ANTISICKLING EFFECT AND ACTIVITY GUIDED FRACTIONATION OF *DICHROSTACHYS CINEREA* (Wight & Arn) ROOT AND *STERCULIA SETIGERA (*Del*)* LEAF METHANOLIC EXTRACTS ON HUMAN SICKLE RED BLOOD CELLS *IN VITRO*

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DEPARTMENT OF BIOCHEMISTRY,

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FEBRUARY, 2016

DECLARATION

I declare that the work in this dissertation entitled "**Antisickling Effect and Activity Guided Fractionation of *Dichrostachys cinerea* (Wight & Arn) Root and *Sterculia setigera* (Del) Leaf Methanolic Extract on Human Sickled Red Blood Cells *in vitro*"** has been carried out by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Baraka Abdullahi

M.Sc/sci/7239/11-12 Signature Date

CERTIFICATION

This dissertation entitled "ANTISICKLING EFFECT AND ACTIVITY GUIDED FRACTIONATION OF *DICHROSTACHYS CINEREA* (Wight & Arn) ROOT AND *STERCULIA SETIGERA* (Del) LEAF METHANOLIC EXTRACT ON HUMAN SICKLED RED BLOOD CELLS *IN VITRO"* by Baraka ABDULLAHI meets the regulations governing the award of the Degree of Masters of Science in Biochemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Almighty Allah (SWT), the most high, the most great.

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ABSTRACT

The *in vitro* antisickling effects of the crude methanolic extract, ethyl acetate, n-hexane, butanol, and aqueous fractions of methanolic extracts of *Dichrostachys cinerea* and *Sterculia setigera* were determined using different concentrates on sodium metabisulphite-induced sickled red blood cells. The result showed that antsickling activity was both concentration and time dependent, with highest activity observed at the longest time interval of 120 min, with the exception of n-hexane concentrate of 0.1mg/ml and aqueous fraction of 0.2mg/ml of *Dichrostachys cinerea* having a percentage unsickling effect of 38.46±1.4% and 33.39±1.4%, respectively. The ethylacetate butanol, n-hexane, and aqueous concentrates of *Dicrostachys cinerea* fractions showed a significant antisickling difference ($p<0.05$) when compared with p-hydroxybenzoic acid, (PABA) as positive control. However, the butanol concentrate of *Sterculia setigera* did not show any significant difference when compared with the positive control. Osmotic fragility test was carried out in saline concentrations of 0.1, 0.2, 0.3, 0.5, 0.6, 0.9ml using extract concentrations of 0.5, 1.0, 2.0, and 2.5mg/ml. A significant difference was observed in percentage inhibition of lysis in ethylacetate fraction of *Sterculia setigera* for all saline concentration when compared with a standard drug, Ibuprofen. For butanolic fraction of *Sterculia setigera*, there is no significant difference in percentage inhibition of haemolysis when compared with Ibuprofen in all tested saline concentrations. N-hexane fraction of *Sterculia setigera* showed a significant difference in percentage inhibition of haemolysis, when compared with Ibuprofen. Also, a significant difference was observed in percentage inhibition of lysis when compared with Ibuprofen. For ethylacetate fraction of *Dicrostachys cinerea*, a significant difference was observed in all tested saline concentrations, when compared with Ibuprofen.

However, in the butanolic fraction, no significant difference in percentage inhibition of haemolysis was observed when compared to Ibuprofen. The n-hexane and aqueous fractions of *Dicrostachys cinerea* showed a significant difference in percentage lysis in all tested saline concentrations when compared with ibuprofen. The percentage methaemoglobin concentration was analysed using extract concentrations of 0.2, 0.4, 0.6, 0.8, and 1mg/ml. results showed that no significant difference was observed in all fractions of both test plants, when compared with the control. Elemental analysis was carried out using an AAS, the highest concentrations of Fe, Zn, Cu, and Cr were observed in aqueous fraction of *Dichrostachys cinerea* with a concentration of 287.20±0.00, 72.60±0.00, 57.20±0.00, 20.80±0.00 respectively, while the ethylacetate fraction has the least concentrations of the elements. Methanolic extracts of *D. cinerea* and *S. setigera* showed the highest antisickling activity. Aqeous fraction of *S. setigera* showed the highest methaemoglobin reduction property, while methanolic extract showed the best methaemoglobin reduction effect. Membrane protection activity was best in butanol fraction of *D. cinerea*, but methanolic extract of *S. setigera* had the highest membrane protection activity. The aqueous fraction of *D. cinerea* had the highest mineral concentration, while butanolic fraction of *S. setigera* had highest concentration of minerals. .

Table of Contents

Title Page .....**Error! Bookmark not defined.**
Declaration iii
Certification iv
Dedication v
Acknowledgements vi
Abstract vii
List of Tables xiii
List of Figures xvi
List of Plates xvii
Appendices xviii

1.0 INTRODUCTION 1
1.1 Overview 1
1.2 Statement of the Research Problem. 3
1.3 Justification for the Research 3
1.4 Hypothesis 4
1.4.1 Null hypothesis 4
1.4.2 Alternate hypothesis 4
1.5 Aim 4

2.0 LITERATURE REVIEW 6
2.1 History of Sickle cell Anaemia 6
2.1.1 Concept of Balanced Polymorphism 8
2.1.2 Distribution of Sickle Cell Anaemia: 10
2.1.3 Pathophysiology of Sickle Cell Anaemia 13
2.1.3.1 Haemoglobin Polymerization. 13
2.1.3.2 Erythrocyte Dehydration 15
2.3.1.3 Increased adhesion of sickle red blood cells to the endothelium 18
2.1.3.4 Painful Crises*:* 21
2.1.3.5 Leg Ulcers. 21

2.1.3.6 *Pulmonary Involvement.* 22
2.1.3.6 Priapism 23
2.1.3.8 *Pregnancy in sickle Cell Anaemia:* 24
2.1.3.9 Chronic End Organ Damage: 25
2.1.4 Oxidative Stress in Sickle Cell Disease 25
2.1.4.1 *Xanthine Oxidase* 26
2.1.4.2 NADPH Oxidase 27
2.1.4.3 Consequences of Oxidative Stress in SCD 27
2.1.4.3.1 Haemolysis: 27
2.1.4.3.2 Red Clood Cell adhesion: 28
2.1.4.3.3 Leucocyte Adhesion: 28
2.1.4.3.4 Platelet adhesion 29
2.1.5 Orthodox Line of Treatment of Sickle Cell Anaemia 29
2.1.5.1 Hydroxyurea 30
2.1.5.2 Bone Marrow Transplantation 30
2.1.5.3 Blood Transfusion: 31
2.1.5.4 Trace element and Sickle Cell Anaemia 32
2.1.6 Potentials of Plants in Sickle cell Anaemia 33

2.2 *Sterculia setigera* 37
2.2.1 Geographical Distribution: 37
2.2.2 Flowering and Fruiting Habit 37
2.2.3 Uses of *Sterculia setigera*: 38
2.2.4 Medicinal Uses of *Sterculia setigera*: 38
2.2.5 Anti-microbial Activity of *Sterculia setigera:* 39

2.3 *Dichrostachys cinerea*: 42
2.3.1 Geographical distribution: 44
2.3.2 Flowering and fruiting habit: 44
2.3.3 Uses of *Dichrostachys cinerea*: 44
2.3.4 Medicinal uses of *Dichrostachys cinerea*: 45

3.0 MATERIALS AND METHODS 47
3.1 Chemicals 47
3.2 Equipment 47
3.3 Plant Sample Collection 47
3.4 Blood Sample 48
3.5 Ethical Approval 48

3.6 Methodology 50
3.6.1 Exraction of the Plant Material 50
3.6.2 Preparation of the Blood Sample 50
3.6.3 Determination of Anti sickling Properties of *Dichrostachys cinerea* and *Sterculia setigera* extracts and Fractions 50
3.6.3.1 Procedure 50
3.6.4 Phytochemical Analysis of *Dichrostachys cinerea* and *Sterculia setigera* methanolic Extracts and fractions. 51
3.6.4.2 Test for Tannins 51
3.6.4.3 Test for Saponins 52
3.6.4.4 Test for Triterpenes and Steroides 52
3.6.4.5 Test for Flavonoids 52
3.6.4.6 Test for Alkaloids 53
3.6.4.7 Test for Cardiac Glycosides 53

3.7 Determination of Methaemoglobin Concentration Reduction Effect of *Dichrostachys cinerea* And *Sterculia setigera* Extracts and Fractions. 53
3.7.1 Procedure: 54

3.8 Bioassay Activity Guided Fractionation 54
3.8.1 Thin Layer Chromatography (TLC) 54
3.8.2 Fractionation 55
3.8.3 Procedure: 55

3.9 Membrane Stability Test 58
3.9.1 Preparation of extract solutions 58
3.9.2 Procedure 58

3.10 Mineral Analysis 59
3.10.1 Principle of Atomic Absorption Spectrophotometry 59
3.10.2 Digestion of Samples 60

3.11 Statistical Analysis 61

4.0 RESULTS 62
4.1 Antisickling Activity Assay of *Dichrostachys cinerea* and *Sterculia setigera* methanolic extracts and fractions: 62
4.2 Phytochemical Screening of *Dichrostachys cinerea* and *Sterculia setigera* Methanolic Extracts and Fractions. 78
4.3 Methaemoglobin concentration analysis: 81
4.4 Membrane Stability Test Result: 85
4.5 Mineral Analysis: 99

5.0 Discussion 102

6.0 Conclusion and Recommendation 110
6.1 Conclusion 110
6.2 Recommendation 111
REFERENCE 112
APPENDICES 125

LIST OF TABLES

Table **Title** **Page**

4.1.1: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with the Methanolic Extract of the Root Bark of *Dichrostachys cinerea* at Various Concentrations and Time Intervals……..64

4.1.2: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with the n-hexane Fraction of Methanolic Extract of the Root Bark *Dichrostachys cinerea* at Various Concentrations and Time-Intervals…….....… 65

4.1.3: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with an Ethylacetate Fraction of the Methanolic Extract of the Root Bark of *Dichrostachys cinerea* at Various Concentrations and Time-Intervals…….....….. 66

4.1.4: The percentage of Human Sickled Red Blood Cells Reversed Following Treatment with a Butanol Fraction of the Methanolic Extract of the Root Bark of *Dichrostachys cinerea* at various concentrations and Time-Intervals…….....… 68

4.1.5: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with an Aqueous fraction of the Methanolic Extract of the Root Bark of *Dichrostachys cinerea* at Various Concentrations and Time-Intervals…….....……..69

4.1.6: The Percentage of Human Sickled Red Blood cells Reversed Following Treatment with the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals……...…….. 71

4.1.7: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with an n-hexane Fraction of the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals…….....….72

4.1.8: The Percentage of Human Sickled Red Blood Cells Reversed following Treatment with an Ethylacetate Fraction of the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals…….....…. 75

4.1.9: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with a Butanol Fraction of the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals…….....……..76

4.1.10: The Percentag of Human Sickled Red Blood cells Reversed Following Treatment with an Aqueous Fraction of the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals…….....…..77

4.2.1: Phytochemical Screening of a Crude Methanolic Extract of the Root Bark of *Dichrostachys cinerea* and of its Various Derived Solvent Fractions…….....…….. 79

4. 1.2: Phytochemical Screening of a Crude Methanolic Extract of the Leaf of *Sterculia setigera* and of its Various Derived Solvent Fractions…….....…….. 80

4.2.1: The Effect of a Methanolic Extract of the Root Bark of *Dichrostachys cinerea* and of its Various Derived Solvent Fractions on Percentage Methaemoglobin, at Different Concentrations. …….....……83

4.3.2: The Effect of a Methanolic Extract of The Leaf of *Sterculia setigera* and of its Various Derived Solvent Fractions on Percentage Methaemoglobin, at Different Concentrations….....…. 84

4.4.1: Effect of The Methanolic Extract Derative of the Root Bark of *Dichrostachys cinerea* on Membrane Stability of Human Erythrocytes…….....… 86

4.4.2: Effect of the n-hexane Fraction Derivative of the Root Bark Methanolic Extract of *Dichrostachys cinerea* on Membrane Stability of Human Erythrocytes.....…87

4.4.3: Effect of the Ethylacetate Fraction Derivative of the Root Bark Methanolic Extract of *Dichrostachys cinerea* on Membrane Stability of Human Erythrocytes…….....….. 89

4.4.5: Effect of the Butanolic Fraction Derivative of the Root Bark Methanolic Extract of *Dichrostachys cinerea* on Membrane Stability

of Human Erythrocytes…….....…….90

4.4.6: Effect of the Aqueous Fraction Derivatives of the Root Bark Methanolic Extract of *Dichrostachys cinerea* on Membrane Stability of Human Erythrocytes…….....……91

4.4.6: Effect of The Methanolic Extract of The Leaf of *Sterculia setigera* on Membrane Stability of Human Erythrocytes…….....…. 93

4.4.7: Effect of the n-hexane Fraction Derivative of the Leaf Methanolic Extract of *Sterculia setigera* on Membrane Stability of Human Erythrocytes……..……94

4.4.8: Effect of the Ethylacetate Fraction Derivative of the Leaf Methanolic Extract of *Sterculia setigera* on Membrane Stability of Human Erythrocytes…….....… 96

4.4.9: Effect of the Butanolic Fraction Derivative of the Leaf Methanolic Extract of *Sterculia setigera* on Membrane Stability…….....…….97

4.4.10: Effect of the Aqueous Fraction Derivative of the Leaf Methanolic Extract of *Sterculia setigera* on Membrane Stability of Human Erythrocytes…….98

4.5.1: The Concentration of Minerals in Various Solvent Fractions of Methanolic Extract of The Root Bark of *Dichrostachys cinerea*…….… 100

4.5.2: The Concentration of Minerals in Various Solvent Fractions of Leaves Methanolic Extract of *Sterculia setigera*……101

LIST OF FIGURES

Figure	Title	Page
1:	Global Distribution of Sickle Cell Anaemia…….....…..12	
2:	The polymerization of deoxy-HbS…….....…..14	
3:	Membrane alterations in the sickle red blood cell…….....….17	
4:	Adhesion of sickle red blood cells to the endothelium and cell activation.....20	
5:	Flow sheet of experimental design for extraction, fractionation, and Antisickling assessment of *Dichrostachys cinerea* and *Sterculia setigera*…….....…49	
6:	Diagramatic Presentation of Fractionation procedure for *Dichrostachys cinerea* and *Sterculia setigera*……..…57	

List of Plates

Plate	Title	Page
1.1:	*Sterculia setigera* in its natural habitat…….....	41
1.2:	*Sterculia setigera* leaves in its natural habitat…….....…	41
2.1:	*Dichrostachys cinerea* in its natural habitat…….....…..	43
2.2:	*Dichrostachys cinerea* and its fruits…….....….	43

Appendices

Appendix **Title** **Page**

I: The percentage mehaemoglobin of various fractions of the root bark of *Dichrostachys cinerea*……125

II: The percentage mehaemoglobin of various fractions of the leaf of *Sterculia setigera*……126

III: The effect of aqueous, ethylacetate, n-hexane, and butanol fraction of the leaf of *Sterculia setigera* on membrane stability ……127

IV: The effect of aqueous, ethylacetate, n-hexane, and butanol fraction of the root bark of *Dichrostachys cinerea* on membrane stability……128

V: TLC plates of 6:4 N-Hexane: Ethylacetate and 8:2 N-Hexane: Ethylacetate

VI: Percentage yields of various fractions of the root bark of *Dichrostachys cinerea*……129.

VII: Percentage yields of various fractions of the leaf of *Sterculia setigera*……130

VIII: Morphology of depranocytes treated with 5mg/ml PABA and Normal saline……132

IX: Morphology of depranocytes treated with 0.3mg/ml Ethylacetate fraction of *Dichrostachys cinerea* and Butanol fraction of *Sterculia setigera* leaf ……133

X: Informed consent form……134

XI: Preparation of stock solution of buffered sodium chloride……138

XII: Preparation of reagents……139

XIII: Ethical clearance certificate……140

CHAPTER ONE

1.0 Introduction

1.1 Overview

From time immemorial, humans have always relied on plants that can meet their basic necessities; such as food, shelter, fuel and health. Of all the numerous uses attached to plants, their therapeutic abilities played an inevitable part in the lives of primitive societies, as they relied on plants for healing ailments. The knowledge of the healing powers of plants was initially passed down orally through generations, and as civilizations grew written records were prepared for the benefit of the population (Malla and Chhetri, 2009). A wide majority of herbal plants possess pharmacologically active compounds that could have potential therapeutic activities against various human, animal and even plant diseases, in addition to their food and nutritional values (Ogbonnia, *et al.,* 2008). World Health Organization reports that 70% – 80% of the world population relies on traditional medicine for primary health care. In recent years, research has been focused to find novel compounds from plant, animal and microbial sources. A review by Elujoba *et al.,* (2005) reiterated that the use of traditional medicine cannot fade out in the treatment and management of an array of diseases in the African continent. In Nigeria, traditional medicine accounts for more than 80% health needs of the rural population. The medicaments are prepared most often from a combination of two or more plant products which often contain active constituents with multiple physiological activities and could be used in treating various disease conditions (Pieme *et al*., 2006).

Sickle cell disorders are a group of related inherited (genetic) disorders of the haemoglobin molecule and they constitute the dominant genetic disorder worldwide (WHO 2006). Sickle cell

disease results from any combination of the sickle cell gene with any other abnormal β-globin gene and hence there are many types of Sickle cell diseases. The most common types include sickle cell anaemia (Hb SS), the sickle beta-thalassemias (Hb Sβº and Hb Sβ+), and haemoglobin SC disease (Hb SC). Sickle cell anaemia (SCA) is a genetic disease resulting from a missense and non-conservative mutation in globin gene chain on chromosome 11 that leads to substitution of a hydrophilic glutamic acid by a hydrophobic valine at the sixth position of the β-chain of haemoglobin, leading to the altering of the expression of the genetic code (Glitz, 2006). This change leads to the production of abnormal haemoglobin (haemoglobin S) which under the deoxygenated state polymerizes, causing the formation of rigid and sickled erythrocytes. The deformity of the sickled erythrocyte results in it's shortened survival since it aggregates to form rod-like polymers which become vulnerable to lysis, hence leading to anaemia with haemoglobin values ranging from 6 to 10 g/L.(Martins, 1981; Karayakin, 1979).

Sickle cell anaemia is associated with high morbidity and mortality among sickle cell sufferers in developing countries (Ogamdi and Onwe, 2000). Sickle cell anaemia (SS) is an inherited disease whose origin and demographic roots may be traced to malaria endemic areas; this includes people whose ancestors come from sub-Saharan Africa, India, Saudi Arabia and Mediterranean countries. In some areas of sub-Saharan Africa, up to 2% of all children are born with the condition. In broad terms, the prevalence of the sickle cell trait (healthy carriers who have inherited the mutant gene from only one parent) ranges between 10% and 40% across equatorial Africa and decreases to between 1% and 2% on the North African coast and <1% in South Africa. Frequencies of the carrier state determine the prevalence of sickle cell anaemia at birth. This distribution reflects the fact that the sickle cell trait confers a survival advantage against

malaria and that selection pressure due to malaria has resulted in high frequencies of the mutant gene especially in areas of high malarial transmission. For example, in Nigeria, which is by far the most populous country in the sub-saharan region, 24% of the population are carriers of the mutant gene and the prevalence of sickle cell anaemia is about 20 per 1000 births. This means that, about 150 000 children are born annually with sickle cell anaemia in Nigeria(WHO, 2006).

1.2 Statement of the Research Problem.

One hundred years after its discovery as a genetically inherited disease (Hyacinth and Hibbert, 2010), finding a widely available remedy for sickle cell anaemia (HbSS) still remains a challenge. Gene therapy and bone marrow transplantation are not only expensive, but they also face incompactibility problems. The proposed compounds such as hydroxyurea that are intended to inhibit the polymerization of haemoglobin S by increasing the concentration of haemoglobin F are reported to be toxic, especially when used for a long time (Mehanna, 2001; Iyamu *et al.*, 2002; Buchanan *et al*., 2004). Presently, the healthcare cost in the management of patients with sickle cell disease is disproportionately high compared with the number of people afflicted by the disease. The group most affected by this high cost is the common people in our localities who belong to the low socio-economic class. Therefore, there is a need for the identification of an indigenous plant with antisickling activity that will provide an affordable medication that can improve the life of sickle cell patients (Samir, 2009).

1.3 Justification for the Research

Considering all genetic disorders to which man is exposed, there is probably no other disease that presents a collection of problems and challenges that is comparable to Sickle Cell Disease. The health care cost of the management of SCD patients is disproportionately high compared to the number of people afflicted by the disease. The common people living in the villages are mostly

peasant farmers who cannot afford the high cost of treatment by Orthodox medicine (Samir, 2009). Due to the debilitating effect and cost of managing the SCD, researchers have been encouraged to engage in studies targeted at determining the efficacy of the use of medicinal plants to tackle the multiple challenges presented in sickle cell disease (Manouk, 2012). The identification of an indigenous plant that has antisickling activity will provide an affordable medication that can improve the life sickle cell patients in Nigeria. This study on the methanolic extracts of *Dichrostachys cinerea* and *Sterculia setigera* will verify scientifically the claim of usefulness of these plants by the traditional medicine in sickle cell patients.

1.4 Hypothesis

1.4.1 Null hypothesis

The partially purified fractions of *Dichrostachys cinerea* root and *Sterculia setigera* leaf do not have effect on human sickle cells.

1.4.2 Alternate hypothesis

The partially purified fractions of *Dichrostachys cinerea* root and *Sterculia setigera* leaf have effect on human sickle cells.

1.5 Aim

To investigate the anti sickling properties of *Dichrostachys cinerea* root and *Sterculia setigera* leaf extracts, *in vitro.*

Specific Objectives

i. To assess the antisickling properties of methanolic extracts of various parts of *Dichrostachys cinerea* and *Sterculia setigera*.

ii. To fractionate the most active part of methanolic extracts of *Dichrostachys cinerea* and *Sterculia setigera* using n-hexane, ethylacetate, butanol.

iii. To determine the antisickling effect of fractions of methanolic extract of *Dichrostachys cinerea* and *Sterculia setigera* affect.

iv. To establish the phytochemical constituents of both methanolic extracts and methanolic extract fractions of the extracts.

v. To assess how methanolic extracts and fractions of *Dichrostachys cinerea* and *Sterculia setigera* affect erythrocyte membrane stability and haemoglobin oxidation.

CHAPTER TWO

2.0 Literature Review

2.1 History of Sickle cell Anaemia

The first case of Sickle Cell Disease (SCD) in the literature was described in a dental student studying in Chicago between 1904 and 1907 (Serjeant, 2001). Coming from the North of the island of Grenada in the eastern Caribbean, he was first admitted to the Presbyterian Hospital, Chicago, in December 1904, and a blood film showed the features characteristic of homozygous sickle cell (SS) disease. It was James Herrick, a Chicago physician who discovered sickle-shaped erythrocytes while examining the student's blood film. The student had been hospitallized for cough and fever. The patient also felt weak, dizzy, and had headache. He had been experiencing palpitation and shortness of breath over a long period of time. Physical examination showed that the patient was physically fit though with yellowish eyes and pale mucus membrane. Blood examination showed that the patient was anaemic. His blood smear contained unusual red cells, which are irregular and large number of thin elongated sickle-shaped forms. The treatment he received was encouraging, consisting of bed rest and nourishing food. One month later, the patient left hospital, less anaemic and feeling much better. However his red blood cells still exhibited a 'tendency' to the peculier crecent-shape. Herrik was confused by the clinical manifestation and laboratory result.

Herrick waited for six years before publishing the case history and then asserted that ' not even a definite diagnosis can be made'. He noted the chronic nature of the disease and the diversity of abnormal physical and laboratory findings; cardiac enlargement, jaundice, anaemia, and

evidence of kidney damage. He concluded that, the disease could not be explained on the basis of an organic lesion in any one organ. He pointed out the abnormal blood picture as the key finding and titled his case report perculiar elongated and sickle-shaped red blood corpsucles in acase of severe anaemia (Serjeant, 2010). The second case, Ellen Anthony, aged 25 years, had already been under observation in the wards of the University of Virginia Hospital from 1907 and the strange blood film sent to pathologists at Johns Hopkins University Hospital was considered an unusual case of pernicious anaemia (Serjeant, 2001). The diagnosis became clear with the publication of Herrick's paper in November, 1910 (Herrick, 1910) and, within 3 months, this second case was reported in February 1911 (Serjeant, 2010). The third case, a woman aged 21 years, reported from Washington University Medical School in 1915, raised suspicions of a genetic basis, as three siblings had died from severe anaemia, and blood from both the patient and her asymptomatic father showed a sickling deformity of the red cells on incubation. The fourth case was a 21-year-old black man in the wards of Johns Hopkins Hospital. It was Mason who noted the similar features of the first four case reports, He was the first to use the term `sickle cell anaemia' and, finding that the cases were all in blacks, he began the popular misconception that the disease was confined to people of African origin (Serjeant, 2001).

The contributions of several workers on the determinants of sickling (Pratima *et al*, 2013), birefringence of deoxygenated sickled cells and the lesser degree of sickling in very young children which implied that it was a feature of adult haemoglobin (Henry, 2002), led Linus Pauling in 1949 to perform Tiselius moving boundary electrophoresis on haemoglobin solutions from subjects with sickle cell anaemia and the sickle cell trait. The demonstration of electrophoretic and, hence, implied chemical differences between normal, sickle cell trait and sickle cell disease led to the proposal that it was a molecular disease (Bruno, 2002). The nature

of this difference was soon elucidated. The haem groups appeared identical, suggesting that the difference resided in the globin, but early chemical analyses revealed no distinctive differences (Beaven and Gratzer, 2015). Analyses of terminal amino acids also failed to reveal differences, although an excess of valine in HbS was noted but considered an experimental error (Havinga, 1953). The development of more sensitive methods of fingerprinting combining high voltage electrophoresis and chromatography allowed the identification of the essential difference between HbA and HbS. This method enabled the separation of constituent peptides and demonstrated that a peptide in HbS was more positively charged than in HbA (Odievre *et al.*, 2011). This peptide was found to contain less glutamic acid and more valine, suggesting that valine had replaced glutamic acid (Bruno, 2006). The sequence of this peptide was shown to be Val-His-Leu-Thr- Pro-Val-Glu-Lys in HbS instead of the Val-His-Leu-Thr-Pro- Glu-Glu-Lys in HbA, a sequence which was subsequently identified as the amino-terminus of the b chain (Eric *et al.,* 2015). This amino acid substitution was consistent with the genetic code and was subsequently found to be attributable to the nucleotide change from GAG to GTG (Serjeant, 2001), thus declaring it a molecular disease.

2.1.1 Concept of Balanced Polymorphism

The maintenance of high frequencies of the sickle cell trait in the presence of almost obligatory losses of homozygotes in Equatorial Africa implied that there was either a very high frequency of HbS arising by fresh mutations or that the sickle cell trait conveyed a survival advantage in the African environment. There followed a remarkable period in the 1950s when three prominent scientists were each addressing this problem in East Africa, Dr Alan Raper and Dr Hermann Lehmann in Uganda and Dr Anthony Allison in Kenya. It was quickly calculated that mutation rates were far too low to balance the loss of HbS genes from deaths of homozygotes (Serjeant,

2001). An increased fertility of heterozygotes was proposed (Thomas and David, 2012) but never convincingly demonstrated. Raper (1949) was the first to suggest that the sickle cell trait might have a survival advantage against some adverse condition in the tropics and Mackey & Vivarelli (1952) suggested that this factor might be malaria. The close geographical association between the distribution of malaria and the sickle cell gene supported this concept (Thomas and David, 2012) and led to an exciting period in the history of research in sickle cell disease. The first observations on malaria and the sickle cell trait were from Northern Rhodesia where Beet (1946, 1947) noted that malarial parasites were less frequent in blood films from subjects with the sickle cell trait. Allison (1954) drew attention to this association, concluding that persons with the sickle cell trait developed malaria less frequently and less severely than those without the trait. This communication marked the beginning of a considerable controversy. Two studies failed to document differences in parasite densities between `sicklers' and `non-sicklers' (Friedman *et al*., 2003; Bouchaud *et al*, 2005) and Beutler *et al* (1955) were unable to reproduce the inoculation experiments of Allison (1954). Raper (1955) speculated that some feature of Allison's observations had accentuated a difference of lesser magnitude and postulated that the sickle cell trait might inhibit the establishment of malaria in non-immune subjects. The conflicting results in these and other studies appear to have occurred because the protective effect of the sickle cell trait was overshadowed by the role of acquired immunity. Examination of young children before the development of acquired immunity confirmed both lower parasite rates and densities in children with the sickle cell trait (Frédéric *et al*., 2010; Bolarinwa *et al*., 2010;Thomas and David, 2012) and it is now generally accepted that the sickle cell trait confers some protection against falciparum malaria during a critical period of early childhood between the loss of passively acquired immunity and the development of active immunity (Serjeant, 2001; Tej and

Rajkumari, 2010). The mechanism of such an effect is still debated, although possible factors include selective sickling of parasitised red cells (Atif *et al.,* 2013) resulting in their more effective removal by the reticulo-endothelial system, inhibition of parasite growth by the greater potassium loss and low pH of sickled red cells (Chikezie, 2009), and greater endothelial adherence of parasitized red cells (Dhananjay, 2001).

2.1.2 Distribution of Sickle Cell Anaemia:

Although sickle cell anaemia is a monogenic disease, its clinical picture is highly heterogeneous with some patients having a mild phenotype and others being severely affected (Ballas, 1991). Many of the factors described as contributing to such heterogeneity are genetically determined (βS haplotypes, concomitant α-thalassemia, fetal haemoglobin levels) (Steinberg, 2005). The βS haplotypes are defined by the nonrandom association of restriction endonuclease cleavage sites around the beta-globin gene cluster. The occurrence of the sickle cell mutation and the survival advantage conferred by malaria together determine the primary distribution of the sickle cell gene. Equatorial Africa is highly malarial and the sickle cell mutation appears to have arisen independently on at least three and probably four separate occasions in the African continent, and the mutations were subsequently named after the areas where they were first described and designated the Senegal, Benin, Bantu and Cameroon haplotypes of the disease (Julio *et al.,* 2006; Graham, 2013; Robert *et al.,* 2007). The disease seen in North and South America, the Caribbean and the UK is predominantly of African origin and mostly of the Benin haplotype, although the Bantu is proportionately more frequent in Brazil (Katsue *et al.,* 2011). It is therefore easy to understand the common misconception held in these areas that the disease is of African origin. However, the sickle cell gene is widespread around the Mediterranean, occurring in Sicily, Southern Italy, Northern Greece and the South Coast of Turkey, although these are all of the

Benin haplotype and so, ultimately, of African origin. In the Eastern province of Saudi Arabia and in central India, there is a separate independent occurrence of the HbS gene, the Asian haplotype. The Shiite population of the Eastern Province traditionally marry first cousins, tending to increase the prevalence of SS disease above that expected from the gene frequency (Elvira *et al*., 2015). Furthermore, extensive surveys performed by the Anthropological Survey of India estimate an average sickle cell trait frequency of 15% across the states of Orissa, Madhya Pradesh and Masharastra which, with the estimated population of 300 million people, implies that there may be more cases of sickle cell disease born in India than in Africa. The Asian haplotype of sickle cell disease is generally associated with very high frequencies of alpha thalassaemia and high levels of fetal haemoglobin, both factors believed to ameliorate the severity of the disease (Bortolini and Salzano, 1999).

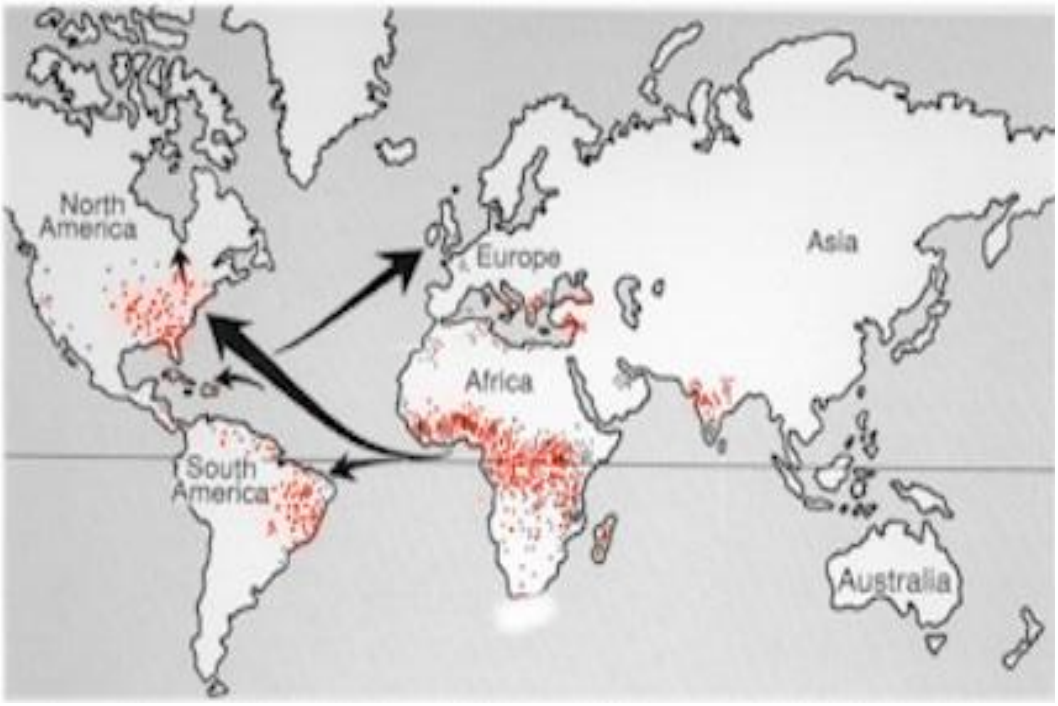


Figure 1: Global distribution of sickle cell anaemia (Vedro and Morrisson, 2002).

2.1.3 Pathophysiology of Sickle Cell Anaemia

2.1.3.1 Haemoglobin polymerization

During the deoxygenation which follows the passage of RBCs in the microcirculation the Hb molecule undergoes a conformational change. In HbS, replacement of the hydrophilic glutamic acid at position 6 in the β-globin chain by the hydrophobic valine residue makes that this last one establishes hydrophobic interactions with other hydrophobic residues on the β-globin chain of another deoxy-HbS molecule.

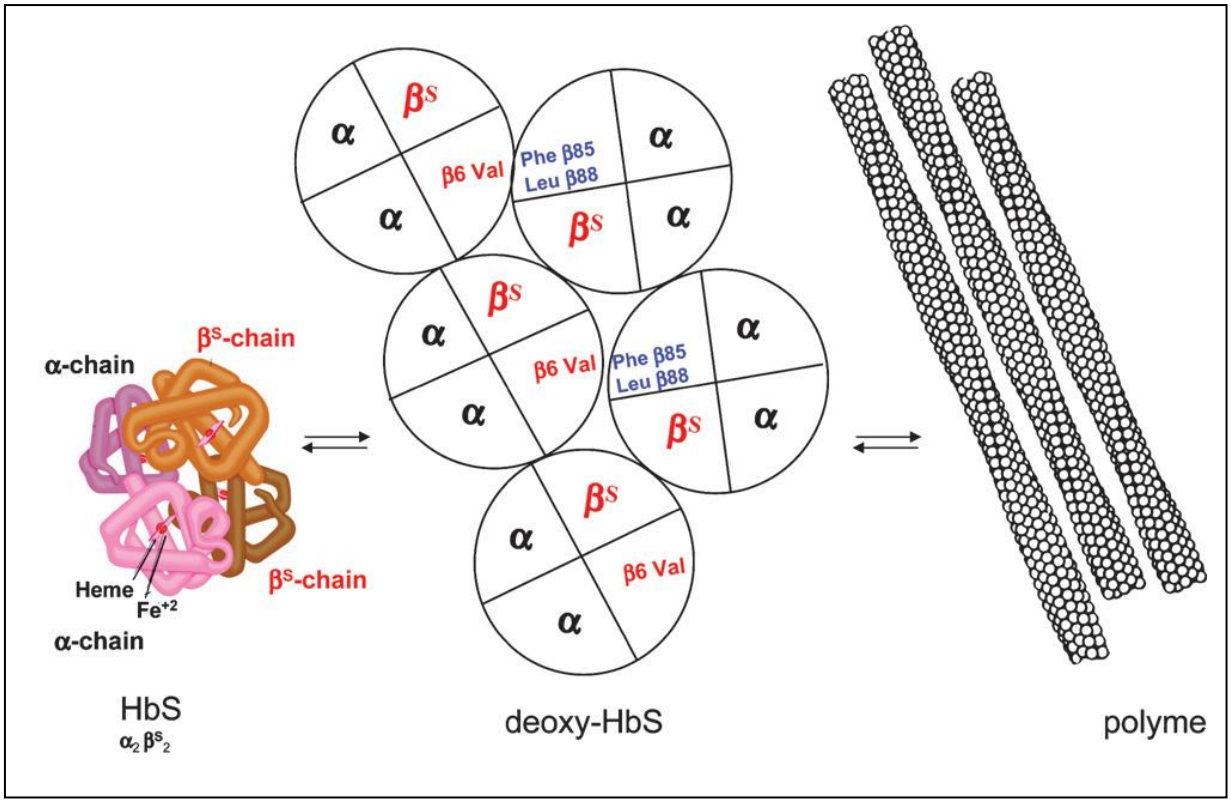


Figure 2: The polymerization of deoxy-HbS (Odievre *et al.*, 2011**)**. The replacement of a glutamic acid by a valine residue at position 6 in the β-globin polypeptide chain characterizes the abnormal haemoglobin of SCD: HbS. At low oxygen pressure, deoxy-HbS polymerises and gets organised in long polymer fibres that deform, stiffen, and weaken the red blood cell. This process represents the basic mechanisms leading to haemolytic anaemia and to vaso-occlusive events in the microcirculation.

A polymer forms and lengthens in helical fibres which, grouped together, stiffen, and induce the characteristic SS-RBC shape change, classically in the shape of a sickle. This process needs a certain time to be primed, the so-called "delay time", which is inversely proportional to the intracellular concentration of HbS (Bernard and Franklin, 2013).

2.1.3.2 *Erythrocyte Dehydration*

Patients with sickle Cell disease have higher proportion of dense dehydrated erythrocytes than normal individuals (Negel and Plat, 2001). This dense cell fraction includes irreversible sickle cells (ISCs) and cells with more extensive membrane damage and thus plays important role in vasoocclusion.The formation of long polymer fibres triggers a cascade of several other cellular abnormalities which participate in the overall pathophysiological mechanism (Fig.2). Dysregulation of cation homeostasis resulting from the activation of some ion channels, such as the K-Cl co-transport system and the Ca-dependent K-channel (Gardos channel) in particular, leads to a loss of potassium and cellular dehydration which, in turn, by increasing the intracellular Hb concentration, favours deoxy-HbS polymerization. Hb becomes denatured and hemichromes concentrate at the internal side of the membrane together with proteins of the cytoskeleton, in particular protein band 3. This process comes along with the loss of heme and with the liberation of Fe3+ which promotes the existence of an oxidizing microenvironment. The normal asymmetry of membrane phospholipids is disrupted with the exposure of anionic phosphatidylserine at the cell surface. Anti-band 3 IgGs accumulate on the protein band 3 aggregates, inducing erythrophagocytosis by macrophages (Stuart and Nagel 2004). Finally, all these membrane changes give rise to the production of microparticles. Stiffening and fragility of SS-RBC explain vaso-occlusion and haemolytic anaemia, respectively. However, if these mechanisms indeed constitute the pathophysiological bases of SCD, they do not explain what

triggers VOCs. In basal conditions, the delay time, necessary for the polymerization of deoxy-HbS is longer than the time of passage of RBCs in the microcirculation.

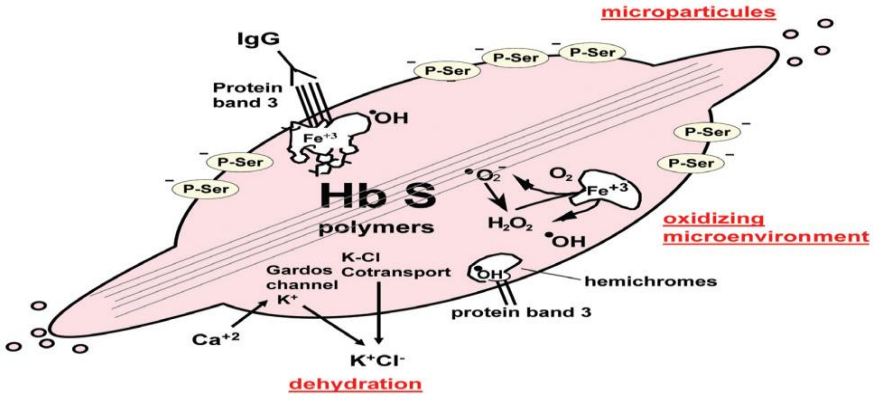


Figure 3: Membrane alterations in the sickle red blood cell (Odievre *et al.*, 2011**)**. Formation of the deoxy-HbS polymer fibres triggers a whole series of changes of the red blood cell membrane. Ion channels are affected and their dysfunction is responsible for a cellular dehydration which, in a vicious circle, favours deoxy-HbS polymerization. Hemichromes are released and lead, in particular, to the formation of protein band 3 aggregates on which anti-band 3 IgGs accumulate. The liberation of heme and Fe3+ favours an oxidizing microenvironment. Exposure of anionic phosphatidylserines at the external side of the membrane creates a procoagulant surface. Finally, microparticles are released**.**

2.3.1.3 *Increased Adhesion of Sickle Red Blood Cells to the Endothelium*

In the eighties and nineties the teams of Hebbel and of Mohandas showed the existence an increased adhesion of the SS-RBCs to the endothelium (Tokumasu and Dvorak, 2003). Unexpectedly, it turned out that rather than the distorted RBCs, the main actors of this abnormal adhesion process are a population of young RBCs, referred to as "stress reticulocytes". These stress reticulocytes, coming out prematurely from the bone marrow because of the anaemic stress, express on their surface adhesion proteins that do normally maintain them in the marrow. Thus VOC seems to be composed of two consecutive steps. The first one involves adhesion of the stress reticulocytes to the endothelium of post-capillary veinules, slowing down the blood flow and thereby inducing and propagating sickling of mature SS-RBCs that are maintained for a longer time in a hypoxic environment. This first step leads to a second one which corresponds to the entrapment of irreversible sickle cells and to the complete occlusion of the micro-vessels (Sergeant, 2001).

The first molecular partners identified as actors of these abnormal interactions on RBCs were the α4β1 integrin or very late antigen-4 (VLA-4) which directly binds to the vascular cell adhesion molecule (VCAM-1) on the endothelial surface, and CD36 which interacts with another CD36 molecule on the endothelium through a molecular bridge formed by a molecule of plasmatic thrombospondin (TSP) (Fig.3). Since then, the picture considerably complexified, with the identification of numerous other receptor/ligand couples on the RBCs, on one side, and on the endothelium on the other, of the involvement of various plasma proteins in addition to TSP, and with the description of an intricate network of probably co-operative and sometimes redundant interactions (Kenneth *et al.,* 2007). Clearly, the situation varies according to the vascular territories, for example VCAM-1 is specific of the endothelium of the microcirculation whereas

von Willebrandt factor probably mediates abnormal cell-cell interactions in large vessels. The endothelium is altered as witnessed by circulating ECs (Hebbel, 2008) and accordingly subendothelial structures are exposed that are also involved. For instance, the basal cell adhesion molecule (Lutheran blood group) Lu/BCAM antigen at the SS-RBC surface interacts with laminin in the subendothelial matrix9. Even though these advances are remarkable, in particular the discovery of the major implication of stress reticulocytes, the SCD basic mechanism, namely deoxy-HbS polymerization, should never be forgotten. Even though other haemolytic anaemias come along with the presence of circulating stress reticulocytes as is the case, for example, of pyruvate kinase deficiency, none of these come along with VOCs. Thus, it is clear that, even though complex abnormal phenomena are at play, HbS is indeed the basic and the sole defect responsible of the SCD pathology, and in fine of the vaso-occlusive events.

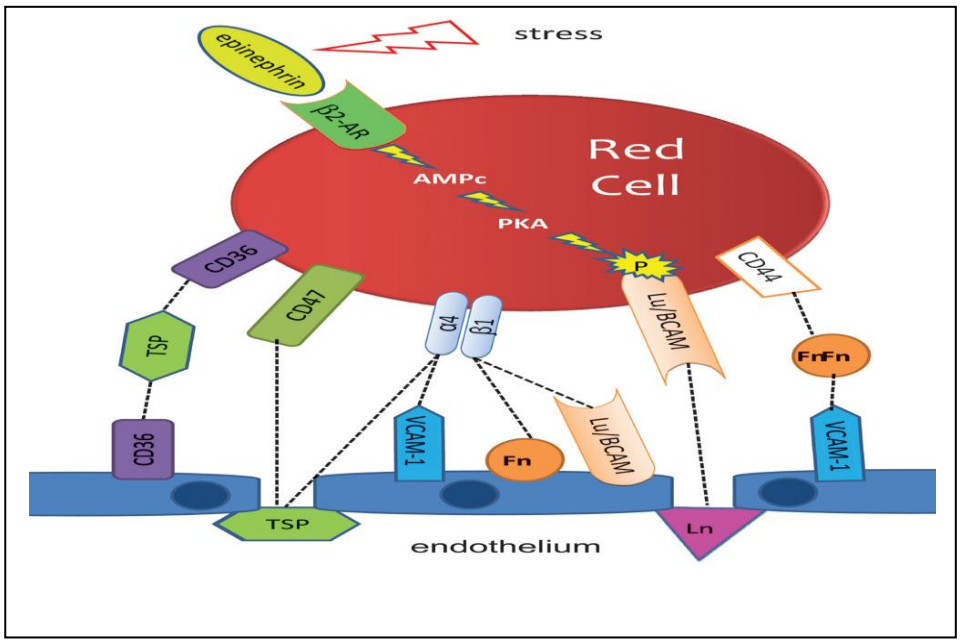


Fig. 4: Adhesion of sickle red blood cells to the endothelium and cell activation (Elion *et al.*, 2010). Simplified scheme of the main interactions involved in the abnormal adhesion of the sickle red blood cells to the endothelium. Locally, endothelial damage exposes sub-endothelial structures that also participate to the adhesion process. Some adhesion proteins are activated by extracellular stimuli. It is the case of the basal cell adhesion molecule (Lutheran blood group) (Lu/BCAM) that expresses its adhesion properties only when phosphorylated via the protein kinase A-dependent (PKA) pathway when the red blood cell is activated by epinephrine. 2-AR, type 2 adrenergic receptor; Fn, fibronectin; TSP, thrombospondin; Ln, laminin; α4β1, α4β1 integrin (or VLA-4), (Elion *et al.*, 2010).

2.1.3.4 *Painful Crises*

The ‘‘painful crisis’’ is currently the most frequent cause of recurrent morbidity in SS disease and accounts for 70±90% of sickle cell-related hospital admissions in the UK and the USA. Considering its high frequency, it is remarkable that systematic studies have only recently detailed risk and precipitating factors (Anyachukwu *et al.*, 2014), clinical features, mechanism (Serjeant, 2001), associated morbidity and prophylaxis (Patrick *et al.*, 2013). Although commonly assumed to be vaso-occlusive in origin (hence the term vaso-occlusive crisis), the frequency of cold as a precipitating factor (Norman *et al.*, 2008), greater prevalence in genotypes with less intravascular sickling (SS disease and homozygous α-thalassaemia and sickle cell-βº thalassaemia), and significantly bilateral and symmetrical distribution are difficult to explain on this basis, leading to the hypothesis that this may represent a steal syndrome (Serjeant, 2001). Many painful crises may be prevented by identifying and avoiding precipitating factors, of which skin cooling is the most frequent in Jamaica. The importance of a high haemoglobin as a risk factor (Anyachukwu *et al.*, 2014) argues for venesection, but currently only anecdotal data are available. Treatment includes rest, reassurance, warmth, rehydration and pain relief. Although most attention has been directed to the pharmacology of pain relief, it is clear that a patient's ability to cope with pain is determined by many factors, of which social and psychological assume particular importance.

2.1.3.5 *Leg Ulcers*

Ulceration around the ankles occurred in all of the first four case reports but, despite a series of cases presented at the Dermatological Societies of Cleveland (Rodrigo *et al.*, 2006; Serjeant, 2001), Bronx, and the Central States , it was not until 1940 that ulceration became recognized as a specific complication of the disease (Serjeant, 2001)). Leg ulcers occur in other haemolytic

syndromes (β thalassaemia, hereditary spherocytosis), suggesting common aetiological factors, although they are almost certainly multifactorial with contributions from venous stasis, local trauma and cutaneous vaso-occlusion producing spontaneous painful deep ulcers suggestive of skin infarction (Serjeant, 2001). The tendency to heal on complete bed rest and deteriorate on prolonged standing are common to venous ulceration. Little progress has been made in the management of this complication which, although rarely causing mortality, is a major contributor to morbidity of the disease especially in areas such as Jamaica, where up to 70% of adult SS patients have been affected (Serjeant, 2001).

2.1.3.6 *Pulmonary Involvement*

This is the major cause of mortality after the age of 2 years, it is surprising that few early papers focused on this area. Pulmonary thrombo-embolism was first reported by Steinberg (1930) and others recorded the increased frequency of thrombi, recanalized thrombi and pulmonary infarcts (Ademola *et al.,* 2012). The pathological processes causing pulmonary pathology include infection, pulmonary infarction, fat embolism (Jo *et al*., 2013) and acute pulmonary sequestration (Serjeant, 2001). Surprisingly, documented infection plays a minor role, bacteria being isolated from 14% of infants and less than 2% of cases aged over 10 years (Fawibe, 2008). Acute pulmonary sequestration may be associated with rapidly deteriorating pulmonary function and a high mortality, which may be reduced by close monitoring using pulse oximetry and emergency exchange transfusion (Serjeant, 2001). Rib or sternal infarction may cause pleuritic pain limiting chest movement and predisposing to secondary pulmonary changes (Jo *et al.,* 2013), the frequency of which may be reduced using incentive spirometry (W.H.O, 2012). This variety of pathological processes and the poor response to therapy that suggested a complex pathology with several components led Charache *et al.,* (1979) to introduce the term `acute chest syndrome' for

all acute pulmonary pathology in sickle cell disease. Recurrent acute chest syndrome may be associated with a progressive deterioration of pulmonary function that contributes significantly to mortality among adults. The frequency and severity of chronic sickle cell lung disease is not widely documented, although the high frequency in Southern California (Roberto and Gladwin, 2005) suggests symptomatic selection or important local factors. Pulmonary hypertension is also an increasingly recognized complication of other chronic hemolytic conditions including thalassemia, paroxysmal nocturnal haemoglobinuria, hereditary spherocytosis and stomatocytosis, and microangiopathic haemolytic anaemias (Aliyu *et al*., 2006). The classic definition of pulmonary hypertension is a mean pulmonary artery pressure (MPAP) > 25 mmHg (or >30 mmHg during exercise) determined by right heart catheterization (Kircher *et al.*, 1990). Retrospective studies performed at a tertiary care sickle cell center in the United States have reported that 20–40% of screened adult SCD patients have moderate to severe pulmonary hypertension with an approximate 20% mortality over a 12-month follow up period. The systolic pulmonary artery pressures can be estimated by echocardiographic measurement of the tricuspid regurgitant jet velocity (V) and estimation of the central venous pressure (CVP).

2.1.3.6 *Priapism*

The first reported case of priapism appears to have been presented at the New York Society for Clinical Psychiatry in 1932 as a `castration fear complex' (Ade and Arthur, 2013), but the association with sickle cell disease was recognized by Diggs & Ching (1934) and has been the subject of several reviews (Serjeant, 2001). However, the high prevalence affecting 40% of post-pubertal males was not appreciated until epidemiological studies in Jamaica that defined two patterns; short lived, nocturnal or stuttering events with normal intervening sexual function, and major attacks lasting more than 24 hours and commonly followed by impotence. The use of

stilboestrol to prevent stuttering attacks and of penile prostheses in the management of impotence followed recognition of the frequency of the problem in Jamaica (Graham, 2013).

2.1.3.8 *Pregnancy in sickle Cell Anaemia*

Early reports stressed the infrequency of pregnancy, the adverse effects of pregnancy on the clinical course of sickle cell disease, and the occurrence of fetal and maternal deaths (Swapnil *et al.,* 2011). In the first major review, Kobak *et al.,* (1941) summarized the outcome in 37 pregnancies among 17 women noting the frequent pre-eclamptic toxaemia, fever, pneumonia, sepsis, high fetal loss and a 33% maternal mortality. Two reports, both in 1949, illustrate the conflicting experience with pregnancy in sickle cell disease. Fouche and Switzer (1949) described pregnancies in six patients from South Carolina, three with toxaemia and four maternal deaths, and argued that the serious outcome justified therapeutic sterilization, whereas Anderson and Busby (1949), reviewing a 20-year experience at Johns Hopkins Hospital, reported 11 deliveries without maternal mortality, concluding that therapeutic abortion and sterilization were rarely indicated. This controversy continues to daunt the clinical practice of obstetrics in mothers with sickle cell disease. As recently as the 1970s, arguments were made in the USA that `the expected rate of reproductive success, when considered in conjunction with the negative attributes concerning motherhood, does not justify a young woman with sickle cell disease being exposed to the risks of pregnancy', advocating primary sterilization, abortion if conception occurs and sterilization for those that have completed pregnancies' (Serjant, 2001). Such recommendations conflict with the improving experience that saw a decline in maternal mortality from 33% between 1924 and 1940 and 11% between 1945 and 1955 to 0% between 1953 and 1972 (Serjeant, 2001). As has often happened with sickle cell disease, published experience is

heavily biased by hospital-dependent severely affected cases, whereas patients with milder clinical courses and repeated uneventful pregnancies may pass unreported.

Controversy has also affected the recommendations for contraception in sickle cell disease, which, although there are almost no published data, leads to patients being refused the most effective contraception, such as the pill, injections of medroxyprogesterone acetate or intrauterine devices, because of theoretical objections on the use of hormonal therapy or risks of intrauterine infection. In addition to being an effective contraceptive, a controlled study of medroxyprogesterone acetate demonstrated beneficial effects on the haematology as well as bone pain (Graham, 2013).

2.1.3.9 Chronic End Organ Damage

The improving survival in SS disease has highlighted the problem of cumulative end organ damage, especially affecting the lungs and kidneys. Recurrent acute chest syndrome may lead to pulmonary fibrosis, pulmonary hypertension and respiratory failure, and chronic renal impairment is an important contributor to death in older patients with SS disease. Glomerular filtration rates, which are supranormal in young children, decline steeply with age resulting in renal impairment that may be underdiagnosed when defined by the range of serum creatinine levels in normal populations. In SS disease, creatinine levels are low and significant renal impairment may be present with creatinine levels above 60 mmol/l. Renal failure is typically clinically silent and may only be manifested by falling haemoglobin levels as a result of low erythropoietin production (Eduard *et al*., 2008) .

2.1.4 Oxidative Stress in SCD

Red blood cells (RBCs) from individuals with sickle cell disease (SCD) are more susceptible to in vivo oxidant damage than are RBC from normal individuals (Serjeant, 2001) RBC oxidant

damage in SCD is due to the inherent instability of haemoglobin **S** (Hb **S) (**Graham, 2013**)** as well as the impaired anti-oxidant defense manifested by the relative decrease in pentose phosphate shunt activity (Obeagu *et al*., 2013). Under normal physiological conditions, anti-oxidant enzymes and oxygen radical scavengers ensure that basal fluxes of ROS do not injure the host organism. Major ROS defence mechanisms include enzymatic (superoxide dismutase (SOD), catalase, glutathione peroxidase) and non-enzymatic systems (reduced glutathione (GSH), ubiquinols, uric acid, vitamins C and E, lipoic acid, selenium, riboflavin, zinc, carotenoids), as well as metal-binding proteins. An oxidatively stressed environment results when the production of ROS overwhelms these endogenous anti-oxidant defence mechanisms and so the membranes is oxidised. Oxidative stress can damage specific molecular targets (lipids, proteins, carbohydrates etc.), resulting in cell dysfunction and/or death. Enzymes that participate in ROS production include xanthine oxidase (XO), NADPH oxidase, nitric oxide synthase (NOS), cytochrome P450, cyclo-oxygenase (COX) and lipoxygenase.

2.1.4.1 *Xanthine Oxidase*

Several lines of evidence support a role for XO in SCD, including increased XO activity in aortic endothelium and attenuation of SCD-associated blood cell–endothelial cell adhesion in the venules of allopurinol-treated SCD mice (Kaul *et al.,* 2004). Although vascular endothelial cells can produce XO and its precursor xanthine dehydrogenase (XD) and hypoxia/re-oxygenation is an established stimulus for XD to XO conversion, it has been suggested that the increased endothelial cell XO activity that accompanies SCD may also reflect the binding of liver-derived XO (Aslan *et al.,* 2001). Forstermann *et al.,* 2006 have proposed that liver I/R resulting from VOC liberates soluble hepatic XO into plasma, where it can bind endothelial cell surfaces in blood vessels of distant organs. The relative importance of soluble circulating XO and

endothelial-associated XO to ROS formation in the microvasculature during SCD remains unclear. Because endothelial cells in some tissues (e.g. brain) exhibit little or no XO activity, whereas other tissues (e.g. intestine) exhibit high endothelial XO activity, the importance of this enzyme as a source of ROS in SCD is likely tissue specific (Wood and Granger, 2007).

2.1.4.2 NADPH Oxidase

Leucocytes, which exist in the blood in higher numbers and produce twice the fluxes of superoxide in SCD, represent potentially important sources of ROS in this disease. The contributions of leucocyte-derived ROS to the haemolysis associated with infections or Vascular Occulative Crises (Ersi *et al*.,2012) and the apparent anti-oxidant effects of hydroxyurea therapy in SCD patients suggests that, like sickle RBC, leucocytes may serve both as targets of (l-selectin activation) and sources of (lipid peroxidation, haemoglobin oxidation) the oxidative environment in SCD. NADPH oxidase, the major superoxide-producing enzyme in leucocytes, is a potentially noteworthy source of ROS in SCD (Benkerrou *et al.,* 2002). Although phagocytic NADPH oxidase has been eliminated as a contributor to the adhesion of sickled RBC in microvessels (Haynes, and Obiako, 2002), recent evidence implicates endothelial cellassociated NADPH oxidase-derived superoxide in the adhesion of leucocytes and platelets in cerebral venules of SCD mice (Wood *et al.*, 2005).

2.1.4.3 Consequences of Oxidative Stress in SCD

2.1.4.3.1 Haemolysis**:**

The enhanced oxidative stress experienced by sickle RBC has both autocrine and paracrine effects that begin with lipid peroxidation mediated senescence and end in lysis of sickle RBC. Sickle cell disease RBC demonstrate increased susceptibility to membrane rigidity and

mechanical instability as a consequence of ROS generation. Attempts at reproducing the sickle RBC phenotype with exogenously supplied superoxide (phenazine methosulphate; PMS) revealed that ROS dose-dependently induce membrane rigidity, reduce RBC elasticity and oxidize membrane-associated heme and thiols (Katherine and Granger, 2007).

2.1.4.3.2 Red Clood Cell Adhesion:

Extracellularly generated superoxide has been shown to promote RBC adhesion and a corresponding auto-oxidation of normal haemoglobin, suggesting that the pro-adhesive phenotype of sickle RBC may be related to oxidative stress. An *in vitro* study noted that adhesion of sickle RBC to cultured human umbilical vein endothelial cells (HUVEC) is linked to increased thiobarbituric acid-reactive substances formation, NF-kB activation and elevated vascular cell adhesion molecule expression in endothelial cells, which were attenuated by treatment with eithersuper oxide dismutase, or catalase (Wood and Granger, 2007).

2.1.4.3.3 Leucocyte Adhesion:

Several groups have reported corresponding increases in superoxide production and numbers of adherent leucocytes in venules of the cremaster and brain of sickled cell mice. Kaul *et al*. noted enhanced leucocyte rolling and adherence in cremasteric venules of unstimulated and hypoxia/re-oxygenation-stimulated sickled cell mice, which were normalized by treatment with sulfasalazine, an NF-kB inhibitor with anti-oxidant properties. A recent study of leucocyte adhesion in the cerebral venules of a chimeric sickled cell mouse, under similar conditions of hypoxia/re-oxygenation stimulation, demonstrated elevated leucocyte adhesion to venular endothelium that was normalized by genetic deficiency of gp91phox (NADPH oxidase) or overexpression of cytosolic sickled cell (SOD1) in vascular endothelium, indicating an important

role for NADPH oxidase-derived superoxide and its products in SCD-associated cerebral vasculopathy (Wood and Granger, 2007).

2.1.4.3.4 Platelet Adhesion:

The recent observation that SCD is associated with adhesion of platelets in the microcirculation is unsurprising given previous reports describing an activated coagulation pathway, increased P-selectin and glycoprotein IIbIIIa expression on circulating platelets, the presence of platelet/RBC aggregates and microthrombi in SCD patients. We recently reported that platelet adhesion in the cerebral microvasculature of SCD is increased four- to fivefold compared with wild-type counterparts and that this exaggerated platelet adhesion in SCD is independent of the platelet phenotype (wild type or sickle), which implicates another cell type in the genesis of the procoagulant phenotype. Furthermore, the adhesion of platelets is normalized in chimeric sickled cell mice with either vascular NADPH oxidase deficiency or genetic overexpression of vascular SOD1 (Wood and Granger, 2007).

2.1.5 Orthodox Line of Treatment of Sickle Cell Anaemia

Until the last decade management was purely preventative or supportive aimed at symptom control which basically is aied at inhibiting sickle cell haemoglobin polymerization, prevention or repair of red cell dehydration and interrupting the interaction of sickle cells with the endothelium(Brugnara and Steinberg, 2002). Apart from stem cell transplant, there is no cure for sickle cell disease. Some of the orthodox modes of treatment include induction of fetal haemoglobin (HbF) using Hydroxyurea (HU), Butyrate or its derivatives, oral administration of Clotrimazole which is a potent Gardos channel inhibitor. The usual target end-point is painful crisis frequency, which has the disadvantage of being multifactorial in origin and subjective in severity. Furthermore, in the established painful crisis, even effective anti-sickling agents such as

oxygen have little effect because the pathology may be irreversible and agents cannot reach the site of pathology. Controlled studies of cyanate showed effective carbamylation of HbS molecules and reduced sickling, but had no effect on pain crisis frequency (Serjeant, 2001) and had potentially serious side-effects.

2.1.5.1 *Hydroxyurea*

Hydroxyurea, an antineoplastic agent was the first approved drug for the causative treatment of sickle cell anaemia (King, 2003). HDU increases fetal haemoglobin which has a higher oxygen carrying capacity and reduces sickling under low oxygen tension, thus improving some aspects of quality of life in patients suffering from moderate to severe SCD (Serjeant, 2001). One potential mechanism by which hydroxyurea induces HbF has been hypothesized to involve the redox inactivation of a tyrosyl radical on ribonucleotide reductase (Darbari *et al*., 2006), an effect that can be mediated by NO and nitrovasodilators (Godwin *et al*., 2010). Hydroxyurea is non-selective, and treatment with HDU is suspected to be associated with side effects including cytotoxicity (or toxicity to cells) and myelosuppression (or reduced production of red blood cells, white blood cells, and platelets), and hydroxyurea can damage DNA ("genotoxic") (Perreault *et al.*, 2008, Friedrisch *et al.*, 2008). The biochemical mechanism by which HDU causes cytotoxicity is currently unclear. It has however, been postulated that HDU –induced organ toxicity could be due to its metabolites particularly Carbamoyl nitroso. Carbamoyl nitroso is easily oxidized to form nitroxyl and nitric oxide. Carbamoyl nitroso may be involved in electron transfer, reactive oxygen species formation, and oxidative stress (Cokic *et al.*, 2003).

2.1.5.2 *Bone Marrow Transplantation*

Bone marrow transplantation (BMT) in SS disease has been proven to be effective in children, it was first reported in an 8-year-old girl with acute leukaemia who was successfully transplanted

with the bone marrow of her AS brother (Serjeant, 2001). Although the indication for this first BMT was the acute leukaemia, the increasing success and lowered morbidity with the procedure has allowed its use in severely affected patients with SS disease (Robert *et al*., 2011). While the survival rate from this procedure is roughly 91% and the cure rate is 82% (Hoppe and walter, 2001). This option is currently limited primarily to children under 16 years of age with severe, pre-existing complications.The major concern in defining its use is the current inability to predict a severe clinical course, especially in children, which limits the counselling options and may contribute to parental refusal. Other problems include the low availability of suitable HLA-matched donors, a relatively high short-term mortality, and complications that may include acute and chronic graft-vs.-host disease, graft rejection in approximately 10% of cases, sometimes marrow aplasia, neurological complications(Jorge *et al.*, 2011).

2.1.5.3 Blood Transfusion

Simple transfusion may be life saving in relieving the acutely lowered haemoglobin of the aplastic crisis or acute splenic sequestration, or in maintaining the chronically lowered haemoglobin in chronic renal failure. Exchange transfusion is used to rapidly replace HbScontaining cells and may have a dramatically beneficial effect in acute pulmonary sequestration of the acute chest syndrome. Chronic transfusion programmes are widely used for a variety of indications of which prevention of recurrent stroke (Graham, 2013) and acute chest syndrome (Fawibe, 2008) are the most established. Although there is little doubt regarding their short-term effectiveness, they may be seriously limited in the long term by problems of increasing red cell alloimmunization (Dipankar *et al*., 2009), non-haemolytic transfusion reactions, delayed haemolytic transfusion reactions, iron accumulation requiring chelation, transfusion acquired infections and venous access (Nirmish *et al*., 2011).

2.1.5.4 *Trace Element and Sickle Cell Anaemia*

Trace elements are essential inorganic molecules found in minute quantities of milligram or microgram per kilogram of body weight. Trace elements include zinc, copper, selenium, manganese, chromium, magnesium, fluorine, cobalt, iron and iodine. Some such as lead, cadmium, arsenic, aluminium and nickel are classified as pharmacologically beneficial and toxic hence monitoring of dosage is required (Burtis *et al*., 2008). People with sickle cell disease suffer from many micronutrient deficiency but preliminary research on dietary habits show that food and nutrient intake by sickle cell patients meet or exceeds recommendation and is not significantly different from healthy controls. This suggests that higher rates of nutrient deficiency may be due to increased needs of many nutrients in sickle cell patients. (Idonije *et al*., 2011). The global use of micronutrients in health care delivery system has taken central stage due to the realization of their importance in disease management. Sickle cell disease is among the disease plaguing a sizeable population of the developing world and the cost implication of its management is very high. Sickle cell disease is characterized by anaemia and immunological disturbances. Free radicals are generated in sickle cell disease; hence a balance between minerals and antioxidants is imperative in maintaining red cell membrane integrity and function (Okpuzor and Okochi, 2009). Protection of red cell membrane from free radical mediated oxidative stress is crucial to the management of sickle cell disease. Minerals such as copper, zinc, iron, chromium, magnesium, selenium, vanadium as well as vitamins like vitaminA, C, E, folate and vitamin B complex have been found to relieve oxidative stress associated with red cell membranes (Suvette *et al*., 2014).

2.1.6 Potentials of Plants in Sickle cell Anaemia

The plant kingdom is considered as a storehouse of traditional medicines that may contribute to development of new medicines for various health problems of human beings and sickle cell disease is no exception. In different parts of the world especially in Africa and Asia with high incidence of the sickle cell disease, the people have learnt to manage the problem using plants which are God's gift of nature. Various advances in scientific research on the use of plants and herbs brought the beneficial aspects of traditional medicine and the rational for their uses to the limelight.

In Nigeria and most parts of developing countries, medicinal plants have been used in the treatment of painful crises associated with sickle cell disease (SCD) especially among the lower socio-economic class who cannot afford the high cost of western medicine as well as traditionalists who simply believe in their efficacy. One of our primary sources of information on the use of medicinal plants is local herb sellers, unorthodox doctors and those whose knowledge of herbs were passed to them by their ancestors. The health care cost of the management of sickle cell disease (SCD) patients is disproportionately high compared to the number of people afflicted by the disease. The common people living in the villages are mostly peasant farmers who cannot afford the high cost of treatment by Orthodox doctors (Okochi and Okpuzor, 2005). Considering all genetic disorders to which man is known to be liable, there is probably no other that presents a collection of problems and challenges quite comparable to SCD and related disorders. Due to the debilitating effect and cost of managing the SCD, research has been on-going to determine the efficacy of the use of medicinal plants to tackle the multiple challenges presented in sickle cell disease.

The use of phytomaterials such as *Piper guineensis*, *Pterocarpa osun*, *Eugenia caryophyllala* and *Sorghum bicolor* extracts for the treatment of sickle cell disease was reported by (Wambebe *et al.*, 2001).

The extract of *Pterocarpus santolinoides* and *Aloe vera* was reported to increase the gelling time of sickle cell blood and inhibits sickling in vitro. This indicates that such plants may indeed have a great potential in the management of sickle cell disorder (Ugbor, 2006). The reversal of sickling by root extracts of *Fagara zanthoxyloides* has also been reported (Adwsina, 2005). *Terminalia catappa* could be an effective antisickling agent inhibiting osmotically induced haemolysis of human erythrocytes (Cock, 2015) in a dose dependent basis. The use of *Scoparia dulcis* (Orhue and Nwanze 2005, Orhue and Nwanze 2006*)* in the management of sickle cell disease by one woman for over two decades and the efficacy of the plant in the management of sickle cell disease was speculated. They therefore, used *Trypanosome brucei* to investigate the effect of the plant on hematological and biochemical indices due to lack of animal models for assessing the effectiveness of the plant extract in sickle cell disease monitoring.Thirteen Congolese plants (Mpiana *et al.,* 2007) were screened for antidrepanocytary activity (anti-sickle cell anaemia) and only twelve of them were reported to possess such properties. These plants are *Alchornea cordifolia, Afromomum albo violaceum, Annona senegalensis, Cymbopogon densiflorus, Bridelia ferruginea, Ceiba pentandra, Morinda lucida, Hymenocardia acida, Coleus kilimandcharis, Dacryodes edulis, Caloncoba welwithsii,* and *Vigna unguiculata.*

The role of crude aqueous extract of *Zanthoxylum macrophylla* roots as an anti-sickling agent was also highlighted (Elekwa *et al.,* 2005) and 2-hydroxybenzoic acid was isolated and identified as the anti-sickling agent obtained from the root of this plant.

Garcinia kola is a popular seed consumed by the locals in Nigeria and it is also known as 'bitter kola'. It has been speculated to be effective in the management of sickle cell disease. An investigation of the aqueous extracts of *Garcinia kola* (Elekwa *et al.,* 2003) to confirm the above claim indicated that it was higher and more effective on membrane stabilization than phenylalanine.

The membrane stabilization activity of aqueous extract of *Zanthoxylum macrophylum* roots (Elekwa *et al.,* 2005) was observed to be lower than phenylalanine which differs from the report on *Garcinia kola*. *Senna alata* and *Senna podocarpa* membrane stabilizing properties (Okpuzor and Adebesin, 2006) had been identified but the stabilizing activity was found to be higher in *Senna alata*. The pharmacological agents that alter membrane stability could be applied in the control of sickling process of erythrocytes, a major physiological manifestation of the sickle cell disease (Kunle and Egharevba, 2013).

Several reports indicate that the membranes of human erythroytes from HbAA, HbAS and HbSS blood types have varying stability as determined from the mean corpuscular fragility (Elekwa *et al.,* 2003), therefore plant extracts that can positively affect the red cell membrane would be useful in sickle cell disease management. Furthermore, it has been suggested (Onah *et al.*, 2002), that the extract of the seed of the *Cajanus cajan* was effective in restoring normal morphology of erythrocytes from blood samples of patients affected by sickle cell anaemia. Thus, Aged garlic could be useful in sickle cell management (Ohnishi and Ohnishi 2001, Moriguchi *et al.,* 2001)

because it has been found to suppress haemolysis and prevented reduced membrane deformability.

Some researchers (Oduola *et al.,* 2006,) investigated the usefulness of *Carica papaya* as an antsickling and found that indeed, the unripe *Carica papaya* has antisickling properties. Some herbal cocktails have been produced and tested for their ability to intervene in sickle cell crises. The drug Nicosan previously NIPRISAN (Nix-0699), which is a product of the extracts of four different plants, (*Piper guineenses* seeds, *Pterocapus osum* stem, *Eugenia caryophyllum* fruit, and *Sorghum bicolor* leaves) were shown to possess anti-sickling properties (Iyamu *et al.,* 2002). Clinical trials of Nix-0699 showed that the drug significantly reduced the number of painful episodes in SCD patients. Nix-0699 was able to improve the survival rates of transgenic cell mice under acute severe hypoxic conditions (Iyamu *et al.,* 2003).

The extracts of the roots of *Cassus populnea L*. CPK (a major constituent of a herbal formula Ajawaron HF used in the management of sickle cell disease in south west of Nigeria), has been examined for its antisickling properties and was found to be efficacious as an anti-sickling agent. The African herbal formula (Okochi, *et al.,* 2003), as a heatinic, increases the haemoglobin, packed cell volume and white blood cell levels. This preparation which has been on sale in Nigerian markets under a new name (Jobelyn or Jubi), helps in bolstering the haemoglobin, pack cell volume and white blood cells whose deficiencies are among the hallmarks of the disease. There are some other drugs prepared from medicinal plants in the market today under various names. The most prominent and widely used of them all is Ciklavit developed by Ekeke after eighteen years of intensive research in collaboration with Neimeth Pharmaceuticals, Lagos, Nigeria.

2.2 *Sterculia setigera*

Sterculia setigera belongs to the family *Sterculia*ceae. The trees are of average height, 16m tall, with a trunk thick at the base and have deciduous leaves (plate 1.1). It grows as high as 35m. From a distance, the trunk is light-grey in color, is pink-red in appearance when sawed and yields white gum. The roots are small with very solid foundations and the young branches have a smooth texture. *S. setigera* leaves are simple, measuring 6-20 cm in length and in width and have 3-5 triangular lobe (plate 1.2). The flowers are unisexual and emerge in clusters, on the edge of a year old twig. The one-meter long calyx is composed of five lance-shaped sepals, hairy on the exterior and green with red streaks. The flowers are 12 mm in diameter and grow out of the previous year's shoots (Touré, 2009).The common names of *S. setigera* names include *kukkuki* in Hausa, *esofunfun* in Yoruba, *bokoci* in Nupe.

2.2.1 Geographical Distribution

Sterculia species are well represented in West Africa. It is found in abundance in the West African region and some east African countries like Sudan. The species is widespread in tropical Africa and is common locally. The natural distribution range stretches from Senegal to Cameroon in West Africa, eastwards to Eritrea, and southwards to Angola. It grows in Savannah type vegetation on a variety of soil types, thriving on poor soils as well as on hilly/stony sites. In Nigeria the plant is found in abundance in the North central and north eastern region (Mshelia *et al.*, 2010).

2.2.2 Flowering and Fruiting Habit

Dioecious Flowering usually occurs during the second half of the dry season, either before or in connection with leaf flushing. The tree flowers in the Sahelian region between February and April with fruit maturation in December.

2.2.3 Uses of *Sterculia setigera*

Sterculia setigera is a multipurpose savanna tree with socio-economic importance due to its gum and cultural importance in sub-Saharan Africa. It is used especially in human nutrition, traditional medicine, and cosmetics (Atakpama *et al.,* 2012). The wood is white and very soft, which makes it unsuitable for fuel wood and charcoal. It is therefore used for non timber forest products (NTFP). It is used for insulation and concealed items in carpentry. The tree produces a water-soluble gum "*karaya gum*" which is mostly extracted from private parkland and in forests in Senegal, the world's second largest exporter after India (Benjamin and Wilshusen 2007). The gum is tapped and used in cooking as an emulsifier, stabiliser and viscosifier; the gum is used medically as a laxative, diuretic and tranquilliser and technically as an adhesive and for glazing pottery. Within these countries, gum exploitation is a valuable source of income for many indigent smallholders. The bark is used for rope making and the bark sap can be made into a refreshing drink. The seeds can be eaten as they have a high crude protein, carbohydrate, fibre, and fat content. Mineral analysis indicated the presence of elements such as iron, calcium, copper, zinc, lead, magnesium, chromium, nickel, cadmium, cobalt, sodium and vitamins thiamine and nicotinicacid. *Sterculia setigera* contains edible oil which can be extracted and used for cooking or for use in the cosmetic industry, the leaves are used as fodder for cattle.

2.2.4 Medicinal Uses of *Sterculia setigera*:

The family of *Sterculia*ceae has over 2500 different species, among them are two species mostly used in folk medicine - *S. setigera,* and *S. tragacantha. Sterculia setigera*, this plant is used in trado-medicine by various indigenous communities. For instance, the Yorubas of Nigeria use a black soap prepared from black powder obtained from burnt mixture of the fruits and seeds in dermatosis (Faruq *et al.*, 2010). In Sudan, cold decoction of the stem bark of *S. setigera* is used

for the treatment of medical conditions such as asthma, bronchitis, wound, fever etc (Mann, *et al*., 2008), while the decoction of leaves is used as pain killers (Sunday *et al*., 2008). The stem bark decoction of *S. setigera* is used for the treatment of toothache, gingivitis sore and abscess (Tapsoba *et al*., 2005). An ethnobotanical survey along the riverside forest of southern blue nile district of Sudan reveals that infusion prepared from the bark of *S. setigera* del. is used to treat jaundice (Tahir *el al.*, 2010), the stem bark decoction is also used for the treatment of diarrhea (Igoli, *et al.*, 2005), In Ghana some communities use the boiled leaves of *S. setigera* to treat malaria (Alex, *et al.*, 2005). In southwest Nigeria the plant is employed for the management of constipation base on ethno-medical information obtain from the region (Lawal, *et al.*, 2010).

2.2.5 Anti-microbial Activity of *Sterculia setigera:*

The anti -bacterial and anti-fungal activities of dried bark, dried fruit and the root of *S. setigera* against common microbes species such as *Bacillus Subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* have been reported **(**Tahir *et al.*, 2010**)**. The anti-viral activity of *S. setigera* against three human and three animal viruses (Poliovirus (type 1), astrovirus, human herpes simplex virus (type 1), equine herpes simplex virus, bovine parvovirus and canine parvovirus have also been reported **(**Fernandes *et al.*, 2012**)**. Investigation on *S. setigera* over the years regarding its therapeutic activity has focused on the seed, gum, root and the bark **(**Falzari *et al.*, 2005**)**. Decoctions of the powdered dried leaves of the plant are commonly used for the treatment of TB, and in HIV/AIDS patients with chronic cough with blood stains (Ibrahim *et al.*, 2012). Ethanol stem bark extract of *S. setigera* was shown to have antibacterial activity on *Pseudomonas aeruginosa* with a minimum inhibitory concentration of 2.0 mg/ml and *Bacillus subtilis* with a minimum inhibitory concentration of 1.0 mg/ml (Kubmarawa, *et al.*, 2007). Crude methanolic extract of *S.*

setigera showed some activity against *Mycobacterium tuberculosis* at a minimum inhibitory concentration of 2500µg/ml (Mann, *et al.*, 2008), petroleum ether, chloroform, methanolic and aqueous crude root bark extract of *S. setigera* showed some good trypanocidal activity against *Trypanasoma brucei* (Atawodi, 2005). Methanolic extract of *S. setigera* showed some minimal activity against minimum lethal dose of *Naja nigricollis* venom, therefore it is not a good treatment for snake bite (Abubakar, *et al.,* 2006).



Plate 1.1; *Sterculia setigera* in its natural habitat (Wouyo *et al*., 2010).



Plate 1.2; *Sterculia setigera leaves* in its natural habitat (Wouyo *et al*., 2010).

2.3 *Dichrostachys cinerea*

Dichrotachys cinerea is commonly called "*Dundu*" among the Hausa speaking people of northern Nigeria and "*Kora*" among the Yoruba speaking people of Western Nigeria (Sunday *et al*., 2008). It is also known as *Vurtuli* in Hindi, *Vidattalai* in Tamil, *Velantarah* in Sanskirit, *kaddad*,in arabic, *mimosa clochette* in french and Sickle bush, bell mimosa or Chinese lantern tree in English (Sunil *et al.,* 2010)**.** The generic name **'***Dichrostachys***'** means '2 coloured spikes, and '*cinerea'* refers to the greyish hairs of the typical subspecies, which is confined to India; from the Greek '*konis'* and the Latin '*cineres'*. The plant is a much – branched thorny shrub, usually attaining a height of up to 5 – 10 m (plate 2.1). The Leaves are bipinnate; rachis 4-8 cm, with 5-15 (max. 19) pairs of pinnae, which each bear (min. 9) 12-22 (max. 41) pairs of leaflets; terminal pair of pinnae shorter, dark green, underside pale. Leaflets are about 8 x 2.5 mm wide. The inflorescence consists of a penduculate spike. The flowers have two sets of colours – pinkish white basally and yellow terminally (Mann *et al*., 2005). Pods are narrow, yellow or brown; generally twisted or spiralled, up to 100 x 15 mm, in dense, stalked, intertwined clusters; indehiscent (plate 2.2). About 4 black seeds with a spot at one end per pod.



Plate 2.1; *Dichrostachys cinerea* in its natural habitat (Ramya and Thaakur, 2009).



Plate 2.2; *Dichrostachys cinerea* and its fruits (Ramya and Thaakur, 2009)

2.3.1 Geographical Distribution

Dichrostachys cinerea penetrates clear-cut areas far into the rainforest zone. In Malaysia, it occurs in areas with strong seasonal climate, usually on poor, occasionally clayey soils, in brushwood, thickets, hedges, teak forest and grassland. It is native in 17 countries namely: Cameroon, Djibouti, Eritrea, Ethiopia, Ghana, Kenya, Madagascar, Malawi, Nigeria, Somalia, South Africa, Sudan, Swaziland, Tanzania, Togo, Uganda, and Zambia. The plant has been found to be exotic in countries such as Angola, Australia, Benin, Botswana, Brunei, Burkina Faso, Burundi, Cambodia, Cape Verde, Central African Republic, Chad, Comoros, Congo, Cote d'Ivoire, Cuba, Democratic Republic of Congo, Egypt, Gabon, Gambia, Guinea, Guinea-Bissau, India, Indonesia, Iran, Laos, Lesotho, Liberia, Malaysia, Mali, Mauritania, Mozambique, Myanmar, Namibia, Niger, Philippines, Rwanda, Sao Tome et Principe, Senegal, Seychelles, Sierra Leone, Thailand, Vietnam, Yemen, Republic of Zimbabwe (Rukangira, 2004).

2.3.2 Flowering and Fruiting Habit

In Indonesia, *D. cinerea* has been found flowering from September to June and fruiting from March to May, sporadically in other months; in southern Africa flowering is from October to February and fruiting from May to September. The structure of the inflorescence suggests pollination by bats. The infructescence has a strong aroma, which probably attracts animals to feed on the pods. A fraction of seeds exhibit polyembryony with usually 2, sometimes 3, or rarely more embryos; the extent of polyembryony seems to be positively correlated with the number of seed produced (Gundidza *et al*., 2011).

2.3.3 Uses of *Dichrostachys cinerea*

The fruits and seeds of *Dichrostachys cinerea* are edible to humans and animals such as Cattle, Camels and game such as Giraffe, Buffalo, *Kudu*, Hartebeest, *Nyala*, red forest *duiker* and

Damara dik-dik feed on the juicy pods that fall to the ground. Such animals also feed on the immature twigs and leaves of the tree which are rich in protein (11-15%) and minerals (Ramaachandra, 2002). As they are rich in nutrients, the plants are often used as fertiliser, particularly in the Sahel region of Africa along river banks. The plant is widely used for soil conservation, particularly in India, for shallow soils, and in arid western and subhumid alluvial plains. The flowers can be a valuable source of honey. The wood is of a dense nature and burns slowly with no toxicity, so it is often used for fuelwood. The species yields a medium to heavy, durable hardwood and is often used in smaller domestic items as walking sticks, handles, spears and tool handles particularly in central Africa (Orwa *et al.,* 2009).

2.3.4 Medicinal Uses of *Dichrostachys cinerea*:

Traditionally, the roots are chewed and placed on the sites of snake bites and scorpion stings. the bark is used for headache, toothache, dysentery, elephantiasis, root infusions are used for leprosy, syphilis, coughs, as an anthelmintic, purgative and strong diuretic, leaves are particularly useful and can be taken to treat epilepsy and can also be taken as a diuretic and laxative, and a powdered form is massaged on limbs with bone fractures. In Siddha medicine of the Tamils in southern India, *Dichrostachys cinerea* is called *vidathther* and used for gonorrhea, syphilis and eczema (Vijayalakshmi *et al*., 2010). In Hausa land it has been used to relieve pain in sickle cell patients (Mal.Y. Shuaibu, 2013, june 17).

Phytochemical studies performed on *D. cinerea* extracts have revealed the presence of tannins, sterols and triterpenes, of reductionist compounds, polyphenols, flavonoids as well as of cardiotonic heterosides α-amyrin, β-amyrin, friedelan-3-β-ol, friedelin, hentriacontan-1-ol, β-sitosterol and octacosan-1-ol (Joshi and Sharma, 2011). Presence of α-amyrin, ceryl-cerotate,

friedelin, lacceric acid, pentacosyl-pentanoate, 6-hydroxy, β- sitosterol, γ-sitosterol, stigmasterol and triacontane have been reported from the heartwood (Jain and Saxena, 2003), many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogeni, antibacterial, and antiviral activities (Sala, 2002). Pharmacological report on *D. cinerea* has shown antibacterial effect (Kambizi and Afolayan, 2001) and antiviral. Several authors have shown that the species inhibit protein farnesyl-transferase activity (Jagadeeshwar *et al.,* 2003). Moreover, chemical studies revealed the presence of a new isomer of mesquitol (a main active principle), which shown free-radical scavenging property and a-glucosidase inhibitory activities (Long *et al.,* 2010). The antibacterial (Ramya and Thaakur, 2009), angiotensin-converting enzyme inhibition (Salah *et al.,* 2001), antilithiatic (Jayakumari *et al.,* 2011), antidiarrhoeal ((Jayakumari *et al.,* 2011), antilice (Vijayalakshmi, 2010), hepatoprotective (Babu *et al.,* 2011) antiurolithiasis and diuretic activity (Jayakumari and Srinivasa, 2007) of different extracts of *D.cinerea* have been reported.

CHAPTER THREE

3.0 Materials and Methods

3.1 Chemicals

All chemicals and reagents used for this study were of analytical grade, purchased from genuine manufacturers and dealers, they include; sodium metabisulphite, p-hydroxybenzoic acid (Sigma Aldrich). Sodium chloride, giemsa stain (Toyochem specialty chemicals, Birmingham,United Kingdom), Immertion oil, methanol, petroleum ether, chloroform, ethyl acetate, n-hexane, sulphuric acid (Sigma-Aldrich, Steinheim,Germany), sodium monohydrogen phosphate, sodium dihydrogen phosphate (Toyochem specialty chemicals, Birmingham,United Kingdom).

3.2 Equipment

The equipments used for this study were of good and sound working condition kept in standard laboratories, they include; centrifuge machine, (Labofuge300, Heraeus, Kendro laboratory,Newton, U.S.A), (Heraeus, Kendro laboratory, Newton, U.S.A), Spectrophotomter (6305, Jenway, Bibbyscientific Ltd-stone-ST15OSA-UK), Soxhlet extractor (Pyrex international cookware, unit1, Hall Dene Way, Seaham Grange industrial Est, Seaham County,Unitd kingdom), Water bath(Grant JB series, B and T, Keison international ltd, Chelmsford, Essex CM1, England), Chromatographic tank,Weighing balance(GF-2000, A&D Company Ltd, Grant Street, Cleona, London), TLC plates(G60 F_{254}, Emerck, Darmstadt, Germany).

3.3 Plant Sample Collection

Fresh parts (root) of *Dichrostachys cinerea* and (leaf) of *Setigera setigera* were collected from Zaria Local Govt Area in Kaduna state. They were authenticated at Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University Zaria, with voucher number

900205 and 365 for *D. cinerea* and *S. setigera*, respectively. Samples were dried in a shade and then pounded with mortar and pestle.

3.4 Blood Sample

Venous blood samples were collected from confirmed consenting HbSS adult patients who attend Ahmadu Bello University Teaching Hospital for medical checkup. Three millilitres blood sample was collected by venipunture into anticuagulated tubes.

3.5 Ethical Approval

The research was approved by the Health Research Ethical Committee (HREC), Ahmadu Bello university Teaching Hospital, Shika via a ref No. ABUTH/HREC/TRG/36 dated 3rd Oct, 2013. Informed consent was obtained from the patients before taking their blood samples.

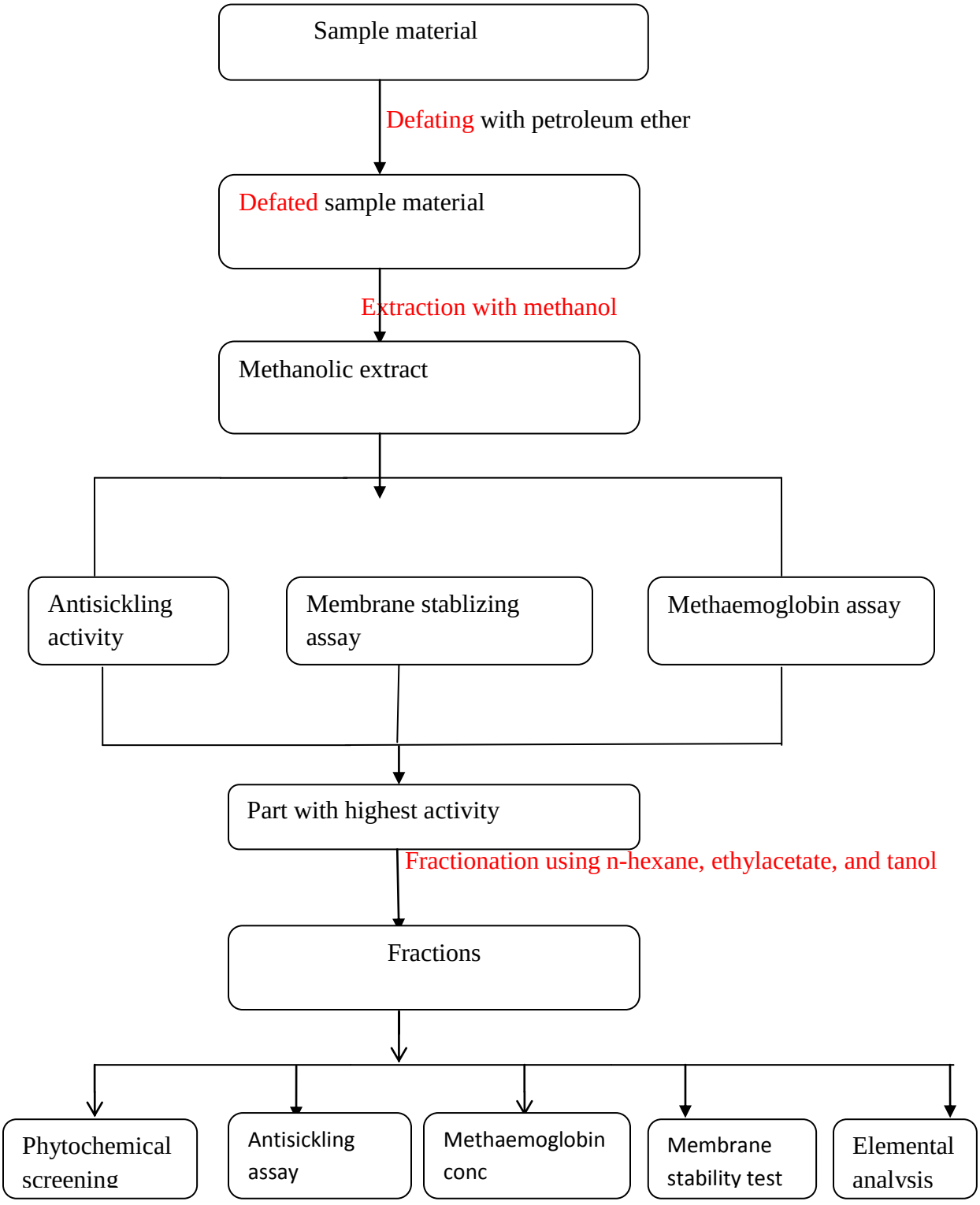


Fig 5: A flow sheet of experimental design for extraction, fractionation, and antisickling assessement of *Dichrostachys cinerea* and *Sterculia setigera*.

3.6 Methodology

3.6.1 Exraction of the Plant Material

Exactly 500g of the sample material was weighed and then loaded into a soxhlet extractor. The Samples were then defated with petroleum ether (40-60) for 8 hours and then exhaustively extracted with methanol.The solvent was evaporated at room temperature.

3.6.2 Preparation of the Blood Sample

Venous blood samples collected into EDTA bottles from sickle cell patients were centrifuged at 3000 × g for 10 minutes to remove the plasma. The resulting packed erythrocytes were washed three times with normal saline equivalent to the volume of the plasma romoved from the blood. The sample were then centrifuged each time for 10min at 3000 × g before removing the supernatant. The washed erythrocytes was then used for the antisickling and membrane stability tests. Whole blood samples were however used in the methaemoglobin concentration assay.

3.6.3 Determination of Anti sickling Properties of *Dichrostachys cinerea* and *Sterculia setigera* extracts and Fractions.

The ability of the plants extract to reverse the sodium metabisulphate induce sickling was estimated using Sodium Metabisulphite test as described by (Imaga *et al*., 2009).

This assay is based on reducing capacity of sodium metabisulphite by removing oxygen content of the haemoglobin which result in the sickling of the haemoglobin.

3.6.3.1 *Procedure*

Some of the washed erythrocytes (0.5ml) was mixed wtih 0.2ml of 2% sodium metabisulphite in test tubes and sealed with parafin. The mixture was then incubated at 37ºC for 30 min. To confirm the induction of sickling, wet preparation was made and observed under the microscpe

at 1,000 magnification .Various concentrations(0.1mg/ml, 0.2mg/ml, 0.3mg/ml) of the extracts and fractions were then added to the test tubes and then incubated again for 120 min at 37ºC. Samples were then taken and then smeared on microcope slide after 0 min, 30 min, 60 min, 90 min and 120 min. Each sample smear was fixed with 98% methanol, dried and stained with Giemsa stain, and each sample was examined under the oil immersion light microscope before counting at least 500 red blood cells in each sample from five different fields of view across the slide. The number of sickled cells was counted and the percentage of reversed cells was determined by the following formula;

$$\% \text{ number of reversed cells} = \frac{\text{Mean number of sickled cells at 0 min} - \text{mean number of sickled cell at time t}}{\text{Mean number of sickled cells at 0 min}}$$

Positive control contains p-hydroxy benzoic acid (5mg/ml), and the negative control contains norrmal saline.

3.6.4 Phytochemical Analysis of *Dichrostachys cinerea* and *Sterculia setigera* methanolic Extracts and fractions

3.6.4.1 Test for Carbohydrates

A) Molisch's test; To 2ml of the extaract, few drops of molisch's reagent was added to form a lower layer with shaking of the tube. A purple ring at the interphase of the liquid indicated presence of carbohydrates (Jaliwala *et al.*, 2011).

3.6.4.2 Test for Tannins

Ferric chloride Test: To 2ml of the extract, distilled water was added followed by a few drops of 10% ferric chloride. A blue green colour was formed which indicated the presence of tannins (Jaliwala *et al.*, 2011).

3.6.4.3 Test for Saponins

Froth test: The extract was dissolved in 3ml of ethanol and mixed with 10ml of distilled water in a test tube. The tube was shaked vigourously and allowed to stand for 30 min. Honey comb froth was observed (Akindakun, 2005).

3.6.4.4 Test for Triterpenes and Steroides

A) Lieberman- Burcherd's Test: A 2ml portion of the extract was mixed with 1ml of acetic anhydride followed by addition of 1ml concentrated sulphuric acid down the side of the test tube. A green layer indicate the presence of terpines.

B) Salkwosky's test: A 2ml portion of the extract was mixed with conc sulphuric acid carefully, a reddish-brown color at inter phase indicated the presence of steroids (Akindakun, 2005).

3.6.4.5 Test for Flavonoids

Sodium hyroxide test;

A) To a 2ml portion of the extract, 5 ml of 10% sodium hydroxide was added. A yellow colouration indicates presence of flavonoids.

B) Schinodo's Test: Small amount of magnesium chips were added to the 2ml of the extract solution followed by a few drops of concentrated hydrochloric acid. Appearence of an orange, pink or red colour indicated the presence of flavonoids (Maras, 2011).

3.6.4.6 Test for Alkaloids

A) Mayer's Test: 1 ml of the extract was treated with few drops of Mayer's reagent giving rise toa cream or pale yellow precipitate which indicate presence of Alkaloids.

B) Dragendoff's Test: Another 1 ml of the extract solution wastreated with a few drops of Dragendroff's reagent giving rise to anorange precipitate indicating the presence of Alkaloids (Jaliwala *et al.*, 2011).

3.6.4.7 Test for Cardiac Glycosides

Keller-Killani Test: To a 2ml of the extract, 2 ml of glacialacetic acid containing one drop of ferric chloride solution was added. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides. A violet ring also appeared below the brown ring, and in the acetic acid layer, a greenish ring formed just gradually throughout thin layer (Jaliwala *et al.*, 2011).

3.7 Determination of Methaemoglobin Concentration Reduction Effect of *Dichrostachys cinerea* And *Sterculia setigera* Extracts and Fractions.

The effect of the extracts on the methaemoglobin concentration was determined by the method of (Paul *et al.*, 2009).

The principle of this method is based on the fact that haemoglobin and methaemoglobin absorb light maximally at diffrent wavelengths, having 540nm and 630nm as their respective wavelengths, hence increase of absorbance at 630nm signify increase in methaemoglobin concentration.

3.7.1 Procedure

The effect of each of the extract and fraction on plasma methaemoglobin concentration was determined by introducing 0.02ml of the specified concentrations (0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml, and 1mg/ml) of each extract and fraction solution into separate test tubes. This was followed by the addition of 5 ml of distilled water and 0.02ml of the blood sample. The mixture was allowed to stand for 60 minutes at room temperature, after which, the absorbance was read at 540 and 630 nm. The control test tube contained 5.0ml of distilled water and 0.02ml of whole blood was added. The percentage plasma methaemoglobin was obtained with the formula;

$$\text{Percentage methaemoglobin} = \frac{(\text{A } 630)2}{(\text{A}540)2 + (\text{A}630)2} \times 100$$

Where A540 and A630 are absorbance at 540 nm and 630 nm respectively.

3.8 Bioassay Activity Guided Fractionation

After a pilot study on the methanolic extracts of the various parts of both plants, the highest antisickling activity was obtained on root bark for *D. cinerea* and on the leaves for *S.setigera*. Therefore the two parts were subjected to bioassay activity guided fractionation as follows;

3.8.1 Thin Layer Chromatography (TLC)

Thin layer chromatography was carried out to determine the solvent system to be use in column chromatography. An aluminium chromatographic plate coated with silica gel was used. The crude extract was dissolved in methanol and then applied to the plates using a micro hematocrit capillary tube. The plates was placed in a chromatographic tank and developed with the following solvent systems;

n-hexane – ethylacetate 6:4

chloroform – ethylacetate 8:2

chloroform- ethyl acetate 9:1

n-hexane – ethylacetate 1:1

n-hexane – ethylacetate 8:2

chloroform - methanol 8:2

Thereafter the plates were removed and air-dried. The spots were developed by spraying the plates with spraying reagent containing 10% sulphuric acid in methanol and heated in an oven. The spots were circled with pencil, and retention factor (R_f) was calculated using the formula below.

$$R_f = \frac{\text{Distance spot travelled up plate from origin}}{\text{Distance solvent travelled up plate from origin}}$$

3.8.2 Fractionation

Based on the TLC, the best solvent system for both plants extracts was the hexane-ethylacetate 6:4, therefore crude methanolic extacts of *Dichostachys cinerea* root *Sterculia setigera* leaves were partitioned with n-hexane, ethylacetate, and butanol, using the method of De *et al*., (2009). Fractions obtained were then subjected to phytochemical screening, membrane stability test, methaemoglobin concentration test, and anti-sickling activity.

3.8.3 Procedure

Exactly 89g of crude methanolic extact of Dichostachys cinerea root and 93g of methanolic extract of *Sterculia setigera* was each weighed out, and they were separately transferred into 1000 ml beakers, A volume of 500ml of distilled water was added to dissolve each of the extracts, and then filtered. The fractionation was carried out by transferring 500ml of the

dissolved methanolic extracts into 1000ml separating funnels and n-Hexane, Ethylacetate, and Butanol were used successfully to fractionate the dissolved extracts. Exactly 200ml of n-Hexane was first added to the solution in the separating funnel and rocked gently, this was allowed to separate, the upper n-Hexane fraction was collected and it was repeated four times making a total of 800ml of n-hexane fraction. These fractions were put together and evaporated to dryness and labelled properly. The same procedure was repeated for Ethylacetate and Butanol, respectively. And the fractions were collected separately and evaporated to dryness and labelled accordingly, yielding a total of 3 fractions. These were kept in a desiccator for further tests.

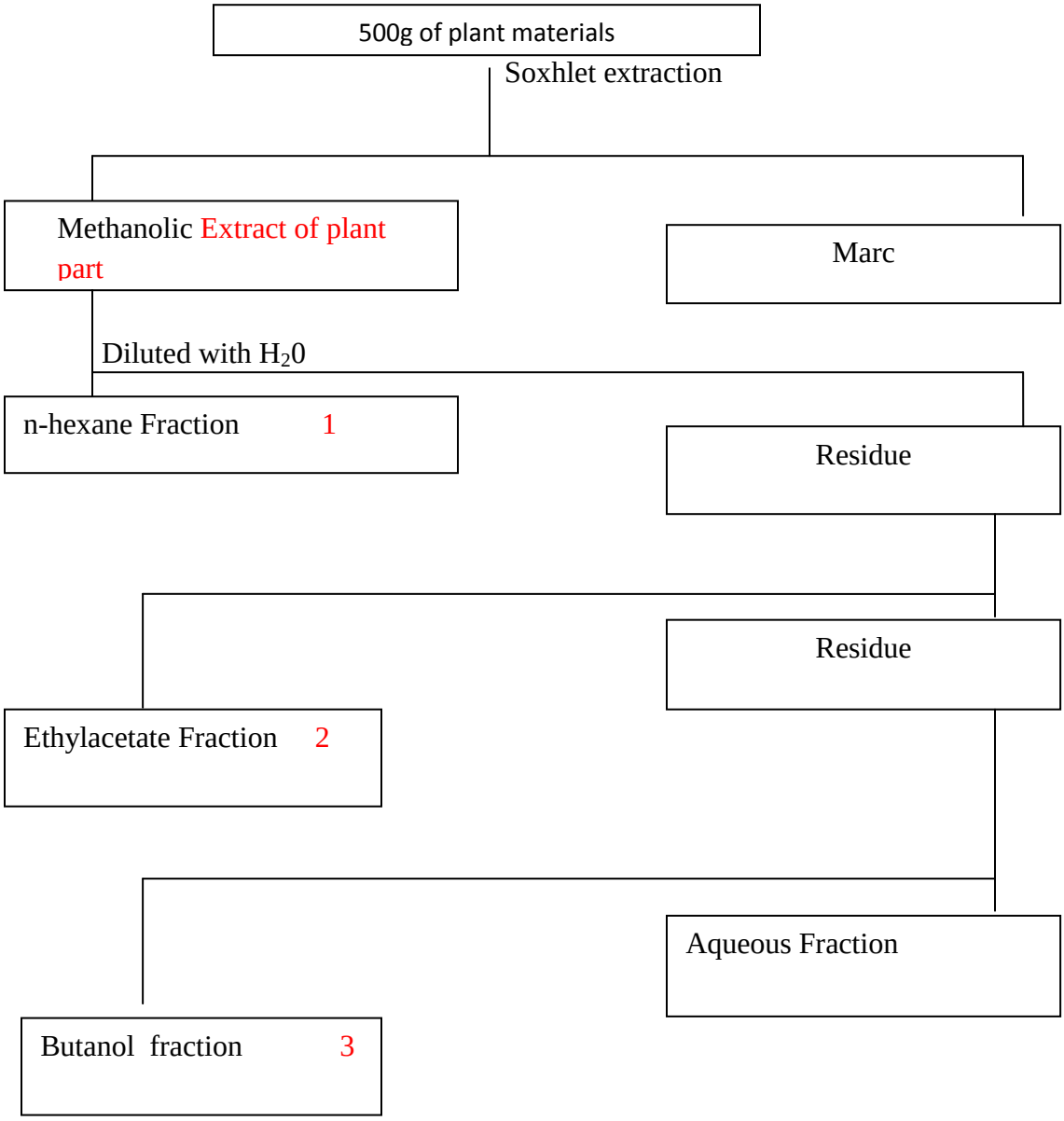


Fig 6: Diagrammatic presentation of fractionation procedure for *Dichrostachys cinerea* and *Sterculia setigera*.

The percentage yields of the methanolic extracts and the fractions were calculated using the formular:

$$\% \text{ yield of methanolic extraction} = \frac{\text{Weight of methanolic extract}}{\text{Weight of plant material}} \times 100$$

$$\% \text{ yield of fractionation} = \frac{\text{Weight of fraction}}{\text{Weight of methanolic extract}} \times 100$$

3.9 Membrane Stability Test

The effect of the plant extract on membrane stability was determined by the method of the standard method described by Mark Layton and David Roper, 2010 .

3.9.1 Preparation of Extract Solutions

About 10mg of various methanolic extract fractions were dissolved in 1ml of normal saline to arrive at a concentration of 10mg/ml, then 0.1ml, 0.2ml, 0.4ml and 0.5ml were collected into separate tubes then 1.9ml, 1.8ml, 1.6ml and 1.5ml 0f nomal saline were added to the test tubes to arrive at a concentration of 0.5mg/ml, 1mg/ml, 2mg/ml, and 2.5mg/ml respectively. A tablet of 500mg ibuprofen was crushed and dissolved in 50ml normal saline to get 10mg/ml, 0.5ml of this solution was then collected into a test tube where 1.5ml 0f nomal saline was added to have a concentration of 2.5mg/ml.

3.9.2 Procedure

About 5.0ml of the prepared saline solutions was put in six different test tubes. To each of the test tubes, 50µl of both extracts and well mixed washed fresh blood sample were added and immediately mixed gently by inverting the tube several times to avoid foaming. The suspension was allowed to stand for 30min at room temperature; it was mixed again and then centrifuged at 1200rpm for 5min. Blanking was done with the supernatant of the test tube having 9.0 hypo

saline solutions. The supernatant of the rest of the test tubes was then each collected into clean test tubes and the light absorbance of the supernatant was then taken using a spectrophotometer at wavelength of 540nm. A positive control of 500mg ibuprofen was used and percentage inhibition of haemolysis was calculated using the formula;

$$\% \text{ inhibition of haemolysis} = \frac{\text{Ab of isosaline soln} - \text{Ab of test sample}}{\text{Ab of isosaline soln}} \times 100$$

3.10 Mineral Analysis

3.10.1 Principle of Atomic Absorption Spectrophotometry

An atom is made up of positively charged nucleus surrounded by a number of negatively charged particles necessary to provide neutrality. These atoms occupy discrete energy levels but it is possible for an electron to be moved from one level to another by introduction of energy. Such transitions will only occur if the available energy is equal to the difference between the two levels. Energy levels and the energies associated with electron transitions are unique for each element. When light (energy) of a characteristic wavelength enters an analytical system, outer shell electrons of corresponding atoms within the light path will be excited as energy is absorbed.

The amount of light transmitted through the system from a source to the detector will be less. The loss of light is proportional to the number of atoms. The measurement of the radiation transmitted (using Beer-Lambert's law) in such a transition form the basis of AAS. Beer Lambert's law relates absorbance, a to the concentration of metallic atoms in the atom cell, c as follows

LogT-1= a b c

Where

a is the absorptivity in grams per litre-centimetre

b is the atom width in centimeters

c is the concentration of atoms

The AAS involves the measurement of the drop in light intensity of initial radiation Io to final radiation I depending on the concentration of the metal. Modern instruments automatically convert logarithmic values into absorbance (Nollet, 2011).

3.10.2 Digestion of Samples

About 10mg of various methanolic extract samples were digested for mineral analysis using the method of Ogunfowokan *et al*., (2009). For each sample, 1 g of sample was digested in Teflon cups with 30 ml aqua-regia (HCl: HNO_3, 3:1) on a thermostatted hotplate at 150ºC. After, about 2 hours of digestion, the Teflon cup with its content was brought down from the hot-plate to simmer. Then, 5ml hydroflouric acid was added and heated further for 30 min. The Teflon cup with the content was allowed to cool down to room temperature and filtered. After which the filtrate was quantitatively transferred into 50ml volumetric flask and made to mark with distilled-deionized water. A blank determination was carried out using the procedure described above without the sample. Chromium, Copper, Iron, Magnesium, and Zinc concentrations were determined using Atomic Absorption Spectrophotometre (AAS) in duplicate.

3.11 Statistical Analysis

The data obtained was statistically analyzed using analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to compare different group means. $P<0.05$ was considered significant in all cases. SPSS package software version 20.0 was used for the data analysis.

CHAPTER FOUR

4.0 Results

4.1 Antisickling Activity Assay of *Dichrostachys cinerea* and *Sterculia setigera* Methanolic Extracts and Fractions

The antisickling activity of the methanolic extract and each of the fractions derived from the root bark of *D. cinerea* and leaves of *S. setigera* was both concentration and time-dependent. The activity at each fraction-concentration increased with time, and showing the highest activity at time interval of 120 minutes and the least activity at 30 minutes for all the fractions.

Table 4.1.1 shows the percentage of reversed red blood cells after being treated with the methanolic extract of the leaves of *Dichrostachys cinerea.* From the result, it was observed that there is a significant increase ($p<0.05$) in the percentage of reversed red blood cells treated with the extract at all concentrations when compared with normal saline treated red blood cells. But 0.2mg/ml of the extract at 30min showed an antisickling activity of 59.52±2.62%, which was not significantly different ($p<0.05$) when compared with p-hydroxy benzoic acid (69.07±0.37%).

Table 4.1.2 shows the percentage of reversed human red blood cells after treatment with different concentrations of the n-hexane fraction of the root bark methanolic extract of *Dichrostachys cinerea.* From the result, there was a significant decrease ($p<0.05$) in the percentage of reversed red blood cells after treatment with the fraction at all concentrations when compared with p-hydroxy benzoic acid, with the highest activity of 16.60±1.61% observed at 0.1mg/ml concentration. There was no significant ($p>0.05$) difference between all the concentrations of the fraction.

Table 4.1.3 Shows the percentage of human sickled red blood cells reversed following treatment with ethylacetate fraction of the root bark methanolic extract of *Dichrostachys cinerea* at

various concentrations (0.1mg/ml, 0.2mg/ml, 0.3mg/ml). It can be observed from the Table that the fraction caused significant difference ($p<0.05$) in the percentage of reversed red blood cells when compared to both p-hydroxy benzoic acid (positive control) and normal saline (negative control), except for 0.3mg/ml at 30, 60, and 90 min which showed no significant difference with the negative control. Again, at 60min time interval, 0.1mg/ml and 0.2mg/ml had no significant difference when compared to p-hydroxy benzoic acid.

Table 4.1.1: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with the Methanolic Extract of the Root Bark of *Dichrostachys cinerea* at Various Concentrations and Time-Intervals.

Incubation Time (Min)	Normal Saline (Control)	Concentrations of Fraction/ Standard (%)			
		0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	3.60±1.47^{a}	28.78±1.25^{b}	59.52±2.62^{c}	50.04±1.67^{b}	67.72±0.03^{c}
60	3.63±1.43^{a}	40.01±1.42^{bc}	61.95±1.51^{c}	55.81±1.40^{c}	78.42±2.81^{d}
90	4.89±2.83^{a}	40.62± 0.48^{bc}	53.51±1.83^{c}	59.37±1.42^{c}	84.96±2.81^{d}
120	1.47±2.89^{a}	52.58±1.43^{c}	69.07±0.37^{c}	65.10±2.86^{c}	93.63±1.48^{de}

The values in the table are the Mean±SD from triplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

Table 4.1.2: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with the n-hexane Fraction of Methanolic Extract of the Root Bark *Dichrostachys cinerea* at Various Concentrations and Time-Intervals.

Incubation Time (Min)	Normal saline (Control)	Concentrations of Fraction/ Standard (%) 0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	8.04±1.46[a]	8.35±1.43[a]	12.57±1.45[a]	16.65±2.87[a]	50.03±0.0[b]
60	8.07±1.43[a]	9.92±1.87[a]	19.01±1.91[a]	10.01±1.46[a]	58.65±2.89[b]
90	10.25±2.81[a]	10.27±1.20[a]	12.59±1.62[a]	11.04±1.20[a]	75.01±2.80[c]
120	10.85±1.82[a]	16.60±1.61[a]	13.61±1.60[a]	16.58±2.81[a]	91.60±1.40[d]

The values in the table are the Mean±SD from duplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

Table 4.1.3: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with an Ethylacetate Fraction of the Methanolic Extract of the Root Bark of *Dichrostachys cinerea* at Various Concentrations and Time-Intervals.

Incubation Time	Normal saline	Concentrations of Fraction/ Standard (%)			
(Min)	(Control)	0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	8.04±1.46^{a}	29.41±2.82^{ab}	42.30±1.29^{b}	8.37±1.42^{a}	50.03±0.71^{b}
60	8.07±1.43^{a}	46.29±1.14^{b}	53.83±1.23^{b}	8.34 ±1.47^{a}	58.65±2.89^{b}
90	10.25±2.81^{a}	29.42±1.82^{ab}	53.80±2.81^{b}	25.01±1.40^{a}	75.01±2.80^{c}
120	10.85±1.72^{a}	41.15±2.81^{b}	57.64±1.25^{b}	41.02±0.76^{b}	91.60±1.40^{d}

The values in the table are the Mean±SD from duplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

Table 4.1.4 Shows the percentage of human sickled red blood cells reversed following treatment with butanolic fraction of the root bark methanolic extract of *Dichrostachys cinerea* at various concentrations (0.1mg/ml, 0.2mg/ml, and 0.3mg/ml) and time intervals. From the table there was a significant ($p<0.05$) decrease in the percentage of reversed red blood cells with highest value of 57.81± 1.49% at 0.2mg/ml concentration as compared with p-hydroxy benzoic acid, however, there was a significant ($p<0.05$) increase in the percentage of reversed red blood cells when compared with normal saline treated red blood cells. Again a significant ($p<0.05$) increase in the percentage of reversed red blood cells was seen 0.3mg/ml concentration when compared with 0.1mg/ml and 0.2mg/ml.

Table 4.1.5 shows the percentage of reversed human red blood cells treated with different concentrations of the aqueous fraction of the root bark methanolic extract of *D. cinerea* at various time intervals. From the result, there was a significant ($p<0.05$) increase in the percentage of reversed cells treated with the fraction at all concentrations and time interval except for an activity of 10.53±4.27% observed at 0.3mg/ml concentration, at 30min time interval, when compared with normal saline treated red blood cells, but compared with p-hydroxy benzoic acid, there was a significant ($p<0.05$) decrease in the percentage of reversed cells treated with the fraction at all concentrations.

Table 4.1.4: The percentage of Human Sickled Red Blood Cells Reversed Following Treatment with a Butanol Fraction of the Methanolic Extract of the Root Bark of *Dichrostachys cinerea* at various concentrations and Time-Intervals.

Incubation Time (Min)	Normal saline (Control)	Concentrations of Fraction/ Standard (%) 0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	8.04±1.46[a]	11.15±1.45[a]	11.52±1.67[a]	20.02±0.71[b]	50.03±0.01[c]
60	8.07±1.43[a]	44.40±1.63[c]	15.79±1.70[a]	26.62±1.28[b]	58.65±2.89[c]
90	10.25±2.81[a]	11.15±1.27[a]	47.30±1.62[c]	20.07±2.89[b]	75.01±2.80[d]
120	10.85±1.82[a]	33.97±1.49[b]	57.81±1.66[c]	38.35±2.80[b]	91.60±1.40[d]

The values in the table are the Mean±SD from duplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

Table 4.1.5: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with an Aqueous fraction of the Methanolic Extract of the Root Bark of *Dichrostachys cinerea* at Various Concentrations and Time-Intervals.

Incubation Time (Min)	Normal saline (Control)	Concentrations of Fractions/ Standard (%) 0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	8.04±1.46[a]	28.50±1.47[a]	66.08±2.82[c]	10.53±1.27[a]	50.03± 0.01[b]
60	8.07±1.43[a]	14.23±1.49[a]	20.05±1.21[a]	31.57±1.43[b]	58.65±2.89[b]
90	10.25±2.81[a]	21.41±1.20[a]	20.01±1.66[a]	47.30±1.06[b]	75.01±2.80[c]
120	10.85±1.82[a]	35.78±2.00[b]	33.39±1.42[b]	47.89±1.21[b]	91.60±1.40[d]

The values in the table are the Mean±SD from triplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

On the other hand, Results of the effect of various concentrations of the methanolic extract of the leaf of *Sterculia setigera* on percentage of reversed human red blood cells at different time intervals were presented in table 4.1.6. Here, there was a significant ($p<0.05$) decrease in the percentage of reversed red blood cells treated with the extract at all concentrations when compared with p-hydroxy benzoic acid, but there was a significant ($p<0.05$) increase in the percentage of reversed red blood cells treated with the extract at all concentrations when compared with normal saline treated blood cells except for 0.1mg/ml and 0.3mg/ml at 30min with an activity of 28.27±5.26%, and 29.68±5.62% respectively.

Table 4.1.7 shows the percentage of reversed human red blood cells treated with various concentrations (0.1mg/ml, 0.2mg/ml, 0.3mg/ml) of the n-hexane fraction of the leaf methanolic extract of *S. setigera* at different time intervals. From the result, it is observed that there was a significant ($p<0.05$) decrease in the percentage of reversed red blood cells treated with the fraction at all concentrations when compared with p-hydroxy benzoic acid except for 0.1mg/ml at 30 and 60min. Also, there was a significant ($p<0.05$) increase in the percentage of reversed red blood cells treated with the fraction at all concentrations and time intervals when compared with normal saline treated blood cells except for 0.1mg/ml at 90min with an activity of 15.39±2.81%, 0.2mg/ml at 30min with an activity of 5.27±1.00%, and 0.3mg/ml at 30min with an activity of 5.83±1.68%. however there is no significant ($p<0.05$) difference in the percentage of reversed red blood cells between 0.2mg/ml and 0.3mg/ml concentrations at all time intervals.

Table 4.1.6: The Percentage of Human Sickled Red Blood cells Reversed Following Treatment with the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals.

Incubation Time (Min)	Normal saline (Control)	Concentrations of Fraction/ Standard (%) 0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	3.60±1.47[a]	28.27±1.26[a]	37.65±2.61[b]	29.68±1.62[a]	67.72±0.03[c]
60	3.63±1.43[a]	38.48±1.44[b]	59.10±1.51[c]	43.20±1.48[b]	78.42±2.81[d]
90	4.89±2.83[a]	39.71±0.49[b]	56.93±1.85[c]	45.61±1.45[b]	84.96±2.81[d]
120	1.47±1.89[a]	51.22±1.43[c]	70.97±0.38[c]	61.75±2.89[c]	93.63±1.48[d]

The values in the table are the Mean±SD from triplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

Table 4.1.7: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with an n-hexane Fraction of the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals.

Incubation Time (Min)	Normal saline (Control)	Concentrations of Fractions / Standard (%) 0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	8.04±1.46[a]	76.04±1.48[d]	5.27±2.00[a]	5.83±1.68[a]	50.03±0.01[c]
60	8.07±1.43[a]	76.31±2.26[d]	21.91±1.48[b]	29.42±2.05[b]	58.65±1.89[c]
90	10.25±2.81[a]	15.39±2.81[a]	21.07±2.89[b]	33.58±1.82[b]	75.01±2.80[d]
120	10.85±1.82[a]	38.44±1.40 [b]	22.61±1.86[b]	49.76±1.49[c]	91.60±1.40[e]

The values in the table are the Mean±SD from duplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

Table 4.1.8 shows the percentage of reversed human red blood cells treated with different concentrations of the ethylacetate fraction of the leaf methanolic extract of *S. setigera* at different time interval. Incubation of the sickle cells with the extract at various concentration for 120 min showed that the anti sickling effect of the fraction had a significant ($p<0.05$) decrease in the percentage of reversed red blood cells treated with the fraction at all concentrations when compared with p-hydroxy benzoic acid treated blood cells except for 0.3mg/ml at 30min with an activity of 86.01±5.69%, which had no significant ($p<0.05$) difference. Again there was a significant ($p<0.05$) increase in the percentage of reversed red blood cells treated with the fraction at all concentrations when compared with normal saline treated with normal saline treated blood cells with the exception of 0.2mg/ml at 30min having an activity of 13.01±5.62%. A significant ($p<0.05$) difference was also seen in the percentage of reversed red blood cells treated with the fraction between all concentrations at all time intervals except for 60min interval where the activities had no significant difference.

Table 4.1.9 shows the percentage of reversed human red blood cells treated with different concentrations of the butanolic fraction of the leaf methanolic extract of *S. setigera* at different time intervals. Here it can be seen that there was a significant ($p<0.05$) decrease in the percentage of reversed cells treated with the extract at all concentrations when compared with p-hydroxy benzoic acid, but there was a significant ($p<0.05$) decrease in the percentage of reversed cells treated with the extract at all concentrations when compared with normal saline treated blood cells except for 0.mg/ml, and 0.3mg/ml at 30min with an activity of 28.27±1.26% and 29.68±5.62% respectively. Also, there was no significant ($p<0.05$) difference between all concentrations at all time intervals except for these concentrations.

Table 4.1.10 shows the percentage of reversed human red blood cells treated with different concentrations of the aqueous fraction of the leaf methanolic extract of *S. setigera* at different time intervals. From the result, there was a significant ($p<0.05$) decrease in the percentage of reversed red blood cells treated with the fraction at all concentrations when compared with p-hydroxy benzoic acid treated red blood cells. However, only 0.3mg/ml at 120min, and 0.1mg/ml at the time interval of 90min and 120min had significant ($p<0.05$) difference when compared with normal saline.

Table 4.1.8: The Percentage of Human Sickled Red Blood Cells Reversed following Treatment with an Ethylacetate Fraction of the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals.

Incubation Time (Min)	Normal saline (Control)	Concentrations of Fraction / Standard (%) 0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	8.04±1.46^{a}	20.05±1.45^{b}	13.01±1.62^{a}	86.01±1.69^{d}	50.03±0.01^{c}
60	8.07±1.43^{a}	20.07±1.48^{b}	30.49±1.48^{b}	26.00±1.42^{b}	58.65±2.89^{c}
90	10.25±2.81^{a}	68.23± 0.41^{cd}	43.53±2.89^{c}	47.82±1.47^{c}	75.01±2.80^{d}
120	10.85±1.82^{a}	69.58±1.40^{cd}	56.67±0.04^{c}	65.29±2.86 ^{c}	91.60±1.40^{e}

The values in the table are the Mean±SD from duplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

Table 4.1.9: The Percentage of Human Sickled Red Blood Cells Reversed Following Treatment with a Butanol Fraction of the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals.

Incubation Time (Min)	Normal saline (Control)	Concentrations of Fractions / Standard (%) 0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	8.04±1.46^{a}	23.00±1.05^{a}	30.40±1.04^{b}	21.73±1.47^{a}	50.03±0.01^{c}
60	8.07±1.43^{a}	30.76±1.09^{b}	52.17±1.48^{c}	30.51±2.80^{b}	58.65±2.89^{c}
90	10.25±2.81^{a}	46.12±1.88^{b}	56.54±1.96^{c}	56.67±1.29^{c}	75.01±2.80^{d}
120	10.85±1.82^{a}	61.55±2.45^{c}	69.52±1.27^{c}	43.92±1.59^{b}	91.60±1.40^{e}

The values in the table are the Mean±SD from duplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

Table 4.1.10: The Percentag of Human Sickled Red Blood cells Reversed Following Treatment with an Aqueous Fraction of the Methanolic Extract of the Leaf of *Sterculia setigera* at Various Concentrations and Time-Intervals.

Incubation Time (Min)	Normal saline (Control)	Concentrations of Fraction / Standard (%) 0.1mg/ml	0.2mg/ml	0.3mg/ml	PABA(5.0mg/ml)
30	8.04±1.46[a]	11.76±2.10[a]	19.02±1.01[a]	8.68±1.83[a]	50.03±0.01[c]
60	8.07±1.43[a]	11.98±1.55[a]	14.26±2.89[a]	13.04±2.89[a]	58.65±2.89[c]
90	10.25±2.81[a]	29.42±1.49[b]	19.22±1.25[a]	13.60±1.04[a]	75.01±2.80[c]
120	10.85±1.82[a]	41.13±2.40[b]	28.51±1.40[a]	46.01±1.49[b]	91.60±1.40[d]

The values in the table are the Mean±SD from duplicate experiments. Values with different superscript vertically shows increase in % of reversedd red blood cells and are significantly different at $p<0.05$.

4.2 Phytochemical Screening of *Dichrostachys cinerea* and *Sterculia setigera* Methanolic Extracts and Fractions.

The results of phytochemical screening of the methanolic extract and fractions of root bark of *D. cinerea* are presented in Table 4.2.1.The result indicated n-hexane fraction recorded no amount of cardiac glycosides, saponins in addition to alkaloids and flavonoids, but the ethylacetate fraction contained phytochemicals like saponins, tannins, carbohydrates, steroids, alkaloids, triterpenes, and flavonoids, but cardiac glycosides were absent. Butanolic fraction also containend carbohydrate, cardiac glycoside, tannins, steroides and triterpenes, and saponins although alkaloids and flavonoids were absent. The aqueous and crude methanolic fractions contained all phytochemicals including cardiac glycosides. However, Anthraquinones were consistently absent in the crude extract and in all the fractions.

Table 4.2.2. shows phytochemical screening on crude methanolic extract and fractions of the leaves of *S. setigera.* The leaves n-hexane fraction showed the presence of flavonoids, steroids & triterpenes, carbohydrate, and cardiac glycosides. Th ethylacetate fraction contained cardiac glycosieds, tannins, flavonoids, carbohydrate, steroides and triterprens, saponins and alkaloids. Butanol fraction showed the presence of all other phytochemicals except for alkaloids. The aqueous fraction contained carbohydrate, cardiac glycoside, flavonoids, tannins, steroids, alkaloids, and triterpenes, though saponinns were absent in froth test. Anthraquinones were however absent in the methanolic extract and in all the fractions.

Table 4.2.1: Phytochemical Screening of a Crude Methanolic Extract of the Root Bark of *Dichrostachys cinerea* and of its Various Derived Solvent Fractions

Photochemical	Ethylacetate	n-hexane	Butanol	Aqueous	Crude Methanol
Carbohydrate	+	+	+	+	+
Alkaloids	+	-	-	+	+
Flavonoids	+	-	-	+	+
Steroids & Triterpenes	+	+	+	+	+
Cardiac glycoside	-	-	+	+	+
Tannins	+	+	+	+	+
Saponins	+	-	+	+	+
Anthraquinone	-	-	-	-	-

KEY; + = Present, - =Absent

Table 4. 3.2: Phytochemical Screening of a Crude Methanolic Extract of the Leaf of *Sterculia setigera* and of its Various Derived Solvent Fractions

Phytochemical	Ethylacetae	n-hexane	Butanol	Aqueous	Crude Methanol
Carbohydrate	+	+	+	+	+
Alkaloids	+	-	-	+	+
Flavonoids	+	+	+	+	+
Steroids & Triterpenes	+	+	+	+	+
Cardiac glycoside	+	+	+	+	+
Tannins	+	-	+	+	+
Saponins	+	-	+	-	+
Anthraquinone	-	-	-	-	-

KEY; + = Present, - =Absent

4.3 Methaemoglobin Concentration Analysis

The effect of various fractions of the root bark methanolic extract of *Dichrostachys cinerea* and leaf methanolic extract of *Sterculia setigera* at different concentrations on the percentage methaemoglobin concentration reduction was inversely proportional to the extracts and fraction concentrations. It is seen here that for all the methanolic extracts and fractions, percentage methaemoglobin concentration decreased with increase in the concentration of both methanolic extracts and methanolic extract fractions.

The percentage of plasma methaemoglobin concentration in the presence of various concentrations (0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml, and 1mg/ml) of crude methanolic extract and various methanolic extract fractions of the root bark of *Dichrostachys cinerea* was presented in table 4.3.1. The methanolic extract showed a significant ($p<0.05$) decrease in the methaemoglobin level at all extract concentrations when compared with the control, but the n-hexane fraction showed no significant ($p<0.05$) decrease in the methaemoglobin level at 0.6mg/ml and 0.8mg/ml with value of 20.59±0.38% and 24.51±0.11% respectively. Similarly, ethylacetae fraction had no significant ($p<0.05$) decrease in the methaemoglobin level at 0.4mg/ml and 0.6mg/ml with value of 20.34±0.01%. However, the butanolic and aqueous fractions significantly ($p<0.05$) decreased the level of methaemoglobin concentration by least concentrations of 10.44±0.21% and 10.18±0.17% respectively when compared with the control.

Table 4.3.2 shows the effect of methanolic extract and the various methanolic extract fractions of the leaf of *Sterculia setigera* at different concentrations on percentage methaemoglobin. It is seen here that for the methanolic extract and all the fractions, there was a significant ($p>0.05$) decrease at all extract concentrations when compared with the distilled water and blood control

with the exceptions of n-hexane and butanolic fractions at 0.2mg/ml having a methaemoglobin concentration value of 20.20±0.12%, and 21.38±0.24% respectively.

Table 4.4.1: The Effect of a Methanolic Extract of the Root Bark of *Dichrostachys cinerea* and of its Various Derived Solvent Fractions on Percentage Methaemoglobin, at Different Concentrations.

Extract Conc.	%Methaemoglobin After Treatment With Methanolic Extract And Their Fractions				
(Mg/ml)	Methanol	n- hexane	Ethylacatate	Butanol	Aqueous
0.2	13.38±0.55^{a}	16.34±0. 29^{ab}	14.87±0.47^{a}	16.01±0.13^{a}	18.64±0.67^{ab}
0.4	12.61±0.86^{a}	11.48±0.12^{a}	20.34±0.01^{b}	10.18±0.17^{a}	11.13±0.32^{a}
0.6	10.20±0.90^{a}	20.59±0.38^{a}	12.51±0.44^{a}	15.13±0.89^{a}	14.89±0.53^{a}
0.8	10.39±0.47^{a}	15.29±0.10^{a}	11.11±0.08^{a}	12.98±0.13^{a}	11.20±0.96^{a}
1	9.52±0.78^{a}	24.51±0.11^{a}	11.64±0.21^{a}	12.88±0.37^{a}	10.44±0.21^{a}
Control	22.51±0.92^{b}	22.51±0.92^{b}	22.51±0.92^{b}	22.51±0.92^{b}	22.51±0.92^{b}

The values in the table are the Mean±SD from duplicate experiments. Values with different superscript vertically shows decrease in % methaemoglobin concentration and are significantly different at $p<0.05$.

Table 4.3.2: The Effect of a Methanolic Extract of The Leaf of *Sterculia setigera* and of its Various Derived Solvent Fractions on Percentage Methaemoglobin, at Different Concentrations

Extract Conc	% Methaemoglobin after Ttreatment with Methanolic Extract and their Fractions				
Mg/ml	Methanol	n- hexane	Ethylacatate	Butanol	Aqueous
0.2	11.68±0.73^{a}	15.55±0.28^{ab}	20.20±0.12^{b}	21.38±0.24^{b}	17.32±0.11^{ab}
0.4	11.25±0.94^{a}	13.48±0.33^{a}	11.81±0.10^{a}	11.17±1.21^{a}	15.88±0.58^{ab}
0.6	10.19±0.67^{a}	11.11±0.66^{a}	15.29±0.21^{ab}	13.69±0.17^{a}	13.23±0.25^{a}
0.8	9.45±0.83^{a}	14.71±2.46^{a}	12.57±0.29^{a}	9.76±0.56^{a}	13.96±0.14^{a}
1	8.21±0.15^{a}	9.61±0.13^{a}	11.81±0.47^{a}	12.64±0.67^{a}	13.00±1.03^{a}
Control	22.51±0.92^{b}	22.51±0.92^{b}	22.51±0.92^{b}	22.51±0.92^{b}	22.51±0.92^{b}

The values in the table are the Mean±SD from duplicate experiments. Values with different superscript vertically shows increase in % methaemoglobin concentration and are significantly different at $p<0.05$.

4.4 Membrane Stability Test Result:

Results from all the fractions of both the root bark of *D. cinerea* and leaf of *S. setigera* exhibit a membrane protection role in a concentration dependant manner for methanolic extracts, methanolic extract fractions and hypotonic buffered saline.

Table 4.4.1 shows the effect of the crude metanolic extract of root bark of *D. cinerea* on membrane stability of human sickled red blood cells exhibiting a highest prevention of haemolysis at 2.5mg/ml extract concentration and 0.9ml saline concentration with an activity of 88.37±0.09% which has no significant difference ($p< 0.05$) with ibuprofen, and lowest activity at 0.5mg/ml and 0.1ml saline concentration having 44.82±0.02% activity. However a significant difference was seen between the 0.1ml and 0.9ml saline concentrations.

Table 4.4.2 shows the effect of n-hexane fraction of the root bark methanolic extract of *D. cinerea* on membrane stability of human sRBC. The highest inhibiton of haemolysis was recorded at 2.5mg/ml and 0.9ml saline concentration with an activity of 79.50±0.04 which has a significant difference ($p<0.05$) when compared with ibuprofen (94.02±0.75) .the least membrane protection role was seen in 0.5mg/ml and 0.1ml saline concentration with an activity of 16.60±0.01%.

Table 4.4.1: Effect of The Methanolic Extract Derivative of the Root Bark of *Dichrostachys cinerea* on Membrane Stability of Human Erythrocytes.

Extract Conc.	Membrane Stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline Volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	44.82±0.02^{a}	81.61±0.07^{bc}	77.58±0.07^{b}	79.09±0.03^{b}	77.20±0.02^{b}	84.13±0.42^{bc}
1.0	65.15±0.09^{ab}	84.38±0.49^{c}	84.88±0.63^{bc}	83.75±0.09^{bc}	83.50±0.04^{bc}	85.76±0.01^{bc}
2.0	74.93±0.04^{b}	76.82±0.02^{b}	76.44±0.33^{b}	87.53±0.23^{c}	85.78±0.28^{bc}	80.22±0.12^{b}
2.5	79.26±0.11^{b}	85.26±0.23^{c}	86.77±0.17^{c}	87.02±0.05^{c}	85.39±0.01^{bc}	88.35±0.09^{c}
Ibuprofen 2.5	96.63±0.71^{c}	96.63±0.71^{c}	96.63±0.71^{c}	96.63±0.71^{c}	96.63±0.71^{c}	96.63±0.71^{c}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

Table 4.4.2: Effect of the n-hexane Fraction Derivative of the Root Bark Methanolic Extract of *Dichrostachys cinerea* on Membrane Stability of Human Erythrocytes.

Extract Conc.	Membrane stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline Volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	16.60±0.01^{a}	21.12±0.05^{a}	25.50±0.03^{a}	35.50±0.03^{a}	34.62±0.09^{a}	40.87±0.01^{ab}
1.0	41.25±0.13^{ab}	44.50±0.03^{ab}	70.37±0.01^{b}	58.12±0.09^{bc}	62.25±0.11^{b}	72.75±0.06^{c}
2.0	52.00±0.08^{b}	56.37±0.05^{b}	65.62±1.03^{b}	66.62±0.05^{bc}	68.22±0.03^{bc}	77.50±0.09^{c}
2.5	68.00±0.87^{b}	69.56±0.07^{b}	73.00±0.12^{c}	71.15±0.05^{c}	72.50±0.04^{c}	79.50±0.04^{c}
Ibuprofen 2.5	94.02±0.75^{d}	94.0±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

Effect of Ethylacetate fraction of methanolic extract of the root bark of *D. cinerea* on membrane stability of human red blood cells is shown in Table 4.4.3. It can be observed that the highest percentage of inhibition of haemolysis was at in 2.5mg/ml and 0.5ml saline concentration which showed an activity of 72.25±0.09 and this activity was significantly different ($p< 0.05$) with ibuprofen whose activity stood at 94.02±0.75%. The least activity was recorded at 0.5mg/ml and 0.1ml saline concentration which only showed membrane protection capacity of 19.87±0.02%.

Table 4.4.4 shows the effect of butanolic fraction of methanolic extract of the root bark of *D. cinerea* on membrane stability of human erythrocytes. Of all the fractions from methanolic extract of the root bark of *D. cinerea,* this fraction the highest activity, exhibiting a highest prevention of haemolysis at 2.5mg/ml and 0.6ml saline concentration with an activity of 90.12±0.01with no significant difference when compared ($p< 0.05$) with ibuprofen with an activity of 94.02±0.75%. The least membrane protection activity was seen in 0.5mg/ml and 0.1ml saline concentration with 18.37±0.00%.

Furthermore, the aqueous methanolic extract fraction of the root bark of *D. cinerea's* effect on inhibition of haemolysis was presented in table 4.4.5. It can be seen here that highest prevention of haemolysis at 2.5mg/ml and 0.9ml and saline concentration with an activity of 88.37±0.09% having no significant difference ($p< 0.05$) with the activity of ibuprofen whose activity is 94.02±0.75%. 25.12±0.01% recorded in 0.5mg/ml and and 0.1ml saline concentration was the least activity of this fraction.

Table 4.4.3: Effect of the Ethylacetate Fraction Derivative of the Root Bark Methanolic Extract of *Dichrostachys cinerea* on Membrane Stability of Human Erythrocytes.

Extract Conc.	Membrane Stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline Volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	19.87±0.02^{d}	42.90±0.01^{cd}	50.37±0.02^{bc}	71.12±0.03^{c}	73.62±0.02^{c}	85.62±0.03^{b}
1.0	28.23±0.03^{d}	42.11±0.06^{cd}	52.28±0.33^{cd}	50.75±0.01^{cd}	78.50±0.00^{bc}	84.65±0.02^{b}
2.0	32.97±0.17^{d}	47.84±0.03^{cd}	60.87±0.01^{c}	84.25±3.21^{b}	69.25±0.01^{c}	65.50±0.01^{c}
2.5	41.25±0.05^{cd}	60.06±0.11^{c}	64.50±0.04^{c}	72.25±0.09^{c}	66.62±0.06^{c}	67.80±0.01^{c}
Ibuprofen 2.5	94.02±0.75^{a}	94.02±0.75^{a}	94.02±0.75^{a}	94.02±0.75	94.02±0.75^{a}	94.02±0.75^{a}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

Table 4.4.5: Effect of the Butanolic Fraction Derivative of the Root Bark Methanolic Extract of *Dichrostachys cinerea* on Membrane Stability of Human Erythrocytes.

Extract Conc.	Membrane Stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline Volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	18.37±0.14^{a}	21.56±0.02^{a}	28.22±0.07^{a}	21.76±0.01^{a}	22.88±0.03^{a}	35.00± 0.01^{ab}
1.0	38.28±0.03^{ab}	53.29±0.06^{b}	53.75±0.07^{b}	36.87±0.11^{ab}	41.37±0.06^{ab}	33.89±0.04^{ab}
2.0	32.78±0.05^{ab}	49.58±0.09^{b}	58.77±0.01^{b}	60.00±0.25^{b}	55.76±0.09^{b}	62.12±0.06^{b}
2.5	40.62±0.01^{ab}	84.12±0.08^{c}	85.50±0.03^{c}	84.00±0.02^{c}	90.12±0.01^{d}	88.50±0.06^{cd}
Ibuprofen 2.5	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

Table 4.4.6: Effect of the Aqueous Fraction Derivatives of the Root Bark Methanolic Extract of *Dichrostachys cinerea* on Membrane Stability of Human Erythrocytes.

Extract Conc.	Membrane stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	25.12±0.01^{a}	34.87±0.02^{b}	33.76±0.04^{ab}	35.12±0.03^{b}	32.25±2.04^{ab}	31.50± 0.04^{ab}
1.0	28.50±0.07^{a}	23.45±0.09^{a}	29.27±0.05^{ab}	29.25±0.07^{ab}	26.37±0.08^{ab}	41.81±0.08^{ab}
2.0	35.23±0.03^{ab}	34.62±0.03^{a}	20.00±0.02^{a}	32.87±0.07^{ab}	48.22±0.03^{b}	47.62±0.09^{b}
2.5	54.29±0.12^{bc}	82.05±0.04^{cd}	86.24±0.19^{cd}	87.12±0.04^{cd}	61.59±0.39^{c}	88.37±0.09^{d}
Ibuprofen 2.5	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

Table 4.4.6 shows the effect of methanolic extract of the leaf of *S. setigera* on membrane stability of human sRBC exhibiting a highest prevention of haemolysis at 2.5mg/ml with an activity of 90.37±0.02% which has no significant difference ($p< 0.05$) with ibuprofen which showed an activity of 96.63±0.71% and lowest activity at 0.5mg/ml having 55.89±0.23%.

The n-hexane methanolic extract fraction effect on membrane stability of human sRBC was displayed in table 4.4.7. From the results this fraction shows the least activity of all the fractions from *S. setigera* having its highest membrane protection effect at extract concentration of 2.5mg/ml, and saline concentration of 0.5ml with 61.25±0.05% inhibition which have a significant difference when compared ($p< 0.05$) with ibuprofen with an activity of 94.02±0.75%. The least activity was recorded in 0.5mg/ml having only 8.66±0.03%.

Table 4.4.6: Effect of The Methanolic Extract of The Leaf of *Sterculia setigera* on Membrane Stability of Human Erythrocytes.

Extract Conc.	Membrane Stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline Volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	55.89±0.23^{a}	57.86±0.03^{a}	64.33±0.08^{a}	72.05±0.04^{b}	77.54±0.07^{b}	84.13±0.12^{bc}
1.0	60.25±0.02^{a}	59.66±0.02^{a}	67.54±0.41^{ab}	87.50±0.34^{bc}	83.50±0.85^{bc}	89.25±0.09^{c}
2.0	77.20±0.05^{b}	87.78±0.41^{c}	86.02±0.03^{bc}	89.59±0.25^{c}	86.27±0.03^{bc}	91.30±0.02^{c}
2.5	75.81±0.11^{b}	84.63±0.09^{bc}	88.66±0.05^{c}	86.27±0.82^{bc}	85.64±0.22^{bc}	78.71±0.03^{b}
Ibuprofen 2.5	96.63±0.71^{c}	96.6±0.71^{c}	96.63±0.71^{c}	96.63±0.71^{c}	96.63±0.71^{c}	96.63±0.71^{c}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

Table 4.4.7: Effect of the n-hexane Fraction Derivative of the Leaf Methanolic Extract of *Sterculia setigera* on Membrane Stability of Human Erythrocytes.

Extract Conc.	Membrane stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline Volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	8.66±0.03^{a}	13.00±0.02^{a}	10.62±0.03^{a}	33.12±0.07^{ab}	48.12±0.02^{b}	61.00±0.08^{c}
1.0	28.00±0.02^{ab}	35.62±0.07^{ab}	29.25±0.04^{a}	24.10±0.10^{a}	41.62±0.26^{ab}	29.12±0.13^{ab}
2.0	48.50±0.01^{b}	53.60±0.05^{b}	52.33±0.08^{b}	52.97±0.04^{b}	48.50±0.03^{b}	47.87±0.05^{b}
2.5	55.50±0.06^{bc}	58.12±0.01^{bc}	51.75±0.05^{b}	61.25±0.05^{c}	55.87±0.06^{bc}	55.12±0.07^{bc}
Ibuprofen (2.5)	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

The results presented in table 4.4.8 shows the effect of ethylacetate fraction of of the leaf the methanolic extract of *S. setigera* on membrane stability of human sRBC. It can be seen from this table that there is a considerable rise in percentage inhibition of haemolysis from the previous fraction, having its highest activity in 2.5mg/ml and and 0.9ml saline concentration with 86.37±0.01% having no significant difference ($p< 0.05$) when compared to ibuprofen (94.02±0.75%). The least membrane protection role was however seen in 0.5mg/ml and and 0.1ml saline concentration with an activity of 27.75±0.01%.

Similarly, Table 4.4.9 Showed the effect of butanolic fraction of the leaf methanolic extract of *S. setigera* on membrane stability of human sRBC exhibiting a highest prevention of haemolysis at 2.5mg/ml and and 0.6ml saline concentration with an activity of 90.37±0.02% which has no significant difference ($p< 0.05$) with ibuprofen which showed an activity of 94.02±0.75% and lowest activity at 0.5mg/ml having 11.50±0.02%.

Table 4.4.10 Showed the effect of aqueous methanolic extract fraction of the leaf of *S. setigera* on membrane stability of human sickle erythrocytes. From the result, The highest protection was observed at 2.5mg/ml concentration and and 0.5ml saline concentration which gives a 89.87±0.04% protection against haemolysis, while the lowest protection was observed at 0.5mg/ml and and 0.1ml saline concentration which gives 21.53±0.09%. while the ibuprofen (positive control) gives 94.02±0.75%, and shows a significant difference ($p< 0.05$) when compared with the fraction at all concentrations.

Table 4.4.8: Effect of the Ethylacetate Fraction Derivative of the Leaf Methanolic Extract of *Sterculia setigera* on Membrane Stability of Human Erythrocytes.

Extract Conc.	Membrane Stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline Volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	27.75±0.01^{a}	47.50±0.16^{ab}	46.25±0.01^{ab}	60.25±0.03^{bc}	58.25±0.04	75.50±0.27^{d}
1.0	36.25±0.11^{ab}	41.82±0.04^{ab}	49.11±0.03^{b}	58.12± 0.02^{b}	60.00±0.11^{bc}	80.00±0.06^{cd}
2.0	53.62±0.02^{b}	60.90±0.06^{bc}	60.01±0.05^{bc}	63.37±0.02^{bc}	81.62±0.04^{d}	78.87±0.13^{c}
2.5	68.37±0.09^{c}	77.22±0.03^{c}	79.93±0.03^{c}	75.12±0.03^{c}	76.87±0.09^{c}	86.37±0.01^{c}
Ibuprofen 2.5	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

Table 4.4.9: Effect of the Butanolic Fraction Derivative of the Leaf Methanolic Extract of *Sterculia setigera* on Membrane Stability.

Extract Conc.	Membrane Stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline Volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	11.50±0.02^{a}	21.12±0.02^{a}	2 7.75±0.08^{a}	34.62±0.03^{ab}	21.75±0.10^{a}	53.75± 0.12^{b}
1.0	56.00±0.05^{b}	63.50±0.03^{bc}	59.50±0.01^{bc}	48.50±0.01^{ab}	60.12±0.03^{b}	74.25±0.05^{b}
2.0	58.12±0.04^{bc}	81.00±0.05^{c}	84.87±0.02^{c}	67.50±0.09^{bc}	78.37±0.21^{c}	89.37±0.11^{cd}
2.5	84.12±0.04^{cd}	85.50±0.01^{cd}	89.25±0.02^{cd}	84.46±0.01^{cd}	90.37±0.02^{d}	90.00±0.02^{d}
Ibuprofen 2.5	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

Table 4.4.10: Effect of the Aqueous Fraction Derivative of the Leaf Methanolic Extract of *Sterculia setigera* on Membrane Stability of Human Erythrocytes.

Extract Conc.	Membrane Stability (% Inhibition of Haemolysis at Different Saline Concentrations)					
	Saline Volumes					
(mg/ml)	0.1ml	0.2ml	0.3ml	0.5ml	0.6ml	0.9ml
0.5	21.53±0.09^{a}	21.62±0.22^{a}	27.00±0.01^{a}	34.62±0.05^{ab}	41.00±0.02^{ab}	77.00±0.01^{b}
1.0	77.87±0.04^{b}	85.50±0.25^{c}	66.12±0.03^{b}	82.00±0.03^{c}	72.87±0.02^{b}	66.37±0.03^{b}
2.0	84.25±0.02^{c}	82.50±0.09^{c}	68.00±0.19^{b}	80.00±0.05^{c}	78.12±0.10^{bc}	76.25±0.05^{b}
2.5	86.87±0.02^{cd}	87.87±0.05^{c}	87.99±0.03^{cd}	89.87±0.04^{cd}	85.37±0.03^{c}	87.87±0.07^{cd}
Ibuprofen 2.5	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}	94.02±0.75^{d}

Values are mean ± SD of the mean of duplicate experiments. Values with different superscript vertically shows increase in % inhibition of haemolysis and are significantly different at $p<0.05$.

4.5 Mineral Analysis

Table 4.5.1 shows the concentration of minerals in various fractions of methanolic extract of root bark of *Dichrostachys cinerea*. From the results, it can be seen that the aqueous fraction of *D. cinerea* has the highest amount of iron with a value of 287.20±0.00ppm, while the least amount was recorded in the ethylacetate fraction with a value of 50.64±0.00ppm. again, the aqueous fraction had the highest zinc concentration of 72.60±0.00ppm. Ethylacetate fraction had the highest concentration of magnesium with a value of 1505.20±0.00ppm. The highest concentration of copper was recorded in the aqueous fraction, n-hxane fraction had 8.00±0.00ppm, while it was absent in both ethylacetate and butanol fractions. For chromium, the highest value was observed in aqueos fraction followed by the the n-hexane fraction, but it was not recorded in ethylacetate and butanol fractions.

Table 4.5.2 shows the concentration of minerals in various fractions of methanolic extract of the leaf of *Sterculia setigera*. Here, it can be seen thatht butanol fraction had the highest concentrations of iron, zinc, and magnesium with values of 233.60±0.00, 365.20±0.00, 141.52±0.00ppm respectively. However, copper and chromium was found to be absent in both ethylacetate fractions.

Table 4.5.1: The Concentration of Minerals in Various Solvent Fractions of Methanolic Extract of The Root Bark of *Dichrostachys cinerea*.

Extract	Elements (ppm)				
Fraction	Iron	Zinc	Magnesium	Copper	Chromium
Ethyl acetate	50.64±0.00	19.28±0.00	1505.20±0.00	0.00±0.00	0.00±0.00
N-hexane	165.60±0.00	12.04±0.00	1272.00±0.00	8.00 ±0.00	15.2±0.00
Butanol	137.60±0.00	20.32±0.00	1419.20±0.00	0.00±0.00	0.00±0.00
Aqueous	287.20±0.00	72.60±0.00	1115.60±0.00	57.20±0.00	20.80±0.00

The values in the table are the Mean±SD from duplicate experiments.

Table 4.5.2: The Concentration of Minerals in Various Solvent Fractions of Leaves Methanolic Extract of *Sterculia setigera*.

Extract	Concentration of Elements (ppm)				
Fractions	Iron	Zinc	Magnesium	Copper	Chromium
Ethyl acetate	102.80±0.00	1383.60±0.00	3.64±0.00	0.00±0.00	1.20±0.00
N-hexane	133.20±0.00	1411.60±0.00	10.96±0.00	0.00 ± 0.00	0.00± 0.00
Butanol	233.60±0.00	3657.20±0.00	141.52±0.00	6.90±0.00	0.00± 0.00
Aquoues	194.80±0.00	1285.20±0.00	75.72±0.00	46.00±0.00	0.00±0.00

The values in the table are the Mean±SD from duplicate experiments.

CHAPTER FIVE

5.0 Discussion

The results of antisickling assay of the different methanolic extracts and methanolic extracts fractions of the root bark of *Dichrostachys cinerea* and leaves of *Sterculia setigera* in this study showed that they exhibited substantial antisickling activity. It was also noticed that the antisickling effect in conformity with earlier reports of Chikezie 2011 was found to be dose and time dependent, the antisickling effect increased as the concentration of the methanolic extract and their fractions increases. The observed increased in antisickling effect could be related to an increase in the concentration of an active substance present in the extracts. The aqueous methanolic extract fraction of *D. cinerea* significantly increased the percentage number of reversed red blood cells to to the highest point of 66.08±2.82% at 0.2mg/ml. The butanol fraction showed highest significant antisickling activity of 57.81±5.66% at 120mins incubation time. The ethylacetate fraction showed highest significant antisickling activity of 41.02±0.76% at 120mins incubation time. While the n-hexane fraction had the highest activity of 16.65±2.87% of reversed sRBC cells at 0.3mg/ml concentration. Of the investigated *D. cinerea* extract and fractions, the aqueous fraction of *D. cinerea* exhibited the highest antisickling activity while the n-hexane fraction exhibited the least activity.

For *Sterculia setigera*, highest antisickling activity was exhibited by ethylacetate methanolic extract fraction having the highest significant antisickling activity of 65.29±2.86% at 120mins incubation time. This is followed by the n-hexane methanolic extract fraction which have 49.76±1.49% of reversed sRBC cells at 0.3mg/ml concentration as the highest activity. The butanol fraction showed highest significant antisickling activity of 46.01±1.49% at 120mins

incubation time. However, the least antisickling activity was exhibited by butanol fraction showing its highest significant antisickling activity of 43.92±4.59% at 120mins incubation time.

The ability of any material to elicit antisickling potential implies that such material would interfere in three different stages of sickling process. Antisickling agents may have the target of modifying at the sickle gene polymerization and red cell membrane levels (Dash, *et al*, 2013). Recently, a good number of studies have been carried out to identify and characterise some antisickling compounds from different plant sources. The most promising were found to be anthocyanins, anthraquinones, steriodal gylcosides, cardiac glycosides, alkaloids, flavonoids, saponins, tannins, phenols, hydroxybenzoic acids, liminoids, 5-hydroxymethyl-2-furfurals (5HMF), isomeric divanilloylquinic acid and certain amino acids such as arginine, tyrosine, aspartic acid and phenylalanine (Ameh, *et al*, 2012;Dash *et al*, 2013). The observed antisickling property in all the methanolic extracts and their fractions may be due to the presence of some secondary metabolite that are present in the fractions like cardiac glycosides, alkaloids, steroides and terpenes. Studies demonstrated that antioxidant molecules were found to be potent inhibitors of sickle cell haemoglobin polymerization, and equally improved the oxidant status of sickle erythrocytes (Imaga et al 2011; Nwaoguikpe and Braide 2012). Moreover, most antioxidants, like the antioxidant metals like zinc, and copper found in this fraction, some amino acids like arginine, leucine may be also localized in this fractions and these may be acted synergistically to elicit such pronounced antisickling response. Alkaloids though not proven yet to possess sickling inhibition activity, has been demonstrated to show other positive biological effect like alleviation of pain that may be of great therapeutic advantage in the management of sickle cell disease.

The results of phytochemical screening of the various methanolic extracts and methanolic extract fractions of the root bark of *Dichrostachys cinerea* and those of the leaves of *Sterculia setigera* presented in table 4.2.1 and 4.2.2 showed that all the methanolic extracts and their fractions of both plants parts contains phytochemicals that mediate some biological activities such as regulation of cell growth and division, stimulation of erythropoesis, stimualtin of production of white blood cells, reduction of inflammation,etc. The methanolic extracts and their fractions contained such phytochemicals like alkaloids, aldehydes/ketones, carboxylic acids,esters, flavonoids, glycosides,saponins,steroid/triterpenes and tannins. The contributions of phytochemicals to the antisickling activity have been reported by some researchers (Adejumo *et al*, 2012). Banso and Adeyemo (2007) also detected that tannins and alkaloids from *Dichrostachys cinerea* possessed antibacterial activities against grampositive bacterial strains more than gram-negative bacteria. The presence of alkaloids in the ethylacetate, and aqueous methanolic fractions of both the root bark of *D. cinerea* and leaf of *S.*setiger*a* is an indication that they may be useful in alleviating some of the symptoms associated with pains (Ejele and Aneke, 2011). Flavonoids act on the gastro-intestinal tract to increase peristalsis (Jeremy, and Spencer 2015). The presence of flavonoids in the ethylacetate, and aqueous fractions of the root bark of *D. cinerea* and ethylacetate, n-hexane, butanol, and aqueous methanolic extract fractions of the leaf of *S. setigera* is evident that they may be useful as a mild laxative especially in cases where sickle cell patients complain of constipation. Tannins are phenolic glycosides and are non-nitrogenous plant constituents with astringent properties on mucous membranes (Eleazu *et al* 2012).The tannins present in butanol, aqueous, ethylacetate, and n-hexane methanolic extract fractions of the root bark of *D. cinerea* and aqueous, butanol, and ethylacetate methanolic extacts fractionsas well as the methanolic extract of the leaves of *S. Setigera* may be useful in cleansing

the surface of chronic skin ulcers that develop as a complication of sickle cell disease (Ejele and Alinnor, 2010). The presence of cardiac glycosides indicated that these glycosides may be potent in managing cardiac insufficiency, coughs and circulatory problems in sickle cell patients.They may also act as good sedatives and have antispasmodic properties (Ejele *et al.*, 2012).

It has been reported that certain saponins and flavonoids exerted profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro,* while tannins and saponins posses ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules (Oyedapo *et al.*, 2004) thus may reduce frequency of sickle cell anaemia crises. In a related develpoment, Ibrahim *et al* ,(2007) reported that saponins, in addition to carboxylic acids and flavonoids may be responsible for the antisickling activity of *H. acida*. The presence of these pyhtochemicals could justify thier use by traditional herbalists in managing sickle cell anaemia.

An increase in Fe^{2+}/Fe^{3+}ratios, or plasma methaemoglobin concentration upon application of a drug or plant extract indicates a reversal of sickling, suggesting conversion of deoxyHbS to oxyHbS (Osuagwu 2010). The findings from the present study showed that, when concentrations of the root bark methanolic extracts and methanolic extract fractions of *D. cinerea* and *S setigera* increases, plasma methaemoglobin concentration significantly decreased. The ethylacetate methanolic extract fraction of *D. cinerea* significantly decreased the percentage of plasma methaemoglobin to 11.64±0.21%, the butanol methanolic extract fraction decreased percentage methaemoglobin to 12.88±0.37, while the aqueous fraction significantly decreased the percentage of methaemoglobin to 10.44±0.21%. Similarly, all the methanolic extract fractions as well as the methanolic extract of the leaf of *S. setigera* showed a significant decrease in the percentage level of methaemoglobin concentration bringing the methaemoglobin levels to

11.81±0.47%, 9.61±0.13%, 12.64±0.67%, and 13.00±1.03% for ethylacetate, n-hexane, butanol, and aqueous methanolic extract fractions respectively.This has shown that the methanolic extract and its fractions are an effective antioxidant on sRBC membrane after incubation for 1 hour. The possible mechanism of action of the methanolic extracts and their fractions may be due to the following reasons; firstly the methanolic extract and their fractions maybe involved in the scavenging of alkoxy or peroxy radicals terminating a chain reaction or decreased initiation of the lipid peroxidation. Secondly, The methanolic extracts and their fractions prevented haemoglobin from oxidizing into methaemoglobin and inhibit the generation of free radicals by improving the metabolic shunt pathway of pentose phosphate in erythrocytes is necessary for the synthesis of NADPH (reducing power) that protects haemoglobin and membrane lipids against oxidation, NADPH is normally defective in sickle erythrocytes. This system reduces Fe^{3+} of the heme to Fe^{2+} and it includes the Nicotinamide Adenine Dinucleotide Phosphate (NADPH), methaemoglobin reductase and cytochrome B5. NADPH enables the synthesis of reduced glutathione (GSH) to reduce the cytotoxic action of hydrogen peroxide (Nanfack *et al* 2013). Thirdly, another likely mechanisms may be due to the fact that the extracts may contain a compound that binds to ferric iron, thereby making it biologically unavailable.

Ethylacetate fractions of the methanolic extracts from both plants had the highest reduction in methaemoglobin concentration in correlation with previous work on *Garcinia xanthochymus* which revealed that the high antioxidant activity of ethylacetate could be attributed to the high content of total phenols In fact, ethylacetate seems to be the solvent that concentrates best phenolic substances of intermediate polarity (Meng *et al* 2012).

On the other hand the untreated blood sample at the same time of incubation gave 22.0±1.10%. This is due to accumulation of the reactive oxygen species within the erythrocytes. Nwaoguikpe

and Braide (2012), have shown that, sickle cells generate about twice the amount of activated oxygen species found in untreated RBCs. The reason for this increase in oxygen radicals is the results of accelerated auto oxidation of HbSS to methaemoglobin a conversion that causes release of heme (Hebbel, 2000).

Plants have principles that possess ability to facilitate the stability of biological membranes when exposed to induce lysis (Oyedapo *et al.,* 2004). Several reports have supported the fact that, the membranes of human erythrocytes Hb AA, HbAS, and HbSS blood types have different stability as evaluated from the mean corpuscular fragilty (Elekwa *et al.,* 2003; Okpuzor *et al.,* 2008; Mpiana *et al*., 2011). Therefore, plant extract that can positively affect the red cell membrane would be useful in the management of sickle cell anaemia. The pharmacological agents that alter membrane stability could be applied in the control of sickling process of the erythrocytes, a major physiological manifestation of the sickle cell disease (Amujuyegbe *et al.,* 2012).

From the study, it was observed that, the methanolic extract and the different methanolic extract fractions of the leaf of *S. setigera* showed an appreciable level of membrane protection agents hypotonic induced lysis at different concentrations. The n-hexane fraction displayed 55.50±0.06% at 2.5mg/ml, the ethylacetate fraction displayed 68.37±0.09% at 2.5mg/ml, the butanolic fraction displayed 84.12±0.04% at 2.5mg/ml, while the aqueous fraction showed 86.87±0.02% also at 2.5mg/ml. moreover, the methanolic extract of the plant also showed 45.3±0.57% protection against induced lysis.

Also, the methanolic extract and the methanolic extract fractions of the root bark of *D cinerea* showed promising cytoprotective effect against hypotonic induced lysis when compared with standard drug ibuprofen. The n-hexane fraction showed 68.00± 0.87% at 2.5mg/ml, the

ethylacetate fraction showed 41.25±2.64% also at 2.5 mg/ml. the butanolic fraction displayed 40.62±0.04% at 2.5mg/ml, while the aqueous fraction showed 54.29±0.12% also at 2.5mg/ml. moreover, the methanolic extract of the plant also showed 45.3±0.57% protection against induced lysis.

Haemolysis of sickle red blood cells decreases with exposure to increasing concentrations of hypotonic saline. This indicates that the methanolic extract and the methanolic extract fractions of both plants improved the ability of SS RBC to take up water without lysis occurring. This stabilisation effect could be explained by noting that the methanolic extract and the methanolic extract fractions rendered the SS RBC capable of withstanding higher concentrations of NaCl by increasing the volume of the RBC, reverting the sickling to produce biconcave cells, and, thereby, maintaining membrane integrity. Such effects have been reported for aqueous extracts of *Zanthoxylum macrophylla* roots *Garcinia kola* seeds and homoserine (Elekwa et al 2003; Elekwa et al 2005).

Another possible mechanism for stabilizing activity of methanolic extracts and the methanolic extract fractions of *S. setigera* and *D cinerea* could be an increase in the surface area to volume ratio of the cells which could be about an expansion of membrane or shrinkage of the cell, and an interaction with membrane protein (Mahat and Patil, 2007, lranlye *et al.,* 2011). Furthermore it has also been shown that, the deformability and cell volume of erythrocytes is closely related to the intracellular content of calcium (Gambline *et al.,* 2009). Hence, it may be speculated that, the cyto protective effect on erythrocyte membrane may be due to the ability of the methanolic extract and the methanolic extract extract to alter the influx of calcium into the erythrocytes. The

observed cytoprotective effect of the extract on HbSS erythrocyte suggests the beneficial effect of the plant to HbSS individuals.

CHAPTER SIX

6.0 Conclusion and Recommendation

6.1 Conclusion

The findings in this research reveal the following results

i. The methanol, butanol, ethylacetate, aqueous, and n-hexane of *D. cinerea* root bark showed a significant percentage increase of reversed cells with the value of 69.07±0.37>57.81±1.66>57.64±1.25>47.89±1.21>16.58±2.81 respectively after 120 min of incubation time.

ii. The methanol, ethylacetate, butanol, n-hexane and aqueous, of *S. setigra* leaf showed a significant percentage increase of reversed cells with the value of 70.97±0.38>69.58±1.66>69.52±1.40>49.76±1.49>46.01±1.49 respectively after 120 min of incubation time.

iii. *Dichrostachys cinrea* root bark showed significant decrease in the concentration of methaemoglobin with the least value of 9.52±0.78, 11.48±0.12, 11.11±0.08, 10.18±0.17 for Methanol, n-hexane, Ethylacetate, Butanol, and Aqueous fractions respectively.

iv. *Sterculia setigera* also shows the percentage decrease of methaemoglobin concentration in the order of aqueous>ethylacetate>butanol>n-hexane>methanol> with the least values of 13.00±1.03>11.81±0.10> 9.76±0.56>9.61±0.13>8.21±0.15respectively.

v. *Dichrostachys cinerea* root bark showed a significant membrane protection in increasing order of n-hexane<ethylacetate<methanol<aqueous<butanol with the highest values of 79.50±0.04<85.62±0.00<88.35±0.09<88.37±0.09<90.12±0.01 respectively.

vi. *Sterculia setigera* leaf showed a significant membrane protection in increasing order of n-hexane<ethylacetate<aqueous<butanol<methanol with the highest values of 61.25±0.05<80.00±0.06<89.87±0.04<90.37±0.02< 91.30±0.02 respectively.

vii. Mineral analysis of the fractions of *D. cinerea* root bark showed that highest concentration of iron, zinc, copper, and chromium was found in the Aqueous fraction. Ethylacetate fraction had the highest concentration of magnesium.

viii. Mineral analysis of the fractions of *S.* se*tigera* leaf showed that highest concentration of iron, zinc, and magnesium was found in the butanol fraction, while aqueous fraction had the highest concentration of copper. Ethylacetate fraction had the highest chromium concentration.

6.2 Recommendation

The findings in this work shows that the methanolic extracts of *D. cinearea* and that of *S. setigera* have antisickling, membrane protection, and methaemoglobin concentration reduction properties and contain some important minerals that are of physiological importance to sickle cell patients and i hereby recommend their use in the management of sickle cell anaemia.

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APPENDICES

Appendix I

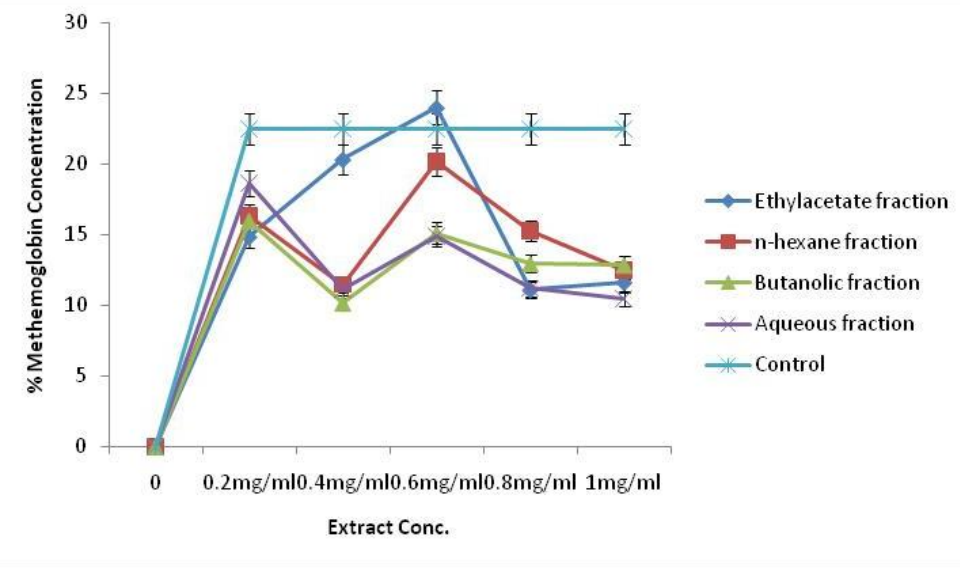


Figure 3: The percentage methemoglobin of various fractions of the root bark of *Dichrostachys cinerea* at different concentrations.

Appendix II

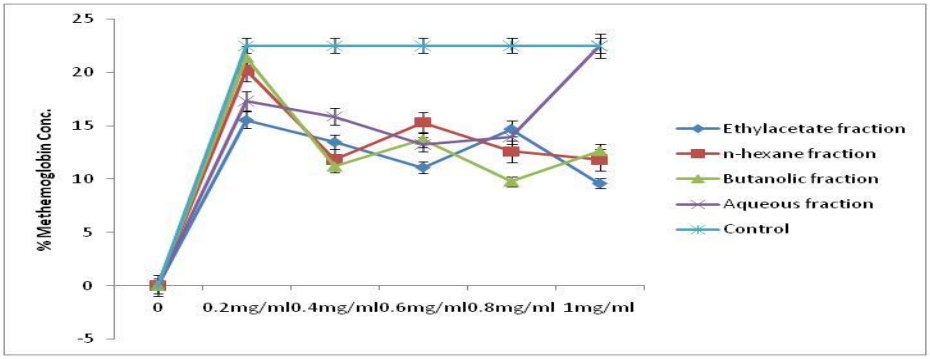


Figure 5: The percentage methemoglobin of various fractions of the root bark of *Sterculia setigera* at different concentrations.

Appendix III

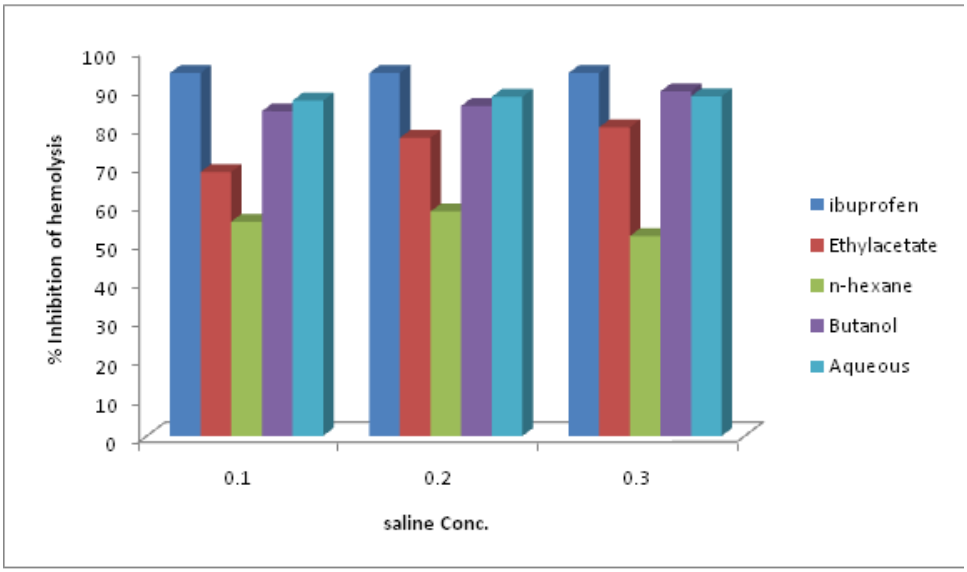


Figure 2: Effect of the Aqueous, ethylacetate, N-hexane, and Butanol Fraction of the Leaves of *Sterculia setigera* on Membrane Stability at Different Saline Concentrations.

AppendiIV

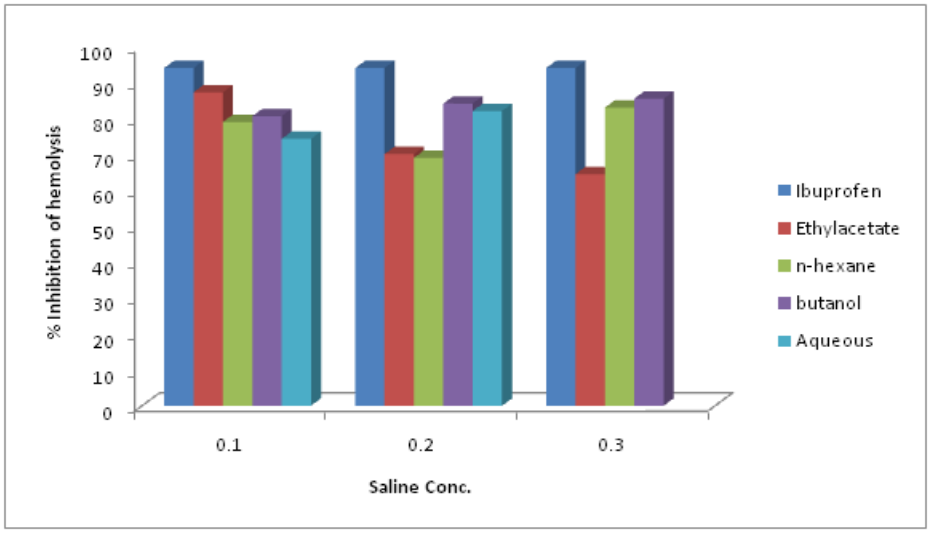


Figure 1: Effect of the aqueous, ethylacetate, n-hexane, and butanol fraction of the root bark of *Dichrostachys cinerea* on membrane stability at different saline concentrations.

Appendix V





TLC plates showing 6:4 n-hexane: ethylacetate system

Appendix VI

Percentage yield of *Dichrostachys cinerea* root fractions

Fractions	Weight (g)	percentage yield (%)
n-hexane	10	11.23
Ethylacetate	24	26.96
Butanol	25	28.08
Aqeuous	30	33.70
Methanol	89	17.80

Appendix VII

Percentage yield of *Sterculia setigera* leaves fractions

Fractions	Weight (g)	percentage yield (%)
n-hexane	16	17.20
Ethylacetate	20	21.50
Butanol	21	22.58
Aqeuous	36	38.71
Methanol	93	18.60

Appendix VIII

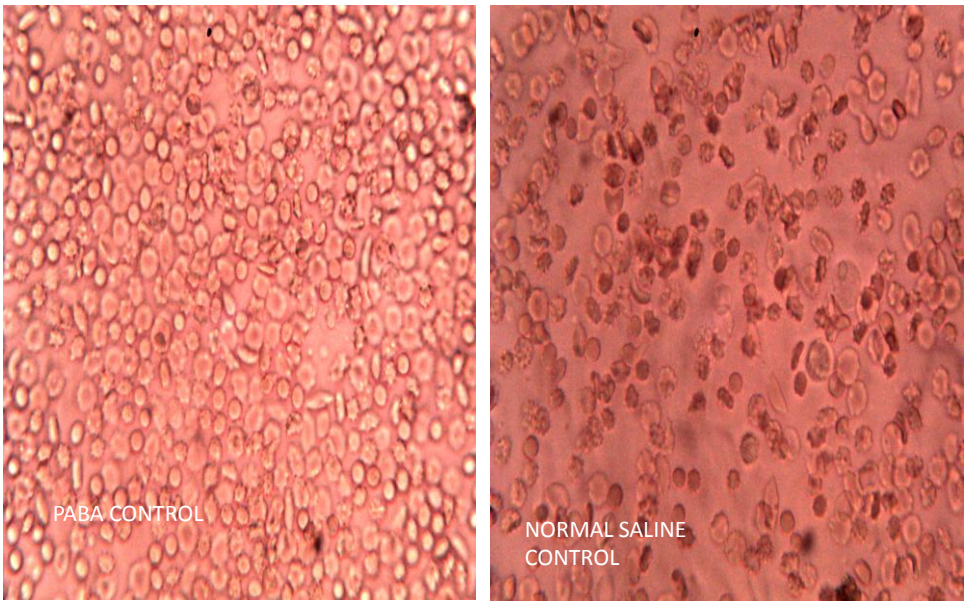


Plate 1: L- R Morphology of drepanocytes treated with 5mg/ml PABA and normal saline respectively .(×1000)

Appendix IX

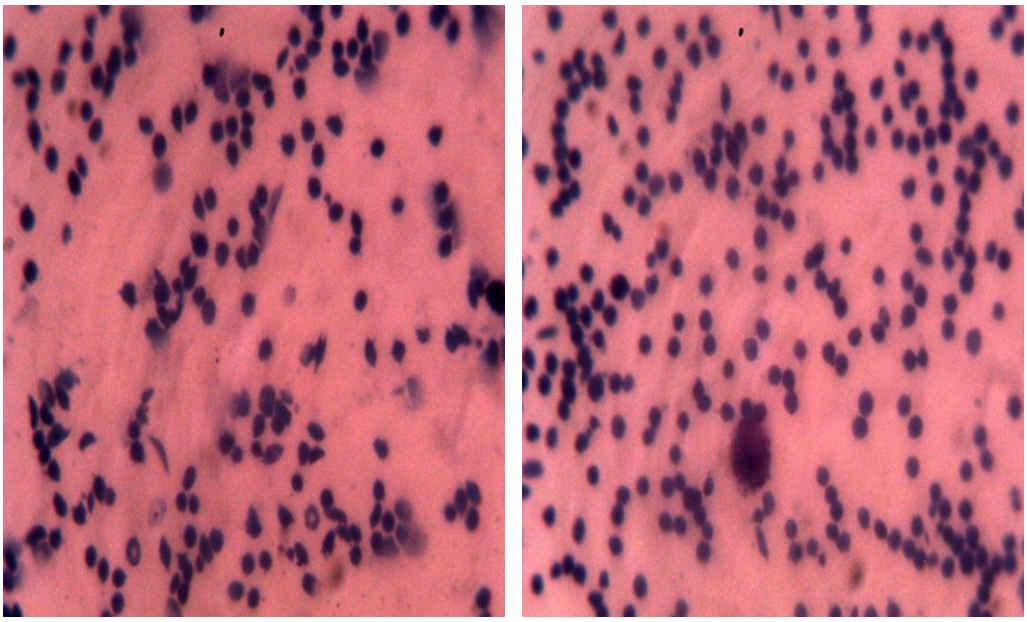


Plate 3: L- R Morphology of drepanocytes treated with 0.3mg/ml ethylacetate fraction of *Dichrostachys* cinerea and Butanol fraction of *sterculia setigera* respectively .(×1000)

Appendix X

INFORMED CONSENT FORM FOR ANTISICKLING EFFECT AND ACTIVITY GUIDED FRACTIONATION OF *DICHROSTACHYS CINEREA* ROOT AND *STERCULIA SETIGERA LEAVES FRACTIONS* ON HUMAN SICKLE RED BLOOD CELLS IN VITRO.

Name of principal investigator: Baraka Abdullahi

Title of research: Antisickling effect and activity guided fractionation of *Dichrostachys cinerea* methanolic extract on human sickle cell in vitro.

Phone number: 081 840 239 3

INTRODUCTION: I am Baraka Abdullahi, a Postgraduate Msc. Biochemistry student from the Department of Biochemistry, Ahmadu Bello University, Zaria. We are doing a research on "antisickling effect and activity guided fractionation of *Dichrostachys cinerea* methanolic extract on human sickle cell in vitro". I will be collecting some blood sample from you, before you decide, you can talk to anyone you feel comfortable with about the research. If there is anything you do not understand, please feel free to ask questions at any point in time.

Purpose of Research: Sickle cell anaemia (also known as sickle cell disorder or sickle cell disease) is a common genetic condition due to a haemoglobin disorder inheritance of mutant haemoglobin genes from both parents. In Nigeria, prevalence of sickle cell anaemia is about 20 per 1000 births. This means that in Nigeria alone, about 150 000 children are born annually with sickle cell anaemia. Although there are current innovations towards the management of sickle cell, there is no sufficient work on some plants that are locally claimed to have antisickling

activity. It is hoped therefore that this research will help improve the understanding of these local plants hence providing a cheaper and safer alternative medicine on the management of sickle cell crisis and complications.

Participant Selection: Adults with sickle cell anaemia attending ABUTH will be invited to participate in the research.

Voluntary Participation: It is entirely your choice whether to participate or not in this research as nothing will change, if you choose not to participate.

Confidentiality: We would not share the identity of the participants in this research. However, the knowledge that is gotten from this research may be published in order that people may learn, improve on the work and further educate/enlighten the sickle cell anaemia community.

Appendix XI

I have read the earlier information, I have had the opportunity to ask questions about it and have been answered satisfactorily. I consent voluntarily to participate as a participant in this research.

Name of participant: ----------

Signature of participant: ----------

Date:----------/----------/----------

Day /month/year

If Illiterate

The consent form has been read to me and I have had the opportunity to ask questions. I confirm and give my consent freely.

Name of participant:----------

Thumb print of participant:

Date:----------/----------/----------

Day /month/year

Name of witness:----------

Thumb print of witness:

Date:----------/----------/----------

Day /month/year

Statement by the Researcher

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

- Some blood samples will be collected from him/her by a phlebotomist in the department of haematology, Ahmadu Bello University Zaria.

I confirm that the participant was given an opportunity to ask questions about the study and all the questions asked by the participant have been answered correctly to the best of my ability .I confirm that the individual has not been coerced into giving consent and the consent has been given freely and voluntarily.

A copy of this informed consent form has been provided to the participant.

Name *of Researcher:*----------

Signature of Researcher:----------

Date:----------/----------/----------

Day/month/year

Appendix XII

Preparation of stock solution of buffered sodium chloride: about 90g of NaCl crystals, 13.65g of Na_2HPO_4, and 2.34g of $NaH_2PO_4.2H_2O$ were dissolved in 1litre of distilled water to make 0.15M solution. Serial dilutions of this stock solution were made by collecting 4.5ml, 3.0ml, 2.5ml, 1.5ml, 1ml, and 0.5ml of this stock solution into a 10ml sample bottle, and 5.5ml, 7ml, 7.5ml, 8.5ml, 9.0ml, 9.5ml, of distill water was added to arrive at hypotonic solutions of 9.0, 6.0, 5.0, 3.0, 2.0 and 1.0 respectively which are used for this experiment.

Appendix XIII

Reagents;

Giemsa stain. 0.68% of Giemsa in methanol/glycerol was diluted with distilled water in the ratio of 1:10.

Mayer's reagent; 1.36g of mercuric chlorate and 5g of potassium iodide was dissolved in 100ml distilled water.

Dragendroff's reagent; 1.27g of iodine and 2g of potassium iodide dissolved in 100ml of distiled water.

Spraying reagent; 10ml of concentrated sulpuric acid dissolved in 90 ml of methanol.