

***In vitro* Evaluation of *Moringa oleifera* Leaf Extracts Used in Managing Sickle Cell Patients in South West Nigeria**

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *Moringa oleifera* Lam. (Moringaceae) cultivated in South West Nigeria has been used locally in the treatment of iron deficiency anaemia, and especially in the management of sickle cell anaemia whose treatment is mainly palliative. *Moringa oleifera* is a nutritious vegetable and attention is now being given to such plant materials which can serve as both food and medicine in the management of sickle cell anaemia. This study therefore investigated the antisickling properties as well as the mode of action of the leaf extract of *Moringa oleifera* grown in South West Nigeria.

Materials and method: The anti-sickling properties of *M. oleifera* leaf ethanol extracts and various fractions were evaluated using nitrogen gas to induce hypoxial state *in vitro*. The mechanisms of action were examined using different *in vitro* models, which include red cell density, membrane stability, haemoglobin HbS polymerization as well as antioxidant assays at various concentrations.

Results: *Moringa oleifera* at 4 mg/mL caused $95.6 \pm 2.47\%$ inhibition and $79.4 \pm 1.93\%$ reversal of HbS sickling and purification of the crude extract enhanced activity. The extract showed a change in density of $14.74 \pm 0.90\%$ and a dose dependent membrane stabilizing property, which was significantly higher ($p < 0.05$) than the positive control. *Moringa oleifera* exhibited metal chelating activity and low inhibition of nitric oxide, which is important in the prevention of the onset of vaso-occlusive crisis in sickle cell patients.

Conclusion: This study therefore justifies the use of *Moringa. oleifera* in the management of sickle cell anaemia and therefore a candidate plant in the development of antisickling drug.

Keywords: Anti-sickling, Polymerization, Membrane stability, Red Cell density

INTRODUCTION

Sickle cell anaemia (SCA) is an inherited chronic haemolytic anaemia in which the red blood cells (RBCs) become crescent in shaped instead of disc-shaped when deoxygenated and have shorter life-span than those with haemoglobin A. It is a genetic

disease caused by abnormal haemoglobin called sickle haemoglobin (Hb S), which polymerizes under deoxygenated conditions and deforms the red blood cells into a 'sickled' shape (Pauling *et al.*, 1949). The commonest symptom of sickle cell disease (SCD) is

pain that is caused by the occlusion of small blood vessels by the sickled cells. Repeated episodes may cause damage to the kidneys, lungs, bones, liver and the central nervous system (CNS) (Whitten and Bertles, 1989). Dense red blood cells play a role in the pathophysiology of SCD acute and chronic organ damage because of heightened tendency to undergo polymerization and sickling because of their higher haemoglobin S concentration. The intracellular total haemoglobin concentration of dense red blood cell (DRBC) is increased from the normal, exhibiting increased rigidity and decreased stability, and include a variable fraction of irreversibly sickled cells (Messmann *et al.*, 1990). Dense dehydrated sickle cells have been shown to contribute disproportionately to impairment of blood flow in the microcirculation and are correlated with biological parameters of haemolysis (Kaul *et al.*, 1983; Bartolucci *et al.*, 2012). Sickle cell disease has no widely available cure. Currently, hydroxyurea is the only FDA approved drug for use in the management of SCD although blood and marrow stem cell transplantations may offer a cure for a small number of people who have SCD. However, the risks and costs of the procedure do not make the procedure readily available. Therefore, researchers continue to look for newer, more cost-effective and accessible modalities of treatment for SCD. Medicinal plants have been used in the management of SCD and patients still depend mostly on ethnomedicinal treatment of the symptoms of the disease.

Moringa oleifera Lam. (Moringaceae) is a popular vegetable widely cultivated in tropical and sub-tropical areas. It is a fast-growing, drought-resistant tree, native to the southern foothills of the Himalayas

METHODOLOGY

Preparation of the Extract

Fresh leaves of *Moringa oleifera* Lam. were harvested at Obafemi Awolowo University, Osun State, South West, Nigeria and the plant identified and authenticated at the Department of Botany Herbarium, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. A voucher specimen was deposited with voucher number IFE17255. The leaves were dried at 40 °C in a hot air oven, milled and weighed. The powder was extracted by cold maceration in absolute ethanol for 72 h after

in northwestern India (Parotta, 1993). In developing countries, Moringa is believed to have the potential to improve nutrition, boost food security, foster rural development, and support sustainable land care (Makkar *et al.*, 1987). The leaves are cooked and used like spinach and are said to be the most nutritious part of the plant, being a significant source of vitamin B6, vitamin C, provitamin A as beta-carotene, magnesium, amino acids, iron and various phenolics (Yang *et al.*, 2006). Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulceration, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine (Anwar *et al.*, 2007; Farooq *et al.*, 2007; Kumar *et al.*, 2010; Das *et al.*, 2012; Kuete *et al.*, 2017). The efficacy of the leaf extract in treating iron deficiency anaemia as well as the inhibitory effect on HbS polymerization had been reported earlier (Onwubiko, 2010; Sindhu *et al.*, 2013; Nwaoguikpe *et al.*, 2015). *Moringa oleifera* is one of various medicinal plants used locally in South West Nigeria for the treatment of anaemia and hence in the management of SCD. Based on this claim, this study was aimed at assessing the *in vitro* anti-sickling properties of *M. oleifera* cultivated in South West Nigeria, using reversal and inhibition models and determining the mechanism of action through its ability to reduce red cell density, membrane stabilization property, inhibition of polymerization and anti-oxidative properties.

which it was filtered and evaporated to dryness *in vacuo* at 40 °C. The crude extract obtained was further fractionated into n-hexane, ethyl acetate and water. All extracts were subjected to antisickling assays.

Collection of Blood Sample

Confirmed Hb SS blood was collected from the Sickle Cell Clinic of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, with

approval from the Ethical and Research Committee of the institution.

Preparation of blood sample for Anti-sickling Assay

Whole blood was centrifuged for 7 min at 2500 rpm and the plasma discarded. The packed red blood cells were washed three times in phosphate buffered saline (PBS) pH 7.0. Plasma suspension (10%) was prepared by adding 2 mL of the packed red cell to 18 mL of PBS.

Anti-sickling assays

Hb S Inhibitory Test

The RBC suspension (5mL) was added to 2 mL of the test extract in boujol bottles. The bottles were covered with parafilm and incubated for 1 h at 37 °C. After this, oxygen-free N₂ gas was bubbled into the cell suspension to cause deoxygenation for 1 h and 200 µL was thereafter fixed in 5% buffered formalin solution (Cyril-Olutayo and Agbedahunsi, 2015).

Hb S Reversal Test

Red blood cell suspension of Hb SS blood (5 mL) was incubated with oxygen free N₂ gas for 1 h at 37 °C in boujol bottles covered with parafilm. Without fixing, 2 mL of the test extract was added, the cell suspension shaken and incubated for another 1 h. After this 200 µL of the cell suspension was fixed in 5% buffered formalin solution (Cyril-Olutayo and Agbedahunsi, 2015).

Slides were prepared from the fixed cells after centrifugation and using a light microscope, 400 cells (both sickled and unsickled erythrocytes) were counted from which the percentage sickle cells was calculated. The experiment was set up in triplicates with PBS as negative control, while para-hydroxyl benzoic acid (PHBA) and vanillin acid were used as positive controls for inhibition and reversal tests, respectively. The extract was tested at various concentrations (1, 2, 4 and 8 mg/mL) to determine the concentration with the optimal activity.

Hb S Polymerization Inhibition

The Hb S polymerization was assessed by the turbidity of the solution at 700 nm, using 2% solution of sodium metabisulphite (Na₂S₂O₂) as deoxygenating agent (Iwu *et al.*, 1988). For the neagive control, 4.4 mL of 2% Na₂S₂O₂, 0.5 mL

normal saline (0.9% NaCl) and 0.1 mL haemoglobin were pipetted into a cuvette, shaken and absorbance reading taken in a spectrophotometer at 700 nm every two min for 30 min. Distilled water was used as blank for the assay. For the test assays, 4.4 mL of 2% Na₂S₂O₂, 0.5 mL of extract and 0.1 mL haemoglobin solution were pipetted in the cuvette and readings taken as above. *P*-hydroxybenzoic acid was used as positive control. The rates of haemoglobin polymerization for the extracts were estimated by the change in optical density (ΔOD) versus time in minutes. The rates were expressed as percentages with respect to the control.

Red Cell Fractionation Assay

The *in vitro* effect of *M. oleifera* ethanol extract on percentage dense cell of the sickle cell sub-population before and after incubation was carried out according to the method of Akinola *et al.* (1992). A red blood cell suspension (10%) of Hb SS red cells (5 mL) was incubated with the extract at 37°C for 1 h, covered with parafilm (Cyril-Olutayo and Agbedahunsi, 2015). After one hour of incubation, the suspension was centrifuged and 40% RBC was thereafter prepared by adding 3 mL PBS to 2 mL packed red blood cells. A discontinuous density gradient method of Mackie *et al.* (1987) was used to provide sufficient cells in each cell fraction. 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 washed Hb SS red cells at approximately 0.40 L/L haematocrit were layered on two discontinuous density gradients in separate tubes. The tubes were centrifuged at 2000 rpm for 40 min using a refrigerated centrifuge 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 counting by the Auto analyser (H18 Light). The assay was carried out using Ciklaviv[®] as positive control.

Membrane Stability Test

Fresh blood sample was collected into anticoagulant bottle containing trisodium citrate (3.8% w/v) and mixed thoroughly to prevent lysing of the red blood cells. This was pipetted into clean centrifuge tubes and then centrifuged at 3000 rpm for 10 min. The supernatants was carefully removed using sterilized pipette and the packed blood re-suspended in fresh isosaline, mixed gently, followed by centrifugation for another 5 min at the same speed as above. A 2% erythrocytes suspension was prepared by diluting 2 mL of packed red blood cells with isosaline (Oyedapo and Famurewa, 1995)

The assay mixture was composed of hyposaline (2 mL), 0.15 M sodium phosphate buffer, pH 7.4 (1 mL), varying volumes of the extract (Ibuprofen as positive control) and 2% v/v erythrocyte suspension (0.5 mL). The final mixture was then made up to 4.5 mL with isosaline. 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 haemoglobin was read at 560 nm using a spectrophotometer. The percentage membrane stability was estimated from the expression:

$$100 - \frac{(\text{Absorbance extract test} - \text{absorbance extract control})}{\text{Absorbance extract control}} \times 100$$

Antioxidant Assays

Total Phenol content

The total phenolic content was estimated using the Folin-Ciocalteu's phenol reagent, which is an oxidizing reagent (Gulcin *et al.*, 2003).

Antioxidant Content

The determination of the antioxidant content is based on the reduction of Molybdenum (VI) to Molybdenum (V) by the extract and the subsequent formation of a green phosphate/Molybdenum (V) complex at an acidic pH (Prieto *et al.*, 1999).

Total Flavonoid Content

Standard quercetin with varying concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL was used as standard in comparison to the sample extract. This experiment was carried out based on the aluminium chloride colorimetric assay method (Miliauskas *et al.*, 2004).

DPPH Assay

The radical scavenging ability of the extracts was determined using the stable radical DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate (Brans-Williams *et al.*, 1995)).

Inhibition of Nitric Oxide Radical

The inhibition of nitric oxide radical activity of the extract was carried out according to the method reported by Marcocci *et al.* (1994). Nitric oxide, generated from sodium nitroprusside (SNP) in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions, which was measured by Griess-Illosvoy reaction.

Ferric Reducing Antioxidant Power (FRAP)

A 300 mM acetate buffer of pH 3.6, 10 mM 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine and 20 mM FeCl₃.6H₂O were mixed together in the ratio of 10:1:1 respectively, to give the working FRAP reagent. A 50 µL aliquot of the extract at 0.1 mg/mL and 50 µL of standard solutions of ascorbic acid (20, 40, 60, 80, 100 µg/mL) was added to 1mL of FRAP reagent in duplicate test tubes. Absorbance measurement was taken at 593 nm exactly 10 min after mixing against reagent blank containing 50 µL of distilled water. All measurements were taken at room temperature with samples protected from direct sunlight. The ferric reducing power was expressed as equivalent concentration (EC) (Benzie and Szeto, 1999).

Ferrous Ion-Chelating Ability Assay

The Ferrous ion-chelating (FIC) assay was carried out according to the method of Singh and (Rajini, 2004).

ABTS⁺ Radical Scavenging Activity

The free radical scavenging activity of extracts was determined by the 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS⁺) radical cation decolourisation assay (Re *et al.*, 1999).

Statistical Analysis

The one way ANOVA was used to detect significant differences and standard errors of the mean values. Level of significance was set at p < 0.05.

RESULTS AND DISCUSSION

Antisickling property

The anti-sickling assay of the crude ethanol extract of *M. oleifera* showed 4 mg/mL, with $95.61 \pm 0.47\%$ inhibition and $79.39 \pm 1.93\%$ reversal, as the optimal

concentration (Figure 1). Fractions viz: n-hexane, ethyl acetate and aqueous of the crude ethanol extract gave high inhibitory and reversal activities (Figure 2). The activities of the fractions were significantly higher ($p < 0.05$) than the positive controls.

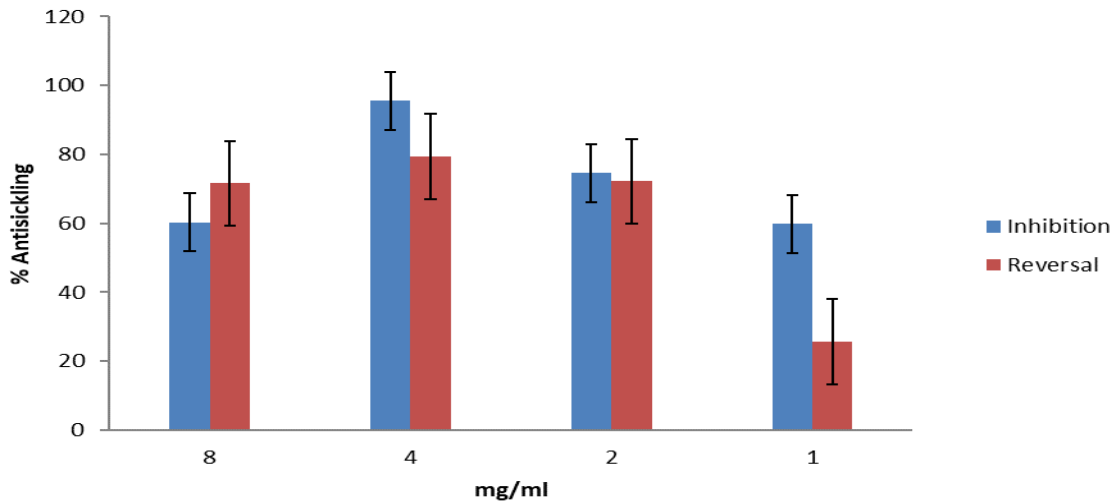


Figure 1: Showing the anti-sickling activities of ethanol extracts of *Moringa oleifera* at various concentrations

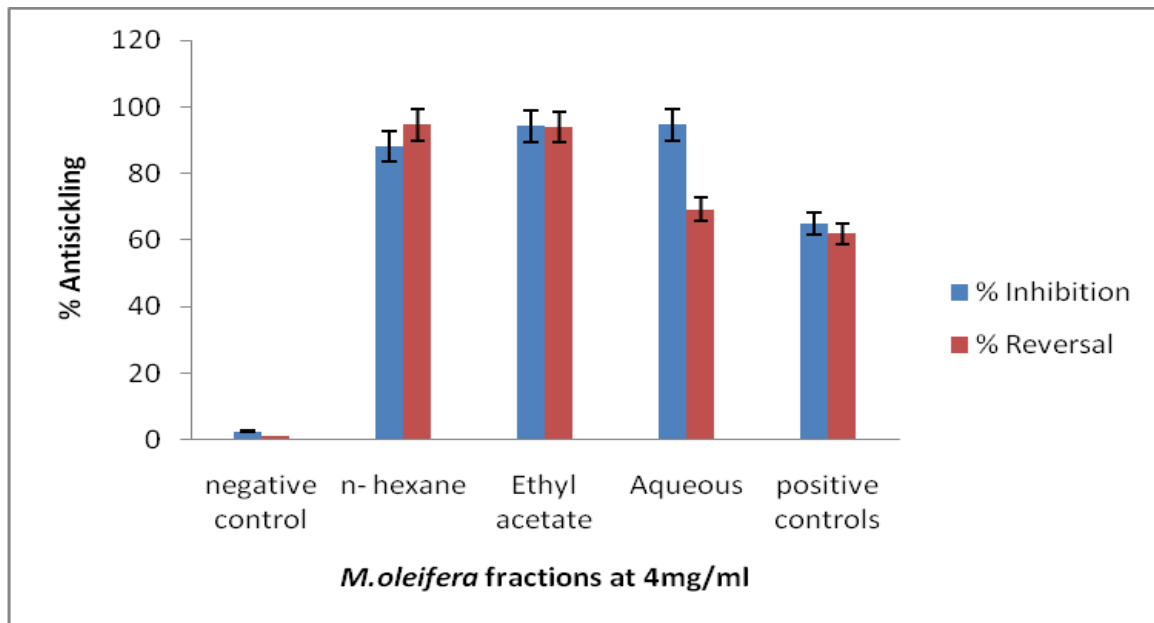


Figure 2: Anti-sickling effects of the different fractions of *M. oleifera* at 4 mg/mL.

Red Cell Fractionation Result

The discontinuous gradient fraction gave four bands, the first contained reticulocytes and cell fragments. The second band (F2) contained young but mature red cells with the lowest density. The third band (F3)

contained cells of intermediate age and density. The fourth band at the bottom (F4) contained the densest cells Figure 3.

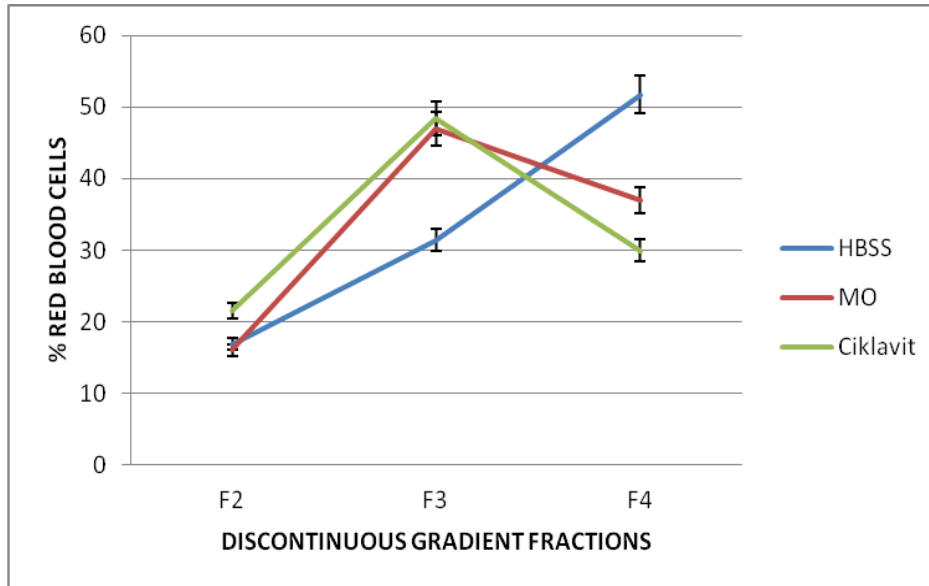


Figure 3: The mean values for percentage red blood cells of control and Hb SS blood treated with MO and Ciklavit® in the discontinuous gradient fractions.

Hb S Polymerization result

Purification of the crude extract increased the rate of decrease in polymerization. Non polar solvents viz n-hexane and ethyl acetate were observed to give the highest inhibition of polymerization (Figures 4 and 5).

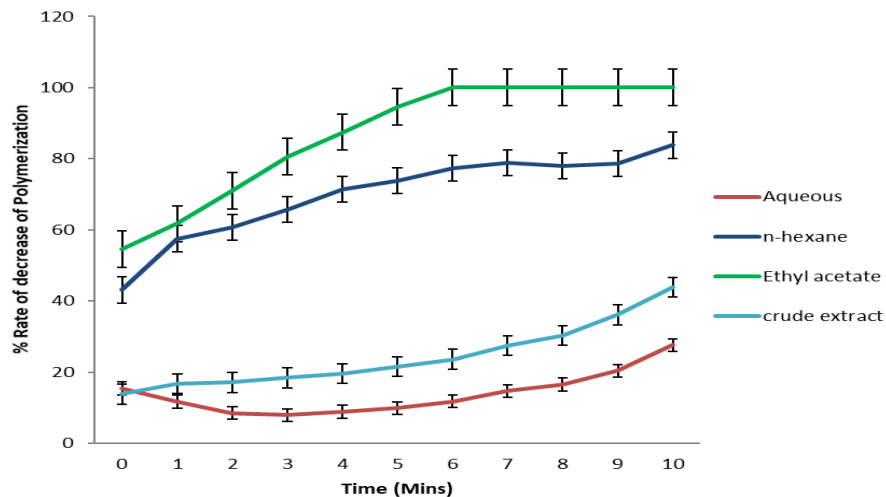


Figure 4: The percentage inhibition of Hb SS polymerization by the various fractions of *M. oleifera* at 2 mg/mL.

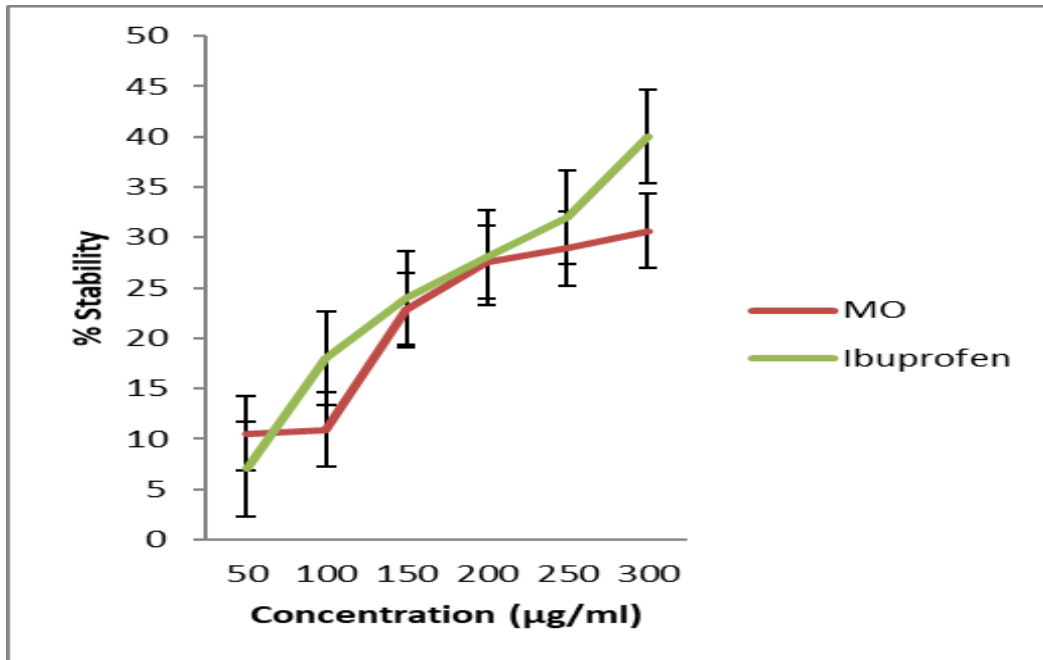


Figure 5: The membrane stability assay of ethanol extract of *M. oleifera* at different concentrations

Membrane Stability Result

The membrane stability property of *M. oleifera* increased as concentration increased (Figure 6).

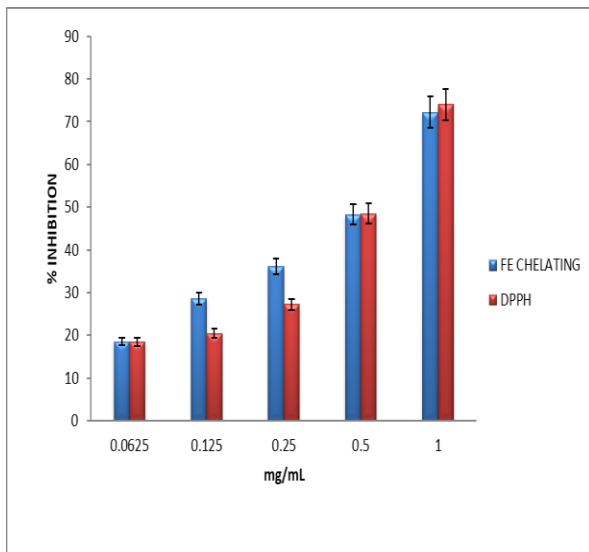


Figure 6: Ferrous ion-chelating and DPPH radical scavenging activities of ethanol extract of *M. oleifera*

Antioxidant Activity

The total phenolic content, total antioxidant, flavonoid content and ferric reducing antioxidant power of MO extract are presented in Table 1. The free radical scavenging activity of *M. oleifera* ethanol extract was determined by the DPPH, Ferrous-ion chelating, ABTS⁺ and Nitric oxide inhibitory assays (Figures 7 and 8). The DPPH assay gave an IC₅₀ of 0.59 ± 0.019 mg/mL with 74% inhibition at 1 mg/mL (Figure 7). The standard (ascorbic acid) however gave an IC₅₀ of 0.01 ± 0.00013. The ferrous ion chelating power of the extract was also high giving 72.2% activity at 1 mg/mL with IC₅₀ of 0.53 ± 0.023. The radical scavenging power of the ethanol extract on DPPH was higher than that reported for the methanol extract (Charoensin, 2014).

Table 1: The *in vitro* Antioxidant contents of *M. oleifera* ethanol extracts

Total phenolic content mg (GAE/ g of the sample (mean ± SEM)	FRAP mg Ascorbic acid equivalent mg/g of the sample (mean ± SEM)	Total antioxidant mg Ascorbic acid equivalent mg/g of the sample (mean ± SEM)	Flavonoid mg Quercetin equivalent mg/g of the sample (mean ± SEM)
54.19±0.19	35.79±0.73	168.9±2.1	280.55±7.000

The antisickling assay of the crude ethanol extract of *M. oleifera* showed 4 mg/mL as the optimal concentration (Figure 1), a reduction in activity was observed at a higher dose. Doses lower than the thresholds are known to produce no response, while those in excess of the threshold exert no additional response and increasing the dose of a drug with a small therapeutic index may increase the probability of toxicity or ineffectiveness of the drug. Fractionation of the crude extracts into n-hexane, ethyl acetate and aqueous enhanced the antisickling property (Figure 2) with the reversal activities of the non-polar fractions significantly higher than that of the crude extract.

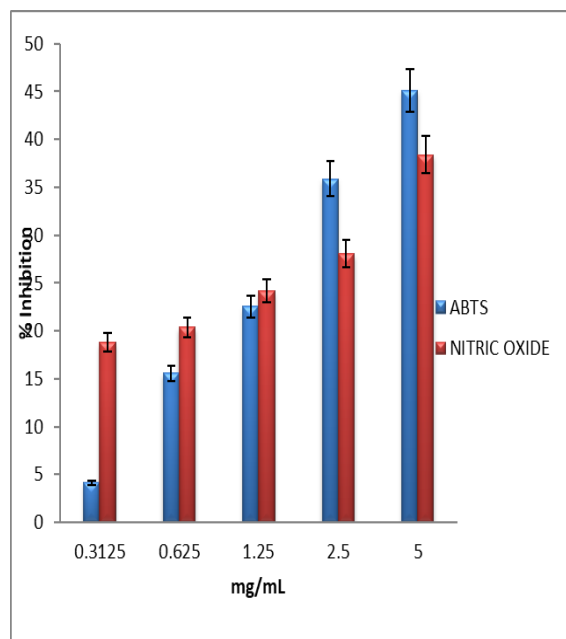


Figure 7: Nitric oxide (NO) inhibitory and ABTS⁺ radical scavenging activities of ethanol extract of *M. oleifera*

The antisickling property of MO extract and fractions at 4 mg/mL was significantly higher ($p < 0.05$) than the positive controls. The effect of MO extract on density of red blood cell gave a mean percentage change in density of 14.74 ± 0.90 , while Ciklavit®, the positive control, gave $21.75 \pm 0.16\%$ (Figure 3). The percentage count of Hb SS red blood cells treated with MO extracts was significantly ($p < 0.05$) lower in the F4 fraction than the F4 fraction of its corresponding untreated Hb SS (negative control). The extract significantly reduced the percentage red blood cells in the F4 dense layer and increased the quantity of red blood cells in the F3 layer of the discontinuous gradient as the density of cells

reduced. This implies that the administration of MO or the positive control Ciklavit® will reduce the density of Hb SS red cells. This reduction in the density is an indication of increased deformability of red blood cells and hence a reduction in polymerization and sickling. This is the first report of a reduction in density of Hb SS red blood cells treated with *M. oleifera*.

The primary cause of the clinical symptomatology of sickle cell anaemia is the intracellular polymerization of sickle haemoglobin (HbS) that occurs when sickle erythrocytes are partially deoxygenated under hypoxic conditions of the microcirculation. This, in turn, makes Hb SS red blood cells deformed resulting in microvascular occlusions and haemolytic anaemia characteristics of sickle cell disorder (Poillon and Kim, 1990). *Moringa oleifera* showed the ability to inhibit polymerization *in vitro*, this result is in conformity with the findings of Nwaoguikpe *et al.* (2015). It was further observed that the ethanol extract decreased the rate of polymerization gradually from time t_4 thereby increasing the rate of decrease in polymerization. The rate of decrease in polymerization was dose dependent. However, further purification was noted to enhance the effect of *M. oleifera* in inhibiting polymerization. The n-hexane fraction gave 83.8% inhibition at time t_{10} (Figures 4), while the ethyl acetate fraction gave 100% inhibition at time t_6 at 2 mg concentrations. This compares favourably with the inhibitory effect of PHBA positive control (87.04%) at 4 mg/mL. Inhibition of polymerization was higher at 2 mg for all fractions tested. The results of the n-hexane and ethyl acetate fractions in the two antisickling models viz inhibition of rate of polymerization and the inhibitory/reversal assay showed similarities in activities.

The mechanism of action of the antisickling activity of *M. oleifera* was further investigated by the membrane stability assay and it was observed that the extracts showed dose dependent membrane stabilizing activity. The ethanol extract of *M. oleifera* exerted minimum and maximum percentage stability activities of $10.54 \pm 0.003\%$ and $30.61 \pm 0.002\%$ at 50 μg and 300 μg , respectively, while Ibuprofen gave $40.0 \pm 0.003\%$ stability at the highest dose tested (Figure 5). The mode of action of the extract 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 or promote dispersal by mutual repulsion of like charges, which are involved in the haemolysis of red blood cells (Oyedapo *et al.*, 2010). Certain saponins, tannins and flavonoids were earlier reported to exert stabilizing effect on lysosomal membrane both *in vivo* and *in vitro*, and tannins and saponins possess ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules (Pathak *et al.*, 1991; El-Shabrany *et al.*, 1997;). The membrane stabilizing activities of the extract of *M. oleifera* might have been aided by the presence of flavonoid, tannins and saponins reported by Kasolo *et al.* (2010) and Nweze and Okafor (2014) to be present in the ethanol extract of *M. oleifera*.

Although the anti-oxidant properties of the leaves of *M. oleifera* had been evaluated extensively, in this study we still went ahead to evaluate these properties based on the location of collection (South West Nigeria) and related the contents to the antisickling properties of the plant. The total flavonoid, total phenol, total antioxidant capacity and ferric reducing ability of plasma (FRAP) of the ethanol extract of *M. oleifera* (Table 1) showed that the leaf extract has high antioxidant contents.

In SCD, sickling leads to the modification of the membrane flexibility, making it more sensitive and fragile towards free radicals or oxidants. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Buyukokuroglu *et al.*, 2001). The *M. oleifera* extracts therefore interfered with the oxidation process by scavenging and reacting with free radicals (Figure 6).

Humans are unable to eliminate the iron released from the breakdown of transfused red blood cells and the excess iron is deposited as hemosiderin and ferritin in the liver, spleen, endocrine organs and

CONCLUSION

Moringa oleifera was found to be a candidate plant for the development of remedy for the management of sickle cell anaemia. *Moringa oleifera* leaf extract and fractions exhibited a high *in vitro* anti-sickling property coupled with its antioxidant contents and free radical scavenging ability. The effect of *M. oleifera* on red cell density, the decrease rate of polymerization after inducing hypoxia and its

myocardium. The accumulation of these toxic quantities of iron causes tissue damage and leads to complications such as heart failure, hypothyroidism, liver failure and ultimately early death (Taher *et al.*, 2006). Chelation therapy reduces iron-related complications by mobilizing tissue iron to form soluble, stable complexes that are then excreted in the faeces and/or urine thereby improving quality of life and overall survival. The MO extracts interfered with the formation of ferrous and ferrozine complex (Aboul-Enein *et al.*, 2003), suggesting that MO has chelating activity and gave dose dependent chelating activities (Figure 6), although the IC₅₀ value of the standard drug EDTA (IC₅₀ 0.07 ± 0.0004) was significantly lower than that of the extract. This activity is very significant because chelation of metal ions by these extracts can be of therapeutic importance in the treatment of iron overload in sickle cell anaemia. In SCD, loss of the biological activity of nitric oxide (NO) could predispose to vaso-occlusive crisis by promoting erythrocyte adhesion, increasing the susceptibility to spasm, thereby obstructing the regional regulation of blood flow (Eberhardt *et al.*, 2003). This is because NO is a potent vasodilator and therefore plays a role in the complications associated with vaso-occlusion (Wood *et al.*, 2008). *Moringa oleifera* extract showed very low inhibition of nitric oxide with IC₅₀ 8.003 ± 0.820 mg/mL (figure 7), this is not comparable with the Gallic acid control with IC₅₀ 0.05 ± 0.001 mg/mL. Several lines of evidence suggest that there is vascular dysfunction and impaired NO bioactivity in Hb SS individuals and continued NO production is important for maintaining cerebral blood flow during experimental stroke in the presence of sickle erythrocytes (French *et al.*, 1997). The low inhibition of NO by *M. oleifera* extract is an important observation, which makes *M. oleifera* a potent plant in the management of SCD.

membrane stability ability might be responsible for its high anti-sickling properties.

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REFERENCES

- Aboul-Enein, A. M., El Baz, F. K., El-Baroty, G. S., Youssef, A. M. and. Abd El-Baky H. H. (2003). Antioxidant activity of algal extracts on lipid peroxidation. *J. Med. Sci.* 3: 87-98.
- Akinola, N. O., Stevens, S. M. E., Franklin, I. M., Nash, B. and Stuart, J. (1992). Subclinical ischaemic episodes during the steady state of sickle cell anaemia. *J. Clin. Pathol.* 45: 902-906.
- Anwar, F., Latif, S., Ashraf, M. and Gilani, A.H. (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother Res.* 21(1):17-25.
- Bartolucci, P., Brugnara, C., Teixeira-Pinto, A., Pissard, S., Moradkhani, K., Jouault, H. and Frederic Galacteros, F.. (2012). Erythrocyte density in sickle cell syndromes is associated with clinical manifestations and hemolysis. *Blood* 120: 3136-3141.
- Benzie, I. F. F. and Szeto, Y. T. (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power (FRAP) assay. *J. Agric. Food. Chem.* 47: 633-636.
- Brans-Williams, W., Cuvelier, M. E. and Berset, C. (1995). The use of free radical method to evaluate antioxidant activity. *Lebensm. -Wiss. u.- Technol* 28: 25-30.
- Buyukokuroglu, M. E., Gulcin, I., Oktay, M. and Kufrevioglu, O. I. (2001). *In vitro* antioxidant properties of dantrolene sodium. *Pharmacol. Res.* 44: 491-495.
- Charoensin, S. (2014). Antioxidant and anticancer activities of *Moringa oleifera* leaves. *J. Med. Plants Res.* 8: 318-325.
- Cyrl-Olutayo, C. M. and Agbedahunsi, J. M. (2015). Effects of the Ethanolic Extract of *Cnidioscolus aconitifolius* (Mill.) I.M. Johnst. on Hb S Red Blood Cells *In Vitro*. *Niger. J. Nat. Prod. Med.* 19: 115-121.
- Das, J., Biswas, S.K., Chowdhury, A., Sharif, S.R. and Hannan, M.A. (2012) *In vitro* Antibacterial and Antifungal Potentials of Petroleum Ether Extract of *Moringa oleifera* *J Pharmacol Toxicol.* 7 (2): 110-113.
- Eberhardt, R., McMahon, L., Duffy, S., Steinberg, M. H., Perrine, S. P., Loscalzo, J., Coffman, J. D. and Vita, J.A. (2003). Sickle cell anemia is associated with reduced nitric oxide bioactivity in peripheral conduit and resistance vessels. *Am. J. Hematol.* 74: 104-111.
- El-Shabrany, O. A., El-Gindi, O. D., Melek, F. R., Abdel-Khalk, S. M. and Haggig M. M. (1997). Biological properties of saponin mixtures of *Fagonia cretica* and *Fagonia mollis*. *Fitoterapia* LXVIII, 219-222.
- Farooq, A., Latif, S., Ashra, F. M. and Gilani, A.H. (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother. Res* 21: 17-25.
- French, J.A., Kenny, D., Paul Scott, J., Hoffmann, R. G., Wood, J. D., Hudetz, A. G. and Hillery, C. A. (1997). Cerebral blood flow in rats after nitric oxide synthase inhibition. *Blood* 12: 4591-4599.
- Gulcin, I., Buyukokuroglu, M. E., Oktay, M. and Kufrevioglu, I. O. (2003). Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. Subsp. Pallsiana (Lamb.) Holmboe. *J. Ethnopharmacol.* 86: 51-58.
- Iwu, M. M., Igboko, A. O., Onwubiko, H. and Ndu, U. E. (1988). Effect of cajaminose from *Cajanus cajan* on gelation and oxygen affinity of sickle cell haemoglobin. *J. Ethnopharmacol.* 20: 99-104.
- Kasolo, J. N., G. S. Bimenya, L. Ojok, J. Ochlang, and J. W. Ogwal-Okeng. (2010). Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. *J. Med. Plant Res.* 4: 753-757.
- Kaul, D. K., Nagel, R. L. and Baez, S. (1983). Pressure effects on the flow behavior of sickle (HbSS) red cells in isolated (ex-vivo) microvascular system. *Microvasc. Res.* 26: 170-181.
- Kuete, V., Karaosmanoglu, O. and Sivas, H., (2017) Anticancer Activities of African Medicinal Spices and Vegetables. In: Medicinal Spices and vegetables from Africa, Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases. Eds.: L.K. Omosa, J.O. Midiwo, V. Kuete, Elsevier UK, 2017, pp 271-297.
- Mackie, L. H., Frank, R.S. and Hochmuth, R. M. (1987). Erythrocyte density separation on discontinuous "Percoll" gradients. *Biorheology* 24: 227-30
- Makkar, H. P. S., Francis, G. and Becker, K. (2007). Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal* 1:1371-1391.
- Marcocci, L, Maguire, J. J., Droy-Lefaix, M. T. and Packer, L. (1994). The nitric oxide scavenging property of Ginkgo biloba extract EGb 761. *Biochem. Biophys. Res. Commun.* 201: 748-755.
- Messmann, R., Gannon, S., Sarnaik, S. and Johnson, R. M. (1990). Mechanical properties of sickle cell membranes. *Blood* 75: 1711-1717
- Miliauskas, G., Venskutonis, P. R. and Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal plants and aromatic plant extract. *Food Chem.* 85:231-237.

- Ndhkala, A. R., Mulaudzi, R., Ncube, B., Abdelgadir, H. A., du Plooy, C. P. and Van Staden, J. (2014). Antioxidant, antimicrobial and phytochemical variations in thirteen *Moringa oleifera* Lam. cultivars. *Molecules* 19:10480-94.
- Nwaoguikpe, R. N., Ujowundu, C. O., Igwe, C. U. and Dike, P. N. (2015). The Effects of *Moringa oleifera* Leaves Extracts on Sickle Cell Hemoglobin. *J. Sci. Res. and Rep.* 4: 123-132.
- Nweze, N. O. and Nwafor, F. I. (2014). Phytochemical, Proximate and Mineral Composition of Leaf Extracts of *Moringa oleifera* Lam. from Nsukka, South-Eastern Nigeria. *J. Pharm. Biol. Sci.* 9:99-103.
- Onwubiko, H. A. (2010). The Anti-Sickling Properties of Ethanol Extracts of *Euphorbia heterophylla* and *Moringa oleifera* leaves. *Plant Products Research Journal* 14: 51-58.
- Oyedapo, O. O., Akinpelu, B. A., Akinwunmi, K. F., Adeyinka, M. O. and Sipeolu, F. O. (2010). Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. *Int J. Plant Physiol. Biochem* 2: 46-51.
- Oyedapo, O. O. and Famurewa, A. J. (1995). Anti-protease and membrane stabilizing activities of extracts of *Fagara zanthoxyloides*, *Ola subscorpioides* and *Tetrapleura tetraptera*. *Int. J. pharmacol.* 33: 65-69.
- Parotta, J. A. (1993). *Moringa oleifera* Lam. Reseda. Horseradish tree. Moringaceae. Horseradish tree family” USDA Forest Service, International Institute of Tropical Forestry. Retrieved 2013: 11-20.
- Pathak, D., Pathal, K. and Singla, A. K. (1991). Flavonoids as medicinal agents:Recent Advances. *Fitoterapia* LXII, 371-376.
- Pauling, L., Itano, H. A., Singer, S. J. and Wells, I.C. (1949). “Sickle cell anemia, a molecular disease,” *Science* 110 (2865): 543–548.
- Poillon, W. N., and Kim, B. C. (1990). 2, 3-Diphosphoglycerate and intracellular pH as interdependent determinants of the physiologic solubility of deoxyhemoglobin S. *Blood* 76: 1028-36.
- Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* 269: 337–341.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice- Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.*, 26: 1231 – 1237.
- Sindhu, S., S. Mangala, and B. Sherry. (2013). Efficacy of *Moringa oleifera* in treating iron deficiency anaemia in women of reproductive age group. *International Journal of Phytother. Res.* 3(4): 15-21.
- Singh, N. and Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem.* 85: 611–616.
- Sudhir, K. P., Mishra, D., Ghosh, G., Chandra, S. and Panda C.S. (2010). Medicinal uses and pharmacological properties of *Moringa oleifera*. *International Journal of Phytomedicine* 2: 210-216
- Taher, A., Isma'eel, H. and Cappellini, M. D. (2006). Thalassemia intermedia: revisited. *Blood Cells Mol. Dis.* 37: 12-20.
- Whitten, C. F. and Bertles, J. F. (1989). Sickle Cell Disease *New York Acad. Sci. volume* 565
- Wood, K. C., Hsu, L. L. and Gladwin, M. T. (2008). Sickle cell disease vasculopathy: a state of nitric oxide resistance. *Free Radic Biol Med.* 44: 1506–28.
- Yang, R. Y., Tsou, S. C. S., Lee, T. C., Chang, L. C., Kuo, G. and Lai, P. Y. (2006). *Moringa*, a novel plant rich in antioxidants, bioavailable iron, and nutrients. *Am. Chem. Soc. Symp. Series*, 925: 224-239.

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