British Journal of Pharmaceutical Research
4(1): 158-171, 2014

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The Effects of Extracts of Christmas Bush (*Alchornea cordefolia*) on Sickle Cell Hemoglobin

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Authors' contributions

It would be noteworthy to mention that some authors had special responsibility in realizing the aims and objectives of the study. Author RNN designed the study, carried out the assays on sickle cell hemoglobin polymerization, the determination of free amino acid concentration and co-ordinated the different contributions from the authors. Author WB performed the proximate and phytochemical analyses. Authors GNO and BOA carried out work on the determination of mineral composition, the Fe^{2+}/Fe^{3+} ratio and the vitamin C concentration of leafy sample while author COU procured the samples and in collaboration with others, performed the statistical analyses. All authors jointly read, corrected and approved the final manuscript.

Research Article

Received 9th April 2013
Accepted 23rd July 2013
Published 7th October 2013

ABSTRACT

The study aims at determining the antisicking effectiveness of the extracts of the leaves of a herbal medicinal plant *Alchornea cordefolia*. The plant is widely distributed in the South-eastern region of Nigeria. The study is designed to involve phytochemical exploration, the nutrient and mineral compositions, the free amino acid, ascorbic acid and the amino acid constituents. Apart from these, the rates of sickle cell hemoglobin polymerization were assayed with different fractions of extracts and compared with the control to ascertain their antisickling effectiveness. Sickle cell blood samples were donated by a total of forty patients (25 males and 15 females) of ages (17-32 years) whose sickle cell status were confirmed by electrophoresis of sickle cell blood by staff of the hematology unit of the

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centre . The donors were co-opted into the study by the personnel of the sickle cell unit of the Federal Medical Centre, Owerri, Nigeria. The determination of the Fe^{2+}/Fe^{3+} ratio was to assess the oxygen affinity of sickle cells or drepanocytes. The antisickling properties of leaf extracts of Christmas bush (*Alchornea cordefolia*) were investigated. Results from phytochemical analysis of crude aqueous extract (CAE) revealed the presence of flavonoids (4.2±0.1%), alkaloids (5.7±0.12%), saponins (4.60±0.10%), tannins (6.50±0.1%), phenol (3.0±0.10%) and oxalate (5.47±0.1%). Proximate composition showed the following results: moisture (11.05±0.0%), ash (6.8±0.1%), crude fat (6.03±0.0%), protein (6.10±0.0%), fiber (24.5±0.2%) and carbohydrates (44.50±0.2%). Assay of mineral composition, revealed a preponderance of such, which include: Potassium (150.30 mg/100g), Sodium (228.20 mg/100g), Calcium (1.60 mg/100g), Magnesium (2.40 mg/100g) and Phosphorus (1.00 mg/100g) of dry weight of sample. The determination of the antisickling effects of the extracts of *Alchornea cordifolia* was assessed based on the inhibition of sickle cell hemoglobin polymerization and the improvement of Fe^{2+}/Fe^{3+} ratio. Fifty grams (50 g) of the powdered sample was used for the batch extraction procedures with chloroform, methanol, butanol and distilled water to obtain the fat soluble fraction (FAS), the butanol soluble(BUS) and water soluble fractions (WAS) respectively. The FAS, BUS and WAS fractions exhibited profound antisickling effectiveness by inhibiting the HbSS polymerization to varying degrees from (47.50% for the BUS to 98.12% for the WAS fractions respectively in 20 min. The WAS and FAS fractions improved the Fe^{2+}/Fe^{3+} ratio remarkably, except the BUS fraction . Thin layer chromatographic (TLC) analysis revealed the following amino acids - Phenylalanine, Alanine, Glutamate, Histidine, etc. The total free amino acid concentration of the fractions were as follows: the FAS (526.8 mg/100g); the WAS (79.33mg/100 g and the BUS (15.65 mg/100 g). The total vitamin C concentration was found to be 1929.18 mg/100 g of sample. *Alchornea cordefolia* leaf extracts, with the preponderance of micro and macro-nutrients, vitamins, amino acids and others, may be very beneficial for the management of sickle cell disease.

Keywords: Sickle cell disease; Alchornea cordifolia; hemoglobih polymerization; $Fe^{2+/}$ Fe^{3+} ratio.

1. INTRODUCTION

Sickle cell disease (SCD) is known to be one disease ravaging most world populations cutting across national and ethnic divide. Sickle cell disease refers to a group of hemoglobinopathies in which, at least one sickle (S) beta –globin gene is inherited together with one type of abnormal hemoglobin. The most common of these diseases are , sickle cell anemia (HbSS), hemoglobin SC (HbSC), hemoglobin Sβ thalassemia, HbSβ thal minor and major[1] respectively .Of all these syndromes, people or patients with sickle cell anemia suffer most severely. A homozygous gene resulting from point mutation in the β globin, a subunit of adult hemoglobin A, is the proximate cause of sickle cell disease (SCD). HbS shows peculiar biochemical characteristics, which lead to polymerization following deoxygenation of the erythrocytes. Studies of the kinetics of HbS polymerization is associated with the reduction in cell ion and water content (cell dehydration), increased red cell density, which further accelerate HbS polymerization [2]. Dense and dehydrated erythrocytes are likely to undergo instant polymerization under conditions of mild hypoxia, due to their high HbS concentration.

Sickle cell disease is caused by the substitution of glutamic acid with a hydrophobic moiety, valine, at the sixth position of β-globin chain of hemoglobin (HbS) [3]. This mutation causes the deformation of erythrocytes, rigidifying their membranes. These changes in the red cell rheology, contribute to the micro-vascular occlusion, tissue ischaemia, and a high degree of morbidity and mortality associated with the disease [4]. The red blood cell membrane's integrity is maintained by hydration and if membrane becomes dehydrated, this impacts on its deformability, which may lead to sickling. Nitric oxide (NO) is believed to play a role in sickle cell disease patients if endogenous (NO_2) production is stepped up, amplifying NO response or decreasing its destruction [5]. Its main mechanism is to act as a vasodilator in the smooth muscles. Due to challenges facing scientists especially in developing countries and world over ; so many sources of nutrients and chemicals, including herbal cocktails have been used to ameliorate the excruciating pathological complications of sickle cell disease[6,7,3]. Dried fish (Tilapia) and Dried Prawn (*Astacus* red) extracts were established in some research findings to possess the capacity to reverse already sickled cells and inhibit hemoglobin polymerization, improve the Fe^{2+}/Fe^{3+} ratio and lower LDH activity in sickle cell blood [8]. Hydroxyurea (HU), Erythropoietin (EP) and Tucaresol preparations have all been found to reduce LDH activity and bilirubin levels in human sera, as well as increase the level of fetal hemoglobin (Hb F) [9]. There have also been reports on effective management of sickle cell disease patients during pregnancy [10] with herbal preparations. In Nigeria and most other parts of the world, medicinal plants have been used in the treatment of painful crises associated with sickle cell disease, especially among the lower socio-economic class that cannot afford the high cost of orthodox drugs. The practice of traditional medicine using medicinal plants is as old as man. This type of health care was described as Herbalism or Botanical medicine [11].

The growing sophistication in life style among world populations,makes it imperative to refer to herbal practice as alternative or complementary medicine, so as to appeal to a cross section of people irrespective of their cultural affiliation. Two third of world population rely entirely on this as their primary form of health therapy and care [12]. A review [13] reiterated that the use of traditional medicine is at its peak and cannot fade out in the treatment and management of a wide array of diseases in the African continent. This was attributed to the socio-economic, socio-cultural heritage, and lack of basic health care personnel. The holistic use of plants in health care, affords the benefit of maximizing all essential ingredients that nature has endowed to humanity. Some of these plant parts possess antioxidant, anti-inflammatory, antimicrobial and anti-adhesion properties, while others may be involved in boosting the immune system; a gamut of substances have been in use for the management of sickle cell disease. The only cure known for the syndrome is the stem cell therapy or the chord gene therapy, which is highly expensive and not within the reach of an average common man. Hence, the continued search for substances that can ameliorate the pathological complications of the disease. *Alchornea cordifolia* belongs to the family, *Euphorbaceae*. It is commonly known as Christmas bush. It occurs from Senegal, East of Kenya, Tanzania, and throughout Central Africa to Angola. It is cultivated in Congo for its menstrual use. *Alchordea cordifolia* is an erect bushy perennial shrub or small tree up to 4 meters high, reproducing from seeds [14]. It has been subjected to intense phytochemical screening to determine its medicinal status and usage.

The plant has been shown to possess antibacterial, antifungal and anti-spasmolytic properties [15,16]. Some workers have shown that the methanol extract of the leaves of *Alchornea cordifolia* possess anti –inflammatory activity when given by intra-peritoneal injection in the egg –albumin, reduces rat paw edema (inhibition of 58.25% for 50mg/kg) body weight by the topical extract [17]. The leaves, roots and stem bark contain terpenoids,

steroidal glycosides, flavonoids, alkaloids, alchroeine and several guanidine alkaloids. The leaves also contain a range of hydroxybenzoic acids, gallic acid, and its ethyl ester, gentisic acid, anthranilic acid and protocatechoic acid as well as ellagic acid (alizarine yellow), a C_{20} homologue of Vanolic acid named, Alchornoic acid, is found in the seed oil. *Alchornea cordifolia* is commonly used as a medicinal plant throughout its area of distribution. The leaves are mostly used, but also the stem bark, stem pith, leafy stem, root bark, roots and fruits enter in local medicines. The leaves as leafy stems are used as infusions or chewed fresh; or taken for antispasmolytic activities to treat a variety of respiratory problems including tonsillitis, sore throat, cough, bronchitis, genito-urinary problems, including veneral diseases , female sterility and intestinal problems, which include: gastric ulceration, diarrhea, amoebic dysentery and helminthiasis [18]. The leaves are equally taken as blood purifier, as a tonic and to treat anemia and epilepsy. The leaves are eaten in West Africa and Congo as an emmenagogue and to facilitate parturition. In Gabon, the leaf extract is used as an abortefacient. A cold infusion of the dried and powdered leaves act as diuretic.Crushed fresh leaves or powdered dried leaves are applied externally as a cicatrisant to wounds, to relieve backache and headache; to fractures to improve healing, to treat eye infections and other numerous sores, abscesses, yaws and filariasis [19].

In Nigeria, the stem bark is thrown into dammed streams as fish poison. In some parts of West Africa, the leaves are used in packing Kola nuts and “Okpeye”, a Nigerian condiment, produced by fermenting the seeds of *Prospis Africana* [14]. *Alchornea cordifolia* extract has been patented for various other uses or applications such as antifouling adjuvant in paints, coating polymers and alchornoic acid was proposed and was proposed for hemi- synthesis of plastics. The use of phyto-materials such as *Piper guinensis*, *Pterocarpus osun* , *Eugenia caryophelbela* and *Sorghum bicolor* extracts for the treatment of sickle cell disease have been reported [20]. The extracts of *Pterocarpus santolinoids* and *Aloe vera* were reported to increase the gelling time of sickle cell blood and inhibit sickling *in vitro*. This indicates that such plants may indeed have great potential in the management of sickle cell disorders [21]. Thirteen Congolese plants were screened for antisickling properties and only five of them were reported to posses such property [17]. The role of crude aqueous extract of *Xanthoxylum macrophylla* roots as antisickling agent obtained from the root of this plant had been highlighted, also 2-Hydroxybenzoic acid was isolated and identified as an antisickling agent from the root of the same plant [22,23,24]; investigated the usefulness of *Carica papaya* seed extract as an antisickling agent and found that indeed, that the unripe seed extracts possess antisickling properties.

The drug Nicosan, previously Nipprixan (NIK-0699), which is a cocktail from four different plants (*Piper guinensis* seeds, *Pterocarpus osun* (stem), *Eugenia carophyllum* fruit, and *Sorghum bicolor* leaves were shown to possess antisickling properties [3]. Clinical trials of Nix- 0699 showed that the drug significantly reduced the number of painful episodes in sickle cell disease patients. Another antisickling preparation, a nutrient CIKLARVIT^{TM} discovered by the duo-Prof. G.I. Ekeke, Department of Biochemistry, University of Port Harcourt and Dr. R.N.Nwaoguikpe, Department of Biochemistry, Federal University of Technology, Owerri, all in Nigeria, is produced and marketed by Niemeth Pharmaceuticals Nigeria, PLC. This preparation has proved to be effective in minimizing most pathological complications of the disease. It is formulated from the seeds of pigeon pea- *Cajaus cajan*; phenylalanine as a predominant antisickling amino acid and Zinc. The main objective of this work is to investigate the antisickling effects/properties of extracts of the leaves of *Alchornea cordifolia* based on the phytochemical, proximate, free amino acid and vitamin C compositions/ concentrations.

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaves of *Alchornea cordifolia* were plucked from the herbarium of Department of Forestry and Wild life of the School of Agriculture and Agricultural Technology, (SAAT) of Federal University of Technology, Owerri. The leaves were authenticated by a plant taxonomist, Mr. Francis Iwunze of the Department of Forestry and Wide life of the university as being the best variety.

2.2 Sample Preparation

The leaves of *Alchornea cordifolia* were plucked, washed under running tap water to remove dust and debris. The leaves were kept at room temperature of 25 ºC for five days to dry. The dried leaves were grinded to powder. The powdered sample was kept in a dessicator until used.

2.3 Batch Extraction Procedures

2.3.1 Extraction of the fat-soluble (FAS) fraction

Fifty grams (50 g) of the powdered plant leaves (*Alchorea cordifolia*) were soaked in 200 ml of dichloromethane of analytical grade for twenty-four hours to de-fat it and in essence to generate the fat- soluble fraction after filtration. The residue after filtration was kept *en vacuo* to dry off the residual volatile solvent.

2.3.2 Methanol extraction process

The residue from the dichloromethane extraction process was soaked in 200 ml of methanol of analytical grade for 24 hours. The solvent was filtered and the filtrate concentrated by rotor evaporation maintained at 40 ºC. The weight and volume of the extracts were recorded.

2.3.3 Butanol-water partitioning

Butanol-water partitioning was done with the methanol soluble extract or the methanol water soluble. Exactly, 50 ml of distilled water and 30 ml of butanol were added to the methanol water -soluble extract in a separating funnel and left to stand for 24 hrs. The two-phase liquid solution was separated after the time limit. These two phase solutions were run into separate containers and labeled appropriately as butanol-soluble (BUS) and water-soluble (WAS) fractions respectively. The BUS and WAS fractions were concentrated using rotor evaporator maintained at 40 ºC and 60 ºC for the butanol and water fractions respectively.

2.4 Determination of the Proximate Composition

The determination of the proximate composition of the leaf was carried by the methods of the Association of Official Analytical Chemists [25].

2.5 Collection of Blood Samples

Blood samples (HbSS) were collected from confirmed patients who attend sickle cell clinic at Federal Medical Centre, Owerri, by a personnel of the Hematology Unit of the Hospital. The donors were informed by the Ethical Committee of the hospital on the need for blood and they consented.

2.5.1 Preparation of blood samples

Portions (0.2 ml) of whole blood was used for the $Fe^{2+/}Fe^{3+}$ ratio determination, while the remaining portions were collected into citrate anticoagulant tubes and kept in a refrigerator at 8 ºC. Erythrocytes were isolated from the blood samples by centrifugation at x 1500 g for 10 minutes, using a bench centrifuge. Following careful siphoning of the plasma with Pasteur pipette, the erythrocytes were by repeated inversion suspended in a volume of isotonic saline (0.9%) equivalent to the volume of the siphoned plasma. The erythrocyte suspension was then frozen at 0 ºC and subsequently thawed to produce a hemolysate from the hemoglobin polymerization experiment.

2.6 Phytochemical Screening and Quantitation

Qualitative and Quantitative screening and determination of the resident phytochemicals were carried out by the methods of the Association of Official Analytical Chemists [25,26] to determine the saponins, flavonoids, tannins, alkaloids etc.

2.7 Determination of Total Free Amino Acid Concentration of the Fractions of Sample

0.1% Ninhydrin in acetone was diluted with distilled water in the ratio 1:4. The water- soluble fraction (WAS) was diluted 1:1 with distilled water; the BUS extract 1:1 with methylated spirit and the FAS, 1:5 with ethanol. Exactly, 20µL each of the diluted extract was added to 4.0 ml portions of the diluted Ninhydrin. The resulting solutions were heated to boiling for 5 minutes, cooled and the absorbance reading taken in a spectrophotometer at 570 nm, using distilled water as blank. The values were extrapolated from a standard curve obtained by treating 20 µL portions of different concentrations (1-20 mg) of Phenylalanine with 4 ml portions of diluted Ninhydrin, treated as above.

2.8 Determination of the Major Amino Constituents of the Extracts by TLC

Thin layer chromatographic (TLC) techniques described in the Official Methods of the Association of Analytical Chemists [25] was used .Solutions of some standard amino acids such as Phe, Ala, Glu, His, Cys, Asp,etc were prepared by dissolving 5 mg each in 1.0 ml portions of 0. 5 M HCl. The resultant solutions were spotted on one side of a thin layer chromatographic plate of dimension 20 x 10 cm using silica gel as the adsorbent. Diluted portions of WAS, FAS and BUS were also spotted on the TLC plates alongside the amino acid standards. The developing solvent was prepared by mixing 30 ml of distilled water, 24 ml of butanol and 6 ml of acetic acid in a ratio of 5:4:1 to give a total of 60 ml. Ninhydrin was used to develop the plates. The relation factor (Rf) values of the standards were recorded and compared with those obtained from the different fractions [27].

2.9 Determination of Ascorbic Acid Concentration of the Water-Soluble (WAS) Fraction

The determination of the Ascorbic acid of the sample was carried out by the methods [28]. Ascorbic acid standard was prepared containing 1 g/dm^3 of the Ascorbic acid such that 1 cm^3= 1 mg vit C. A burette was filled with a solution of 2, 6-Dichlorophenolindophenol (DCPIP) of 0.01%. 10 cm^3 of the Ascorbic acid was acidified with two or three drops of dilute HCl. The indophenols solution was titrated into the Ascorbic acid solution until there was a permanent pink solution. If X cm^3 of the indophenol were required, 1 cm^3 of indophenol solution is equivalent to 10 mg vitamin C/X. Having standardized the indophenol solution, 10 cm^3 of the test solution (extract) was taken and treated the same.

2.10 Sickle Cell Hemoglobin Polymerization Inhibition Experiment

The original methods [7,29,30], were used for HbSS polymerization inhibition experiment. Sickle cell hemoglobin polymerization was assessed by the turbidity of the polymerizing mixture at 700 nm, using 4.4 ml of 2% Sodium metabisulphite ($Na_2S_2O_3$) as the reductant or deoxygenating agent; 0.5 ml normal saline and 0.1 ml hemoglobin solution were introduced into the cuvette by means of a pipette, shaken and inserted into a spectrophotometer and absorbance reading taken at 2 minutes intervals for 30 minutes. This represented the control. Distilled water was used as blank for all assays. For the test assay, 4.4 ml of 2% Sodium metabisulphite solution, o.5 ml antisickling agent and 0.1 ml hemoglobin solution were assayed as above. The rates of hemoglobin polymerization were calculated. The relative percent polymerization and relative percent inhibition were determined with respect to the control. These were all determined from the formula below [7].

$$Rp = \text{Final OD-Initial OD/time}$$
$$Rp = ODf - ODi / t = \Delta OD / t$$

Where Rp =rate of polymerization; ODf= final Absorbance

ODi = initial Absorbance; t= time in minutes.

2.11 Determination of Fe^{2+}/Fe^{3+} Ratio of Sickle Cell Blood

The Fe^{2+}/Fe^{3+} ratio was determined by the method [31]; while the oxygen affinity of hemoglobin and methemoglobin were measured at 540 nm and 630 nm respectively. The approach employs lyzing 0.02 ml whole blood in 5.0 ml distilled water and 0.02 ml normal saline. The absorbance of Hb (hemoglobin) and mHb (methemoglobiin) were determined to give % Hb and % mHb respectively. This represents the control. In determining the effect of the extract on the ratio; 0.02 ml of the extract was added to 5.0 ml of distilled water and 0.02 ml of whole blood added and incubated for 60 minutes in a test tube. The procedure above was repeated to determine the % Hb and % mHb. The analysis gives the relative percent increase or decrease in the ratio [32].

2.12 Determination of the Mineral Content of *Alchornea cordiofolia*

Plant materials were washed with lukewarm water and dried at room temperature of 25^{0}C. The washed dried materials were ground to powder and used for dried ashing [33]. The powdered plant material was taken in a pre-cleaned and constantly weighed silica crucible,

heated in a muffle furnace at 400 0C, till there was no evolution of smoke. The crucible was cooled at room temperature in a desiccators and the carbon-free ash was moistened with conc. sulphuric acid and heated on a heating mantle till fumes of sulphuric acid ceases to evolve. The crucible with the sulphuric acid was then heated in a muffle furnace at 600 0C to a constant weight obtained between 2-3 hrs. One gramme (1 g) of the sulphated ash obtained was dissolved in 100 ml of 5% HCl to obtain the solution for determination of mineral elements using Atomic Absorption Spectrophotometry (AAS) and Flame Photometry (FPM).Standard solutions of each element was prepared and calibration curve drawn for each element using AAS/FPM [34].

2.13 Statistical Analyses of Results

Statistical analysis of results is multifaceted. Some results are expressed in percentages, Mean and Standard deviations, as well as, ONE-WAY-ANOVA.

3. RESULTS

The results of all analysis are shown in Tables 1-6 and Fig. 1. Table 1 shows the quantitative phytochemical composition of the crude aqueous extract of *Alchornea cordifolia* expressed as percentage composition, with mean and standard deviation. Fig. 1 shows the proximate composition of the leaves. Values are expressed as percentages. Table 2 shows the total free amino acid and Vitamin C concentrations, expressed in mg/100 g of sample. Table 3 shows the mineral composition of the leaves. Table 4 depicts the in vitro effects of the extracts on the Fe^{2+}/Fe^{3+} ratio. Values are expressed in percentage and ONE-WAY-ANOVA. Table 5 shows the effects of the extracts on the inhibition of Hb SS polymerization. Table 6 shows some amino acids present in the leaves, determined by TLC. Results are expressed in percentages (%), mg/100 g of sample and analyzed using ONE-WAY ANOVA.

Table 1. Quantitative phytochemical composition of the crude aqueous extract (CAE) of sample. Values expessed in (mg %)

Phytochemicals	Flavonoids	Alkaloids	Saponins	Tannins	Phenols
Concentration (mg %)	4.20±0.1	5.70±0.12	4.60±0.10	6.50±0.1	3.0±0.0

The values in the table are the Mean ±SD from triplicate determinations .

Table 2. Total free amino acid and vitamin C concentrations of the WAS, BUS and FAS extracts of *Alchornea cordifolia* expressed in mg/100g of sample

Fraction of sample	Vol. of extract	Dilution factor	Total volume	Conc. (mg/ml)	Conc. mg/80 g	Total A.A.(mg/100g)
FAS	5.0	6.0	30.0	8.78	263.4±0.00	526.80±0.00
BUS	24.0	2.0	48.0	0.16	7.824±0.00	15.65±0.00
WAS	74.0	2.0	148.0	0.27	39.66±0.00	79.33±0.00
Vitamin C	**vol. of extract(ml)**	**dilution factor**	**(mg/ml)**	**mg/ 80g**	**Total Vitamin C (mg/100 g**	
Values	74.00	10.00	2.09±0.01	1543.34±1.10	1929.18±0.20	

The values in the table are the Mean ± SD from triplicate determinations.

Table 3. Mineral elements identified in the leaves of *Alchornea cordifolia*

Mineral (s)	Na	Ca	Mg	K	P
Conc.mg/100g	228.20±0.01	1.60±0.0	2.40±0.0	150.30±0.01	1.00±01

Values are Mean ± SD from triplicate determinations.

Table 4. *In vitro* effects of the fractions, FAS, BUS and WAS on the Fe^{2+}/Fe^{3+} ratio of sickle cell blood (HbSS) at 4.80 µM Phenylalanine equivalence

Sample	Fraction	Final assay	conc. (µM) % Hb	% mHb	Fe^{2+}/Fe^{3+}	(%) increase/ decrease
HbSS blood	Control	-----	93.43±0.1	6.57±0.1	14.20±0.1	0.00±0.0
A. cordifolia	WAS	4.80	93.86±0.0	6.14 ±0.0	15.30±0.0	7.75±0.2
A. cordifolia	FAS	4.80	93.59±0.1	6.41±0.1	14.60 ±0.0	2.82±0.1
A. cordifolia	BUS	4.80	90.09±0.1	9.91±0.2	9.09±0.0	-36.0±0.0

The values in the table are the Mean± SD from triplicate determinations.

Table 5. The rates of polymerization, relative percent polymerization and relative percent inhibition of HbSS polymerization by different fractions of the extracts of *Alchornea cordifolia* at 4.80 µM Phe equivalence

Sample	Fraction	final assay conc. (µM)	Rates of polymerization	Relative % polymerization	relative % inhibition/ enhancement
HbSS Blood	---		^{c}0.0240±0.0	^{c}100.00±0.0	^{c} 0.00±0.0
A. cordifolia	WAS	4.80	^{a} 0.0025±0.1	^{a}1.88±0.0	^{a} 98.12±0.0
A. cordifolia	FAS	4.80	^{a} 0.0007±0.1	^{a}2.71±0.1	^{a}97.29±0.1
A. cordifolia	BUS	4.80	^{a} 0.0126 ±0.1	^{b}52.50±0.0	^{a}47.50±0.0

Values in the table are the Mean ± SD from triplicate determinations. Values with the same superscript are statistically the same and different from others along the rows and not the columns, at p≤0.05.

Table 6. Major amino acids identified by TLC in the different fractions of the samples

Sample	Fractions	Amino acids identified
Alchornea cordifolia	WAS	Gly , His, Glu, Ala, Lys
	FAS	Phe
	BUS	Met, Cys, Lys, Glu

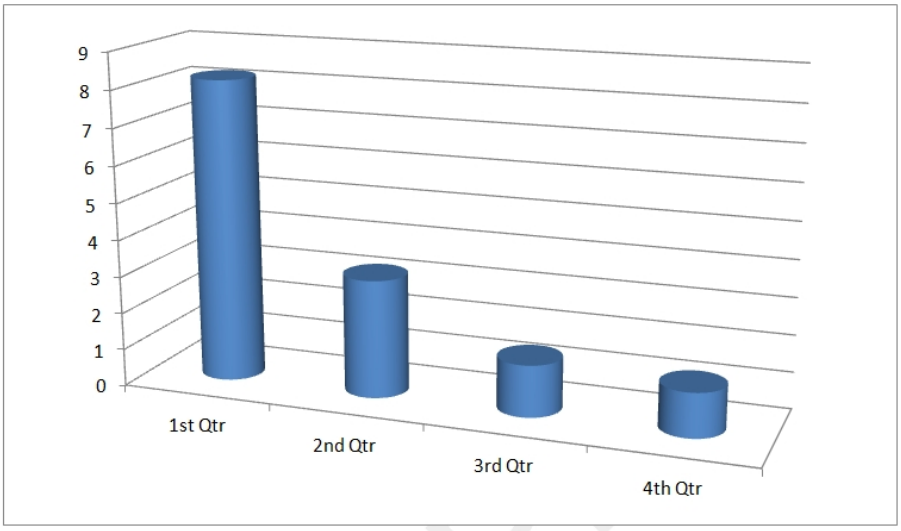

Fig. 1. Proximate composition of the leaves of *Alchornea cordifolia*. 1st Qtr=CHO(45%), 2ndQtr=Crude fiber(20.50%),3rdQtr=moisture(11.50%), 4thQtr=Ash(6.8%,CP(6.10%).Fat (6.03%)

4. DISCUSSION

From the results on tables 1-6, it can be inferred that the water soluble extract (WAS), the fat- soluble(FAS) extract and the butanol- soluble BUS) extracts of the leaves of *Alchornea cordifolia* posses profound antisickling potency. Table 1 shows the quantitative phytochemical composition, showing the presence of saponins,flavonoids and alkaloids,thus, pointing to the medicinal value of the plant. The presence of flavonoids in most medicines and extracts has been attributed to their synergistic effect drugs eliciting high potency by the potentiation of other metabolites, drugs, vaccines, resident [11]. Saponins have equally been found to posses stimulating effect both in vaccines and conventional extracts. Many researchers have reported the presence of anthocyanidine as constituent of the leaf [17]. Anthocyanins present in the crude extracts of the plant have attracted attention of researchers on their outstanding antisickling effect, thus supporting the claims of ethno-practitioners, hence suggesting a possible correlation between chemical composition and medicinal/therapeutic application by ethno- medicinal practitioners.

Table 2 shows the total vitamin C of the water- soluble (WAS) fraction free amino acid concentration of the extracts, which value is very outstanding (1929.175mg/100g of sample. The antisickling effectiveness of vitamin C, had already been demonstrated [35,36]. Table 2 equally reveals the total free amino acid concentration of FAS, BUS and WAS fractions. The FAS fraction showed the highest value of 526.8 mg/100 g, followed by the WAS (79.328 mg/100g and the BUS, 15.648 mgl100 g respectively. It can be recalled that in Table 6, the FAS fraction contain only phenylalanine, possibly at very high concentration. Phenylalanine is a standard antisickling amino acid, which exhibits synergistic activity in any extract with other antisickling components, drugs and nutrients when compounded together, and has found pronounced role in the management of sickle cell disease [37,38,29]. Some researchers have reported on the anti-anemic properties of the leaf extract. This may be deduced from the high content of free amino acids of which many are essential amino acids.

The different leaf extracts showed significant anti –anemic activity by increasing the level of Hb and iron in the blood after oral administration to anemic rats [39]. It has been found that crude leaf extracts coagulate blood plasma in vitro. The high tannin content was thought to be responsible for this activity. Table 4 shows the total free amino acid content of the different leaf extracts. Here also, the highest concentration was observed in the FAS fraction, followed by the water soluble (WAS) fraction and finally, the BUS fraction. It can be seen that the leaf is a rich source of protein and amino acids and has been implicated in the treatment of anemia in rats [37]. A glance at Fig. 1, shows the proximate composition of the leaf, this equally reflects the nutritional role of the plant. Table 5 shows the in vitro effect of the extracts on the oxygen affinity of the erythrocytes by improving the Fe^{2+}/Fe^{3+} ratio. A cursory glance on the table shows that the extracts relatively improved the ratio, with the WAS fraction having more pronounced effect than the FAS fraction. More notable is the fact that the BUS fraction decreased the ratio by the value (-36%). The negative value would therefore imply a reversion from antisickling to sickling, a condition not consistently favorable to sickle cell disease patients.

Table 5 focuses on the antisickling effectiveness of the extracts based on their capacity to inhibit sickle cell hemoglobin polymerization. It could seen from the table that all fractions of the extract are potent inhibitors, exhibiting the act very remarkably, with the WAS fraction inhibiting 98.12% of the process, followed by the FAS 97.29%) and the BUS (47.50%). The BUS fraction is not actually rich in antisickling amino acids and by its hydrophobic nature cannot diffuse easily into the heme pocket, a site for polymerization of sickle blood cells [29].

The methanol extract of the leaves have been found to have a mild relaxing effect on smooth muscles *in vitro*, which is attributed to the flavonoid- quercetin and its derivatives. The extract of the root significantly delayed the effect of histamine-induced bronchio-constriction, characterized by shortness of breath in guinea pigs. The crude methanol extract of the leaves and several fractions of it have shown anti-inflammatory action on the Croton- oil induced ear edema test in mice [40]. The medicinal roles of various plant parts and extracts are not in doubt as have been published by many authors. We are equally delighted by stumbling over these facts on the antisicking effectiveness of the WAS and FAS fractions. Although, there is a report on the anti-anemic role of leaves extract of the plant. It would therefore be of good dividend to humanity to derive this benefit therapeutically from this all abundant plant. Table 6 shows the major amino acids identified by TLC in the fraWAS and FAS fractions showed the presence of Phe, an antisickling amino acid in all fractions [35,37]. This amino acid was not identified in the BUS fraction and its absence in this fraction must have accounted for the glaring results obtained from the fraction, especially in the hemoglobin polymerization experiment .The plant *Alchornea cordifolia* is endowed with abundant secondary metabolites which may have acted synergistically to elicit the reactions and results obtained in this work. The leaf extracts may be used nutritionally and therapeutically in the management of sickle cell disease and other similar syndromes.

5. CONCLUSION

The *in vitro* antisickling and nutritionsl properties of leaf extracts of *Alchornea cordifolia* were investigated. The leaves extracts exhibited high antisickling potency by inhibiting sickle hemoglobin gelation remarkably. The WAS extract exhibited more pronounced potency than other fractions. The extracts were equally able to improve the oxygen affinity of the drepanocytes except the BUS fraction. This inaction was attributed to its poor diffusibility into the hemoglobin molecule and its hydrophobic property. Nutritionally, the free amino

acids concentrations were high and proximate compositions revealed the presence of proteins, fiber, carbohydrates, lipids and ash. Phytochemical analysis indicated high levels of secondary metabolites such as flavonoids, saponins, alkaloids, tannins and others. The mineral content was ideal, higher than the recommended dietary allowance. *Alchornea cordiofolia* leaves nonetheless, would be very beneficial for the management of sickle cell disease and other syndromes of related etiology.

ACKNOWLEDGEMENT

To arrive at this level of work is always an uphill task which required many committed hands. We must not fail to acknowledge the contributions of the personnel of the Hematology Unit of the Federal Medical Center, Owerri, Nigeria, for granting us the permission to use and the provision of sickle cell blood for the research. I must not fail to thank members of the research unit for their effort in providing the fund for the work within a minimum space of time. I wish also to acknowledge the contribution of Miss Nancy Akagha of the ICT unit of the University library, for her contribution in typesetting the manuscript.

COMPETING INTEREST

There were no competing interests in the work. Every member worked assiduously to achieve the aim and objectives of the work. It was just like a bee-hive session throughout the duration of study. Authors have unequivocally declared that no competing interests existed in the course of the work.

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