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Full Length Research Paper

Antisickling activities of two ethnomedicinal plant recipes used for the management of sickle cell anaemia in Ibadan, Nigeria

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Two plant recipes used in the management of Sickle Cell Anaemia (SCA) by the indigenous people of Ibadan, Nigeria were studied for their antisickling activities. Using methanolic extracts of powdered plant parts, *in vitro* studies antisickling activities of the extracts were evaluated using p-hydroxybenzoic acids and normal saline as controls. The method employed involved the inhibition of sodium metabisulphite induced sickling of HbSS red blood cells, collected from confirmed non-crises sickle cell patients. Extracts of Recipe 1 (consisting of 28 plants) and 2 (consisting of 7 plants) showed antisickling activities; 63.4 and 78.8% inhibition, respectively, at 180 min incubation. The confirmation of the antisickling activity in the two recipes justifies their use by indigenous people. Phytochemical screening of the extracts showed that they contained similar secondary metabolites except that anthraquinones were absent from Recipe 2.

Key words: Antisickling activities, ethnophytomedicines, sickle cell anaemia.

INTRODUCTION

The use of natural products in attempts at inhibiting sickling could be as old as when the sickle cell (SC) disease was discovered. Folkloric history has indicated attempts made by inhabitants using plant derived recipes in parts of Nigeria to treat what they described as "fever of crises", shifting joint pains, exacerbations especially during rainy seasons and "constant abnormality of the blood," though relatively few have been validated scientifically. Very few ethnomedicinal remedies for the treatment of sickle cell anaemia have been reported in the literature due to secrecy attached to the treatments of these disease. An example of treatment used by indigenous people of Southwestern Nigeria for the treatment of sickle cell is reported by Abimbola Sodipe (1985). It includes six pints of cow's urine and six bottles of dry gin. The two combinations are used to soak four well ground leaves of *Nicotiana tabacum*. One desert spoonful of rock salt is added to the mixture in a big bottle corked and kept for 2 days. The patient takes a tot 3 times a week (Abimbola Sodipe, 1985). Thomas and Ajani (1987) noted the use of an aqueous extract of the unripe fruit of pawpaw (*Carica papaya*) as a home remedy in Southwestern Nigeria for patients during crises. Evaluation of a 48 h aqueous extract showed that it was capable of inhibiting and reversing sickling of HbSS red blood cells. The possible antisickling compounds were suspected to be organic acids released from esters during fermentation of the fruits for 48 h as a 24 h extract was not active (Thomas and Ajani, 1987).

An investigation of the use of *Adansonia digitata* bark during crises showed that the plant can revert sickling but demonstrated only little inhibitory activity (Adesanya et al., 1988). Also, the aqueous decoction of the seeds of *Cajanus cajan* as a home remedy in Southwestern Nigeria was found to reverse the sickling of HbSS blood and significantly inhibited sickling (Ekeke and Shode, 1985). Bioassay guided extraction and column fractionation of the seed extract yielded an active fraction which delayed gelation of HbS and increased its affinity for oxygen.

The anti-sickling property of *Zanthoxylum xanthoxyl-*

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oides roots was discovered when it was observed that an aqueous extract preserved the colour of red blood cells during a screen for its antimicrobial activity (El-Said et al., 1971). The extract was later shown to revert sickled HbAS and HbSS and crenated HbAA red blood cells to normal *in vitro* (Sofowora et al., 1979). The activity was also demonstrated in the root of other *Zanthoxylum* species: *Zanthoxylum gilleti* was found to be just as active as *Z. xanthoxyloides* (Adesanya and Sofowora, 1983). This and previous observations led to the postulation of a membrane based activity earlier reported for the extracts (Sofowora et al., 1979). Activity directed fractionation of the aqueous extract located the ether fraction as the active fraction. Although, an attempted preliminary clinical trial on sickle cell anaemia patients with the extract was plagued with a high drop-out rate, the result obtained seem to indicate a significant diminution of painful episodes in treated individual (Isaacs-Sodeye et al., 1975). Active principles were found to be hydroxylbenzoic acid derivatives; vanillic acid and p-hydroxybenzoic acid.

In a screen of substances known to bind proteins, a leaf extract of *Lawsonia inermis* was found to inhibit sickling and to increase the oxygen affinity of HbSS blood (Chang and Suzuka, 1982). The plant is used as a hair condition and ethnomedically to treat yellow fever, pains and blood related diseases like jaundice (Bhat et al., 1990).

The fact that the seeds of *Cajanus cajan* accumulate phenylalanine, an aromatic amino acid known to possess sickling activity suggests that other plant parts could contain this acid or other amino acids which are known to have antisickling activity. The aromatic amino acids tyrosine and tryptophan, as well as small peptides containing these amino acids, have antisickling activity (Dean and Schecter, 1978; Noguchi and Schechter, 1978; Ekeke and Shode, 1990). Moody et al. (2003) reported that the aqueous extracts of the reddish brown freshly fallen leaves of *Terminalia catappa* were able to exhibit antisickling activity on sodium metabisulphite induced sickling.

As traditional healers claim to have potent herbs for managing sickle cell disease it was thought desirable to verify their claim. This study was aimed at collecting and identifying plant species constituting two different recipes mostly used with acclaimed success by traditional healers, evaluating the plant recipes *in vitro* for antisickling activities by testing their extracts on blood samples from sickle cell patients, and carrying out phytochemical tests of the two recipes.

MATERIALS AND METHODS

Plant materials

Two frequently used recipes consisting of roots, barks and leaves were bought from herbal market in Ibadan, Oyo State Nigeria and identified at the Herbarium (UIH) of the Department of Botany, University of Ibadan.

Preparation of plant materials for analysis

Each recipe was dried separately in a cool dry room for 3 weeks until completely dry and then powdered using a blender. The powdered samples were stored in containers and labeled. Water soluble fractions of the powdered recipes were obtained and then tested for antisickling activity.

Extraction of the water soluble fractions

Water soluble fraction was chosen for extraction because of the mode of preparation stipulated by the herb seller which is by decoction with clean water. 50 g of each of the powdered plant materials was put into 250 ml conical flasks and 300 ml of methanol was poured over it and left to stand for 5 days with intermittent stirring using a spatula. The conical flasks were covered with foil paper. After 5 days, the extracts were filtered and evaporated to dryness on a hot water bath and then dispensed into labeled sample vials and stored in the refrigerator at 4°C for subsequent use and to prevent spoilage and degradation.

Evaluation of plant samples extracts for antisickling activity

Blood collection: 4 – 5 ml of blood was obtained by venipuncture from each of 10 confirmed sicklers (HbSS) not in crises from Adeoyo Hospital, HbSS Clinic, Ring Road, Ibadan. The subjects, who aged between 17 – 24 yrs and of both sexes, were in reasonably good health. Blood was collected in sodium EDTA bottles and the content thoroughly mixed by gently rolling the bottle. All experiments were performed with fresh blood.

Procedure for antisicking activity evaluation: The evaluation of the 2 different methanolic extracts for antisickling activities was carried out using a modified method of Sofowora et al. (1979). Vein punctured blood samples from sickle cell anaemia patients not in crises were collected into EDTA bottles, and centrifuged to remove the serum. The resulting packed erythocytes were washed 3 times with 1 ml sterile normal saline per 5 ml of blood. The samples were then centrifuged each time for 5 min at a speed of 2000 revolution per minute to remove the supernatant. 0.5 ml of the washed erythrocytes were mixed each with 0.5 ml of the different extracts in uncovered test tubes and mixed together. Samples were taken from the different mixtures and the remaining mixtures incubated at 37°C for 3 h while shaking occasionally.

0.2 ml of 2% sodium metabisulphite were added to deoxygenate the system, mixed thoroughly and sealed with liquid paraffin. Samples were taken in duplicates from the different mixtures at 0 min after which the systems were incubated again at 37°C and the samples taken again at 45 min interval until 5 readings were obtained.

Each sample was smeared on a microscope slide, fixed with 95% methanol, dried and stained with giemsa stain. Each sample was examined under the oil immersion light microscope and counting at least 500 red blood cells in each sample from five different fields of view across the slide. The numbers of both sickled and unsickled red blood cells were counted and the percentage of unsickled cells determined. Two types of controls were employed in this biological testing. A positive control using p-hydroxybenzoic acid (5 mg/ml) a compound known to reverse the sickling in HbSS blood cells. The negative control involves the use of normal saline. Each set-up in the experiment was replicated twice. The blood sample collected from a particular patient was used for testing of each set of experiment.

Table 1. Plants constituting Recipe 1.

Botanical names	Part used	Chemical constituents**
Uvaria chamae P. Beauv	Root	-
Euphorbia laterifolia Shum & Thoun	Stem	Resin: Euphorbon, tirucallol
Securidaca longepedunculata Fres	Root	Methylsalicylate, Valerianate
Mangifera indica Linn	Bark	Mangiferin, Tannins, Resins
Vitellaria paradoxa Gaertn. F.	Bark	Fixed oil
Calliandra portoricensis (Jacq)	Root	-
Zanthophyllum xanthoxyloides (Lam.) Watern.	Root	P. hydroxybenzoic acid, Artarine, Fagaramide
Terminalia superba Engl & Diels	Bark	Resins, Tannins
Khaya ivorensis A. Chev.	Bark	Khayasine, Calicedrin, Nimbosterol
Olax subscorpoidea Oliv.	Root	Mono & Poly unsaturated acids, Saponin
Microdesmis keayana J. Leonard	Root	-
Cassia fistula Linn	Root	Sennapicrin, Antraquinones
Lophira alata Banks ex Gaertn f.	Bark	Fixed oil (Mene oil)
Uvaria afzeliit Sc. Elliot	Root	Tannin
Detarium microcarpum Guill & Perr	Bark	Detaric acid, Gum-resin
Tetrapleura tetraptera (Schum & Thonn) Taub	Fruit	Mimosine, Saponin
Alstonia boonei (Dewild)	Bark	Echitamine, Alstonine Reserpine
Plumbago zeylanica Linn	Root	Plumbago = Plumbagin, Quinone
Rauvolfia vomitoria Afzel	Root	-
Gongronema latifolium Benth	Root	Condurangoside
Lannea welwitschii (Hiem) Engl.	Bark	-
Citrus medica Linn	Root	-
Anogeissus leiocarpus (DC.) Guill & Perr.	Bark	20% Uronic acid, 17% Tannin
Chasmanthera dependens (Hochst)	Root	Beberine
Xylopia aethiopica (Dunal) A.R.ch	Fruit	Annonacein, Resin, Reberoside
Terminalia sp	Bark	Tannins, Resins
Mondia whitei (Hoof.k) Skeels	Root	-

** Derived from Gill (1992) and Oliver-Bever (1960).

Table 2. Plants constituting Recipe 2.

Botanical names	Part used	Chemical constituents**
Vernonia amygdalina Dcl	Leaves	Vernodalin, Vernomygdin
Garcinia cola Heckel	Root	Kolaviron, Apigenin, Ametoflavone
Mangifera indica Linn.	Bark	Quercertin, Methysalicylate
Terminalia catappa Linn.	Leaves	Tannin
Newbouldia laevis Seem.	Bark	Tannin
Z. xanthoxyloides (Lam.) Waterm.	Bar & Root	Fagarol Pseudofagarol, P. hydroxybenzoic acid
Capsicum frutescens Linn.	Fruit	Capsaicin

**Derived from Gill (1992) and Oliver-Bever (1960).

Phytochemical tests

The two recipes were screened for their phytochemical constituents. Powdered samples were used to test for alkaloids, saponins, tannins, anthraquinones, cardiac glycosides (cardenolides) following established protocols (Adesanya and Sofowora, 1993).

The botanical names, plant parts, used and known chemical constituents of the plants making up Recipes 1 and 2 are shown in Tables 1 and 2. Based on the information derived from the herb sellers the recipes are to be boiled (decocted) with ordinary water. The dosage was given as half of a stainless cup (100 ml) 3 – 5 times daily.

RESULTS AND DISCUSSION

Table 3 shows the effect of the methanolic extracts of the

Table 3. Antisickling activities of methanolic extracts of Recipes 1 and 2 using p-hydroxybenzoic acid and normal saline as controls.

Time of incubation (min)	% of unsickled red blood cells					
	R1	PHBA 1	NS1	R2	PHBA 2	NS2
0	43.6	36	62.4	23.8	43	56
45	53.8	61.4	56.4	57.6	63	31.8
90	57.8	79.6	53.4	62.8	68.4	26.2
135	59.8	82.8	37.4	68.6	69	22
180	63.4	52.1	22.6	78.8	78.2	27.4

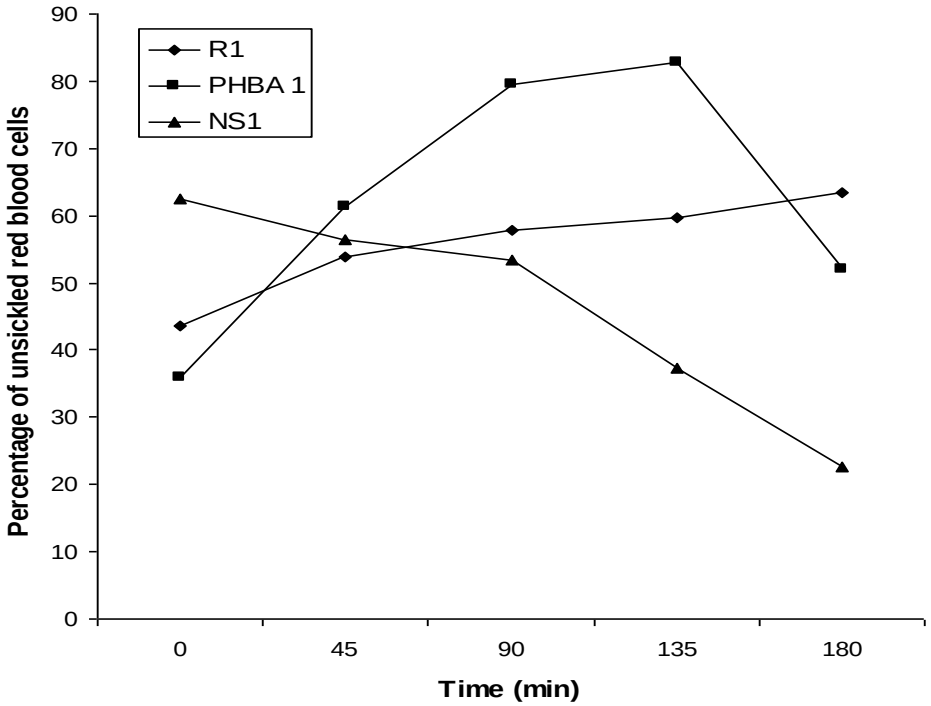

Figure 1. Effect of extract of Recipe 1 (R1) on sickled red blood cells. PHBA = p-hydroxybenzoic acid (+ve controls) and NS = normal saline (-ve controls).

two recipes in inhibiting red blood cell sickling. Recipe 2 was more active with 78.8% inhibition at 180 min than Recipe 1 which showed 63.4% inhibition also at 180 min.

As shown in Figures 1 and 2, p-hydroxybenzoic acid control demonstrated more sickling inhibition than the methanolic extracts of the recipes while in Figure 2, the metabolic extract of recipe 2 showed a slightly higher ability to inhibit sickling (78.2%). Control normal saline showed relatively no inhibition and so majority of the cells still remained sickled after incubation even up to 180 min.

Phytochemical screening showed the presence of alkaloids, saponins, cardiac glycosides and tannins in both recipes. However, the presence of anthraquinones was recorded only in recipe 1 while it was absent in recipe 2.

The results obtained in the present study show that Recipes 1 and 2 exhibited substantial antisickling activity. The methanolic extracts of these two recipes showed significant inhibitory effect on sodium metabisulphite induced sickling. Recipe 1 exhibited a maximum 63.4% inhibition at 180 higher degree of inhibition of 82.8% at 135 min of incubation. The methaolic extract of recipe 2 showed a better activity which was sustained even after 180 min of incubation. Recipe 2 showed a more significant inhibitory effect on sickling with a maximum of 78.8% at 180 inhibition. A high degree of inhibition was noticed at 45 min incubation which increased rapidly to 78.8% at 180 min compared with 78.2% inhibition demonstrated by p-hydroxybenzoic acid at the same time of 180 min. Thus recipe 2 could be a better remedy for sicklers than the standard P-hydroxybenzoic acid. The present result agrees with the statement made by Ekeke and Shode (1985) that the efficacy of an antisickling agent, whether *in vitro* must be assessed by a set of

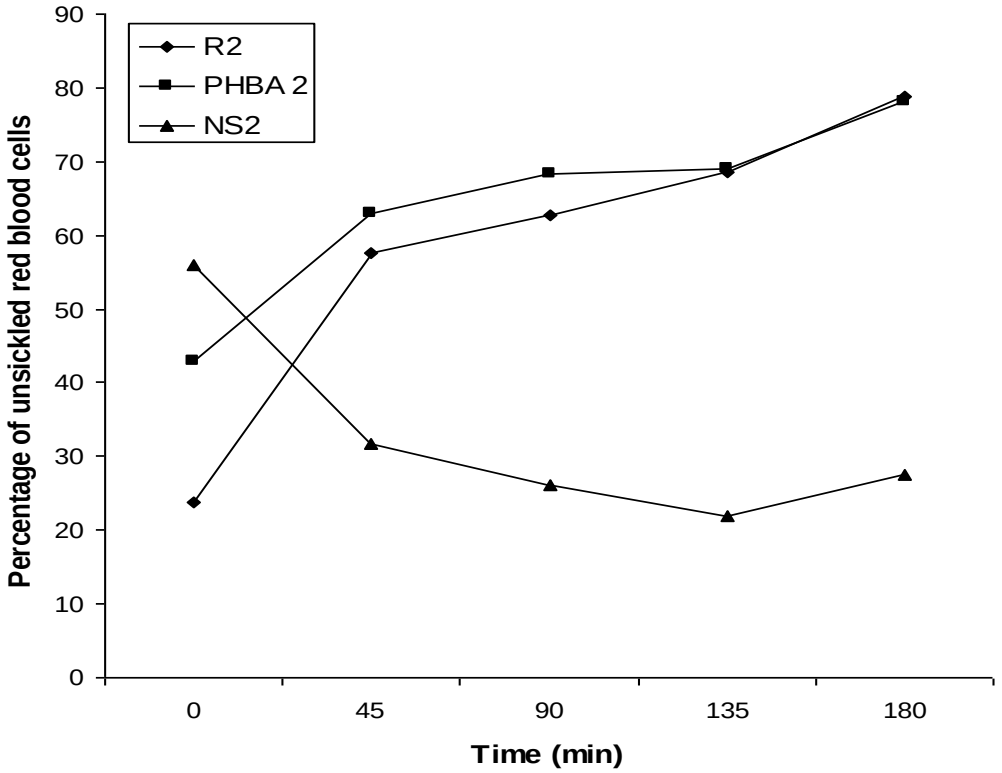

Figure 2. Effect of extract of Recipe 2 on sickled red blood cells. PHBA = p-hydroxybenzoic acid (+ve controls) and NS = normal saline (-ve controls).

Table 4. Phytochemical screening of Recipe 1 and 2.

Secondary metabolite	Recipe 1	Recipe 2
Alkaloids		
Meyer's reagent	+	+
Dragendoff's reagent	+	+
Wagner's reagent	+	+
Anthraquinones	+	-
Saponin	+	+
Cardiac glycosides		
Keller Killiani test	+	+
Kedde test	+	+
Tannins	+	+

+ = Present; - = absent

reproducible criteria. It must act effectively and rapidly, especially in cases of severe crises.

Although, Sofowora et al. (1979) identified an anti-sickling factor in *Fagara xanthoxyloides* and later isolated and characterized it, there is as yet no such record of similar observation for any other plant species in Recipes 1 and 2. In comparison with Recipe 2, antisickling component of *Fagara xanthoxyloides* unsickled 20% of sickled red blood cells after 45 min of incubation and 74% of sickled red blood cells after 180 min of incubation (Sofowora et al., 1979).

Mangifera indica and *Z. xanthoxyloides* are common to both Recipes 1 and 2. However, the bark is used in both recipes in case of *M. indica* while both bark and root of *Z. xanthoxyloides* are used in recipe 2; only the root is used in recipe 1. The higher percentages of use of both plants in recipe 2 and the inclusion of reddish brown, freshly fallen leaves of *Terminalia catappa* (Moody et al., 2003) in Recipe 2 may be responsible for its having higher antisickling activity than Recipe 1. It is possible that apart from these two plant species which are common to both recipes and the presence of *T. catappa* in Recipe 2, other plants present in both recipes may contribute to their activity.

Phytochemical screening of recipes 1 and 2 for secondary metabolites reveals the presence of alkaloids, glycosides, saponins and tannins. Anthraquinones were present in Recipe 1 but was absent in Recipe 2. Alkaloids were nerve stimulants, convulsants and muscle relaxant (Kenner and Yves, 1996). The presence of alkaloids in the two recipes is an indication that they may be useful in alleviating some of the symptoms associated with pains. Anthraquinones act on the gastro-intestinal tract to increase the peristalsis action. Anthranols, anthrones, oxanthronea and dianthrones are all derivatives of anthraquinones (Evans, 1989). The presence of anthraquinones in recipe 1 is an indication that it may be useful

as a mild laxative especially in cases where SC patients complain of constipation. Tannins are phenolic derivatives and are non-nitrogenous plant constituents with astringent properties on mucous membranes. The tannins present in the two recipes make it useful in bathing or cleansing the surface of the skin ulcers that develop as a result of sickle cell disease. The presence of cardiac glycosides indicates that they may be potent in curing cardiac insufficiency, coughs and circulatory problems. Also, they may act as good sedatives and have antispasmodic properties (Kenner and Yves, 1996).

Dennis and Roberts (1990) attempted an explanation of the antisickling activity of plant species on sickled erythrocytes. They were of the view that it may be due to inhibition of Ca^{2+} activated K^+ channel. Activation of this channel results in K^+ and water loss from sickled erythrocytes with subsequent dehydration which brings about increase in intracellular concentration of HbS leading to polymerization of deoxy HbS with its associated painful episodes. Inhibition of this pathway increases K^+ cell content, rehydration of red blood cells and an increase in haemoglobin level. This approach results in cell swelling, decreased HbS concentration and decreased sickling. Results obtained from the experiment for sickling reversion indicates that *in vitro* action of the extracts of Recipes 1 and 2 is rapid and probably helps in the inhibition of the sickling pathway such that potassium cell content is increased and rehydration is increased. More than 50% of sickled erythrocytes were reverted at 180 minutes in both cases. If this action can be reproduced *in vivo*, then, the recipes may well hold a lot of promise in the treatment of the disease. Depending on its half-life, it would also be expected that its periodic administration would reduce both the frequency and duration of crises. The present study has demonstrated that the extracts from Recipes 1 and 2 could significantly inhibit sickling in the presence of sodium metabisulphite. The use of sodium metabisulphite in sickling induction is probably a more drastic approach than what actually happens in the vascular system of humans. In that case, the extracts may perform its antisickling action more efficiently under *in vivo* conditions than has hitherto been demonstrated.

Recipe 2 is composed of seven plant species. Of these, *Z. xanthoxyloides* (Fagara) and *T. catappa* have been reported to have antisickling activity (Sofowora et al., 1979; Moody et al., 2003). *Newbouldia laevis* stem bark extract was reported by Olajide et al. (1997) to have exhibited anti-inflammatory and analgesic properties which would take care of the severe pains experienced by sicklers. As investigated by Akinpelu (1998), *Vernonia amygdalina* leaves showed antimicrobial activity. Since people with Sickle Cell Anaemia (SCA) often have infections due to a depressed immune system (especially in childhood), the inclusion of *V. amygdalina* is justified. *Garcinia kola* (root) is a remedy for inflammations and respiratory tract infections (Gill, 1992) hence beneficial for management of SCA. *Capsicum frutescens* contains "capsaicin" which acts as a carminative and counter-irritant (Oliver Bever, 1960) while the inclusion of *M. indica* a very good haematinic (Aboaba, 2002) will improve the anaemic condition of sicklers. All of the foregoing shows why recipe 2 gave the better result. It is therefore recommended as a good candidate for managing SCA.

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