Chapter 5

DISCUSSION
5.1. PROLOGUE
5.2. PHYTOCHEMISTRY & ANTISICKLING ASSAY
5.3. CONCLUSION
Plants with medicinal properties are of great use in community health. The phytochemicals present in the plants are the causative factors behind its medicinal properties. These phytochemicals differ in their composition in various parts of the plants itself. Therefore, there may be a difference between the potency of the whole plant extracts and the extracts from its constitutive parts, as the concentration of many phytochemicals vary from part to part. The phytochemicals found in the plant extracts falls into the following categories—alkaloids, tannins, flavonoids, cardiac glycosides and phenolic compounds. The ethno medicinal plants are very important in the pharmacological novel drug discovery because they have the potential to treat diseases like, cancer, hypertension, diabetes, arthritis etc., without any side effects. In India, about 50,000 plant species have been identified, out of which about 10 thousand plants are recognized for their medicinal value.

There is an urgent need to identify more and more species of plants for their medicinal properties. The present studied and validated the phytochemistry and antisickling activity of two plants *viz.*, *Carica papaya* L. and *Cajanus cajan* L. along with their antisickling and sickling inhibiting properties.

The potential of phytochemicals as an antisickling agent is discussed in the light of the results obtained in various assays like, proximate analysis, qualitative and quantitative phytochemistry; thin layer chromatography (TLC), antisickling activities and sickling reversal activities have been assessed individually for leaves, stems and seeds of these two plants. Furthermore, the antisickling effects found in various concentrations of extracts of different parts of these plants has been discussed. This section discusses the results of phytochemistry in the light of phytochemicals reported in other plant species till date. Attempts to validate the results on the basis of the individual properties of different phytochemicals have been made and a detailed hypothesis on the
phenolic compounds along with the relation between the anti-oxidant activity and antisickling activity has been presented.

### 5.2. PHYTOCHEMISTRY & ANTISICKLING ASSAY

Medicinal inclinations showed by the plants are because of their underlying phytochemicals. The phytochemicals responsible for medicinal properties needs to be identified and characterised to manage specific disease conditions and symptoms. It is generally accepted that phytochemicals responsible for antioxidant activity in plants may also serve as potential antisickling agents. The phytochemical zanthoxylol, which is a derivative of butyric acid and 1-hydroxylbenzoic acid, the active phytochemicals of the plant *Fagara zanthoxyloides* which has been proposed to be the key reason behind the antisickling activity of this plant (Sofowora *et al.*, 1979). Many other reports pertaining to the antisickling activity of plant extracts have been attributed to the phytochemicals present. Phytochemicals like, phenols, alkaloids, flavonoids, saponins, tannins, anthocyanins, anthraquinones, cardiac glycosides, hydroxybenzoic acid, co-enzyme Q10, eugenol, eugenyl acetate, β-caryophyllene, gallotannic acid, 2-hydroxy-1,4-napthaquinone, isoplumbagin, zinc, iron, germanium, allantoin, ecdysteroids, pfaffic acid, pfaffic acid glycosides, saponins, stigmasterol, sitosterol, arginine, lysine, serine, tyrosine, aspartic acid, and phenylalanine have been reported to be behind the antisickling activities of their respective plants (Waller *et al.*, 1978; Thomas and Ajani, 1987; Adesanya *et al.*, 1988; Marlier *et al.*, 1993; Mazzanti and Braghirol, 1994; Noronha, 1997; Moody *et al.*, 2003; Elekwa *et al.*, 2003; Oduola *et al.*, 2006; Mpiana *et al.*, 2007; Mpiana *et al.*, 2007; Okpuzor *et al.*, 2008; Li *et al.*, 2010; Gupta *et al.*, 2010; Okwu and Ukanwa, 2010; Otunola *et al.*, 2010; Nwaoguikpe *et al.*, 2010; Malviya *et al.*, 2011; Ijomone *et al.*, 2011; Afolabi *et al.*, 2012; Soladoye and Chukwuma, 2012; Simeone *et al.*, 2012; Gbadamosi *et al.*, 2012; Sulaiman and Gopalakrishnan, 2013; Gbadamosi *et al.*, 2013). The major activity of
A medicinal plant against various ailments is a function of the amount of phytochemicals it can produce and as such would produce definite physiological actions in the human body system. Tannins are used as astringent medicine for the treatment of intestinal disorder, such as dysentery and diarrhea and it occurs in a high percentage. They also react with protein to form stable cross link polymers, which transforms animal skin into leather. They are relevant to the food processing, fruit ripening, manufacture of cocoa and wines. Saponins are used medically for the treatment of increased blood cholesterol and are beneficial to patients with arteriosclerosis and hypertension and in the control of post-menopausal syndrome (Akpuaka, 2009). However, these compounds were studied for their in vitro anti-sickling properties and their exact mechanism of action is yet to be studied.

Results of the phytochemical estimation when seen extract wise, revealed maximum presence of phytochemicals in the ethanolic extract of leaves; chloroform extract of seeds and ethanolic and methanolic extracts of stem, in the plant *Cajanus cajan* L. In the other plant, *Carica papaya* L. maximum phytochemicals were observed to be present in the methanolic extracts of leaves, ethanolic extract of seeds and methanolic, chloroform and petroleum ether extracts of the stem.

Qualitative phytochemical analysis of both the plants in the present study showed the presence of tannins, saponins, phenols, alkaloids, and cardiac glycosides as the major compounds. In the quantitative analysis of the plant *Cajanus cajan* L., the leaves extracts showed the presence of phenol 16.61%; tannins 0.49%; alkaloids, 2.65%; flavonoids 4.77% and saponins 5.97%. In the seeds extract the secondary metabolites were found to be phenol 3.82%; tannins 0.23%; alkaloids 2.65%; flavonoids 2.11% and saponins 6.23%, whereas the results for the stem showed the presence of phenol 14.19%; tannins 0.22%; alkaloid 2.51%; flavonoids 5.44% and saponins 4.98%. The other plant, *Carica papaya*
showed the presence of phenol 13.42%; tannins 0.17%; alkaloids, 2.99%; flavonoids 5.08% and saponins 7.41% in the leaves extract, whereas in the extracts of seeds the quantities of phytochemicals detected were, phenols 12.11%; tannins 0.12%; alkaloids 6.96%; flavonoids 3.38% and saponins 4.84%. The stem extract showed the presence of phenol 4.78%; tannins 0.04%; alkaloid 1.5%; flavonoids 2.88% and saponins 2.85%. Maximum phenolic content was found in leaves extracts (134.20 ± 3.36 mg/g) followed by seeds (121.40 ± 1.02 mg/g) and stems (47.80± 2.46 mg/g).

Precisely, during the quantitative analysis the most active phytochemical was noted were phenolic compounds in the leaves and stems of *C. cajan*; while the stem extracts revealed saponins as the most active phytochemical. On the other hand, *C. papaya* showed higher quantity of phenolic compounds in all of the parts studied. Hence, clearly the phenolic compounds are the most active compounds in both these plants studied. Phenolic compound is a major component of the methanolic extracts. Results of the phenolic compounds in the present study is in accordance with the works of earlier workers (Hall, 1997; Slinkard and Singleton, 1977; Velioglu *et al*., 1988; Shahidi and Wanasundara, 1992; Tsajimoto *et al*., 1993; Kahkonen *et al*., 1999; Singleton *et al*., 1999; McDonald *et al*., 2001; Zheng *et al*., 2003; Cai *et al*., 2004; Miroslawa and Goslinksa, 2004; Beta *et al*., 2005; Hassimotto *et al*., 2005; Huang *et al*., 2005; Sreeram *et al*., 2005; Espinosa-Alonso *et al*., 2006; Harish and Shivanandappa, 2006; Kruawan and Kangsadalampai, 2006; Malencic *et al*., 2007; Othman *et al*., 2007; Pourmorad *et al*., 2006; Sasidharan *et al*., 2007; Silva et al., 2007; Tawaha *et al*., 2007; Atiкур Rahman *et al*., 2008; Bozin *et al*., 2008; Karagozler *et al*., 2008; Kamilolu *et al*., 2009; Arash Rafat *et al*., 2011; Mohammad *et al*., 2011; Rajan *et al*., 2011; Subramanion *et al*., 2011; Anjali and Sheetal, 2013; Hamdoon A. Mohammed *et al*., 2013). Phenolic compounds are a class of antioxidant agents which serve as free radical terminators and chelators (Kessler *et al*., 2003; Om Prakash and Yamini, 2007).
In plants, phenolic compounds serve as defensive barriers against environmental stress, harmful radiations, pest attacks and injury (Chung et al., 2003; Zulak et al., 2006; Diaz Napal et al., 2010; Kennedy and Wightman, 2011). Structure of phenolic compounds is characterized by the presence of an aromatic ring in association with hydroxyl groups (Chirinos et al., 2009). Total plant phenolics is constituted by simple phenols, coumarins, lignins, lignans, condensed and hydrolyzable tannins, phenolic acids and flavonoids (Soto-Vaca et al., 2012). They are the omnipresent major groups of plant metabolites. They prevail as esters, glycosides or amides. Phenolic compounds are antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protective and are known for their inhibition of angiogenesis and cell proliferative activities (Han et al., 2007). They may vary in the phenolic acids as well as the number and location of hydroxyl groups on the aromatic ring (Pereira et al., 2009). Phenolic acids prevail as hydroxycinnamic and hydrocarbons acid. Among the hydroxycinnamic acid derivatives there are ferulic, caffeic, p-coumaric and sinapic acids, whereas the hydroxybenzoic acid derivatives contain gallic, vanillic, syringic and protocatechuic acids.

Phenols occur in the cell wall as well. Cell wall phenols are compounded as complexes with other cell constituents and are insoluble in nature. Cell wall phenolics belong to two classes, viz., lignins and hydroxycinnamic acids (Baucher, 1998; Vanholme et al., 2010). In the cell wall phenols act as anti-stress and are protective in nature. Tannins can be divided into 2 groups, hydrolyzable tannins and condensed tannins, and have great potential to form oxidative linkages to other plant molecules. Free phenolic acids and their derivatives such as, hydroxy benzoic acid and gallate, aromatic amino acids such as phenylalanine, tyrosine and tryptophan, phenylpropanoids such as coumaric acid and its derivatives are known to possess antisickling activity (Ekeke and Shode, 1990; Dean and Schechter, 1978a, b; Noguchi and Schechter, 1978). Phenylalanine has been found to be a potent inhibitor of HbS gelation and acts
by competing for the protein-protein contact sites within the HbS polymer, which may be the mechanism of action of other antisickling amino acids and peptides (Dean and Schechter, 1978a, b; Noguchi and Schechter, 1978). Phenolic acids, such as hydroxy benzoic acid, have been proposed to show their antisickling properties by acting as membrane active agents. The p-hydroxybenzaldehyde found in Sorghum bicolor L. increases oxygen affinity of HbS as archetypal of all aldehydes (Beddell et al., 1975). Vanillin, which occurs in over 79 plant genera, is well known for inhibit gelation of haemoglobin and markedly increases oxygen affinity of both normal haemoglobin (HbA) and HbS (Sofowora, 2008; Zaugg et al., 1977). The stilbene, resveratrol, found naturally in many plants including peanuts, is believed to be more active as an antioxidant than vitamin C and E (Chanvitayapongs et al., 1997). It possesses similar activity to hydroxyurea and acts by inhibiting ribonucleotide reductase and inducing haemoglobin production in the cell (Rodrigue et al., 2001). Resveratrol inhibits lipid peroxidation of LDL and store-operated Ca2+ channels (SOCC) thereby preventing Ca2+ inflow in thrombin-stimulated human platelet leading to regulated platelet adhesion and intravascular clotting (Dobrydneva et al., 1999; Rotondo et al., 1998; Soleas et al., 1997). Its action has also been associated with inhibition of nitric oxide vascular dependent action in SCA (Bradamante et al., 2003). Thus, most plants rich in these simple phenolics may exhibit their pharmacological antisickling property by the synergistic actions of these chemical components.

The naphthoquinone, lawsone, is responsible for the antisickling action of Lawsonia inermis. The compound decreases the blood oxygen saturation level required for 50% sickling and lowers the partial pressures at the organs thereby increasing the oxygen affinity of the HbSS red blood cell (Chang and Suzuka, 1982). Plumbagin and other derivatives of naphthoquinones may act in a similar manner. The sesquiterpenoid, β-caryophyllene, the major terpenoid compound found in Eugenia caryophyllata, Canabis sativa, Piper guineense and most
essential oils may also possess useful pharmacological activity in SCD management (Ameh et al., 2012). E. caryophyllata which is particularly rich in eugenol and β-caryophyllene is an integral part of an active herbal recipe used in the management of SCD. β-caryophyllene has been found to bind selectively to cannabinoid receptor type 2 (CB2) which is expressed in the immune system, hematopoietic cells and peripheral nerve terminals where it plays a significant role in pain control (Ameh et al., 2012). C. saliva seed oil and leaves, richer in β-caryophyllene, is consumed by SCD patients for pain relief in North America (Ameh et al., 2012; Lynch et al., 2006). C. sativa has been found to lower intraocular pressure and reduce sleep disturbance by changing blood flow pattern in the brain and limbs, which may be the basis of action in SCD cases (Colasanti, 1986; Rog et al., 2005 and Berlach et al., 2006).

Importance of phenolic compounds in rendering antioxidant properties to medicinal plants have been reported by many workers (Brown and Rice-Evans, 1998; Krings and Berger, 2001.). Phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. serve as potential anti-oxidants in plants (Ali et al., 2008). Tannins bind to proline rich protein and is detrimental to protein synthesis.

Plants flavonoids are phenolic substances (hydroxylated) were found to resist pesticide infection when tested in vitro. This activity is rendered in their capacity to complex with extracellular and soluble proteins and to compound with the bacterial cell wall (Marjorie, 1996). Phenolic substances are potent antioxidants and anti-carcinogens (Salah et al., 1995; Del-Rio et al., 1997 and Okwu, 2004). Total phenolic content of the C. papaya and C. cajan in all parts, particularly in the leaves is encouraging. Correlating these results with the results of other workers suggests that presence of large quantities of phenolic compounds is directly related to the antioxidant activities of the plants studied. There are various reports which assessed the phenolic contents of various plants and attributed the high activity of phenolic compounds to the antioxidant
properties (Amin and Yazdanparast, 2007; Pan et al., 2008; Nagendra et al., 2009; Demla and Verma, 2012). This indicates that the plants rich in anti-oxidant properties may also possess antisickling properties. Sickling process of the erythrocytes can be altered by the oxygenation and rehydration of its outer membrane; from here it follows that the plants with antioxidant properties may also act as potential antisickling agents.

Individually, flavonoids include flavones, flavanones, flavanols, isoflavonols/isoflavones, diflavanones, anthocyanins, for example, quercetin, keamferol, apigenin, luteolin, cajarin, rutin, vavain, isohamnetin, etc. The cellular mechanisms of action of bound plasma flavonoids are still not clear. What is clear is that this group of compounds exhibits huge number of biological/pharmacological activities ranging from antioxidant, antipyretic, anticancer, antiviral, antifungal and antimicrobial to immuno-modulatory. Generally, flavonoids are known to act as free radical scavengers, modulator of enzymatic activities, an inhibitor of cellular proliferation, as well as possessing antibiotic, anti-allergenic, anti-diarrheal, anti-ulcer and anti-inflammatory activities. They scavenge peroxyl radicals, alkyl peroxyl radicals, superoxide hydroxyl radicals, and peroxynitrite in aqueous and organic environments, which mitigate against lipid peroxidation, change in cellular osmotic pressure and subsequent inflammation and cell death (Mira et al., 2002; Nijveldt et al., 2001; Guthrie et al., 2000; Ng et al., 2000; Duthie and Crozier, 2000; Chen et al., 1996; Sanz et al., 1994).

Flavonoids have also been reported to interfere with nitric-oxide synthase responsible for the production of nitric oxide which forms peroxynitrite with free radicals. Peroxynitrite can directly oxidize low density lipoproteins may cause permanent damage to the cell membrane. Quercetin and some other flavonoids can interfere with the xanthine oxidase pathway and help to reduce the generation of superoxide free radicals by the enzymic process. Lipid
peroxidation also occurs in the presence of iron and reactive oxygen species. Hence, iron chelators and stabilisers, like quercetin, contribute to reducing the free radical peroxidation process (Nijveldt et al., 2001). Flavonoids have been shown to decrease adhesion of leukocytes to endothelial wall during ischemia and inflammation reducing the stimulation of degranulation of the neutrophil and enhancing spontaneous flow of blood. Nijveldt et al. (2001) also showed that flavanoids may prevent the degranulation of mast cells due to the intonation of calcium channel modulation in the cell membrane. They also act as anti-thrombotic agents due to their inhibiting action on the cyclooxygenase and lipoxygenase pathways. Arachidonic acid released during inflammation is catabolized by platelets into prostaglandins, endoperoxides and thromboxane, resulting in activation and aggregation of platelets. Quercetin and kaempferol were said to resist platelet aggregation (Tzeng et al., 1991). Platelet anti-aggregatory effect of flavonoids is ascribed to the inhibition of thromboxane synthesis. Flavonoids affect arachidonic acid metabolism by specifically blocking cyclooxygenase or lipoxygenase, or both enzymes. Most of the activities of flavonoids are considered to be due to the free radical scavenging ability and interference with enzymes functions (Nijveldt et al., 2001). Some of these processes are implicated in the metabolism of sickle red blood cells resulting in the observed crises.

Another class of phenolic compounds, the coumarins (phenylpropanoids) derived from a branch of the phenylalanine metabolism pathway that leads ultimately to furanocoumarin (psoralin) synthesis, is another class of bioactive compounds. Although limited data are available to demonstrate the antioxidant activity of coumarins, some of them have been shown to possess anticarcinogenic and antithrombotic activities (Gunatilaka et al., 1994; Fernandez-Puntero et al., 2001; Ng et al., 2000). It is possible that they exhibit their antisickling activity by mimicking the actions of free phenolic acids. The highly acetylated β (1,4)-linked polysaccharide, acemannan, which is an immuno-modulator, antitumor,
antiviral and antimicrobial, is believed to act through various mannose mediated mechanisms (Ramamoorthy and Tizard, 1998; Kahlon et al., 1991a, b). Monosaccharides of Acemannan are mannose, glucose and galactose, which are among the eight essential glyconutrients necessary for efficient cell functioning. Acemannan is believed to influence the production of glycoproteins and glucolipids in the liver needed for effective cell communication. Acemannan has been shown to facilitate communication between cells at a distance by stimulating the release of cytokines (Marshall, 1993). It has also been demonstrated to activate macrophages and induce cellular immune response including recognition of foreign antigens (viruses, bacteria and cancer), capture and removal of microorganisms, production of antibody and wound healing. This activity of Acemannan is ascribed, in part, to the recognition of terminal mannose by macrophages as a foreign substance (Zhang and Tizard, 1996). The compound has been reported to increase the cytotoxic T-lymphocyte cell in mixed lymphocyte culture (Womble and Helderman, 1992). Thus, many of the activities of the compound as an antitumor or anticancer, antidiabetic, wound healing, antiviral, antibacterial, anti-psoriasis and immuno-modulatory, are primarily through stimulation of glycoprotein and glycolipid levels.

Limonoids are a group of highly oxygenated triterpenoids present mainly in the Rutaceae and Meliaceae families. Research on these compounds has shown that some limonoids could induce the detoxifying enzyme glutathione S-transferase (GST) in the liver of mice and rats (Lam et al., 1989). Hence, limonoids could induce the xenobiotic enzyme GST, against endogenous peroxide inducing xenobiotics and peroxidized lipids, enabling them ready elimination from the body and prevention of further propagation in the autooxidation chain-reaction process. Citrus limonoids were also shown to inhibit the formation of chemically-induced neoplasia in the oral cavity, oesophagus or forestomach, small intestine, colon, lung and skin of laboratory animals (Lam et al., 2000; Miller et al., 2000). Limonoids have also been shown to
exhibit some antioxidant activity despite the fact that less than that observed for flavonoids and hydroxycoumarins. They also inhibit the proliferation of breast cancer cells when cultured (Yu et al., 2005; Tian et al., 2001; Guthrie et al., 2000). Some limonoids are antimalarial and some antimalarials have been recorded to be effective in managing SCD (Chikezie et al., 2011, 2010, 2009 a & b; Chikezie, 2009, 2008).

Though the link between regional malaria prevalence and SCD prevalence rate has been established and malaria is considered to be worsening SCD prevalence, the molecular relationship between the two diseases is still unclear. Elevated oxygen tension in the blood reduces sickling of HbS but also enhances malaria parasite survival in the red blood cells, while the reverse is also true. Malaria incidence almost always results in SCD crisis for HbSS patients. The relationship between malaria and SCD crisis may lie in the change in cellular metabolism of HbS erythrocytes and other factors that may influence the immune system of sufferers. More work should be done on the effect of the malaria parasite in HbS erythrocytes and other blood parameters of SCD patient. Rapid breakdown of the RBC by the parasite leading to high concentration of Fe2+, which could act as a pro-oxidant in the blood could be a factor, and may be accountable for the ability of iron chelators to help reduce crises (Okpuzor et al., 2008).

Most alkaloids, including the piperine and guanidine alkaloids possess a wide range of pharmacological and biological activities, which include anticancer, anti- malarial, antibacterial, antiviral, antifungal, cardiotonic, antidiabetic, antioxidant, immuno-modulatory and psycho- active among others (Evans, 2002). Many alkaloids exact their actions by interference with various enzymic processes although the mechanism of action may differ from one alkaloid to another (Okwute and Egharevba, 2013). For instance, canarosine, a guanidine alkaloid from Canavalia rosea has been shown to inhibit dopamine
receptor binding (Pattamadiloka et al., 2008). Other compounds from plants showing anti-sickling properties belong to the furan, amides, amino acids, urea, guanidine, cysteine sulfoxide, carotenoid groups. Most of these compounds are abundant in pi-electrons and function as antioxidants. It is also possible that some like allantoin which contains the urea moiety, may act by inducing the synthesis of foetal haemoglobin like hydroxyurea or resveratrol. Compounds with the guanidine moiety may exhibit parallel action like arginine, while others with amide groups like the piperamides or amide-alkaloids and cysteine sulfoxides, may exhibit actions like the active amino acids such as lysine, arginine and phenylalanine. The furan group appears to be a very potent antioxidant moiety. It is found in 5HMF and ascorbic acid. Hence, it may play an important role in the activity of the parent compound. It should be noted that the actions of most active agents against SCD including hydroxyurea, 5HMF, amino acids etc, are primarily based on their antioxidant property. These compounds which have proven pharmacological activities such as anticancer, antitumor, anti-inflammatory, antibacterial, antimalarial, antifungal, antiviral, antidiabetic, immune modulatory and antioxidant activity may exhibit most of their activity by free radicals scavenging and prevention of LDL peroxidation.

The key factor for a new drug discovery is the preliminary information regarding the chemical constituents eventually comes from the qualitative phytochemical screening of plant extracts. In the present study, qualitative tests for different extracts of C. cajan and C. papaya revealed an array of phytochemicals including phenolic compounds. Plants have diverse chemical composition and related physiology for its separate parts. This results in the presence of different phytochemicals in its different parts. Therefore, specific plant parts used in the management of specific disease conditions could yield favourable results in comparison to using whole plant extracts.
Results of the antisickling activities in the present study revealed the sickling inhibitory activities of *Cajanus cajan* L. leaves in increasing order for various concentrations (concentration: activity) as - 10.0:68.45; 5.0:62.48; 2.5:58.17; 2.49.43; 1.5:41.42; 1:36.79; 0.5:34.43; 0.1:30.93. Seeds - 2.0:70.82; 2.5:56.63; 1.5:54.79; 5.0:47.39; 1.0:41.42; 10.0:41.01; 0.5:35.19; 1.0:29.2. In the stem extracts inhibition activity (IA) observed were, 10.0: 65.18; 5.0: 62.61; 2.5: 59.41; 2.0: 52.41; 1.5: 45.1; 1.0:39.54; 0.5: 31.3; 0.1: 27.9. Maximum IA of 68.45% was for *Cajanus cajan* L. leaves in a concentration of 10.0 mg/ml; seeds showed a IA of 70.82% in a concentration of 2.0 mg/ml; while the stems showed a IA of 65.18% in a concentration of 10.0 mg/ml. Whereas in *Carica papaya* L. the inhibitory activities of leaves extracts in increasing order of various concentrations (concentration: activity) are- 1.0:71.80; 1.5:62.03; 2.0:58.20; 0.5:53.90; 2.5:48.23; 5.0:45.36; 10.0:39.37; 0.1: 38.46. Seeds- 2.5:68.50; 2.0:61.67; 1.5:57.41; 1.0:53.21; 0.5:44.54; 10.0:37.71; 0.1:34.45; 5.0: 44.44. Stems-10.0:68.33; 5.0:62.48; 2.5:58.16; 2.0:49.22; 1.5:41.82; 0.1:41.82; 1.0:36.68; 0.5:34.21. Maximum IA of 71.80% of *Carica papaya* L. leaves in a concentration of 1.0 mg/ml; seeds showed a IA of 68.50% in a concentration of 2.5 mg/ml; while the stems showed a IA of 68.33% in a concentration of 10.0 mg/ml. Results of the antisickling activities in the present study revealed the reversal of sickled erythrocyte activity (RA) of *Cajanus cajan* L. in increasing order for various concentrations (concentration: activity) as- Leaf extracts- 10.0:72.13; 5.0:67.98; 2.5:63.53; 2.0:54.05; 1.5: 47.42; 1.0:41.59; 0.5:39.62; 0.1:36.55. Seed extracts- 10.0:70.96; 5.0:61.46; 2.5:56.71; 2.0:51.03; 1.5:49.66; 1.0:39.24; 0.5:32.94; 0.1:30.06. Stem extracts- 10.0:63.24; 5.0:57.72; 2.5:52.71; 2.0:46.26; 1.5:41.14; 1.0:35.41; 0.5:32.75; 0.1:27.22. The Reversal of sickled erythrocyte for different concentrations of extracts revealed maximum reversal activity (RA) of 72.13% in *Cajanus cajan* L. leaves in a concentration of 10.0 mg/ml; seeds showed a RA of 70.96% in a concentration of 10.0 mg/ml; while the stems showed a RA of 63.24% in a concentration of 10.0 mg/ml. RA for *Carica papaya* L in increasing order for various concentrations (concentration: activity) is, Leaf extracts- 10.0:72.43; 5.0:65.58; 2.5:63.37; 2.0:56.83; 1.5:50.20;
The Reversal of sickled Erythrocyte for different concentrations of extracts revealed maximum reversal activity (RA) of 72.43% in *Carica papaya* L. leaves in a concentration of 10.0 mg/ml; seeds showed a RA of 65.96% in a concentration of 10.0 mg/ml; while the stems showed a RA of 62.97% in a concentration of 10.0 mg/ml.

Both plants showed antisickling activities in the inhibitory and reversal tests, in various concentrations. The antisickling assays specified that there was a linear correlation within the parts studied and both the inhibitory and reversal activities shown by both these plants was quite encouraging. The oxygen level in vitro experiments is low due to the addition of sodium metabisulphite. The medium is also acidic and dehydration conditions prevail, which finally leads to sickling of HbS. Blood samples are subject to oxidative stress upon treatment with sodium metaphysical which creates hypoxia conditions resulting in the formation of sickle cells. Chikezie (2011) opined that under the influence of sodium metabisulphite the haemoglobin molecules undergo massive congregation and they are readily polymerized, which augments the sickle cell formation. The anti-sickling activity observed could be linked to their ability either to inhibit in vitro polymerization of haemoglobin or to some structural modification linked to the environment of haemoglobin by the extracts (Bianchi *et al.*, 2007).

The management of SCD could be sought in the numerous methods which could prevent polymerization of erythrocytes into sickle cells. The haemoglobin molecule was found to resist polymerization and changing its shape into a sickle by all or in association of one of the following conditions- (a) The propensity and proficiency of the biomolecule to bind to the deoxygenated haemoglobin molecules (Abdulmalik *et al.*, 2005; Bianchi *et al.*, 2007) (b)
Alteration of amino acids which are responsible for the quaternary structures of haemoglobin molecules including their active and contact sites (Oyewole et al., 2008) (c) Offer consistency and stability to the haemoglobin molecule (Oyewole et al., 2008; Ibraheem et al., 2010; Chikezie, 2011). Therefore, antioxidants present in the antisickling agents are essential and important as they are capable of attaining on or all of the above mentioned conditions.

Stuart et al. (1994) and Imaga et al. (2011) reported various phytochemicals which acted as strong antioxidant molecules and at the same time they were also found to be potential antisickling agents.

Consequently, varied antisickling activity showed by the extracts in different concentration in the present study can be ascribed to the presence of varying degrees of antioxidants (polyphenols, alkaloids and flavanoids etc) in the extracts of different plants parts, in different concentrations. Another factor could be the affinity showed by the antioxidant molecule with haemoglobin molecule’s binding site; which is the key factor determining the success of the concerned molecule as a potent antisickling agent. Usually, most of the anti-sickling agents prefer the RBC membrane as a binding site to the haemoglobin molecule. The anti-radical and anti-oxidant properties of the plants extracts along with their antisickling potential lies in their capability to deliver hydrogen atoms to the haem molecule of haemoglobin (Kasi et al., 2008). Phytochemical acting as potent antioxidants have been proved also to possess anti-sickling (reversal and inhibitory) properties and can well be used as a curative agent for the management of SCD (Tatum and Chow, 1996; Kannat et al., 2007). Therefore, the antioxidant property of a phytochemical can be asserted to be directly proportional to its anti-sickling effect, as it can reduce the amount of oxidative stress which is the key factor behind the formation of sickle cells, critical during the crisis stage of the SCD. Antioxidants also contribute towards maintaining the membrane stability of the erythrocytes.
Thus, there is a strong possibility that during the “crisis stage” of the SCD, when the patient is subjected to oxidative stress, antioxidants (=antisickling agents) can come into play by scavenging the free radicals present in the system. Additionally, the phenolics as antioxidants can help in scavenging of free radicals, which can cause damage to the cell. This scavenging and free radical accepting attitude of the phenolic compounds makes it an inexorable phytochemicals for the management of SCD. Alkaloids are widely used in the treatment of various ailments like, malaria, cold, cough, hypertension, diabetes as well as cancer (Akpuaka, 2009). Phenolic compounds act as an antiseptic in surgical procedures, as an oral analgesic, in drug production, in cosmetics, to embalm bodies etc. Hence, alkaloids and phenolic compounds possess immense medicinal properties and could also act as potential antisickling agents. However, further studies to isolate and characterize these phytochemicals responsible for antisickling activities should be the major thrust area.

The constituents of the extracts of the plant are highly oxygenated due to the presence of poly hydroxyl constituents of the plant such as gallic acid, methyl gallate, myricetin, quercetin, afzelin and isoquercetin (Yoshiki et al., 1996); myricetin 2', 3'-di-O-galate and quercetin 3'-O-methyl ether (Sofowora et al., 1979). Hence they have an opportunity of possessing antioxidant and anti sickling properties. Caffeic acid which is reported to be present in the plant was known to exhibit antioxidant property in vitro and in vivo and also poses immuno-modulatory and anti-inflammatory activities (Olthof et al., 2001). This is similar to p- hydroxy benzoic acid earlier reported being the active antisickling principles of Zanthoxylum xathoxyloides (Sofowora et al., 1979). Finally, the presence of these compounds in the extracts of Cajanus cajan and Carica papaya could be responsible for the observed antisickling activity. The presence of phenolic compounds found in these plants could also contribute to the antisickling property of the plant because phenols and gallic acid have been reported to protect human cells against oxidative damage. Amino acids present
in plants with anti-oxidant properties may contribute towards the anti-sickling activities shown by the plants (Noguchi, 1977). It has been reported that amino acids may play a major role in the gelation kinetics and the solubility of sickled cells (Igbal and Kazi, 1980). They further observed that the aromatic amino acids, phenylalanine, tyrosine and tryptophan were significantly more active as antisickling agents than other amino acids. Duke (1992a) opined that the amino acids, glycine and phenylalanine, were the antisickling agents, while Ekeke and Shode (1990) reported that phenylalanine was the most prominent antisickling constituent in seeds. The three aromatic amino acids already reported by Igbal and Kazi (1980): tyrosine, phenylalanine and tryptophan, are present in *C. papaya* unripe fruit (Duke 1992a). It could therefore be suggested that these amino acids might be part of the antisickling components of the leaf, stem and seeds of *C. papaya*. Furthermore, Ohnishi and Ohnishi (2001) studied the *in vitro* mechanism of dense cell formation caused by deoxy-cycling and found that nutritional antioxidants could inhibit the formation of dense cells when they are employed in the combination of vitamin C, vitamin E and aged garlic extracts. Many constituents of *Carica papaya* fruit including vitamin C, beta-carotene, citric acid, gamma-terpinene, lycopene, methionine, alanine, sucrose and tartaric acid have been reported to have antioxidant activities (Duke 1992b) which could also contribute to its observed antisickling properties.
5.3. CONCLUSIONS

The present study validates the extracts of leaves, seeds and stems of two plants, *Carica papaya* L. and *Cajanus cajan* L. to possess various phytochemicals as well as antisickling activity. The antisickling activity observed in these plants may be because of their underlying phytochemicals. A detailed thin layer chromatography (TLC) pattern obtained in this study in different extracts provides ample insight into the total number of phytochemicals present in each of the individual parts studied. In both plants most of the parts showed maximum presence of phytochemicals and significant antisickling activity.

The results attained in these phytochemical and antisickling assays can 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 results further support the view that phytochemicals with antioxidant properties can well act as potential antisickling agents. Therefore, there is a possibility that all the plants possessing anti-oxidant effects may act as antisickling agents also. However, further studies on the patterns of antisickling assays have to be ascertained for these plants.

In, both of the plants studied, the nutritive value is also high as evident from proximate assays. Hence, on the basis of the results obtained by the proximate, phytochemical and antisickling assays, we conclude that both of these plants, *Carica papaya* L. and *Cajanus cajan* L. can be used as a nutritive as well as an antisickling agent during the pharmacological formulation of novel drugs which can be used the management if Sickle Cell Disease (SCD).