PRELIMINARY STUDIES ON SAPONINS EXTRACTS OF TETRACAPIDIUM CONOPHORUM NUT (HUTCH AND DALZ)

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ABSTRACT

Preliminary studies on saponins of *Tetracarpidium conophorum* nut (conophor nut) was carried out. Result of phytochemical screening revealed the presence of alkaloids, tannins, phlobatannins, coumarins, and saponins. Haemolytic activity of crude and purified saponins were tested using human blood groups A, B, AB and O. The highest titre value was observed with blood group A and O with crude and purified saponins respectively. It was suggested that the formation of complex between saponins and cholesterol caused permeability of the membrane, which was responsible for their haemolytic activity.

Key words: Conophor nut, saponins, Haemolysis.

INTRODUCTION

Tetracapidium conophorum belongs to the family *Euphorbiaceae*, mainly found in tropical Africa (Hutchinson and Dalziel, 1978). It is grown in the traditional farming system of the low land humid tropics of Nigeria 4°15'N of the equator (Okafor, 1983). The fruit is a four-winged ribbed capsule, containing subglobose seeds with thin brown shell and yellowish Kernel (Hutchinson and Dalziel, 1978). Conopor nut yields a drying oil and contain 60% linolenic acid which predisposes it for use as edible oil, soap making and in varnish/lacquer industries (Ogunsua and Adebona, 1983) The nuts are also eaten locally and the leaves as vegetable with rice (George, 1974). The nut was chemically analysed to contain 53.38% moisture, 23.5% crude protein, 5.0% crude fibre,2.9% ash, and 47.4% fat (Adesioye, 1991).

Although the nut has been used for food, no attempt was made to screen for the presence of saponins and to evaluate some of its biological activities. In recent years the presence of saponins in plants intended for human or animal consumption has attracted increasing interest, and have been shown to possess both beneficial and deleterious properties (Oakenfull and Sidhu, 1987: Scott and Eagleson, 1988). The term saponins generally refers to a group of natural products which have in common the properties o forming foam when shaken with water, bitter taste, and haemolysing red blood cells (Milgate and Robert, 1995). In the present study the phytochemical

screening of conophor nut was conducted, the saponins of conophor nut purified and the haemolytic activity of the crude and purified saponins evaluated using different human blood groups.

MATERIALS AND METHODS

The nuts were collected fresh from Omi Adio near Ibadan Oyo State, Nigeria and subjected to further treatment.

Different human blood groups were collected from healthy donors (within the College of Medical sciences University of Maiduguri) into clean sterile tube containing EDTA (5mg/ml blood). Blood samples were centrifuged at 3,000 rpm for 5min. the supernatant was discarded and packed cells washed five times with PBS pH 7.2 using packed cells/12ml PBS (Ralston, 1976).

Octadecyl saline (C_{18}) bonded to silica gel, the matrix used for reverse phased flash chromatography, was supplied as a gift from Dr. Keith R. Price of the Agricultural and Food Research Council (AFRC) Institute of Food Research, Norwich Laboratory U.K.

Plant Extraction/Purification

The nut was treated using the method of Joslyn (1970). The ground sample was sieved with 0.025mm sieve (Endecott, London) and aliquot were weighed into Whatman thimbles (60mmx26mm) and then plugged with glass wool. The dried plant part 1kg was extracted in 50g portion as described by Adams and Mc Chesney, (1983) and Oleszek, (1988). Isolation of crude saponins was done based on the method of Kitagawa *et al.*, (1976). The crude saponins was purified using reverse phase flash chromatography column (9.5x1.0cm) Octadecyl silane bonded to silica gel and eluted with 2ml of different grades of methanol in water (v/v) Curl *et al* (1985); Price *et al.*, (1987) and Oleszek, (1988). Saponins were detected by spraying with Liebermann Buchards reagent using TLC as Described by Oleszek (1988).

Phytochemical Screening

Both aqueous and methanolic extracts of conophor nut were used for the phytochemical screening. The methods described by Trease and Evans, (1978) were used to screen for the presence of alkaloids, combined anthraquinones, tannins, and phlobatannins. Anthraquinones was screened using the method of Shellard, (1957), coumarins by Feigl (1960) and saponins by Oleszek, (1988) and Odebiyi and Sofowora (1978).

Haemolytic Activity

The method used was a modification of the agglutination procedure of Gordon and Marquandt (1974). A positive pattern indicating full haemolysis (FH) appeared as a circular big spot of red solution covered by a small zone (if any). While a negative pattern indicating the absence of haemolysis appeared as a uniform small spot of erythrocyte at the bottom of the well surrounded by a big concentric clear zone. In instances where the clump of erythrocyte appeared as a large and non-uniform spot, partial haemolysis (PH) was recorded (Marquandt, 1974).

RESULTS AND DISCUSSION

Results of the phytochemical screening have been summarised in Table 1.

Table 1: Phytochemical Screening

Test	Methanolic extract	Aqueous extract
Alkaloids	+	+
(i) Mayer's	+	+
(ii) Wagner's	+	+
Tannins	+	+
Phlobatannins	+	+
Anthraquinones	-	-
Combined Anthraquinones	-	-
Coumarins	+	+
Saponins		
(i) Liebermann-Buchard's	+	+
(ii) Foaming activity	+	+

Key:- + = Positive
- = Negative

The phytochemical screening indicated the probable presence of alkaloids, tannins, phlobatannins, coumarins and saponins, while anthraquinone and combined anthraquinones were probably absent. Presence of some of these natural products in plant materials have been reported (Caudrado *et al.*, 1995; Kamis, 1997; Banso and Olutimayin, 2001). Also the presence of some of these natural products have been reported in other members of the family Euphorbiaceae such as Acalypha, Euphoria and Ricinus (Rizk and Al-Nownai, 1989). Some of these natural products are therapeutic agents and have been identified in medicinal plants (Ebena *et al.*, 1991).

The haemolytic activity of crude and purified saponins was tested using human erythrocyte blood groups A, AB, B, and O (Table 2).

Table 2: Haemolytic activities of crude and purified saponins

Extract	Pattern of haemolysis	*Titre Blood Group			
		A	AB	B	O
M/PBS	FH	2^2	2^6	2^2	2^2
	PH	2^3	2^{10}	2^8	2^3
PBS	FH	2^1	2^4	2^2	2^0
	PH	2^2	2^7	2^5	2^1
PS	FH	2^1	2^5	2^3	2^1
	PH	2^5	2^8	2^4	2^3

Key:- FH - Full haemolysis
PH - Partial haemolysis

*Titre - Defined as the reciprocal of greatest dilution at which haemolysis occurred.

Both full and partial haemolysis were observed. The highest titre value was observed with blood group AB in both cases, while lowest titre value was observed with blood groups A and O. The result is in agreement with the findings of Kamis, (1997) in which higher titre was reported with blood group AB and lower values with blood group O. Cheeke (1971), earlier suggested that the formation of a complex between saponins and cholesterol causes permeability of the membrane which was responsible for their haemolytic activity. Relationship between haemolytic activity and the structure of saponins, reveals higher contents of medicagenic acid as well as higher ratio of sapongenin to sugar (Gestetner, *et al.,* 1971).

In view of the haemolytic effect of the saponins of conophor nut, proper cooking of the nut should be carried out before consumption. Conophor nut is usually consumed in its cooked form, which perhaps explains why toxic effects have not been reported among humans consuming the nut. However, it might be possible that animals consuming the nuts are likely to suffer mild anaemia. We are currently studying that.

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