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

A. Egunyomi1*, J. O. Moody2 and O. M. Eletu1

1Department of Botany and Microbiology, University of Ibadan, Nigeria.
2Department of Pharmacognosy, University of Ibadan, Nigeria.

Accepted 27 October, 2008

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-crisis 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, ethnomedicines, 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-
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 seems to indicate a significant diminution of painful episodes in treated individual (Isaacs-Sodeye et al., 1975). Active principles were found to be hydroxybenzoic 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 Schecter, 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 antisickling 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 erythrocytes 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.

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

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

Table 2. Plants constituting Recipe 2.

<table>
<thead>
<tr>
<th>Botanical names</th>
<th>Part used</th>
<th>Chemical constituents**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vernonia amygdalina Dcl</td>
<td>Leaves</td>
<td>Vernodalin, Vernomygdin</td>
</tr>
<tr>
<td>Garcinia cola Heckel</td>
<td>Root</td>
<td>Kolaviron, Apigenin, Amentoflavone</td>
</tr>
<tr>
<td>Mangifera indica Linn</td>
<td>Bark</td>
<td>Quercertin, Methylsalicylate</td>
</tr>
<tr>
<td>Terminalia catappa Linn</td>
<td>Leaves</td>
<td>Tannin</td>
</tr>
<tr>
<td>Newbouldia laevis Seem.</td>
<td>Bark</td>
<td>Tannin</td>
</tr>
<tr>
<td>Z. xanthoxyloides (Lam.) Water.</td>
<td>Bar &amp; Root</td>
<td>Fagarol Pseudofagarol, P. hydroxybenzoic acid</td>
</tr>
<tr>
<td>Capsicum frutescens Linn</td>
<td>Fruit</td>
<td>Capsaicin</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Time of incubation (min)</th>
<th>% of unsickled red blood cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
</tr>
<tr>
<td>0</td>
<td>43.6</td>
</tr>
<tr>
<td>45</td>
<td>53.8</td>
</tr>
<tr>
<td>90</td>
<td>57.8</td>
</tr>
<tr>
<td>135</td>
<td>59.8</td>
</tr>
<tr>
<td>180</td>
<td>63.4</td>
</tr>
</tbody>
</table>

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 met abolic 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 metholic 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
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 deriva-
tives 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
erthrocytes. 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 asso-
ciated 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 experim-
ent for sickling reversion indicates that \textit{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 \textit{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 \textit{in vivo} conditions than has hitherto been
demonstrated.

Recipe 2 is composed of seven plant species. Of these, \textit{Z. xanthoxyloides} (Fagara) and \textit{T. catappa} have been
reported to have antisickling activity (Sofowora et al.,
1979; Moody et al., 2003). \textit{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), \textit{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 \textit{V. amygadalin}a is justified.
\textit{Garcinia kola} (root) is a remedy for inflammations and
respiratory tract infections (Gill, 1992) hence beneficial
for management of SCA. \textit{Capsicum frutescens} contains
“capsaicin” which acts as a carminative and counter-
irritant (Oliver Bever, 1960) while the inclusion of \textit{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.

REFERENCES
Abimbola Sodipe RO (1985). Traditional treatment of Hypertension
Stroke Asthma Sickle Cell Anaemia Small Pox and Diabetes. The
State of Medicinal Plants in Nigeria. Proceedings of a Conference of
Pharmacy at University of Ife. pp. 66-72.
Aboaba TR (2002). Uses, Sourcing and Conversation of some
medicinal plants in Southern Nigeria. Unpublished Ph.D. Thesis of
the University of Ibadan. p. 341.
Adesanya SA, Idowu TB, Elujoba AA (1988). Antisickling activity of
\textit{Adansonia digitata}. Plant. Med. 54: 374.
Adesanya SA, Sofowora EA (1983). Biological standardization of
increases the oxygen affinity of sickle cell blood. Biochem. Biophys.
Dean J Schechter AN (1978). Sickle cell anaemia: molecular and
cellular basis of therapeutic approaches. N. Engl. J. Med. 299: 863-
870.
Dennis D, Robers A (1990). Trease and Evans Pharmacognosy. The
cajan} Plant. Med. 6: 504-507.
Ekeke GL, Shode FO (1990). Phenyllalanine is the predominant
antisickling agent in \textit{Cajanus cajan} seed extract. Plant. Med. 56: 41-
43.
El-Said F, Fadulu SO, Kuye JO, Sofowora EA (1971). Native cures in
Nigeria II: The antimicrobial properties of buffered extracts of chewing
sticks. Lloydia, 34: 72-174
Evans WM (1989). In Trease and Evans, Pharmacognosy. The Alden
Benin, Nigeria, p. 276.
Isaacs-Sodeye WA, Sofowora EA, Williams AO, Marquis VO, Adekunle
AA, Anderson CO (1975). Extracts of \textit{Fagara zanthoxyloides} root in
sickle cell anaemia: clinical trial and toxicology. Acta haematologica,
53: 158-164.
Professional Perspective, p. 487.
activity of \textit{Terminalia catappa} leaves harvested at different stages of
Noguchi CI, Schechter AN (1978). Inhibition of gelation by amino acids
and related compounds. Biochemistry, 17: 5455-5459.
Olajide OA, Awe SO, Makinde JM (1997). Pharmacological studies on
Cambridge University Press, p. 466.
Sofowora EA, Isaacs-Sodeye WA, Ogunkoya LO (1979). Isolation and
Characterization of an antisickling agent from the root of \textit{Fagara
anthoxyloides} African Medicinal Plants. Proceedings of a
pawpaw fruit (\textit{Carica papaya}). Trans. Royal Soc. Trop. Med. Hyg. 81:
510-511.