Search for antisickling agents from plants

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ABSTRACT

The sickle cell disease is fatal in nature. Thousands of children are dying off due to this health problem throughout the globe. Due to the rapid development of diagnosis and clinical managements such patients are living up to a respectable age. But as there is no permanent cure the patients are suffering from bone and joint pain, jaundice, hepato-splenomegaly, chronic infections etc. The main physiological complicacy is due to the polymerization of sickle hemoglobin (HbS), (sickling process) inside the red blood cell (RBC) of these patients during deoxygenating state. The change of RBC from spherical to sickle shape is due to the polymerization of mutant hemoglobin (HbS) inside the RBC and membrane distortion during anoxic condition. The mechanism and the process of sickling are very complex and multifactor in nature. To get rid from such complicacies it is necessary to suitably and accurately stop the sickling of RBC of the patients. The potential anti-sickling agents either from natural sources and/or synthetic molecules may be helpful for reducing the clinical morbidity of the patients. A lot of natural compounds from plant extracts have been tried by several workers in recent past. Most of the studies are based on *in vitro* red cell sickling studies and their mode of action has not been properly understood. Although, few studies have been *in vivo* in nature pertaining to transgenic sickle animal model, there is paucity of data on the human studies. The result of such studies although has shown some degree of success, a promising anti-sickling agent is yet to be established.

Key words: Antisickling, plants, red blood cell, sickle

INTRODUCTION

Sickle cell disease (SCD) is a genetic blood disorder characterized by red blood cells (RBCs) that assume an abnormal rigid sickle shape. Sickling decreases the RBC's flexibility and results in a risk of various complications. It is the most prevalent human hereditary disorder with prominent morbidity and mortality.[1] It is due to the change of an amino acid in position six within the beta globin chain of hemoglobin molecule whereby glutamic acid, a polar amino acid is replaced by valine, a non-polar amino acid.[2,3] The amino acid change is due to the defective gene (mutation) in chromosome 11. At low oxygen tension, the mutant hemoglobin polymerizes inside the RBCs into a gel or further into fibers leading to a drastic decrease in the red cell deformability. Polymerization and precipitation of sickle hemoglobin (HbS) within the erythrocytes cause the change of shape from the normal spherical form into the one resembling a sickle, hence the name sickle cell. A single nucleotide substitution (Thymine [T] for Adenine [A]) allows HbS to polymerize when deoxygenated, since valine can dock with the complimentary sites on the adjacent globin chains.[4] The presence of sickle shaped RBCs in human blood was first reported by Herrick (1910).[5]

The SCD has been reported to wide spread in Africa, Jamaica, Central India, Saudi Arabia, Greece and Italy and also among the Negroids of America and Britain. SCD affects millions of people throughout the world.[6] The clinical symptoms of patients suffering from the disease vary widely. Some lead a normal life while others suffer from a variety of life threatening complications. The main clinical symptoms are anemia, mild jaundice, repeated vaso-oclusive crises, hepatosplenomegaly, acute chest syndrome, bone and joint pain and growth retardation.[1,7]

SCD widely has no cure. However, treatment can help to relieve symptoms and reduce the complications. Infants who have been diagnosed with SCD through newborn screening are treated with antibiotics to prevent infections. Blood

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transfusions are commonly used to treat worsening of anemia and vaso-oclusive complications. A sudden worsening of anemia due to an infection or enlarged spleen is a common reason for a blood transfusion. Blood and marrow stem cell transplants may offer a cure for a small number of economically fortunate patients.[8] Gene therapy is being experimentally successful to some extent in transgenic animal models and few studied patients so far.[9]

The health-care cost of the management of SCD patients is disproportionately high compared to the number of people afflicted by the disease. The individuals affected by this disease are mostly below poverty line and unable to afford the high-cost of treatment. Due to the debilitating effect and cost of managing the SCD, research has been going on to determine the efficacy of certain natural and artificial agents having anti-sickling effect with a aim to have a suitable and permanent cure for this health problem.

SICKLING OR POLYMERIZATION OF HbS

The fundamental cause of SCD is the decreased deformability of the sickled RBCs produced by gelation of hemoglobin S molecules during de-oxygenation. The deformation of sickle red cells upon complete de-oxygenation is due to the intracellular HbS polymerization. The gelation or polymerization is initiated by nucleation of a single polymer. Two types of HbS nucleation, i.e., homogeneous and heterogeneous have been observed. The nucleation is due to the aggregation of hemoglobin S molecules. Once a certain size or the critical nucleus is reached other monomers of HbS add endlessly to form a very large polymer. Then due the heterogeneous nucleation new polymers are formed on the surface of the pre-existing polymer. Individual polymer is a fiber which is made up of 14 inter-wined helical strands of HbS molecules of seven inter-wined double strands. In each molecule, one of the two β^6 Valines of the α_2 β_2 tetramer is involved in an intermolecular contact with its neighbor in the double strand.[4] The polymerization of deoxy-HbS leads to erythrocyte deformation from a biconcave morphology into the sickle shape. Two types of contacts occur between deoxygenated HbS tetramers in the double-stranded fibers. The contacts along the long axis of the fiber are termed axial contacts, whereas contacts along the sides of tetramers are termed lateral contacts. The amino acid Valine at beta six positions plays a crucial role in the lateral contact by interacting with the hydrophobic beta 85th Phenylalanine and beta 88th Leucine on a neighboring tetramer. The axial contact is the interaction of the beta 22nd Glutamic acid with an alpha 20th Histidine on an adjacent tetramer.[10]

PLANT BASED NATURAL ANTI-SICKLING AGENTS

Natural plant extracts and products discovered from medicinal plants have provided numerous drugs which are being used clinically for different diseases. In spite of various challenges encountered in the medicinal plant-based drug discovery, natural products isolated from plants will remain an essential component in the search for further new medicines. Although, drugs for many diseases have been discovered from medicinal plants but still there is no promising drug for the treatment of SCD. However, several recent reports have been documented regarding the *in vitro* and *in vivo* anti-sickle cell properties of the extracts from different medicinal plants [Table 1].

To have potential anti-sickling agents the candidate molecule/s would have to interfere in three different stages of sickling process. Anti-sickling agents could be targeted to modify at the sickle gene level, the HbS polymerization level and the red cell membrane level!

The traditional healers without any scientific background have made use of nature's abundant resources to manage SCD with a promising degree of success (??) over time. There are many plants which have already been used as a source for the treatment of SCD in various parts of the world.

The use of phytomaterials of *Piper guineense*, *Pterocarpa osun*, *Eugenia caryophyllala* and *Sorghum bicolor* extracts for the treatment of SCD has been reported.[11] The extracts of *Pterocarpus santolinoides* and *Aloe vera* have also been reported to increase the gelling time of sickle cell blood and inhibit sickling *in vitro*.[12,13] *A. vera*, also known as the true or medicinal aloe, is a species of succulent plant that, it is presumed, originated in the southern half of the Arabian Peninsula, Northern Africa, the Canary Islands, and Cape Verde. The species is frequently cited as being used in herbal medicine. Many scientific studies of the use of *A. vera* have been undertaken, some of them conflicting. The phytochemical and anti-sickling properties of the leaf and gel extracts of *A. vera* plant were studied. The phytochemical composition of the gel and leaf extracts revealed the presence of alkaloids, flavonoids, saponins, tannins and phenols at various concentrations. The determination of anti-sickling effects of these extracts was directed towards the inhibition of sickle cell polymerization and improvement of Fe^{2+}/ Fe^{3+} ratio of Sickle hemoglobin (HbS) in the presence of the extracts.[14]

The reversal of sickling by root extracts of *Fagara zanthoxyloides* has also been reported.[15] Fractionation of an aqueous extract of root bark from *Fagara xanthoxyloides* by column chromatography on Diethylaminoethyl cellulose (DEAE)-A-50, utilizing an elution gradient of pH 7.5-5.0, yielded five fractions. All fractions reported to reversed metabisulfite-induced sickling *in vitro* of erythrocytes homozygous for HbS. Only the fraction containing the least anti-sickling activity also contained alkaloids.[16]

Table 1: List of plants and their parts having antisickling properties

Name of the plant and parts used	Sickling/ Polymerization test	Description	Results	Ref. No.
Cyperus esculentus (seeds)	Both	Methanol and aqueous extracts were used.	Methanol extract showed pronounced antisickling and anti-gelation activity.	17
Xylopia aethiopica (spice) *Monodora myristica* (spice)	Both	Crude aqueous extract and Batch Process extraction were used.	Crude Aqueous Extraction exhibited profound antisickling and polymerization activities.	18
Alchornea cordifolia (leaves-anthocyanins)	Sickling	Ethanol and aqueous extract was carried out.	Aqueous extract showed a higher antisickling activity.	19
Hymenocarida acida (leaves-saponins)	Sickling	Ethanol extracts at 0.5, 1.0 and 2.0% w/v were taken.	The activity was found to be dose dependent.	20
Parquetina nigrescens (leaves and stem)	Sickling	Percentage reversal and inhibition of sickling parameters were analyzed	untreated SS cell suspensions had over 65% sickle cell	21
Vigna unguiculata (anthocyanins)	Sickling	Aqueous and ethanol extracts were tested for their antidrepanocytary activity.	Both ethanol and aqueous extracts showed pronounced antisickling activity.	33,34
Cajanus cajan (beans)	Sickling	Ethanol and methanol extracts were carried out for antisickling activity.	Both showed significant antisicikling activity.	22
Khaya senegalensis (stem, bark and leaves)	Sickling	Aqueous extract was prepared for the activity.	It showed profound antisickling activity.	23
Cissus populnea (roots)	Sickling	Cold methanol and aqueous extracts were carried out.	Aqueous extract showed a higher antisickling activity.	24
Allium sativum (bulb)	Sickling			25-29
Morinda lucida (anthocyanins)	Sickling	Aqueous and ethanol extracts were carried out.	Ethanol extract was found to exhibit highest antisickling activity.	30
Carica papaya (ripe fruit) *Sorghum bicolor* (leaves)	Sickling	Aqueous extracts of fermented mixture of the two were performed and was incubated for 5 days for antisickling test.	The day 5 aqueous extract was found to have the highest antisickling properties	31
Nigella sativa (oil extract)	Sickling		The oil extract has significant antisickling activity.	32
Aloe vera (leaves and gel)	Both			14
Fagara zanthoxyloides (roots)	Sickling			15,16
Entadrophragma utile (bark) *Chenopodium ambrosioides* (leaves) *Petiveria alliacea* (roots)	Sickling	Crude methanol and aqueous fractions were carried out	The extracts of the three plants at 1.0 and 0.1mg/ml were observed to exhibit significant antisickling activity.	36
Anacardium occidentale (leaves) *Psidium guajava* (leaves) *Terminalia catappa* (leaves)	Polymerization		Polymerization of deoxy HbS molecules was seen after 60s.	37
Plumbago zeylanica (roots) *Uvaria chamae* (roots)	Sickling	Crude methanol and aqueous extract were used for antisickling activity.	P.zeylanica showed a significantly higher antisickling activity.	38
Garcinia kola (leaves, seeds and seed pods)	Sickling	Crude methanol and aqueous fractions were carried out.	The leaf extracts exhibited the greatest antisickling activity.	35
Piper guineensis, *Pterocarpa osun*, *Eugenia caryophyllala and Sorghum bicolor* (Leaves)	Sickling			11
Pterocarpus santolinoides and Aloe vera (leaves)	Sickling			12,13
Afromomum albo violaceum (leaves) *Cymbopogon densiflorus* (leaves) *Bridelia ferruginea* (leaves) *Ceiba pentandra* (leaves) *Coleus kilimandcharis* (leaves) *Dacryodes edulis* (leaves) *Caloncoba velwitchia* (leaves) *Annona senegalensis* (leaves) *Percea Americana* (leaves)	Sickling	Aqueous and ethanol extracts were tested for their antidrepanocytary effects.	Antidrepanocytary activity was found in atleast one extract in all the tested plants.	25

Cyperus esculentus (chufa sedge, yellow nutsedge, tigernut sedge, earth almond) is a species of sedge native to warm temperate to subtropical regions of the Northern Hemisphere, often cultivated for its edible tubers (tigernuts). It is widely consumed in Southern Nigeria by SCD and healthy persons alike, and there are undocumented and unverified claims of health improvements in SCD persons who consumed these seeds regularly. Methanol and aqueous extracts of the plant were prepared and these were used for the anti-sickling experiment. Results of the gelation experiment revealed that both the methanol and aqueous extracts of *C. esculentus* possess anti-sickling activity with methanol extract having a more pronounced anti-HbS gelation activity.[17]

Spices give good flavor and aroma to the food and add greatly to the pleasure of eating. They also have very good healing and remedial values. The aroma of these herbal spices is due to volatile essential oil, pungency due to alkaloid like substances and color due to fat or water soluble pigments. The anti-sickling effects of two indigenous spices *Xylopia aethiopica* and *Monodora myristica* have been investigated. Two hundred grams (200 g) of each powdered sample was divided into two equal parts. One part was used for crude aqueous extraction (CAE) and the other, for batch process extraction, with chloroform, methanol, butanol, and water to yield; the fat-soluble (FAS), butanol-soluble (BUS) and water-soluble (WAS) extracts respectively. The FAS, BUS, CAE and WAS fractions exhibited profound anti-sickling effect by inhibiting HbSS polymerization to varying degrees from 70% for FAS to 90% for CAEs. The CAE and WAS fractions were equally able to improve Fe^{2+}/Fe^{3+} ratio for CAEs and 13-100% for WAS fractions respectively. These fractions also reversed already sickled erythrocytes, with the WAS fractions having less time than the CAE fractions. Thin layer chromatographic analysis showed that the extracts generally contain some anti-sickling amino acids such as Arg, Tyr and Asp at varying concentrations. The total free amino acid concentrations of the samples revealed high concentrations of such, with the CAE fractions of *X. aethiopica* and *M. myristica* having concentrations of 1028 mg and 1680 mg/100 g of samples respectively. Results suggest that these spices when used in combination with other nutritious regimen like fruits, fish and legumes, might be a promising option for the effective management of SCD and a gamut of its pathophysiological complications.[18]

Alchornea cordifolia is a shrub or small tree distributed throughout tropical Africa. *In vitro* anti-sickling activity of ethanolic and aqueous extract of the leaves of this plant has been evaluated. The aqueous extract showed higher anti-sickling activity. The dried and powdered plant material has repeatedly extracted by cold percolation using 95% ethanol and water for 48 h. The fractions were filtered and the solvent was evaporated. Extraction of anthocyanins and alkaloids was then done with distilled water and diethyl ether. Blood sample was added to the plant extract with different concentrations using physiologic solution as dilution solvent. A normalization of erythrocytes of sickle blood samples treated with *A. cordifolia* extract indicated the influence of the extract on the sickling of cells. This normalization increases with the extract concentration. Taking into consideration both the chemical screening results and the solubility of different chemical groups it was found that the anti-sickling activity of *A. cordifolia* aqueous extract would be due to the presence of anthocyanins or alkaloids present in this plant.[19]

Hymenocardia acida is a small shrub. The leaves of this plant are commonly used alone or in combination with other plant parts to manage SCD. The phytochemical screening revealed the presence of carbohydrates, tannins, flavonoids, saponins, alkaloids, cardiac gylcosoids, resins, steroids and terpenes in the leaves of this plant. Ethanol extracts of the leaves have been observed to reverse sickled human RBCs and this activity was found to be dose dependent. Saponins have been shown to possess anti-sickling activity. The RBC have been observed to change from sickle shape to normal biconcave cells and later observed to increase in size after 30 min.[20]

Parquetina nigrescens has been reported to be an herbal remedy for the management of SCD. A study has been carried out to screen the leaves and stem for anti-sickling activity, erythrocyte membrane stabilizing effects and any end organ toxicity. Air dried leaves and stems were grounded and an aliquot was extracted using petroleum ether and aqueous methanol as solvents. The extract was stored in freeze dried form and used for the anti-sickling experiments, osmotic fragility and toxicological assessment. The effect of varied concentrations of *P. nigrescens* plant extracts on the RBC membranes analyzed using the osmotic fragility test revealed appreciable membrane protective effects of the herb and its inhibitory action on hemolysis of RBCs.[21]

Boiled and crude ethanolic extracts of edible *Cajanus cajan* beans were prepared and used for *in vitro* studies involving blood samples obtained from confirmed sickle cell (HbSS) patients. It was demonstrated that the extracts were able not only to inhibit sickling in sodium metabisulphite solution but also quickly reverted to normal morphology of already sickled erythrocytes. There was also a perceptible improvement in the general morphology of the sickle cell erythrocytes after coming into contact with the plant extracts. The amounts of phenylalanine and hydroxybenzoic acid in a *C. cajan* methanolic extract were estimated. Results showed that the amount of phenylalanine and hydroxybenzoic acid per gram weight of bean was 4.92 mg ± 0.13 mg and 21.0 μg ± 3.0 μg respectively. Sickling inhibition was observed to be efficient with the extract which contains a mixture of phenylalanine (0.69 mg/ml) and p-hydroxybenzoic acid (10.5 μg/ml) equivalent to those found in bean extract. The additive anti-sickling effect of both compounds can be

therapeutically exploited for the treatment of SCD. The aqueous methanol extract (3:1, v/v) of the seeds of *C. cajan* was investigated for anti-sickling properties. The extract possessed significant anti-sickling activity and was found to be concentration dependent. Phytochemical screening of the extract revealed the presence of free amino acids, phenolic compounds, tannins, globulins and saponins.[22]

Khaya senegalensis (also called African mahogany) is a species of plant in the Meliaceae family. In West Africa it naturally occurs in gallery forests but is most often found as a shade tree of the old colonial streets. The bark is often harvested as a traditional medicine. Aqueous extracts of the stem, bark and leaves of *K. senegalensis* exhibited a strong anti-sickling activity. The bioassay was based on sickle cells counting, before and after de-oxygenation, in blood samples taken from patients with severe sickle cell anemia and pre-incubated with the drugs to be tested. The main active constituent was identified as a rearranged limonoid. In comparison with pentoxifylline used as standard, the *in vitro* anti-sickling activity of this limonoid was much higher at any concentrations and incubation conditions.[23]

The anti-sickling activities of the extracts of the roots of a plant *Cissus populnea* L. (CPK) (a major constituent of a herbal formula (HF) Ajawaron used in the management of SCD in South-West Nigeria) has been examined. Phytochemical examination of the extract showed the presence of anthraquinone derivatives, steroidal glycosides and cardiac glycosides. Alkaloids and tannins were completely absent in the CPK extracts. Evaluation of the anti-sickling activity involved the use of both positive (p-hydroxybenzoic acid, 5 μg/mL) and negative control (normal saline) for each set of experiments aimed at the inhibition of sodium metabisulphite-induced sickling of the HbSS RBCs obtained from confirmed non-crisis state sickle-cell patients. The chloroform and water partitioned fractions of the cold methanol extracts of CPK exhibited a 62.2% and 52.9% inhibition of sickling, respectively, at 180 min. The HF aqueous extract showed the highest anti-sickling activity on a weight by weight basis of all the extracts and fractions tested, giving a 71.4% inhibition of sickling at the end of 180 min incubation when compared with the normal saline control. The maximum percentage inhibition of sickling exhibited by the p-hydroxybenzoic acid control was 46.0% at 90 min incubation.[24]

Allium sativum, commonly known as garlic, is a species in the onion family Alliaceae. Garlic has been used throughout history for both culinary and medicinal purposes. The garlic plant's bulb is the most commonly used part of the plant. One of the garlic formulations Aged Garlic Extract (AGE) has been reported to exert an anti-oxidant effect *in vitro* on sickle RBCs.[25] Recent studies have shown that the oxidative phenomenon plays a significant role in the pathophysiology of SCD.[26] The oxidant stress may contribute to the sickling process with formation of dense cells, development of vaso-occlusion and shortened RBC survival.[27] The oxidant damage in sickle RBC is most likely the consequence of the inherent instability of hemoglobin S, which results in a concomitant increase in free radical generation in association with impaired antioxidant defense.[28] With sustained intracellular production of oxygen free radicals, the three dimensional structure of hemoglobin is affected sufficiently to lower its solubility. These factors lead to the formation of Heinz bodies that are aggregates of insoluble hemochromes. The Heinz bodies which adhere to the RBC membrane may themselves cause significant damage to the membrane.[29] AGE therapy was associated with the decrease in Heinz bodies in sickle RBC in each patient. AGE has been shown to significantly improve erythrocyte deformability through stabilization of erythrocyte membranes in non-sickle RBC. This phenomenon was attributed to the anti-oxidant activities of AGE. Thus AGE is a relatively harmless agent for the management of SCD.

Anthocyanins are water-soluble vacuolar pigments that may appear red, blue or purple. Anthocyanin extracts from a Congolese plant *Morinda lucida* was found to be responsible for the inhibition of the sickling process. The RBCs were observed to change from the sickled shape to normal biconcave cells in the presence of two percent sodium metabisulfite. The treated Sickle (SS) RBCs demonstrated a remarkable similarity to normal blood values. Sodium citrate suspension of blood samples were used to evaluate the anti-sickling activities of the plant extracts. All experiments were carried out with freshly collected blood. Anti-sickling activity of plant extract was evaluated by preparing an *in vitro* anti-sickling assay, in which 2% sodium metabisulfite pre-treated blood samples is put in contact with plant extracts at different concentrations. Both aqueous and ethanolic extracts have shown a sickling reversal activity. *M. lucida* ethanolic extract was found to exhibit the highest anti-sickling activity.[30]

Carica papaya is native to the tropics of the Americas, and was first cultivated in Mexico several centuries before the emergence of the Mesoamerican classic cultures. *S. bicolor*, commonly called sorghum and also known as durra or jowari, is a grass species cultivated for its edible grain. Sorghum originated in northern Africa, and is now cultivated widely in tropical and subtropical regions. Fermented mixture of dried unripe fruit pulp of *C. papaya* and dried *S. bicolor* leaves showed anti-sickling properties. Both were mixed in equal proportions in distilled water at room temperature and the experiment was carried out using sodium metabisulphite sickled RBCs. The aqueous extracts were obtained and were used for anti-sickling assays. The extracts were incubated for five days and the highest anti-sickling properties was found in the day five extract with 93% inhibitory and 84% reversal activities. Anti-sickling properties of *C. papaya* fruit pulp in distilled water, methanol,

and chloroform using sodium metabisulphite sickled RBCs were also tested. The highest anti-sickling potencies of 87% inhibitory and 74% reversal activities were obtained from the 5-day fermentation products at the optimum concentration of 2.5 mg/ml. The methanol extract gave 64% inhibitory and 55% reversal activities while the chloroform extract was inactive. The amino acids, phenylalanine, tyrosine and glycine already reported in the unripe fruit of *C. papaya*, are the possible anti-sickling components and responsible for their anti-sickling activity.[31]

Nigella sativa has been reported to have calcium antagonist and antioxidant activities, both of which play a role in the management of the disease. This study aimed to investigate the *in vitro* anti-sickling effect of extracts from *N. sativa*. A total of 3 ml of venous blood was collected from each patient recruited for the study and divided into six tubes with heparin. The blood was mixed with 0.5 ml of 0.1%, 0.05% or 0.01% v/v of the oil extract of *N. sativa*. A slide was prepared by spreading a drop of treated blood, covered with a cover slide to ensure the complete de-oxygenation condition. The separation of irreversibly sickled cells (ISCs) was performed by a density gradient centrifugation method. The 0.1% v/v concentration of the oil extract of *N. sativa* resulted in an approximately 80 percent reduction in the formation of sickle cells. The 0.05% v/v concentration of *N. sativa* produced an intermediate effect, while the 0.01% v/v concentration had no effect on the formation of sickle cells. The 0.1% v/v concentration of the fixed oil of *N. sativa* led to a considerable reduction in the formation of ISCs. Thus it can be concluded that the fixed oil extracted from *N. sativa* seeds has an *in vitro* anti-sickling activity.[32]

Aqueous and ethanol extracts from 13 Congolese plants have been evaluated for their anti-depranocytary (anti-sickle) activity. Twelve of these plants exhibited significant activities. These plants were *A. cordifolia, Afromomum albo violaceum, Annona senegalensis, Cymbopogon densiflorus, Bridelia ferruginea, Ceiba pentandra, M. lucida, H. acida, Coleus kilimandcharis, Dacryodes edulis, Caloncoba velwitchia and Vigna unguiculata.* The dried and powdered plant material was repeatedly extracted by cold percolation with 95% EtOH and water for 48 h. Fractions were then filtered and the solvent was evaporated under reduced pressure using a rotary evaporator. All tested plants had at least one extract with anti-drepanocytary activity. Anti-drepanocyatary activity of *V. unguiculata* and *H. acida* were more pronounced than those of other plant extracts.[33] *V. unguiculata* is one of the most important food legume crops in the semi-arid tropics covering Asia, Africa, Southern Europe and Central and South America. The aqueous and ethanol extracts have been evaluated *in vitro* for their anti-sickling activity on sickle blood erythrocytes using Emmel's test. Phytochemical screening revealed the presence of anthocyanins, tannins, alkaloids and steroids. The ethanolic extract was more active than the aqueous extract. The anti-sickling activity was dose-dependent. Anthocyanin extracts was found to be responsible for the inhibition of the sickling process.[34]

Crude methanol extracts and the aqueous fractions of the leaf, seed and seed pod of *Garcinia kola* were carried out using p-hydroxybenzoic acid and normal saline as positive and negative controls respectively. The leaves exhibited greatest anti-sickling activity whilst the seed pod had the least anti-sickling activity. However the activity of the methanol extract did not differ significantly. The results confirm a preliminary confirmation for the effectiveness and use of *G. kola* in the management of SCD and its implication in drug development.[35]

The root, leaf and bark of *Petiveria alliacea*, *Chenopodium ambrosioides* and *Entandrophragma utile* respectively are used for the management of SCD. *In vitro* anti-sickling activity of these plant parts were evaluated using p-hydroxybenzoic acid and normal saline as positive and negative controls respectively. The extracts or fractions of three plants exhibited significant anti-sickling activity.[36]

Anacardium occidentale, *Psidium guajava* and *Terminalia catappa* are found to alter polymerization of sickle cell hemoglobin. Spectrophotometric method was used to monitor the level of polymerization of hemolysate HbS molecules treated with sodium metabisulfite in the presence of separate aqueous extracts of *A. occidentale*, *P guajava* and *T. catappa*. The extracts of the three medicinal plants caused significant reduction in polymerization of deoxy HbS molecules. Aqueous extract of *P. guajava* exhibited the highest capacity to reduced polymerization of deoxyHbS molecules.[37]

The roots of *Plumbago zeylanica* (Plumbaginaceae) and *Uvaria chamae* (Annonaceae) have been used in folklore medicine in the management of sickle cell disease (SCD) in South-West Nigeria. Using both crude methanol extract and its aqueous fraction, *in vitro* anti-sickling activities of these plant parts were evaluated using p-hydroxybenzoic acid and normal saline as positive and negative controls respectively. Extracts of *P. zeylanica* had a significantly higher anti-sickling activity.[38]

In an attempt to find other effective agents with less adverse effects, the anti-sickling effect of NIPRISAN (Nix-0699) a product of the extracts of four different plants, (*P. guineenses* seeds, *Pterocapus osum* stem, *Eugenia caryophyllum* fruit, and *S. bicolor* leaves) was investigated. It was found that Nix-0699, an ethanol/water extract from indigenous plants, has a strong anti-sickling effect. The concentration of Nix-0699 required to inhibit 50% of erythrocyte sickling was about 0.05 mg/ml. As for the kinetics of polymerization, addition of 0.05 mg/ml. Nix-0699 caused a six fold prolongation of the delay time prior to deoxy-HbS

polymerization when compared with that of untreated HbS samples. The solubility of deoxy-HbS significantly increased upon treatment with Nix-0699. Analysis of the effect of Nix-0699 on the HbS oxygen affinity indicated that the drug slightly shifted the oxygen-dissociation curve of HbS toward the left without any apparent change in the Hill coefficient. These results suggest that the anti-sickling properties of Nix-0699 may involve direct interaction with Hb molecules.[39-41]

Anthocyanins from *Justicia secunda* were found to possess anti-sickling activity. Treated sickle (SS) RBCs recovered a normal, classical biconcave form with a radius of 3.3 μm ± 0.3 μm, similar to that of normal erythrocytes. The solubility of sickle de-oxyhemoglobin increased and the osmotic fragility of sickle red cell (drepanocytes) decreased upon treatment with anthocyanin extracts.[42]

Anti-sickling properties of three plant leaf extract such as Cashew *(A. occidentale),* Guava *(P. guajava),* and Indian almond (*T. catappa)* have been reported recently by Chikezie (2011).[43]

Methanolic extracts from *S. monostachyus*, *C. papaya* seed oil and *I. involucrata* exhibited particular anti-sickling properties coupled with the potential to reduce stress in sickle cell patients. Each plant individually or in combination may be useful for the management of SCD.[44]

The mixture of aqueous extracts of *Raphiostylis beninensis, Croton zambesicus, Lonchocarpus cyanescens, U. chamae, M. lucida* and *X. aethiopica* showed obvious *in vitro* anti-sickling effect as per the study of Avaligbe *et al*. (2012).[45] They have also showed a dose dependant anti-sickling effect of different plant extract.

CONCLUSION

Recently a good number of studies have been done for identification and characterization of potential anti-sickling agent/compound from various plants. The most promising compounds ware found to be Anthocyanins a water-soluble vacuolar pigments, Anthraquinone derivatives, Steroidal glycosides, Cardiac glycosides, Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Hydroxybenzoic acid, Limonoids, 5-hydrooxymethyl-2-furfural (5HMF), a naturally occurring aromatic aldehyde, Isomeric divanilloylquinic acids and certain amino acids like Arginine, Tyrosine, Aspartic acid, and Phenylalanine. Although the reported compounds were having *in vitro* anti-sickling effects their mode of action and mechanism has not been properly assed yet.

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