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The Antisickling Properties of *Aristeus Antennatus* Extracts in the Management of Sickle Cell Disease

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Abstract

The antisickling effect of dried *Aristeus antennatus* was investigated to ascertain the efficacy of the extract of the sample to inhibit polymerization of sickle cell haemoglobin (HbSS), and improve the F_e^{2+}/F_e^{3+} ratio of sickle cell blood. The sample was first dried, ground into powder and soaked in chloroform to defat it in order to produce the fat soluble fraction (filtrate). The defatted residue was air-dried and later soaked in methanol for 24 hours to obtain the methanol soluble fraction. The fat soluble fractions (FAS) and methanol soluble fractions (MAS) were able to inhibit HbSS polymerization to varying degrees from 58% for FAS and 69% for MAS. Phytochemical analysis of the sample revealed the presence of proanthocyanin, quinine, saponin, isoflavone, spartein, phytate, cyanogenic glycoside, catechin, flavone, tannin, oxalate, alkaloids and others, at varying concentrations. Amino acid composition was determined for the sample using the amino acid analyzer (PTH), which revealed a preponderance of both essential and non-essential amino acids of which some were antisickling amino acids at varying concentrations. The identified amino acids include: Phenylalanine, Histidine, Leucine, Lysine, Tryptophan, Isoleucine, Valine, Methionine, Proline, Arginine, Tyrosine, Serine and others. However, the FAS and MAS fractions of the sample *Aristeus antennatus* extract improved F_e^{2+}/F_e^{3+} ratio significantly. *Aristeus antennatus* extracts with the preponderance of micro and macro nutrients, vitamins, antioxidants, amino acids and others, may be very beneficial for the management of sickle cell disease.
Keywords: Sickle cell disease; *Aristeus antennatus*; hemoglobin polymerization, F_e^{2+}/F_e^{3+} ratio.

1. Introduction

Sickle cell disease, also known as sickle cell anemia, is a genetic blood disorder resulting from a point mutation in the beta-globin chain, leading to the replacement of the amino acid glutamic acid, a hydrophilic moiety by valine, a hydrophobic moiety, at the sixth position of the beta-chain of hemoglobin [1]. Sickle cell disease refer to a large group of hemoglobinopathies, in which at least one sickle cell gene of the beta-globin chain is inherited together with an abnormal gene. It is a severely malignant disorder associated with protean clinical manifestations and decreased life expectancy. The loss of charge on the glutamic acid and its substitution for valine, resulted in an abnormal hemoglobin molecule with severe hematological consequences [2, 3]. Sickle cell haemoglobin (HbS) is a structural variant of normal adult haemoglobin (HbA) [4]. SCD affects 20–25 million people globally, and 50–80% of infants born with SCD in Africa die before the age of 5 years [5]. It is estimated that 240,000 children are born

with SCD annually in sub-Saharan Africa [6]. The United Nations General Assembly has recognized SCD as a global public health concern due to high morbidity and mortality rate caused by the disease and the significant social and economic impact that results (United Nations General Assembly, 2009).

Hemoglobin is an oxygen-binding protein found in erythrocytes which transports oxygen from the lungs to tissues. Each hemoglobin molecule is a tetramer made of four polypeptide globin chains (α2β2). Each globin subunit contains a heme moiety formed from an organic protoporphyrin ring and a central iron ion in the ferrous state (Fe^{2+}). The iron molecule in each heme moiety can bind and unbind oxygen, allowing for oxygen transport in the body. The most common type of hemoglobin in the adult is HbA, which comprises two alpha-globin and two beta-globin subunits. Different globin genes encode each type of globin subunit [7, 8].

2. Materials and Methods

2.1. Materials and Reagents

Weighing balance, test tubes, centrifuge, separating funnel, water bath, BUCK M910 Gas chromatography, rotary evaporator, Applied Biosystem phenylthiohydantoin (PTH) amino acid analyzer (model 120A), Whatman filter paper (No1), Kjelcihal digestion flask, volumetric flask, Markham distillation apparatus, Bunsen burner, Oven, plastic specimen bottles, Extraction thimble, Anti-bumping granules, Pasteur pipette.

Hexane, buthanol, chloroform, methanol, ethanol, sodium sulphate, concentrated sulphuric acid, copper sulphate, selenium oxide, distilled water, sodium hydroxide, boric acid, bromocresol green/methyl red, hydrochloric acid, acetate buffer (pH 2.0), sodium chloride. All reagents used in the study were of standard analytical grade.

2.2. Sampling and Sample Preparation

Sample collection is an essential process for good laboratory practice. The sample container was washed thoroughly with detergent, rinsed with water and then with distilled water and air-dried. The air-dried plastic containers were covered with air tight covers in order to avoid contamination of the samples. Aristeus antennatus (Shrimps), were sourced from local market in Obalende market in Lagos, and Relief market and Ekeonunwan market in Imo State respectively.

Buthanol, chloroform, methanol, ethanol, and hydrochloric acid, were sourced from Finlab Chemicals Limited in Owerri, Imo State. Concentrated sulphuric acid, copper sulphate, selenium oxide, sodium hydroxide, boric acid, and methyl red, were sourced from the department of pharmacognosy, University of Lagos, Lagos State. Sodium matabisulphite was sourced from Chemi-Science in Owerri, Imo State.

100g of dried Aristeus antennatus, were ground to powder using a grinder, and transferred into a sample container and labelled.

2.3. Collection and Preparation of Blood Samples

Blood samples were collected from confirmed HbSS patients who attend clinic at Federal Medical Centre, Owerri by a personnel of the Haematology unit. The patients willingly consented to the exercise after having explained to them the relevance of the research project to their health. The blood sample was collected in an ethylenediaminetetraacetic acid (EDTA) bottle to prevent the blood from coagulation.

Portions (0.20ml) of the whole blood samples were used for the Fe^{2+}/Fe^{3+} ratio, while the remaining portions were collected into EDTA anticoagulant tubes. Erythrocytes were isolated from the blood samples by centrifugation at 10,000rpm for 20minutes using 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% NaCl) equivalent to the siphoned plasma. The erythrocyte suspension was then frozen at 0oC, for 3 days and subsequently thawed to produce a haemolysate for the hemoglobin polymerization experiment.

2.4. Extraction of Fat-Soluble Fractions (FAS)

The amount of 100g each of the grinded samples of Aristeus antennatus was soaked in 200ml of chloroform for twenty-four (24) hours to defat the sample in order to generate the fat-soluble fraction by filtration. The residue was kept for methanol extraction while the filtrate was subsequently evaporated en vacuo and the resulting fat-soluble (FAS) extract was weighed and volume recorded.

2.5. Methanol Extraction Process

The residue from the chloroform extraction was air dried and soaked in 200ml of methanol (MeOH) of analytical grade for 24 hours. The solvent for the sample was filtered and the filtrate subjected to evaporation en vacuo, using rotor evaporator. The weight and volume of the methanol extract were recorded.

2.6. Determination of Amino Acid Profile and Nitrogen

The amino acid profile in the known sample was determined using methods described by Benitez, (1989). The known sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Applied Biosystem PTH Amino Acid Analyser.

A small amount (150mg) of the ground sample was weighed, wrapped in Whatman filter paper (No 1), and put in the Kjelcihal digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$), and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added. The flask was then put in

kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected. The distillate was then titrated with standardized 0.01N Hydrochloric acid to a grey coloured end point. The percentage Nitrogen in the original sample was calculated using the formula:

% Nitrogen = (a-b) x 0.01 x 14 x V x 100/ W x C

Where; a = Titre value of the digested sample, b = Titre value of the blank sample, v = volume after dilution (100ml), w = weight of dried sample (mg) of Aristeus antennatus, c = aliquot of the sample used (10ml), 14 = nitrogen constant in mg.

2.7. Determination of Free Amino Acid Concentration of the Extracts

0.1% Ninhydrin in acetone was diluted with distilled water in the ratio 1:4. The water-soluble (MAS) extract was diluted in the ratio of 1:1 with water and the FAS extract was diluted 1: 5 ratio with ethanol. Exactly 20µl each of the diluted extracts was added to 4ml portion of the diluted Ninhydrin. The resultant solutions were heated to boiling for five (5) minutes, cooled and the absorbance read in a spectrophotometer (Spectronic 20DR) at 570nm using distilled water as blank. The values were extrapolated from a standard curve obtained by treating 20µl portions of different concentrations (1-20mg/ml) of phenylalanine with 4ml portions of diluted Ninhydrin, and boiling for five minutes, the absorbance taken as above and a plot of concentration made against the absorbance.

2.8. Determination of the Fe2+/Fe3+ Ratio

The determination of the Fe2+/Fe3+ was carried out by the method described by Davidson, et al., 1974, while oxygen affinity of hemoglobin and met-hemoglobin were measured at 540nm and 630nm respectively. The approach employs lysing 0.02ml whole blood in 5.0ml of distilled water and 0.02ml normal saline. The absorbance of hemoglobin (Hb) and methemoglobin (mHb) were measured at 540nm and 630nm to determine the % Hb and % mHb respectively. This represents the control. In determining the effect of the extract on Fe2+/Fe3+ ratio; 0.02ml of each extract was added to 5.0ml of distilled water and 0.02ml of blood added and incubated for 60 minutes in a test tube.

2.9. Sickle Cell Hemoglobin Polymerization Inhibition Experiment

Sickle cell hemoglobin polymerization inhibition experiment was carried out by the original method of [9, 10] and Nwaoguikpe, *et al.* [11] for HbSS polymerization experiment. This method is used to assess the sickle cell hemoglobin polymerization, by the turbidity of the polymerizing mixture at 700nm, using 2% solution of sodium matabisulphite as reductant or deoxygenating agent [9].

4.4ml of 2% sodium matabisulphite (Na2S2O3), 0.5ml normal saline (0.9% NaCl), and 0.1ml hemoglobin were pipetted into a cuvette, shaken and a spectrophotometer (Unicam Spectronic 20-DR) was used to read the absorbance at 700nm, every 2 minutes for 30minutes. This was used as the control, and distilled water used as blank for all assays. For the test assay, 4.4ml of 2% sodium matabisulphite, 0.5ml of the extract Aristeus antennatus, and 0.1ml hemoglobin (HbSS) solution were pipetted into the cuvette, inserted into the spectrophotometer, readings taken at absorbance of 700nm every 2 minutes for 30 minutes. The rate of hemoglobin polymerization for the control, extracts or fractions were estimated by calculating the tangent of a plot of average change in extinction or change in optical density (ΔOD700nm) versus time in minutes. The rates were equally expressed as percentages with respect to control.

The rate of polymerization can be calculated from the formula,

Rp = Final OD – Initial OD/ Time.

i.e. $Rp = \frac{ODF - ODI}{T}$

Where, Rp = rate of polymerization, ODF = final optical density, ODI = initial optical density and T= time of assay in minutes.

2.10. Determination of Vitamin A

Determination of vitamin A was carried out by the method of [12]. 2g of the sample was weighed into a flat bottomed reflux flask. 10ml of distilled water was added, shake carefully to form a paste. 25ml of alcoholic potassium hydroxide (KOH) solution was added and a reflux condenser attached. The above mixture was heated in boiling water bath for 1 hour with frequent shaking. The mixture was cooled rapidly and 30ml of water was added. The hydrolysate obtained was transferred into a separating funnel. The solution was then extracted three times with 250ml of chloroform. 2g of anhydrous sodium sulphate (Na2SO4) was added to the extract to remove any traces of water. The mixture was then filtered into 100ml volumetric flask and made up to the mark with chloroform. Standard solution of β-carotene, vitamin A of range 0 to 50µg/ml with chloroform made by dissolving 0.003g of standard β-carotene in 100ml of chloroform. The above gradient of different standard solution prepared were determined with reference to their absorbance from which average gradient was taken to calculate vitamin A (β-carotene in µg/100g). Absorbance of the standard was read in the spectrophotometer (Metrolin Spectronic 21D model) at a wavelength of 328nm.

Calculation

Vitamin A (µg/100g) = Absorbance of sample x DF/ weight of sample
Where DF = Dilution factor

2.11. Determination of Vitamin C

The determination of the ascorbic acid (vitamin C) concentration of the extract was carried out by the methods of Lambert and Muir, (1974).

Ascorbic acid standard was prepared containing 1g/dm3 of the ascorbic acid (vitamin C), so that 1cm3/mg vitamin C equals 1g/dm3. A burette was filled with a solution of 2,6-dichlorophenol indophenol of 0.01%. Two or three drops of dilute hydrochloric acid (HCl), was used to acidify 10cm3 of the ascorbic acid. The indophenol solution was run into the ascorbic acid solution until there is a permanent pink solution. If Xcm3 of the indophenol are required, 1cm3 of indophenol solution is equivalent to 10mg/x vitamin C. Having standardized the indophenol solution, 10cm3 of the test solution (extract) was taken and treated in a similar way.

2.12. Determination of Vitamin E

Vitamin E was determined as described earlier by Amadi, *et al.* [13]. 1g of the sample was weighed into a conical flask with reflux condenser. 10ml of absolute alcohol and 20ml of 1M alcoholic sulphuric acid was added. The condenser and conical flask were wrapped in aluminium foil and refluxed for 45 minutes. After cooling, 50ml of distilled water was added to the mixture and transferred to a separating funnel with 50ml water. The unsaponifiable matter was extracted with 30ml diethyl ether. The combined ether extract was washed free from acid and was dried over anhydrous sodium tetraoxosulphate (VI) acid. The residue obtained was immediately dissolved in 10ml absolute alcohol. Aliquots of solutions of the sample and standards (0.3-3.0mg vitamin E) transferred into 20ml volumetric flasks; 5ml alcohol was added, followed by 1ml HNO3 concentrated trioxo-nitrate (V) acid. The flasks were placed on a water bath at 90oC for 3 minutes from the time the alcohol begins to boil. It was cooled rapidly under running water and adjusted to volume with absolute alcohol. The absorbance was read at 470nm on a metrohm spectronic 21D spectrophotometer, against a blank containing 5ml absolute alcohol and 1ml concentrated HNO3 treated in a similar manner.

$$\text{Vitamin E (µg/100g)} = \frac{\text{Absorbance of sample x Gradient factor x Dil. Factor}}{\text{Weight of sample}}$$

2.13. Determination of Carotenoids and Lycopene

Total carotenoids and lycopene were estimated by the method described by Zakaria, *et al.* [14].

The experiment was carried out in the dark to avoid photolysis of carotenoids once the saponification was complete. 0.5g of the sample (Aristeus antennatus) was homogenized and saponified with 2.5ml of 12% alcoholic potassium hydroxide in a water bath at 60oC for 30 minutes. The saponified extracts was transferred to a separating funnel containing 10-15ml of petroleum ether and mixed well. The lower aqueous layer was then transferred to another separating funnel and the upper petroleum ether layer containing the carotenoids was collected. The extraction was repeated until the aqueous layer became colourless. A small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of the petroleum ether extract was noted. The absorbance of the yellow colour was read in a spectrophotometer (Genesys 10-S, USA) at 450nm and 503nm using petroleum ether as blank. The amount of total carotenoid and lycopene was calculated using the formulae,

$$\text{Amount of total carotenoid} = \frac{\text{A450 x volume of the sample x 100 X 4}}{\text{Weight of the sample}}$$

$$\text{Amount of lycopene} = \frac{\text{3.12 x A503 x volume of the sample x 100}}{\text{Weight of the sample}}$$

The total carotenoid and lycopene were expressed as mg/g of the sample

2.14. Qualitative and Quantitative Phytochemical Analysis of Extract by GC

The qualitative and quantitative phytochemical analysis by gas chromatography as described by Kelly and Nelson [15].

1g of the sample Aristeus antennatus was weighed and transferred to a test tube and 15ml of ethanol added. The test tube was allowed to stand in a water bath at 60oC for 60 minutes. After the reaction time, the reaction product contained in the test tube was transferred to a separating funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water and 3ml of hexane, were all transferred to the funnel. This extract was combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution dried with anhydrous sodium sulphate, and the solvent evaporated. The sample was solubilized in 1000µl of hexane of which 200µl was transferred to a vial for analysis.

2.15. Quantification by Gas Chromatography

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250µm x 0.15µm) was used. The injector temperature was 280oC with splitless injection of 2µl of sample and a linear velocity of 30cms-1. Helium 5.0pa.s was the carrier gas with a flow rate of 40ml/min or 40mlmin-1. The oven operated initially at 200oC, it was heated to 330oC at a rate of 3oC min-1 and was kept at this temperature for 5minutes. The detector operated at a

temperature of 320oC. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals is expressed in µg/g.

2.16. Determination of Alkaloids of the Extracts

The alkaloid content of Aristeus antennatus was determined gravimetrically by the methods of Harborne [16]. A quantity of 5 gram of Aristeus antennatus was weighed using a weighing balance, and dispersed into 50ml of 10% acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for 4 hours before filtering the mixture. The filtrate was then evaporated to one quarter of its original volume on a hot plate. This was followed by drop-wise addition of concentrated ammonium hydroxide, in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried in an oven at 60oC for 30 minutes, transferred into desiccators to cool and reweighed until a constant weight was obtained. The constant weight was recorded. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed.

$$\% \text{ Alkaloids} = \frac{W2 - W1}{\text{Weight of sample}} \times 100$$

Where W1 = weight of the filter paper

W2 = weight of the paper + alkaloid precipitate

2.17. Determination of Cholesterol using Liebermann-Burchard Reagent (LB) (Campbell, et al., 2005)

To 500ml amber glass bottle fitted with a polyseal cap, add 220ml of cold acetic anhydride and 200ml of glacial acetic acid. A room temperature rise by inversion and 30ml of cold concentrated sulphuric acid added. The reagent is ready for immediate use and is stable for more than six months at 4oC in the dark. When stored at room temperature, the reagent shows signs of darkening (deterioration) after 1- 2 weeks.

3. Results

The results of all analysis are shown in tables 1– 6

Table-1. Amino Acid composition of Aristeus antennatus

Amino Acid (g/100g protein)	***Aristeus antennatus***
Leucine	9.50 ± 0.15*
Lysine	7.53 ± 0.12*
Isoleucine	5.50 ± 0.09*
Phenylalanine	4.61 ± 0.08*
Tryptophan	1.10 ± 0.01
Valine	4.60 ± 0.09*
Methionine	2.94 ± 0.03*
Proline	3.25 ± 0.09*
Arginine	11.20 ± 0.13*
Tyrosine	3.44 ± 0.06*
Histidine	2.43 ± 0.04*
Cystine	1.12 ± 0.02*
Alanine	4.90 ± 0.09
Glutamic acid	13.02 ± 0.14*
Glycine	7.89 ± 0.11*
Threonine	4.61 ± 0.10*
Serine	3.29 ± 0.08*
Aspartic acid	11.91 ± 0.13*

Table-2. Result of the Free Amino Acid concentration of Aristeus antennatus.

	Prawn(MAS)	**Prawn(FAS)**
Total Free Amino Acid (ug/ml)	11.90 ± 1.56^{a}	9.40 ± 0.81^{b}

Table-3. Antioxidant content of Aristeus antennatus extracts.

Antioxidant	Prawn
Vitamin A (mg/100mg)	25.02 ± 3.01
Vitamin C (mg/100g)	ND
Vitamin E (mg/100g)	630.59 ± 4.98#
Carotenoid (mg/g)	110.40 ± 3.42*
Lycopene (mg/g)	77.38 ± 4.25*
Catalase (U/g)	0.32 ± 0.01*
Peroxidase (U/L)	0.004 ± 0.000
Cholesterol (mg/100ml)	13.82 ± 1.10

The value in the table above are the mean ± SD from triplicate determinations.

Table-4. Phytochemical composition of Aristeus antennatus extract.

Phytochemicals	Prawn
Proanthocyanin (ppm)	4.91 ± 0.02#
Narigenin (µg/ml)	4.67 ± 0.05
Lunamarine (µg/ml)	10.95 ± 0.10
Quinine (µg/ml)	5.41 ± 0.05#
Sapogenin (µg/ml)	4.89 ± 0.07#
Kaempferol (µg/ml)	ND
Anthocyanin (µg/ml)	ND
Isoflavone (µg/ml)	27.10 ± 0.15*
Spartein (µg/ml)	5.27 ± 0.06#
Phytate (µg/ml)	2.31 ± 0.02#
Cyanogenic glycoside (ppm)	7.09 ± 0.05#
Catechin (ppm)	10.99 ± 0.09#
Flavone (µg/ml)	4.16 ± 0.03#
Proanthocyanidin (µg/ml)	ND
Resveratol (ppm)	ND
Epihedrine (µg/ml)	23.16 ± 0.10*
Ribalinidine (µg/ml)	22.93 ± 0.09*
Oxalate (ppm)	1.62 ± 0.01#
Tannin (µg/ml)	3.13 ± 0.04*
Steroid (ppm)	ND
Flavonones (ppm)	ND
Alkaloids (%)	16.85 ± 0.13#

The values in the table above are the result of phytochemical analysis by GC.

Table-5. Effect of FAS and MAS extracts of Aristeus antennatus on Fe2+/Fe3+ ratio of HbSS.

Sample	Fraction	Conc (%Hb)	Conc. (%mHb)	Fe^{2+}/Fe^{3+}	%Increase/ Decrease
HbSS Control (Normal Saline)		79.74 ± 0.12[a]	20.26 ± 0.11 [a]	3.94 ± 0.01[a]	0.00 ± 0.00[a]
HbSS Standard (phenylalanine)		87.67 ± 0.11[b]	12.33 ± 0.09 [b]	7.11 ± 0.02 [b]	80.46 ± 0.01[b]
Prawn	MAS	89.46 ± 0.14[c]	10.54 ± 0.07 [c]	8.49 ± 0.03 [c]	115.48 ± 0.02[c]
Prawn	FAS	90.92 ± 0.12[e]	9.08 ± 0.09 [e]	10.01 ± 0.04 [e]	154.06 ± 0.04 [e]

The value in the table above are the mean ± SD from triplicate determinations.

Table-6. Effect of FAS and MAS extracts of Aristeus antennatus on Haemoglobin Polymerization Inhibition of HbSS.

Sample	Fraction	Rate of polymerization	Relative % polymerization	Relative % Inhibition
HbSS (Control)		0.0081	100.00 ± 0.00[a]	0.00 ± 0.00
HbSS (phenylalanine)		0.0060	74.07 ± 0.02 [b]	25.93 ± 0.00
Prawn	MAS	0.0025	30.86 ± 0.01[c]	69.14 ± 0.00
Prawn	FAS	0.0034	41.98 ± 0.02[e]	58.03 ± 0.00

The value in the table above are the mean ± SD from triplicate determinations.

4. Discussion

The nutritional approach to the management of sickle cell disease has been the most modern and effective process adapted in the management of the syndrome. Numerous research studies have furnished humanity with dependable information on the deficiencies of various nutrients some of which are worsened by the sickling saga.

The amino acid composition of the extracts (Aristeus antennatus) was investigated to ascertain the ability of the extracts to inhibit sickle cell haemoglobin polymerization. The antisickling amino acids identified in the Aristeus

antennatus extracts were phenylalanine, Arginine, and Lysine. These amino acids have been reported to possess antisickling activities. Noguchi and Schechter [10]; [17, 18]. Phenylalanine is a standard antisickling amino acid, which exhibits synergistic activity in any extract with other antisickling components, nutrients and drugs when combined together, and has found pronounced role in the management of sickle cell disease [19-21].

The mean values for the antisickling amino acids in the extracts of Aristeus antennatus are: phenylalanine: 4.61±0.08, Lysine: 7.53±0.12, and Arginine: 11.20±0.13 respectively. The antioxidant composition of Aristeus antennatus extract was also investigated. The extract was found to be rich in antioxidants Vitamin A, vitamin E, Carotenoids, Lycopene, Catalase and Peroxidase. Previous studies have shown that the antioxidant vitamins, A, and E are potent inhibitors of sickle cell hemoglobin polymerization [21]. The antioxidant vitamin E content in Aristeus antennatus has a mean value of 630.59±4.98, which is significantly high. There is a non-detectable trace of vitamin C anti-oxidant in Aristeus antennatus. Thus, Aristeus antennatus extract has a high vitamin E content but no vitamin C. The phytochemical composition of Aristeus antennatus extracts were also investigated using Gas Chromatography (GC). Proanthocyanin, Quinine, Sapogenin, Spartein, Phytate, Cyanogenic glycoside, Catechin, were found in Aristeus antennatus with mean value of 4.91±0.02, 5.41±0.05, 4.89±0.07, 5.27±0.06, 2.31±0.02, 7.09±0.05 and 10.99±0.09 respectively. Flavone has a mean value of 4.16±0.03 for Aristeus antennatus. Oxalate has a mean value of 1.62±0.01 for Aristeus antennatus. Alkaloid has a mean value of 16.85±0.13 for Aristeus antennatus extract. These mean values show that Aristeus antennatus extracts contain significant amount of Phytochemicals, Proanthocyanin, Quinine, Sapogenin, Spartein, Phytate, Cyanogenic glycoside, Catechin, Flavone, Oxalate and Alkaloid which improves their nutritional values. The effect of FAS and MAS extracts of Aristeus antennatus on Fe2+/Fe3+ ratio of HbSS were investigated. Fe2+/Fe3+ ratio is a measure of the conversion of metHb to Hb. It is also a measure of oxidant status and improvement of the oxygen affinity of erythrocytes. Phenylalanine, used as a standard, has a percentage increase of Fe2+/Fe3+ by

80.46 ± 0.01 when compared with the control of 0.00 ± 0.00 thereby showing significant difference. When compared with the standard, it was shown that the samples; Aristeus antennatus (MAS), and Aristeus antennatus (FAS) have values 115.48 ± 0.02, and 154.06 ± 0.04 respectively, and are significantly higher than the standard and control. However, this study shows that the Aristeus antennatus extract improved Fe2+/Fe3+ ratio. Aristeus antennatus (MAS), and Aristeus antennatus (FAS), were also seen to inhibit hemoglobin polymerization to about 69.14%, and 58.03% respectively.

The results from the hemoglobin polymerization experiment showed that the FAS and MAS fractions of the samples inhibited polymerization of HbSS. This result is in agreement with results of previous researches, where the samples were shown to inhibit polymerization of HbSS. [18, 21-23] These extracts have proved very effective in inhibiting sickle cell hemoglobin polymerization. The nutritional approach to the management of sickle cell disease is novel and remains in vogue, with promising approach in the management of sickle cell disease. There is no doubt that genetic abnormality can be managed successfully only by nutrition in the near future as the knowledge of the pathophysiology of the disease become more apprehensive to nutritionists and other scientists. Although there hasn't been any permanent cure for sickle cell disease, the nutritional and naturopathic approaches to the management of the syndrome have yielded tremendous, sustainable and reliable benefits which has motivated researchers to focus back to nature in finding a lasting solution to this debilitating syndrome by the application gamut of natural substances such as amino acids and phytonutrients [10], [17, 20, 22].

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