**Scholars Journal of Applied Medical Sciences (SJAMS)** **ISSN 2320-6691**
**Sch. J. App. Med. Sci., 2013; 1(3):191-198**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

## Research Article

# The Antisickling Potentials of Four Curcubits (*T. Occidentalis*, C. *Maxima; C. Sativus* and *C.Lonatus*)

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**Abstract:** The antisickling potentials of four curcubits (*T.occidentalis* , *C. maxima* , *C. sativus* and *C* . *lonatus* and their extracts were investigated to ascertain the ability of the extracts of the samples to inhibit sickle cell hemoglobin polymerization and improve the $Fe^{2+}/Fe^{3+}$ ratio of sickle cell blood. Phytochemical analysis of the samples revealed the presence of flavonoids, phenols, alkaloids, tannins and saponins, all present at varying concentrations. The seeds of the sample (*T.occidentalis*) were first dehulled and dried in an oven at 40 $^0$C, ground into powder and finally soaked in chloroform of analytical grade to defat the sample and in essence to produce the fat-soluble extract (FAS). The defatted residue was dried *en vacuo* , soaked in 200 ml methanol of analytical grade for 48 hrs to generate the methanol soluble fraction (MSF),which was concentrated by rotor evaporator, set at 45 $^0$C. This was finally fractionated in a mixture of BuOH/$H_2O$ (1:1) to give the butanol-soluble (BUS) and the water-soluble (WAS) fractions respectively .The samples of *(C.maxima, C. sativus* and *C. lonatus*) of weights 600 g each ,were washed, sliced and blended to homogenous powders .Ten (10 ml) milliliters of 10 % Alum solution were added to each of the later extracts, then refrigerated at 8 $^0$C for 24 hours before filtration to generate the crude aqueous extracts (CAEs). The fat-soluble (FAS),the butanol-soluble BUS, and the water- soluble( WAS) extracts of *T.occidetalis*, were able to inhibit HbSS polymerization to varying degrees from 60.42 % for the BUS to 95.00 % for the WAS of *T* . *occidentalis* .The hemoglobin polymerization inhibition assay results for the CAEs are as follows: C. *lonatus* (97.1 %), *C. sativum* (97.1 %) and *C. maxima* (94.58 %) respectively. The vitamin C concentrations of the samples ranged from 9.50 mg/100g for the WAS fraction of *T. occidentalis* to 288.08mg/100 g for C. *lonatus* . Nutritionally, the different samples were found to be rich sources of free amino acids, having concentrations that ranged from 15.80 mg/100g for the BUS fraction of *T. occidentalis* to 36553.40 mg/ 100g for *C. lonatus.* Thin layer chromatographic analysis revealed the following amino acids: Arg, Phe, Lys and others, present in most of the fractions. The $Fe^{2+}/Fe^{3+}$ analysis of the extracts on sickle cell blood revealed an increase from 8.61% for the FAS fraction of *T. occidentalis* to 167.5 % for the WAS fraction of the same sample. The curcubits (*T. occidentalis*, *C. lonatus*, *C. sativus* and C. *maxima* studied, exhibited high level potency in inhibiting sickle cell hemoglobin polymerization, improvement in $Fe^{2+}/Fe^{3+}$ ratio, and providing the sickle cell disease patients with adequate nutrients and phytochemicals, for a stable healthy status .The cucurbits are another important nutritional and dietary regimen for effective management of sickle cell disease.
**Keywords**: Curcubits, hemoglobin polymerization, $Fe^{2+/}$ $Fe^{3+}$ ratio, sickle cell disease.

## INTRODUCTION

Sickle cell disease, otherwise called sickle cell anemia is a genetic blood disorder arising from a point mutation in the β-globin gene that leads to the replacement of glutamic acid (a hydrophilic moiety) residue by valine (a hydrophobic moiety) at the sixth position of the β-chain of hemoglobin [1]. This inherited disorder leads to the production of hemoglobin S (HbSS). There are equally other hemoglobin variants, such as HbC, Hb E, Hb D, Hb AS, HbAC, HbAS [2].

Hemoglobin is an iron containing protein found in the erythrocytes. It transfers oxygen from the lungs to all other parts of the human body, releasing it to cells and tissues. At low oxygen tension, the sickle hemoglobin polymerizes within the red cells into a gel-like rigid fiber form, blocking the vasculature, leading to ischemia and red cell deformability [3]. The blockage leads to fatal crises known as sickle cell disease (SCD) [4] .Challenges emanating from the search for drugs and or nutrients to cure or manage the syndrome necessitated the trial and application of many substances especially in developing countries where incomes are low and adequate medical care is grossly lacking. Herbal medicines or alternate and complementary medicine becomes the most available protocol. Herbal medicines have been known to man for ages. It can be phyto-medicines that employ various plant parts or the wholesome use of a plant, which possess healing properties. In Nigeria and in most developing countries, medicinal plants have been used in the treatment of pain crises associated with sickle cell crises. In recent years, these active principles in plants have been extracted and used in different forms such as infusions, syrups, decoctions, infused oils, essential oils, ointments and creams [5-7].

Various approaches have been adopted in an effort to find agents that can inhibit polymerization or gelation of sickle cell hemoglobin and hence prevent or ameliorate the excruciating pathological complications of the disease [8]. In many protocols, carbon monoxide and Sodium nitrate were used to reduce the amount of deoxyHbS ; however, report indicates that these exercises or approaches did not yield the much needed benefit [8]. Hydroxyurea (HU), became the first drug used to prevent further complications of the syndrome. It was found to be potentially mutagenic and carcinogenic [9]. Patients randomized to hydroxyurea (HU), had fewer pain episodes, less acute chest pain syndromes and less transfusion requirements. This agent received rapid approval for use in sickle cell anemia in the USA and elsewhere. Generally, the actual mechanism of action of HU is to increase levels of fetal hemoglobin (HbF) in most sickle cell disease patients. Other drugs like NICOSSAN (previously (NIPRISSAN (NIX-0699), a product of extracts from four plants were shown to posses antisickling properties [8].

The role of nutrition in the management of sickle cell disease appears revolutionary in the ardent search for antisickling agents. This approach has proved worth-while. It was found earlier that some nutrients were lacking in most if not all sickle cell patients, such as vitamin C, Zinc, amino acids, and vitamin D [9]. It was reported that nutritional supplementation improves the prognosis of the syndrome and eventually reduces the pathological complications of the disease. An antisickling nutrient " CIKLERVIT $^{TM}$) was discovered by Professor G.I.Ekeke and Dr. R.N.Nwaoguikpe, all from Nigeria, now produced and marketed by NIEMETH PHARMACEUTICAL PLC. This preparation, like other preparations (DISCOVITE$^{TM}$) from USA) was formulated from edible legumes and foodstuffs. These nutrients posses the mechanism of inhibiting sickle cell hemoglobin polymerization and improving the oxygen affinity of the erythrocytes.Hence, the search for a therapeutic bullet will knock out completely, this syndrome. The current work focuses on assaying the nutritional and antisickling potentials of the extracts of four curcubits (*Telferia occidentalis*, *Curcubit maxima*, *Curcumis sativum* and *Curcubit lonatus*. *Curcubit maxima* known locally as UGBOGORO (among the Igbo of South-East Nigeria) is a gorge-like squash belonging to the genus *Curcubita*, which is a member of the family *Curcubitaceae* and consist of 118 genera and 825 species. The nutritional composition is variable ranging from carbohydrates, fats, proteins, amino acids to vitamins. Studies have shown that the pumpkin posses high medicinal values. The leaves are hematinic, analgesic and also possess dietary benefits. These curcubits are rich sources of vitamins and antioxidants, known to protect cells and tissues against free radicals [10, 11].

**CUCUMBER** (*Curcumix sativus*)

The cucumber is a widely cultivated plant in the gourd family *Curcubitaceae*, and has a family *Curcumis*, which belongs to the species *sativus*. The plant is a creeping vine which bears cylindrical edible fruits when ripe. However, cucumber usually contains more than 90 % water and has countless health benefits as well as cosmetic properties. It is an excellent source of vitamin C, folic acid, and potassium, when not peeled. The skin is rich in fiber and contains variety of minerals such as Magnesium and Silicon. Cucumber has a cleaning property that removes accumulated waste and toxins from the body. It eliminates uric acid and is more beneficial to rheumatic conditions caused by excess uric acid deposition in the body. *Cucumis sativus* is very beneficial in the treatment of diabetes mellitus, gout eczema, chest, lung and stomach problems. The potassium content of cucumber helps in the regulation of blood pressure and promotes flexibility of the muscles. The magnesium content helps in smooth blood circulation, relaxes muscles and nerves.

**WATER MELON** (*Citrullus lonatus*)

It is a member of the family *Curcubitaceae* and a vine-like plant, originally from South Africa. Its fruit also called Water melon, is a special type, referred to by botanists as pepo ; a berry, which has a thick rind (excess epicarp) and a fleshy succulent mesocarp and endocarp). The water melon fruit is loosely considered a type of melon, although not in the genus Cucumis; It has a smooth exterior rind (green, yellow, and sometimes, white) and a juicy sweet interior (usually deep red pink, but sometimes orange yellow and even green, when not ripe [12].

**FLUTED PUMPKIN *(Telferia occidentalis )***

*Telferia occidentalis* popularly called Ugu in Igbo of the South-Eastern Nigeria. It is primarily grown as leafy vegetable although the fruits or seeds are edible. Nigerian researchers have demonstrated how diets rich in pumpkin seeds and leaves could be used to increase hematological indices, improve sperm count (quality), reduce blood glucose level and inhibit cancer growth. Simple chromatographic analysis shows that it contains phytochemicals such as potassium, copper, edible oils, linoleic acid, oleic acid and vitamin E [13].

## MATERIALS AND METHODS

### Plant Materials

The following plant parts were purchased from a metropolitan market in Owerri municipality. These include: *Curcubita maxima* (pumpkin leaves), *Citrillus lonatus* (Water melon (fruits), *Cucumis sativus*(fruits) (Cucumber), *Telferia occidentalis* , seeds).

### Preparation of samples

The samples were assigned to four groups, namely: A (*Telferia occidentalis*), B (*Citrillus lonatus*), C (*Curcubita maxima*) and D (*Cucumis sativus*). Two hundred grams (200 g) of each sample were washed

under tap water to remove debris . The samples were reweighed to ensure accuracy of the mass of samples. The samples were blended after cutting them into tiny bits into a homogenous mixture. These were filtered and volume of filtrate measured and recorded. Ten milliliters (10 ml) of 10% Alum solution were run into each of the filtrates and kept in a refrigerator at 8 $^0$C for 24 hrs. The filtrates were removed from the refrigerator after 24 hrs, filtered with Whatman No 1 filter paper. The filtrates were kept in sealed jars in the refrigerator at the same temperature until used.

**Collection of sickle cell blood**

Two milliliters (2 ml) of sickle cell blood were collected from each of the sickle cell disease patients who attend sickle cell Clinic at the Federal Medical Centre, Owerri .The request for blood was made after discussing with donors on the nature and benefits of the research work. This exercise was carried out by the head of the Hematology Unit of the medical centre. Portions of whole blood (0.02 ml) were used for the $Fe^{2+}/Fe^{3+}$ ratio, while the remaining portions of blood were kept in the freezer for freeze thawing to hemolyze the erythrocytes and subsequently release the hemoglobin (Hb).

**Extraction of Crude Aqueous Extracts (CAES)**

The various samples of weight, 500 g were washed in running tap water, sliced into bits and blended to give a homogenate. Ten (10 ml) of 10 % Alum solution was added to the extract and left in the refrigerator for 24 hrs. The solution was filtered with Whatman No 1 filter paper and centrifuged at 15000 X g for 10 minutes to obtain a clear solution devoid of debris, mucilage and cells.

**Extraction of Fat-Soluble Fraction (FAS)**

One hundred grams (100 g) of the powdered sample of *Telferia occidentalis* was soaked in 200 ml of chloroform for 48 hours to de-fat them and in essence to generate the fat-soluble fraction (FAS). The residue from the above process was dried *en vacuo* and kept in a dessicator for the butanol- soluble fraction (BUS).The resulting fat-soluble fraction was weighed and the volume recorded.

**Extraction of The Methanol- Water Soluble Fraction (MWS)**

The dried evaporated residue from the chloroform extraction was later soaked in 200 ml of methanol of analytical grade for 48 hrs. The mixture was filtered and the filtrate concentrated by rotor evaporation maintained at 45 $^0$C. The final volume of the methanol-water soluble was recorded and the filtrate kept in a dessicator until used.

**Butanol- Water Partitioning**

Butanol partitioning was done with the methanol-water soluble fraction. Exactly, 40 ml of distilled water, 40 ml of butanol were added to the methanol-water soluble and the two phase mixture allowed to separate on a clamped separating funnel mounted for 24 hours. The separated samples -the water-soluble fraction (WAS) and the butanol-soluble fraction (BUS) were concentrated by rotor evaporation maintained at 60 $^0$C and 45 $^0$C respectively.

**Phytochemical Screening and Quantitation**

Qualitative and quantitative analyses of the samples were carried out by the methods of the Association of Official Analytical Chemists [4, 14, 15].

**Sickle Cell Hemoglobin Polymerization Inhibition Experiment**

The original methods of [16-18] were used for HbSS experiment. HbSS polymerization was assessed by the turbidity of the polymerizing solution at a wavelength of 700nm, using 4.4ml of 2% solution of Sodium metabisulphite ($Na_2S_2O_3$) as a deoxygenating agent or a reductant. One half (0.5 ml) milliliters of normal saline (0.9% NaCl) solution and 0.1 ml hemoglobin were pippetted into a cuvette, shaken and inserted into the spectrophotometer and absorbance readings taken at 2 min. intervals for 30 mins. This served as control. Distilled water was used as blank for all assays. For the test, 4.4 ml of 2% solution of sodium metabisulphite , 0.5ml of each extract nd 0.1 ml hemoglobin (HbSS) solution were pippetted into the cuvette and readings taken as above. The rate of hemoglobin polymerization for all assays was estimated,using the relationship below.

$$Rp = \frac{ODf - OD\,i}{t_m - t_0} = \frac{\Delta OD}{\Delta t}$$

where ODf= final Absorbance at maximum time($t_m$)
ODi = initial Absorbance at time zero ($t_0$)
t = time in minutes
Rp = rate of polymerization

**Determination of Total Free Amino Acid Concentration of the Extracts**

0.1% Ninhydrin solution was diluted with distilled water in the ratio 1:4. The water-soluble (WAS) fractions were diluted 1:1 with distilled water; the BUS extract 1:1 with methylated spirit, and the FAS extract, 1:5 with ethanol. For the crude aqueous extracts (CAEs) , the values were extrapolated from a standard curve obtained by treating different portions (1-20 mg/ml) of Phenylalanine with 4ml portions of diluted ninhydrin. The resultant solution was heated to boiling for 5 min, cooled and absorbance taken from a spectrophotometer (Unicam Spectronic 20 DR) at 570 nm, using distilled water as blank.

**DETERMINATION OF AMINO ACID CONSTITUENTS OF EXTRACTS**

Thin layer chromatographic techniques were used as described in the Official methods of Analysis of the Association of Analytical Chemists [13]. Solutions of

standard amino acids were prepared by dissolving 5 mg of each standard amino acid in 1.0 ml portions of 0.1MHCl. The resultant solutions were spotted on one side of thin layer at the longer side of a chromatographic plate of dimensions 20 X10 cm, using silica gel as adsorbent .Diluted portions of the CAE,WAS,BUS and FAS extracts were also spotted on the TLC plate alongside the amino acid standards. The developing solvent was prepared by mixing 24 ml of butanol , 6 ml of acetic acid and 30 ml of distilled water to give a total volume of 60 ml in a ratio of 4:1:5. The relation factor ( Rf ) or values of the standards were recorded and compared with those of FAS, BUS, CAE and CAE extracts respectively to give the amino acids identified.

**Determination of the Ascorbic acid Concentration of the Extracts**

The determination of the Ascorbic acid Concentration of the Extract was carried by the methods of [18]. Ascorbic acid standard was prepared containing 1 g/dm3 of vitamin C. A burette was filled with a solution of 2,6-Dihlorophenolindophenol (DCPIP) of concentration 0.01%. Ten milliliters (10 ml) of Ascorbic acid was acidified with two drops of dilute HCl in a beaker. The indophenol solution then titrated against the Ascorbic acid until a permanent pink color develops. If X $cm^3$ of the indophenol are required, then 1 $cm^3$ of the indophenol solution is equivalent to 10 mg Vitamin C. Having standardized the indophenol solution, 10 $cm^3$ of the test solution (extract) was taken and treated as above.

**Determination of the $Fe^{2+}/Fe^{3+}$ ratio**

In the determination of the effects of the extracts on the $Fe^{2+}/Fe^{3+}$ ratio of sickle cell blood, 0.02 ml of normal saline was added to 5.0 ml of distilled water and 0.02 ml of whole blood (HbSS) incubated in a test–tube mounted on a rack for 1 hour. The percent Hb (% Hb) and (% mHb) were determined spectrophotometrically at 630 nm and 540 nm respectively. In the test assay, the normal saline was replaced by 0.02 ml of the extract or antisickling agent, [20,21]

**RESULTS**

The results of all analyses are shown in tables 1-7

**Table1: Results of qualitative phytochemical analyses**

| Sample | Tannins | Saponins | Flavonoids | Phenol | Alkaloids |
|---|---|---|---|---|---|
| *T. occidentalis* | + | + | + | + | + |
| *C. lonatus* | + | + | + | + | + |
| *C. sativus* | + | + | + | + | + |
| *C.maxima* | + | + | + | + | + |

**Table2: Quatitative Phytochemical Composition of Samples . Values are expressed as Percentage (%)**

| Sample | Tannins | Saponins | Flavonoids | Phenol | Alkaloids |
|---|---|---|---|---|---|
| *T. occidentalis* | $0.48\pm0.00^a$ | $0.24\pm0.02^b$ | $0.33\pm0.01^a$ | $0.32\pm0.00^a$ | $0.30\pm0.00^a$ |
| *C. lonatus* | $1.54\pm0.12^b$ | $0.11\pm0.01^c$ | $0.16\pm0.00^c$ | $0.16\pm 0.00^c$ | $0.13\pm0.01^c$ |
| *C. sativus* | $2.21\pm0.01^a$ | $0.23\pm0.02^b$ | $0.29\pm0.02^b$ | $0.22\pm0.00^b$ | $0.18\pm0.01^c$ |
| *C.maxima* | $0.58\pm0.01^c$ | $0.52\pm0.01^a$ | $0.23\pm0.01^b$ | $0.19\pm0.01^b$ | $0.24\pm0.01^b$ |

Values in the table are the Mean ± SD from triplicate determinations. Values with the same superscript are significantly related along the columns and not the rows at $p\leq0.05$

**Table 3: Total Vitamin C Concentration of the Extracts. values are expressed in mg/ 100 g of Sample**

| Sample | Fraction | Dilution factor | Volume of extract | Vit.C(mg/ml) | vitC(mg/200g) | Vit.C (mg/100 g) |
|---|---|---|---|---|---|---|
| *T. occidentalis* | WAS | 10.0 | 95.0 | 2.000 | 19.00 | 9.50 |
| *C. lonatus* | CAE | 10.0 | 650.0 | 8.864 | 576.16 | 288.08 |
| *C. sativus* | CAE | 10.0 | 445.0 | 7.210 | 320.85 | 160.48 |
| *C.maxima* | CAE | 10.0 | 254.0 | 3.911 | 99.34 | 49.67 |

**Table 4: Amino acids identified by TLC in the different fractions of the samples**

| Sample | Fraction | Amino acids identified |
|---|---|---|
| *T. occidentalis* | FAS | Arg,His,Phe,Glu,Met,Asp |
| *T. occidentalis* | BUS | Tyr,Phe,Met |
| *T. occidentalis* | WAS | Asp,Cys,Tyr,Gly,Phe,Arg |
| *C. lonatus* | CAE | Asp,Tyr,Glu,Ser,Ala,Ile,Phe,Lys,Arg |
| *C. sativus* | CAE | Tyr,Arg,Phe,Asp,Glu,His,Lys,Met,Thr |
| *C.maxima* | CAE | Phe,Asp,Arg,Met,His, Thr |

**Table 5: Total free Amino Acid Concentration of the Extracts expressed in mg/100 g of Sample**

| Sample/Fraction | Vol. of extract | Dilution factor | Amino acid conc.(mg/ml) | Total FAA conc.mg/50g | Total FAA conc.mg/100 g |
|---|---|---|---|---|---|
| *T. occidentalis*(FAS) | 21.0 | 6.0 | 0.1 95±0.1 | 24.57±0.1 | 49.14±0.1 |
| *T. occidentalis*(BUS) | 25.0 | 2.0 | 0.158±0.0 | 7.90±0.0 | 15.30±0.0 |
| *T.occidentalis*(WAS) | 95.0 | 2.0 | $3.586\pm0.1^{c}$ | $681.34\pm0.2^{c}$ | $1362.68\pm0.2^{c}$ |
| *C. lonatus* (CAE) | 650.0 | -- | $28.118\pm0.0^{a}$ | $18276.70\pm0.1^{a}$ | $36553.40\pm0.0^{a}$ |
| *C. sativus* (CAE) | 445.0 | -- | $18.822\pm0.0^{b}$ | $8375.79\pm0.1^{b}$ | $16751.52\pm0.1^{b}$ |
| *C.maxima* (CAE) | 254.0 | -- | $17.823\pm0.1^{b}$ | $4527.04\pm0.0^{b}$ | $9054.08\pm0.0^{b}$ |

The values in the table are the Mean± SD from triplicate determinations. Values with the same superscript are significantly related along the rows at $p\leq0.05$.

**Table 6: In vitro effect of the fractions on the $Fe^{2+}/Fe^{3+}$ ratio of sickle cell hemoglobin**

| Sample | Fraction | % Hb | % mHb | $Fe^{2+}/Fe^{3+}$ | % Increase/Decrease |
|---|---|---|---|---|---|
| Control (HbSS) | - | 93.43±0.0 | 6.57±0.1 | 14.29±0.1 | 0.00±0.0 |
| *T. occidentalis* | FAS | $95.54\pm0.1^{b}$ | $4.46\pm0.0^{b}$ | $21.42\pm0.0^{b}$ | $50.04\pm0.0^{b}$ |
| *T. occidentalis* | BUS | $93.94\pm0.1^{c}$ | $6.06\pm0.0^{c}$ | $15.52\pm0.0^{c}$ | $8.61\pm0.0^{c}$ |
| *T.occidentalis* | WAS | $97.46\pm0.2^{a}$ | $2.54\pm0.0^{a}$ | $38.22\pm0.0^{a}$ | $167.46\pm0.0^{a}$ |
| *C. lonatus* | CAE | $97.06\pm0.0^{a}$ | $2.94\pm0.0^{a}$ | $33.01\pm0.0^{a}$ | $131.0\pm0.0^{a}$ |
| *C. sativus* | CAE | $96.67\pm0.1^{a}$ | $3.33\pm0.1^{a}$ | $31.89\pm0.0^{a}$ | $123.16\pm0.1^{a}$ |
| *C.maxima* | CAE | $94.01\pm0.0^{c}$ | $5.99\pm0.1^{c}$ | $15.69\pm0.0^{c}$ | $9.80\pm0.0^{c}$ |

The values in the table are the Mean± SD from triplicate determinations. Values with the same superscript are significantly related along the rows and columns at $p\leq0.05$.

**Table 7: The rates of polymerization, the relative percent polymerization and the relative percent inhibition of sickle cell hemoglobin at a final assay concentration of 16.58µM Phe Equivalence**

| Sample | Fraction | final assay Conc(µM) | rate of polymerization | relative % polymerization | relative % inhibition |
|---|---|---|---|---|---|
| Control (HbSS) | - | 16.58 | 0.0240 | 100.0±0.0 | 0.00±0.0 |
| *T. occidentalis* | FAS | 16.58 | 0.0014 | $5.83\pm0.1^{a}$ | $94.17\pm0.1^{a}$ |
| *T. occidentalis* | BUS | 16.58 | 0.0095 | $39.58\pm0.2^{b}$ | $60.42\pm0.0^{b}$ |
| *T.occidentalis* | WAS | 16.58 | 0.0012 | $5.00\pm0.0^{a}$ | $95.00\pm0.0^{a}$ |
| *C. lonatus* | CAE | 16.58 | 0.0007 | $2.917\pm0.1^{a}$ | $97.083\pm0.1^{a}$ |
| *C. sativus* | CAE | 16.58 | 0.0006 | $2.917\pm0.1^{a}$ | $97.083\pm0.1^{a}$ |
| *C.maxima* | CAE | 16.58 | 0.0013 | $5.416\pm0.0^{a}$ | $94.584\pm0.0^{a}$ |

The values in the table are the Mean±SD from triplicate determinations. Values with the same superscript are significantly the same along the rows and columns at $p\leq0.05$

## DISCUSSION

From the analyses of phytochemical composition, vitamin C concentration, free amino acid concentration of the Curcubits, it can be seen that the curcubit extracts are rich sources of phytochemicals , vitamin C and amino acids. Table 7 shows the potentiality of the extracts bbeing used as antisickling agents .They profoundly inhibited sickle cell hemoglobin polymerization to varying degrees from 60% for the BUS fraction of *T.occidetalis* to 97.083% for *C. lonatus* and *C. sativus* respectively. The presence of flavonoids in most medicines, drugs, vaccines and crude extracts has been attributed to their synergistic effect with other drugs [22]. The preponderance of vitamin C in all samples is to promote antisickling potecy of the samples since this vitamin has been found to be highly deficient in sickle cell disease patients and also a documented antisickling agent [23, 24].

Table 5 shows the free amino acid concentration of the samples .The values for *C. lonatus*, *C. sativus* , *C. maxima* are very high. This shows that these samples apart from their antisickling effectiveness, can also be of nutritional relevance in the management of sickle cell disease and other related syndromes. The following amino acids were identified by TLC and these included: Asp, Tyrosine, Phe, Arg, Asp [16, 25]. Equally important are some of the essential amino acids such as –His, Methionine , leucine , some of which their relevance in nutrition cannot be under-rated . Table 6 shows the *in vitro* effect of the extracts on the $Fe^{2+/}Fe^{3+}$ ratio, a measure of the oxygen affinity of the erythrocytes. Under hypoxic condition, this ratio decreases resulting in sickle cell crises. Pathologically, this can be used to monitor the prognosis of the treatment as well as the complications of the syndrome. It can be seen that the extracts remarkably improved the ratio with the following values: For *T.occidentalis* (167.46%); for *C. lonatus* (131.0%) and *C. sativus* (123.16%) respectively; *T. occidentalis* scored highest. This vegetable plant parts (seeds and leaves) have been regarded as blood builder. Table 7 shows the results of hemoglobin polymerization inhibition experiment. Results from the study show that the relative % polymerization for all fractions of the samples were able to inhibit HbSS polymerization to varying degrees. For example *C. sativus* exhibited a 97.08 % inhibition level followed by *C. lonatus* 97.08 % and the WAS fraction of *T. occidentalis* (95.00 %).

Astonishingly, *C.maxima* and the FAS extract of *T. occidentalis* equally exhibited profound antisickling effectiveness with values (94.58 % and 94.47 %) respectively. It might intrigue researchers and other scientists by the action of the FAS, which is non – polar and its diffusibility into the Hb molecule .This might be explained by the presence of many antisickling amino acids detected by TLC, such as Phenylalanine , Arginine , and others which must have acted synergistically to elicit such high potency reaction. The amino acid , Arginine has been reported by many researchers as an important antisickling amino acid acting on the synthesis of NO, a vasodilator, which relaxes smooth and endothelial cells[27] .The curcubits like edible legumes have elicited profound antisickling effectiveness. Nutritionally, they have proved to be rich in nutrients, including amino acids, minerals and vitamin C. Their actions may be likened to that of Ciklavit™, an antisickling nutrient, marketed in Nigeria, formulated from an edible legume , *Cajanus cajan*, Phe, Zn and minerals [28]. The antisicklig effect of antioxidants have been reported by many researchers [29]. These have been found to reduce the reductant effects of free radicals produced during sickling.These vegetables unlike other vegetables, provide high oxidative potentials, thus improving the oxygen affinity of the sickled erythrocytes [30]. Erythropoietin is such an important antioxidant that has been therapeutically employed in the management of sickle cell disease [31,32] . Its mechanism of action is to increase the plasma level of HbF( fetal hemoglobin), which inhibits fiber formation. Other medicines such as Tucaresol, has equally been used, as it also increases the oxygen affinity of the erythrocytes [33].

The vitamin C content of the curcubits is worth mentioning. Apart from the antioxidant role of the vitamin, it has been widely reported that it affects autonomic and cardiac response to change in posture when supplemented to sickle cell disease patients [34, 35]. These extracts have proved very effective in inhibiting sickle cell hemoglobin polymerization, improvement of $Fe^{2+/}Fe^{3+}$ ratio, reversing already sickled erythrocytes by increasing the oxygen affinity and like Ciklavit™, may normalize most excruciating complications of sickle cell disease in patients taking the nutrients. The nutritional approach to the management of sickle cell disease is novel and remains the current and the most promising approach in the management of sickle cell disease. There is no doubt that the Curcubits would nonetheless, prove worthwhile as frontline nutrients in the management of sickle cell disease

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