

## Full Length Research Paper

# The phytochemical, proximate and amino acid compositions of the extracts of two varieties of tiger nut (*Cyperus esculentus*) and their effects on sickle cell hemoglobin polymerization

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The phytochemical, proximate composition, amino acid profile and the effects of the extracts of two varieties of *Cyperus esculentus* (tiger nuts) on sickle cell blood were investigated. One hundred grams (100g) powdered samples of each fraction of the two varieties of the plant were used for batch extraction procedures to generate the crude aqueous extracts (CAEs) and the methanol water soluble fractions (MWS) used for hemoglobin polymerization and the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  determinations. The following nutrients and anti-nutrients were identified and quantified with their values expressed in (%) for the proximate composition and mg/100g for the anti-nutrients compositions. Four varieties of the nut were used for the work based on size and texture and these were designated as follows :- (large (L) and small (S) varieties; wet (W) and dry (D). The results of the proximate composition for the four (4) samples were as follows: ash  $1.80 \pm 0.1$ (W),  $2.68 \pm 0.2$  (D); moisture-  $42.80 \pm 0.2$ (W),  $32.16 \pm 0.2$ (D); crude fiber-  $18.0 \pm 0.1$ (W),  $21.36 \pm 0.1$ (D); crude lipid-  $14.10 \pm 0.0$ (W),  $19.67 \pm 0.2$ (D); crude protein-  $4.82 \pm 0.1$  (W),  $7.94 \pm 0.2$ (D) and carbohydrates-  $18.44 \pm 0.0$ (W),  $16.19 \pm 0.1$ (D). For the small seeded variety: ash-  $1.75 \pm 0.1$ (W),  $1.79 \pm 0.2$  (D); moisture-  $24.22 \pm 0.1$ (W),  $9.7 \pm 0.0$ (D); crude fiber-  $15.22 \pm 0.1$ (W),  $15.60 \pm 0.1$ (D); crude lipid-  $11.50 \pm 0.0$ (W),  $27.54 \pm 0.0$ (D); crude protein-  $3.63 \pm 0.1$ (W),  $3.94 \pm 0.1$ (D) and carbohydrates-  $16.39 \pm 0.1$ (W),  $15.60 \pm 0.0$ (D). The phytochemicals showed the trend: saponins-  $0.76 \pm 0.02$  (L) and  $0.65 \pm 0.01$  (S); cyanogenic glycosides-  $1.07 \pm 0.2$ (L) and  $0.85 \pm 0.2$ (S); phytate-  $2.35 \pm 0.2$  (L) and  $2.12 \pm 0.1$  (S); oxalates-  $0.50 \pm 0.2$ (L) and  $0.42 \pm 0.1$ (S). The total free amino acid concentration of the four samples was equally determined and the result expressed in mg/100g of sample. For the large variety (L)-  $169.6 \pm 0.0$ (W) and  $161.20 \pm 0.0$  (D); the small variety (S)-  $165.60 \pm 0.1$ (W) and  $160.40 \pm 0.1$  (D). There is no significant difference between the free amino acid concentrations of the two varieties and samples at  $p \leq 0.05$ . Hemoglobin polymerization inhibition assay showed the following result for the relative percent inhibition by the CAE and MWS fractions respectively of the large and small varieties. For the (L) variety, CAE (85.20%) and MWS (86.20); for the (S) variety- CAE (84.06%) and MWS (85.51%). When compared with the values of the standard antisickling agents- Phe (85.47) and ascorbic acid (84.89); there is no significant difference in the relative percent inhibition by these samples at  $p \leq 0.05$ . The amino acid profiles of the two varieties were the same and only varied in the relative concentrations of the wet and dry samples. Results showed the presence of the following major antisickling and essential amino acids at various concentrations expressed in mg/ 100 g. The antisickling amino acids included: Phe, Lys, Arg, Histidine, Serine, and Aspartate –  $2.37$ (W) and  $2.37$  (D);  $4.16$  (W) and  $4.78$  (D);  $18.29$ (W) and  $20.17$  (D);  $1.94$  (W) and  $2.32$  (D);  $2.27$  (W) and  $2.37$  (D);  $5.73$  (W) and  $6.86$  (D). Some essential amino acids identified included: valine ( $3.14$  (W) and  $2.50$  (D); methionine-  $0.73$  (W) and  $0.89$  (D); leucine -  $3.71$  (W) and  $4.01$  (D) and Isoleucine-  $2.07$  (W) and  $1.79$  (D). The CAEs and MWSs of the large and small varieties improved the oxidant status of sickle cell erythrocytes by increasing the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio remarkably. The fractions of the two varieties increased the ratio to varying degrees. For the L variety, the CAE and MWS values were: 72.19% and 41.96%. while for the small variety; 57.73% and 36.74%. It can be deduced that the two varieties of *Cyperus esculentus* studied are very nutritious and exhibited high antisickling effectiveness by inhibiting sickle cell hemoglobin gelation and improving the oxidant status of the erythrocytes. These extracts can be of immense nutritionally and therapeutic relevance in the management of nutritionally related syndromes like anemia, kwashiorkor and moreover sickle cell disease.

**Keywords:** Phytochemical composition, proximate values, amino acid profile, hemoglobin polymerization inhibition,  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio, *Cyperus esculentus*.

## INTRODUCTION

Tiger nut (*Cyperus esculentus*) belongs to the family *Cyperaceae* and the order, *Commelinales*. It is found worldwide in warm and temperate zones, occurring in Southern Europe and Africa. Tiger nut can be taken by diabetics for its content of sucrose and starch and its high content of arginine which stimulates the production of insulin (Belewu and Belewu, 2007). It can also be cooked, dried and powdered and may be used in confectionary to make biscuits with a delicious nut-like flavor. Mixing the ground tubers with cinnamon, sugar, vanilla and the cream, makes it a refreshing beverage. The roasted tubers are a substitute for coffee (Okafor et al., 2003). Tiger nuts (*Cyperus esculentus*) have long been recognized for their health benefits as they are rich in fiber, protein and natural sugars, minerals (phosphorus, potassium) and vitamins E and C (Belewu and Belewu, 2007). *Cyperus esculentus* is a popular plant seed in Nigeria and known by different names by different ethnic groups. In Hausa, it is known as “aya”, in Yoruba “ofio” and in Ibo “aki-Hausa”. It grows well in the middle belt of Nigeria (Okafor et al., 2003), where three varieties are cultivated. These varieties include; the black, brown and yellow that has two varieties, the large and small. The yellow is larger in size, more attractive in color and has fleshier body (Belewu and Belewu, 2007; Umerie et al., 1997; Belewu and Abodurin, 2006). It is believed that they help prevent thrombosis/cancer and activates blood circulation. They are equally thought to be beneficial to diabetics and in cholesterol lowering activities and as such implicated in the reduction of colon cancer (Burr et al., 2004). The nuts were found to be ideal for children, the elderly and for sportsmen and women (Martinez, 2003). The inclusion of 33.3% tiger nut in the diet of cockerel starter (marsh) was reported (Bamgbose et al., 2003). The extract from tiger nut is a product of plant origin with high nutritional and health properties which can be used as milk substitute (Nwokolo, 1985). Tiger nuts are regarded as digestive, tonic, having heating and drying effects on the digestive system and alleviates flatulence. The tubers are said to be aphrodisiac, carminative, diuretic, emmanagogue and stimulating. In Ayurvedic medicine, they are used for the treatment of flatulence, indigestion, colic, diarrhea, dysentery, debility and excessive thirst (Ghanson, 2008; Agoha, 2003). The tubers contain up to 30% of non-drying oil which is used for cooking and for making soap.

Sickle cell disease is an inherited disorder of the  $\beta$ -globin chain of human erythrocytes, characterized by life long anemia and recurrent painful episodes. It is a potentially lethal disease with several clinical manifestations. The HbS syndrome results from the substitution of a hydrophilic amino acid, glutamic acid by a hydrophobic moiety, valine at the sixth position of the  $\beta$ -globin chain of hemoglobin. The highest prevalence of the hemoglobinopathy is found in black Africa with sickle

cell trait, HbAS, HbSC and HbSS predominating. It is also found in some Mediterranean countries such as Greece, Italy, Israel, as well as Saudi Arabia and India (Cotram et al., 1999). The relationship between sickle cell disease and nutrition has been reviewed (Cotram et al., 1999; Charache, 1981; Reed and Ortiger, 1987). However, sickle cell disease remains one of the major chronic diseases in which the role of nutrition in its etiology has not been systematically addressed. Several investigators have commented on the abnormally low level of certain micronutrients in sickle cell blood (Ekeke, 2001) and some dietary constituents such as thiocyanate, phenylalanine and ascorbic acid as being definitely beneficial in ameliorating the symptoms (Agbai, 1986; Maenthalsong et al., 2007; Uzoegwu, 1996). This work focuses on the possible beneficial effects of the extracts of two varieties of tiger nut based on their dietary constituents, phytochemicals and antisickling potentials of their extracts in inhibiting hemoglobin polymerization and improving the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio. These extracts would nonetheless ameliorate some of the hematological and vascular complications of the syndrome on sickle cell disease patients and other patients afflicted with similar syndromes.

## MATERIALS AND METHODS

### Materials

Four hundred grams (400g) each of the two varieties of *Cyperus esculentus* (Large (L) and Small (S) of the yellow species) were bought from a local market in Owerri metropolis, capital city of Imo State, Nigeria. The samples were authenticated by a crop scientist at the Department of Crop Science and Technology of the School of Agriculture, Federal University of Technology, Owerri, as being the best variety.

### Design of the experiment

Two varieties of the best (yellow) species of *Cyperus esculentus* were bought for the work. The two varieties selected included the large and small sized varieties of the yellow species. Each of these varieties was prepared to get two additional samples- the wet and dry fractions or samples. Equal weights (100 g) of each powdered sample was used to generate the crude aqueous extracts (CAE) and the methanol water soluble (MWS) for the polymerization inhibition experiment and the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio determinations respectively. These four samples were used for the determinations of the total free amino acid concentrations and proximate composition.

## METHODS

### Extraction of *Cyperus esculentus*

One hundred and fifty grams (150 g) of each of the two varieties

were taken, dried in an oven at 60 °C for 48 hours. One hundred grams (100 g) from each dried sample were powdered into flour. One hundred grams (100 g) of fresh samples of the two varieties were homogenized and all samples used for batch extraction procedures to generate the crude aqueous extract (CAEs) and the methanol water soluble (MWS)

#### **Extraction of the crude aqueous extracts (CAEs) and the methanol water soluble (MWSs) fraction**

The methanol water soluble (MWS) fraction was obtained by soaking 100 g of each sample with 200 ml of 60% methanol of analytical grade in a 300 ml corked volumetric flask, for the wet samples of the large (L) and the small (S) varieties. The crude aqueous extracts (CAEs) were generated by soaking 100 g of the powdered dried samples of both the large (L) and small (S) varieties with 200 ml distilled water containing 0.9% NaCl (normal saline) for 48 hours in a refrigerator. The decoctions were filtered using Whatman filter paper No.1 and the filtrates concentrated using rotor evaporator. The extracts were standardized against L-phenylalanine to a concentration of 100 µM phenylalanine equivalence of the extracts and used for various assays.

#### **Determination of proximate composition of the wet (W) and dry (D) samples**

The recommended methods of the Association of Official Analytical Chemists (AOAC, 1990) were used for the determination of moisture, crude fiber, crude protein (NX 6.25), crude lipid, ash and carbohydrates respectively. Crude protein was determined by the Macro-Kjeldal method using 1.0 g samples. Ash was determined by the incineration of 1.0 g sample of each variety in a muffle furnace maintained at 550 °C for 6 hrs (until ash was obtained). Crude lipid (ether extract) was determined by exhaustively extracting 5.0 g of the sample with petroleum ether by use of Soxhlet apparatus. The level of carbohydrate was obtained by the difference method by subtracting the sum of the protein, lipid and ash from the total dry matter. The calorific value was calculated by multiplying the mean values of the crude protein, fat and carbohydrates by Atwater factors of 4, 9 and 4 respectively, taking the sum of the products and expressing the results in kilocalorie (kcal) (Edem et al., 1990).

#### **Determination of total free amino acid concentration of the samples.**

The free amino acid concentrations of the extracts were determined with ninhydrin reagent using phenylalanine as standard and reading the developed color at 570 nm and extrapolating the values from a standard curve of phenylalanine. Ninhydrin in acetone (0.1%) was diluted with distilled water in the ratio 1:4; the MWS and CAE 1:1 with distilled water.

Exactly, 20µL each of the diluted extracts was added to 4 ml portions of the diluted ninhydrin. The resulting solutions were heated to boiling for 5 minutes, cooled and the absorbance read in a spectrophotometer at 570 nm using distilled water as blank (Edem et al., 1990; Lowry et al., 1951)

#### **Determination of the major amino acid constituents of the samples**

This was carried out with the aid of the Technicon Sequential Multisample Amino acid Analyzer (TSM) (Yemm and Cocking, 1954). Technicon Instruments Corporation, Dublin-Ireland at the Post graduate Laboratory, Zoology Unit, University of Jos, Nigeria.

#### **Preparation of blood samples**

Blood samples were collected from confirmed HbSS (homozygous) sickle cell disease patients who attend sickle cell disease clinic at the Federal Medical Center, Owerri, Nigeria. Permission for the use of blood was granted by the bio-ethics committee of the hospital. Portions of HbSS blood were collected into citrate tubes. Erythrocytes were isolated from the blood samples by centrifugation at a gravitational force of 1500x g for 15 minutes using the bench centrifuge (Nickel-Electro 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%NaCl) equivalent to the siphoned plasma. The erythrocyte suspension was freeze thawed at 0°C to produce a hemolyzate for the hemoglobin polymerization experiment.

#### **Sickle cell hemoglobin polymerization experiment**

HbSS polymerization was assessed by the turbidity of the polymerizing mixture at 700 nm using 2% Sodium metabisulphite as reductant or deoxygenating agent (Iwu et al., 1988). 4.4 ml of 2% solution of sodium metabisulphite ( $\text{Na}_2\text{S}_2\text{O}_3$ ), 0.5 ml normal saline and 0.1ml of antisickling agent were pipetted into a cuvette shaken and the absorbance read at 700 nm every two minutes for 30 minutes. This represented the control. Distilled water was used as blank in all assays. In the main assay, 4.4 ml of 2% solution of sodium metabisulphite, 0.5 ml of extract and 0.1 ml of hemoglobin solution (HbSS) were pipetted into a cuvette and the optical density reading taken as above. The rates of hemoglobin polymerization were calculated from the formula of average change in optical density/absorbance against time in minutes (Nwaoguikpe, 1999).

$$Rp = \frac{OD_t - OD_i}{t}$$

Rp = rate of polymerization,  $OD_t$  = final absorbance /optical density at time, t

t = time of assay in minutes.

$$Rp = \frac{\Delta OD}{t}$$

$OD_i$  = initial absorbance/ optical density at zero time

Where,

#### **Determination of $\text{Fe}^{2+}$ / $\text{Fe}^{3+}$ ratio.**

The determination of the  $\text{Fe}^{2+}$ /  $\text{Fe}^{3+}$  ratio was carried out by the methods [26, 27].

#### **Statistical Analysis**

Two statistical methods were used in the analyses of results and these include: (a) Mean and Standard deviation (b) One way ANOVA

## **RESULTS**

The results of all analyses and determinations are shown in tables 1-6 respectively.

**Table 1.** Phytochemical composition of the large (L) and small (S) varieties of *Cyperus esculentus* expressed in percent (%)

Sample	oxalate	phytate	saponin	tannins	cyanogenic glycozides
<i>Cyperus esculentus</i> (L)	0.50±0.2	2.35±0.2	0.76±0.2	9.50±0.2	1.07±0.2
<i>Cyperus esculentus</i> (S)	0.42±0.1	2.12±0.1	0.65±0.1	7.22±0.1	0.85±0.2

Values in the table are the Mean± SD from triplicate determinations. There is no statistical difference in the values of the same phytochemicals present in the two varieties of the nut studied at  $p \leq 0.05$

**Table 2.** Proximate composition of both large and small; wet and dry samples of *Cyperus esculentus*

Samples	Fraction	Ash	Moisture	Crude fiber	Crude lipid	Crude protein	CHO	Energy(Kcal/100mg)
<i>Cyperus esculentus</i>	(L) wet	1.80±0.1	42.80±0.2	18.0±0.1	14.10±0.0	4.82±0.1	18.44±0.2	213.9
<i>Cyperus esculentus</i>	(L) dry	2.68±0.2	32.16±0.2	21.36±0.0	19.67±0.1	7.94±0.1	16.19±0.2	317.61
<i>Cyperus esculentus</i>	(S) wet	1.75±0.1	24.22±0.1	15.27±0.1	11.50±0.0	3.65±0.2	16.39±0.1	183.50
<i>Cyperus esculentus</i>	(S) dry	1.79 ±0.0	9.7±0.1	15.60±0.0	27.54±0.0	3.94±0.1	15.60±0.0	326.02

The results in the table are the Mean ± SD from triplicate determinations. There is significant difference in the proximate compositions of the large and small varieties of *Cyperus esculentus*. Values with the same superscript are significantly different along the columns and rows at  $p \leq 0.05$

**Table 3.** Total free amino acid concentration of the different samples of two varieties: Large (L) and small (S) expressed in mg/100g sample.

Samples	fractions	Vol. of extract (ml)	Amino acid concentration (mg/ml)	Total free amino acid ( mg/100g)
<i>Cyperus esculentus</i> (L)	Wet	80.0	<sup>a</sup> 2.13±0.2	<sup>a</sup> 169.60±0.1
<i>Cyperus esculentus</i> (L)	Dry	72.0	<sup>a</sup> 2.38±0.0	<sup>a</sup> 165.60±0.1
<i>Cyperus esculentus</i> (S)	Wet	65.0	<sup>a</sup> 2.48±0.1	<sup>a</sup> 161.20±0.1
<i>Cyperus esculentus</i> (S)	Dry	60.0	<sup>a</sup> 2.20±0.0	<sup>a</sup> 160.49±0.1

The values in the table are the Mean ± SD from triplicate (n=3) determinations. The values with the same superscript are significantly the same along the columns and rows at  $p \leq 0.05$ . There is no statistical difference in the total free amino acid concentrations of the samples and varieties assayed. The values with the same superscript are significantly the same along the rows and columns.

**Table 4.** The rates of polymerization, the relative percent polymerization and the relative percent inhibition of HbSS by different fractions (CAE and MWS) of the large (L) and the small (S) varieties of *Cyperus esculentus* at a final assay concentration of 100 µM phenylalanine concentration of the extracts.

Sample	fraction	final assay Conc. µM	rate of polymerization	relative % polymerization	relative % inhibition
L- Phenylalanine	-----	100	0.0175±0.1	14.49±0.1	85.51±0.1
Ascorbic acid	-----	100	0.0183 ±0.0	15.15 ±0.2	84.85±0.1.
<i>Cyperus esculentus</i> (L)	CAE	100	0.0180±0.0	14.90±0.0	85.10±0.0
<i>Cyperus esculentus</i> (L)	MWS	100	0.0167±0.1	13.02±0.1	86.98±0.2
<i>Cyperus esculentus</i> (S)	CAE	100	0.0193±0.2	15.98±0.2	84.02±0.1
<i>Cyperus esculentus</i> (S)	MWS	100	0.0175±0.1	14.49±0.1	85.51±0.1

The values in the table are the Mean ± SD from triplicate (n=3) determinations. There is no significant difference in the relative percent inhibitions of HbSS polymerization by the extracts of the samples as antisickling agents at  $p \leq 0.05$ ; although the MWS fractions of both varieties inhibited the polymerization process better than the CAE fractions

**Table 5.** Amino acid compositions of the wet and dry samples of the large (L) sized variety of *Cyperus esculentus* showing results from the multi-sample amino acid analyzer, values expressed in mg/100 g of sample.

Amino acids identified	wet (mg)	dry (mg)	Amino acids identified	wet (mg)	dry (mg)
Lysine	4.16	4.78	Proline	2.02	2.34
Histidine	1.94	2.32	Glycine	4.13	3.19
Arginine	18.29	20.17	Alanine	3.17	3.55
Aspartate	5.73	6.86	Cysteine	2.05	2.32
Threonine	2.77	3.00	Valine	3.14	2.50
Serine	2.27	2.37	Methionine	0.73	0.89
Glutamic acid	7.25	7.98	Leucine	3.71	4.01
Isoleucine	2.07	1.79	Tyrosine	0.81	0.96
Phenylalanine	2.37	2.37	Norleucine	---	---

The data in the table were generated from the technicon sequential multisample (TSM- 1) amino acid analyzer. The high level of some antisickling amino acids like arginine, tyrosine, histidine, phenylalanine, lysine, serine and others shows the high nutritious and anti-gelation effects respectively.

**Table 6.** The effect of the CAE and MWS fractions of the large (L) and small (S) varieties of *Cyperus esculentus* on the  $Fe^{2+}/Fe^{3+}$  ratio of HbSS blood at 13 $\mu$ M phenylalanine equivalence of the extracts

Sample	fraction	% Hb	%m Hb	$Fe^{2+}/Fe^{3+}$	% increase in ratio
Control (HbSS) blood	----	92.56 $\pm$ 0.2	7.44 $\pm$ 0.1	<sup>b</sup> 12.44 $\pm$ 0.1	<sup>b</sup> 0.00 $\pm$ 0.0
L-Phenylalanine	----	96.42 $\pm$ 0.1	3.58 $\pm$ 0.1	<sup>a</sup> 26.93 $\pm$ 0.2	<sup>a</sup> 116.0 $\pm$ 0.0
Ascorbic acid	-----	95.50 $\pm$ 0.1	5.0 $\pm$ 0.1	<sup>b</sup> 17.18 $\pm$ 0.1	<sup>b</sup> 38.60 $\pm$ 0.1
<i>Cyperus esculentus</i> (L)	CAE	95.54 $\pm$ 0.1	4.46 $\pm$ 0.1	<sup>a</sup> 21.42 $\pm$ 0.1	<sup>a</sup> 72.19 $\pm$ 0.1
<i>Cyperus esculentus</i> (L)	MWS	94.64 $\pm$ 0.2	5.36 $\pm$ 0.2	<sup>b</sup> 17.65 $\pm$ 0.1	<sup>b</sup> 41.96 $\pm$ 0.2
<i>Cyperus esculentus</i> (S)	CAE	95.00 $\pm$ 0.1	5.00 $\pm$ 0.2	<sup>a</sup> 19.00 $\pm$ 0.2	<sup>a</sup> 57.73 $\pm$ 0.1
<i>Cyperus esculentus</i> (S)	MWS	94.45 $\pm$ 0.1	5.55 $\pm$ 0.1	<sup>b</sup> 15.00 $\pm$ 0.1	<sup>b</sup> 36.74 $\pm$ 0.1

The values in the table are the Mean $\pm$  SD from triplicate determinations. The values with the same superscript are statistically related along the columns and the rows at  $p \leq 0.05$ .

Table 1 shows the phytochemical / antinutrient composition of two varieties: Large (L) and Small (S). Values are recorded in mg/100 g of substance. Table 2 shows the proximate composition of the wet and dry samples of the two varieties of *Cyperus esculentus*. Table 3 depicts the total free amino acid concentration of the wet and dry samples of the large and small varieties with values expressed in mg/100 g of sample. Table 4 shows the rate of polymerization, the relative percent polymerization and the relative percent (%) inhibition at 100 $\mu$ M Phenylalanine equivalence. Table 5 shows the amino acid profile of the wet (W) and dry (D) samples of the large variety of *Cyperus esculentus*. Table 6 shows the effect of the CAE and MWS extracts of the large and small varieties on the  $Fe^{2+}/Fe^{3+}$  ratio showing relative (%) increase in the ratio.

## DISCUSSION

Results from various assays showed the presence of high levels of protein, lipid, crude fiber and carbohydrates in the samples, all point to the nutritious nature and the

energy value of this all important tuber/ nut, highly consumed in Nigeria and other countries of the world. This finding is shown in table 2. The proximate compositions showed a higher protein, crude fiber, lipid in the dried samples of both the large and smaller species. Contrarily, the moisture and carbohydrate contents were higher in the wet varieties than in the dry varieties. This can be ascribed to the effect of heat in concentrating the samples by the reduction of the moisture content. Hence, the values obtained for these entities – protein for the large and dried compared with the wet (7.94 mg (D) and 4.82 (W); crude lipid (19.67 and 14.1); crude fiber (21.36 and 18.0); for the small sized variety-crude protein (3.94 and 3.65); crude fiber-(15.60 and 15.27); crude lipid (27.54 and 11.50). For the carbohydrates of large species, the results for the wet and dry varieties-(18.44 (W) and 16.19 (D); for the small varieties, the results for the wet and dry samples are samples (16.39 and 15.60). The moisture content of the two varieties decreased appreciably in both the large and small varieties. The decrease in the carbohydrate content may be attributed to the lability of the sugars in the sample which are easily destroyed. The ash content

was equally increased after ashing. The results of total free amino acid concentration ranged from 160.45 mg/100 g for the dried small seeded variety to 169.60 mg/100 g for the wet large seeded variety. There is no significant difference in the total free amino acid concentration of the varieties and samples. This finding points to genetic descent of the two varieties with homologous protein translational machinery. In table 4, the relative % polymerization and the relative % inhibition are displayed showing the effects of the CAE and MWS fractions of both the large and small seeded varieties of *Cyperus esculentus*. The MWS fractions of both varieties were more efficacious in inhibiting sickle cell hemoglobin polymerization than the CAE fractions. This may be due to the presence of liposoluble metabolites in addition to other water soluble compounds like sugars, water soluble vitamins and minerals (Monago and Uwakwe, 2009). Moreover, the MWS fractions also appeared to be more concentrated than the CAE fractions because of the higher volatility of methanol compared to aqueous extract. Statistically, there is no significant difference between the relative percent inhibitions of both fractions at the concentrations used in the analysis.

The amino acid content of the varieties were the same but the only observed variation was in the individual amino acid concentrations between the wet and dry samples of the large variety, except phenylalanine, a known antisickling agent, having the same concentration of 2.37 mg/100 g in both the wet and dry samples of the large variety (Ekeke and Shode, 1990). The amino acid profile shows a preponderance of both essential and non-essential ones including the antisickling amino acids. This finding is similar to what some workers observed in some beans species and Bambara groundnuts (Nwaoguikpe, 2008). A known antisickling amino acid, arginine was found at very high concentrations in both the wet (18.29 mg/100 g) and dry samples (20.17 mg/100 g) of the large variety. The effect of the extracts (CAE and MWS) of the large and small varieties on the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio of sickle cell blood was assayed. The CAE fractions of both the large and small varieties were more effective in the improvement of the ratio than the MWS fractions of the varieties. This may be accounted for by the solubility of the CAE fraction and its diffusibility into the hemoglobin molecule. Moreover, most antioxidants like vitamin C, flavonoids and even antioxidant metals like zinc, copper and enzymes may be found in this soluble fraction. Most of the basic amino acids such as arginine, lysine, serine, including carbohydrates such as glucose, may be localized in this fraction and these may have acted synergistically to elicit such pronounced antisickling response. The values for the relative percent increase in the ratio were as follows: for the CAEs, the trend is from 57.0 % for the small variety to 72.19 % for the large seeded one. The MWS values were 36.74% for the small variety to 41.96% for the large seeded one. Statistically, there is pronounced

difference in the relative improvement in the ratio at  $p \leq 0.05$ . In comparing the effects of the CAE and MWS fractions on hemoglobin polymerization inhibition and the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio; it would appear that the converse is the case in each of these situations. Although, other workers have not reported extensively on the effects of these samples on the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio; the result here agrees with the findings of Monago and Uwakwe (Monago and Uwakwe, 2009). Many workers have reported the role of soluble fibers in this nut to increase the transit time through the gut, slowing the emptying of stomach and equally reducing the absorption of glucose (Nwaoguikpe and Uwakwe, 2005). *Cyperus esculentus* species have high fiber content and this is consistent with the findings as reported by some researchers (Swaminathan, 2002). It has equally been reported that the extracts of this nut can lower blood glucose levels and unequivocally finds application in the management of diabetes mellitus (Umerie and Enebeli, 1997). Many workers have implicated the high level of arginine in the nut, which invariably has been ascribed the role of plant insulin. Based on the functionality of its diverse natural constituents, the presence of nutrients, phytochemicals, amino acids and moreover, their pronounced antisickling potency in inhibiting sickle cell hemoglobin polymerization, the improvement in the oxidant status of sickle erythrocytes (improving the oxygen affinity); the extracts of these varieties of *Cyperus esculentus*, would nonetheless, proffer profound nutritional and antisickling effectiveness for the effective treatment and management of anemia, kwashiorkor, thalassemia, sickle cell disease (SCD) and its complications.

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