

## Potentials of ethnobotanicals and nutraceuticals in the management of sickle cell disease

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### Abstract

Plants are the richest resource of drugs of traditional systems of medicine, nutraceuticals, food supplements, pharmaceutical intermediates, and chemical entities for synthetic drugs due to their therapeutic alkaloidal, flavonoid and terpenoid content. The entire world is very rich in all levels of biodiversity namely, species diversity, genetic diversity and habitat diversity. A vast array of plant species is known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in trend even before ancient civilization. Herbal medicine is still the mainstay of about 75% of the whole population in rural Africa, and a major part of traditional therapy involves the use of plant extract and their active constituents. The reliance could be attributed to our socio-cultural, socio-economic heritage, lack of basic health care and personnel in every nook and cranny of rural communities. Sickle cell disease (SCD) is one of the most prevalent hereditary disorders with prominent morbidity and mortality. Most of the 2.4 million Nigerians with sickle cell trait belong to lowest cadre of the society; consequently, they cannot afford the high cost of orthodox management of sickle cell disease. As a result, they rely on natural products such as herbs to alleviate the numerous symptoms presented in SCD. The main objective of this chapter is to highlight the potentials of Phyto materials and nutraceuticals in the management of SCD.

**Keywords:** Sickle Cell; Genetic; Defects; Ethnobotanicals; Nutraceuticals; Traditional Systems

### 1. Introduction

Sickle cell anaemia, drapanocytosis or sickling anaemia is among the common genetic disorder in Sub-Saharan Africa and Middle East responsible for high mortality, this disorder was initially thought to exist in tropical and Mediterranean regions [1]. Sickle cell disease (SCD) was first discovered by a Chicago physician, Dr James B. Herrick in 1904 when he examined a 20-year-old black student from West Indies [2]. Normal red blood cells move through small vessels in the body to deliver oxygen and food nutrient. Sickled red blood cells, however, tend to obstruct the blood flow causing poor blood microcirculation [3]. The red cell membrane of sickle haemoglobin (HBSS) is osmotically and mechanically more fragile than those of haemoglobin AA(HbAA), hence sickle red blood cells are easily destroyed and removed from circulation in the spleen thus causing anaemia and subsequent splenomegaly [4].

Over 50 million people are affected throughout the world [5] African continent remains the most affected by this disorder with the highest prevalence in its West and Central parts. Migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased in recent decades and in some European countries In Nigeria, more than 3% of its population is affected [6], about 80% of children suffering from

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drepanocytosis that do not receive regular medical care, die before the age of five [7]. Drepanocytosis is a genetic disease in which the SS individual possesses an abnormal beta globin gene. A single base substitution in the gene encoding the human B-globin subunit results in the replacement of B- 6 glutamic acid by valine, which leads to the devastating clinical manifestations of sickle cell disease [8] This substitution causes a drastic reduction in the solubility of sickle cell haemoglobin when oxygenated [9] under these conditions, the HbSS molecules polymerize to form long crystalline intracellular mass of fibres which are responsible for the deformation of the biconcave disc shaped erythrocyte into a sickle shape. Sickling of blood cell disease (SDC) appears to be unsatisfactory; patients suffer from painful crisis, acute chest syndrome and malfunctioning of organs including the spleen, heart and brain as well as from degeneration of the bone [4].

Patients with acute manifestations may have prolonged and repeated hospitalization leading to inferior quality of life and profound psychological impact. Multiple organ systems may be involved leading to splenic infarction, leg ulcers, pulmonary hypertension, strokes, retinopathy, and a vascular necrosis [10]. HbSS individuals have reduced life span, with an average life expectancy of 40 to 50 years [11]. Even though there is no cure for SCD, good management involves proper diet, folic acid supplementation and adequate intake of fluid. Recently, bone marrow transplantation is being implemented though it is expensive for the African poor population. More critical concern is the issue of the use of a virus in the transplant processes. Fetal haemoglobin synthesis stimulants such as hydroxyurea are toxic [12], more so, gene therapy is under investigation at various research centres. The low-income people living in the rural communities who cannot afford the excessive cost of orthodox medicines have resorted to using plant nutraceuticals; and other natural phytochemicals as a line of treatment for their ailments [13]. Nutrigenomics, a process of using nutrients to either enhance or suppress phenotypic outcomes without distorting DNA sequences has now provided a beam of hope in this direction. This epigenetic event is thought to occur through the various processes of DNA and histone modifications.

Most of the proposed therapies for sickle cell anaemia (SCA) appear to be unsatisfactory, bone marrow transplantation is expensive for African poor populations, foetal haemoglobin synthesis stimulants such as hydroxyurea are toxic and repeated transfusions constitute high risk of human immunodeficiency virus (HIV) infection rate [12]. The health cost of the management of SCD patients is disproportionately high compared to the number of people afflicted by this disorder. The common people living in the villages are mostly peasant farmers who cannot afford the high cost of treatment by orthodox medicine. Due to the debilitating effect and the cost of managing SCD, research has been ongoing to determine the efficacy of the use of medicinal plants to tackle the multiple challenges presented in SCD [13].

The use of natural products in attempts at inhibiting sickling could be as old as when SCD was discovered (14). Folkloric history has indicated attempts made by inhabitants using plant derived recipes in parts of Nigeria to treat what they described as “fever of crisis”, shifting joint pains, exacerbations especially during rainy seasons and constant abnormality of the blood [14]. Very few ethnobotanical remedies for the treatment of Sickle Cell Anaemia (SCA) have been reported in literature due to the secrecy attached to the treatments of this disease. Recent discoveries of anti-sickling Phyto remedies that are cheaper and less toxic alternative therapies for SCD include: *Piper guinesis*, *Pterocarpus osun*, *Eugenia caryophylla* and *Sorghum bicolor* extracts [15].

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## 2. Phyto-remedies for SCD Management

Phytochemicals are non-nutritive chemicals that contain protective, disease preventive compounds [16 – 18], which are naturally occurring compound in fruits and vegetables, legumes and grains. These compounds are associated with prevention and treatment of diseases such as cancer, diabetes, cardiovascular diseases, and hypertension [19 – 21]. The result of quantitative phytochemical analyses of ethnobotanicals used in the management of SCD indicates the presence of tannins, phenols, flavonoid, alkaloid and saponin in concentrations stated therein [19]. Tannins have been reported to provide protection against microbial degradation of dietary protein [22]. Tannins also act as an antioxidant; may inhibit enzymes that activate carcinogens [23]. On the other hand, alkaloids, a group of nitrogenous compounds are reported to interfere with cell divisions [22]. Therefore, this suggests that these extracts may help to punctuate the undue proliferation of mutated hemoglobin in SCD, by halting the proliferation [24]. The presence of alkaloids in the extracts may have pharmacological effects on animals. Conine, an alkaloid is reported to be effective in preventing blood loss during cuts and also brings about blood clot [25]. Alkaloids such as vinorelbine, melphalan and temozolomide are used for treatment of induced anaemia [26]. Tubocurarine and procenbazine have been used in tincture used in wounds apart from their use as neuro-blockers [25]. The main component of tincture is the oxygen carrying capacity that increases the circulation of blood [27], by implication, alkaloids which are present in the extract of the botanicals used in SCD can enhance oxygen carrying capacity which is a key factor in SCD pathophysiology. The antioxidant properties of the extracts could also be useful to SCD patients by acting as free radical sink.

Similarly, saponins can control human cardiovascular disease and reduce cholesterol levels [22]. It has also been reported to cleanse and purify blood [28]. The presence of saponins in *Eremomastax polysperma* may be one of the reasons for its name as a blood tonic and use as a spiritual soap in some part of Ghana. *Duranta ripens* has an elevated level of saponins, hence can also serve as a cleanser and tonic for blood, this can be useful in combating anemia which is prevalent in SCD. The presence of both phenols and flavonoids in the plants is important. The levels of these two phytochemicals are important in antisickling formulations. Both flavonoids and phenols are known to possess antioxidant activities. Antioxidants neutralize highly unstable and extremely reactive molecules, called free radicals, which attack the cells of the human body [29]. Free radical is responsible for a variety of health problems noticed in SCD, heart disease and aging. Anti-oxidation is one of the ways by which the extracts achieve their result [30;31]. Oxidation of RBC has been implicated in sickle cell polymerization due large to release of oxygen per unit time. Therefore, the presence of antioxidant nutrients in the extracts can help mop the excess oxygen released in SCD, thereby reducing the severity of SCD crises. This could be one of the possible modes through which the plant extract reduces the severity of painful crises in SCD patients when administered with the extracts by traditional herbalists. Despite the presence of phytochemicals of medicinal importance, the plant samples also showed some quantities of anti-nutrients particularly oxalate, thiocyanide and phytate. This is likely to raise safety concerns on the consumption of these herbs. Oxalate, cyanide and phytate present in the plants with varying concentrations. Cyanide has been reported to confer some level of stability to people affected by SCD in the tropics because of the consumption of tubers with reasonable levels of cyanide [32]. Therefore, the presence of cyanide is a two-edge sword of a sort. Luckily, the level of the anti-nutrient is within range of safety. Phytate and oxalate are known to militate against absorption of certain minerals in the body.

In SCD, metabolic changes occur leading to anemia, anemia is a life-threatening feature in SCD. There is increased turnover of hemopoietic cells due to chronic hemolysis resulting in increases in nutrient and energy demand [33]. Some minerals have been found beneficial in the control of anemia under this condition. These include iron (Fe), copper (Cu), Zinc (Zn). The biochemical evidence for diagnosing zinc deficiency in patients with SCD includes low zinc concentrations in plasma, erythrocytes, hair, lymphocytes and granulocytes and low activities certain zinc-dependent enzymes, such as carbonic anhydrase in rbc, alkaline phosphatase in neutrophils, and thymidine kinase in newly synthesizing skin connective tissue and collagen [34]. Iron is very important in the synthesis, copper and zinc play very important roles in iron metabolism. The biological importance of iron in mammals is well known, therefore its importance in this situation of hemolysis cannot be over emphasized considering its role in oxygen transport as well as in many metalloenzymes involved in oxidative phosphorylation [33].

Copper is known to be essential in the proper functioning of different metalloenzymes which include ceruloplasmin involved in iron metabolism. The chemical analyses of the antisickling extracts of these plants revealed the presence of these minerals. The consumption of the plant extracts may provide the body with adequate supplementation of the loss micronutrient. Various incompletely understood roles have been postulated for selenium in human metabolism. Selenium plays crucial role in cytochrome p450 system in pancreatic function, in DNA repair and enzyme activation, in immune system function and in detoxifying heavy metals. The macro-minerals of calcium and magnesium are also very important especially in maintaining membrane integrity. Phytate is present in some of these extracts, such presence may militate against the absorption of calcium, therefore need for calcium supplementation will rise. The global use of micronutrient in healthcare delivery has taken Centre stage due to the realization of their importance in disease management and the protection of red cell membranes from free radical. Mediated oxidative stress is crucial to their management. Minerals such as copper, iron, magnesium and selenium have been found to relieve oxidative stress associated with certain disease situations [33]. These local sources of plants including fruits could be good sources of trace elements essential to man [35].

Anaemia is one of the major health problems affecting the tropical and sub-tropical regions of the world. Each year, there are more than 245 million cases of anaemia, majority of which are young children and women [36]. A wide variety of supplements are available for the treatment and prevention of anaemia. Iron fortification of foods and beverages was another alternative to treat and prevent anaemia, it has been a common practice to add micronutrients to different foods such as cereals, dairy products, snacks

The incidences of anaemia in SCD due to increase loss RBC, resulting from haemolysis is of great concern. The results of hematological studies of *Eremomastax polysperma* used by traditional herbal practitioners in Akwa Ibom State, Nigeria, revealed that the extracts affected WBC, HCT, MCV, RBC, NEUT, MCHC, count and lymphocyte significantly [37]. WBC is the first line of the body defense against invading organisms. This suggests that the extract of the plants contains agent that stimulate the production of these hematological indices. The presence of such agents had been reported in for *Viscum album* (Mistletoe) and other commonly prescribed medicinal plants. [38]. The increased levels of Hb implies that there is a positive change in oxygen carrying capacity of the blood and transferring of respiratory gases [39]. WBC

differentials are the indicators of the ability of the organism to fight infection which is a common issue with SCD. The increase RBC which is supported by the improved levels of MCH and MCHC are positive clues to the alleviation of anaemia by the extracts. These parameters are used mathematically to define the concentration of Hb and to suggest the restoration of oxygen carrying capacity of the blood. Though the mode of action is not known, however, it may be attributed to the ability of the extracts to lower lipid peroxidation level that causes hemolysis of erythrocytes [40; 41], therefore could inhibit peroxidation of polyunsaturated fatty acids in the cell membrane hence hemolysis of RBC which is reported that the plant extracts possessed compounds such as phenols, flavonoids, and tannins which are strong antioxidants. Lymphocyte and platelet count of wistar albino rats fed with aqueous extract of *Passiflora edulis* showed significant increases. This is also supported by the findings that *Hermania incana* also produced mild changes in haematological indices of rats through sensitization of bone marrow [40].

Orthodox medicines for management of SCD have been suggested to grossly affect the levels of some plasma proteins which might result due to thiocyanate ingestion [42]. Decrease in albumin has been observed in serum of patients with tissue inflammation and damages [43].

The extracts significantly reduced TC and TG when compared with those animals in the control group. These decreases might be due to the presence of hypolipidemic agents in the extracts. [44] earlier reported that ascorbic acid increases cholesterol transformation to its degradation product, bile acids by stimulating  $7\alpha$ -hydroxylase responsible for the conversion of cholesterol to hydroxycholesterol. Increased clearance of the end product of cholesterol catabolism due to absorption by dietary fibre may be another mechanism by which the extracts help in lowering cholesterol levels of the animals compared with the control groups. [45], had earlier reported that bile acid absorption by dietary fibre in vitro has also been reported for many fibre types. In addition, polyphenols such as flavonoids and tannins have been shown to have numerous health protective benefits of which include lowering of blood lipids. Moreso, it has been reported that several plant sterols reduce serum cholesterol by inhibiting cholesterol absorption in the intestine (46). It can therefore be deduced from the proximate and phytochemical analysis that the presence in the plant extracts may interact in a synergistically to impart hypolipidemic properties of the extract. Changes in the levels blood cholesterol may be an indirect indicator of liver functions.

Hypocholesterolemia, and to a lesser extent hypertriglyceridemia have been document in SCD cohorts. Decreased TC and LDL-C has also been documented in patients with SC [47]. TC, in particular LDL-C in SCD is consistent with the low levels of total cholesterol and the virtual absence of atherosclerosis among SCD patients. Increased TG levels in serum Lipids are generally characterized by insolubility in aqueous or polar solvents but highly soluble non-polar or organic solvents. Biochemical reactions and transportations of molecules generally occur in the aqueous medium. Hence, lipids are normally combined with specific proteins to form structures called lipoproteins which possess substantial degree of hydrophilicity. Low density lipoproteins (LDL), high density lipoproteins (HDL) and chylomicrons which are basically TG are integral part of serum lipoproteins [48]. Except for HDL, high level of all lipids in the blood is arguably a high-risk factor in the onset of cardiovascular disorders. High serum concentrations of TG and LDLs have been reported to cause atherosclerosis and coronary heart diseases (CHDs) [49]. Lipoproteins and albumins in plasma can contribute fatty acids to red blood cells for incorporation into membrane phospholipids [50], but RBC membranes are not TG rich. Interestingly, chronic intermittent or stable hypoxia just by exposures to high altitudes, with no underlying disease, is sufficient to increase TG levels in healthy subjects [51]. Thus, it is possible that hypoxia in SCD may contribute at least to observe increase in serum TG, therefore, the extracts if administered on SCD patients may help in lowering their TG levels.

Therefore, the analgesic property of the extracts may be attributed to the use of these phytochemicals in SCD management. Moreso, recent studies suggest that inflammatory tissue damage is due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites. There are also reports on the role of flavonoid, a powerful antioxidant [52]. In analgesic activity primarily by targeting prostaglandins [53]. Acetylation appears to occur at specific sites on the protein. The compound significantly inhibits the gelation of cell free dexoy Hbs and erythrocyte sickling [54].

During inflammation, activation of mast cells, macrophages, eosinophils, and neutrophils are known to produce ROS such as super oxide radicals ( $O_2^\ominus$ ) with NADPH playing a role [55]. These free radicals can also act as secondary messengers, thereby provoke the production of other mediators involved in the inflammatory response. Bruneton, [56] had shown that presence of tannins, flavonoids, saponins, triterpenoids and steroids to possess anti-inflammatory, analgesic and antioxidant effects. Most of the aforementioned plant secondary metabolites are present in the extracts investigated in the current study. The anti-inflammatory effects of the extracts were time dependent. Most of the extracts act maximally between two and three hours from the time of administration. *Duranta ripens*, 250mg/kg b.wt

and *Eromomastax poylsperma* (green bark), 500mg/kg b.wt were able to match the potency of the standard drug used as positive control at the third hour of administration (75%) [37].

The possession of antisickling potential by the candidate molecule(s) implies that such material would interfere in three different stages of sickling process. Antisickling agents could be targeted to modify at the sickle gene, polymerization and red cell membrane levels [57]. Recently, a good number of studies have been done for identification and characterization of potential antisickling compounds from different plant sources. The most promising were found to be anthocyanins, anthraquinones, steroidal glycosides, cardiac glycosides, alkaloids, flavonoids, saponins, tannins, phenols, hydroxybenzoic acids, liminoids, 5-hydroxymethyl-2-furfurals (5HMF), isomeric divanilloylquinic acid and certain amino acids like arginine, tyrosine, aspartic acid and phenylalanine [2; 57; 58]. Even though, *in vitro* antisickling activity were observed with these compounds, their mode of action and the mechanism through which these actions are exerted is yet to be properly elucidated. [59] Have shown that *Cajanus cajan* exerts significant inhibitory effect on sickling. The extract was found to reverse sickling in a dose dependent manner. They also report an average half-life of the extract indicative of a reasonable duration of action of the extract [8].

## 2.1. Nutrition and Genetic Diseases

Several therapies have been proposed and many chemical substances investigated for their possible role in the management of SCD. However, this haemoglobin disorder remains one chronic disease in which the role of nutrition in its aetiology has not been systematically addressed [60]. Many investigations have been carried out on the role of some dietary supplements, such as thiocyanate [61]. Different species of legumes abound in the tropical Africa, which are very rich sources of proteins and amino acids. Some of these amino acids such as phenylalanine, lysine, arginine and glutamine have antisickling properties [60]. This has led to the formation of an antisickling preparation 'ciklavit™' in combination with other food extracts is used in Nigeria and other West African countries for the management of SCD [62].

Some measure of success has been recorded in the use of mineral elements such as zinc, in the management of SCD [34]. Results of laboratory investigations have shown that blood concentration of zinc is significantly lower ( $P < 0.05$ ) in sickle cell patients (SCD) than in people with normal haemoglobin (HbAA), and that zinc level drops even lower during sickle cell crises [34;63]. The biochemical evidence for zinc deficiency in patients with SCD includes low zinc concentration in plasma, erythrocyte, hair, lymphocytes and granulocytes [64;65]. Similarly, low activities of zinc dependent enzymes such as carbonic anhydrase, alkaline phosphate and thymidine kinase have also been observed [34]. A higher-than-normal activity of plasma ribonuclease in patients with SCD is also seen because zinc is known to inhibit the activities of this enzyme [34]. Zinc supplementation in patients with SCD resulted in a significant improvement in the secondary sexual characteristics, normalisation of plasma ammonia concentrations and the reversal of abnormalities in adaptation to darkness [64;66].

We eat a complex mixture of foods, which contain a host of different nutrients and other bioactive compounds. Intricate biochemical processes extract energy and other useful components, enabling us to keep our bodies and minds functioning effectively. In the past, many food compounds were dismissed, having no obvious nutritional role [67]. We now know they have a range of biological effects. For example, eating diets rich in foods containing plant polyphenols (e.g apples, onions, carrots and tomatoes) reduce the risk of developing gastrointestinal tract cancers; consumption of cooked tomato sauces reduces a man's likelihood of developing prostate cancer [67], and a high folate intake reduces plasma homocysteine, which is an independent risk factor for cardiovascular disease (CVD) as well as preventing neural tube defects in the developing foetus [67].

Some people have or acquire a substantial risk of disease (e.g breast cancer), which may or may not be affected by diet. For majority, however, simply keeping our weight down, not smoking, not consuming alcohol in excess and taking regular exercise will be sufficient to keep our risk low. Nevertheless, as we get older, our bodies are less effective at avoiding disease; our immune systems are less able to detect and mount a defence; our DNA becomes more error prone, and our proteins are less functional. The resulting breakdown in cellular structure and function leads to those diseases we associate with old age like cancer, CVD, type II diabetes, cataract and arthritis. Poor diet can accelerate this degeneration whilst the majority of studies support those diets rich in fruits and vegetables, cereals and plant oils, reduces risks. Thus, diets and risk of disease are ultimately associated not only in terms of excess but also in promoting optimal health [67].

### 2.1.1. Nutrigenomics

Nutrigenomics is the science of how bioactive chemicals in foods and supplements alter the molecular expression and structure of an individual's genetic make-up [68]. Thus, by identifying individual predispositions for chronic diseases

and the potential for individual response to dietary intake, Nutrigenomics brings together the science of bioinformatics, nutrition, molecular biology, genomics, epidemiology, and molecular medicine. This term nutrigenomics encompasses the field of genomics, epigenomics, post-translational modifications, proteomics, and metabolomics. The great challenges of the 21<sup>st</sup> century will be to integrate this scientific understanding of nutrigenomics into the provisions of diet, lifestyle, and drug recommendations to an individual in maintenance of healthy lifestyles and diseases prevention rather than development of diagnostics kits and drugs to identify and attempt to cure them once they have emerged.

The sum of this knowledge will be a better understanding of the influence of inheritance and environment on individual health and performance we will better understand what role diet and environment play on gene expression and what limitations gene expression imposes on an individual response. This opens the opportunity to have personalized medicine and personalized nutrition.

## 2.2. Antisickling Effects of Dietary Thiocyanate

The rarity of SCA in Africans was in the past attributed to an unknown environmental protective factor [61]. Song noted that the active form of SCA is quite rare, while the harmless heterozygous sickle cell trait (SCT) occurs more frequently in Africans than in African Americans in the United States [61]. He pointed out that genetic theory of Neel and Pauling does not seem to explain the relationship between SCT and SCA [70]. Song then proposed that a protective factor is present in regions of Africa with negligible SCA aetiology. The protective factor against SCA was subsequently identified as the thiocyanate (SCN) which is found in the African yam (*Dioscorea sp*) and cassava (*Manihot utilissima*) [71].

Lower levels of SCN are found in non-staples in the American diet, in carrots, cabbage and radishes [72]. According to Houston [71], a daily African meal is estimated to have some levels of SCN than that of the diet of African Americans. The relative SCN-deficiency of American staple foods allows for the full and predictable incidence of SCA [61]. Jamaicans experience the same nutritional prophylaxis against SCA as do Africans because the cassava diets are present in Jamaica. Serjeant and co-researchers have reported that SCA is remarkably mild in Jamaica and that the homozygous sickle haemoglobin phenotype is compatible with survival into and beyond middle age [73]. They noted that the benign clinical course of SCA in Jamaicans must be because of the presence of an unknown indigenous protective factor, as patients with mild SCA suffer severe crises when they immigrate to the United States and are relieved on return to Jamaica [61].

Cassava, a staple of many households in the “tropics” is among the cyanogenic plants with the highest SCN levels [71]. In Africa, a popular cassava staple product, “garri” contains about 60mg of SCN-for each 100g of cassava. In Jamaica and other West Indian Islands “farine” is a well-known cassava derived food (74); hence cassava, common to tropical countries, provides a major amount of SCN needed for prophylactic control of SCA.

Thiocyanate is a naturally occurring compound in body fluids-blood, urine, saliva, sweat and tears [75]. In plants, the parent compounds are non-toxic sugary compounds called cyanogenic glycosides termed nitrilosides (or vitamin B<sub>17</sub>) [75]. Nitrilosides are initially hydrolyzed to hydrogen cyanide (HCN) by  $\beta$ -glucosidase, an enzyme produced by intestinal bacteria as well as body tissues. The released HCN, in the presence of a sulphur donor (cysteine and methionine), is converted to a non-toxic SCN – by rhodanese, an enzyme found in various organs and tissues of the body, with the highest concentration in the liver [61]. The released HCN is used also by the liver for the production of vitamin B<sub>12</sub>, cyanocobalamin from provitamin B<sub>12</sub>, hydroxycobalamin [75]. The liver is thus the organ most responsible for the formation of SCN-and vitamin B<sub>12</sub> from HCN metabolic pool. High levels of preformed SCN-as found in some plants, arise from similar enzymatic hydrolysis of nitrilosides.

In 1932, Torrance and Schnabel used potassium thiocyanate (KSCN) to successfully resolve two cases of sickle cell crises [76]. It was observed that KSCN promptly and effectively eliminated pain and discomfort in the patient. The KSCN dosage administered was less than half the effective dosage of cyanate, a well-known antisickling agent [61].

Oxidation of the SCN-ion to cyanate is catalysed in the erythrocytes by haemoglobin which acts as a peroxidase (77). In this reaction, the intermediate product, HCN, is spontaneously converted to cyanate. Cyanate inhibits sickling of erythrocyte irreversibly *in vitro* and extends the lifespan of erythrocyte to almost normal *in vivo* [78]. In preliminary clinical trials with thiocyanate, at the University of Rockefeller, Gillette and Colleagues observed a decrease in haemolytic anaemia in patients with the HbSS phenotype as shown by a significant increase of haemoglobin and haematocrit in patients with oral cyanate regimen [78].

Cyanate, the product of SCN-oxidation in erythrocyte, binds to amino terminal valine residues of haemoglobin, such a binding reaction, called carbamylation, occurs at the active site of error on the sickle haemoglobin molecule and thus correct it [61]. Carbamylation increases oxygen affinity of HbS, thus increasing the proportion of oxygenated conformers

of HbS that cannot sickle. The electrophoretic mobility of HbA, and one with HbS treated with cyanate shows two bands; one band with the mobility of HbS instead of just one band for HbS found in untreated HbSS phenotypes [79]. Carbamylation causes a structural modification of the amino terminal of HbS resulting in a protein variant with the functional characteristics of the normal haemoglobin [80].

### 2.3. Pyrroloquinoline Quinone

Pyrroloquinoline quinone (PQQ) was discovered in 1979 by J. G. Hauge as the third redox cofactor after nicotinamide and flavin in bacteria. The enzymes containing PQQ are called “quino proteins”. Glucose dehydrogenase, one of the quinoprotein, is used as a glucose sensor subsequently, PQQ was found to stimulate growth in bacteria [81].

PQQ and CoQ<sub>10</sub> promote antioxidant and mitochondrial health through different mechanisms, providing multifunctional support for cardiovascular health and neuroprotection. [82]. Rucker, and colleagues reported that mice deprived of PQQ showed various abnormalities and suggested that PQQ may also have an important nutritional role in other mammals [83;84]. It was further suggested that PQQ a vitamin [85]. It was recently shown that PQQ may stimulate the growth of plants in hydroponic culture and may be the causative factor in plant growth stimulation by a strain of *Pseudomonas fluorescens* bacterium [86]. PQQ as a prosthetic group on glucose dehydrogenase has been utilized at the anode in an enzyme-based fuel system [87]. As an antioxidant, PQQ helps mop up excessive oxygen released by sickled cells thereby relieving the cells of oxidant stress.

### 2.4. Use of Micronutrients in the Management of Anaemia in Sickle Cell Disease

Maintaining the membrane integrity is key strategy in the control of anaemia in SCD. Disruption of membrane integrity crises from fragility, dehydration as well as increased production of ROS. Chronic haemolysis leads to loss of Hb. These metabolic changes lead to depletion of essential nutrients and micronutrients required for proper cell function.

Some minerals and vitamins have been found beneficial in the control of anaemia under this condition. These include Iron (Fe), Copper (Cu), zinc (Zn) and folate [88]. Prasad first reported zinc deficiency in adult patients with SCD [33]. Iron is very important in the synthesis of Hb while Cu and Zn play very important roles in Fe metabolism [88]. The biological importance of Fe in mammals is well known. Therefore, its importance in this situation need not be overemphasized considering its role in oxygen transport as well as in many metalloenzymes involved in oxidative phosphorylation.

Copper is known to be essential in the proper functioning of different metalloenzymes which include ceruloplasmin involved in Fe metabolism. Deficiency of Cu is known to cause anaemia. The mechanism of copper deficiency induced anaemia is not well understood [89]. Previous studies suggest that the Cu-containing enzyme, ceruloplasmin, may have specific role, probably related to its function in mobilization of stored Fe in the liver which makes Fe available for haemoglobin synthesis [89]. However, it has been observed that in copper deficiency-induced anaemia, in spite of elevated Fe level in the liver, the rate of haemoglobin synthesis remains significantly reduced [89].

Like copper, the mechanism through which Zn exerts its effect in correcting anaemia in SCD is not well understood, but it is known that the proteins making up the cytoskeleton of cell membranes acquire some abnormal configurations and often get irreversibly damaged. Zn prevents the formation of such irreversibly damaged sickle cells [89].

Furthermore, it has been proposed that the role of Zinc in the management SCA centres on its calcium antagonism. Zinc is known to inhibit the activity of calmodulin which activates the calcium-ATPase that controls the calcium pump system of the erythrocytes. During sickling, there is an influx of calcium into the erythrocytes, and this occurs probably because calmodulin is so over activated that the membrane is destroyed. It is suggested, therefore, that during zinc therapy, its anti-calcium action produces antisickling effect [89].

Zinc deficiency in adult with SCD was first reported in 1975 [34]. Several clinical manifestations of this disorder were subsequently related to Zn deficiency [90]. These manifestations include growth retardation, hypogonadism in males, hyperammonaemia, abnormal dark adaptation and cell mediated immune disorder [34]. The biochemical evidence for diagnosing Zn deficiency in patients with SCD includes low Zn concentrations in plasma, erythrocytes, hair, lymphocytes granulocytes and low activities of certain Zn – dependent enzymes such as carbonic anhydrase in RBCs, alkaline phosphatase in neutrophils and thymidine kinase in newly synthesizing skin connective tissue and collagen [34]. A higher-than-normal activity of plasma ribonuclease in patients with SCD also indicate Zn deficiency because Zn is known to be an inhibitor of this enzyme [90].

Hyperammonaemia was formerly reported in Zn deficient patients with SCD. This has to do with role of Zn in urea cycle enzymes. Prasad and colleagues indicated a decreased activity of ornithine carbamoyl transferase and increased activities of glutamate dehydrogenase and carbamoyl phosphate synthase-I in liver of Zn-deficient rats [34]. A significant increase in the activity of AMP deaminase—an enzyme involved in the purine catabolic pathway; in the muscle of the zinc deficient rats was also observed. The alternation may also contribute hyperammonaemia in Zn-deficient animals and humans [91]. Zinc supplementation in patients with SCD resulted in significant improvement in secondary sexual characteristics, in the normalization of plasma ammonia concentration adaptation [91].

#### 2.4.1. Micronutrients in the Control of Sickling and Reactive Oxygen Generation in Sickle Cell Disease

The sickle erythrocytes are fragile and dehydrated. They require a delicate balance of minerals and antioxidants to maintain hydration and membrane integrity [92]. It has been shown that Mg is effective in reducing not only the painful episodes in SCD but also affects the hydration of RBC. Brugnara [93] has reported that K-Cl cotransport is a major determinant in the dehydration of erythrocytes in SCD [91]. Reduced erythrocyte-Mg content with normal serum Magnesium is observed as a common feature of HbSS. When the internal Mg of the erythrocytes is increased, the activity of K-Cl cotransport is markedly decreased. Therefore, blockage of this pathway by intracellular Mg could result in decreased dehydration and sickling *in vivo* [93].

Among the many important functions of Mg is its involvement, along with calcium, in the organization of membranes. Both cations are known to act as bridges between the neighbouring carboxylate groups in lipoproteins and such bridges stiffen the cell membranes [89]. The generation of ROS which is a steady state cellular event in normal respiring cells, is exacerbated in SCD as in many other diseases uncontrolled production of ROS often leads to damage of cellular macromolecules such as lipids, proteins, and DNA as well as other antioxidant molecules [94]. Protection of red cell membranes from free radical mediated oxidative stress is crucial to the successful management sickle cell crisis. Certain minerals, Fe, Cu, Mg, Se as well as some antioxidant vitamins, have been found to effectively relieve the oxidative stress that prevails in SCD.

## 2.5. Vitamins and Sickle Cell Disease

Various authors have reported the effect of some supplements in the management of SCD [34;64;66;95;97]. Ckiyton and Machlin studied the effect of vitamin E supplementation in the management of SCD, they found that inadequate amount of vitamin E in the plasma and RBCs of sickle cell patients contribute significantly to the sickling process and concluded that vitamin E possessed a potential as a natural therapy capable of normalizing the RBCs in sickle cell patients. Furthermore, the authors showed that the percentage of RBCs, which sickled irreversibly, was between 5 and 30%, and varied from one patient to another. In some instances, the vitamin E supplement reduced the percentage of irreversibly sickled RBCs by more than half. Among the patients who received daily doses of vitamin E for 6-35 weeks, the average number of irreversibly sickled cells decreased from 25 to 11%[(96].

Nutritional deficiencies of folic acid and vitamin B<sub>12</sub> are well described acquired causes of elevated plasma homocysteine concentrations [98;99]. Although folic acid deficiency is a well-recognized complication of SCD, [100]. Many authors do not recommend giving routine folic acid supplements since most patients have plasma and red cell folate concentrations within the usual reference range [101]. The concentration of folate in the blood cells is a more sensitive marker of folic acid deficiency than is the plasma concentration.

Vitamin A is necessary for normal immune function. Its deficiency is characterized by widespread immunological effects including pathological alternations is mucosal surfaces, impaired antibody responses, changes in lymphocyte populations and altered T-and B-cell function, compromised function of neutrophils, macrophages and natural killer (NK) cells, altered immune response [102] Vitamins A, E, carotenoids, Vitamins B, C and folic acid have been modulatory effects on pathogenesis in patients [33]. These nutrients are well known to have antioxidant properties.

#### 2.5.1. Amino Acids and Sickle Cell Disease

The role of amino acids in health and nutrition is well documented. Amino acids are the building block of proteins, glycoproteins, peptide hormones and immunoglobulin. Amino acids are classified as either essential or non-essential based on their relevance in human nutrition and physiology. The essential amino acids are those that cannot be synthesized endogenously and must be obtained from the diet. The non-essential amino acids are those the body can synthesize from various precursors and metabolites in the human system.

The food we eat contains nutritionally useful quantities of most of the essential amino acids including the sulphur containing ones. Recent research findings have revealed an excellent free amino acid content vis-à-vis the protein



concentration of some edible vegetables [103]. Some aromatic acids such as Phe, Tyr, Trp, and others like Lys, Arg, Ser and Glu have found to possess antisickling effects [104;106]. Moreover, these amino acids especially Phe, have been found to possess remarkable antisickling effects and the ability to reverse already sickled erythrocytes even at very low concentrations. This has led to the formulation of an antisickling preparation “Ciklervit TM in combination with other food extracts is used in Nigeria and other West African countries for the management of SCD [62].

The erythrocyte redox environment may contribute to the increased oxidative stress, haemolysis and decreased nitric oxide bioavailability observed in pulmonary hypertension, a common complication of haemolytic disorders [107]. Glutamine plays an additional antioxidant role through preservation of intracellular nicotinamide adenine dinucleotide (NADPH) levels required for glutathione recycling [107]. Glutathione is synthesized from glutamate, cysteine and glycine via reactions catalysed by two cytosolic enzymes, gamma-glutamyl cysteine ligase and GSH synthetase. The intracellular GSH concentration is the result of a dynamic balance between the rate of intracellular GSH consumption and efflux [107].

When Hebbel and colleagues first reported an increase in endothelial adhesion of sickle RBC three decades ago, one of the major factors involved in the pathophysiology of Vaso-occlusion started to unfold [108]. Since then, several endothelial adhesion molecules are abnormally expressed in both RBC and endothelial cells of SCD patients. A role for L-glutamine in SCD was conceived when Zerez and colleagues looks closely into the redox process in sickle RBC focusing on NAD which is a potent antioxidant [109]. As NAD is a major antioxidant molecule in RBC, the phenomenon of increased synthesis for NAD with decreased NAD redox potential was interpreted as a compensatory mechanism of sickled RBC in the presence of increased oxidant stress [109].

The presence of increase oxidant stress and oxidant susceptibility of sickle cell has been described independently by numerous investigators [110]. Additional studies suggested that supplementation with the precursor of NAD, L-glutamine, may further enhance NAD redox system [111]. With 30g of L-glutamine supplementation, there was improvement in NAD redox potential in 7 out of 7 patients. In addition, there were consistent reports of improved general clinical condition in such areas as energy level and chronic pain levels [110]. Furthermore, a short observation period of approximately 3 months suggested a decrease in the incidence of vaso-occlusive painful crises among those patients.

Sequel to this observation, the investigators proceeded to study the effect of L-glutamine therapy on endothelial cell adhesion of sickle RBC. The results indicated that L-glutamine therapy improved the endothelial adhesion of sickle RBC [110]. At present, the exact mechanism by which L-glutamine effectively decreases, the endothelial adhesion of sickle RBC is not clear. However, there is evidence suggesting that one of the ways L-glutamine may benefit sickle RBC is by improvement of NAD redox potential [112]. This may prevent oxidant damage to RBC which may result in stimulation of inflammation and expression of adhesion molecules [110]. In addition, L-glutamine may provide material to maintain the integrity of sickle RBC. It has been reported that sickle cell children require increased amino acid consumption, especially glutamine [113]. L-glutamine is an inexpensive compound widely consumed as part of diet or as a dietary supplement. There is essentially no major adverse effect when used as an oral agent both in research and non-research setting [114;115].

N-acetylcysteine (NAC) could cause significant diminishment in the *in vitro* formation of ISCs at concentrations greater than 250µmol/L [116]. NAC can block ISC formation by converting it back to biconcave cells. This is due in part to its ability to block dense cell formation [116]. Cysteines and cysteine-cysteamine mixed disulphides. NAC is an antioxidant and is converted to reduce glutathione. It was clear that NAC had the ability to block the formation of dense cells and ISCs *in vitro* and that it has at least two targets; channels involved in cell dehydration and membrane skeletal β-actin.

Dense cell formation is caused by water loss from sickle cells, in response to K<sup>+</sup> leakage. The cation transport system that appears to be involved in dehydration of SS erythrocyte are the K<sup>+</sup>- Cl<sup>-</sup> cotransporter, the Ca<sup>2+</sup> activated K<sup>+</sup> channel and the deoxygenation induced Na<sup>+</sup> K<sup>+</sup> and Ca<sup>2+</sup> leak pathways [117]. Therefore, these are the most likely targets of NAC protection of oxidative damage in SCD, leading to decreased dense cell formation. Because dense cells contain the highest percent ISCs, this supplies a partial explanation of NAC's effect on ISC formation. But it does not get directly at the molecular mechanism of NAC effect on ISC formation. Dense cells have decreased foetal haemoglobin (HbF) and increased HbS concentration leading to greater polymerization of HbSS [118]. This would cause many of the high-density SS erythrocyte to become sickled in shape. But what causes the cell to lock into an irreversibly sickled shape cannot be explained by dehydration alone [117].

First, some ISC's in blood from SS subjects are low density cells (117). Furthermore, the substitution of NO<sub>3</sub><sup>-</sup> for Cl<sup>-</sup> in the *in vitro* deoxygenation – reoxygenation cycling experiments results in a high concentration of low density ISC's

[119]. These low density ISC's show slow dissociation of their membrane skeleton at 37°C [119]. Therefore, the formation of ISC's can be uncoupled from cellular dehydration of SS erythrocyte. While dense cell fractions contain many sickled cells, Goodman suggests that the locking of the ISC requires modification in the membrane skeleton [120]. The C<sub>284</sub> C<sub>373</sub> disulphide bridge in  $\beta$ -action is the key determinant of the slow dissociation of the ISC membrane skeletal component and therefore the inability of the ISC to remodel its shape [121]. It was therefore proposed that reducing agents and antioxidants might be effective in blocking ISC formation *in vitro* of the clinically relevant antioxidants that have been tested, NAC was the most potent in inhibiting ISC formation *in vitro*, furthermore, the NAC-linked reduction in ISC's was accompanied by an increase in  $\beta$ -action thiols [116].

NAC, as the N-acetyl derivative of L-cysteine is an antioxidant [122]. It is highly permeable to cell membranes and within the cytoplasm, is converted to L-cysteine which is a precursor of GSH [123]. It, therefore, could protect thiols from oxidative damage both by its antioxidant capacity as well as raising the levels of GSH. Indeed, adult respiratory distress syndrome (ARDS) patients treated with NAC (70mg/kg) showed a 30% increase in erythrocyte GSH in 1 day and a 47% increase after 4 days with drugs administered every 8 hours [124].

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### 3. Toxicity of antisickling agents

Even though SCD has been studied extensively, there has not been a universally acceptable therapeutic agent for the treatment of this disorder. Some of the antisickling agents investigated exhibit varying degrees of toxicity, cause haemolysis of the sickle red blood cell at their effective dose levels and are therefore unsuitable for clinical use [125;126].

Thiocyanate is used as an antithyroid drug and is about 100 times less toxic than cyanate. It is an ionic inhibitor that interferes with the concentration of iodine by the thyroid gland [127]. Hydroxyurea is a cytotoxin agent which has a specific effect on dividing cells. The major toxic symptom in animals surviving acute toxicity dose is myelosuppression, which is rapidly reversed [128]. It has been used in the treatment of chronic myelogenous leukaemia and other myeloproliferative disorders [129]. Animal studies suggested that tellurite ingestion might affect the conversion of squalene to cholesterol thereby interfering with neurotransmission via demyelination [130].

*In vitro* experiments and few case studies have revealed that thiocyanate and tellurite are able to prevent, and reverse sickling in SCD patients and thereby help in ameliorating the painful crises associated with the disease [131;132]. These therapies are being given to patients in the absence of adequate toxicity data or any animal toxicity studies that describe their safety at their therapeutic dose. Hydroxyurea has been approved for the management of ailment, but many uncertainties remain about its long-term benefits and adverse effects as varying degree of toxicity were reported in patients who received the drug [133].

Herbal medicines have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased. Experimental screening method is important to ascertain the safety and efficacy of traditional and herbal products and to establish the active component of the herbal products. Due to the presence of anti-nutrients in the botanical's safety concerns were raised, therefore, acute, and sub-acute toxicity studies of the plant materials used in the management of SCD. Evaluation of some of the herbal materials indicated that *Eremomastax polysperma* were highly safe going by the LD<sub>50</sub> of over 5,000mg/kg body weight, while *Duranta ripens* and *Piper guineensis* though safe had LD<sub>50</sub> less than 1000mg/kg. It can therefore be concluded that the active ingredient(s) present in *Eremomastax polysperma* is devoid of acute oral toxicity [19]. The popular perception that natural product does not present toxic effects might be explained by the concept of intrinsic nature of those products, which contains different compounds, usually in low concentrations, the opposite of synthetic drugs whose toxicity is more prone to appear in acute assays [134].

Body weight differences is an indicator toxicity, low body weight of animals administered drugs is synonymous with the toxicity of such agent. However, improved body weight reveals that such agent(s) is not injurious to the animals. The presence of protein, lipids, carbohydrate, and moisture in the plant materials can provide the nutrients required for growth and development resulting in increased body weight. Conventional antisickling agent can cause significant growth depression due to loss of appetite or mal absorption [135]. Hydroxyurea and tellurite were reported to cause gastrointestinal irritation and may therefore hinder intestinal absorption of food [130]. In contrast, natural products contain agents which induce appetite, other substances include vitamin c, folate and are themselves nourishing.

Therefore, multiple dose studies are necessary to assure the safety of natural products. On the other hand, clinical observations of acute assays are valuable tools to define the doses to be assessed in the multiple dose experiments along with pharmacological studies in animals and in humans.

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#### 4. Conclusion

This genetic disorder commonly affects people living in the villages who are mostly peasant farmers who cannot afford the prohibitive cost of treatment by orthodox medicine. Due to the debilitating effect and the cost of managing SCD, this vulnerable group resort to alternative therapies using the rich natural biodiversity of plants in their disposal. Often, without knowledge of the effective dose regimen and adverse health effects of such materials. Research has been ongoing to determine the efficacy of medicinal plants to tackle the multiple challenges presented in SCD.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

The authors declare that there is no conflicting interest concerning the publication of this article.

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