	22.67 ± 0.17 (µg Cholest/g)
Tannin	4.62 ± 0.05 (µg CE/g)
Phenol	800.60 ± 2.08 (mg GAE/g)
Glycosides	17.78 ± 0.46 (µg SE/g)
Saponin	17.78 ± 0.46 (µg SE/g)

Values a are a mean a of a three a replicates a ± a SD

In-Vitro Antisickling Effect of Aqueous Stem Bark Extract of *Zanthoxylum Zanthoxyloides*

The *in-vitro* sickling reversal test of the extract demonstrated that the extract significantly reduced the percentage of sickled erythrocyte from 100 % to 41.89 % (as shown in table 3) which corresponds to 58.11 % reduction in sickling.

Table 3: Percentage Sickling Reversal Effect of Aqueous Stem Bark Extract of *Zanthoxylum Zanthoxyloides*

Extracts	% Sickling reversal	% Polymerization Inhibition
Sickled control	100 ± 0.01[a]	
Z. zanthoxyloides aqueous stem bark extract	41.89 ± 1.43[b]	

Values are mean of 3 replicates ± SEM. Values a with a different a superscripts a down a the a same a column a are a significantly a different a (p a < a 0.05)

Percentage Polymerization Inhibition of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides*

The extract significantly inhibited haemoglobin polymerization from 100 % to 54.04 % as shown in table 4.

Table 4: Percentage Polymerization Inhibition of Aqueous Stem Bark Extract of *Zanthoxylum Zanthoxyloides*

Samples	% Polymerization Inhibition
Negative Control	100 ± 0.00[a]
Z. zanthoxyloides aqueous stem bark extract	54.04 ± 1.20[b]

Values are mean of 3 replicates ± SEM. Values a with a different a superscripts a down a the a same a column a are a significantly a different a (p a < a 0.05)

Osmotic Fragility Test of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides*

The extract was able resist haemolysis when exposed to different salt concentration, the rate of haemolysis reduced as the salt concentration increased as shown in figure 1.

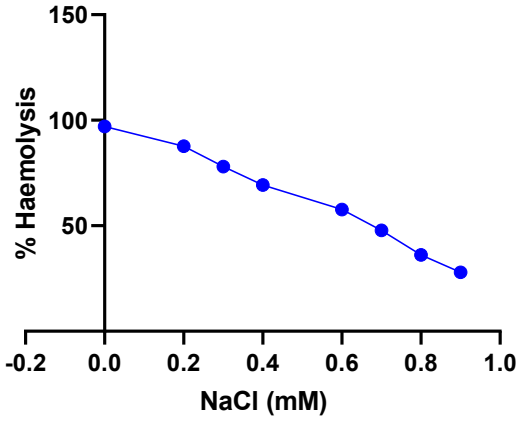

Figure 1: Effect of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides* on Haemolysis

In-Vitro Antioxidant Effect of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides*

Radical Scavenging Activity of 2, 2-Diphenyl-1-picryhydrazyl (DPPH)

Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides* according to figure 2 possesses radical scavenging activity in a dose-dependent manner at a lower significant rate (P<0.05) when compared with the standard. The extract inhibits DPPH at a percentage between 13.61 % at 7.8125 and 71.36 % at 1000 µg/ml against the standard between 46.19 and 88.08 % at the same concentrations. This indicates that increasing the concentration of the extract enhanced its ability to neutralize DPPH free radicals.

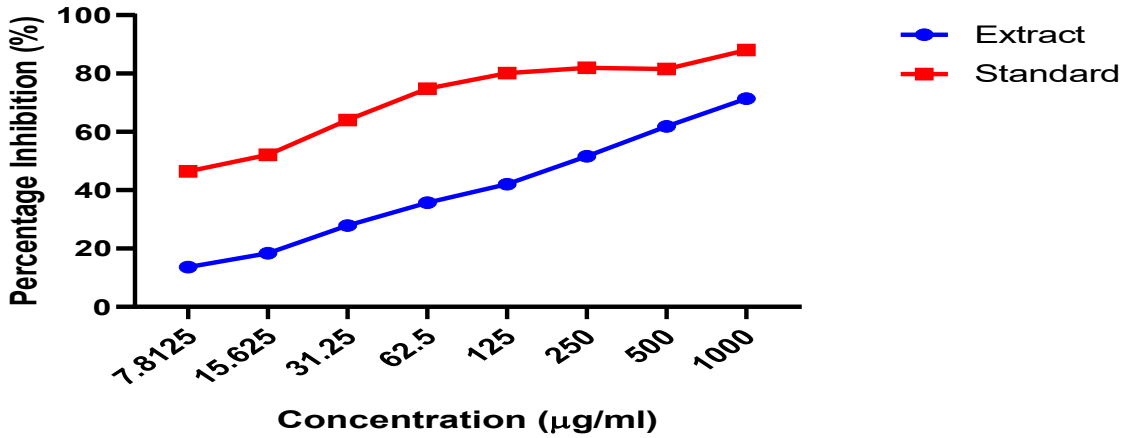

Figure 2: Effect of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides* on Radical Scavenging Activity of 2, 2-Diphenyl-1-Picryhydrazyl (DPPH)

Ferric Reducing Antioxidant Power (FRAP)

The effect of *Zanthoxylum zanthoxyloides* aqueous stem bark extract and the standard was 1.574 ± 0.041 and 2.637 ± 0.047 mmol Fe^{2+} /g respectively as shown in figure 3. This showed that the extract possesses reducing power activity but at a lower significant ($P < 0.05$) rate compared to the standard.

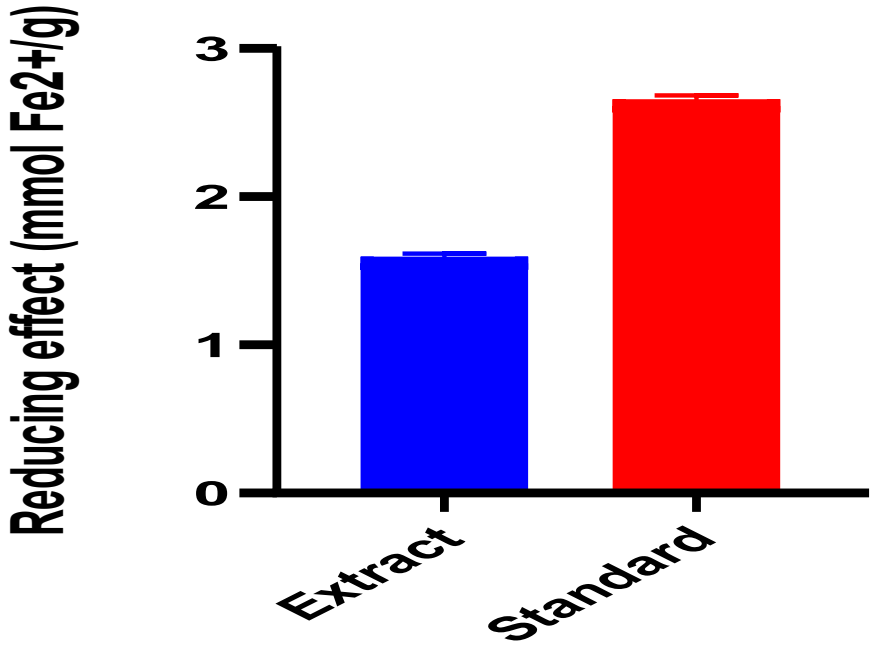

Figure 3: Effect of Aqueous Stem Bark Extarct of *Zanthoxylum zanthoxyloides* on Ferric Reducing Antioxidant Power (FRAP)

Radical scavenging activity of Hydroxyl ion (OH^-)

The percentage inhibition of hydroxyl ion by *Z. zanthoxyloides* aqueous extract just like other oxidative stress markers increased with increase in concentration. The inhibition increases from 23 to 67 %, at concentrations 7.8125 to 1000 µg/ml respectively. However, the standard exhibited significant ($p < 0.05$) higher inhibition as it increased from 40 to 92 % at the same concentration as the extract (figure 4).

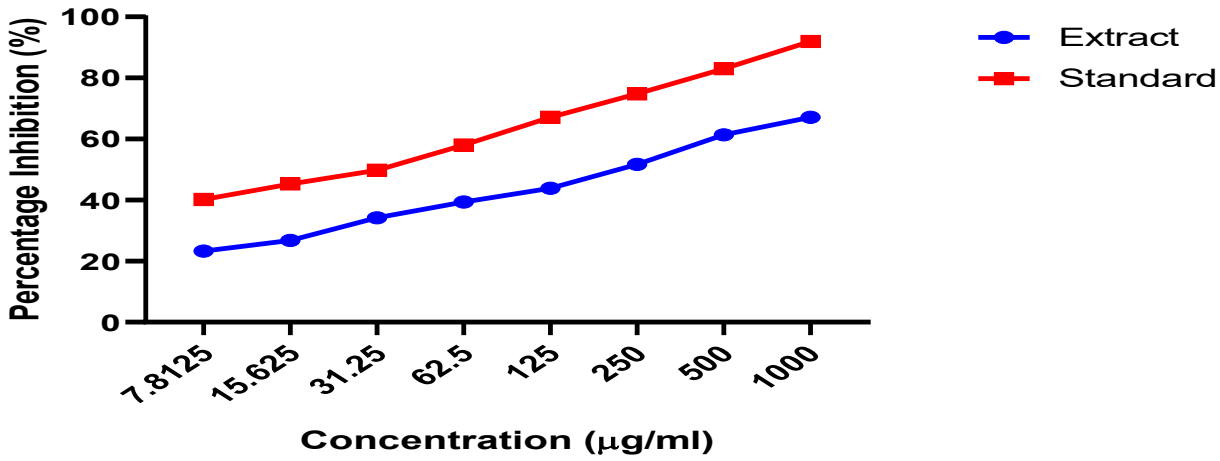

Figure 4: Effect of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides* on Radical Scavenging Activity of Hydroxyl Ion (OH^-)

In-vitro Anti-inflammatory Properties of Aqueous Stem Bark Extract of *Zanthoxylum Zanthoxyloides*

Percentage Inhibition of Heat Induced Haemolysis

From figure 5, it is observed that both the extract and the standard exhibited concentration-dependent increase in inhibition of haemolysis. The extract inhibited heat induced haemolysis from 18 % at 7.8125 µg/ml, to 64 % at 1000 µg/ml. The standard showed more inhibitory effect as it inhibited haemolysis from 34 to 90.5 % at the same concentration range. Thus, the extract showed an appreciable anti-haemolytic effect, although its capacity is significantly ($P < 0.05$) lower than the standard.

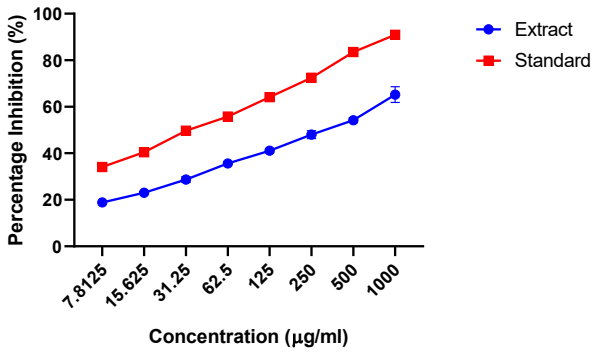

Figure 5: Effect of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides* on Percentage Inhibition of Heat Induced Haemolysis

Percentage Inhibition of Proteinase

Zanthoxylum zanthoxyloides aqueous stem bark extract inhibited proteinase activity in a concentration-dependent manner (20 % at 7.8125 to 63 % at 1000 µg/ml). The rate of inhibition was significantly lower ($p < 0.05$) than that of the standard which inhibited the enzyme from 38 % at 7.8125 to 89 % at 1000 µg/ml (figure 6).

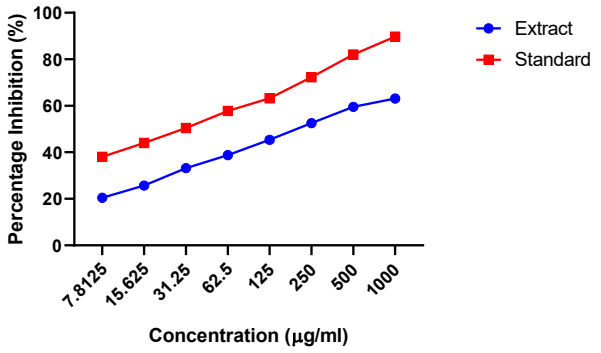

Figure 6: Effect of Aqueous Stem Bark Extract of *Zanthoxylum zanthoxyloides* on Proteinase Inhibition

Percentage Inhibition of Protein Denaturation

The percentage inhibition of protein denaturation by aqueous stem bark extract of *Zanthoxylum zanthoxyloides* as shown in figure 7 increased as the concentration increases from 22 % at 7.8125 to 60 % at 1000 µg/ml. The standard however showed significantly higher inhibition than the extract as it inhibited protein denaturation from 36 to 92 % at the same concentration range.

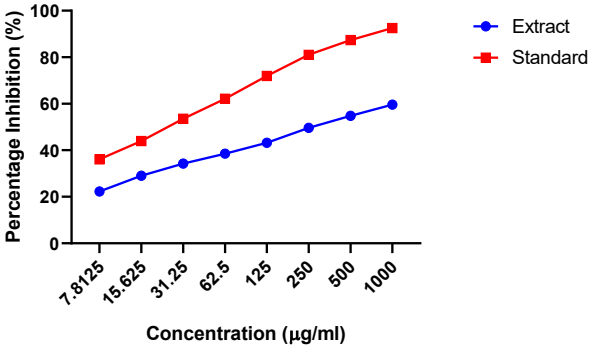

Figure 7: Effect of *Zanthoxylum zanthoxyloides* on Percentage Inhibition of Protein Denaturation

DISCUSSION

Zanthoxylum zanthoxyloides over the years has been proven to be effective in the management and treatment of several health conditions including dental problem, and sickle cell diseases. This is owing to the various phytochemical constituents of the plant. In this study, the aqueous stem bark extract of the plant was found to contain alkaloids, flavonoids, saponins, steroids, and terpenoids which suggests its biological importance. This is consistent with the previous report conducted by Haidara *et al.* (2025), Ozigis *et al.* (2023), Togola *et al.* (2023), and Tougoma *et al.* (2021). However, the variance in the exact quantity of each phytochemical compound is influenced by the different methods of extraction, analytical method used, geographical origin, and plant maturity.

The presence of various bioactive compounds which are believed to prevent haemoglobin polymerization, and stabilize erythrocyte membrane has been exploited for the management of sickle cell disease. In

this *in-vitro* study, the aqueous extract of the stem bark of the plant was able to reverse sickling, inhibit haemoglobin polymerization, and prevent haemolysis. This is consistent with the previous study reported by Ouattara *et al.* (2009) who observed that divanilloylquinic acids including burkinabins A, B, and C isolated from the root bark exhibits antisickling activity, although, there is no confirmation that the aqueous stem extract has the same constituent as the root bark. The systematic review study carried out by Amponsah *et al.* (2024) also reported similar antisickling effect confirming the efficacy of the current study.

The presence of some important phytochemicals indicates the antioxidant power of the extract, this was observed in the radical scavenging activity of the extract on DPPH, the result confirmed that *Z. zanthoxyloides* contains bioactive constituents capable of donating electrons to stabilize the radicals. This reports is supported by Ouedraogo *et al.* (2024) who reported antioxidant activity in both stem and root bark extracts of *Z. zanthoxyloides*. Similarly, Togola *et al.* (2023), reported same effect in the hydroethanolic fruit and bark extracts of the plant. This reports suggests and supports the presence of phytochemicals such as flavonoids, tannins, and alkaloids which contribute to the antioxidant activity of the plant. In addition, the observed reducing activity of the *Z. zanthoxyloides* stem bark aqueous extract demonstrated its antioxidant potential, this supports its possible contribution to protection against oxidative processes. This result is consistent with the study reported by Ogunbolude *et al.* (2014) who also identified appreciable ferric-reducing capacity in the stem extracts of *Z. zanthoxyloides* but at a lower rate compared to the standard. In contrast, Oudreogo *et al.* (2024) reported the ferric-reducing antioxidant power to be higher in *Z. zanthoxyloides* than standard, this contradiction might be related to the influence of extraction solvent and plant part evaluated. This antioxidant ability of the plant has been associated with some bioactive compounds which contribute to antioxidant activity through electron and hydrogen donation mechanism.

The anti-inflammatory effects of the extract were also evaluated, one of which is proteinase inhibition. Proteinases break down proteins, but excessive proteolytic activity can contribute to tissue injury during inflammation, this study observed that the aqueous stem bark extract under the tested concentrations were able to inhibit proteinase from 20 to 63 % which is lower than the activity of the standard at the same concentration. This inhibitory activity observed is consistent with earlier report of Oyedapo and Famurewa (1995), who also reported anti-protease activity in an alkaline extract of *Fagara zanthoxyloides* also known as *Zanthoxylum zanthoxyloides*.

To further assess the anti-inflammatory effect of the extract, heat induced haemolysis was carried out and it was observed the extract stabilized erythrocyte membrane by 67 % at 1000 µg/ml. This result is similar to the study carried out by Oyedapo and Famurewa (1995) who worked on membrane stabilizing activity of extracts of *Fagara zanthoxyloides*, where they observed that the extracts stabilized human red blood cell membrane against both hypotonic and heat induced haemolysis, *Z. zanthoxyloides* showed the highest membrane stabilization among the plants tested. In addition, the extract was able to stabilize protein structure and inhibit denaturation. Togola *et al.* (2023), also reported similar effect in a study carried out with aqueous trunk-bark extract of *Zanthoxylum zanthoxyloides*, although the percentage of inhibition by the extract was not significantly different from the standard as the extract rate of inhibition was 85.12 ± 0.44 % while the standard was 93.33 ± 1.53 %.

CONCLUSION

It can be concluded from this study that owing to the bioactive constituents found in the aqueous stem bark extract of *Zanthoxylum zanthoxyloides,* it was able to reverse some sickled erythrocyte, prevent haemoglobin polymerization, reduce haemolysis, inhibit free radicals, and possesses anti-inflammatory capacity. Hence, the plant can be regarded as a promising source of bioactive compounds for the development of potential therapeutic agent for sickle cell diseases. However, further *in-vivo* and clinical studies are required to establish its therapeutic efficacy and safety.

CONFLICT OF INTEREST

There was no any form of conflict of interest between the authors.

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