



***In-vitro* Antisickling Activity of *Pergularia daemia*, *Canna indica* and *Petiveria alliacea* Plants used in the Treatment of Sickle Cell Anaemia in Edo State, Nigeria**

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Abstract

Sickle cell disease is a genetic blood disorder that affects the shape and transportation of the red blood cells (RBCs) in blood vessels, leading to various clinical complications. Available medicines for treating the disease are insufficiently effective, toxic or expensive. Therefore, there has been a pressing need for effective and inexpensive therapeutic agents from indigenous medicinal plants. *Petiveria alliacea*, *Canna indica* and *Pergularia daemia*, respectively were evaluated for their cationic constituents as a measure for their antisickling activity in sickle cell anaemia disorder. The three medicinal plants were extracted separately with methanol solvent using maceration method for 72 hours. The extracts were concentrated using a rotary evaporator (Model RE, 200, USA). Phytochemical screenings were conducted using standard method, while other portions of the extract were subjected to dry ash digestion for determination of mineral elements by emission flame photometry (EFP) and atomic absorption spectrophotometry (AAS). Evaluation of the antisickling activity of the extracts was done using the sodium metabisulphite (SMBS) test. Eugenol, terpenoids and alkaloids were present in the three plant extracts, while K^+ values recorded for *Petiveria alliacea*, *Canna indica* and *Pergularia daemia* were 54.30 mg kg^{-1} , $180.10 \text{ mg kg}^{-1}$ and 28.30 mg kg^{-1} , respectively. Other mineral elements detected in the three plants were Cu^{2+} , Zn^{2+} , Fe^{2+} and Mg^{2+} . The leaves extract of *Canna indica* and *Pergularia daemia* at high dose of $300 \mu\text{g/mL}$ caused significant reductions of sickle red blood cells from 15% to 6% and 15% to 1%, respectively at 90 minutes of the antisickling test. The research confirmed that extracts of both *Canna indica* and *Pergularia daemia* used in this study have significant antisickling properties in *invitro* studies than *Petiveria alliacea*.

Keywords: Antisickling activity, *Petiveria alliacea*, *Canna indica*, *Pergularia daemia*, sickle cell, phytochemicals, minerals.

Introduction

The use of medicinal plants as first line of medication for the treatment of various diseases cannot be over stressed. They have been used by human beings to treat a wide range of ailments for thousands of years (Sofowora 1982). Anti-sickling activity of the

red blood cells of humans have been reported for different medicinal plants among which are: *Zanthoxylum* (Fagara) (Sofowara 1982), *Rauwolfia vomitoria* stem bark and *Nicotiana tabacum* (Gills 1992), while antimalarial potencies have been reported for different medicinal plants including *Carica papaya*,

Citrillus vulgaris, stem bark of *Astonia*, leaves of *Mangifera indica*, *Cymbopogon citratus* and *Morinda lucida* (Gills 1992). *Stigmaphyllon ovatum* has also been reported to indicate antimalarial potency (Iyekowa and Edema 2017, Iyekowa et al. 2021, Rasoanaivo et al. 1992). Microbial infections have been treated with *Alchornea cordifolia* (Iyekowa et al. 2016), cancer with soursop and *Stigmaphyllon ovatum* (Elemike et al. 2019), asthma with *Phyllanthus amarus* (Iyekowa et al. 2019) and pain with *Crinum jagus* bulb (Iyekowa and Oderanti 2023). Anaemia, a deficiency in oxygen-carrying erythrocytes, is the most common form of anaemia for sickle cell patients. Initially, the observed sickling is reversible upon re-oxygenation of the system. However, repeated oxygenation and de-oxygenation cycle lead to irreversible sickle cell (Sofowora 2008). Sickle cell disease (SCD) is an inherited disorder of haemoglobin. It is characterized by lifelong haemolytic anaemia and a wide variety of painful and debilitating vaso-occlusive events, which occurs in 70000 to 80000 Americans of African, Mediterranean, or Middle Eastern extraction (Steinberg 1999). The term sickle cell disorder refers to states in which the red cells undergo sickling when they are deoxygenated. The sickle cell diseases are those disorders in which sickling produces prominent clinical manifestations. Included are sickle cell-haemoglobin C disease (haemoglobin SC disease), sickle cell-haemoglobin D disease (haemoglobin SD disease), sickle cell β thalassemia and sickle cell anaemia. The latter term is reserved for the homozygous state for the sickle cell gene (Reid et al. 1995)

Haemoglobin, the red blood pigment is a protein whose major function is to transport oxygen throughout the body. A molecule of haemoglobin is $\alpha_2 \beta_2$ tetramer; that is, it consists of two identical α chains and two identical β chains. Haemoglobin is contained in the erythrocytes of which it forms about 33% by weight in normal individuals, a concentration that is nearly the same as it has in the crystalline state. Sickle cell disease is an important genetic cause of haemolytic

anaemia, a form of anaemia due to increased erythrocyte destruction, instead of the reduced mature erythrocyte production seen with iron, folic acid and vitamin B₁₂ deficiency (Atimati et al. 2013). Sickle cell anaemia is a molecular disease, which occurs as a result of the presence of mutant haemoglobin. Sickle cell anaemia is inherited according to the laws of Mendelian genetics. The haemoglobin of individuals who are homozygous for sickle cell anaemia is almost entirely sickle cell haemoglobin (Hbs). In contrast, individuals heterozygous for sickle cell anaemia have haemoglobin that is 40% Hbs. Such persons, who are said to have the sickle cell trait, lead a normal life though their erythrocytes have a shorter lifetime than those of normal individuals. From the long fibrous aggregates characteristics of this disorder, sickle cells impede the flow of blood in the capillaries such that in a sickle cell crisis, the blood flow in some areas may be completely blocked thereby giving rise to extensive tissue damage and excruciating pain (Voet and Voet 2004).

Iron deficiency is the most common cause of chronic anaemia, like other forms of chronic anaemia, iron deficiency anaemia leads to pallor, fatigue, dizziness, exertion dyspnoea and other generalized symptoms of tissue hypoxia. Iron forms the nucleus of the iron-porphyrin heme ring which together with globin chains form haemoglobins. Haemoglobins reversibly build oxygen and provide the critical mechanism for oxygen delivery from the lungs to other tissues in the absence of adequate iron, small erythrocytes with insufficient haemoglobin are formed, giving rise to microcytic hypochromic anaemia. Iron-containing heme is also an essential component of myoglobin, cytochrome and other proteins with diverse biological functions (Bertram et al. 2012). In individuals with the inherited disease sickle-cell anaemia, many erythrocytes assume an irregular crescent-like shape under conditions of low oxygen concentration typical of the capillaries. This sickling increases the erythrocyte rigidity which hinders their free passage through the capillaries. Moreover,

individuals with sickle-cell anaemia suffer from severe haemolytic anaemia (a condition characterized by red cell destruction) because the increased mechanical fragility of their erythrocytes halves the normal 120-day lifeline of these cells. The debilitating effect of this disease is such that before the latter half of the twentieth century, individuals with sickle-cell anaemia rarely survived to maturity (Carson-DeWitt 2013).

Sickle-cell anaemia is a life-threatening and painful disease. People with sickle-cell anaemia suffer from repeated crises brought on by physical exertion. They become weak, dizzy, and short of breath, and they also experience heart murmurs and an increased pulse rate. The haemoglobin content of their blood is only about half the normal value of 14 to 17.5 g/dL because sickled cells are very fragile and rupture easily; this results in anaemia (lack of blood). In Nigeria, NIPRISAN™ is a herbal drug developed by the National Institute for Pharmaceutical Research and Development (NIPRD), and widely in use in Nigeria, India and the United States of America, which has shown great indication for success (Ameh et al. 2012). This may have been possible due to synergistic effects of the chemical components of the constituent herbs. Hence, the observed therapeutic effects of this phytomedicine may be as a result of combined antisickling, antipolymerization, antidehydration and antioxidants effects from the component plants, which is typical of most herbal remedies.

Advance developments and researches in medicine have upheld the treatment and management of sickle cell disease to include significant increases in life expectancy of this group of patients. Improved public health, neonatal screening, parental and patient education, advances in red cell transfusion medicine, iron chelation therapy, penicillin prophylaxis for children, pneumococcal immunization, and hydroxyl urea therapy have all likely contributed to this effect on longevity (Bunn 1997). This prophylactic intervention is highly expensive for many poor patients of Africa and demands complex clinical management and attention. In fact, without major breakthroughs in gene therapy or bone marrow transplantation that make these treatments applicable to a large number of patients, drug intervention will remain the major therapeutic option for sickle cell disease (Isoa 2009). A number of phytomedicines have been used in the treatment and prevention of SCD in Africa in general and in Nigeria in particular.

Various researches on medicinal plants by traditional herbal practitioners in Nigeria to find alternative, cheaper and less toxic therapies, led to the scientific discovery of antisickling potentials of some medicinal plants like *Cajanus cajan* seeds, *Zanthoxylum zanthoxyloides* (Fagara) root, *Carica papaya* unripe fruit and leaves; and *Parquetina nigrescens* whole plant extracts which boost blood volume (Oduola et al. 2006, Imaga 2010). Some medicinal plants with potential antisickling properties are shown in Figure 1.



Petiveria alliacea



Canna indica



Pergularia daemia

Figure 1: Medicinal plants with potential antisickling properties (Source: Ananth et al. 2021, Kumbhar et al. 2018).

Bioactive chemical constituents of most antisickling herbs are the phenylalanine and hydroxyl benzoic acid components (Onah et al. 2002). Other constituents include

anthraquinone derivatives, steroidal glycosides, cardiac glycosides, hydroxyl urea, lysine and arginine (Folashade and Omoregie 2013). See Figure 2.

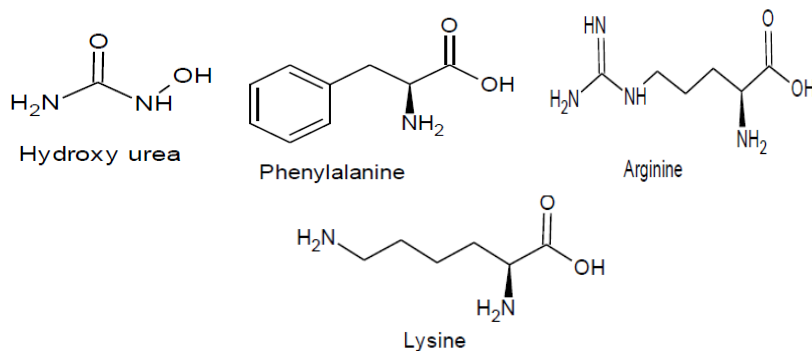


Figure 2: Chemical constituents with antisickling properties.

In developing countries where the use of herbal remedies is at its peak, the potential benefits of using medicinal plants in the management of sickle cell disease should not be under estimated.

Among the medicinal plants used in combination for the treatment of SCD in Edo State of Nigeria are *Petiveria alliacea*, *Canna indica* and *Pergularia daemia*.

Researchers have reported the antisickling activities of some medicinal plants. Egunyomi et al. (2009) reported that the aqueous extracts of the reddish brown freshly fallen leaves of *Terminalia catappa* were able to exhibit antisickling activity on sodium metabisulphite induced sickling. Nwaoguikpe et al. (2010) reported that Aloe vera plant extracts with the preponderance of nutrients, phytochemicals, amino acids and other compounds can be very beneficial in the management of sickle cell disease, while Onyegeme-Okerenta et al. (2019) investigated the anti-sickling properties of aqueous leaf extracts of *Physalis angulata* and *Dennettia tripetala* on homozygous sickle cell erythrocyte. From their findings, antisickling effect showed that, 1 mL of 100 mg/ mL concentration of *D. tripetala* and *P. angulata* with the sickle red blood cell (RBC), in the ratio of 1:1, reduced the abnormal cell to 13, 9 and 6% with *D. tripetala* and 11, 8 and 6% with *P. angulata* at a time interval of 20, 40 and 60 minutes, respectively. This led to their conclusion that *D. tripetala* leaf extract and *P. angulata* have antisickling potentials and may be used for therapeutic management of sickle cell anaemia. Similarly, the use of phytomaterials

of *Piper guineense*, *Pterocarpus osun*, *Eugenia caryophyllata* and *Sorghum bicolor* extracts for the treatment of SCD has been reported (Mehanna 2001). The extracts of *Pterocarpus santolinoides* and *Aloe vera* have also been reported to increase the gelling time of sickle cell blood and inhibit sickling in *in-vitro* (Boudreau and Beland 2006, Ejele and Njoku 2008). Some herbal cocktails have been produced and tested for their ability to intervene in sickle cell crises. The drug Nicosan previously NIPRISAN (Nix0699), which is a product of the extracts of four different plants (*Piper guineense* seeds, *Pterocarpus osun* stem, *Eugenia caryophyllus* fruit, and *Sorghum bicolor* leaves) were shown to possess anti-sickling properties (Iyamu et al. 2012). Clinical trials of Nix-0699 showed that the drug significantly reduced the number of painful episodes in SCD patients.

Pergularia daemia, family Apocynaceae (locally called, Utazi in Igbo) is a high climbing vine plant that scrambles over the ground for twines into other plants for supports. The stems are somewhat woody at the base. The plant has a range of traditional medicinal uses as well as supplying food as vegetables and fibres. The roots and leafy twigs are traded for medicinal uses in local markets of Africa. Because of its sweet scented flowers and its climbing habit, the plant is cultivated as a pergola ornamental in tropical gardens (Karthishwaran et al. 2010). It is a highly toxic plant, especially the aerial plants, due to the presence of numerous cardenolides and cardenolid glycosides; these have digitalis-like cardio activity (Bhat

1995). The latex in the plants is poisonous. It is used as fishing and hunting poison, and is added to water to poison animals.

Terpenoids, flavonoids, sterols and cardenolides are among the chemicals that have been isolated from the leaves, stems, roots, seeds or fruits (Bhat 1995). Traditionally, it has been used as an anthelmintic, laxative, antipyretic, expectorant, besides treatment of infantile diarrhoea, malarial intermittent fevers, toothaches and colds (Bhat 1995). Studies have shown hepatoprotective, antifertility, anti-diabetic, analgesic, and anti-inflammatory properties of substances in its aerial parts (Bhat 1995).

Petiveria alliacea, family Phytolaccaceae, locally called “Awogba” (Yoruba), “Ebenmbara” (Ibibio) is a herbaceous perennial herb with medicinal properties. *Petiveria alliacea* is popularly known as guinea hen weed in English and ‘anami’ in most part of South America countries. It is a perennial short plant with a deep tap root and height of about 0.5–1.5 metres. *Petiveria alliacea* has a wide range of therapeutical properties. Both roots and leaves are used topically and orally in the form of a crude extract or infusion and the roots have been identified as the most active parts of the plant (Lopes-Martins et al. 2002). It is considered an antispasmodic diuretic, menstrual promoter, stimulant and sweet promoter. Herbalists and natural health practitioners use it for oedema, arthritis, malaria rheumatism, poor memory, topical analgesic and anti-inflammatory for skin afflictions. A fraction of *Petiveria alliacea* leaves and stems were also discovered to induce in vitro cell death and in vivo tumour regression in a murine breast cancer model (Hernández et al. 2014). Compounds isolated and reported for *Petiveria alliacea* include flavonoids as astilbin, myricitrin, engeletin, triterpenes and several sulfur-containing amino acids in the roots (Kubec et al. 2002).

Canna indica, family Cannaceae, locally called “Ebesalebo” (Edo), “Gwangwama” (Hausa), “Manyagoloagede” (Igbo) and “Majesinmi” (Yoruba), is a perennial plant chimp of stems 150–300 cm tall with large

leaves up to 50 cm long and 25 cm wide. The stems arise from a large, thick and tuber like rhizome (Darsini et al. 2015). The plant is widely grown as ornamental and selected forms are cultivated for their edible roots. The plant is used in the treatment of women’s complaint and as antipyretic (Darsini et al. 2015).

An infusion of the rhizome is said to be febrifuge and stimulant, whilst a decoction is said to be diaphoretic and diuretic (Darsini et al. 2015). The phytochemical analysis of *Canna indica* showed that it contained various phytochemicals, including alkaloids, cardiac glycoside, anthocyanin pigments, flavonoids, steroids, terpenoids, tannins, phlobatannins, saponins, carbohydrates, proteins, oils and many other chemical compounds (Kumbhar et al. 2018). The pharmacological studies revealed that the plant exerted anthelmintic, anti-bacterial, anti-microbial, antiviral, anti-diabetic, antidiarrheal, anti-inflammatory, analgesic, immune modulating antioxidant, cytotoxic, haemostatic, hepatoprotective and molluscidal activity (Al-Snafi 2015).

Materials and Methods

Plant collection

Fresh leaves of *Pergularia daemia*, *Canna indica* and *Petiveria alliacea* were collected from Eyaen, Benin City, Edo State, Nigeria. They were identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria with herbarium voucher number UBHM 0278. The plants were dried for 28 days and pulverized into powder using mechanical grinding machine.

Extraction

Five hundred grams (500 g) of each pulverized plant were macerated in 1 L of methanol solvent respectively for 72 hrs. The mixture was sieved using porcelain cloth and further using No. 1 Whatman filter paper number 4. The filtrate was concentrated using rotary evaporator and the crude concentrate was stored in the refrigerator below 7 °C until required for further experiments.

Phytochemical screening

Phytochemical screenings were performed on the extracts using standard procedures by Sofowora (1993) and Trease and Evans (1989).

Test for glycosides: 1 mL of the plant extract was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1 ml of conc. H₂SO₄. A brown ring is required for the presence of glycosides.

Test for saponins: 0.5 g of plant extract was shaken with water in a test-tube and observed for frothing. Saponin rein Weiss (supplied by Merck) was used as a standard.

Test for flavonoids: 2 mL of the plant extract was boiled with distilled water and filtered. 5 mL of 20% NaOH and few drops of dilute HCl were added to the solution. Formation of a colourless solution is indicative of a positive test.

Test for phenolic compounds: 1 mL of the plant extract was added to 5 mL of 90% ethanol. In addition, 1 drop of 10% FeCl₃ was added. A pale-yellow colouration of indicative of positive test.

Test for tannins: To 2 mL of the plant extract, 10 mL of distilled water was added and boiled for 5 minutes and then filtered into halves. To about 2 drops of the filtrate, ferric (FeCl₃) solution was added; formation of a bluish precipitate is required for hydrolysable tannins.

Test for eugenols: 2 mL of the plant extract was mixed with 5% KOH solution. The aqueous layer was separated and filtered. Few drops of dilute HCl were added to the filtrate. A pale yellow precipitate is indicative of a positive test.

Test for steroids: 2 mL of acetic anhydride was added to 0.5 g plant extract in 2 mL of dilute H₂SO₄. A colour change from violet to blue or green is required for the presence of steroids.

Test for terpenoids (Salkowski test): 5 mL of plant extract was mixed in 2 mL of chloroform and 3 mL of conc. H₂SO₄ were carefully added down the side of the inner wall of test tube to form a layer. A reddish brown colouration of the inter-phase is required for the presence of terpenoids.

Test for alkaloids: 2 mL of picric acid was added to the plant extract. A yellowish precipitate test is a positive test.

Determination of mineral and selected heavy metal contents

The following mineral elements were analysed for each sample using Atomic Absorption spectrophotometer (AAS) and Emission Flame Photometry (EFP): Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Cu²⁺ according to standard methods (AOAC 2015).

Examination of anti-sickling activity of plant extracts

Blood collection: 5 mL of blood sample was obtained by vein puncture from one (1) confirmed sickle cell patient (HbSS) not in crises from University of Benin Teaching Hospital (UBTH), Ugbowo, Benin City, Nigeria. The erythrocytes were isolated from whole blood by centrifuging at 1500 x g for 15 minutes. The plasma was siphoned out carefully from the sickle cell sediment using Pasteur pipette. The patient had not been transfused for twelve months before the antisickling analysis, with Hb AA blood. All antisickling experiments were carried out with freshly collected blood. Only one type of human blood sample was used at a time for the three plant extracts. In order to confirm the patient's sickle cell nature, the HbSS blood samples were first characterized by haemoglobin electrophoresis on cellulose acetate gel (Mpiana et al. 2010). The sample was found to be sickle cell blood and was then stored at ± 4 °C in a refrigerator. Only blood samples presenting good sickling rate (≥ 90%) were selected for antisickling activity.

Procedure for antisickling activity evaluation: Sodium metabisulphite (SMBS) test

The evaluation of the three (3) different methanol extracts for antisickling activities was carried out using a modified method of Sofowora and Isaacs (1971). Vein punctured blood samples from sickle cell anaemia patients not in crisis were collected into EDTA bottles and centrifuged to remove the

serum. The resulting packed erythrocytes were washed 3 times with 1 mL sterile normal saline per mL blood. The samples were then centrifuged each time for 5 min at a speed of 2000 revolution per minute to remove the supernatant, 0.5 mL of the washed erythrocytes were mixed each with, 0.5 mL of the different extracts in uncovered test tubes and mixed together. Samples were taken from the different mixtures and incubated at 37 °C for 3 h while shaking occasionally. About 0.2 mL of 2% sodium metabisulphite were added to deoxygenate the system, mixed thoroughly, sealed with liquid paraffin, samples were incubated again at 37 °C and samples taken at 45 min intervals until 5 readings were obtained.

Each sample was smeared on a microscope slide, fixed with 95% methanol, dried and stained with Leischman's stain. Each sample was examined under the oil. Immersion light microscope was used to count at least 500 red blood cells in each sample from five different fields of view across the slide. The numbers of sickle and

unsickle red blood cells were counted. Negative control was achieved by sodium metabisulphite (5 mg/mL) as a reductant or deoxygenating agent (Iwuet al.1988). Each set in the experiment was replicated twice.

Calculation of percentage of sickled red blood cell

The following formula was used in calculation of percentage of sickled red blood cell:

$$\begin{aligned} \% \text{ sickled red blood cell} \\ = \frac{\text{Sickle cells}}{\text{RBC count}} \times \frac{100}{1} \end{aligned} \quad (1)$$

5 mg.mL⁻¹ = 500 mg.L⁻¹ RBC = Red blood cell.

Results and Discussion

Phytochemical constituents

The phytochemical constituents detected qualitatively in the three plant extract are shown in Table 1.

Table 1: Phytochemical constituents of methanol extract of *Pergularia daemia*, *Petiveria alliacea* and *Canna indica*

S/N	Phytochemical constituents	<i>Petiveria alliacea</i>	<i>Canna indica</i>	<i>Pergularia daemia</i>
1	Glycosides	+	+	-
2	Saponins	+	+	-
3	Flavonoids	-	-	+
4	Phenolics	-	+	+
5	Tannins	-	-	-
6	Eugenol	+	+	+
7	Steroid	-	-	-
8	Terpenoids	+	+	+
9	Alkaloids	+	+	+

Key: + = present - = absent.

Eugenols, terpenoids and alkaloids (Table 1) were present among the three extracts, but glycosides and saponins were only detected in *Petiveria alliacea* and *Canna indica* in the methanol extract. *Petiveria alliacea* has been reported to contain alkaloids and glycosides, while *Canna indica* leaves were found to contain saponins, tannins, limonene, amyridin and phellandrine (Gills 1992). *Petiveria*

alliacea have been reported also to contain isothiocyanates; polyphenols and tannins (Kubec et al. 2002, Kubec and Musah 2001). However, tannins were not detected in this study and this may be attributed to the method of extraction.

Glycosides are used in the treatment of heart disease. Saponins decrease blood lipids glucose response. Phenolics have been

reported to be responsible for their chemo preventive properties (antioxidant, anticarcinogenic or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle (Doughari 2012).

The three plant extracts contain Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Fe^{2+} , Zn^{2+} and Cu^{2+} at different concentrations (Table 2). However, potassium and calcium had more concentrations in the three plant extracts than other elements analysed in this study. The K^+ content was higher than the Ca^{2+} contents in the three extracts. While Cu^{2+} was detected as the lowest among the three plant extracts.

Research has shown that K^+ , Na^+ , Ca^{2+} and water are the major electrolytes involved in the electrolytes imbalance theory, which by osmotic and diffusion processes could correct the imbalance and reverse the physiological processes observed during sickling of red blood cells of sickle cell patients (Joiner et al. 1998). Loss of K^+ in the blood stream has been demonstrated to cause dehydration in sickle cell patients, but the high values of K^+ obtained for the plant extracts in this research will be of immense benefits to the patients in preventing dehydration of the cells which normally triggers improper flow of the blood leading to pains (De Franceschi et al. 1997).

Table 2: Cationic analysis of plant extracts

S/N	Parameters (mg.kg^{-1})	<i>Petiveria alliacea</i>	<i>Canna indica</i>	<i>Pergularia daemia</i>
1	Na^+	0.50	0.50	0.40
2	K^+	54.30	180.10	28.30
3	Ca^{2+}	48.30	55.30	12.20
4	Mg^{2+}	2.90	3.10	2.20
5	Fe^{2+}	2.80	2.70	1.90
6	Zn^{2+}	0.60	0.80	1.00
7	Cu^{2+}	0.03	0.02	0.03

Research has shown that cationic Ca constitutes a large proportion of the bone, human blood and extracellular fluids. It is necessary for the normal functioning of cardiac muscles, blood coagulation and the regulation of cell permeability. More so, calcium is also involved in sickle cell disease relative to the hydration of sickle cells. Since, the membrane of sickle cells is generally more rigid than that of normal erythrocytes; a decrease in the efflux of Ca^{2+} out of the cell is observed. Thus, calcium from these plants could therefore disrupt the trans membrane transport of Ca^{2+} ions to the membrane of sickle cells and improve cell hydration, which would be beneficial for sickle cell patients and would enhance the antisickling activity of these plants (Brugnara et al. 1993). Ca^{2+} has also been found to play significant roles in low K^+ and Na^+ permeability of erythrocyte membrane, which helps to maintain the normal rate of cation leakage

from the cell. Indeed it had been suggested that sickling of red cells could be reversed if excess Ca^{2+} in the red cells is pumped out (Okpuzor and Adebessin 2006).

The magnesium content was found to be 2.90 mg/kg, 3.10 mg/kg and 2.20 mg/kg, respectively for *Petiveria alliacea*, *Canna indica* and *Pergularia daemia* leaves extracts. In humans, Mg is required in the plasma and extracellular fluids, where it helps maintain osmotic equilibrium. It reduces the number of abnormal erythrocytes in sickle cell disease and improves the hydration of the normal red blood and sickle cells. However, sickle cell patients have a magnesium deficiency (Franceschi et al. 2000) and this deficit causes red blood cell dehydration. Thus, Mg supplementation in drugs and herbal formula is required to reduce the number of abnormal erythrocytes and improves the hydration of red blood cells in sickle cell patients (Brugnara et al. 1993). More so, low levels of

total magnesium in sickle erythrocytes have been associated with increased sickling due to red cell dehydration and hence, increased polymerization (De Franceschi et al. 1997). Excess copper may contribute to free radical production and oxidative damage in HbSS (Natta et al. 1992). In this study, the concentrations of Cu in the three plants extracts were the lowest and this suggests that the plants are valuable for the management of sickle cell crisis. Zinc concentrations (Table 2) were fairly higher than that of Cu and this also contributes to the wellbeing of sickle cell patients because zinc supplementation in patients with SCD resulted in significant improvements in secondary sexual characteristics in the normalization of plasma ammonia concentrations and in the reversal of abnormalities of dark adaptation (Nwaoguikpe and Braide 2012). Iron content is beneficial for sickle cell patients because it increases haemoglobin levels, but the low level of iron in the plant extracts is very beneficial to the patients because large proportion of anaemia cases does not respond to iron supplementation from drugs since sickle cell patients have more iron contents in their blood (Gupta 2017).

Figure 3A shows an optical micrograph of the sickle cell blood alone (control group), while Figures 3B, 3C and 3D are the photomicrographs of sickle cell blood in the presence of methanol extracts of *Petiveria alliacea*, *Pergularia daemia* and *Canna indica*, respectively. Figure 3B showed total haemolysis (destruction of red cells) of the sickle blood at the initial (100 µg/mL) start of the experiment (time, 0 minute) rather than causing reversal of the sickle cells. Thus, the antisickling test was discontinued for *Petiveria alliacea*. For Figures 3C and 3D, there were observed modifications of the sickle cells at 90 minutes duration in the presence of *Pergularia daemia* and *Canna indica*, respectively. This antisickling behaviour for *Pergularia daemia* and *Canna indica* have been reported for leaves, stems, roots and whole plants of *Pergularia daemia* (Sahu et al. 2012). In this research, *Pergularia daemia* extract showed the best reversal of sickle cells at 90 minutes duration (Figure 3C). Mpiana et al. (2010) have also reported that medicinal plants containing anthocyanins, phenolics and terpenoids show antisickling activity, and in this study, *Pergularia daemia* and *Canna indica* indicated these phytochemicals.

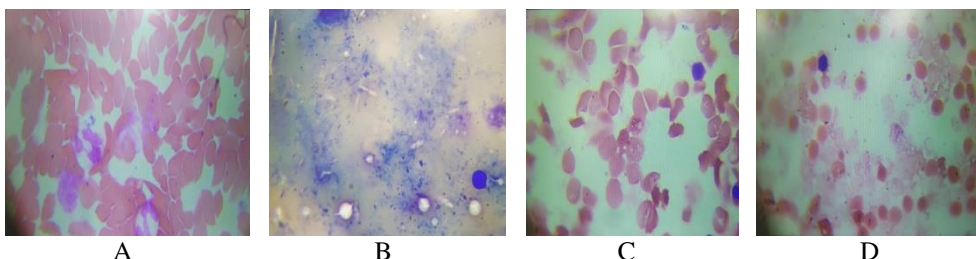


Figure 3: A: Optical microphotography of untreated HbSS blood erythrocytes (control), NaCl 0.9%, 2% Na₂S₂O₅, (Magnification x 100). Plate B: Optical Micrograph of 100 µg/mL of methanol extract of *Petiveria alliacea* on sickle cell blood at 0 mins (Magnification x 100). Plate C: Optical micrograph of 300 µg/mL of methanol extract of *Pergularia daemia* on sickle cell blood at 90 minutes (Magnification x 100). Plate D: Optical micrograph of 300 µg/mL of methanol extract of *Canna indica* on sickle cell blood at 90 mins (Magnification x 100).

In Figures 4 and 5, the bar charts show the % sickle cell against concentrations of *Pergularia daemia* and *Canna indica* adopted

for the antisickling activity up to 90 minutes, respectively.

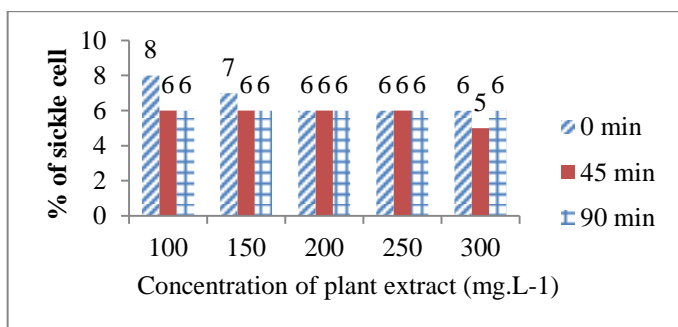


Figure 4: % sickle cell against concentrations of methanol extract of *C. indica*.

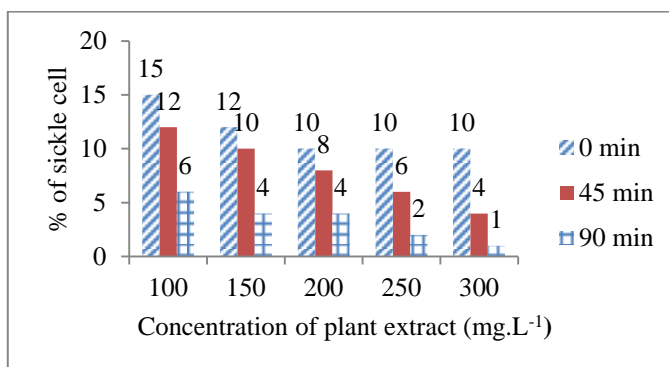


Figure 5: % sickle cell against concentrations of methanol extract of *Pergularia daemia*.

The leaves of *C. indica* (Figure 4) at high dose of 300 mg/mL caused a significant reduction in percentage of sickle red blood cells from 8% to 6%, while *Pergularia daemia* (Figure 5) reversed sickle cell from 15% to 1%. Thus *Pergularia daemia* showed the highest antisickling activity in this study.

Conclusion

The antisickling effects of methanol extract of *Canna indica* and *Pergularia daemia* in *in-vitro* studies have shown a relatively high satisfactory antisickling activity and this corroborates their traditional uses in the treatment of sickle cell anaemia in Edo State, Nigeria. Thus, this research provides a scientific basis for the use of the plants as antisickling agents and further clinical research into their *in-vivo* studies and active principles is required.

Conflict of Interest: The authors declare no conflict of interest.

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