OBAFEMI AWOLOWO UNIVERSITY

STUDIES ON THE ANTI-SICKLING ACTIVITIES OF SOME NIGERIAN MEDICINAL PLANTS

BY

FAPOJUWO, OMOLOLA TEMITOPE

B. Pharm. (Ife)

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (PHARMACOGNOSY)

IN

DEPARTMENT OF PHARMACOGNOSY,

FACULTY OF PHARMACY,

OBAFEMI AWOLOWO UNIVERSITY,

ILE-IFE, OSUN STATE,

NIGERIA.

MAY, 2015

OBAFEMI AWOLOWO UNIVERSITY

AUTHORIZATION TO COPY

OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE

HEZEKIAH OLUWASANMI LIBRARY

POSTGRADUATE THESIS

AUTHOR: FAPOJUWO, OMOLOLA TEMITOPE

TITLE: STUDIES ON THE ANTI-SICKLING ACTIVITIES OF SOME NIGERIAN MEDICINAL PLANTS.

DEGREE: M.Sc. (PHARMACOGNOSY)

YEAR: 2015

I, Miss Omolola Temitope FAPOJUWO, hereby authorize the Hezekiah Oluwasanmi Library to copy my thesis in whole/part, in response to request from individual researcher or organisation for the purpose of private study or research.

SIGNATURE DATE

CERTIFICATION

This is to certify that Miss Omolola Temitope Fapojuwo of the Department of Pharmacognosy, Faculty of Pharmacy carried out this research under the supervisions of Professor A.A. Elujoba and Dr J.M.Agbedahunsi. This was in accordance with the requirement for the award of Masters of Science (M.Sc.) in Pharmacognosy, Obafemi Awolowo University, Ile-Ife.

Professor A.A. Elujoba ----------

Supervisor's NameDate

Dr J.M.Agbedahunsi ----------

Co-Supervisor's Name Date

Drug Research and Production Unit,

O.A.U., Ile-Ife.

Prof. C. A. Adebajo----------

Head, Department of Pharmacognosy. Date

DEDICATION

I dedicate this project to GOD ALMIGHTY who is my strength, shield, pillar and fortress.

ACKNOWLEDGEMENT

I am eternally grateful to God for being my help and guide all through the course of this project.

I am grateful to my supervisor, Prof. A.A.Elujoba;co-supervisor,Dr J.M. Agbedahunsiand also Mrs C.M. Cyril-Olutayofor their invaluable contributions,care, support and patience during the course of this research.

I acknowledge TetFund for the research support through the grant given, to the Drug Research and Production Unit, to execute this project.

I appreciate the help and support of the Head of Department Prof. C. A. Adebajo, Dr.O. A.Igbeneghu, and the entire staff of the Department of Pharmacognosy, especially Mr.Ogunlowo, Mr.Oluwaniyi, Mr. Fadare, Mr.Adeyemi and also the entire staff of the Drug Research and Production Unit, especially Mr.Omodara for their time and support even when the environment was very uncondusive.

My sincere love and gratitude goes to YemiFabusuyi, huge thanks for your help and support, and all my colleagues:AkinwunmiOlubunmi, AkinrotohunOluwafunmilola and all others. I am thankful for the love, care, help, prayers and moral support you gave me through the course of this project. A great chunk of encouragement came from you.

My unfailing love and gratitude go to my sweet hearts Jide and Jeffrey, my family for the love, care, prayers and support given me. You got my back at all times in every way. What would I do without you?

I am thankful to you all and I love you all.

Fapojuwo Omolola Temitope.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

Abstract

The study screened some medicinal plants used in the ethnomedicinal management of sickle cell anaemia (SCA), fractionated the most active plant extract(s) and determined the fraction(s) with the highest anti-sickling activities. This was with a view to providing scientific information on the activities of the plants as anti-sickling agents.

The medicinal plants: *Anthocleista vogelii* Planch,*Cassia sieberiana* DC, *Harungana madagascariensis* Lam. ex. poir, *Mimosa pudica* Linn, *Morinda lucida* Benth, *Peperomia pellucida* Linn, *Senna alata* (L.) Roxb and *Spondias mombin* Linn, were collected, extracted and screened for anti-sickling properties. Three extractive methods were used viz: maceration

with 70 % cold ethanol in water (v/v), Soxhlet extraction with 70 % ethanol in water (v/v) and aqueous extraction (decoction). The extracts obtained were tested for inhibitory or reversal activities of erythrocytes (HbSS) *in vitro* at low oxygen tension using sodium metabisulphite. For the inhibitory activities: whole blood, phosphate buffer and extract was mixed together, liquid paraffin was added to the surface of the mixture and incubated after which sodium metabisulphite was added and further incubated. For the reversal activities: whole blood, phosphate buffer and sodium metabisulphite was mixed together, liquid paraffin was added to the surface of the mixture and incubated after which the extract was added and further incubated. Theaqueous extract of the whole fruit and the 70 % (v/v) ethanol Soxhlet extract of the pericarp of *C. sieberiana* were each fractionated using Vacuum Liquid Chromatography (V.L.C). All the fractions were screened for their anti-sickling properties. Thin Layer Chromatography (T.L.C) of the fractions in various solvent systems, followed by detection with chromogenic spray reagents was carried out.

The results obtained for inhibitory anti-sickling activities were as follows: 70 % (v/v) cold ethanol extract of *P. pellucida* leaf (54.1 %), 70 % (v/v) ethanol soxhlet extracts of *M. lucida* leaf (51.5 %) and *C. sieberiana* (seed, 55.6 % and whole fruit, 55.1 %). The 70 % (v/v) ethanol soxhlet extract of *C. sieberiana* pericarp gave 71.3 % inhibitory activity while for the reversal anti-sickling activities, the results were as follows: 70 % (v/v) ethanol extract of *A. vogelii* stem bark (61.1 %),70 % (v/v) ethanol Soxhlet extracts of *H. madagascariensis* (52.1 %),*S. alata* leaf (84.0 %), and aqueous extract of *C. sieberiana* whole fruit (90.2 %). Following the VLC of *C. sieberiana* whole fruit aqueous extract which exhibited the highest reversal anti-sickling activity, the dichloromethane fraction of its aqueous extract gave 79.6 % inhibitory and 70.1 % reversal activities, respectively. The ethyl acetate fraction of the 70 % (v/v) ethanol Soxhlet extract of its pericarp gave 87.1 % inhibitory and 79.7 % reversal

activities. These two fractions exhibited the most considerable anti-sickling activities among the other fractions and the crude extracts of the plants.

The study concluded that there is scientific justification for the folkloric use of *C. sieberiana,A. vogelii*, *S. alata*, *P. pellucida, H. madagascariensis* and *M. lucida* in the management of SCA.

Keywords : ethnomedicinal, sickle cell anaemia, scientific information, metabisulphite, Soxhlet extraction, *sieberiana* pericarp

Supervisor: Prof. A.A.Elujoba

xvii, 135p

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Sickle cell anaemia is a genetic disease condition, an inherited disorder in which the quality of haemoglobin is defective. It is an autosomal dominant anaemia characterised by crescentric or sickle-shaped erythrocytes with accelerated haemolysis. It stems out from inadequate oxygen transport by an abnormal type of haemoglobin molecule in the red blood cells. Sickle cell anaemia is characterised by abnormal haemoglobin which results in the abnormally shaped (sickled) red blood cells. Haemoglobin is the protein molecule in the red blood cell that carries oxygen from the lungs to the body tissues and it plays an important role in maintaining the shape of the red blood cell. Normal red blood cells are disc shaped and look like doughnuts without holes in the center but this shape is distorted in sickle cell anaemia manifestations. The iron rich protein gives blood its red colour (Eaton and Hofrichter, 1987).

The sickle cell disease was first described by a Chicago physician, James B. Herrick, who noted in 1910 that a patient of his from the West Indies had an anaemia characterized by unusual red cells that were "sickle" shaped. Sickle cell disease also known as drepanocytosis is prevalent among the people of African ancestry and among the Indians. It occurs as a result of the copulation of parents with homozygous sickle cell gene (SS) or the heterozygous gene (AS) (Pauling and Itano, 1949).

Today, search remains in progress for the cure of sickle cell disease besides the current forms of management such as non-therapeutic, chemotherapeutic, surgical and ethnomedical forms aimed

at ameliorating the pain during sickle cell crises. The most effective method of management is still the method of advocacy and genetic counseling through public and private enlightenment. Since a large population of the sufferers exist coupled with the fact that only a few will heed to advice, it is very important to research into the drug development from herbs that can reverse or inhibit the sickling process (Modell and Darlison, 2008).

Thus, this research was designed to investigate the anti-sickling properties of *Anthocleista vogelii* Planch (Gentiaceae), *Cassia sieberiana* DC (Leguminoseae), *Dioclea reflexa* Hook. F (Papilionaceae), *Harungana madagascariensis* Lam. ex. Poir (Hypericaceae), *Lecanioidiscus cupanioides* Planch ex Benth (Sapindaceae), *Mimosa pudica* Linn (Mimosaceae), *Morinda lucida* Benth (Rubiaceae), *Peperomia pellucida* Linn (Piperaceae), *Senna alata* (L.) Roxb (Leguminoseae) and *Spondias mombin* Linn (Anacardiaceae).

1.2. GENETIC / MOLECULAR NATURE OF SICKLE CELL ANAEMIA.

Sickle cell anaemia is a genetic disorder and a type of anaemia in which the blood contains a lower than normal number of red blood cells. Red blood cells are made in the spongy marrow inside the large bones of the body and the bone marrow is always making new red blood cells to replace the old ones. The name haemoglobin reflects the fact that each subunit of haemoglobin is a globular protein with an embedded haem group. In adult humans, the most common haemoglobin is a tetramer (contains 4 subunit proteins) called haemoglobin A, consisting of two alpha and two beta subunits non-covalently bound. This is denoted as $\alpha 2\beta 2$. The subunits are structurally similar and about the same size. The haem group consists of an iron atom held in a heterocyclic ring, known as porphyrin. This iron atom is the site of oxygen binding. The iron atom binds equally to all four nitrogen's in the center of the ring, which lie in one plane. Oxygen is then able to bind to the iron center perpendicular to the plane of the porphyrin ring. A

decreased level of haemoglobin, with or without an absolute decrease of red blood cells, leads to symptoms of anaemia (Pauling and Itano, 1949).

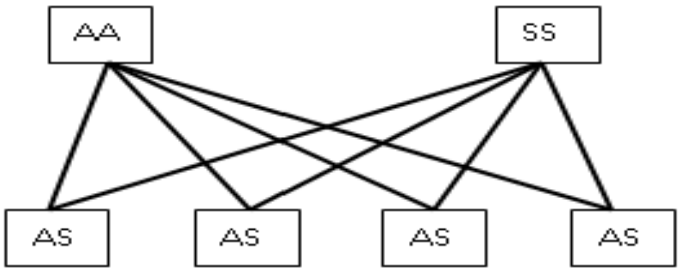
The deoxyribonucleic acid (DNA) molecule is the fundamental genetic material that determines the arrangement of the amino acid building blocks in all proteins. Segments of DNA that code for particular proteins are called genes. Each haemoglobin variation is the product of an altered gene. The variant genes are called alleles. The gene that controls the production of the beta globin subunit of haemoglobin is located on one of the 46 human chromosomes (chromosome #11). Humans have twenty-two identical chromosome pairs (the twenty-third pair is the unlike X and Y chromosomes that determine a person's sex). One of each pair is inherited from the father, and one from the mother. Occasionally, a gene is altered in the exchange between parent and offspring. This event, called mutation, occurs extremely rarely. Mutations in the gene for the haemoglobin protein result in a group of hereditary diseases termed the haemoglobinopathy; one of the most common examples is sickle-cell disease. The sickle cell mutation reflects a single change in the amino acid building blocks of the oxygen-transport protein, haemoglobin. The alpha subunit of haemoglobin is normal in people with sickle cell disease. The beta subunit has the amino acid valine at position 6 instead of the glutamic acid in a normal individual. Hence, it is the change of an amino acid in position six within the beta globin chain of haemoglobin molecule whereby glutamic acid, a polar amino acid is replaced by valine, a non-polar amino acid (Pauling and Itano, 1949; Ingram, 1957) that results in this genetic disorder. The amino acid change is due to the defective gene (mutation) in chromosome 11 and the alteration is the main basis for all the problems that occur in people with sickle cell disease. The inheritance of sickle cell disease depends totally on the genes of the parents. If only one of the beta globin genes is the "sickle" gene and the other is normal, the person is a carrier for sickle cell disease (HbAS).

The condition is called sickle cell trait. With a few rare exceptions, people with sickle cell trait are completely normal. If both beta globin genes code for the sickle protein, the person has sickle cell disease. Sickle cell disease is determined at conception, when a person acquires his/her genes from the parents. Sickle cell disease cannot be caught, acquired, or otherwise transmitted. Also, sickle cell trait does not develop into sickle cell disease. The condition is passed to offspring only if both parents carry the genetic trait and statistically one in four of their children will be a 'sicklier' (Figure 1 ***(i), (ii), (iii)***).

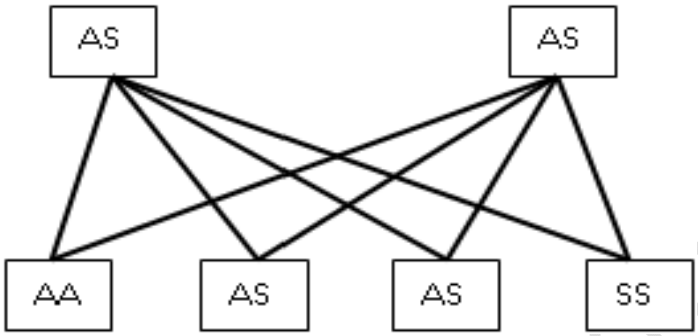
1.3. THE SICKLING PROCESS.

Ordinarily, the haemoglobin molecules exist as single, isolated units in the red blood cell, whether they are bounded to oxygen or not. Normal red blood cells maintain a basic disc shape, whether they are transporting oxygen or not. The red blood cells with mutated haemoglobin molecules exist as isolated units in the red cells when they are bounded to oxygen, whereas when the oxygen is released in the peripheral tissues, the molecules tend to stick together and form long chains or polymers. These rigid polymers distort the cell and cause it to bend out of shape. While most distorted cells are simply shaped irregularly, a few have a crescent-like appearance under the microscope. These crescent-like or "sickle shaped" red cells gave the disorder its name. Normal haemoglobin exists as solitary units whether oxygenated or deoxygenated. At low oxygen tension, the mutant haemoglobin polymerizes inside the Red Blood Cells (RBCs) into a gel or further into fibres leading to a drastic decrease in the red cell deformability.

(*i*)



(ii)



(iii)

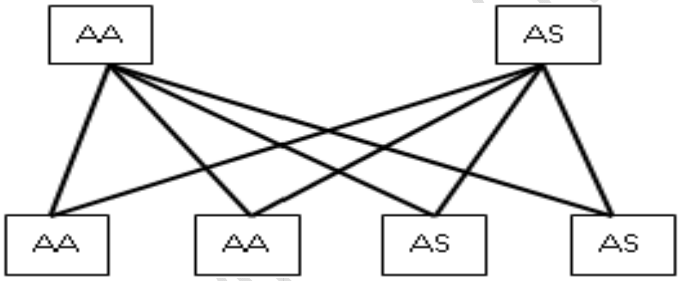


FIGURE 1. Some examples of Inheritance Patterns for Sickle Cell Anaemia Genotype.

KEY: A-Normal Haemoglobin, S-Abnormal Haemoglobin, AA-Normal, AS-Carrier of Sickle Cell Trait, SS-Patient with SCD.

Polymerization and precipitation of sickle haemoglobin (HbS) within the erythrocytes cause the change of shape from the normal spherical form into the one resembling a sickle, hence the name sickle cell. A single nucleotide substitution (Thymine [T] for Adenine [A]) allows HbS to

polymerize when deoxygenated, since valine can dock with the complimentary sites on the adjacent globin chains (Eaton and Hofrichter, 1987).

The fundamental cause of SCD is the decreased deformability of the sickled RBCs produced by gelation of haemoglobin S molecules during de-oxygenation. The deformation of sickle red cells upon complete de-oxygenation is due to the intracellular HbS polymerization. The gelation or polymerization is initiated by nucleation of a single polymer. Two types of HbS nucleation, i.e., homogeneous and heterogeneous have been observed. The nucleation is due to the aggregation of haemoglobin S molecules. Once a certain size or the critical nucleus is reached other monomers of HbS add endlessly to form a very large polymer. Then due to the heterogeneous nucleation new polymers are formed on the surface of the pre-existing polymer. Individual polymer is a fibre which is made up of 14 inter-wined helical strands of HbS molecules of seven inter-wined double strands. In each molecule, one of the two β^6 valines of the α_2 β_2 tetramer is involved in an intermolecular contact with its neighbour in the double strand (Eaton and Hofrichter, 1987). The polymerization of deoxy-HbS leads to erythrocyte deformation from a biconcave morphology into the sickle shape. Two types of contacts occur between deoxygenated HbS tetramers in the double-stranded fibres. The contacts along the long axis of the fibres are termed axial contacts, whereas contacts along the sides of tetramers are termed lateral contacts. The amino acid valine at beta six position plays a crucial role in the lateral contact by interacting with the hydrophobic beta 85 th phenylalanine and beta 88 th leucine on a neighbouring tetramer. The axial contact is the interaction of the beta 22nd glutamic acid with an alpha 20th histidine on an adjacent tetramer (Levasseur *et al.,* 2004). Plate 1 shows the changes that occur as sickle or normal red cells release oxygen in the microcirculation. The upper panel shows that normal red cells retain their biconcave shape and move through the smallest blood vessels (capillaries)

without problem. In contrast, the haemoglobin polymerizes in sickle red cells when they release oxygen, as shown in the lower panel. The polymerization of haemoglobin deforms the red cells. The problem, however, is not simply one of abnormal shape. The membranes of the cells become rigid due to repeated episodes of haemoglobin polymerization/depolymerisation as the cells pick up and release oxygen in the circulation. These rigid cells fail to move freely through the small blood vessels, blocking local blood flow to a microscopic region of tissue. The incidence of anaemia in sickle cell disease is caused by red blood cell destruction, or haemolysis. The production of red cells by the bone marrow increases dramatically, but is unable to keep pace with the destruction. Red cell production increases by five to ten-folds in most patients with sickle cell disease. The average half-life of normal red cells is about 40 days with life span of about 120 days. In patients with sickle cell disease, this value can fall to as low as four to five days as the life span for the sickle cell is about 10-20 days (Pauling and Itano, 1949).

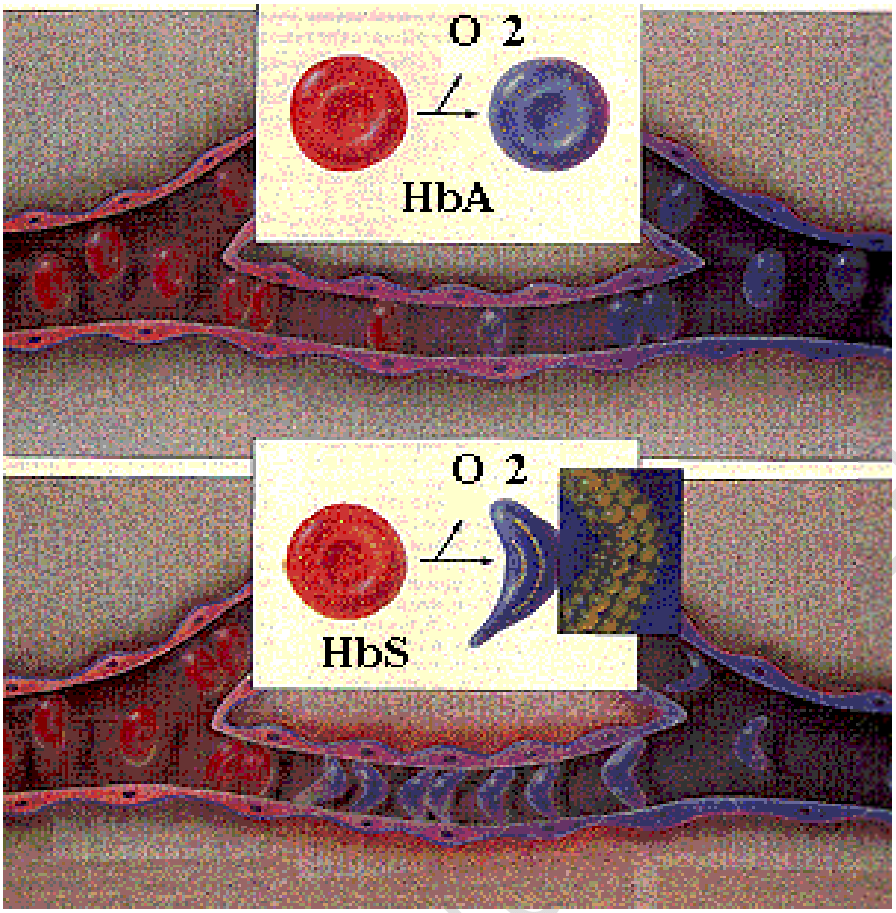


Plate 1. Capillary Flow of Normal (upper panel) and Sickled red blood cells (lower panel) (Heidi, 2001).

1.4. PREVALENCE OF SICKLE CELL ANAEMIA.

Sickle cell disease is seen most commonly in people of African ancestry and in the tribal peoples of India. Africans have peculiar names describing the symptoms of the disease in their tribal

languages such as “Chwechweechwe” by the Ga tribe in Ghana, “Adep” by the Banyangi tribe in Cameroun, and “Aromoleegun” by the Yoruba in Nigeria (Konotey-Ahulu, 1974). Clinically significant sickle cell syndromes also occur in people of the Middle Eastern background but the disease is most prevalent in the black races. Though sickle cell anaemia affects millions of people worldwide, it is most common in people whose families come from Africa, South or Central America (especially Panama), Caribbean islands, Mediterranean countries (such as Turkey, Greece, and Italy), and Saudi Arabia. It affects about 8 % of the U.S. black population depicting it affects African Americans. The disease occurs in about 1 out of every 625 African American births. Sickle cell anaemia also affects Hispanic Americans. The disease occurs in 1 out of every 36,000 Hispanic American births. It has been established that about 24 % of the entire population of Nigeria or 1 in 4 Nigerian men and women are healthy carriers of the sickle cell trait. About 2 % of all babies born to Nigerian parents have sickle cell anaemia. However, this prevalence is only at birth and progressively decreases through late childhood, adolescence and adulthood (WHO, 1994). Two per hundred births translates to over 150,000 births annually of children with sickle cell anaemia (Luzzatto, 1975; Evans, 1944).

The prevalence of sickle cell anaemia among the blacks has been attributed to the prevalence of malaria among the blacks. The distribution of indigenous sickle cell disorder coincides with the present or past distribution of *Plasmodium falciparum* malaria. However, it does not occur in the cooler, drier climates of the highland regions of the world. Neither does the gene for sickle haemoglobin. The immune system, the human defence mechanism, and pathogenic organisms have been in constant struggle over the ages, with the outcome being health due to immunity or illness. Over time, in an attempt to defend and adapt to the environment and to pathogenic attack, a few spontaneous alterations occur in DNA called mutations. Sickle haemoglobin

provides the best example of a change in the haemoglobin molecule that impairs malaria growth and development. Sickle cell trait provides a survival advantage over people with normal haemoglobin in regions where malaria is endemic. The protective effect of HbAS was remarkably specific for falciparum malaria (Williams *et al*., 2005). However, the sickle cell trait provides neither absolute protection nor invulnerability to the disease. Rather, people (and particularly children) infected with *Plasmodium falciparum* are more likely to survive the acute illness if they have sickle cell trait. This is because possession of the sickle cell trait (HbAS) confers a natural protection from malaria deaths. In any unprotected community in Nigeria, many children with HbAA will succumb to malaria before the age of 5 years. Those with the sickle cell trait will have milder malarial syndromes and survive the infection. Thus, a higher proportion of carriers would live to reproduce and pass on the sickle gene to their offspring. Those who inherit HbAS are thus better fitted for survival in this environment than those who inherit HbAA. Malaria does not select for sickle cell disease but for the sickle cell trait. A person with sickle cell disease is at an extreme survival disadvantage because of the problems the disease comes with.

A study of population genetics has shown that the eradication of falciparum malaria, by eliminating the reproductive advantage of HbAS carriers, predictably leads to a gradual dilution of the S gene pool within a population. Hence, in South Africa and southern Mozambique, both of which lie within the temperate non-malaria zone of sub-Saharan Africa, the S gene frequency is so low that sickle cell anaemia is not perceived there as a problem. Carriers of the trait (HbAS) do not exceed 0.3 % of the Bantu population in these areas in contrast to the much higher prevalence among Bantus in northern Mozambique and in countries lying to the north of the Zambezi River. The same argument is advanced for the lower S gene frequencies in African-

Americans (AS 8 %) and African -West Indians (AS 10 %). Thus, the ultimate control of the S gene within a population is linked to the eradication of malaria in that population. The problem in the affected countries in Africa is that malaria is not being controlled. Professor Luzzatto has estimated that, even if malaria were controlled, it would still take some 300 years for the gene frequency to be reduced by half. As a strategy for reducing the incidence of sickle cell anaemia, it is too slow to be appealing to countries in which sickle cell disorder is a significant public health problem (Evans, 1944; Altmann, 1945; Bernstein, 1969; Luzzatto, 1975).

In summary, Sickle cell trait (genotype HbAS) confers a high degree of resistance to severe and complicated malaria (Allison, 1964; Willcox *et al.,* 1983; Hill *et al.,* 1991; Aidoo *et al.,* 2002) yet the precise mechanism remains unknown. To some extent it almost certainly relates to the peculiar physical or biochemical properties of HbAS red blood cells: invasion, growth, and development of *Plasmodium falciparum* parasites are all reduced in such cells under physiological conditions in vitro and parasite-infected HbAS red blood cells also tend to sickle, a process that may result in their premature destruction by the spleen (Luzzatto *et al.,* 1970; Friedman, 1978; Pasvol *et al.,* 1978; Roth *et al.,* 1978; Shear *et al.,* 1993). Sickle cell trait is the genetic condition selected for in regions of endemic malaria thus the prevalence of sickle cell disease which is a necessary consequence of the existence of the trait condition because of the genetics of reproduction.

1.5. SIGNS, SYMPTOMS AND COMPLICATIONS OF SICKLE CELL ANAEMIA.

The symptoms of sickle cell anaemia arise as a result of vaso-occlusion in the small blood vessels by the sickled cells due to insufficient oxygen supply to the tissues at the body extremities. The symptoms and complications of this condition vary genetically from patient to patient, some patients at some point need to be hospitalized while others have mild symptoms.

Sickle cell anaemia is present at birth, but many infants don't show any signs until after 4-6 months of age or more in some patients. The most common signs and complications are linked to anaemia and pain, others are;

- Fatigue.
- Shortness of breath.
- Headache.
- Chest pain.
- Dizziness.
- Pale skin.
- Coldness of hands and feet.
- Sudden pain all through the body.

The pain is termed crisis and could either be acute or chronic, affecting the joints, bones and other organs. Acute pain is sudden and can range from mild to very severe. The pain usually lasts from hours to a few days and chronic pain often lasts for weeks to months. Chronic pain can be hard to bear and mentally draining and may severely limit the patients' daily activities. All the people who have sickle cell anaemia have crises at some point in their lives. Crisis could also be described as haemolytic, characterised by rapid destruction of the red blood cells or aplastic, an abrupt arrest of red blood cell production (Whitten and Bertles, 1989). Some have these crises less than once a year while others may have 15 or more crises in a year and because sickle cells block the blood vessels, in various organs and other parts of the body and due to repeated crisis, complications such as the following could arise;

- Splenic crisis.
- Acute chest syndrome.
- Pulmonary arterial hypertension.
- Hand and foot syndrome.
- Stroke.
- Delayed growth and puberty in children.
- Eye problems.
- Priapism.
- Gallstones.
- Ulcers on the leg.
- Multiple organ failure.

Sickle cell patients especially infants and young children are prone to various infections such as pneumonia, influenza, meningitis and hepatitis (Gaston *et al.,* 1986; Vichinsky *et al.,* 2000).

1.6. DIAGNOSIS OF SICKLE CELL DISEASE.

Sickle cell disease can be diagnosed *in utero* before a child is born (prenatal diagnostics), newborns, or older persons through haemoglobin electrophoresis, isoelectric focusing, high-performance liquid chromatography or DNA analysis. In general, since the various tests have comparable accuracy, the testing method of choice should be selected on the basis of local availability and cost.

Solubility testing methods (Sickledex, Sick-lequik) and sickle cell preparations are inappropriate diagnostic techniques. Although these tests identify sickle haemoglobin, they miss haemoglobin

C and other genetic variants. Furthermore, solubility testing is inaccurate in the newborn, in which fetal haemoglobin is overwhelmingly predominant. Solubility testing methods also fail to detect sickle haemoglobin in persons with severe anaemia. Although haemolysis is a feature of all forms of sickle cell disease, patients with haemoglobin SC disease or sickle β^{+}-thalassemia may not have significant anaemia. Thalassemia is suspected if microcytosis or hypochromia is present in the absence of iron deficiency. A haematologist can assist in the often difficult task of determining the exact type of sickle cell disease, especially in the presence of rarer haemoglobin variants. If both parents are accessible, studies of parental blood can aid in the diagnosis of sickle cell disease in the child. DNA analysis provides the most accurate diagnosis in patients of any age, but it is still relatively expensive and unaffordable (Dierdorf, 1996).

1.7. MANAGEMENT OF SICKLE CELL ANAEMIA.

Sickle cell anaemia is not known to have any major cure. The major essence of management has been to make life easier and more comfortable for the patients; helping them to manage their pains. Educating the general public i.e. genetic counseling, is of utmost importance as the prevention of HbS carriers from marrying each other would prevent the propagation of the genotype HbSS. Prospective HbS couples should be advised against marrying each other, having children of HbSS and the consequences of refusal. Studies are being carried out all over the world to find the most suitable prophylaxis against painful and complicated crises.

The reversal of the sickling process has been difficult up to date and as such more emphasis is placed on prevention. Measures to protect the patient with sickle cell disease from a potential vaso-occlusive crisis include ensuring adequate hydration, anticipating blood loss, blood transfusion and fluid shifts, providing systemic oxygenation and avoiding acidosis, maintenance

of normothemia and meticulous positioning in order to prevent circulatory stasis (Dierdorf, 1996).

There are 2 forms of management;

1.7.1. Non Therapeutic Management.

With good health care and lifestyle, many people who have sickle cell anaemia can live productive lives. People with sickle cell anaemia are advised to adopt a healthy life style. They are advised also to avoid alcohol, smoking and also maintain a good eating habit. Plenty of water is needed to prevent dehydration. To prevent complications, patients should avoid extremes of heat and cold, decongestants such as pseudoephedrine. Patients can manage pain by taking a hot bath, using a heating pad and getting enough rest. Counseling and self-hypnosis may help (Muskiet *et al.,* 1991).

Diet is also very important in the management of sickle cell anaemia. A lot of important body constituents are lacking in sickle cell patients hence the severity of their crisis. Examples include vitamins B_6, B_{12}, C and E, magnesium and zinc. The consumption of foods and fruits that contain all the above help to prolong the time between episodes of crisis and examples of sources are cassava, yams, sweet potatoes, corn, lima and soya beans oils, fish oils, carrots, tomato; fruits such as red grapes, grapes, tangerine, oranges, eucalyptus, spruce, peanuts, e.t.c.; some of these sources are known to be rich in antioxidants. Grape fruits and tangerine seeds can inhibit lipid peroxidation. The citruses contain considerable amounts of limonenes, monoterpenes, carotinoids, and flavonoids which prevent various diseases such as anaemia, cardiovascular diseases, cancer (Muskiet *et al.,* 1991) and other complications of sickle cell anaemia. Retarded growth is a major problem with children with sickle cell anaemia; zinc deficiency is associated with growth retardation and there is some evidence that people with

sickle cell anaemia are more likely than others to be deficient in the mineral zinc and as such, zinc supplementation at nutritional doses has been suggested for children with sickle cell anaemia (Zemel *et al.,* 2002). Thiocyanate has been proven to greatly reduce the sickling of red blood cells and it might completely prevent sickling in many individuals. A diet high in organic iron, cleansing natural chlorophyll, and the anti-sickling nutrient, thiocyanate has been said to reduce the episodes of crisis. Foods and herbs high in natural iron include: green leafy vegetables such as greens and green leaf lettuce (not ice berg), yellow vegetables such as squash, whole grain products made from grains such as spelt, barley, millet, sorghum and oats, legumes, lentils, kidney beans, dandelion, burdock, yellow dock root, strawberry fruit, and cayenne. African yam and cassava are believed to be the richest sources of thiocyanate; other sources of thiocyanate are: millet, buckwheat, lima beans, greens, cabbage, carrots, cashew nuts, cauliflower, strawberries, lentils, broccoli, chickpeas, plantain (a type of banana), and sorghum. Foods that are high in nitric oxide, or the amino acid L-Arginine, help to relax the blood vessels and are very beneficial (Ana Kirk, 2010).

1.7.2. Therapeutic Management.

The therapeutic management of sickle cell anaemia could be

- Chemotherapeutic.
- Surgery and Transfusion.
- Ethnomedical.

When considering the chemotherapeutic and ethnomedicinal forms of therapeutic management, the genetic characteristics of the biological test system in this case the red blood cell should be considered as studies in pharmacogenetics has shown that the effect of any plant, plant extract, synthetic or semi-synthetic product or combination of any of the above would depend on the these genetic characteristics. The genetic variation in the red blood cell with respect to the enzyme Glucose-6-Phosphate Dehydrogenase (G6PD), that is, red blood cells or erythrocytes with different variants of this enzyme behave differently and the differences are not confined to the physiology of the cell but also to the susceptibility of the cell to infections and drugs. It was observed that patients who developed acute haemolytic anaemia after taking certain antimalarial drugs such as primaquine, had much lower than normal level of Glucose-6-Phosphate Dehydrogenase i.e. they were G6PD deficient (Luzzato *et al.,* 1970).

Variance in G6PD include differences in the structure of the enzyme and enzyme properties, differences in the gene for this enzyme, since the level of the enzyme is genetically determined by a sex linked gene, and how it affects the enzyme itself, the red blood cells that carry the enzyme and the people that carry the red blood cell. Many other gene products inside or on the membrane of the red blood cell could exhibit genetic variation and thus affect or determine the patients susceptibility to drugs and medicinal agents (Luzzatto *et al.,* 1970).

1.7.2.1. Chemotherapeutic Management.

Antibiotics are given to most children with sickle cell until age 5, e.g. penicillin to prevent infections. It is necessary to give supplemental folic acid for erythropoiesis and thus replenish the depleted folate stores secondary to haemolysis. Folic acid, an important cofactor for RBC production, is necessary for proper nucleotide metabolism. Reactivation of fetal hemoglobin (HbF) expression is an important therapeutic option in patients with hemoglobin disorders. In

sickle cell disease (SCD), an increase in HbF inhibits the polymerization of sickle haemoglobin and the resulting pathophysiology. During the last two decades, considerable efforts have been focused on the pharmacological induction of HbF in patients with haemoglobin disorders. Multiple drugs including 5-azacytidine (and decitabine), hydroxyurea, butyrate and erythropoietin were shown to induce HbF *in vivo* in animal models and in patients with these disorders. In spite of the incomplete understanding of the mechanisms of induction of HbF by these agents, considerable progress has been made in developing these agents as drugs that induce HbF in patients with SCD. Drugs that are presently used include;

- 5-Azacytidine: 5-Azacytidine was the first prototype of an agent that induces HbF by targeting epigenetic silencing. It was shown to induce very high levels of HbF in anemic baboons. This agent was never tested in large-scale clinical trials due to concerns about its potential carcinogenic effects, since a previous study conducted in laboratory rats showed that it increased the incidence of tumors (Carr *et al*., 1988).
- Decitabine: Decitabine (trade name Dacogen), or 5-aza-2'-deoxycytidine, is a drug used for the treatment of myelodysplastic syndromes (a class of conditions where certain blood cells are dysfunctional) and for acute myeloid leukemia (AML). Chemically, it is a cytidine analog. It hypomethylates DNA by inhibiting DNA methyltransferase (Kantarjian *et al.,* 2003; Kantarjian *et al.,* 2006). It is known to increase haemoglobin F levels which are a type of haemoglobin that carries more oxygen. The increase in the level of HbF was associated with significant improvement in several parameters that are important in the pathophysiology of vaso-occlusion such as red blood cell adhesion, endothelial damage and activation of the coagulation pathway. An early study reported that this agent was not carcinogenic in the rat model (Carr *et al*., 1988). More interestingly, recent studies have shown that treatment of mice with a genetic disposition

for colon or lung cancer with decitabine results in a marked reduction in tumor formation (Laird *et al.,* 1995; Belinsky *et al.,* 2003). Thus, these studies suggested that decitabine may provide potential chemoprevention for certain cancers. Larger and longer-term studies are clearly needed to confirm the safety and efficacy of decitabine in patients with SCD.

- Butyric acid: This is a food additive that may increase normal haemoglobin in the blood (Laird *et al.,* 1995).

- Nitric oxide: This is known to make sickle cells less sticky and also keep blood vessels open. People with sickle cell anaemia have low blood levels of nitric oxide. Inhaled nitric acid is still being investigated for its role in the prevention and treatment of vaso-occlusion due to sickled cells (Laird *et al.,* 1995).

- Hydroxyurea: This is an orally active drug used to prevent painful crisis and induce HbF levels. It increases total and fetal haemoglobin in children with sickle cell disease. It also reduces levels of circulating leukocytes, which decreases the adherence of neutrophils to the vascular endothelium. In turn, these effects reduce the incidence of pain episodes (Heeney *et al.,* 2008) and acute chest syndrome episodes (Brawley *et al.,* 2008). Hydroxyurea is a potentially leukemogenic and carcinogenic agent. Children, in a study by a cooperative group, were reported to have remained on hydroxyurea for more than a year with only minor adverse effects, but potential complications from long-term use are not yet known. Considerable effort is being expended to identify agents whose ultimate effect interferes with the sickling process and prevents the many complications of sickle cell disease (Laird *et al.,* 1995).

Pain management, a major challenge in SCD includes 4 stages: assessment, treatment, reassessment, and adjustment. While considering the severity of pain and the patient's past response, the following consistent protocols are used to relieve the patient's pain. The goals of

treatment are symptom control and management of disease complications. Treatment strategies include the following 7 goals:

- Management of vaso-occlusive crisis
- Management of chronic pain syndromes
- Management of chronic haemolytic anemia
- Prevention and treatment of infections
- Management of the complications and the various organ damage syndromes associated with the disease
- Prevention of stroke
- Detection and treatment of pulmonary hypertension

The following classes of anti-inflammatory drugs are administered to manage pain;

- Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, diclofenac e.t.c.
- Acetaminophen (paracetamol).
- Narcotics e.g. meperidine, morphine (preferably Diamorphine, fentanyl, hydromorphone, and methadone), oxycodone, e.t.c. narcotic abuse and addiction are considerable issues.

All chemotherapeutic agents come with side effects and as such other ways of managing the condition are necessary (Muskiet *et al.,* 1991).

1.7.2.2. Surgery and Transfusion

Research on bone marrow transplant and gene therapy is ongoing. Gene therapy continues to be studied as a way to inactivate the sickle gene, to increase expression of the gene for haemoglobin F or to introduce genes whose products can inhibit the polymerization of haemoglobin S. Bone marrow transplant involves a transplant from a closely matched donor usually a family member but the procedure carries a lot of risks. Allergenic bone marrow transplantation (BMT) can cure SCD, but it is difficult to decide which patients should be offered BMT. The risk-to-benefit ratio must be assessed carefully for each patient. With the advent of cord blood stem cell transplantation and with the development of less immunoablative conditioning regimens, perhaps BMT will gain wider acceptance and use. Long-term follow-up is problematic, and the procedure is expensive and not widely available. Another serious barrier to the use of BMT is the frequent lack of a human leukocyte antigen–matched sibling as donor in the individual case (Walters *et al.,* 1996).

The use of cord-blood stem cells might prevent some of the problems of bone marrow transplantation (Brichard *et al.,* 1996). Non-ablative marrow infusion, rather than total marrow replacement, also shows promise. Other treatment modalities currently being studied include arginine butyrate to enhance fetal haemoglobin production; poloxamer 188, a nonionic surfactant, to reduce the length of pain crises (Adams-Graves *et al.,* 1997) and nitric oxide, to manage acute chest syndrome (Head *et al.,* 1997).

Acute red cell exchange transfusion is indicated in the following situations: Acute infarctive stroke, severe acute chest syndrome, multi-organ failure syndromes, right upper quadrant syndrome, priapism that does not resolve after adequate hydration and analgesia. Transfusion-related complications include alloimmunization, infection, and iron overload. Treatment of iron overload is becoming easier with the new oral chelators. Desferrioxamine is an efficient

chelator. However, it needs to be given parenterally or subcutaneously by prolonged infusion and nearly every day (5 days a week), which has limited its effectiveness in many patients (Cappellini *et al.,* 2008). Deferasirox has a capacity similar to desferrioxamine in chelating iron, but it is administered orally. Renal toxicity might be a limiting factor in its use, but it is generally safe. Deferiprone does not seem to be as effective as the other 2 agents but can be taken orally and selectively removes cardiac iron. It might be most effective when used with desferrioxamine or deferasirox. It is used as a second-line therapy. A novel new iron chelator is being developed but is still in the clinical testing phase (Rienhoff *et al.,* 2011). Erythrocytapheresis is an automated red cell exchange procedure that removes blood that contains HbS from the patient while simultaneously replacing that same volume with packed red cells free of HbS (Das *et al*., 2008). Transfusion usually consists of sickle-negative, leuco-reduced, and phenotypically matched blood for red cell antigens C, E, K, Fy, and Jkb. The procedure is performed on a blood cell processor (pheresis machine) with a continuous-flow system that maintains an isovolemic condition. RBCs are removed and simultaneously replaced, with normal saline followed by transfused packed RBCs along with the patient's plasma. The net RBC mass/kg is calculated for each procedure based on the measured hematocrit of the transfused and removed blood and the total RBC volume transfused. Erythrocytapheresis thus has the advantage of controlling iron accumulation in patients with SCD who undergo long-term transfusion, as well as the ability to achieve adequate Hb and HbS concentrations without exceeding the normal concentration. This precision is achieved because, before the start of the transfusion, the computer in the pheresis machine calculates the expected amount of packed RBCs required to obtain a specific post-transfusion haemoglobin level, using various physiologic parameters (e.g., height, weight, Hb level). Furthermore, erythrocytapheresis requires less time than simple transfusion of similar blood volumes. Although erythrocytapheresis itself is more expensive than simple transfusion,

the additional costs associated with simple transfusions (i.e. those of chelation and organ damage due to iron overload) make erythrocytapheresis more cost-effective than simple transfusion programs. Central venous access devices can safely be used for long-term erythrocytapheresis in patients with SCD with a low rate of complications (Das *et al.,* 2008).

1.7.2.3. Ethnomedicinal Management of SCD

Nature has been known to provide solutions to various ailments, infections and non-infectious diseases. It is a widely known and accepted fact that a large segment of the population in tropical countries rely on traditional medicine for their health needs. The practices are well established in China, India, Europe, and to some extent Africa (Oduola *et al.,* 2006).

The use of natural products in treatment and management of sickle cell disease could be as old as the disease, though very few ethnobotanical remedies for its treatment have been reported in the literature due to secrecy attached to the indigenous knowledge of treatments of the disease (Egunyomi *et al.,* 2009). However the efficacy of some ethnobotanical remedies in the management of sickle cell disease has been reported.

Many plants are used in ethnomedicine for the management of sickle cell disease. These include *Acacia xanthophloea* (Leguminosae) (Kunle and Egharevba 2013), *Carica papaya* (Caricaceae) (Oduola *et al.,* 2006; Ogunyemi *et al.,* 2008), *Sorghum bicolor* (Poaceae) (Ogunyemi, 2009), *Lawsonia inermis* (Lythraceae) (Kunle and Egharevba, 2013), *Aloe vera* (Xanthorrhoeaceae) (Nwaoguikpe *et al.,* 2010), *Petiveria alliacea* (Phytolaccaceae), *Chenopodium ambrosioides* (Chenopodiaceae) and *Entandrophragma utile* (Meliaceae) (Adejumo *et al.,* 2011b).

Anacardium occidentale, *Psidium guajava* and *Terminalia catappa* were found to alter polymerization of sickle cell haemoglobin (Adejumo *et al.,* 2011). Spectrophotometric method was used to monitor the level of polymerization of haemolysate HbS molecules treated with

sodium metabisulphite in the presence of separate aqueous extracts of *A. occidentale*, *P. guajava* and *T. catappa*. Aqueous extract of *P. guajava* exhibited the highest capacity to reduce polymerization of deoxy HbS molecules (Chikezie *et al.,* 2011). The mixture of aqueous extracts of *Raphiostylis beninensis, Croton zambesicus, Lonchocarpus cyanescens, Uvaria chamae, Morinda lucida* and *Xylopia aethiopica* showed obvious *in vitro* anti-sickling effect (Avaligbe *et al*., 2012). The reversal of sickling by the root extracts of *Zanthoxylum zanthoxyloides* has also been reported (Sofowora *et al.,* 1971). Fractionation of an aqueous extract of root bark from *Z. zanthoxyloides* by column chromatography on Diethylaminoethylcellulose (DEAE)-A-50, utilising an elution gradient of pH 7.5-5.0, yielded five fractions. All fractions were reported to reverse *in vitro* metabisulfite-induced sickling of erythrocytes homozygous for HbS (Abu *et al.,* 1981). *Nigella sativa* has been reported to have calcium antagonist and antioxidant activities, both of which play a role in the management of the disease. The study aimed to investigate the *in vitro* anti-sickling effect of extracts from *N. sativa*. A total of 3 mL of venous blood was collected from each patient recruited for the study and divided into six tubes with heparin. The blood was mixed with 0.5 mL of 0.1 % (v/v), 0.05 % (v/v) or 0.01 % (v/v) of the oily extract of *N. sativa*. A slide was prepared by spreading a drop of treated blood, covered with a cover slide to ensure the complete de-oxygenation condition. The separation of irreversibly sickled cells (ISCs) was performed by a density gradient centrifugation method. The 0.1 % (v/v) concentration of the oily extract of *N. sativa* resulted in an approximately 80 % reduction in the formation of sickled cells. The 0.05 % (v/v) concentration of *N. sativa* produced an intermediate effect, while the 0.01 % (v/v) concentration had no effect on the formation of sickle cells. The 0.1% (v/v) concentration of the fixed oil of *N. sativa* led to a considerable reduction in the formation of ISCs. Thus it can be concluded that the fixed oil extracted from *N. sativa* seeds has an *in vitro* anti-sickling activity (Ibraheem *et al.,* 2010).

Table 1: Some plants with reported anti-sickling activities.

PLANT MATERIAL	DESCRIPTION	RESULTS	REFERENCE
Cyperus esculentus (seeds)	methanol and aqueous extracts	methanol extract showed pronounced anti-sickling activity	Nwaoguikpe, 2010
Xylopia aethiopica (spice)	aqueous extract	profound anti-sickling activity	Uwakwe and Nwaoguikpe, 2008
Alchornea cordifolia (leaf-anthocyanins)	ethanol and aqueous extracts	aqueous showed higher anti-sickling activity.	Mpiana *et al.,* 2007
Hymenocardia acida (leaf - saponins)	ethanol extracts at 0.5, 1.0 and 2.0% (w/v)	activity was found to be dose dependent.	Ibrahim *et al.,* 2007
Vigna unguiculata (anthocyanins)	ethanol and aqueous extracts	both showed pronounced anti-sickling activities	Mpiana *et al.,* 2008
Cajanus cajan (beans)	ethanol and methanol extracts	both showed pronounced anti-sickling activities	Osuagwu, 2010
Khaya senegalensis (leaves, stem and bark)	aqueous extract	profound anti-sickling activity	Fall *et al.,* 1999
Cissus populnea (root)	cold aqueous and methanol extracts	aqueous showed higher anti-sickling activity.	Moody *et al.,* 2003
Morinda lucida (anthocyanins)	ethanol and aqueous extracts	ethanol showed higher anti-sickling activity.	Mpiana *et al.,* 2010
Carica papaya (unripe fruit) & *Sorghum bicolor*	aqueous extract of fermented mixture was incubated for 5 days	day 5 aqueous extract showed the highest anti-sickling activity.	Ogunyemi *et al.,* 2009
Entadrophragma utile (bark)	aqueous and methanol extracts	significant anti-sickling activities	Adejumo *et al.,* 2011b
Garcinia kola (leaf, seed and seed pod)	aqueous and methanol extracts	leaf extract exhibited higher anti-sickling activity	Adejumo *et al.,* 2011
Plumbago zeylanica (roots)	aqueous and methanol extracts	significant anti-sickling activity	Adejumo *et al.,* 2010

1.8. LITERATURE SURVEY ON THE PLANT SPECIES STUDIED.

1.8.1. *Peperomia pellucida* L. (Piperaceae)

Scientific Name

Peperomia pellucida Linn.

Synonyms

Peperomia exigua, peperomia translucens, piper pellicudum L.

Family

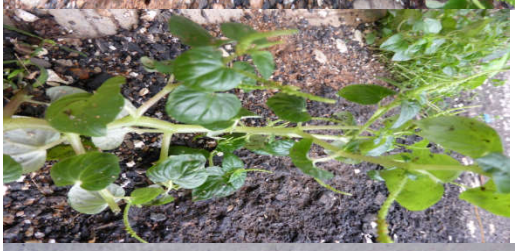
Piperaceae

Common Names

Renren (Yoruba), Shiny bush, pepper elder, man-to-man, rat-ear, Clearweed (English), pansit pansitan, konsaka wiwiri, càng cua, pak krasang, olasiman ihalas, suna-kosho, rangu-rangu, coracaozinho, lingua de sapo (Gill, 1992).

Botanical Description

Peperomia pellucida grows to one foot tall, stem initially erect, smooth, no hair, leaves fleshy and heart-shaped, smooth like candle wax, shiny light green, translucent, resemble pepper leaves but smaller. It has a very small bisexual flower growing from cord-like spikes from the leaf axils. Fruit is very small, round to oblong, ridged, green turning to black. It has one single seed with longitudinal ribs and ladder-like reticulation. The plant has a mustardy odour (Gill, 1992).



Origin and Distribution

Peperomias are herbs of tropical and subtropical regions. Most of them occur in Central and Northern South America. Fewer species are known from Africa, Asia, and Oceania. Different endemic species are known from the islands of the Indian Ocean, the Pacific, and the Caribbean. It has been reported that Peperomia is native to tropical America and Asia. It is well represented and naturalized in India too. Although a lot of them grow as epiphytes in rainforest habitats, others are succulents found in the high Andes. It can be found in lightly shaded and damp areas such as nooks, walls, yards, and even on roofs. Peperomia is the largest genus of the family of the Piperaceae (Bojo, 1994).

Major Ethnomedicinal Uses

It is used ethnomedicinally for its anti-inflammatory and analgesic properties. A solution of the fresh juice of stem and leaves is used against eye inflammation. It is also used in the treatment of cough, fever, common cold, headache, sore throat and diarrhea. It is used to treat kidney and prostate problems, high blood pressure, abscesses, furuncles, gout, rheumatic pains, arthritis and conjunctivitis (Bojo, 1994).

Pharmacological Properties

This plant has been reported to have analgesic activity; antibacterial activity against *Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*, and antifungal activity (Bojo, 1994). Oral administration of the extract of *P. pellucida* in rats has been confirmed (Arrigoni *et al.,* 2001) to interfere with the synthesis of prostaglandin, thus acting as an anti-inflammatory agent. The oral administration of the extract in rats had analgesic activity (Aziba *et al.,* 2001).

The extract of whole plant of *P. pellucida* has been reported to check the growth of Chloroquine-resistant *Plasmodium falciparum* Indo- strain by 95%. It has also been reported that this type of extract causes total lyses of *Leismania braziliensis*, L., *L. donovani*; and *L. amazonensis*. Animals given *P. pellucida* aqueous extract 5 g/kg for 14 days showed no adverse reactions or changes in behavior or weight. No clinical data have been reported on human toxicity (Aziba *et al.,* 2001).

Chemical Constituents

Seeds of *P. pellucida* yield an essential oil. This oil has been reported to contain as many as 71 chemical compounds. Major chemical constituents of the essential oil are sesquiterpenes. Some major categories of compounds isolated from the plant body of different species of Peperomias have flavonoids like acacetin, apigenin, isovitexin, and pellucidatin; phytosterols like campesterol and stigmasterol; essential oils like hydrozylated sesquiterpene; carotol. The plant has also been reported to contain peperomines that are reported to have cytotoxic or anti-cancer properties. Besides these, the plant extract also contains arylpropanoides like apiols having anti-fungal activities. Five compounds ***(1–5)***, including two secolignans, two tetrahydrofuran lignans, and one highly methoxylated dihydronaphthalenone, were isolated from the whole plant. These compounds were accompanied by the known peperomins A, B, C, and E, 7,8-*trans*-8,8‘-*trans*-7‘,8‘-*cis*-7,7‘-bis(5-methoxy-3,4-methylene dioxyphenyl)-8-acetoxymethyl-8‘-hydroxymethyl tetra hydrofuran, 7,8-*trans*-8,8‘-*trans*-7‘,8‘-*cis*-7-(5-methoxy -3,4-methylenedioxyphenyl)-7‘-(4-hydroxy-3,5-dimethoxy phenyl)-8,8‘di acetoxy methyltetrahydrofuran, sesamin, and isoswertisin (Aquil *et al.,* 1993, Aquil *et al.,* 1994).

1.8.2. Harungana *madagascariensis* Lam. ex. Poir. *(Hypericaceae)*

Scientific Name

Harungana madagascariensis Lam. ex Poir.

Synonyms

Harungana madagascariensis Choisy, *Harungana paniculata* (Pers.) Lodd ex Steud (Gill, 1992).

Family

Hypericaceae

Common Names

English- blood tree, orange-milk tree, dragon's blood tree, haronga, *Harungana,* French- bio harongue" (Dalziel, 1957). Among the Igbo - uturu, among the Yoruba and Bini (South-West Nigeria), the plant is known as Arunje, Aroje, elepo and itue, Hausa- alillibar (Gill, 1992).

Origin and Distribution

The plant is native to Central African Republic, Congo, Democratic Republic of Congo, Ethiopia, Kenya, Lesotho, Madagascar, Namibia, Sierra Leone, South Africa, Sudan, Swaziland, Tanzania and Uganda (Gill, 1992).

Botanical Description

It is a shrub or tree up to 12 m (exceptionally 27 m) high, much branched, evergreen, with scaly bark and orange or blood-red sap. It has young stems densely covered with rusty stellate or dendroid hairs. Leaves petiolate; petioles up to 27 mm long; blades lanceolate to ovate, ranging from 6.5 x 4.5 cm and 8.5 x 3.5 cm to 20 x 10 cm, shortly acuminate, rounded (rarely broadly cuneate, truncate or cordate) at the base, with about 14 parallel lateral veins on each side of the

midrib, glabrescent and dark glossy green above, pallid below with short glandular or rusty stellate indumentum; young leaves densely rusty on both surfaces. Inflorescence is a large many-flowered corymbose-cymose panicle; pedicels and calyx rusty. Flowers are sweet-scented. Sepals, ovate-elliptic, about 2 mm long, with a few longitudinal linear glands and gland dots. Petals, ovate-elliptic, up to 3 mm long, with 2–4 gland dots near the apex, white. Stamens 3–4 per bundle; filaments glabrous. Staminodes fleshy, glabrous. Drupe spherical, about 4 mm diameter; pericarp crustaceous, yellow or orange; pyrenes each 0–2-seeded. Seeds about 2 mm long (Gill, 1992).

Major Ethnomedicinal uses

In African traditional medicine, different parts are highly valued for the treatment of diverse human diseases. For example, in Sierra Leone, the red juice is employed to arrest postpartum bleeding (Olagunju *et al.,* 2004). The pounded bark along with *Pentaclethrra macrophylla* is equally employed in treating leprosy while the red sap from the stem bark is drunk as a remedy for tapeworm infection, craw-craw or as dressing materials for wounds among Ghanians. Decoction of the root and stem bark is also used as remedy for dysentery, bleeding piles, trypanosomiasis, fever, cold and cough (Gill, 1992). The plant exudate is used by the Ondo people (South-West Nigeria) to cure acute enteritis, scabies, and jaundice (Gill, 1992). The boiled decoction of the leaves is equally reputed for the treatment of malaria (Agbor *et al.,* 2007). Recently, the antifungal and antibacterial activities of different extracts of the stem bark were reported (Iwalewa *et al.,* 2007; Agbor *et al.,* 2007).

Plate 3: Leaves and Fruit of *Harungana madagascariensis* Lam. ex. *Poir. (Hypericaceae).*

Collection site: Opposite O.A.U. International school O.A.U. Ile-Ife.

Gbadamosi *et al.* (2012), reported the use of the stem bark in the management of SCD. Among Yoruba herbalists (South West, Nigeria), the aqueous root decoction of the plant is employed in the treatment of suspected liver or kidney diseases (Adeneye *et al.,* 2008).

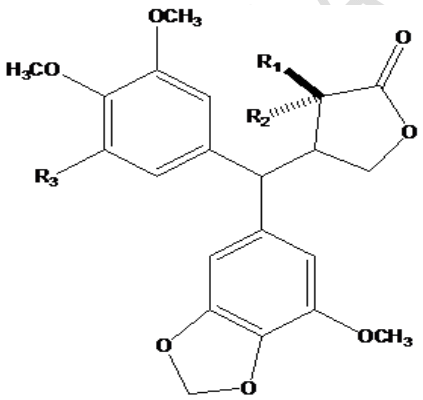
Pharmacological Properties.

In one instance, leaf aqueous extract of the plant was reported to show antioxidant (Kouam *et al.,* 2005) and antimicrobial activities (Okoli *et al.,* 2002), while a diminution of oral bacteria was also reported by Moulari *et al*., 2006 in an *in vitro* study. The stem bark extract of *H. madagascariensis* exhibited significant anti-protozoan effects against *Trichomonas* and *Plasmodium* both *in vivo and in vitro* (Iwalewa *et al.,* 2008). *Bacillus subtilis, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* showed susceptibility to the cold and hot aqueous extracts while *Staphylococcus aureus* showed susceptibility only to the hot aqueous extract. The ethanol extract of the stem bark was found to reduce glucose level in diabetic animals and oedema size formation in the right hind paw of rats (Iwalewa *et al.,* 2008). The polyphenolic extract of the stem bark was observed to have putative antiamoebic and spasmolytic activities *in vitro* (Tona *et al.,* 2000). Both α-pinene and β-caryophyllene are useful as antimicrobial, anaesthetic and anti-inflammatory agents, while germacrene D exhibits insecticidal properties (Mozuraitis *et al.,* 2002). The stem bark has been reported to have anti-anaemic properties (Iwalewa *et al.,* 2009).

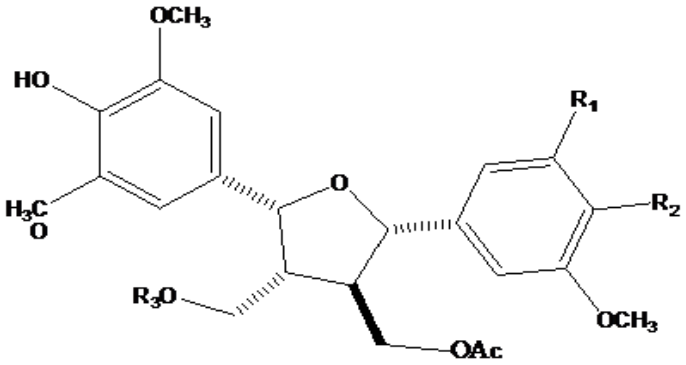
Chemical Constituents

Constituents common to three oils obtained from the leaf, stem bark and fruits of *H. madagascariensis* included α-pinene, α-copaene, β-elemene, α-humulene, β-selinene, d-cadinene and caryophyllene oxide in different quantities. Among the less frequently occurring monoterpenes, linalool (2.2 % in leaf), β-pinene (1.9 % in the stem bark) and *trans*-ocimene (4.1 % in fruit) were noticed. The minor sesquiterpenes in 1.4-4.4 % yields found in the oils were α-copaene, β-elemene, viridiflorene, germacrene A, 7-epi-α-selinene, d-cadinene, caryophyllene oxide, epi-α-muurolol and α-α-cadinol for leaf; β-pinene, β-elemene, β-selinene, α-selinene for

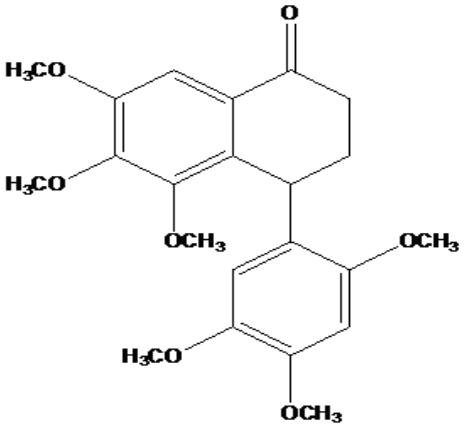
stem bark; and *trans*-ocimene, α-copaene, β-elemene, β-acoradiene, γ-muurolene, germacrene D, β-selinene, α-selinene, epizonarene, α-and d-cadinene, caryophyllene oxide and α-α-cadinol for fruit. Non-terpene components in the aliphatic category (0.1-3.1 %) occurring largely in the fruit oil, and fatty acid derivatives (0.1 %) in the stem bark oil were also present. Two prenylated anthronoids, harunmadagascarins A and B, were isolated from the stem bark of *Harungana madagascariensis* along with six known compounds including two anthronoids: harunganol B, one benzophenone: three pentacyclic triterpenes: friedelin, lupeol and betulinic acid ***(6)***. Harunmadagascarins A and B were characterized as 8,9-dihydroxy-4,4-bis-(3,3-dimethylallyl)-6-methyl-2,3-(2,2-dimethylpyrano)anthrone and 8,9-dihydroxy-4,4,5-tris-(3,3-dimethylallyl)-6-methyl-2,3-(2,2-dimethylpyrano) anthrone, respectively (Kouam *et al.,* 2005**)**. Kengaguinine, Kenganthranols A, B, C and D, Bazouanthrone, a novel anthrone derivative has been isolated from the root bark, together with betulinic acid ***(6)***, feruginin A, harunganin ***(7)***, haninganol A ***(8)***, harunganol B ***(9)***, harungin anthrone ***(10)*** and friedelane-3-one (Lenta *et al.,* 2007).



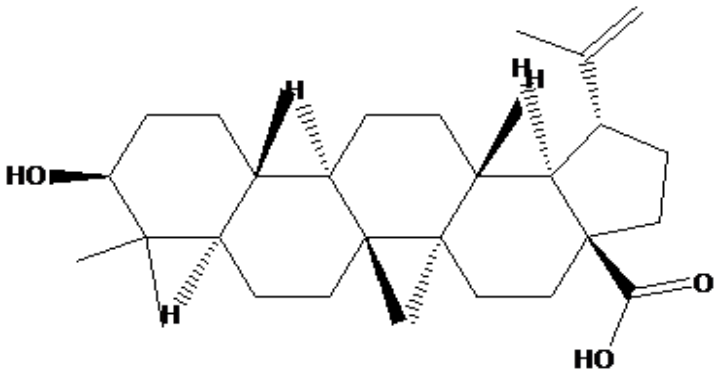
	R_1	R_2	R_3	
(1)	CH_2	CH_2	OCH_3	Peperomin G
(2)	H	CH_3	OH	Peperomin H



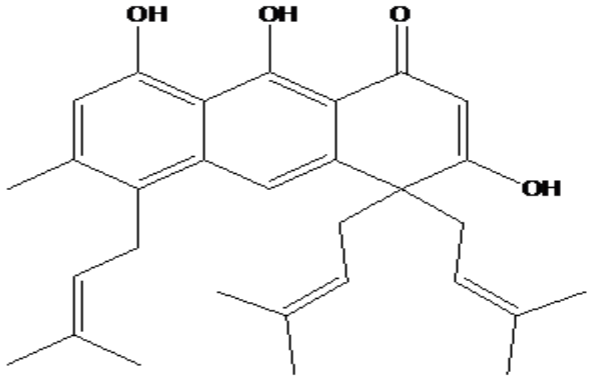
	R_1	R_2	R_3	
(3)	OCH_2O	OCH_2O	H	Peperomin A
(4)	OCH_3	OH	Ac	Peperomin E



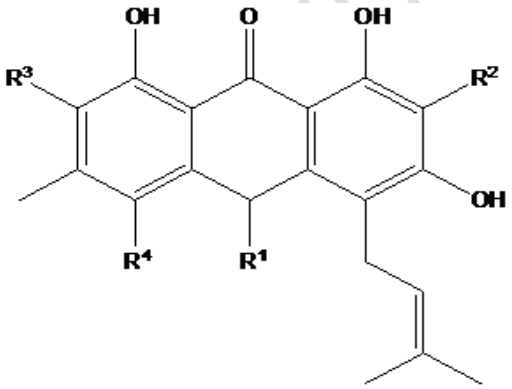
Peperomin B *(5)*



betulinic acid *(6)*



Harunganin *(7)*



	R^1	R^2	R^3	R^4
Harunganol A *(8)*	H	H	H	Prenyl
Harunganol B *(9)*	H	H	Prenyl	Prenyl
Harongin anthrone *(10)*	H	Prenyl	H	Prenyl

1.8.3. *Senna alata* (L.) Roxb (Leguminoseae)

Scientific Name

Senna alata (L.) Roxb.

Family

Leguminosae-Caesalpinioideae.

Synonyms

Cassia alata L., *Cassia alata* L. var. *perennis* Pamp., *Cassia alata* L. var. *rumphiana* DC., *Cassia bracteata* L.f., *Cassia herpetica* Jacq., *Cassia rumphiana* (DC.) Bojer, *Herpetica alata* (L.) Raf (Gill, 1992).

Common Names

Asunwon oyinbo (Yoruba), Ogala (Igbo), dadmari / candlebrush (English).

Origin and Distribution

Senna alata is native to South America, but has been planted widely for medicinal and ornamental purposes and is now pantropical. In many countries, including most countries of tropical Africa, it has become naturalized and is often considered a weed (Gill, 1992).

Botanical Description

It is a shrub up to 2–5 m tall. Leaves are arranged spirally, paripinnately compound with 8–20 pairs of leaflets; stipules triangular, 7–10 mm long; petiole 2–3 cm long; leaflets oblong-

elliptical, 5–15 cm × 3–7 cm, base and apex obtuse, mucronate, hairy on midrib, veins and margin. Inflorescence, an erect, terminal raceme 20–50 cm long, many-flowered; bracts elliptical, orange, enclosing flower buds. Flowers, bisexual, zygomorphic, 5-merous; sepals oblong, 10–20 mm × 6–7 mm, orange-yellow; petals ovate-orbicular, 16–24 mm × 10–15 mm, bright yellow; stamens 10, the 2 lower ones largest with filaments 4 mm long and anthers 12–13 mm long, 5 medium-sized, 3 short and rudimentary; ovary superior, woolly, recurved, style slender, short. Fruit, a winged pod 10–15 cm × 1.5–8 cm, wings 4–8 mm large, black, glabrous, and dehiscent, up to 50-seeded. Seeds quadrangular, flattened, 7–8 mm × 5–8 mm, shiny. The shrub stands 3–4 m tall, with leaves 50–80 cm long. The inflorescence looks like a yellow candle. The fruit shaped like a straight pod is up to 25 cm long. Its seed are distributed by water or animals. The leaves close in the dark. *Senna alata* is propagated by seed or cuttings. The seed pods are nearly straight, dark brown or nearly black, about 15 cm long and 15 mm wide. On both sides of the pods there is a wing that runs the length of the pod. Pods contain 50 to 60 flattened, triangular seeds. Soaking the seeds overnight before sowing improves germination. The active constituents are probably most abundant prior to flowering, at which time the leaves are preferably collected (Gill 1992).

Major Ethnomedicinal Uses

In Nigeria, *S. alata* formerly *Cassia alata* is used in the management of skin diseases such as eczema, scabies and ringworm (Sofowora, 1986). The decoction of the leaves, roots, and flowers is given for the treatment of venereal diseases in women. The leaf infusion or decoction is also given as a mild laxative and in large doses it acts as a purgative (Trease and Evans, 1996). Like many other members of the genus *S. alata* contains anthraquinone derivatives, responsible for the laxative properties (Elujoba *et al.,* 1989).

Plate 4*: Senna alata* Linn. (Caesalpiniaceae).

A: Whole plant, B: Flower, C: Leaves, D: Fruits. Collection site: Medicinal Plant Garden (O.A.U. Ile-Ife).

The main medicinal uses of *S. alata* are as a laxative or purgative and in the treatment of skin problems. For laxative purposes usually a decoction of the leaves is drunk, and less often the flowers, roots or the stem are used. Skin problems treated with *S. alata* include ringworm, favus and other mycoses, impetigo, syphilis sores, psoriasis, herpes, chronic lichen planus, scabies, rash and itching. Skin problems are most often treated by applying leaf sap or by rubbing fresh leaves on the skin. Other ailments treated in tropical Africa with *S. alata* include stomach pain during pregnancy, dysentery, haemorrhoids, blood in the urine (schistosomiasis, gonorrhoea), convulsions, heart failure, oedema, jaundice, headache, hernia, one-sided weakness or paralysis. A strong decoction of the dried leaves is used as an abortifacient. In veterinary medicine too, a

range of skin problems in livestock is treated with leaf decoctions. Such decoctions are also used against external parasites such as mites and ticks. The seeds are a source of gum. The young pods are eaten as a vegetable, but only in small quantities. Toasted leaves are sometimes used as a coffee substitute. *Senna alata* can become a weed in pastures; it is not eaten by livestock and is reported to be poisonous, especially for goats. The roots and the bark are reported to be used for tattooing. It is widely appreciated as a garden ornamental and bee forage. It has been reported to be used in the management of SCA (Okpuzor *et al.,* 2009).

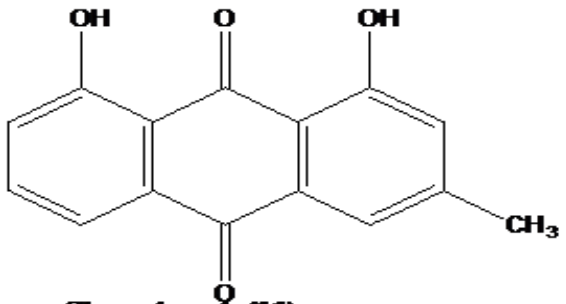
Pharmacological Properties

The aqueous and methanol extracts of the leaves have been reported to have antimicrobial activity. Extracts of the petals have bactericidal activity against gram-positive bacteria but not against gram-negative bacteria. It has been reported to have laxative activity attributed to the presence of anthraquinones. Anthraquinone aglycone from glycosidic fraction extracted from the leaves exhibited superior *in vitro* activity, both qualitatively and quantitatively against clinical strain of dermatophytes in comparison with anthraquinone aglycones extract, anthraquinone glycosides extract, anthraquinone aglycones from crude ethanol extract, and crude ethanol extract of the leaf (Mansuang *et al.,* 2010). The leaf and stem extracts have been reported to possess larvicidal potential which can be exploited in mosquito vector control (Edwin *et al.,* 2011). The hexane, chloroform, and ethylacetate extracts of the leaves were tested for analgesic activity. The hexane extract exhibited analgesic activity (Edwin *et al.,* 2011). The membrane stabilizing property of the extracts of the leaves has been documented (Okpuzor *et al.,* 2009).

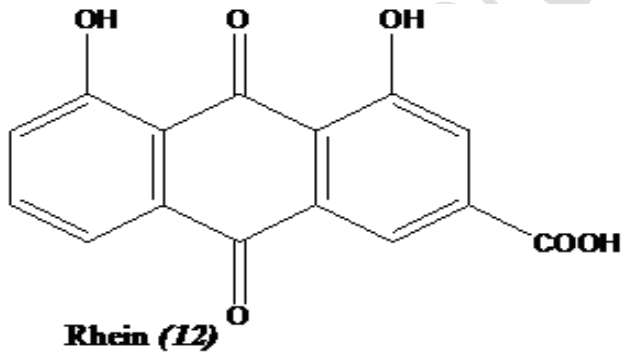
Chemical Constituents

From the leaves of *S. alata* a number of anthraquinone derivatives have been isolated, such as chrysophanol ***(11)***, Rhein ***(12)***, 1,5,7-trihydroxy-3-methylanthraquinone ***(13)***, flavonol such as

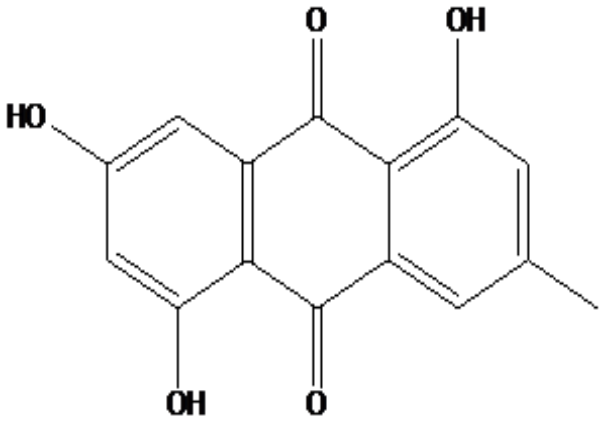
kaempferol ***(14)***, and other anthraquinones such as emodin ***(15)***, aloe-emodin ***(16)*** and 1,3,5,8-tetrahydroxy-6-methoxy-2-methylanthraquinone ***(17)*** as well as the alkaloid tyramine and the common steroid β-sitosterol. The bark contains tannins. The petals contain anthraquinones, glycosides, steroids, tannins and volatile oil. Chrysophanol ***(11)*** and emodin ***(15)*** can be produced *in vitro* by using root cultures of *Senna alata* (Moriyama *et al.,* 2003).



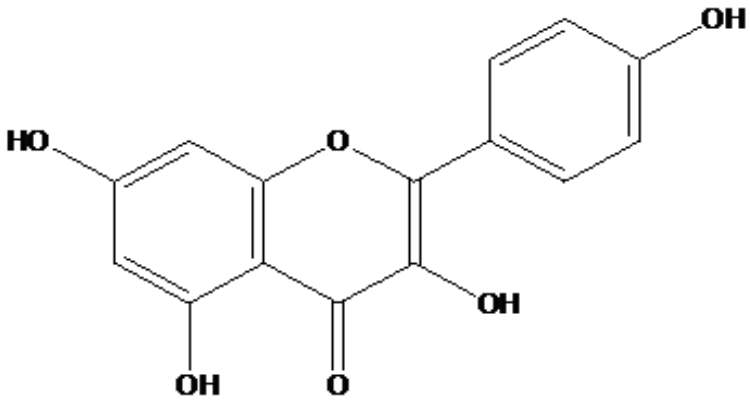
Chrysphanol *(11)*



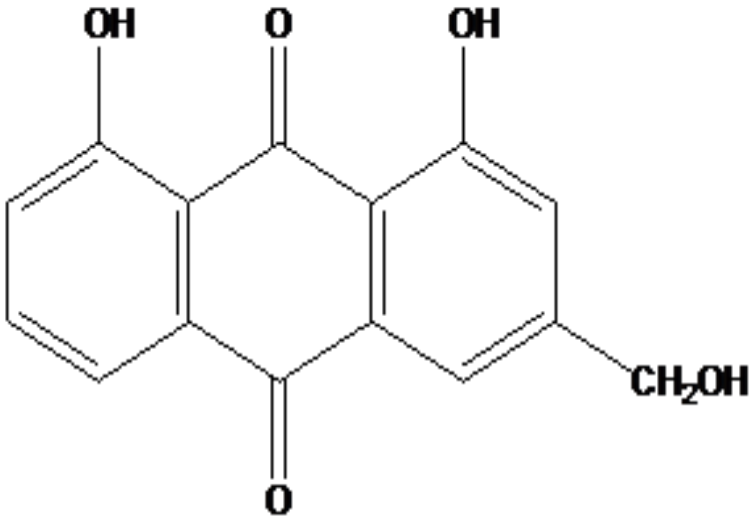
Rhein *(12)*



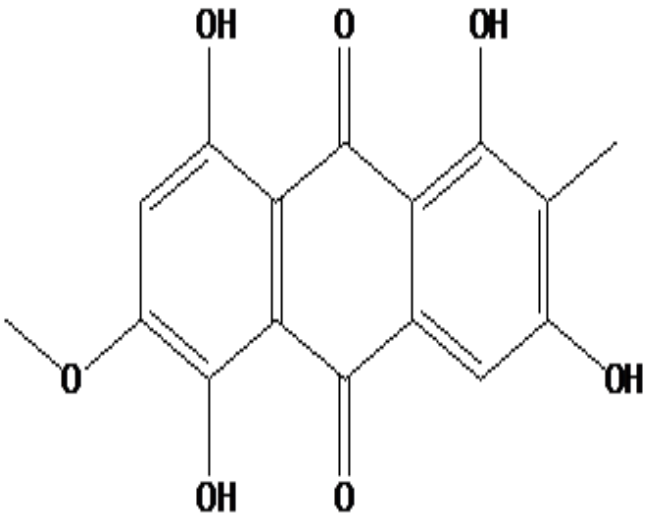
1,5,7-trihydroxy-3-methylanthraquinone *(13)*



kaempferol *(14)*



Aloe-emodin *(16)*



1,3,5,8-tetrahydroxy-6-methoxy-2-methylanthraquinone *(17)*

1.8.4. *Dioclea reflexa* Hook. f. (Fabaceae)

Scientific Name

Dioclea reflexa Hook.f.

Synonyms

Dioclea fergusonii Thwaites, *Dioclea javanica* Benth.

Family

Papilionaceae-Leguminosae

Common Names

Marbles vine; marble plant; bulls' eye (English). Aarin pupa, epe, "Agbaarin" (Yoruba) (Burkill, 1995).

Botanical Description

In Nigeria, the plant is cultivated and also grows wild. It is propagated from seeds (Oladosu *et al.,* 2006). In Eastern Nigeria, it is cultivated as a cover crop and the seed is commonly used as a soup thickener (Burkill, 1995).

Origin and Distribution

D. reflexa is native to west-central tropical Africa: Cameroon; Equatorial Guinea; Gabon; Sao Tome and Principe; Zaire (w.) West Tropical Africa: Benin; Cote D'Ivoire; Ghana; Guinea-Bissau; Liberia; Nigeria; Sierra Leone; Togo South Tropical Africa: Angola (Burkill, 1995).

Plate 5: Seeds of *Dioclea reflexa* Hook .f. (Fabaceae), (bought from New market Ile-Ife) and authenticated by Mr. Ibhameselbhor at IFE herbarium.

Major Ethnomedicinal Uses

The gelling potential and the folkloric use of the seed flour as a soup thickener make it a material of interest as a food and pharmaceutical excipient. It is used in the management of arthritis, rheumatism, cutaneous, subcutaneous parasitic infections; generally healing and pulmonary troubles. The seed powder, in Senegal, is used in preparation with palm oil to cure rheumatism and itching. It is also used in Congo for treating cough. A roasted seed, ground and mixed with kaolin is used in the treatment of asthma (Faleye, 2012).

Pharmacological Properties

Dioclimidazole from the seeds showed a significant anticholinesterase activity and has better zones of inhibition in antibacterial assay when compared with 2.0 g/mL gentamycin (Oladosu *et al.,* 2006). Extracts of *D. reflexa* showed broad spectrum antibacterial activity (Ogundare and Olorunfemi, 2007).

Chemical Constituents

The phytochemical screening conducted on the crude extracts of the seeds revealed the presence of phlobatannin, saponin, steroid, cardiac glycosides, alkaloid and flavonoids (Ajayi *et al.,* 2012). Dioclimidazole is a new natural compound, which appeared as a cream precipitate. The methanol extract of the seeds contain stigmasterol, taraxasterol (Oladosu *et al.,* 2006).

1.8.5. *Morinda lucida* Benth (Rubiaceae)

Scientific Name

Morinda lucida Benth.

Family

Rubiaceae

Common Names

Brimstone tree (English); uhon (Ivory Coast); hojologbo (Sierra Leone); oruwo, eruwo (Yoruba), eze ogu, ngisi (Nigeria) (Burkill, 1997).

Origin and Distribution

Senegal to Sudan, Southward to Angola and Zambia, Liberia and Tanzania.

Botanical Description

It can grow into a tree of 15 m and 40 cm; tree of the coastal zone and coastal savannas, but also growing in the northern savannas. This distinctive tree has light coloured, scaly or fissured bark and dark shiny leaves on the upper surface. The shiny leaves are the origin of the latin name 'lucida'. Unlike most members of Rubiaceae the stipules fall very early, leaving a clear scar. It is an evergreen shrub or small to medium-sized tree up to 18(–25) m tall, with bole and branches often crooked or gnarled; bark smooth to roughly scaly, grey to brown, often with some distinct purple layers. Leaves are opposite, simple and entire; stipules ovate or triangular, 1–7 mm long, falling early; petiole up to 1.5 cm long; blade elliptical, 6–18 cm × 2–9 cm, base rounded to cuneate, apex acute to acuminate, shiny above, sometimes finely pubescent when young, later only tufts of hairs in vein axils beneath and some hairs on the midrib. Inflorescence a stalked head 4–7 mm in diameter, 1–3 at the nodes opposite a single leaf; peduncle up to 8 cm long bearing at base a stalked cup-shaped gland (Burkill, 1997).

Plate 6: *Morinda lucida* Benth. (Rubiaceae).
A: Whole Plant, B: Stem, C: Leaves, D: Flowers, E: Fruit. Collection site: along the Teaching and Research Farm road O.A.U. Ile-Ife.

Flowers are bisexual, regular, 5-merous, heterostylous, fragrant; calyx cup-shaped, c. 2 mm long, persistent; corolla salver-shaped, c. 1.5 cm long, white or greenish yellow, lobes ovate-lanceolate, up to 5 mm × 2.5 mm; ovary inferior, 2-celled, style 8–11 mm long with 2 stigma lobes 4–7 mm long; stamens 5, inserted in the corolla throat, with short filaments. Fruit is a drupe, several together arranged into an almost globose succulent syncarp 1–2.5 cm in diameter, soft and black when mature, yellowish and soft (Burkill 1997).

Major Ethnomedicinal Uses

In West Africa *M. lucida* is an important plant in traditional medicine. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during childbirth. The plant is also employed in cases of diabetes, hypertension, cerebral congestion, dysentery, stomach-ache, ulcers, leprosy and gonorrhoea. In Nigeria, it is one of the 4 most used traditional medicines against fever. In Côte d'Ivoire a bark or leaf decoction is applied against jaundice and in DR Congo it is combined with a dressing of powdered root bark against itch and ringworm. In Agri-horticulture it is used as an ornamental and is cultivated or partially tended. The leaves are used as an ingredient of "fever teas", which are usually taken for the traditional treatment of malaria. The plant is also used as a general febrifuge, analgesic and laxative. A weak decoction of the stem bark is used for the treatment of severe jaundice. Alcoholic extract of the leaf is used as a stimulant and used in the management of arthritis, rheumatism, leprosy, pulmonary troubles (Burkill, 1997; Awe *et al*., 1998) and management of SCA (Okpuzor, 2008; Mpiana *et al.,* 2010).

Pharmacological Properties

Obih *et al.,* (1985) reported the antimalarial activity of *M. lucida* against *Plasmodium berghei berghei* in mice. Anthraquinones isolated from the leaves of *M. lucida* have been shown to possess potent *in vitro* activity against *Plasmodium falciparum* (Koumaglo *et al.,* 1992). The leaf extract of the plant was reported to possess trypanocidal (Asuzu *et al.,* 1990), antimalarial activities (Makinde *et al.,* 1985, Tona *et al.,* 1999) and aortic vasorelaxant effect (Ettarh *et al.,* 2004). Oliver-Bever documented the use of a weak decoction of the stem bark to treat severe jaundice. The leaf extract has also been reported to have a strong oral hypoglycemic property (Olajide *et al.,* 1999; Adeneye *et al.,* 2008). Adeneye and Agbaje (2008) attributed this property to increased peripheral utilization of glucose. The leaf extract has also been documented to possess reversible antispermatogenic activities in rats (Raji *et al.,* 2005), anticholinesterase activity (Elufioye *et al.,* 2013), hypoglycemic, and trypanocidal activities (Taofeeq *et al.,* 2010). According to reports by Adomi and Umukoro (2010), the ethanol crude root bark extract, at a concentration of 1000 mg/mL, showed antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*.

Chemical Constituents

From the wood and bark of *M. lucida* 18 anthraquinones have been isolated, including the red colorants 1-methylether-alizarin, rubiadin and derivatives, lucidin, soranjidiol, damnacanthal, nordamnacanthal, morindin, munjistin and purpuroxanthin. Two anthraquinols, oruwal and oruwalol, have also been found; these give a yellow colour and possibly are intermediates in the formation of anthraquinones. In addition to anthraquinones, tannins, flavonoids and saponosides have been isolated (Koumaglo *et al.,* 1992). The major constituents of *M. lucida* extract are various types of alkaloids-anthraquinones and anthraquinols. Adewunmi and Adesogan (1984)

isolated and characterized two compounds, oruwalol and oruwal, and ten anthraquinones from the stem of the plant.

1.8.6. *Anthocleista vogelii* Planch (Gentianaceae)

Scientific Name

Anthocleista vogelii Planch.

Synonyms

Anthocleista buchneri Gilg, *A. auriculata* De Wild.

Family

Loganiaceae (APG: Gentianaceae).

Common Names

Cabbage tree (English), apa oro Sapo/Shapo (Yoruba), kwari (Hausa) (Gill 1992).

Botanical Description

Anthocleista vogelii is a small to medium-sized tree up to 20 m tall; bole up to 55 cm in diameter, sometimes with stilt roots; twigs with 2–4 divergent spines confluent at base. Leaves opposite, simple and entire, almost sessile; blade oblong-ovate to oblanceolate, 15–45 cm × 6–24 cm, in young plants up to 150 cm × 45 cm, base cuneate, auricled, apex rounded, margin recurved, papery or leathery. Inflorescence an erect terminal dichasial cyme 30–50 cm long, many-flowered; peduncle and branches yellowish green or orange, thickened at the nodes. Flowers bisexual, regular; sepals 4, free, orbicular or broader than long, outer ones 4–12 mm long, inner ones about twice as long; corolla with cylindrical tube, 12–18 mm long, lobes 13–16, oblong-lanceolate, 12–19 mm long, spreading, creamy to pale yellow; stamens as many as corolla lobes and alternating with them, exserted, filaments partly or entirely fused, anthers whitish green; ovary superior, ovoid-cylindrical to ovoid-conical, 5–7 mm × 3–6 mm, 4-celled,

stigma obovoid-cylindrical, apically 2-lobed. Fruit an ellipsoid berry 2.5–4.5 cm × 2–3.5 cm, rounded at apex, thick-walled, green or yellowish, many-seeded. Seeds obliquely ovoid-globose, 2–2.5 mm × 1.5–2 mm, dark brown. The leaves are collected from young trees or by climbing older ones. The bark is obtained by slashing or peeling with a cutlass. The roots are dug up when the soil is workable. The collected material is dried in the sun and kept in wrappers or it is pounded and made into balls. Sometimes, the plant material is powdered when dry. The wood is whitish, soft and perishable. In Nigeria, it flowers from October to February and from March to May; it fruits from November to March (Leeuwenberg, 1961).

Origin and Distribution

It occurs from Sierra Leone east to Kenya, and south to Zambia, Angola and Nigeria.

Major Ethnomedicinal uses

The stem, root, bark and leaves of *A. vogelii* are used to treat malaria, jaundice, diabetes and abscesses. The Ibibios of Southern Nigeria use the leaves and stem bark as malarial remedy.

It is widely used in West Africa as a strong purgative and diuretic. A root decoction is commonly taken to treat constipation, to regulate menstruation and as an abortifacient. It is used as a wash, bath or vapour bath to treat leprosy, venereal diseases, oedema and scrotal elephantiasis. In Sierra Leone a decoction of the roots with lemon is taken to treat hepatitis, while a decoction of dry fallen leaves is taken to treat jaundice. In Nigeria the bark and seed are used as an antipyretic and tonic. The seed is also used as a purgative. In Congo fresh twig bark with manioc is eaten raw to treat aspermia. A stem bark decoction is taken to treat hernia and a root decoction is taken to treat stomach-ache in women, ovarian problems, venereal diseases, hernia, bronchitis and fever, and also as purgative and to induce labour. Sap of young leaves,

root powder or bark pulp is used to treat sores, abscesses, as a haemostatic and for cicatrization (Burkill, 1995).

Plate 7: *Anthocleista vogelii* Planch. (Gentianaceae).

A: Plant showing leaves and fruits, B: juvenile plant. Collection site: along road 2 to staff quarters.

The sap is applied topically to treat otitis or ophthalmia. A plaster of pulp of terminal buds is used to draw out thorns or splinters and is applied to snakebites. The bark is noted for its anti-pyretic, tonic and purgative properties and a decoction of the leaves is known to prevent malaria and alleviate symptoms of malaria such as fever. It is also used to treat jaundice and as haemostatic (Burkill, 1995).

Pharmacological Properties

Reports of antibacterial and wound healing activities, in vitro anthelmintic activity and antiplasmodial activity have been published (Rai *et al.,* 1989; Abuh, 1990; Olukoya, 1993). Tests with aqueous, hexane, acetone and methanol extracts of the stem bark in rats showed potent anti-ulcer properties, which could explain the traditional use in the treatment of stomach-ache (Ateufack *et al.,* 2006).

Chemical Constituents

Phthalide and xanthones have been isolated from *A. vogelii*. The major xanthone is decussatin (1-hydroxy-3, 7, 8-trimethoxyxanthone) and it also contains minor compounds such as 1, 7-dihydroxy-3, 8-dimethoxy-xanthone ***(18)*** and swertiaperennin (1, 8-dihydroxy-3, 7-dimethoxy-xanthone), Stigmasterol (stigmasta-5, 22-dien-3-beta-ol) and hexadecanoic acid (Tene *et al*., 2008; Alaribe *et al.,* 2012). The stem bark contains the alkaloid fagaramide while the stem bark and wood contain several xanthones (Okorie, 1976). It contains the closely related secoiridoid glycosides, secologanic acid, vogeloside, and sweroside (Chapelle, 1976).

1.8.7. *Mimosa pudica* L. (Fabaceae)

Scientific Name

Mimosa pudica Linn.

Synonyms

Mimosa tetrandra Humb. & Bonpl. ex Willd., *Mimosa pudica* L. var. *tetrandra* (Willd.) DC., *Mimosa unijuga* Duch. & Walp., *Mimosa pudica* L. var. *unijuga* (Duch. & Walp.) (Gill, 1992).

Family

Leguminosae/ Fabaceae - Mimosoideae

Common Names

Mimosa, sensitiva, sensitive plant, dorme, dormidera, humble plant, marie-honte, mayhont, morivivi, honteuse, sleeping grass, ti mawi, touch-me-not, adormidera, feuilles honte, honte, quitem tranquille, memalu (modesty), puteri malu (modest princess). Patanmo-yoruba (Gill, 1992).

Botanical Description

It is an annual or perennial that normally grows to 50-70 cm tall (but can be up to 1 m tall), and often takes the form of a straggling prickly sub-shrub. Its stems have sparse prickles, 2-2.5 mm long, or are sometimes bristly, or can also be almost hairless. The leaves are alternate, bipinnate (twice compound), do not have prickles and are very sensitive to touch. The rachis (axis of the compound leaf) is 1.5-5.5 cm long, and the pinnae (primary divisions of the compound leaf) are subdigitate (almost finger-like projections). There are 10-26 pairs of leaflets (the smallest segments of the leaf) per pinna, which are 6-15 x 1.2-3 mm and linear oblong (Burkill, 1995)

Plate 8: *Mimosa pudica* Linn. (Mimosaceae).
A: Whole plant, B: thorny Stem, C: Compound leaves. Collection site - Kosere road Ile-Ife.

The flowers are lilac or pink (the colour mainly the stamen filaments) and are held in ovoid, stalked heads of 1-1.3 x 0.6-1 cm. A cluster of 1-5 flower heads is borne in the leaf axil. The calyx is minute, about 0.2 mm long. The corolla is 2-2.3 mm long, and contains four stamens. The pods are 1.8 cm x 3-5 mm, densely bristly, clustered, along their margins. It is grown for its curiosity value- the fern like leaves close up and droop when touched, usually re-opening within minutes. It has prickly stems and small, fluffy, ball shaped pink flowers in summer. It grows to a height of 5 ft and spreads around 3 ft- a perennial plant, it grows to a height of 0.5m with a spread of 0.3m. In some areas this plant is becoming a noxious weed. The stem is erect, slender and branching. The leaves are bipinnate, fern like and pale green- closing when disturbed. The flowers are pale lilac pink, occurring in globose heads and appearing in summer. Indigenous to

the northern hemisphere, it is adaptable to most soils in an open, sunny position, and is drought and frost tender. Due to its ability to fix nitrogen from the air it does well on poor soils. "Sensitive Plant" folds up its leaves when touched or exposed to a flame. This plant requires a medium light exposure, an evenly moist soil, and temperatures between 60 and 85 degrees (Barneby 1991). One should use caution when handling seedlings because the plant dislikes root disturbance. Mimosa may be difficult to grow and is sensitive to over watering. It is often a major weed of agricultural fields and plantations. However, it is also sometimes valued as a forage plant (Barneby, 1991).

Origin and Distribution

M. pudica is native to South America and Central America. It has been introduced to many other regions and is regarded as an invasive species in Tanzania, South Asia and South East Asia and many Pacific Islands. It is regarded as invasive in parts of Australia and is a declared weed in the Northern Territory and Western Australia although not naturalized there. Control is recommended in Queensland. It has also been introduced to Nigeria, Seychelles, Mauritius and East Asia but is not regarded as invasive in those places (Burkill, 1995).

Major Ethnomedicinal Uses

It has been used widely in traditional medicine. Pulped leaves are used in India on glandular swellings and in the Republic of the Congo (Congo-Brazzaville) the entire plant is pulped and rubbed onto people suffering pains in their body sides and kidneys. In Senegal, the leaves are used for lumbago and nephritis. All parts of the plant have been used to combat glandular tumours and uterine cancer. In India leaf-sap is applied for sinus disorders, and rubbed onto sores and piles (Burkill 1995). It is used as an anti-diarrhoeal and anti-convulsant. The leaves

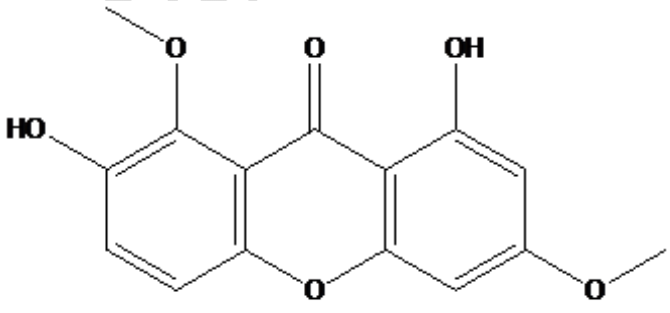
and roots are used in treatment of piles and fistula. Paste of leaves is applied to hydrocele. Cotton impregnated with juice of leaves is used for dressing (Shahriar *et al.,* 2014).

Pharmacological Properties

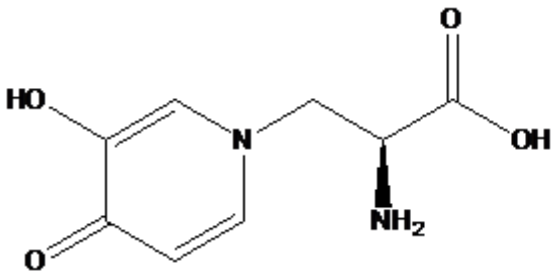
It contains the alkaloid mimosine (a hydroxamino acid of aromatic nature), which in large doses is toxic to humans and animals and may depress growth in mammals if eaten in large quantities (Restivo, 2005). The membrane stabilizing activity and thrombolytic and cytotoxic effect of extracts from the leaves on brine shrimps have been reported (Shahriar *et al.,* 2014).

Chemical Constituents

It contains the toxic alkaloid mimosine, which has been found to also have antiproliferative and apoptotic effects (Restivo, 2005). Aqueous extracts immobilize the filarial form larvae of *Strongyloides stercoralis* in less than one hour (Robinson, 1990). Aqueous extracts of the roots of the plant have shown significant neutralizing effects in the lethality of the venom of the monocle cobra (*Naja Kaouthia*). It appears to inhibit the myotoxicity and enzyme activity of cobra venom. It also contains ascorbic-acid, crocetin, crocetin-dimethyl-ether, D-glucuronic-acid, D-xylose, linolenic-acid, mucilage, oleic-acid, norepinephrine, palmitic-acid, sitosterol, mimosine ***(19)*** (Bisby, 1994).



1, 7-dihydroxy-3, 8-dimethoxy-xanthone (18)



Mimosine (19)

1.8.8. *Lecaniodiscus cupanioides* Planch ex. Benth (Sapindaceae).

Sc

ientific Name

Lecaniodiscus cupanioides Planch ex Benth.

Family

Sapindaceae

Common Names

Ukpo (Igbo), Utantan (Edo), Kafi-nama-zaki (Hausa) and Akika/ Aka (Yoruba) (Burkill, 2000).

Botanical Description

It is a small tree 12 m tall, with spreading crown; branches dull brown; twigs initially pubescent and glandular. Petiole 4–12 cm long, flattened; rhachis 18–26 cm long; petiolules 2–8 mm long; leaflets in 5–7 pairs, ovate-elliptic, elliptic or oblaceolate, the lowermost relatively broader in relation to its length, the middle and upper leaflets 9–18 cm long, 3–9 cm wide, shortly acuminate, the base obtuse, glabrous; lateral nerves in 10–15 pairs. Inflorescence-axis 7–11 cm

long, all parts initially golden-pubescent. Male flowers: pedicels 5–6 mm long; calyx ovoid-spherical in bud, 3.2 mm long, the lobes very short, then opening nearly to the base and reflexed; disk conspicuous, fleshy; stamens exerted. Female flowers: pedicels 2–3 mm long, not accrescent in fruit; calyx ovate-oblong in bud, as in the male, scarcely visibly lobed at this stage, later opening fully and reflexed; disk obscured by the pinkish- or brownish-pubescent ovary; staminodes rudimentary, the anthers degenerate. Fruit is orange when fresh, drying orange-ferruginous, asymmetrically ovoid, 2–2.2 cm long, 1.5–1.7 cm wide, indumentum velvety; seed slightly compressed, 1.1 cm long, 1.2 mm wide (Burkill, 2000).

Plate 9: *Lecaniodiscus cupanioides* Planch. (Sapindaceae).
A: Whole plant, B: Stem, C: Leaves, D: Fruits.
Collection site: behind Computer building O.A.U. Ile-Ife.

Origin and Distribution

A tree to 12 m high, or more, low-branching spreading crown, of forest outliers in the savannah zone, over the Region from Senegal to West Cameroons, and across Africa to Sudan, Uganda and Angola (Burkill, 2000).

Major Ethnomedicinal uses

The bark and leaves are used as a febrifuge, laxative and in pulmonary troubles such as for cough and broncho-pneumonial infections (Burkill, 2000). The bark and root are also used as sedatives, on wounds, boils, burns and bruises, tooth ache, fever and abdominal swelling caused

by liver abscess (Gill, 1992; Iwu, 1993). Flower is used in food sauces, condiments, spices, flavourings and fruit-pulp is eaten. The leaf is also used in food poisoning. The bark is made into a pulp for inhalation or into a snuff to relieve headache, sinusitis and nasal congestion in head-colds, also otitis (ear infection) and ophthalmia (eye problem). The fresh bark is used to treat ear ache. A bark-decoction is applied externally as a general reconstituent or as a revulsive for pains in the chest, bronchitis, pleurisy and kidney-pains. The crushed bark is used in frictions to treat pruritus, whilst a wash made from the bark is applied to the skin of people with small-pox. The bark sap is used as an enema for treating kidney-pains (Fasola and Egunyomi, 2005). The leaves are antibacterial and rubefacient. The leaves and the fruit are pounded together with white clay and then rubbed on the body as a treatment for fever. They are used on skin-eruptions and internally for worms and pains, while crushed up they are pasted over the stomach as a treatment for difficult childbirth. The fruit is considered anthelmintic (Burkill, 2000).

Pharmacological Properties

The aqueous root extract of the plant has been reported to prevent strychnine-induced seizure, prolonged the latency and reduced severity of PTZ (pentylenetetrazole) and picrotoxin induced seizure. Pharmacological studies have also shown that the plant possessed analgesic and hepatotoxic properties (Yemitan and Adeyemi, 2004; Yemitan *et al.,* 2005). The leaf extract exhibited strong DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethylbenzo thia zoline-6-sulfonic acid) radical scavenging activity greater than BHT (butylated hydroxytoluene) and comparable to ascorbic acid. A leaf extract has been shown to be an effective antibiotic on a range of bacteria including *Staphylococcus aureus* and *Streptococcus pyrogens* (Sofidiya *et al.,* 2008). An aqueous extract of the root has been shown to have CNS depressant activity. In trials, it protected mice from strychnine-induced convulsions (Yemitan *et*

al., 2005). The aqueous pod extract was found to exhibit a substantial inhibitory action on both the laboratory strain and a clinical isolate of *Candida albicans* obtained from an AIDS patient (Okore *et al.,* 2007). Mikhail *et al.,* 2013 reported that oral administration of aqueous root extract of *L. cupanioides* exhibited antimalarial activity in *P. berghei*–infected mice. The extract at 50 mg/kg body weight showed the best action. The effect of the extract compared favorably with chloroquine and artesunate.

Chemical Constituents

Phytochemical investigation of the stems of *L. cupanioides* Planch afforded two triterpenoid saponins identified as 3-O- [Î±-L-arabinofuranosyl-(1â†'3)- Î±-L-rhamnopyranosyl- (1â†'2) - Î± -L-arabinopyranosyl-]-hederagenin and 3-O- [Î± -L-arabinopyranosyl- (1â†'3)- Î± -L-rhamnopyranosyl (1â†'2)- Î± -L-arabinopyranosyl-]-hederagenin. The compounds exhibited antifungal activity against *C. albicans, C. neoformans* and *A. fumigates* (Adesegun *et al.,* 2008). The results of the phytochemical analysis of the root extract showed the presence of alkaloids (2.37 %), saponin (0.336 %), tannin (0.012 %), phenol (0.008 %), and anthraquinone (0.002 %) (Mikhail *et al.,* 2013).

1.8.9. *Spondias mombin* Linn (Anarcadiaceae)

Scientific Name

Spondias mombin Linn.

Synonyms

Mauria juglandifolia Benth. *Spondias aurantiaca* Schum and Thonn. *Spondias brasiliensis* Mart. and Engl. *Spondias lucida* Salisb. *Spondias lutea* L. *Spondias lutea* var. glabra Engler

Spondias lutea var. maxima Engler *Spondias myrobalanus* L. *Spondias nigrescens* Pittier *Spondias pseudomyrobalanus* Tuss. *Spondias radlokoferi* J. D (Burkill 2000).

Family

Anacardiaceae

Common Names

Iyeye- fruit, ekika/okika- tree (Yoruba), tsaadar Lamarudu (Hausa); ijikara (Igbo), hog plum, mombin plum, yellow mombin, (English) (Gill, 1992).

Botanical Description

Spondias mombin is a tree to 30 m high; bark greyish-brown, thick, rough, often deeply grooved, with blunt, spine like projections; trunk with branches 2-10 m above ground level to form a spreading crown up to 15 m in diameter and forming an open or densely closed canopy, depending on the vigour of the individual; seedlings with deep taproot, probably persisting in mature tree, which also possesses a shallower root system near the surface. Leaves alternate, once pinnate with an odd terminal leaflet; stipules absent; rachis 30-70 cm long; leaflets 5-10 pairs, elliptic, 5-11 x 2-5 cm; apex long acuminate, asymmetric, truncate or cuneate; margins entire, glabrous or thinly puberulous. Inflorescence a branched, terminal panicle with male, female and hermaphrodite flowers; sepals 5, shortly deltoid, 0.5-1 cm long; petals 5, white or yellow, oblong, 3 mm long, valvate in bud, becoming reflexed; stamens 10, inserted beneath a fleshy disc; ovary superior, 1-2 mm long; styles 4, short, erect. Fruit an ovoid or ellipsoid drupe, 3-4 x 2-2.5 cm in diameter; dull light orange to yellow or brown; in clusters of 1-20; epicarp thin, enclosing a juicy orange or yellow mesocarp 3-6 mm thick; endocarp large, with a soft,

fibrous, grooved coat surrounding 4-5 small seeds. The tree is tolerant of most soil types and rainfall patterns. *S. mombin* is severely damaged by freezing temperatures. It is generally found in the terra firma forests; trees may be found in drier areas as well as along high fertile floodplains, where they are waterlogged for 2 or 3 months of the year. Mean annual rainfall: over 1 500 mm (Gill, 1992). It does well in a great variety of soils, such as sandy soils, gravelly or heavy clays, but best results are usually obtained in rich, moist, and relatively heavy soil. Flowering occurs during the dry season and some ripe fruit can be found on the tree most of the year. Fruiting usually starts at about 5 years of age, although well-kept cuttings may produce earlier. Ripe fruits are usually collected from the ground, but this must be done rapidly before they rot or are eaten by animals. Propagation is by seed, or more frequently by large cuttings, often 50-100 cm long and 5-10 cm thick (wood from the previous season or older); patch budding has been used as well. Fresh seeds germinate well. Seeds germinate within 35-75 days.

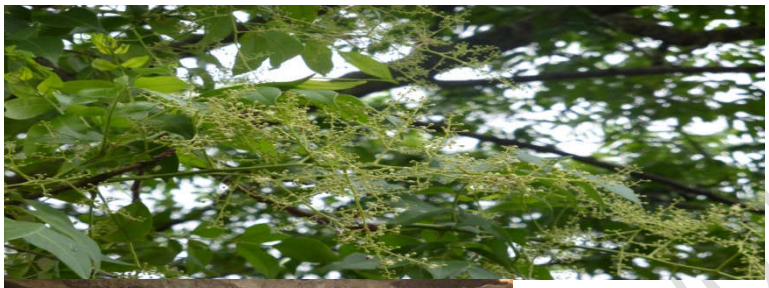


Plate 10: *Spondias mombin* Linn. (Anacardiaceae).
A: Whole plant, B: Stem-bark, C: Compound leaf, D: Flowers.
Collection site: Ede road Ile-Ife.

Origin and Distribution

It is native to Central America and northern South America and can be found under semi-wild cultivation in most lowland areas of the American tropics. The species probably originated in the Amazon Basin, as it is commonly encountered in most of the lowland forests of the region. It is also well known in northeast Brazil and the West Indies. The tree is widely cultivated and naturalized in tropical Africa. It occurs in a great variety of humid tropical climates, often in secondary vegetation derived from evergreen lowland forest or semi-deciduous forest. It has been introduced to most tropical locations and performs well under varied conditions (Burkill, 2000).

Major Ethnomedicinal uses

Ripe fruits are eaten out-of-hand, or stewed with sugar. The extracted juice is used to prepare ice cream, cool beverages and jelly. Young leaves are cooked as greens. The fruit juice is drunk as a diuretic and febrifuge. The decoction of the astringent bark serves as an emetic, a remedy for diarrhoea, dysentery, haemorrhoids and a treatment for gonorrhoea and leucorrhoea; and, in Mexico, it is believed to expel calcifications from the bladder. The powdered bark is applied on wounds. A tea of the flowers and leaves is taken to relieve stomach ache, biliousness, arthritis, cystitis, eye and throat inflammation. The juice of crushed leaves and the powder of dried leaves are used as poultices on wounds and inflammations. The gum is employed as an expectorant and to expel tapeworms (Taylor, 2004; Uchendu and Chowdhary, 2004).

Pharmacological Properties

Most of the effects observed with extract of *S. mombin* may be attributed to the phenols, tannins, anthraquinones and flavonoids present in the plant These active compounds have been reported to have several activities like antibacterial, anti-inflammation, haemostatic, anti-microbial, antioxidant, abortifacient activitiy, purgative, hypnotic, anti-diarrhoeal, anti-helmintic, anti-malarial, wound-healing, enzyme inhibiting activity, increased capillary permeability in rats, anti-free radical action, anti-aging, reduced glutathione synthesis (Ayoka *et al.,* 2008) and anticholinesterase activities (Elufioye *et al.,* 2010). The methanol and ethanol extracts at 50 and 100 mg doses were more potent as an anxiolytic than diazepam. It blocked picrotoxin-induced, convulsions. The methanol, ethanol and aqueous extracts showed sedative activity, decreased amphetamine and, apomorphine-induced, stero typed behavior. The aqueous and chloroform extract have been found to have some level of antifungal activity against some strains of fungi e.g. *Aspergillus niger, Penicillium* species and *Microsporum* species. No toxicity has been noted

for the aqueous, methanol and ethanol extract of the leaf when tested on mice (orally and intraperitorially) and rat (intraperitorially) at 5 mg/kg and 200 mg/kg (Taylor, 2004; Uchendu and Chowdhary, 2004).

Chemical Constituents

Phytochemical screening and subsequent quantification revealed the presence of bioactive compounds tannins 3.82 %; saponins 7.60 %; flavonoids 3.00 %, alkaloids 6.00 % and phenols 1.00 % proanthocyanins. Tests for the presence of vitamin showed that the plant leaves contained ascorbic acid **(**Abo *et al.,* 1999; Edeoga and Eriata, 2001). Moronkola *et al.,* (2003) reported more than 54 essential oils, chiefly caryophyllene as most abundant compound, delta cadinine, alpha-muurolene, alpha-gurjunene, 5-isocedranol and –cadinene. Augusto *et al.,* (2000) reported alcohols, esters, carbonyl compounds and terpernoids. Lemos *et al.,* (1995) reported that the leaf oil was rich in 3-hexenol and beta-caryophyllene.

1.8.10. *Cassia sieberiana* DC (Leguminoseae)

Scientific classification:

Kingdom: Plantae.
Division: Eudicots
Class: Rosids
Order: Fabales
Family: Leguminosae-Caesalpinioideae
Genus: Cassia
Species: *Cassia sieberiana* DC.

Synonyms

Cassia kotschyana Oliv.

Common Names

Drumstick tree (English), gama fada, Marga (Hausa), margaje (fulani), kiskatigrai (kanuri) Apagban (Edo), Kuhuwa (Tiv), Efo, ifo, Aridan tooro (Yoruba) (Keay *et al.,* 1964).

Origin and Geographic Distribution

Cassia sieberiana is distributed from Nigeria, Senegal and Gambia east to DR Congo and Uganda. In West Africa *C. sieberiana* flowers in March–April, just before the rainy season when the trees are leafless. In Uganda flowering is in June–August, during the rainy season, when new leaves have formed. The fruits ripen in August–October in West Africa and in September–February in Uganda. It is also found in most parts of Nigeria. In the North-West it is found in places like Zamfara, Zurumi and forest reserve near Sokoto. It is found widely distributed in Borno, Yobe, Bauchi and some part of Adamawa State in the North eastern part of Nigeria. It is found in Agodi in Ibadan Oyo State, Ile-Ife- Osun state and Awka near Onitsha in the South–west and South-east respectively (Keay *et al.,* 1964).

Botanical Description

C. sieberiana occurs in tree or shrub savannah with less than 800 mm annual rainfall. Acid sandy soil is preferred. It is a shrub or small tree up to 15(–20) m tall; bole short, twisted; bark fissured, grey to brown, with blackish stripes; young branches densely shortly hairy. Leaves are arranged spirally, paripinnately compound with 5–14 pairs of leaflets; stipules narrowly triangular, c. 2 mm; leaflets elliptical to ovate, 3.5–10 cm × 2–5 cm, apex rounded to acute, shortly hairy. Inflorescence is an axillary pendulous raceme up to 35(–45) cm long; bracts soon falling. Flowers are bisexual, slightly zygomorphic, 5-merous; sepals are elliptical, 5–8 mm long, slightly hairy; petals oblong to almost circular, 2–3.5 cm long, bright yellow during the dry season, which is from February through March. Flowers are arranged either upright or in pendulous racemes ranging from 30-50cm (Keay *et al.,* 1964). There are five sepals with 5

bracts.; stamens 10, free, 3 lower ones fertile, hooked at base, much longer than the petals, 4 middle ones fertile, short, 3 upper ones rudimentary; ovary superior, sessile, style slender, much longer than the petals. Fruit a, cylindrical pod 40–60(–90) cm × c. 1.5 cm, transversely partitioned, dehiscent by 2 valves, black, many-seeded with seeds embedded in yellow pulp. Seeds are ellipsoid, 8–9 mm long, rusty to dark brown and glabrous. Seedling is with epigeal germination. *C. sieberiana* is mainly propagated by seed. Ripe, fresh seeds have nearly 100% viability. One kg contains 7,000–16,500 seeds. Treatment with sulphuric acid is recommended before sowing older seeds.

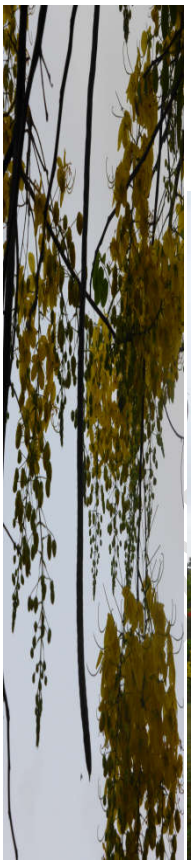


Plate 11: *Cassia sieberiana* DC. (Caesalpiniaceae).
A: Whole plant, B: Stem-bark, C: Leaves, D: Flowers, E: Seeds, F: Fruit. Collection site: Along road 2 O.A.U. Ile-Ife.

Pods of *Cassia sieberiana* are harvested by hand and the seeds are extracted manually as well. For harvesting the roots, the plant has to be dug up. After harvesting, the seeds need to be stored in a dry place. Storage in the pods is also feasible, but in that case extra care must be taken to prevent insect damage (Van der Maesen, 2007).

Major Ethnomedicinal uses

Leaves, roots and pods of *C. sieberiana* are widely used in traditional medicine (Ayantunde *et al.,* 2009). The entire plant is purgative and diuretic while in Senegal an infusion is given against all children's diseases. In Uganda powder of different plant parts is applied to teeth to cure toothache; when mixed with butter it is used to treat skin diseases. Previous ethnobotanical studies, undertaken by Asase and co-workers, indicated that *C. sieberiana* also has antimalarial activity (Asase *et al.,* 2005). In Senegal and Burkina Faso a steam bath of leafy twigs boiled in water is prescribed to help against malaria attacks and fever; the liquid should also be drunk. An infusion of the leaves sweetened with honey is taken against stomach-ache, ulcers and diarrhoea. The leaves help with the symptoms of arthritis and rheumatism. Boiled and squeezed fresh leaves are applied as poultice in pleurisy or burns. In Uganda diarrhoea, dysentery and vomiting are treated by a decoction of leaves. Gonorrhoea in women is treated by taking leaf powder with food. In Benin the twigs are used to treat sleeping sickness. Further unpublished reports indicate that the leaves are used with the leaves of other medicinal plants such as *Nauclea latifolia* and *Annona arenaria* to alleviate drepanocytosis acute symptoms (Madusolumuo *et al.,* 1999). In Uganda, diarrhoea, dysentery and vomiting are treated by a decoction of the bark. The wood is suitable for making furniture, tools, construction and railway sleepers. It is used as firewood, but it is considered inferior because it produces a lot of smoke. The root wood is used in Sierra Leone and Burkina Faso as chewing sticks. The tree is planted as an ornamental and as an

avenue tree. The yellow pulp around the seeds and an infusion of the pods is taken as a laxative. The seeds and roots are used as fish poison in Côte d'Ivoire and Nigeria. Specifically, cassia seeds assist in expelling internal heat, detoxification, weight reduction as well as the clearing off of acne. However, these factors are yet to be evaluated extensively in Nigeria (Olapade *et al.,* 2013). The roots are used as a diuretic and vermifuge and in the treatment of elephantiasis, leprosy, diarrhoea, haemorrhoids, dysentery, and venereal diseases. It can also be used to help symptoms related to the menstrual cycle and as an analgesic. The roots, boiled in water, are used to treat dropsy and bloody dysentery. Herd's men in some countries including Nigeria use the stem bark in the treatment of jaundice. The aqueous extracts of the roots, stem bark and the fruit pulp have been used traditionally in North-eastern Nigeria for the treatment of inflammatory conditions, tiredness and joint pains (Madusolumuo *et al.,* 1999). Other uses include improvement of lactation after child birth and treatment of rheumatic condition. In Côte d'Ivoire the decoction is taken in large doses to treat intestinal worms including tapeworms, although this is risky. An infusion of the root bark is employed against venereal diseases, sterility and dysmenorrhoea. After soaking the roots in water, the liquid is used for a bath against tiredness and for body massage. A decoction of the roots is considered an aphrodisiac. In Burkina Faso a pinch of powdered dried decorticated roots taken at the end of each meal is said to prevent malaria. Crushed roots are rubbed on the temples to treat headache. Debarked roots are boiled with bark of *Terminalia macroptera* Guill. & Perr. to combat eczema. In Burkina Faso capsules made from the root bark are prescribed against Aids. The extracts are used to treat fever, malaria, diuretics, diarrhoea, leprosy, bilhazia, stomach pains and as a dewormer (Tamboura *et al.,* 2005).

Major Pharmcological Properties

Previous studies showed that ethanol root extract of *C. sieberiana* had an antiparasitic effect, myorelaxant and antispasmodic activity. The ethanol and aqueous root extracts possessed analgesic, anti-inflammatory, antiparasitic, myorelaxant and antispasmodic activities (Duwiejua *et al.,* 2008; Sy *et al.,* 2009). The anti-nociceptive activity of the ethyl acetate extract of the root bark has been reported (Donkor *et al.,* 2013).

Recently, pharmacological studies showed that it has antimicrobial and antifungal activities (Asase *et al.,* 2008). It was also shown that its extracts had antimicrobial activity against *Neisseria gonorrhoeae, Herpes simplex virus* type I and African swine fever virus (Silva *et al.,* 1997a, b). The leaf extracts of *C. sieberiana* and *S. alata* inhibited *P. aeruginosa, B. cereus* and *S. aureus* at 45 mg/mL; at 90 mg/mL, the activity of the extracts was comparable to that of ampicillin, as well as that of gentamycin against *P. aeruginosa*. Its pod extract was more active than the pod extract of *C. alata* for antibacterial activity (Abo *et al.,* 1998).

The antioxidant properties of the whole extract of the root bark may explain its utilization and benefits in traditional medicine to alleviate pathological conditions. Since its roots extract possessed significant antioxidant and gastric cytoprotective prostaglandin properties as well as serum secretory phospholipase A_2 inhibitory activity which could be due to its content of polyhydroxy and/or phenolic substances. This may justify its use as an anti-ulcerogenic agent in traditional medicine in West Africa (Nartey *et al.*, 2012).

Toxicity

Neuwinger, (1996) reported that in many West African regions, from Senegal/ Gambia/Guinea to Nigeria, *C. sieberiana* is very well known as a very active poison, widely used for hunting and fishing or as arrow poison. In Sudan, it is well known that *C. sieberiana* will never be eaten by any animal, even when they are in danger of acute starvation. The age of the plant and the vegetal part used could also explain the variability and high amount of toxicity of *Cassia* gender

members (El Sayed *et al.,* 1983), although many species are used as food or medicine, without any toxicity (Salunkhe *et al.,* 1982).

The acute toxicity study of *C. sieberiana* leaf aqueous extract revealed an intraperitoneal lethal median dose LD_{50} of 960 mg/kg, and clinical signs, gross and microscopic lesions that were dose-related. This suggests that the extract is moderately toxic to albino rats. Clarke and Clarke, (1975) and Biu *et al.,* (2010) suggested that substances with LD_{50} of between 500 and 5000 mg/kg are moderately toxic, and should be used in veterinary practice with some degree of caution. The toxicity of plant extracts are generally related to their phytocomponents such as glycosides, saponins, terpenes, flavonoids, tannins and alkaloids. These substances have been shown to be present in high levels in *C. sieberiana* (Mavar-Manga, 2006; Asase *et al.,* 2008). Oral administration of *C. sieberiana* stem bark extract resulted in significant hepatotoxicity and nephrotoxicity (Obidah *et al.,* 2009). Obidah and his co-workers (Obidah *et al.,* 2009) reported that oral administration of aqueous extract of *C. sieberiana* stem bark to rats resulted in hepatotoxicity at lower dose levels of 20- 60 mg/kg and nephrotoxicity at higher doses of 180 mg /kg. Tamboura *et al.,* (2005) found that the LD_{50} of the aqueous leaf extract in mice was 24.4 mg/kg. Acute toxicity test of *C. sieberiana* roots extract showed no sign of toxicity up to a dose level of 2000 mg/kg body weight p.o (Nartey *et. al*, 2012). Oral toxicity of the aqueous extract of the root bark of *C. sieberiana* in rodents is low (oral $LD_{50} > 5000$ mg/kg). However, the extract may have deleterious effects on the liver at high doses on prolonged administration. These findings support its safe ethnomedicinal use at moderate doses (Donkor *et al.,* 2014).

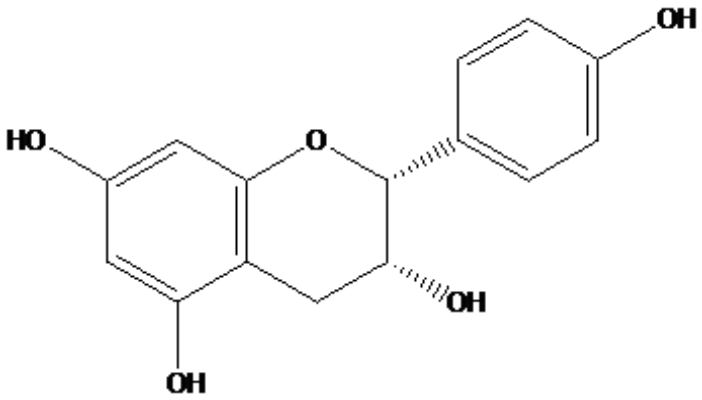
Toma *et al.,* 2009 reported that despite the fact that the calculated LD_{50} of the fruit of *C. sieberiana* indicated a low toxicity, the result of an oral acute toxicity study showed that the LD_{50} of the extract is 1950 mg/kg, indicating that the extract is of low toxicity, using the extract at a high dose such as 400 – 1600 mg/kg body weight for a long period can cause liver damage.

Therefore, the plant should be taken at a very low dosage (below 200 mg/kg body weight) and should not be taken over a long period of time.

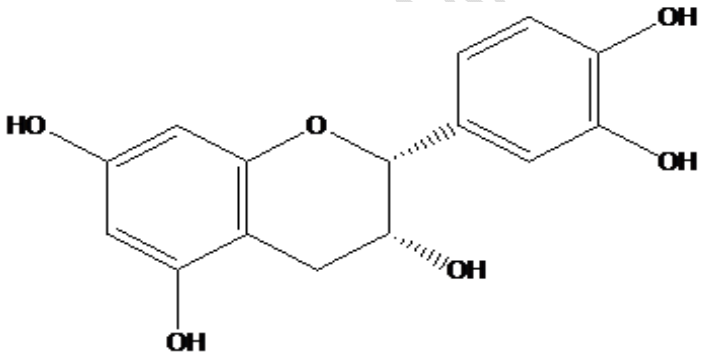
Chemical Constituents

Cassia species are rich sources of polyphenolics and anthraquinone derivatives, flavonoids and polysaccharides and tannins (Deshpande and Bhalsing, 2011). The phytoconstituents of *C. sieberiana*, which are phenols, anthraquinones, alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants (Hafiza *et al.,* 2002). As one of these phytoconstituents, saponins have been reported to exhibit haemolytic and foaming activity, antifungal, anti-inflammatory, fungistatic, molluscidal (Ajayi *et al.,* 2011). The occurrence of tannins as one of the phytochemicals/ antinutrients might result in lowering the food value of the seed (Rao and Prahavathi, 1982). It is reported that tannins lower the digestibility of grain legumes (Odo *et al.,* 2005). The presence of anthraquinones glycosides are responsible for many of the medicinal properties observed in the plant however, anthraquinones have not been isolated (Akomolafe *et al.,* 2003). The root of this plant also contains many other natural products such as anthracenic derivatives and tannins. Flavonoids have been found previously in the roots of *C. sieberiana* through classical isolation and characterization methods. In *C. sieberiana* roots the main compound (-)-epiafzelechin ***(20)***, with antioxidant activity isolated from the ethanol root extract, was slightly more effective than the crude extract in a DPPH assay. The bark extracts contain calcium oxalates, tannin sterols, phenolic compounds leucopelargonicol, epicatechin ***(21)*** and triterpenoids such as sitosterol ***(22)***, stigmasterol ***(23)*** were found (Tamboura *et al.*, 2005). Olapade *et al.,* (2012) studied changes in some physico-chemical properties of the seed during roasting. This study showed that roasting had a significant effect on moisture content, caffeine content, weight loss, colour and swelling of the seed while it had non-significant effect on pH, total titratable acidity, acetic acid, and total soluble solid of the seed. Seed was found to be

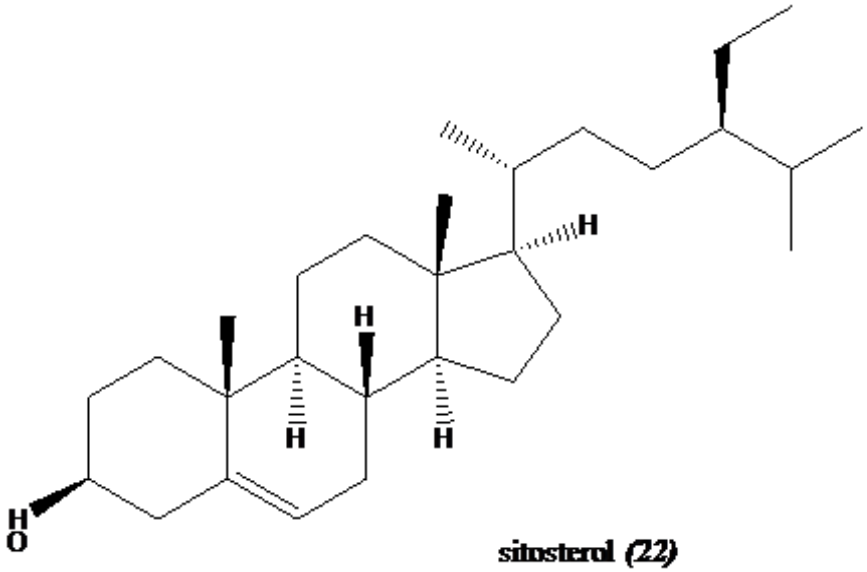
moderately high in crude protein (23.72 %) and crude fibre (10.75 %) and also in potassium (252.33 mg/L) and magnesium (52.68 mg/L). Amongst the phytochemical constituents of seed are tannins, flavonoids, cardiac glycosides, alkaloids, phenols, and oxalate. Saponins were not detected (Olapade *et al.,* 2012). Toma *et al.,* 2009 reported that the pods pulp of *C. sieberiana* contained tannins, alkaloids, saponins, steroids, flavonoids, phlobatannins, cardiac glycosides, cyanogenic glycosides and reducing sugars.



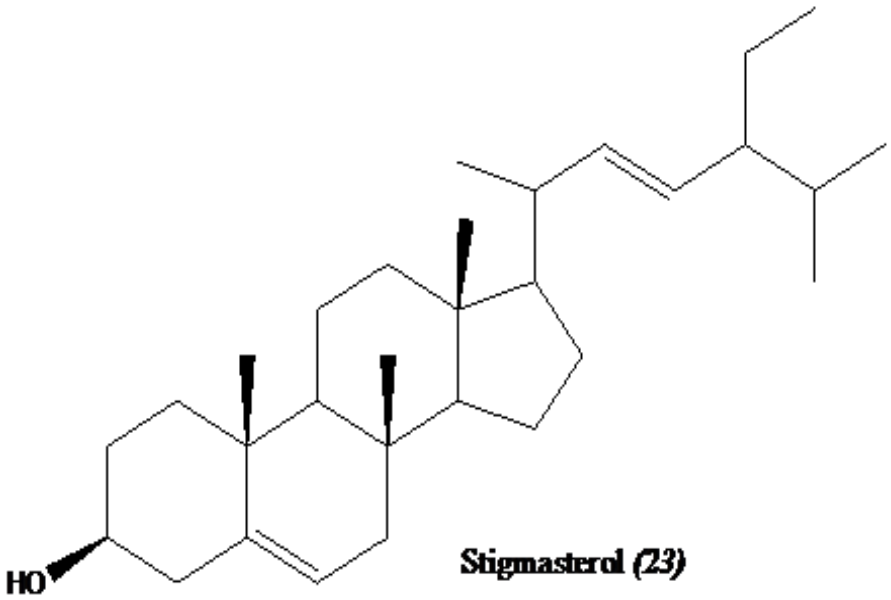
(-)-Epiafzelechin *(20)*



epicatechin *(21)*



sitosterol *(22)*



Stigmasterol *(23)*

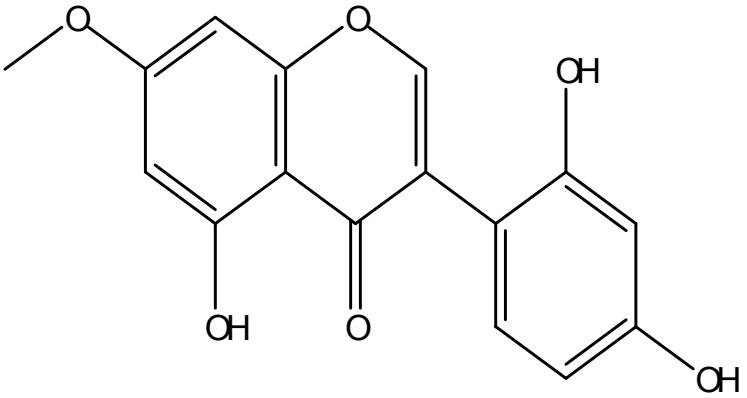
Figure 2a: (1-23): Structures of compounds present in medicinal plants.

1.9. COMPOUNDS WITH ANTI-SICKLING ACTIVITIES

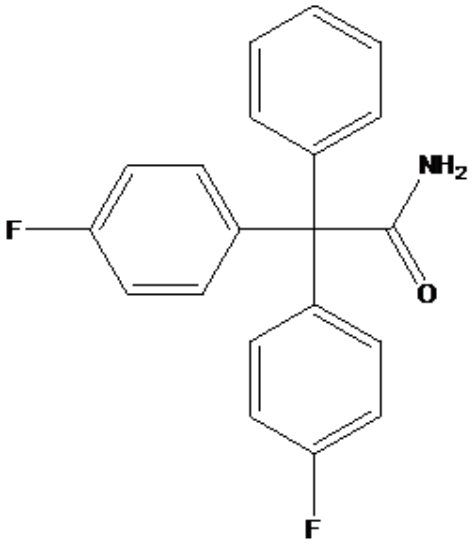
It is known that phytochemicals confer pharmacological relevance on plants. The growing interest in herbal medicine (Atawodi, 2005) demands information on various plant preparations used in the treatment of diseases (Sofowora, 1991). Scientific evaluation of medicinal plants is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of either herbal preparations or conventional drugs of plant origin.

Recently a good number of studies have been done for identification and characterization of potential anti-sickling agents/compounds from various plants. The most promising compounds were found to be anthocyanins, water-soluble vacuolar pigments, anthraquinone derivatives, steroidal glycosides, phenols, senicapoc ***(24)***, 5,2',4'-trihydroxy-7-methoxyisoflavone ***(25)***, gallate ***(26)***, lawsone ***(27)***, emodin ***(28)***, resveratrol ***(29)***, 4-hydroxybenzoic acid ***(30)***, limonoids, 5-hydroxymethyl-2-furfural (5hmf), a naturally occurring aromatic aldehyde, isomeric divanilloylquinic acids, vanillic acid ***(31)*** and certain amino acids like arginine, tyrosine, aspartic acid, and phenylalanine (Kunle and Egharevba, 2013). Divanilloylquinic acid, zanthoxylol ***(32)***, 2-hydroxy methyl benzoic acid ***(33)*** isolated from root bark of *Z. zanthoxyloides* possessed anti-sickling properties (Sofowora *et al.,* 1979). The reported compounds are having *in vitro* anti-sickling effects, their modes of action and mechanism have not been properly assayed (Dash *et al.,* 2013). Using two new techniques of nuclear magnetic resonance spectroscopy, investigation on the binding sites of L-phenylalanine, L-tryptophan, L-valine, and p-bromo benzyl alcohol to sickle haemoglobin has been carried out. With the exception of L-valine, all of these molecules are known to inhibit the polymerization of deoxy Hb S. Also, for the compounds with anti-sickling activity, there are at least two binding sites to the Hb S molecule, one at or near the heme pockets of the alpha and beta chains and the other one in the vicinity of the beta 6 mutation

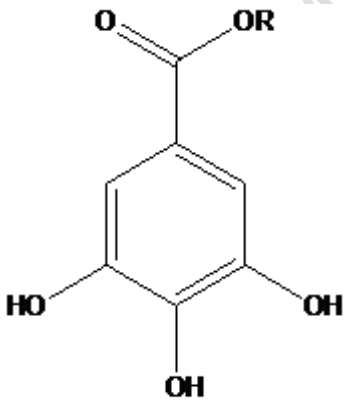
site (Ho and Russu, 1987). A large variety of compounds have been found to inhibit the polymerization of deoxy- Hb S either by reacting covalently to modify the HbS molecule or by non-covalent interactions. Among the non-covalent agents, inhibition of polymerization has been demonstrated for aromatic amino acids, alkyl ureas (Elbaum *et al.,* 1974), phenyl derivatives (Behe and Englander, 1979), benzyl esters of aromatic and hydrophobic amino acids (Gorecki *et al.,* 1980) and benzyloxy and phenoxy acids (Abraham *et al.,* 1984). In addition, phenyl alanine containing di- and tripeptides and various oligopeptides have been shown to inhibit HbS polymerization (Noguchi and Schechter, 1978; Gorecki *et al.,* 1980; Franklin *et al.,* 1983).



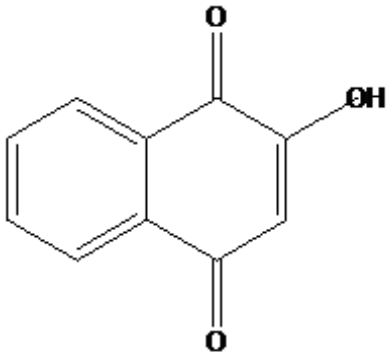
5,2',4'-trihydroxy-7-methoxyisoflavone *(25)*



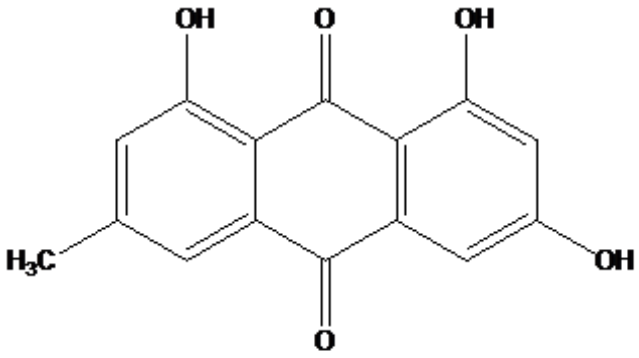
Senicapoc *(24)*



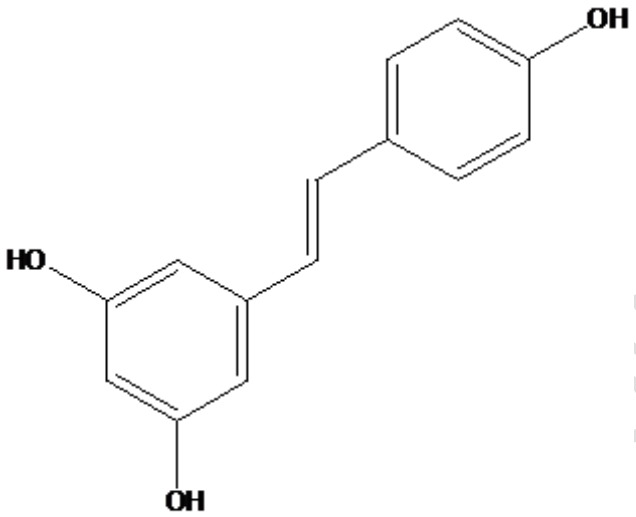
Gallate *(26)*



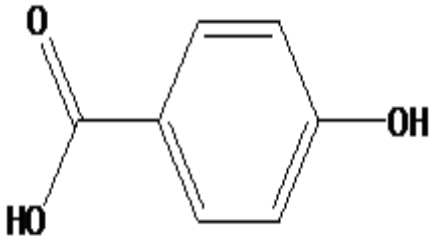
2-hydroxy-1,4-napthoquinone (Lawsone) *(27)*



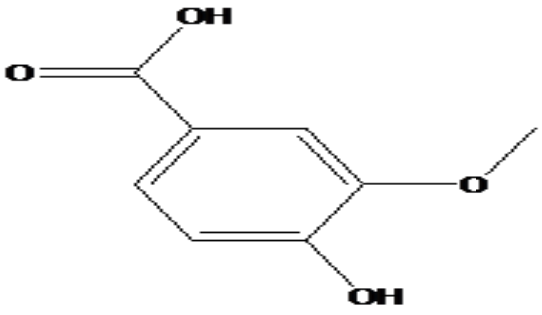
6-methyl-1,3,8-trihydroxyanthraquinone (Emodin) (28)



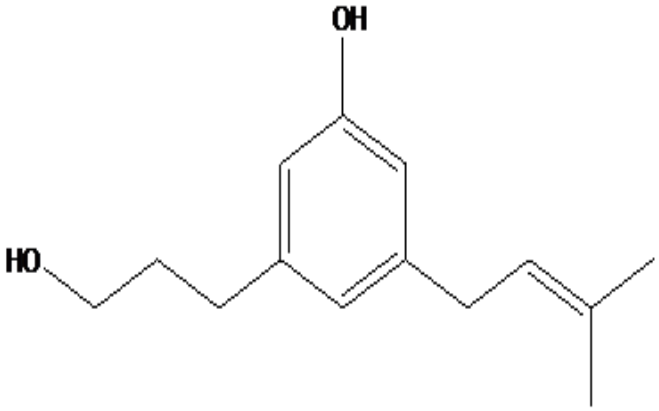
Resveratrol (29)



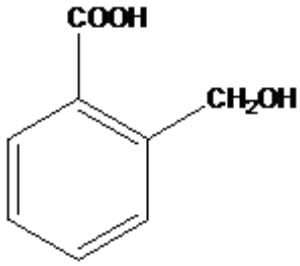
4-hydroxybenzoic acid (30)



vanillic acid (31)



Xanthoxylol (32)



2-Hydroxymethylbenzoic acid (33)

Figure 2b: (24-33): Structures of phyto-compounds with anti-sickling activities.

1.10. Commercially-Available Herbal Products for the Management of Sickle Cell Disease.

Different products have been developed and are being sold commercially for the management of sickle cell anaemia. Examples among others include:

- DREPANOSTAT developed from the extract of *Zanthoxylum zanthoxyloides* in Togo and Benin Republics.

- FACA, a mixture of *Fagara* and *Calotropis procera* used in Burkina Faso.
- SOLAMIN®, a honey based herbal preparation consisting of *Andrographis paniculata, Citrus aurantifolia* and *Allium sativum*. It is produced by Esoma Herbals Limited in Nigeria. Recommended dosage is set at 5mg/kg.
- CIKLAVIT®, marketed by Neimeth Pharmaceuticals Nigeria, is an extract of *Cajanus cajan* (Oniyangi and Cohall, 2010).
- SICULINE, by the Village Chemist, Obafemi Awolowo University, a mixture of *Carica papaya* and *Sorghum bicolour*; and
- NIPRISAN®, now NICOSAN®, developed by the National Institute of Pharmaceutical Research and Development, Abuja Nigeria. In an attempt to find other effective agents with less adverse effects, the anti-sickling effect of NIPRISAN (Nix-0699) a product of the extracts of four different plants, (seeds *of Piper guineenses*, stem parts of *Pterocapus osun*, fruit of *Eugenia caryophyllum*, and leaves of *S. bicolor*) was investigated. It was found that Nix-0699, an ethanol/water extract from indigenous plants, has a strong anti-sickling effect. The concentration of Nix-0699 required to inhibit 50% of erythrocyte sickling was about 0.05 mg/mL. As for the kinetics of polymerization, addition of 0.05 mg/mL. Nix-0699 caused a six fold prolongation of the delay time prior to deoxy-HbS polymerization when compared with that of untreated HbS samples. The solubility of deoxy-HbS significantly increased upon treatment with Nix-0699. Analysis of the effect of Nix-0699 on the HbS oxygen affinity indicated that the drug slightly shifted the oxygen-dissociation curve of HbS toward the left without any apparent change in the Hill coefficient. These results suggest that the anti-sickling properties of Nix-0699 may involve direct interaction with Hb molecules (Wambebe *et al.,* 2001; Iyamu *et al.,* 2002; Iyamu *et al.,* 2003).

1.11. Models of Anti-sickling Test.

The basis of any treatment and evaluation lies in finding effective assay methods and models to project potential drugs for clinical trials. The most common assay methods attempt to observe the ability of the compound to inhibit or reverse sickling of erythrocytes *in vitro* at low oxygen tensions (Dean and Schechter, 1978). Low oxygen tensions can be brought about by nitrogen gas, sodium metabisulphite and sodium dithionite.

1.12. Methods of Anti-sickling Assays.

- Blood-agar plate test (Fadulu, 1977).

Sheep blood is dispersed in agar in a standard agar plate. On heating in an oven at 70° C for 15 minutes, the red Plates turn rust brown. If a drop of a solution of an effective anti-sickling drug is placed on the plate prior to heating, the blood under that spot remains red while the rest of the plate turns brown.

- Modified method of Sofowora *et al.,* (1979).

Vein-punctured blood samples from sickle cell anaemia patients not in crises were collected into EDTA bottles, and centrifuged to remove the serum. The resulting packed erythrocytes were washed 3 times with 1 mL sterile normal saline per 5 mL of blood. The samples were then centrifuged each time for 5 min at a speed of 2000 revolution per minute to remove the supernatant. 0.5 mL of the washed erythrocytes were mixed each with 0.5 mL of the different

extracts in uncovered test tubes and mixed together. Samples were taken from the different mixtures and the remaining mixtures incubated at 37°C for 3 h while shaking occasionally. 0.2 mL of 2 % sodium metabisulphite were added to deoxygenate the system, mixed thoroughly and sealed with liquid paraffin. Samples were taken in duplicates from the different mixtures at 0 min after which the systems were incubated again at 37°C and the samples taken again at 45 min interval until 5 readings were obtained. Each sample was smeared on a microscope slide, fixed with 95 % methanol, dried and stained with giemsa stain. Each sample was examined under the oil immersion light microscope and counting at least 500 red blood cells in each sample from five different fields of view across the slide. The numbers of both sickled and unsickled red blood cells were counted and the percentage of unsickled cells determined. Two types of controls were employed in this biological testing, a positive control using p-hydroxybenzoic acid (5 mg/mL) a compound known to reverse the sickling in Hb SS blood cells and a negative control involving the use of normal saline. Each set-up in the experiment was replicated twice. The blood sample collected from a particular patient was used for testing of each set of experiment (Egunyomi *et al.,* 2009).

- Method by Oduola *et al.,* (2006).

Inhibitory activity of extract.

A drop of blood from a sickle cell patient (SS) was mixed with a drop of freshly prepared 2 % sodium metabisulphite on a clean slide, mixed well and cover slipped (Barbara, 1980). The cover slip was gently pressed to remove excess mixture, the excess mixture was removed with cotton wool and the edges of the cover slip sealed with Vaseline to prevent air from going in.

The slide was incubated at 37°C for 30 min and then viewed under a microscope. Similar slides as described above were prepared and a drop of saline extract from each of the bottles with different concentration of the extract or the aqueous, fractions or isolates was added to each of the slides. The slides were incubated at 37°C for 30 min and viewed under a microscope.

Reversal activity of the extract

A drop of blood from a sickle cell patient (SS) and a drop of freshly prepared 2 % sodium metabisulphite were mixed together on a clean glass slide and covered with a cover slip. The edges were sealed with Vaseline after gently pressing to remove excess mixture. The slide was incubated at 37°C for 30 min and viewed under a microscope. A drop of saline extract or the aqueous, fractions and isolates of the fruit was then added to the mixture on the slide, mixed and covered with a cover slip. The slide was incubated at 37°C for another 30 min. The slide was viewed under a microscope.

1.13 Justification of Study

The 63rd session of the UN General Assembly in December 2008 adopted a resolution on the "recognition of sickle-cell anaemia as a public health problem," and urged Member States and UN organizations to raise awareness of Sickle Cell Disease (SCD) on June 19 of each year.

Despite the fact that the UN has called for global efforts "to bring the disease out of the shadows," relatively little attention has been given to assessing the burden of SCD and taking actions to reduce its effects in Africa, where about 85 % of children with SCD are born (Modell and Darlison, 2008).

There is the need for continuous research to discover new drugs that will be used to manage the symptoms and complications associated with the disorder. Medicinal plants are continually being investigated in this regard.

An ethno-botanical survey was conducted by the Drug Research and Production Unit of the Obafemi Awolowo University, Ile-Ife in 2012 on medicinal plants used in the management of SCD in South-western region of Nigeria from which some of the following plants with high frequency of usage and reports on some pharmacological activities associated with SCD crises were selected; *Peperomia pellucida* Linn, *Mimosa pudica* Linn, *Spondias mombin* Linn, *Lecanioidiscus cupanioides* Planch ex Benth, *Anthocleista vogelli* Planch, *Morinda lucida* Benth, *Cassia sieberiana* DC, *Harungana madagascariensis* Lam. ex. poir, *Senna alata* (L.) Roxb and *Dioclea reflexa* Hook. f.

1.14. Objectives of the Study

The main objective of this study is to determine the anti-sickling activities of the ten selected Nigerian medicinal plants employed in the management of SCD. The specific objectives are to:

- screen some medicinal plants used in the ethnomedical management of sickle cell anaemia;
- fractionate the extract(s) of the plant with the highest anti-sickling activities; and
- determine fraction(s) with the highest anti-sickling activities.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1. Collection and Identification of Plant materials and processing.

The plants parts were all collected from different locations within the campus of Obafemi Awolowo University Ile-Ife (Table 2) except for the seeds of *D. reflexa* which was bought from the herbal shop (New Market Ile-Ife). Each plant was identified by the plant curator, Mr. G. Ibhameselbhor, Department of Botany, O.A.U. and deposited at the IFE herbarium. Their herbarium numbers are documented below (Table 2).

The fresh leaf samples were separately oven dried at 40ºC and powdered using grinding machine (Christy), while the stem bark, pericarp, seed, whole fruit and root parts were also separately washed, oven dried at 60°C and powdered using grinding machine (Christy).

2.2. Methods of Extraction.

2.2.1. 70% (v/v) cold ethanol extracts.

Each powdered plant material (200 g) was macerated with 70 % (v/v) ethanol at room temperature, soaked and shaken in a mechanical shaker at intervals for 3 days, filtered and concentrated to dryness *in vacuo*, using a rotary evaporator (Buchi), to yield the 70 % (v/v) cold ethanol extracts (CEE).

Table 2: List of plant species investigated and their herbarium numbers.

	Plant Name	Plant Family	Part used	Month of Collection	Herbarium number
1	*Anthocleista vogelii* Planch.	Gentiaceae	Bark	November, (2013)	IFE-16773
2	*Cassia sieberiana* DC.	Leguminoseae	Leaf, Fruit, Bark	July, (2013) & February, (2014)	IFE-17381
3	*Dioclea reflexa* Hook. f.	Papilionaceae	Fruit	Bought	FPI 1762
4	*Harungana madagascariensis* Lam. ex. Poir.	Hypericaceae	Leaf	July, (2013)	IFE-17382
5	*Lecanioidiscus cupanioides* Planch. ex. Benth.	Sapindaceae	Leaf	November, (2013)	IFE-16822
6	*Mimosa pudica* Linn.	Mimosaceae	Leaf	May, (2013)	IFE-16932
7	*Morinda lucida* Benth.	Rubiaceae	Leaf	November. (2013)	IFE-16931
8	*Peperomia pellucida* Linn.	Piperaceae	Leaf	October, (2013)	IFE-16778
9	*Senna alata* (L.) Roxb.	Leguminoseae	Leaf	November, (2013)	IFE-16787
10	*Spondias mombin* Linn.	Anarcadiaceae	Leaf, Root	November, (2013)	IFE-16229

2.2.2. 70 % (v/v) ethanol Soxhlet extracts.

Each powdered plant material (100 g) was weighed using a chemical balance (Mettler); extracted with 70 % (v/v) ethanol using the Soxhlet apparatus and filtered. The filterate was concentrated to dryness *in vacuo*, using a rotary evaporator (Buchi), to yield the 70 % (v/v) ethanol Soxhlet extract (SEE).

2.2.3. Bulk extraction of *Cassia sieberiana* pericarp.

The powdered pericarp of *C. sieberiana* (2 kg) was weighed using a chemical balance (Mettler); extracted with 70 % (v/v) ethanol using the Soxhlet apparatus and the resulting extract was concentrated to dryness *in vacuo* using a rotary evaporator (Buchi), to yield the bulk extract of *C. sieberiana* pericarp for the fractionation experiments.

2.2.4. Aqueous extracts (decoction)

The aqueous extracts were prepared by boiling 200 g of each powdered plant material for 3 hours under reflux, allowed to cool, filtered and concentrated to dryness *in vacuo* using a rotary evaporator (Buchi), to give the dried aqueous extract (AQE).

2.2.5. Aqueous extraction (decoction) of *C. sieberiana* whole fruit.

The powdered whole fruit of *C. sieberiana* (1 kg) was boiled for 3 hours under reflux, allowed to cool, filtered and concentrated to 1.2 L *in vacuo* at 50°C using a rotary evaporator (Buchi). The resulting extract was freeze-dried at the Central Science Laboratory Obafemi Awolowo University thus bringing the extract to complete dryness, to yield the bulk aqueous extract of *C. sieberiana* whole fruit for the VLC fractionation experiment.

2.3. Chemicals

Ethanol, Methanol (Fluka), n- Hexane, Benzene, Dichloromethane, Ethyl acetate (BDH), Benzene, Chloroform (Sigma-Aldrich), Formaldehyde, Phosphate buffered saline pellets pH 7.0, Sodium metabisulphite (Hopkins and Williams), Tween-80, para- hydroxybenzoic acid (PHBA), Vanillic acid, Concentrated sulphuric acid. All solvents were distilled before use. All solutions were prepared with distilled water.

2.3.1. Preparation of Reagents.

2.3.1.1. Sodium metabisulphite solution (2 % w/v):

Sodium metabisulphite powder (2 g) was dissolved in distilled water and the solution was made up to 100 mL to give 2 % w/v solution. Fresh preparations were made for each use.

2.3.1.2. Phosphate buffered saline solution (pH 7.0):

A phosphate buffered saline pellet, was dissolved in a little quantity of distilled water and made up to 100 mL distilled water. The pH was adjusted using a pH meter.

2.3.1.3. Buffered formalin solution (5 % v/v):

From a stock solution of 40 % (w/v) formalin, 12.4 mL was taken and diluted up to 100 mL with phosphate buffered saline solution.

2.3.1.4. Preparation of Reference Standards (experimental controls):

A 4 mg/mL p-hydroxybenzoic acid (PHBA) solution was prepared as a positive control for the reversal anti-sickling activity while a 4 mg/mL solution of vanillic acid was prepared as a positive control for the inhibitory anti-sickling activity. Phosphate buffer saline was used as negative control. Where Polysorbate 80 commercially known as Tween 80 was used to dissolve the organic residues obtained from the fractionation experiments, a 0.1 % (w/v) of Polysorbate 80 was used as negative control.

2.3.1.5. Potassium hydroxide 5 % (w/v):

Potassium hydroxide pellets (5 g) was dissolved in 100 mL of absolute ethanol.

2.3.1.6. Ferric chloride 2 % (w/v):

Iron (III) Chloride (2 g) was dissolved in 100 mL of methanol. .

2.4. Anti-sickling Experiments

2.4.1. Preparation of test extracts/fractions for Anti-sickling experiments.

For each of the plant extract/fraction, a stock solution of 4 mg/mL was prepared by dissolving 40 mg of the extract/fraction in 10 mL of distilled water and used for anti-sickling experiments. For each of the extracts of *C. sieberiana* pericarp, seeds, whole fruits, the 4 mg/mL stock solution was further diluted serially to make concentrations of 2 mg/mL, 1 mg/mL and 0.5 mg/mL for antisickling experiments. A 0.1% (v/v) of Polysorbate 80 commercially known as Tween 80 was used to solubilize the fractions in distilled water.

2.4.2. Collection of blood samples for Anti-sickling experiments.

Blood samples were collected from confirmed sickle cell patients in steady state at the Obafemi Awolowo University Teaching Hospitals Complex at the Haematology outpatient clinic after taking ethical clearance from the ethical clearance committee of the Obafemi Awolowo University Teaching Hospital Ile-Ife. Using new disposable needles and syringes, 5 mL of blood was drawn by vein-puncture from each patient into EDTA (ethylene diamine tetra acetic acid) bottles. The fresh blood samples collected were always used within the first 24 hours of collection (Ogunyemi *et al.,* 2008).

2.4.3. Inhibitory Anti-sickling Assay (Modified method of Sofowora *et al.*, 1979).

HbSS whole blood (0.2 mL in triplicates) was placed in a test tube and to each test tube, 0.2 mL of freshly prepared phosphate buffer solution (pH 7.0) and 0.2 mL of the test sample/fraction were added. This was mixed carefully together and the mixture covered with 1mL liquid paraffin to prevent aeration or oxygenation. It was then incubated in a thermostated water bath (Tecam) at 37°C for 4 hours. At the end of the incubation period, 0.6 mL of freshly prepared 2 % (w/v) sodium metabisulphite solution was carefully added to the mixture under the liquid paraffin layer and then gently mixed by rolling the test tube between the two palms followed by further incubation for another 1.5 hours at 37ºC in a water bath (Tecam). After this, the liquid paraffin was carefully removed using a Pasteur pipette and the red blood cells in the remaining mixture were fixed with 3 mL of 5 % (v/v) buffered formalin solution. Cells were counted using a

microscope at a magnification of 1000 (using × 100 objective × 10 eye piece) and 5 fields of view were counted per slide.

Each experiment was carried out in triplicates. The negative control consisted of 0.2 mL phosphate buffered solution and/or 0.2 mL polysorbate 80 used in place of the test samples in the mixture. Vanillic acid solution and Ciklavit (0.2 mL) was used in place of the test samples in the positive controls for inhibitory activity. All results were subjected to statistical analysis using GraphPad Instat version 3.01.

2.4.4. Reversal Anti-sickling Assay (Modified method of Sofowora *et al.*, 1979).

HbSS whole blood (0.2 mL in triplicates) was placed in a test tube, 0.2 mL of phosphate buffer solution was added and the mixture was covered with 1 mL liquid paraffin to prevent aeration and oxygenation. Freshly prepared 2 % (w/v) sodium metabisulphite solution (0.6 mL) was carefully added to the blood layer under the liquid paraffin and then gently mixed by rolling the test tube between the two palms. The mixture was then incubated for 1.5 hours at 37ºC in a water bath (Tecam). At the end of the incubation period, 0.2 mL of the extract solution or fraction was carefully added under the liquid paraffin into the blood layer and incubated for another 6 hours at 37ºC in a water bath (Tecam). The liquid paraffin was carefully removed using a Pasteur pipette and the remaining mixture was fixed with 3 mL of 5 % (v/v) buffered formalin solution. Cells were counted using a microscope at a magnification of 1000 (using × 100 objective / × 10 eye piece) and 5 fields of view were counted per slide. Each experiment was carried out in triplicates. The negative control consisted of 0.2 mL phosphate buffered solution and/or 0.2 mL polysorbate 80 was used in place of the test samples in the mixture. Ciklavit and p- hydroxy

benzoic acid (0.2 mL each) was used in place of the test samples in the positive controls for reversal activity.

2.4.5. Method of calculation (Ogunyemi *et al.*, 2008).

% Sickled = × 100

% Inhibition or Reversal = × 100

Where % SN = % sickled in negative control

% ST= % sickled in test sample/fraction

2.4.6 Vanillic acid-, p-Hydroxybenzoic acid-, Ciklavit-, equivalents (Adesanya, 1980)

2.4.6.1 Calculation of vanillic acid-equivalents (VAE) and percentage vanillic acid activities (VAA).

The vanillic acid-equivalent (VAE) of any anti-sickling plant material at 4 mg/ mL is the relative anti-sickling activity of that plant in relation to the anti-sickling activity of vanillic acid at the same concentration when tested under the same experimental conditions of inhibition of HbSS as described in this work. Similarly, the % vanillic acid activity (VAA) is the percentage of the anti-sickling activity of vanillic acid at 4 mg/mL possessed or demonstrated by the plant under investigation when treated under the same experimental conditions. Both VAE and VAA can give an assessment of how much VA is more or less potent than the plant material under investigation using the following formulae:

Vanillic acid – equivalent (VAE) =

where IH test is the inhibitory activity of test sample and IH VA is the inhibitory activity of vanillic acid.

Thus, the percentage vanillic acid activity (VAA) = VAE × 100 %

2.4.6.2 Calculation of p-Hydroxybenzoic acid-equivalents (PHBAE) and percentage p-Hydroxybenzoic acid activities (PHBAA).

The p-hydroxybenzoic acid-equivalent (PHBAE) of any anti-sickling plant material at 4 mg/ mL is the relative anti-sickling activity of that plant in relation to the anti-sickling activity of p-hydroxybenzoic acid at the same concentration when tested under the same experimental conditions of reversal of HbSS as described in this work. Similarly, the % p-hydroxybenzoic acid activity (PHBAA) is the percentage of the anti-sickling activity of p-hydroxybenzoic acid at 4 mg/mL possessed or demonstrated by the plant under investigation when treated under the same experimental conditions. Both PHBAE and PHBAA can give an assessment of how much PHBA is more or less potent than the plant material under investigation using the following formulae:

p- Hydroxybenzoic acid equivalent (PHBAE) =

where RE test is the reversal activity of test sample and RE PHBA is the reversal activity of p-hydroxybenzoic acid.

Thus, the percentage p-hydroxybenzoic acid activity (PHBAA) = PHBAE × 100 %

2.4.6.3 Calculation of Ciklavit-equivalents (CE) and percentage Ciklavit activities (CA)

The Ciklavit-equivalent (CE) of any anti-sickling plant material is the relative anti-sickling activity of that plant material at 4 mg/mL in relation to the anti-sickling activity of 0.2 mL of

Ciklavit when tested under the same experimental conditions of inhibition/reversal of HbSS as described in this work. Similarly, the % Ciklavit activity (CA) is the percentage of the anti-sickling activity of Ciklavit possessed or demonstrated by plant under investigation when treated under the same experimental conditions. Both CE and CA can give an assessment of how much CA is more or less potent than the plant material under investigation using the following formulae:

Ciklavit-equivalent (CE) =

Ciklavit-equivalent (CE) =

where IH test is inhibitory activity of test sample and IH Cik- inhibitory activity of Ciklavit; RE test- reversal activity of test sample, RE Cik- reversal activity of Ciklavit.

Thus, the percentage Ciklavit activity (CA) = CE × 100 %

2.5. Vacuum Liquid Chromatographic (VLC) Procedure.

The 70 % ethanol soxhlet extract of *C. sieberiana* pericarp (80 g) which gave the best inhibitory activity, was mixed with TLC grade silica gel (20 g) and fractionated using TLC grade silica gel (280 g) in a sintered-glass funnel. The solvents used are: n-hexane (100 %), n-hexane: dichloromethane (1:1), dichloromethane (100 %), dichloromethane: ethyl acetate (1:1), ethyl acetate (100 %), ethyl acetate: methanol (1:1) and methanol (100 %) in order of increasing polarity to give 7 fractions (fractions A-G). The same was repeated for the aqueous extract of the whole fruit of *C. sieberiana* (80 g) which gave the best reversal activity to give 7 fractions (fractions H-N). The VLC fractions were concentrated *in vacuo* and their yields determined

(Table 4 and 5). Each fraction was screened for anti-sickling activities using the method as shown in 2.3.3.-2.3.5.

2.6. Thin Layer Chromatography (T.L.C).

Precoated silica gel Plates (Silica gel 60 F_{254} Merck) 0.25 mm thick, were used for T.L.C. Solutions of each of the fractions and methanol solutions of their corresponding crude extracts were spotted with capillary tube on the precoated silica gel sheets. The Plates were carefully placed in a saturated chamber containing appropriate solvent systems: chloroform: methanol (7:3) (v/v), dichloromethane: methanol (8:2) (v/v) and benzene: ethyl acetate: acetic acid (7:2:1) (v/v/v). After full development, the Plates were removed, air dried, visually inspected and then examined under ultraviolet light at 254 nm and 366 nm using Ultraviolet lamps (Gallenkamp). The Plates were sprayed with chromogenic agents namely: 20 % (w/v) sulphuric acid, Vanillin-sulphuric acid, 5 % (w/v) alcoholic KOH, 2 % (w/v) alcoholic $FeCl_3$ and Draggendorf's spray reagent, followed by colour development at 110°C.

CHAPTER THREE

3.0 RESULTS

3.1 Plant Extraction

Table 3 below shows the different yields of the extracts i.e. 70 % (v/v) cold ethanol extracts (CEE), 70 % (v/v) ethanol Soxhlet extracts (SEE) and aqueous extracts (decoctions) (AQE).

3.2. Vacuum Liquid Chromatography (VLC) of *C. sieberiana*.

Tables 4 and 5 show the yields obtained and volumes of solvents used in the VLC fractionation of the extracts of the pericarp and ripe fruit of *C. sieberiana*. Different solvents were used, ranging from non-polar to moderately polar to polar solvents leading to separation of the constituents based on their relative polarity.

3.2.1. VLC fractionation of 70 % (v/v) ethanol Soxhlet extract of *C. sieberiana* pericarp

The 70 % (v/v) ethanol soxhlet extract *C. sieberiana* pericarp, which gave the highest inhibitory anti-sickling activity, was fractionated and many constituents were observed (Table 4).

3.2.2. VLC fractionation of the Aqueous extract (decoction) of *C. Sieberiana* mature ripe fruit.

The aqueous extract of the ripe fruit of *C. sieberiana* which gave the highest reversal anti-sickling activity was also fractionated (see Table 5).

Table 3: Percentage yields of different plant extracts.

	NAME	Yield (%)		
		CEE	SEE	AQE
•	*Anthocleista vogelii* stem bark	2.85	8.93	7.13
•	*Cassia sieberiana* leaf	4.63	20.50	12.60
•	*Cassia sieberiana* pericarp	2.28	7.10	6.85
•	*Cassia sieberiana* seed	1.60	5.70	5.40
•	*Cassia sieberiana* unripe fruit	3.05	8.30	10.50
•	*Cassia sieberiana* mature ripe fruit	2.10	9.40	7.60
•	*Cassia sieberiana* stem bark	4.18	17.30	13.70
•	*Dioclea reflexa* seed	1.73	7.50	9.10
•	*Harungana madagascariensis* leaf	5.80	29.10	25.30
•	*Lecanioidiscus cupanioides* leaf	5.10	26.00	19.40
•	*Peperomia pellucida* whole plant	2.82	8.35	8.10
•	*Mimosa pudica* leaf	2.58	8.50	9.65
•	*Morinda lucida* leaf	5.65	28.50	19.20
•	*Senna alata* leaf	6.79	35.00	21.43
•	*Spondias mombin* root	1.23	3.63	1.83
•	*Spondias mombin* leaf	4.90	18.50	10.70

KEY: CEE- 70 % (v/v) cold ethanol extract; SEE- 70 % (v/v) ethanol Soxhlet extract; AQE- aqueous extract (decoction).

Table 4: Percentage yields of VLC fractions from the 70 % (v/v) ethanol Soxhlet extract of *C. sieberiana* pericarp.

Fractions	Eluate (L)	yield (g)	Yield (% w/w)
A	1	0.5	0.63
B	2	1.07	1.34
C	2.5	2.86	3.58
D	3.5	3.04	3.80
E	2.85	1.82	2.28
F	5.5	28.95	36.19
G	8.65	10.37	12.96
Total yield (% w/w)			60.78

KEY: A: n-hexane fraction, B: n-hexane: dichloromethane (1:1) fraction, C: dichloromethane fraction, D: dichloromethane: ethyl acetate (1:1) fraction, E: ethyl acetate fraction, F: ethyl acetate: methanol (1:1) fraction, G: methanol fraction.

Table 5: Percentage yields of VLC fractions from the aqueous extract (decoction) of *C. sieberiana* mature ripe fruit.

Fractions	Eluate (L)	Yield (g)	Yield (% w/w)

H	1.5	0.17	0.21
I	1.7	0.25	0.31
J	4	0.59	0.74
K	7.5	1.43	1.79
L	5.2	2.17	2.71
M	8.4	26.03	32.54
N	11	12.5	15.63
Total yield (% w/w)			53.93

KEY: H: n-hexane fraction, I: n-hexane: dichloromethane (1:1) fraction, J: dichloromethane fraction, K: dichloromethane: ethyl acetate (1:1) fraction, L: ethyl acetate fraction, M: ethyl acetate: methanol (1:1) fraction, N: methanol fraction.

3.3. Results of Anti-Sickling Activities.

3.3.1. Inhibitory activities of 70 % (v/v) cold ethanol extracts at 4 mg/mL.

The 70 % (v/v) cold ethanol extracts of *P. pellucida, D. reflexa*, and *C. sieberiana* pericarp exhibited comparable inhibitory activities, which were also comparable to vanillic acid and Ciklavit (reference standards) as shown in Figure 3. The % inhibitory activities were 54.1 %, 44.7 %, 54.9 %, 54.0 % and 64.1 % respectively.

3.3.2. Reversal activities of 70 % (v/v) cold ethanol extracts at 4 mg/mL.

The 70 % (v/v) ethanol extract of *Anthocleista vogelii* exhibited the highest reversal activity, comparable to PHBA and Ciklavit (reference standards) as seen in Figure 4. The % reversal activities were 61.1 %, 69.2 % and 76.9 % respectively.

Figure 3: % Inhibitory activities of 70 % (v/v) cold ethanol extracts at 4 mg/mL.

L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit; RF- Reference standards, (vanillic acid and Ciklavit); * significant at $p < 0.05$. Results are expressed as Mean ± SEM. Bars with similar letters depict non-significance in anti-

sickling activity. S.m- *Spondias mombin*; C.s- *Cassia sieberiana*, A.v- *Anthocleista vogelii*, M.l- *Morinda lucida*. H.m- *Harungana madagacariensis*, S.m- *Spondias mombin*, L.c- *Lecanodiscus cupanioides*, M.p *Mimosa pudica*,, D.r- *Dioclea reflexa*, P.p- *Peperomia pellucida*.

Figure 4: % Reversal activities of 70 % (v/v) cold ethanol extracts at 4 mg/mL.

L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit, PHBA- para-hydroxybenzoic acid; RF- Reference standards- (PHBA and Ciklavit); * significant at $p < 0.05$; Results are expressed as MEAN ± SEM. Bars with similar letters depict non-significance in anti-sickling activity. S.m- *Spondias mombin*; C.s- *Cassia sieberiana*, A.v- *Anthocleista vogelii*, M.l- *Morinda lucida*. H.m- *Harungana madagacariensis*, S.m- *Spondias mombin*, L.c- *Lecanodiscus cupanioides*, M.p *Mimosa pudica*,, D.r- *Dioclea reflexa*, P.p- *Peperomia pellucida*.

3.3.3. Inhibitory activities of 70 % (v/v) ethanol Soxhlet extracts at 4 mg/mL.

The 70 % (v/v) ethanol soxhlet extracts of *H. madagascariensis* (L), *A. vogelii*, *M. lucida*, mature ripe fruits and seeds of *C. sieberiana* and *S. mombin* all exhibited comparable inhibitory activities with vanillic acid. The % inhibitory activities were 47.5 %, 51.5 %, 55.1 %, 55.6 % and 56.6 % respectively. *C. sieberiana* pericarp (71.3 %) exhibited the highest activity and better than vanillic acid (64.1 %) as seen in Figure 5.

3.3.4. Reversal activities of 70 % (v/v) ethanol Soxhlet extracts at 4 mg/mL.

The 70 % (v/v) ethanol soxhlet extract of *S. alata* (84.0 %) exhibited the highest reversal activity. It gave a higher value compared to Ciklavit (76.9 %) and p-hydroxybenzoic acid (69.2 %) the reference standards as seen in Figure 6.

Figure 5: % Inhibitory activities of 70 % (v/v) ethanol Soxhlet extracts at 4 mg/mL.

L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit; RF- Reference standards- (Vanillic acid and Ciklavit); * significant at $p < 0.05$; Results are expressed MEAN ± SEM. Bars with similar letters depict non-significance in anti-sickling activity. S.m- *Spondias mombin*; C.s- *Cassia sieberiana*, A.v- *Anthocleista vogelii*, M.l-

Morinda lucida. H.m- *Harungana madagacariensis*, S.m- *Spondias mombin*, L.c- *Lecanodiscus cupanioides*, M.p *Mimosa pudica*,, D.r- *Dioclea reflexa*, P.p- *Peperomia pellucida*.

Figure 6: % Reversal activities of 70 % (v/v) ethanol Soxhlet extracts at 4 mg/mL.

L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit, PHBA- para-hydroxybenzoic acid; RF- Reference standards- (PHBA and Ciklavit); * significant at $p < 0.05$; Results are expressed as MEAN ± SEM. Bars with similar letters depict non-significance in anti-sickling activity. S.m- *Spondias mombin*; C.s- *Cassia sieberiana*, A.v- *Anthocleista vogelii*, M.l- *Morinda lucida*. H.m- *Harungana madagacariensis*, S.m- *Spondias mombin*, L.c- *Lecanodiscus cupanioides*, M.p *Mimosa pudica*,, D.r- *Dioclea reflexa*, P.p- *Peperomia pellucida*.

3.3.5. Inhibitory activities of aqueous extracts (decoctions) at 4 mg/mL.

The pericarp and mature ripe fruit of *C. sieberiana* showed the highest inhibitory activities and had values comparing favourably with Ciklavit and vanillic acid, (reference standards) as seen in Figure 7. The % inhibitory activities were 53.9 %, 58.5 %, 64.1 % and 54.0 % respectively.

3.3.6. Reversal activities of aqueous extracts (decoctions) at 4 mg/mL.

The mature ripe fruit of *C. sieberiana* showed the highest reversal activity (90.2 %) and had higher values than the reference standards Ciklavit (76.9 %) and p-hydroxybenzoic acid (69.2 %) as seen in Figure 8.

Figure 7: % Inhibitory activities of the aqueous extracts (decoctions) at 4 mg/mL.

L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericap, MF- mature ripe fruit, IF- unripe fruit; RF- Reference standards- (Vanillic acid and Ciklavit); * significant at $p < 0.05$; Results are expressed ± SEM. Bars with similar letters depict non-significance in anti-sickling activity. S.m- *Spondias mombin*; C.s- *Cassia sieberiana*, A.v- *Anthocleista vogelii*, M.l- *Morinda lucida*. H.m- *Harungana madagacariensis*, S.m- *Spondias mombin*, L.c- *Lecanodiscus cupanioides*, M.p *Mimosa pudica*,, D.r- *Dioclea reflexa*, P.p- *Peperomia pellucida*.

Figure 8: % Reversal activities of the aqueous extracts (decoctions) at 4 mg/mL.

L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit, PHBA- para-hydroxy benzoic acid; RF- Reference standards- (PHBA and Ciklavit); * significant at $p < 0.05$; Results are expressed MEAN ± SEM. Bars with similar letters depict non-significance in anti-sickling activity. S.m- *Spondias mombin*; C.s- *Cassia sieberiana*, A.v- *Anthocleista vogelii*, M.l- *Morinda lucida*. H.m- *Harungana madagacariensis*, S.m- *Spondias mombin*, L.c- *Lecanodiscus cupanioides*, M.p *Mimosa pudica*,, D.r- *Dioclea reflexa*, P.p- *Peperomia pellucida.*

3.3.7. Effects of concentration on the anti-sickling activities of *C. sieberiana* extracts.

Due to the consistent anti-sickling activity exhibited by the pericarp and mature ripe fruits of *C. sieberiana* at 4 mg/mL, other concentrations were tested. There was a significant increase in inhibitory activity from 2 mg/mL to 4 mg/mL. There was no statistically significant difference in activity, from 0.5 mg/mL to 2 mg/mL. The confidence limit was 95%. The results are as shown in Figure 9. There was no significant difference in reversal activities with increase in concentration at $P < 0.05$ and 95% confidence limit with the 70 % (v/v) ethanol soxhlet extract of the pericarp and decoction of the fruit of *C. sieberiana* while the reversal activity of the seed was significantly different. Better reversal activities were obtained at 1 – 2 mg/mL of the seed extract as seen in Figures 9 and 10.

3.3.8. Anti-sickling Activities of VLC fractions from the 70 % (v/v) ethanol Soxhlet extract of the pericarp and aqueous extract (decoction) of the whole mature ripe fruits of *C. sieberiana* at 4 mg/mL.

The dichloromethane fraction of the aqueous extract of the ripe fruit and the ethyl acetate fraction of the ethanol extract of the pericarp exhibited profound anti-sickling activities as seen in Table 6

Figure 9: Effects of concentration on the inhibitory activities of *C. sieberiana* extracts.

SES- 70 % (v/v) ethanol Soxhlet extract of seed; SEP- 70 % (v/v) ethanol Soxhlet extract of pericarp; WF- aqueous extract (decoction) of mature ripe fruit; Reference standards (RF)- (vanillic acid and Ciklavit). Results are expressed as MEAN ± SEM. Bars with similar letters depict non-significance in anti-sickling activity.

Figure 10: Effects of concentration on the reversal activities of *C. sieberiana* extracts.

SES- 70 % (v/v) ethanol Soxhlet extract of seed; SEP- 70 % (v/v) ethanol Soxhlet extract of pericarp; WF- aqueous extract (decoction) of mature ripe fruit; Reference standards (RF)- (PHBA and Ciklavit). Results are expressed MEAN ± SEM. Bars with similar letters depict non-significance in anti-sickling activity.

Table 6: % Anti-sickling activities of VLC fractions from 70 % (v/v) ethanol Soxhlet extract of the pericarp and aqueous extract of the whole mature ripe fruit of *C. sieberiana* at 4 mg/mL.

Extracts	VLC Fractions	% Inhibitory Activity ± SEM	% Reversal Activity ± SEM
70 % (v/v) ethanol soxhlet extract of *C. sieberiana* pericarp	A	11.86 ± 2.00^{c}	11.94 ± 1.07^{c}
	B	14.81 ± 4.72^{c}	19.48 ± 2.60^{c}
	C	7.22 ± 1.06^{c}	48.36 ± 3.48^{d}
	D	$18.55 \pm 3.77^{d\,c}$	72.63 ± 3.91^{e}
	E	87.07 ± 2.28^{e}	79.74 ± 2.44^{e}
	F	83.13 ± 1.80^{e}	14.42 ± 1.69^{c}
	G	34.60 ± 9.77^{d}	12.27 ± 2.12^{c}
Aqueous extract (decoction) of ripe fruit of *C. sieberiana*	H	32.51 ± 1.90^{d}	10.11 ± 0.62^{a}
	I	91.50 ± 1.24^{e}	18.76 ± 0.97^{b}
	J	79.64 ± 1.60^{e}	70.13 ± 2.11^{e}
	K	47.24 ± 4.29^{d}	83.36 ± 1.82^{e}
	L	7.78 ± 1.05^{b}	46.28 ± 2.22^{c}
	M	9.07 ± 1.09^{b}	11.80 ± 0.84^{a}
	N	16.80 ± 1.71^{c}	13.20 ± 1.47^{a}

Results are expressed MEAN ± SEM**.** Similar letters depict non-significance in anti-sickling activity. Different superscript letters depict significant increase in anti-sickling activity from a-e.

KEY A/H: n-hexane fraction, B/I: n-hexane: dichloromethane (1:1) fraction, C/J: dichloromethane fraction, D/K: dichloromethane: ethyl acetate (1:1) fraction, E/L: ethyl acetate fraction, F/M: ethyl acetate: methanol (1:1) fraction, G/N: methanol fraction.

3.3.9. Comparative Vanillic acid-, p-Hydroxybenzoic acid-, Ciklavit-, equivalents.

Tables 7-12 show the degree to which the test extracts were as active as the positive controls: vanillic acid, p-hydroxybenzoic acid and Ciklavit.

For the inhibitory activities of 70 % (v/v) cold ethanol extracts, *C. sieberiana* pericarp had a vanillic acid-equivalent of 1.02 and percentage vanillic acid activity of 102 %, while *P. pellucida* had a vanillic acid-equivalent of 1.00 and percentage vanillic acid activity of 100 % (Table 7). For the inhibitory activities of the aqueous extracts, *C. sieberiana* ripe fruit had a vanillic acid-equivalent of 1.00 and *C. sieberiana* pericarp had a vanillic acid-equivalent of 1.08 with 100 % and 108 % vanillic acid activities respectively, while for the reversal activities, *C. sieberiana* ripe fruit had a PHBA-equivalent of 1.30 with PHBA activity of 130 % and Ciklavit-equivalent of 1.17 with Ciklavit activity of 117 % (Tables 9 and 10). For the inhibitory activities of the 70 % (v/v) ethanol soxhlet extracts, *C. sieberiana* pericarp had a vanillic acid-equivalent of 1.32 with vanillic acid activity of 132 %and Ciklavit-equivalent of 1.11 with Ciklavit activity of 111 % while for the reversal activities, *Senna alata* had a PHBA-equivalent of 1.21 with PHBA activity of 121 % and Ciklavit-equivalent of 1.09 with Ciklavit activity of 109 % (Tables 11 and 12).

3.4. Photomicrographs of anti-sickling activities of *C. sieberiana*.

Photomicrographs (Plates 12 and 13) showing sickled red blood cells from the negative control with 92% sickled cells (12a and 13a), 90 % reversal anti-sickling activity of aqueous extract of *C. sieberiana* mature ripe fruit (12b) and 92 % inhibitory anti-sickling activity of the n- hexane: dichloromethane (1:1) fraction (13b) of the aqueous extract of *C. sieberiana* mature ripe fruit.

Table 7: Comparative Vanillic acid-equivalents (VAE) and Ciklavit-equivalents (CE) for inhibitory activities of 70 % (v/v) cold ethanol extracts.

Plant	VAE	VAA (%)	CE	CA (%)
Spondias mombin (L)	0.13	13	0.11	11
Cassia sieberiana (MF)	0.21	21	0.18	18
Anthocleista vogelli (B)	0.23	23	0.19	19
Cassia sieberiana (B)	0.26	26	0.22	22
Senna alata (L)	0.30	30	0.25	25
Cassia sieberiana (IF)	0.32	32	0.27	27
Cassia sieberiana (S)	0.38	38	0.32	32
Morinda lucida (L)	0.47	47	0.40	40
Harungana madagascariensis (L)	0.50	50	0.42	42
Cassia sieberiana (L)	0.57	57	0.48	48
Spondias mombin (R)	0.72	72	0.61	61
Lecanodiscus cupanioides (L)	0.78	78	0.65	65
Mimosa pudica (WP)	0.83	83	0.69	69
Dioclea reflexa (S)	0.83	83	0.70	70
Peperomia pellucida (WP)	1.00	100	0.84	84
Cassia sieberiana (P)	1.02	102	0.86	86
vanillic Acid	1.00	100	0.84	84

Ciklavit	1.19	119	1.00	100

KEY: L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit; RF- Reference standards- vanillic acid and Ciklavit; VAA- % vanillic acid activity and CA- % Ciklavit activity; VAE- vanillic acid-equivalent and CE- Ciklavit-equivalent.

Table 8: Comparative p-Hydroxybenzoic acid-equivalents (PHBAE) and Ciklavit-equivalents (CE) for reversal activities of 70 % (v/v) cold ethanol extracts.

Plant	PHBAE	PHBAA (%)	CE	CA (%)
Peperomia pellucida (WP)	0.00	0	0.00	0
Cassia sieberiana (IF)	0.04	4	0.04	4
Dioclea reflexa (S)	0.05	5	0.04	4
Cassia sieberiana (P)	0.09	9	0.08	8
Cassia sieberiana (L)	0.09	9	0.08	8
Lecanodiscus cupanioides (L)	0.12	12	0.11	11
Mimosa pudica (WP)	0.13	13	0.12	12
Cassia sieberiana (S)	0.14	14	0.13	13
Cassia sieberiana (B)	0.20	20	0.18	18
Harungana madagascariensis (L)	0.23	23	0.20	20
Cassia sieberiana (MF)	0.23	23	0.20	20
Spondias mombin (L)	0.33	33	0.30	30
Senna alata (L)	0.38	38	0.34	34
Morinda lucida (L)	0.42	42	0.38	38
Spondias mombin (R)	0.42	42	0.38	38
Anthocleista vogelli (B)	0.88	88	0.79	79
PHBA	1.00	100	0.90	90

Ciklavit	1.11	111	1.00	100

KEY: L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit, PHBA- para-hydroxy benzoic acid; PHBAE- para-hydroxy benzoic acid-equivalents; PHBAA- % para-hydroxy benzoic acid activity; CA- % Ciklavit activity and CE- Ciklavit-equivalent.

Table 9: Vanillic acid-equivalents (VAE) and Ciklavit-equivalents (CE) for inhibitory activities of Aqueous extracts (decoctions).

Plant	VAE	VAA (%)	CE	CA (%)
Spondias mombin (L)	0.14	14	0.12	12
Senna alata (L)	0.15	15	0.12	12
Cassia sieberiana (IM)	0.15	15	0.13	13
Perperomia pellucida (WP)	0.17	17	0.15	15
Cassia sieberiana (L)	0.17	17	0.15	15
Lecanodiscus cupanioides (L)	0.19	19	0.16	16
Anthocleista vogelli (B)	0.21	21	0.18	18
Dioclea reflexa (S)	0.23	23	0.19	19
Mimosa pudica (WP)	0.24	24	0.20	20
Morinda lucida (L)	0.32	32	0.27	27
Cassia sieberiana (B)	0.38	38	0.32	32
Cassia sieberiana (S)	0.48	48	0.40	40
Spondias mombin (R)	0.49	49	0.41	41
Harungana madagascariensis (L)	0.77	77	0.65	65
Cassia sieberiana (MF)	1.00	100	0.84	84
Cassia sieberiana (P)	1.08	108	0.91	91

vanillic acid	1.00	100	0.84	84
Ciklavit	1.19	119	1.00	100

KEY: L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit; RF- Reference standards- vanillic acid and Ciklavit. VAA- % vanillic acid activity and CA- % Ciklavit activity; VAE- vanillic acid-equivalent and CE- Ciklavit-equivalent.

Table 10: Comparative p-Hydroxybenzoic acid-equivalents (PHBAE) and Ciklavit-equivalents (CE) for reversal activities of Aqueous extracts (decoctions).

Plant	PHBAE	PHBAA (%)	CE	CA (%)
Cassia sieberiana (IM)	0.08	8	0.08	8
Senna alata (L)	0.12	12	0.11	11
Spondias mombin (R)	0.23	23	0.21	21
Anthocleista vogelli (B)	0.24	24	0.22	22
Cassia sieberiana (S)	0.27	27	0.24	24
Spondias mombin (L)	0.27	27	0.24	24
Lecanodiscus cupanioides (L)	0.44	44	0.39	39
Mimosa pudica (WP)	0.46	46	0.42	42
Cassia sieberiana (B)	0.54	54	0.48	48
Cassia sieberiana (L)	0.54	54	0.49	49
Dioclea reflexa (S)	0.55	55	0.50	50
Cassia sieberiana (P)	0.61	61	0.55	55
Morinda lucida (L)	0.63	63	0.57	57
Perperomia pellucida (WP)	0.71	71	0.64	64
Harungana madagascariensis (L)	0.75	75	0.68	68
Cassia sieberiana (MF)	1.30	130	1.17	117

PHBA	1.00	100	0.90	90
Ciklavit	1.11	111	1.00	100

KEY: L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit, PHBA- para-hydroxy benzoic acid; PHBAE- para-hydroxy benzoic acid-equivalents; PHBAA- % para-hydroxy benzoic acid activity; CA- % Ciklavit activity and CE- Ciklavit-equivalent.

Table 11: Comparative Vanillic acid-equivalents (VAE) and Ciklavit-equivalents (CE) for inhibitory activities of 70 % (v/v) ethanol Soxhlet extracts.

Plant	VAE	VAA (%)	CE	CA (%)
Spondias mombin (R)	0.19	19	0.16	16
Lecanodiscus cupanioides (L)	0.22	22	0.18	18
Dioclea reflexa (S)	0.22	22	0.18	18
Mimosa pudica (WP)	0.25	25	0.21	21
Cassia sieberiana (B)	0.33	33	0.28	28
Senna alata (L)	0.34	34	0.29	29
Cassia sieberiana (IF)	0.36	36	0.30	30
Cassia sieberiana (L)	0.49	49	0.41	41
Perperomia pellucida (WP)	0.50	50	0.42	42
Harungana madagascariensis (L)	0.73	73	0.61	61
Anthocleista vogelli (B)	0.88	88	0.74	74
Morinda lucida (L)	0.95	95	0.80	80
Cassia sieberiana (MF)	1.02	102	0.86	86
Cassia sieberiana (S)	1.03	103	0.87	87
Spondias mombin (L)	1.05	105	0.88	88

Cassia sieberiana (P)	1.32	132	1.11	111
vanillic acid	1.00	100	0.84	84
Ciklavit	1.19	119	1.00	100

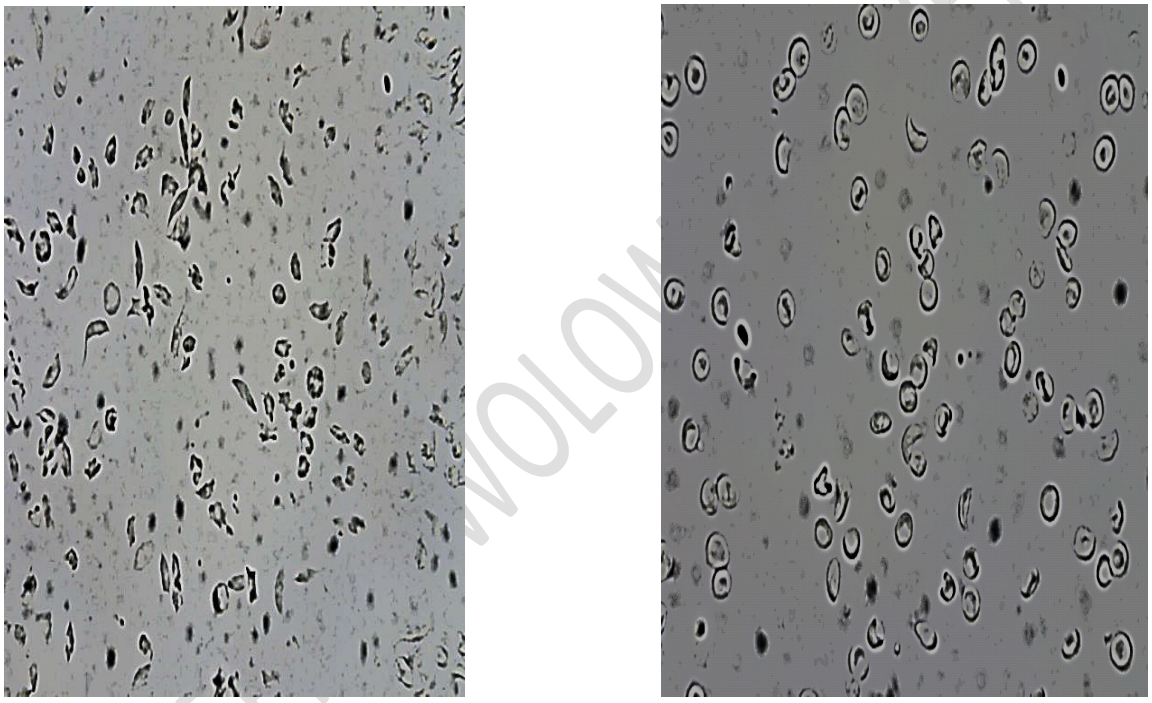
KEY: L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit; RF- Reference standards- vanillic acid and Ciklavit. VAA- % vanillic acid activity and CA- % Ciklavit activity; VAE- vanillic acid-equivalent and CE- Ciklavit-equivalent.

Table 12: Comparative p-Hydroxybenzoic acid-equivalents (PHBAE) and Ciklavit-equivalents (CE) for reversal activities of 70 % (v/v) ethanol Soxhlet extracts.

Plant	PHBAE	PHBAA (%)	CE	CA (%)
Anthocleista vogelli (B)	0.04	4	0.03	3
Cassia sieberiana (L)	0.04	4	0.04	4
Cassia sieberiana (B)	0.07	7	0.06	6
Mimosa pudica (WP)	0.13	13	0.12	12
Spondias mombin (L)	0.13	13	0.12	12
Cassia sieberiana (IF)	0.16	16	0.15	15
Spondias mombin (R)	0.17	17	0.15	15
Lecanodiscus cupanioides (L)	0.17	17	0.15	15
Peperomia pellucida (WP)	0.20	20	0.18	18
Dioclea reflexa (S)	0.25	25	0.22	22
Harungana madagascariensis (L)	0.43	43	0.39	39
Cassia sieberiana (S)	0.52	52	0.47	47
Morinda lucida (L)	0.57	57	0.51	51
Cassia sieberiana (MF)	0.74	74	0.66	66
Cassia sieberiana (P)	0.78	78	0.70	70

Senna alata (L)	1.21	121	1.09	109
PHBA	1.00	100	0.90	90
Ciklavit	1.11	111	1.00	100

KEY: L- leaf, B- stem bark, S- seed, R- root, WP- whole plant, P- pericarp, MF- mature ripe fruit, IF- unripe fruit, PHBA- para-hydroxy benzoic acid; PHBAE- para-hydroxy benzoic acid-equivalents; PHBAA- % para-hydroxy benzoic acid activity; CA- % Ciklavit activity and CE- Ciklavit-equivalent.



Control (12a) Test (12b)

Plate 12: Photomicrographs showing sickled red blood cells from the negative control with 92% sickled cells (12a) and 90 % reversal anti-sickling activity of aqueous extract of *C. sieberiana* mature ripe fruit (12b) at 4 mg/mL.

RBCs -Sickled red blood cells; RBCU- Unsickled red blood cells.

Control (13a) Test (13b)

Plate 13: Photomicrograph showing sickled red blood cells from the negative control with 92% sickled cells (13a) and 92 % inhibitory anti-sickling activity of the n- hexane: dichloromethane (1:1) fraction (13b) of the aqueous extract of *C. sieberiana* mature ripe fruit at 4 mg/mL

RBCs -Sickled red blood cells; RBCu- Unsickled red blood cells (sickling inhibited).

3.5 Thin Layer Chromatography of VLC fractions

Tables 13 and 14 show the various colours observed from the Thin Layer Chromatography of VLC fractions obtained from 70 % (v/v) ethanol soxhlet extracts of *C. sieberiana* pericarp and VLC fractions obtained from the aqueous extract of *C. sieberiana* ripe fruit.

Colours observed under the UV lamp depict the compounds have chromophores and the colours vary depending on the nature of the groups attached to the chromophore.

Sulphuric acid (20 % v/v) spray shows the presence of organic compounds. Vanillin- sulphuric acid spray detects the presence of steroids and terpenes (green, yellow e.t.c.). $FeCl_3$ spray detects the presence of phenolic acids, phenolics such as flavonoids; coumarins (black, blue, blue- black). Alcoholic KOH spray detects the presence of anthraquinones (orange- red, pink)

Table 13: Thin Layer Chromatography of VLC fractions obtained from the 70 % (v/v) ethanol Soxhlet extract of *C. sieberiana* pericarp.

Fraction	R_f	Daylight	UV 254nm	UV 366nm	Sulphuric acid	Vanillin sulphuric acid	Alc. KOH	$FeCl_3$
A	0.87	-	-	blue	yellow	yellow	-	-
	0.90	-	-	Purple	yellow	yellow	-	Black
	0.94	-	-	purple	yellow	yellow	-	-
B	0.75	-	-	purple	-	-	-	-
	0.77	-	-	purple	Brown	Brown	pink	-
	0.78	-	-	brown	Yellow	Yellow	Pink	Black
	0.83	-	green	red	Yellow	Yellow	Yellow	Black
	0.87	-	brown	purple	Yellow	Yellow	Yellow	-
	0.93	-	brown	purple	Brown	Brown	-	-
C	0.31	-	-	Brown	-	Brown	-	-
	0.36	-	-	Brown	-	-	-	-
	0.42	-	-	Blue	-	-	-	-
	0.48	-	-	Blue	Brown	-	-	-
	0.56	-	-	Purple	Brown	Brown	-	-
	0.60	Brown	-	Green	Yellow	Brown	-	Brown
	0.72	Orange	-	green	Yellow	Yellow	Red	-
	0.86	Orange	green	red	Yellow	-	Red	Brown
	0.93	Orange	brown	purple	Brown	Yellow	-	-
D	0.08	Brown	-	Brown	Red	-	-	-
	0.16	Brown	-	Brown	Red	-	Red	Blue
	0.22	-	-	Blue	Brown	Brown	-	-

	0.28	-	-	green	Brown	Brown	-	-
	0.34	Brown	Brown	brown	Brown	Brown	-	-
	0.36	Brown	Brown	brown	Brown	Brown	-	Blue black
	0.40	-	-	brown	Orange	Orange	-	-
	0.45	-	-	Purple	Orange	Orange	-	-
	0.48	brown	-	Brown	Brown	Brown	-	-
	0.51	Orange	Brown	Purple	brown	brown	-	-
	0.53	Orange	Brown	Blue	Brown	Brown	Red	-
	0.55	Orange	Brown	Brown	Brown	Brown	-	-
	0.59	Orange	Brown	Blue	Yellow	Brown	Pink/ Red	Blue
	0.63	Yellow	Brown	Blue	Yellow	Purple	Pink/ Yellow	-
	0.65	Yellow	Brown	brown	Yellow	Blue	Pink	-
	0.69	Yellow	Green	Brown	Yellow	Green	-	-
	0.81	Green	Green	Green	Yellow	Yellow	-	Blue
	0.90	Green	Brown	Brown	-	Green	-	-
	0.02	Brown	Brown	Brown	Brown	-	-	-
E	0.06	Brown	Brown	Brown	Brown	-	-	-
	0.10	Brown	Brown	Red	Brown	-	-	Black
	0.17	-	-	Purple	brown	-	-	-
	0.24	-	-	Brown	Brown	Blue	Red	-
	0.30	Brown	-	Blue	Brown	Brown	-	Blue black
	0.40	Green	-	Brown	Brown	Brown	-	Black
	0.48	-	-	Purple	yellow	Purple	Brown	-
	0.60	-	-	Brown	brown	Brown	-	-
	0.66	-	-	Brown	Yellow	Brown	-	-
	0.72	-	-	Purple	Yellow	Blue	-	-
	0.84	Yellow	Green	Green	Yellow	Blue	-	-
	0.90	Yellow	Brown	Red	Yellow	Yellow	-	Blue
F	0.02		-	Brown	Brown	Brown	-	-
	0.07		-	Brown	Brown	Brown	-	-
	0.12	Green	-	Blue	Blue	Blue	-	-
	0.24	brown	-	Blue	Blue	Blue	-	-
	0.34	-	-	Purple	Brown	Brown	-	-
	0.46	-	-	Blue	Brown	Brown	-	-
	0.58	-	-	Purple	Brown	Brown	-	-
	0.84	-	-	Red	Yellow	Yellow	-	-
	0.90	-	--	Red	Yellow	Yellow	-	-
G	0.90	-	-	Brown	Brown	Brown	-	-

Adsorbent-Silica on T.L.C. plate

KEY

A: n-hexane fraction of the 70 % (v/v) ethanol soxhlet extract of the pericarp,

B: n-hexane: dichloromethane (1:1) fraction of the 70 % (v/v) ethanol soxhlet extract of the pericarp,

C: dichloromethane fraction of the 70 % (v/v) ethanol soxhlet extract of the pericarp,

D: dichloromethane: ethyl acetate (1:1) fraction of the 70 % (v/v) ethanol soxhlet extract of the pericarp,

E: ethyl acetate fraction of the 70 % (v/v) ethanol soxhlet extract of the pericarp,

F: ethyl acetate: methanol (1:1) fraction of the 70 % (v/v) ethanol soxhlet extract of the pericarp,

G: methanol fraction of the 70 % (v/v) ethanol soxhlet extract of the pericarp

Table 14: Thin Layer Chromatography of VLC fractions obtained from the aqueous extract (decoction) of *C. sieberiana* ripe fruit.

Fractions	Rf	Day light	UV 254nm	UV 366nm	Sulphuric acid	Vanillin sulphuric acid	Alc. KOH	$FeCl_3$
H	0.04	-	-	yellow		-	-	-
	0.15	-	-	-		-	-	-
	0.27	-	-	Brown	Brown	Brown	-	-
	0.37	-	-	Brown	Brown	Brown	-	-
	0.49	-	-	Brown	Brown	Brown	-	-
	0.55	-	-	Brown	Brown	Brown	-	-
	0.61	-	-	Brown	Brown	Brown	-	-
	0.73	-	-	Brown	Brown	Brown	-	-
	0.81	-	-	-		-	-	-
	0.87	-	-	Purple		-	Yellow	-
	0.95	-	purple	Blue	Green	-	Pink	Brown
I	0.90	-	-	-		-	-	Brown
	0.94	-	Green	Blue	Yellow	Yellow	Pink	-
	0.99	Orange	Green	Green	Yellow	Yellow	-	-
J	0.09	-	-	Brown	-	-	-	-
	0.20	-	-	Brown	Brown	-	-	-
	0.30	-	-	Brown	Brown	-	-	-
	0.40	-	-	Brown	Brown	-	-	-
	0.45	-	-	Brown	Brown	-	-	-
	0.55	-	-	Brown	Brown	-	-	-
	0.65	-	Blue	Red	Brown	Blue	-	-
	0.70	-	Purple	-	-	-	Green	-
	0.83	-	Violet	Blue	-	-	Pink	-

	0.88	Green	Green	Green	Green	Yellow	Pink	Brown
	0.94	Orange	Blue	Purple	Green	-	-	-
	0.98	Green	Blue	Orange	Yellow	-	-	-
K	0.04	-	brown		Brown			-
	0.09	-	Brown	-	Brown	-	Brown	-
	0.15	-	Brown	-	Brown	-	Brown	-
	0.17	-	Brown	-	Brown	Brown	Brown	-
	0.21	-	Brown	-	Green	Brown	Brown	Black
	0.27	Brown	Brown	-	Brown	Brown	Brown	Black
	0.32	Brown	Blue	Purple	Brown	Green	Blue	
	0.35	Brown	Blue	Purple	Brown	Brown	Pink	
	0.40	Brown	Brown	Red	Green	Brown	Pink	
	0.45	Brown	Brown	Blue	Green	Brown	Blue	
	0.51	Green	Purple	Brown	Green	Green	-	Blue black
	0.57	Green	Green	Orange	Green	Green	-	-
	0.64	-	Purple	-	Yellow	Green	-	Black
	0.73	Orange	Purple	Purple	Brown	Green	-	-
	0.83	-	Green	orange	Brown	Yellow	Green	-
	0.87	Green	Blue	Orange	Brown	Brown	-	-
	0.89	Green	Blue	Yellow	Yellow	Yellow	Blue	Black
	0.95	Orange	Green	Orange	Yellow	Yellow	-	Brown
	0.98	Green	Green	Red	Red	-	-	-
L	0.02	-	Brown	Brown	Brown	Brown	Blue	-
	0.12	-	Brown	Brown	Green	Green	Blue	-
	0.22	-	Blue	-	Green	Green	-	-
	0.29	-	Green	-	Red	Red	-	-
	0.35	-	Blue	-	Red	Red	-	-
	0.40	-	Blue	-	-	Brown	-	-
	0.44	-	Green	Blue	-	Green	-	-
	0.49	-	-	Blue	-	Green	-	Black
	0.63	-	-	Orange	-	Red	-	-
	0.82	-	-	Blue	Brown	Red	Green	-
	0.91	Orange	Green	Red	Yellow	Yellow	Blue	Black
M	0.06	-	Brown	Blue	Brown		-	-
	0.10	-	Brown	Blue	Brown		Blue	-
	0.16	-	Green	Blue	Red		-	-
	0.20	-	Blue	Blue	Green		-	-
	0.40	-	Green	-	-		-	-
	0.51	-	Green	-	-		-	-
	0.67	-	Blue	-	-		-	-
	0.93	-	-	-	-		-	Black
	0.98	-	-	-	-		-	-
N	0.04	-	brown	Blue	Brown		-	-
	0.09	-	Brown	Blue	Brown		-	Black

	0.17	-	Green	Blue	Green		-	-
	0.24	-	Green	Blue	-		-	-
	0.43	-	Brown	Blue	-		-	-

Adsorbent-Silica on T. L. C. plate

KEY: H: n-hexane fraction of the aqueous extract of mature ripe fruits,

I: n-hexane: dichloromethane (1:1) fraction of the aqueous extract of mature ripe fruits,

J: dichloromethane fraction of the aqueous extract of mature ripe fruits,

K: dichloromethane: ethyl acetate (1:1) fraction of the aqueous extract of mature ripe fruits,

L: ethyl acetate fraction of the aqueous extract of mature ripe fruits,

M: ethyl acetate: methanol (1:1) fraction of the aqueous extract of mature ripe fruits,

N: methanol fraction of the aqueous extract of mature ripe fruits

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1. Extraction Procedures

The efficiency and percentage yield in an extraction process is influenced by a number of factors: the method used, the morphological part being extracted, the nature of the chemical constituents present in the plant material and the extracting solvent. Hence, the percentage yields obtained in 70 % (v/v) ethanol extraction were lower than the yields obtained from the soxhlet and decoction extraction process. Also, ethanol has a higher extractive power than water hence the percentage yields of the extracts obtained from the 70 % (v/v) ethanol soxhlet extraction was higher than those from the aqueous extraction (Table 3). Furthermore, Soxhlet extraction provides a continuous and exhaustive percolation of the solvent in the plant material, while reflux decoction method is generally of shorter duration with non-exhaustive extraction. Ethanol as a solvent therefore gave mostly higher yields with greater extractive power than water (Table 3) since alcohol would extract both the polar and non-polar constituents from the plant materials while water would extract only the polar substances.

4.2. Anti-sickling Experiments and Bioactivity Outcome.

The most common assay methods are designed to investigate the ability of the extracts to inhibit or reverse sickling of erythrocytes obtained from SCA patients in steady state, under a low oxygen tension (Dean and Schechter, 1978), artificially simulated or induced with sodium metabisulphite solution (2 % w/v). In the present work, phosphate buffer solution (pH 7) was used as a physiological medium for the RBC while incubation was carried out at 37°C to simulate the normal body temperature under which condition each extract was studied for inhibitory and reversal anti-sickling activities. Vanillic acid (4-hydroxy-3-methoxybenzoic acid)

was used as a reference compound for the inhibitory activity while p-hydroxy benzoic acid was used as reference compound for the reversal activity (Qin *et al.,* 2008). It is recalled that the anti-sickling activity of vanillin has earlier been reported by Abraham *et al.* (1999). Ciklavit, another reference standard is a formulated anti-sickling aqueous decoction of *Cajanus cajan* seed, commercially available as a herbal drug in Nigeria (Ekeke and Shode, 1985).

According to the Nigerian folkloric records, the inhabitants have used plant recipes to treat what they described as "fever of crises", manifesting as joint pains and exacerbations occurring especially during rainy seasons and "abnormality of the blood," of which only relatively few have been validated scientifically. Very few ethno-medicinal remedies for the treatment of sickle cell anaemia have been reported in the literature due to secrecy attached to the treatments of this disease and even among the available folkloric records, only a few have been validated scientifically (Egunyomi *et.al.,* 2009). From the present work (Figures 3-8), only about 5 out of 10 plants investigated, possessed significant anti-sickling activities while from the literature, a few of the others have been reported to possess biological activities closely related to haematological welfare such as boosting the level of red cells and white blood cells. Many of these activities are useful supportive measures in the management of SCA. For example, the aqueous and ethanol extracts of *S. mombin* leaf have been reported to have haematinic properties (Adeyemi and Gbolade, 2006; Asuquo *et al.,* 2013), thus supporting its ethnomedical uses in the management of sickle cell anaemia. The extracts of *S. mombin* leaf and root exhibited no appreciable inhibitory or reversal anti-sickling activity in this present study (Figures 3-8), except the soxhlet extract of its leaf which gave an inhibitory activity of 56 %. Extracts of *M. lucida* have been reported to show anti-inflammatory, antimalarial and analgesic activities (Lawal *et al.,* 2012) and can alleviate the sickle cell anaemia symptoms of fever, malarial and pain

episodes during vaso-occlusive crises. *L. cupanioides, M. pudica* and *D. reflexa* have similarly shown no appreciable anti-sickling activities except for their 70 % (v/v) cold ethanol extracts which exhibited marginal inhibitory activity comparable to that of the pericarp of *C. sieberiana* (Figure 3 and 4). Their 70 % (v/v) ethanol soxhlet extracts gave very low activities confirming that even the inhibitory agents were destroyed by the soxhlet extraction process. The leaf and stem bark of *C. sieberiana* did not exhibit appreciable anti-sickling activities (Figures 3-6). However, Buratai *et al.* (2011) reported a significant increase ($p<0.05$) in the mean packed cell volume (PCV), red blood cell and white blood cell counts dose- dependently as well as non-dose dependent increase in the mean haemoglobin concentration (MHC) of albino rats treated with graded doses of *C. sieberiana* leaf extract. The beneficial effects on the haematopoietic values caused by *C. sieberiana* leaf have been reported by Jain, (1993). In the whole, all of such biological effects are strongly supportive in SCA management.

4.2.1 Influence of extraction methods on the anti-sickling activities.

Soxhlet and reflux extractions essentially involve heat application and hence are destructive to thermolabile active components in the plant material, for example, the inhibitory activity of *A. vogelii* stem bark was observed to be unaffected by heat suggesting that the constituents responsible for their inhibitory activity were thermosTable while those responsible for reversal activity were thermolabile (Figures 11 and 12). The compounds responsible for the inhibitory and reversal activities of *C. sieberiana* pericarp and mature ripe fruit, readily go into alcohol and water, with or without heat suggesting the presence of thermostable polar substances. On the other hand, the inhibitory activity of *P. pellucida* whole plant was grossly affected by heat suggesting that the compound(s) responsible for the activity were thermolabile while constituents responsible for reversal activity are thermostable polar compound(s), *S. alata*

contained no inhibitory constituents but reversal constituents which were thermostable (Figures 11 and 12). Therefore, the type of extraction method and solvent used could determine the physical and chemical properties of the extracts obtained (Abah and Egwari, 2011).

4.2.2. Inhibitory anti-sickling activities.

The 70 % (v/v) cold ethanol extracts of *L. cupanioides* leaf, *M. pudica* whole plant, *D. reflexa* seed, *P. pellucida* whole plant and *C. sieberiana* pericarp showed comparable inhibitory activities (Figure 3). Their activities were comparable to vanillic acid which is the appropriate reference standard (Table 7). Similarly, the 70 % (v/v) ethanol soxhlet extracts of *H. madagascariensis* leaf, *A. vogelli* stem bark, *M. lucida* leaf, mature ripe fruit of *C. sieberiana*, *C. sieberiana* seed, *S. mombin* leaf and *C. sieberiana* pericarp all exhibited comparable inhibitory anti-sickling activity (Figure 5) with the highest value produced by the pericarp of *C sieberiana* (71%). The aqueous extract of both the mature ripe fruit and pericarp of *C. sieberiana* also exhibited comparable inhibitory activities (Figure 7) but the activities were lower than those produced by their 70 % (v/v) ethanol soxhlet extracts (Figures 5 and 7). The inhibitory anti-sickling activity of the ripe fruits of *C. sieberiana* was best among all the plants as its seeds, pericarp and ripe fruit showed good inhibitory activity (Figures 3-8).

Figure 11: Effect of the extraction methods on the inhibitory activities at 4 mg/mL.

MF- mature ripe fruit, P- pericarp. WP- whole plant, B-stem bark, L- leaf, RF- Reference standard (Vanillic acid and Ciklavit). * significant at $p < 0.05$; Results are expressed MEAN ± SEM. Bars with similar letters depict non-significance in anti-sickling activity

Figure 12: Effect of the extraction methods on the reversal activities at 4 mg/mL.

MF- mature ripe fruit, P- pericarp. WP- whole plant, B-stem bark, L- leaf, PHBA- para-hydroxybenzoic acid, RF- Reference standard (PHBA and Ciklavit). * significant at $p < 0.05$; Results are expressed MEAN ± SEM. Bars with similar letters depict non-significance in anti-sickling activity.

4.2.3. Reversal anti-sickling activities.

At 4 mg/mL, the 70 % (v/v) ethanol extract of the stem bark of *A. vogelii* gave the highest reversal activity (61.09 %), which is comparable to that of the reference standards, PHBA and Ciklavit while its 70 % (v/v) ethanol soxhlet extract resulted in the least reversal activity (2.49 %). The aqueous extract did not give any significant activity. It is possible that the chemical constituents responsible for the activity were thermolabile (Figures 11 and 12). The aqueous extracts of *C. sieberiana* pericarp, *H. madagascariensis, P. pellucida* and *M. lucida* gave comparable marginal reversal activities (Figure 6). Thus, *C. sieberiana* pericarp (inhibitory) and ripe fruit (reversal) have proven the most active among the 16 plant materials investigated in this research.

The genus *Cassia*, commonly acclaimed for their laxative properties due to the contents of anthracene derivatives, appeared to have demonstrated relevance in SCA and related research studies. *C. auriculata* and *C. fistula* have been reported to have haematinic effects (Kainsa *et al.,* 2012) while the root of *C. fistula* is a component of a preparation reported to possess a reversal activity (Egunyomi *et al.,* 2009). The membrane stabilizing properties of *S. alata* and *S. podocarpa* had been reported by Okpuzor and Adebesin (2006) with a more profound activity in *S. alata*. The pharmacological agents that alter cell membrane stability can play a useful role in

the prophylaxis of the sickling process, a major physiological manifestation of the sickle cell disease (Dean and Schechter, 1978).

In this study, the 70 % (v/v) ethanol soxhlet extract of *S. alata* gave a reversal activity (84.04 %), which is even higher than that of the reference compound PHBA but comparable to Ciklavit, a commercially available herbal preparation at the same concentration of 4 mg/mL (Figure 6).

Various parts of *C. sieberiana* have exhibited significant reversal activities particularly the aqueous extract of the ripe fruit followed by the 70 % (v/v) ethanol soxhlet extract of the pericarp. For example, the aqueous extract of the ripe fruit of *C. sieberiana* exhibited 91 % reversal activity at 4 mg/mL while the aqueous extract of the pericarp was observed to have 42 % activity (Figure 8). The 70 % (v/v) ethanol soxhlet extracts of the ripe fruit and pericarp of *C.sieberiana* gave reversal activities (Figure 6) which were not significantly different from each other at $P < 0.05$. Previously, the decoction of *C. sieberiana* has been reported to be used ethnomedicinally in the management of fever, tiredness, inflammatory conditions and joint pains (Madusolumuo et al., 1999) all of which are well-documented symptoms associated with sickle cell anaemia.

Due to the outstanding inhibitory activity exhibited by *C. sieberiana* pericarp, and reversal activity of its ripe fruit, studies were carried out on varying concentrations of their extracts. The 70 % (v/v) ethanol soxhlet extract of the pericarp and the seed, gave significant increases in inhibitory activities from 2 mg/mL to 4 mg/mL whereas no significant difference was observed from 0.5 mg/mL to 2 mg/mL at 95 % confidence limit. On the other hand, no significant concentration effects were observed in the reversal activity at ($P < 0.05$) of soxhlet extract of the

pericarp, whereas a significant difference was observed in the reversal activity of the seed (Figures 9 and 10).

Similarly, the ripe fruit of *C. sieberiana* gave a dose-dependent increase in the inhibitory activities from 2 mg/mL to 4 mg/mL whereas no significant difference in activity was observed from 0.5 mg/mL to 2 mg/mL (Figure 9) at 95% confidence limit. The reversal activity was observed for the aqueous extract of the ripe fruit except at 1 mg/mL and 2 mg/mL were comparable. The reversal activity of the seeds at 2 mg/mL is not significantly different from that of the ripe fruit at 4 mg/mL ($P < 0.05$) and there was no significant difference in inhibitory activity at 4 mg/mL among the extracts of the seeds, pericarp and ripe fruit (Figure 10).

4.3. Vacuum Liquid Chromatography (VLC) of *C. sieberiana*.

Vacuum Liquid Chromatography, a separation technique involving the use of a short chromatographic column and pressure was used to carry out the fractionation of *C. sieberiana* extracts. It allows for effective preliminary separation of the chemical constituents at a comfortable speed. The process can help to narrow down the large groups or classes of chemical constituents some of which would be responsible for the observed anti-sickling activities in order to facilitate the process of isolation.

It was observed that the anti-sickling activity of the 70 % (v/v) ethanol soxhlet extract of *C. sieberiana* pericarp was greatly enhanced by this preliminary purification exercise. Consequently, the 100 % ethyl acetate and ethyl acetate: methanol (1:1) from the VLC experiment exhibited the highest inhibitory anti-sickling activities of 87 % and 83 % respectively while the dichloromethane: ethyl acetate (1:1) and 100 % ethyl acetate fractions exhibited the highest reversal activities 73 % and 80 %, respectively. The ethyl acetate (100 %)

fraction of the 70 % (v/v) ethanol soxhlet extract of the pericarp exhibited the highest inhibitory (87.1 %) and reversal activities (79.7 %) (Table 15). Hence, the 100 % ethyl acetate and ethyl acetate: methanol (1:1) fractions showed significantly higher activities than the crude extract. More importantly while the crude extract of *C. sieberiana* pericarp gave inhibitory activity of 71 % and reversal activity of 54 %, its 100 % ethyl acetate fraction gave higher values for both inhibitory (87 %) and reversal activities (79.7 %) among other fractions.

Table 15: Comparative Anti-sickling activities of crude extracts and active VLC fractions from the 70 % (v/v) ethanol Soxhlet extract of the pericarp and aqueous extract of the ripe fruit of *C. sieberiana* at 4 mg/mL.

Extract	Fractions	% Inhibitory Activity ± SEM	% Reversal Activity ± SEM
70 % (v/v) ethanol Soxhlet extract of *C. sieberiana* pericarp	CDP	71.30 ± 6.05	53.68 ± 3.83
	D	18.55 ± 3.77	72.63 ± 3.91
	E	87.07 ± 2.28	79.74 ± 2.44
	F	83.13 ± 1.80	14.42 ± 1.69
Aqueous extract (decoction) of *C. sieberiana* ripe fruit	CDF	53.98 ± 3.75	90.17 ± 1.36
	I	91.50 ± 1.24	18.76 ± 0.97
	J	79.64 ± 1.60	70.13 ± 2.11
	K	47.24 ± 4.29	83.36 ± 1.82

KEY: CDP-Crude extract of *C. sieberiana* pericarp; CDF- Crude extract of *C. sieberiana* ripe fruit. D/K- dichloromethane: ethyl acetate (1:1) fraction, E- ethyl acetate fraction, F: ethyl acetate: methanol (1:1) fraction, I- n-hexane: dichloromethane (1:1) fraction, J- dichloromethane fraction.

In phytochemistry, purification enhances potency and reduces therapeutic doses. The n-hexane: dichloromethane (1:1) and 100 % dichloromethane fraction of the decoction of the ripe fruit exhibited enhanced and indeed the highest inhibitory anti-sickling activities of about 92 % and 80 % respectively (Table 15). The 100 % dichloromethane and dichloromethane: ethyl acetate (1:1) fractions gave the highest reversal anti-sickling activities 70 % and 83 % respectively. The 100 % dichloromethane fraction of the aqueous extract of the ripe fruit exhibited both inhibitory and reversal anti-sickling activities of 80 % and 70 % respectively, which were not significantly different from one other at $P < 0.05$ (Table 15).

Ogunlana *et al.,* (2008) reported that *M. lucida* possessed antioxidant activities which, were directly attributed to the content of phenolic compounds. Many plants with phenolic moieties exhibit antioxidant activities (Kahkonen *et al*., 1999; Frankel *et al*., 1995) which had been linked to RBC membrane stabilization such as supportive of prophylaxis in SCA crises by offering protection against the free radical-mediated oxidative injuries, representing an important role in the mechanism of anti-sickling activity (Ohnishi *et al*., 2001). The *L. cupanioides* ethanol leaf extract has been reported, by Salami *et al.,* 2014 to have the potentials for inhibiting, *in vivo,* antioxidant enzymatic activity. Flavonoids have been referred to as "nature's biological response modifiers" due to their inherent ability to modify the body's reactions to pathogens, allergens and carcinogens (Onyeama *et al.,* 2012). Flavonoids are significantly recognized for their anti-oxidant activities as well which is directly related to free- radical prophylaxis on RBC membrane distortion in SCA manifestation, anticarcinogenic, antimicrobial and antitumor properties (Manikandan *et al.,* 2006). Tannins are diverse organic compounds producing physiological astringent properties that hasten wound healing, ameliorate inflamed mucus membrane and have haemostatic properties. They are well known also for their anti-oxidant as well as for soothing

relief, skin regeneration, anti-inflammatory and diuretic activities (Okwu and Okwu, 2004). Alkaloids have been reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pain, develop resistance against disease and endurance against stress many of which will have positive roles in alleviating SCA crises. Glycosides stimulate various physiological actions, while orally ingested saponins are active immune boosters (Atawodi, 2005; Ojo *et al.,* 2012; Sandabe *et al.,* 2006) enhancing the body's resistance to opportunistic infections in SCA. Invariably, the 10 plants with or without significant anti-sickling properties in this study possess many of these secondary metabolites of favourable implications in SCA management.

4.4. Thin Layer Chromatography (T.L.C.).

The active constituents of plants are the reasons for the observed pharmacological actions in plant extracts. Phytochemical evaluation of medicinal plants is essential for herbal drug research and development which may include the evidence-base assurance of safety, efficacy and pharmacology associated with their use (Toma *et al*., 2009; Ullah *et al*., 2011). In respect of the plants investigated in this work, *P. pellucida* contains phytochemicals such as terpenes, alkaloids, tannins and saponins but not steroids, phlobatannins and flavonoids. *Harungana madagascariensis* contains all of these phytochemicals also, except phlobatannins. The stem-bark of *A.vogelii* has been reported to contain carbohydrates, saponins, flavonoids, terpenes, sterols and phenols while, reducing sugar, tannin, phlobatanins, and glycosides were found to be absent (Jegede *et al*., 2011). *Senna alata* leaf contained a chemical called "adenine" a compound with an effective platelet aggregating inhibitor (reduces sticky blood and arterial plaque) which can cause resistance of the blood cells against aggregation and eventual vaso-occlusion. Other chemicals include chrysoeriol -7-O- (2"-O-Beta -D -mannopyranosyl) -beta-dallopyranoside,

kaempferol, kaempferol 3 - O -gentiobioside, naringenin, quercetin, and rhamnetin - 3 -O - (2"-O -beta-D-mannopyranosyl) -beta-D-allopyranoside. The presence of adenine and these flavonoids in *S. alata* may explain its wide use in ethnomedical practice for the treatment of sickle cell anaemia in south western Nigeria (Borbalan *et al.,* 2003). Many biological activities can be rationalized to have support roles in the management of SCA

Fluorescence of T.L.C spots is an important phenomenon in chromatography inherent in chromophoric chemical constituents present in plants while some plant constituent's fluoresce in the visible range in day light and with the aid of chemical reagents. Others fluoresce in the ultraviolet region. For example, alkaloids like berberine, which do not visibly fluoresce in day light. If the substances themselves do not naturally fluoresce, they may often be converted into fluorescent derivatives by applying different reagents as a spray reagent hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Upendra *et al.,* 2012). The T.L.C exercise in this work, gave different chromatographic spots which were visualised by colour formation with the aid of detecting or spray reagents (Tables 13 and 14). Plant phenolics are secondary metabolites with diverse chemical nature and biological activity potentials namely: phenolic acids, flavonoids, tannins, coumarins, lignans, and amino acids, xanthones and stilbenes e.t.c. Many of these classes of phenol can occur in plants as glycosides while others appear inbound (Liu, 2004; Harborne, 1980). The VLC fractions gave positive reaction to $FeCl_3$ spray as follows: blue, blue-black or black indicating; the presence of phenolics (Tables 13 and 14). On spraying the T.L.C Plates with vanillin-sulphuric acid, various colours were obtained indicating the presence of terpenes and steroids. The fractions did not show positive results for the presence of alkaloids on spraying with draggendorfs spray reagent (Tables 13 and 14).

Coumarins give blue, purple, green and yellow colouration under UV at 366nm depending on the substituents or side chains attached to the parent coumarin nucleus e.g. hydroxy or alkoxy or methoxy groups (Murray, 1989). Since strong reducing agents (e.g. sodium metabisulphite) can induce sickling (Ekeke and Shode, 1986; Oduola *et al.,* 2006; Okpuzor *et al.,* 2008; Ejele and Njoku, 2008), substances such as quinones which are strong oxidising agents may be considered for reversal of the sickling phenomenon. Elujoba and Sofowora, (1977) have reported that the compounds exhibiting the anti-sickling properties appear to possess the following groups in common: a single benzene ring, a carboxylic acid group, at least one electron rich groups e.g. a hydroxyl or an amino group in the benzoic acid nucleus. Many of the constituents present in the plants studied possess this group of compounds including anthracene derivatives, anthrones, anthranols, present in the *Cassia* species studied. The n-hexane: dichloromethane (1:1) and 100 % dichloromethane fractions showed positive results for the presence of flavonoids and anthraquinones on spraying with 10 % alcoholic KOH (Tables 13 and 14). Further purification experiments would be required on the 100 % dichloromethane fraction of the aqueous extract of the ripe fruit and the 100 % ethyl acetate fraction of the 70 % (v/v) ethanol soxhlet extract of its pericarp which gave very high inhibitory and reversal anti-sickling activity with a view to isolating and characterizing the active constituents responsible for the observed activities.

Different mechanisms of action have been postulated to explain the anti-sickling activities exhibited by various extracts and isolated compounds. (Rumen, 1975, Noguchi, 1978; Abraham *et al.,* 1982; Iwu *et al.,* 1988). Many of the current works on the development of specific therapies for sickle cell anaemia are related to haemoglobin modifiers, cell membrane modifiers and genetic modifiers among others (Abraham *et al.,* 1982). HbS polymerization and the sickling process are intimately linked to the intracellular concentration of HbS measured as the mean

corpuscular haemoglobin concentration [MCHCs]. Therefore, any compound that increases cell ion content and causing cell swelling will decrease MCHC and inhibit or retard cell sickling. On the other hand, a drug that causes the cells to loose ions and shrink will increase MCHC, enhance polymerization and support sickling. Some agents increase oxygen affinity (e.g, cyanate). (Abraham *et al.,* 1999), while vanillin may be acting to decrease HbS polymerization by a dual mechanism of action: allosteric modulation to a high-affinity HbS molecule and by stereospecific inhibition of HbS polymerization. There are several compounds such as amino acids, which prevent sickling by acting on the erythrocyte membrane, causing an increase in the cell volume or swelling of the erythrocyte and thus reducing the intracellular haemoglobin concentration below its minimum gelling concentration (Rumen, 1975; Noguchi 1978; Iwu *et al.,* 1988; Abraham *et al.,* 1982).

4.5. CONCLUSION

The Soxhlet extract of *C. sieberiana* pericarp (71.3 %) exhibited the highest inhibitory activity while the aqueous extract of *C. sieberiana* ripe fruits (90.2 %) exhibited the highest reversal activity followed by the 70 % (v/v) ethanol Soxhlet extract of *S. alata* leaf (84.0 %). The VLC dichloromethane fraction of the aqueous extract of the ripe fruit (79.6 % inhibitory, 70.1 %

reversal) and the ethyl acetate fraction of the 70 % (v/v) ethanol Soxhlet extract of the pericarp (87.1 % inhibitory, 79.7 % reversal) exhibited significantly higher activities than their crude extracts and any other fraction, respectively. Hence, herbal formulations containing either of these two fractions can be developed for SCA management and can also serve as the raw materials for the isolation and characterization of the active constituent(s), responsible for the observed anti-sickling activities.

REFERENCES

Abah, S. E. and Egwari, L. O. (2011). Methods of Extraction and Antimicrobial Susceptibility Testing of Plant Extracts. *Afr. J. Basic & Applied Sci;* **3**(5): 205-209.

Abo, K. A., Adeyemi, A. A. and Jegede, I. A. (1999). Standardization and utilization of herbal medicines: challenges of the 21st century. Proceedings of 1st International Workshop on Herbal Medicinal Products, Ibadan, Nigeria, 22-24 November, 1999 pp. 171-177.

Abo, K. A., Ogunleye, V. O. and Ashidi, J. S. (1999). Antimicrobial potential of *Spondias mombin*, *Croton zambesicus* and *Zygotritonia crocea*. *Phyto Res;* **13**: 494 – 497.

Abraham, D. J., Mehanna, A. S. and Williams, F. L. (1982). Design, synthesis, and testing of potential anti-sickling agents. Halogenated benzyloxy and phenoxy acids. *J. of Med. Chem*; **25**(9): 1015–1017.

Abraham, D. J., Kennedy, P. E., Mehanna, A. S., Patwa, D. C. and Williams, F. L. (1984). Design, synthesis, and testing of potential anti-sickling agents. Structure-activity relationships of benzyloxy and phenoxy acids. *Amer. Chem. Soc. J. Med. Chem.* **27**: 967-978.

Abraham, D. J., Mehanna, A. S., Wireko, F. C., Whitney, J., Thomas, R. P. and Orringer, E. P. (1991). Vanillin, a potential agent for the treatment of sickle cell anaemia. *Blood* ;**77** (6):1334-1341.

Abu, S., Anyaibe, S. and Headings, V. (1981). Chromatographic fractionation of anti-sickling agents in *Fagara xanthoxyloides*. *Acta Haematol*; **66**:19-26.

Abuh, F. Y., Wambebe, C., Rai, P. P. and Sokomba, E. N. (1990). Hypoglycaemic activity of *Anthocleista vogelii* (Planch) aqueous extract in rodents. *Phyto. Res.* **4**: 20–24.

Adams-Graves, P., Kedar, A., Koshy, M., Steinberg, M., Veith, R. and Ward, D. (1997). RheothRx (poloxamer 188) injection for the acute painful episode of sickle cell disease: a pilot study. *Blood*.; **90**:2041–6.

Adefemi, O. A., Elujoba, A. A. and Odesanmi, W. O. (1988). Evaluation of the toxicity potential of *Cassia podocarpa* with reference to official *senna*. *West Afri. J. Pharmacol. Drug Res*. **8**: 41-48.

Adejumo, O. E., Ayoola, M. D., Kolapo, A. L., Orimoyegun, V. O. and Olatunji, P. O. (2011). Anti-sickling activities of extracts of leaf, seed and seed pod of *Garcinia kola* Heckel. *Afr J. Pharm. Pharmacology*. **5**: 48-52.

Adejumo, O. E., Kolapo, A. L. Roleola, O. P. and Kasim, L. S. (2010). In vitro anti-sickling activities and phytochemical evaluation of *Plumbago zeylanica* and *Uvaria chamae*. *Afr. J. of Biotech.*; **9**: 9032-6.

Adejumo, O. E., Owa-Agbanah, I. S., Kolapo, A. L. and Ayoola, M. D. (2011b). Phytochemical and anti-sickling activities of *Entandrophragma utile, Chenopodium ambrosioides* and *Petiveria alliacea*. *J. of Med. Plant Res.*; **5**: 1531-5.

Adeneye, A. A. and Agbaje, E. O. (2008). Pharmacological evaluation of oral hypoglycemic and antidiabetic effects of fresh leaf ethanol extract of *Morinda lucida* Benth in normal and alloxan-induced diabetic rats. *Afr. J. Biomed. Res*; **11**(1):65–71.

Adeneye, A. A., Olagunju, J. A., Benebo, A. S., Elias, A. S., Adisa, A. O., Idowu, B. O., Oyedeji, M. O., Isioye, E. O., Braimoh, O. B., Oladejo, O. O. and Alana, E. O. (2008).

Nephro-protective effects of the aqueous root extract of *Harungana madagascariensis* (L.) in acute and repeated dose acetaminophen renal injured rats. *Int. J. of Applied Res. in Nat. Prod;* **1**(1): 6 – 14.

Adesanya, S. A., Olugbade, T. A., Odebiyi, O. O. and Aladesanmi, A. J. (1992). Antibacterial alkaloids in *Crinum jagus*. *Int. J. of Pharm*; **30** (4):303–307.

Adesegun, A. H., Coker, A. B. and Hamann, M. T. (2008). Antifungal triterpenoid saponins from *Lecaniodiscus cupanioides. Res. J. of Phyto*; **2** (2): 93-99.

Adewunmi, C. O. and Adesogan, E. K. (1984). Anthraquinones and oruwacin from *Morinda lucida* as possible agents in fasciolasis and schistosomiasis control. *Fitoterapia*; **55**: 259–63.

Adeyemi, A. A. and Gbolade, A. (2006). Anti-anaemic activity of *Spondias mombin* and *Khaya grandifoliola* aqueous extracts on rat. *J. Pharm. and Bio;* **3** (2): 94-97.

Adeyemi, O. O. (2006). Effects of aqueous extract of *Baphia nitida* on isolated cardiac tissues. *Phyto. Res*., **6**(6): 318321

Adeyemi, O. O., Yemitan, O. K. and Adeogun, O. O. (2004). Analgesic Activity of the aqueous root extract of *Lecaniodiscus cupanioides*. *W Afr J of Pharm and Drug Res*; **20**(1-2): 10-14.

Adomi, O. P. and Umukoro, E. G. (2010). Antibacterial Activity of Aqueous and Ethanol Crude extracts of the root bark of *Alstonia boonei* and Preliminary phytochemical test of *Morinda lucida*. *J of Med Plants Res;* **4**(8): 644-648.

Agbor, G. A., Kuate, D. and Oben, J. E. (2007). Medicinal plants can be good source of anti-oxidants: case study in Cameroon. *Pakistani J of Bio Sci;* **10**(4): 537 – 544.

Aidoo, M., Terlouw, D. J., Kolczak, M. S., McElroy, P. D. and Kuile, F. (2002). Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet* **359**: 1311–1312.

Ajayi, I. A., Ajibade, O. O. and Oderinde, R. A. (2011). Preliminary phytochemical analysis of some plant seeds. *Res. J. of Chem. Sci;* **1**(3): 58-62.

Ajayi, O. B. and Adefioye, A. A. (2012). Comparative study on chemical compositions, phytochemical screening and physico-chemical properties of the seeds of *Dioclea reflexa*. *Ultra Chemistry*; **8**(2), 251-264.

Akah, P. A. and Nwambie, A. I. (1994). Evaluation of Nigerian traditional medicines; Plants used for rheumatic (Inflammatory) disorders. *J. Ethnomedicinal*, **42** (3): 179-182.

Akanmu, M. A. (1999). Evaluation of the toxicity potential of *Cassia podocarpa* and *Cassia fistula* fruits. M.Sc. Thesis Obafemi Awolowo University, Ile-Ife, Nigeria. 19-20 pp.

Akanmu, M. A., Iwalewa, E. O., Elujoba, A. A. and Adelusola, K. A. (2005). Toxicity Potentials of *Senna podocarpa* (Guill.Et Perr.) Lock Pods in Rodents. *Afr. J. Trad. CAM*; **2** (3): 274 – 281.

Akomolafe, R. O., Adeoshun, I. O., Elujoba, A. A., Iwalewa, E. O. and Ayoka, A. O. (2003). Effects of *Cassia sieberiana* leaf extracts on the intestinal motility of rat. *Afr. J. Bio. Res*; **6**(3), 141-145.

Alaribe, S.A., Coker, A. B., Shode, O. I., Ayoola, G., Adesegun, S. A., Bamiro, J., Anyim, E. I. and Anyakora, C. (2012). Antiplasmodial and phytochemical investigations of leaf extract of *Anthocleista vogelii* (Planch). *J. Nat. Prod*; **5**: 60-67.

Allison, A.C. (1964). Polymorphism and natural selection in human populations. *Cold Spring Harb Symp Quant Biol* **24**: 137–149.

Altmann, A. (1945). The sickle cell trait in the South African Bantu. *South Afr. J of Med*; **19**:457.

Amujoyegbe, O. O., Agbedahunsi, J. M., Akinpelu, B. A. and Oyedapo, O. O. (2012). *In vitro* evaluation of membrane stabilizing activities of leaf and root extracts of *Calliandra portoricensis (*JACQ) benth on sickle and normal human erythrocytes. *Int. Res. J of Pharm. and Pharma*. **2**(8): 198-203.

Aquil, M., Khan, I.Z. and Ahmad, M. B. (1993). Flavonoids from *Peperomia pellucida*. *Sci Phys Sci*; **5**: 213-215.

Aquil, M., Rahman, F. A. and Ahmad, M. B. (1994). A new flavonol glycoside from *Peperomia pellucida*. *Sci Phys Sci*; **6**:141-143.

Arbonnier, M. (2004). Trees, shrubs and lianas of West African dry zones. CIRAD, Margraf Publishers Gmbh, MNHN, Paris, France. 573 pp.

Arrigoni-Blank, M. F., Oliveira, R. L. and Mendes, S. (2002). Seed Germination, Phenology, and anti-dermatogenic activity of *Peperomia pellucida* (L.) *HBK BMC Pharmacology*; **2**: 12-19.

Asakura, T., Onishi, T., Friedman, S. and Schwartz, E. (1974). Abnormal Precipitation of Oxyhaemoglobin S by Mechanical Shaking. *Proc Nat. Acad Sci*; **71**: 1594-8.

Asase, A., Kokubun, T., Grayer, R. J., Kite, G., Simmonds, M. S. J., Oteng- Yeboah, A. A. and Odamtten, G. T. (2008). Chemical Constituents and Antimicrobial Activity of Medicinal

Plants from Ghana: *Cassia sieberiana*, *Haematostaphis barteri, Mitragyna inermis* and *Pseudocedrela kofschyi*. *Phyto. Res.* **22**: 1013−1016.

Asase, A., Oteng-Yeboah Alfred, A., Odamtten George, T. and Simmonds, M. S. (2005). Ethnobotanical study of some Ghanaian anti-malarial plants. *J. of Ethnopharmacology*, **99**:273–279.

Asuquo, O. R., Theresa, E. B., Paul, U. B., Out, M. E. and Patrick, E. E. (2013). Haematinic potential of *Spondias mombin* leaf extract in wistar rats. *Advances in Bioresearch*, **4** (2): 53 - 56

Asuzu, I. U. and Chineme, C. N. (1990). Effect of *Morinda lucida* leaf extract on *Trypanosoma brucei* infection in mice. *J. of Ethnopharm*; **30** (3):307–312.

Atawodi, S. E. (2005). Antioxidant potentials of African Plants. *Afr. J of Biotech*; **4** (2):128-133.

Atawodi, S. E. (2005). Comparative In vitro Trypanocidal Activities of Petroleum Ether, Chloroform, Methanol and Aqueous Extracts of Some Nigerian Savannah Plants. *Afri. J. Biotechnol*; **4** (2): 177-182.

Ateufack, G., Nguelefack, T. B., Wabo, H. K., Watcho, P. Tane, P. and Kamanyi, A. (2006). Antiulcer effects of the aqueous and organic extracts of the stem bark of *Anthocleista vogelii* in rats. *Pharm Bio;* **44**(3): 166–171.

Atindehou, K. K., Koné, M., Terreaux, C., Traoré, D., Hostettmann, K. and Dosso, M. (2002). Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast. *Phytotherapy Research*; **16**(5): 497–502.

Augusto, F., Valente, A. L. P., Tada, E. S. and Rivellino, S. R. (2000): Screening of Brazilian fruit aromas using solid-phase micro extraction-gas chromatography-mass spectrometry. *J of Chrom;* **873** (1), 117 – 127.

Avaligbe, C. T., Gbenou, J. D., Kpoviessi, D. S., Gbaguidi, F., Moudachirou, M. and Accrombessi, G. C. (2012). Assessment of anti-sickling properties of extracts of plants used in the traditional treatment of sickle cell disease in Benin. *Eur J Sci Res*; **87**:100-108.

Awe, S.O., Olajide, O. A., Adeboye, J. O. and Makinde, J. M. (1998). Some pharmacological studies on *Morinda lucida*. *Ind J of Pharmacol;* **30**(1): 38–42.

Awe, S. O. and Makinde, J. M. (1998). Evaluation of sensitivity of *Plasmodium falciparum* to *Morinda lucida* leaf extract sample using rabbit in vitro microtest techniques. *Ind J of Pharmacol;* **30**(1): 51–53.

Ayantunde, A. A., Hiernaux, P., Briejer, M., Udo, H. and Tabo, R. (2009). Uses of local plant species by agropastoralists in South-western Nigeria. *Ethnobotany Research and Applications*; **7**: 53-066.

Ayoka, A. O., Akomolafe, R. O., Akinsomisoye, O. S. and Ukponmwan, O. E. (2008) Medicinal and Economic Value of *Spondias mombin*. *Afr J of Biomed Res*; **11**(2):129-136

Ayoka, A. O., Akomolafe, R. O., Iwalewa, E. O. and Ukponmwan, O. E. (2005). Studies on the Anxiolytic effect of *Spondias mombin* L. (Anacardiaceae) Extracts. *Afr. J. Trad. CAM*; **2** (2): 153 – 165.

Ayoka, A. O., Akomolafe, R. O., Iwalewa, E. O., Akanmu, M. A. and Ukponmwan, O. E. (2006): Sedative, antiepileptic and antipsychotic effects of *Spondias mombin* L. (Anacardiacaea) in mice and rats. *J. of Ethnopharm;* **103**: 166 – 175.

Aziba, P. I., Adedeji, A., Ekor, M. and Adeyemi, O. (2001). Analgesic activity of *Peperomia pellucida* aerial parts in mice. *Fitoterapia*; **72**:57-58.

Ballas, S. K. and Marcolina, M. J. (2006). Hyperhaemolysis during the evolution of uncomplicated acute painful episodes in patients with sickle cell anaemia. *Transfusion*. **46** (1): 105-110.

Barneby, R. (1991). Sensitivae censitae: a description of the genus Mimosa Linnaeus (Mimosaceae) in the New World. New York Botanical Garden, New York.

Bayma, J. D., Arruda, M. S., Muller, A. H., Arruda, A. C. and Canto, W. C. (2000). A dimeric Ar C2 compound from *Peperomia pellucida*. *Phytochem;* **55**: 779-782.

Behe, M. J. and Englander, S. W. (1979). Mixed gelation theory. Kinetics, equilibrium and gel incorporation in sickle haemoglobin mixtures. *J Mol Biol*.; **133** (1):137-60.

Belinsky, S. A., Klinge, D. M. and Stidley, C. A. (2003). Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer. *Cancer Res*.; **63**:7089-7093.

Berhaut, J. (1975). Flore illustrée du Sénégal. Dicotylédones. Volume 4. Ficoidées à Légumineuses. Gouvernement du Sénégal, Ministère du Développement Rural et de l'Hydraulique, Direction des Eaux et Forêts, Dakar, Senegal. 625 pp.

Bernstein, R. E. (1969). Sickle haemoglobin in South Africa. *South Afr Med J*; **43**:1455-56.

Bisby, F. A., Buckingham, J. and Harborne, J. B. (1994). Phytochemical Dictionary of the Leguminosae. ILDIS. Chapman & Hall, London.

Biu, A. A., Yusufu, S. D. and Rabo, J. S. (2010). Acute Toxicity Study on Neem (*Azadirachta indica*, Juss) Leaf Aqueous Extract in Chicken (Gallus gallus domesticus). *African Scientist;* **11**: 241−244.

Bojo, A. C., Albano-Garcia, E. and Pocsidio, G. N. (1994). The anti-bacterial Activity of *Peperomia pellucida* (L.) HBK (Piperaceae). *Asia Life Sci*; **3**: 35-44.

Borbalan, A. M., Zorro, L., Guillen, D. A. and Barroso, C.G. (2003). Study of the Polyphenol Content of Red and White Grape Varieties by Liquid Chromatography - mass spectrometry and its Relationship to Antioxidant Power. *J. Chromatogr. A*; **1012**: 31-38.

Borges, A. and Desforges, J. F. (1967). Studies of heinz body formation. *Acta Haematol*; **37**:1-10

Brawley, O.W. Cornelius, L. J. Edwards, L. R. Gamble, V. N. Green, B. L. and Inturrisi, C. (2008). National Institutes of Health Consensus Development Conference statement: Hydroxyurea Treatment for Sickle Cell Disease. *Ann Intern Med*.17; **148**(12):932-8.

Brichard, B., Vermylen, C., Ninane, J. and Cornu, G. (1996). Persistence of Fetal Haemoglobin Production after Successful Transplantation of Cord Blood Stem Cells in a Patient with Sickle Cell Anemia. *J Pediatr*; **128**:241–3.

Buratai, L. B., Biu, A. A. and Hauwa, M. M. (2011). Studies on the effects of aqueous leaf extract of *Cassia sieberiana* D.C. (Caesalpiniaceae) on Haematological parameters of albino rats. *Savannah J of Agr*; **6**:58-62.

Burkill, H. M. (1985). The useful plants of West tropical Africa. 2nd Edition. Volume 1, Families A–D. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 960 pp.

Burkill, H. M. (1995). The useful plants of West tropical Africa. 2nd Edition. Volume 3, Families J–L. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 857 pp.

Burkill, H. M. (1997). The useful plants of West tropical Africa. 2nd Edition. Volume 4, Families M–R. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 969 pp.

Burkill, H. M. (2000). The Useful Plants of West Tropical Africa. 2nd Edn., Royal Botanical Garden, Kew, 293-294pps.

Cappellini, M. D. and Piga, A. (2008). Current status in iron chelation in haemoglobinopathies. *Curr Mol Med*; **8**(7):663-74.

Carr, B. I., Rahbar, S., Asmeron, Y., Riggs, A. and Winberg, C. D. (1988). Carcinogenicity and haemoglobin synthesis induction by cytidine analogues. Br J Cancer; **57**:395-402.

Catalfamo, J. L., Martin Jr, W. B. and Birecka, H. (1982). Accumulation of alkaloids and their necines in *Heliotropium curassavicum, Heliotropium spathulatum* and *Heliotropium indicum*. *Phytochemistry;* **21** (11): 2669–2675.

Chapelle, J. P. (1976). Vogeloside and secologanic acid, secoiridiod glucosides from *Anthocleista vogelii*. *Planta Medica*; **29**(2): 268–274.

Che, Q. M. (1991). Isolation of human intestinal bacteria capable of transforming barbaloin to aloe-emodin anthrone. *Planta medica*, **57**:15–19.

Chikezie, P. C. (2011). Sodium metabisulphite induced polymerization of sickle cell haemoglobin incubated in the extracts of three medicinal plants (*Anacardium occidentale*, *Psidium guajava* and *Terminalia catappa*). *Afr. J Biotechnol*; **10**:6154-61.

Clarke, E. G. and Clarke, M. I. (1979). Factors affecting the actions of poison. In: Veterinary Toxicology, Bailliere Tindall, London. 9−13 pps.

Dalziel, J. M. (1937). The useful plants of West tropical Africa, Crown agents for the colonies, London, 612pp.

Dalziel, J. M. and Hutchinson, J. D. (1958). Flora of West Tropical Africa, London, Vol. 1 part 2, 450-455pp.

Das, B. B., Sobczyk, W., Bertolone, S. and Raj, A. (2008). Cardiopulmonary stress testing in children with sickle cell disease who are on long-term erythrocytapheresis. *J Pediatr Hematol Oncol*.; **30**(5):373-7.

Dash, B. P., Archana, Y., Satapathy, N. and Naik, S. K. (2013). Search for anti-sickling agents from plants. Phcog Rev;**7**: 53-60.

Dean, J. and Schechter, A. N. (1978). Sickle cell anemia: Molecular and cellular basis of therapeutic approaches. *New Engl J Med*.; **229**: 753-755.

Dean, J. and Schechter, A. N. (1978). Sickle cell anaemia: molecular and cellular basis of therapeutic approaches. *N. Engl. J. Med*. **299**: 863-870.

Deshpande, H. A. and Bhalsing, S. J. (2011). Phytochemical analysis of *Cassia obtusifolia, Cassia auriculata, Tephrosia purpurea, Helictres isora* and *Centella asiatica*. *Int J of Pharma and Bio Sciences;* **2**(3): B363-367.

Dierdorf, S. (1996). Anaesthesia for patients with rare and coexisting diseases. Barash P. Cullen B., Stoelting R, eds. Clinical Anaesthesia 3rd ed. Philiadelphia, Pa: Lippincott-Raven; 414-416.

Donkor, K., Okine, N. K., Wonder, K. M. and Abotsi, E. W. (2014). Acute and Sub-Chronic Toxicity Studies of Aqueous Extract of Root Bark of *Cassia Sieberiana* D.C. in Rodents. *J of Applied Pharm. Sci;* **4** (04); 084-089

Donkor, K., Okine, N. K., Abotsi, W. K. and Woode M. E. (2013). Anti-inflammatory and Anti-nociceptive effects of ethyl acetate fraction of root bark of *Cassia sieberiana* D. C. in murine models. *Pharmacologia*; **4**:301-310.

Duvall, C. S. (2006). Origin of the tree *Spondias mombin* in Africa. *J of His Geography;* **32**: 249-266.

Duweijua, M., Weremfo, A. and Abassah- Oppong, S. (2007). Toxicological evaluation of ethanol root extract of *Cassia sieberiana* (DC) in rats. *Bio. Biotech. Res. Asia;* **4**(2): 23-28.

Duwiejua, M., Panyin, A. B., Weremfo, A., Woode, E. and Ansah, C. (2008). Antinociceptive activity of the ethanol extract of the root bark of *Cassia sieberiana* (Fam. Caesalpinaceae). *J of Pharm & Bio*; **4**:49-58.

Eaton, W. A. and Hofrichter, J. (1987). Haemoglobin S gelation and sickle cell disease. *Blood* ;**70**: 1245-66.

Edeoga, H. O. and Eriata, D. O. (2001). Alkaloid, tannin and saponin contents of some Nigerian medicinal plants. *J of Med and Arom Plant Sci*; **23** (3): 344 – 349.

Edwin, A. K., Edwin, F. and Etwire, V. (2011). Controlling sickle cell disease in Ghana—ethics and options. *Pan African Med J;* **10**: 14–22.

Egunyomi, A., Moody, J. O. and Eletu, O. M. (2009). Anti-sickling Activities Ethnomedicinal of Two Plant Recipes used for Sickle Cell Anaemia in Ibadan, Nigeria. *J of Afr Biotech*; **8**(1): 20-25.

Ejele, A. E. and Njoku, P. C. (2008). Anti-sickling Potential of *Aloe vera* Extract. *J. Sci. of Food and Agric*; **88**: 1482 – 1485.

Ekeke, G. I. and Shode, F. (1986): Anti-sickling potential of *Cajanus cajan* Seed Methanol-Water Soluble Extract. *Planta Medica*; **56**: 41-43.

Ekeke, G. L. and Shode, F. O. (1985). The Reversion of Sickle Cell by *Cajanus cajan*. *Plant. Med*; **6**: 504-507.

El Sayed, N. Y., Abdelbari, E. M., Mahmoud, O. M. and Adam, S. E. (1983). The toxicity of *Cassia senna* to Nubian goats. *Vet. Rec . Q*; **5**(2): 80-85.

Elbaum, D., Nagel, R. L., Bookchin, R. M. and Herskovits, T. T. (1974). . Effect of alkylureas on the polymerization of hemoglobin S. *Proc natn. Acad. Sci.* U.S.A; **71**:4718.

Elufioye, T. O., Obutor, E. M., Agbedahunsi, J. M. and Adesanya, S. A. (2013). Cholinesterase inhibitory activity of *Morinda lucida*. *J. of Med Plants Res;* **7**(12): 734-737.

Elufioye, T. O., Obutor, E. M., Sennuga, A. T., Agbedahunsi, J. M. and Adesanya, S. A. (2010). Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some selected Nigerian medicinal plants. *Rev. bras. Farmacogn;*. **20** (4):472-477.

Elujoba, A. A. and Sofowora, E. (1977). Detection and estimation of total acid in the anti-sickling fraction of *Fagara* species. *Planta Med;* **32**: 54–59.

Elujoba, A. A. and Iweibo, G. O. (1988).*Cassia podocarpa* as substitute for official senna. *Planta Medica;* **54**: 372.

Elujoba, A. A. and Ogunti, E. O. (1993). Laxative activity of *Cassia alata*. *Fitoterapia;* **5**: 437-439.

Elujoba, A. A., Abere, A. T. and Adelusi, S. A. (1999). Laxative activities of Cassia pods sourced from Nigeria. *Nig. J of Nat Prod and Med;* **3**: 51–53.

Elujoba, A. A., Ajulo, O. O. and Iweibo, G. O. (1989). Chemical and Biological analysis of Nigeria Cassia species for laxative activity. *J. Pharm. Biomedical Analysis;* **12**:1453-1457.

Elujoba, A. A., Ogunti, E. O., Soremekun, R. D. and Iranloye, T. A. (1994). The Pharmacognosy and Dosage formulation of *Cassia podocarpa* leaf with reference to Senna. *J. Pharm. Sci. & Pharm. Pract*; **2**: 14-18.

Ettarh, R. R. and Emeka, P. (2004). *Morinda lucida* extract induces endothelium-independent and independent relaxation of rat aorta. *Fitoterapia*; **75**(3-4):332–336.

Evans, R. W. (1944). The sickling phenomenon in the blood of West African natives. Trans Roy Soc Tropical Med; **37**:281-6.

Fadulu, A. (1977). Ethyl-Alcohol Extract from *Fagara zanthoxyloides* Root: *In vitro* Effect on Red Blood Cells, *Faculty Research J, Texas Southern Univ*; **1**: 20-31.

Faleye, F. J. (2012). Steroids constituents of *Dioclea reflexa*. *J of Pharm and Scientific Innovation*; **1** (3): 89-90.

Fall, A. B., Vanhaelen-Fastré, R., Vanhaelen, M., Lo, I., Toppet, M. and Ferster, A. (1999). In vitro anti-sickling activity of a rearranged limonoid isolated from *Khaya senegalensis*. *Planta Med;* **65**: 209-12.

Fasola, T. R. and Egunyomi, A. (2005). Nigerian usage of bark in phytomedicine. *Ethnobotany Research and Applications*; **3**: 73–78.

Frankel, E. N. (1995). Natural and biological antioxidant in food and biological systems, their mechanism of action, applications and implications. *Lipid Technol*; **1**: 77 - 80.

Franklin, F. C., Lehrbachp. H. R., Lurz, R., Rueckertb, B. and Timmisk, N. (1983). Localization and Functional Analysis of Transposon Mutations In Regulatory Genes of the Tol Catabolic Pathway. *J of Bact*; **154**:676- 685.

Friedman, M. J. (1978). Erythrocytic mechanism of sickle cell resistance to malaria. *Proc Natl Acad Sci.* **75**: 1994–1997.

Gaston, M. H., Verter, J. I. and Woods G. (1986). Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. *N Engl J Med*; **314**: 1593-1599.

Gbadamosi, I., Adeyemi, S., Adeyemi, A. and Moody, J. (2012). In vitro anti-sickling activities of two indigenous plant recipes in Ibadan, Nigeria. *Int J of Phytomedicine,* **4** (2), 205 - 211.

Gill, L. S. (1992): Ethno-medical uses of plant in Nigeria UNIBEN Press 46 p.

Gill, L. S. 1992. Ethnomedical Uses of Plants in Nigeria. Benin: Uniben Press, 130 p.

Gill, L. S. (1992). Ethnomedical uses of plants in Nigeria. Benin: University of Benin press; p. 276.

Gorecki, M., Votano, J. R. and Alexander, R. (1980). Peptide inhibitors of sickle haemoglobin aggregation: effect of hydrophobicity. *Biochemistry*; **19** (8):1564–1568.

Hafiza, M. A., Parveen, B., Ahmad, R. and Hamid, K. (2002). Phytochemical screening of Medicago sativa and Zinnia elegans. *On line J of Biol Sci*; **2**(2): 130-132.

Haller, J. S. (1990). A drug for all seasons, medical and pharmacological history of aloe. *Bulletin of the New York Academy of Medicine*; **66**:647–659.

Harborne, J. B. (1980). In Secondary plant products. Encyclopedia of plant physiology. Bell EA, B.V. Charlwood, Springer-Verlag-Berlin-Heidelberg; **8**:329-402.

Head, C. A., Brugnara, C., Martinez-Ruiz, R., Kacmarek, R. M., Bridges, K. R. and Kuter, D. (1997). Low concentrations of nitric oxide increase oxygen affinity of sickle erythrocytes *in vitro* and *in vivo*. *J Clin Invest*; **100**: 1193–1198.

Heeney, M. M. and Ware, R. E. (2008). Hydroxyurea for children with sickle cell disease. *Pediatr Clin North Am*; **55** (2):483-501.

Heidi, M. (2001). New Advances in the Treatment of Sickle Cell Disease: Focus on Perioperative Significance. *Amer Ass N Anae J*; **69** (4): 281-287.

Herrick, J. B (1910). Peculiar elongated and sickle shaped red blood corpuscles in a case of severe anaemia. *Arch Intern Med*; **6**: 517-21.

Hill, A. V., Allsopp, C. E., Kwiatkowski, D., Anstey, N. M. and Twumasi, P. (1991). Common west African HLA antigens are associated with protection from severe malaria. *Nature;* **352**: 595–600.

Ho, C. and Russu, I. M. (1987). Proton NMR. studies of the molecular basis for the anti-sickling activity of non-covalent anti-sickling compounds. *Prog Clin Biol Res*; **240**: 59-66.

Houghton, P. J., Agbedahunsi, J. M. and Adegbulugbe, A. (2004). Cholinesterase inhibitory properties of alkaloids from two Nigerian *Crinum* species. *Phytochemistry*; **65**(21): 2893-2896.

Ibraseem, N. K., Ahmed, J. H. and Hassan, M. K. (2010). The effect of fixed oil and water extracts of *Nigella sativa* on sickle cells: An in vitro study. *Singapore Med J*; **51**:230-234.

Ibrahim, H., Sani, F. S., Danladi, B. H. and Ahmadu, A. A. (2007). Phytochemical and anti-sickling studies of the leaf of *Hymenocardia acida* Tul (Euphorbiaceae). *Pak J Biol Sci*; **10**:788-91.

Ingram, V. M. (1957). Gene mutations in human haemoglobin: The chemical difference between normal and sickle cell haemoglobin. *Nature*; **180**: 326-328.

Inngjerdongen, K., Nergård, C.S., Diallo, D., Mounkoro, P.P. and Paulsen, B.S. (2004). An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa. *J of Ethnopharm;* **92**: 233–244.

Irina, M., Russu, A. K., Lin, L. C., Chao, P. Y. and Chien, H. (1986). Molecular basis for the anti-sickling activity of aromatic amino acids and related compounds: a proton nuclear magnetic resonance investigation. *Biochemistry*; **25** (4):808–815

Ishii, O., Tanizawa, H. and Takino, Y. (1990). Studies of *Aloe* III. Mechanism of laxative effect. *Chemical and Pharmaceutical Bulletin*; **38**:197–200.

Iwalewa, E. O., Omisore, N. O., Adewunmi, C. O., Gbolade, A. A., Ademowo, O. G., Nneji, C., Agboola, O. I. and Daniyan, O. M. (2008). Anti-protozoan activities of *Harungana madagascariensis* stem bark extract on trichomonads and malaria. *J of Ethnopharm*; **117** (3):507-11.

Iwalewa, E. O., Omisore, N. O., Adewunmi, C. O., Oluborode, I. O., Fatokun, O. A., Taiwo, B. J. and Daniyan, O. M. (2009). Elemental composition and anti-anaemic property of *Harungana madagascariensis* stem bark extract. *Bangladesh J of pharmacol*; **4**:115-121.

Iwalewa, E. O., Suleiman, M. M., Mdee, L. K. and Eloff, J. N. (2007). Antifungal and antibacterial activities of six different extracts of *Harungana madagascariensis* stem bark. *Planta Medica;* **73**(9): 890.

Iwalewa, E. O., Adewale, I. O., Taiwo, B. J., Arogundade, T., Osinowo, A., Daniyan, O. M. and Adetogun, G. E. (2008). Effects of *Harungana madagascariensis* Stem Bark Extract on the Antioxidant Markers in Alloxan Induced Diabetic and Carrageenan Induced Inflammatory Disorders in Rats. *J. of Comp and Int Med*; **5**(1): 1553-3840.

Iwu, M. M., Igboko, A. O., Onwubiko, H. and Ndu, U. E. (1988). Effect of cajaminose from *Cajanus cajan* on gelation and oxygen affinity of sickle cell haemoglobin, *J. of Ethnopharm*; **23**(1):99–104.

Iwu, M. M. (1993). Handbook of African medicinal plants. CRC Press, Boca Raton, Florida, United States. 464 pp.

Iyamu, E. W., Turner, E. A. and Asakura, T. (2002). In vitro effects of NIPRISAN (Nix-0699): A naturally occurring, potent anti-sickling agent. *Br J Haematol*; **118**: 337-43.

Iyamu, E. W., Turner, E. A. and Asakura, T. (2003). NIPRISAN (Nix-0699) improves the survival rates of transgenic sickle cell mice under acute severe hypoxic conditions. *Br J Haematol*; **122**: 1001-1008.

Jain, S. K. and Shohet, S. B. (1984). A novel phospholipid in irreversibly sickled cells: Evidence for in vivo peroxidative membrane damage in sickle cell disease. *Blood*; **63**: 362-367.

Jegede, I. A., Ibrahim, J. A. and Kunle, O. F. (2011) . Phytochemical and pharmacognostic studies of the leaf and stem-bark of *Anthocleista vogelii* (Planch). *J of Med. Plants Research*; **5**(26): 6136-6139.

Kahkonen, M. P., Hopia, A. I., Vourela, H. J., Rauha, J., Pihlaja, K., Kajala, T. H. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **47**:3954-3962.

Kantarjian, H., Issa, J. P. and Rosenfeld, C. S. (2006). "Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study". *Cancer;* **106** (8): 1794–1803.

Kantarjian, H. M., O'Brien, S. and Cortes, J. (2003). "Results of decitabine (5-aza-2'deoxycytidine) therapy in 130 patients with chronic myelogenous leukemia". *Cancer;* **98** (3): 522–528.

Kar. B. C. (1991). Sickle cell disease in India. *J Assoc Physicians India*; **39**: 954-60.

Keay, R. W., Omochie, C. F. and Standfield, D. P. (1964). Nigerian Trees. Nigerian National Press Ltd. Apapa, 10-69 pp.

Konotey-Ahulu, F.I.D. (1974). The Sickle Cell Disease, *Arch, Intern. Med*; **133**: 611-61.

Kouam, S. F., Ngadjui, B. T., Krohn, K., Wafo, P., Ajaz, A. and Chowdhary, M. I. (2005). Prenylated anthronoid antioxidants from the stem bark of *Harungana madagascariensis*. *Phytochemistry*; **66** (10):1174-9.

Kouam, S. F., Yapna, D. B., Krohn, K., Ngadjve, B. T. Ngoupayo, J. Chowdhary, M. I. and Schulz, B. (2007). Antimicrobial prenylated anthracene derivatives from the leaf of *Harungana madagascariensis*. *J of Nat Prod*; **70** (4):600-603.

Koumaglo, K., Gbeassor, M., Nikabu, O., De Souza, C. and Werner, W. (1992). Effects of three compounds extracted from *Morinda lucida* on *Plasmodium falciparum*. *Planta Med*; **58**(6): 533–534.

Kritikar, K. R., and Basu, B. D. (2003). Indian medicinal plants Illustrations. Volume-5, Second edition, published by oriental enterprises, 1394-96 pp.

Kuku, A., Stoppini, M., Cobianchi, A., Minetti, G., Balduini, C. and Aboderin, A. (2000). The complete primary structure of a mannose/glucose specific lectin from the seeds of *Dioclea reflexa* Hook, F. *Nig. J. Biochem. Mol. Biol*; **15**: 115-119.

Kunle, O. F. and Egharevba, H. O. (2013). Chemical constituents and biological activity of medicinal plants used for the management of sickle cell disease –A Review; *J Med Plants Res*; **7**(48):3452-3476.

Kweifio-Okai, G. (1991). Anti-inflammatory activity of a Ghanaian anti-arthritic herbal preparation II. *J of Ethnopharmacol;* **33**(1-2): 129-133.

Kweifio-Okai, G., Bird, D., Field, B., Ambrose, R., Carrol, A. R., Smith, P. and Valdes, R. (1995). Antiinflammatory activity of a Ghanaian anti-arthritic herbal preparation. *J. Ethnopharmacol;* **46**: 7-15.

Lachant, N. A. and Tanaka, K. R. (1986). Antioxidants in sickle cell disease: The *in vitro* effects of ascorbic acid. *Am J Med Sci*; **292**:3-10.

Laird, P. W., Jackson-Grusby, L. and Fazeli, A. (1995). Suppression of intestinal neoplasia by DNA hypomethylation. *Cell*; **81**:197-205.

Lawal, H. O., Etatuvie, S. O. and Fawehinmi, A. B. (2012). Ethnomedicinal and Pharmacological properties of *Morinda lucida*. *J of Nat Prod*; **5**:93-99

Leeuwenberg, A. J. M. (1961). The Loganiaceae of Africa. 1. *Anthocleista. Acta Botanica Neerlandica;* **10**: 1–53.

Lemos, T. L., Nogueira, P. C., Alencar, J. W. and Craveiro, A. A. (1995). Composition of the leaf oils of four *Spondias* species from Brazil. *J of Essent Oil Res;* **7** (5): 561 – 563.

Lenta, B. N., Ngouela, S., Boyom, F. F., Tantangmo, F., Raymond, G., Tchouya, F., Samo, E., Gut, J., Rosentha, P. J. and Connolly, J. D. (2007). Antiplasmodial activity of some constituents of the root bark of *Harungana madagascariensis* LAM (Hyperiacacea). *Chem and Pharm Bull*; **55**(3):464-467.

Levasseur, D. N., Ryan, T. M., Reilly, M. P., McCune, S. L., Asakura, T. and Townes, T. M. (2004). Recombinant human haemoglobin with anti-sickling properties greater than fetal haemoglobin. *J Biol Chem*; **279**: 27518-24.

Liu, R.H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J. Nut*; **134**: S3479-3485.

Luter, L., Onaji, A. R., Galadima, A. and Okoronkwo, M. U. (2012). Phytochemical Screening and Anti-Microbial Activity Studies of the Root Extract of *Anthocleista djalonensis* (Cabbage Tree). *International J of Chem*; **4** (4); 37-44.

Luzzatto, L., Nwachuku-Jarrett, E. S. and Reddy, S. (1970). Increased sickling of parasitised erythrocytes as mechanism of resistance against malaria in the sickle-cell trait. *Lancet;* **1**: 319–321.

Luzzatto, L. (1975). Genetic counselling in haemoglobinopathies. *Dokita*; **7**: 65-68.

Madusolumuo, A. M., Nadro, S. M. and Wurochekke, U. A. (1999). Antihepatotoxic properties of *Cassia sieberiana* in acetaminophen treated rats. *Nig. J. Biochem. Mol. Biol*; **14**: 21-25.

Makinde, J. M. and Obih, P. O. (1985). Screening of *Morinda lucida* leaf extract for malarial action on *Plasmodium berghei* in mice. *Afr J Med and Med Sc*; **14**(1-2):59–63.

Manikandan, L., Senthilkumar, G. P., Rajesh, L. T. and Suresh, R. 2006. Cancer chemopreventive agents from medicinal plants. In: Trivedi, P.C (ed.). Medicinal Plants: Ethnobotanical approach. Agrobios, India. 410pp.

Mansuang Wuthi-udomLert (2010). *In vitro* evaluation of antifungal activity of anthraquinone derivatives of *Senna alata*. *J Health Res*; **24**(3): 117-122.

Mavar-Manga, H., Chapon, D., Hoet, S., Block, S., De Pauw-Gillet, M. C. and Quetin-Leclercq, J. (2006). N_1, N_2, N_3, Tri-isiso pentenyl-1-guanidine and N_1, N_2 di-isopentenyl guanidine, two cytotoxic alkaloids from *Alkomea cordifolia* Müll. Arg. (Euphorbiaceae) root bark. *Natural Products Communications*; **12**: 1097−1100.

Mikhail, O. N., Taoseed, A. A. and Musbau, A. A. (2013). Phytochemical analysis and antimalarial activity aqueous extract of *Lecaniodiscus cupanioides* root. *J of Trop Med*. Volume 2013, Article ID605393, 4 pages.

Misra, S. N (1979). Antifungal activity of leaf extract of some higher plants. *Acta Botanica Indica*; **7**:147-150.

Modell, B. and Darlison, M. (2008). Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ*; **86**: 480–487.

Moody, J. O., Ojo, O. O., Omotade, O. O., Adeyemo, A. A., Olumese, P. E. and Ogundipe, O. O. (2003). Anti-sickling potential of a Nigerian herbal formula (ajawaron HF) and the major plant component (*Cissus populnea* L. CPK). *Phytotherapy Research*; **17**: 1173-6.

Moreira, A. R., Castelo-Branco, C. C., Monteiro, A. C. O., Tavares, R. O. and Beltramini, L. M. (1998). Isolation and partial characterization of a lectin from *Artocarpus incise* seed. *Phytochem;* **47**:1183-1188.

Moriyama, H., Iizuka, T., Nagai, M., Miyataka, H. and Satoh, T. (2003). Anti-inflammatory activity of heat-treated *Cassia alata* leaf extract and its flavonoid glycoside. Yakugaku Zasshi, *J Pharm Soc Japan*; **123**: 607-611.

Moronkola, D. O., Adeleke, A. K. and Ekundayo, O. (2003). Constituents of the *Spondias mombin Linn* and the comparison between its fruit and leaf essential oils. *J Essen Oil Bearing Plants*; **6** (3), 148 – 152.

Morton, J. (1987): *Yellow mombin*. In Fruits of warm climates. Julia F. Morton, Miami, Florida. 245 – 248 pp.

Moulari, B., Lboutounne, H., Chaumont, J. P., Guillaume, Y., Millet, J. and Pellequer, Y. (2006). Potentiation of the bactericidal activity of *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae) leaf extract against oral bacteria using poly (D, L-lactide-co-glycolide) nanoparticles: *In vitro* study. *Acta Odontol Scand*; **64**:153-8.

Mozuraitis, R., Stranden, M., Ramirez, M. I., Borg-Karlson, A. K. and Mustaparta, H. (2002). (-)-Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. *Chem Senses*; **27**:505-9.

Mpiana, P. T., Mudogo, V., Ngbolua, K. N., Tshibangu, D. S., Atibu, E. K., Kitwa, E. K. and Kanangila, A. B. (2008). *In vitro* anti-sickling activity of anthocyanins extracts of *Vigna unguiculata* (L.) Walp. *Recent Pro Med Plants Chem Med Value*; **25**: 91-8.

Mpiana, P. T., Mudogo, V., Tshibangu, D. S., Ngbolua, K. N., Shetonde, O. M. and Mangwala, K. P. (2007). *In vitro* anti-sickling activity of anthocyanins extracts of a congolese plant: *Alchornea cordifolia M. Arg J Med Sci*; **7**: 1182-6.

Mpiana, P. T., Ngbolua, N. N., Bokota, M. T., Kasonga, T. K., Atibu, E. K., Tshibangu, D. S. T. and Mudogo, V. (2010). *In vitro* effects of anthocyanin extracts from *Justicia secunda* Vahl on the solubility of Haemoglobin S and membrane stability of sickle erythrocytes. *Blood Transfus;* **8**(4): 248 -254.

Mpiana, P. T., Tshibangu, D. S., Shehonde, O. M. and Ngbolua, K. N. (2007). *In-Vitro* antidrepanocytary activity (anti-sickle cell anaemia) of some Congolese plants. *Phytomedicine;* **14** (2-3): 192 -195.

Murray, R. D. H. (1989). Coumarins. *Nat Prod. Rep;* **6**: 591-624

Muskiet, F. A., Muskiet, F. D. and Meiborg, G. (1991). Supplementation of patients with homozygous sickle cell disease with zinc, alpha-tocopherol, vitamin C, soybean oil, and fish oil. *Am J Clin Nutr* ; **54**:736-744.

Nadkarni, A. K. Indian Materia Medica, Volume-1, Third edition 2005, Published by Popular Prakashan, Bombay.1402pp.

Nartey, E. T., Ofosuhene, M., Kudzi, W. and Agbale, C. M. (2012). Antioxidant and gastric cytoprotective prostaglandins properties of *Cassia sieberiana* roots bark extract as an anti-ulcerogenic agent. *BMC Complement Altern Med*. **20**; 12-65.

Neuwinger, H. D. (1996). African ethno botany: poisons and drugs. Chapman & Hall. 280-296pp.

Neuwinger, H. D. (2000). African traditional medicine: a dictionary of plant use and applications. Medpharm Scientific, Stuttgart, Germany. 589pp.

Njoku, P. C. and Akumefula, M. I. (2007). Phytochemical and Nutrient Evaluation of *Spondias mombin* Leaf. *Pakistan J of Nutr*; **6**: 613-615.

Noguchi, C. T. (1978). "Inhibition of sickle haemoglobin gelation by amino acids and related compounds," *Biochemistry*; **17**(25): 5455–5459.

Noguchi, C. T. and Schechter, A. N. (1977). "Effects of amino acids on gelatin kinetics and solubility of sickle haemoglobin," *Biochemical and Biophysical Research Communications*; **74**(2):637–642.

Noguchi, C. T. and Schechter, A. N. (1978). Inhibition of gelation by amino acids and related compounds. *Biochemistry*; **17**: 5455-5459.

Nwaoguikpe, R. N. (2010). The phytochemical, proximate and amino acid compositions of the extracts of two varieties of tiger nut (*Cyperus esculentus*) and their effects on sickle cell haemoglobin polymerization. *J Med Med Sci*; **1**: 543-549.

Nwaoguikpe, R. N., Braide, W. and Ezejiofor, T. I. (2010). The effect of *Aloe vera* plant (*Aloe barbadensis*) extracts on sickle cell blood (HbSS). *Afr. J of Food Sci and Tech*; **1**:58-63.

Obidah, W., Sa'ad, U. A. and Wurochekke, A. U. (2009). Toxic effects of aqueous stem bark extract of *Cassia sieberiana* on some biochemical parameters in rats. *Afr. J. Biochem. Res*; **3** (5): 229-231.

Obih, P. O., Makinde, J. M. and Laoye, J. O. (1985): Investigations of various extracts of *Morinda lucida* for antimalaraial actions on *Plasmodium berghei berghei* in mice. *Afr J Med Med Sci*; **14**: 45–9.

Odo, M. O., Ngele, S. and Okeuze, I. E. (2005). Evaluation of nutritional composition and anti-nutrients in African white star apple (*Chrysophyllum albidum*) seed. *Nig. Food J;* **23**: 252-255.

Odugbemi, T. and Akinsulire, O. (2006). Medicinal Plants by Species names. In: (Odugbemi T, ed) Outlines and Pictures of Medicinal Plants from Nigeria. University of Lagos Press, Nigeria, 73-116 pp.

Oduola, T., Adeniyi, A. A., Ogunyemi, E. O., Bello, I. S. and Idowu, T. O. (2006). Anti-sickling agent in an extract of unripe pawpaw (*Carica papaya*): Is it real? *Afr. J of Biotech;* **5** (20):1947-1949.

Ogundare, A. O. and Olorunfemi, O. B. (2007). Antimicrobial Efficacy of the Leaf of *Dioclea reflexa, Mucuna pruriens, Ficus asperifolia* and *Tragia spathulata*. *Res. J of Micro*; **2**: 392-396.

Ogundare, O. A. (2009). The antimicrobial pattern and phytochemical properties of the leaf extracts of *Senna podocarpa*. *Afr. J. Microbiol. Res;* **3**(7): 400-406.

Ogunlana, O. E. and Farombi, O. E. (2008). *Morinda lucida*: Antioxidant and reducing activities of crude methanol stem bark extract. *Adv. Natl. Appl. Sci*; **2**(2):49-54.

Ogunyemi, C. M., Elujoba, A. A. and Durosinmi, M. A. (2008). Anti-sickling Properties of *Carica papaya* Linn. *J of Nat. Prod;* **1**: 56-66.

Ogunyemi, C. M., Elujoba, A. A. and Durosinmi, M. A. (2009). Anti-sickling properties of the fermented mixture of *Carica papaya* Linn and *Sorghum bicolor* (L.) Moench. Afr. *J. Pharm. Pharmacology*; **3**(4):140-143.

Ojo, N. A., Adawaren, E. O., Tijjani, M. B., Chiroma, M., Simon, J., Afisu, B., Mogbojuri, O. M. and Wakil, A. M. (2012). Acute Toxicity and Effects of Ethanol Extract of *Tefairia occidentalis* Leaf on Blood Glucose Level in Normal Rats. *Vom. J. Vet. Sc;* **9**: 25-31.

Okoli, A. S., Okeke, M. I., Iroegbu, C. U. and Ebo, P. U. (2002). Antibacterial activity of *Harungana madagascariensis* leaf extracts. *Phyto. Res*; **16**(2):174-9.

Okore, C. M., Ugwu, P. O. and Oleghe Akpa, P. A. (2007). Selective Anti-Candidal action of crude aqueous pod extract of *Lecaniodiscus cupanioides*: A Preliminary Study on *Candida albicans* obtained from an AIDS Patient; **2** (2):043-046,

Okorie, D. A. (1976). A new phthalide and xanthones from *Anthocleista djalonensis* and *Anthocleista vogelii*. *Phytochemistry;* **15**: 1799–1800.

Okpuzor, J. and Adebesin, O. Membrane stabilizing effect and anti-sickling activity of *Senna podocarpa* and *Senna alata*. 31st congress for European biochemical societies, Instanbul, Turkey, July 25-29, 2006.

Okpuzor, J., Ogbunugafor, J., Kareem, G. K. and Igwo-Ezipke, M. N. (2009). *In vitro* investigation of antioxidant phenolic compounds in extracts of *Senna alata*. *Res. J Phytochem*; **3**: 68-76.

Okwu, D. E. (2007). Nigerian Medicinal and Aromatic Plants. *Sci Biotechnol*; **1**(1):90-96

Okwu, D. E. (2001). Evaluation of chemical composition of indigenous species and flavoring agents. *Global J. Pure Appl Sci*; 458-459 pp.

Okwu, D. E. and Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *Afr. J of Biotech*; **5**(4): 357-361.

Okwu, D. E. and Okwu, M. E. (2004). Chemical Composition of *Spondias mombin* Linn. Plant Parts. *J. Sustain. Agric Environ*; **6**(2):140 – 147.

Oladimeji-Salami, J. A., Akindele, A. J. and Adeyemi, O. O. (2014). Effects of ethanol dried leaf extract of *Lecaniodiscus cupanioides* on antioxidant enzymes and biochemical parameters in rats. *J. Ethnopharmacology*; **155**(3):1603-8.

Oladosu, I. A., Echeme, J. O. and Zubair, M. F. (2006). Anticholinesterase and Antibacterial Activities of Dioclimidazole from *Dioclea reflexa* seeds. Fitoterapia; **77**: 571-575.

Olagunju, J. A., Ogunfeibo, A. B., Ogunbosi, A. O. and Taiwo, O. A. (2004). Biochemical changes elicited by isosaline leaf and stem bark extracts of *Harungana madagascariensis* in the Rat. *Phytotherapy Research;* **18**: 588 – 591.

Olajide, O. A., Awe, S. O., Makinde, J. M. and Morebise, O. (1999). Evaluation of the anti-diabetic property of *Morinda lucida* leaf in Streptozocin-diabetic Rats. *J. of Pharm*; **51**(11):1321–1324.

Olapade, A. A., Ajayi, O. A. and Ajayi, I. A. (2014). Physical and chemical properties of *Cassia sieberiana* seeds. *International Food Res. J*; **21**(2): 767-772.

Olapade A. A., Akinoso, R. and Oduwaye, A. O. (2012). Changes in some physicochemical properties of *Cassia sieberiana* seeds during roasting. *Nig. Food J*; **30**(1): 26- 34.

Oliver-Bever, B. (1986). Medicinal Plants in Tropical West Africa. Cambridge: Cambridge University Press; 89–90 pp.

Oliver-Bever, B. (1983): Medicinal plants in tropical west Africa. III. Anti-infection therapy with higher plants. *J. Ethnopharmacology*; **9**: 1 – 83.

Oliver-Bever, B. (1986). Propagation and management, functional uses of Medicinal plants In: medicinal plant tropical West Africa. Cambridge University Press. 89-90 pp.

Olukoya, D. K., Idika, N. and Odugbemi, T. (1993). Antibacterial activity of some medicinal plants from Nigeria. *J of Ethnopharmacol;* **39**: 69–72.

Oniyangi, O. and Cohall, D. H. (2013). Phytomedicines (medicines derived from plants) for sickle cell disease. Cochrane Database of Systematic Reviews 2013, Issue 1. Art. No.: CD004448. DOI: 10.1002/14651858.CD004448.pub4.

Onyeama, H. P., Ibekwe, H. A., Ofemile, P. Y., Peter, A., Ahmed, M. S. and Nwagbo, P. O. (2012). Screening and Acute toxicity studies of *Calliandra portoricensis* (Eri agbo in Igbo) used in the treatment of snake bite in South-eastern Nigeria. *Vom J. Vet. Sc*; **9**: 17-24.

Owoyale, J. A., Olatunji, G. A. and Oguntoye, S. O. (2005). Antifungal and antibacterial activities of an alcoholic extract of *Senna alata* leaf. *J of App Sci & Environ Man*: **9**(3):105–107.

Pasvol, G., Weatherall, D. J. and Wilson, R. J. (1978). Cellular mechanism for the protective effect of haemoglobin S against *P. falciparum* malaria. *Nature*; **274**: 701–703.

Pauling, L. and Itano, H. A. (1949). Sickle cell anaemia a molecular disease. *Science*; 110:543-8. phenylalanine. *Nig. J. Bchm. Mol. Biol;* **25**(2): 68-71.

Poorter, L., Bongers, F., Kouamé, F. and Hawthorné, W. (2004). Biodiversity of West African forests. In: Ancological Atlas of Woody Plants Species, CABI Publishing, Cambridge, USA, 521 p.

Qin, Y. M., Xing Q. Y., Fang, Z. X., Chen, J. C., Gui –Hua, X. and Liu, L. D. H (2008). Phenolic compounds and antioxidant activity of extracts from ultrasonic treatment of *Satsuma Mandarin* (*Citrus unshiu* Marc.) peels. *J Agric Food Chem*; **56**:5682-5690.

Quadri, O. N. and Taofeek, O. A. (2012). Aqueous root extract of *Lecaniodiscus cupanioides* restores the alterations in testicular parameters of sexually impaired male rats. *As Pac J Reprod*. 120-124 pp.

Rai, P. P., Wambebe, O. C. and Abuh, F. Y. (1989). Some pharmacological actions of *Anthocleista vogelii*. *Planta Medica*; **55**: 661.

Raji, Y. and Bolarinwa, A. F. (1997). Antifertility activity of *Quassia amara* in male rats – *in vivo* study. *Life Sci*; **64**: 1067–74.

Raji, Y., Udoh, U. S., Mewoyeka, O. O., Ononye, F. C. and Bolarinwa, A. F. (2003). Implication of reproductive endocrine malfunction in male antifertility efficacy of *Azadirachta indica* extract in rats. *Afr J Med Sci*; **32**: 159–65.

Rao, B. S. and Prahavathi, N. T. (1982). Tannin content of food commonly consumed in India and its influence on ionized iron. *J Sci Food Agri*; **33**: 89-96.

Restivo, A. L., Brard, C., Granai, O. and Swamy, N. (2005). Antiproliferative effect of Mimosine in ovarian cancer. *J Clin Oncol*; **23**(16): 3200.

Reynolds J. E. F. Martindale, the extra pharmacopoeia, 30th ed. London, Pharmaceutical Press, 1993.pp903.

Rienhoff, H. Y., Viprakasit, V. and Tay, L. (2001). A phase 1 dose-escalation study: safety, tolerability, and pharmacokinetics of FBS0701, a novel oral iron chelator for the treatment of transfusional iron overload. *Haematologica*; **96**(4):521-5.

Robinson, R. D., Williams, L. A., Lindo, J. F., Terry, S. I. and Mansingh, A. (1990). Inactivation of *strongyloides stercoralis* filariform larvae *in vitro* by six Jamaican plant extracts and three commercial anthelmintics. *West Indian Med. J*; **39** (4): 213–217.

Ross, I. A. (2003). Medicinal plants of the world. Chemical constituents, traditional and modern uses. Volume 1. 2nd Edition. Humana Press, Totowa NJ, United States. 489 Pp.

Roth, E. F., Friedman, M., Ueda, Y., Tellez, I. and Trager, W. (1978). Sickling rates of human AS red cells infected *in vitro* with *Plasmodium falciparum* malaria.. *Science;* **202**: 650–652

Rumen, N. M. (1975). Inhibition of sickling in erythrocytes by amino acids. *Blood*; **45**:(1):45–48.

Salunkhe, D. K., Jaghar, S. S., Kadam, S. S. and Chavan, J. K. (1982). Chemical, biochemical and biological significance of polyphenols in cereals and legumes. *Crit. Rev. Food. Sci. Nutr;* **17**(3): 277-305.

Sandabe, U. K., Onyeyili, P. A. and Chibuzo, G. A. (2006). Phytochemical screening and effect of aqueous extract of *Ficus sycomorus* L. (Moraceae) stem bark on muscular activity in laboratory animals. *J. Ethnopharmacol*; **104**: 283-285

Shahriar, M., Tanjida, A., Shahjabeen, S., Rumana, A. and Mohiuddin, A. B. (2014). Phytochemical screenings, membrane stabilizing activity, thrombolytic activity and cytotoxic properties of leaf extracts of *Mimosa pudica. Int J Pharm*; **4**(2):155-158.

Shear, H. L., Roth, E. F., Fabry, M. E., Costantini, F. D. and Pachnis, A. (1993). Transgenic mice expressing human sickle hemoglobin are partially resistant to rodent malaria. *Blood.* **81**: 222–226.

Silva, O., Barboza, S., Diniz, A., Valdeira, L. and Gomes, E. (1997). Plant extracts antiviral activity against Herpes simplex virus type I and African swine fever virus. *Int. J. Pharm*; **35**(1): 12-16.

Silva, O., Ferreira, E., Vaz-Pato, M. and Gomes, E. (1997). Guinea-Bissau's plants: *in vitro* susceptibility studies on *Neisseria gonorrhoea*. *Int. J. Pharm*; **35**(5): 323-328.

Silva, O., Duarte, A., Cabrita, J., Pimentel, M., Diniz, A. and Gomes, E. (1996). Antimicrobial activity of Guinea-Bissau traditional remedies. *J Ethnopharmacol*; **50**: 55–59.

Sofidiya, M. O. (2008). Antioxidant and Antibacterial Properties of *Lecaniodiscus cupanioides*. *Res J Micro*; **3** (2): 91 – 98.

Sofowora, E. A. (1991). Medicinal plants and traditional medicine. John Wiley & Sons. Pages 66-79.

Sofowora, E. A. and Isaacs, W. A. (1971). Reversal of Sickling and Crenation in Erythrocytes by the Root Extract of *Fagara zanthoxyloides*. *Lloydia* 1971; **34**: 383-385.

Stone, B. C. (1970). The flora of Guam. *Micronesica*; **6**:1-659.

Sunday, A. A., Coker, A. B. and Hamann, M. T. (2008). Antifungal Triterpenoid Saponins from *Lecaniodiscus cupanioides*. *Res J Phytochem*; **2**: 93-99.

Sushma, K., Praveen, K. and Poonam, R. (2012). Pharmacological potentials of *Cassia auriculata* and *Cassia fistula* Plants: A Review. *Pakistan J of Bio Sci;* **15**: 408-417.

Sy, G. Y., Fall, A. D., Diatta, W., Gueye, M., Badji, K. and Bassène, E. I. (2009). Analgesic and Anti-inflammatory activity of aqueous root extract of *Cassia sieberiana* DC (Caesalpiniaceae). *Afr. J. Pharm. Pharmacol*; **3**:651- 653.

Tamboura, H. H., Bayala, B., Lompo, M., Guissou, I. P. and Sawadogo, L. (2005). Ecological distribution, morphological characteristics and acute toxicity of aqueous extract of *Holarrhena floribunda* (G.DON) Durand and Schinz, *Leptadenia hastata* (PERS.) Decne and *Cassia sieberiana* (DC) used by veterinary healers in Burkina Faso. *Afr. J. Trad. Compl. Altern. Med*; **2**(1); 13-24.

Taofeeq, O., Ibrahim, B., Ganiyu, A., Abdul-Waseed, A., Gassa, R. and Godwin, A. (2010). Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to *Morinda lucida* leaf extract. *North Am. J. Med. Sci*; **2**:230-233.

Taylor, L. (2004). The healing power of rainforest herbs: A guide to understanding and using herbal medicinals. Square One Publishers Inc; 1–2 pp.

Tene, M., Tane, P., Kuiate, J., Tamokou, J. and Connolly, J. D. (2008): Anthocleistenolide, A new rearranged Nor-Secoiridoid derivative from the stem bark of *Anthocleista vogelii*. *Planta Medica*; **74**: 80-83.

The Wealth Of India, A Dictionary of Indian Raw Materials and Industrial Products Vol. V (H-k) first supplement series, National Institute of science Communication and information resources, New Delhi: CSIR Publication;1997, 31 pp.

Thin-Layer Chromatography, Revised and Expanded By Bernard Fried, Joseph Sherma CRC Press, Jan 4, 1999 - Science - 512 pp.

Thiocyanate: An All Natural Cure for Sickle Cell Anaemia? Anti-Sickling Medicine in African Yams and Cassava by Ana Kirk. November 4, 2010.

Toma, L., Karumi, Y. and Geidam, M. A. (2009). Phytochemical screening and toxicity studies of the aqueous extract of the pods pulp of *Cassia sieberiana* DC. (Cassia kotchiyana Oliv.). *African J. Pure and Applied Chemistry*; **3**(2): 026-030.

Tona, L., Ngimbi, N. P., Tsakala, M., Mesia, K., Cimanga, K., Apers, S., De Bruyne, T. Pieters, L., Totte, J. and Vlietinck, A. J. (1999). Antimalarial activity of 20 crude extract from nine African medicinal plants used in Kinshasha. *Congo J Ethnopharm*; **68** (1-3):193–203.

Tona, L., Kambu, K., Ngimbi, N., Mesia, K., Penge, O., Lusakibanza, M., Cimanga, K., De Bruyne, T., Apers, S., Totte, J., Pieters, L. and Vlietinck, A. J. (2000). Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. *Phytomedicine*; **7** (1):31–38.

Toruan-Purba, A. V. (1999). Senna Miller. In: de Padua, L.S., Bunyapraphatsara, N. & Lemmens, R.H.M.J. (Editors). Plant Resources of South-East Asia No 12(1). Medicinal and poisonous plants 1. Backhuys Publishers, Leiden, Netherlands. 442–447 Pp.

Tyler, V. E., Bradley, L. R. and Robbers, J. E. (1988). Pharmacognosy, 9th ed. Philadelphia, Lea & Febiger; 62–63 pp.

Uchendu, C. N. and Chowdhary, M. I. (2004). The in vitro effects of butanolic leaf extract of *Spondias mombin* on rat uterine muscle. *Nig J Experimen and Appl Biol*; **5**(1):109–113.

Ullah, N., Khurram, M., Khan, A. F., Khayyam, S. U., Amin, M. U., Ullah, S., Najeeb, U., Muhammad, S., Hussain, J. and Khan, M. A. (2011). Estimation of Phytochemical and

Antimicrobial Activities of *Mentha spicata* from Southern Districts of Pakistan. *J. Applied. Pharm. Sc*; **1**(7): 81-84

Upendra, J. (2012). Assessment of Nutritive Values, Phytochemical Constituents and Biotherapeutic Potentials of *Epiphyllum Oxypetalum*. *Int J Pharm Pharm Sci*; **4** (5):421-425

Uwakwe, A. A. and Nwaoguikpe, R. N. (2008). In vitro anti-sickling effects of *Xylopia aethiopia* and *Monodora myristica*. *J Med Plant Res*; **2**: 119-24.

Van der Maesen, L. J. G. (2007). *Cassia sieberiana* DC. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes médicinales 1. [CD-Rom]. PROTA, Wageningen, Netherlands.

Vichinsky, E. P., Neumayr, L. D. and Earles, A. N. (2000). Causes and outcomes of the acute chest syndrome in sickle cell disease. National Acute Chest Syndrome Study Group. *N Engl J Med*; **342**:1855-65.

Walters, M. C., Patience, M., Leisenring, W., Eckman, J. R., Scott, J. P. and Mentzer, W. C. (1996). Bone marrow transplantation for sickle cell disease. *N Engl J Med*; **335**:369–76.

Wambebe, C., Khamofu, H., Momoh, J. A., Ekpeyong, M., Audu, B. S. and Njoku, O. S. (2001). Double-blind, placebo-controlled, randomised cross-over clinical trial of NIPRISAN in patients with Sickle Cell Disorder. *Phytomedicine*; **8**: 252-61.

Whitten, C. F. and Bertly, J. F. (1989). Sickle Cell Disease. *New York Acad. Sci*; **565**: 104-106.

Willcox, M., Bjorkman, A., Brohult, J., Pehrson, P. O. and Rombo, L. (1983). A case-control study in northern Liberia of *Plasmodium falciparum* malaria in haemoglobin S and beta-thalassaemia traits. *Ann Trop Med Parasitol*; **77**: 239–246.

Williams, T. N., Mwangi, T. W., Wambua, S., Alexander, N. D., Kortok, M., Snow, R. W. and Marsh, K. (2005). Sickle cell trait and the risk of *Plasmodium falciparum* malaria and other childhood diseases. *J Infect Dis.*; **192**(1):178-86.

World Health Organisation (1994). Guidelines for the control of haemoglobin disorders Sardinia. WHO 1994.

Yemitan, O. K. and Adeyemi, O. O. (2005). CNS depressant activity of *Lecaniodiscus cupanioides*. *Fitoterapia*; **76**(5):412-418.

Yusuf, M., Begum, J., Hoque, M. N. and Chowdhury, J. U. (2009). *Medicinal Plants of Bangladesh. BCSIR, Chittagong*; **79** (42):213-221.

Zemel, B. S., Kawchak, D. A. and Fung, E. B. (2002). Effect of zinc supplementation on growth and body composition in children with sickle cell disease. *Am J Clin Nutr*; **75**: 300-307.

APPENDIX I

Inhibitory anti-sickling activities of aqueous extracts (decoctions)

Plant	% Inhibitory Activity
Spondias mombin (L)	7.64 ± 1.67
Senna alata (L)	7.95 ± 1.32
Cassia sieberiana (IM)	8.11 ± 1.21
Perperomia pellucida (WP)	9.42 ± 1.60
Cassia sieberiana (L)	9.43 ± 6.07

Lecanodiscus cupanioides (L)	10.28 ± 2.03
Anthocleista vogelli (B)	11.25 ± 1.87
Dioclea reflexa (S)	12.22 ± 1.42
Mimosa pudica (WP)	13.05 ± 2.42
Morinda lucida (L)	17.12 ± 1.05
Cassia sieberiana (B)	20.68 ± 2.62
Cassia sieberiana (S)	25.85 ± 2.63
Spondias mombin (R)	26.50 ± 0.67
Harungana madagascariensis (L)	41.78 ± 1.72
Cassia sieberiana (MF)	53.98 ± 3.75
Cassia sieberiana (P)	58.46 ± 2.96
vanillic acid (RF)	54.01 ± 2.54
Ciklavit (RF)	64.19 ± 3.45

KEY

L- leaf, S- seed, B-stem bark, R- root, P-pericap, MF- mature ripe fruit, IM- unripe fruit, WP- whole plant. RF-reference standard.

APPENDIX II

Reversal anti-sickling activities of aqueous extracts (decoctions)

Plant	% Reversal Activity
Cassia sieberiana (IM)	5.78 ± 1.81
Senna alata (L)	8.62 ± 1.11
Spondias mombin (R)	16.11 ± 1.40
Anthocleista vogelli (B)	16.84 ± 2.22

Cassia sieberiana (S)	18.53 ± 2.67
Spondias mombin (L)	18.82 ± 0.73
Lecanodiscus cupanioides (L)	30.30 ± 1.25
Mimosa pudica (WP)	32.10 ± 1.35
Cassia sieberiana (B)	37.16 ± 1.17
Cassia sieberiana (L)	37.60 ± 4.37
Dioclea reflexa (S)	38.35 ± 2.36
Cassia sieberiana (P)	42.09 ± 2.11
Morinda lucida (L)	43.68 ± 1.76
Perperomia pellucida (WP)	49.31 ± 2.07
Harungana madagascariensis (L)	52.11 ± 1.49
Cassia sieberiana (MF)	90.17 ± 1.36
PHBA(RF)	69.21 ± 1.25
Ciklavit (RF)	76.88 ± 1.31

KEY

L- leaf, S- seed, B-stem bark, R- root, P-pericap, MF- mature ripe fruit, IM- unripe fruit, WP- whole plant. RF-reference standard.

APPENDIX III

Inhibitory anti-sickling activities of 70 % (v/v) cold ethanol extracts

Plant	% Inhibitory Activity
Spondias mombin (L)	6.91 ± 5.71
Cassia sieberiana (MF)	11.32 ± 4.15
Anthocleista vogelli (B)	12.40 ± 4.02

Cassia sieberiana (B)	13.88 ± 2.19
Senna alata (L)	16.15 ± 2.88
Cassia sieberiana (IF)	17.08 ± 1.63
Cassia sieberiana (S)	20.47 ± 5.78
Morinda lucida (L)	25.59 ± 12.72
Harungana madagascariensis (L)	27.10 ± 11.79
Cassia sieberiana (L)	30.60 ± 1.33
Spondias mombin (R)	38.89 ± 7.25
Lecanodiscus cupanioides (L)	41.93 ± 6.12
Mimosa pudica (WP)	44.60 ± 1.68
Dioclea reflexa (S)	44.65 ± 7.64
Peperomia pellucida (WP)	54.13 ± 3.45
Cassia sieberiana (P)	54.93 ± 8.70
vanillic acid (RF)	54.01 ± 2.54
Ciklavit (RF)	64.19 ± 3.45

KEY

L- leaf, S- seed, B-stem bark, R- root, P-pericarp, MF- mature ripe fruit, IM- unripe fruit, WP- whole plant. RF-reference standard.

APPENDIX IV

Reversal anti-sickling activities of 70 % (v/v) cold ethanol extracts

Plant	% Reversal Activity
Peperomia pellucida (WP)	0.09 ± 1.18
Cassia sieberiana (IF)	2.96 ± 1.65

Dioclea reflexa (S)	3.32 ±1.13
Cassia sieberiana (P)	5.90 ± 4.37
Cassia sieberiana (L)	6.12 ± 1.68
Lecanodiscus cupanioides (L)	8.11 ± 2.33
Mimosa pudica (WP)	9.29 ± 3.01
Cassia sieberiana (S)	9.78 ± 1.28
Cassia sieberiana (B)	14.12 ± 10.36
Harungana madagascariensis (L)	15.59 ± 5.47
Cassia sieberiana (MF)	15.69 ± 3.11
Spondias mombin (L)	22.81 ± 7.19
Senna alata (L)	26.35 ± 2.18
Morinda lucida (L)	28.92 ± 9.12
Spondias mombin (R)	29.31 ± 8.10
Anthocleista vogelli (B)	61.09 ± 5.68
PHBA(RF)	69.21 ± 1.25
Ciklavit (RF)	76.88 ± 1.31

KEY

L- leaf, S- seed, B-stem bark, R- root, P-pericarp, MF- mature ripe fruit, IM- unripe fruit, WP- whole plant. RF-reference standard.

APPENDIX V

Inhibitory anti-sickling activities of 70 % (v/v) ethanol Soxhlet extracts

Plant	% Inhibitory Activity
Spondias mombin (R)	10.38 ± 2.10

Lecanodiscus cupanioides (L)	11.65 ± 1.03
Dioclea reflexa (S)	11.75 ± 5.45
Mimosa pudica (WP)	13.70 ± 1.48
Cassia sieberiana (B)	17.90 ± 2.01
Senna alata (L)	18.49 ± 6.92
Cassia sieberiana (IF)	19.33 ± 3.19
Cassia sieberiana (L)	26.30 ± 2.11
Perperomia pellucida (WP)	26.96 ± 5.98
Harungana madagascariensis (L)	39.19 ± 8.89
Anthocleista vogelli (B)	47.48 ± 2.39
Morinda lucida (L)	51.45 ± 0.92
Cassia sieberiana (MF)	55.07 ± 6.05
Cassia sieberiana (S)	55.59 ± 13.04
Spondias mombin (L)	56.61 ± 7.40
Cassia sieberiana (P)	71.30 ± 6.05
vanillic acid (RF)	54.01 ± 2.54
Ciklavit (RF)	64.19 ± 3.45

KEY

L- leaf, S- seed, B-stem bark, R- root, P-pericarp, MF- mature ripe fruit, IM- unripe fruit, WP- whole plant. RF-reference standard.

APPENDIX VI

Reversal anti-sickling activities of 70 % (v/v) ethanol Soxhlet extracts

Plant	% Reversal Activity

Anthocleista vogelli (B)	2.49 ± 1.77
Cassia sieberiana (L)	2.96 ± 2.59
Cassia sieberiana (B)	4.77 ± 0.94
Mimosa pudica (WP)	9.03 ± 3.80
Spondias mombin (L)	9.29 ± 2.70
Cassia sieberiana (IF)	11.27 ± 1.77
Spondias mombin (R)	11.68 ± 4.90
Lecanodiscus cupanioides (L)	11.73 ± 2.39
Perperomia pellucida (UP)	13.85 ± 0.69
Dioclea reflexa (S)	17.21 ± 0.63
Harungana madagascariensis (L)	29.81 ± 2.37
Cassia sieberiana (S)	36.15 ± 3.29
Morinda lucida (L)	39.45 ± 0.63
Cassia sieberiana (MF)	51.07 ± 1.99
Cassia sieberiana (P)	53.68± 3.83
Senna alata (L)	84.04 ± 4.65
PHBA (RF)	69.21 ± 1.25
Ciklavit (RF)	76.88 ± 1.31

KEY

L- leaf, S- seed, B-stem bark, R- root, P-pericarp, MF- mature ripe fruit, IM- unripe fruit, WP- whole plant. RF-reference standard.