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PHYTOCHEMICAL AND ANTI-SICKLING PROPERTIES OF HYMENOCARDIA ACIDIA (*Tul*)

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Summary:The plant *Hymenocardia. acidia* is a member of the family *Euphorciaceae*. The various morphological parts are used in Nigeria for the treatment of eye infections, diarrhoea, dysentery, rheumatic pains and most importantly sickle cell anaemia. The present study aims at unveiling the reality or otherwise of its anti-sickling claim. To this end, the plant was processed accordingly and assayed for anti-sickling activity using a standard method. The stem-bark and leaves reversed sickling red blood cells in a dose dependent manner. Moreover, this study also revealed the presence of tannins, flavonoids, saponins, alkaloids, resins, steroids, terpenes, etc. Thus the use of the plant in traditional medicine practice in the treatment of sickle cell disorder might be justified.

Key Words: *Hymenocardia acidia*, traditional medicine practice, anti-sickling activity, red blood cells.

Introduction

The plant, *Hymenocardia acida* (Tul) belongs to the family *Euphorbiaceae*. It is commonly known as *Jan yaro* (Hausa), Yawa *satioje* (Fulani), *Ikalaga* (Igbo) or *Orupa* (Yoruba) in Nigeria. The plant is a shrub about 6m (20ft) 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 S. Leone, Togo and Nigeria (Dalziel, 1937; Keay *et al*, 1964).

In Akwa Ibom State, the leaves and stem are used in the treatment of eye infection and sickle cell anaemia. The root decoction is used for fever, conjunctivitis, trachoma and as aphrodisiac, the leaves and stem infusion for respiratory diseases, diarrhoea and dysentery. The powdered leaves are used as snuff or applied locally for various pains e.g. headache, rheumatic pains and toothaches (Irvine, 1961). The leaf infusion has been reported to be useful in the oral treatment of urinary tract infections, anaemia, diabetes and topical application for skin diseases in Northern Nigeria (Muanza *et al*, 1994).

Several biological activities have been associated with this plant. Some of these include; the dried leaves have inhibitory activity against, *Streptococcus mutans* M.I.C. 125mcg/ml, *Klebsiella pneumoniae* M.I.C. 250mcg/ml, *Staphylococcus aureus* M.I.C. 500mcg/ml, (Muanza *et al*, 1994). The dried root had inhibitory activity against *Shigella dysenteriae* M.I.C. 12.5mg/ml, *Staphylococcus aureus* M.I.C. 21.5mg/ml, *Escherichia coli* M.I.C 25mg/ml, *Pseudomonas aeruginosa* M.I.C. 25mg/ml, *Salmonella Typimurium* M.I.C 25mg/ml, *Streptococcus faecalis* 3.13mg/ml, *Vibrio cholera* 6.25mg/ml, *Candica albicans* 25mg/ml (Silva *et al*, 1996).

The plant extract is believed to posses weak cytotoxic activity against breast cancer, CNS cancer and lung cancer and no inhibitory activity against HIV (Muanza *et al*, 1995). This study aims at determining the bioactive ingredients and anti-sickling potentials of the stem and leaves of *Hymenocardia acida* (Tul).

Materials and Methods

Plant Collection and Preparation

The plant, *Hymenocardia acida* was collected from Eket Forestry Reserve in Akwa Ibom State Nigeria. It was identified on the field using descriptions given in a monograph (Dalziel, 1937; Keay *et al*, 1964) and was authenticated by the taxonomist of the department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria.

The different morphological parts were separately processed; the leaves were dried for two weeks and crushed into powder while the stem bark were cut into small pieces, crushed, processed into fine powder and dried in an oven at 40-45^{0}C for 48 hours. The powders were then extracted with ethanol and evaporated to dryness using Rotary-evaporator.

Phytochemical Screening

The powders were treated separately. Each was suspended into methanol and, or distilled water as the case may be to investigate the presence or otherwise of potential bioactive ingredients in the parts (leaves and stem bark) using standard methods (Evans, 1996; Balbaa, 1976; Sofowora, 1993).

Anti-Sickling Activity

The anti-sickling activity was determined using the methods described by Fasanmade and Olaniyi (1991) and Sofowora (1979). 1ml of blood was collected in EDTA bottle from sickle cell patient and washed three times in phosphate buffered saline (PBS). The erythrocytes were then suspended in PBS such that the concentration of the erythrocytes was diluted 10-fold. This stock solution was used in experiment I-III.

Control Experiment: 0.5ml of washed erythrocyte was mixed with 0.5ml of 2% sodium metabisuphite and 0.5ml of PBS. A few drops of this on a slide (sealed with a cover slip) was observed under the microscope and the percentage sickling determined at intervals of 0, 15, 30 and 60 mins by counting different views (Fasannmade and Olaniyi, 1991).

Inhibition studies:

0.5ml of washed erythrocytes was mixed with 0.5ml of 2% Sodium metabisulphite and 0.5ml of 5% extract. A few drops of this was observed on a slide and the percentage sickling determined at the following interval 0, 15, 30, 60 mins by counting from different views (Fasannmade and Olaniyi, 1991). A total of five hundred cells at least were counted for each sample from different fields of view (Sofowora, 1979). 0.5ml of washed erythrocytes was mixed with 0.5ml of 2% sodium metabisulphite. This was incubated in a water bath for 30 mins to ensure complete sickling of erythrocytes and 0.5ml of 0.5%w/v extract was then introduced. A few drops of this were observed under the microscope at time intervals of 0, 15, 30, 60 mins counting was done from different fields of view ((Fasannmade and Olaniyi, 1991). A control experiment of this was conducted using 0.5ml PBS in place of 0.5ml extract and same procedure repeated as in the sample ((Fasannmade and Olaniyi, 1991). This procedure was repeated for 1.0%w/v and 2%w/v levels of extract.

Table 1: *The chemical constituents of Hymenocardia acida (Tul)*

Constituents	*Stem bark*	*Leaves*
Carbohydrates	++	++
Tannins	++	++
Flavonoids	+++	+++
Saponins	++	++
Anthraquinones	+++	+++
Cyanogenic glycosides	-	-
Cardiac glycosides	++	++
Terpenes	+	+
Steroids	+	+
Resins	-	-

'absent, '+present, '++present in large quantity, '+++ present in very large quantity.

Anti-Sickling Activity

The stem bark and leaves were found to possess anti-sickling activity. This activity was found to be dose dependent (tables 2, 3, 4, 5, 6). The RBC were observed to change from the sickled shape to normal biconcave cells and was observed to increase in size after 30 mins.

Results

Phytochemical Screening

The leaves were found to contain carbohydrate, alkaloids, tannins, flavonoids, cardiac glycosides, saponins, terpenes and steroids. Anthraquinones and cyanogenic glycosides were absent (table 1).

The stem bark was found to contain carbohydrate, alkaloids, tannins, flavonoids, cardia glycosides, saponins, anthraquinones, terpenes and steroids. Resins and cyanogenic glycosides were absent (table 1).

Table 2: *Inhibition of Sickling – Stem Bark* of *H. acida.*

Concentration/Time Of exposure	*No. of RBC observed*	*No. of Sickle cell Before extract*	*No. of sickle cell after extract*
Control	530-590	330-370	330-370
0.5%w/v			
0 mins	530-590	330-370	230-300
15 mins	530-590	330-370	-
30 mins	530-590	330-370	-
60 mins	530-590	330-370	-
1.0%w/v			
0 mins	530-590	330-370	230-300
15 mins	530-590	330-370	-
30 mins	530-590	330-370	-
60 mins	530-590	330-370	-
2.0%w/v			
0 mins	530-590	330-370	230-300
15 mins	530-590	330-370	-
30 mins	530-590	330-370	-
60 mins	530-590	330-370	-

Table 3: Inhibition of Sickling-Leaves

Concentration/Time of exposure	*No. of RBC observed*	*No. of Sickle cell before extract*	*No. of sickle cell after extract*
Control	530-590	330-370	330-370
0.5%w/v			
0 mins	530-590	330-370	320-350
10 mins	530-590	330-370	250-260
30 mins	530-590	330-370	180-210
60 mins	530-590	330-370	150-170
1.0%w/v			
0 mins	530-590	330-370	200-240
10 mins	530-590	330-370	130-150
30 mins	530-590	330-370	100-120
60 mins	530-590	330-370	80-100
2.0%w/v			
0 mins	530-590	330-370	120-180
10 mins	530-590	330-370	80-130
30 mins	530-590	330-370	40-70
60 mins	530-590	330-370	-

Table 4: Result of Effects of Plant Aqueous Extract on Erythrocyte Sickling at 2%W/V Concentration

Time Interval (Mins)	*Observations*	
	STEM-BARK	*LEAVES*
0	The cells were dispersed and they appeared quite healthy.	The cells appeared in clusters of about 11-16 cell in a group but they were completely healthy.
15	The cells appeared to have increased in size more than that of the leaf under same time interval and are not clustered.	The cells appeared to have increased in size with the clusters reducing to about 3.4 and 8 cells in a cluster. They were all normal cells.
30	The cells increased to almost double their size and were normal. The biconcave shape is clearly visible.	The cells were still in group much more bigger that before and appeared healthier.
60	The cells appeared in groups of 2,3,4 and 5 but were same as that observed under 30 mins interval.	The cells were same as observed at time 30 mins interval.

Control: The cells were dispersed with very few clusters visible; there was about 40% sickling.

Discussion

The extracts were observed to inhibit further sickling of the red blood cells. The extracts of the stem bark and 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 haematinics, analgesics and fluid infusion. Most of the plants screened, which have been claimed to have anti-sickling activity, were discovered otherwise although some might possess analgesics and/or anti-inflammatory activities (Balbaa, 1976; Sofowora, 1993). A few have been discovered to have anti-sickling activity like the Fagara, zanthozyloides (Adesanya *et al*, 1988), which is not available in all parts of the country. Therefore, the discovery of other plants with anti-sickling activity will supplement it.

These activities of this extracts could be linked with the presence of saponins, phenolic compounds and cyanogenic glycosides, which have been reported to have anti-sickling activity (Balbaa, 1976). From the above results, the plant *Hymenocardia acida* might be a potential anti-sickling agent for the treatment of sickle cell disorder.

Table 5: Result of Effects of Plant Aqueous Extract on Erythrocyte Sickling At 1%W/V Concentration

Time Interval (Mins)	*Observations*	
	STEM-BARK	*LEAVES*
0	The cells were of the same size as the control but the sickle cells were very few compared to the control.	The cells were same as that of the control with few sickle cells
15	The cells appeared to have increased in size and were more dispersed than the control. No sickled cells were seen.	The cells were of same size as that of the control. No sickled cells were seen.
30	The cells increased to have increased in size and are more dispersed than the control. No sickled cell was seen.	The cells appeared to have decreased in size and as such were not clearly visible. The few cells seen appeared to be in clusters of many cells packed together.
60	The cells appeared much more larger than before and some appeared to be in clusters of 6,8, and 4 cells in a group.	The cells appeared to have decreased in size and as such were not clearly visible. The few cells seen appeared to be in clusters of many cells packed together.

Control: The cells were dispersed and there was about 50% sickling. The healthy cells were of normal size and shape while the sickled ones were either completely sickled or partially sickled.

Table 6: The Effect of Plant Ethanol Extract on Erythrocyte Sickling at Concentration 0.5%

Time Interval (Mins)	*Observations*	
	STEM-BARK	*LEAVES*
0	The cells appeared to be in dispersed form with some few sickled ones seen.	The cells appeared smaller than those seen using the bark extract. The number of sickled cells was comparable to that of the bark.
15	The cells appeared dispersed and are mostly healthy looking with about 10 sickled cells visible. There seems to be an increase in size of cells.	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	The cells were same size and are normal looking cells. There were about 2 sickled cells visible.	There was no change in size of the cells but the number of sickled cells has decreased to about 7 cells.
60	The cells observed were all of normal shape and there was no change in size of the cells.	The cells were same as observed under 30 mins interval, but no sickled cells were seen.

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