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The biochemical effect of *Moringa oleifera, Alchornea Cordifolia* and their combination leaf methanol extract on lactate dehydrogenase levels in sickle cell disease

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Abstract

The effect of *Moringa oleifera*, *Alchornea cordifolia* leaf methanol extracts and their combination on lactate dehydrogenase were investigated to ascertain the ability of both plants and their combination in reducing lactate dehydrogenase in sickle cell blood. The proximate analysis, phytochemical and antioxidant effect of *Moringa oleifera* and *Alchornea cordifolia* leaf methanol extract were investigated. *Moringa oleifera* and *Alchornea cordifolia* leaves were sourced from a horticulturist in a horticulture garden along Okigwe road in Owerri, Imo State, Nigeria. Their voucher specimens were identified, classified and authenticated by a professional taxonomist at the Department of Biological Sciences, University of Agriculture and Environmental Sciences, Umuagwo, Imo State. The samples were first dried at room temperature to constant weight, ground into powder and soaked in methanol for 24 hours to obtain the methanol soluble fraction. The samples were concentrated at different concentrations of 1000 µg/ml, 2000 µg/ml and 4000 µg/ml doses for the lactate dehydrogenase activity, and 100, 200 and 400 mg/dose for polymerization inhibition activities. Blood sample were collected from confirmed HbSS patients who attend clinic at Federal University Teaching Hospital, Owerri by personnel of the Hematology Units. Amino acid compositions and phytochemical properties were determined for the samples, using gas chromatography which revealed a preponderance of both essential and non-essential amino acid and phytochemical compounds of which some were antisickling in nature. The different concentrations of *Alchornea cordifolia* and *Moringa oleifera* leaf methanol extracts and their combination were able to reduce the lactate dehydrogenase enzymes in the blood significantly when compared to the control and standard. Ciklavit™ was used as the standard for this study.

Keywords: Sickle Cell Disease; Amino Acids; Phytochemicals *Alchornea cordifolia*; *Moringa oleifera*; Lactate Dehydrogenase

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, and 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 haemoglobin molecule with severe hematological consequences (Azubuike and Nkangininema, 1999; Denise, 1992). Sickle cell haemoglobin (HbS) is a structural variant of normal adult haemoglobin (HbA) (Chakravorty and 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

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years (Aygun and 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).

Lactate dehydrogenase (LDH) is an enzyme whose major role is to catalyze the conversion of pyruvate to lactate during anaerobic condition, thereby contributing to energy production by the oxidation of NADH to NAD+ and vice versa (Nelson et al., 2017; Farhana and Lappin, 2023). Lactate dehydrogenase act as a biological marker to tissue damage and so elevated levels of LDH in the blood is an indication of tissue damage (Farhana et al., 2023). LDH-1 and 2 are different isoenzyme forms that are predominant in the heart and red blood cells, with LDH-2 levels higher in the heart and rbc, than LDH-1 (Farhana et al., 2023). Sickle cell disease patients have high levels of LDH in their blood owing to their frequent rbc breakdown (hemolysis) as a result of anaemia.

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 biochemical effect of both plants and their combination methanol leaf extracts on lactate dehydrogenase levels in sickle cell disease.

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* and *M. 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 M. oleifera and *Alchornea cordifolia* leaf each were collected, dried and ground to powder using a grinder. The powdered samples were 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 Fe2+/Fe3+ 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 samples of *M. oleifera* and *A. cordifolia* leaves were soaked in 200 ml of methanol (MeOH) of analytical grade for 24 hours. The solvents were filtered and the filtrate subjected to evaporation en-vacuo to concentrate the filtrate. The weight and volume of the methanol extracts were recorded.

2.6. Proximate Analysis of the sample

2.6.1. Moisture Content (AOAC 1990) Procedure

Approximately 1-2 g each of the samples was weighed into petri dish. The weight of the petri dish and sample were noted before drying. The petridish and samples were put in the oven and heated at 105^{o}c for 2hr 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;

W1 = weight of petridish and sample before drying
W2 = weigh of petridish and sample after drying.

2.6.2. Ash content (AOAC, 1990)

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. Procedures

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

Calculations

$$\% \text{ Ash content} = \frac{W_{1-}W_2}{W_{1-}W_2} \times 100 \ldots\ldots\ldots Eqn.2$$

Where

- W1 = weight of empty platinum crucible
- W2 = weight of platinum crucible and sample before burning
- W3 = weight of platinum and ash.

3.1. Crude Fibre (AOAC 1990)

3.1.1. 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.

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

3.1.2. 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.

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 300 ml 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 – 110°c for 1hour, and transferred from the oven into a desiccator and allow to cool; then weighed.

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

3.1.3. 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.

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 5 ml was transferred to the Kjeldhal distillation apparatus, followed by 5 ml 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

$$\% \text{ Nitrogen} = \text{Titre value} \times 0.001 \times 14 \times 4 \ldots\ldots\ldots \text{Eqn. 5}$$

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

% Protein = % Nitrogen x 6.25

Carbohydrate determination

(Differential method)

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

3.2. Phytochemical analysis

3.2.1. Phytochemical quantitative and qualitative analysis

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

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.

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

3.3. Extraction of phytochemicals (*A. cordifolia* and *M. oleifera* leaves)

A. cordifolia and *M. oleifera* leaf samples (1 g) were 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 tubes were allowed to react in a water bath at 60^{o}c for 60 minutes. After the reaction time, the reaction product contained in the test tubes were transferred to a separating funnel. The tubes were washed successfully with 20 ml of methanol, 10ml of cold water and 3 ml of hexane, were transferred to the funnel. These extracts were combined and washed three times with 10ml of 10 %v/v methanol aqueous solution. The solution as dried with anhydrous sodium sulphate, and the solvents were evaporated. The samples were solubilized in 1000 µl of hexane of which 200 µl were transferred to a vial for analysis.

3.4. 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 (15m x

250 µm x 0.15 µm) was used. The injector temperature was 280^{o}c with splitless injection of 2 µl of sample and a linear velocity of 30 cms-1. Helium 5. 0 pa. s was the carrier gas with a flow rate of 40 ml/min or 40 ml min1. The oven operated initially at 200^{o}c, it was heated to 330^{o}c at a rate of 3^{o}c min-1 and was kept at this temperature for 5 minutes. The

detector operated at a temperature of 320^{o}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.

3.5. Determination antioxidant vitamins

3.5.1. 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 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 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.

3.5.2. 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 1cm^3/mg vitamin C equals 1g/dm^3. A burette was filled with a solution of 2,6 dichlorophenolindophenol 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, 1 cm^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.

3.5.3. 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 50ml 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^{o}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 5ml absolute alcohol and 1ml concentrated HNO_3 treated in a similar manner.

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

3.6. Determination of antioxidant effects

3.6.1. Determination of Catalase (A. cordifolia and M. oleifera)

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* and *M. oleifera* leaves were 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 240nm by 0.05 units.

3.6.2. Determination of Peroxidase (A. cordifolia and M. oleifera)

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.

3.7. Determination of Lactate dehydrogenase (LDH) activity

3.7.1. Collection of blood samples (Elekwa, 2004)

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 to 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.

The LDH activity was measured according to the method of Rec (1970) and Rec (1972).

Principle

LDH catalyzes the reduction of pyruvate to lactate - with the simultaneous release of NAD+ group. The rate of reduction of pyruvate, measured by determining the rate of decrease in absorbance at 340nm, is directly proportional to the LDH activity.

$$\text{Pyruvate} + \text{NADH} + \text{H} + \text{LDH} \quad \text{Lactate} + \text{NAD} +$$

Reagent Contents: Reagent 1a, Buffer (50mmol/L phosphate buffer, 0.6mmol/L pyruvate),

Reagent 1b: NADH (0.18mmol/L)

Procedure

The reagents were brought to room temperature, and reagents were pipetted into labelled cuvettes as follows

	Reagent blank	**Sample**	**Extract**
Sample	-	0.1 ml	0.1 ml
Reagent	3.0 ml	3.0 ml	3.0 ml
Extract	-	-	0.1 ml
Distilled water	0.2 ml	0.1 ml	-
Total	3.2ml	3.2ml	3.2ml

They were mixed, initial absorbance read against the blank after 0.5 Min and timer started simultaneously. And read again after 1, 2 and 3 minutes.

Calculations

$$\text{LDH activity } \left(\frac{\text{U}}{\text{L}}\right) = \frac{\Delta\text{A } 340\text{nm}}{\text{min}} \times 4921$$

Where; ΔA= Change in absorbance, min = minute

3.8. Sickle Cell Haemoglobin Polymerization Inhibition Experiment (*M. oleifera* and *A. cordifolia*)

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 ($Na_2S_2O_3$), 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 extracts (*M. oleifera* and *A. cordifolia*), 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 (ΔOD 700 nm) 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.

3.9. Determination of Alkaloids of the Extracts

The alkaloid content of *M. oleifera* and *A. cordifolia* were determined gravimetrically by Achi and Ohaeri, (2012).

3.9.1. 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^{0}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 samples each were 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 solutions were filtered, and the filtrates were 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 extracts to precipitate the alkaloids. They're resulting solutions were allowed to settle, and the precipitates were 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 *M. oleifera* and *A. cordifolia* leaf methanol extract

Parameter (%)	*Moringa oleifera*	*Alchornea cordifolia*	t-value	p-value	Comment
Moisture	11.29 ± 0.09	8.20 ± 0.04	44.37	0.0005	Significant
Fat	5.59 ± 0.02	1.89 ± 0.03	145.10	<0.0001	Significant
Ash	7.30 ± 0.06	3.71 ± 0.04	70.41	0.0002	Significant
Fibre	6.59 ± 0.04	7.30 ± 0.02	22.45	0.0020	Significant
Protein	11.49 ± 0.08	12.37 ± 0.05	13.19	0.0057	Significant
Carbohydrate	57.76 ± 0.05	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)	*Moringa oleifera*	*Alchornea cordifolia*	t-value	p-value	Comment
Artemetin	0.77 ± 0.04	2.64 ± 0.03	52.89	0.0004	Significant
Retusin	0.73 ± 0.02	1.23 ± 0.02	25.00	0.0016	Significant
Catechin	8.44 ± 0.02	5.72 ± 0.06	60.82	0.0003	Significant
Ellagic acid	10.80 ± 0.05	0.38 ± 0.01	289.00	<0.0001	Significant
Vanillic acid	2.31 ± 0.03	2.27 ± 0.01	1.789	0.2155	Not Significant
Naringenin	0.70 ± 0.02	0.34 ± 0.01	22.77	0.0019	Significant
Apigenin	0.98 ± 0.04	4.03 ± 0.04	76.25	0.0002	Significant
Hesperidin	0.71 ± 0.01	1.77 ± 0.02	67.04	0.0002	Significant
Isorhamnetin	2.56 ± 0.01	1.29 ± 0.01	127.00	<0.0001	Significant
Maricetin	1.08 ± 0.03	0.27 ± 0.01	36.22	0.0008	Significant
Epicatechin	1.87 ± 0.01	2.05 ± 0.02	11.38	0.0076	Significant

Daidzein	1.11 ± 0.02	0.63 ± 0.01	30.36	0.0011	Significant
Genistein	1.74 ± 0.02	1.12 ± 0.01	39.21	0.0006	Significant
Lunamarin	0.47 ± 0.02	0.47 ± 0.01	0.00	1.000	Not Significant
Gallocatechin	1.24 ± 0.03	0.33 ± 0.01	40.70	0.0006	Significant
Resveratrol	2.60 ± 0.06	1.90 ± 0.02	15.65	0.0041	Significant
Tangeretein	1.77 ± 0.02	1.19 ± 0.02	29.00	0.0012	Significant
Naringin	1.37 ± 0.01	1.74 ± 0.01	37.00	0.0007	Significant
Silymarin	85.82 ± 0.05	158.95 ± 0.07	1202.00	<0.0001	Significant

Value is mean ± standard deviation of duplicate determination

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

Parameter (mg/100g)	***Moringa oleifera***	***Alchornea cordifolia***	**t-value**	**p-value**	**Comment**
Threonine	18.69 ± 0.09	16.37 ± 0.03	34.58	0.0008	Significant
Isoleucine	38.19 ± 0.06	30.08 ± 0.02	181.30	<0.0001	Significant
Leucine	1.82 ± 0.01	1.60 ± 0.07	4.400	0.0480	Significant
Aspartate	2.12 ± 0.04	0.97 ± 0.01	39.44	0.0006	Significant
Lysine	4.91 ± 0.04	3.43 ± 0.02	46.80	0.0005	Significant
Methionine	1.18 ± 0.01	0.91 ± 0.03	12.07	0.0068	Significant
Glutamate	5.64 ± 0.05	2.75 ± 0.04	63.83	0.0002	Significant
Phenylalanine	4.21 ± 0.05	1.52 ± 0.02	70.64	0.0002	Significant
Histidine	2.07 ± 0.02	1.17 ± 0.03	35.30	0.0008	Significant
Arginine	18.64 ± 0.09	9.16 ± 0.04	136.10	<0.0001	Significant
Tyrosine	5.54 ± 0.04	3.81 ± 0.03	48.93	0.0004	Significant
Tryptophan	7.42 ± 0.03	1.77 ± 0.02	221.60	<0.0001	Significant
Cysteine	16.60 ± 0.07	1.32 ± 0.06	234.40	<0.0001	Significant

Value is mean ± standard deviation of duplicate determination

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

Parameter	***Moringa oleifera***	***Alchornea cordifolia***	**t-value**	**p-value**	**Comment**
Alkaloid (mg/g)	9.89 ± 0.19	6.21 ± 0.06	26.12	0.0015	Significant
Catalase (IU/ml)	0.03 ± 0.00	0.04 ± 0.01	1.000	0.4226	Not significant
Peroxidase (U/mg protein)	2.06 ± 0.22	1.02 ± 0.06	6.450	0.0232	Significant
Carotenoids (mg/g)	7.10 ± 0.96	3.85 ± 0.66	3.945	0.0587	Not significant
Vitamin E (mg/100g)	67.75 ± 3.38	31.56 ± 1.80	13.37	0.0056	Significant
Vitamin A (µg/g)	1887.27 ± 4.15	2116.36 ± 6.56	41.74	0.0006	Significant
Vitamin C (mg/100g)	297.6 ± 1.60	837.00 ± 4.50	159.70	<0.0001	Significant

Value is mean ± standard deviation of duplicate determination

Table 5 Lactate dehydrogenase activity (U/L) of sickle cell haemoglobin exposed to extracts of M. oleifera and A. cordifolia leaf samples

	LDH (U/L)
HbSS	290.59 ± 3.86a
HbSS + CIKLAVIT™	260.00 ± 5.98b
HBSS + M. oleifera 1000µg/ml	209.02 ± 4.18c
HBSS + M. oleifera 2000µg/ml	196.27 ± 3.33d
HBSS + M. oleifera 4000µg/ml	196.27 ± 3.12e
HBSS + A. cordifolia 1000µg/ml	112.16 ± 1.56f
HBSS + A. cordifolia 2000µg/ml	58.63 ± 1.27g
HBSS + A. cordifolia 4000µg/ml	43.33 ± 3.67h
HBSS + Combination 1000µg/ml	152.94 ± 2.64i
HBSS + Combination 2000µg/ml	79.02 ± 3.19j
HBSS + Combination 4000µg/ml	68.82 ± 3.11k

Table 6 Rate of sickle cell haemoglobin polymerization inhibition effect of methanol extracts of *A. cordifolia* and *M. oleifera* leaf extracts

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
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
100mg MO + AC	0.0029	35.80 ± 0.60	64.20 ±0.15
200mg MO + AC	0.0029	35.80 ± 0.56	64.20 ± 0.19
400mg MO + AC	0.0028	34.57 ± 1.32	65.43 ± 0.12

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* and *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 *A. cordifolia* and *M. oleifera* 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, and 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, and 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, and 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, and Xie, 2013; Wang, Feng, Li, Chen, Wang, Lan, Tang, Jiang, Zheng, and 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, and 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 and Carroll, 2000; Wilcox, Borradaile, Dreu and Huff, 2001; Borradaile, Dreu, Barrett, and Huff, 2002; Borradaile, Dreu, Barrett, Behrsin, and Huff, 2003; Allister, Borradaile, Edwards, and Huff, 2005; Nahmias, Goldasasser, Casali, Daan, Takaji, Raymond, and 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 and 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* and *M. oleifera* leaf extracts 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* and *M. oleifera* leaf extracts 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, and Mukherjee, 2016).

Alchornea cordifolia and *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 and 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.

Moringa oleifera and *Alchornea cordifolia* methanol leaf extracts both separately and in their combinations, were able to significantly reduce LDH levels in sickle cell disease patients, when compared to the standard (Ciklavit™). This positive effect of both leaves, may be as a result of their antioxidative effects of the natural compounds like polyphenols (quercetin, kaempferol, naringenin, silymarin, baicalin, butein, epicatechin, resveratrol, etc.), capable of inhibiting lactate dehydrogenase levels and activities, by binding to its active site (Han, Lee, Park, Ha, and Chung, 2023).

Antioxidant vitamins like vitamin c and vitamin E contained in contained in *Moringa oleifera* and *Alchornea cordifolia* methanol leaf extract may reduce LDH levels, potentially by decreasing oxidative stress and muscle damage. The above explanation conforms to previous research findings which showed that the antioxidative effects of vitamin C and vitamin E may play a role in the reduction of lactate dehydrogenase (Yanardag, Ozlem, and Bolkent, 2007; Traber and Stevens, 2011).

6. Conclusion

The work examined the biochemical effect of Moringa oleifera, Alchornea cordifolia and their combination leaf methanol extract on lactate dehydrogenase levels in sickle cell disease. The different concentrations of Alchornea cordifolia and Moringa oleifera leaf methanol extracts and their combination were able to reduce the lactate dehydrogenase enzymes in the blood significantly when compared to the control and standard. CiklavitTM was used as the standard for this study. The outcome of this study showed that both plant leaves and their combination 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] 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.

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

[14] 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

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

[16] Elekwa, I., Monanu, M.O., and Anosike, E.O. (2004). Effect of aqueous extracts of Garcinia Kola seeds on membrane stability of HbAA, HbAS, and HbSS human erythrocytes. Global Journal of Medical Science, 2003 Vol.2(2). Published May 21, 2004. Doi: 10.4314/gjms.v2:2.10116.

[17] Farhana, A., and Lappin, S.L. (2023). Biochemistry, Lactate Dehydrogenase. National Center for Biotechnology Information, 2023, May. Retrieved from, https://www.ncbi.nlm.nih.gov/books/NBK557536/#. StatPearls Publishing LLC. Bookshelf ID: NBK557536. PMID: 32491468.

[18] 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.

[19] Han, J.H., Lee, E., Park, W., Ha, K., and Chung, H. (2023). Natural compounds as lactate dehydrogenase inhibitors: potential therapeutics for lactate dehydrogenase inhibitors-related diseases. *Frontiers in Pharmacology*, 2023, Oct. 17; 14:1275000. Doi: 10.3389/fphar.2023.1275000. PMCID: PMC 10616500. PMID: 37915411.

[20] 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.

[21] 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

[22] Kauadio, I.S., Gunes, A.K., Quattara, K.E., Jozsef, J., Zoltan, C., Katalin, G., and Gokhan, Z. (2021). Deeper Insights on *Alchornea cordifolia* (Schumach & Thonn) Mull. Arg. Extracts. Chemical Profile, Biological Abilities, Network Analysis and Molecular Docking. *Journal of Biomolecule*. 2021; 11, 219. Doi:103390/biom 11020219.

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

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

[25] 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.

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

[27] 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.

[28] 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.

[29] 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.

[30] 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.

[31] 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.

[32] 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.

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

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

[35] 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.

[36] 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.

[37] 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.

[38] 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.

[39] 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.

[40] 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.

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

[42] 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.

[43] 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.

[44] 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.

[45] 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.

[46] 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.

[47] Rec. G.S.C.C., (1970). Determination of Lactate Dehydrogenase Activity using UV method. *Journal of Clinical Chemistry and Clinical Biochemistry,* 8, 658.

[48] Rec. G.S.C.C., (1972). Optimized standard colorimetric methods. Serum Alkaline phosphatase (DGKC). *Journal of Clinical Chemistry and Clinical Biochemistry,* 10, 182-182.

[49] 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.

[50] 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.

[51] 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.

[52] 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.

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

[54] 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.

[55] 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.

[56] 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.

[57] 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.

[58] Yanardag, R., Ozlem, O., and Bolkent, S. (2007). Combined Effect of Vitamin C, Vitamin E, and Sodium Selenate Supplementation on Absolute Ethanol-Induced Injury in Various Organs of Rats. *International Journal of Toxicology* 2007, Nov. vol 26(6). https://doi.org/10.1080/10915810701707296.

[59] 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.