

Full Length Research Paper

Phytochemical and antisickling activities of *Entandrophragma utile*, *Chenopodium ambrosioides* and *Petiveria alliacea*

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The root, leaf and bark of *Petiveria alliacea*, *Chenopodium ambrosioides* and *Entandrophragma utile* respectively are used by traditional healers for the management of sickle cell disease (SCD) in some parts of South West Nigeria. *In-vitro* antisickling activities of these plants' parts were evaluated using p-hydroxybenzoic acid and normal saline as positive and negative controls respectively. Methodology involved the inhibition of sodium metabisulphite induced sickling of HbSS erythrocytes, collected from a confirmed sickle-cell disease (SCD) volunteer in steady state using both crude methanol extract and its aqueous fractions. The extracts/fractions of the three plants at 1.0 and 0.1 mg/ml were observed to exhibit significant ($p < 0.05$) antisickling activity while lysis of erythrocytes occurred at 10.0 mg/ml. Phytochemical screening of the plant extracts revealed the presence of saponnins, tannins and alkaloids. Therefore, the use of the plants by the traditional medical practitioners in the treatment of SCD is justified. The implication of the results obtained in drug development for SCD management is discussed.

Key words: Antisickling activity, *Chenopodium ambrosioides*, *Entandrophragma utile*, *Petiveria alliacea*, phytochemicals, sickle cell disease.

INTRODUCTION

Sickle cell disease (SCD) is known to be one of the diseases ravaging most world populations cutting across nations and ethnic divide. It has an interesting, but alarming statistics. About 89% of sicklers worldwide are in Africa, of which Nigeria alone constitutes 25%. Europe has the least, 0.1%, while the USA, Asia and the Mediterranean have 3.5% each (Ameh et al., 2009). In a related statistics, Moody et al. (2003) had earlier stated that in Nigeria, up to 3% of the population suffers from SCD.

SCD is caused by the substitution of amino acid, glutamic acid with valine at the sixth position of the

beta-globin chain of hemoglobin S (HbS) (Iyamu et al. 2003) and different amino acids can be substituted at the same time (Hartwell et al., 2000). The variants of SCD include those that produce prominent clinical manifestations as seen in sickle cell anaemia (HbSS), sickle cell HbC disease, sickle cell α -thalasemia, while sickle cell trait (HbAS), which has never been considered a disease, has one abnormal gene (Konety-Ahulu, 1974). The consequence of the abnormal hemoglobin in SCD variants is such that; under hypoxic conditions, deoxy-HbS molecules polymerise, forming rigid, sickled cells. This in turn causes the deformation of the normal disc biconcave Red blood cell (RBC). Due to the polymerization of the sickled cells, the red cell membrane loses its functional abilities which results in loss of K^+ and water and a corresponding gain of Na^+ . Small blood

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vessels are blocked by the clumping of sickled RBCs, preventing blood supply to various organs. Deoxygenation in tissue capillaries causes damage to its endothelium, leading to exudation of plasma into the surrounding soft tissue (Fleming, 1982).

The discovery that sickle cell anaemia is a molecular abnormality laid the foundation for the sophisticated molecular and biochemical studies being carried out in the quest for the answer to the problem of sickle cell anaemia. Most current works on the development of specific therapy for sickle cell anaemia that goes beyond supportive factors involve strategies that interfere with the primary pathogenetic pathway of sickle cell anaemia. Such approaches include the development of Hemoglobin modifiers, membrane modifiers and genetic modifiers to mention a few. Okpuzor et al. (2008) stated that some of the orthodox modes of treatment of SCD include induction of fetal hemoglobin (HbF) using HydroxyUrea (HU), Butyrate or its derivatives, oral administration of Clotrimazole which is a potent Gardos Channel inhibitor; blood transfusion and Haematopoietic cell Transplantation (HCT). Although, the successful use of HU was reported in children, however, side effects or poor drug efficacy of some of these agents poses problems for many patients (Iyamu et al., 2003). In addition, the high cost of HCT is hardly affordable by most sickle-cell disease patients in Nigeria and other developing countries.

In developing countries where the use of herbal remedies is at the peak, the potential benefits of using medicinal plants in the management of sickle cell disease should not be underestimated. This becomes more plausible on the understanding that folkloric history has indicated attempts made by inhabitants using plant derived recipes in parts of Nigeria to treat SCD (Egunyomi et al., 2009). This position has been strengthened with the development of Niprisan from a South-west Nigeria traditional recipe by the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria to alleviate the sufferings of SCD patients.

The recipes used by traditional medical practitioners (TMPs), in the management of SCD in Nigeria vary from one region to another, and this is mainly dependent on the availability of efficacious medicinal plants. Aside the need for scientific validation of some of these recipes; scientific investigations on them could also lead to the development of other eff herbal based drugs that would be useful to myriads of sufferers of SCD the world over. In this regard, the present work focuses for the first time to the best of our knowledge on the scientific investigation of three plants that are commonly used in preparing one of such traditional recipes by TMPs, in Ogun State Nigeria.

The investigated plants are *Entandrophargma utile* (*Meliaceae*), *Chenopodium ambrosioides* (*Chenopodiaceae*) and *Petiveria alliacea* (*Phytolaccaceae*).

MATERIALS AND METHODS

Plant collection and preparation

A frequently used recipe consisting of root of *P. alliacea* leaves of *C. ambrosioides* and bark of *E. utile* was obtained from a TMP in Ago-Iwoye, Ogun State, Nigeria and its various plant constituents were each identified by the plant taxonomist at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The plant materials were then obtained dried from local herb sellers in Ibadan, Oyo State, Nigeria and powdered using mortar and pestle. The powdered samples were stored in airtight containers and properly labeled for further study.

Extraction

Each of the dried powdered material (500 g) was extracted with 2 L of methanol by cold extraction for 7 days in large amber bottles with intermittent shaking. At the end of seven days, the crude methanol extract was filtered and the filtrate evaporated to dryness in a water bath. A portion of the crude methanol extract (30 g) was dissolved in a methanol-water mixture (3:1) and poured into a separate funnel clamped to a retort stand. This mixture was then extracted with aliquots of chloroform to obtain a chloroform soluble fraction and a water-soluble fraction of the crude methanol extract. The water soluble fraction obtained was concentrated using a rotatory evaporator to dryness. Thereafter, both crude methanol extract and aqueous soluble fraction were serially diluted with normal saline (0.9% NaCl) to give 10, 1.0 and 0.1 mg/ml solutions which were used for subsequent antisickling assay.

Phytochemical tests

Powdered samples of each of the plant materials were used to test for alkaloids, saponins, tannins, combined and free anthraquinones using established protocols (Adesanya and Sofowora, 1983).

Blood collection and preparation

Blood (0.5 ml) was obtained by venipuncture from one volunteer who was a confirmed sickle cell disease patient (HbSS) with informed consent. The volunteer, a patient attending the Haematology Day Care Unit of the Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria was in steady state. Blood was collected into sodium EDTA bottles and the content thoroughly mixed by gently rolling the bottle. The blood sample was centrifuged to remove serum and the packed erythrocyte obtained was washed with normal saline as described by Egunyomi et al. (2009).

Bioassay of plant extracts for antisickling activity

The bioassay of both crude methanol extract and aqueous fraction of the three plant materials for antisickling activity was carried out using two approaches, namely: inhibition of sickling (antisickling) and reversal of sickled erythrocytes.

The evaluation of antisickling activities of the extracts/fractions was carried out using a modified method of Sofowora et al. (1979).

Table 1. Result of screened phytochemicals for *Entandrophragma utile*, *Chenopodium ambrosioides* and *Petiveria alliacea*.

Plant specimen	Combined anthraquinones	Free anthraquinones	Alkaloids	Saponins	Tannins
<i>Entandrophragma utile</i>	-	-	+	+	+
<i>Chenopodium ambrosioides</i>	-	-	+	+	+
<i>Petiveria alliacea</i>	-	-	+	+	+

Table 2. Antisickling effect (% inhibition of sickling) of methanol and aqueous fraction of *Entandrophragma utile* bark.

Time of Incubation (min)	Normal saline	p-Hydroxy benzoic acid (PHBA)	Methanol extract concentration (mg/ml)			Aqueous fraction concentration (mg/ml)		
			10.0	1.0	0.1	10.0	1.0	0.1
0	-(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
30	-(2)	60(48)	- (-)	52(65)	60(66)	- (-)	60(63)	56(65)
60	-(4)	68(50)	- (-)	64(64)	68(64)	- (-)	68(63)	62(64)
90	-(5)	54(53)	- (-)	69(63)	79(63)	- (-)	69(62)	64(63)
120	-(8)	48(55)	- (-)	63(62)	84(60)	- (-)	75(62)	70(62)

Values in brackets are percentage sickling reversal of 2% metabisulphite induced sickled cells.

The washed erythrocytes (0.5ml) were mixed with 0.5 ml of the concentration of the test extracts/fractions in uncovered test tubes. Samples were taken from the different mixtures and the remainder incubated at 37°C for 3 h with occasionally shaking. Five drops of sodium-metabisulphite (2%) were added to the mixture and mixed thoroughly and sealed with liquid paraffin. Samples were then taken in duplicates from the different mixtures at 0 min after which the systems were incubated at 37°C. Samples were again taken at 30 min interval until four more readings were taken. Procedure for smear preparation and counting of sickled and non-sickled cells was as described by Egunyomi et al. (2009). Two types of controls were employed in this bioassay: a positive control using p-hydroxybenzoic acid (5 mg/ml) and normal saline as the negative control. The formula of Moody et al. (2003) was used to calculate percentage inhibition of sickling.

Evaluation of different plant extracts/fractions for sickling reversal activity was carried out according to the procedure of Oduola et al. (2006). The washed erythrocytes(0.5ml) was mixed with 0.5 ml of freshly prepared sodium metabisulphite (2%) in a clean test tube and incubated at 37°C for 30 min. A drop of the mixture was viewed under the microscope. Equal volumes of normal saline/extract/fraction were added to the blood-metabisulphite mixture in separate test tubes and mixture was incubated at 37°C for another 30 min. Samples were taken at 0 min and at 30 min interval up to 2h. The procedure described by Egunyomi et al. (2009) was used for smear preparation and counting of sickled and non-sickled cells.

Statistical analysis

Data obtained were expressed as means. The statistical significance of differences was assessed using analysis of variance (ANOVA). A two-tailed p value of 0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the results of screened phytochemicals for the investigated plant materials. All the three specimens contained saponins, alkaloids and tannins while both free and combined anthraquinones were absent. The results presented in Tables 2, 3 and 4 indicate that the tested plant parts of *E. utile*, *C. ambrosioides* and *P. alliacea* demonstrated antisickling effects which compared favourably with the exhibited antisickling activity of the positive control (PHBA). The observed antisickling activity in the present study was concentration, plant specimen and the extracting solvent dependent. At the tested highest concentration (10 mg/ml), both the methanol extract and aqueous fraction were cytotoxic, causing lysis of erythrocytes. The antisickling activity of crude methanol extract of *E. utile* was significantly higher ($P < 0.05$) at 0.1 mg/ml concentration, while its aqueous fraction exhibited a significantly higher ($P < 0.05$) antisickling activity at 1.0 mg/ml concentration.

The methanol extract of *C. ambrosioides* leaves and *P. alliacea* root had a significantly higher ($P < 0.05$) antisickling activity compared to the aqueous fraction. In this regard, the observed antisickling activity of the methanol extract was higher at 1.0 mg/ml concentration while the aqueous fraction exhibited greater antisickling potential at lower concentration of 0.1 mg/ml. Of the three investigated plants, it is inferred that *C. ambrosioides* leaves had a significantly higher antisickling activity while *P. alliacea* root had the least. Both the methanol and aqueous fraction of the tested plant organs exhibited

Table 3. Antisickling effect (% inhibition of sickling) of methanol and aqueous fraction of *Chenopodium ambrosioides* leaves.

Time of Incubation (min)	Normal saline	p-Hydroxy benzoic acid (PHBA)	Methanol extract concentration (mg/ml)			Aqueous fraction concentration (mg/ml)		
			10.0	1.0	0.1	10.0	1.0	0.1
			0	-(0)	0(0)	0(0)	0(0)	0(0)
30	-(2)	60(48)	- (-)	60(62)	68(64)	- (-)	52(60)	60(64)
60	-(4)	68(50)	- (-)	77(58)	74(63)	- (-)	70(60)	70(63)
90	-(5)	54(53)	- (-)	79(54)	69(63)	- (-)	69(60)	79(63)
120	-(8)	48(55)	- (-)	76(52)	67(62)	- (-)	64(57)	76(62)

Values in brackets are percentage sickling reversal of 2% metabisulphite induced sickled cells.

Table 4. Antisickling effect (% inhibition of sickling) of methanol and aqueous fraction of *Petiveria alliacea* root.

Time of Incubation (min)	Normal saline	p-Hydroxy benzoic acid (PHBA)	Methanol extract concentration (mg/ml)			Aqueous fraction concentration (mg/ml)		
			10.0	1.0	0.1	10.0	1.0	0.1
			0	-(0)	0(0)	0(0)	0(0)	0(0)
30	-(2)	60(48)	- (-)	68(64)	52(66)	- (-)	44(66)	50(58)
60	-(4)	68(50)	- (-)	70(63)	70(64)	- (-)	40(-)	49(60)
90	-(5)	54(53)	- (-)	59(63)	59(64)	- (-)	36(-)	49(62)
120	-(8)	48(55)	- (-)	52(62)	52(62)	- (-)	33(-)	39(62)

Values in brackets are percentage sickling reversal of 2% metabisulphite induced sickled cells.

reversal of sickling activities which favourably compared with that exhibited by the positive control. However; the trend of sickling reversal differed from the observed antisickling activity. For instance, the tested concentration and the solvent used in extraction had no significant effect ($p > 0.05$) on the observed sickling reversal activity. In addition, the sickling reversal activity was only observed for aqueous fraction of *P. alliacea* root at the lowest tested concentration of 0.1 mg/ml.

DISCUSSION

The investigated plant parts revealed the presence of alkaloids, tannins and saponins while free and combined anthraquinones were absent. This result is similar to that reported by Egunyomi et al. (2009) for two traditional recipes used to manage SCD in south-west Nigeria. In a related development, Ibrahim et al. (2007) reported that saponins, in addition to carboxylic acids and flavonoids may be responsible for the antisickling activity of *H. acida* leaves. Additionally, alkaloids are nerve stimulants, convulsants and muscle relaxants (Kenner and Yves, 1996) hence; the presence of alkaloids in the investigated plant parts is an indication that they may be useful in alleviating some of the symptoms associated with pains.

The results of antisickling assay of the extract/fraction of the three plant organs in the present study showed that

they exhibited substantial antisickling activity. This may offer a rational explanation for the use of these plants in managing SCD by traditional healers. Another salient contribution of the present study to knowledge is its report on cytotoxicity of the investigated plant part at high concentration (10 mg/ml). This information appears to be vital as dosage standardization hardly characterize traditional medicine practice in a developing country like Nigeria.

Of the three investigated plant parts, the root of *P. alliacea* exhibited the least antisickling activity. The result of the present study may possibly be revealing the role/contribution of the root of *P. alliacea* in the traditional recipe used to manage SCD. Its traditional use as remedy for arthritis and rheumatism has been validated by clinical research confirming its pain-relieving and anti-inflammatory properties (Moody et al. 2003). Ibrahim et al. (2007) stated that more than one constituent are usually responsible for the antisickling activity of medicinal plants, hence the isolation of anyone of them can be used to formulate a standard preparation. Given that the investigated plants are abundant in the equatorial Africa, further work should be done to isolate and characterize the various compounds responsible for their antisickling activity in a bid to develop a herbal based medication for the management of SCD, which is endemic in Africa thereby enhance their health and prolong life.

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