Chapter 1

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1.1. General Introduction

Since early times, plants have been noted for their numerous lifesaving and therapeutic properties (Olagunjua *et al.*, 2009). Lifestyle and eating habits alterations among the people make it imperative to refer to herbal medicine as an alternative or complimentary therapeutic measure. Nearly 70% world populations (mainly in developing countries) depend on ancient customary medical cures as their principal therapeutic method. (Sarkadi *et al.*, 1979) and (Bewaji *et al.*, 1985). Various herbs are also a part of socio-cultural and socio-economic heritage. Even in contemporary times rural populations turn to herbal medicine as the most preferred therapeutic source.

Phytochemicals in plant extracts have healing and curing properties which are exploited by the customary therapists. A substance found in medicinal plants, containing the healing property of plants is recognized as the active principle (Ballas and Marcolina, 2006). It differs from plant to plant and examples of active principles include: anthraquinones, flavonoids, glycosides, saponins, tannins etc. Plants also contain other compounds such as morphine, atropine, codeine, steroids, lactones and volatile oils, which possess medical values for the treatment of different disease. In recent years, these active principles have been extracted and utilised in different forms such as infusions, syrups, concoctions, decoctions, infused oils, essential oils, ointments and creams (Walters *et al.*, 2000; Sofowora, 1993). Since most plants have medicinal properties, it is of utmost importance that their efficacy and toxicity risks are evaluated (Olagunjua *et al.*, 2009).

Sickle cell disease (SCD) is documented as a disease affecting most of the portions of the world irrespective of caste and creed. The initial record of the disease is from a medical student who complained of difficulty in breathing, cardiac tremors with painful episodes of the abdomen and body. The shape of the red blood cells served to describe his observations (Vichinsky, 2002).
According to reports, Africa is seen as the origin of sickle cell disease, and those afflicted with the disease are huge (Herrick, 1910; Nwaoguikie and Uwakwe, 2005). The crisis part of the sickle cell patients is characterized by severe pain and breathlessness making it one of the most dreaded of all genetic diseases (Herrick, 1910; Nwaoguikie and Uwakwe, 2005; Goldberg et al., 1992; Roopen et al., 1996). Sickle cell crises have been studied in the highest priority by researchers all around the world in order to explore effective therapy towards solving the sickle cell disease problem. Consequently, anti-sickling effects of different substances have been studied. The potential of dried fish and shrimp extracts to inhibit polymerization of sickle cell hemoglobin (HbS), improve the iron status and lower the activity of lactate dehydrogenase (LDH) in the blood plasma. LDH is a sensitive signal of haemolysis and its level in sickle cell blood determines the severity of crises (Nwaoguikie and Uwakwe, 2005). Many chemicals such as Hydroxyurea and erythropoietin concoctions have been found to reduce LDH enzyme activity and bilirubin level in the serum, with an increase in the level of foetal hemoglobin (HbF) (Goldberg et al., 1992; Roopen et al., 1996). Further, there are reports on operational management of SCD patients during pregnancy (Mou Sun and Wilburn, 2001; Hassell, 1990).

In developing countries, medicinal plants have served in treating sickle cell crises associated morbidity among the under privileged classes of the society. Traditional healers and local folks are known to own knowledge of the use and healing properties of various herbs common to their locality. There is likewise a huge need to gather such knowledge from diverse geographical parts to compile all information in the form of a database which could be utilized by researchers all over the world.
1.2. Sickle cell disease, its clinical manifestations and the physiology of reversal of the sickled erythrocytes

Sickle cell disease (SCD) is the result of a single base mutation of adenine to thymine, which results in a substitution of valine for glutamic acid at the sixth codon of the β-globin chain (βS) (Koch et al., 2002; Iyamu et al., 2003) with the possibility for various amino-acids to be get transposed simultaneously (Hartwell et al., 2000). Sickle cell disease includes those that produce protuberant medical expressions as is evident during sickle cell anemia, sickle cell disease, sickle cell trait and an array of other related haemoglobinopathies (Iyamu et al., 2003; Hartwell et al., 2000). Pathophysiology of sickle cell anaemia, sickle cell disease, sickle cell trait and an array of other related haemoglobinopathies are now known to the scientific community. Due to polymerization of the sickled cells, the red cell membrane loses its functional abilities which results in loss of potassium and water and a corresponding gain of sodium ion. Increased intracellular free calcium occurs during sickling (Brugnara et al., 1993), resulting in a loss of potassium with accompanying movements of chloride and water. Blood vessels are clogged by the clunking of sickled erythrocytes, deterring blood supply to various organs. The process of de-oxygenation in tissue capillaries causes damage to its endothelium, leading to exudation of plasma into the surrounding soft tissue. This is characteristic of the soft tissue swelling seen in most sickle cell disease patients (Olufunmilayo et al., 2010).

Sickle cell anemia is an autosomal genetic disorder caused by a point mutation in the β globin chain of hemoglobin on the eleventh chromosome by replacing glutamic acid (more polar amino acid) with valine (less polar amino acid) at the sixth position. The association of two wild type globin subunits with two mutant β-globin subunits forms hemoglobin S, which polymerizes under low oxygen conditions causing distortion of red blood cells and a tendency for them to lose their elasticity.
The outer membrane of the erythrocytes possesses a compact identity which is preserved by the hydration and in the case of outer membrane dehydration; it loses its identity and acquires the shape of a sickle. Nitric oxide is believed to play a part in sickle cell disease. It was pointed out that it may be beneficial to SCD patients if endogenous nitrous oxide production is boosted. In turn, boosting the nitrous oxide response or lowering it demolition (Mack and Kato, 2006). This is to avoid hemoglobin released as a result of haemolysis using the nitrous oxide and activating a flow of events that finally hinder blood supply.

Sickle cell disease has a variety of phenotypes which makes it quite confusing to formulate a diagnosis. Phenotypic expression is strikingly different among different patients (Ballas, 2002). Symptoms of sickle cell disease are assorted and wide-ranging and can be recognized into the following categories- a) anemia b) painful consequences, and c) organ failure. When oxygen tension lowers, the HbS molecules undergo nucleation, expansion and ensuing arrangement of the molecule into parallel microfibrils resulting in membrane deformations along with damage. The hemoglobin from these cells forms relatively insoluble polymer under hypoxia condition, creating a crescent-shaped erythrocytes cell which may lead to micro-vascular occlusion leading to severe crises and casualty. The polymerization and occlusion are particularly supported by the stage of increased dehydration during crisis (Afolabi et al., 2012; Mehanna, 2001). Primarily, this perceived sickling is reversible upon re-oxygenation of the system; nevertheless, repeated oxygenation and de-oxygenation cycles in the system lead to irreversible sickle cells (Sofowora, 2008).

The polymerized HbS stretches the cell membrane interfering with the Ca$^{2+}$ activated, Mg$^{2+}$ dependent ATPase (Ca$^{2+}$ pump) responsible for maintaining membrane integrity. Declining Ca$^{2+}$ pump efficiency may result in premature ageing and formation of rigid sickle cells. The increased level of ATP required for maintaining the cellular integrity leads to the accumulation of 2, 3-diphosphoglycerate (DPG), which binds with hemoglobin, facilitating rapid
release of oxygen from the blood cell and partial hypoxia condition in the cell. Low oxygen tension and subsequent absorption of carbon dioxide creates local acidosis, early sickling, high blood viscosity and enhanced adhesion of deformed cells with slower blood flow and in vivo clotting. This phenomenon subsequently leads to haemolysis and micro vascular occlusion, tissue/organ infarction and painful crises (Sofowora, 2008).

The cell undergoing sickling loses K\(^+\) and gains Na\(^+\) ions without any significant change in total cation level. Sickle cell formations have also been reported to be enhanced during low Zn\(^{2+}\) and increased Ca\(^{2+}\) levels (Brewer and Oelshelegel, 1974; Jasen et al., 1973). Corpuscular fragility tests have shown that the stability of erythrocyte membranes in human beings varies with HbAA, HbAS and HbSS blood types (Ibeh et al., 1992 & Elekwa et al., 2003). Hence, there is a possibility that the phytochemical which bring about changes in membrane stability can well be used for alleviating the process of sickle formation in the erythrocytes (Dean and Schechter, 1978). The common variants of sickle cell disease are homozygous sickle cell disease (hemoglobin SS disease) and doubly heterozygous sickle cell disease (hemoglobin AS disease) (Benton et al., 2007; Brugnara et al., 1993) resulting in a loss of potassium with accompanying movements of chloride and water. A characteristic soft tissue swelling is further seen in most sickle cell disease patients (Olufunmilayo et al., 2010). The outer membrane of the erythrocytes possesses a compact identity which is preserved by the hydration and case of outer membrane de-hydration; it loses its identity and gains the shape of a sickle.

Choking of blood and injured organs results in the experience of painful happenings or “crises”. Sickle cell crises may be caused by blood vessel occlusion, triggered by membrane deformation (Ohnishi et al., 2000). SCD patients suffer from a variety of ailments which includes acute chest syndrome (ACS) which is one of the reasons for hospital admissions (Quinn and Buchanan, 1999), stroke (Adams, 2000), and acute splenic sequestration (Edmond et al.,
1985; Sumner, 2000; Svarch et al., 2001). Other clinical manifestations of this condition are hyposthenuria, priapism, vascular necrosis, proliferative retinopathy, aplastic crises, cholelithiasis, delayed growth and sexual maturation, chronic pulmonary disease and chronic nephropathy (Bianchi et al., 2007). However all these clinical features of sickle cell disease do not appear until after the first sixth months of life, at which time most of the HbF has been replaced by HbS.

A number of plant products have been reported, that could serve as agents that alter membrane stability. Some of these have been implicated in the management of several human ailments including sickling and sickle cell disease (Parpart et al., 1945; Uphof, 1968; Sofowora and Isaacs-Sodeye, 1971 & 1975; Willis, 1973; Iwu, 1985 and Ekeke and Shode, 1990).

**Zanthoxylum macrophylla** roots extract contains 2-hydroxy-methylbenzoic acid and which was reported to possess antisickling properties and was widely used in the management SCD (Sofowora et al., 1979). Elekwa et al. (2003) reported that extracts from *Garcinia kola* (Heckel) seeds can inhibit the sickling process of erythrocytes in various genotypes of SCD. In addition, further studies on the antisickling properties of plant extracts are immensely needed to develop novel strategies towards the management of SCD.

### 1.3. Plant Extracts used as Therapeutic Measures

Plant extracts are found to have properties which prevent the erythrocytes from deforming and losing its integrity. In the treatment of SCD, it is required that one focuses on the ways of inhibiting sickle cell hemoglobin polymerization, prevention or repair of red cell dehydration and interrupting the interaction of sickle cells with the endothelium (Brugnara et al., 1993; Charache et al., 1995; Kinney et al., 1999; Claster and Vichinsky, 2003). Lower drug response in SCD patients is considered to be a major factor which hinders in the discovery of an effective therapy (Iyamu et al., 2003). Hydroxyurea is a known inhibitor of sickle
cell polymerization and various drugs containing it has an inability to increase fetal hemoglobin concentration (Mcgoron et al., 2000; Sauntharajah et al., 2008).

Recent therapy focuses on the erythrocytic rehydration (Mcgoron et al., 2000; Stocker et al., 2003). Management of sickle cell disease (SCD) hence, involves substances which have an ability to rehydrate the erythrocytes and furthermore, preventing it from losing its shape. The anti-sickling properties of certain amino acids such as phenylalanine, alanine, lysine, arginine etc., have also been reported (Ekeke and Shode, 1990; Oyewole et al., 2008). Various plant extracts were reported to lower the painful episodes and related complications of SCD (Okochi and Okpuzor, 2005 and Ugbor, 2006). In another report, the importance of thiocyanate, hydroxyurea, and tellurite as potent antisickling agents possessing immense potential to inhibit erythrocyte deformations was mentioned. Blood transfusion is also carried out in the management of sickle cell disease and can help the process to a great extent (Sofowora and Issac-Sodeye, 1971; Mgbemene and Ohiri, 1999). One of the drawbacks of blood transfusion was it lowered the macrophage count in patients (Orhue et al., 2005; Orhue and Nwanze, 2006). Phytochemical found in extracts of *Piper guinensis*, *Pterocarpa osun*, *Eugenia caryophyllala* and *Sorghum bicolor* can serve as potential antisickling agents (Onah et al., 2002). *Pterocarpus santolinoides* and *Aloe vera* extracts were found to be useful in crisis management and extracts of *Fagara zanthoxyloides* and *Terminalia catappa* extracts were reported to possess potential reversal properties (Ohnishi et al., 2001; Moriguchi et al., 2001; Oduola et al., 2006), *Solaria dulcis* (Thomas and Ajani, 1987; Moody et al., 2003), was also used in SCD management. The following plants were also found to possess potential antisickling properties – *Alchornea cordifolia*, *Afromomum alboviolaceum*, *Annona senegalensis*, *Cymbopogon densiflorus*, *Bridelia ferruginea*, *Ceiba pentandra*, *Morinda lucida*, *Hymenocardia acida*, *Coleus kilimandcharis*, *Dacryodes edulis*, *Caloncoba welwithsii*, *Vigna unguiculata* and *Adansonia digitata* L. (Bambacaceae) (Vanhaele-Fastre et al., 1999).
Root extracts of *Zanthoxylum macrophylla* was also reported possessing antisickling properties (Hayashi, 1987).

The potential of *Cajanus cajan* in the management of sickle cell anemia was also reported (Kawasaki, 1987 and Hayashi, 1990). Likewise, garlic was also suggested possessing the same properties (Ahmed and Jakupovic, 1990; Ohnishi and Ohnishi, 2001).

Potential of the fruits of *Carica papaya* for the sickle cell reversal was shown by (Abdulmalik *et al*., 2005; Adesina, 2005). Furthermore, extracts from *Piper guineenses* seeds, *Pterocapus osum* stem, *Eugenia caryophyllum* fruit, and *Sorghum bicolor* leaves were found to possess properties for reversal of sickling of erythrocytes (Iyamu *et al*., 2002; Iyamu *et al*., 2003).

### 1.4. Phytochemicals responsible for the Anti-sickling properties

Following reports on the antisickling potentials of plant extracts, efforts to identify the causative agent behind it were initiated. There are arrays of reports on the various phytochemical constituents of different plant extracts. The plants *Cissus populnea* L., *Khaya senegalensis*; *Scopari adulcis*; was reported possessing anthraquinones, steroidal glycosides and cardiac glycosides along with akaloids and tannins (Agbai, 1986; Abraham *et al*., 1991; Oyedapo and Famurewa, 1995; Takasu *et al*., 2002; Brugnara and Steinberg, 2002; Ouattara *et al*., 2004; Abdulmalik *et al*., 2005; Adesina, 2005).

Other free amino acids, ascorbic acid, organic acids, lipophilic amino acids, carboxylic acid and amino acids (Phenyl alanine, alanine, glutamate, histidine, arginine, lysine, tyrosine and aspirin were reported as antisickling agents (Elekwa *et al*., 2005; Ogunyemi *et al*., 2007; Uwakwe *et al*., 2008; Nwaichi *et al*., 2013; Nwaoguikpe *et al*., 2013; Nwaoguikpe *et al*., 2014; Kototenyiwa, 2014).
In the course studying the importance of various phytochemicals in the management of SCD, many phytochemicals and concoctions from combination of different plant extracts were identified. Among the important ones are the divanilloylquinic acids and 2-dihydroxymethyl benzoic acid isolated and identified from *Fagara* (Ojo *et al.*, 2006; Adaikpoh *et al.*, 2007). The potential of garlic fermented extracts in the treatment and management of SCD was also demonstrated (Rajaprabhu *et al.*, 2007). Phytochemicals such as thiocyanate were also studied and reported it to possess potential antisickling properties (Adebanjo and Adewunmi, 1983; Rajaprabhu *et al.*, 2007; Semiz and Sen, 2007; Adejumo *et al.*, 2011).

Various plants were also found to possess an array of phytochemicals responsible for the reversal of sickled erythrocytes. Some of them are *M. charantia; Cymbropogon citratus; Camellia sinensis; Scopari adulcis;* and *Picrorhiza kurroa* (Brugnara, 2000; Chevalier, 2000; Cyril-Olutayo *et al.*, 2009; Egunyomi *et al.*, 2009; Oyewole and Malomo, 2009; Ameh *et al.*, 2009; Adejumo *et al.*, 2011;) Phynyle alanin was reported as antisickling agent.

1.5. Current Status of Research in the Field

Overall reports on reversal of suckled erythrocytes are meager globally except in the African countries where a prevalence of Sickle Cell Disease (SCD) is quite high, resulting in quite a significant number of deaths each year.

However, the SCD is incurable; management therapy is primarily concentrated towards reversal of sickled erythrocytes during the crisis stage of the disease. Works on the reversal of sickling activity has been done by – Herrick 1910; Sofowora and Issac-Sodeye, 1971; Konety-Ahulu, 1974; Adebanjo and Adewunmi, 1983; Bewaji *et al.*, 1985; Edmond *et al.*, 1985; Agbai, 1986; Thomas and Ajani, 1987; Adesanya *et al.*, 1988; Hassell, 1990; Ekeke and Shode, 1990; Ahmed and Jakupovic, 1990; Goldberg *et al.*, 1992; Sofowora, 1993; Brugnara *et al.*, 1993; Oyedapo and Famurewa, 1995; Svarch *et al.*, 1996; Quinn and
Apart from these works, there are no available reports on the antisickling properties of plant extracts in our country. Furthermore, there are absolutely no reports from Chhattisgarh or Central India.

Therefore, the prospects for further research in this area in our country have to be assessed and promoted for managing sickle-cell anaemia.
1.6. Management of Sickle Cell Disease (SCD)

In spite of the fact that the molecular nature of Sickle Cell Disease (SCD) has been adequately studied, we are yet to develop a permanent cure to the disease condition. Numerous approaches have been tailored in an effort to find agents that inhibit the polymerization of haemoglobin and hence prevent or reduce the occurrence of crises in sickle cell disease (Akojie and Fung, 1992; Iyamu et al., 2002). Amidst scarce hope towards the development of a permanent cure to the disease, different practices have been commissioned in an attempt to find agents that inhibit the polymerization of haemoglobin which eventually prevent or reduce the occurrence of crises in sickle cell disease (Akojie and Fung, 1992; Iyamu et al., 2002).

Instantaneous emphasis has also been on developing antisickling agents which act by blocking or inhibiting activities in the HbS leading to its polymerisation with loss of its shape and functionality (Chikezie et al., 2011). One such instance was when oxygen, Carbon monoxide and sodium nitrite were used to decrease the amount of deoxy-haemoglobin. Stress has also been on developing antisickling agents which act by blocking or inhibiting activities in the HbS which eventually cause polymerisation (Chikezie et al., 2011). Therapeutic line of attack for sickle cell anemia since the early times of discovery in 1910 have been on prophylactic measures to mitigate the agonizing “crises stage” by administering analgesics, antipyretics, intravenous fluids or hydrants or anti-dehydrants, oral antibiotics such as penicillin and the anti-cancer drug hydroxyurea as inducer of foetal haemoglobin. Chemicals like the butyrates and decitabines have been engaged for the induction of foetal haemoglobin (Sauntharajah et al., 2008; Stocker et al., 2003; McGoron et al., 2000). Fetal hemoglobin is alleged to impede the polymerized globin chains and has also the ability to prevent polymerisation of the HbS. Lactate dehydrogenase (LDH) inhibitors such as hydroxyurea, erythropoietin and tucaresol preparation have
been found to be effective in SCD crises management (Okpuzor et al., 2008). Hydroxyurea has already been approved by the United States Food and Drug Administration as anti-sickling agent/drug for the management of SCD (Mehanna, 2001). Further efforts towards finding a treatment led to of SCD led to the development of bone-marrow transplant or hematopoietic cell transplantation (HCT). Treatment with HCT was later found to be a costly affair with insubstantial therapeutic potentials. Another line of therapy focused on finding new-fangled anti-sickling agents which was able to unambiguously bind to HbS. These attempts steered the advance of a few anti-sickling agents viz., 5-hydroxymethyl-2-furfural (5HMF) (Abdulmalik et al., 2005), certain amino acids such as phenylalanine, lysine and arginine (Anosike et al., 1991), 2-imidazolines such as clotrimazole (Chang et al., 1983), and other Gardos channel inhibitors like senicapoc [bis(4-fluorophenyl) phenyl acetamide], dimethyl adipate, vaso-active molecules like endothelins were also subsequently developed (Nagalla and Ballas, 2010). Gardos channel inhibitors like clotrimazole, magnesium and ICA-17043 acted by preventing dehydration (Okpuzor et al., 2008). De-oxygenation leads to increased membrane permeability for Mg^{2+} resulting in a net loss of intracellular Mg^{2+}. The cadence of the activities of the three membrane-based ATPases (Na^{+}, K^{+} and Ca^{2+}) have been shown to be significantly lower in sickle cell disease patients’ (HbSS) erythrocytes, while Mg^{2+} ATPases was shown to be significantly higher in HbSS compared to normal patient (HbAA) erythrocytes. There are a few reports which have shown Ca^{2+} to play a significant role in the lowering of K^{+} and Na^{+} permeability of the erythrocyte membrane; which assists to conserve the normal rate of cation outflow from the cell. Furthermore, the most crucial proposition was that, the sickled erythrocytes can well be reversed if excess Ca^{2+} in the red cells was propelled out of the system (Okpuzor et al., 2008). There are also few reports on the ability of some anti-malarial drugs and oral iron chelators to which can alter some of the individual red blood cell elements which eventually leads to transformed physicochemical properties, which may be helpful in the
management of the disease (Gibson et al., 1998; Chikezie, 2009, 2008; Chikezie et al., 2011, 2010, 2009a, b). However, all these procedures are yet to give the much desired beneficial effects, hence there is an immense need for further research in this field (Iyamu et al., 2002).

The use of herbs for the treatment of patients with (SCD) is a widespread practice in many parts of the world, especially in the rural communities where SCD is endemic. Current indigenous drug development processes also use active crude extracts (ACEs) from medicinal plants from proven traditional recipes in their formulations. An example is NIPRISAN™, a widely used herbal drug developed by the National Institute for Pharmaceutical Research and Development (NIPRD); Web URL- http://www.commonwealthofnations.org/partner/national_institutefor_pharmaceutical_research_and_development/.

This drug is prescribed to the SCD patients in our country India, South African countries and the United States of America, which has great potential for the management of SCD without any side effects (Ambebe et al., 2001; Ameh et al., 2012).

The success of NIPRISAN™ may be attributed to the synergistic effect of the phytochemical present in its constituent herbs. Hence, the remarked therapeutic effect of this phyto-medicine may be a result of collective antisickling, antipolymerisation, antidehydration and antioxidants effects possessed by the phytochemicals of the component plants, which are a characteristic feature of most herbal cures.

Numerous authors and researchers have reported several herbal recipes and formulas from medicinal plants for managing SCD. These medicinal plants have been demonstrated to own diverse activities in managing SCD condition. These activities may range from antisickling, anti-aggregating, antipolymerisation, radical scavenging or antioxidant, anti-inflammatory, analgesic, anti-pyretic, anti-dehydrating to anti-osmotic effects, etc. All these properties add
up to give a supplementary stable and endurable patient condition (Dong et al., 1998; Nagalla and Ballas, 2010; Chikezie et al., 2011; Ameh et al., 2012; Afolabi et al., 2012 and Sulaiman and Gopalakrishnan, 2013).

It thus follows that the identification of and characterisation of phytochemicals with antisickling inclinations from plants has become vital in developing effective SCD management strategies.

### 1.7. Plan and Scope of the Present Study

Phytochemicals with antisickling activities are of paramount importance for designing and implementation of the current SCD management strategies. Many plants possess antisickling propensities, in due course, it has to be explored and their respective active principles need to be identified; particularly in a country like India where the incidence of SCD is high. In our country plants have not been tested for antisickling activities till date. Most of the studies on phytochemical analysis and all of the studies on antisickling properties are from foreign countries and on exotic plant species. In a country like India, where the incidence of SCD is quite high among some communities, with its rich and diverse plant species, there is an urgent need to undertake studies pertaining to the antisickling properties and phytochemistry of native species.

The present study aims to validate the extracts of leaves, seeds and stems of two plants, *Carica papaya* L. and *Cajanus cajan* L. which, in the preliminary studies were found to possess various phytochemicals as well as antisickling activity. A detailed thin layer chromatography (TLC) study would provide an ample insight into the total number of phytochemicals present in each of the individual parts proposed.

The results thus attained could provide important data towards classification of extracts according to their total phytochemical content and antisickling potential; for different individual parts viz., leaves, stem and seeds.
The Proximate assay proposed in the study could provide important insight into the total composition of these plants.

The outcome of the present study could well serve towards the formulation of novel drugs which can be used in the management if Sickle Cell Disease (SCD).

Therefore, the present study was planned to analyse the phytochemistry of two plants, *viz.*, *Carica papaya* and *Cajanus cajan* with their antisickling and sickling inhibiting properties. Furthermore, proximate assay, qualitative and quantitative phytochemical analysis, thin layer chromatography (TLC), antisickling and sickling reversal activities will be assessed individually for leaves, stems and seeds of these two plants.