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## Studies on the effect of a nutritious vegetable, *Telfairia occidentalis*, on HbSS blood

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### ABSTRACT

Medicinal plants have been used traditionally in Africa, especially Nigeria, in the management of sickle cell disorder (SCD) whose treatment has been mainly palliative. The antisickling properties of ethanol extract of *Telfairia occidentalis* Hook, F. (TO) (Family Cucurbitaceae) leaf was tested *in vitro* at concentrations 1, 2, 4, 8 and 16 mg/mL using inhibitory and reversal models. Nitrogen gas was used to induce hypoxia for 1 h. The effect of TO on red cell density and cell membrane were also determined. The methanol sub fraction of TO extract was subjected to GC/MS to identify some of the active compounds. The TO gave antisickling activities of  $78.84 \pm 1.34\%$  inhibition and  $95.4 \pm 0.81\%$  reversal, which are significantly ( $p < 0.05$ ) higher than that of Ciklavit<sup>®</sup>. The TO extract gave a change in density of  $17.83 \pm 0.77\%$  and a dose dependent activity on RBC membrane. Methyl 9-cis 11- trans-octadecadienoate; 1, 4- benzenedicarboxylic acid; 9, 12-octadecadienoic acid (Linoleic acid); and hexadecanoic acid methyl ester (palmitic acid); were identified from TO ethanol leaf extract for the first time using GC/MS. This study authenticated the ethnomedicinal claims of the use of *T. occidentalis* in the management of sickle cell disorder.

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### 1. Introduction

Sickle cell disease (SCD) is a genetic disorder of the hemoglobin molecule. This disorder is heritable and the affected hemoglobin is unable to transport oxygen to the tissues properly because it is defective.<sup>1</sup> The disorder expresses itself in the homozygous state as hemoglobin S (HbSS) and is referred to as sickle cell anemia (SCA). The management of sickle cell anemia has been a real challenge since its discovery in 1910 by Dr. Herrick. This disorder occurs because of a mutation in the hemoglobin gene characterized as a glutamine-to-valine substitution at the sixth residue of the  $\beta$ -globin polypeptide.<sup>2</sup>

The genetic mutation occurred in many malaria endemic areas of Africa and Asia and resulted in the different haplotypes which include: the Benin, Senegal, Cameroun, Bantu, and Arabian. In Africa, the highest prevalence of sickle-cell trait occurs between latitudes 15° North and 20° South, ranging between 10% and 40%

of the population in some areas. Prevalence levels decrease to between 1% and 2% in North Africa and to less than 1% in Southern Africa. In countries such as Cameroon, Republic of Congo, Gabon, Ghana, and Nigeria, the prevalence is between 20% and 30% while in some parts of Uganda it is as high as 45%. In countries where the trait prevalence is above 20%, the disease affects about 2% of the population.<sup>3</sup> In Nigeria, the most severe haplotype, the Benin haplotype, is most prevalent, whilst the Arabian haplotype with high HbF levels are the least severe.<sup>4,5</sup> About 150 000 children are born annually in Nigeria with SCD and 50% of these children will die before their 10th birthday. Approximately 7% of the world's populations are healthy carriers of a gene for sickle-cell disease or thalassaemia.<sup>6</sup>

The management of SCD is mainly palliative since it has no cure outside hematopoietic stem cell transplantation for now. Palliative care includes rehydration, pain management, blood transfusion, and prevention of complications. The use of hydroxyurea revolutionized the management of SCD but it is not without its side effects and complications.<sup>7</sup> Life expectancy is shortened in sickle cell disorder, with studies reporting an average life expectancy of 42 and 48 years for males and females, respectively.<sup>8</sup>

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Therefore there is a need to investigate the antisickling properties of more medicinal plants for their efficacy and use in the management of the disease. The antisickling effects of several medicinal plants such as *Zanthoxylum xanthoxyloides*<sup>9</sup>, *Cajanus cajan*<sup>10</sup>, *Carica papaya*,<sup>11</sup> *Cnidioscolus aconitifolius*<sup>12</sup> amongst others, have been investigated and efforts are being made by scientists to either isolate active components for drug production or formulate them into dosage forms.

*T. occidentalis* Hook F. (Cucurbitaceae) (TO) is an edible vegetable grown in West Africa as a leaf vegetable and for its edible seeds and occurs in its cultivated form in various parts of Nigeria. The plant is a drought-tolerant, dioecious perennial that is usually grown trellised, a climber attaining up to 1000 ft in length. It is widely cultivated for its palatable and nutritious leaves, which are mainly consumed as vegetables.<sup>13</sup> In Nigeria, the herbal preparation of the plant has been employed in the treatment of anemia, chronic fatigue and diabetes.<sup>14</sup> The infusion of the leaves is given as blood tonic to help convalescents, in the treatment of malaria and loss of appetite.<sup>15</sup> The consumption of the leaf of *T. occidentalis* enhances various hematological parameters and therefore improves the physiological and nutritional status of its consumers. The aqueous extract of *T. occidentalis* leaf was reported to reverse sickling with very low membrane stability property.<sup>16</sup> The leaves are good sources of K, Cu, Fe, and Mn; and moderate sources of Mg and Zn, which are essential in human and animal nutrition.<sup>17</sup> Total amino acid in *T. occidentalis* was 455.3 mg/g with total essential amino acid of 256.1 mg/g or 56.3%, showing the plant proteins to be high in essential amino acids. Aqueous extract of the leaf contain 5.07 mg/100 mL and 40 mg/100 mL of Vitamins E and C, respectively.<sup>18</sup> Palmitoleic acid (16.62 %) and elaidic acid (0.85 %) are the predominant omega 9 fatty acid present in the leaf.<sup>19</sup>

From ethnobotanical survey carried out in this study, *T. occidentalis* was found to be one of the plants used to ameliorate anemic conditions in sickle cell patients hence this study is aimed at studying the ethanol extract and fractions of *T. occidentalis* to determine its antisickling properties and mode of action using different *in vitro* antisickling models.

## 2. Materials and methods

### 2.1. Ethnobotanical survey

Ethnobotanical survey was carried out in six senatorial districts of Ondo and Osun states southwest Nigeria, on the herbs used locally in the management of sickle cell anemia. *T. occidentalis* was selected based on its frequency of use as blood tonic in the treatment of anemia in sickle cell management. *T. occidentalis* Hook. F. was identified and authenticated by G. Ibhanebor and B.E Omomoh at the IFE Herbarium, OAU, Ile-Ife with voucher number IFE 17254.

### 2.2. Preparation of extracts

The fresh leaves of *T. occidentalis* were collected at Owena town, Osun State, Nigeria. The leaves were separated from their twigs dried in a hot air oven at 40 °C and then powdered using the grinding machine. The dried powder (500 g) of the plant was extracted by macerating in absolute ethanol for 72 h at room temperature as prepared traditionally in south west Nigeria. Each

extract was filtered, evaporated to dryness *in vacuo* at 40 °C and kept in the refrigerator for the assays. Different concentrations viz: 1, 2, 4, 8, and 16 mg/mL were prepared and used for the antisickling assay to determine the optimal dose and EC<sub>50</sub>.

### 2.3. Blood collection

Venous blood was collected into ethylene diamino tetraacetic acid bottles from confirmed sickle cell anemia patients between ages 12–30 years in steady state, who attend regular hematology clinic at the Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria. Ethical clearance with reference number IRB/IEC/0004553 was given by the Ethical and Research committee of the Obafemi Awolowo University Teaching Hospital Complex for this project. The blood was mixed carefully but properly to avoid clotting and fresh blood samples were always used within the first 48 h of collection.

### 2.4. Antisickling assays

#### 2.4.1. Inhibitory antisickling assay procedure

Whole blood (5 mL) was centrifuged for 7 min and the plasma discarded. The packed red blood cell (RBC) was washed three times in phosphate buffered saline (PBS) (pH 7.0). A 10% red blood cell suspension was prepared and 5 mL of the suspension was added to 2 mL of the TO extracts in boujol bottles. The bottles were covered with parafilm and incubated for 1 h at 37 °C. After this, N<sub>2</sub> gas was bubbled into the mixture for 60 min at 37 °C to induce deoxygenation.<sup>20</sup> 200 µL of the solution was fixed after 1 h in 5% formalin.

#### 2.4.2. Reversal antisickling assay procedure

Red blood cell suspension (10%) of HbSS red cells (5 mL) was incubated while N<sub>2</sub> gas was bubbled in for 1 h to cause deoxygenation at 37 °C covered with parafilm. Without fixing, 2 mL of the TO extract (under parafilm) was added, shaken and incubated for another 1 h. After this, aliquot 200 µL of the solution was fixed in 5% formalin.

For both reversal and inhibitory tests, 2 mL phosphate buffered saline was used as the negative control while 0.6 mL Ciklaviv<sup>®</sup> concentrate made up to 2 mL with distilled water was used as the positive control. Ciklaviv<sup>®</sup> is a nutraceutical used for the management of sickle cell anaemia with *Cajanus cajan* as the basic component and phenylalanine the active constituent.<sup>21</sup> The antisickling assays were carried out in triplicates and at the different concentrations.

#### 2.4.3. Counting of cells

Slides were prepared from the fixed cells after centrifugation. An aliquot of the fixed cells was taken with the use of capillary tube, dropped on a microscope slide and carefully covered with a cover slip. The slide was placed under a bright field microscope and red blood cells (500 sickled and unsickled erythrocytes) were counted at x 400 magnification. The percentage sickle red blood cells were calculated as shown below.

$$\% \text{ Sickled cells} = \frac{\text{No of Sickled cells}}{\text{Total no of cells}} \times 100$$

The percentage inhibition/reversal was thereafter calculated from the percentage sickled cells using the formula:

$$\% \text{ Inhibition or Reversal} = \frac{\% \text{ sickled cells of control} - \% \text{ sickled cells of sample}}{\% \text{ sickled cells of control}} \times 100$$

### 2.5. Red cell fractionation assay

The *in vitro* effect of *T. occidentalis* extracts on percentage dense cell of the sickle cell sub-population before and after incubation was determined by the red cell fractionation assay. 5 mL of 10% HbS RBC suspension was incubated with 2 mL of TO extract (4 mg/mL) at 37 °C for 1 h covered with parafilm. After 1 h of incubation, the suspension was centrifuged and 40% RBC was thereafter prepared by adding 3 mL PBS to 2 mL of the treated red blood cells.

A discontinuous density gradient method was used to provide sufficient cells in each cell fraction.<sup>22,23</sup> The stock gradient was composed of different concentrations of percoll and 50% w/v sodium diatrizoate (adjusted to pH 7.4) in a ratio of 9:2. The 100% stock was diluted with phosphate buffered saline (pH 7.4) to provide three different concentrations (52%, 60% and 66% v/v) with medium densities of 1.084, 1.096 and 1.104 respectively. Aliquots (2 mL) of each concentration were layered on top of one another in a 10 mL tube with the most dense layer (66%) at the bottom. Aliquots (1.5 mL) of the 40% HbSS RBC above were layered on two discontinuous density gradients in separate tubes. The tubes were centrifuged at 450 g for 40 min using a refrigerated centrifuge (KR-4i) at 12 ± 2 °C. Each fraction was pooled with the corresponding fraction in the duplicate tube and washed separately three times in buffer. Each pooled fraction was then re-suspended in an equal volume of buffer for analysis by the H18 light Auto analyzer. The assay was carried out in triplicates and Ciklavit<sup>®</sup> was used as positive control.

### 2.6. Membrane stabilizing assay

Fresh blood samples were collected into citrate anticoagulant bottle and mixed carefully to prevent lysing of the red blood cells.<sup>24</sup> The blood samples were then poured into clean centrifuge tubes and centrifuged at 3000 rpm for 10 min. The supernatant was carefully removed using sterilized pipette and the packed blood cells re-suspended in fresh isosaline, mixed gently and centrifuged for another 5 min at the same speed as above. A 2% erythrocyte suspension was prepared by diluting 2.0 mL of packed red blood cells with isosaline.

The assay mixture was composed of hyposaline (2 mL), 0.15 M sodium phosphate buffer, pH 7.4 (1 mL), varying concentrations of TO extract (50, 100, 150, 200, 250, 300 µg/mL)<sup>24</sup> and 2% v/v erythrocyte suspension (0.5 mL) and the final mixture was then made up to 3.0 mL with isosaline. Ibuprofen was used as positive control. The reaction mixture was incubated at 56 °C for 30 min, cooled under running water, centrifuged at 5000 rpm, and the supernatant collected. The released hemoglobin was read at 560 nm spectrophotometrically. The assay was done in triplicates and the percentage membrane stability was estimated from the expression:

$$\% \text{ Membrane Stability} = 100 - \left( \frac{\text{absorbance of drug}_{\text{test}} - \text{absorbance of drug}_{\text{control}}}{\text{Absorbance of blood}_{\text{control}}} \right) \times 100$$

### 2.7. Chromatographic techniques

Vacuum liquid chromatography (VLC) using solvents of increasing polarity (n-hexane, dichloromethane (DCM), ethyl acetate and methanol) was carried out on the crude extract of TO. The resultant

fractions were tested for their antisickling properties and the fraction with the highest activity was further purified by subjecting it to analytical thin layer chromatography using precoated silica gel TLC plate (G60 PF<sub>254</sub>), preparative thin layer chromatography and GC/MS. The sub-fractions were each tested for their antisickling activities. The solvent system ethyl acetate: methanol: water (ratio 10:1.4:1) was used.

### 2.8. GC/MS analysis

The GC-MS analysis of the preparative TLC sub-fraction (B4-6) of TO was performed on an Agilent technologies model 7890 Series GC System equipped with an Agilent technologies 5975 MS detector (EI mode, 70 eV). A column type DB-5 (5% phenyl methyl siloxane) with a length of 30 m, an inside diameter of 0.25 µm and a film thickness of 320 µm, was used. The temperature of the column was programmed to increase after 5 min from 50 °C to 280 °C at the rate of 5 °C/min after which the run was left for 15 min at 280 °C. Helium was used as a carrier gas at a flow rate of 0.5 mL/min. The injector and detector temperatures were 300 °C and 250 °C, respectively. The components of the sub-fraction (B4-6) were identified on the basis of comparison of mass spectra from NIST Standard Reference Database 1A (NIST/EPA/NIH Mass Spectral Database (NIST 11) and NIST Mass Spectral Search Program (Version 2.0 g), Agilent Technologies, Inc. ChemStation Version).

### 2.9. Statistical analysis

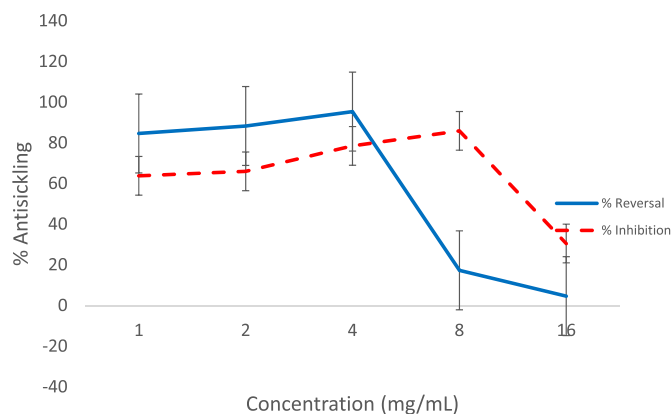
Each test was performed in triplicates and the results expressed as mean values and standard error of mean or standard deviation as indicated. Results were subjected to analysis of variance (ANOVA) using SPSS and the level of significance set at  $p < 0.05$ .

## 3. Results and discussion

*T. occidentalis* was selected from the outcome of the ethnobotanical survey based on its frequency of use as blood tonic in the treatment of debility in the management of sickle cell anemia. A total of 146 plants from 48 families were surveyed.

The pathophysiology of SCD is a consequence of hemoglobin polymerization and its deleterious effects on RBC shape, deformability, adhesion, density and membrane.<sup>25</sup> Specifically, the polymerization and deformability of sickle RBC have been identified as critical biophysical factors involved in vaso-occlusion and research has been ongoing to determine the efficacy of natural products such as medicinal plants chemical constituents and nutritional supplements in the management of sickle cell disorder. Some researchers have earlier reported and argued in favor of nutrition in the management of SCD as malnutrition is one of the complications of HbSS.<sup>26</sup> The reports of feeding high protein and L-arginine supplements to sickle mice

and n-3 fatty acids to HbSS men showed significant reductions in oxidative stress, pain episodes, inflammation, red cell density and improved micro-vascular function.<sup>27</sup> *T. occidentalis* is a nutritious edible vegetable with positive erythropoietic effect and commonly administered to SCD patients in ethno-medicine

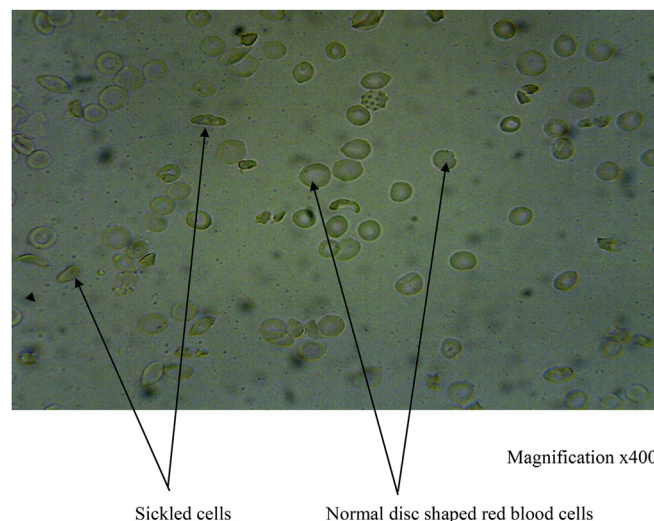


**Fig. 1.** Antisickling activities of *Telfairia occidentalis* at various concentrations.  $n = 3$ ; Mean values  $\pm$  SEM.

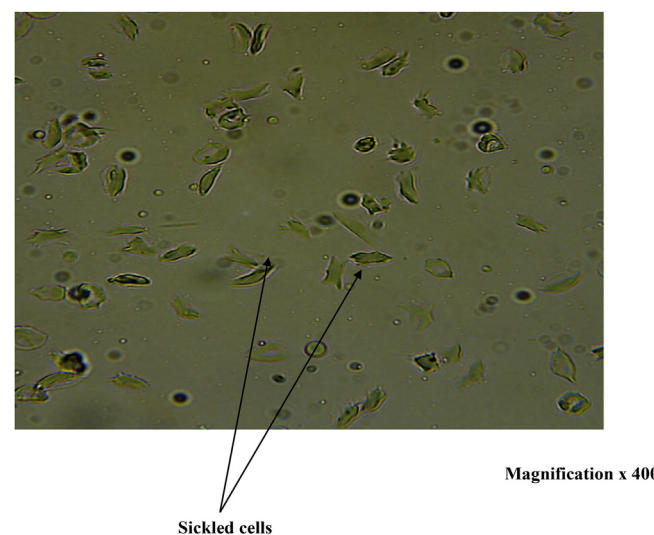
to combat anemia. The leaf which is rich in proteins, minerals (such as iron, potassium, sodium, phosphorus, calcium and magnesium), antioxidants, vitamins (such as thiamine, riboflavin, nicotinamide and ascorbic acid) and phyto-chemicals such as phenols,<sup>28</sup> is cooked as soup or squeezed in water or alcohol and administered to patients with low PCV to boost blood in south-west Nigeria.

There was a steady increase in activities from 1 mg/mL to 4 mg/mL for both inhibitory and reversal activities. However a reduction was observed as concentration increased from 4 mg/mL to 16 mg/mL (Fig. 1).

*T. occidentalis* ethanol extract at 4 mg/mL gave the highest antisickling properties, hence 4 mg/mL can be said to be the optimal dose for activity. This activity can be explained by the dose-response relationship which describes a range of doses over which response occurs,<sup>29</sup> and helps determine the dose necessary to achieve the desired effect. Increasing the dose of a drug with a small therapeutic index increases the probability of toxicity or ineffectiveness of the drug.<sup>30</sup> Hence 4 mg/ml which was observed to give the highest inhibitory and reversal activities can be said to be the threshold of the antisickling activity since a reduction in activity was produced at higher concentrations. The  $EC_{50}$  value was 6.73 mg/mL for inhibition and 3.32 mg/mL for reversal. TO extract at 4 mg/mL gave a significantly ( $p < 0.05$ ) higher inhibitory and reversal antisickling activities than Ciklavit<sup>®</sup>, positive control ( $64.2 \pm 1.65\%$  and  $76.88 \pm 1.34\%$  inhibition and reversal respectively) at same concentration. Nwaoguikpe,<sup>31</sup> reported 75.27% and 69.63% hemoglobin polymerization inhibition of the crude aqueous and water-soluble extracts of *T. occidentalis* respectively using the spectrophotometric method. The reversal effects of the methanol and aqueous extracts of the leaves of TO was reported by Atabo et al.<sup>16</sup> The extractives used were different and the activities lower than that of the ethanol extract reported in this study. The results from this study using the reversal and inhibitory antisickling model however gave much higher antisickling activities at lower concentration and indicated that the ethanol extract of TO can be used as prophylactic drug in offsetting sickling crisis as well as be administered during crisis to reduce vaso occlusion and hence pain. Amino acids, iron as well as the vitamin C, had been implicated for antisickling activities,<sup>31,32</sup> and these together with the antioxidant contents of TO might be responsible for the high antisickling property (see Plates 1–4).



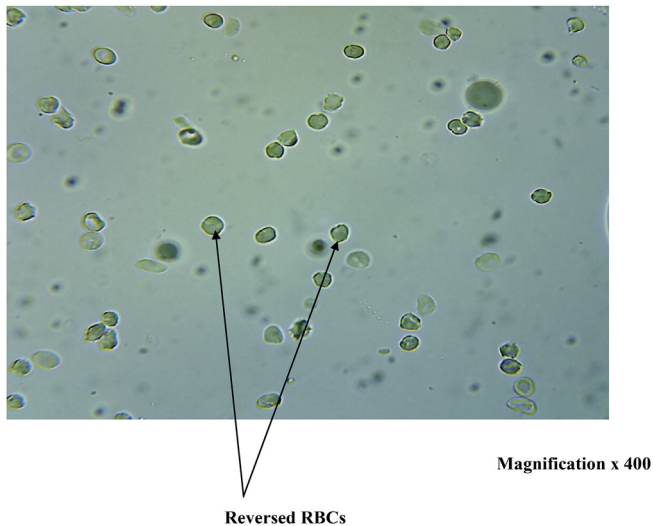
**Plate 1.** Photomicrograph of sample blood film of an HbSS patient in steady state.



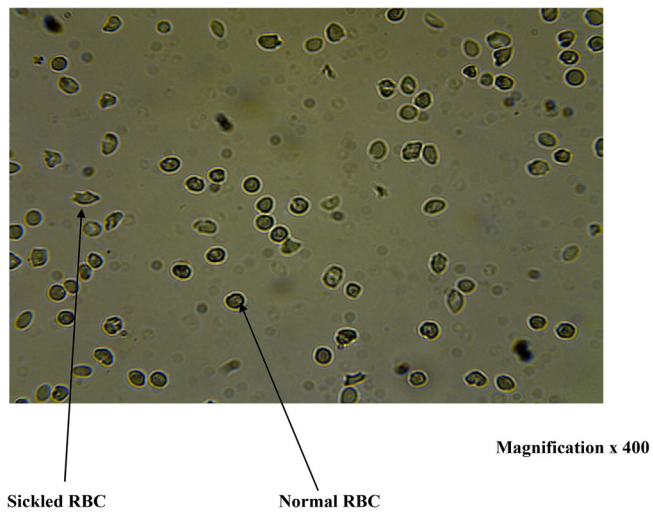
**Plate 2.** Photomicrograph of sickled cells (95.0%) in an HbSS blood sample following 1 h deoxygenation with nitrogen gas (Negative control).

The high antisickling property can also be attributed to the effects of TO extract on the density of red blood cells. The deformability of red blood cell decreases progressively with increasing cell density,<sup>33</sup> and attention should be given to any drug that reduces the density of red blood cells thereby increasing cell deformability. The density of HbAA blood cells is normal and no F4 layer (dense cell layer) was observed in the discontinuous gradient layers before and after incubation with TO and Ciklavit<sup>®</sup>.

There was no significant difference ( $p < 0.05$ ) between HbAA red blood cells treated with the extracts and the HbAA control. On the other hand, HbSS RBCs were found in the F4 layer of the discontinuous gradient. HbSS RBCs incubated with TO as well as Ciklavit<sup>®</sup> gave a significant ( $p < 0.05$ ) reduction in the red cell count of the cells in the F4 layers (Fig. 2). This reduction caused an increase in the blood cells in the F3 layers; this implies a reduction in cell density. The percentage of dense cells in the F4 layer of HbSS blood control was

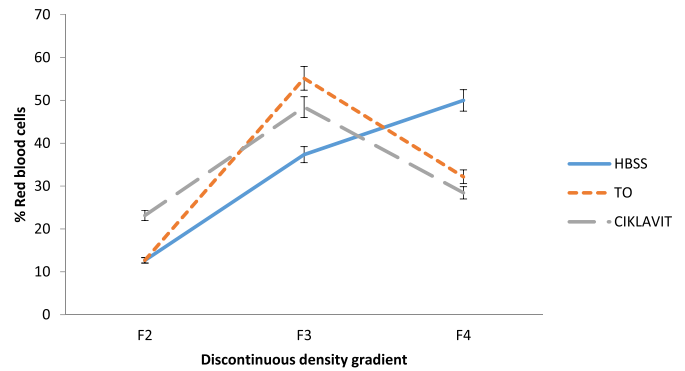


**Plate 3.** Photomicrograph of reversed HbSS red blood cells after treatment with *T. occidentalis* at 4 mg/mL.

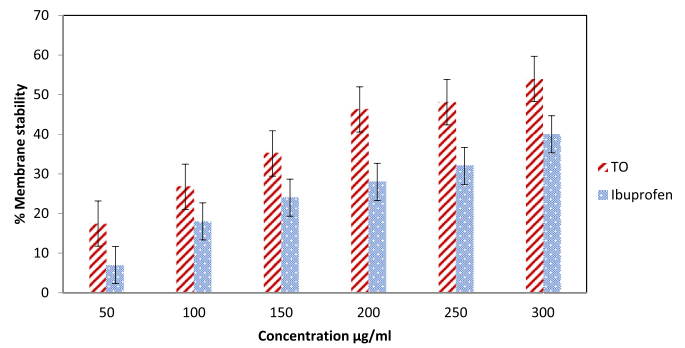


**Plate 4.** Photomicrograph of the inhibition of sickling of HbSS red blood cells by *T. occidentalis* at 4 mg/mL.

50 ± 0.78% while that treated with TO extract was 32.17 ± 0.77%. The mean percentage change in density of HbSS blood treated with TO was therefore 17.83 ± 0.77%. Since F2 layer contained young matured red cells; F3 layer cells of intermediate age and density; and F4 layer contained the most dense (rigid) cells, TO extract was able to reduce the density of HbSS red blood cells thereby reduced rigidity and increased deformability of red blood cells. The effect of TO on density of red cells compared favorably with that observed for Ciklavit®. The mean percentage change in density of HbSS blood treated with Ciklavit® was 21.57 ± 0.16%. The K<sup>+</sup> efflux from red blood cell (RBC) of SCD causes erythrocyte dehydration thereby decreasing intracellular water content and increasing mean corpuscular hemoglobin concentration.<sup>34</sup> Dense red blood cells include variable fraction of irreversible sickle cells and exhibit increased rigidity and decreased stability. Hence any drug whose mechanism of action includes reduction of cell density thereby increasing cell deformability will be



**Fig. 2.** The effect of *T. occidentalis* extract and Ciklavit® on HbSS red blood cell density using the discontinuous density gradient. F2 layer: contained young matured red cells, F3 layer: contained cells of intermediate age and density, F4 layer: contained the most dense (rigid) cells.



**Fig. 3.** Red cell Membrane stability activities of *T. occidentalis* and Ibuprofen control. Data are presented as mean ± SEM; n = 3; level of significance at p < 0.05.

**Table 1**  
Antisickling activities of the VLC fractions of TO.

Fractions	% Inhibition	% Reversal
n-hexane	25.34 ± 2.19	32.31 ± 2.83
DCM	83.52 ± 0.35	40.26 ± 1.31
Ethyl acetate	42.88 ± 5.55	51.15 ± 0.78
Methanol	85.52 ± 1.70	63.57 ± 4.50

useful in the treatment of SCD. This study is the first to investigate the effects of medicinal plants on the density of red blood cells.

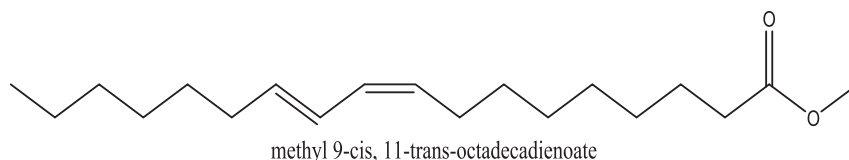
The effect of TO extract on red blood cell membrane stability was observed to increase with increased concentration, i.e. the activity was dose dependent (Fig. 3).

Although Ibuprofen (positive control) also showed a dose dependent activity, the membrane stability effect of TO leaf extract was significantly higher (p < 0.05) than that of Ibuprofen. This result showed that TO extract has the ability to protect sickle red blood cell membrane thereby preventing sickling. Since the membrane of the red blood cell plays many roles that aid in regulating their surface flexibility, deformability, adhesion to other cells and immune recognition,<sup>35</sup> the use of TO as an antisickling agent will promote flexibility of sickle red blood cells and prevent loss of cellular deformability which can compromise the ability of the red cell to optimally perform its function of oxygen delivery to the tissues. The mode of action of the

**Table 2**  
Antisickling activities of the sub-fractions of the methanol fraction of TO.

Sub-fractions	Yield (mg)	% Inhibition	% Reversal
A1-3	400	42.88 ± 3.04	31.57 ± 5.37
B4-6	460	64.95 ± 2.0	61.84 ± 3.03
C7-9	420	48.15 ± 1.81	39.90 ± 2.73

extracts could be connected with binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells



thereby preventing physical interaction with aggregating agents which are involved in the hemolysis of red blood cells.

The yield of the ethanol extract of TO was: n-hexane (17.70 g), dichloromethane (DCM) (9.80 g), ethyl acetate (4.96 g) and methanol (3.97 g). The results of the inhibitory and reversal antisickling tests of the VLC fractions of TO, were presented in Table 1.

The DCM and methanol fractions gave high inhibitory activities while only the methanol fraction gave a high reversal activity of 63.57 ± 4.50% (Table 1). The antisickling properties of the sub-fractions A1-3, B4-6 and C7-9 obtained after purification of the methanol fraction are shown in Table 2. The antisickling properties of TO can be attributed to polar compounds present in the methanol fraction.

The sub-fraction B4-6 with the highest antisickling property was further purified by subjecting it to GC/MS analysis, four compounds were identified. The compounds were identified by comparing with reference spectra registered in spectral libraries and the similarity estimated for each reference. They included: methyl 9-cis 11- trans-octadecadienoate (1); 1,4-

activities exhibited. The antifungal, antioxidant, antimalarial and hypoglycemic activities of palmitic acid had been reported while the use of linoleic acid (polyunsaturated Omega-6-fatty acid) along with omega-3 fatty acids play a crucial role in brain function, and normal growth and development.<sup>37</sup> The role of omega-3 fatty acid in sickle cell cannot be overemphasized.

**Compound 1:**

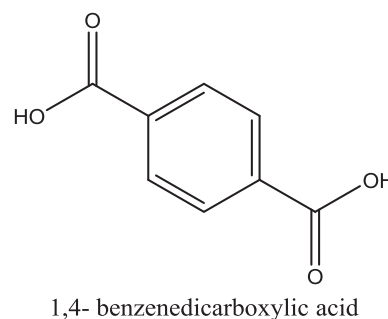
Methyl 9-cis 11- trans-octadecadienoate.

Molecular formula: C<sub>19</sub>H<sub>34</sub>O<sub>2</sub>; Molecular weight: 294.47 g/mol.

**Compound 2:**

1, 4-benzenedicarboxylic acid.

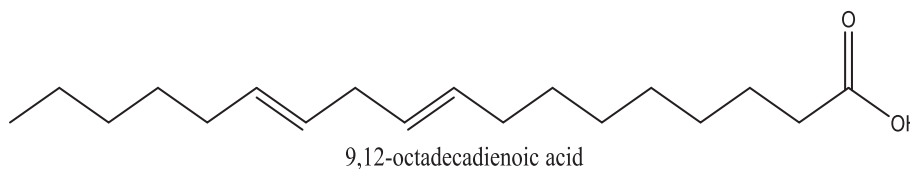
Molecular formula: C<sub>8</sub>H<sub>6</sub>O<sub>4</sub>; Molecular weight: 166.13 g/mol.



**Compound 3:**

9, 12-octadecadienoic acid (Linoleic acid).

Molecular formula: C<sub>18</sub>H<sub>32</sub>O<sub>2</sub>, Molecular weight: 280.44 g/mol.



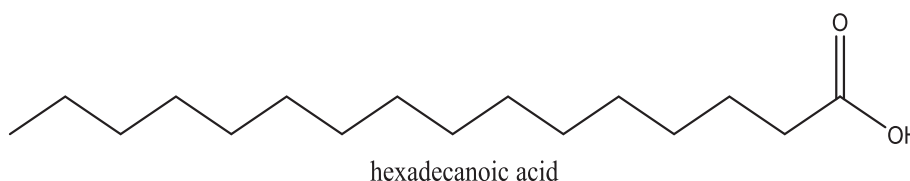
benzenedicarboxylic acid (2); 9, 12-octadecadienoic acid (Linoleic acid) (3); and hexadecanoic acid methyl ester (palmitic acid) (4). Linoleic and palmitic acids were common lipids identified by GC/MS analysis of the DCM fraction of *T. occidentalis* seeds.<sup>36</sup> However,

**Compound 4:**

Hexadecanoic acid.

Molecular formula: C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>.

Molecular weight: 256.42 g/mol.



this is the first time the compounds are being identified in ethanol extract of *T. occidentalis* leaves. The presence of these compounds in the sub-fraction B4-6 might be responsible for the antisickling

**4. Conclusion**

*T. occidentalis* is a nutritious vegetable in Nigeria and contains

nutrients, vitamins, proteins, antioxidants to mention a few. This vegetable is given locally in southwest Nigeria to anemic individuals to increase their PCV and could be used as a target in the prevention and amelioration of SCD crisis in infants and adults. Therefore a treatment using this vegetable that prevents and reverses sickling of RBC, stabilizes the RBC membrane and reduces HbS red cell density will be most desirable.

### Conflict of interest

The authors wish to draw the attention of the Editor to the fact that we declare that there is no known conflict of interest associated with this publication. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all authors.

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