The Antioxidant, Sickle cell reversal, and Hemoglobin S Polymerization Inhibitory Activities of *Brillantaisia owariensis* Leaf Extracts

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Abstract- Sickle cell disease (SCD) represents a significant healthcare challenge in Africa, where conventional therapies such as hydroxyurea and hematopoietic stem cell transplantation are frequently limited by high costs, systemic toxicity, and poor accessibility. This study investigates the antisickling potential of *Brillantaisia owariensis*, a medicinal plant traditionally utilized in Southern Nigeria for managing anemia and SCD complications, despite a lack of prior scientific validation. Sequential ethanol and n-hexane leaf extracts of *B. owariensis* were evaluated for their antioxidant capacity (DPPH, Fe^{2+} chelation, Nitric oxide scarvenging and TAC) and *in vitro* antisickling effects, specifically HbS polymerization inhibition and sickled erythrocyte reversal. The results demonstrated that *B. owariensis* leaf extracts possess potent antioxidant and nitric oxide modulatory activities, alongside a significant capacity to inhibit polymerization and restore sickled erythrocytes to their normal morphology. These findings provide preliminary scientific evidence supporting the traditional use of *B. owariensis* and underscore its potential as a viable candidate for antisickling therapy.

Keywords: **Antioxidant potential, Antisickling activity, *Brillantaisia owariensis,* Hemoglobin S polymerization, Traditional medicine.**

I. INTRODUCTION

Red blood cells (erythrocytes) are known to perform critical gas exchange, transporting oxygen from the lungs to systemic tissues and returning carbon dioxide from the body tissues to the lungs for exhalation [1], [2]. This process of oxygen transportation relies on the cell's biconcave morphology and membrane flexibility, allowing seamless passage through narrow microvascular networks [3]. However, in individuals with sickle cell disease, these red blood cells are reported to lose their deformability, becoming rigid and crescent-shaped, which severely impairs physiological function [4], [5]. Hence, sickle cell disease is known as an inherited hematological disorder marked by the presence of an abnormal hemoglobin S (HbS) [6]. The sickled condition is reported to arise from a single point nucleotide substitution (GAG to GTG) in the β-globin gene, replacing polar glutamic acid with hydrophobic valine [7], [8]. Under low oxygen tension, this substitution promotes abnormal intermolecular interactions, causing HbS to polymerize into rigid, fibrous structures [9]. The resulting cellular deformation is said to result in several complications such as microvascular occlusion, tissue ischemia, chronic anemia, and recurrent vaso-occlusive crises [10]–[12].

Globally, Sub-Saharan Africa is reported to bear the highest burden of SCD, accounting for 75–80% of all cases [13]. The disease presents a massive public health challenge in this region, where limited healthcare resources, inadequate screening, and delayed diagnoses significantly impact the quality of life and survival rates [14].

Brillantaisia owariensis (P. Beauv) Family Acanthaceae

Brillantaisia owariensis (P. Beauv.), a member of the Acanthaceae family, is a herbaceous perennial shrub native to tropical Africa, with a broad distribution across Central and West Africa [15], [16]. This species holds significant importance in indigenous traditional medicine, particularly in Southern Nigeria, where it is utilized to manage anemia and complications associated with sickle cell disease. Despite its widespread traditional use, there is currently a lack of documented scientific evidence to substantiate these local claims. Consequently, this study aims to conduct a preliminary investigation into the plant's antisickling properties and is designed to evaluate its potential therapeutic effects by assessing antioxidant capacity, in-vitro sickle cell reversal, and hemoglobin S (HbS) polymerization inhibition.

II. MATERIALS AND METHOD

Materials

(All the chemicals used were of analytical grade and were not further purified)

Analytical weighing balance (Ohaus, USA), UV-visible Spectrophotometer (Perkins, USA), Electronic oven (B.Bran Scientific & Instrument Company, England), Hot water bath (Lifecare Medical LTD, Norway), Whatmann No. 1 filter paper, Capillary spotter (Drummond Scientific CO. Broomail, USA), Forceps, Test tubes, Pipette, Volumetric flask, Beaker, Masking tape, Iodine flask, pH meter, Venipuncture supplies(needles, syringes), Wax/Vaselinemixture, Sodium EDTA Bottles, Fisherbrand pipette, Microscope slides, Cover slips,, Microscope (Cottingen, Germany), Mortar, Pestle, Centrifuge (B.Bran Scientific & Instrument Company, England) Incubator (Biobase Biolin Co. LTD China), Blood samples from volunteers (confirmed sicklers, HbSS). Dipotassium hydrogen phosphate (BDH Chemical Ltd. England), Potassium dihydrogen phosphate(Hopkins and Williams Ltd., England), Nitroprusside, Sulphanilamide, Phosphoric acid(Molychem India), 1-Naphthylethylenediamine dihydrochloride (Molychem India), Ascorbic acid, Tris-hydrochloride(LOBA Chemie Mumbai, India), Phenanthrolinechloride(LOBA Chemie Mumbai, India), EDTA(Molychem India), Acetone(Hopkins and Williams Ltd., England),Sodium metabisulphite (Molychem India),Leishman's stain (Pathozyme Biomedicals, India) Absolute Methanol(Hopkins and Williams Ltd., England), P-hydroxybenzoic acid (LOBA Chemie PVT. LTD India), Normal saline.

Collection and Preparation of Plant Extract

The fresh leaves of *Brillantaisia owariensis* P. Beauv. (Family: Acanthaceae) were collected from Otuasega community, Ogbia Local Government Area, Bayelsa State, Nigeria (Latitude 4.92520°N, Longitude 6.40310°E). The plant sample was identified and authenticated by Dr. Ayo Oyedeji, a botanist of the Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, and a voucher specimen was deposited at the university herbarium. The leaf sample were carefully selected to remove debris and damaged portions, washed with running tap water, thereafter rinsed with distilled water, and air-dried under shade for 14 days. The dried leaves were then placed in the oven set at 50°C for 30 minutes and pulverized into a fine powder. Approximately 300 g of the pulverized leaves was macerated in 70% ethanol (3L) for 72 hours with intermittent agitation. The resulting extract was filtered using Whatman No. 1 filter paper, and the solvent was removed by evaporation using water bath set at 45°C. The extract obtained was weighed, and percentage yield was determined. A similar procedure was carried out for n-hexane extracts

In-vitro Anti-oxidant Assay

The antioxidant potential of the leaf extracts was evaluated using several *in vitro* assays, as described below:

DPPH Radical Scavenging Assay

The DPPH radical chelation activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay outlined in [17], in which various concentrations (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) of the extracts and ascorbic acid standard were prepared. The respective sample (0.5 mL) was mixed with 0.5 mL Ethanolic DPPH solution and incubated in the dark for 30 minutes and absorbance values determined at 516nm.

Ferrous Ion (Fe^{2+}) Chelation Assay

The Fe^{2+} chelation capacity was evaluated using the modified method of Minotti and Aust [18] with modifications by Puntel et al. [19], which employed the use of reaction mixtures containing 160 µL of 0.1 M Tris–HCl (pH 7.4), 200 µL normal saline, varying concentrations of the extracts and EDTA standard (0 – 1 mg/mL), and 150 µL of freshly prepared 500 µM $FeCl_2 \cdot 4H_2O$ were mixed together and incubated for 5 minutes. After which, 13 µL of 0.25% w/v 1,10-phenanthroline was added, and absorbance reading was recorded at a wavelenght of 510 nm. EDTA served as the standard.

Total Antioxidant Capacity (Phosphomolybdenum Method)

The procedure outlined by Perito [20], was employed in the determination of TAC. Different concentrations (0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) of the standard (ascorbic acid), and test samples (plant extracts) were prepared. To 0.1 mL of each sample, was added 1 mL of reagent solution containing 0.6 M sulfuric acid, and 28 mM sodium phosphate, and 4 mM ammonium molybdate was added. The test tubes containing the mixtures were sealed appropriately and incubated in a boiling water bath set at 95°C for a duration of 90 minutes. Thereafter, the samples were allowed to cool at room temperature and absorbance was measured at 695 nm. Ethanol was employed as the blank in this assay. The antioxidant capacity was expressed as mg of ascorbic acid standard (equivalents) per gram of dried extract.

Nitric Oxide Scavenging/Generation Activity (Griess Reaction)

The nitric oxide scavenging activity was assessed following a modified method by Marcocci et al. [21]. Different concentrations of the Gallic acid standard and extracts at 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL were prepared and to each sample, 5 mM sodium nitroprusside in phosphate-buffered saline (pH 7.4) 0.5 mL was added and incubated at ambient temperature for a period of 30 minutes. After incubation, the nitrite formed was measured using the Griess Reagent, which consists of 1% sulphanilamide in 5% phosphoric acid and 0.1% 1-naphthylethylenediamine hydrochloride in water. The absorbance values was determined using a UV-visible spectrophotometer.

In-vitro Antisickling Assay

Sickle Cell Reversal Activity

After obtaining ethical approval from the Faculty of Pharmacy Research and Ethics Committee, Niger Delta University, Bayelsa State, Nigeria, and informed consent from the sickle cell disease patient's. Blood samples (5ml) were collected using a venipuncture into a sodium EDTA bottle. The blood was then mixed thoroughly by rolling the bottle gently. a drop of blood was then mixed with one drop of 2 % sodium metabisulphite solution on a microscope slide using a Fisher brand pipette. The blood-$Na_2S_5O_3$ mixture was gently spread on the microscope slide to make a smear size of about 3cm, covered with a cover slip and the edge sealed with wax/vaseline mixture. This was then examined under a microscope with the dry objective. Sickle cell present were counted and the percentage of sickling determined.

Procedure for Sickle Cell Reversal Assay

The modified method described by Sofowora et al. [22], was utilized in the assessment of the sickle cell reversal assay. The blood samples collected were first subjected to centrifugation at 2000 rpm, this aid the separation of the serum. the packed erythrocytes were washed thrice with sterile normal saline 1ml per 5 mL of blood. Each washing step involved centrifugation of the blood at 2000 rpm for 5 minutes, and subsequent removal of supernatant. For the assay, the erythrocytes washed 0.2ml was mixed with the respective plant extract 0.2ml in an uncovered test tubes, followed by incubation at a temperature of 37°C for a period of 3 hours with intermittent gentle shaking. 2% sodium metabisulphite 0.08ml was added to the respective test tube, and the tubes were gently rolled to ensure uniform mixing. Triplicate samples were collected at 0 minutes and subsequently at 30-minute intervals until three time points were recorded. At each time point, samples were smeared onto microscopic slides, fixed using 95% methanol, dried under air, and stained using Leishman's stain.

Slides were viewed under an oil immersion objective (×100), and three fields were counted per slide, with approximately 300 erythrocytes per field. Cells exhibiting biconcave or disc-like shapes were classified as normal healthy cells, whereas starlike, wrinkled, or elongated cells were considered sickled.

Test Control

The two-control employed in this assay are; P-hydroxybenzoic acid (PABA), having a concentration ranging from (0.2, 0.4, 0.6, 0.8, to 1 mg/mL,) represents the positive control, and Normal saline (N/S) used as the negative control.

Sickle Cell Hemoglobin Polymerization Inhibition Assay

The method outlined by Noguchi and Schetcher [23] was employed in the determination of HbSS polymerization inhibition activity of the plant extracts. The Polymerization rate was examined by measuring the turbidity of the solutions (polymerization mixture) using a spectrophotometer at a wavelenght of 700nm. a deoxygenating agent known as Sodium metabisulphite was utilized in this procedure [24]. the deoxygenating agent, 2% sodium metabisulphite 4.4ml was transferred into a test tube, thereafter, normal saline 0.5ml was added. Subsequently, the sickle cell hemoglobin solution 0.1ml was introduced into the test tube, the resulting mixture was gently mixed. The mixture was then monitored using a spectrophotometer, with absorbance readings taken at two-minute intervals over a 30-minute period. This setup served as the negative control for the assay. the test samples comprises of PABA, n-hexane extract, and Ethanol extract, all having same concentrations (0, 200, 400, 600, 800 and 1000µg/ml). 2% sodium metabisulphite 4.4ml was added into a test tube followed by the transfer of the respective test sample 0.5ml, into the test tube. Subsequently, sickle cell hemoglobin (HbSS) solution 0.1ml was added. The absorbance reading for the respective mixture were measured at a time interval of two minutes over a period of 30 minutes. The rate of hemoglobin polymerization for each test sample was calculated based on the change in optical density (OD) over time and expressed in percentage that is relative to the negative control.

III. RESULTS

The % DPPH Scarvenging activity was calculated using the equation below, with the results displayed in the accompanying bar chart below presented as Fig.1:

$$\% \text{ DPPH Scarvenging activity} = \frac{\text{Acontrol} - \text{Atest}}{\text{Acontrol}} \times 100$$

Where Atest = Absorbance of the plant extract or standard sample

Acontrol = Absorbance of the control

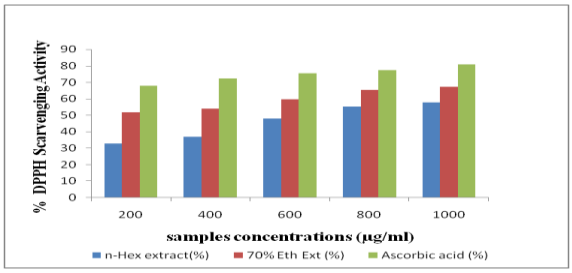

Fig.1: 1,1-Diphenyl-2-picrylhydrayl (DPPH) radical scarvenging activities of the leaf extracts of *B. owariensis*

The % Fe^{2+} Chelation was calculated using the equation below, with the results presented in the accompanying bar chart presented as Fig.2:

$$\%\ Fe^{2+}\ \text{Chelation} = \frac{\text{Atest} - \text{Acontrol}}{\text{Acontrol}} \times 100$$

Where Atest = Absorbance of the plant extract or standard sample

Acontrol = Absorbance of the control

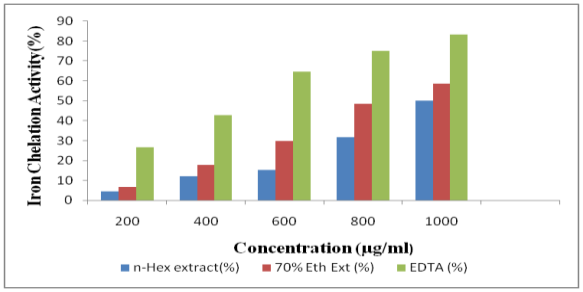

Fig.2: Ferrous ion (Fe^{2+}) chelating activity of the leaf extracts of *Brillantaisia owariensis.*

The values obtained for the total antioxidant capacity of both the ethanol and n-Hexane extracts of *Brillantaisia owariensis* is presented in the bar chart below (Fig.3:).

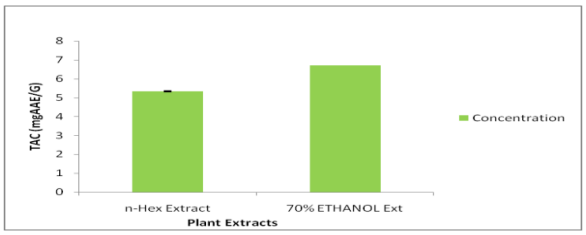

Fig.3: Total antioxidant capacity of the leaf extracts of *B. owariensis*

The % Nitric Oxide Scarvenging activity was calculated using the equation below, with the results shown in the accompanying bar chart presented as Fig.4:

%Nitric Oxide Scarvenging activity = Acontrol – Atest X 100 /Acontrol

Where Atest = Absorbance of the plant extract or standard

Acontrol = Absorbance of the control.

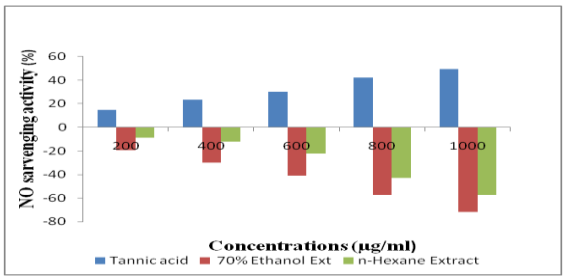

Fig.4: Nitric oxide inhibiting/generating activity of the leaf extracts of *Brillantaisia owariensis*

The percentage Sickling was derived using the equation below and the results presented in the accompanying bar chart presented as Fig.5:

Percentage (%) of sickling = (Number of sickled cells X 100) / Total cells.

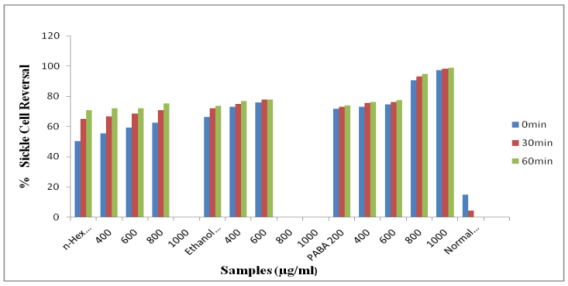

Fig.5: Sickle cell reversal activity of the leaf extracts of *B. owariensis.*

The % HbS polymerization inhibition activity was calculated using the equation below:

% Inhibition = $((\Delta D)/OD_0)\times 100$

Change in optical density (ΔD) = (D_0 -D_1)

Where D_0 = initial density

D_1 = final density.

and the results depicted in the accompanying bar chart presented as Fig.6:

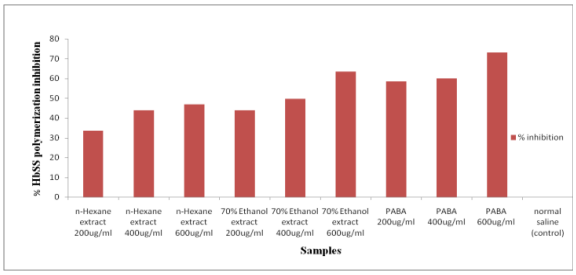

Fig.6: Sickle cell hemoglobin polymerization inhibiting activity of the leaf extracts of *B. owariensis*

IV. DISCUSSION

The findings from the DPPH radical scavenging assay presented in Fig.1 demonstrated that the plant extracts exhibited appreciable free radical scavenging activity, with efficacy reaching approximately 83.1% relative to ascorbic acid. This result suggests a strong capacity of the extracts to neutralize reactive oxygen species, which are key contributors to oxidative stress and cellular damage as reported by Ere et al., [25]. Such antioxidant activity is closely associated with anti-inflammatory effects and may play significant role in mitigating the severity of painful vaso-occlusive episodes observed in sickle cell disease (SCD) as stated by Pavitra et al., and Chauhan & Zennadi, [26], [27]. Similarly, the result of the Fe^{2+} chelation assay as depicted in Fig. 2 revealed that the plant extracts possessed notable metal chelating activity, achieving up to 70.35% of the activity of the EDTA standard. This indicates a potential role in reducing iron overload, a common complication in SCD patients thereby limiting excessive iron accumulation. This suggest that the extracts may help prevent oxidative tissue damage and subsequent end-organ complications according to Pavitra et al., [26]. The total antioxidant capacity (TAC) values shown in Fig.3 further support the strong antioxidant potential of the plant extracts, with values ranging from 5.33 to 6.71 mg AAE/g, based on established TAC classification ranges for ascorbic acid standard: (0.1 – 1.0 mg AAE/g: low; 1.0 – 5.0 mg AAE/g: moderate; 5.0 – 10.0 mg AAE/g: high; and > 10.0 mg AAE/g: very high), these values places the extracts within the

high antioxidant capacity category. This result indicates that the plant extracts may contribute significantly to reducing oxidative stress and inflammation, thereby potentially decreasing the frequency and severity of pain crises, improving red blood cell (RBC) integrity, and preventing organ damage in SCD [26], [27]. The result of the nitric oxide (NO) scavenging assay shown in Fig. 4 revealed that the plant extracts exhibited a pro-oxidant effect, with the ethanolic extract reaching −71.8% at its peak concentration. This observation suggests that the plant extracts may enhance nitric oxide bioavailability, supporting its critical role in vasodilation, improved blood flow, reduction of vaso-occlusive events, and enhanced RBC deformability as highlighted by Ere et al., [25]. Additionally, as noted, improved nitric oxide dynamics may contribute to reduced inflammation and oxidative stress, thereby limiting erythrocyte sickling, according to the findings of Almeida et al., and Gladwin et al., [28], [29]. Furthermore, the leaf extracts appears to demonstrate significant biological activity in hematological models as result from the sickle cell reversal assay presented in Fig. 5 showed that the plant extracts promoted the conversion of sickled erythrocytes toward a more normal biconcave morphology, with efficacy up to 78.6% relative to (PABA). The absence of reversal activity in the negative control confirms the specificity of the observed effects. This result suggest that the plant extracts may posses capacity to reverse red blood cell sickling, indicating potential for improving RBC function, reducing hemolysis, and enhancing oxygen delivery. While the hemoglobin S (HbS) polymerization inhibition assay as shown in Fig. 6 further demonstrated that the plant extracts effectively inhibited hemoglobin polymerization *in vitro* with efficacy reaching 75.3% relative to Para-amino benzoic acid (PABA). As expected, the negative control (normal saline) showed no activity. These findings suggest that the extracts may interfere with the polymerization process of deoxygenated HbS, thereby preventing erythrocyte sickling, reducing hemolysis, and minimizing vaso-occlusive crises as reported by Almeida et al., and Camacho et al., [28], [30]. Overall, the results of this study demonstrated that *Brillantaisia owariensis* exhibits considerable antioxidant, anti-inflammatory, HbS polymerization inhibitory, and sickle cell reversal activities, thereby highlighting its notable antisickling potential. The consistently superior performance of the ethanolic extract across multiple assays provides strong scientific support for the traditional use of ethyl alcohol as the solvent of choice in herbal extraction by the local population. These findings provide preliminary scientific evidence supporting the traditional use of *B. owariensis* in the management of sickle cell disease and anemia. Further studies are, however, necessary to establish its safety profile owing to the cytotoxicity observed at higher concentrations above 800µg/ml, as well as to isolate and characterize the bioactive constituents responsible for these effects.

V. CONCLUSION

Brillantaisia owariensis could be said to possess significant antisickling properties which are relevant to the management of sickle cell disease, Since the extracts exhibited the capacity to reduce oxidative stress, inhibit hemoglobin S polymerization, and promoted the reversal of sickled erythrocytes towards a normal morphology. This finding provide scientific support to the claims made by locals on the use of the *B.owariensis* in mitigating sickle cell disease complications. The superior performance of the ethanolic extracts justifies the preference for ethanol as the extraction solvent, consistent with local practices. However, the observed cytotoxicity at higher concentrations is a red flag highlighting the need for caution regarding over dosage in traditional use.

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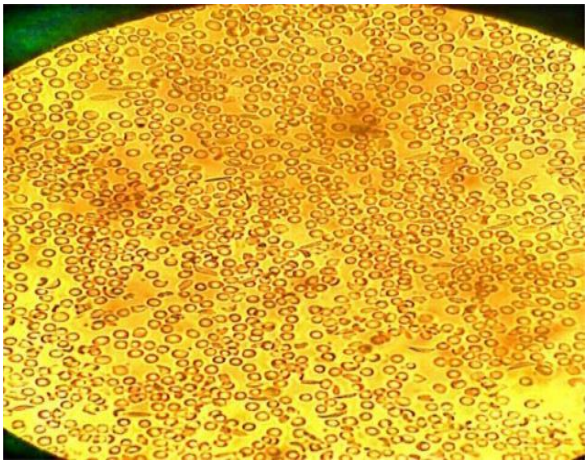
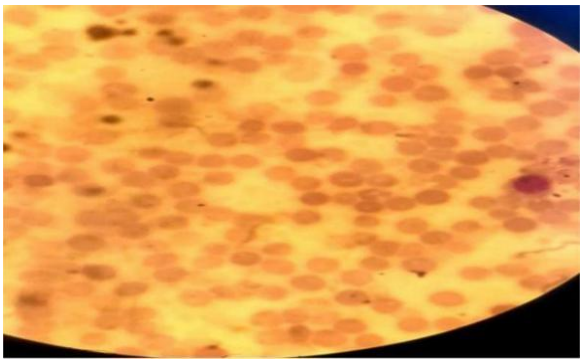
APPENDIX

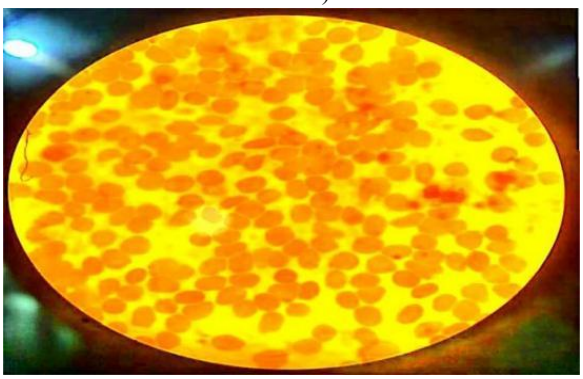
Microscopic slides from the in-vitro sickle cell reversal assay.



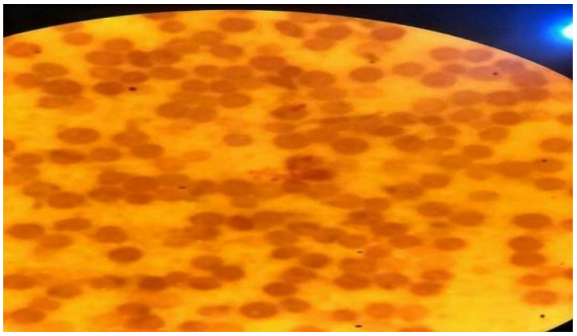
1 drop of blood + 1 drop of 2% $Na_2S_2O_5$



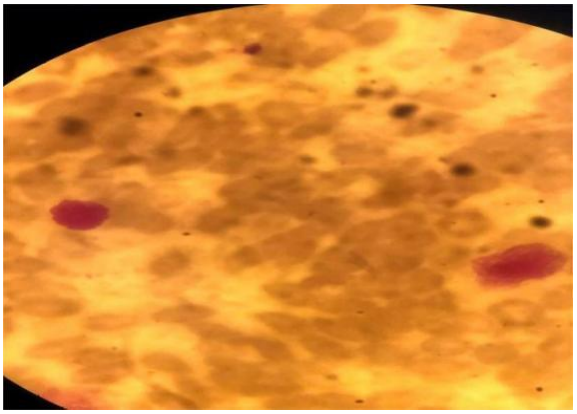
HbSS Blood treated + PABA 1000µg/ml(T = 60mins)



HbSS blood treated with n-Hexane Extract 800µg/ml(T = 60mins)



HbSS blood treated with 70% Ethanol Extract 600µg/ml(T = 60mins)



HbSS blood treated with Normal saline(T = 60mins)