



(RESEARCH ARTICLE)



## Proximate analysis, phytochemical content and the antioxidant effect of *Moringa oleifera* leaf methanol extract in sickle cell disease management

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International Journal of Science and Research Archive, 2025, 16(01), 334-348

Publication history: Received on 26 May 2025; revised on 30 June 2025; accepted on 03 July 2025

Article DOI: <https://doi.org/10.30574/ijrsra.2025.16.1.1963>

### Abstract

The proximate analysis, phytochemical and antioxidant effect of *Moringa oleifera* leaf methanol extract were investigated and their effects on sickle cell hemoglobin polymerization observed. *Moringa oleifera* leaf was sourced from a horticulturist in a horticulture garden along Okigwe road in Owerri, Imo State, Nigeria. The voucher specimen for *Moringa oleifera* was identified, classified and authenticated by a professional taxonomist at the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, Imo State University Owerri, Imo State. The sample was first dried at room temperature to constant weight, ground into powder and soaked in methanol for 24 hours to obtain the methanol soluble fraction. Amino acid composition was determined for the sample, using gas chromatography which revealed a preponderance of both essential and non-essential amino acids of which some were antisickling amino acids of varying concentrations. All values were expressed in g/100 g of sample. The identified amino acids include: Phenylalanine, Histidine, Leucine, Lysine, Tryptophan, Isoleucine, Valine, Methionine, Proline, Arginine, Tyrosine, Serine and others. The different concentration of *Moringa oleifera* leaf methanol extracts were able to inhibit HbSS polymerization at varying degrees, when compare to the normal control and Ciklavit™, which was used as a standard. The results of the percentage polymerization inhibition showed that 100 mg/dose, 200 mg/dose and 400 mg/dose of a *Moringa oleifera* leaf methanol extracts inhibited sickle cell polymerization by  $53.09 \pm 1.00$ ,  $53.09 \pm 1.02$  and  $50.62 \pm 0.66$  respectively. *Moringa oleifera* leaf methanol extracts is nutritionally rich in both essential and non-essential amino acids, phytochemicals and antioxidants enzymes and vitamins, that may possess anti-oxidative properties that may have contributed in no small measure to the high antisickling profile of the extract especially in terms of maintaining a healthy  $Fe^{2+}/Fe^{3+}$  ratio and reduction in hemoglobin polymerization from 61.73 % (Ciklavit™) standard to 46.9185 % (100 mg/dose) *Moringa oleifera* leaf methanol extracts. *Moringa oleifera* leaf should be employed in the management of sickle cell disease and other related diseases like anemia and thalassemia.

**Keywords:** Sickle Cell Disease; Amino Acids; Phytochemicals; *Moringa oleifera*; Hemoglobin Polymerization;  $Fe^{2+}/Fe^{3+}$  Ratio

### 1. Introduction

Sickle cell disease, also known as sickle cell anaemia, is a genetic blood disorder from a point mutation in the beta-globin chain, leading to the replacement of the amino acid glutamic acid, a hydrophilic moiety by valine, a hydrophobic moiety, at the sixth position of the beta-chain of haemoglobin (Nwaoguikpe, Obiekwe, & Emejulu, 2023). Sickle cell disease refers to a large group of haemoglobinopathies, in which at least one sickle cell gene of the beta-globin chain is inherited together with abnormal gene. It is a severely malignant disorder associated with protean clinical manifestations and decreased life expectancy. The loss of charge on the glutamic acid and its substitution by valine, resulted in an abnormal

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haemoglobin molecule with severe haematological consequences (Azubuike and Nkanginema, 1999; Denise, 1992). Sick cell haemoglobin (HbS) is a structural variant of normal adult haemoglobin (HbA) (Chakravorty & Williams, 2015). SCD affects 20–25 million people globally, and 50–80 % of infants born with SCD in Africa die before the age of 5 years (Aygun & Odame, 2012). It is estimated that 240,000 children are born with SCD annually in sub-Saharan Africa (Makani et al., 2011). The United Nations General Assembly has recognized SCD as a global public health concern due to the morbidity and mortality caused by the disease and the significant social and economic impact that results. Most sickle cells possess the ability to revert to the discocyte shape when oxygenated, but some cannot revert to their earlier morphology and are referred to as “irreversible sickle cell” (ISC). These irreversibly sickled cells are the result of recurrent sickling episodes (Nwaoguikpe et al., 2010).

Hemoglobin is an oxygen-binding protein found in erythrocytes which transports oxygen from the lungs to tissues. Each hemoglobin molecule is a tetramer made of four polypeptide globin chains. Each globin subunit contains a haeme moiety formed of an organic protoporphyrin ring and a central iron ion in the ferrous state ( $Fe^{2+}$ ). The iron molecule in each haeme moiety can bind and unbind oxygen, allowing for oxygen transport in the body. The most common type of hemoglobin in the adult is HbA, which comprises two alpha-globin and two beta-globin subunits. Different globin genes encode each type of globin subunit (Hafen, and Sharma, 2019, Yostina, and Paul, 2019).

Amino acid has a well-documented role in health and nutrition. They are known to be building blocks of macromolecules like; proteins, peptides, glycoproteins, immunoglobulin and hormones (Nelson and Cox, 2017). Amino acids are classified as essential and non-essential based on their relevance in physiology and human nutrition. The essential amino acids are those that cannot be synthesized in vivo and must be obtained from the diet. The essential amino acids are: Arginine, valine, histidine, isoleucine, leucine, lysine, threonine, methionine, phenylalanine, and tryptophan. The non-essential amino acids are those synthesized by the body from various metabolites and precursors in the human system. Recent research findings have shown tremendous free amino acid content in protein concentration of small edible plants (Nwaoguikpe et al., 2008; Nwaoguikpe, 2009). Aromatic amino acid such as phenylalanine, tryptophan and tyrosine as well as other amino acids like arginine, serine, glutamine and lysine have shown to be very efficacious in the management of sickle cell diseases (Ekeke and Shode, 1990; Nwaoguikpe, 1999; Uwakwe and Nwaoguikpe, 2005). The amino acid phenylalanine has been found to possess remarkable antisickling effects and the ability to reverse already sickled erythrocytes even at very low concentration. The role of amino acids in nutrition and in the management of sickle cell disease remains very remarkable. That a health issue resulting from amino acid substitution in the globin chain can be effectively managed with amino acid extraneously administered from the amino acid and protein rich foods remain an interesting area for research. The main objective of this work is to demonstrate the proximate analysis, phytochemical content and the antioxidant effect of *Moringa oleifera* leaf methanol extracts in the management of sickle cell disease with respect to their hemoglobin polymerization inhibition effects, phytochemical and antioxidant content and their ability to improve  $Fe^{2+}/Fe^{3+}$  ratio.

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## 2. Materials and methods

### 2.1. Materials

Digital UV-Visible Spectrophotometer model 752N, analytical weighing balance- Kern PT 320 (Mettler-Wagen, Switzerland), Water bath (Grant, England), Bench centrifuge (Search Tech, China), Automatic pipettes (Teco diagnostics, USA), Digital pH meter (Labtech India), and Deep freezer (Hisense, H250), test tubes, separating funnel, BUCK M910 Gas chromatography, rotary evaporator, Applied Biosystem phenylthiohydantoin (PTH) amino acid analyzer (model 120A), Whatman filter paper (No1), Kjeldhal digestion flask, volumetric flask, Markham distillation apparatus, Bunsen burner, Oven, plastic specimen bottles, Extraction thimble, Anti-bumping granules ETC was used for this study.

### 2.2. Reagents

The kits employed in the study will include: ALT test kit (Randox, UK), AST test kit (Randox, UK), Alkaline phosphatase test kit (Randox, UK), Bilirubin test kit (Biosystems, Spain), Urea test kit (Biosystems, Spain), Sodium test kit (TECO Diagnostic, USA),

Potassium test kit, Creatinine test kit (Biosystems, Spain), Chloride test kit (TECO Diagnostic, USA), Bicarbonate test kit (TECO Diagnostic, USA), hexane, butanol, chloroform, methanol, ethanol, sodium sulphate, concentrated sulphuric acid, copper sulphate, selenium oxide, distilled water, sodium hydroxide, boric acid, bromocresol green/methyl red, hydrochloric acid, acetate buffer (pH 2.0), sodium chloride. Buthanol, methanol, ethanol, and hydrochloric acid, of

analytical grades, was sourced from Finlab Chemicals limited in Owerri, Imo State. Concentrated sulphuric acid, copper sulphate, selenium oxide, sodium hydroxide, boric acid,

Sodium metabisulphite and methyl red, of analytical grades, was sourced from Finlab Chemicals limited and Chemi-Science both in Owerri, Imo State. Twin-80 was sourced from the department of Biochemistry, Federal Polytechnic Nekede, Imo State. All other chemicals and reagents to be used were of analytical grade.

### 2.3. Sampling and sample preparation

Sample collection is an essential element for good laboratory practice. The sample container was washed thoroughly with detergent, rinsed with water and then with distilled water and air-dried. The air-dried plastic containers were covered with air tight covers in other to avoid contamination of the samples. *Moringa oleifera* leaf was sourced from a horticulturist in a horticulture garden along Okigwe road in Owerri, Imo State, Nigeria.

Methanol, ethanol, and hydrochloric acid, were sourced from Finlab Chemicals Limited in Owerri, Imo State. One hundred grams (100 g) of *Moringa oleifera* leaf was collected, dried and ground to powder using a grinder. The powdered sample was put in an airtight container and labelled.

### 2.4. Collection and preparation of blood samples

Blood samples were collected from confirmed HbSS patients who attend clinic at Federal University Teaching Hospital, Owerri by a personnel member of the Hematology Units. The patients willingly consented to the exercise after having explained to them the relevance of the research project to their health. The blood was collected in an ethylenediaminetetraacetic acid (EDTA) bottle to prevent the blood from coagulation.

Portions (0.20 ml) of the whole blood samples were used for the Fe<sup>2+</sup>/Fe<sup>3+</sup> ratio, while the remaining portions were collected into EDTA anticoagulant tubes. Erythrocytes were isolated from the blood samples by centrifugation at 10,000 rpm for 20minutes 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 siphoned plasma. The erythrocyte suspension was then frozen at 0oC, and subsequently thawed to produce a haemolysate for the haemoglobin polymerization experiment.

### 2.5. Methanol Extraction Process

One hundred grams (100 g) of the powdered sample of *Moringa oleifera* leaf was soaked in 200 ml of methanol (MeOH) of analytical grade for 24 hours. The solvent was filtered and the filtrate subjected to evaporation en-vacuo to concentrate the filtrate. The weight and volume of the methanol extract was recorded.

## 3. Proximate Analysis of the sample

### 3.1. Moisture Content (AOAC 1990) Procedure

Approximately 1-2 g of the sample was weighed into petri dish. The weight of the petri dish and sample was noted before drying. The petridish and sample was put in the oven and heated at 105°C for 2 hr. the result noted and heated another 1 hr. until a steady result was obtained and the weight was noted. The drying procedure continued until a constant weight was obtained.

$$\% \text{ moisture content} = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100 \dots \dots \text{Eqn. 1}$$

Where W1 = weight of petridish and sample before drying

W2 = weigh of petridish and sample after drying.

Ash content (AOAC, 1990)

#### 3.1.1. Principle

The ash of foodstuff is the inorganic residue remaining after the organic matter has been burnt away. It should be noted, however, that the ash obtained is not necessarily of the composition as there may be some from volatilization.

### 3.1.2. Procedures

Empty platinum crucible was washed, dried and the weighted. Approximately 1- 2 g of sample was weighed into the platinum crucible and placed in a muffle furnace at 550°C for 3 hours. The sample was cooled in a desiccator after burning and weighed.

Calculations

$$\% \text{ Ash content} = \frac{w_3 - w_1}{w_2 - w_1} \times 100 \dots \dots \text{Eqn. 2}$$

Where

W1 = weight of empty platinum crucible

W2 = weight of platinum crucible and sample before burning

W3 = weight of platinum and ash.

## 3.2. Crude Fibre (AOAC 1990)

### 3.2.1. Procedure

Two grams (2 g) of the material was defatted with petroleum ether (if the fat content if more than 10 %). This was boiled under reflux for 30 minutes with 200ml of a solution containing 1.25 g of H<sub>2</sub>SO<sub>4</sub> per 100 ml of solution. The solution was filtered through linen and then washed with boiling water until the washings are no longer acid.

The residue was transferred to a beaker and boil for 30 minutes with 200 ml of a solution containing 1.25 g of carbonate free NaOH per 100 ml. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible. This was dried in an electric oven and weighed, incinerate, cool and weighed.

The loss in weight after incineration  $\times 100$  is the percentage of crude fibre

$$\% \text{ crude fibre} = \frac{\text{weight of fibre}}{\text{Weight of sample}} \times 100 \dots \dots \text{Eqn. 3}$$

## 3.3. Crude fat

### 3.3.1. Soxhlet fat extraction method

This method was carried out by continuously extracting a food with non- polar organic solvent such as petroleum ether for about 1 hour or more.

### 3.3.2. Procedure

Two hundred and fifty milliliters (250 ml) clean boiling flasks in oven at 105 – 110 °C for about 30 minutes, was transferred into a desiccator and allow to cool. the cooled boiling flasks was labelled and weighed. The boiling flasks were filled with about 300ml of petroleum ether (boiling point 40 – 60 °C). The extraction thimble was plugged lightly with cotton wool, and the Soxhlet apparatus was assembled and allowed to reflux for about six (6) hours. The thimble was removed with care, the petroleum ether in the top container of the set was collected and drained into a container for reuse. When flask is almost free of petroleum ether, it was removed and dry at 105 °C - 110 °C for 1hour, and transferred from the oven into a desiccator and allow to cool; then weighed.

$$\% \text{ fat} = \frac{\text{Weight of flask + oil} - \text{weight of flask}}{\text{Weight of sample}} \dots \dots \text{Eqn. 4}$$

### 3.4. Crude proteins (AOAC, 1990)

#### 3.4.1. Principle

The method is the digestion of sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia. This is retained in the solution as ammonium sulphate. The solution was made alkaline, and then distilled to release the ammonia. The ammonia was trapped in dilute acid and then titrated.

#### 3.4.2. Procedure

Zero point five (0.5 g) of sample was weighed into a 30 ml Kjeldhal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stoppered and shaken. Then 0.5 g of the Kjeldhal catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appears.

The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling, was made up to 100 ml with distilled water added to avoid caking and then 5ml was transferred to the Kjeldhal distillation apparatus, followed by 5ml of 40% sodium hydroxide. One hundred milliliter (100 ml) receiver flask containing 5 ml of 2 % boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methylene blue was added under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution and distillation will commence immediately until 50 drops will get into the receiver flask, after which it was titrated to pink color using 0.01 N hydrochloric acid.

Calculations

$$\% \text{ Nitrogen} = \text{Titre value} \times 0.01 \times 14 \times 4 \dots \dots \text{Eqn. 5}$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25 \dots \dots \text{Eqn. 6}$$

Carbohydrate determination

(Differential method)

$$100 - (\% \text{Protein} + \% \text{Moisture} + \% \text{Ash} + \% \text{Fat} + \% \text{Fibre}) \dots \dots \text{Eqn. 7}$$

## 4. Phytochemical analysis

### 4.1. Phytochemical quantitative and qualitative analysis

The phytochemical qualitative and quantitative analysis by gas chromatography as described by Kelly et al., (2014).

#### 4.1.1. Principle

When the vapor of a sample mixture moves between the stationary phase (liquid) and the mobile phase (gas), the different component of the sample mixture will separate according to their partition coefficient between the gas and liquid stationary phase.

$$\text{Partition coefficient (kg)} = \frac{\text{Concentration of solution in liquid(w/cc)}}{\text{Concentration of solute in gas(w/cc)}} \dots \dots \text{Eqn. 8}$$

### 4.2. Extraction of phytochemicals (*Moringa oleifera*)

*Moringa oleifera* leaf sample (1 g) was weighed and transferred in a test tube and 15 ml of methanol added and 10 ml of 50 %m/v potassium hydroxide was also added. The test tube was allowed to react in a water bath at 60°C for 60 minutes. After the reaction time, the reaction product contained in the test tube was transferred to a separating funnel. The tube was washed successfully with 20 ml of methanol, 10 ml of cold water and 3 ml of hexane, was transferred to the funnel. This extract was combined and washed three times with 10ml of 10%v/v methanol aqueous solution. The solution as dried with anhydrous sodium sulphate, and the solvent was evaporated. The sample was solubilized in 1000 µl of hexane of which 200 µl was transferred to a vial for analysis.

### 4.3. Quantification by gas chromatography (GC-FID)

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15 m x 250  $\mu\text{m}$  x 0.15  $\mu\text{m}$ ) was used. The injector temperature was 280 °C with splitless injection of 2  $\mu\text{l}$  of sample and a linear velocity of 30  $\text{cm s}^{-1}$ . Helium 5.0 pa. s was the carrier gas with a flow rate of 40ml/min or 40mlmin<sup>1</sup>. The oven operated initially at 200 °C, it was heated to 330 °C at a rate of 3 °C min<sup>-1</sup> and was kept at this temperature for 5 minutes. The detector operated at a temperature of 320 °C. phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals is expressed in  $\mu\text{g/g}$ .

### 4.4. Determination antioxidant vitamins

#### 4.4.1. Determination of vitamin A

Determination of vitamin A was carried out by the method described by Delia and Mieko, (2003).

Five grams (5 g) of the sample was homogenized using acetone 30 ml each, with the aid of pestle and mortar. The solution was filtered after washing. The filtrate was then extracted three times with 250 ml of petroleum ether, using separating funnel. Two layer of both aqueous and solvent were obtained. The upper layer which contains the vitamin A was washed very well with distilled water, in other to removed residual water and was then transferred into a 50 ml volumetric flask through a separating funnel, and made up to the mark with petroleum ether. The absorbance of the solution was read using spectrophotometer at wavelength of 450 nm.

#### 4.4.2. Determination of vitamin C

The determination of the ascorbic acid (vitamin C) concentration of the extract was carried out by the methods of Lambert and Muir, (1974).

#### 4.4.3. Principle

This method is based on the measurement of the extent to which the indophenol dye is decolorized by ascorbic acid in biological fluids. Since reduction of the dye is instantaneous but reduction of dye with interfering reducing substance is slow, the decrease in color intensity with time is determined. This permit correction for reduction of the dye by substance other than ascorbic acid. This method estimates the reduced form of vitamin C.

Procedure. Ascorbic acid standard was prepared containing 1  $\text{g/dm}^3$  of the ascorbic acid (vitamin C), so that 1 $\text{cm}^3$ /mg vitamin C equals 1  $\text{g/dm}^3$ . A burette was filled with a solution of 2,6dichlorophenolindophenol of 0.01 %. Two or three drops of dilute hydrochloric acid (HCl), was used to acidify 10  $\text{cm}^3$  of the ascorbic acid. The indophenol solution was run into the ascorbic acid solution until there is a permanent pink solution. If x $\text{cm}^3$  of the indophenol are required, 1 $\text{cm}^3$  of indophenol solution is equivalent to 10  $\text{mg/x}$  vitamin C. Having standardized the indophenol solution, 10  $\text{cm}^3$  of the test solution (extract) was taken and treated in a similar way.

#### 4.4.4. Determination of vitamin E

Vitamin E was determined as described earlier by Amadi et al., (2012). One gram (1 g) of the sample was weighed into a conical flask with reflux condenser. 10 ml of absolute alcohol and 20 ml of 1M alcoholic sulphuric acid was added. The condenser and conical flask were wrapped in aluminum foil and refluxed for

45 minutes after cooling. 50 ml of distilled water was added to the mixture and transferred to separating funnel with 50 ml water. The unsaponifiable matter was extracted with 30 ml diethyl ether. The combined ether extract was washed free from acid and was dried over anhydrous sodium tetraoxosulphate (VI) acid. The residue obtained was immediately dissolved in 10 ml absolute alcohol. Aliquots of solutions of the sample and standards (0.3-3.0 mg vitamin E) were transferred into 20 ml volumetric flasks; 5 ml alcohol was added, followed by 1ml  $\text{HNO}_3$  concentrated Trioxo-nitrate (V) acid. The flasks were placed on a water bath at 90 °C for 3 minutes from the time the alcohol begins to boil. It was cooled rapidly under running water and adjusted to volume with absolute alcohol. The absorbance was read at 470 nm on a metrohm spectronic 21D spectrophotometer, against a blank containing 5 ml absolute alcohol and 1ml concentrated  $\text{HNO}_3$  treated in a similar manner.

$$\text{Vitamin E} \left( \mu \frac{\text{g}}{100\text{g}} \right) = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dil.Factor}}{\text{Weight of sample}} \dots \dots \text{Eqn. 10}$$

## 5. Determination of antioxidant effects

### 5.1. Determination of Catalase (*Moringa oleifera*)

Catalase activity was assayed following the method of Luck, (1974).

#### 5.1.1. Principle

The UV absorption of hydrogen peroxide can be measured at 240nm, whose absorbance decreased when degraded by enzyme catalase. From the decrease in absorbance, the enzyme activity can be calculated.

#### 5.1.2. Reagents

Phosphate buffer; 0.067 M (pH 7.0), hydrogen peroxide (2 mM) in phosphate buffer

#### 5.1.3. Procedure

##### PREPARATION OF ENZYME EXTRACT

A 20 % homogenate of the sample *Moringa oleifera* leaves was prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay.

##### ASSAY

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) phosphate buffer (3.0ml) was taken in an experimental cuvette, followed by the rapid addition of 40 µl of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm in a spectrophotometer (Genesys 10-S, USA). The enzyme solution containing H<sub>2</sub>O<sub>2</sub>- free phosphate buffer served as control.

One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

### 5.2. Determination of Peroxidase (*Moringa oleifera*)

The method proposed by Reddy et al., (1995) was adopted for assaying the activity of peroxidase.

#### 5.2.1. Principle

In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub>. The oxidation of pyrogallol or dianisidine to a colored product called purpurogalli can be followed spectrophotometrically at 430nm.

#### 5.2.2. Reagents

Pyrogallol; 0.05 M in 0.1 M phosphate buffer (pH 6.5), H<sub>2</sub>O<sub>2</sub>; 1 % in 0.1 M phosphate buffer, pH 6.5.

#### 5.2.3. Procedure

##### Preparation of enzyme extract

A 20 % homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from the various samples, clarified by centrifugation and the supernatant was used for the assay.

##### Assay

To 3.0 ml of pyrogallol solution, 0.1 ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5 ml of H<sub>2</sub>O<sub>2</sub> was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer (Genesys 10-S, USA). One unit of peroxidase is defined as the change in absorbance/minute at 430 nm.

### 5.3. Determination of the Fe<sup>2+</sup>/Fe<sup>3+</sup> Ratio

The determination of the Fe<sup>2+</sup>/Fe<sup>3+</sup> was carried out by method described by Davidson, et al. 1974, while oxygen affinity of haemoglobin and met-haemoglobin were measured at 540 nm and 630 nm respectively. The approach employs lysing 0.02 ml whole blood in 5.0ml of distilled water and 0.02ml normal saline. The absorbance of haemoglobin (Hb) and met-

haemoglobin (mHb) were measured at 540 nm and 630 nm to determine the % Hb and % mHb respectively. This represents the control. In determining the effect of the extract on Fe<sup>2+</sup>/Fe<sup>3+</sup> ratio; 0.02 ml of each extract was added to 5.0 ml of distilled water and 0.02 ml of blood added and incubated for 60 minutes in a test tube.

#### 5.4. Sickle Cell Haemoglobin Polymerization Inhibition Experiment

The sickle cell haemoglobin polymerization inhibition was carried out by the original method of Noguchi et al., (1978); Iwu et al., (1988) and Nwaoguikpe et al., (1999) used for HbSS polymerization experiment. This method is used to assess the sickle cell haemoglobin polymerization, by the turbidity of the polymerizing mixture at 700 nm, using 2 % solution of sodium matabisulphite as reductant or deoxygenating agent (Iwu, et al., 1988).

Portion 4.4 ml of 2 % sodium matabisulphite (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>), 0.5 ml normal saline (0.9 % NaCl), and 0.1 ml haemoglobin were pipetted into a cuvette, shaken and a spectrophotometer (Unicom Spectronic 20-DR) was used to read the absorbance at 700 nm, every 2 minutes for 30 minutes. This was used as the control, and distilled water used as blank for all assays. For the test assay, 4.4 ml of 2 % sodium matabisulphite, 0.5 ml of the extract (*Moringa oleifera*), and 0.1 ml haemoglobin (HbSS) solution were pipetted into the cuvette, and readings taken at absorbance of 700 nm every 2 minutes for 30 minutes. The rate of haemoglobin polymerization for the control, extracts or fractions was estimated by calculating the tangent of a plot of average change in extinction or change in optical density ( $\Delta OD_{700 \text{ nm}}$ ) versus time in minutes. The rate was equally expressed as percentages with respect to control.

The rate can be calculated from the formula,

$$R_p = \frac{\text{Initial OD}}{\text{Time. i. e. } R_p} = \frac{OD_f - OD_i}{T}$$

Where, R<sub>p</sub> = rate of polymerization, OD<sub>f</sub> = final optical density, OD<sub>i</sub> = initial optical density and T= time of assay in minutes.

#### 5.5. Determination of Alkaloids of the Extracts

The alkaloid content of *Moringa oleifera* was determined gravimetrically by Achi and Ohaeri, (2012).

##### 5.5.1. Plant Extract

The extraction method used in the study was maceration extraction method which involves simple soaking, filtering and then evaporations. 200 g of the ground plant material was weighed into a conical flask (Pyrex/England) and 1000 ml of methanol (BDH/England) was added, ensuring that the solvent properly cover the plant material. The mixture was allowed to stand for 48 hours with occasional stirring. After 48 hours the mixture was filtered using filter paper (Whatman/England) and the filtrate was evaporated in a water bath (50 °C, Gallenkamp/Germany) to obtain a jelly-like extract which was stored in a refrigerator (Haier Thermocool/England) for further use.

Five grams (5 g) of the sample was weighed into a 250 ml beaker (Pyrex/England), and 200 ml of 10% acetic acid (BDH/England) in ethanol (BDH/England) was added and then covered and allowed to stand for 4 hours. The solution was filtered, and the filtrate was concentrated in a water bath (Gallenkamp/Germany) to about three-quarter of the original volume. Dilute ammonia hydroxide (BDH/England) solution was added drop wise to the extract to precipitate the alkaloids. They're resulting solution was allowed to settle, and the precipitate was filtered and weighed using an analytic weighing balance (Metler/USA) to a constant mass (Achi and Ohaeri, 2012).

## 6. Results

Results are shown in tables 1 to 6

**Table 1** Proximate composition of *Moringa oleifera* leaf methanol extract

Parameter (%)	<i>Moringa oleifera</i>	t-value	p-value	Comment
Moisture	11.29 ± 0.09	44.37	0.0005	Significant
Fat	5.59 ± 0.02	145.10	<0.0001	Significant
Ash	7.30 ± 0.06	70.41	0.0002	Significant
Fibre	6.59 ± 0.04	22.45	0.0020	Significant
Protein	11.49 ± 0.08	13.19	0.0057	Significant
Carbohydrate	57.76 ± 0.05	144.20	<0.0001	Significant

Value are mean ± standard deviation of duplicate determination

**Table 2** Phenolic profile of *Moringa oleifera* leaf methanol extract

Parameter (mg/100 g)	<i>Moringa oleifera</i>	t-value	p-value	Comment
Artemetin	0.77 ± 0.04	52.89	0.0004	Significant
Retusin	0.73 ± 0.02	25.00	0.0016	Significant
Catechin	8.44 ± 0.02	60.82	0.0003	Significant
Ellagic acid	10.80 ± 0.05	289.00	<0.0001	Significant
Vanillic acid	2.31 ± 0.03	1.789	0.2155	Not Significant
Naringenin	0.70 ± 0.02	22.77	0.0019	Significant
Apigenin	0.98 ± 0.04	76.25	0.0002	Significant
Hesperidin	0.71 ± 0.01	67.04	0.0002	Significant
Isorhamnetin	2.56 ± 0.01	127.00	<0.0001	Significant
Maricetin	1.08 ± 0.03	36.22	0.0008	Significant
Epicatechin	1.87 ± 0.01	11.38	0.0076	Significant
Daidzein	1.11 ± 0.02	30.36	0.0011	Significant
Genistein	1.74 ± 0.02	39.21	0.0006	Significant
Lunamarin	0.47 ± 0.02	0.00	1.000	Not Significant
Gallocatechin	1.24 ± 0.03	40.70	0.0006	Significant
Resveratrol	2.60 ± 0.06	15.65	0.0041	Significant
Tangeretin	1.77 ± 0.02	29.00	0.0012	Significant
Naringin	1.37 ± 0.01	37.00	0.0007	Significant
Silymarin	85.82 ± 0.05	1202.00	<0.0001	Significant

Value are mean ± standard deviation of duplicate determination

**Table 3** Amino Acid composition of *Moringa oleifera* leaf methanol extract

Parameter (mg/100g)	<i>Moringa oleifera</i>	t-value	p-value	Comment
Threonine	18.69 ± 0.09	34.58	0.0008	Significant
Isoleucine	38.19 ± 0.06	181.30	<0.0001	Significant
Leucine	1.82 ± 0.01	4.400	0.0480	Significant
Aspartate	2.12 ± 0.04	39.44	0.0006	Significant
Lysine	4.91 ± 0.04	46.80	0.0005	Significant
Methionine	1.18 ± 0.01	12.07	0.0068	Significant
Glutamate	5.64 ± 0.05	63.83	0.0002	Significant
Phenylalanine	4.21 ± 0.05	70.64	0.0002	Significant
Histidine	2.07 ± 0.02	35.30	0.0008	Significant
Arginine	18.64 ± 0.09	136.10	<0.0001	Significant
Tyrosine	5.54 ± 0.04	48.93	0.0004	Significant
Tryptophan	7.42 ± 0.03	221.60	<0.0001	Significant
Cysteine	16.60 ± 0.07	234.40	<0.0001	Significant

Value are mean ± standard deviation of duplicate determination

**Table 4** Chemical content and antioxidant enzyme activity of *Moringa oleifera* leaf samples

Parameter	<i>Moringa oleifera</i>	t-value	p-value	Comment
Alkaloid (mg/g)	9.89 ± 0.19	26.12	0.0015	Significant
Catalase (IU/ml)	0.03 ± 0.00	1.000	0.4226	Not significant
Peroxidase (U/mg protein)	2.06 ± 0.22	6.450	0.0232	Significant
Vitamin E (mg/100g)	67.75 ± 3.38	13.37	0.0056	Significant
Vitamin A (µg/g)	1887.27 ± 4.15	41.74	0.0006	Significant
Vitamin C (mg/100g)	297.6 ± 1.60	159.70	<0.0001	Significant

Value are mean ± standard deviation of duplicate determination

**Table 5** The Fe<sup>2+</sup>/ Fe<sup>3+</sup> Ratios of sickle cell blood exposed to extract of *Moringa oleifera* leaf

Group	%Hb	%mHb
Normal saline	84.88 ± 1.09 <sup>de</sup>	15.11 ± 0.11 <sup>f</sup>
Ciklavit (Standard)	89.94 ± 1.02 <sup>d</sup>	10.06 ± 0.17 <sup>d</sup>
100mg <i>M. Oleifera</i>	74.31 ± 2.24 <sup>a</sup>	25.69 ± 0.15 <sup>a</sup>
200 mg <i>M. Oleifera</i>	76.97 ± 2.15 <sup>ab</sup>	23.03 ± 0.14 <sup>b</sup>
400mg <i>M. Oleifera</i>	62.62 ± 1.96 <sup>c</sup>	37.38 ± 0.17 <sup>c</sup>

Values are mean ± standard deviation (n = 3). Values with different superscript letters per column are statistically significant (p<0.05)

**Table 6** Rate of sickle cell haemoglobin polymerization inhibition effect of methanol extracts of *Moringa oleifera* leaf

Group	Rate of polymerization	Relative % polymerization	Relative % inhibition
Normal saline (Control)	0.0081	100.00 ± 0.00	0.00 ± 0.00
Ciklavit (Standard)	0.0050	61.73 ± 1.09	38.27 ± 0.02
100mg MO	0.0038	46.91 ± 1.36	53.09 ± 1.00
200 mg MO	0.0038	46.91 ± 1.92	53.09 ± 1.02
400mg MO	0.0040	49.38 ± 1.18	50.62 ± 0.66

Values are mean ± standard deviation (n = 3). Values with different superscript letters per column are statistically significant (p<0.05)

## 7. Discussion

The nutritional approach to the management of sickle cell disease has been the most effective and modern process imbibed in the management of the syndrome. Many research studies have provided humanity with reliable statistics on the deficiencies of various nutrients, some of which are exacerbated by the sickling chronicle.

The proximate analysis of the extract of *M. oleifera* showed that the leaves contained nutritive compounds like proteins, fats, and carbohydrates which are important for the overall nutritional health of the body. This is in line with previous researches by Ogundele et al., 2017 and Jules et al. 2020. Compounds like crude fibre, known for its crucial function of adding bulk to the diet, improve bowel movement and prevent constipation, is very beneficial to sickle cell patients. Constipation can impact sickle cell patients by causing discomfort, worsening existing sickle cell crises, and negatively affecting other aspects of inpatient care (Nationwide Children's Hospital, 2010). Constipation can trigger or worsen painful abdominal cramps and crises, making it difficult for patients to manage their pain and potentially leading to confusion with sickle cell crises (NCH, 2010).

The phytochemical composition of *M. oleifera* leaf extract showed that the leaves contained kaempferol, quercetin, flavone, luteolin, daidzein, artemetin, retusin, catechin, allagic acid, vanillic acid, naringenin, apigenin, hesperidin, isorhammetin, maricetin, epicatechin, daidzein, genistein, lunamarin, gallic acid, resveratrol, and tangeretin, all with antioxidative properties, have been observed to play key roles in managing sickle cell crises. Kaempferol, a derivative of the aromatic amino acid phenylalanine, is a flavonol found in many fruits, vegetables and herbs, processes antioxidative and anti-inflammatory properties (Ruying, Long, Yuling, & Hui, 2024; Nitesh, et al., 2024). The antioxidant effect may play key role in inhibiting lipid peroxidation of the red blood cell, thereby reducing hemolysis of the red blood cell (Ruying, et al. 2024). It enhances the activity of antioxidant enzymes like catalase and peroxidase, and reduces inflammation and oxidative stress (Ruying, et al. 2024).

Quercetin, also a plant flavonol from the flavonoid group of polyphenols, also found in fruits, vegetables, leaves, seeds and grains, has also been observed to have antioxidative, antimicrobial, antiviral, anticancer and anti-inflammatory effects. Quercetin has been shown to have cardiovascular benefits such as lowering blood pressure and reducing cholesterol levels (Fatemeh, & Milad, 2023). The latter is important to sickle cell disease patients as cholesterol reduces the oxygen carrying capacity of haemoglobin, thereby inducing sickling (Marcela, Manal, Maria, & Fernandez, 2017; Fatemeh, et al. 2023). This finding also conforms to previous research work of Olayemi et al., 2022.

Flavones like luteolin, apigenin and tangeretin possess high antioxidant, anti-inflammatory, neuroprotective, antibacterial and antiviral effect. Apigenin for instance has been observed to have, in addition to its antioxidative properties, hepatoprotective effect, by enhancing glutathione (GSH) reductase activity and reducing the content of GSH and malondialdehyde (Yang, Wang, Xue, Gu, & Xie, 2013; Wang, Feng, Li, Chen, Wang, Lan, Tang, Jiang, Zheng, & Liu, 2024). Malondialdehyde may be directly involved with conditions that can lead to hepatomegaly (Yang, et al. 2013).

Tangeretin processes cholesterol lowering effect as well as antioxidative and anti-inflammatory properties, all of which play an important role in sickle cell disease management (Ashrafizadeh, Ahmadi, Mohammadinejah, & Afshar, 2020).

Naringenin also has high antioxidative activities and is known to reduce oxidative damage to DNA in vitro. Other biological properties of naringenin are anti-inflammatory, and antimicrobial effect. Naringenin is also known to inhibit very low-density lipoprotein (VLDL) secretion both in vivo and in vitro, as well as cholesterol (Kurowska, Borradaile, Spence & Carroll, 2000; Wilcox, Borradaile, Dreu & Huff, 2001; Borradaile, Dreu, Barrett, & Huff, 2002; Borradaile, Dreu,

Barrett, Behrsin, & Huff, 2003; Allister, Borradaile, Edwards, & Huff, 2005; Nahmias, Goldasasser, Casali, Daan, Takaji, Raymond, & Martin, 2008). Lunamarine, the major constituent of *Boerhavia diffusa* leave is a quinolone alkaloid as detected by electronic and infra-red spectral studies (Rani, Asha, Linda, Williams & Abraham 2022). Lunamarine is also known for its antioxidative, anticancer, and anti-inflammatory properties (Ojukwu, Onyegbule & Umeyor, 2021). Lunamarine along with antimalarial compound like artemetin, may play effective role in reducing anaemia in sickle cell patients.

Ellagic acid and Vanillic acid found in *Moringa oleifera* leaf extract has been demonstrated to possess antioxidative, anticancer, anti-inflammatory, antimicrobial, and also protect against oxidative stress (Wang, Ren, Li, Song, Chen, and Ouyang, 2019). This research finding on the phytochemical composition of *Moringa oleifera* leaf extract conformed to previous studies by Don-Lawson, and Okah, 2019; Olayemi et al., 2022 and David et al., 2024.

Antioxidants can be defined as compounds that act in a cell as redox couples to scavenge reactive oxygen species (ROS) and to maintain cells in a more reduced redox state. Therefore, all antioxidants are considered reducing agents. This is because they can donate electrons, effectively reducing other molecules while being oxidized themselves. This electron donation is the key to their ability to neutralize free radicals and prevent oxidation in a variety of chemical and biological context (Whayne, Saha, & Mukherjee, 2016).

*M. oleifera* leaf extracts have been demonstrated to contain high quantity of antioxidant vitamins. These vitamins are vitamins A, E and C. Apart from their oxidative properties, vitamin E helps strengthens the immune system against viruses and bacteria, helps in the formation of red blood cells and widen blood vessels, which may help sickle cell disease patients in having more red blood cells and significantly reduced sickle cell blood from clogging the veins respectively (National Institute of Health, 2021; Kubala, 2024; The Nutrition Source, 2025). Vitamin C on the other hand, help in the absorption of iron which is need by sickle cell disease patients, and vitamin c also helps in the reduction of phytate that may chelate irons (NIH, 2021; Markell & Siddiqui, 2022). The antioxidative properties of these vitamins are in line with previous studies of Nitesh et al., 2021; Kanadio et al., 2021; Mojisola et al.,2022; Nwaoguikpe et al. 2023.

Catalase and Peroxidase are antioxidant enzymes that helps reduce oxidative stress in sickle cell disease patients by scavenging reactive oxygen species (ROS), and maintaining the cell in a more reduce redox state (Nelson and Cox, 2017). The high availability of antioxidant vitamins and enzymes contained in the leaf of *M. oleifera*, show promise to sickle cell disease patients who have low antioxidant capacity in combatting the reactive oxygen species (ROS) emanating from various sources.

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## 8. Conclusion

The work examined the proximate analysis, phytochemical content and the antioxidant effect of *Moringa oleifera* leaf methanol extract in sickle cell disease management. The *Moringa oleifera* leaf methanolic extracts contained antisickling amino acids: phenylalanine, lysine and arginine, thus making both plants potent for sickle cell disease management. The antioxidant concentration of catalase, peroxidase, carotenoids, phenolic compounds, vitamins C, A and E, of *Moringa oleifera* leaf methanolic extracts may show promise in reducing oxidative stress that may result to sickle cell crises, as well as a potent inhibitor of sickle cell haemoglobin polymerization. The outcome of this study showed that *Moringa oleifera* leaf methanolic extracts may show promise in the management of sickle cell disease.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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