



The Effects of *Moringa oleifera* Leaves Extracts on Sickle Cell Hemoglobin

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Authors' contributions

It would be interesting to note that some authors had special responsibility in realizing the aims and objectives of the study. Author RNN designed the study, carried out the assays on sickle cell hemoglobin polymerization and co-ordinated the different sections of the work. Author PND carried out the determination of free amino acid concentrations, the total ascorbic acid content and the collection, identification and preparation of plant materials. Author CUI prepared the sample for amino acid analysis by TSM. He also carried out the phytochemical analysis and the determination of Fe²⁺/Fe³⁺ ratio. Author COU carried out the assay on proximate composition and statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Sickle cell disease (SCD) caused by hemoglobin mutant called hemoglobin S or HbS, which originated from the replacement of a hydrophilic amino acid residue-glutamic acid by a hydrophobic moiety valine, at the sixth position of the β -chain of hemoglobin molecule. This loss of charge causes the erythrocytes to sickle under hypoxic conditions. The phytochemical, proximate and amino acid compositions of the leaves extracts of the plant *Moringa oleifera* were assayed to determine their antisickling effects on sickle cell hemoglobin. The proximate composition of the leaves showed the data:moisture (6.0 \pm 1.2mg/100g); crude protein (28.0 \pm 1.0mg/100g); carbohydrate (48.10 \pm 0.13mg/100g); crude fiber (10.05 \pm 0.01mg/100g); ash(9.30 \pm 0.01 mg/100g) ; pH 6.25 and crude fat (2.5 \pm 0.0 mg/100g). The different extracts including the crude aqueous extracts (CAE) revealed a preponderance of the following phytomedicines:-alkaloids

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(4.0 ± 0.01 mg/100g), flavonoids (0.4 ± 0.0 mg/100g), saponins (8.0mg/100g) and phenols (1.10 ± 0.10 mg/g). The antisickling effects of the different fractions of the leaves exhibited pronounced antisickling effectiveness by inhibiting sickle cell hemoglobin polymerization from 88.80% for the WAS(water-soluble) fraction to 98.35% for the FAS(fat-soluble) fraction. The BUS (butanol-soluble) fraction inhibited the process to 98.35%. The following antisickling amino acids were equally identified by TSM (technicon sequential multisample) amino acid analyzer at different concentrations: arginine (6g/100g); histidine(2.1g/100g);lysine (4.3/100g); tryptophan 1.9g/100g; phenylalanine 6.4g/100g. The total free amino acid concentration of the fractions (WAS, FAS, BUS) were high and expressed as follows: 13.57 mg/100g; 450.78 mg/100g and 49.76 mg/100g respectively. The total ascorbic (Vitamin C) acid concentration of the (crude aqueous extract) CAE was 4512 mg/100 g of sample. The Fe^{2+}/Fe^{3+} ratio showed improvement from 5.48% for the WAS fraction to 18.89% for the FAS fraction. Based on the preponderance of phytonutrients, the proximate composition of the leaves, the antisickling effectiveness of the fractions based on hemoglobin polymerization inhibition experiment, the presence of antisickling and essential amino acids; *Moringa oleifera* leaves extracts may present future therapeutic hope for the effective management of sickle cell disease and other related nutritional syndromes.

Keywords: Sickle cell disease; hemoglobin polymerization; *Moringa oleifera*; Fe^{2+}/Fe^{3+} ratio.

1. INTRODUCTION

Sickle cell disease (SCD) or sickle cell anemia (SCA) or drepanocytosis, is an autosomal recessive blood disorder, characterized by red blood cells that assume abnormal, rigid sickle shape. Sickling of erythrocytes or gelation decreases the flexibility of red blood cells and results in a risk of various complications. The sickling of erythrocytes occur as a result of hypoxia, which is a consequence of point mutation in the β -globin chain of normal hemoglobin. Life expectancy is shortened. Sickle cell disease occurs more commonly among people of African stock or whose ancestors lived in tropical and Sub-Saharan regions of the world, where malaria is endemic. Specifically, humans with one of the two alleles show less severe symptoms when infested with malaria. HbS (sickle cell hemoglobin) polymerization is associated with a reduction in cell ion and water content (cell dehydration), increased red cell density, which further accelerate HbS polymerization [1,2,3,4].

Dense dehydrated erythrocytes are likely to undergo instant polymerization in conditions of mild hypoxia due to their high HbS concentration; and HbS polymers may be formed under normal oxygen pressure. Medical advances in the management of sickle cell disease patients have led to significant increase in life expectancy. This improved longevity is likely the result of early neonatal screening, patient and parental education, improved public health advances on red blood cell transfusion medicine, penicillin prophylaxis for children, pneumococcal

vaccinations, nutrition of affected patients and hydroxyurea therapy [1]. To date, allogenic bone marrow transplant remains the only available cure, although this form of therapy is limited by the availability of potential donors and by its toxicity [1]. To this end, there has been a constant search for novel therapies that may be accessible to majority of sickle cell disease patients. Many therapeutic approaches and protocols have been adopted involving many chemicals, herbal preparations, nutrients and induction processes to improve the health of sickle cell patients as well as the reduction of many pathophysiological complications of the syndrome. There is therefore an uphill task in stumbling over a therapeutic bullet that can knock out the sickle cell syndrome from patients. For instance, fetal hemoglobin (HbF) is a potent inhibitor of HbS polymerization. This is because, neither HbF($\alpha_2\gamma_2$) nor the hybrid tetramer ($\alpha_2\gamma\beta$) are incorporated into the polymer phase of gelation. Multiple epidemiological studies show that the level of HbF predicts clinical severity in patients with SCD. Hydroxyurea, a ribonucleotide reductase inhibitor is the only drug approved by the US Food and Drug Administration for treating SCD. It has been shown to reduce the frequency of hospitalization, acute painful episodes, acute chest syndromes and blood transfusion in severe sickle cell anemia patients. In Nigeria, many herbal extracts for example, NipprisanTM [5] has been tested and proved to elicit remarkable action on sickle cell gelation and whose main mechanism is to stabilize the erythrocyte membrane by providing electrolyte homeostasis. Other plants include: *Adesonia digitata* (bark), *Bryophyllum pinatum*

(leaves), *Cajanus cajan* (seeds), *Carica papaya* (fresh unripe seeds), *Zanthoxylum xanthoxyloides*, aged garlic extracts, edible legumes, some of which mitigate sickling by the provision of cations, nutrients, antisickling amino acids and vitamins [6,7,8,9] that function to ameliorate the syndrome.

Apart from all these, an antisickling nutrient "CIKLAVIT™" is in use for the management of sickle cell disease, discovered in Nigeria by Prof G.I Ekeke and Dr. R.N. Nwaoguikpe; produced and marketed by Neimeth Pharmaceutical PLC. It contains amino acids, food extracts and zinc. Traditional medicine or ethno-medicine is more available and affordable in Africa because of the high cost of orthodox medication and the failure rate of these drugs in ameliorating the pathophysiological complications of some disease conditions including the sickle syndrome. Almost 24 million Nigerians are carriers and sufferers of sickle cell disease [10].

Moringa oleifera (Moringaceae) possess high nutritional components. In ancient Egypt, *Moringa roil* (known as "BAK") was used by the more wealthy to anoint the body and to keep skin supple. Egyptian medicare incorporated moringa oil into remedies for stomach ailments, headache and earaches. In central Africa, it is used to treat skin infections. Currently, moringa is used raw, unprocessed by many countries from Australia, Fiji, Senegal to Brazil. Moringa is a remarkable tree whose leaves gram for gram have seven times the vitamin C found in oranges, four times the vitamin A of carrots, three times the iron of spinach, four times as much calcium as milk [11]. It is on record that moringa trees have been used to combat malnutrition especially among infants and nursing mothers. In fact, the nutritional properties of moringa are now so well known that there seems to be little doubt of the substantial health benefits [11,12]. The leaves of *Moringa oleifera* have a wide range of beneficial effects. It was reported to prevent gastric ulceration, immune-modulatory and wound healing properties [13,14]. Report has it that leaf extracts prepared using ethanol or methanol, inhibit microsomal lipid peroxidation. *Moringa oleifera* also showed radio protective potential as the leaf extract protect bone marrows against radiation induced marrow damage. It has also been reported that extract from *Moringa oleifera* is able to protect the liver against acetaminophen and carbon tetrachloride induced damage [15].

Irrespective of the amount of reported uses of *Moringa oleifera*, the leaves have been reported as the most nutritious part of the plant, being a significant source of B-vitamins, vitamin C, pro-vitamin A, β -carotene, and vitamin K. Not much information is available on the antisickling properties of this plant. Hence, we are prompted to investigate the antisickling effects of the leaves extracts on sickle cell hemoglobin.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material used in the work are the leaves of *Moringa oleifera* which were plucked fresh in a village Akokwa, in Ideato North LGA of Imo State, Nigeria at the local government headquarters where the trees grow luxuriantly. The leaves were identified and authenticated as being the best variety by a plant taxonomist, Mr. Francis Iwunze, at the Department of Forestry and Wildlife of the School of Agriculture and Technology (SAAT), of the Federal University of Technology, Owerri Nigeria.

2.2 Sample Preparation

Fresh leaves were washed under running tap water, dried at room temperature of 27°C, weighed and blended into powder, to a total weight of 300 g.

2.3 Preparation of Crude Aqueous Extract (CAE)

This was prepared by soaking 50 g of the powdered sample with 150 ml of distilled water for 24 h in a refrigerator, maintained at 8°C. The filtrate was kept in a corked volumetric flask in a refrigerator until used [16].

2.4 Batch Extraction Procedures

2.4.1 Extraction of the fat-soluble (FAS) fraction

One hundred (100 g) grams of the powdered plant leaves were soaked in 200 ml of Dichloromethane of analytical grade in a 500 ml conical flask, corked for 24h until filtered. This is in essence to defat the sample and to generate the fat-soluble (FAS) fraction. The residue after filtration was dried *en vacuo* to evaporate the available residual volatile solvent [17]

2.4.2 Methanol solvent extraction process

The residue from the dichloromethane extraction process was soaked in 200 ml of methanol of analytical grade for 24 h. The solvent was filtered off and the filtrate concentrated by rotor evaporator maintained at 30 °C. The weights and volumes of the extracts were recorded. The extract was corked and kept in the refrigerator at 8 °C [17] until used.

2.4.3 Butanol-water partitioning

Butanol -water partitioning was carried out with the methanol water soluble (MWS) in a separating funnel, left standing for 24 h. The two-phase liquid solution was separated into two different fractions, labeled appropriately as butanol-soluble (BUS) and water-soluble (WAS) fractions respectively. The two fractions were concentrated by rotor evaporator [17].

2.5 Phytochemical Screening and Quantitation

Qualitative and quantitative screening (assays) were carried out on the sample to determine the resident phytochemicals or anti-nutrients such as: saponins, tannins, alkaloids, flavonoids and phenols, by the methods [18,19].

2.6 Collection of Blood Samples

Blood sample (HbSS) was collected from confirmed sickle cell disease patients who attend sickle cell clinic by the personnel of the Hematology Unit of the Federal Medical Centre, Owerri, Nigeria. The donors were informed by the ethical committee of the hospital on the need for the blood samples and they consented.

2.6.1 Preparation of blood samples

Portions of whole blood (0.2 ml) was used for the Fe^{2+}/Fe^{3+} ratio determination while the remaining portions were collected into citrate anticoagulant tubes, kept in a refrigerator at 8 °C. Erythrocytes were isolated from the blood samples by centrifugation at X 1500 g for 10 minutes, using bench centrifuge. Following careful siphoning of the plasma with Pasteur pipette; the erythrocytes were by repeated inversion suspended in a volume of isotonic saline (0.9%NaCl) equivalent to the volume of the siphoned plasma. The erythrocyte suspension was then frozen at 0 °C and subsequently thawed to produce a

hemolysate for the hemoglobin polymerization experiment.

2.7 Proximate Composition of *Moringa oleifera* Leaves

Proximate analysis was carried out according to the methods described by the Association of Official Analytical chemists [18] to determine the moisture content, crude protein, carbohydrate, ash, pH and crude fat compositions respectively.

2.8 Determination of Total Free Amino Acid Concentrations of the Fractions of the Sample

0.1% Ninhydrin in acetone was diluted with distilled water in the ratio 1:4. The water-soluble fraction (WAS) was diluted 1:1 with distilled water; the butanol-soluble fraction (BUS) diluted 1:1 with methylated spirit and the fat-soluble (FAS) fraction, 1:5 with ethanol. Exactly, 20 μ L each of the diluted extracts was added 4.0 ml portions of diluted Ninhydrin. The resulting solutions were heated to boiling for 5 min, cooled and the absorbance reading taken at 2 min intervals for 30 minutes in a spectrophotometer at 570 nm, distilled water as blank. The values were extrapolated from a standard curve obtained by treating 20 μ L portions of different concentrations of Phenylalanine (1-20 mg) with 4.0 ml portions of diluted Ninhydrin treated as above.

2.9 Determination of the Major Amino Acid Constituents of *Moringa* Leaves by TSM

The amino acid profile of the powdered leaves sample was determined using the methods described by Speckman and colleagues [20]. The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotor evaporator and loaded into the Technicon Sequential Multisample (TSM) Amino Acid Analyzer.

2.10 Determination of the Ascorbic Acid Concentration of the Crude Aqueous Extract (CAE) of the Leaves

The determination of the Ascorbic acid concentration of the leaves was carried out by the method [21]. Ascorbic acid standard was prepared containing 1 g/dm³ of Ascorbic acid

such that 1 cm³ = 1 mg vitamin C. A burette was filled with a solution of 2, 6-Dichlorophenolindophenol (DCPIP) of 0.01%. Ten milliliters (10 ml) of Ascorbic acid standard was acidified with 2 or 3 drops of dilute HCl. The indophenol solution was titrated into the Ascorbic acid solution until there was a permanent pink solution.

If X cm³ of the indophenol solution were required, therefore 1 cm³ of the indophenols is equivalent to 10 mg Vitamin C/Xcm³. Having standardized the indophenols solution, 10 cm³ of the test solution (CAE) was taken and treated as above.

2.11 Sickle Cell Hemoglobin Polymerization Inhibition Experiment

The original methods [17,22,23] were used for HbSS polymerization inhibition experiment. Sickle cell hemoglobin polymerization was assessed by the turbidity of the polymerizing mixture at 700 nm, using 4.4 ml of 2% Sodium metabisulphite as the reductant or deoxygenating agent. 0.5 ml normal saline and 0.1 ml hemoglobin solution were introduced into the cuvette by means of a pipette, shaken and inserted into a spectrophotometer and absorbance readings taken at 2 min intervals for 30 min. This represents the control. Distilled water was used as blank in all assays. For the test assay, 4.4. ml of 2% Sodium metabisulphite solution, 0.5 ml antisickling agent and 0.1 ml hemoglobin solution were assayed as above. The rates of hemoglobin polymerization were calculated. The relative percent polymerization and relative percent inhibition were determined with respect to the control. These parameters were all determined from the formula below [17].

$$\text{Rate of Polymerization} = \frac{\text{Final OD} - \text{Initial OD}}{\text{time}}$$

Where

Rp= rate of polymerization
t= time in minutes

$$Rp = \frac{OD_f - OD_i}{t} = \frac{\Delta OD}{t}$$

ODf= final optical density/absorbance
ODi= Initial optical density/absorbance

2.12 Determination of *In vitro* effects of the Extracts on Fe²⁺/Fe³⁺ Ratio of Sickle Cell Blood

The Fe²⁺/Fe³⁺ ratio was determined by the method [24], while the oxygen affinity of hemoglobin and methemoglobin were measured at 540 nm and 630 nm respectively. The approach employs lving 0.02 ml of whole blood in 5.0 ml distilled water and 0.02 ml normal saline. The absorbance of Hb (hemoglobin) and mHb (methemoglobin) were determined to give % Hb and % mHb respectively. This represents the control. In determining the effect of the extracts (antisickling agent) on the ratio; 0.02 ml of the extract was added to 5.0 ml of distilled water and 0.02 ml of whole blood added and incubated for 60 min in a test tube. The procedure above repeated to determine the % Hb and % mHb respectively. Further analysis gives the relative percent increase or decrease in the ratio [17].

2.12 Statistical Analysis of Results

Statistical analysis of results is multifaceted. Some results are expressed in percentages, mean and standard deviations as well as ONE-WAY ANOVA. The level of significance was taken at p≤0.05.

3. RESULTS

The results of all determinations and assays are shown in Tables 1-7.

Table 1. The table shows the results of quantitative phytochemical composition of the crude aqueous extract (CAE) of leaves of *Moringa oleifera*. Values in the table are expressed in mg/g of sample

Phytochemical	Flavonoid	Alkaloid	Saponin	Tannin	Phenol
Concentration(mg/g)	0.04±0.00	4.0±0.01	8.0±0.12	0.65±0.20	1.10±0.10

The values in the table are the Mean±SD from triplicate determinations

Table 2. The table depicts the proximate composition of moringa leaves, showing the various parameters assayed. Values are expressed in mg/g of sample

Parameters	Moisture	Crude protein	Carbohydrate	Crude fiber	Ash	Crude fat	pH
Conc.(mg/g)	6.0±1.2	28.0±1.0	48.10±0.11	10.05±0.01	9.30±0.01	2.50±0.00	6.10±0.00

The values in the table above are the Mean±SD from triplicate determinations

Table 3. Shows the total free amino acid concentrations of various fractions of the sample. The parameters assayed are shown in the table. Values are expressed in mg/100 g of sample

Sample/fraction	Conc.mg/ml	Volume of extract	Dilution factor	Total volume	Total free a.a mg/50g	Total free a.amg/100 g
FAS	7.513	5.0	6.0	30.0	225.39	450.78
BUS	0.053	54.0	2.0	108.0	5.72	11.44
WAS	0.622	20.0	2.0	40.0	24.88	49.76

Total free amino acid conc. for FAS + BUS+ WAS 511.98 mg/100g

Table 4. Shows the various amino acids and their concentrations identified by TSM (technicon sequential multi-sample (amino acid) analyzer

Amino acid	Symbol	Concentration (g/100g)
Lysine	Lys	4.13
Histidine	His	2.00
Arginine	Arg	5.80
Tryptophan	Trp	1.70
Methionine	Met	1.60
Threonine	Thr	4.70
Leucine	Leu	8.70
Isoleucine	Ile	6.00
Valine	Val	6.80
Phenylalanine	Phe	6.20

Table 5. Summarizes the total ascorbic acid concentration of the CAE of the leaves, values are expressed in mg/100 g except volumes that are expressed in milliliters (ml)

Fraction	Volume(ml)	Dilution factor	Vit. C (mg/ml)	Vit C (mg/50g)	Vit. C (mg/100g)
CAE	47.0	10.0	2.0856	980.232	1960.464

Table 6. Is a table of hemoglobin polymerization inhibition experiment showing the rates of polymerization, the relative percent polymerization and the relative percent inhibition of HbSS by the fractions of the sample at 5.0 µM Phe equivalence

Sample	Fraction	Final Assay Conc. µM	Rate of polymerization	Relative % polymerization	Relative % Inhibition
<i>Moringa oleifera</i> leaves	Control	-----	0.0079	100.00	0.00
	L-Phe	4.85	0.00010 ^a	1.50 ^a	98.50 ^a
	WAS	4.85	0.00087 ^b	11.20 ^b	88.80 ^b
	FAS	4.85	0.00013 ^a	1.65 ^a	98.35 ^a
	BUS	4.85	0.00030 ^a	4.18 ^a	95.82 ^a

Values with the same superscript within the rows and columns are statistically the same at p≤0.05

Table 7. Shows the *In vitro* effect of FAS, BUS and WAS fractions of sample on Fe²⁺/Fe³⁺ ratio of HbSS blood at 4.85µM Phe equivalence

Sample	Fraction	Final assay conc. µM	%Hb	%mHb	Fe ²⁺ /Fe ³⁺	% increase
HbSS blood	Control	-----	88.0	12.0	7.3	0.00
Vitamin C	AQ	4.85	92.0	8.0	11.5	57.53
<i>Moringa</i>	WAS	4.85	89.0	11.0	8.1	10.97
<i>oleifera</i>	FAS	4.85	90.0	10.0	9.0	20.34
	BUS	4.85	89.0	11.0	8.1	10.97

4. DISCUSSION

The results of all analyses are shown in Tables 1-7. In Table 1, the result of quantitative phytochemical analysis is displayed showing the presence of alkaloids (4.0±0.12mg/g), saponins (8.0±1.0 mg/g), flavonoid (0.40±0.0mg/g) and phenols (1.10±0.10). It has been reported that the antisickling activity of any medicinal plant is linked to the phytochemicals present in the plant, most of which are antioxidants [25]. For example, 1-Hydroxybenzoic acid, an alkaloid, isolated from *Fagara xanthoxyloides* is a known antisickling agent. *Moringa oleifera* leaves have been reported to contain some organic acids which possess mild antisickling activity [26,27]. Apart from phytochemicals and antioxidants, the plant has been reported to contain over 46 antioxidants and 36 anti-inflammatory compounds. The presence of flavonoids in most medicines and extracts has been linked to their synergistic effect with other drugs. Saponins have equally been found to possess stimulating effect both in vaccines and extracts [28]. The high preponderance of phytochemicals in the leaves of this plant may be responsible for the antisickling activity of the extracts. Table 2 shows the proximate composition of the leaves. The moisture content of 6.0±1.2 mg/g, shows that the plant leaves as juicy and hence, used as vegetable for preparing soups and sauce by some consumers. The protein content of 28.0±0.02 mg/g is remarkable. It can serve as a rich source of protein in nutrition comparable to those of leguminous seeds of two varieties of *Vigna unguiculata*; the carbohydrate content of 48.10±0.12 mg/g, shows that the vegetable is a source of energy and can provide high calories in diets. No wonder the plant is often referred to as a miracle plant. Others include, crude fat, crude fiber and Ash; all having outstanding roles in nutrition. The free amino acid concentration of the leaves is shown in Table 3. Values in the table reflect the actual role of the plant as a rich protein source. This value corroborates with the data in Table 2 on proximate analysis, showing

high crude protein value. Table 4 shows the amino acid content of the leaves, identified by Amino Acid Analyzer. The following antisickling amino acids were identified with high concentrations- Lysine(4.13 g/100g), Histidine (2.00 g/100g), Arginine, one of the most potent antisickling amino acids (5.8 g/100g), which has been implicated in the production of NO(nitric oxide); L-arginine is a vasodilator, utilized in the treatment of pulmonary hypertension in SCD patients[19] and Phenylalanine (6.2 g/100g), is predominant antisickling amino acid both *in vitro* and *in vivo* [10]. Some of these amino acids such as Histidine, Alanine, Arginine, Tryptophan, possess the triple role of being anti-inflammatory, antioxidant and antisickling [17,25,26].

Table 5 shows the vitamin C concentration of the leaves, which is very outstanding. It also shows the antioxidant status of the leaves. Sick cell disease is a stress loaded problem and requires potent antioxidants which scavenge free radical generated in situ. Vitamin C in various *in vitro* experiments has exhibited high antisickling effectiveness by inhibiting sickle cell hemoglobin polymerization and the improvement of Fe²⁺/Fe³⁺ratio [17,29]. Many studies have validated the combination of some micronutrients like Zinc and an antioxidant Vitamin C as antisickling agent with therapeutic application in the management of sickle cell disease [29,30]. Many workers reported on the antisickling role of *Moringa oleifera* seed and flower extracts and in combination with aged garlic extract (AGE), *Mormadica charratia* and other extracts from Curcubits [31]. Table 6 shows the rate of polymerization and the percent inhibition of HbSS polymerization by various fractions of the leafy sample. When compared with Phenylalanine, a standard antisickling amino acid; the WAS, the FAS and BUS fractions showed remarkable antisickling effectiveness by inhibiting the process to varying percentages of 88.80%,98.35% and 95.82% respectively. It is quite outstanding that *Moringa oleifera* leaves extracts can inhibit sickle cell hemoglobin polymerization effectively. The antisickling

effectiveness of Moringa leaf extracts is comparable to those of the Curcubits [31]. Table 7 shows the *in vitro* effects of the various fractions on Fe²⁺/Fe³⁺ ratio of HbSS blood. The extracts were able to improve the oxygen affinity of the erythrocytes and equally reversed the sickling or gelation process. Improvement in oxygen affinity is a positive indicator of antisickling effectiveness. Also the antioxidant role of *Moringa oleifera* leaf extract had been found to mitigate in memory impairment and in Neurodegenerative disorders [32]. There is no doubt that with the preponderance of antisickling amino acids, the high vitamin C concentration, polyphenols, flavonoids, tannins, alkaloids and other phytochemicals [33,34,35]; Moringa leaves extracts when properly formulated, may be a promising therapeutic protocol for the effective management of sickle cell disease and also, a positive indicator in the nutritional approach to the management of the syndrome.

5. CONCLUSION

The research work epitomized and revealed the medicinal and nutritious use of the leaves of *Moringa oleifera*. Based on various assays carried out, the leaves extracts were able to inhibit sickle cell hemoglobin polymerization, improve the oxidant status of erythrocytes by increasing the Fe²⁺/Fe³⁺ ratio. The preponderance and the high concentrations of amino acids, some of which have previously been shown to be antisickling, the high concentration of Ascorbic acid, the proximate and phytochemical compositions; all point to the fact that the plant really possesses nutritional and medicinal benefits, which can be therapeutically beneficial in the management of sickle cell disease(SCD).

ETHICAL APPROVAL

All authors hereby declare that the approval for the use of human blood to generate hemoglobin for the research was approved by two ethical committees. Firstly, the ethical committee of the federal medical centre, Owerri in collaboration with its Hematology Unit, got the expressed consent of sickle cell disease patients who attend clinic at the centre. Finally, the federal University of Technology, Owerri, ethics committee on the use of animal/human specimens for research, granted the authors the permission to use sickle cell blood. All experiments were examined and approved by the university ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Atuga KI. Novel therapies in sickle cell disease. Hematology. 2009;1:54-64.
2. Eaton WA, Hofrichter J. Sickle cell hemoglobin polymerization. Advance Protein Chemistry. 1990;40:63-279.
3. Steinberg MH. Management of sickle cell disease. New England Journal of Medicine. 1990;340:1021-1030.
4. Ballas SK, Smith ED. Red blood cell changes during the evolution of sickle cell painful crisis. Blood. 1992;79:2154-2163.
5. Wanbebe C, Khamofo H, Momoh JA. Double blind placebo controlled, randomized crossover clinical trial of NIPPRISAN in patients with sickle cell disease. Phytomedicine. 2001;8(40):252-261.
6. Segun F, Odukova AO, Moody OJ. Management of sickle cell in Nigeria with medicinal plants: Cationic evaluation of extracts on the efficacy. Journal of Biological Sciences. 2006;6(1):100-102.
7. Oduola T, Adeniyi FAA, Ogunyemi EO, Bello IS. An antisickling agent in an extract of unripe pawpaw. Is it real?. African Journal of Biotechnology. 2006;5(20):1947-1949.
8. Glader BE, Nathan DG. Cation permeability alterations during sickling relationship to cation composition and cellular hydration of irreversibly sickled cells. Blood. 1978;51:983-989.

9. Ekeke GI, Uwakwe AA and Nwaoguikpe RN. Edible legumes as nutritionally beneficial antisickling agents. Nigerian Journal of Biochemistry and Molecular Biology, 2001;16:31-35.
10. Adeleke AD. Hemoglobinopathies. In paediatrics and child health in a tropical region. Azubuike JC and Nkanginieme KED (eds.) African Educational Services, Owerri, Nigeria. 1999;194-197.
11. Ekeke GI, Shode FO. Phenylalanine is the predominant antisickling agent in *Cajanus cajan* seed extract. Plant Medica. 1990;56:41-43.
12. Bosch CH. *Moringa stenopetala* (Baker F) [Internet]. Record from Protabase Grubben,GJH and Denton,OA(eds).PROTA(Plant Resources of Tropical Africa/Resources. Wageningen, Netherlands; 2004
13. Abubakar AM, Nur ASP, Palanisamy A, Faridah A, Shanda F. *In vitro* wound healing effects of *Moringa oleifera*. Biomed Research International; 2013.
14. Rath BS, Bodham Kar SL, Bahati AM. Evaluation of aqueous leaves extract of *Moringa oleifera* Lam for wound healing in albino rats. Indian Journal of Exptal. Biology. 2006;44(11):898-901.
15. Fakarazi S, Sharifudin SA, Arulselvan P. *Moringa oleifera* hydroethanolic extracts effectively alleviate acetaminophen induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules. 2012;17(7):8334-8350.
16. Nwaoguikpe RN, W Braide, GN Okwu, Anyanwu BO, Ujowundu CO. Effects of extracts of Christmas Bush (*Alchornea cordifolia*) on sickle cell Hemoglobin. British Journal of Pharmaceutical Research. 2014;4(1):158-171.
17. Nwaoguikpe RN, Ekeke GI, Uwakwe AA. The effects of extracts of some foodstuff on Lactate Dehydrogenase activity and hemoglobin polymerization of sickle cell blood (HbSS). Ph D thesis, University of Port-Harcourt, Nigeria; 1999.
18. AOAC. Official methods of analysis of the association of analytical chemists, 15th edition, Washington DC,USA; 1990.
19. Harborne JB. Phytochemical methods. A guide to modern technique of plant analysis, Chapman and Hull, London; 1983.
20. Speckman DH, Stein EH, Moore S. Automatic recording apparatus for use in the chromatography of amino acids. Analytical Chemistry. 1958;30:1191.
21. Lambert J, Muir TA. Estimation of Vitamin C .In Practical Chemistry, 3rdedn. Heinman Publishers International Books, London; 1974.
22. Iwu MN, Igboko AO, Onwubiko H and Ndu UE. Effects of *Cajanus cajan* on gelation kinetics and oxygen affinity of sickle cell hemoglobin. Journal Ethnopharmacology. 1988;20:99-104.
23. Noguchi CT, Schetcher AN. The inhibition of sickle cell hemoglobin polymerization by amino acids and related compounds. Biochemistry. 1978;17:5455-5459.
24. Davidson J, Henry JB. Clinical Diagnostics by Laboratory methods. Todd-Sanford, W.B. Saunders, Philadelphia Chemistry (Tietz N., ed.), W.B. Saunders CO, Philadelphia; 1974.
25. Ohnishi ST, Ohnishi T, Ogunmola GB. Sickle cell anemia: A potential nutritional approach for a molecular disease. Nutrition. 2000;16:330-338.
26. Sofowara EA, Isaac-Sodeye WA, Ogunkoya LO. Isolation and characterization of an antisickling agent. In Sofowara EA (editor). African Medicinal Plants; 1979.
27. Gopalan BV, Rama S. Anti-inflammatory and Antioxidant properties of leaves of *Moringa oleifera*. International Journal of Pharmacognosy. 1996;34:207-212.
28. Bamishaiye EI, Olayemi FF, Awagu EF, Bamishaiye OM. Proximate and Phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. Advanced Journal of Food Science and Technology. 2011;3(4):233-237.
29. Lachant NA, Tanaka KR. Antioxidants in sickle cell disease: The *In vitro* effects of ascorbic acid. American Journal of Medical Sci. 1986;292:3-10.
30. Nwaoguikpe RN, Ejele EA. Amino acid profile of some indigenous antisickling plant extracts and their hemoglobin polymerization inhibition .Nigerian Journal of Biochemistry and Molecular Biology. 2010;25(2):53-59.
31. Mahuys S, Dipesh Kr Das, Sanjukta D, Santinath G, Sanjit D. Amelioration of Ionizing radiation induced lipid peroxidation in mouse liver by *Moringa oleifera* Lam leaf extract. Indian Journal of Experimental Biology. 2012;50:209-215.

32. Chatchada S, Jintanaporn W, Supraporn M, Wepawee T. *Moringa oleifera* mitigates memory impairment and Neurodegeneration in animal model of age-related dementia. *Oxidative Medicine and Cellular Longevity*. 2013;(2013). Article ID695936-45.
33. Ijeoma AU, Ugwuona FU, Abdullahi H. Phytochemical Composition and Antioxidant Properties of *Hibiscus sabdariffa* and *Moringa oleifera*. *Nigerian Journal of Agriculture and Environment*. 2012;8(1):10-16.
34. Nweze EL, Okafor JL, Njoku O. Methanolic extract of *Treme guinensis* (Schum and Thonn) and *Moringa oleifera* used in Nigerian Herbal Practices. *Biological Review*. 2004;21:39-48.
35. Olufinmilayo EA, Adelodun I, Kolapo L, Kintomuwa A. *Moringa oleifera* Lam (Moringaceae) grown in Nigeria. *In vitro* antisickling activities in red blood cells. *Journal Pharm. and Allied Sciences*. 2012;4(2):118-122.

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