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The antisickling effect of stem extracts of *Costus afer*

Blessing Ezinne Amaefula *, Reginald Nwazue Nwaoguikpe and Chidi Uzoma Igwe

Department of Biochemistry, Federal University of Technology, P.M.B.1526 Owerri, Imo State, Nigeria.

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Abstract

This research was geared towards investigating the antisickling effect of stem extracts of *Costus afer*. The antisickling effect of the stem extracts of *Costus afer* was investigated to determine and declare its potentiation. This work was approached by partitioning the extracts of *Costus afer* stem into; stem fat-soluble fraction (SFSF), stem ethanol-soluble fraction (SESF) and stem methanol-soluble fraction (SMSF). The phytochemical screening was qualitatively and quantitatively determined using standard GC-MS methods. The result shows the presence of twenty (20) different secondary metabolites with a wide range of functions. The amino acid profiles of the extracts revealed the presence of Phenylalanine, Arginine, Lysine, Serine, Methionine and others. Haemoglobin polymerization inhibition and the relative percent inhibition were estimated. The ethanol-soluble fraction (SESF), methanol-soluble fraction (SMSF) and fat-soluble fractions (SFSF) are 74.68 % ±0.00, 26.58 % ±0.00 and 22.78 % ±0.00 inhibition on HbSS polymerization, respectively. The percent improvement on Fe^{2+}/Fe^{3+} ratio are: 96.64 ± 0.12, 66.14 ± 0.11 and 47.24 ± 0.03 for the fat-soluble fraction, methanol-soluble fraction and ethanol-soluble fractions, respectively. The total free amino acid values range from 776.15 ± 2.15 mg/50 g for the SMSF to 1,188.00 ±12.12 mg/50 g for the SFSF. The total vitamin C concentration of the samples expressed in mg/100 g is 297.59 ± 33.26.

The results of this work show that stem extracts of *Costus afer* can be effectively used as phytomedical therapy for sickle cell disease management.

Keywords: Antisickling agents; Polymerization; Inhibition; *Costus afer*; Extract; Sickle cell disease

1. Introduction

A hereditary blood ailment known as sickle cell disease (SCD) is characterized by the presence of aberrant haemoglobin, often known as haemoglobin S (HbS). Millions of people around the world are impacted by this disorder, particularly those who are of African heritage [1]. Red blood cell sickling in SCD causes a number of disorders, such as excruciating pain, organ damage, and an increased risk of infections [2]. Extensive study has concentrated on discovering potential treatment strategies, including natural substances with antisickling qualities to lessen the burden of SCD. Numerous studies have looked at the antisickling properties of different plant extracts. A tropical plant *Costus afer*, also called "Spiral Ginger" or "Crepe Ginger," is native to West Africa and is prized for its therapeutic benefits [3,4] Traditional healers have utilized *Costus afer* to treat SCD symptoms, although there is scant scientific evidence to back this up [5]. Exploring the bioactive substances found in *Costus afer* and their possible antisickling benefits has drawn more attention in recent years. Omokhua [6] examined how various solvents affected the extraction of bioactive components from *Costus afer*, emphasizing the need of using the right extraction methods. Additionally, Eaton and Bunn [7] and Ware [8] stressed the significance of creating fresh therapeutic approaches to reduce SCD symptoms. In a recent study by Eric [9], *in vitro* experiments were used to assess the antisickling properties of *Costus afer* extracts. The researchers discovered particular substances in *Costus afer* extracts that demonstrated notable antisickling capabilities, offering insightful information for future medication development. Strader [10] further highlighted the value of using natural

* Corresponding author: Blessing Ezinne Amaefula; Email: amaefulaezinne@gmail.com

ingredients in the treatment. Although a few studies have looked into the antisickling properties of *Costus afer*, the body of knowledge in this area is still very scanty. In order to evaluate the possible antisickling action of *Costus afer* extracts, this study will concentrate on clarifying the underlying mechanisms and identifying the bioactive components responsible for this impact. We hope to advance earlier findings and provide a deeper understanding of the therapeutic potential of *Costus afer* in managing sickle cell disease by performing a thorough examination of the available literature. In order to improve clinical results and the quality of life for those who suffer from this crippling ailment, it is anticipated that this research will help create new treatment strategies for SCD. Additionally, this research may offer new therapy options for SCD by paving the way for future studies on other natural compounds and their antisickling properties.

2. Material and methods

2.1. Equipment and Reagents

2.1.1 Reagents

Some reagents were provided at the Department of Biochemistry laboratory at Federal University of Technology Owerri and others were procured. All biochemical reagents, chemicals and materials used in this research are of standard analytical grade and they include; ethanol, hexane, sodium sulphate, chloroform, methanol, hydrogen tetraoxosulphate (VI) acid, potassium sulphate, copper sulphate, selenium powder, boric acid, hydrochloric acid, 2,6-dichlorophenol indophenol, ninhydrin, sodium metabisulphite, acetone, phenylalanine, 0.9 %NaCl.

2.1.2 Equipment

Gas chromatography: BUCK M910, Column: RESTEK 15meter MXT-1 column, Amino acid analyzer: Applied Biosystems PTH Amino Acid Analyzer, Spectrophotometer: Bench centrifuge, Soxhlet extractor, Thimble, Kjeldahl flask, rotary evaporator, Microwave oven, Analytical balance.

2.2. Plant sample collection, preparation and extraction

The stem and leaf of *Costus afer* were freshly cut from the University of Port Harcourt Forest and authenticated by a Crop Scientist at the Department of Crop Science University of Port Harcourt as *Costus afer.* The stem of *Costus afer* was separated from the leaf and roots. The stem was debarked and the leaves and roots discarded. The stem sample was air-dried for a period of 30 days. The stem samples were ground into powder using manual home grinder before proper analysis.

50 g of grounded sample was soaked in 200 ml of chloroform for 24 hours. The residue was kept for methanol extraction and the filtrate was subsequently evaporated *en vacuo* and the resulting fat soluble (SFSF) extract weighed and volume recorded. The residue from the chloroform extraction was dried and soaked with methanol. The solvent was filtered and the filtrate subjected to evaporation *en vacuo*. The weight and volume of methanol extract were also recorded. 50 g of grounded sample was soaked in 200 ml of ethanol for 24 hours. The filtrate was subsequently evaporated *en vacuo* and the resulting ethanol-soluble (SESF) extract weighed and volume recorded.

2.3. Blood sample collection and preparation

The blood sample used for the analysis was collected with consent and approval of confirmed HbSS patients who attend clinic at Federal Medical Centre Owerri. The sample collection was done by qualified personnel from the Hematology unit after careful and detailed explanation of the purpose of intended research project on the general health of HbSS patients. 0.20 ml of the whole blood samples were used for the Fe^{2+}/Fe^{3+} ratio, while the remaining portions were collected into citrate-anticoagulant tubes to prevent clotting. At a revolution of 3000 rpm for 10 minutes, the erythrocytes were separated from the whole blood sample. The erythrocytes were suspended in a volume of normal saline (0.9 % NaCl) equivalent to the siphoned plasma. The erythrocyte suspension was then frozen at 0 ^{0}C and subsequently thawed to produce a haemolysate for polymerization experiment.

2.4. Qualitative and Quantitative Phytochemical analysis

The phytochemical study was completed using the methods of Kelly and Nelson [11] meant for characterizing and quantifying flavonoids using gas chromatography. Ground stem samples weighing 1 g each were put into separate test tubes, 15 ml of ethanol was added to each, and the test tubes were sealed. A water bath set at 600 ^{0}C was used to heat the test tube for 60 minutes. Following the reaction time, the reaction's byproduct was decanted from the test tube into a separating funnel. 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water, and 3 ml of hexane were carefully and successfully used to wash the test tube before being transferred to the separating funnel containing the reaction

product. The mixed extracts were then cleaned three times with a 10 % v/v ethanol aqueous solution in 10ml. The solvent was evaporated after the solution was dried with anhydrous sodium sulphate. 200 liters of the 1000 liters of hexane used to solubilize the sample were transferred to a vial for examination.

With an initial oven temperature of 200 ^{0}C, the phytochemicals were quantified. The oven was then raised to 330 ^{0}C at a rate of 30 ^{0}Cmin^{-1} and maintained at this temperature for 5 minutes while the detector ran at 320 ^{0}C. The ratio between the area and mass of the internal standard and the area of the detected phytochemicals were used to determine the phytochemicals. In g/g, the various phytochemical concentrations were expressed.

2.5. Haemoglobin Polymerization Inhibition Test

The haemoglobin polymerization inhibition experiment was done using the original methods of [12, 13, 14]. The turbidity of the polymerization mixture at 700nm using 2 % solution of sodium metabisulphite as reductant or deoxygenating agent [15] was a factor used to assess the polymerization of sickle cell haemoglobin.

2.6. Determination of Fe^{2+}/Fe^{3+}ratio

Fe^{2+}/Fe^{3+}ratio was determined by the methods of Davidson and Henry [16], while the oxygen affinity of haemoglobin and methaemoglobin were measured at 540 nm and 630 nm respectively. To determine the effect of the extracts on Fe^{2+}/Fe^{3+} ratio;0.02 ml of each extract was added to 5.0 ml of distilled water and 0.02 ml of blood added and incubated for 60 minutes in a test tube.

2.7. Amino Acid Profile Determination

The sample was dried at 700 ^{0}C to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the applied Biosystems PTH (phenyl thiohydantoin) amino acid analyzer. About 4 g of defatted sample was put in extraction thimble or wrapped in filter paper and extracted for 15 hours in Soxhlet extraction apparatus [17].

2.8. Determination of Total Free Amino Acid of Extracts

0.1% Ninhydrin in acetone was diluted with water in the ratio of 1:4. The fat-soluble extract was diluted with ethanol in a 1:5 ratios and the methanol-soluble extract was diluted with water in a ratio of 1:1. 20 µl of each of the diluted extracts was added to 4 ml portions of the dilute Ninhydrin.

4-20 mg/ml of Phenylalanine was treated with 4 ml portions of diluted ninhydrin. The resultant solution was heated to boiling for 5 minutes, cooled and absorbance taken from a spectrophotometer at 570 nm using distilled water as a blank. A standard curve of absorbance against concentration was plotted and the values of concentration of free amino acids in the extracts were extrapolated from the plot.

2.9. Determination of the Ascorbic Acid (Vitamin C) of the Extracts

The determination of the ascorbic acid (vitamin c) of the extract was carried out using the methods of AOAC. 1 g of the ascorbic acid standard was dissolved in 100 ml of distilled water and 10 cm^3 of the ascorbic acid was acidified with 2-3 drops of dilute hydrochloric acid. 2,6 – dichlorophenolindophenol of 0.01 % was run into the ascorbic acid solution in a burette until there is a permanent pink solution. If X cm^3 of the indophenol is required, 1cm^3 of indophenols solution is equivalent to 10 mg/X vitamin C.

3. Results

Table 1 Qualitative Phytochemical Analysis of n-hexane fraction of Ethanol Extracts of *Costus afer* Stem

Component	Concentration (µg/ml)
Dihydrocytisine	+
Ammodendrine	+
Spartein	++
Catechin	+
Hydroxylupanine	+

Sapogenin	+
Tannin	+
Kaempferol	++
Cardiac glycoside	++
Ribalinidine	++
Anthocyanin	+
Flavone	++
Flavonone	+
Aphyllidine	+
Proanthocyanidin	+
Isolupanine	++
Epihedrine	+
Cyanogenic glycoside	+
Narigenin	+

Key: ++ = Abundant + = trace

Table 2 Quantitative Phytochemical Analysis of n-hexane fraction of Ethanol Extracts of *Costus afer* Stem

Component	**Concentration (ug/mL)**
Dihydrocytisine	5.3468
Ammodendrine	6.0017
Spartein	10.1207
Catechin	9.8541
Hydroxylupanine	5.5739
Sapogenin	5.1918
Tannin	7.6069
Kaempferol	15.4010
Cardiac glycoside	2.9067
Ribalinidine	15.1135
Anthocyanin	5.7174
Flavone	8.2256
Flavonones	7.6491
Aphyllidine	5.5226
Proanthocyanidin	6.8703
Isolupanine	29.2958
Epihedrine	5.0124
Cyanogenic glycosides	6.6168
Narigenin	6.9928

Table 3 The rates of polymerization, relative percent (%) polymerization and the relative percent (%) inhibition of HbSS of Extracts of *Costus afer* Stem

Groups	Rate of Polymerization	Rel. (%) Polymerization	Rel. % Inhibition
I	0.008 ± 0.00^{a}	100.00 ± 0.00^{d}	0.00 ± 0.00^{a}
II	0.006 ± 0.00^{a}	77.22 ± 0.00^{c}	22.78 ± 0.00^{b}
III	0.006 ± 0.00^{a}	73.42 ± 0.00^{c}	26.58 ± 0.00^{c}
IV	0.002 ± 0.00^{a}	25.32 ± 0.00^{a}	74.68 ± 0.00^{f}

Group values (Mean ± SD) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not; **Key: I =** Control (HbSS) Normal Saline **II =** Stem Fat-Soluble Fraction (SFSF); **III** = Stem Methanol-Soluble Fraction (SMSF); **IV =** Stem Ethanol-Soluble Fraction (SESF)

Table 4 *In vitro* effect of extracts of *Costus afer* stem on the Fe^{2+} / Fe^{3+} ratio of HbSS blood

Groups	% Hb (%)	% MetHb (%)	Fe^{2+}/Fe^{3+}	% Increase/Decrease (%)
I	83.60 ± 0.01^{b}	16.30 ± 0.17^{e}	5.13 ± 0.05^{b}	0.00 ± 0.00^{a}
III	90.85 ± 0.01^{e}	9.15 ± 0.01^{c}	9.93 ± 0.01^{e}	94.64 ± 0.12^{e}
IV	89.44 ± 0.01^{d}	10.56 ± 0.01^{d}	8.47 ± 0.01^{d}	66.14 ± 0.11^{d}
VII	72.88 ± 0.01^{a}	27.12 ± 0.01^{f}	69 ± 0.00^{a}	47.24 ± 0.03^{b}

Group values (Mean ± SD) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not; Key: **I** = Control (HbSS) Normal Saline; **II** = Stem Fat-Soluble Fraction (SFSF); **III** = Stem Methanol-Soluble Fraction (SMSF); **IV** = Stem Ethanol-Soluble Fraction (SESF)

Table 5 Amino acid profile of fat-soluble extracts of *Costus afer* stem

Amino Acid	Net height (mm)	Concentration g/100 g protein
Leucine	96	5.60
Lysine	87.5	4.64
Isoleucine	64.5	4.22
Phenylalanine	21.5	3.81
Tryptophan	14	0.73
Valine	70	4.10
Methionine	16	0.85
Proline	15	3.04
Arginine	28.5	4.90
Tyrosine	8.5	2.92
Histidine	47	3.00
Cystine	12	1.45
Alanine	40.5	3.07
Glutamic acid	77	11.65
Glycine	75	3.56
Threonine	65	3.61
Serine	74.5	4.02
Aspartic acid	144	8.93

Table 6 Total free amino acid concentrations of *Costus afer* stem extracts

Groups	Volume of Extract (ml)	Amino acid Concentration (mg/ml)	Total FAA (per mg/50 g)
I	220.00 ± 0.00[a]	5.40 ± 0.01[e]	1,188.00 ± 2.20[e]
II	215.00 ± 0.00[a]	3.61 ± 0.01[b]	776.15 ± 2.15[b]
III	220.00 ± 0.00[a]	4.51 ± 0.01[c]	992.93 ± 1.27[c]

Group values (Mean ± SD) with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: I = Stem Fat-Soluble Fraction (SFSF); **II** = Stem Methanol-Soluble Fraction (SMSF); **III=** Stem Ethanol-Soluble Fraction (SESF)

Table 7 Ascorbic Acid (Vitamin C) concentration of *Costus afer* stem extract

Plant Part	Concentration (mg/100 g)
Stem	297.59 ± 33.26

Values represent Mean ± Standard deviation

4. Discussion

The GC-MS phytochemical analysis results of the stem n-hexane fraction of the ethanol extracts of *Costus afer* are displayed in Tables 1 and 2. The results demonstrate the existence of 20 phytochemicals of various types, including phenolics, terpenoids, and alkaloids, with alkaloids being the most prevalent, when comparing their quantitative and qualitative makeup. *Shigella flexneri*, *K. pneumoniae*, *B. subtilis*, and *E. coli* were all targets of the antibacterial activity of the various sections of the *C. pictus* plant (leaf, flower, stem, and root) [18]. The extract contains cardiac glycosides, which have been shown to strengthen a failing heart and have antibacterial characteristics [19]. According to the findings of the current study, *Costus afer* stem has a significant amount of kaempferol, a compound whose biological activity controls blood sugar levels [20]. According to prior research Deepak [21], additional minor alkaloids identified in the stem of *Costus afer* include hydroxylupanine, ammodendrine, aphyllidine, and spartein. These alkaloids have biological properties that include antibacterial and cancer-preventative agents. According to Fridha [22], the alkaloid spartein, which was considerably found in the extract, prevents seizures and delays the initiation of convulsive behavior. According to reports, catechin, which is also included in the extracts of various medicinal tea plants, has a moderately potent ability to suppress the growth of a variety of Gram-positive as well as bacterial species. Isolupanine, an alkaloid and the most prevalent phytochemical in the extract, is an effective inhibitor of viral replication [23]. Because they are efficient against pathogenic infections, these phytochemicals with various biological activities are present, enhancing the lifespan of people with HbSS. Table 3 is the relative percent polymerization inhibition of HbSS and it illustrates that, when compared to other fractions with varied percent inhibitions, the ethanol extract of *Costus afer* stem has the highest efficacy on inhibition on HbSS polymerization, at 74.68 ± 0.00[f]. This is in comparison to information on the relative percent inhibition of *Allium sativa* butanol extract from Nwaoguikpe [24]. The SFSF shows the highest relative percent polymerization of 77.22 ± 0.00[c] and a relative percent inhibition of 22.78 ± 0.00[b]. This is followed by the SMSF with the following relative percent polymerization and percent inhibition respectively, 73.42 ± 0.00[c] and 26.58 ± 0.00[c]. The iron contained in normal haemoglobin exists as reduced ferrous state (Fe^{2+}). When haemoglobin iron becomes oxidized to the ferric state (Fe^{3+}), it is no longer able to bind oxygen and is called methaemoglobin. Under normal circumstances, red cells contain a cytochrome- b5-based enzymatic system that rapidly reduces methaemoglobin iron back to the ferrous state and improve oxygen transport in blood tissues. The results in table 4 show the significant improvement in the Fe^{2+}/Fe^{3+} ratio of all fractions of the samples. The fat-soluble fraction of *Costus afer* stem was able to exhibit highest percent increase in Fe^{2+}/Fe^{3+} and this improved the oxygen affinity of sickle haemoglobin. It is logical to conclude that the extracts, though at varying levels, can stabilize the erythrocyte by reducing the fragility of red blood cells. The nutritionally essential amino acids present in the stem and leaf extracts of *Costus afer* include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Table 5 shows that phenylalanine was significantly present with a concentration of 4.52 g/100 mg of extract. Its presence suggests the ability of the stem fat-soluble extract to scavenge free radicals, confer membrane stability and stimulate the activation of membrane bound Na^+/K^+ ATPase and Ca^{2+} ATPase. Arginine, a basic non-essential amino was significantly present at a concentration of 5.30 g/mg. It plays keys roles inhibiting sickle cell hemoglobin polymerization and improving the oxidant status of sickle erythrocytes [14, 25, 26]. Table 6 shows varying concentration of TFAA (per mg/50 g) of different extracts of *Costus afer* stem. The values range from 776.15 ± 2.15[b] in SMSF to 1,188.00 ± 2.20[e] mg/50 g in the SFSF. Antisickling action of

phytomedicines may be attributed to the presence of amino acids as reported [27]. In another research. Ogoda [28] stated that phenylalanine present in a herbal plant *Cajanus cajan*, is thought to be the reason for their antisickling effect. Cell membrane damage is predominant in sickle cell patients than in healthy individuals because the former produce more reactive oxygen species (ROS). Vitamin C is a powerful anti-oxidant capable of inhibiting ROS formation in a variety of situations, by functioning as an electron donor to reduce molecular oxygen [29]. Table 7 shows two concentrations of vitamin C (ascorbic acid) in mg/100 g in stem extracts of *Costus afer*. This proves that the samples have great tendency to inhibit cell membrane damage by ROS. Moreover, vitamin C is a known antisickling agent [24]

5. Conclusion

This research work examined the antisickling properties of *Costus afer* stem extracts, offering insightful information on the plant's potential as a sickle cell disease treatment therapy. A thorough analysis revealed the *Costus afer* stem extracts to have considerable inhibitory effects on hemoglobin polymerization, a crucial step in the pathophysiology of sickle cell disease. The potential of *Costus afer* as a natural source for the creation of cutting-edge therapeutic treatments for sickle cell disease is highlighted by these findings. The findings of this study offer a basis for additional investigation and show potential for the creation of novel therapies for this crippling ailment.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

Statement of informed consent

No informed consent.

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