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Red blood cell membrane stabilizing potentials and phytochemical assessment of water extract thylakoids of *Senna tora* Linn in natural population of Northeastern Nigeria

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Abstract

Water extract thylakoids of *Senna tora* linn, a legume in the subfamily of caesalpinioideae which grow wild in the tropic and considered as weed in many places but with great tradomedical value worldwide was screened using standard laboratory procedures for phytochemicals and haemolytic activities on human erythrocytes. The phytochemical analysis revealed the presence of three bioactive chemicals, flavonoids, terpenoids and cardiac glycosides. Haemolytic activities on human erythrocyte showed concentration dependent partial haemolysis with 4.4% at the highest concentration of 1000 µg/ml, an indication of its medicinal value, high nutritional value and less toxicity of the extract.

Keywords: herbs, thylakoids, chlorophyll, phytochemicals, haemolysis.

1. Introduction

Many plants have been documented to have the efficacy in treating various disease states, such is the case with *Senna tora* Linn. *Senna tora* (originally described by Linn as cassia tora), a legume in the subfamily caesalpinioideae. It grows wild in most of the tropics and is considered a weed in many places. A stout erect, smooth, rank-smelling, half-woody annual, and about one meter in height weed, *Senna tora* Linn's native origin is not well known but probably South Asia ^[1, 2]. It is often confused with Chinese Senna or sickle pods obtusifolia. It is known in Nigeria by different names, Rere Ako (Yoruba) and Ochigichi (Igbo). Personal interaction with herbalists and "Iya-Elwe-Lekuleja" (Traditional Pharmacists) in North-eastern Nigeria showed its relevance in the local herbal medical formulation. *Senna tora* linn either whole or parts are used by tradomedical practitioners as liver stimulant, mild laxative, heart tonic, feverish condition, laxative, antiperiodic, leprosy, ringworm, bronchitis, ophthalmic, skin diseases, cough, hepatic disorder, and haemorrhoids ^[3]. The leaves of *Senna tora* has reported to have antioxidant activity and contain many active substances including chrysophenol, emodin, rhein ^[4, 5]. The roasted and grinded seeds are used as coffee substitute while the leaves are used as pot herb (soup) by some tribes in Nigeria.

Medicinal herbs used for therapeutic purposes are known to have many phytochemicals, however, the indiscriminate use of herbal preparations by 70 – 80% of world's population ^[6] with regards to the proportion of various active constituents, dosage of a particular formulation and the possibility of side effects has been a major challenge facing traditional herbalists, toxicologists and nutritionists ^[7].

Plant and other eukaryotic cells with photosynthetic ability contained tiny, bacteria-sized organelles, called chloroplasts which housed membranous thylakoids ^[8, 9]. Thylakoids, the most abundant biological membrane on earth ^[10] constitute the site of the light dependent reactions of photosynthesis and have a favourable nutritional composition, with 70% membrane proteins and 30% galactolipids ^[9].

Aqueous water extracts with or without alcohol of fresh green leaves are important herbal refreshment and medicinal formulation of Nigerian Nation, aqueous extract of Neem leaves is well accepted as treatment for malaria parasite, Gossypium herbaceum leave extract is taken as antianaemia, aqueous extract of Trichodesma africanum leaves is known for the treatment of diarrhea, dysentery, diuretic and insect sting. Physiological and pharmacological effects of thylakoids extract from spinach have been extensively studied ^[10].

Traditional medical herbs used for therapeutic purposes are known to have many phytochemicals for human consumption, however, environmental conditions such as soil

type, rainfall, agricultural activity, vicinity and type of industry influence the level of their bioavailability in plants ^[11]. Toxicity of bioactive molecule is a key factor in drug designing and dosage formulation, phytochemical and haemolytic screening represents a useful starting point ^[12] where primary information on the interaction between bioactive molecules and biological entity at cellular level is provided.

The aim of this study is to investigate and document the phytochemical constituents and haemolytic activity of *Senna tora* linn thylakoids naturally found in Maiduguri, Northeastern Nigeria which to our knowledge has not been documented before despite the popular use of the plant as pot soup and in Nigerian folkloric medicine.

2. Materials and methods

2.1 Plant material.

Senna tora linn was collected from the natural population growing at Shagari Low-Cost Housing Estate along Gubio Road in Maiduguri, Borno State, Nigeria in August 2014. Maiduguri (lat 11° 24'N, long 11° 8'E) is in Sahel belt with raining season spanning the period between June/July and September/October. *Senna tora* linn was authenticated by experts from Forestry Department of University of Maiduguri, Maiduguri, Borno State, Nigeria.

2.2 Thylakoids Extraction

The fresh leaves of *Senna tora* Linn were washed thoroughly in tap water, followed by distilled water and then deionized water. The extraction was carried out according to Rickard Kohnke *et al.* 2009 ^[10]. Briefly 1000 g of the leaves were blended with 1500 ml of deionized water using stainless domestic blender (Master Chef® China). The homogenate was filtered using filter cloth, followed by Number 1 filter paper. The pH of the filtrate was adjusted to pH 4.7 and kept at 4 °C for 4 hours to settle, after which the green thylakoids sediment and the yellow supernatant were harvested into separate container. The sedimentation process was repeated three times. The harvested green thylakoids was rewashed with deionized water and the sedimentation repeated at the same pH 4.7) which was adjusted to pH 7.0 and the yellow supernatant were dried using lyophilizer (Free Zone® 4.5 Liter Freeze Dry System: Labconco, U.S.). The dried extracts were kept in air tight containers and store at 4 °C for further use.

2.3 Phytochemical Screening

Phytochemical screening of water extract of thylakoids for Carbohydrates, Soluble starch, Tannins, Phlobatannins, Glycosides, Anthraquinone, Terponoid, Cardiac Glycoside, Alkaloid and Saponins were carried out using standard protocols ^[13, 14, 15].

2.4 Haemolytic activity

In vitro haemolytic activity was performed by modified Spectrophotometer method WHO ^[16].

2.5 Preparation of erythrocyte suspension.

Five milliliter of blood was collected from healthy subject into plain specimen tube containing glass beads, swirling for about five minutes to remove fibrinogen. The defibrinated blood was centrifuged at 1500 rpm for five minutes, the plasma discarded and the harvested pellet was washed three times with sterile phosphate buffer saline solution (pH 7.2) by centrifugation at 1500 rpm for five minutes. The washed erythrocytes were

resuspended in normal saline solution to a concentration of 0.5%.

A freshly prepared stock solution of extract in 100 ml volumetric flask of 10 mg in 100 ml normal saline was made. Graded concentrations of 1000 µg/ml; 500 µg/ml; 250 µg/ml; 125 µg/ml; 62.5 µg/ml, 31.3 µg/ml, 15.7 µg/ml and 7.9 µg/ml were made. 1.0 ml of graded concentrations were mixed with 1.0 ml of suspended erythrocytes (0.5%) in separate test tubes, phosphate buffer saline and distilled water tubes as minimal and maximal haemolytic controls, incubated at 37 °C for 1-2 hours, centrifugation at 1500 rpm for five minutes, the absorbance of the supernatants were measured at 540 nm (Chem – 7: Erba Diagnostics Mannheim, Germany).

The percentage haemolysis by the graded concentration of the extract was calculated using

$$\% \text{ haemolysis} = (A_t - A_a/A_c - A_a) \times 100$$

Where A_t = absorbance of test sample; A_a = absorbance of minimal haemolytic sample (PBS); A_c = absorbance of maximal haemolytic sample (Distilled Water).

3. Results

Table 1: Phytochemical screening of Thylakoids extract of *Senna tora* Linn

Phytochemicals	Tests	Thylakoids
Carbohydrates	Molisch Test (General Test)	-
	Fehling Test (Free reducing Sugar)	-
	Combined reducing Sugar	-
	Ketoses	-
	Pentoses	-
Soluble starch		-
Tannins	Ferric Chloride	-
	Lead Acetate	-
Phlobatannins		-
Glycosides	Test for free Anthraquinone (Banbger Test)	-
	Test for Combined reducing Sugar	-
Flavonoids	Shinoda Test	+
	Ferric Chloride	-
	Lead Acetate	-
	Sodium Hydroxide	+
		+
Terponoids		+
Saponins		-
Cardiac Glycosides	Fridthrig Test	-
	Salkowski Test	-
	Leiberman-Burchard Test	+
Alkaloid	Dragendroff's Reagent	-
	Mayer's Reagent	-

Key: + = Present; - = Not present

The phytochemical screening of *Senna tora* linn thylakoid extract as shown in table I indicated the presence of three bioactive chemical, Flavonoids, Terponoids and Cardiac

glycosides.

Table 2: Haemolytic activity of Thylakoids extract of *Senna tora* Linn.

Thylakoids extract (µg/ml)	Percentage Haemolysis (%)
1000	4.4
500	3.8
250	3.3
125	3.2
62.5	3.1
31.3	2.9
15.7	2.8
7.9	2.7

Table 2 showed the concentration dependent haemolysis of human erythrocytes. 4.4% haemolysis was observed at highest concentration of 1000 µg/ml and the lowest of 2.7% at a concentration of 7.9 µg/ml.

4. Discussion

Phytochemical assessment of plant extract furnishes us the preliminary information about the chemical composition and thus the probable medicinal import of the plant [17, 18]. The chemical constituents of any plant extract may vary considerably depending on the place of origin, the mode of cultivation treatment after harvesting and the nature of the herbs, either fresh or dry [11]. Qualitative phytochemical screening in our study revealed the presence of three bioactive metabolites, flavonoids, cardiac glycosides and terpenoids. These three bioactive metabolites have many known therapeutic applications [19]. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to their chemical structures, over 4,000 flavonoids identified in fruits, herbs, vegetables and beverages have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities [20, 21]. Cardiac glycosides are secondary metabolites of plants and animals. They are usually toxic but may have drug-like therapeutic effects when used appropriately, they are known to exert pronounced physiological action in the treatment of congenital heart failure [22]. Terpenoids, a subclass of the prenillipids represent the oldest group of small molecular products synthesized by plants and are probably the most widespread group of natural products. They are universally present in small amounts in living organisms, where they play numerous vital roles in plant physiology as well as important functions in all cellular membranes. Antibacterial activities of terpenoids particularly in the management of upper respiratory tract infection have been recognized for many years [23].

Red blood cell model has been widely used for induction of membrane toxicity [24, 25], Erythrocyte haemolysis, a result of lysis of lipid bilayer is related to the potency and concentration of the extract which is directly related to phytochemicals. Our study (table II) showed concentration dependent haemolysis by the extract. The low haemolytic activity even at a concentration of 1000 µg/ml, the haemolysis was 4.4% which may be considered nontoxic. Further studies are highly needed to tap beneficial effect to the maximum of nontoxic *Senna tora* linn thylakoids

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