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Phytochemical and Antisickling Studies of the Leaves of *Hymenocardia acida* Tul (Euphorbiaceae)

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Abstract: The leaves of *Hymenocardia acida* are commonly used in Northern Nigeria alone or in combination with other plant parts to manage sickle cell disease. Phytochemical screening and antisickling studies were carried out. The phytochemical screening revealed the presence of carbohydrates, tannins, flavonoids, saponins, alkaloids, cardiac glycosides, resins, steroids and terpenes. The leaves ethanol extracts at 0.5, 1.0 and 2.0% w/v were observed to reverse sickled human Red Blood Cells (RBC) using microscopic technique. The antisickling activity was found to be dose dependent. The fractions containing flavonoids, saponins and carboxylic acids were found to be responsible for reversal of the sickled RBC. Therefore, the use of the plant by the traditional medical practitioners in the treatment of sickle cell anaemic patients is justified.

Key words: *Hymenocardia acida*, leaves, phytochemical, reversed sickled RBC

INTRODUCTION

The plant *Hymenocardia acida* Tul belongs to the family Euphorbiaceae. It is commonly known as Jan yaro (Hausa), yawa satoje (Fulani), Ikalaga (Igbo), or Orupa (Yoruba) in the specified Nigeria languages. It is usually a shrub about 6 m (20 ft) high with twisted branches and orange-brown bark (Dalziel, 1937; Keay *et al*., 1964). It is widely spread in Tropical Africa and commonly found in the Savannah forest. It has been found in Senegal, Sera Leone, Togo and Nigeria (Dalziel, 1937; Keay *et al*., 1964).

The plant has been reported to have varying traditional medicinal uses (Irvine, 1961). It has been shown to have antimicrobial (Muanza *et al*., 1994), but has no inhibitory activity against HIV (Muanza *et al*., 1995).

In Northern Nigeria, the leaves and stem are used in the treatment of eye infection and sickle cell anaemia (oral communication).

Sickle anaemia is a genetic disease. In Nigeria up to 3% of the population suffer from the disease (Moody *et al*., 2003). In the search for alternative to *Fagara zanthoxyloides* in the treatment of sickle cell anaemia (Sofowora, 1979), *Hymenocardia acida* is one of the most commonly used plant for the management of this disease in Northern Nigeria. *Fagara zanthoxyloides* is not commonly found in Northern Nigeria. There are few drugs (e.g., 2-hydroxymethylbenzoic acid, depanostat®) in orthodox medicine that act directly on the sickled cell to alleviate the disease condition. Mostly haematinics, antibiotics, analgesics, water and/or blood infusion are used.

This research aims at determining the chemical constituents and antisickling activity of the leaves of *Hymenocardia acida*.

MATERIALS AND METHODS

Plant collection and preparation: The branches of the *Hymenocardia acida* were collected from bushes of Dajin Tohu around Shika Dam, Zaria. It was identified on the field using descriptions given in the monograph (Dalziel, 1937; Keay *et al*., 1964). It was authenticated at the Herbarium, Biological Sciences, Ahmadu Bello University (A.B.U), Zaria. The voucher specimen number given was (7108) identified by A.O. Ohieri.

The leaves of *H. acida* were separated from the branches, dried under the shade for 2 weeks and then in the oven at 40°C for 24 h. The dried leaves were powdered using mortar and pestle and sieved using sieve mesh size 20.

The following studies were conducted at the Department of Pharmacognosy and Drug Development, A.B.U, Zaria. Nigeria.

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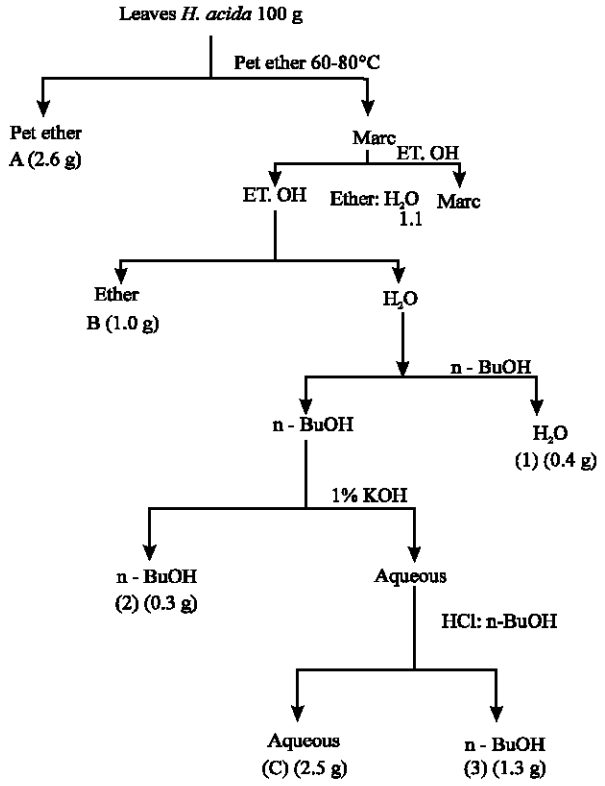
Phytochemical screening: The leaves were tested for the presence of chemical constituents such as carbohydrates, tannins, resins, balsams, glycosides, terpenes and steroids (Evans, 1996; Balbaa, 1976; Sofowora, 1993).



Scheme I: Extraction of the leaves of *Hymenocardia acida*. Key: Pet. Ether-Petroleum ether, H_2O-Water, Et. OH-Ethanol, n-BuOH-n-butanol, HCl-hydrochloric acid

Extraction of the leaves of *H. acida*: Fifty grams of the powdered leaves was extracted by maceration with ethanol. The extract was concentrated in vacuo and used to test for the antisickling activity.

The fractionation was carried out as given on scheme I (Woo *et al*., 1980). Fractions B, 1, 2, 3 and C resulting from the ethanolic extracts were tested for antisickling activity. The residues were dissolved in water or their respective solvents used for fractionations.

Antisickling activity: The antisickling activity (Reversal of sickled RBC) was determined using standard methods (Sofowora, 1979; Fasanmade; Olaniyi, 1991).

RESULTS

Phytochemical screening: The phytochemical screening revealed the presence of carbohydrates, tannins, flavonoids, saponins, cardiac glycosides. terpenes, steroids and alkaloids in the leaves of *H. acida*.

The chemical test of the various fractions revealed the following compounds A-terpenes, steroids and alkaloids; B-carboxylic acid; 1-Saponins and flavonoids; 2-saponins; 3-saponins; C-flavonoids.

Antisickling activity: The leaves were found to possess antisickling activity. The activity was found to be dose dependent (Table 1-4). The RBC were observed to change from the sickled shape to normal biconcave cells and later observed to increase in size after 30 min (Table 2-4).

All the various fractions (B, 1, 2, 3, C) soluble in water were observed to reverse sickling. The ether soluble fraction causes deformation of the cells. They were seen

Table 1: Inhibition of sickling-leaves of *H. acida*

Concentration	No. of RBC observed	No. of sickle cells before drug	No. of sickle cell after drug	Percentage inhibition of sickling
Control	530-590 (560)	30-370 (350)	330-370 (350)	-
0.5%w/v				
0 min	530-590 (560)	330-370 (350)	20-28 (24)	93.1
10 min	530-590 (560)	330-370 (350)	11-15 (13	96.3
30 min	530-590 (560)	330-370 (350)	6-8 (7)	97.9
60 min	530-590 (560)	330-370 (350)	-	100.0
1.0%w/v				
0 min	530-590 (560)	330-370 (350)	20-24 (22)	93.8
10 min	530-590 (560)	330-370 (350)	-	100.0
30 min	530-590 (560)	330-370 (350)	-	100.0
60 min	530-590 (560)	330-370 (350)	-	100.0
2.0%w/v				
0 min	530-590 (560)	330-370 (350)	23-30 (26.5)	92.5
10 min	530-590 (560)	330-370 (350)	-	100.0
30 min	530-590 (560)	330-370 (350)	-	100.0
60 min	530-590 (560)	330-370 (350)	-	100.0

Key - Absence of sickled cells, Average values in bracket, %w/v Percentage weight by volume, min minutes

Table 2: Results of effect of the plant ethanol extract on erythrocyte sickling at 2% concentration

Time interval (min)	Observation leaves
0	The cells appeared in clusters of about 11-16 cells in a group but they were completely normal
10	The cells appeared to have increased in size with the clusters reducing to about 3, 4 and 8 cells in a cluster. They are all normal cells.
30	The cells were still in group much bigger than before and normal.
60	The cells were same as observed at the 30 mins interval

Table 3: The effect of plant ethanol extract on erythrocyte sickling at 1% concentration

Time interval (min)	Observation leaves
0	The cells were normal with few sickle cells
10	The cells seen were of same size as the control with no sickled cell seen.
30	The cells were of the same size as that of the control. No sickled cell was seen.
60	The cells appeared to have decreased in size as such were not clearly visible. The few cells seen appeared to be in cluster of many cells packed together

Table 4: The effect of plant ethanol extract on erythrocyte sickling at 0.5% concentration

Time interval (min)	Observation leaves
0	The cells appeared smaller than normal.
10	There seems to be not much change in the size of the cells but the number of sickled cells appeared to have decreased to about 13 cells.
30	There was no change in size of the cells but the numbers of sickled cells have decreased to about 7 cells.
60	The cells were same as observed under 30 min interval, but no sickled cells were seen

to be irregularly shaped and smaller in size. The clusters of RBC observed with ethanol extract were not observed with the fractions.

DISCUSSION

The ethanolic extracts of the leaves were seen to reverse the sickling of RBC. Many drugs given to patients in the management of sickle cell conditions in our hospitals do not have effect on the sickle cells. The treatments are usually supportive with haematenics (Sofowora, 1979), analgesics and fluid infusion. Some plants have been discovered to have antisickling activity like the *Fagara zanthoxyloides* (Sofowora, 1979). *F. zanthoxyloides* is not available in northern part of Nigeria. Therefore the discovery of other plants with antisickling activity will supplement it.

The study revealed that the leaves of *H. acida* contained various chemical constituents which led to the fractionation of the plant leaves extract.

All the fractions tested (B, 1, 2, 3 and C) were found to reverse the sickling of RBC. Therefore carboxylic acids, flavonoids and saponins are responsible for the antisickling activity of the leaves of *H. acida*. The fractions did not produce clusters of reversal sickled cells as with the ethanol extracts. This shows the purification and isolation will enhance the antisickling activity of the constituents. Saponins have been shown to possess antisickling activity (Evans, 1996; Moody *et al*., 2003). Zanthoxylol, a butyric acid derivative and 1-hydroxylbenznoic acid were isolated from *Fagara zanthoxyloides* which are responsible for the antisickling activity of this plant (Sofowora, 1979). This research has shown that more than one constituent is responsible for the antisickling activity. Isolation of anyone of them can be used to formulate a standard preparation. Scientific evidence has been provided for the use of this plant in the management of sickle cell anaemia. Further work needs to be done to isolate and characterize the various compounds responsible for the antisickling activity.

CONCLUSIONS

The leaves were found to contain carbohydrates, tannins, glycosides (saponins flavonoids), terpenes, alkaloids and steroids. Also, it could reverse sickled red blood cells. The fractions containing saponins, flavonoids and carboxylic acid were found to be responsible for this activity. Therefore, from the above results; the plant *Hymenocardia acida* might be potential sources of antisickling agent for sickle cell patients.

This justifies the use of plant in the management of sickle cell disease.

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