International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Proximate analysis, phytochemical content and the antioxidant effect of *Alchornea Cordifolia* methanol leaf extract in sickle cell disease management

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International Journal of Science and Research Archive, 2025, 16(01), 319-333

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

Article DOI: https://doi.org/10.30574/ijsra.2025.16.1.1962

Abstract

The proximate analysis, phytochemical and antioxidant effect of *Alchornea cordifolia leaf methanol extract* were investigated and their effects on sickle cell hemoglobin polymerization observed. *Alchornea cordifolia leaf* was sourced from a horticulturist in a horticulture garden along Okigwe road in Owerri, Imo State, Nigeria. The voucher specimen was identified, classified and authenticated by a professional taxonomist at the Department of Biological Sciences, University of Agriculture and Environmental Sciences, Umuagwo, 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 *Alchornea cordifolia* 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 *Alchornea cordifolia* leaf methanol extracts inhibited sickle cell polymerization by 51.85 ± 1.08, 43.21 ± 0.56 and 35.81 ± 0.58 respectively. *Alchornea cordifolia* 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 improvement in the F_e^{2+}/F_e^{3+} ratio from 84.88% to 87.91% and reduction in hemoglobin polymerization from 61.73% (Ciklavit™) standard to 48.15 % (100 mg/dose) *Alchornea cordifolia* leaf methanol extracts. *Alchornea Cordifolia* 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 *Alchornea Cordifolia*; Hemoglobin Polymerization; F_e^{2+}/F_e^{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 Nkangininema, 1999; Denise, 1992). Sickle 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 *Alchornea Cordifolia* 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.

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 matabisulphite 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. *Alchornea cordifolia 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 *Alchornea cordifolia 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 personnel 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^{2+}/Fe^{3+} 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 20 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 siphoned plasma. The erythrocyte suspension was then frozen at 0 °C, 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 *Alchornea cordifolia* 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.

2.6. Proximate Analysis of the sample

2.6.1. 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 1hr until a steady result was obtained and the weight was noted. The drying procedure continued until a constant weight was obtained.

$$\% \text{ moisture content} = \frac{w_1 - w_2}{\text{Weight of sample}} \times 100 \ldots\ldots\ldots \text{Eqn.1}$$

Where W_1 = weight of petridish and sample before drying

W_2 = weigh of petridish and sample after drying.

2.7. Ash content (AOAC, 1990)

2.7.1. 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.

2.7.2. 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 ℃ for 3 hours. The sample was cooled in a desiccator after burning and weighed.

Calculations

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

Were

W_1 = weight of empty platinum crucible
W_2 = weight of platinum crucible and sample before burning
W_3 = weight of platinum and ash.

2.8. Crude Fibre (AOAC 1990)

2.8.1. 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 200 ml of a solution containing 1.25 g of H_2SO_4 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 x 100 is the percentage of crude fibre.

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

2.9. Crude fat

2.9.1. 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.

2.9.2. Procedure

Two hundred and fifty milliliters (250 ml) clean boiling flasks in oven at 105 – 110 ℃ 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 300 ml of petroleum ether (boiling point 40 – 60 ℃). 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 ℃ - 110 ℃ for 1hour, and transferred from the oven into a desiccator and allow to cool; then weighed.

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

2.10. Crude proteins (AOAC, 1990)

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.

2.10.1. 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 100ml with distilled water added to avoid caking and then 5 ml 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 20 cm 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

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

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

Carbohydrate determination

(Differential method)

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

3. Phytochemical analysis

3.1. Phytochemical quantitative and qualitative analysis

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

3.1.1. 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.

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

3.2. Extraction of phytochemicals (*Alchornea* leaves)

Alchornea cordifolia leaf sample (1 g) was weighed and transferred in a test tube and 15 ml of methanol added and 10ml of 50 %m/v potassium hydroxide was also added. The test tube was allowed to react in a water bath at 60 ℃ 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 10 ml 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.

3.3. 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 µm x 0.15 µm) was used. The injector temperature was 280 °C with splitless injection of 2µl of sample and a linear velocity of 30cms^{-1}. Helium 5. 0pa.s was the carrier gas with a flow rate of 40 ml/min or 40mlmin^{1}. The oven operated initially at 200 °C, it was heated to 330 °C at a rate of 3^{o}C min^{-1} and was kept at this temperature for 5minutes. 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 µg/g.

3.4. Determination antioxidant vitamins

3.4.1. 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 30ml 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 layers 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 50ml 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 450nm.

3.4.2. 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).

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 g/dm^3 of the ascorbic acid (vitamin C), so that 1 cm^3/mg vitamin C equals 1 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 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 cm^3 of the indophenol are required, 1cm^3 of indophenol solution is equivalent to 10 mg/x vitamin C. Having standardized the indophenol solution, 10 cm^3 of the test solution (extract) was taken and treated in a similar way.

3.4.3. 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 1 M 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 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 metrohmspectronic 21D spectrophotometer, against a blank containing 5 ml absolute alcohol and 1ml concentrated HNO_3 treated in a similar manner.

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

3.5. Determination of antioxidant effects

3.5.1. Determination of Catalase (*Alchornea* leaves)

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

Principle

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

Reagents

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

Procedure

Preparation of enzyme extract

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

ASSAY

Hydrogen peroxide (H_2O_2) phosphate buffer (3.0 ml) 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_2O_2- free phosphate buffer served as control.

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

3.5.2. Determination of Peroxidase (*Alchornea cordifolia*)

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

Principle

In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts H_2O_2 to H_2O and O_2. The oxidation of pyrogallol or dianisidine to a colored product called purpurogalli can be followed spectrophotometrically at 430 nm.

Reagents

Pyrogallol; 0.05 M in 0.1 M phosphate buffer (pH 6.5), H_2O_2; 1 % in 0.1 M phosphate buffer, pH 6.5.

Procedure

Preparation of enzyme extract

A 20 % homogenate was prepared in 0.1 M 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_2O_2 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.

Determination of the Fe^{2+}/Fe^{3+} Ratio

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

represents the control. In determining the effect of the extract on Fe^{2+}/Fe^{3+} 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.

3.5.3. 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 700nm, using 2% solution of sodium matabisulphite as reductant or deoxygenating agent (Iwu et al., 1988).

Portion 4.4 ml of 2 % sodium matabisulphite ($Na_2S_2O_3$), 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 (*Alchornea cordifolia*), and 0.1ml haemoglobin (HbSS) solution were pipetted into the cuvette, and readings taken at absorbance of 700nm 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 (ΔOD700nm) versus time in minutes. The rate was equally expressed as percentages with respect to control.

The rate can be calculated from the formula,

$$\text{Rp} = \frac{\text{Final OD} - \text{Initial OD}}{\text{Time. i. e. Rp}} = \frac{\text{ODF} - \text{ODI}}{\text{T}}$$

Where, Rp = rate of polymerization, OD_F = final optical density, OD_I = initial optical density and T= time of assay in minutes.

3.5.4. Determination of Alkaloids of the Extracts

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

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 ℃, 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).

4. Results

Results are shown in tables 1 to 6

Table 1 Proximate composition of *Alchornea Cordifolia* leaf methanol extract

Parameter (%)	*Alchornea cordifolia*	t-value	p-value	Comment
Moisture	8.20 ± 0.04	44.37	0.0005	Significant
Fat	1.89 ± 0.03	145.10	<0.0001	Significant
Ash	3.71 ± 0.04	70.41	0.0002	Significant
Fibre	7.30 ± 0.02	22.45	0.0020	Significant
Protein	12.37 ± 0.05	13.19	0.0057	Significant
Carbohydrate	66.53 ± 0.07	144.20	<0.0001	Significant

Value are mean ± standard deviation of duplicate determination

Table 2 Phenolic profile of *Alchornea cordifolia* leaf methanol extract

Parameter (mg/100g)	*Alchornea cordifolia*	t-value	p-value	Comment
Artemetin	2.64 ± 0.03	52.89	0.0004	Significant
Retusin	1.23 ± 0.02	25.00	0.0016	Significant
Catechin	5.72 ± 0.06	60.82	0.0003	Significant
Ellagic acid	0.38 ± 0.01	289.00	<0.0001	Significant
Vanillic acid	2.27 ± 0.01	1.789	0.2155	Not Significant
Naringenin	0.34 ± 0.01	22.77	0.0019	Significant
Apigenin	4.03 ± 0.04	76.25	0.0002	Significant
Hesperidin	1.77 ± 0.02	67.04	0.0002	Significant
Isorhamnetin	1.29 ± 0.01	127.00	<0.0001	Significant
Maricetin	0.27 ± 0.01	36.22	0.0008	Significant
Epicatechin	2.05 ± 0.02	11.38	0.0076	Significant
Daidzein	0.63 ± 0.01	30.36	0.0011	Significant
Genistein	1.12 ± 0.01	39.21	0.0006	Significant
Lunamarin	0.47 ± 0.01	0.00	1.000	Not Significant
Gallocatechin	0.33 ± 0.01	40.70	0.0006	Significant
Resveratrol	1.90 ± 0.02	15.65	0.0041	Significant
Tangeretein	1.19 ± 0.02	29.00	0.0012	Significant
Naringin	1.74 ± 0.01	37.00	0.0007	Significant
Silymarin	158.95 ± 0.07	1202.00	<0.0001	Significant

Value are mean ± standard deviation of duplicate determination

Table 3 Amino Acid composition of *Alchornea cordifolia* leaf methanol extract

Parameter (mg/100g)	*Alchornea cordifolia*	t-value	p-value	Comment
Threonine	16.37 ± 0.03	34.58	0.0008	Significant
Isoleucine	30.08 ± 0.02	181.30	<0.0001	Significant
Leucine	1.60 ± 0.07	4.400	0.0480	Significant
Aspartate	0.97 ± 0.01	39.44	0.0006	Significant
Lysine	3.43 ± 0.02	46.80	0.0005	Significant
Methionine	0.91 ± 0.03	12.07	0.0068	Significant
Glutamate	2.75 ± 0.04	63.83	0.0002	Significant
Phenylalanine	1.52 ± 0.02	70.64	0.0002	Significant
Histidine	1.17 ± 0.03	35.30	0.0008	Significant
Arginine	9.16 ± 0.04	136.10	<0.0001	Significant
Tyrosine	3.81 ± 0.03	48.93	0.0004	Significant
Tryptophan	1.77 ± 0.02	221.60	<0.0001	Significant
Cysteine	1.32 ± 0.06	234.40	<0.0001	Significant

Value are mean ± standard deviation of duplicate determination

Table 4 Chemical content and antioxidant enzyme activity of *Alchornea cordifolia* leaf samples

Parameter	*Alchornea cordifolia*	t-value	p-value	Comment
Alkaloid (mg/g)	6.21 ± 0.06	26.12	0.0015	Significant
Catalase (IU/ml)	0.04 ± 0.01	1.000	0.4226	Not significant
Peroxidase (U/mg protein)	1.02 ± 0.06	6.450	0.0232	Significant
Carotenoids (mg/g)	3.85 ± 0.66	3.945	0.0587	Not significant
Vitamin E (mg/100g)	31.56 ± 1.80	13.37	0.0056	Significant
Vitamin A (µg/g)	2116.36 ± 6.56	41.74	0.0006	Significant
Vitamin C (mg/100g)	837.00 ± 4.50	159.70	<0.0001	Significant

Value are mean ± standard deviation of duplicate determination

Table 5 The Fe^{2+}/ Fe^{3+} Ratios of sickle cell blood exposed to extract of *Alchornea cordifolia* (AC) leaf

Group	%Hb	%mHb
Normal saline	84.88 ± 1.09^{ad}	15.11 ± 0.11^{a}
Ciklavit (Standard)	89.94 ± 1.02^{af}	10.06 ± 0.17^{b}
100mg AC	87.91 ± 3.10^{bd}	12.09 ± 0.16^{e}
200mg AC	87.67 ± 4.02^{e}	12.33 ± 0.18^{f}
400mg AC	68.67 ± 5.10^{ef}	31.33 ± 0.13^{fg}

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 *Alchornea* 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 AC	0.0039	48.15 ± 0.39	51.85 ± 1.08
200mg AC	0.0046	56.79 ± 0.65	43.21 ± 0.56
400mg AC	0.0052	64.19 ± 1.55	35.81 ± 0.50

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

5. 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 *A. cordifolia* 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 *A. cordifolia* leaf extract showed that the leaves contained kaempferol, quercetrin, flavone, luteolin, daidzein, artemetin, retusin, catechin, allagic acid, vanillic acid, naringenin, apigenin, hesperidin, isorhammetin, maricetin, epicatechin, daidzein, genistein, lunamarin, gallocatechin, resveratrol, and tangeretein, 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). 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).

Naringinin 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. Naringinin is also known to inhibit very low-density lipoprotein (VLDL) secretion both invivo and invitro, 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 *Alchornea cordifolia* 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 *Alchornea cordifolia* 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).

Alchornea Cordifolia 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 (Traber et al, 2011; 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 & Siddiqu, 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 *Alchornea cordifolia* leaf, show promise to sickle cell disease patients who have low antioxidant capacity in combatting the reactive oxygen species (ROS) emanating from various sources.

6. Conclusion

The work examined the proximate analysis, phytochemical content and the antioxidant effect of Alchornea cordifolia leaf methanol extract in sickle cell disease management. The Alchornea cordifolia 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 Alchornea cordifolia 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 Alchornea cordifolia leaf methanolic extracts may show promise in the management of sickle cell disease.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Azubuike, C.J. and Nkangininema, K.E.O. (1999). Haemoglobinopathies. In Pediatrics and Child health in a tropical region. Publishers, African Educational Services, Owerri, Pp. 194-213.

[2] Aygun, B., and Odame, I. (2012). A global perspective on sickle cell disease pectiatric blood and cancer, 59(2), 386-390. http://dx.doi.org/10.1002/pbc.24175. PubMed PMID: 2011585449 Revision date: 20120928 Publicate type: Journal article.

[3] AOAC (Association of Official Analytical Chemists), (1990) Official Method of Analysis of the AOAC (Q. Horwitz Editor Eighteen Edition, Washington; D.C., AOAC.

[4] Amadi B. Agomuo E. Duru M. and Adindu E. (2012); Chemical profile of an anti-malarial concoction "Udu" used in Umunchi autonomous community in Isiala Mbano LGA of Imo State, Nigeria. *Journal of Emerging Trends in Engineering and Applied science*, vol.3, no.3

[5] Achi, N.K. and Ohaeri, O.C. (2012). Acute and subacute toxicity studies of Sansevieria liberica aqueous leaf extracts. *Pharma Sci Monit*., 3(4): 1938-1951.

[6] Ashrafizadeh, M., Ahmadi, Z., Mohammadinejad, R., and Afshar, E.G. (2020). Tangeretin: a mechanistic review of its pharmacological and therapeutic effects. Journal of Basic and Clinical Physiology and Pharmacology, 2020, April. 31(v). doi:10.1515/jbcpp-2019-0191

[7] Allister, E.M., Borradaile, N.M., Edwards, J.Y., Huff, M.W. (2005). Inhibition of microsomal triglyceride transfer protein expression and apolipoprotein B-100 secretion by the citrus flavonoid neringenin and by insulin involving activation of the mitogen-activated protein kinase pathway in hepatocytes. *Diabetes*, 2005;54: 1676-1683.

[8] Borradaile, N.W., Dreu, L., Barrett, P., Huff, M.W. (2002). Inhibition of hepatocyte Apo B secretion by neringenin enhanced rapid intracellular degradation independent of reduced microsomal cholesteryl esters. *Journal lipid Res,* 2002; 431544-1554.

[9] Borradaile, N.W., Dreu, L., Barrett, P., Behrsin, C.D., Huff, M.W. (2003). hepatocyte Apo B-containing lipoprotein secretion is decreased by the grapefruit flavonoid, neringenin via inhibition of MTP-mediated microsomal triglycerides accumulation. *Biochemistry,* 2003; 42: 1283-1291.

[10] Chakravorty, S. and Williams, T.N. (2015). Sickle Cell disease: A neglected chronic disease of increasing global health importance. *Archives of Diseases in children*, 100(1), 48-53.

[11] David, N.E., Anderson, C.A., and Inemesit, N.B. (2024). Ethnobotany and Phytochemical Composition of *Alchornea Cordifolia* in Abia State, Nigeria. *International Journal of Traditional and Complementary Medicine*. 2024, vol.5(1). Doi:10.53811/ijtemr.1332261.

[12] Davidson J and Henry J.B. (1974). Clinical Diagnostics by Laboratory methods. Todd-Sanford- W.B. Saunders, *Philadelphia*, 1974: 112, 1380.

[13] Denise, M. Harmening (1992). Hemolytic anemia; Intra-corpuscular defects. In clinical Hematology and Fundamentals of Hemospasis, (2nd edition), Publishers, F.A. Davis Company Philadelphia, Pp. 142-162.

[14] Delia, B., and Mieko, K. (2004). Harvestplus Handbook for Carotenoid Analysis. Washington, D.C. pp.34- 38.E.

[15] Don-Lawson, C., and Okah, R. (2019). Analysis of *Moringa oleifera* (leaves and flowers) to determine some phytochemicals and functional groups. Direct Research Journal of Chemistry and Material Science, 2019 Nov. vol. 6(3). Pp45-49. https://doi.org/10.26765/DRJCMS2019371853. ISSN. 2354-4163

[16] Ekeke, G.J., and Shode, F.O. (1990). "Phenylalanine is the predominant antisickling agent in Cajanus cajan seed extract" Plant media, 56: 41-43.

[17] Fatemeh, A., and Milad, H. (2023). Recent Advances in Potential Health Benefits of Quercetin. *Pharmaceutical (Basel),* 2023 Jul. 18: 16(7): 1020. Doi: 10.3390/ph16071020. PMCID: PMC10384403. PMID: 37513932.

[18] Hafen B.B, and Sharma S. (2019): Oxygen Saturation; study guide from StatPearls Publishing, Treasure Island (FL), 25 Sep. 2018. PMID: 30247849.

[19] Iwu M.N, Igboko A.O, Onwubiko H and Ndu U.E; Effect of Cajanus cajan on gelation and oxygen affinity of sickle cell hemoglobin. Journal Ethno pharmacology, 1988; 22:99-101.

[20] Jules, M.K., Clement, L.I., Emmanuel, M.L., Damian, S.T., Dorothy, D.T., Koto, K.M., …, and Pius, T.M. (2020). Mineral Content and Antisickling Activity of *Annona senegalensis*, *Alchornea Cordifolia*, and *Vigna unguiculata* Used in the Management of Sickle Cell Disease in the Kwali

[21] Kelly, D.A. and Nelson, R. (2014). Characterization and quantification by gas chromatography of flavonoids. *J. Braz. Chem. Soc*. Vol. 25.

[22] Kubala, D. (2024). What are the benefits of vitamin E. *Healthline* 2024, Nov. Retrieved from, www.healthline.com/health/all-about-vitamin-e?

[23] Kurowska, E., Borradaile, N., Spence, J.D., Carroll, K.K. (2000). Hypocholesterolemic effects of dietary citrus juices in rabbits. *Nutrition Res* 2000;20: 121-129.

[24] Lambert J and Muir T.A; Estimation of Vitamin C. In Practical Chemistry, 3rd edition. Heinemann Educational Books, London, 1974: 447-448.

[25] Luck, H. (1974). Estimation of catalase, methods in enzymatic analysis. 2nd edition (Bergmeyer, H.u., ed), pp.885-888, verlag Chemie, Weinheim and Academic press, New York.

[26] Makani, J., Cox S.E, Soka, D., Komba, A.N., Oruo, J., Mwamtemis, H, et al. (2011). Mortality in Sickle Cell anemia in Africa; A prospective cahort study in Tanzaria. Plos ONE, 6(2), e1499. http://dx.doi.org/10.1371/journalpone.0014699. Epub 2011/02/02. Pubmed PMID 21358818: Pubmed central PMCID: PMCPMC3040170.

[27] Marcela, V.J., Manal, M.A., and Fernandez, M.L. (2017). Bioactive compounds in *Moringa oleifera* leaves against chronic disease. *Antioxidants(Basel)*. 2017 Nov. 16; 6(4): 91. Doi: 10.3390/antiox6040091.

[28] Markell, M., and Siddiqu, H.A. (2022). Vitamins and trace elements. In: McPherson RA, Pincus MR, eds. Herry`s Clinical Diagnosis and Management by Laboratory Methods. 24th ed. Philadelphia, PA: *Elsevier*; 2022: Chap. 27.

[29] Mojisola, C.C., and Idowu, O.O. (2022). *Moringa oleifera* Extracts Regulates the Expression of Some Sickle Cell Related Genes in Normal Wister Rats. *Journal of Pharmacy and Pharmacognosy Research*, 2022, 10(5), 837-846. Doi:10.56499/ ippres22.1389-10.5.837 ISSN:0719-4250.

[30] Nahmias, Y., Goldwasser, J., Casali, M., Daan, V.P., Takaji, W., Raymond, T.C., Martin, L.Y. (2008). Apolipoprotein B-dependent hepatitis C Virus secretion is inhibited by the grapefruit flavonoid neringenin. *Hepatology*, vol 47, issue 5/p. 1437-1445. Doi.org/10.1002/hep.22197.

[31] National Institute of Health (2021). Vitamin C, fact sheet for health professionals. Retrieved from https://ods.od.nih.gov/factsheets/vitamin C-Health professional/.

[32] National Institute of Health (2021). Vitamin E, fact sheet for health professionals. Retrieved from https://ods.od.nih.gov/factsheets/vitamin E-Health professional/.

[33] Nationwide Children`s Hospital (2010). Sickle cell patients should be better monitored for constipation prevention. *ScienceDaily.* 2010, June 16. Retrieved May 20, 2025 from www.sciencedaily.com/releases/2010/06/100616102858.htm.

[34] Nelson, D.L and Cox, M.M. (2017). Lehninger Principles of Biochemistry. (Seventh Ed), W.H. Freeman and Company, One New York Plaza, Suite 4500, New York, NY 10004-1562. Pp 158.

[35] Nitesh, B., Nitin, I., Srelakshmi, V.P., and Haranath, D. (2021). Phytochemical Analysis of *Moringa leifera* Leaves Extract by GC-MS and Free Radical Scavenging Potency for Industrial Application. *Saudi Journal of Biological Sciences*, 2021, 28, 6915-6928. Doi: 10.1016/j.jsbs.2021.07.075.

[36] Noguchi, C.T and Schechter A.N. (1978). The inhibition of sickle cell hemoglobin polymerization by amino acids and related compounds. *Journal Biochemistry*, 1978; 17:5455-5459.

[37] Nwaoguikpe, R.N., Obiekwe, L.C., and Emejulu, A.A. (2023). The Antisickling Properties of *Aristeus antennatus* Extract in the Management of Sickle Cell Disease. *Sumerianz Journal of Biotechnology*, 2023, vol.6, no.1, pp.11-18. Doi: https://doi.org/10.47752.

[38] Nwaoguikpe R.N. (2010). The Phytochemical, Proximate and Amino acid compositions of the extracts of two varieties of figernut (*Cyperus esculentus*) and their effects on sickle cell hemoglobin polymerization.

[39] Nwaoguikpe, R.N and Anyalogbu, E.A. (2008). The prevalence of Sickle Cell Disease (SCD) and Hemoglobin genotype distribution in Awka, capital city of Anambra State, Nigeria. *International Journal of Biotechnology and Allied Science*, 3(1): 383-386.

[40] Nwaoguikpe, R.N. (2009). The antisickling effects of some edible vegetables. *International Journal of Biological and Chemical Science*, 2009: 3(5): 1005-1010.

[41] Nwaoguikpe R.N, Ekeke G.I, and Nwakwe, A.A (1999). The effect of extract from some food stuffs on Lactate Dehydrogenase (LDH) activity and Hemoglobin polymerization of sickle cell blood. Ph.D thesis, University of Port Harcourt, Nigeria.

[42] Ogundele, D.T., Oludele, E.O., Oladejo, A.A. (2017). Phytochemical Constituents, Proximate composition and Mineral Analysis of Aqueous and Ethanolic Stem bark, Seed extracts and Plant parts of *Moringa oleifera*. *Journal of Applied Life Sciences International* 10(4):1-7. Doi:10.9734/JALSI/2017/30694.

[43] Ojukwu, U.S., Onyegbule, F.A., and Umeyor, C.E. (2021). GC-FID Phytochemical analysis of selected antidiabetic herbal preparations sold in Onitsha Metropolis, Anambra State, Nigeria. *Journal of Medicinal Plant Studies*, 2021: 9(4), pp 60 – 67. https//doi.org/10.22271/plants.2021.v9.i4a.1313.

[44] Olayemi, A., Rafael, B., Sumana, B., Alan, C., Robert, J.N., Ana, W., …., and Luis, A.J. (2022). Isolation and Characterization of Quercitrin as a Potent Anti-Sickle Cell Anemia Agent from *Alchornea Cordifolia*. *Journal of Clinical Medicine*. 2022, 11, 2177. Doi: 10.3390/jcm 11082177.

[45] Rani, A.T., Asha, T., Williams, L., and Abraham, J. (2022). Effect of Lunamarine, the major Constituent of *Boerhaavia diffusa* Leave Extract on the Corrosion Inhibition of Mild Steel in Hydrochloric acid; Computational modelling, Surface screening and Electroanalytical studies. *Journal of Bio Tribo- Corrosion,* 2022, Aug. 18. https://doi.org/10.1007/s40735-021-00616-2.

[46] Reddy, K.P., Subhani, S.M., Khan, P.A., and Kumar, K.B., (1995). Effect of light and benzyladenine and desk treated growing leaves changes in the peroxidase activity. *Cell Physiology*, 26:984.

[47] Ruying, T., Longfei, l., Yuling, L., and Hui, L. (2024). Bibliometric and visual analysis of global publications on kaempferol. *Frontiers Nutrition*. 2024, Aug. 16. Vol. 11. https://doi.org/10.3389/fnut.2024.1442574.

[48] Traber, M., and Stevens, J.F. (2011). Vitamin C and E: Beneficial effects from a mechanistic perspective. *Free Radical Biology and Medicine,* 2011, Sept. 1, vol. 51(5), pp1000-1013. https://doi.org/10.1016/j.freeradbiomed.2011.05.017.

[49] Uwakwe, A.A and Nwaoguikpe, R.N. and: The antisickling effects of dried fish (Tilapia) and dried Prawn (*Astacus red*). *Journal Applied Sciences and Environmental Management*. 2005; 9(3): 115-119.

[50] The Nutrition Source (2025). Vitamin E. Harvard School of Public Health 2025, Feb. Retrieved from https://nutritionsource.hsph.harvard.edu/vitamin-2/

[51] Wilcox, L.J., Borradaile, N.W., Dreu, L., Huff, M.W. (2001). Secretion of Hepatocyte ApoB is Inhibited by the Flavonoids, neringenin and hesperidin, via reduced activity and expression of ACAT 2 and MTP, *Journal Lipid, Res* 2001; 42: 725-734.

[52] Wang, C., Feng, X., Li, W., Chen, L., Wang, X., Lan, Y., Tang, R., Jiang, T., Zheng, L., and Liu, G. (2024). Apigenin as an emerging hepatoprotective agent: current status and future perspective. Front *Pharmacol*. 2024 Dec. 19; 15:1508060. Doi:10.3389/fphar.2024.1508060. PMCID: PMC 11693974. PMID:39749193.

[53] Wang, Y., Ren, F., Li, B., Song, Z., Chen, P., and Ouyang, L. (2019). Ellagic Acid Extracts Anti-tumor Effects via the PI3K Signaling Pathway in Endometrial Cancer. *Journal Cancer*, 2019: 10:3303. Doi:10.7150/jca.29738.

[54] Whayne, T.F., Saha, S.P., and Mukherjee, D. (2016). Antioxidants in the Practice of Medicine; What should the clinician know? *Cardiovasc Hematol Disord Drug Targets*, 2016; 16(1): 13-20. Doi: 10.2174/1871529x16666160614015533. PMID: 27296476.

[55] Yang, J., Wang, X.Y., Xue, J., Gu, Z.L., and Xie, M.L. (2013). Protective effect of apigenin on mouse acute liver injury induced by acetaminophen is associated with increment of hepatic glutathione reductase activity. *Food Funct*, 2013, 4(6), 939-943. Doi:10.1039/c3fo60071h.

[56] Yostina F, Bowman N.S, and Paul L. (2019) Biochemistry, Hemoglobin synthesis; study guide from StatPearls Publishing, *Treasure Island (FL)*, 07 Feb. 2019. PMID: 30725597.