British Journal of Pharmaceutical Research
2(2): 80-88, 2012

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of the Amino Acid Profile and Haemoglobin Polymerization Inhibition Potential of Some Nigerian Legumes

O. A. Ojiako[1*], K. Anugweje[2], C. U. Igwe[1] and C. S. Alisi[1]

[1]Functional Foods Research Group, Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
[2]Department of Health Services, University of Port Harcourt, Port Harcourt, Nigeria.

Research Article

Received 26th September 2011
Accepted 17th January 2012
Online Ready 28th March 2012

ABSTRACT

The amino acid profile and the effects of the seed extracts of *Sphenostylis sternocarpa*, *Monodora myristica* and *Mucuna sloanei* were studied based on their ability to inhibit haemoglobin polymerization and improve the Fe^{2+}/Fe^{3+} ratio of sickle cell erythrocytes. The samples were fractionated into crude aqueous extract (CAE), fat-soluble (FAS), butanol-soluble (BUS) and water-soluble (WAS) fractions. The CAEs of the samples ranked highest in amino acid content in the range of *S. sternocarpa* (7.12 ± 0.00 g/100g)>*M. myristica* (6.00 ± 0.15 g/100g)>*M. sloanei* (3.56 ± 0.21 g/100g). The amino acids identified in appreciable quantities in the seed samples included Phe, Leu, Val, Ile, His, Arg, Tyr, Met, among others. The extracts inhibited polymerization to varying degrees with CAE of both *S. sternocarpa* and *M. myristica*, as well as the WAS of *M. myristica* eliciting significantly ($p<0.05$) high percent inhibition of polymerization when compared with Phe standard. The extracts improved the Fe^{2+}/Fe^{3+} ratio of HbSS blood from 1.36% for CAE of *M. sloanei* to 85.04% for CAE of *S. sternocarpa*; and from 11.03% for WAS of *S. sternocarpa* to 36.08% for WAS of *M. sloanei*. These legumes could, therefore, have immense nutritional and therapeutic importance in the management of sickle cell disease and other related diseases.

Keywords: Sickle cell disease; Fe^{2+}/Fe^{3+} ratio; legumes.

**Corresponding author: Email: okeyojiako@yahoo.com;*

1. INTRODUCTION

Sickle Cell Disease (SCD) is one of the diseases ravaging most world populations cutting across nations and ethnic divide, but most common in people of African ancestry. Although, it is common in most parts of Africa, it is also prevalent in the Mediterranean countries but with wide variation in its prevalence. The frequency of the disease in Africa is not fully established but the carrier rate has been estimated to be in the range of 25 – 40% of the population (Nwaoguikpe and Ejele, 2010).

Sickle cell disease is a general name for all diseases associated with the haemoglobin molecule, hence the name haemoglobinopathies. The commonest haemoglobinopathy is Sickle Cell Anaemia (SCA), which is caused by a point mutation in the β-globin gene of Red Blood Cell (RBC) haemoglobin. As a result of this mutation, valine (a non-polar amino acid) is inserted into the β-globin chain in place of glutamic acid (an electronically charged amino acid). The variants of SCD include those that produce prominent clinical manifestations as seen in sickle cell anaemia HbSS, sickle cell HbC disease, sickle cell α- and β- thalassemia. Sickle cell trait (HbAS), which has never been considered a disease, has one abnormal gene (Okpuzor et al., 2008).

The mutation in HbS causes the RBCs containing them to become stiff and sometimes sickle-shaped when they release their load of oxygen. The sickle cell mutation produces a 'sticky' patch on the surface of the β-chains when they are not complexed with oxygen. Because other molecules of sickle cell Hb also develop the sticky patch, they adhere to each other and polymerize into long fibers that cause the deformation of the normal disc biconcave RBC into a sickle shape (Iyamu et al., 2003). Due to polymerization of the sickled cells, the RBC membrane loses its functional abilities which results in loss of K^+ and water and a corresponding gain of Na^+. Increased intracellular free Ca^{2+} occurs during sickling (Brugnara et al., 1993), resulting in a loss of K^+ with accompanying movements of Cl^- and water. Small blood vessels are blocked by the clumping of sickled RBCs, preventing blood supply to various organs. As a result, those with the disease suffer painful 'crises' in their joints and bones. They may also suffer stroke, blindness or damage to the lungs, kidneys or heart (Okpuzor et al., 2008).

In the treatment of SCD, attention has been focused on the inhibition of sickle cell haemoglobin polymerization, prevention or repair of red cell dehydration and interruption of the interaction of sickled cells with the endothelium (Brugnara and Steinberg, 2002). Some of the orthodox modes of treatment include induction of faetal haemoglobin (HbF) using hydroxyurea, erythropoietin, butyrate or its derivatives, oral administration of clotrimazole, which is a potent Gardos channel inhibitor, blood transfusion and haematopoietic cell transplantation (Okpuzor et al., 2008). Thiocyanate rich foods, nutritional supplements, food extracts, phytochemicals and synthetic compounds have been tested *in vitro* and *in vivo* on their possible roles in the management of SCD (Nwaoguikpe, 2008). The antisickling properties of certain amino acids such as phenylalanine, alanine, lysine, arginine, etc, have also been reported with a postulation that the sickling phenomenon is one variant of metabolic crises exerted by free radicals (Osuagwu, 2010).

Different traditional plants have been used for various purposes including food security, wound healing etc (Ojiako et al, 2010, Alisi et al., 2011). Several other plants including different species of legumes available in tropical Africa have also been reported to be rich sources of proteins and amino acids. Some of those amino acids have been demonstrated to have antisickling potentials. The antisickling mechanisms of action and the bioactive

principles of some legumes have also been described (Iwu et al., 1988; Osuagwu, 2010) even though there are also reports of some indigenous fruits that exacerbate the sickling phenomenon (Nwaoguikpe and Braide, 2011). Furthermore, legumes have been noted to be rich sources of minerals such as Ca, Mg, Zn, P and K. Some of these have also been reported to play significant roles in SCD therapy (Okpuzor et al., 2008). These facts are only a part of the reasons for the current study. We are interested in studying the amino acid profile and haemoglobin polymerization inhibition effects of the leguminous seeds of *Sphenostylis sternocarpa*, *Monodora myristica* and *Mucuna sloanei* which are common traditional legumes grown and consumed in most parts of Nigeria and have been reviewed copiously in some of our earlier works (Ojiako et al., 2010).

Further to the study of the possible antisickling potential of these legumes, we are looking at the larger picture of oxidative stress and its associated avalanche of free radical species which can be reduced by legumes with antioxidant potentials. A well established phenomenon associated with sickle cell anaemia is blood system hyper-oxidation (Osuagwu, 2010) which leads to a chain of events culminating in the generation of reactive oxygen species (ROS). Incidentally, the same ROS and other non-oxygen free radicals are implicated in the pathogenesis of several other disorders including diabetes, Alzheimers's disease, autism, schizophrenia and even anxiety (Hovatta et al., 2005). If the studied legumes can be used in the management of sickle cell crises which is associated with the generation of free radicals, they may also find possible application in food security efforts to manage hunger and even in sports as ergogenic supplements that can enhance athletic performance. These other applications are however, to be tried out in further studies.

2. MATERIALS AND METHODS

2.1 The Legume Seeds

The seeds of *Sphenostylis sternocarpa*, *Monodora myristica* and *Mucuna sloanei* were purchased form a local market in Owerri Metropolis of Imo State, Nigeria. The seed samples were authenticated by a Crop Scientist at the Department of Crop Science and Technology, Federal University of Technology, Owerri, Nigeria. The seeds were washed with clean water, drained of the water and then oven-dried to a constant weight, ground to a fine powder and then stored in a refrigerator at 4℃ until required for use, as in our previous work (Ojiako et al., 2010).

2.2 Preparation of the Seed Sample Extracts

Twenty-five grams (25g) of each powdered sample were soaked in 50ml of chloromethane for 72 hours and filtered. The filtrate was concentrated by rotor evaporation to obtain the fat soluble extract (FAS). The residues obtained were dried at 40℃ and re-suspended in 50ml of methanol for 72 hours after which it was filtered and the filtrate taken through a butanol/water (1:1) partition in a separating funnel to obtain the butanol soluble (BUS) and water soluble (WAS) fractions. The respective extracts were each concentrated by rotor evaporation. Similarly, 25g of each of the powdered sample were soaked in 50ml of distilled water for 72 hours. These were later filtered and the filtrate concentrated by rotor evaporation at 80℃ to obtain the crude aqueous extract (CAE). The respective extracts were labeled and stored in a refrigerator at 4℃ until required for analysis.

2.3 Determination of Total Free Amino Acid Concentration of Extracts

Each of the WAS, CAE, BUS and FAS extracts were diluted 1:1 with distilled water and their total free amino acid concentration determined spectrophotometrically at 570nm using diluted ninhydrin in acetone (0.1%) solution (Nwaoguikpe and Ejele, 2010).

2.4 Determination of Amino Acid Profile of the Extracts

The CAE extracts of each legume seed were each dried in an oven at 50℃ until it reached a constant weight. The dried extracts were defatted, hydrolyzed and evaporated in a rotary evaporator and then 5 – 10 µl of each processed sample was loaded into a Technicon Sequential Multisample Amino Acid Analyzer (TSM) to obtain the amino acid profile of each extract (Nwaoguikpe and Ejele, 2010).

2.5 Determination of Antisickling Effects of the Extracts

2.5.1 Preparation of erythrocyte haemolysate

Three milliliters (3ml) of blood sample was collected by venepuncture into a citrate container from a confirmed homozygous sickle cell disease patient attending the sickle cell clinic at the Federal Medical Centre, Owerri, Nigeria. Written informed consent was obtained from the subject and all other practises complied with the hospital's Ethical Committee standards. Such standards include hospital staff explaining the benefits of the study to the subject as well as collecting the samples themselves even after the approval of the study.

About 0.2ml portions of the HbSS blood sample was centrifuged (Nickel-Electro Centrifuge) at 1500 x g for 15 minutes to sediment the erythrocytes. After careful siphoning of the plasma with a pasteur pipette, the erythrocytes were washed thrice with isotonic saline (0.9% NaCl) solution, and then re-suspended in a volume of the saline solution equivalent to the siphoned plasma volume. The erythrocyte suspension was then frozen at 0℃ and subsequently thawed to produce a haemolyzate for the haemoglobin polymerization study.

2.5.2 Determination of plant extract haemoglobin polymerization inhibition potential

The rate of inhibition of sickle cell haemoglobin (HbSS) polymerization by the extracts were carried out by monitoring with time the turbidity of the polymerizing mixture at 700nm using 2% solution of sodium metabisulphite as a deoxygenating agent (Iwu et al., 1988; Nwaoguikpe and Ejele, 2010).

2.5.3 Determination of the Fe^{2+}/Fe^{3+} ratio of sickle cell blood

The Fe^{2+}/Fe^{3+} ratio of the sickle cell blood was determined by the methods of Davidson and Henry (1974) and Virgil and George (1976). The procedure involves the lysing of 0.02cm^3 of whole blood from each patient in 5cm^3 of distilled and deionized water and then determining the absorbance of hemoglobin and methemoglobin at their characteristic wavelengths of 540nm and 630nm, respectively.

2.6 Statistical Analysis

The data generated were analyzed using One-way ANOVA and Tukey test on a computer-based software, GraphPad Prism version 5.0. Values for $p<0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

Table 1 presents the total free amino acid contents (g/100g) of the leguminous seed extracts. It shows that the crude aqueous extracts (CAEs) have the highest contents of amino acids. Furthermore, the CAE of *S. sternocarpa* ranked highest in free amino acid content (7.12 ± 0.00 g/100g), followed by that of *M. myristica* (6.00 ± 0.15 g/100g) and then *M. sloanei* (3.56 ± 0.21 g/100g). This result corroborated earlier reports of appreciable quantities of total protein and amino acids in the seeds of most legumes including *S. sternocarpa* and *M. myristica* (Ojiako et al., 2010).

Table 1. Total free amino acid concentrations (g/100g) of the sample fractions

Samples	Fractions			
	CAE	**WAS**	**BUS**	**FAS**
S. sternocarpa	7.12 ± 0.10	0.36 ± 0.01	2.15 ± 0.11	1.14 ± 0.20
M. myristica	6.00 ± 0.15	0.18 ± 0.01	3.60 ± 0.14	1.37 ± 0.12
M. sloanei	3.56 ± 0.21	0.30 ± 0.02	4.80 ± 0.10	2.73 ± 0.10

Values are mean ± standard of triplicate determinations; CAE, crude aqueous extract; WAS, water soluble fraction; BUS, butanol soluble fraction; FAS, fat soluble fraction.

Table 2 shows the amino acid profile of the seed samples. A study of the amino acid distribution of the seeds show that they are rich sources of both essential and non-essential amino acids. This is in tandem with reported high content of Phe, Leu, Val, Ile and other important amino acids in legumes such as *S. sternocarpa*, *M. myristica*, *Cajanus cajan*, Telferia occidentalis, Solanum melongena, etc (Ojiako et al., 2010; Nwaoguikpe and Ejele, 2010; Uwakwe and Nwaoguikpe, 2008). The results from the table also revealed the presence in the leguminous seeds of high contents of some antisickling amino acids such as Phe, Lys, Leu, Asp, Ser, Arg and Tyr (Nwaoguikpe and Ejele, 2010). These amino acids especially Phe, like other aromatic amino acids, have been suggested to have antisickling potentials (Noguchi and Schechter, 1978). Ekeke and Shode (1990) postulated that Phe is the main active chemical agent in *Cajanus cajan* aqueous extract, responsible for its antisickling effect. This effect of Phe has been suggested to be due to its free radical scavenging potential and its ability to supply reducing power by dehydrogenation. Furthermore, Phe has effect on membrane stability and stimulates the activation of membrane bound Na^+/K^+ and Ca^{2+} ATPase activities (Elekwa et al., 2005).

One of the areas of focus in the management of SCD is inhibition of sickle cell haemoglobin polymerization. Thus, it has been hypothesized that an ideal antisickling drug or agent should significantly inhibit polymerization of the abnormal sickle haemoglobin HbS (Nwaoguikpe and Ejele, 2010). The CAE fractions from *S. sternocarpa* and *M. myristica* as well as WAS from *M. myristica* exhibited the highest level of inhibition of HbS polymerization at 99.10%, 96.30% and 94.30% respectively, which compared favouably and significantly ($p<0.05$) with that of Phe (Table 3), a well researched standard antisickling agent (Ekeke and Shode, 1990; Osuagwu, 2010).

Table 2. Amino acid profile of the crude aqueous extract of the legumes expressed in g/100g protein

Amino Acid	*S. sternocarpa*	*M. myristica*	*M. sloanei*
Lysine	5.19	3.62	6.38
Histidine	3.15	2.21	3.28
Arginine	4.06	4.14	6.13
Aspartic acid	8.65	7.17	9.62
Threonine	3.09	2.54	3.31
Serine	2.77	4.12	3.25
Glutamic acid	12.65	9.09	13.48
Proline	3.46	2.24	3.26
Glycine	3.70	3.12	3.55
Alanine	4.71	4.48	4.10
Cystine	1.13	0.72	1.06
Valine	4.80	3.41	4.85
Methionine	0.94	0.83	1.04
Isoleucine	3.14	3.30	3.67
Leucine	7.37	6.61	7.20
Tyrosine	3.17	2.38	2.54
Phenylalanine	4.63	4.28	5.66

These results, coupled with the over 50% inhibition potential (although significantly, $p<0.05$, lower than that of Phe) observed for the WAS of both *M. sloanei* (70.20%) and *S. sternocarpa* (59.00%), may be attributed to the polar nature of these fractions and their ability to diffuse into the haemoglobin molecule to bind at the heme pocket, thereby obstructing the 'sticky patches' of the sickle cell Hb molecules (Noguchi et al., 1982). This will prevent polymerization of Hb molecules into long fibers that would have caused deformation into sickle shapes of the normal disc biconcave shape of RBCs (Iyamu et al., 2003). On the other hand, the BUS and FAS fractions of the seeds elicited significantly ($p<0.05$) low percent inhibition of polymerization in comparison with that of Phe standard. This may be attributed to the non-polar nature of their extracts.

Table 4 shows the effect of the sample extracts on the Fe^{2+}/Fe^{3+} ratio of sickle cell blood. The ratio measures an increase or decrease in the oxygen affinity of erythrocytes. An increase in Fe^{2+}/Fe^{3+} ratio upon application of a drug or extract is an indication of reversal of sickling suggesting conversion of deoxyHbS to oxyHbS. Thus an ideal antisickling agent, apart from inhibiting polymerization, would also increase the oxygen affinity of the haemoglobin molecule (Osuagwu, 2010). Data generated from this study (Table 4) show that the CAE and WAS extracts of all the leguminous seed samples significantly ($p<0.05$) increased the Fe^{2+}/Fe^{3+} ratios of the HbSS blood, except for the CAE of *M. sloanei*. These observations translated to appreciable percentage increases ranging from 1.36% for CAE of *M. sloanei* to 85.04% for CAE of *S. sternocarpa* in comparison with the Fe^{2+}/Fe^{3+} ratio of the control HbSS blood. The ability of these seeds to improve the Fe^{2+}/Fe^{3+} ratio of HbSS erythrocytes may be attributed to the presence in these seeds of vitamins C and E, minerals and phytochemicals like saponins, tannins, and alkaloids, reported to exhibit antisickling potential (Ejele and Njoku, 2008).

Table 3. The rates of polymerization, the relative % polymerization and the relative % inhibition of HbSS by the legumes sample fractions at 100mM phenylalanine equivalence

Sample	Fraction	Concentration (mM)	Rate of Polymerization	Relative % Polymerization	Relative % Inhibition
HbSS Blood	--	--	2.50×10^{-2}	100.00 ± 0.00	0.00 ± 0.00
L-Phe	--	100.00	1.30×10^{-3}	5.76 ± 0.10	94.96± .10
S. sternocarpa	CAE	100.00	2.00×10^{-4}	0.86± 0.10^{c}	99.10± $.11^{b}$
	WAS	100.00	6.00×10^{-3}	41.01 ± 0.21^{b}	59.00±0.01^{c}
	BUS	100.00	1.50×10^{-2}	88.50 ± 0.12^{b}	11.00 ± 0.10^{c}
	FAS	100.00	7.00×10^{-3}	46.90 ± 0.20^{b}	53.10 ± 0.12^{c}
M. myristica	CAE	100.00	1.00×10^{-3}	3.70 ± 0.01^{c}	96.30 ± 0.10^{b}
	WAS	100.00	2.00×10^{-3}	5.70 ± 0.21^{a}	94.30 ± 0.20^{a}
	BUS	100.00	6.13×10^{-3}	51.41 ± 0.16^{b}	49.00 ± 0.11^{c}
	FAS	100.00	1.56×10^{-2}	89.02 ± 0.12^{b}	11.00 ± 0.01^{c}
M. sloanei	CAE	100.00	1.20×10^{-2}	88.16 ± 0.17^{b}	11.90 ± 0.10^{c}
	WAS	100.00	3.12×10^{-3}	29.80 ± 0.10^{b}	70.20 ± 0.10^{c}
	BUS	100.00	6.10×10^{-3}	51.40 ± 0.11^{b}	48.60 ± 0.20^{c}
	FAS	100.00	2.86×10^{-3}	16.02 ± 0.10^{b}	84.00 ± 0.12^{c}

Values are mean ± standard of triplicate determinations; [a] Non-significantly ($p>0.05$), [b] significantly ($p<0.05$) higher, and [c] significantly ($p<0.05$) lower than Phe value; CAE, crude aqueous extract; WAS, water soluble fraction; BUS, butanol soluble fraction; FAS, fat soluble fraction.

Table 4. The effects of the legume sample fractions on Fe^{2+}/Fe^{3+} ratio of sickle cell (HbSS) blood at a concentration of 20mM Phenylalanine equivalence

Sample	Fraction	% Hb	% mHb	Fe^{2+}/Fe^{3+} Ratio	% Increase
HbSS Blood	--	93.71 ± 1.00	6.29 ± 0.10	14.90 ± 0.12	0.00 ± 0.00
S. sternocarpa	CAE	96.50 ± 0.10	3.50 ± 0.20	27.57 ± 0.10^{b}	85.04 ± 0.00^{b}
	WAS	94.30 ± 0.20	5.70 ± 0.11	16.54 ± 0.00^{b}	11.03 ± 0.02^{b}
M. myristica	CAE	94.40 ± 0.10	5.60 ± 0.21	16.86 ± 0.10^{b}	13.14 ± 0.10^{b}
	WAS	94.79 ± 0.20	5.21 ± 0.10	18.19 ± 0.20^{b}	22.11 ± 0.02^{b}
M. sloanei	CAE	93.79 ± 0.21	6.21 ± 0.20	15.10 ± 0.17^{a}	1.36 ± 0.10^{b}
	WAS	95.30 ± 0.10	4.70 ± 0.20	20.28 ± 0.20^{b}	36.08 ± 0.10^{b}

Values are mean ± standard of triplicate determinations; [a] Non-significantly ($p>0.05$), [b] significantly ($p<0.05$) higher, and [c] significantly ($p<0.05$) lower than Phe value; CAE, crude aqueous extract; WAS, water soluble fraction; BUS, butanol soluble fraction; FAS, fat soluble fraction.

4. CONCLUSION

From this study, the extracts of these leguminous seeds exhibited both the potential of inhibiting polymerization of sickle cell haemoglobin and increased the oxygen affinity of sickle cell erythrocytes. Thus, they can be recommended for the management of sickle cell disease and can also be used to combat hunger since they are readily available sources of protein and amino acids. They may also delay fatigue and so find applications in sports. This is more so, especially as the nutritional compositions of some of these legumes as well as processing methods that can reduce their antinutrient content have been studied (Ojiako et al., 2010).

ACKNOWLEDGEMENTS

(A brief acknowledgement section may be given after the conclusion section just before the references. The acknowledgments of people who provided assistance in manuscript preparation, funding for research, etc. should be listed in this section.)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Alisi, C. S., Ojiako, O. A., Osuagwu, C. G. Onyeze, G.O.C. (2011). Free radical scavenging and in vitro antioxidant effects of ethanol extract of the medicinal herb *Chromolaena odorata* Linn. Brit. J. Pharm. Res., 1(4), 141-155.

Brugnara, C., Steinberg, M.H. (2002). Developing treatment for sickle cell disease. Expert opinion on Invest. Drugs, 11(5), 645-659.

Brugnara, C., De Franceshi, L., Alper, S.L. (1993) Inhibition of Ca^{2+} dependent K^{+} transport and cell dehydration in sickle erythrocytes by CLT and other imidazole derivatives. J Clin. Invest., 92, 520-526.

Davidson, J., Henry, J.B. (1974). Clinical diagnostics by laboratory methods. Todd-Sanford. W.B. Saunders, Philadelphia. Pp.112, 1380.

Ejele, A.E., Njoku, P.C. (2008). Antisickling potential of Aloe vera extract. J Sci Food & Agric., 88, 1482-1485.

Ekeke, G.I., Shode, F.O. (1990). Phenylalanine is the predominant antisickling agent in *Cajanus cajan* seed extract. Planta Medica, 56, 41-43.

Elekwa, I., Monanu, MO., Anosike, EO. (2005). In vitro effects of aqueous extracts of *Zanthxylum macrophyla* roots on adenosine triphosphates from human erythrocytes of different genotypes. Biokemistri, 17(1), 19-25.

Hovatta, I.,Tennant, R.S., Helton, R., Marr, R.A., Singer, O., Redwine, J.M., Ellison, J.A., Schadt, E.E., Verma, I.M., Lockhart, D.J., Barlow, C. (2005). Glyoxalase 1 and glutathione reductase 1 regulate anxiey in mice. Nature, 438, 662-666.

Iwu, M.N., Igboko, A.O., Onwubiko, H., Ndu, U.P. (1988). Effects of *Cajanus cajan* on Gelation and Oxygen Affinity of Sickle Cell Hemoglobin. J. Ethnopharm., 22, 99-104.

Iyamu, E.W, Turner, E.A, Asakura, T. (2003). Niprisan (Nix-0699) improves the survival rates of transgenic sickle cell mice under acute severe hypoxic conditions. Brit. J. Haematol., 122(6), 1001-1008.

Noguchi, C.T., Schechter, A.N. (1978). The inhibition of sickle cell hemoglobin and related compounds. Biochem., 17, 5455-5459.

Noguchi, C.T., Ackermann, S., Dimai, J., Sciller, P.W., Schechter, A.N. (1982). The effect of phenylalanine analogs on the solubility of deoxyHbS. A model class of gelation inhibitors. Mol. Pharm., 23, 106-110.

Nwaoguikpe R.N., Braide, W. (2011). Some fruit juices as environmental sickling agents. World J. Med. Sci., 6 (2), 61-67.

Nwaoguikpe, R.N. (2008). Effects of the extracts of African yam beans (*Sphenostylis stenocarpa*), Hyacinth beans (*Lablab purpureus*) and Bambara groundnut (*Voandzeia subterrenea*) on the polymerization and Fe^{2+}/Fe^{3+} ratio of sickle cell blood. Nig. J. Bchm. Mol. Biol., 23(2), 46-50.

Nwaoguikpe, R.N., Ejele, E.A. (2010). Amino acid profile of some anti-sickling plant extracts and their haemoglobin polymerization inhibition. Nig. J. Bchm. Mol. Biol., 25(2), 53-59.

Ojiako, O.A., Igwe, C.U., Agha, N.C., Ogbuji, C.A, Onwuliri, V.A. (2010). Protein and Amino acid compositions of *Sphenostylis stenocarpa, Sesanum indicum*, *Monodora myristica* and *Afzelia africana* seeds from Nigeria. Pak. J. Nut., 9(4), 368-372.

Okpuzor, J., Adebesin, O., Ogbunugafor, H., Amadi, I. (2008). The potential of medicinal plants in sickle cell disease control: A review. Int. J Biomed. Health Sci., 4(2), 47-55.

Osuagwu, C.G. (2010). Mechanism of the antisickling effects of *Cajanus cajan* and phenylalanine. Nig. J. Bchm. Mol. Biol., 25(2), 68-71.

Uwakwe, A.A., Nwaoguikpe, R.N. (2008). In vitro antisickling effects of *Xylopia aethiopica* and *Monodora myristica*. J. Med. Plants Res., 2(6), 119-124.

Virgil, F.F., George, G.K. (1976). Biochemical aspects of hematology. In: Fundamentals of Clinical Chemistry. Teitz, N.W. ed. W.B. Saunders Co., Philadelphia, Pp. 411-417.