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The effect of aloe vera plant (*aloe barbadensis*) extracts on sickle cell blood (hbss)

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The phytochemical and antisickling properties of the leaf and gel extracts of the Aloe vera plant were studied. A large quantity of the plant was collected from the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Nigeria, Nsukka and authenticated by a taxonomist at the Department of Crop Science and Technology of the Federal University of Technology, Owerri as being of good quality. The phytochemical composition of the gel and leaf extracts revealed the presence of alkaloids, flavonoids, saponins, tannins and phenols at different concentrations . The determination of the antisickling effects of these extracts was directed towards the inhibition of sickle cell polymerization and the improvement of the Fe²⁺/Fe³⁺ ratio of HbSS in the presence of the extracts. Most of the antisickling amino acids identified in the extracts included: phenylalanine, arginine , tyrosine, aspartic acid , histidine and others, all at high concentrations. The relative percent inhibition of sickle hemoglobin polymerization by the extracts ranged from 77.93% for the CAE of the gel to 80.86% for that of the leaf . The Ascorbic acid concentrations of the extracts were remarkable ranging from 2.028±0.1 mg/ml for the gel to 3.631±0.1 mg/ml for the leaf. The CAE fraction of both gel and leaf improved the Fe²⁺/Fe³⁺ ratio from 46.98% for the gel to 78.0% for the leaf extracts respectively .The total vitamin C concentration of the samples expressed in mg/100g sample are : 202.8±0.1 for the gel and 199.7±0.1 for the leaf. There is no significant difference in the concentrations of the CAE fractions of the leaf and gel. Aloe vera plant extracts with the preponderance of nutrients, phytochemicals, amino acids and other compounds can be very beneficial in the management of sickle cell disease.

Keywords: Aloe vera extract, sickle cell blood, hemoglobin polymerization, Fe²⁺/Fe³⁺ ratio

INTRODUCTION

Aloe vera (*Aloe barbadensis*) known as "secret plant" originated from ancient Egypt. The plant has been presented as a constituent of many phytochemicals, vitamins , nutrients and anti-nutrients found in foods (Maenthalsong et al.,2007). Aloe vera contain ,flavonoids, saponins, tannins, alkaloids and other compounds which exhibit medicinal effects for the treatment of various diseases including severe burn, psoriasis, skin infections, eczema,just to mention but few (Reynolds and Dweck, 1999) . It has been found to be rich in anthraquinones , polysacchandes , salicylic acid , vitamins B₁₂, B₁, B₆, C,

niacinamde, choline, calcium, Iron, lecithin , magnesium, potassium, zinc and others. Aloe gel is one of the most remarkable healing substances known (Vogler and Ernest,1999) . Glucommanans constitute the principal component of the gel. Some other components consists of glucose and mannose only , or glucose, mannose and glucuronic acid ; others are acetylated derivatives. Other polysaccharides found in the plant include galactogalacturans(galactose+galacturonicacid), galactomannan and galactoglucoarabinomannan .The glycoprotein fraction of the gel may also influence the immune system since this fraction has been shown to posses proliferative promoting activity on human and hamster cells(Boudreau and Baland,2006) .When

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applied topically, it hastens skin regeneration, the healing of cuts and burns. The plant extract is purgative and is used in the preparation of ointments, lotions, drinks, jellies and creams. It is used in the treatment of many diseases like ulcer and leukemia. It is also effective against neuro ectodermal tumors and can also be used as a detoxifier since one of its biologically active components is "Acetylated mannose or Acemannan" which is an immune stimulant with activities against some viruses causing flu, measles and also effective at the early stages of the HIV/AIDS infection. Aloe actually accelerates fibroblast development which is necessary for collagen repair and wrinkle prevention (Jeffrey et al., 2004). Aloe vera gel is also used as beverage commonly sold as "aloe juice". Aloe gel mixed with water, citric acid, fruit juice and preservatives is marketed as "aloe juice", a digestive and folk remedy for arthritis and ulcer. The gel contains 75 different nutrients and over 200 ingredients. It also contains twenty amino acids including seven of the essential amino acids. The essential fatty acids in Aloe vera are beneficial, not only nutritional, but as anti-inflammatory agent. Fermented aloe gel with honey and spices is known as "Kumaryasava" in India and is used as tonic for anemia, poor digestive function and liver disorders (Azububike et al., 1999). Sickle cell disease arose among the population of central and West Africa. It is an inherited blood and genetic disorder arising from the substitution of an hydrophilic amino acid (glutamic acid) at the sixth position on the β -chain of hemoglobin by an hydrophobic amino acid, valine. This resulted to a loss in electric charge and an abnormality of the shape of the erythrocytes. Hemoglobin S ($\alpha_2 \beta_2 6\text{val}$) differs from HbA in the solubility of the erythrocytes (Roderick et al., 1996). Sickling of red cells occur as a result of polymerization of de-oxygenated HbS molecules that become stacked linearly in the vasculature. Initially, the process is reversible when the hemoglobin is re-oxygenated but by repeated sickling, membrane damage occurs, resulting in the formation of irreversible sickled cells (ISC) which loose water and potassium ions (K^+). Clinical symptoms occur in homozygotes which develop about the sixth month of life. There are chronic hemolytic anemia, recurrent painful episodes and vaso-occlusive crises which occur due to sickled erythrocytes blocking the vasculature, leading to tissue ischemia and infarction; most commonly affecting the bones, liver, spleen, lungs, brain and retina; leg ulceration and priapism are common and may occur in the male. Patients are prone to serious bacterial infections, particularly pulmonary septicemia, meningitis, osteomyelitis (Roderick et al., 1996; Azubuike and Nkangineme, 1999). The management of sickle cell disease has met great challenges. Different substances including nutrients and chemotherapeutic agents have been tested on patients based on their ability to normalize the sickle erythrocytes and improve the oxidant status of red cells (Ekeke et al., 2000; 2001; Uwakwe and Nwaoguikpe, 2008; Nwaoguikpe and Uwakwe, 2005). The

search for the treatment of sickle cell disease is unending, hence, the need for a researches that mirror or reflect back to nature. The miraculous and overwhelming therapeutic effects of this secret plant had been reported since medieval times. We were therefore prompted to investigate the antisickling effects of aloe vera plant extracts based on their *in vitro* effects on sickle cell hemoglobin polymerization and their ability to improve the oxidants status of sickle cell erythrocytes.

MATERIALS AND METHODS

MATERIAL

In addition to the laboratory reagents, the following chemicals and reagents were used for the work. Dichlorophenolindophenol, sodium metabisulphite, ninhydrin reagent, normal saline, 10% alum solution and a spectrophotometer (Unicam Spectronic 20-DR). Six hundred grams (600 g) of the Aloe plant were purchased from the Herbarium of the department of Pharmacognosy, Faculty of Pharmacy, University of Nigeria, Nsukka. and authenticated as being of high quality by a Horticulturist at the Crop Science and Technology department of the Federal University of Technology, Owerri, Nigeria.

METHODS

Extraction of the aloe vera gel sample

Four hundred grams (400 g) of the Aloe vera plant were collected, dissected and the gel collected into a large vat. The gel changed to liquid of volume 200 ml of liquid extract which was filtered using Whatman filter paper No 1. The filtrate was stored in a refrigerator at 8°C until used.

Extraction of aloe vera leaf sample

Aloe vera plant leaf of weight (400 g) was washed, chopped/macerated and blended into a homogenate. 10 ml of 10% alum solution was added to the extract and left in a refrigerator at 8°C for 24 hours and filtered. The filtrate was kept in the refrigerator until used.

Determination of the ascorbic acid (vit.c) concentration of the aloe vera extracts

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

Collection of blood sample

5ml of sickle cell blood was collected from confirmed HbSS donors who attend clinic at the Federal medical centre, Owerri. The patients willingly consented to the exercise after having explained to them the relevance the research project or work to their health. Portions of the whole blood (0.02ml) were used for $\text{Fe}^{2+}/\text{Fe}^{3+}$ experiment while the remaining portions were stored in the freezer for thawing to hemolyze the erythrocytes and subsequently release the hemoglobin.

Table 1. Phytochemical analysis of the *Aloe vera* crude aqueous extract (CAE) of the plant.

Phytochemical	Result
Tannins	+
Saponins	+
Anthraquinones	+
Flavonoids	+
Alkaloids	+
Phenols	+

Table 2. Quantitative phytochemical analysis of the crude aqueous extract of *Aloe vera* plant

Phytochemical	Tannins	Saponins	Flavonoids	Alkaloids	Phenols
Percent(%) composition	69.91±1.0	25.10±0.1	45.0±1.0	26±0.1	32.27±1.0

Determination of the amino acid profile of the sample

The crude aqueous extract (CAE) prepared from the plant was first dried in an oven at 50 °C for 1 hour. The amino acid profile of the extract was determined and before the determination, the sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multisample Amino acid Analyzer (TSM-1). The volume of the sample loaded was between 5-10 µL. This was dispensed into the analyzer where the hydrolyzate was separated into basic, acidic and neutral amino acids.

Sickle cell hemoglobin polymerization inhibition experiment

The original methods of Noguchi and Schetcher, 1978 and Iwu et al., 1988 were used for HbSS polymerization experiment. HbSS polymerization was assessed by the turbidity of the (polymerizing mixture) at 700 nm, using 2% solution of sodium metabisulphite as reductant or deoxygenating agent (Iwu et al., 1988). 4.4 ml of 2% sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_3$) . 0.5 ml normal saline, (0.9% NaCl) and 0.1 ml hemoglobin were pipetted into a cuvette, shaken and absorbance read in a spectrophotometer (Unicam-Spectronic 20-DR) at 700 nm every two minutes for 30 minutes. This represented the control. Distilled water was used as blank for all assays. For the test assay, 4.4 ml of 2% $\text{Na}_2\text{S}_2\text{O}_3$, 0.5 ml of each extract and 0.1 ml hemoglobin (HbSS) solution were pipetted into the cuvette, and readings taken as above. The rates 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 ($\Delta\text{OD}_{700 \text{ nm}}$) versus time in minutes. The rates were equally expressed as percentages with respect to control.

Phytochemical screening

Phytochemical screening and quantitation were carried out by the established methods of Harborne et al., 1995 and the tests involved those of alkaloids, saponins, flavonoids, anthraquinones, tannins etc.

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

In determining the effect of the extracts on the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio, 0.02 ml of normal saline was added to 5.0 ml of distilled water and 0.02 ml of whole blood (HbSS) were added and incubated for 1 hour. The percent (%) metHb and %Hb were determined spectrophotometrically at 630 nm and 540 nm respectively after reading the absorbances at these wavelengths. In the real assay, the normal saline was replaced with 0.02 ml of the extract or antisickling agent (Davidson and Henry, 1974; Vigil and George, 1976).

RESULTS

The results are shown in tables 1 – 6. Table 1 shows the results of qualitative screening of crude aqueous extracts (CAEs) of *Aloe vera* plant. Table 2 shows the result of quantitative analysis of the phytochemicals present in the CAEs fraction of the leaf. Table 3 shows the vitamin C concentration of the leaf and gel fractions of *Aloe vera* plant. Table 4 shows the rates of hemoglobin polymerization and the relative percent inhibition of sickle cell hemoglobin (HbSS) by the gel and leaf extracts compared with control and some notable antisickling agents (Ascorbic acid and phenylalanine) as standards. Table 5 depicts the effects of CAEs of the gel and leaf on the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio of sickle cell blood

DISCUSSION AND CONCLUSION

From the results in the tables, it can be seen that the fractions (the gel and the leaf possess profound antisickling potency. In table 1, the presence of some phytochemicals like tannins, saponins, flavonoids, alkaloids and anthraquinones in high concentrations is a strong indication of the medicinal value of this plant. It has been reported widely by many workers of the

Table 3. Total vitamin C concentration of the CAEs of the leaf and gel fractions expressed in mg/100g of sample .

Sample(s)	Fraction	Volume of extract(ml)	Vitamin C concentration mg/ ml	VitaminC mg/200g	Total vitamin C (mg/100g)
Gel	CAE	200.0	2.028±0.1	404.6±0.1	202.08±0.1
Leaf	CAE	A11.0	3.631±0.1	399.4±0.1	199.7±0.1

The values of vitamin C concentration in the table are the Mean ± S.D from triplicate determinations.

Table 4: The rates of polymerization and the relative percent inhibition of HbSS at 100µM Ascorbic acid equivalence of the extracts of *Aloe barbadensi*.

Sample	Fraction	Final assay Conc.(µM)	Rate of Polymerization	Relative % Polymerization	Relative inhibition %
HbSS	Control	-----	0.03485	100.0	0.00
Ascorbic acid	-----	100.0	0.003227	9.26±0.11 ^d	90.74±0.10 ^d
Phenylalanine	-----	100.0	0.001625	4.66±0.12 ^c	95.34±0.11 ^c
ALOE GEL	CAE	100.0	0.000667	1.91±0.10 ^b	98.09±0.20 ^b
ALOE LEAF	CAE	100.0	0.000269	0.77±0.01 ^a	99.23±0.11 ^a

The values in the table are the Mean ± SD from n=3 determinations. Values with the same superscript are significantly related along the rows at 95% confidence level or 5% of significance (p≤ 0.05)

Table 5: *In vitro* effect of the CAEs extracts of the gel and the leaf on the Fe²⁺/ Fe³⁺ ratio of HbSS blood.

Sample	% Hb	% mHb	Fe ²⁺ /Fe ³⁺	% Increase
Control (HbSS)	92.26±0.2	7.74±0.2	11.92±0.1	0.00±0.00
Ascorbic acid	93.60±0.1	6.40±0.0	14.69±0.2	18.21±0.01
Phenylalanine	94.30±0.1	5.70±0.1	16.54±0.2	38.76±0.11
GEL(CAE)	94.60±0.0	5.40±0.0	17.52±0.0	46.98±0.1
LEAF(CAE)	95.50±0.0	4.50±0.0	21.22±0.1	78.0±0.0

The values in the table are the Mean ± SD from triplicate determinations. All samples showed increase in the Fe²⁺/ Fe³⁺ ratio.

medicinal value of Aloe vera since ancient times (Volgler, 1999). Table 6 shows the amino acid composition of the entire plant extract (gel and leaf) and the protein concentration of the extracts . Most of the antisickling amino acids identified include: phenylalanine, arginine, histidine, lysine, tyrosine and others , present at very high concentrations. Table 2 shows quantitatively, the phytochemical composition of the plant extracts expressed in percent(%) . It can be seen that tannins ranked highest, 69.91% followed by flavonoids, 45.0%.The presence of flavonoids in most medicines and extracts have been attributed to their synergistic effect with other drugs, thus enhancing or potentiating the effect of the extracts (Evans, 2005). Saponins have equally been found to have stimulating effect both in vaccines and conventional extracts and tend to improve the potency of vaccines (Evans, 2005). Many researchers have reported the presence of anthraquinones as a constituent of the gel (Reynold and Dweck, 1999). A

member of the tannin family (tetragalloylquinic acid) has been indicated as an HIV reverse transcriptase inhibitor, now used in the management of AIDS (Evans, 2005). Table 3 depicts the vitamin C concentrations of the gel and leaf extracts. The vitamin C concentrations of the two fractions are significantly the same with the following values for the CAE of gel (202 mg/100g); while that of the leaf approximates to 200mg/100g. The antisickling effectiveness of vitamin C had already been documented. (Ekeke et al.,2001 ; Nwaoguikpe and Braide,2009; Nwaoguikpe, 2009;) . Table 4 shows the result of hemoglobin polymerization inhibition experiment and the relative % inhibition by the extracts. The leaf extract had a relative percent inhibition of 99.23±0.1 followed by the gel, 98.09±0.01 . It can also be seen from the table that the extracts inhibited polymerization better than L-Phenylalanine and Ascorbic acid respectively. There is no doubt, probably because of the preponderance of

Table 6: Amino acids identified in the entire plant extract and their various concentrations with a total protein concentration of 47.17g/100g of sample.

AMINO ACIDS	CONC.(g/100g)	AMINO ACID	CONC.(g/100g)
Lysine	3.08	Alanine	2.79
Histidine	2.37	Cysteine	0.66
Arginine	5.88	Methionine	0.53
Aspartic acid	5.96	Isoleucine	1.89
Serine	2.06	Leucine	5.61
Glutamic acid	5.64	Tyrosine	1.91
Proline	0.89	Phenylalanine	2.85
Glycine	1.96	Valine	3.18
Threonine	1.94		

some obscure organic compounds present in the plant whose exact functions remain unraveled. Moreover, the presence of twenty amino acids in the plant is another landmark. Most antisickling amino acids (phenylalanine, arginine, serine, histidine) function synergistically to elicit their effectiveness (Noguchi and Schechter, 1978; Nwaoguikpe, 1993). Table 5 shows the *in vitro* effect of the extracts on Fe²⁺/Fe³⁺ ratio of sickle cell blood. The samples increased the Fe²⁺/Fe³⁺ ratio remarkably better than vitamin C, a standard antisickling agent. However, the leaf exhibited a better increase in the Fe²⁺/Fe³⁺ ratio than the gel with the values: 78.0% for the leaf and 22.69% for the gel. This might be as result of the presence of phytochemicals in the leaf extract. We therefore conclude that Aloe vera plant extracts studied would be very beneficial in the management of sickle cell disease.

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