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Full Length Research Paper

In vitro* antisickling activities and phytochemical evaluation of *Plumbago zeylanica* and *Uvaria chamae

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The roots of *Plumbago zeylanica* (Plumbaginaceae) and *Uvaria chamae* (Annonaceae) have been used in folklore medicine in the management of sickle-cell disease (SCD) in South-West Nigeria. Using both crude methanol extract and its aqueous fraction, *in vitro* antisickling activities of these plant parts were evaluated using p-hydroxybenzoic acid and normal saline as positive and negative controls, respectively. Phytochemical screening of the investigated plant specimens revealed the presence of flavonoids, saponins, alkaloids, tannins, cardiac glycosides free and combined anthraquinones. Extracts/fractions of *P. zeylanica* had a significantly higher ($p < 0.05$) antisickling activity at the tested concentrations of 10.0, 1.0 and 0.1 mg/ml. Therefore, the use of these plants by the traditional medical practitioners in the treatment of SCD in Ogun State, Nigeria is justified. The implication of these results is in defining the role of each plant specimen in traditional recipes for SCD management and drug development is presented.

Key words: Antisickling activity, phytochemicals, *Plumbago zeylanica*, sickle cell disease, *Uvaria chamae*.

INTRODUCTION

Sickle cell anemia is a genetic blood disorder arising from a point mutation in the β -globin gene that leads to the replacement of glutamic acid residue by valine at the sixth position of the β -chain of haemoglobin. At low oxygen tension, the mutant haemoglobin, sickle haemoglobin, polymerises inside the red blood cell into a gel or further into fibres leading to a drastic increase in the red cell deformability. As a result, micro-vascular occlusion arises which may lead to serious, sometimes fatal crises (Mehanna, 2001).

Various approaches have been adapted in an effort to find agents that inhibit the polymerization of haemoglobin and hence prevent or reduce the occurrence of crises in sickle cell disease (SCD) (Iyamu et al., 2002; Akojie and Fung, 1992). In this regard, oxygen, carbon monoxide and sodium nitrite were used to reduce the amount of deoxy haemoglobin. Iyamu et al. (2002) however,

reported that these approaches did not give the much needed beneficial effects. In the recent years, bone marrow transplantation has been found to be an efficient way of treating SCD. However, the cost implications, availability of necessary expertise and the problems of finding suitable donors constituted a major setback to this approach in developing countries (Mpiana et al., 2007). Currently, in clinical practice, clotrimazole, hydroxyurea and erythropoietin are used in SCD management, but the side effects of these drugs limit their clinical use (Rifai et al., 1995; Mehanna, 2001; Eliot et al., 2006).

On the understanding that herbal remedies and medicinal plants products from indigenous flora have long been used in folk medicine in the management of SCD, it appears that proper and in-depth scientific investigation on such medicinal plants could be of tremendous help in developing efficacious and safer drugs for SCD treatment. The use of phytomaterials such as *Piper guinensis*, *Pterocarpa osun*, *Eugenia caryophylla* and *Sorghum bicolor* extracts for the treatment of SCD has been reported by Wambebe et al. (2001). The extracts of

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Pterocarpus santolinoides and *Aloe vera* were reported to increase the gelling time of sickle cell blood and inhibit sickling *in vitro* (Ugbor, 2006). Sofowora and Isaac-Sodeye (1971) reported the reversal of sickling by root extracts of *Fagara zanthoxylloides*. *Terminalia catappa* could be effective antisickling agents that inhibit osmotically induced haemolysis of human erythrocytes (Mgbemene and Ohiri, 1999).

Research on phytomedicine for the treatment of SCD has led to the development of Niprisan (a herbal based drug) which has been patented by the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and produced to meet increasing global demand by sufferers of SCD. This development indicates that more of such herbal based drugs could be consequent upon scientific investigations on plants that are used in folklore medicine. Current report indicates that roots of *Uvaria chamae* (Annonaceae) and *Plumbago zeylanica* (Plumbaginaceae) are part of the constituents that are used in making traditional recipe for the management of sickle cell anemia (Egunyomi et al., 2009). *U. chamae*, commonly known as finger root or bush banana is a climbing large shrub or small tree native to tropical West and Central Africa. The common name refers to the fruit growing in its small bunches; the fruit is edible and widely eaten. *U. chamae* is a medicinal plant used throughout its range to treat fevers and has antibiotic properties (Iwu, 1993; Bongers et al., 2005). *P. zeylanica* (commonly called Leadwort) is native to West Africa where its folklore medicinal uses include the treatment of arthritis, rheumatism, haemorrhoids, leprosy, stomach troubles, cutaneous and subcutaneous parasitic infection to mention a few (Burkill, 1985).

In the parlance of herbal therapy, Gillete et al. (2004) opined that all components of a therapeutic mixture of plants are necessary. However, the need for the role definition of each component of such traditional recipe could not be overemphasized on the roadmap of upgrading such traditional recipe. In this regard, the present work focused on phytochemical screening and evaluation of antisickling activities of *Plumbago zeylanica* and *Uvaria chamae*. This is with a view of gaining insight on the possible role that these plants might be playing in the recipe for SCD management which contains them.

MATERIALS AND METHODS

Plant collection and preparation

Different plant parts such as leaves, stems, roots and fruits of *P. zeylanica* and *U. chamae* were collected from Iperu Remo, Ogun State, Nigeria. They were identified by plant taxonomist at the Forestry Research Institute of Nigeria, (FRIN), Ibadan, Nigeria. The voucher specimen numbers given were 107863 for *P. zeylanica* and 106767 for *U. chamae* respectively. All plant parts were collected for identification purpose, only the roots were used for the study. The dried roots were powdered using mortar and pestle. The powdered samples were stored in airtight containers and properly labeled for further work.

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; Harbone, 1998).

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 was concentrated using rotary evaporator. In another setup, dried powdered materials were soaked in distilled water for 3 days using the same proportion used for methanol extraction. The filtrates obtained thereafter were evaporated to dryness to obtain aqueous extract. Thereafter, both crude methanol extract and aqueous extract 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.

Blood collection and preparation

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

Bioassay of plant extracts for antisickling activity

Bioassay of crude methanol extract and aqueous extract of the two plant materials for antisickling activity was carried out within two approaches, namely: inhibition of sickling (antisickling) and reversal of sickled erythrocytes.

Evaluation of antisickling activities of the extracts/fractions was carried out using a modified method of Sofowora et al. (1979). The washed erythrocytes (0.5 ml) were mixed with 0.5 ml of the concentration of the test extracts/fractions in uncovered test tubes and mixed together. Samples were taken from the different mixtures and the remaining was incubated at 37°C for 3 h, while shaking occasionally. Five drops of sodium metabisulphite (2%) were added to the mixture, 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. The samples were taken again at 30 min intervals until four more readings were taken. The procedure for smear preparation and counting of sickled and unsickled 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 at 5 mg/ml and normal saline as negative control. The percentage inhibition of sickling was calculated using the formula of Moody et al. (2003).

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 (0.5 ml) 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 volume of normal saline/extract/fraction was added to the blood-metabisulphite mixture in a different test tube and incubated at 37°C for another 30 min. Samples were taken at 0 min and at 30 min interval for up to 2 h. The procedure

Table 1. Results for screened phytochemicals of roots of *P. zeylanica* and *U. chamae*.

Plant part	Combined anthraquinones	Free anthraquinones	Cardiac glycosides	Flavonoids	Alkaloids	Saponins	Tannins
<i>P. zeylanica</i>	+	+	-	+	-	+	+
<i>U. chamae</i>	+	+	+	+	+	-	-

described by Egunyomi et al (2009) was used for smear preparation and counting of sickled and unsickled 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 less than 0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the results for screened phytochemicals of the investigated plant materials. The two specimens contained flavonoids, free and combined anthraquinones, while *P. zeylanica* contained saponins and tannins in addition and *U. chamae* contained cardiac glycosides and alkaloids. In Tables 2 and 3, the antisickling activities of both methanol and aqueous extracts of *P. zeylanica* and *U. chamae* are presented. At the tested concentrations of 10 and 1 mg/ml, both the methanol and aqueous extracts of *P. zeylanica* exhibited antisickling activity that compared favourably with that exhibited by p-hydroxy benzoic acid (PHBA) (positive control). This exhibited antisickling activity was concentration dependent. Also, the antisickling activity exhibited by the aqueous extract of *P. zeylanica* was significantly higher ($p < 0.05$) at the three tested concentrations of 10, 1 and 0.1 mg/ml when compared to its methanol extract. In a trend that contrasts the antisickling activity of *P. zeylanica*, *U. chamae* had a very weak antisickling activity. Even at the tested highest concentration of 10 mg/ml, both the methanol and aqueous extracts of the root of this plant exhibited antisickling activity that was significantly lower ($p < 0.05$) than that exhibited by the positive control whose concentration was 5 mg/ml.

The result of sickling reversal bioassay shown in Tables 2 and 3 revealed a trend which was similar to that of the presently reported antisickling activities of both *P. zeylanica* and *U. chamae*. The sickling reversal activities of the investigated plant parts were concentration-dependent and plant specimen-dependent. For the *P. zeylanica*, the solvent used in extraction had no significant effect ($p > 0.05$) on the exhibited sickling reversal activity.

DISCUSSION

The results of screened phytochemicals for roots of the

two plants investigated revealed the presence of free and combined anthraquinones, saponins, alkaloids, tannins, flavonoids and cardiac glycosides. This result is in agreement with the report of Egunyomi et al. (2009), with an exception of tannins and flavonoids which were not reported to be present in a traditional recipe which had *P. zeylanica* and *U. chamae* as its constituents. However, this present report further indicates the specific phytochemicals that are being contributed by each of the *P. zeylanica* and *U. chamae*. Anthraquinones act on the gastro-intestinal tract to increase the peristalsis action (Evans, 1989). In cases where sickle cell patients complain of constipation, the anthraquinones present in the investigated plant may be useful as a mild laxative. Kenner and Yves (1996) stated that cardiac glycosides have antispasmodic properties and act as good sedatives. The contribution of *U. chamae* in respect of cardiac glycosides is an indication that a recipe that contain *U. chamae* root may be potent to relieve symptoms of cardiac insufficiency, coughs and circulatory problems.

Result of the antisickling assay of root of *P. zeylanica* and *U. chamae* establish their abilities to inhibit sickling under hypoxic condition, thus justifying their use in folklore medicine for SCD management. However, the present study indicates further that the antisickling potential of the extracts of the investigated plant decreased after 1 h when compared to that of the positive control whose antisickling potential was maintained throughout the 2 h incubation. Consequently, results from this study suggest that the antisickling activity of *U. chamae* was lower than that of *P. zeylanica*. However, the weakness of the former in terms of lower antisickling activity was compensated for in terms of presence of phytochemicals such as cardiac glycosides and alkaloids which were absent in the latter. Alkaloids are nerve stimulants, convulsants and muscle relaxant, while cardiac glycosides act as good sedatives and have antispasmodic properties (Kenner and Yves, 1996). It then becomes evidently clear that each constituent of the traditional recipe used in SCD management have a peculiar role that it is performing. This corroborates the opinion of Gillete et al. (2004) that all components of a therapeutic mixture of plants are necessary.

Niprisan (a herbal based drug for SCD management) was developed from four plants: *P. guineensis*, *P. osun*, *E. caryophylla* and *S. bicolor*. Results from the present study are possibly suggesting the roles played by *P. zeylanica* and *U. chamae* are playing in the traditional recipe of which they are constituents. More of such study

Table 2. Antisickling effect (% inhibition of sickling) of methanol and aqueous extract of root of *P. zeylanica*.

Time of incubation (min)	Normal saline	p-Hydroxy benzoic acid (PHBA)	Methanol extract concentration (mg/ml)			Aqueous extract 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)	48 (55)	64 (48)	56 (40)	16 (33)	68 (50)	60 (40)	20 (32)
60	- (4)	50 (59)	73 (51)	66 (41)	35 (34)	77 (52)	69 (44)	39 (40)
90	- (5)	53 (62)	64 (54)	58 (44)	17 (38)	69 (55)	61 (51)	23 (46)
120	- (8)	55 (63)	45 (60)	39 (57)	9 (51)	51 (58)	45 (55)	18 (51)

Values in brackets are percentage sickling reversal of 2% metabisulphite induced sickled cells. All values are means of triplicate determinations.

Table 3. Antisickling effect (% inhibition of sickling) of methanol and aqueous fraction of root of *U. chamae*.

Time of incubation (min)	Normal saline	p-Hydroxy benzoic acid (PHBA)	Methanol extract concentration (mg/ml)			Aqueous extract 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)	48 (55)	36 (26)	20 (12)	10 (5)	20 (18)	16 (6)	6 (0)
60	- (4)	50 (59)	52 (28)	39 (18)	15 (5)	39 (21)	20 (6)	7 (1)
90	- (5)	53 (62)	35 (28)	23 (20)	10 (8)	23 (23)	15 (8)	5 (2)
120	- (8)	55 (63)	18 (30)	18 (22)	9 (9)	18 (26)	9 (9)	3 (3)

Values in brackets are percentage sickling reversal of 2% metabisulphite induced sickled cells. All values are means of triplicate determinations.

should be undertaken on other constituents of traditional recipes on a road map of development of other phyto-pharmaceuticals for SCD management.

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