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ANTISICKLING ACTIVITY OF THE FRESH AND DRIED ROOTS OF *CISSUS POPULNEA* GUILL. ET PERR (VITACEAE)

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ABSTRACT

Research into plants with claimed traditional use in the management of sickle cell anaemia constitutes a useful research strategy in the search for new antisickling drugs and templates. The root of *Cissus populnea* has been used traditionally in the management of sickle cell disease (SCD). Phytochemical screening of the fresh and powdered sample of the root of *C. populnea* (CP) was done using standard methods followed by *in vitro* antisickling evaluation of extracts, solvent-partitioned fraction (ethylacetate) and the vacuum liquid chromatographic fractions (VLC) of the fresh and dried roots of *C. populnea* with sodium metabisulphite induced sickling of HbSS erythrocytes. *p*-hydroxybenzoic acid and normal saline were used as positive and negative controls respectively. Phytochemical screening revealed the presence of tannins, saponins and cardiac glycosides. The result of this study showed that the extracts and solvent-partitioned fraction (ethylacetate) from the root of CP have antisickling property with a higher activity for the ethylacetate partitioned fraction of the dried roots compared to that of the fresh roots. The VLC fractions exhibited higher activity than the crude extracts; fraction 3 (FR3) had the maximum activity of 96.4% while 88.6% activity was demonstrated for the standard drug (PABA) at an incubation time of 45 min. This study has thus provided scientific evidence for the traditional use of CP in the management of SCD.

INTRODUCTION

Sickle cell disorder is a common genetic condition due to a haemoglobin disorder resulting from inheritance of mutant haemoglobin genes from both parents. This disorder is caused by a point mutation in the c-globin chain of haemoglobin, causing the hydrophilic amino acid glutamic acid to be replaced with the hydrophobic amino acid valine at the sixth position [1]. Sickle cell disorder results in anaemia and crisis that could be of many types, including the vaso-occlusive, a plastic sequestration, hyper-haemolytic and other crises [2]. Over the years, several research works have been done and chemical agents for inhibiting sickle of normal cell or reversing sickle shaped red cell *in vitro* have been proposed. The use of phytomedicines such as *Piper guineensis, Pterocarpus osun, Eugenia caryophyllala* and *Sorghum bicolor* extracts for the management of sickle cell disease have been reported [3]. Aqueous extract of the leaves of *Terminalia catappa* has been reported to exhibit antisickling activity on sodium metabisulphite induced sickling [4]. *Xylopia aethiopica* was also found active as an antisickling agent *in vitro* [5]. CP, commonly found in the Savannah is a long climber and has been credited with anti-diabetic property [6], anti-trypanosomal property, antibacterial activity [7] and source of gum powder [8]. In a Nigerian ethno-medicine, it is an ingredient in an anti-sickling preparation [4]. CP is traditionally used in Republic of Benin as a diuretic and aphrodisiac [9]. In south eastern Nigeria, the root is used to cure sore breast of women at birth and the gum or whole root is used as traditional soup thickener [10]. This study was designed to evaluate the antisickling activities of CP as claimed in traditional folklore.

RESULTS AND DISCUSSION

The normal saline had percentage sickling of 71.0% within the first 90 min while *p*-hydroxybenzoic acid (PABA) showed a slight increase in the first 45 min and a consistent reduction in the last 135 min. The 70% distilled ethanol extract of the dried roots (A) had the highest activity (at 100 mg/ml) of 37.2% in the first 45 min, a decrease to 19.0% at 90 min and a gradual increase to 51.2% in the next 90 min when compared with the activity of other extract and fractions. At 100 mg/ml, ethylacetate partitioned fraction of A (C), showed an increase in % sickling of 46.6% in the first 45 min which decrease to 27.4% at 90 min and increased to 61.2% in the 90 min. The 70% distilled ethanol extract of the fresh roots (D) showed % sickling (at 100 mg/ml) of 39.2% in the first 45 min, a decrease to 21.4% at 90 min and then increased to 57.6% in the next 90 min while the ethylacetate partitioned fraction of D (E), showed an increase of 54.0% in the first 45 min, a decrease to 28.4% at 90 min and rose to 63.4% in the next 90 min.

At 200 mg/ml, the 70% ethanol extract of the dried roots (A) showed the highest activity of 29.2% within the first 45 min, which was reduced to 11.6% at 90 min and gradually increase to 46.2% in the next 90 min. Ethyl acetate partitioned fraction of A (C) at 200 mg/ml, showed % sickling increase of 42.2% in the first 45 min, a decrease to 22.0% at 90 min and increased to 57.4% in the next 90

min. The 70% ethanol extract of the fresh roots (D) showed 33.4% sickling of cells in the first 45 min which decrease to 17.8% at 90 min and then increased to 50.4% in the next 90 min. The ethyl acetate partitioned fraction obtained from the fresh root (E), showed an increase of 40.6% in the first 45 min, a decrease to 25.0% at 90 min and rose to 62.6% in the next 90 min (Table 1).

Comparing the anti-sickling activity of the extracts of dried and fresh roots of *C. populnea*, the dried roots exhibited more activity than the fresh at both concentrations of 100 mg/ml and 200 mg/ml. This is indicative of the possible chemical inter-conversion reactions that takes place in plants during the process of drying (air drying), which may be responsible for the higher anti-sickling activity of the constituents in the dried roots. More so, the ethylacetate partitioned fraction of the dried roots has a higher activity than the ethylacetate partitioned fraction of the fresh roots.

Table 2 shows the anti-sickling activity of the VLC fractions at 100 mg/mL concentration. Fraction 1 (FR1) showed percentage sickling of 17.2% in the first 45 min, then decreases to 15.8% at 90 min and a gradual increase to 20.0% in the next 90 min. In fraction 2 (FR2), 9.0% in the first 45 min, an increase to 19.2% at 90 min and further increased to 20.0% in the next 90 min. Fraction 3 (FR3) showed 3.0% in the first 45 min of incubation, increases to 8.6% at 90 min and sustaining its anti-sickling effect at 7.4% in the next 90 min. Fraction 4 (FR4) presented 14.8% in the first 45 min, decreased to 11.4% at 90 min and increased to 31.0% in the next 90 min.

Table1: Antisickling activity of extracts and partitioned fraction of *C.populnea* at 100and 200mg/ml

	% Sickled red blood cells									
Time (Minutes)	0		45		90		135		180	
Conc. in (mg/ml)	100	200	100	200	100	200	100	200	100	200
A	22.2	19.6	37.2	29.2	19.0	11.6	48.2	42.2	51.2	46.2
B	43.3	40.0	58.0	55.8	33.8	29.4	59.0	54.2	65.2	65.2
C	28.0	22.2	46.6	42.2	27.4	22.0	57.0	55.0	61.2	57.4
D	27.6	25.6	39.2	33.4	21.4	17.8	53.4	50.4	57.6	54.0
E	32.6	29.2	54.0	50.6	28.4	25.0	57.6	54.2	63.4	62.6
PABA (5 mg/ml)	25.2		40.6		25.0		23.6		24.0	
NS	76.4		64.4		61.6		71.0		54.6	

Key:

A - 70% distilled aqueous ethanol extract of Dried plant, B -70% undistilled aqueous ethanol extract of Dried plant, C – Ethylacetate partitioned fraction of A, D - 70% distilled aqueous ethanol extract of fresh plant, E – Ethylacetate partitioned fraction of D, PABA = Parahydroxybenzoic acid (Reference standard), NS = Normal saline (Control)

Table2: Anti-sickling activity of vacuum liquid chromatographic fractions of 70% distilled ethanolic extract of dried *C. populnea* (% sickled) at 100 mg/ml

	% Sickled red blood cells				
Time (Minutes)	0	45	90	135	180
FR1	22.6	17.2	15.8	20.0	20.0
FR2	22.0	9.0	19.2	20.0	20.0
FR3	3.0	3.0	8.6	6.2	7.4
FR4	18.8	14.8	11.4	33.2	31.0
PABA(5mg/ml)	11.0	10.8	2.8	2.6	2.0
N. SALINE	90.2	94.8	95.8	97.8	97.7

Key:

FR1 - Fraction 1, FR2 - Fraction 2, FR3 - Fraction 3, FR4 - Fraction 4, PABA- Parahydroxybenzoic acid (Reference standard), N. saline - Normal saline (Control)

Percentage inhibition of sickling of the ethanolic extracts and fractions of the root extract of CP at 100 mg/ml and 200 mg/ml are shown in Figure 1 and 2. Figure 3 shows Percentage inhibition of sickling induced by the VLC fractions at 100 mg/ml. The ethanolic extract obtained from the dried CP sample at 200 mg/ml showed 81.1% inhibition of sickling at 90 min. The ethanolic extract and fractions obtained from the fresh CP sample respectively produced 64.3% (at 90 min) and 96.4% (at 45 min). Unlike the ethanolic extract and fractions examined in this study, the PABA sustained its activity till the end of the experiment (Figure 3). PABA would therefore appear to be much longer-acting than the extracts and fractions, although, fraction 3 (FR3) produce a percentage inhibition of 92.4% at 135th min. Interestingly, the fractions exhibited higher antisickling activity than the ethanolic extracts, especially the FR3 fraction which had the maximum activity at incubation time of 45 min (96.4%) when compared with 88.6% (45 min) of PABA). This therefore suggest that the fractions are more active than the crude extract and confirmed the anti-sickling potential of CP used as a major plant component in a Nigerian herbal formula (Ajawaron HF) [4].

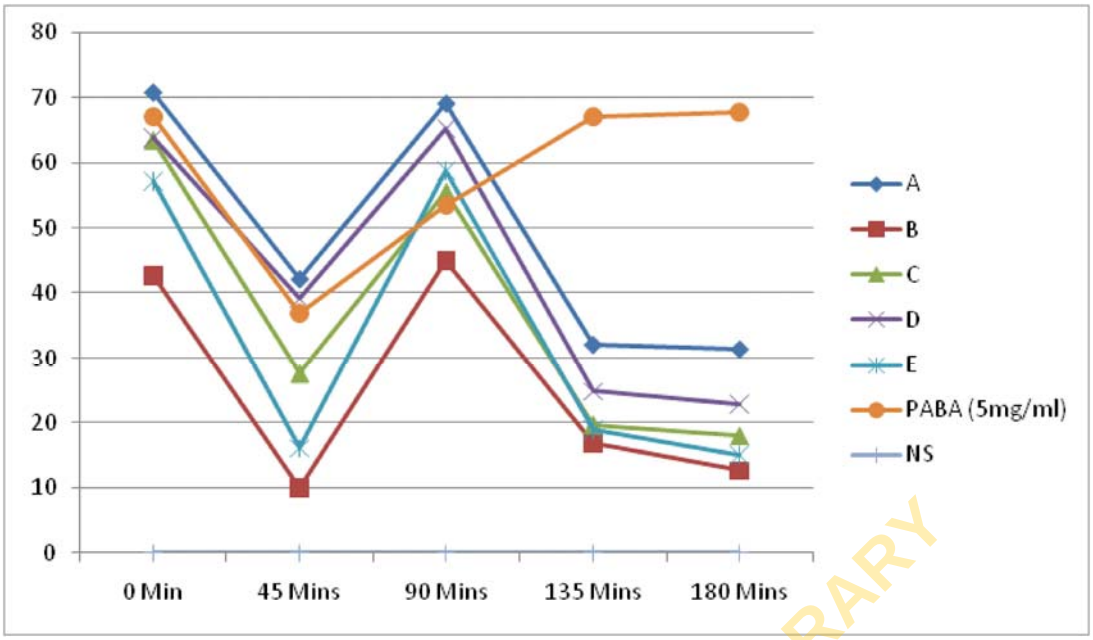

Figure1: Percentage inhibition of sickling by extracts and partitioned fraction of *C. populnea* at 100 mg/ml

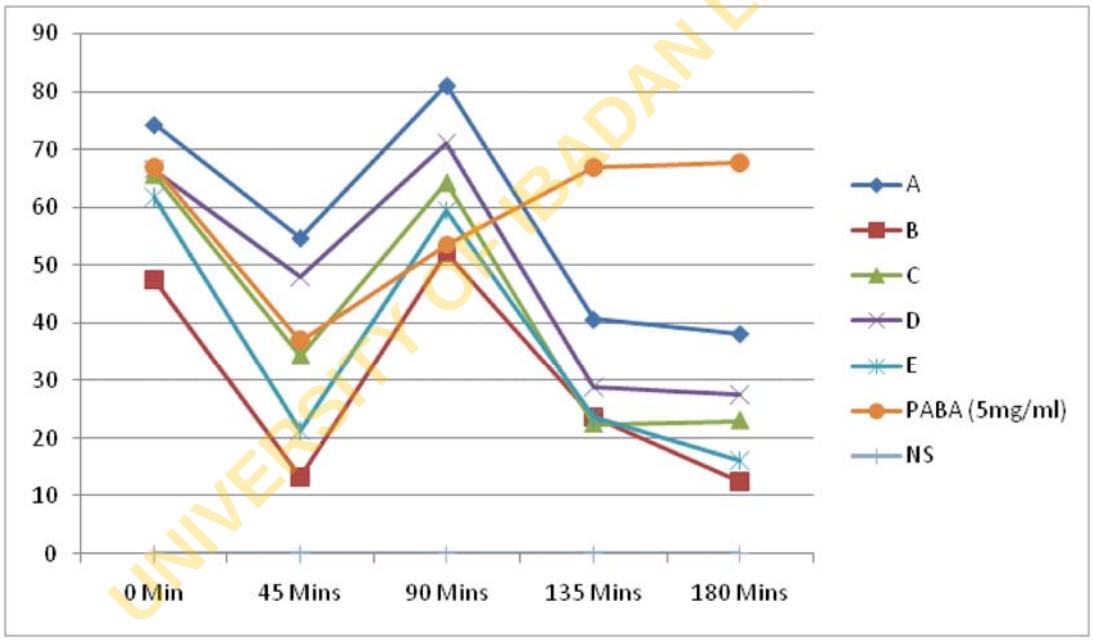

Figure2: Percentage inhibition of sickling by extracts and partitioned fraction of *C. populnea* at 200 mg/ml

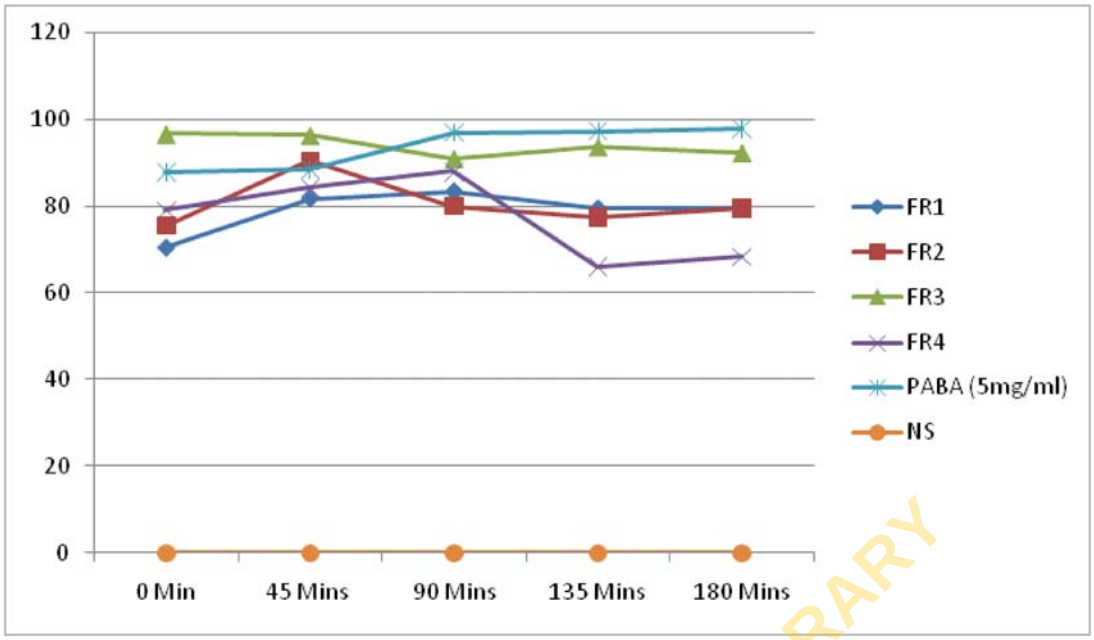

Figure 3: Percentage inhibition of sickling by vacuum liquid chromatographic fractions (100 mg/ml) of 70% distilled ethanol extract of dried roots

CONCLUSION

This study has provided scientific explanation of the folkloric use of the plant CP. However, further work is required on the aqueous ethanol extract of the roots. Particular attention should be paid to the sickling activity within 0-90 min of extract administration. This may reveal the peak action of the active anti-sickling principles in the roots. Isolation and characterization of more interesting compound from 70% aqueous ethanol fraction of the dried root should be pursued.

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh root of CP was collected along Eruwa road (15 km to Eruwa village), Ibadan, Oyo State on 21st December, 2008. The plant specimen was identified and authenticated at the forest Herbarium, Ibadan (FHI 108782), Nigeria where a voucher specimen of the plant red sample was deposited. Freshly crushed and dried powdered root of CP was prepared for extraction.

Extraction of plant materials

Fresh roots of CP (5 kg) chopped into small pieces was extracted in 5 liters of 70% aqueous ethanol by cold extraction for 72 hours. The ethanolic extract was evaporated to dryness in vacuum. 30.1 g of the ethanolic extract was partitioned into ethylacetate, butanol and water and each of the fractions was separately concentrated in vacuum. Dried powdered roots of CP (1.5 kg) were equally extracted with 4 liters of 70% aqueous ethanol using the same procedure as described above.

Fractionation of extracts

The vacuum liquid chromatographic technique was adopted in the fractionation of CP ethanolic extract. This involved the use of silica gel 60 G with particle size of 5-40 µm, and solvent systems n-hexane/ethyl acetate/methanol/water in gradient elution. All fractions collected were analyzed by thin layer chromatography (TLC) (silica gel 60 G: solvent systems chloroform, hexane/ methanol 9:1, chloroform/hexane 9:1) leading to 4 pooled chromatographic fractions FR1, FR2 and FR3 (Table 2).

Preliminary chemical screening

Preliminary phytochemical screening was carried out on fresh and dried roots samples of CP using standard procedures [11, 12, 13, 14).

Collection of blood sample

Fresh blood samples (5 ml) each were collected from confirmed sickle cell anaemia patients who were in a steady state and attending the routine clinic at Haematology Day Care Unit of University College Hospital, Ibadan, Oyo State. The blood samples were collected into an EDTA bottles.

Antisickling activity of extracts and fractions

Antisickling activities of the extract and solvent-partitioned fractions was determined using modified method of [15]. Venipuncture blood samples obtained from sickle cell anaemia patients who were not in crises was centrifuged at 4,000 revolutions per minute (rpm) for 10 mins and the serum was removed using a Pasteur pipette. Equal volume of normal saline was added and

centrifuged at the same revolution per minute and the serum was again removed. The above procedure (washing of erythrocytes) was carried out thrice. 0.5 ml of the washed erythrocyte was measured into small test tubes and equal volume of plant extracts and solvent-partitioned fractions (100 and 200 mg/ml) or 100 mg/ml chromatographic fractions were added, mixed together using a vortex mixer for 10 seconds and incubated for 3 hrs at 37°C while shaking occasionally. After incubation, the system was deoxygenated by adding 0.2 ml of freshly prepared 2% sodium metabisulphite, mixed thoroughly and sealed with 3 drops of liquid paraffin. Experimental blood samples were prepared in triplicate and incubated again at 37°C. 5 readings were obtained at different time point starting from 0 min to the 45th min. Para-hydroxybenzoic acid (5 mg/mL) serve as positive control and normal saline was used as negative control.

The count of cells under high power magnification (×100) was carried out in other to obtained relative number of sickled and non-sickled cells. The percentage mean sickled as well as percentage inhibition for each extract and fractions were determined.

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